

Summary and future research

A substantial advancement has been achieved in screening the hyperaccumulating efficiency of *Pityrogramma calomelanos* var. *austroamericana* and *Pteris vittata* for the phytoremediation of As-contaminated sites. The phytoextraction potential of a lesser-known As-hyperaccumulator, *P. calomelanos* var. *austroamericana* was compared against the well-known *P. vittata* by conducting a field trial at a highly variable As-contaminated cattle-dip site (Chapters 4 and 5). *P. calomelanos* var. *austroamericana* performed significantly (> 2-fold) better than *P. vittata* in terms of accumulating As in fronds, producing larger amounts of aboveground (frond) biomass and subsequently reduced more As content in the soil, over the 27 month experimental period. These results demonstrate that this species is better suited for phytoremediation of As than *P. vittata* under the experimental conditions at the studied site. The research also demonstrates that field experiment data should be considered when evaluating the phytoextraction potential of plants for the remediation of contaminated soils. Further research is warranted to understand the As accumulation behaviour of these fern species at As-contaminated sites with variable soil properties. Since performance of these plants in the field may be a cumulative function of soil (e.g. pH, extent of As contamination, chemical association of As and its availability), environmental (e.g. rainfall, temperature, weeds) and plant (e.g. root morphology, distribution and density, mycorrhizal association of roots) factors. These aspects should be considered in future phytoremediation research studies.

Although *P. calomelanos* var. *austroamericana* has shown tremendous potential for extraction of As from the soil with high contents of As ($> 1000 \text{ mg kg}^{-1}$ As), however, there are certain additional factors in agricultural soils that need to be considered. For example, it is important to investigate the effects of phosphate and sulfate on the phytoavailability of As in soil under field conditions and to examine the nutritional significance of such effects on plant growth.

Despite the progress in the identification of As-hyperaccumulating ferns and understanding the soil-plant processes affecting the plant accumulation of As, research is required to safely dispose large quantities of As-containing frond biomass that could be produced from large scale field trials. Research is also warranted to examine the transport, handling and storage of As loaded fronds to reduce the risk of secondary pollution.

The use of geostatistical approach was an important advancement to describe the spatial trend and variability in soil As content over a small area adjoining the cattle-dip site. The model-based geostatistical method provided guidelines for design-based sampling schemes, such as simple random sampling (Chapter 3). Such methodology could also be useful for monitoring the changes in soil contaminant level at a site after (phyto)remediation activities (Chapter 5). Using the linear mixed model, it was recommended that a generic sampling scheme requiring 5 samples would be adequate to statistically assess the contamination level at the site; and 15 samples would be sufficient to determine the effect of phytoremediation on As content in soil (e.g. in Chapter 5). For future studies other options, such as stratified random sampling could be explored that can further improve the efficiency of the sampling scheme. Given the significance of the trend parameters, i.e. decrease in As concentration in soil with distance

from the cattle-dip and the highly variable nature of As, a stratified random sampling scheme can be employed where the strata are delineated based on distance from the dip bath.

The solid-phase speciation and phytoavailability of As in historically contaminated soils from As^{III}-containing pesticides and herbicides, and soils spiked with As in the laboratory, were determined using a sequential extraction procedure (SEP), X-ray absorption near edge structure (XANES) spectroscopy and plant uptake experiment (Chapter 7). The results revealed that combination of SEP and XANES spectroscopy is a powerful approach in delineating chemical (labile, sorbed, mineral bound), and mineral (scorodite, orpiment) forms, and oxidation states (As^V/As^{III}) of As in contaminated soils. Arsenic was predominantly found in As^V form and it was present in association with the amorphous Fe oxides. However, significant proportion of As was detected in soluble and exchangeable pools, which was only detected by the SEP. In addition, the plant As uptake data reflected that As in exchangeable and amorphous Fe oxides bound forms was phytoavailable and might present potential risks to the environment. The detailed knowledge obtained on the speciation of As in soil in this work is imperative for the reclamation and management of the As-contaminated soils.

While a significant progress was made in understanding the solid-phase speciation of As in soils at disused cattle-dip sites; further research is needed to investigate the factors controlling the site-specific variation in As at these sites. For example, the role of microbes and soil properties in the oxidation-reduction reactions of soil As is unclear and require further investigations. Also, μ -XANES and EXAFS should be used to understand soil environmental processes at fine scale because in certain cases bulk measurement methods may not provide accurate solid-state speciation and minor phases may be overlooked.

Mid infrared (MIR) spectroscopy in combination with partial least-squares (PLS) regression enabled the estimation of total As content in soil; the model predicted the total soil As in a separate set of unknown samples with an acceptable reliability (Chapter 6). This is a rapid, inexpensive, and easy-to-use procedure to estimate total soil As that could be useful in some situations. For example, for a routine estimation of total As involving large number of soil samples at a given site, to monitor changes in soil As content at a given site (Chapter 5) and to estimate total As in soil at highly variable sites (Chapters 3,4). In this study, As was predominantly present in adsorbed form; further research is required to build a more robust prediction model that is able to estimate total soil As at different sites, where As may be present in variable concentrations and in both adsorbed and mineral forms.

From the research presented in this thesis, it is evident that the phytoextraction of As contaminated soils using As-hyperaccumulating fern species offers a good promise for the remediation and restoration of As-contaminated sites. Further research should also focus on technology for increasing plant biomass of hyperaccumulating ferns so that the phytoremediation could be routinely used in the large scale remediation of contaminated sites.