

Arsenic hyperaccumulation by ferns: A field study in northern NSW

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Abstract

Historical applications of arsenic-based pesticides to control cattle ticks has resulted in large expanses of As contaminated dip sites across Australia. A field experiment was conducted to evaluate the extraction of As using As hyperaccumulating ferns, *Pityrogramma calomelanos* (L.) Link var. *austroamericana* (Domin) Farw. (Gold dust fern) and *Pteris vittata* L. (Chinese brake fern), at a disused As contaminated cattle dip site at Wollongbar, in northern New South Wales (NSW), Australia. Arsenic concentrations in the fronds of *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* were 1262–3941 mg/kg and 775–2569 mg/kg dry weight (DW), respectively. Our results showed that both ferns successfully accumulated As under field conditions, however, As removal rate and bioaccumulation factor was higher in Gold dust fern (3–5) than in Chinese brake fern (1–3).

Key Words

Hyperaccumulation, cattle dip sites, phytoremediation, phosphate extractable, contamination

Introduction

Arsenic has been classified as a toxic and carcinogenic metalloid which exists in the environment in both organic and inorganic forms. The inorganic form of As is supposed to be more common in soils and found in two main oxidation states, arsenate (As^V) and arsenite (As^{III}), the later being more toxic and available than As^V (Masscheleyn *et al.*, 1991). Both natural processes and anthropogenic activities are responsible for the release of As in soil and water bodies. Mining, smelting, use of pesticides and herbicides in agriculture, wood treatment with CCA and tanning operations are some of the major human activities causing As contamination in soil (Smith *et al.*, 1998).

Arsenic-based pesticides have been widely used at the cattle dip sites throughout the world including Australia. From the early 1900s to 1955, prior to the introduction of dichlorodiphenyltrichloroethane (DDT), more than 1600 dips were constructed in northern NSW Australia, where arsenicals were used as the dipping solution to control the cattle ticks (Smith *et al.*, 1998). Soil As contamination around such sites needs attention due to the presence of toxic and bioavailable forms of As.

Remediation of the contaminated soils includes the excavation, capping, chemical immobilization and phytoremediation or phytoextraction (Gonzaga *et al.*, 2006). Phytoextraction of the As contaminated soils, using ferns as hyperaccumulators, has emerged as an effective remediation strategy, which provides a cost-effective and environmental friendly option as compared to other remediation methods (Kertulis-Tartar *et al.* 2006). The bioaccumulation factor (BF; ratio of the As concentration in fronds to total As in soil) and translocation factor (TF; ratio of As concentration in fronds to total As in roots) determine the capability and efficiency of the plants to remove the As from soil (Ma *et al.*, 2001; Gonzaga *et al.*, 2006).

Pteris vittata L. (Chinese brake fern) is a well known As hyperaccumulator that can survive in soil with As concentrations of up to 1500 mg/kg and accumulate As in fronds > 3000 mg/kg DW (Tu and Ma 2002; Kertulis-Tartar *et al.* 2006). Other *Pteris* and non-*Pteris* ferns (e.g. *Pteris longifolia*, *Pteris cretica* and *Pityrogramma calomelanos*) have also been identified to accumulate As (Francesconi *et al.*, 2002; Wei *et al.*, 2007). Recently, Kachenko *et al.*, (2007) found that *P. calomelanos* var. *austroamericana* (Domin) Farw. (Pteridaceae) can accumulate As in fronds up to 16 415 mg/kg DW in fronds. To our knowledge, there has been no research on the phytoremediation capacity of Gold dust fern under field conditions. The study aims to (1) determine the capability of *P. calomelanos* var. *austroamericana* and *P. vittata* for As hyperaccumulation in the field (2) compare BFs of *P. calomelanos* var. *austroamericana* and *P. vittata* under field conditions.

Methods

Two fern species, *P. vittata* and *P. calomelanos* var. *austroamericana* were selected for the field experiment. *Pteris vittata* was obtained from the Randwick City Council Nursery, NSW while *P. calomelanos* var. *austroamericana* was propagated from spores under controlled glasshouse conditions. After eight months of growth, uniform ferns were ready for transplanting in the field.

The field site was located at the Environmental Centre of Excellence, Wollongbar in northern NSW, Australia. It is a disused cattle dip site, where As-based pesticides were used to control the cattle ticks (DIPMAC, 1992). The soil around the dip site was contaminated with variable and high concentration of As from the dipping process and disposal of waste material from the dip.

The study area was selected on the basis of preliminary soil sampling at 0–10 cm depth for total soil As distribution in the area. The selected area was sprayed with Roundup® weed-killer and cleared mechanically prior to planting. In January 2009, fern species were transplanted into hand-excavated holes in two separate plots of 3.15 m² size each, keeping plant to plant distance of 30 cm to give 42 ferns per plot. The plants were watered twice a day using drip irrigation system. Black nylon weed mat was used to minimise the weed growth and a shade cloth was erected over the area to protect ferns from direct sun light exposure.

An intensive soil sampling was done in June 2009, to collect the soil samples at 0–20 cm, 20–40 cm and 40–60 cm depths using a hand-driven soil corer. After five months period, the ferns were harvested at the fresh fronds tip, thoroughly washed and dried in a fan-forced oven at 70 °C for 48–72 h until a constant weight was obtained.

Soil samples were air dried and ground to obtain < 2 mm fraction which was used to determine the various soil properties as given in Table 1. Sub-samples of soil (< 200 µm) were used to determine the total soil As concentration. Soil samples were digested with a mixture of hydrofluoric and other mineral acids at 125 °C and diluted with HCl and E-pure water (Huang and Fujii, 1996). Fern samples were digested in a mixture (1:1) of nitric and perchloric acids at 120–180 °C and diluted with E-pure water (Miller 1998). The digests were analysed for As using a Varian Vista AX CCD inductively coupled plasma atomic emission spectrometer.

Potassium dihydrogen phosphate solution (0.5 M KH₂PO₄) was used to extract the specifically sorbed (bioavailable) As pool in soil with a 1:25 soil to solution ratio and 4 h shaking time (Alam *et al.*, 2007). The As concentration in the phosphate extracts was analysed using a Varian hydride-generation atomic absorption spectrometer.

Table 1. Properties of soil at the Wollongbar experimental site.

pH (1:5 CaCl ₂)	EC (1:5) (dS/m)	CEC (mmol _c /kg)	Total carbon (%)	DCB Fe (g/kg)	DCB Al (g/kg)	Sand (%)	Silt (%)	Clay (%)
4.82	0.11	87.5	4.5	158.9	13	16	40	44

Results

During the course of the experiment, all the ferns survived without symptoms of As phytotoxicity. In both species As concentration in fronds increased with the increasing levels of soil As, which indicated the capability of Chinese brake fern and Gold dust fern to grow and tolerate in soils with the high levels (313–1903 mg/kg) of As. The total As concentration in soil was in the range of 393 to 1903 mg/kg for the plot where Chinese brake fern was grown, while it varied between 313 and 1486 mg/kg for Gold dust fern (Table 2). Arsenic concentrations in fronds of the Chinese brake fern and Gold dust fern were in the range of 775–2569 mg/kg DW and 1262–3941 mg/kg DW, respectively (Table 2). Bioaccumulation factor (based on total soil As) for Chinese brake fern and Gold dust fern were between 1–3 and 3–5, respectively. Arsenic concentration in fronds as a function of total and extractable soil As illustrated higher accumulation in Gold dust fern as compared to Chinese brake fern (Figure 1). These results are consistent with the previous studies on Chinese brake fern grown under field or controlled conditions (Zhao *et al.*, 2002; Wei and Chen, 2006). The higher concentration of As and BF in Gold dust fern suggest that this species is more efficient in As extraction under field conditions. Our results are opposite to the earlier studies that have examined As

accumulation in these two species under glasshouse conditions. Kachenko *et al.* (2007) observed a higher As accumulation capacity for the Chinese brake fern with no toxicity symptoms at 100–500 mg/kg applied As, while Gold dust fern showed toxicity with As concentrations in fronds >3008 mg/kg DW. Similarly, Xu *et al.* (2009) compared the hyperaccumulation potential of the two fern species in four different soils in a pot experiment, and observed higher As concentrations for Chinese brake fern than the Gold dust fern. At the time of sampling growth of Gold dust fern was better than the Chinese brake fern. To compare the total biomass of both species, one average plant from each of species was harvested above soil. The total biomass of the Gold dust fern (80.04 g DW) was almost two times greater than the Chinese brake fern (39.49 g DW).

Table 2. Total and phosphate extractable As concentration in soil and total As concentration in fronds of Chinese brake fern and Gold dust fern.

Fern species	Total soil As (mg/kg)	Phosphate extractable As (mg/kg)			As in fronds (mg/kg)
		0–20 cm	20–40 cm	40–60 cm	
Gold dust fern					
Range	313–1486	21–117	29–104	13–53	1262–3941
Mean	753	51	54	28	2617
Median	734	45	52	25	2589
SD (±)	284	23	19	11	764
Chinese brake fern					
Range	393–1903	25–90	23–105	12–141	775–2569
Mean	909	51	53	42	1501
Median	878	52	45	34	1339
SD (±)	354	17	21	31	500

Wei and Chen (2006) observed that the As accumulation rate and BFs of the Chinese brake fern were relatively less than the Cretan brake fern (*Pteris cretica*) on a contaminated mining site, suggesting that As accumulation by Chinese brake fern in the field depends on the various soil properties. In this study, it is not immediately evident why As concentration in the Gold dust fern was higher than the Chinese brake fern, however, we cannot exclude the role of soil properties and field conditions (as compared to glasshouse) affecting the growth behaviour of ferns (Wei and Chen 2006; Xu *et al.* 2009).

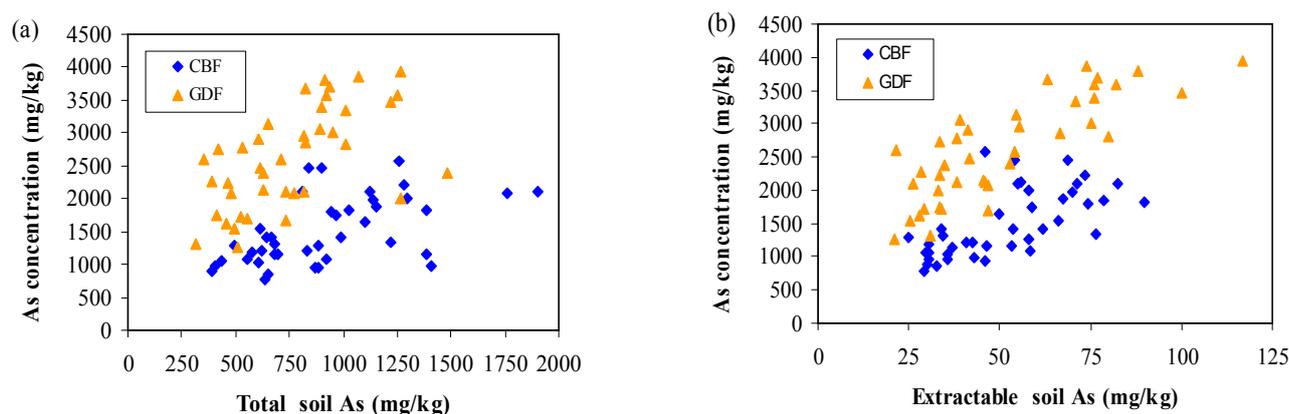


Figure 1. Total As concentration in Chinese brake fern (CBF) and Gold dust fern (GDF) as a function of (a) total soil As and (b) phosphate extractable soil As at 0–20 cm depth.

Arsenic concentration in the Gold dust fronds was better correlated with the total soil As ($r^2 = 0.59$) than the Chinese brake fern ($r^2 = 0.55$). Furthermore, there was a stronger relationship between the As concentration in the Gold dust fern and phosphate extractable soil As at 0–20 cm ($r^2 = 0.67$) and 20–40 cm ($r^2 = 0.53$) depths than the Chinese brake fern ($r^2 = 0.42$ and 0.14, respectively).

Conclusion

Both Gold dust fern and Chinese brake fern accumulated high levels of As in fronds under field conditions. On the basis of the total As concentrations in fronds and BFs, we conclude that the Gold dust fern is more efficient in As hyperaccumulation than the Chinese brake fern, and thus better suited for the remediation of As contaminated dip sites in NSW.

Acknowledgements

Nabeel gratefully acknowledges the Higher Education Commission of Pakistan for the award of PhD scholarship. The authors are thankful to the NSW Environmental Trust for providing the funding for the research project. We would also like to thank George Nastase, Adam Mitchell, Joshua Rust, Victor and Desmond at DPI, Wollongbar for their assistance in the field work.

References

- Alam MGM, Tokunaga S, Stagnitti F (2007) Removal of arsenic from contaminated soils using different salt extractants. *Journal of Environmental Science and Health, Part A* **42**, 447-451.
- DIPMAC (1992) Report on the management of contaminated waste at the cattle dip sites in north eastern New South Wales, Govt of NSW, Sydney, Australia.
- Francesconi K, Visoottiviset P, Sridokchan W, Goessler W (2002) Arsenic species in an arsenic hyperaccumulating fern, *Pityrogramma calomelanos*: a potential phytoremediator of arsenic-contaminated soils. *Science of the Total Environment* **284**, 27-35.
- Gonzaga MIS, Santos JAG, Ma LQ (2006) Arsenic phytoextraction and hyperaccumulation by fern species. *Scientia Agricola* **63**, 90-101.
- Huang PM, Fujii R (1996) Selenium and arsenic. In 'Methods of Soil Analysis. Part 3, Chemical Methods'. (Ed. DL Sparks) pp. 793-831. (Soil Science Society of America Inc: Wisconsin, USA).
- Kachenko AG, Bhatia NP, Singh B, Siegele R (2007) Arsenic hyperaccumulation and localization in the pinnule and stipe tissues of the gold-dust fern *Pityrogramma calomelanos* L. Link var. *austroamericana* (Domin) Farw. using quantitative micro-PIXE spectroscopy. *Plant and Soil* **300**, 207-219.
- Kertulis-Tartar GM, Ma LQ, Tu C, Chirenje T (2006) Phytoremediation of an arsenic-contaminated site using *Pteris vitrata* L.: A two-year study. *International Journal of Phytoremediation* **8**, 311-322.
- Ma LQ, Komar KM, Tu C, Zhang WH, Cai Y, Kennelley ED (2001) A fern that hyperaccumulates arsenic - A hardy, versatile, fast-growing plant helps to remove arsenic from contaminated soils. *Nature* **409**, 579-579.
- Masscheleyn PH, Delaune RD, Patrick WH (1991) Effect of redox potential and pH on arsenic speciation and solubility in a contaminated soil. *Environmental Science & Technology* **25**, 1414-1419.
- Miller RO (1998) Nitric-perchloric acid wet digestion in an open vessel. In 'Handbook of Reference Methods for Plant Analysis'. (Ed. YP Kalra) pp. 57-61. (CRC Press: Boca Raton, FL).
- Smith E, Naidu R, Alston AM (1998) Arsenic in the soil environment: A review. *Advances in Agronomy*, **64**, 149-195.
- Tu C, Ma LQ (2002) Effects of arsenic concentrations and forms on arsenic uptake by the hyperaccumulator ladder brake. *Journal of Environmental Quality* **31**, 641-647.
- Wei CY, Chen TB (2006) Arsenic accumulation by two brake ferns growing on an arsenic mine and their potential in phytoremediation. *Chemosphere* **63**, 1048-1053.
- Wei CY, Wang C, Sun X, Wang WY (2007) Arsenic accumulation by ferns: a field survey in southern China. *Environmental Geochemistry and Health* **29**, 169-177.
- Xu W, Kachenko AG, Singh B (2009) Effect of soil properties on arsenic hyperaccumulation in *Pteris vittata* L. and *Pityrogramma calomelanos* (L.) Link var. *austroamericana*. *International Journal of Phytoremediation* (in press).
- Zhao FJ, Dunham SJ, McGrath SP (2002) Arsenic hyperaccumulation by different fern species. *New Phytologist* **156**, 27-31.

The close correlation between extracted arsenic and extracted iron/aluminum suggested that arsenic was removed via the dissolution of Fe/Al oxides in soils. The fractionation of arsenic in four contaminated soils was investigated using a modified sequential extraction method. The Feature Paper can be either an original research article, a substantial novel research study that often involves several techniques or approaches, or a comprehensive review paper with concise and precise updates on the latest progress in the field that systematically reviews the most exciting advances in scientific literature. This type of paper provides an outlook on future directions of research or possible applications.

New South Wales' premier has warned residents to brace for a lockdown extension as the state struggles to contain a growing outbreak of Covid-19. A woman in her 90s died as the state recorded another daily record of 77 cases. "Tragically, we've seen one older person die and I want to extend my deepest condolences to their families and loved ones," NSW State Premier Gladys Berejiklian told a daily press conference on Sunday. The victim was a woman in her 90s who apparently contracted the disease in her household and died at Liverpool Hospital on Saturday. It is the first officially reported coronavirus-related death in Australia in 2021. NSW recorded 77 new locally acquired cases of COVID-19 in the 24 hours to 8pm last night. pic.twitter.com/jLeeE5CAMD.

Arsenic contamination of soils is a global environmental, agricultural, and health issue given to the toxic and carcinogenic nature of As. Several anthropogenic activities, such as mining and... Since the discovery of As-hyperaccumulator, *Pteris vittata* L., several other As-hyperaccumulating fern species have been identified in *Pteris* and *Pityrogramma* genera which demonstrated the ability to remove As from soil. Arsenic concentrations in non-*Pteris* ferns were generally much lower than those in *Pteris* ferns, with 0.81, 1.32, 3.59, 10.7, 6.17, 24.3 mg kg⁻¹ in the fronds of *Blechnum orientale*, *Dicranopteris dichotoma*, *Pteridium aquilinum* and *Cyclosorus acuminatus*, respectively. For *P. vittata*, the As bioaccumulation factor (ratio of As in fronds to that in soils) changed, whereas the As translocation factor (ratio of As in fronds to that in roots) remained unchanged among the different sites. Arsenic hyperaccumulation by ferns: A field study in northern NSW. N. K. Niazi, B. Singh, L. Zwieten, Anthony G. Kachenko. *Geography*. Within New South Wales the rainforest vary considerably in their structural and floristic composition. This is the gully rainforest community which is found along creeksides through the better class coastal eucalypt forests in northern New South Wales. Structurally and floristically this is intermediate between the subtropical and submontane rainforest type. It is the climax vegetation for the gullies. but by repeated logging and burning tends to be replaced by the Flooded Gum (*Eucalyptus grandis*) a socrition.