

# VARIABILITY, SPECIATION AND PHYTOREMEDIATION OF SOIL ARSENIC AT CATTLE DIP SITES IN NSW, AUSTRALIA

**NABEEL KHAN NIAZI**  
M.Sc. (Hons) Agri

A THESIS SUBMITTED TO THE FULFILMENT OF THE REQUIREMENTS  
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

Faculty of Agriculture, Food and Natural Resources  
The University of Sydney, Sydney  
New South Wales  
Australia



## **CERTIFICATE OF ORIGINALITY**

I hereby declare that the text of this thesis is my own work, and that, to the best of my knowledge and belief, it contains no material that has been previously published or written by another person, nor any material that has been accepted as part of the requirements for any other degree or diploma in any university or other institute of higher learning, unless due acknowledgement has been made.

I also declare that the intellectual content of this thesis is original and the result of my own research and to the best of my knowledge and belief, any assistance I received in the experimentation presented, and all sources of information cited have been duly acknowledged.

---

Nabeel Khan Niazi

Declared at The University of Sydney  
this 31st day of  
August 2011

**TO  
MY PARENTS  
AND FAMILY**

## ABSTRACT

Arsenic (As) contamination of soils is a major environmental problem due to its toxic and carcinogenic nature. Historical use of As-containing pesticides has resulted in the contamination of soils with high and variable concentrations of As in many parts of Australia. Phytoremediation using As-hyperaccumulating ferns can be potentially utilised as an environmental friendly and low-cost remediation technology to phytoextract As from soils at sites containing elevated and varying concentration of As.

The spatial variability of total and phosphate-extractable As concentrations was evaluated in soil adjacent to a cattle-dip site located at Wollongbar in northern NSW, Australia. The results from the linear mixed model showed that total (0–0.2 m) and phosphate-extractable (0–0.2, 0.2–0.4 and 0.4–0.6 m depths) As concentrations in the soil adjacent to the dip site varied greatly and increased significantly ( $P = 0.004–0.048$ ) toward the dip site, indicating that As variability in soil was spatially correlated with distance from the dip. The data suggest that 5 samples should be required to assess the soil contamination level (mean =  $826 \text{ mg kg}^{-1}$ ) and 15 samples would be required to evaluate the effects of phytoremediation of As-contaminated site. The proposed guidelines on sampling requirements are important to estimate the variability in As contamination levels around other cattle-dip sites and to monitor changes in soil As content from phytoremediation activities.

Ensuing study compared the phytoremediation potential of *Pityrogramma calomelanos* var. *austroamericana* (gold dust fern) against the well-known *Pteris vittata* (Chinese brake fern) over a 27-month duration grown at the cattle-dip site described earlier. The ferns were planted in January 2009 and harvested following 10, 22 and 27 months of growth. After 10 months of

growth (short-term data), *P. calomelanos* var. *austroamericana* produced significantly higher frond dry biomass, possessed higher frond As concentration and removed more As in fronds (mean = 130 g plant<sup>-1</sup>, 887 mg kg<sup>-1</sup> and 124 mg plant<sup>-1</sup>, respectively;  $P < 0.05$ ) than *P. vittata* (mean = 81 g plant<sup>-1</sup>, 674 mg kg<sup>-1</sup>, 57 mg plant<sup>-1</sup>). Further samplings up to 27 months (long-term data) confirmed the earlier results that the mean frond dry biomass, As concentration and As uptake were significantly higher in *P. calomelanos* var. *austroamericana* than *P. vittata*. In the three harvests over the 27-month period, *P. calomelanos* var. *austroamericana* removed (8,053 mg As) 2.65 times higher As than *P. vittata* (3,042 mg As). For the surface (0–20 cm) and subsurface (40–60 cm) layers, the mean total soil As content was significantly ( $P < 0.05$ ) reduced by 49% and 63%, respectively, using *P. calomelanos* var. *austroamericana*; and 17% and 15%, respectively, by *P. vittata* ( $P > 0.05$ ). 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<sup>-1</sup>) limit in the surface and subsurface soils, whereas *P. vittata* would require 13–15 years to achieve this target. The field experiment results suggest that *P. calomelanos* var. *austroamericana* is better suited than *P. vittata* for the phytoremediation of As-contaminated soils under the experimental conditions existing at the site.

The potential of mid infrared (MIR) spectroscopy in combination with partial least squares (PLS) regression was investigated to estimate the total As content in a large number of soil samples collected from a highly variable As-contaminated dip site. The MIR-PLS calibration model developed excluding spectral outliers ( $n = 149$ ) was robust with an acceptable reliability (coefficient of determination;  $R^2_c = 0.73$ ; residual prediction deviation;  $RPD_c = 1.94$ ) to estimate total soil As content. The validation of calibration model using a separate set of unknown soil samples ( $n = 149$ ; validation set) showed  $R^2_v$  and  $RPD_v$  values of 0.63 and

1.66, respectively. The results indicate an acceptable prediction of total As content in unknown samples, suggesting that MIR-PLS based model is capable of estimating total soil As and possibly be used in certain situations; for example to estimate soil As concentration at a highly variable site, where a large number of samples needs to be analysed.

The solid-phase speciation and plant availability of As in contaminated soils was determined using combination of a sequential extraction procedure (SEP), X-ray absorption near edge structure (XANES) spectroscopy and As plant uptake using *Brassica juncea* as a test plant. Arsenic was found to be predominantly associated with amorphous Fe oxides in arsenate ( $\text{As}^{\text{V}}$ ) form; in few samples As was present in arsenite ( $\text{As}^{\text{III}}$ ) form. The concentration of As in plant shoots showed significant ( $P < 0.001\text{--}0.05$ ) correlations with the exchangeable As ( $r = 0.85$ ), and amorphous Fe oxides associated As evaluated by the SEP ( $r = 0.67$ ) and XANES spectroscopy ( $r = 0.51$ ). The results suggest that As in these fractions is readily available for plant uptake and may pose potential risk to the environment. Such detailed analysis for As speciation and phytoavailability is vital for the management and rehabilitation of As-contaminated soils.

\* \* \*

## ACKNOWLEDGEMENTS

I am thankful to the Higher Education Commission (HEC) of Pakistan for awarding me a PhD scholarship to start my postgraduate studies here at The University of Sydney in Australia. I am highly grateful to my supervisor, Associate Professor Balwant Singh who always travelled above and beyond his responsibility as a supervisor in all aspects of my candidature. He has been deeply involved and dedicated in my research endeavours at all times – from the very first day when I met him in Pakistan and until the end of my thesis submission. I thank you for your sincere and substantial efforts to initiate this project, guiding and encouraging me, and most importantly, for maintaining a high level of contact with me throughout the last years of my PhD. I must say that I have learnt it from you to offer high level of passion and devotion to *science*. I would like to sincerely thank Dr Lukas Van Zwieten (Associate Supervisor) for his substantial contribution in successfully continuing the phytoremediation experiment at the field site in Wollongbar, northern New South Wales (NSW). I am thankful to Lukas and his colleagues, Stephen Kimber, Joshua Rust, Scott Petty, Victor Warren, Desmond Cook and George Nastase for their help during the field work at the site. My heartfelt thanks to Dr Anthony G. Kachenko (Nursery and Garden Industry Australia) who introduced me to the intriguing world of ferns and offered his sincere contribution in my field experiment. I am grateful to Dr Thomas F. A. Bishop (The University of Sydney) who assisted me in learning geostatistics and provided me with useful suggestions for the work I did in my PhD research. My sincere thanks to Dr Pushan Shah (Environmental Protection Authority, Adelaide) who came with me (and Balwant) to use synchrotron at the Australian National Beamline Facility at Tsukuba, Japan. Both Balwant and Pushan introduced me to the fascinating world of synchrotron science and I feel honoured to have worked with such passionate scientists, and to have had the opportunity to conduct a beamline experiment and

visit the Australian Synchrotron. My thanks to Dr Michael Cheah who assisted at the beamline for spectroscopy work. The bulk of this research would not have been possible without generous funding from several sources. I would like to acknowledge the financial support for this project by the NSW Government through its environmental trust. I wish to thank the Australian Synchrotron Research Program, for enabling me to travel to the Australian National Beamline Facility in Tsukuba (Japan) for performing my experiment (Project AS093/ANBF1851).

I am thankful to Dr Edith Lees for providing constructive comments on review chapter of this thesis. My thanks go to Dr Euan Smith (University of South Australia, Adelaide) for providing me three soil samples from As-contaminated soils in South Australia, and Dr Budiman Minasny (The University of Sydney) for assistance in MIR data analysis. Thanks are extended to the technical staff, Ms Loraine Watson and Ms Iona Georgy for their assistance. I wish to thanks Tom Savage for his invaluable assistance in ICP-AES analyses, and Michael Nelson and Peter Geelan-Small who provided much needed statistical advice. Many thanks to the administration staff, Ms Pamela Stern, Ms Prue Winkler, Ms Fortunée Cantrell and Ms. Nancy Cheng for providing me with overwhelming levels of logistical support throughout the course of my study. I am also thankful to all my friends at FAFNR and in my research group for their assistance whenever I needed.

I am thankful to Almighty God Who has blessed me with this much of strength, passion and courage to complete my studies. I am thankful to my wife, Irshad Niazi for her tremendous support who with her own PhD (and our little son, Aaish Khan) assisted and encouraged me in the last few months of my thesis writing. And finally, I would like to convey my deepest love and heartfelt thanks to my parents, brothers and sister.



## PUBLICATIONS

### Refereed scientific journals (Published/submitted)

1. Niazi NK, Singh B, Van Zwieten L, Kachenko AG (2011) Phytoremediation potential of *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* L. grown at a highly variable arsenic contaminated site. *International Journal of Phytoremediation* 13 (9):912–932.
2. Niazi NK, Singh B, Shah P (2011) Arsenic speciation and phytoavailability in contaminated soils using a sequential extraction procedure and XANES spectroscopy. *Environmental Science & Technology* 45 (17):7135–7142.
3. Niazi NK, Bishop TFA, Singh B, (2011) Evaluation of spatial variability of soil arsenic adjacent to a disused cattle-dip site, using model-based geostatistics. *Environmental Science & Technology* 45 (24):10463–10470.
4. Niazi NK, Singh B, Van Zwieten L, Kachenko AG (2011) Phytoremediation of an arsenic contaminated site using *Pteris vittata* L. and *Pityrogramma calomelanos* var. *austroamericana*: a long-term study. *Environmental Science and Pollution Research* (Submitted).

### Refereed scientific journals (In preparation)

5. Niazi NK and Singh B (2011) Using mid infrared (MIR) spectroscopy and partial least-squares regression to predict total soil arsenic content at a highly variable site. *Environmental Science & Technology* (In preparation).

### Conference paper (refereed)

6. Niazi NK, Singh B, Zwieten LV and Kachenko, AG (2010) Arsenic hyperaccumulation by ferns: a field study in northern NSW. *Published paper in the Proceedings of the 19<sup>th</sup> World Congress of Soil Science on Soil Solutions for the Changing World*. Brisbane, Australia, 1–6 August 2010, pp. 5–8.

### Commentary (Article published in a refereed journal)

7. Niazi NK and Kachenko AG (2011) Letter to the Editor regarding, “First evidence on different transportation modes of arsenic and phosphorus in arsenic hyperaccumulator *Pteris vittata*” by Lei et al. (2012). *Environmental Pollution* (Article in press) doi: 10.1016/j.envpol.2011.11.031.

### Conference proceedings (abstracts)

8. Niazi NK, Singh B, Shah, P (2010) Speciation and bioavailability of arsenic in contaminated soils using X-ray absorption near edge structure spectroscopy and a sequential extraction procedure. *Proceedings of ConSoil International Conference on the Management of Groundwater, Soil & Sediments*. Salzburg, Austria, 21–24 September, 2010 (Oral presentation).
9. Niazi NK, Bishop T, Singh B, (2010) Comparative study for the arsenic hyperaccumulation by ferns: a model-based geostatistical approach. *Proceedings of ConSoil International Conference on the Management of Groundwater, Soil & Sediments*. Salzburg, Austria, 21–24 September, 2010 (Poster presentation).
10. Niazi NK, Singh B, Zwieten LV and Kachenko, AG (2010) A comparative study to evaluate the phytoremediation potential of two fern species for the remediation of an arsenic contaminated site under field conditions. *7<sup>th</sup> International Conference on Phytotechnologies*. Parma, Italy, 26–29 September, 2010 (Oral presentation).\*
11. Niazi NK, Singh B, Bishop, T (2010) A geostatistical model based approach to evaluate spatial variability of arsenic in soil and to compare arsenic hyperaccumulation efficiency of two fern species. *7<sup>th</sup> International Conference on Phytotechnologies*. Parma, Italy, 26–29 September, 2010 (Poster presentation).\*
12. Niazi NK, Singh B, Zwieten LV and Kachenko, AG (2011) Phytoremediation potential of *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* L. grown at a highly variable arsenic contaminated site. *Proceedings of the International Annual Conference of ASA, CSSA and SSSA*. Fundamental for Life: Soil, Crop & Environmental Sciences. San Antonio, Texas, USA, 16–19 October, 2011 (Oral presentation).
13. Niazi NK, Bishop TFA and Singh B (2011) Using model-based geostatistics to develop design-based sampling guidelines for estimating arsenic contamination around cattle-dip sites. *Proceedings of Pedometrics- Innovations in Pedometrics*. Czech Republic, 31<sup>st</sup> August–2 September, 2011 (Poster presentation).

### Poster presentations

14. Niazi NK, Singh B, Zwieten LV and Kachenko, AG (2010) Field evaluation of hyperaccumulating ferns to remediate an arsenic contaminated soil. *Nursery & Garden Industry National Conference*. Darwin, Australia, 19–22 April 2010.
15. Niazi NK, Singh B, Bishop T, Zwieten LV and Kachenko, AG (2010) Environmentally friendly approach to clean-up arsenic contaminated soils. *The 'Centenary Research Symposium' of The Faculty of Agriculture, Food and Natural Resources, The University of Sydney*, 4 June, 2010.

**\* The 3<sup>rd</sup> best prize was awarded for the poster and oral presentation at the 7<sup>th</sup> International Conference on Phytotechnologies, Parma, Italy, 26–29 September, 2010.**

## TABLE OF CONTENTS

|                          |              |
|--------------------------|--------------|
| <b>ABSTRACT</b>          | <b>iii</b>   |
| <b>ACKNOWLEDGEMENTS</b>  | <b>vi</b>    |
| <b>PUBLICATIONS</b>      | <b>viii</b>  |
| <b>TABLE OF CONTENTS</b> | <b>x</b>     |
| <b>LIST OF FIGURES</b>   | <b>xviii</b> |
| <b>LIST OF TABLES</b>    | <b>xxii</b>  |

## CHAPTER 1

---

### **General introduction** **1**

|            |                     |          |
|------------|---------------------|----------|
| <b>1.1</b> | <b>Introduction</b> | <b>1</b> |
| <b>1.2</b> | <b>Aims</b>         | <b>4</b> |
| <b>1.3</b> | <b>Objectives</b>   | <b>4</b> |
| <b>1.4</b> | <b>References</b>   | <b>5</b> |

---

**CHAPTER 2**

|   |           |
|---|-----------|
| <b>Review of literature</b>   | <b>8</b>  |
| <b>2.1 History of As and its position in the periodic table</b>       | <b>8</b>  |
| <b>2.2 Distribution of As in the soil environment</b>                 | <b>9</b>  |
| <b>2.3 Sources</b>  | <b>16</b> |
| 2.3.1 Natural sources   | 16        |
| 2.3.2 Anthropogenic sources   | 17        |
| 2.3.2.1 Mining and smelting activities                                | 18        |
| 2.3.2.2 Industrialisation and urbanisation                            | 18        |
| 2.3.2.3 Irrigation  | 19        |
| 2.3.2.4 Agriculture activities  | 19        |
| <b>2.4 Speciation of As in soil</b>                                   | <b>21</b> |
| 2.4.1 Sequential extraction procedure                                 | 22        |
| 2.4.2 X-ray absorption fine structure (XAFS) spectroscopy             | 23        |
| 2.4.3 Combination of SEP and XAFS spectroscopy                        | 24        |
| <b>2.5 Availability of As in soil</b>                                 | <b>25</b> |
| <b>2.6 Arsenic accumulation in plants</b>                             | <b>30</b> |
| 2.6.1 Tolerance strategies in plants                                  | 31        |
| 2.6.2 Arsenic uptake mechanisms                                       | 34        |
| 2.6.3 Factors affecting plant As uptake                               | 36        |
| <b>2.7 Remediation of As-contaminated soils</b>                       | <b>40</b> |
| 2.7.1 Phytoremediation  | 41        |
| <b>2.8 Phytoextraction of As using hyperaccumulating fern species</b> | <b>44</b> |
| 2.8.1 Background on <i>P. calomelanos</i> var. <i>austroamericana</i> | 48        |
| <b>2.9 References</b>   | <b>50</b> |

## CHAPTER 3

### **Evaluation of spatial variability of soil arsenic adjacent to a cattle-dip site** **64**

---

|                                      |           |
|--------------------------------------|-----------|
| <b>Abstract</b>                      | <b>64</b> |
| <b>3.1 Introduction</b>              | <b>65</b> |
| <b>3.2 Materials and methodology</b> | <b>67</b> |
| 3.2.1 Study area and dataset         | 67        |
| 3.2.2 Geostatistical analysis        | 69        |
| 3.2.3 Sample size estimation         | 72        |
| <b>3.3 Results and discussion</b>    | <b>74</b> |
| 3.3.1 Spatial variability in soil As | 74        |
| 3.3.2 Estimation of sample size      | 82        |
| <b>3.4 Conclusions</b>               | <b>86</b> |
| <b>3.5 References</b>                | <b>88</b> |

---

**CHAPTER 4**

|   |  |            |
|---|--|------------|
| <b>Phytoremediation potential of <i>Pteris vittata</i> L and <i>Pityrogramma calomelanos</i> var. <i>austroamericana</i> at a highly variable arsenic contaminated site – short-term data</b> |  | <b>91</b>  |
| <b>Abstract</b>   |  | <b>91</b>  |
| <b>4.1 Introduction</b>   |  | <b>92</b>  |
| <b>4.2 Materials and methodology</b>  |  | <b>94</b>  |
| 4.2.1 Fern species  |  | 94         |
| 4.2.2 Experimental Site   |  | 94         |
| 4.2.3 Experimental set up   |  | 94         |
| 4.2.4 Soil and plant sampling   |  | 97         |
| 4.2.4.1 Total and phosphate-extractable As in soil  |  | 97         |
| 4.3 Statistical analysis  |  | 98         |
| 4.4 Calculations to estimate the remediation time-frame based on As uptake data   |  | 99         |
| <b>4.5 Results</b>  |  | <b>101</b> |
| 4.5.1 Soil As concentrations  |  | 101        |
| 4.5.1.1 Total As concentration in the surface soil  |  | 101        |
| 4.5.1.2 Phosphate-extractable soil As concentrations for three depths   |  | 101        |
| 4.5.2 First plant sampling  |  | 103        |
| 4.5.3 Major plant harvest   |  | 105        |
| 4.5.3.1 Fern dry matter yield   |  | 105        |
| 4.5.3.2 Fern As concentration   |  | 107        |
| 4.5.3.3 Fern As uptake  |  | 107        |
| 4.5.3.4 Fern bioconcentration factors (BFs)   |  | 110        |
| <b>4.6 Discussion</b>   |  | <b>112</b> |
| <b>4.7 Time estimation for remediation</b>  |  | <b>116</b> |
| <b>4.8 Conclusions</b>  |  | <b>118</b> |
| <b>4.9 References</b>   |  | <b>119</b> |

---

**CHAPTER 5****Phytoremediation of an arsenic contaminated site using *P. vittata* L. and *P. calomelanos* var. *austroamericana* – long-term data** **123**

---

|  |            |
|--|------------|
| <b>Abstract</b>  | <b>123</b> |
| <b>5.1 Introduction</b>  | <b>124</b> |
| <b>5.2 Materials and methodology</b>   | <b>126</b> |
| 5.2.1 Fern species and experimental site   | 126        |
| 5.2.2 Ferns transplantation and experimental set up                              | 126        |
| 5.2.3 Frond harvests   | 126        |
| 5.2.4 Soil sampling  | 127        |
| <b>5.3 Statistical analysis</b>  | <b>127</b> |
| <b>5.4 Results and discussion</b>  | <b>128</b> |
| 5.4.1 Frond harvests   | 129        |
| 5.4.1.1 Dry matter yield   | 129        |
| 5.4.1.2 Arsenic concentration in ferns   | 129        |
| 5.4.1.3 Fern As uptake   | 131        |
| 5.4.2 Total and phosphate-extractable soil As concentrations<br>for three depths | 135        |
| <b>5.5 Time estimation for remediation of the experimental site</b>              | <b>138</b> |
| <b>5.6 Conclusions</b>   | <b>139</b> |
| <b>5.7 References</b>  | <b>140</b> |



---

**CHAPTER 6****Mid infrared reflectance spectroscopy and partial least-squares regression to predict total As content in soil** **143**

---

|  |            |
|--|------------|
| <b>Abstract</b>  | <b>143</b> |
| <b>6.1 Introduction</b>                                      | <b>144</b> |
| <b>6.2 Materials and methodology</b>                         | <b>147</b> |
| 6.2.1 Soil samples   | 147        |
| 6.2.2 Soil digestion to measure total As                     | 147        |
| 6.2.3 MIR spectroscopy                                       | 148        |
| 6.2.4 Principal component analysis and partial least-squares | 148        |
| <b>6.3 Results and discussion</b>                            | <b>153</b> |
| 6.3.1 MIR spectral features                                  | 153        |
| 6.3.2 Principal component analysis                           | 157        |
| 6.3.3 Calibration model using MIR spectra and PLS regression | 157        |
| 6.3.4 Validation of MIR-PLS model                            | 159        |
| <b>6.4 Conclusions</b>                                       | <b>160</b> |
| <b>6.5 References</b>  | <b>162</b> |

---

**CHAPTER 7****Speciation and phytoavailability of arsenic in contaminated soils  
using a SEP and XANES spectroscopy** **166**

---

|  |            |
|--|------------|
| <b>Abstract</b>  | <b>166</b> |
| <b>7.1 Introduction</b>  | <b>167</b> |
| <b>7.2 Materials and methodology</b>   | <b>169</b> |
| 7.2.1 Soil sampling  | 169        |
| 7.2.2 Physicochemical analysis   | 171        |
| 7.2.3 Phytoavailability pot experiment   | 171        |
| 7.2.4 Sequential extraction procedure  | 174        |
| 7.2.5 XANES spectroscopy   | 175        |
| 7.2.6 Data analysis  | 177        |
| <b>7.3 Results and discussion</b>  | <b>178</b> |
| 7.3.1 Total As in soil   | 179        |
| 7.3.2 Arsenic fractionation in soils using a SEP   | 179        |
| 7.3.3 Arsenic speciation using XANES spectroscopy  | 183        |
| 7.3.4 Arsenic phytoavailability to <i>B. juncea</i> in relation to SEP<br>and XANES spectroscopy | 190        |
| <b>7.4 Conclusions</b>   | <b>194</b> |
| <b>7.5 References</b>  | <b>196</b> |

**CHAPTER 8****Summary and future research** **201**

---

**APPENDICES** **205**

---

---

## LIST OF FIGURES

### CHAPTER 2

---

|                   |   |           |
|-------------------|---|-----------|
| <b>Figure 2.1</b> | Global As cycle (adapted from Matschullat 2000).  | <b>10</b> |
| <b>Figure 2.2</b> | A model for determining available As concentration in soil (modified from Morel 1997).  | <b>27</b> |
| <b>Figure 2.3</b> | A dose response curve for the essential and non-essential (As in this thesis) elements in plants (modified after Morel 1997).   | <b>31</b> |
| <b>Figure 2.4</b> | (a,c,e) Tolerance strategies in plants in relation to increasing heavy metal(loid) (As in this study) concentrations in soil; (modified after Baker 1981); (b,d,f) the plant growth in response to increasing heavy metal(loid) concentrations (in soil and plant), are also presented (Kachenko 2008). | <b>32</b> |
| <b>Figure 2.5</b> | Generalised pattern of As uptake strategies by terrestrial plants (modified from Fitz and Wenzel 2006).   | <b>33</b> |

### CHAPTER 3

---

|                   |  |           |
|-------------------|--|-----------|
| <b>Figure 3.1</b> | Schematic representation of the soil sampling scheme around a cattle dip at the experimental site. The filled circles show the intensive soil sampling scheme ( $n = 102$ ) performed to evaluate the spatial variability of As in soil. The internodal distance between soil cores reduced to 0.25 m (as shown in figure) due to the presence of a buffer region.   | <b>69</b> |
| <b>Figure 3.2</b> | The frequency distribution and summary statistics of the untransformed data ( $\text{mg kg}^{-1}$ ) for (a) total As at 0–0.2 m depth, and phosphate-extractable As at (b) 0–0.2 m (c) 0.2–0.4 m, and (d) 0.4–0.6 m depths; (e) the log-transformed data for phosphate-extractable As 0.4–0.6 m depth ( $\log, \text{mg kg}^{-1}$ ).   | <b>75</b> |
| <b>Figure 3.3</b> | The semivariogram models fitted with spatial trend by REML for mapping of total and phosphate-extractable soil As at the studied depths, using the given parameters; (a) spherical model (untransformed data, $\text{mg kg}^{-1}$ ); (b,c) exponential models (untransformed data, $\text{mg kg}^{-1}$ ); (d) exponential model ( $\log$ -transformed data, $\log(\text{mg kg}^{-1})$ ). The parameters are; $C_1$ = partial sill (variance); $\phi$ = distance parameter (range); $C_0$ = nugget. The filled circles represent the experimental semivariogram obtained using method of moments. | <b>76</b> |
| <b>Figure 3.4</b> | The maps showing the spatial variability of (a) total As at 0–0.2 m depth generated using a spherical model (untransformed data); and phosphate-extractable As for (b) 0–0.2 m and (c) 0.2–0.4 m depths using an exponential model (untransformed data); (d) 0.4–0.6 m depth using an exponential model ( $\log$ -transformed data were back-transformed).   | <b>79</b> |
| <b>Figure 3.5</b> | The regression and reference lines showing the distribution of observed and predicted values for the total and phosphate-extractable As at the investigated depths, where the variogram models were fitted based on the spatial (northings and eastings) trend (in Figure 3.3); (a,b,c) untransformed data ( $\text{mg kg}^{-1}$ As) (d) $\log$ -transformed data ( $\log, \text{mg kg}^{-1}$ As). The leave-one-out cross validation (LOOCV) indices are also presented, ME = mean error; MSE = mean square error; MSDR = mean squared deviation ratio.   | <b>81</b> |

- 
- Figure 3.6** The semivariogram models fitted without spatial trend by REML using the given parameters; (a,b) spherical models (untransformed data, mg kg<sup>-1</sup>); (c) exponential model (untransformed data, mg kg<sup>-1</sup>); (d) exponential model (log-transformed data, log(mg kg<sup>-1</sup>)). The parameters are;  $\beta$  = estimated mean;  $C_1$  = partial sill (variance); and  $\varphi$  = distance parameter (range) and  $C_0$  = nugget. **83**
- Figure 3.7** The regression and reference lines showing the distribution of observed and predicted values for the total and phosphate-extractable As at the investigated depths, where the variogram models were fitted without spatial trend (see in Figure 3.6); (a,b,c) untransformed data (mg kg<sup>-1</sup> As), (d) log-transformed data (log, mg kg<sup>-1</sup> As). The leave-one-out cross validation (LOOCV) indices are also presented; ME = mean error; MSE = mean square error; MSDR = mean squared deviation ratio. **84**
- Figure 3.8** The number of samples ( $n$ ) required to estimate the spatial mean of total As (mg kg<sup>-1</sup>) in soil with a known error and confidence interval (CI) at 95% confidence level (CL) around mean (untransformed data). **85**

## CHAPTER 4

---

- 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. **96**
- Figure 4.2** The relationship between total soil As concentration and the frond dry matter yield of (a) *P. calomelanos* var. *austroamericana* and (b) *P. vittata*. **106**
- 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. **108**
- 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. **108**
- 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*. **109**
- 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. **111**
- 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. **117**

---

## CHAPTER 5

---

- 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. *austramericana*) 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. **134**

---

## CHAPTER 6

---

- Figure 6.1** The absorbance spectra of a soil sample in the MIR spectral region (4000–400  $\text{cm}^{-1}$ ). The blue colour line represents the raw absorbance spectrum; red colour spectrum is first derivative of the raw spectrum after removing the baseline. **149**
- Figure 6.2** Principal component analysis (PCA) performed on the (first derivative pre-treated) absorbance spectra of all soil samples ( $n = 304$ ). The green filled circles are the 6 spectral outliers in the soil samples. PC-1 = principal component-1; PC-2 = principal component-2. **150**
- Figure 6.3** Frequency distribution (histograms and box-plots) of the measured total As concentration in soil samples used for the MIR-PLS regression analysis for the development of (a) final calibration model; and for (b) validation of the calibration model. **151**
- Figure 6.4** (a) The mid infrared (MIR) absorbance spectra of the six soil samples collected from the surface (0–20 cm) and the subsurface (20–40, 40–60 cm) depths; and (b) the MIR partial least-squares (PLS) coefficients obtained in the calibration model ( $n = 149$ ), in relation to the wavenumber (4000–400  $\text{cm}^{-1}$ ) used for the MIR spectroscopy. **154**
- Figure 6.5** The measured values of total soil As concentration are plotted against the MIR-PLS model predicted As values; (a) preliminary calibration model including the 6 spectral outliers ( $n = 155$ ); (b) validation of the preliminary calibration model; (c) final calibration model developed excluding the 6 spectral outliers spectral data ( $n = 149$ ) using the calibration set data as shown in Figure 6.3a; (d) validation of the calibration model constructed with  $n = 149$  samples. The  $R^2_c$  and  $R^2_v$  are coefficient of determination in the calibration and validation sets, respectively; SECV = standard error of cross validation; SEP = standard error of prediction;  $\text{RPD}_c$  and  $\text{RPD}_v$  = residual prediction deviation in the calibration and validation sets, respectively. **158**

---

## CHAPTER 7

---

- Figure 7.1** Locations of the As-contaminated cattle-dip sites, railway corridor and As spiked soil samples collected from New South Wales (NSW) and South Australia (SA) states of Australia. **170**
- Figure 7.2** Sequential extraction procedure applied to the cattle-dip sites, railway corridor and As spiked soil samples ( $n = 18, 3$  and  $11$ , respectively). Means of each extracted fraction are presented as percent (%) of the total soil As concentration present in the contaminated and As spiked soils. The residual fraction of As was calculated by subtracting sum of the four fractions from the total As concentration in each of the soils. The bars represent  $\pm$ SD around the mean values. **178**

- Figure 7.3** Arsenic K-edge XANES spectra of (a) reference compounds, and (b) selected soil samples from the cattle-dip sites, railway corridor and As spiked soils. Solid (–), dotted (...) and dashed (---) lines drawn at 11875.7, 11872.2 and 11868.7 eV represent the position of white lines for arsenate ( $\text{As}^{\text{V}}$ ), arsenite ( $\text{As}^{\text{III}}$ ), and orpiment ( $\text{As}_2^{\text{III}}\text{S}_3$ ), respectively. The white line energy values (eV) are given in the parentheses for each of the reference compound. **184**
- Figure 7.4** Arsenic K-edge XANES spectra for Boorie (black and purple lines) and Wollongbar (red and green lines) soil samples analysed after Step-II and Step-III of the SEP. The solid (–) line is drawn at 11875.7 eV represent the position of white line for arsenate ( $\text{As}^{\text{V}}$ ). **188**
- Figure 7.5** Arsenic concentration in dry shoots of *B. juncea* in relation to the sequentially extracted (a) exchangeable As (Step-I); (b) specifically sorbed As (Step-II); (c) amorphous Fe oxides bound As (Step-III); (d) crystalline Fe oxides bound As (Step-IV); and (e) As determined using XANES associated with amorphous Fe oxides. Cattle-dip sites and railway corridor soils (●); As spiked soils (●). The correlations are presented excluding the As spiked soils data due the considerable differences in ageing time. **191**
- Figure 7.6** The relationship of amorphous (ammonium oxalate extractable) Fe concentration with the amorphous Fe oxides associated As in the studied soils determined using (a) SEP in Step-III, and (b) XANES spectroscopy. **193**

---

## LIST OF TABLES

### CHAPTER 2

---

|                  |  |           |
|------------------|--|-----------|
| <b>Table 2.1</b> | Review of As concentrations in different materials on earth; adapted from (Smith et al. 1998; Mandal and Suzuki 2002). | <b>11</b> |
| <b>Table 2.2</b> | Arsenic minerals in the soil environment; adapted from (Mandal and Suzuki 2002).                                       | <b>14</b> |
| <b>Table 2.3</b> | Arsenic concentrations in soil adjoining the cattle-dip sites in New South Wales (NSW), Australia (Smith et al. 2006). | <b>20</b> |
| <b>Table 2.4</b> | Review of the confirmed As-hyperaccumulating species.  | <b>46</b> |

### CHAPTER 3

---

|                  |   |           |
|------------------|---|-----------|
| <b>Table 3.1</b> | Fixed effect parameters for total and phosphate-extractable As in soil at the different depths. | <b>77</b> |
|------------------|---|-----------|

### CHAPTER 4

---

|                  |  |            |
|------------------|--|------------|
| <b>Table 4.1</b> | Physico-chemical properties of the soil from the experimental site at Wollongbar in northern NSW.  | <b>95</b>  |
| <b>Table 4.2</b> | Total and phosphate-extractable soil As concentrations ( $\text{mg kg}^{-1}$ ) 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 <i>t-test</i> .  | <b>102</b> |
| <b>Table 4.3</b> | The frond As concentration, dry matter yield and As uptake of <i>P. vittata</i> and <i>P. calomelanos</i> var. <i>austroamericana</i> . The differences between the two fern species are evaluated using ANCOVA and <i>t-test</i> .  | <b>104</b> |
| <b>Table 4.4</b> | The bioconcentration factors of <i>P. vittata</i> and <i>P. calomelanos</i> var. <i>austroamericana</i> were calculated based on the total and phosphate-extractable soil As concentrations. The differences between both the fern species are evaluated using <i>t-test</i> . | <b>105</b> |

### CHAPTER 5

---

|                  |  |            |
|------------------|--|------------|
| <b>Table 5.1</b> | Dry matter yield ( $\text{g plant}^{-1}$ ), As concentration ( $\text{mg kg}^{-1}$ DW) and As uptake ( $\text{mg plant}^{-1}$ ) measured in fronds of <i>Pityrogramma calomelanos</i> var. <i>austroamericana</i> and <i>Pteris vittata</i> after first (10 month in December 2009), second (22 month in November 2010) and third (27 month in April 2011) harvests. | <b>130</b> |
| <b>Table 5.2</b> | 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.                               | <b>136</b> |

---

**CHAPTER 7**

---

|                  |   |            |
|------------------|---|------------|
| <b>Table 7.1</b> | Physico-chemical properties of soil samples from historically As-contaminated cattle-dip and railway corridor sites and soils spiked with As in the laboratory.   | <b>172</b> |
| <b>Table 7.2</b> | Detail for the extraction steps used in the sequential extraction procedure employed for the As speciation of As-contaminated cattle-dip sites, railway corridor and As spiked soils.                                   | <b>175</b> |
| <b>Table 7.3</b> | Arsenic distribution in various phases as determined by the SEP showing the As distribution in cattle-dip sites, railway corridor and As spiked soil samples ( $n = 32$ ).  | <b>180</b> |
| <b>Table 7.4</b> | Linear least-squares combination fits of XANES spectra of reference compounds with those of soil samples ( $n = 30$ ) analysed. Fit results correspond to XANES spectra of selected soil samples plotted in Figure 7.2. | <b>186</b> |