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Arsenic and Heavy Metal Accumulation by *Pteris vittata* L. and *P. umbrosa* R. Br.

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Abstract This study compared the accumulation of arsenic, copper and chromium by *Pteris vittata* and *Pteris umbrosa* grown in a glasshouse in soil from a timber treatment facility. Soil was collected from three locations. Accumulation (as percentage removed) varied between these soils but was not related to soil concentration. *P. vittata* was more efficient than *P. umbrosa*, both in accumulating As and metals in the below-ground plant parts and in translocating As to the fronds. Under the experimental conditions, only *P. vittata* could be effectively used in soil from one location for phytoremediation purposes.

Keywords Arsenic · Heavy metal · Fern · *Pteris vittata* · *Pteris umbrosa* · Phytoremediation

The application of preservants to wood products has resulted in contamination of soil and water in and around timber treatment facilities. The treatment solution of choice historically has been the timber preservant CCA, consisting of Cu, Cr and As. All of these metals pose toxic risks to ecological systems (Hapke 1991); in addition, Cr (III) (Franchini and Mutti 1985) and As (Mandal and Suzuki 2002) have documented carcinogenic effects on humans. At present, there are eleven licensed timber preservation facilities in NSW, Australia (EPA 2003). Common practice is to contain contaminated soil in situ or remove it to hazardous waste landfill sites. Phytoremediation initiatives to ameliorate contaminated areas may provide low-impact, economically viable and ecologically desirable alternatives to landfill (Salt et al. 1995). The contaminant of major concern on CCA treatment sites is As, since it is toxic at lower exposure concentrations than Cr or Cu. With the recent discovery of As hyperaccumulating plant species (Visoottiviseth et al. 2002; Zhao et al. 2002, Ma et al. 2001b), phytoremediation has become a feasible As management and remediation option.

The mechanisms for As hyperaccumulation have been studied extensively, predominantly in the brake fern, *Pteris vittata* (Poynton et al. 2004; Lombi et al. 2002; Zhang et al. 2002). It was found in these studies that As accumulated predominantly in the above-ground biomass which can be easily harvested. This makes it an excellent candidate for phytoextraction. Results have shown bioconcentration factors (BCF; ratio of above-ground biomass to soil As concentration) of up to 126 (Ma et al. 2001a). However, both the BCF's and the translocation factors (TF; generally ratio of As concentration of above:below ground biomass) from various experiments are highly variable. Both BCFs and TFs tend to increase with increasing exposure due to relatively greater uptake and translocation of As at lower exposure concentrations (Koller et al. 2007). Equally, BCFs and TFs are higher in spiked soils and in glasshouse experiments than in aged contaminated soils, and in the field, where contaminants are less bioavailable due to surface complexation (Goh and Lim 2005). Experiments combining exposure to As and other metals (Cr, Cu) have found that the latter may suppress As accumulation (An et al. 2006; Fayiga and Ma 2006; Fayiga et al. 2004). More recently, other species of fern from the same genus have shown promise as As hyperaccumulators, including the Australian native fern *Pteris umbrosa* (Koller et al. 2007; Zhao et al. 2002). This species exhibited similar uptake

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and translocation of As to *P. vittata* in a study using spiked general purpose compost (Zhao et al. 2002).

The relative accumulation and phytoremediation potential of the established hyperaccumulating fern *P. vittata* and the Australian native fern *P. umbrosa* were compared by quantifying the ability of these two species to extract As from contaminated soils from an Australian industrial site under glasshouse conditions. The uptake of Cu and Cr were also investigated.

Materials and Methods

Sandy clay loam soils were collected from a CCA-treatment plant. "Control" soil was collected from the up-hill periphery of the site where contamination was expected to be minimal. "Drain" soil was collected from a drain at the downstream side of a sedimentation pond for runoff from the area of the CCA timber preservation process. "Dredge" soil came from stockpiled soil that had been dredged from the sedimentation pond about 18 months previously. Each soil was mixed well before use. Five sub-samples of soil (ca. 800 g each) were collected and air-dried for chemical analysis.

The ferns used in this study are both from the family Pteridaceae. *Pteris umbrosa* R.Br. occurs naturally on the east coast of Australia, in Queensland, NSW and Victoria. *Pteris vittata* L. is a cosmopolitan species rare in Australia, occurring in Western Australia, Northern Territory, Queensland, NSW and Victoria (Harden 1990).

Four-month old ferns were purchased from a commercial nursery. They were maintained in the glasshouse with minimum/maximum temperatures of 18°C and 28–30°C, respectively, and natural day-length. After 14 days of acclimatisation, plants were removed from nursery soil, roots washed with water, fresh weights recorded (32.2 ± 6.1 g) and each fern transplanted into separate 150 mm pots containing 1 kg of moist site soil (N = 5, per species, per treatment). Five grams of slow-release (270 d Nutracote® Type 27; 18N-2.6P-6.6K TE) were added to each pot. The pots were returned to the glasshouse, placed in individual trays (130 × 130 mm) and watered into trays to maintain soil at field capacity. The position of pots was randomised on the benches at 6-weekly intervals. Plants were harvested after 16 weeks.

At harvest, ferns were gently removed from their pots and roots washed in water to remove adhering soil. Excess water was removed and whole plants were weighed. Fronds were dissected at the base of the stipes and fronds separated into green and dead. Each fraction was washed in de-ionised water and placed in a paper bag, as was the remaining below-ground portion of the plant consisting of rhizome and adventitious roots. This separation makes sense from a phytoremediation perspective, since the above ground portion is easily harvested whereas the below-ground portion would remain in the soil.

Plant samples were dried at 60°C for 48 h, weighed, ground in a micro-hammer mill (Culatti AG, Zurich) with a sieve size of 0.8 mm, and stored in sealed 70 mL polypropylene containers. Soil samples were air-dried and homogenized in a mortar and pestle.

Hot acid digestion of vegetation and soil followed the method of Cai et al. (2000). Samples were analysed without further dilution or addition of internal standards on a ThermoFinnigan Element 2 – High Resolution Inductively Coupled Mass Spectrometer (ICP-MS) with detection limits of 0.028 ppb, 0.045 ppb, and 0.011 ppb for As, Cr and Cu, respectively.

For QA/QC, each group of digests (28) contained a blank sample, a reference material, a sample spiked with 1 μL of 1000 mg L^{-1} As, Cr and Cu, a digest replicate and an analysis replicate. The recovery rates from the spiked samples were 78% for Cr, 72% for Cu and 69% for As.

The carbon content, pH, conductivity and redox potential were analysed according to Rayment and Higginson (1992). Particle size was determined by sieving (Bowles 1986).

Differences among treatments were analysed by one- and two-way analyses of variance (ANOVA), using STATISTICA (StatSoft 1995), with species and soil type as factors. Homogeneity of variance was tested using the Cochran C statistic. In the absence of homogeneity, data were log transformed ($X' = \ln(X + 1)$) (Zar 1999). Post-hoc comparisons were performed by Tukey's honest significant difference tests (StatSoft 1995).

Results and Discussion

The soils collected were from a low impact site (control) and two high impact sites (dredge and drain). Acid-extractable concentrations of As and Cu in the drain and dredge soils were similar, for Cr they were higher in the drain soil. All concentrations were significantly lower in the control soil (Table 1). The levels of Cu and Cr were within the acceptable range for soils (Gardea-Torresdey et al. 2005), whereas As was elevated (Adriano 2001).

The levels of contaminants in the ferns were elevated above the background ranges of 0.1–40 μg g^{-1} for As (Adriano 2001), 5–25 μg g^{-1} for Cu and 0.2–5 μg g^{-1} for Cr (Gardea-Torresdey et al. 2005). There was no significant difference between species in the Cu and Cr concentrations of the below-ground parts, with fern below-ground parts from control soils having significantly lower levels than fern below-ground parts grown in drain soil (Table 1). Most of the accumulated Cu and Cr remained in

Table 1 Results for one- and two-way ANOVAs of various factors examined with respect to As, Cu and Cr accumulation by *P. umbrosa* and *P. vittata* grown in three soils (N = 5)

Analysis	Factor	Effect	F	*p*	Posthoc
One-way	Soil As	Soil	394.8	**0.000**	Drain = dredge > control
	Soil Cu	Soil	433.8	**0.000**	Drain = dredge > control
	Soil Cr	Soil	300.9	**0.000**	Drain > dredge > control
Two-way	Below-ground parts As	Species	0.073	0.400	
		Soil	3.78	**0.038**	Drain > control
		Interaction	0.30	0.700	
	Below-ground parts Cu	Species	3.45	0.076	
		Soil	4.75	**0.018**	Drain > control
		Interaction	1.51	0.240	
	Below-ground parts Cr	Species	0.67	0.420	
		Soil	4.47	**0.020**	Drain > control
		Interaction	1.07	0.360	
	TF As	Species	3.63	0.069	
		Soil	4.91	**0.016**	Drain > control ≥ dredge
		Interaction	3.34	0.051	
	BCF As	Species	46.21	**0.000**	*P. vittata* > *P. umbrosa*
		Soil	9.6	**0.001**	Control ≥ drain > dredge
		Interaction	0.79	0.469	
	As conc of green fronds	Species	70.37	**0.000**	*P. vittata* > *P. umbrosa*
		Soil	10.48	**0.001**	Drain > dredge ≥ control
		Interaction	0.39	0.681	

The bold values of *p* which differ significantly at the 5% level

the below-ground parts, with concentrations in the fronds of both ferns <10 μg g^{-1}, a level associated with micronutrients (Lasat 2000).

The As concentrations in the below-ground parts of *P. umbrosa* were lower than in *P. vittata* (Fig. 1), although due to high variation (especially in control soils) this was not significant via a two-way ANOVA (Table 1). Overall, As concentrations were higher in drain soil than in control soil (Table 1). Concentrations in the below-ground parts were at or below levels in the soil, except for As in *P. vittata* below-ground parts grown in control soil (Fig. 1).

The TFs for As were similar for *P. vittata* and *P. umbrosa* but differed among soils, being significantly higher in drain soil than in the other soils (Table 1). The BCFs were significantly higher for *P. vittata* than *P. umbrosa* (Table 1) and were significantly lower in the dredge soil than in other soils for both species (Table 1). BCF for *P. umbrosa* was greater than one for the control soil only. In contrast, the BCFs for *P. vittata* ranged form two to ten (Table 1). The distribution of As in plant parts was similar in both species: green fronds > below-ground parts > senesced fronds (Fig. 2), indicating that translocation of As occurs from below-ground parts to fronds and from senescing fronds to green ones. The variation in the As concentration of dead fronds (32–71%) was much larger than in green fronds (9–32%) and below-ground parts (2–29%).

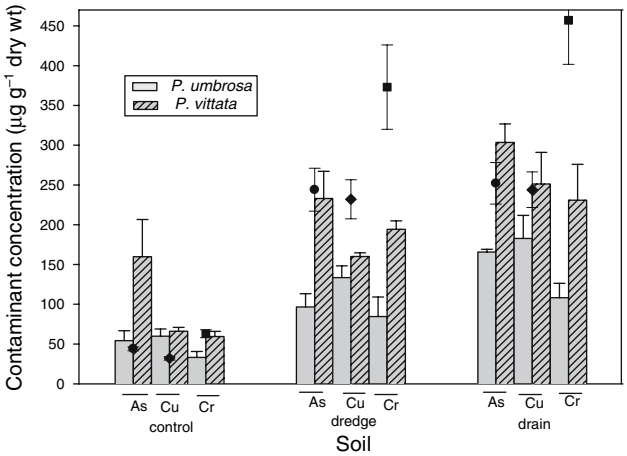

Fig. 1 As, Cu and Cr concentrations (μg g^{-1} dry wt) of *P. umbrosa* and *P. vittata* below-ground parts grown in three different soils in the glasshouse for 4 months. Soil concentrations (μg g^{-1} dry wt) of As (●), Cu (◆) and Cr (■) are also shown (mean ± SE; N = 5, except for *P. vittata* in control and dredge soils, due to the death of two plants, N = 3)

The As concentration of green fronds of *P. vittata* was significantly higher than that of *P. umbrosa* (Table 1) and

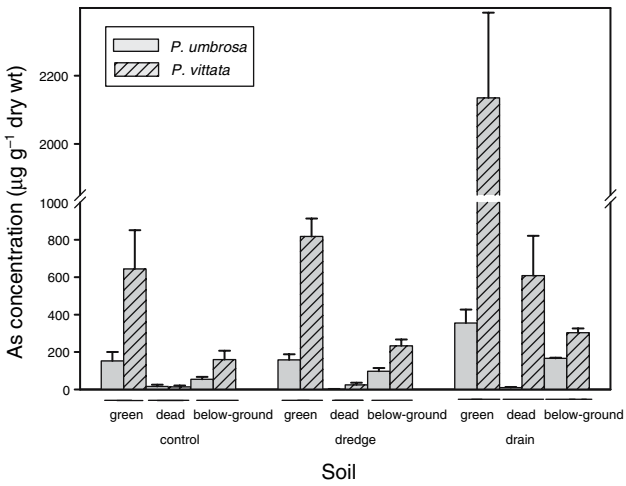

Fig. 2 Arsenic concentration (µg g^{-1} dry wt) of green and dead fronds and of below-ground parts of *P. umbrosa* and *P. vittata* grown in three soils in the glasshouse for 4 months. (mean ± SE; N = 5, except for *P. vittata* in control and dredge soils due to two deaths, N = 3)

differed between soils in the order drain > dredge ≥ control (Table 1; Fig. 2).

Phytoextraction efficiency is not only indicated by the degree of hyperaccumulation, but also by the biomass produced. Both ferns contained over half of the accumulated As in the fronds, 56.8 ± 4.8% for *P. umbrosa* and 57.1 ± 9.0% for *P. vittata*, although the As content in *P. vittata* fronds was about four times higher than of *P. umbrosa*. The relatively high below-ground biomass of *P. vittata* led to an above-ground to below-ground ratio <1, ranging from 0.3 to 0.6, the range between soils being mainly due to the variability in biomass of below-ground parts (Table 2). A great variation in above-ground to below-ground biomass for *P. vittata* has been found previously in ferns grown at various sites in China (Liao et al. 2004). The below-ground biomass of *P. umbrosa* was always less than that of *P. vittata*, allowing root contact with a smaller volume of soil which may partially explain the lower As uptake for *P. umbrosa*. In addition, the frond biomass of *P. umbrosa* exceeded that of *P. vittata*, so As translocated from the below-ground parts was distributed in a larger volume, thus further decreasing the concentration.

Although the senesced fronds made up between 40% and 49% of the above-ground biomass, they contained only between 1% and 10% of the As taken up by the plants (Table 2). The exception were the dead fronds of *P. vittata* plants grown in drain soil which contained 20% of the accumulated As. Thus under certain circumstances it may be possible to drastically reduce the amount of disposable material by harvesting green fronds only.

The As concentrations of the soils did not show a measurable decrease in the pots containing ferns. Estimates of pot soil As levels before and after plant growth showed that *P. vittata* removed 4.6 % and *P. umbrosa* 1.5 % of the As present in control soil, followed by drain soil (3.1 and 0.8% for *P. vittata* and *P. umbrosa*, respectively) and dredge soil (1.8 and 0.4%) (Table 2). Overall, *P. vittata* plants removed three to four times more As than *P. umbrosa* plants. The number of harvests required to remove enough As from contaminated soils to reach safe levels

Table 2 Arsenic partitioning and growth of *P. umbrosa* and *P. vittata* plants raised in contaminated soil samples as specified (mean ± SE)

	Control		Dredge		Drain	
Parameter	*P. umbrosa* N = 5	P. vittata N = 3	*P. umbrosa* N = 5	*P. vittata* N = 3	*P. umbrosa* N = 5	*P. vittata* N = 5
BCF	2.1 ± 0.6	9.8 ± 4.6	0.3 ± 0.1	2.1 ± 0.1	0.8 ± 0.1	6.0 ± 1.0
TF	1.8 ± 0.3	2.4 ± 0.8	1.0 ± 0.2	2.3 ± 0.4	1.2 ± 0.2	5.0 ± 0.7
As content (µg)						
Below-ground parts	243.9 ± 103.4	921.5 ± 364.8	504.0 ± 184.3	2467.2 ± 799.3	663.2 ± 104.4	2033.7 ± 714.2
Green fronds	402.7 ± 97.6	1159.6 ± 295.1	461.1 ± 106.0	1537.9 ± 157.7	891.9 ± 177.7	3504.5 ± 577.4
Senesced fronds	40.2 ± 25.5	20.6 ± 6.7	4.85 ± 1.56	43.9 ± 36.3	16.4 ± 6.1	683.7 ± 226.4
Total per plant	686.8	2101.7	970.0	4049.0	1571.5	6221.9
Total As in pot (mg)	45	45	220	220	200	200
No. harvests[a]	12	5	382	113	176	38
Dry wt (g)						
Below-ground parts	4.22 ± 1.24	5.46 ± 0.87	4.56 ± 1.10	10.24 ± 2.15	3.99 ± 0.58	6.30 ± 2.10
Green fronds	3.10 ± 0.81	1.78 ± 0.28	3.31 ± 0.69	2.00 ± 0.39	2.75 ± 0.56	1.68 ± 0.25
Senesced fronds	2.42 ± 0.42	1.72 ± 0.97	2.43 ± 0.65	1.06 ± 0.28	1.82 ± 0.48	1.12 ± 0.16
Above-ground to: below-ground ratio DW	1.39 ± 0.19	0.58 ± 0.13	1.27 ± 0.30	0.30 ± 0.18	1.09 ± 0.12	0.48 ± 0.12

[a] Number of harvests of fronds required to reduce soil As concentration to 40 µg g^{-1}; assumes constant uptake

(40 μg g^{-1}) in the current field context ranges from 38 for *P. vittata* in drain soil to 382 for *P. umbrosa* in dredge soil, both based on frond concentrations (Table 2). The time and effort involved would thus render this approach prohibitive for these particular soil conditions. The total amounts removed were about 1.7 times higher in dredge soil than control soil and 1.5 times higher in drain soil than dredge soil. This pattern is inconsistent with that for the acid-extractable As, indicating that As bioavailability differed between soils. This may be affected directly by variations in soil chemistry, or indirectly by differences in plant response to soil characteristics. In this case, neither BCFs nor TFs were strongly related to soil As concentrations.

The amount of As available for plant uptake may depend on the rate of replenishment of As in the rhizosphere soil solution from the bulk soil (Fitz et al. 2003), which is partly determined by the physicochemical properties of the soil (Adriano 2001). However, soil characterisation revealed no differences in these properties as indicated by pH (7.44–7.74), carbon content (3.2–5.6%), Fe concentration (8.9–12.6 mg g^{-1}) and particle size distribution as determined by sieving (data not shown). Redox potential of the drain soil however was lower in situ (0.09 mV) than of both dredge and control soils (310 mV), indicating anoxic/reduced conditions in drain soil. Arsenic speciation under anoxic conditions would predict a greater proportion of As(III) than As(V). Arsenic (III) is generally more mobile/bioavailable than As(V) (Adriano 2001), which may explain greater As accumulation to plants grown in drain soil.

As bioavailability may also be modified by plant processes. *P. vittata* has been shown to increase soil pH (Silva Gonzaga et al. 2006) and vary its secretion of organic carbon (phytic and oxalic acids) in response to As exposure (Tu et al. 2004). As uptake may be enhanced by addition of chelating agents such as EDTA (Wongkongkatep et al. 2003). However, due to the differences in soil characteristics and the dynamic behaviour of plants, soil and site specific trials are recommended.

In conclusion, *P. vittata* accumulated a greater proportion of Cu, Cr and As from the soil and translocated more As than the Australian native, *P. umbrosa*. The difference was especially pronounced in anoxic soil, where the bioavailability of metals may have been greater. Neither species significantly decreased the As burden in the soil in one harvest, although with higher plant density and additional harvests, the As load of the soil may be reduced.

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