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Potential of *Pteris vittata* L. for phytoremediation of sites co-contaminated with cadmium and arsenic: The tolerance and accumulation

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Abstract

Field investigation and greenhouse experiments were conducted to study the tolerance of *Pteris vittata* L. (Chinese brake) to cadmium (Cd) and its feasibility for remediating sites co-contaminated with Cd and arsenic (As). The results showed that *P. vittata* could survive in pot soils spiked with 80 mg/kg of Cd and tolerated as great as 301 mg/kg of total Cd and 26.8 mg/kg of diethyltriaminepenta acetic acid (DTPA)-extractable Cd under field conditions. The highest concentration of Cd in fronds was 186 mg/kg under a total soil concentration of 920 mg As/kg and 98.6 mg Cd/kg in the field, whereas just 2.6 mg/kg under greenhouse conditions. Ecotypes of *P. vittata* were differentiated in tolerance and accumulation of Cd, and some of them could not only tolerate high concentrations of soil Cd, but also accumulated high concentrations of Cd in their fronds. Arsenic uptake and transportation by *P. vittata* was not inhibited at lower levels (⩽20 mg/kg) of Cd addition. Compared to the treatment without addition of Cd, the frond As concentration was increased by 103.8% at 20 mg Cd/kg, with the highest level of 6434 mg/kg. The results suggested that the Cd-tolerant ecotype of *P. vittata* extracted effectively As and Cd from the site co-contaminated with Cd and As, and might be used to remediate and revegetate this type of site.

Key words: arsenic (As); cadmium (Cd); Chinese brake (*Pteris vittata* L.); hyperaccumulator; phytoremediation; tolerance; uptake

Introduction

Large areas of land contaminated with Cd were caused by anthropogenic activities such as mining and mineral processing of metallic ores, waste disposal, phosphate fertilizer application and wastewater irrigation (McGrath *et al*., 2001; Udom *et al*., 2004; Lei *et al*., 2005). Soil Cd contamination is a great threat to human health since Cd is easily extracted by plants from the environment compared with other non-essential elements, and transferred to human food chain from the soils (Lindén *et al*., 2003). Arsenic, also a highly toxic element, is a ubiquitous contaminant of global concern. As sites of As contamination often co-exists higher levels of other heavy metals such as Cd, Cu, Pb, Cr and Zn (Groudev *et al*., 2001; Kim *et al*., 2003), there is an increasing awareness of soils contaminated with As or co-contaminated with As and other metals (Loska *et al*., 2004; Liao *et al*., 2005) and the requirement for developing approaches for some remediation of the co-existing contaminants.

Phytoremediation is emerging as a potential cost-effective solution for contaminated soils through the use of plants to remove pollutants (Salt *et al*., 1995). Vázquez *et al*. (2006) studied the phytoextraction of Cd and As in Cd and As co-contaminated soils by lupin (*Lupinus albus*), however, they did not recommend the plant for Cd and As phytoremediation due to its low removal, 1.04 g/(hm^2·a) for Cd and 8.06 g/(hm^2·a) for As. *Pteris vittata* L. (Chinese brake), an As-hyperaccumulator discovered by Chen's group (Chen and Wei, 2000; Chen *et al*., 2002a) has the ability to hyperaccumulate As in its fronds. In addition, it has characteristics of fast growing, high biomass production, wide geographic distribution and perennial. It has great potential for remediating As-contaminated sites (Chen *et al*., 2002b). A field phytoremediation of As-contaminated farmland using *P. vittata* has been successfully carried out in Chenzhou City, southern China since 2001, with the highest efficiency of As removal being 7.84% after seven months of experiment (Liao *et al*., 2004). Our previous studies indicated that *P. vittata* had the potential to phytoremediate or revegetate soils co-contaminated with As and Zn (An *et al*., 2006). Fayiga *et al*. (2004) showed the capability of *P. vittata* in hyperaccumulating As from soils was adversely impacted in the presence of Cd spiked with 50 and 200 mg/kg. Lei *et al*. (2005) found that the *P. vittata* growing in the Caishan tailing pond, Shizhuyuan mining areas could concentrate Cd in the roots up to 365 mg/kg, which was 21.5 times above the highest concentration of Cd in other fourteen

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Table 1 Properties of the soil used in the greenhouse experiment

Properties	Value
pH	7.9
Organic matter content (g/kg)	14.4
Total N (g/kg)	1.1
Olsen-P (mg/kg)	19.0
NH_4OAc-extractable K (mg/kg)	85.2
Total Cd (mg/kg)	0.08
Total As (mg/kg)	5.5

plant species investigated. Whether *P. vittata* can be used to remediate or revegetate mine tailings and soils co-contaminated with Cd and As depends on its Cd tolerance and accumulation. However, so far there is little information available on the tolerance and accumulation of Cd under As and Cd co-contaminated soil. Therefore, in this study, a field investigation and a greenhouse experiment were carried out to verify whether this plant has the potential to remediate sites polluted with Cd and As.

1 Materials and methods

1.1 Descriptions of field sites

Both *P. vittata* and soil samples were collected from twenty-two sites in southern China where *P. vittata* is widely distributed. Sites 1–7 were located in Guangxi Province and Sites 8–22 in Hunan Province. Site 1 was located on farmland, Sites 2 and 4 in sewage ditches, Site 3 on a domestic garbage dump, Site 5 on farmland near a Mn mine area, Site 7 on a sunken riverbed, and Sites 6 and 8–22 around mining, metal refinery or tailing areas.

1.2 Greenhouse experiment

Spores of *P. vittata* collected from arsenic mining areas in Shimen County, Hunan Province were scattered onto a moist soil in a seedbed covered with a plastic cling film to maintain moisture. After the spores germinated and grew into sporelings with true leaves, the plants were watered and fertilized as needed.

The soil used for cultivation was a loam cinnamon soil (Typical Agric-Udic-Luvisols) collected from the top layer (0–20 cm) of farmland in Beijing, China, then air-dried and passed through a 2-mm sieve for the greenhouse experiment. Soil pH was measured using a pH meter in a ratio of soil: solution =1:2.5 (w/v). Important properties of the soil determined according to the methods described by Page *et al*. (1982) are listed in Table 1. Basal fertilizers of 0.4 gN/kg as $(NH_4)_2SO_4$, 0.2 gP/kg and 0.25 gK/kg as KH_2PO_4, and 400 mg As/kg as $Na_2HAsO_4{\cdot}7H_2O$ were mixed with the soil, and then 0.5 kg of the soil was placed into each plastic pot. Cadmium as $CdCl_2$ was added to the pots at concentrations of 0, 10, 20, 60, 80 mg/kg, respectively. Each treatment was replicated four times. After an equilibration time of 1 month, 4 plants with 3 or 4 fronds were transplanted into each pot, and were grown in a greenhouse with temperatures between 22°C (night) and 30°C (day), with a 12-h light period. After fifteen weeks of growth, the plants were harvested.

1.3 Sampling and analysis

P. vittata grown in the greenhouse experiment and collected from the sites studied were separated into rhizoids and fronds. They were washed with tap water, and then rinsed with deionized water. Plant samples were dried at 60°C for 48 h, and weighed. Topsoil samples (0–20 cm) from the field in which the plants were growing were also collected, air-dried, ground using an agate mortar, sieved through a 2-mm screen, then pulverized and passed through a sieve of 0.149 mm for analysis.

The soils were digested with HNO_3-H_2O_2 (Chen *et al*., 2002a), and the plants were digested with HNO_3-$HClO_4$ (Liao *et al*., 2004). Diethyltriaminepenta acetic acid (DTPA)-extractable Cd was extracted by 0.005 mol/L diethylenetriamine pentaacetic acid, 0.01 mol/L $CaCl_2$ and 0.1 mol/L triethanolamine buffered at pH 7.3 (Bailey *et al*., 1995). Cadmium was determined by a graphite furnace atomic absorption spectrophotometer (Vario 6, Analytic Jena, German), and As was analyzed using a hydrogen generation-atomic fluorescence spectrometer (HG-AFS) (AFS-2202, Beijing Haiguang Instrumental Co., China). Blank and standard reference materials for plant (GBW-07603) and soil (GBW-07401) samples obtained from the China National Center for Standard Reference Materials were included for the Quality Assurance/Quality Control program.

1.4 Data analysis

Data were statistically performed with one-way ANOVA using SPSS 13.0 for Windows, and means among treatments for a given property were subjected to LSD test at $P < 0.05$.

2 Results

2.1 Soil Cd concentration at sampling sites

Great variations in Cd concentrations were found in the soils from the investigated sites, ranging from 0.17 to 301 mg/kg (Table 2). Most of the total soil Cd concentrations at the sampling sites were higher than the background soil Cd concentrations (arithmetic means) of 0.27 mg/kg in Guangxi Province and 0.13 mg/kg in Hunan Province, China (CNEMC, 1990), which indicated slight or great elevations of soil Cd levels at these sites. The soil As concentrations varied in the range of 7.6–32.2 mg/kg at most of the sites that were far away from polluted areas. However, for Sites 11–22, high As concentrations usually occurred together with high Cd concentrations in soils from the same site (Table 2). These results indicated that *P. vittata* could grow well at sites seriously co-contaminated with As and Cd, indicating its capability for revegetation of sites co-contaminated with As and Cd.

The DTPA-extractable soil Cd was positively correlated with the total soil Cd ($P < 0.05$). The maximum concentration of DTPA-extractable Cd was 26.8 mg/kg at Site 15, and the value at Site 22 was 13.1 mg/kg and this accounted for 13.2% of the total soil Cd. The soil pH of the sites varied from 4.6 at Site 17 to 8.4 at Site 19, indicating that

Table 2 Soil pH and concentrations of Cd and As in soils and *Pteris vittata* of different field sites

Site No.	pH	Soil As (mg/kg)	Soil Cd (mg/kg)		Plant As (mg/kg)		Plant Cd (mg/kg)		Cd	
			Total	DTPA-Cd	Rhizoid	Frond	Rhizoid	Frond	BF	TF
1	7.6	15.4	1.42	0.70	ND	6.53	ND	0.06	0.04	
2	8.0	23.2	0.54	0.09	ND	150	ND	0.07	0.13	
3	7.6	25.3	0.49	0.07	6.78	52.9	0.03	0.08	0.17	2.5
4	7.7	20.4	0.30	ND	29.5	210	0.14	0.22	0.74	1.5
5	7.9	7.6	2.43	0.25	115	94.7	0.10	0.13	0.05	1.3
6	6.7	21.9	1.13	0.09	31.3	212	0.11	0.11	0.1	1.0
7	5.2	13.9	0.17	0.06	ND	90.8	ND	0.01	0.03	
8	7.6	19.3	3.06	0.14	363	257	0.12	0.12	0.04	1.02
9	7.8	8.8	1.71	0.05	225	269	3.87	3.31	1.9	0.86
10	7.4	15.7	1.52	0.18	7.85	27.1	3.80	3.74	2.5	0.98
11	7.0	1547	25.4	4.18	1074	617	4.61	4.69	0.18	1.0
12	7.3	9406	4.81	2.77	845	388	13.3	8.02	1.7	0.60
13	7.2	313	0.90	0.43	785	1123	3.04	3.45	3.8	1.1
14	8.0	5.5	5.47	0.18	1033	683	4.00	4.81	0.88	1.20
15	7.8	32.2	301	26.8	19.4	1572	263	20.1	0.07	0.08
16	8.1	572	10.7	1.23	1068	294	ND	11.4	1.1	14.8
17	4.6	9118	43.8	2.02	97.1	2362	2.27	2.93	0.07	1.3
18	6.6	1466	22.3	1.22	2067	2680	3.61	5.33	0.24	1.5
19	8.4	932	17.2	0.58	726	304	6.83	15.3	0.89	2.3
20	6.2	30.0	6.97	0.91	35.0	28.0	6.43	10.8	1.5	1.7
21	8.1	694	122	5.12	13.5	87.2	19.6	16.5	0.14	0.84
22	7.1	920	98.6	13.1	1930	93.3	160	186	1.9	1.2

ND: not detected; BF (bioconcentration factor) means the ratio of the Cd concentration in fronds of *P. vittata* to that in soil; TF (translocation factor) stands for the ratio of the Cd concentration in fronds to that in rhizoids of *P. vittata*.

most of the soils were alkaline (Table 2).

2.2 Cd uptake and transportation by *P. vittata* in the field

Cadmium concentrations in *P. vittata* grown at field sites varied substantially with changes in the soil Cd concentrations (Table 2). There was a significant positive correlation between soil DTPA-extractable Cd and the rhizoid Cd concentration ($P < 0.05$). Both rhizoid and frond Cd concentrations of the plants at Sites 1–8 with lower soil Cd concentrations were less than 1.0 mg/kg, and most of the rhizoid Cd concentrations of the plants grown at mining or refinery areas were more than 3.0 mg/kg, while the frond Cd concentrations at some sites were approximately 10.0 mg/kg. The maximum rhizoid Cd concentration was 263 mg/kg at Site 15, and the frond Cd concentration reached 186 mg/kg at Site 22 (Table 2). On the other hand, *P. vittata* grew well in the soil with 9406 mg/kg of As, and had high capacity to accumulate As in the frond with the highest As level of 2680 mg/kg.

Bioconcentration factors (BFs) for Cd in *P. vittata* grown at Sites 1–8 were less than 1.0 (Table 2). There were also low BFs for Cd at severely contaminated sites, i.e. Sites 17 and 21. However, soil Cd could be effectively accumulated by *P. vittata* at Sites 9, 10, 13 and 20, where the BFs were greater than 1.0, and the maximum value was as high as 3.8. *P. vittata* also effectively transport Cd from rhizoids to fronds. The translocation factors (TFs) for Cd at most of the sites were close to 1.0. The BF and TF at the heavily Cd-contaminated Site 22 were 1.9 and 1.2, respectively (Table 2).

2.3 Cadmium accumulation of *P. vittata* in the greenhouse

Addition of Cd to the soil under greenhouse conditions

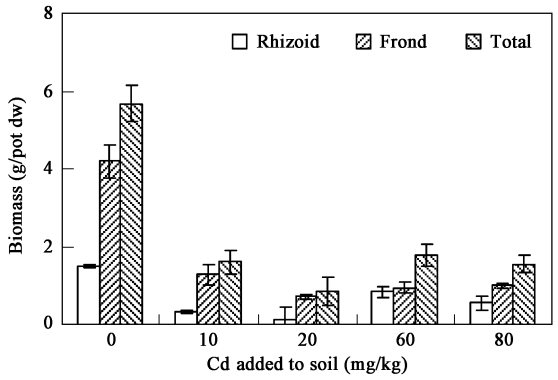

Fig. 1 Biomass of *P. vittata* after transplanting for 15 weeks in soils spiked with 400 mg/kg of As and various concentrations of Cd. Values are presented as means ± standard deviations for 4 replicates.

obviously inhibited the growth of *P. vittata*, and its biomass sharply declined in pot experiment (Fig.1). Compared to the treatment without Cd addition, the dry weights of rhizoid, frond and total biomass were decreased by 77.9%, 69.7% and 71.8%, respectively in the treatment with the addition of 10 mg Cd/kg (Fig.1). These values of the other treatments with Cd addition were also lower than those of the control (Fig.1). The results show that all the treatments with different concentrations of Cd added to soil in this experiment had a strong toxicity to the sporelings of *P. vittata*.

The addition of Cd to pot soil in the greenhouse experiments could promote Cd uptake by *P. vittata*. Rhizoid and frond Cd concentrations were 3.76 and 0.37 mg/kg in the control, and increased to 7.35 and 1.37 mg/kg in the treatment with the addition of 10 mg Cd/kg (Fig.2a). Rhizoid Cd concentrations were significantly enhanced with increase of added Cd concentrations over the range of 10–20 mg/kg, while the difference in frond Cd con-

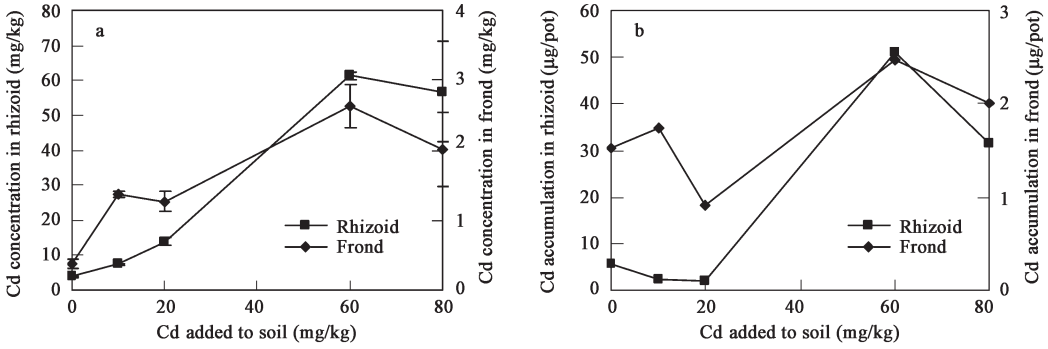

Fig. 2 Cd concentrations (a) and accumulations (b) in *P. vittata* after transplanting for 15 weeks in soils spiked with 400 mg/kg of As and various concentrations of Cd. Values are means ± standard deviations for 4 replicates.

centrations is not significant ($P > 0.05$). Rhizoid and frond Cd concentrations increased to 61.2 and 2.63 mg/kg, respectively when the concentration of Cd added was 60 mg/kg, and then decreased with the addition of 80 mg Cd/kg (Fig.2a). Cadmium taken up by the plant was predominantly retained in the rhizoid, and the TF of Cd was reduced with increasing concentrations of added Cd. The TF was 0.19 for the treatment of addition 10 mg Cd/kg and decreased to 0.04 with 60 mg Cd/kg.

The Cd accumulations (Cd concentration × biomass) in *P. vittata* shifted with the added Cd concentrations. The plant in the soil added with 20 mg Cd/kg showed lower Cd accumulation than the other Cd-spiked soils, while the maximum frond and rhizoid Cd accumulations were 2.47 and 51.0 μg/pot, respectively for the treatment with the addition of 60 mg Cd/kg (Fig.2b).

2.4 Effects of Cd on As accumulation by plants under greenhouse condition

As compared to the control, rhizoid As concentrations increased by 48.0% and 23.0% for the treatments with the additions of 60 and 80 mg Cd/kg, respectively (Fig.3a), and frond As concentrations were increased remarkably with the addition of lower concentrations of Cd (⩽20 mg/kg); these were enhanced 38.5% and 103.8% in the soils spiked with 10 and 20 mg Cd/kg, respectively, reaching to 6434 mg/kg at 20 mg Cd/kg. However, for the treatments with higher concentrations of Cd addition (⩾60 mg/kg), frond As concentrations decreased by 25.6% at 80 mg Cd/kg in comparison to the control (Fig.3a).

The TF of As in the treatments spiked with lower Cd concentrations (⩽20 mg/kg) was much greater than that of the control (Fig.3b). The TF was 75.4% higher at 10 mg Cd/kg than the control, and decreased visibly with higher concentrations of Cd applied to the soil, suggesting that application of lower Cd concentrations favored the transportation of As from rhizoid to frond. Although addition of Cd to soil might disturb metabolisms in *P. vittata*, As uptake and transportation was not inhibited by lower addition concentrations of Cd.

The amount of As accumulated in fronds of *P. vittata* reduced markedly due to the decrease in its biomass for all the treatments spiked with various Cd concentrations. The As accumulation of *P. vittata* was much higher in the control than those in the Cd-amended soils, and decreased by 57.8% and 82.3% in the treatments with addition of 10 and 80 mg Cd/kg, respectively (Fig.3b), showing that the efficiency of As accumulation in *P. vittata* was adversely affected by Cd.

3 Discussion

The field investigation showed that *P. vittata* grew well at many field sites where the total Cd concentration in soil was much higher than 10 mg/kg, greatly exceeding the ranges which were considered toxic to normal plants (Dong and Chen, 1982). Cd-hyperaccumulating plant species such as Brassicaceae (*Thlaspi caerulescens*), amongst higher plants, are almost the only ones that can grow in soil solutions containing Cd concentrations as high as 35 μmol/L (3.9 mg/L) (Brown *et al*., 1994). No visible symptoms of Cd toxicity in *P. vittata* at field Sites 11, 15, 21 and 22 heavily contaminated with Cd, where the highest total soil concentrations of Cd and DTPA-extractable Cd were 301 and 26.8 mg/kg (238 μmol/L), respectively (Table 2), representing 1115 times of their soil background Cd levels for Guangxi and 2315 times for Hunan (CNEMC, 1990). Out of the 22 sites investigated, soil Cd concentrations at 12 sites were higher than 3 mg/kg of Australia Ecological Investigation Levels for Soils (NEPC, 1999). Furthermore, the pot experiment showed that this plant survived in the soil added with 80 mg Cd/kg, though its growth was inhibited. Our previous field survey also showed that *P. vittata* grew well at contaminated sites with high level of Cd (32.1 mg/kg) (Lei *et al*., 2005). So this plant growing in the polluted site exhibited strong Cd adaptability.

There were significant variations in Cd uptake and transportation for populations of *P. vittata*. Concentrations of Cd in the fronds of this plant grown at Sites 11, 15, 21 and 22 were 4.69, 20.1, 16.5 and 186 mg/kg, respectively with corresponding TFs of Cd of 1.02, 0.08, 0.84 and 1.16 (Table 2). Cadmium has strong toxicity to plants, and the normal range of Cd concentration in leaf tissues (dry weight) of some plant species is approximate 0.05–0.2 mg/kg, and the excessive or toxic concentrations are 5–10 up to 30 mg/kg (Kabata-Pendias and Pendias, 2001). The frond Cd concentrations in

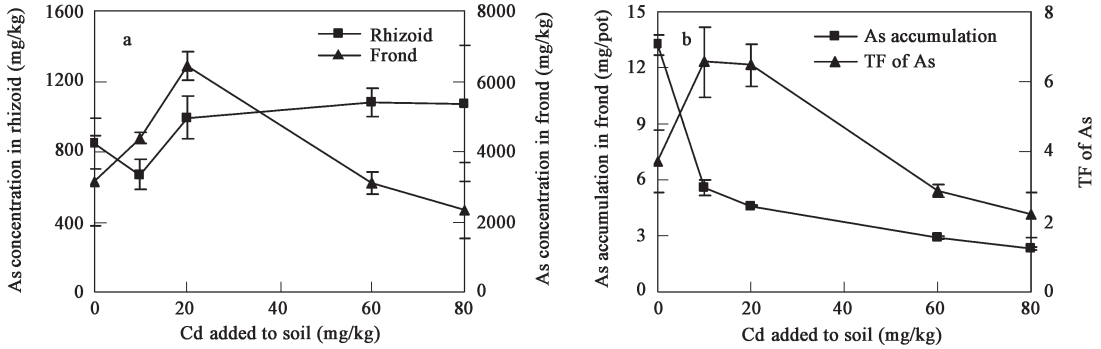

Fig. 3 As concentrations (a) and accumulations (b) in *P. vittata* after transplanting for 15 weeks in pot soils spiked with 400 mg/kg of As and various concentrations of Cd. Values are means ± standard deviations for 4 replicates.

the plants collected from many sites investigated were higher than 5 mg/kg (Table 2), showing that *P. vittata* had exceptional ability to tolerate and accumulate Cd in the fronds. Heavy metals act as a powerful selective force for the evolution of tolerant populations of plants (McNeilly and Bradshaw, 1968). Meerts and Van Iascker (1997) reported that non-metallicolous populations (*T. caerulescens* Subsp. *T. caerulescens*) from the Crand-Duchy of Luxembourg have a lower tolerance and a higher zinc accumulation capacity compared with metallicolous population (*T. caerulescens* Subsp. *calaminare*) of Prayon and Plombières in Belgium. Escarré *et al*. (2000) also showed a higher Cd hyperaccumulation capacity for the metallicolous ecotype compared to the non-metallicolous populations from southern France. Therefore, populations of *P. vittata* growing at Cd-contaminated sites might evolve into Cd-tolerant ecotypes. However, addition of 10 mg Cd/kg under greenhouse conditions with DTPA-extractable Cd of 4.3 mg/kg inhibited the growth of *P. vittata*. (Fig.1), and the frond Cd concentration was only 2.6 mg/kg, with a low BF and TF for addition of 60 mg Cd/kg (Fig.2). However, Fayiga *et al*. (2004) reported that the total biomass of *P. vittata* growing in the soil spiked with 50 mg/kg of Cd increased by 125% compared to the treatment without Cd addition in greenhouse experiments. They speculated that the stimulation of plant growth by Cd might result from added N nutrition since Cd was added as nitrate salts. In our previous study, however, there were no significant differences of biomass of *P. vittata* between treatments spiked with 0.1 mol Ca/L in the form of $Ca(NO_3)_2$ and those applied with Ca as $CaCl_2$ in solution under sand culture (Liao *et al*., 2003). It was implied that the plants propagated from spores collected from sites with low Cd concentrations such as As mine areas had less tolerance to Cd than those grown on Cd-contaminated sites. The results from the greenhouse experiment and field investigation suggest that ecotypical differences in Cd tolerances of *P. vittata* to Cd be present.

At some sites heavily contaminated with As, i.e. Sites 13, 15, 17 and 18, the As concentrations in the frond were far above 1000 mg/kg, with the highest As concentration of 2680 mg/kg under total soil As level of 1466 mg/kg (Table 2), which also support the demonstration that this plant has high capacity to concentrate As in the fronds (Chen and Wei, 2000; Chen *et al*., 2002a). In addition, the greenhouse experiment results showed that addition of low Cd concentrations (⩽20 mg Cd/kg) to soil increased the frond As concentration. The field investigation results indicated that some ecotypes of *P. vittata* tolerated high concentrations of soil Cd and As; on the other hand, *P. vittata* grown at Site 22 could hyperaccumulate Cd (186 mg/kg) in its frond, exceeding the standard of 100 mg/kg for a Cd-hyperaccumulator (Baker *et al*., 1994; Brooks *et al*., 1998). *P. vittata* can grow up to 2 m height with biomass production reaching as high as 36 t/hm^2 (fresh weight) under the favorable environmental conditions in the field (Chen *et al*., 2002a), which has the greater harvestable biomass compared to most documented Cd hyperaccumulators such as *T. caerulescens* with only small aboveground biomass (Cobbett and Meagher, 2002). These results imply that some ecotypes of *P. vittata* offer a great potential for phytoremediation of Cd contaminated soils. Hence, it is feasible to select Cd-tolerant ecotypes of *P. vittata* to phytoremediate and revegetate As and Cd co-contaminated sites. Our previous study demonstrated that this plant could not only tolerate high As but also high Zn levels in growing soils. It grew healthily in the soil applied with 1000 mg/kg of Zn (An *et al*., 2006). The high tolerance of *P. vittata* to Cd and the other heavy metal is important characteristics required for phytoremediation of multiple-metal contaminated soils. The mechanism for the co-tolerance of *P. vittata* to Cd and the other metal toxicities remains to be studied in the future.

4 Conclusions

The present results from a field investigation and greenhouse experiments results show great ecotypical differences in the tolerance of *P. vittata* to Cd. Some of the ecotypes had a high tolerance to Cd, which could grow under very high level of soil Cd (301 mg/kg) and As (9118 mg/kg). The plants grown at As and Cd co-polluted soils effectively absorbed and transported Cd from soil, with a frond Cd concentration of 186 mg/kg and a TF of 1.2. The pot experiment showed that *P. vittata* also could survive in the soil added with 80 mg/kg of Cd. Furthermore, addition of lower concentrations of Cd obviously increased the frond As concentrations. Therefore, a Cd-

tolerant ecotype of *P. vittata* could be used to remediate sites co-contaminated with Cd and As.

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