RP 6 - Phytoextraction of Metals: Investigation of Hyperaccumulation and Field Testing

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Executive Summary

The remediation of heavy metal and metalloid contaminated soil is of considerable national importance in the UK. This is because of the potential adverse effects these contaminants may pose to food quality, soil and human health and the environment. In response, there have been numerous technologies developed to remediate contaminated soil. A relatively new technology is phytoextraction, an *in situ* remediation technique that uses hyperaccumulator plants to extract contaminants from soils and accumulate them in the harvestable parts of the plant which can then be removed from site. Phytoextraction has been considered as an environmentally sustainable, low-input approach for remediation of contaminated soils. However, it is also a relatively new technology and there are still a number of aspects of the mechanisms of metal/metalloid uptake that are poorly understood that require investigation.

In this investigation a field trial was undertaken to evaluate the ability of two hyperaccumulators of arsenic (As), *Pteris vittata* and *Pteris cretica*, to extract As from contaminated soils. In addition, a series of complementary laboratory experiments were undertaken to explore the mechanisms of As accumulation in these ferns. The field trial demonstrated that both *P. vittata* and *P. cretica* could grow in the climatic conditions of southwest England, although *P. vittata* did not survive winter. Furthermore *P. vittata* and *P. cretica* could both accumulate large amounts of As in their fronds (437 and 2366 mg As kg^{-1} respectively). However, the total amount of As that was extracted by either species was < 1% of the total soil As content. The relatively small proportion of bioavailable As, and more importantly the low plant biomass yield, likely contributed to the low amount of As that was extracted from the soil. Laboratory experiments indicated that compared to non-accumulators, *P. vittata* had a very efficient root uptake, root to shoot translocation and hyper-tolerance to As. It was also observed that while soil amendments such as lime and phosphorus could increase As concentration in

The start of a harvest: *Pteris cretica* in foreground, *Pteris vittata* in background.

soil solutions, they had no effect on As uptake by *P.vittata*. It was also found that co-contamination of soil with metals such as Cu and Zn may negatively affect plant growth and decrease As uptake in *P.vittata*. In addition, compared to other hyperaccumulators, *P. vittata* roots do not actively forage for As in soil. The occurrence of symbiotic fungi (mycorrhiza) do not enhance As uptake by either *P.vittata* or *P.cretica*.

Field tests were also conducted to evaluate the phytoextraction potential of two other hyperaccumulator plants. *Thlaspi caerulescens* and *Arabidopsis halleri*, for extracting cadmium (Cd) and zinc (Zn) from soils previously contaminated with varying amounts of heavy metals. Both *T. caerulescens* and *A. halleri* were able to hyperaccumulate Cd and Zn from contaminated soils. However *T. caerulescens* produced a greater biomass, accumulated higher Cd and Zn concentrations in their shoots and consequently extracted a greater proportion of metals from the soil than *A. halleri*. On average, for plots where Cd or Zn exceeded limits set by the Commission of the European Communities Directive for sludge-treated agricultural soils, i.e. 3 and 300 mg kg^{-1} respectively, two crops of *T. caerulescens* extracted 3.9% of the total soil Cd content and 0.6% of the soil Zn, compared to < 0.1% of the soil Cd or Zn for a

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single crop of *A. halleri*.

The costs and feasibility of phytoextraction were reviewed for the two field trials undertaken in this project. The literature would indicate that incineration and pyrolysis appear to be the most promising techniques for post harvest disposal or possible recovery of metal from biomass grown on contaminated soils. However both these technique have yet to be fully tested on a commercial scale. Results from the field trials undertaken in the present project would indicate that there is little value in trying to recover As, Zn and Cd from the biomass for purely economic reasons. The estimated costs of disposal of plant material containing "hazardous" concentrations of heavy metal/metalloids to landfill sites are between £100-£150 per tonne. Along with the other costs associated with phytoremediation such as biomass production and pre-treatment of biomass, phytoremediation remains cheaper than many other remediation technologies currently available, but may take more time.

Conclusions

- On an arsenic (As) contaminated site, a field trial demonstrated that the As hyperaccumulators *P. vittata* and *P. cretica* could both be grown in the climatic conditions of southwest England; however *P. vittata* did not survive the winter.

- *Pteris vittata* accumulated up to 4371 mg As kg $^{-1}$ and *P. cretica* 2366 mg As kg $^{-1}$ in their fronds when grown on a soil with extractable and total As concentrations of 1.7 and 471 mg kg $^{-1}$ respectively.

- Average bioconcentration (plant shoot:soil as ratio) values were 9 for *P. vittata* and 4 for *P. cretica*.

- The total amount of As extracted from soil in two harvests ranged between 2.1 and 3.9 kg ha^{-1} for *P.vittata* and 0.35 and 0.09 kg ha^{-1} for *P. cretica*.

- Results indicate that *Pteris vittata* removed 0.51% of the total soil As burden in the soil compared to 0.038% for *P. cretica*.

- Low biomass yield was the main reason for the low extraction of As in the field trial.

- Complementary glasshouse studies showed that *P. vittata* was able to hyperaccumulate As from soil contaminated in the field; however bioaccumulation values varied between soils with a bioaccumulation factor > 10 observed in only one out of the five soils tested.

- The occurrence of co-contamination of Zn and Cu at high levels in some soil greatly affected growth and As uptake in *P. vittata*.

- *Pteris vittata* took up As twice as fast as the non-hyperaccumulator *P. tremula* and was more efficient at transporting As from roots to fronds.

- *Pteris vittata* was also more tolerant of high As additions to the external medium than *P. tremula*, with As concentrations up to 500 mg kg^{-1} having no effect on biomass compared to *P. tremula* which did not survive beyond as As concentration of 100 mg kg^{-1}.

- Despite the addition of phosphate increasing the concentration of soluble As in soil pore water, it had no significant effect on As uptake by *P. vittata*. Arsenic uptake was most likely to have been decreased by competition between P and As during root uptake processes.

- Arsenic "hot spots" in soil did not affect the rooting distribution of *P. vittata*, indicating *P. vittata* does not have the ability to actively forage for this element in soil.

- Despite *P. vittata* not foraging for As, its roots do not appear to actively avoid As "hot spots", which is a positive trait for a hyperaccumulator.


Close up of *Pteris vittata.*

• At the field trial site, on average, 27% of *P. cretica* roots contained symbiotic mycorrhizal fungi, whereas no significant mycorrhizal infection was observed for *P. vittata*.

• Inoculation of *P. vittata* and *P. cretica* with Arbuscular Mycorrhiza (AM) fungi has no significant effect on either biomass yield or plant As concentrations.

• In a field trial, the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri* were both able to hyperaccumulate Cd and Zn from contaminated soils.

• *Thlaspi caerulescens* produced a greater biomass, accumulated higher Cd and Zn concentrations in their shoots and consequently extracted a greater proportion of metal from the soil than *A. halleri*.

• On average, for plots where Cd or Zn exceeded limits set by the Commission of the European Communities Directive for sludge-treated agricultural soils, *T. caerulescens* extracted 3.9% of the total soil Cd content and 0.6% of the total soil Zn content, compared to <0.1% of the soil Cd or Zn by *A.halleri* in one year.

• An assessment of the costs associated with growing hyperaccumulator plants indicated that these were similar to normal crops.

• The biomass produced after harvest requires specialist treatment prior to disposal or recovery of the As, Zn or Cd extracted by the plant.

• Incineration and pyrolysis appear to be the most promising techniques for either post harvest biomass disposal or recovery of metal extracted from soil using hyperaccumulators.

• Results of biomass yields and the contaminant concentrations measured in plants from the field trials in the present investigation would indicate that there is little value in trying to recover the As, Zn or Cd from the biomass for purely economic reasons.

• The estimated costs of disposal of plant residues containing "hazardous" concentrations of heavy metal/metalloids to landfill sites are between £100-150 per tonne. Along with the other costs associated with phytoremediation such as biomass production and pre-treatment of biomass, phytoremediation remains cheaper than many other remediation technologies currently available but may take more time. Therefore, the most suitable applications of phytoextraction may be where either the concentration of contaminants is not far above acceptable values or for areas where time is not an issue (McGrath and Zhao, 2003).

• The findings from the present study and those from past research would indicate that phytoextraction using hyperaccumulators as a remediation option for Cd, Zn and As contaminated soils is most likely to be successful under the following conditions:

i. When the plant biomass production can be maximised;

ii. The soil contaminant is in bioavailable forms;

iii. Most of the soil contamination is within the plant rooting zone, which for the hyperaccumulators tested in the present study is < 20 cm soil depth;

iv. The magnitude of soil contamination is low to moderate;

v. Generally there is no co-contamination with metals such as Cu which can result in plant toxicity and hence reduced phytoextraction.

- In terms of future research needs, more work is still required to optimise agronomic practices such as the length of the crop growing season, planting density, irrigation, fertilisation and pest control to maximise the biomass yield and hence the phytoextraction potential of hyperaccumulators.

- Considerable variation exists within accumulator plants of the same species, in terms of yield and metal concentrations. Work is required to identify the best genotypes of these species and test them in the field.

- Hyperaccumulator plants are often small and slow growing species. These two properties limit their ability to extract metal/metalloids from soils. To improve their potential for metal phytoextraction, the transfer of hyperaccumulator traits from small and slow growing hyperaccumulator species to fast growing, high biomass producing, non-accumulator plants has been proposed. Unfortunately, the success of conventional crossing to improve efficiency has been hampered by the sexual incompatibility between hyperaccumulator and crop plants. As an alternative, genetic manipulation (GM) has been investigated because it offers the opportunity for direct gene transfer, thus circumventing limitations imposed by sexual incompatibility. However, there is still a large amount of research required to identify the genes responsible for important traits such as metal transport, storage and tolerance, and expressing them in suitable high biomass crops. A further limitation to the widespread application of genetically manipulated hyperaccumulators in the UK at the present time, may be the low public acceptance of GM technology. Clearly this would need to be overcome if genetically manipulated hyperaccumulators are to be routinely used to remediate contaminated soils.

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