Phytoremediation potential of *Pteris vittata* L. and *Pityrogramma calomelanos* var. *austroamericana* at a highly variable arsenic contaminated site — short-term data

**ABSTRACT**

This study examined the phytoextraction potential of two arsenic (As) hyperaccumulators, *Pteris vittata* L. and *Pityrogramma calomelanos* var. *austroamericana* at a historical As-contaminated cattle-dip site in northern New South Wales (NSW), Australia. Total As concentration in the surface soil (0–20 cm) showed a better spatial structure than phosphate-extractable As in the surface and subsurface soil at this site. *P. calomelanos* var. *austroamericana* produced greater frond dry biomass (mean = 130 g plant$^{-1}$) than *P. vittata* (mean = 81 g plant$^{-1}$) after 10 month of growth. Arsenic concentration and uptake in fronds were also significantly higher in *P. calomelanos* var. *austroamericana* (mean = 887 mg kg$^{-1}$ and 124 mg plant$^{-1}$) than in *P. vittata* (mean = 674 mg kg$^{-1}$ and 57 mg plant$^{-1}$). The results showed that under the field conditions and highly variable soil As at the site, *P. calomelanos* var. *austroamericana* performed better than *P. vittata*. It is predicted that *P. calomelanos* var. *austroamericana* would take approximately 100 years to reduce the total As to below 20 mg kg$^{-1}$ at the site compared to ≥ 200 years estimated for *P. vittata*. However, long-term data are required to confirm these observations under field conditions.

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2 This chapter has been published in *International Journal of Phytoremediation*, 13 (9): 912–932 (2011) under the title ‘Phytoremediation potential of *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* L. grown at a highly variable arsenic contaminated site’. Authors are Nabeel K. Niazi, Balwant Singh, Lukas Van Zwieten and Anthony G. Kachenko.
Chapter 4: Phytoremediation using *P. vittata* and *P. calomelanos* var. *austroamericana*

### 4.1 INTRODUCTION

Arsenic is highly toxic and carcinogenic, and therefore the restoration of As-contaminated sites is imperative (Smith et al. 1998; Mandal and Suzuki 2002). From early 1900s to 1955, As-based pesticides were used to control ticks in cattle at the historical cattle-dip sites in NSW, Australia. This practice led to As contamination of over 1600 dip sites along the eastern coast of Australia with many located in northern NSW (Smith et al. 1998). Arsenic concentration in soils around these cattle-dip sites is extremely variable and high, with levels up to 14,000 mg kg$^{-1}$ (McLaren et al. 1998; Kimber et al. 2002).

Phytoextraction using hyperaccumulating ferns has emerged as an efficient, environmental friendly and cost-effective, *in situ* remediation technology for As-contaminated sites (Ma et al. 2001; Gonzaga et al. 2006; Kertulis-Tartar et al. 2006). Plants that accumulate > 1000 mg As kg$^{-1}$ dry weight (DW) in fronds and produce large quantities of aboveground biomass are considered suitable for phytoextraction of As-contaminated soils (Gonzaga et al. 2006). *Pteris vittata* (Chinese brake fern) is a well-recognised As-hyperaccumulator which can accumulate > 3000 mg kg$^{-1}$ of As in fronds on a dry weight (DW) basis from the As-contaminated soils (Ma et al. 2001; Tu and Ma 2002; Kertulis-Tartar et al. 2006). Other *Pteris* (e.g. *Pteris longifolia*, *Pteris cretica* and *Pteris umbrosa*) and non-*Pteris* ferns species (e.g. *Pityrogramma calomelanos*, *Pityrogramma calomelanos* var. *austroamericana*) have also been identified to hyperaccumulate As (Francesconi et al. 2002; Zhao et al. 2002; Kachenko et al. 2007; Wei et al. 2007). *Pityrogramma calomelanos* var. *austroamericana* (Gold dust fern), a fern species naturalised in Australia, has been reported to accumulate up to 16,415 mg As kg$^{-1}$ DW in fronds (Kachenko et al. 2007). Xu et al. (2010) compared the phytoremediation potential of *P. vittata* and *P. calomelanos* var. *austroamericana* grown in
four As-contaminated soils with contrasting properties and found that *P. vittata* generally possessed higher aboveground biomass and As accumulation than *P. calomelanos* var. *austroamericana*.

Few studies have reported As extraction potential of these ferns under field conditions (Salido et al. 2003; Kertulis-Tartar et al. 2006). In a field study on an As-contaminated orchard site As concentration in the fronds of *P. vittata* ranged from 1,000–2,740 mg As kg\(^{-1}\) DW (Salido et al. 2003). The authors suggested that 8 years would be required to decrease the concentration of acid extractable As in soil from a mean value of 82 to 40 mg As kg\(^{-1}\), the limit set by EPA. However, some other studies indicate a relatively poor performance of *P. vittata* for As phytoremediation (Reichmann et al. 2004; Wei and Chen 2006; Shelmerdine et al. 2009). Reichmann et al. (2004) suggested that unfavourable environmental conditions, such as drought, made it difficult for *P. vittata* to have practical applications for the phytoextraction of As in Australian conditions.

This study was undertaken to evaluate the phytoextraction potential of *P. calomelanos* var. *austroamericana* and *P. vittata* at an As-contaminated cattle-dip site containing high and extremely variable As concentrations. *P. calomelanos* var. *austroamericana* was investigated as part of the study as this species has shown a consistent As accumulation pattern in previous glasshouse studies (Kachenko et al. 2007; Kachenko et al. 2010) and is well adapted to field conditions in eastern Australia (Ashley et al. 2003). To the best of knowledge, no field study has been performed to evaluate the phytoextraction efficiency of *P. calomelanos* var. *austroamericana*. Geostatistical methods have been previously used to estimate the distribution of metal(loid)s on large scales (Lark and Cullis 2004; Aelion et al. 2009); and
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such methods could be useful in describing the spatial variation in soil As in the vicinity of the cattle-dip sites. Therefore, the objectives of the present study were to (1) determine the spatial distribution of As in soil employing geostatistical methods; and (2) compare the phytoremediation potential of *P. calomelanos* var. *austroramicana* against the well-known As hyperaccumulator, *P. vittata* under field conditions.

### 4.2 MATERIALS AND METHODOLOGY

#### 4.2.1 Fern species

*P. calomelanos* var. *austroramicana* was propagated from spores and nourished under controlled glasshouse conditions for seven months, while *P. vittata* plants with 5–6 fronds were obtained from the Randwick City Council Nursery, NSW, Australia.

#### 4.2.2 Experimental Site

The field site is a disused cattle-dip site located at the Wollongbar Primary Industries Institute in northern NSW, Australia (28.82° S, 153.397° E) (site history and map in Appendix 2). The soil at the site has acidic pH (4.82), low cation exchange capacity (87.5 mmol c kg⁻¹), and high contents of free Fe (15.9%), organic carbon (4.5%) and clay (44%) (Table 4.1).

#### 4.2.3 Experimental set up

The experimental area was selected based on a preliminary soil sampling (0–10 cm depth) to determine the total As distribution in soil (52–725 mg kg⁻¹; *n* = 30) around the dip site. The site was prepared in January 2009 (Appendix 2), uniform ferns of *P. calomelanos* var. *austroramicana* and *P. vittata* at the 5–6 fronds stage, were transplanted in the field into
hand-dug holes (~10 cm deep × 10 cm wide). The ferns were planted in a 30 × 30 cm grid pattern in two separate plots of equal size (3.15 m²) (Figure 4.1).

Table 4.1 Physico-chemical properties of the soil from the experimental site at Wollongbar in northern NSW.

<table>
<thead>
<tr>
<th>Soil property</th>
<th>Values (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (1:5, 0.01 M CaCl₂)</td>
<td>4.82±0.05</td>
</tr>
<tr>
<td>EC (dS m⁻¹)ₐ</td>
<td>0.11±0.03</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>4.5±0.02</td>
</tr>
<tr>
<td>CEC (mmol c kg⁻¹)ᵇ</td>
<td>87.5±1.02</td>
</tr>
<tr>
<td>DCB Fe (%)ᵇ⁻ᶜ</td>
<td>15.9±0.12</td>
</tr>
<tr>
<td>DCB Al (%)ᵇ⁻ᶜ</td>
<td>1.6±0.05</td>
</tr>
<tr>
<td>Sand (%)</td>
<td>16</td>
</tr>
<tr>
<td>Silt (%)</td>
<td>40</td>
</tr>
<tr>
<td>Clay (%)</td>
<td>44</td>
</tr>
</tbody>
</table>

ₐElectrical conductivity (1:5, soil:water ratio); ᵇcation exchange capacity; ᶜDithionite citrate-bicarbonate extractable Fe and Al.

Forty-two ferns of each species were planted in each plot and a buffer strip of 1 m was kept between the two plots. A drip irrigation system was installed to irrigate ferns for 5 minutes, twice a day at 12 hour intervals using a timer. Shade cloth was raised over the experimental area to protect the ferns from excessive sunlight and a black nylon weed mat was used to minimise the weed growth within the experimental area (Appendix 2). The ferns were supplied with basic plant nutrients using a slow-release fertiliser (4–5 g plant⁻¹, Osmocote® plus), containing N:P:K of 16:4.4:10 wt/wt %.
Figure 4.1 A schematic representation of the experimental site at Wollongbar along a cattle-dip bath. The area planted with *P. vittata* (○) and *P. calomelanos* var. *austroamericana* (●). In June 2009, two soil cores were taken around each fern and mixed together to get a composite soil sample, as indicated in this figure.
4.2.4 Soil and plant sampling

In June 2009, soil samples \( n = 102 \) were taken using a hand-driven stainless steel soil corer at 0–20, 20–40 and 40–60 cm depths. Two soil cores were extracted around each fern and mixed together to get a representative composite sample and in addition 18 soil cores were collected from the buffer area between the two plots (Figure 4.1).

At the same time, preliminary plant sampling of fern tips (i.e. the apex portion of fern fronds) was undertaken to evaluate plant As uptake.

4.2.4.1 Total and phosphate-extractable As in soil

The soil samples were air dried and sieved (< 2 mm). Sub-samples (~ 15 g) were taken and ground finely to obtain soil fraction of < 200 \( \mu \)m which was used for measuring total (0–20 cm depth) and phosphate-extractable As (0–20, 20–40 and 40–60 cm depths) concentrations in soil.

For total As (0–20 cm depth), soil samples (~ 0.25 g) were digested in a mixture of concentrated hydrofluoric (HF), sulphuric (H\(_2\)SO\(_4\)), perchloric (HClO\(_4\)), nitric (HNO\(_3\)) and 1.2 M hydrochloric (HCl) acids (Huang and Fujii 1996). Briefly, the samples were soaked in HNO\(_3\) and HCl overnight. The soaked samples were then mixed with H\(_2\)SO\(_4\), HF and HClO\(_4\) and heated at 120 °C overnight until the mixture was dried. After cooling for 5 minutes, the digest was dissolved by adding 25 mL of 6 M HCl and final volume was made to 50 mL using E-pure® water.

To evaluate bioavailable fraction of As, soil samples (~ 1.0 g) from three depths were shaken in polythene tubes with 25 mL of 0.5 M potassium dihydrogen phosphate (KH\(_2\)PO\(_4\)) solution at room temperature (Alam et al. 2007). After 4-hours shaking, the samples were centrifuged
at 3000 rpm for 10 minutes and filtered (Whatman® no. 1). The extracts were acidified by adding 1 M HCl and analysed for As using a Varian® Spectraa 220Z hydride-generation atomic absorption spectrometer (HG-AAS). The relative standard deviation (RSD) for HG-AAS analysis was < 2%.

In December 2009, after 10 month of growth, aboveground biomass (fronds) was harvested leaving fiddle-heads and some young fronds to facilitate regrow the ferns for the subsequent harvests. The harvested material was thoroughly washed using a series of steps involving 2–3 rinses with tap water followed by a rinse with a 0.1 M HCl solution and a final thorough washing with deionised water. The fronds were dried in a fan-forced oven at 70 °C for 72 hours. The dry matter yield was recorded and the samples were ground (< 1 mm) and digested in a mixture (1:1) of concentrated HNO₃ and HClO₄ acids (Miller 1998) (Appendix 5).

The soil and plant digests were analysed for As using an inductively coupled plasma atomic emission spectrometer (ICP-AES). Reagent blanks and National Institute of Standards and Technology reference plant material (pine needles no. 1575) and soils (Montana 2710 and San Janquin 2709) were used for quality assurance to check the efficiency of the digestion procedures and ICP-AES analyses. Recovery of As was within ±10% of recommended values. The RSD for ICP-AES analysis was < 3%.

### 4.3 STATISTICAL ANALYSIS

The correlations, t-test and geostatistical analysis for soil and plant data were performed using R version 2.10.1(R Development Core Team 2008). Ordinary kriging was used to krig the data and generate the spatial maps (see Appendix 2 for these maps). The spatial analysis of
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Variance (ANOVA) (Lark and Cullis 2004) and *t-test* were used to evaluate differences between the concentrations of total and phosphate-extractable As in soils of Plot A and Plot B.

There were 37 and 40 plants of *P. calomelanos* var. *austroamericana* and *P. vittata* at the time of harvest, respectively. Prior to analysis, the total dry matter yield and As uptake of *P. calomelanos* var. *austroamericana* were normalised to 40 plants using the mean for comparing the performance of the two species. The performance of *P. vittata* and *P. calomelanos* var. *austroamericana* was compared employing analysis of covariance (ANCOVA) and *t-test*. ANCOVA neutralised the confounding effect of total soil As and distinguished between the frond As concentration, As uptake and dry matter yield of the two fern species. The bioconcentration factors (BFs) were calculated by dividing the As concentration in fern fronds to the As concentration in soil.

Remediation estimates for the two fern species were made for a period of 10 and 20 years for total As and for 4 years for phosphate-extractable As using their respective As uptake data (Appendix 2; Section 4.4 next). The spatial maps were generated to show the remediation trends over the estimated time scale using the method, as described earlier (Chapter 3).

### 4.4 Calculations to Estimate the Remediation Time-Frame Based on As Uptake Data

To estimate the As removal by ferns over time, it was assumed that both fern species extracted As from a depth of 0–60 cm and depleted uniform amount of As to this depth. At the harvest ferns roots were observed to occur at depth of up to 60 cm. Since the total As was available for the surface layer (0–20 cm); the total As concentration at 20–40 and 40–60 cm
The soil bulk density ($\rho$, kg m$^{-3}$) for each depth was determined in triplicate by taking soil core using a stainless steel corer (see Appendix 2). Every single fern was assumed to occupy equal soil volume, since the ferns were planted on a grid (see Figure 4.1). From measured bulk density values for each depth, amount of the total and phosphate-extractable As in soil were calculated for three depths using the following equation:

$$As_d (g) = V_d (m^3) \times C_d (g \ kg^{-1}) \times \rho_d (kg \ m^{-3})$$

Where $As_d$ is the calculated amount of As at each depth, $V_d$ is the soil volume occupied by every single fern (see Appendix 2), $C_d$ is the soil As concentration at each location for each layer and $\rho_d$ is the bulk density measured for each depth (Appendix 2). The amount of As calculated for each layer ($As_d$, at 0–20, 20–40 and 40–60 cm depths) was summed to obtain the total amount of As contained in 0–60 cm depth.

Using As uptake data for the two species, remediation estimates for the fern species were calculated for a period of 10 and 20 years for total As and for 4 years for phosphate-extractable As. The spatial maps were generated to show the remediation trends of both ferns over the estimated time scale.
4.5 RESULTS

4.5.1 Soil As concentrations

4.5.1.1 Total As concentration in the surface soil

The concentration of total As in soil in the surface samples was highly variable in both the plots, as described earlier (see Appendix 2) (Figure 3.4a; Chapter 3). The average As concentration was significantly ($P < 0.05$; $t$-test) higher in Plot A (909 mg kg$^{-1}$) than in Plot B (753 mg kg$^{-1}$). Spatial ANOVA results also showed that the estimated mean for total As at 0–20 cm depth was significantly ($P < 0.05$) higher in Plot A (1058 mg kg$^{-1}$) than in Plot B (714 mg kg$^{-1}$) (Table 4.2). The exponential model was better to fit the variogram for total As concentration (at 0–20 cm depth), and showed a practical range of approximately 7 m (Appendix 2).

4.5.1.2 Phosphate-extractable soil As concentrations for three depths

The spatial maps indicated a higher variability in phosphate-extractable As concentration for the first two depths (0–20 and 20–40 cm) than the lowest layer (40–60 cm) (Table 4.2 and Appendix 2) (see Figure 3.4b,c,d in Chapter 3). Spatial ANOVA estimated mean for phosphate-extractable As in the surface layer (0–20 cm) was significantly ($P < 0.01$) higher in Plot A (72 mg kg$^{-1}$) as compared to Plot B (38 mg kg$^{-1}$) (Table 4.2). For the 20–40 and 40–60 cm depths, no significant differences were found for phosphate-extractable As between the two plots (Table 4.2).
Table 4.2 Total and phosphate-extractable soil As concentrations (mg kg\(^{-1}\)) in Plot A and Plot B (n = 42 per plot). The statistical differences between the two plots, for total and phosphate-extractable As, are presented based on spatial ANOVA and \(t\)-test.

<table>
<thead>
<tr>
<th>Plot</th>
<th>Total soil As</th>
<th>Phosphate-extractable As</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0–20 cm</td>
<td>0–20 cm</td>
</tr>
<tr>
<td>Plot A ((P. \text{vittata}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>393–1903</td>
<td>25–90</td>
</tr>
<tr>
<td>Mean</td>
<td>909</td>
<td>51</td>
</tr>
<tr>
<td>Median</td>
<td>734</td>
<td>45</td>
</tr>
<tr>
<td>SD ((\pm))</td>
<td>354</td>
<td>17</td>
</tr>
<tr>
<td>(\beta)</td>
<td>1058</td>
<td>72</td>
</tr>
<tr>
<td>Plot B ((P. \text{calomelanos}) var. (\text{austroamericana}))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>313–1486</td>
<td>21–117</td>
</tr>
<tr>
<td>Mean</td>
<td>753</td>
<td>52</td>
</tr>
<tr>
<td>Median</td>
<td>878</td>
<td>52</td>
</tr>
<tr>
<td>SD ((\pm))</td>
<td>284</td>
<td>23</td>
</tr>
<tr>
<td>(\beta)</td>
<td>714</td>
<td>38</td>
</tr>
<tr>
<td>Significance ((P))</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spatial ANOVA</td>
<td>0.038</td>
<td>0.001</td>
</tr>
<tr>
<td>(t)-test</td>
<td>0.028</td>
<td>0.938</td>
</tr>
</tbody>
</table>

\(\beta\) = means estimated using spatial ANOVA

For the phosphate-extractable As at 20–40 and 40–60 cm depths, exponential model best fitted the variogram, while for the 0–20 cm layer spherical model was best to fit the variogram (Appendix 2). The practical range for phosphate-extractable As was 2.5, 2.3 and 1.5 m for 0–20, 20–40 and 40–60 cm depths, respectively.
Phosphate-extractable As constituted ≤ 14% of the total soil As in the two plots (Table 4.2). In the deepest soil layer (40–60 cm), the concentration of extractable As was lower than the upper two layers. The t-test showed no significant (P > 0.80) differences between phosphate-extractable As concentration at 0–20 and 20–40 cm depths for the two plots, however, for the lowest depth (40–60 cm) mean As concentration was significantly (P < 0.05; t-test) higher in Plot A than in Plot B.

4.5.2 First plant sampling

Field observations indicated that both fern species established successfully in the experimental plots and grew without showing phytotoxicity symptoms (Appendix 2). *P. calomelanos* var. *austroamericana* was more dense and vigorous in growth than *P. vittata*. More new crosiers and young fronds emerged from *P. calomelanos* var. *austroamericana* plants as compared to *P. vittata* during the experimental period. The ferns nearer to the plot boundary demonstrated more vigorous growth than those located towards the centre of the plot. This behaviour was more evident in *P. calomelanos* var. *austroamericana* (Plot B) and may have resulted in the higher mortality in this species.

The preliminary data for As in frond tips show that significant amounts of As have been accumulated by both fern species in the five-month period (Table 4.3). The average As concentration in *P. calomelanos* var. *austroamericana* (2,616 mg kg⁻¹ DW) fronds was significantly greater (P < 0.001; t-test) than that of *P. vittata* (1,502 mg kg⁻¹ DW) (Table 4.3). The high variability in total soil As can create a confounding effect on plant As concentration, therefore, it was vital to isolate the effect of variable soil As concentration for comparing frond As concentration in both species, using ANCOVA.
Chapter 4: Phytoremediation using *P. vittata* and *P. calomelanos* var. *austroamericana*

Table 4.3 The frond As concentration, dry matter yield and As uptake of *P. vittata* and *P. calomelanos* var. *austroamericana*. The differences between the two fern species are evaluated using ANCOVA and *t*-test.

<table>
<thead>
<tr>
<th>Ferns species</th>
<th>As in frond tips</th>
<th>Major harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>at the first sampling</td>
<td>Ferns dry matter yield</td>
</tr>
<tr>
<td></td>
<td>(mg kg(^{-1}) DW)</td>
<td>(g plant(^{-1}))</td>
</tr>
<tr>
<td><em>P. vittata</em> (Plot A)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>771–2570</td>
<td>15–175</td>
</tr>
<tr>
<td>Mean</td>
<td>1502</td>
<td>81</td>
</tr>
<tr>
<td>Median</td>
<td>1340</td>
<td>80</td>
</tr>
<tr>
<td>SD (±) (n = 40)</td>
<td>500</td>
<td>38</td>
</tr>
<tr>
<td><em>P. calomelanos</em> var. <em>austroamericana</em> (Plot B)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>1260–3940</td>
<td>10–555</td>
</tr>
<tr>
<td>Mean</td>
<td>2616</td>
<td>130</td>
</tr>
<tr>
<td>Median</td>
<td>2588</td>
<td>105</td>
</tr>
<tr>
<td>SD (±) (n = 40)</td>
<td>760</td>
<td>109</td>
</tr>
<tr>
<td>Significance (p)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANCOVA</td>
<td>0.014</td>
<td>0.065</td>
</tr>
<tr>
<td><em>t</em>-test</td>
<td>&lt; 0.001</td>
<td>0.012</td>
</tr>
</tbody>
</table>

The results from ANCOVA confirmed that frond As concentration in *P. calomelanos* var. *austroamericana* was significantly (*P* < 0.05) higher than *P. vittata* (Table 4.3). The BFs based on total and extractable soil As were significantly (*P* < 0.001; *t*-test) higher for *P. calomelanos* var. *austroamericana* than *P. vittata* (Table 4.4).
Table 4.4 The bioconcentration factors of *P. vittata* and *P. calomelanos* var. *austroamericana* were calculated based on the total and phosphate-extractable soil As concentrations. The differences between both the fern species are evaluated using *t*-test.

<table>
<thead>
<tr>
<th>Fern species</th>
<th>Bioconcentration factors&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Total soil As</th>
<th>Phosphate-extractable soil As</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. vittata</em> (Plot A)</td>
<td>Range</td>
<td>0.7–3</td>
<td>0.3–1.3</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>1.8</td>
<td>0.78</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>1.7</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>SD (±)</td>
<td>0.5</td>
<td>0.24</td>
</tr>
<tr>
<td><em>P. calomelanos</em> var. <em>austroamericana</em> (Plot B)</td>
<td>Range</td>
<td>1.6–7</td>
<td>0.4–2.34</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>3.7</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>3.6</td>
<td>1.2</td>
</tr>
<tr>
<td></td>
<td>SD (±)</td>
<td>1.2</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Significance (*p*)

| t-test                          | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

<sup>a</sup>Ratio of As concentration in fronds to As concentration in soil

### 4.5.3 Major plant harvest

#### 4.5.3.1 Fern dry matter yield

The *t*-test showed that mean frond dry biomass of *P. calomelanos* var. *austroamericana* (130 g plant<sup>−1</sup>) was significantly (*P* < 0.05) higher than *P. vittata* biomass (81 g plant<sup>−1</sup>) (Table 4.3). However, ANCOVA analysis revealed a non-significant (*P* > 0.05) difference between frond dry biomass of the two fern species. *P. calomelanos* var. *austroamericana* produced a total frond biomass of 5,189 g (Plot B; *n* = 40, normalised) which is 1.61 times greater than that of *P. vittata* (3,223 g dry biomass; *n* = 40). A significant (*P* < 0.05) positive correlation
occurred between the total soil As in the surface soil and the frond dry matter yield of \textit{P. calomelanos} var. \textit{austroamericana} ($r = 0.39$) and \textit{P. vittata} ($r = 0.33$) (Figure 4.2). The frond dry biomass was also positively ($P < 0.05$) correlated with As concentration in fronds of \textit{P. calomelanos} var. \textit{austroamericana} ($r = 0.35$) and \textit{P. vittata} ($r = 0.37$) (Figure 4.2a,b).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image}
\caption{The relationship between total soil As concentration and the frond dry matter yield of (a) \textit{P. calomelanos} var. \textit{austroamericana} and (b) \textit{P. vittata}.}
\end{figure}
4.5.3.2 Fern As concentration

The average frond As concentration was significantly (\(P < 0.001; \text{t-test}\)) higher in \(P.\) calomelanos var. austroamericana (887 mg kg\(^{-1}\) DW) than in \(P.\) vittata (674 mg kg\(^{-1}\) DW) (Table 4.3). These results were also verified from ANCOVA that illustrated a significant (\(P < 0.001\)) difference between the frond As concentration of the two fern species (Table 4.3). The frond As concentration in \(P.\) calomelanos var. austroamericana and \(P.\) vittata was significantly (\(P < 0.001\)) correlated with the total soil As concentration (0–20 cm depth) and the relationship was stronger for \(P.\) calomelanos var. austroamericana (\(r = 0.68\)) (Figures 4.3a and 4.4a).

The frond As concentration in both fern species was also significantly correlated with the phosphate-extractable As in soil in the top two layers for \(P.\) calomelanos var. austroamericana and the top and bottom layers for \(P.\) vittata (Figures 4.3b–d and 4.4b–d).

4.5.3.3 Fern As uptake

The average frond As uptake of \(P.\) calomelanos var. austroamericana (124 mg plant\(^{-1}\)) was significantly (\(P < 0.001; \text{t-test}\)) higher than \(P.\) vittata (57 mg plant\(^{-1}\)). ANCOVA results also showed a significant (\(P < 0.05\)) difference between the frond As uptake of the two fern species, however, the probability was declined from \(P < 0.001\) in \(t\)-test to < 0.05 for ANCOVA (Table 4.3). The simple ANCOVA model showed that \(P.\) calomelanos var. austroamericana removed 2.7 times more As than \(P.\) vittata after 10 month of growth.
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Figure 4.3 Arsenic concentration in *P. calomelanos* var. *austroamericana* fronds in relation to the (a) total soil As at 0–20 cm depth and phosphate-extractable As at (b) 0–20 cm (c) 20–40 cm, and (d) 40–60 cm depths.

Figure 4.4 Arsenic concentration in the fronds of *P. vittata* in relation to the (a) total soil As at 0–20 cm depth and phosphate-extractable As at (b) 0–20 cm (c) 20–40 cm, and (d) 40–60 cm depths.
Figure 4.5 The frond dry biomass of *P. calomelanos* var. *austroamericana* and *P. vittata* in relation to the fronds As concentration (a and b) and As uptake (c and d), respectively. The relationship between the frond As concentration and As uptake is also presented (e) *P. calomelanos* var. *austroamericana*, and (f) *P. vittata*. 
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The spatial distribution of frond As uptake (and frond As concentration) (Figure 4.6a,b) illustrates that As uptake (and concentration) in *P. calomelanos* var. *austroamericana* is higher than that of *P. vittata*. *P. calomelanos* var. *austroamericana* ferns extracted a total of 4.98 g As from Plot B, that is 2.1 times greater than the total As removed by *P. vittata* (2.30 g As) in Plot A.

The frond As uptake for *P. calomelanos* var. *austroamericana* and *P. vittata* increased significantly with the increasing As concentration in soil ($r = 0.56$ and 0.54, respectively; $P < 0.001$). For both fern species, frond As uptake was significantly ($P < 0.001$) correlated with the frond dry matter yield and As concentration (Figure 4.5c–f).

### 4.5.3.4 Fern bioconcentration factors (BFs)

The mean BFs (based on the total and phosphate-extractable soil As) were significantly ($P < 0.001$; *t*-test) higher in *P. calomelanos* var. *austroamericana* (1.3 and 20, ranging from 0.4–2.2 and 6–45, respectively) than in *P. vittata* (0.78 and 14, ranging from 0.3–1.4 and 6–22) (Table 4.4).
Figure 4.6 Spatial distribution of (a) As concentration, and (b) As uptake in *P. vittata* (Plot A) and *P. calomelanos* var. *austroamericana* (Plot B) after 10 months of growth.
4.6 DISCUSSION

The total and phosphate-extractable As data for soil showed a spatial heterogeneity; and the experimental area close to the dip bath showed relatively higher and more variable As concentration in soil. These trends could be attributed to the dipping process, pumping-out of the dipping fluid from the dip bath and disposal of As-concentrated dip sediment around the cattle-dip (Kimber et al. 2002; Okonkwo 2007). Phosphate-extractable As in soil generally increased down the soil profile up to 40 cm depth and then decreased below this depth. Arsenic movement down the profile was possibly due to the mixing of soil in the past. The restricted flow below 40 cm depth was possibly due to the presence of a hard pan below this layer recognised during the soil-sampling. It is possible that this compaction occurred due to high movements of cattle activity in the vicinity of the dip bath. Kimber et al. (2002) reported a limited movement of soil As below 50 cm depth in heavy texture soils around cattle-dip sites. The high adsorption capacity of Fe oxides rich soil (15.9%) could also be another main mechanism, which hinders leaching of As to the deeper layers in the soil (Smith et al. 1998; Goldberg and Johnston 2001).

The higher practical range for total As (7 m) than the phosphate-extractable As (1.5–2.5 m for three depths) indicated that total As in the surface soil showed a stronger autocorrelation among the measured values and had a better spatial structure than the phosphate-extractable As (Lark and Cullis, 2004). These results also indicated that phosphate-extractable As varied over shorter distances than the total As. This is the first report which demonstrates that kriging-based geostatistical methods could be useful in evaluating the small scale variation in soil As in a confined area. These methods should be further explored to determine variability pattern of As in soil adjacent to other cattle-dip sites for monitoring and management.
purposes (presented in Chapter 3).

The *t*-test may not be appropriate for grid soil sampling. In grid (systematic) sampling, it cannot be assumed that the samples are at random and independent of each other. Instead, they are spatially correlated (Lark and Cullis 2004). The spatial ANOVA took into account the spatial variability in soil As concentration to differentiate between the two plots for total and extractable As. The results from spatial ANOVA for phosphate-extractable soil As at 0–20 and 40–60 cm depths were dissimilar to those obtained by *t*-test. This revealed that the spatial dependence might appear to affect the distribution of As adjacent to the dip bath and should be considered with higher importance during data interpretation.

Arsenic is a toxic pollutant and is not a plant nutrient. However, *P. vittata* has been reported to propagate efficiently in As-contaminated or spiked soils, producing significant quantities of frond biomass (Tu and Ma 2002; Kertulis-Tartar et al. 2006; Xu et al. 2010). The growth stimulation in *P. vittata* has been attributed to the production of low-molecular-weight thiols upon exposure to As, which help in the detoxification of As in this fern (Cai et al. 2004; Zhang et al. 2004). Recently, Kachenko et al. (2010) reported that As\textsuperscript{III}–S\textsuperscript{2−} compounds might be involved in the biochemical reduction of As\textsuperscript{V} to As\textsuperscript{III} during the transport of As from root to shoot in *P. calomelanos* var. *austroamericana*. However, further research is needed to confirm the physiological mechanisms for As tolerance by *P. calomelanos* var. *austroamericana*.

Fern frond biomass and As concentration are considered important elements for efficient extraction of As from contaminated soils (Ma et al. 2001; Gonzaga et al. 2006). The spatial
variability in frond As concentration of both fern species was attributed to the varying As concentrations in soil. The results from this field study illustrated that the total frond dry biomass and frond As concentration of \textit{P. calomelanos} var. \textit{austroamericana} were greater than that of \textit{P. vittata}. These findings are contrary to an earlier glasshouse study that reported greater frond biomass and As accumulation in \textit{P. vittata} than \textit{P. calomelanos} var. \textit{austroamericana} (Xu et al. 2010). The results from our current field study suggest that \textit{P. calomelanos} var. \textit{austroamericana} was better suited to subtropical field conditions than \textit{P. vittata}. These results also suggest that field studies are imperative in assessing the performance of hyperaccumulating plants due to complex soil and environmental influences. Although \textit{P. calomelanos} var. \textit{austroamericana} is naturalised in the region of the experimental site (Ashley et al. 2003), the current study could not clarify the factors that contributed to the increased frond biomass production for this species. Reichmann et al. (2004) suggested that unfavourable growing conditions, such as drought and weeds can decrease the hyperaccumulation efficiency of \textit{P. vittata} under field conditions. Wei and Chen (2006) showed that the mean As concentration in \textit{P. cretica} fronds was relatively higher (418 mg kg\textsuperscript{-1} DW) than \textit{P. vittata} (269 mg kg\textsuperscript{-1} DW); and they contributed this to the differences in plant species and soil properties. Our results suggest that the plant species differences were more significant than the soil properties since the two species were grown in the same soil.

Arsenic concentration in fern fronds of \textit{P. vittata} and \textit{P. calomelanos} var. \textit{austroamericana} in the present study was lower than reported values in previous field studies (Salido et al. 2003; Kertulis-Tartar et al. 2006). Kertulis-Tartar et al. (2006) reported an average frond As concentration of 3,186 mg kg\textsuperscript{-1} DW in \textit{P. vittata} when grown on a sandy (88\%) and alkaline (pH 7.4) soil contaminated with As. Similarly, As concentration $\geq 2,000$ mg kg\textsuperscript{-1} DW were
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reported for \textit{P. vittata} ferns growing in soil with pH $> 6$ (Salido et al. 2003). The lower frond As concentration in our study could be attributed to low As availability in soil due to the properties, such as low pH (4.82), very high free Fe (15.9\%) and clay (44\%) contents. These soil characteristics play a crucial role in availability of As (Smith et al. 1998; McLaren et al. 2006). Low soil pH and high free Fe content result in increasing As sorption on the surface of Fe oxyhydroxides and can limit As bioavailability (Smith et al. 1998; Goldberg and Johnston 2001). Xu et al. (2010) also reported the lowest frond As concentration in \textit{P. vittata} and \textit{P. calomelanos} var. \textit{austroamericana} in the soil containing free Fe of 7.06\% and pH of 5.5. Phosphate-extractable As concentrations are $\leq 14\%$ of the total soil As which further suggest that reduced bioavailability of As in soil at this site may have decreased As translocation in the studied fern species.

The fern As uptake is related to the frond As concentration and dry biomass (Ma et al. 2001). The higher As uptake by \textit{P. calomelanos} var. \textit{austroamericana} than \textit{P. vittata} in this field study contradicts with the results of a glasshouse study reported by Xu et al. (2010). A stronger relationship between the frond As uptake and dry matter yield of \textit{P. calomelanos} var. \textit{austroamericana} implied that in this species dry biomass was the dominant factor to maximise the As depletion from soil. In contrast, a stronger correlation between frond As uptake and As concentration in \textit{P. vittata} indicated that frond As concentration appeared to affect the As removal efficiency of this species.

In addition to the frond As concentration, biomass production and As uptake, BFs for \textit{P. calomelanos} var. \textit{austroamericana} were greater than those of \textit{P. vittata}. This was also in contrast with the earlier findings of Xu et al. (2010), who reported higher BFs of \textit{P. vittata}
than *P. calomelanos* var. *austrroamericana*, when ferns were grown in three As spiked soils in the glasshouse. The average BF based on total soil As for *P. vittata* was < 1 in this field study. In previous pot trials, *P. vittata* possessed BF generally > 10 (Ma et al. 2001; Zhao et al. 2002), where soluble form of As was added and As was equilibrated for a relatively short time in the spiked soils. However, Wei and Chen (2006) reported BFs from 0.06–7.4 for *P. vittata* growing at a mining site and suggested that soil properties played a significant role in the As accumulation of this fern species. Wei et al. (2006) suggested that ageing of As in soil at an Au-mineralization site reduced its plant availability and declined the mean BF for *P. vittata* to 0.9. The soil at the experimental site of our study had high As sorption capacity due to the high free Fe content and low pH of the soil, and the sorbed As has stabilized due to the long ageing time (> 40 years) in the soil. The mean BF of *P. calomelanos* var. *austrroamericana* was 1.3 supporting the argument that most of the As was strongly bound to Fe oxides and only a small fraction (≤ 14%) was in the bioavailable As pool. It was suggested that BFs based on the bioavailable or extractable soil As should be considered under field conditions (Wei and Chen 2006; Wei et al. 2006) and our results support this view.

### 4.7 Time estimation for remediation

The remediation time was estimated for the study site based on the As uptake by the two fern species (see detail in Appendix 2). The remediation assessment based on phosphate-extractable soil As over 4 years time scale reveals that some of the area (at 0–60 cm depth) under *P. calomelanos* var. *austrroamericana* (Plot B) would be completely remediated and majority of the area would reach at the required Ecological Investigation Level (EIL) value of 20 mg kg$^{-1}$ (Figure 4.7g vs. 4.7e (see figure on next page)) (NEPM 1999). In contrast, *P. vittata* would take almost double time (or more) to meet this requirement. *P. calomelanos* var. *austrroamericana* would take approximately 100 years to remediate the 0–60 cm soil in Plot B
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to bring the total As level below the EIL value of 20 mg kg\(^{-1}\), whereas *P. vittata* would need twice the time to that taken by *P. calomelanos* var. *austroamericana* ferns to achieve this target. The phytoextraction efficiency of both the fern species and their remediation trend should be explored on long term basis to confirm these assessments under field conditions.

![Figure 4.7](image)

**Total As in soil at 0–60 cm depth (g)**

(a) Initial (b) After 10 months (c) After 10 years (d) After 20 years

**Phosphate extractable As in soil at 0–60 cm depth (g)**

(e) Initial (f) After 10 months (g) After 4 years

Figure 4.7 The initial amount of As contained in 0–60 cm of Plot A (*P. vittata*) and Plot B (*P. calomelanos* var. *austroamericana*) of the experimental area (a) total As, (e) phosphate-extractable As. The maps showing the (b) total and, (e) phosphate-extractable As after 10 months of ferns growth. The remediation estimates made for the total As over a period of (c) 10 years, (d) 20 years, and for phosphate-extractable As for a period of (g) 4 years, are also presented.
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### 4.8 CONCLUSIONS

Geostatistical methods adequately described the spatial distribution in soil As concentration and suggest that As in the soil surrounding the cattle-dip was spatially correlated with the distance from the dip bath. Total As had a better spatial structure than phosphate-extractable As in a confined area around the cattle-dip. The results also demonstrated that the extent of variation in phosphate-extractable soil As was greater than the total As in the top layer.

The frond As concentration, dry matter yield, As uptake and BFs were greater for *P. calomelanos* var. *austroamericana* than *P. vittata*. The results from this field study show that *P. calomelanos* var. *austroamericana* was better suited than that of *P. vittata* for the remediation of this As-contaminated site. The study demonstrates that field experiment data should be considered when evaluating the phytoextraction potential of plants for remediation of contaminated soil. This study should be expanded to other As-contaminated sites to further understand the As accumulation behaviour of these fern species for soils with variable properties.
4.9 REFERENCES


Chapter 4: Phytoremediation using *P. vittata* and *P. calomelanos* var. *austroamericana*


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