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Phytoremediation of an arsenic contaminated site using *P. vittata* L. and *P. calomelanos* var. *austroamericana* – long-term data

ABSTRACT

This field study investigated the phytoremediation potential of arsenic (As) hyperaccumulating fern species, *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* over 27 month duration at a disused As-contaminated cattle-dip site located at Wollongbar, New South Wales (NSW), Australia. Ferns planted in January 2009 were harvested following 10, 22 and 27 months of growth. The average frond dry biomass, As concentration and As uptake were significantly greater in *P. calomelanos* var. *austroamericana* than *P. vittata*, at all three harvests (1.6–4.3, 1.3–1.5 and 2.2–5.7 times, respectively; $P < 0.001–0.05$). After 27 month of growth, *P. calomelanos* var. *austroamericana* removed total 8,053 mg As (i.e. cumulative over three harvests) in Plot B (25.4 kg As ha^{-1}) that was 2.65 times higher than that depleted by *P. vittata* (3,042 mg As in Plot A (9.7 kg As ha^{-1})). For the surface (0–20 cm) and subsurface (40–60 cm) layers, the mean total soil As content was significantly reduced by 49% and 63% (753–385 and 324–121 mg kg^{-1}; $P < 0.05$), respectively, using *P. calomelanos* var. *austroamericana*; and 17% and 15% (909–754 and 323–276 mg kg^{-1}; $P > 0.05$) using *P. vittata*. It is estimated that *P. calomelanos* var. *austroamericana* would take approximately 6 years to decrease mean total As content below the ecological investigation level (EIL; 20 mg kg^{-1}) in the surface and subsurface soils, whereas *P. vittata* would require 13–15 years to achieve this target.

5.1 INTRODUCTION

Arsenic (As) is an extremely toxic and carcinogenic element (Smith et al. 1998; Vangronsveld et al. 2009). Pesticides containing As were historically used to control cattle ticks at disused cattle-dip sites across large expanses of New South Wales (NSW), Australia, between early 1900s and 1955 (Smith et al. 1998). Application of arsenical pesticides at these sites has inadvertently led to the contamination of surrounding soils with elevated (> 1,000 to 3000 mg kg^{-1}) and highly variable concentrations of As (Smith et al. 1998; Kimber et al. 2002) (Chapters 3 and 4). Given the toxic and mobile forms of As present in soil around the cattle-dip sites (Chapter 7), immediate attention is required for the restoration of these sites using a suitable remediation strategy.

Phytoremediation of As-contaminated sites using As-hyperaccumulating ferns has emerged as an environmental friendly, economically viable and efficient remediation technology (Gonzaga et al. 2006; Kertulis-Tartar et al. 2006; Vangronsveld et al. 2009). Several ferns in the *Pteris* genus (e.g., *Pteris vittata*, *Pteris longifolia*, *Pteris cretica* and *Pteris umbrosa*) have been reported as As-hyperaccumulators (Ma et al. 2001; Zhao et al. 2002; Wei et al. 2007). *P. vittata* (Chinese brake fern) is a well-known As-hyperaccumulator which can accumulate > 3,500 mg kg^{-1} As in fronds on a dry weight (DW) basis (Ma et al. 2001; Tu and Ma 2002; Kertulis-Tartar et al. 2006). In non-*Pteris* fern species, *Pityrogramma calomelanos* (silver fern) and *Pityrogramma calomelanos* var. *austroamericana* (gold dust fern) have also been reported as As-hyperaccumulators (Francesconi et al. 2002; Kachenko et al. 2007). Few studies have explored the phytoextraction efficiency of these ferns under field conditions (Salido et al. 2003; Kertulis-Tartar et al. 2006). In a field study on a copper-chromium-arsenate (CCA) contaminated site, Kertulis-Tartar et al. (2006) reported that *P. vittata*

reduced the mean total As content in the surface (0–15 cm) soil from 190 to 140 mg kg^{-1} over 24 month of experimental period. They estimated that *P. vittata* would take 8 years to reduce the total As content in soil below 3.7 mg kg^{-1}, the limit set by Florida Department of Environmental Protection for the remediation As-contaminated sites. Similarly, in another field study by Salido et al. (2003) on an As-contaminated (orchard) site *P. vittata* was reported to accumulate As in the fronds efficiently, with As concentrations of up to 2,740 mg As kg^{-1} DW. The authors estimated that 8 years would be required to reduce the acid extractable As concentration in soil by 50%, i.e. from a mean value of 82 to 40 mg As kg^{-1}, the limit set by EPA. Conversely, in a field study Reichmann et al. (2004) the authors indicated that the performance of *P. vittata* to accumulate As from an As-contaminated soil decreased after subsequent frond harvests and the fern species could not regrow well in the field. The authors attributed this to the inefficiency of *P. vittata* to compete with weeds and suggested that this ferns might not have practical applications for the phytoremediation of As in Australian conditions.

The present study is an extension of our earlier work reported in Chapter 4. Therefore, the present study was undertaken to compare the phytoremediation potential of *P. calomelanos* var. *austroamericana* and *P. vittata* over a period of 27 months. The objectives of this study were to (1) evaluate the phytoremediation efficiency of *P. calomelanos* var. *austroamericana* against the well-known As-hyperaccumulator, *P. vittata* after three harvests of fronds, and (2) estimate the remediation time based on reduction in the total soil As concentration after 27 month duration using the two fern species.

5.2 MATERIALS AND METHODOLOGY

5.2.1 Fern species and experimental site

Two As-hyperaccumulating fern species, *P. vittata* and *P. calomelanos* var. *austroamericana* were used in this study. *P. vittata* was obtained from the Randwick City Council Nursery (Sydney, Australia) and *P. calomelanos* var. *austroamericana* was propagated from spores in the glasshouse.

The experimental site is a disused cattle-dip site at Wollongbar Agricultural Institute in northern NSW, Australia (28° 49′ 12.0″ S, 153° 23′ 49.2″ E) where As-based pesticides were used to treat ticks in livestock in the past. The soil properties at this site have been discussed in Chapter 4.

5.2.2 Ferns transplantation and experimental set up

In January 2009, forty-two uniform (5–6 fronds stage) plants of *P. vittata* and *P. calomelanos* var. *austroamericana* were transplanted in the field in two separate plots (Plot A and Plot B, respectively) of equal size (3.15 m^2). The ferns were planted on a 30 × 30 cm grid leaving a buffer strip of 1 m between the two plots. Further details on the experimental design, area selection and field-site set up have been described in Chapter 4.

5.2.3 Frond harvests

During the experimental period, fronds of both fern species were harvested at 10 (December 2009), 22 (November 2010) and 27 months (April 2011) experimental duration. At each harvest, young fronds and fiddle-heads were left to facilitate regrow for successive frond harvests. The harvested fronds were thoroughly washed using a three step washing procedure (tap water-0.1 M HCl-deionised water) as described in Chapter 4 and dried in a fan-forced

oven at 70 ºC for 72 hours. The dry matter yield was recorded and the samples were ground (< 1 mm) prior to digestion in a mixture (1:1) of concentrated HNO_3 and $HClO_4$ acids (Miller 1998) as described earlier.

5.2.4 Soil sampling

The soil samples were taken using a hand-driven stainless steel soil corer to a depth of 60 cm and divided into three sections to obtain soil samples for 0–20, 20–40 and 40–60 cm depths. The soil sampling was undertaken at two stages: initial sampling was performed in June 2009 as described before in Section 4.2.4 (Chapter 4). The final sampling was done in April 2011, 15 samples were randomly selected in each of the experimental plot (n = 15 samples per plot), as this much number of samples were suggested to be enough to statistically assess the change in soil As content after the phytoremediation efforts (see Chapter 3). Total and phosphate-extractable As concentrations were determined in soils as described earlier in Section 4.2.4.1 (Chapter 4).

The soil and plant digests were analysed for As using an ICP-AES (Varian® Vista AX CCD). Arsenic in the phosphate extracts was analysed using a Varian® Spectra 220Z HG-AAS spectrometer (detail in Chapter 4).

5.3 STATISTICAL ANALYSIS

The R version 2.10.1 was used for all geostatistical analyses (R Development Core Team 2008). Geostatistical analysis including spatial maps of total As in soil, spatial analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were performed as described in Chapters 3 and 4. Spatial ANOVA was used to differentiate between the total and phosphate-extractable soil As concentrations in Plot A and Plot B. ANCOVA was used to compare the

performance of *P. vittata* and *P. calomelanos* var. *austroamericana* in terms of their dry matter yield, frond As concentration and As uptake. The bioconcentration factors (BFs; concentration of As in fern fronds to concentration of As in soil) were also calculated based on the total and phosphate-extractable soil As. The significant differences between the mean BFs of the two fern species were evaluated using *t-test* (Chapter 4).

To test significance difference between the mean soil As concentrations (total and phosphate-extractable) determined in June 2009 (initial) and April 2011 (final, after fronds harvest) for three depths a 95% confidence interval was calculated around the mean total As values for June 2009 and April 2011 data set using method described in Chapter 3 (Sections 3.2.2 and 3.2.3).

5.4 RESULTS AND DISCUSSION

Both *P. calomelanos* var. *austroamericana* and *P. vittata* established successfully in the experimental plots at the studied field site, and showed no plant toxicity symptoms during the 27 month experimental period. After each frond harvest, *P. calomelanos* var. *austroamericana* ferns were observed to re-establish more rapidly and successfully as compared to *P. vittata*. Similarly, more fiddleheads and juvenile fronds were observed in *P. calomelanos* var. *austroamericana* than in *P. vittata* (Appendix 2). During the 27 month experimental period, it was observed that *P. vittata* was less efficient in competing with the weeds as it has a less dense canopy than that of *P. calomelanos* var. *austroamericana*.

5.4.1 Frond harvests

5.4.1.1 Dry matter yield

At all three harvests, the dry biomass yield of *P. calomelanos* var. *austroamericana* fronds was significantly higher (ANCOVA; $P < 0.001–0.05$) than that of *P. vittata* (Table 5.1). The average frond dry biomass was 130, 151 and 68 g plant^{-1} for *P. calomelanos* var. *austroamericana* and 81, 39 and 16 g plant^{-1} for *P. vittata* at the 10, 22 and 27 month harvests, respectively (Table 5.1). Higher frond biomass produced at the second harvest in *P. calomelanos* var. *austroamericana* reflects the longer growing time between harvests (12 months vs. 10 month). Conversely, *P. vittata* frond dry matter yield decreased at the second harvest and is in agreement with the field observations suggesting *P. vittata* was less suited to the field conditions at this site. The 27 month harvest yielded the least biomass in both fern species possibly due to the short growth period (5 month) between harvest 2 and 3. *P. calomelanos* var. *austroamericana* yielded a total frond dry matter of 12,147 g in Plot B (38.6 ton ha^{-1}) over the 27 month experimental period which is 2.36 times greater than that of *P. vittata* (5,151 g dry biomass in Plot A, i.e. 16.4 ton ha^{-1}).

5.4.1.2 Arsenic concentration in ferns

At all three harvests, the mean frond As concentration was significantly (ANCOVA; $P < 0.001–0.05$) higher in *P. calomelanos* var. *austroamericana* (887, 423 and 581 mg kg^{-1} DW, respectively) than in *P. vittata* (674, 292 and 401 mg kg^{-1} DW) (Table 5.1). In both fern species, the frond As concentration was highly variable at the three harvests and ranged from 82–1,545 mg kg^{-1} DW in *P. calomelanos* var. *austroamericana* and 103–1,307 mg kg^{-1} DW in *P. vittata* (Table 5.1). This was attributed to the varying As concentration in soil at this site, as described in Chapters 3 and 4.

Table 5.1 Dry matter yield (g plant^{-1}), As concentration (mg kg^{-1} DW) and As uptake (mg plant^{-1}) measured in fronds of *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* after first (10 month in December 2009), second (22 month in November 2010) and third (27 month in April 2011) harvests.

Fern species	Ferns harvest	Frond As concentration		Frond dry matter yield		Ferns As uptake	
		Mean ± SD	Range	Mean ± SD	Range	Mean ± SD	Range
P. vittata	2009 (n = 40)	674 ± 226	417–1307	81 ± 38	15–175	57 ± 44	9–229
(Plot A)	2010 (n = 38)	292 ± 144	103–648	39 ± 32	2–146	14 ± 20	1–95
	2011 (n = 29)	401 ± 174	187–920	16 ± 18	2–86	7 ± 9	1–37
P. calomelanos var.	2009 (n = 37)	887 ± 252	360–1,545	130 ± 109	10–555	124 ± 147	5–857
austroamericana	2010 (n = 35)	423 ± 137	82–710	151 ± 91	13–457	64 ± 43	1–210
(Plot B)	2011 (n = 30)	581 ± 205	274–1203	68 ± 33	16–134	40 ± 24	6–96
Significance (P) (ANCOVA)	2009	< 0.001		> 0.05		< 0.05	
	2010	< 0.05		< 0.05		< 0.001	
	2011	< 0.05		< 0.001		< 0.001	

5.4.1.3 Fern As uptake

The average frond As uptake at all three harvests was significantly (ANCOVA; $P < 0.001–0.05$) higher in *P. calomelanos* var. *austroamericana* (124, 64 and 40 mg plant^{-1}, respectively) than in *P. vittata* (27, 14 and 7 mg plant^{-1}) (Table 5.1). *P. calomelanos* var. *austroamericana* fronds removed total 8,053 mg As (25.4 kg As ha^{-1}; i.e. cumulative As over three harvests) from soil in Plot B after 27 month of growth , which is 2.65 times greater than the total As depleted by *P. vittata* (3,042 mg As (9.7 kg As ha^{-1})) in Plot A.

The higher frond biomass observed in *P. calomelanos* var. *austroamericana* at 22 and 27 months is in agreement with the results presented in Chapter 4 where 1.61 times greater frond biomass was reported in *P. calomelanos* var. *austroamericana* than in *P. vittata* after 10 month of growth. The results suggested that the subtropical field conditions appeared to be better suited for *P. calomelanos* var. *austroamericana* than *P. vittata*. In the current study, decreasing trend in the frond biomass yield in *P. vittata* could be attributed to competition of weeds, which was evident from the field observations during the experimental period. In a previous study, Reichmann et al. (2004) also indicated that the phytoextraction efficiency of *P. vittata* might decline due to unfavourable growing conditions, such as drought and weeds under field conditions.

The concentration of As in the fronds of *P. vittata* was lower than the values reported in previous field studies (Salido et al. 2003; Kertulis-Tartar et al. 2006). In the study by Kertulis-Tartar et al. (2006), *P. vittata* was grown on a CCA contaminated site in soil with alkaline pH (7.4) and sandy texture (88%); the mean As concentration in fronds of *P. vittata* was 3,186 mg kg^{-1} DW. In the present study, lower frond As concentration in the two fern species could

be attributed to the low soil pH (4.80) and very high free Fe concentration (16%) at this site (Chapter 4). Arsenic sorption on the surface of Fe oxyhydroxides increases at low soil pH and in the presence of high free Fe can reduce the bioavailability of As in soil (Smith et al. 1998; de Mello et al. 2007; Pan et al. 2010). The presence of lower (≤ 13.4% of total soil As) phosphate-extractable soil As concentrations in both plots at the studied site also supports the argument that Fe oxyhydroxides decreased the bioavailability of As in soil and resulted in reduced translocation of As to the studied fern species. The decreasing trend in the frond As concentration of both fern species at the studied site is also in agreement with this view.

The frond dry biomass and As concentration are used to calculate the fern As uptake (i.e. amount of As extracted by ferns from soil) (Ma et al. 2001; Xu et al. 2009). *P. calomelanos* var. *austroamericana* showed higher frond As concentration and produced significantly greater amount of frond dry biomass than *P. vittata* after all three harvests (Table 5.1). This resulted in the greater As uptake in *P. calomelanos* var. *austroamericana* than in *P. vittata*, suggesting that this species is favourable in the phytoremediation of As at this site.

For *P. vittata*, the production of low-molecular-weight thiols has been reported to detoxify As in the fern while plants were exposed to As-rich environment or contaminated soil (Cai et al. 2004; Xu et al. 2010). Whereas in *P. calomelanos* var. *austroamericana* formation of arsenide–sulfide (As^{III}–S^{2-}) compounds have been investigated to be responsible for the biochemical reduction of As^{V} to As^{III} during As transportation from root to shoot, which could play a pivotal role in the detoxification of As in this species (Kachenko et al. 2010). In the current study, both fern species were grown at same site; hence the significant differences found between performance of the two fen species could be attributed to disparities in

physiological mechanisms involved in the detoxification of As after being uptaken by plants (ferns). However, further studies are warranted (e.g. using X-ray absorption spectroscopy) to elucidate the physiological functions responsible for As tolerance in *P. calomelanos* var. *austroamericana*.

For all three harvests, the average BFs (based on the total and phosphate-extractable As in soil) in *P. calomelanos* var. *austroamericana* (1.3, 0.71, 0.82 and 21, 10, 13, respectively) were significantly ($P < 0.001$; *t-test*) higher than in *P. vittata* (0.80, 0.34, 0.45 and 14, 6, 8). Low BFs in both fern species were attributed to decreased plant (fern) availability of As at the studied site possibly due to the long ageing period of > 40 years, very high free Fe and low soil pH as discussed earlier in Chapter 4. The data revealed that extractable (bioavailable) As in the top-soil was ≤ 11% in the two plots and also support the earlier explanation. Therefore, using BFs based on phosphate-extractable As are meaningful under the field conditions as suggested in previous studies (Chapter 4) (Wei and Chen 2006; Wei et al. 2006).

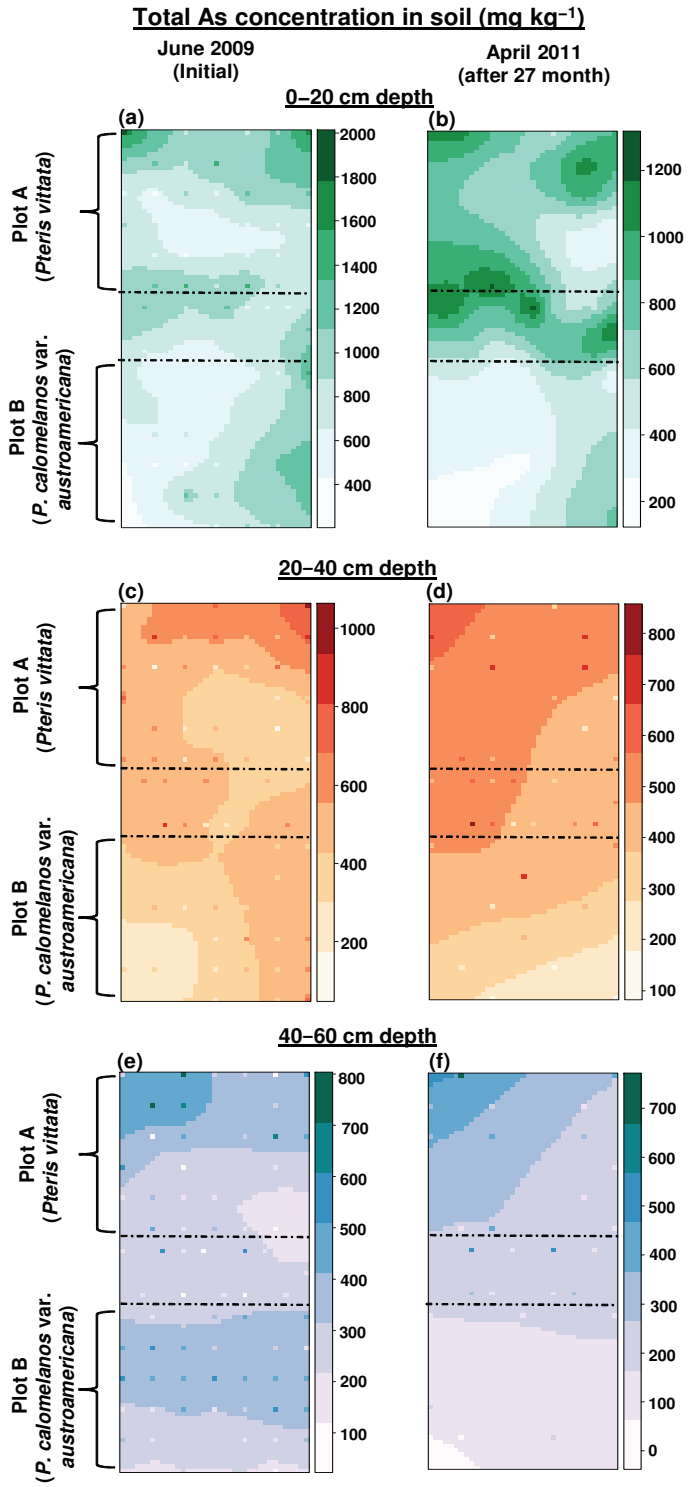

Figure 5.1 The spatial variability maps of As showing remediation trend in the total soil As concentration for three depths in Plot A (*P. vittata*) and Plot B (*P. calomelanos* var. *austroamericana*) of the experimental area; (a,c,e) initial As concentration in soil determined in June 2009; (b,d,f) final As concentration in soil measured in April 2011, after 27 month of fern growth. The two experimental plots were separated by a buffer strip as shown in the figure above. The data presented here correspond to the mean total soil As concentration in Table 5.2.

5.4.2 Total and phosphate-extractable soil As concentrations for three depths

The initial and final (27 month) total As concentration in soil was spatially heterogeneous (Figure 5.1). The spatial variability pattern in soil As concentration adjacent to the cattle-dip site has been previously described (Chapter 3) in a geostatistical context. The concentration of As in soil was found to be spatially correlated with distance from the cattle-dip; the variability in soil As content was reported to be associated with the cattle dipping process, disposal of the As-contaminated dip sediment in the vicinity of the cattle-dip and pumping-out of the dipping fluid from the dip bath (Chapter 3).

After 27 month, the concentration of total soil As in Plot B (*P. calomelanos* var. *austroamericana* plot) decreased significantly ($P < 0.05$) in the surface (0–20 cm) and the lowest (40–60 cm) depths; however a slight ($P > 0.05$) reduction was observed for the second layer (20–40 cm) (Table 5.2; Figure 5.1a,b). For Plot A (*P. vittata* plot), the mean total As concentration was reduced ($P > 0.05$) in the surface (0–20 cm) and the lowest (40–60 cm) soils after 27 month duration, however, no reduction was observed in the second (20–40 cm) layer (Table 5.2; Figure 5.1c–f).

The mean total soil As concentration in the top-soil after 27 month was reduced by 17% in Plot A (*P. vittata* plot) and 49% in Plot B (*P. calomelanos* var. *austroamericana* plot) (909–754 and 753–385 mg kg^{-1}, respectively; Table 5.2; Figure 5.1a,b). For the second layer (20–40 cm), no decline in average total soil As concentration was observed in Plot A, while 11% decrease was depicted in Plot B (see Table 5.2; Figure 5.1c,d). In Plot A and Plot B, the average total As content in the soils at the lowest (40–60 cm) depth was reduced by 15% and 63% after 27 month duration, respectively (Figure 1e,f).

Table 5.2 The concentrations of total and phosphate-extractable As in soil in Plot A and Plot B of the experimental area (*n* = 42 per plot for initial sampling; *n* = 15 per plot for final sampling (as mentioned below). Initial As concentrations in soil were determined before harvesting and final after three harvests of fern fronds.

Plots	Soil depth (cm)	Total As in soil[a] (mg kg^{-1})		Significance (*P*)[b]	Phosphate-extractable As in soil[a] (mg kg^{-1})		Significance (*P*)[b]
		June 2009	April 2011		June 2009	April 2011	
Plot A (*P. vittata*)	0–20	909 ± 354	754 ± 291	> 0.05	51 ± 17	66 ± 16	< 0.05
	20–40	499 ± 204	503 ± 175	> 0.05	53 ± 21	69 ± 20	< 0.05
	40–60	323 ± 173	276 ± 170	> 0.05	42 ± 31	37 ± 27	> 0.05
Plot B (*P. calomelanos* var. *austroamericana*)	0–20	753 ± 284	385 ± 169	< 0.05	52 ± 23	51 ± 17	> 0.05
	20–40	396 ± 117	352 ± 166	> 0.05	54 ± 19	51 ± 14	> 0.05
	40–60	324 ± 133	121 ± 55	< 0.05	29 ± 11	21 ± 10	< 0.05
Significance (*P*)[c]	0–20	< 0.05	< 0.001	–	> 0.05	< 0.05	–
	20–40	> 0.05	< 0.05	–	> 0.05	< 0.05	–
	40–60	> 0.05	< 0.01	–	> 0.05	< 0.05	–

[a] The values are presented as mean ± SD; [b] 95% confidence interval was calculated around the mean soil As concentrations determined in June 2009 (initial) and April 2011 (final after three harvests).

[c] Spatial ANOVA was used to differentiate between soil As concentration in Plot A and Plot B (Statistical analysis section, Chapter 4).

These results confirmed that greater amount of soil As was depleted by *P. calomelanos* var. *austroamericana* than using *P. vittata*. The decrease in total soil As concentration after the 27 month period for the two experimental plots is also evident in Figure 5.1, particularly for the surface (0–20 cm) and the deepest (40–60 cm) layers in Plot B. The variability in the total soil As concentration appeared to be slightly reduced in both plots for the studied depths (Figure 5.1).

After 27 month, the mean phosphate-extractable As concentration in soil showed a significant reduction ($P < 0.05$) of 28% only in Plot B for the lowest depth (29–21mg kg^{-1}); however negligible change was observed for the first two depths (Table 5.2). Conversely, the average phosphate-extractable As concentration in Plot A significantly ($P < 0.05$) increased for the first two depths, and reduced by 8% for the lowest (40–60 cm) layer.

The results showed that both fern species decreased the concentration of total As in soil at the studied site from the surface (0–20 cm) and the lowest (40–60 cm) layers, indicating that roots of the two fern species could extract As at the lowest depth. In the As-hyperaccumulating fern species, excretion of root exudates such as low-molecular-weight organic acids have been reported to be associated with the solubilisation of As in the rhizosphere soil; thereby increasing the available As content in soil for fern uptake (Gonzaga et al. 2006; Gonzaga et al. 2009). In the current study, both *P. calomelanos* var. *austroamericana* (Plot B) and *P. vittata* (Plot A) roots could possibly use the same mechanism to enhance the available As concentration in the soils and accumulate As in the aboveground biomass. However, the rate of As accumulation seemed to be higher in *P. calomelanos* var. *austroamericana* than in *P. vittata* which resulted in greater decrease in soil

As content using this fern species. The higher decrease in the phosphate-extractable soil As content using *P. calomelanos* var. *austroamericana* in Plot B than by *P. vittata* in Plot A could possibly support the earlier suggestion.

These findings are in agreement with those of Kertulis-Tarter et al. (2006), who also reported 26% and 12% decline in the total soil As concentrations for 0–15 and 30–60 cm depths, respectively, using *P. vittata* for 24 month at an As-contaminated (CCA) site. In the study by Kertulis-Tarter et al (2006), maximum decrease (43%) in the total soil As concentration was found at 15–30 cm depth using *P. vittata*. In the current study, no reduction was observed for the total soil As content at the second (20–40 cm) depth in Plot A (*P. vittata*); and 11% As was reduced in the soil in Plot B by *P. calomelanos* var. *austroamericana* at this depth. Immediately, it is unclear what factors caused reduced (or no) depletion of As by both fern species from soil at the second depth in their respective plots. Further detailed examinations are required to elucidate the root distribution, density and morphology of these fern species grown at the field site.

5.5 TIME ESTIMATION FOR REMEDIATION OF THE EXPERIMENTAL SITE

The remediation time was estimated for the studied site based on decrease in the mean total soil As concentration in both plots after 27 month duration (i.e. mean reduction in total soil As after 27 months (mg kg^{-1}) = initial As – final As concentration after 27 months). In Plot B, *P. calomelanos* var. *austroamericana* would take 5 years for the top-soil (0–20 cm) and 4 years for the deepest layer (40–60 cm) to remediate the site and reduce the total As content below the required ecological investigation level (EIL) of 20 mg kg^{-1} in Australia (NEPM 1999). In Plot A, *P. vittata* would take 2.9 and 4 times (13 and 15 years) more time than *P. calomelanos* var. *austroamericana* to achieve this target for the top and the lowest soil depths,

respectively. For 20–40 cm depth, remediation time-estimation was only possible for area under *P. calomelanos* var. *austroamericana* (Plot B), as no decrease in soil As occurred in Plot A using *P. vittata*, as mentioned earlier (see Table 5.2). The data suggest that 20 years would be required to reduce total soil As in the second layer below 20 mg kg^{-1} in Plot B using *P. calomelanos* var. *austroamericana*.

The remediation estimates are based on the assumption that both fern species would show the same As-accumulation trend over time as it was observed during this study for the 27 month period. Our remediation assessments are based on the mean total soil As content and they may result in the underestimation or overestimation of the remediation time at the site. Since As concentrations in soil at the studied site were highly variable (Figure 5.1); therefore, it is expected that some areas in the two experimental plots would be completely remediated before the estimated period of remediation. Similarly, some of the area under both fern species would need longer than the estimated period for remediation.

5.6 CONCLUSIONS

The results revealed that *P. calomelanos* var. *austroamericana* extracted (2.65 times) higher amounts of As from soil in Plot B than that of *P. vittata* in Plot A, resulting in greater reduction in the mean total soil As content for three depths. This study shows that *P. calomelanos* var. *austroamericana* performed much better than *P. vittata* for the remediation of As in soil at the studied site. It is evident from the experimental data that long-term field-based studies are imperative in evaluating the phytoremediation potential of hyperaccumulating ferns, and should be extended to the other As-contaminated dip sites containing varying soil As concentrations and properties.

5.7 REFERENCES

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