Environ Sci Pollut Res (2014) 21:325–336
DOI 10.1007/s11356-013-1895-3

RESEARCH ARTICLE

Phytoremediation potential of *Pteris vittata* L. under the combined contamination of As and Pb: beneficial interaction between As and Pb

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Received: 28 February 2013 / Accepted: 30 May 2013 / Published online: 14 June 2013

Abstract The frequent co-existence of arsenic (As) and lead (Pb) necessitates the investigation of clean-up technologies for multi-metal(loid)s. Field survey and hydroponic experiments were conducted to elucidate the co-accumulation of As and Pb in *Pteris vittata* L. The *P. vittata* population isolated from a Pb–Zn mine in Yunnan province, China is a potential extractor of As and Pb co-contamination. Hydroponic experiment found that the highest frond As and Pb concentrations in mining population of *P. vittata* reached 12.2 and 0.99 g kg^{-1}, respectively. The interaction between As and Pb in *P. vittata* was further more disclosed. Pb (2 mg L^{-1}) improved the frond As concentration by 60 to 150 % in mining populations of *P. vittata*. Micro-X-ray absorption spectroscopy indicated that under the combined exposure of As and Pb, the As content in the rhizoid epidermis increased by about 10-fold, and the As(V) percentage increased in each rhizoid tissue, as compared with that under As exposure alone. The co-absorption of As and Pb on the epidermis and the enhanced transportation of As(V) from epidermis into the rhizoid were suggested to contribute to the increased As accumulation.

Keywords Arsenic · Interaction · Lead · Mining population · *Pteris vittata* L. · Phytoremediation

Responsible editor: Elena Maestri

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Introduction

The heavy metal and metalloid contamination of soil and water is a widespread problem; the co-contamination of lead (Pb) and arsenic (As) is a particular challenge because of their high toxicity (Patra et al. 2004). Pb and As are regarded as toxic to organisms even in small amounts (Mandal and Suzuki 2002). The co-existence of multiple heavy metals was reported to increase their toxicity to animals (McDermott et al. 2011; Norwood et al. 2007). The simultaneous presence of Pb and As at high concentrations in the environment is a common phenomenon (Vaca-Escobar et al. 2012; Vaughan 2006) that results from non-ferrous mining and metallurgical activities, as well as the application of $PbHAsO_4$ as a pesticide (Nikolaidis et al. 2010; Wolz et al. 2003).

Several technologies have been developed to remedy contaminated soils (Kwon et al. 2010; Olguin 2010); among these, phytoextraction has emerged to be an economical and environment-friendly alternative (Memon and Schroder 2009; Schwitzguebel et al. 2011). Phytoextraction uses specially selected metal-accumulating plants, named as hyperaccumulators, to remove toxic metals from soil (Salt et al. 1995). Most of the known hyperaccumulators can only tolerate and accumulate a single toxic element (van der Ent et al. 2013). If the selected plant cannot adapt to multiple contaminants at high concentrations, both the plant growth and the cleanup of toxic metals or metalloids through phytoextraction can be negatively influenced (Fayiga and Ma 2006). This phenomenon highlights the need to investigate the interaction between contaminants in plants and plant response to multi-contaminants.

A field survey showed that in a Pb–Zn mine area, Northern Vietnam, the frond As and Pb concentrations of the known As hyperaccumulator *Pteris vittata* L. were as high as 3,750 and 1,020 mg kg^{-1}, respectively (Nguyen et al. 2011). Pot experiments showed that *P. vittata* has a strong

tolerance to Pb, Cd, and Ni with the biomass reaching 19 g pot^{-1} after 8 weeks of culture (Fayiga et al. 2004, 2007; Xiao et al. 2008). These reports implied the potential application of *P. vittata* to clean up Pb–As contaminated sites. However, the interaction between Pb and As remains unclear.

To date, the number of studies on the co-accumulation of more than one toxic element is limited. The most popular multi-element hyperaccumulators are the *Thlaspi* and *Sedum* species, as well as *Potentilla griffithii*, which are capable of accumulating Zn and Cd (Qiu et al. 2011; Zhang et al. 2011). The mechanisms of multi-element co-accumulation and detoxification include the increased availability of metals and the compartmentalization of metals in vacuoles, which are primarily caused by the chemical similarity between Zn and Cd (Leitenmaier et al. 2011; Qiu et al. 2011; Zhang et al. 2011).

Given that As exists predominantly in anionic forms ($H_2AsO_4^-$ and $HAsO_4^{2-}$) in soil under oxic conditions, the interaction between As and other cationic heavy metals differs from that between Cd and Zn. A pot experiment (Fayiga et al. 2004) showed that the As uptake in *P. vittata* was decreased with the increasing Cd concentrations. By contrast, Drava et al. (2012) found that Cd increased the As accumulation by 46.2 to 75.7 % in a hydroponic system. The negative role of Cd in As uptake in the pot experiment may have come from the decreased availability of As in soil due to the addition of Cd.

For the interaction between As and Pb, only a pot experiment was conducted, which found that although the uptake of As was not significantly affected by exposure to 50 mg Pb kg^{-1}, the translocation of As from the rhizoids to fronds was increased (Fayiga et al. 2004). The possibility of similar characteristics between Pb and Cd remains unclear. To the best of our knowledge, a possible mechanism for the interaction between As and cationic heavy metals has not been proposed.

This study conducted a hydroponic experiment to elucidate the interaction between Pb and As in *P. vittata*. Populations growing on contaminated sites may be genetically distinct from populations of the same species grown in sites with low concentrations of heavy metals (Wan et al. 2013). Thus, special attention was given to the differences between mining and non-mining populations of *P. vittata* in terms of the As and Pb uptake. Considering that the reduction of As(V) to As(III) in *P. vittata* rhizoids is one of the most important hyperaccumulation mechanisms (Duan et al. 2005; Pickering et al. 2000), the transformation process of As in *P. vittata*'s rhizoids was studied in detail.

Materials and methods

Field survey

A field survey was performed in Southern China, which has a history of producing nonferrous metals such as Pb, Zn, and As. The characteristics of these sites are described in Table 1. CK1 and CK2 were uncontaminated farmlands in Guangxi and Hunan provinces, China, respectively. LZ2 and LZ3 were contaminated Pb–Zn mining areas near CK1 and CK2, respectively. LZ1 was in the vicinity of a third Pb–Zn mining area in Yunnan province, China, whereas AS1 was close to an As smelter in Hunan province, China. Corresponding uncontaminated sites with *P. vittata* growing were not identified for LZ1. Guangxi, Hunan and Yunnan provinces are all located in Southern China, with the typical climate type being subtropics monsoon climate, continental subtropical monsoon humid climate, and plateau monsoon climate, respectively.

Three *P. vittata* plants were collected from each site, with their corresponding soil samples. The plants were separated into rhizoid (including root and rhizome) and frond (including petiole and pinnae) samples and then flushed with tap water for 1 h followed by three rinses using deionized water. Samples were dried at 60 °C for the subsequent analysis of As and Pb.

Hydroponic experiment

The spores of three *P. vittata* populations collected from sites CK1, AS1, and LZ1, as well as that from the As-tolerant fern *Adiantum capillus-veneris* L. cultivated in greenhouse (Li et al. 2009), were germinated to reproduce via spores for sexual propagation. The loamy cinnamon soil (Agric-Udic-Luvisols) collected from Beijing, China was used for germination. This soil contained 18.5 g kg^{-1} organic matter, 0.88 g kg^{-1} total P, 1.04 g kg^{-1} total N, 14.2 cmol kg^{-1} CEC, 35.9 mg kg^{-1} total Pb, and 8 mg kg^{-1} total As. Precultured sporophytes with a uniform height of ~10 cm were transferred to a modified Hoagland nutrient solution containing, in moles per liter: K_2SO_4 1.875×10^{-4}, $MgSO_4$ 1.625×10^{-4}, KCl 0.25×10^{-4}, $Ca(NO_3)_2$ 0.5×10^{-3}, KH_2PO_4 0.625×10^{-4}, H_3BO_3 0.25×10^{-5}, $MnSO_4$ 0.25×10^{-6}, $CuSO_4$ 0.25×10^{-7}, $ZnSO_4$ 0.25×10^{-6}, $(NH_4)_6MoO_4$ 1.25×10^{-9}, and Fe-EDTA 0.25×10^{-4} (pH adjusted to ~6.0). The chemicals used in the nutrient solution were of analytical or trace metal grade (Sinopharm Chemical Reagent, Beijing, China).

After acclimatization to the hydroponic system for 2 weeks, young sporophytes of *P. vittata* and *A. capillus-veneris* were transferred to nutrient solution containing 5 mg As L^{-1} (66.7 μM As) as $Na_2HAsO_4\cdot7H_2O$ or of 1 mg Pb L^{-1} (4.8 μM Pb) as $Pb(NO_3)_2$ for the As and Pb treatments, respectively. The plants were harvested after 7 days of treatment, washed with tap water, rinsed thrice with deionized water, and dried at 60 °C for the subsequent analysis of As and Pb. The mining populations of *P. vittata* (AS1 and LZ1) were further tested for the interactions between As and Pb. As and Pb were added separately or simultaneously at concentrations of 0, 1, 5, and 8 mg As L^{-1} (0, 13.3, 66.7, and 106.8 μM As) as $Na_2HAsO_4\cdot7H_2O$ or with 0, 1, and 2 mg Pb L^{-1} (0, 4.8,

Table 1 Soil pH and Pb, As concentrations in soil and *P. vittata* at the investigated sites

Site	Site description	Geographical coordinates	Soil						*P. vittata*						
			pH	Total As	Water soluble As	Total Pb	DTPA-extractable Pb		Rhizoid As	Frond As	TF of As	BF of As	Rhizoid Pb	Frond Pb	TF of Pb	BF of Pb
CK1	Farmland	E 108°14′27.4″; N 24°48′12.5″	6.7 b	25 e	0.05 d	94 c	0.7 d		6.8 d	53 d	7.8 b	2.1 b	69 c	83 c	1.2 bc	0.9 ab
CK2	Farmland	E 113°08′12″; N 25°36′28″	7.5 a	7.6 e	n.d.	55 c	0.6 d		120 c	95 d	0.8 c	12.5 a	92 c	87 c	0.9 c	1.6 a
AS1	Near an arsenic smelter	E 113°09′05″; N 25°48′04″	7.7 a	310 d	16 c	210 b	13 c		790 b	1100 b	1.4 c	3.6 b	170 b	330b	2.0 b	1.6 a
LZ1	Lead–zinc mine area	E 103°15′05″; N 23°23′04″	6.5 b	570 c	22 b	1700 a	32 c		1110 a	290 c	0.3 d	0.5 c	110 c	550 a	5.2 a	0.3 b
LZ2	Lead–zinc mine area	E 108°15′03″; N 24°44′04″	6.3 b	930 b	16 c	2000 a	99 b		730 b	300 c	0.4 cd	0.3 c	290 a	530 a	1.8 b	0.3 b
LZ3	Lead–zinc mine area	E 112°23′01″; N 26°18′02″	7.4 a	9100a	31 a	2400 a	210 a		97 c	2400 a	24a	0.3 c	290 a	270 b	0.9 c	0.1 b

The units of the As and Pb concentrations in soil and plants are in milligrams kilograms per dry weight. Different superscript letters within a column indicate significant differences among populations ($P<0.05$, $n=3$)

n.d. not detected

and 9.6 μM Pb) as $Pb(NO_3)_2$. The As and Pb concentrations of the hydroponic solution were determined using the Geochem software (Diatloff et al. 1993), which showed that 95.8 % of As and 89 % of Pb in the culture solution existed as free ions, i.e., $HAsO_4^{2-}$ (or $H_2AsO_4^-$) and Pb^{2+}, respectively. The dosing concentrations were chosen based on two additional rules and the pre-experiment results: (1) As concentrations used were not toxic to *P. vittata*; (2) more concentrations of As than Pb were chosen because the focus was the influence of Pb on As accumulation. After acclimatization, the AS1 and LZ1 populations were transferred to the different treatments. The plants were harvested after 4 weeks of treatments, separated into fronds and rhizoids, washed with tap water, rinsed thrice with deionized water, and dried at 60 °C for the subsequent analysis of As and Pb.

In the above two experiments, phosphate was excluded from the culture solution to avoid its precipitation with Pb. Hydroponic solutions were not aerated but renewed every 2 days to keep enough oxygen and nutrient components. Usually a decrease below 3 mg L^{-1} of dissolved oxygen in the nutrient solution inhibits root growth (Bonachela et al. 2010; Gislerod and Adams 1983). And the pre-experiment found that after 3 days, the DO content decreased to ~3.5 mg L^{-1}. So the solution renewal period was set as 2 days. In the pre-experiment, there was no significant difference ($P>0.05$, $n=4$) in root growth between the aerated treatment and the treatment without aeration but with solution renewed every 2 days. The water loss due to transpiration was replenished every day. Each replicate was composed of two plants growing in a 500-mL hydroponic container. Each treatment had four replicates. The experiments were conducted in a greenhouse at 22 to 27 °C under a 12-h light period with a light intensity of 300 μE m^{-2} s^{-1} and a relative humidity of 65 to 70 %.

The LZ1 and AS1 populations treated with 5 mg As L^{-1} and 1 mg Pb L^{-1} were used for micro-X-ray fluorescence (μ-XRF) and micro-X-ray absorption near-edge structure (μ-XANES) analyses. Fresh rhizoid samples were placed in aluminum foil containers with embedding medium (optimum cutting temperature compound, Sakura, Japan), quickly frozen at −30 °C, and sliced into 20-μm-thick sections using a cryomicrotome (Figocut 2700 Reichert-Jung, Nussloch, Germany). Sections were attached to sample holders by polyethylene film and freeze-dried under vacuum (<20 Pa) at −50 °C for 48 h and then stored in a −30 °C freezer prior to the μ-XRF and μ-XANES analyses.

XRF scanning was performed using the 4W1B beamline at the Beijing Synchrotron Radiation Facility (Lei et al. 2008). The electron storage ring was operated at 2.2 GeV. Samples were fixed in a high precision sample positioning stage, with 1 μm/step in three dimensions, driven by computer-controlled stepping motors. The sample profile was at a 45° angle with the beam direction and the distance between the sample and detector fixed at 50 mm. The As K-edge (11,867 eV) was

obtained by μ-XANES using the 15U beamline at the X-ray microbeam station of the Shanghai Synchrotron Radiation Facility, China. The electron storage ring was operated at 3.5 GeV. The distance from the sample to the detector was fixed at 50 mm. The spatial resolution was ~5 μm. Aqueous solutions of analytical quality reagents (Alfa Aesar, Tianjin, China), sodium arsenite and sodium arsenate dibasic, were employed as reference compounds for inorganic As(III) and As(V), respectively. As(III)-tris-glutathione (As-GSH), synthesized by adding a 10-fold molar excess of reduced L-GSH (Alfa Aesar, Tianjin, China, 97 %) to a solution of sodium arsenite, was used to model As coordinated to three sulfhydryl groups (−SH) (Pickering et al. 2000).

Chemical analysis

The soil samples were air-dried, ground, and sieved (to <2 mm). Soil pH was determined in a 1:2.5 soil/water mixture (Wei and Chen 2006) and soluble soil As determined using a 1:5 soil/water mixture (Huq et al. 2003). The CEC was determined by the neutral ammonium acetate method (Rhoades 1982), and the total organic matter was calculated using the Walkley–Black method (Nelson and Sommers 1982). Total P and total N in soil were determined using the titrimetric and gravimetric method with ascorbic acid (John 1970) and Kjeldahl method (Anantakrishnan and Srinivasa Pai 1952), respectively. The diethylene triamine pentaacetic acid (DTPA)-extractable Pb concentration was used to assess the bioavailability of Pb (Dai et al. 2004). For the total As and Pb concentrations, the soil samples were grounded and sieved to 0.2 mm and then digested according to the US Environmental Protection Agency method 3050B. Dried plants were digested with $HNO_3/HClO_4$ (4:1, *v*/*v*) for the analysis of As and Pb concentrations.

The Pb concentration was determined using inductively coupled plasma–mass spectrometry (ICP–MS; ELAN DRC-e; PerkinElmer, USA). ^{89}Y was used as internal standard for ICP–MS. The As concentrations were quantified using hydride generation atomic fluorescence spectrometry (HG-AFS; AFS-2202; Beijing Haiguang Corp., China). The detection limit of AFS for As is 0.09 μg L^{-1}. Inductively coupled plasma–optical emission spectrometry (5300DV, PerkinElmer, USA) was used to measure the concentrations of potassium (K), phosphorus (P), calcium (Ca), iron (Fe), magnesium (Mg), and manganese (Mn).

External calibration and the analysis of blanks and standard reference materials together with the samples were conducted to ensure the analysis accuracy. The external standards were produced using a solution with the same acid concentration as the samples. The As and Pb concentrations of the blank were 0.09±0.01 and 4±1 μg kg^{-1} (n=3), respectively. Standard reference materials for plants (GBW-07603) were obtained from the National Institute of Metrology of People's Republic of China (the former China National Standard Materials Center) and included in the analysis for quality control. The reference As and Pb concentrations were 1.25±0.15 and 47±3 mg kg^{-1}, respectively, and the measured As and Pb concentrations of reference samples (n=3) ranged from 1.12 to 1.35 mg kg^{-1} and from 45 to 50 mg kg^{-1}, respectively.

Data analysis

The bioaccumulation factor (BF) was calculated as the ratio of the frond-to-soil As or Pb concentration. The translocation factor (TF) was defined as the ratio of the frond-to-rhizoid As or Pb concentration. The hydroponic data were presented as the mean±standard deviation (n=4), while the field data were presented as the mean (n=3).

To describe the effects of Pb and As in the culture solution on the uptake of these two elements, the following general linear model (Drava et al. 2012) was adopted:

$$Z = Z_0 + aX + bY + cX^2 + dY^2 + fXY, \quad (1)$$

where X and Y are the As and Pb concentrations in the solution, respectively; a and b are the estimated effects of the As and Pb treatments, respectively; c and d are the quadratic effects, whereas f is the interaction between the treatments. Z is the frond As or Pb concentration of *P. vittata*.

ANOVA and correlation analysis were conducted using the SPSS package (SPSS Inc., Chicago, IL, USA, Release 13.0). Pairwise comparisons were conducted using the least significant difference method with a significance level of P<0.05. The three-dimensional curved surface of the model was fitted via nonlinear surface fitting using Origin Pro 8.0 (OriginLab, USA).

Results

Variation in the As and Pb uptake among *P. vittata* populations in the field

In these six investigated sites, the lowest soil As concentrations was 7.6 mg kg^{-1}, found in the non-contaminated farmland CK1, while the highest was 9,100 mg kg^{-1}, found in the Pb–Zn mine area LZ3 (Table 1). The water soluble As concentration followed the same trend of the total As. *P. vittata* was the dominant species in AS1 and a common species in the remaining sites. *P. vittata* from the mining area displayed higher As concentrations in its rhizoids and fronds, as compared with those from non-mining areas. The highest frond As concentration and TF of 2,400 mg kg^{-1} and 24, respectively, were observed in LZ3, which also had the highest soil As and Pb concentrations. The As concentration

was higher in the fronds than that in the rhizoids for populations CK1, AS1, and LZ3.

The lowest soil Pb concentrations was 55 mg kg^{-1} found in the non-contaminated farmland CK2, while the highest was 2,400 mg kg^{-1} found in the Pb–Zn mine area LZ3 (Table 1). The highest Pb availability was also observed at site LZ3, with a DTPA-extractable Pb concentration of 210- mg kg^{-1}. The corresponding Pb concentration in *P. vittata* from non-contaminated farmlands (CK1 and CK2) was lower than those from contaminated sites. LZ1, although it did not have the highest soil Pb concentration, displayed the highest frond Pb concentration (550 mg kg^{-1}) and TF of Pb (5.2).

Uptake of Pb and As in several populations of *P. vittata*

The *P. vittata* plants grew normally in the different treatments, with no significant differences among populations in terms of the biomass during a 7-day hydroponic experiment ($P>0.05$, data not provided). *P. vittata* displayed a higher As concentration than *A. capillus-veneris* in both the rhizoids and fronds (Fig. 1a). *P. vittata* from the non-contaminated site CK1 accumulated more As than the other two populations. The difference between the frond As concentrations of CK1 and AS1 was significant ($P<0.05$). The As concentration in the fronds was higher than that in the rhizoids for all *P. vittata* populations, but was lower in the fronds than in the rhizoids of *A. capillus-veneris*.

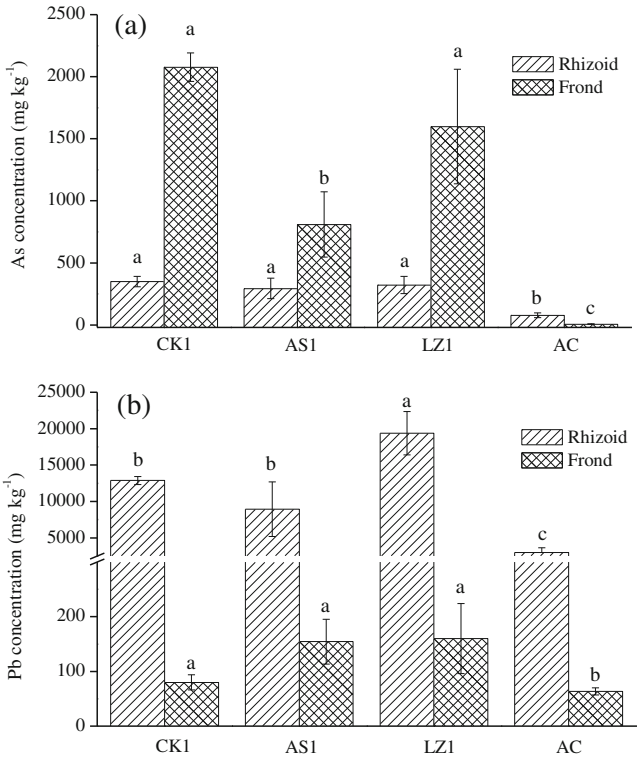

Fig. 1 As (**a**) and Pb (**b**) concentrations of *P. vittata* populations in a hydroponic system. CK1, AS, and LZ1 are the spore-beginning sexual reproduction offspring of three *P. vittata* L. populations collected from sites CK1 (uncontaminated farmland), AS1 (near an arsenic smelter), and LZ1 (lead–zinc mine area); AC is the spore-beginning sexual reproduction offspring of an arsenic tolerant fern *A. capillus-veneris* L. For each organ, *different letters* indicate significant differences between treatments ($P<0.05$, $n=4$)

P. vittata had higher Pb concentrations in the rhizoids and fronds than *A. capillus-veneris* (Fig. 1b). The *P. vittata* populations from the contaminated sites (AS1 and LZ1) accumulated more Pb (~150 mg kg^{-1}) than those from the non-contaminated sites (CK1, <100 mg kg^{-1}). Likewise, LZ1 displayed significantly higher rhizoid Pb concentrations than the other plant populations ($P<0.05$). All plants exhibited lower Pb concentrations in the fronds than in the rhizoids.

Interaction between Pb and As in mining populations of *P. vittata*

Given that AS1 and LZ1 had the highest TF values in the field and showed greater Pb uptake during the hydroponic experiment, these two populations may have higher potential for the extraction of As and Pb. Therefore, AS1 and LZ1 were selected to investigate the interaction between As and Pb, thereby providing information for the phytoextraction of As and Pb.

The *P. vittata* plants grew well in solutions containing high concentrations of As and Pb. Certain amounts of As or Pb in the solution increased the biomass of *P. vittata* (Tables 2 and 3). The highest biomass for the LZ1 population was achieved by the treatment containing 5 mg As L^{-1} with 2 mg Pb L^{-1}, whereas the treatment containing 5 mg As L^{-1} with 1 mg Pb L^{-1} produced the highest biomass in the AS1 population. The LZ1 population displayed higher biomass under co-exposure to higher concentrations of As and Pb, as compared with the AS1 population.

The concentrations of As and Pb in fronds significantly depended on the supplied As and Pb concentrations ($P<0.05$). The significance of the regression between the supplied As or Pb and the uptake of these elements by *P. vittata* is summarized in Table 4. The fitted curves are presented in Figs. 2 and 3. The addition of Pb increased the frond As concentration of both populations in all four As treatments. Compared with the controls, treatment with 2 mg Pb L^{-1} increased the frond As concentration by 120, 150, and 60 % in the LZ1 plants treated with 2, 5, and 8 mg As L^{-1}, respectively. The highest As concentration was obtained from the treatment with 2 mg Pb L^{-1} and 8 mg As L^{-1}; this concentration was 1.22 % of the total dry weight (Table 2). The presence of Pb facilitated the uptake of As in the AS1 population to a lesser extent. The highest frond As concentration was 0.77 % of the total dry weight (Table 3).

P. vittata demonstrated its Pb-accumulating ability, with the highest frond Pb concentration close to the threshold

Table 2 Concentrations of As, Pb, P, and K in the fronds and rhizoids of LZ1 population under different treatments for 4 weeks (n=4)

Treatment	Biomass (g)	Concentration by dry weight in the fronds				Concentration by dry weight in the rhizoids			
		As (g kg^{-1})	Pb (g kg^{-1})	P (g kg^{-1})	K (%)	As (g kg^{-1})	Pb (g kg^{-1})	P (g kg^{-1})	K (%)
As0Pb0	0.23±0.01	0.010±0.001	0.010±0.007	2.0±0.2	0.4±0.1	0.011±0.004	0.03±0.01	0.81±0.06	0.25±0.07
As0Pb1	0.21±0.01	0.026±0.004	0.49±0.07	2.0±0.1	0.7±0.1	0.012±0.005	16±0.5	1.3±0.4	0.59±0.12
As0Pb2	0.32±0.01	0.28±0.03	0.78±0.06	3.5±0.1	2.4±0.6	0.014±0.004	27±0.4	1.7±0.3	0.67±0.14
As1Pb0	0.41±0.03	1.8±0.1	0.03±0.02	4.0±0.1	4.2±0.3	0.27±0.08	0.04±0.01	1.3±0.5	1.1±0.03
As1Pb1	0.58±0.04	3.5±0.1	0.54±0.01	4.7±0.5	4.8±0.2	0.28±0.07	26±2	1.0±0.5	0.90±0.05
As1Pb2	0.40±0.04	4.0±0.1	0.88±0.11	8.0±0.1	7.3±0.6	0.13±0.02	29±0.3	1.2±0.4	0.76±0.16
As5Pb0	0.43±0.04	2.3±0.6	0.08±0.02	4.1±0.9	4.7±1.1	0.33±0.09	0.05±0.01	0.98±0.12	0.72±0.14
As5Pb1	0.31±0.01	3.2±0.7	0.56±0.01	6.4±0.7	6.1±0.5	0.23±0.09	25±0.7	1.2±0.6	0.42±0.13
As5Pb2	1.5±0.8	5.7±0.5	0.86±0.02	3.8±0.3	4.2±0.2	0.69±0.15	31±2	1.2±0.3	0.87±0.17
As8Pb0	0.19±0.01	7.7±0.2	0.14±0.09	3.8±0.4	5.2±0.4	0.50±0.02	0.06±0.01	0.87±0.16	0.78±0.16
As8Pb1	0.57±0.02	9.2±0.9	0.66±0.04	4.1±0.1	4.3±0.2	0.39±0.02	20±1.2	0.89±0.35	0.96±0.17
As8Pb2	0.24±0.01	12.2±0.6	0.99±0.18	6.0±0.2	5.7±0.6	0.49±0.05	30±1.0	1.2±0.3	0.84±0.09

value of Pb hyperaccumulator (1,000 mg kg^{-1}; Fig. 3). The frond Pb concentration apparently increased in the LZ1 population when the supplied Pb concentration was increased. Such increase was not evident in the AS1 population. The LZ1 population demonstrated a higher ability for Pb uptake than the AS1 population. With the increased As concentration in the solution, the Pb uptake in *P. vittata* was slightly increased. However, this increase was to a lesser extent than the influence of Pb on As accumulation. The highest Pb concentration was found in the LZ1 population treated with 2 mg Pb L^{-1} and 8 mg As L^{-1} (0.99 g kg^{-1}; Table 2).

The rhizoid As concentrations were much lower than the frond As concentrations in both populations, thereby implying the strong translocation ability of As in *P. vittata*. The Pb treatment (2 mg Pb L^{-1}) significantly increased the rhizoid As concentration in the LZ1 population treated with 5 mg As L^{-1} and the AS1 population treated with 8 mg As L^{-1} (P<0.05).

The Pb concentration in the rhizoids of *P. vittata* was much higher than that in the fronds (Tables 2 and 3). The LZ1 population showed higher rhizoid Pb concentration than the AS1 population. The presence of As increased the accumulation of Pb in the rhizoids of both populations, with LZ1 displaying greater increase. The highest rhizoid Pb concentration was found in the LZ1 population treated with 2 mg Pb L^{-1} and 5 mg As L^{-1}, which was 3.07 % of the total dry weight (Table 2).

Table 3 Concentrations of As, Pb, P, and K in the fronds and rhizoids of AS1 population under different treatments for 4 weeks (n=4)

Treatment	Biomass (g)	Concentration by dry weight in the fronds				Concentration by dry weight in the rhizoids			
		As (g kg^{-1})	Pb (g kg^{-1})	P (g kg^{-1})	K (%)	As (g kg^{-1})	Pb (g kg^{-1})	P (g kg^{-1})	K (%)
As0Pb0	0.17±0.01	0.09±0.07	0.03±0.01	2.0±0.1	0.31±0.04	0.11±0.01	0.08±0.02	1.3±0.1	0.28±0.02
As0Pb1	0.29±0.01	0.15±0.03	0.16±0.02	3.6±0.2	0.59±0.02	0.14±0.03	5.9±0.6	2.6±0.2	0.64±0.20
As0Pb2	0.18±0.01	0.16±0.04	0.35±0.03	6.2±0.4	1.5±0.4	0.11±0.03	6.6±0.3	1.2±0.2	0.67±0.13
As1Pb0	0.48±0.03	0.65±0.01	0.03±0.01	4.6±0.2	1.0±0.2	0.13±0.01	0.06±0.01	1.5±0.1	0.56±0.02
As1Pb1	0.42±0.04	1.4±0.1	0.18±0.05	6.9±0.4	1.5±0.2	0.18±0.01	7.9±0.5	2.1±0.2	0.81±0.19
As1Pb2	0.30±0.04	1.5±0.9	0.35±0.06	5.7±0.5	2.5±0.7	0.13±0.01	8.6±0.4	3.4±0.2	1.0±0.1
As5Pb0	0.28±0.04	2.8±0.2	0.03±0.02	3.7±0.3	2.5±0.8	0.22±0.02	0.08±0.03	1.4±0.1	0.39±0.07
As5Pb1	0.57±0.01	4.0±0.1	0.16±0.04	5.1±0.1	2.1±0.8	0.31±0.07	5.3±0.3	3.6±0.3	0.53±0.04
As5Pb2	0.54±0.08	3.2±0.1	0.29±0.04	6.0±0.3	1.8±0.1	0.21±0.04	9.3±0.6	3.8±0.6	0.55±0.11
As8Pb0	0.26±0.01	5.4±0.6	0.02±0.01	3.5±0.2	3.4±0.4	0.46±0.01	0.06±0.02	2.7±0.2	0.71±0.14
As8Pb1	0.35±0.02	7.7±0.2	0.12±0.02	4.6±0.8	3.7±0.9	0.54±0.04	5.7±0.8	1.8±0.1	1.6±0.4
As8Pb2	0.18±0.01	6.3±1.1	0.25±0.04	5.5±0.1	1.4±0.1	0.61±0.07	10±0.2	1.7±0.2	0.99±0.16

Table 4 ANOVA results (significance of the model coefficients)

As/Pb concentration in frond	*a*	*b*	*c*	*d*	*f*	R^2
As in LZ1	NS	NS	*	NS	*	0.869
As in AS1	NS	*	*	*	NS	0.958
Pb in LZ1	*	NS	*	*	NS	0.991
Pb in AS1	*	NS	*	NS	*	0.995

The letters *a* and *b* indicate the linear effects of As (a) and Pb (b) treatments, respectively, whereas *c* and *d* are quadratic effects of the As (c) and the Pb (d) treatments, respectively, and *f* is the interaction term

NS not significant

*Significant $P<0.05$

The K concentration in LZ1 and AS1 followed the same trend as the As concentration (Tables 2 and 3). The As and K concentrations in the fronds of the AS1 population were significantly correlated ($P<0.05$). In fronds of the LZ1 population, the correlation between As and K was significant when the samples with frond As concentrations higher than 8,000 mg kg^{-1} were excluded. Pb and P followed a similar relationship, with a significant correlation between the Pb and P concentrations in the fronds of the AS1 population ($P<0.05$). No significant relationship has been found between As and other elements, including Ca, Fe, Mg, and Mn (data not provided).

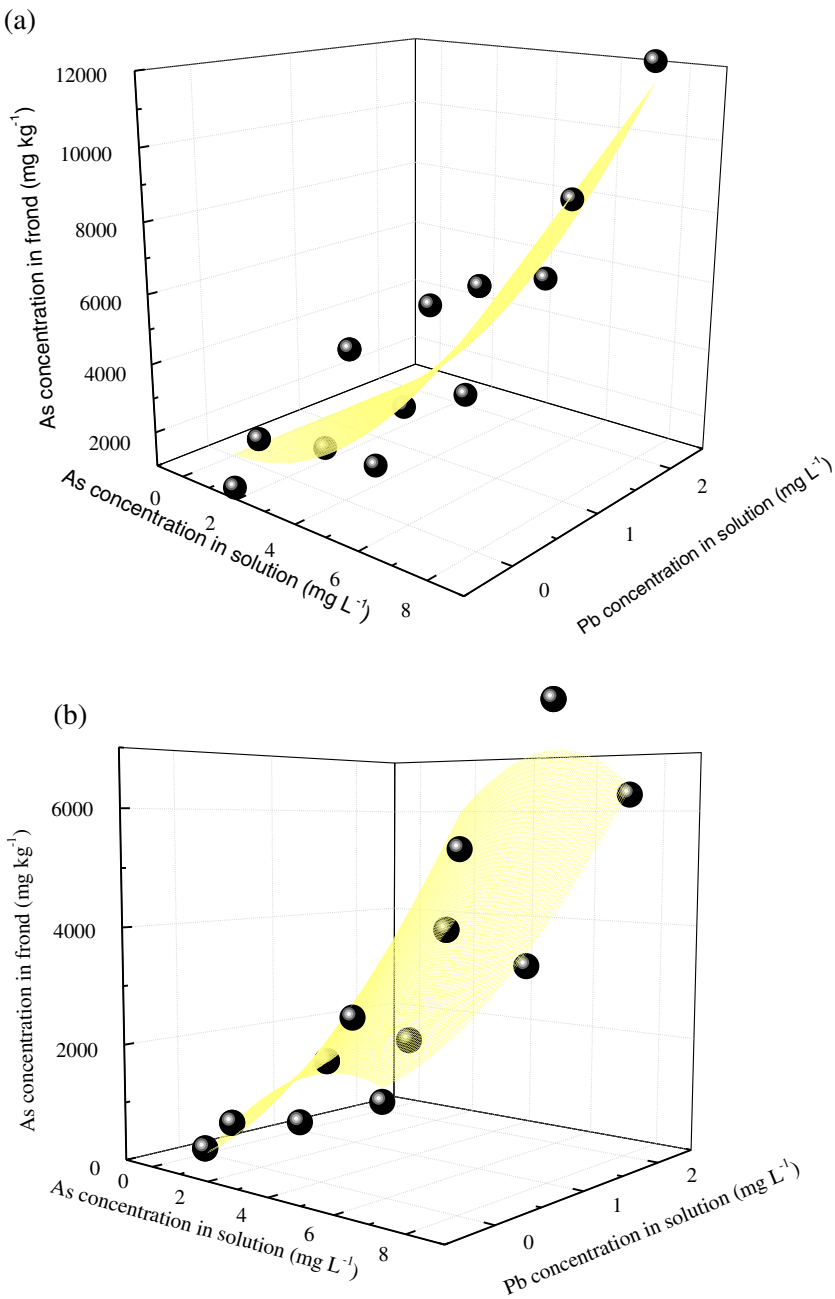

Fig. 2 Effects of Pb on the uptake of As in the fronds of *P. vittata* populations: **a** LZ1 and **b** AS1

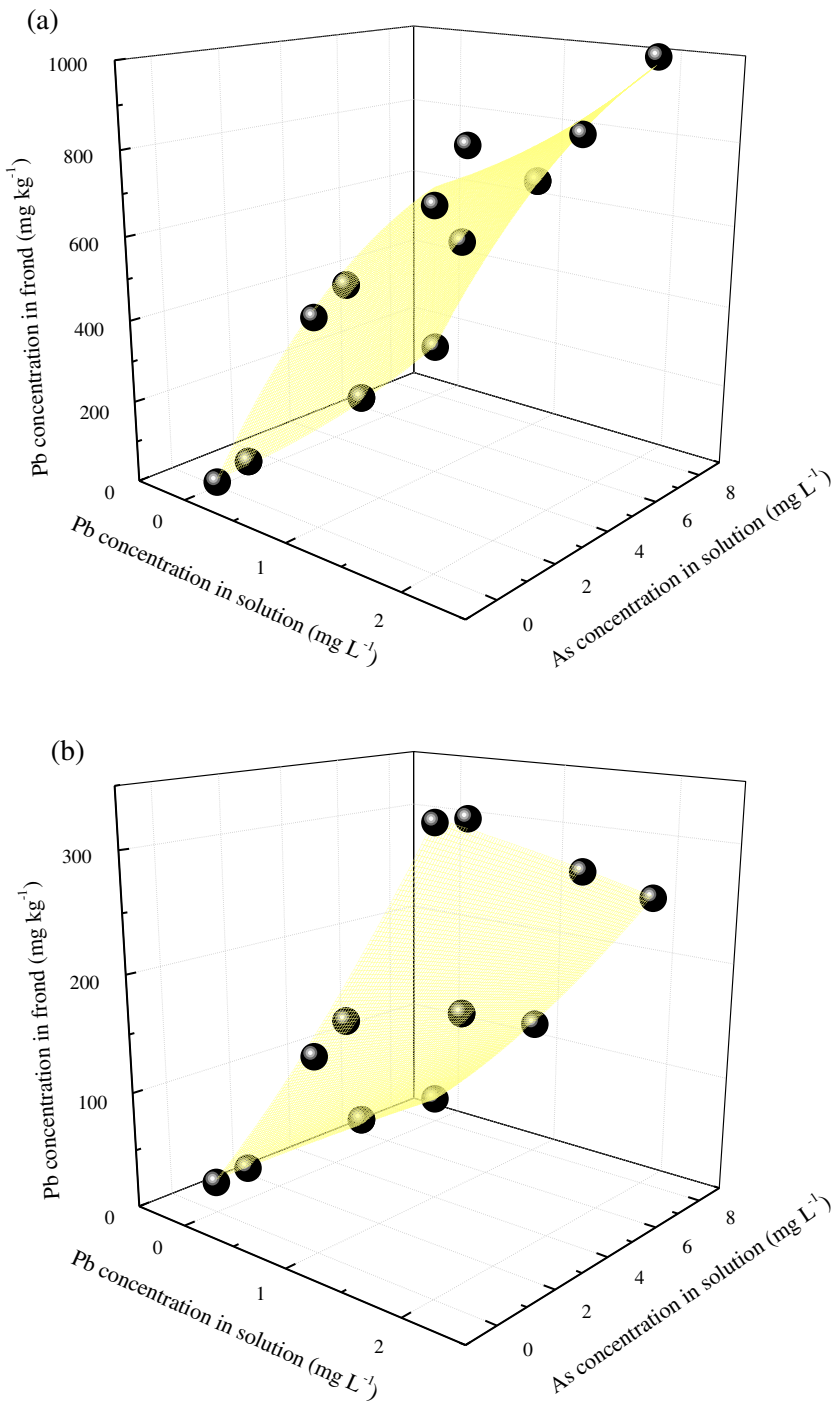

Fig. 3 Effects of As on the uptake of Pb in the fronds of *P. vittata* populations: **a** LZ1 and **b** AS1

Microdistribution of the As concentration and speciation in the rhizoids of *P. vittata* mining populations

The microdistribution of As revealed that the As concentration in *P. vittata* under As exposure alone followed this order: endodermis>vascular bundle>cortex=epidermis in the LZ1 and AS1 populations (Fig. 4). The As content in the rhizoid epidermis of the LZ1 population was greatly improved by approximately tenfold upon the combined exposure of As and Pb (Fig. 4a). For the AS1 population, co-exposure to As and Pb increased the As content in the epidermis and cortex but decreased the As in the endodermis. The rhizoid vascular bundle and endodermis displayed the highest As content for both the As and As–Pb treatments in the AS1 population (Fig. 4b).

For both treatments in these two populations, the percentage of As(V) in the rhizoid decreased from the outermost epidermis to the innermost vascular bundle. This decrease was accompanied by an increase in the As(III) percentage (Fig. 5), thereby revealing the reduction of As(V) to As(III).

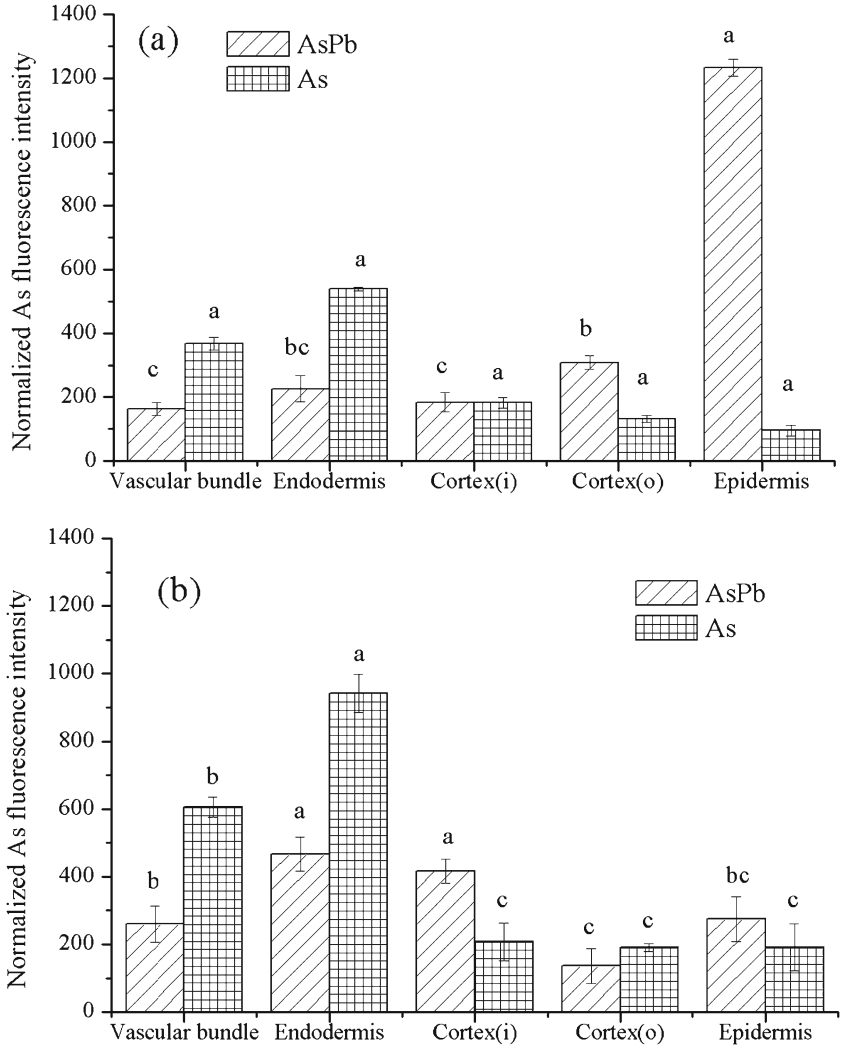

Fig. 4 Distribution of As in the rhizoids of *P. vittata* from the **a** LZ1 and **b** AS1 populations in a hydroponic system. Cortex(i) indicates cortex tissues close to the endodermis while cortex(o) indicates cortex tissues close to the epidermis. For each treatment, *different letters* indicate significant differences between different tissues ($P<0.05$, $n=4$)

The co-exposure of As and Pb in the LZ1 population increased the percentage of As(V) in every rhizoid tissue, which was accompanied by a decrease in the As(III) percentage. The percentage of As(III)–SH likewise increased in the cortex, endodermis, and vascular bundles (Fig. 5a, b). Similarly, the percentage of As(V) in the rhizoid of AS1 population increased with the decrease in the As(III) percentage under the co-exposure of As and Pb. Between the two populations, LZ1 displayed higher As(V) but lower As(III) percentages than the AS1 population under both treatment conditions.

Discussion

Pb facilitates the uptake of As

The hydroponic experiment showed that the application of Pb increased the As concentration in the fronds of the LZ1 and AS1 populations, particularly in the LZ1 population from the Pb–Zn mining area. This increased As accumulation may be attributed to both the improved absorption of As by the rhizoids and the enhanced translocation of As from the rhizoids to the fronds.

The microdistribution of As concentrations indicated that Pb increased the absorption of As on the epidermis of the *P. vittata* rhizoids and that more than 90 % of Pb was restricted to the epidermis (data not provided), similar to the behavior of hyperaccumulator *Brassica juncea* (Meyers et al. 2008). The sequestration of Pb in epidermis may have resulted from the displacement of Ca on cell wall by Pb since Pb has higher binding capacity to pectins (the main cell wall constituents) than Ca (Brunner et al. 2008). This displacement of Ca by Pb may have increased the membrane permeability (Sharma and Dubey 2005), allowing the easier movement of As across the cell membranes. A similar correlation has been found between Pb and As in the roots of *Rubus ulmifolius* growing on contaminated soil (Marques et al. 2009).

The micro-distribution of As speciation indicated that a higher percentage of As(V) appeared in the rhizoid tissues under the combined exposure of As and Pb, as compared with that under As exposure alone. The higher percentage of As(V) may be an oxidative response of *P. vittata* to Pb stress

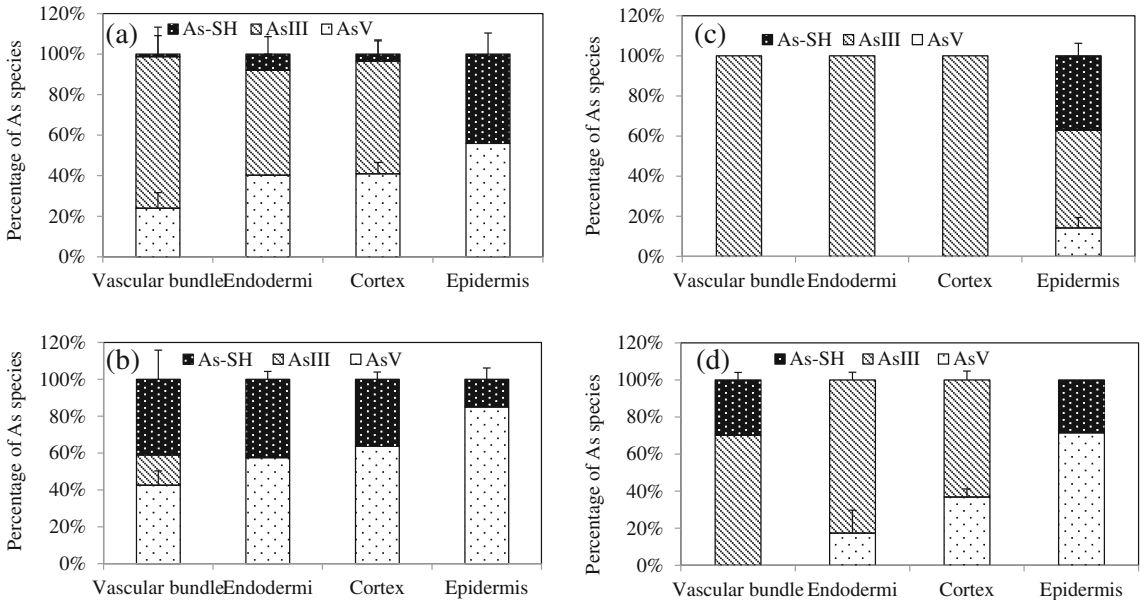

Fig. 5 Micro-speciation of As in the rhizoids of the **a**, **b** LZ1 and **c**, **d** AS1 *P. vittata* populations under **a**, **c** sole exposure to 5 mg As L^{-1} or **b**, **d** co-exposure to 5 mg As L^{-1} and 1 mg Pb L^{-1}

(Huang et al. 2011). The reduction of As(V) to As(III) in the rhizoids of *P. vittata* has been well-known as an essential process for the As hyperaccumulation in *P. vittata* (Duan et al. 2005; Huang et al. 2008). By contrast, the current study found that higher As uptake in *P. vittata* was accompanied by a higher As(V) percentage. One possible explanation for the increased As uptake accompanied by an unexpected increase in As(V) is that the barrier to the apoplasmic transportation of As(V) was broken by the co-exposure to As and Pb. However, under environmental stress, the plant is more likely to improve the strength of the barrier than break it (Stolarikova et al. 2012; Vatehova et al. 2012). Another possible explanation is the appearance of a novel As transporter or the dominance of an original subsidiary transporter. A previous study on the Pb uptake of *Lespedeza* species suggested that Pb may form complexes with phosphorus and sulfur compounds in the roots (Zheng et al. 2012). In the current study, P displayed a similar trend with Pb in plants. An apparent increase in the percentage of As–SH was observed in the rhizoids. The addition of Pb likely induced the appearance of a considerable amount of P and –SH, which facilitated the uptake of As by sharing the transporters with P (Lei et al. 2012; Xue et al. 2012) or forming an As–SH complex through the special transporters (Araki et al. 2011), respectively. The increased cell membrane permeability due to Pb stress (Sharma and Dubey 2005) may have also played a role.

Differences among populations

Pb tolerance The current study showed that perennial *P. vittata* from mining areas with high concentrations of Pb in soil may have evolved into Pb-tolerant populations with a genetically stable Pb tolerance. Kabata-Pendias and Pendias (1984) stated that soils with a total Pb concentration higher than 400 mg kg^{-1} or a DTPA-extractable Pb concentration higher than 100 mg kg^{-1} are toxic to plants and that the approximate concentration of excessive or toxic Pb in plants is 30~300 mg kg^{-1}. In the present study, the indexes of several mining populations were exceeded in the field, thereby implying the Pb tolerance of mining populations, as confirmed in the hydroponic experiment.

Compared to the controls, the biomass of mining populations that received the Pb treatment did not decrease. By contrast, the data showed an increase, which coincided with the findings of Fayiga et al. (2004). The *P. vittata* populations from the Pb–Zn mine area could tolerate higher amounts of Pb than populations from other sites. Zhang et al. (2012) found that the co-contamination of As and Pb was toxic to *P. vittata*, thereby inducing oxidative stress and adverse effects on the leaf ultrastructure. The difference between the previous studies and our study may be explained by the different populations studied. Similar differences were found in the Cd tolerance of *P. vittata* between different populations (Fayiga et al. 2004; Xiao et al. 2008). Furthermore, the simultaneous increase in the K and As concentrations confirmed that K was simultaneously taken up by *P. vittata* with As (Chen et al. 2005), which may contribute to the increased biomass under Pb and As treatments.

Pb uptake In the field survey, the highest frond Pb concentration was 549 mg kg^{-1}. Several *P. vittata* populations had TFs higher than 1 for Pb, with the highest value of 5.2. The frond Pb concentration of *P. vittata* was proportional to the Pb concentration in the substrate under both field and hydroponic conditions. The highest frond Pb concentration was almost

1,000 mg kg^{-1}, after the 4-week growth in the hydroponic solution containing 2 mg Pb L^{-1}. This concentration (frond concentration of 1,000 mg kg^{-1}) is the threshold concentration of a Pb hyperaccumulator. Therefore, the LZ1 population of *P. vittata* may be a potential Pb hyperaccumulator. However, as van der Ent et al. (2013) indicated, the results from hydroponic experiments sometimes can be very different from the real condition. The confirmation of Pb hyperaccumulation by *P. vittata* under a more real circumstance needs further investigation.

The *P. vittata* population from the Pb–Zn mine with the higher soil Pb concentration had accumulated more Pb than that from the As smelter area with lower soil Pb concentration. Plants from Pb-contaminated sites had higher Pb uptake than those from the uncontaminated sites, thereby implying that the long-term acclimation of *P. vittata* to elevated Pb concentrations in the soil improved its ability for Pb uptake. The results indicated that, unlike the congenital As accumulation (Wan et al. 2013; Wu et al. 2009), Pb accumulation of *P. vittata* may be an adaptive trait. These findings were similar to those reported by Wu et al. (2007, 2009).

The feasible use of aquatic plants to remove metals and metalloids from contaminated water has been suggested (Rahman and Hasegawa 2011; Schroder et al. 2007). After the 4-week incubation in the nutrient solution with 5 mg As L^{-1} and 2 mg Pb L^{-1}, the Pb concentration in rhizoids was as high as 3.07 % of the dry weight, whereas the As concentration in the frond reached 1.22 % of the dry weight. The results indicated the potential of *P. vittata* for the economical remediation of As- and Pb-bearing wastewater.

Conclusions

The co-exposure of As and Pb increased the frond As concentration by 60 to 150 % in *P. vittata* populations from mining sites, as compared with that under As exposure alone. The *P. vittata* populations from mining sites displayed high tolerance to Pb and As. A strong ability to accumulate Pb and As was observed in these plants in hydroponic culture, with the highest As and Pb concentration in shoots reaching 12.2 and 0.99 g kg^{-1}, respectively. These traits indicate that the *P. vittata* populations in mining areas are appropriate materials for cleaning up As and Pb co-contamination.

Acknowledgments Financial support was provided by the National Natural Science Foundation of China (grant nos. 41271478, 41071215, and 41271479) and China Postdoctoral Science Foundation (no. 2013M530718).

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