RESEARCH PROJECT REPORT: RP6

PHYTOEXTRACTION OF METALS:

INVESTIGATION OF HYPERACCUMULATION

AND FIELD TESTING

CONTAMINATED LAND: APPLICATIONS IN REAL ENVIRONMENTS

CL:AIRE

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Contaminated Land: Applications in Real Environments (CL:AIRE)

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PHYTOEXTRACTION OF METALS: INVESTIGATION OF HYPERACCUMULATION AND FIELD TESTING

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Contaminated Land: Applications in Real Environments (CL:AIRE)

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This is a CL:AIRE Research Project Report. Publication of this report fulfils CL:AIRE's objective of disseminating and reporting on remediation research. This report describes research into the phytoextraction of arsenic, cadmium and zinc from contaminated soils using hyperaccumulator plants. It is not a definitive guide to the application of phytoremediation technology. CL:AIRE strongly recommends that individuals/organisations interested in using this technology retain the services of experienced environmental professionals.

EXECUTIVE SUMMARY

The remediation of heavy metal and metalloid contaminated soils 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 the winter. Furthermore *P. vittata* and *P. cretica* could both accumulate large amounts of As in their fronds (4371 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 concentrations in soil solution, 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 appear to 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 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 techniques 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 metals/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.

ACKNOWLEDGEMENTS

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ABBREVIATIONS

Al	aluminium
AM	arbuscular-mycorrhiza
As	arsenic
Cd	cadmium
Cu	copper
DM	dry matter
EDTA	ethylenediamine tetra-acetic acid
Fe	iron
FW	fresh weight
$g\ kg^{-1}$	gram per kilogram
g	gram
Hg	mercury
HGAAS	hydride generation atomic absorption spectrometry
ICP-AES	inductively coupled plasma atomic emission spectrometry
K	potassium
m	metre
$mg\ kg^{-1}$	milligram per kilogram
mm	millimetre
Mn	manganese
N	nitrogen
$nmol\ g^{-1}$	nanomole per gram
OM	organic matter
P	phosphorus
ppm	parts per million
S	sulphur
$t\ ha^{-1}$	tonnes per hectare
Ti	titanium
UK	United Kingdom
v/v	volume per volume
w/w	weight per weight
Zn	zinc
$\mu g\ L^{-1}$	microgram per litre
μM	micromole
$\mu mol\ m^{-2}\ s^{-1}$	micromole per square metre per second
°C	degrees Celsius

1. INTRODUCTION

1.1   BACKGROUND

There is great interest surrounding issues of soil contamination by a large range of pollutants including heavy metals such as cadmium (Cd) and zinc (Zn) and metalloids such as arsenic (As). The accumulation of these pollutants in soil is of interest because of the adverse effects these contaminants may pose to food quality, soil and human health and the environment.

Plant uptake of heavy metals/metalloids from soil and their entry into the food chain varies depending on the type of metals/metalloids in the soil. This can be explained to a large extent by the soil–plant barrier concept described by Chaney and Giordano (1977) which suggests that soil or plant barriers limit the transmission of metals/metalloids through the food chain, either by soil chemical processes that limit metal solubility or by plant senescence from phytotoxicity of metals/metalloids taken up by the plants. For example, the soil barrier limits transmission of metals/metalloids such as titanium (Ti) and mercury (Hg) that are very insoluble and/or strongly adsorbed to soil or in plant roots, whereas the plant barrier limits transmission of heavy metals/metalloids such as Zn and As that result in plant phytotoxicity before they reach concentrations in the edible parts of plants often considered harmful to humans. In contrast, heavy metals such as Cd are not limited by soil or plant barriers and may accumulate to concentrations in plants which may exceed food regulations and constitute a risk to humans. However, variation in plant physiology and rhizosphere biochemistry between different plant species means that metal phytotoxicity is species specific.

In response to the negative impacts that heavy metals/metalloids in soils may pose such as plant uptake and risk to soil and human health, there has been ongoing development of a variety of technologies to remediate polluted soils. However, many of the proposed remediation technologies are engineering-based, considered expensive, and often produce secondary wastes. Recently however, more environmentally sustainable, *in situ*, low-input approaches for cleaning up metal and metalloid contaminated soils have been proposed, such as phytoremediation (McGrath *et al.*, 2002). Phytoremediation can be loosely defined as the use of plants to improve the environment. One aspect of phytoremediation is phytoextraction, an emerging remediation technology that uses hyperaccumulator plants to extract heavy metals or metalloids from contaminated soils and accumulate them in the harvestable parts of the plants which can then be removed from site. These are plants where metal/metalloid uptake is not limited by plant barriers and they accumulate and tolerate very high concentrations.

To be classified as a hyperaccumulator, a plant must meet a number of criteria including; (i) possessing metal/metalloid concentrations in their leaves >100 times higher than 'non-accumulator' plants (ii) having leaf:soil bioconcentration factors > 1 (iii) having enhanced transport of the contaminant from root to shoot and (iv) possess tolerance to both high internal and external concentrations of metals/metalloids (McGrath *et al.*, 2002).

The hyperaccumulation of heavy metals and metalloids is a rare phenomenon in terrestrial higher plants. To date, some 400 taxa of hyperaccumulator species have been identified, however about three-quarters of these are nickel (Ni) hyperaccumulators (Baker *et al.*, 2000). Because there are many Ni hyperaccumulators which are part of the well-studied serpentine flora, "phytomining" of Ni has been researched in other parts of the world (Angle *et al.,* 2001; Broadhurst *et al.,* 2004). However, Ni is not a widespread contaminant in the UK (McGrath and Loveland, 1992). In contrast, Baker *et al.* (2000) listed only 11 taxa of Zn hyperaccumulator plants, the best known examples being members of the Brassicaceae family, *Thlaspi caerulescens* and *Arabidopsis halleri* (McGrath *et al.*, 2002). While *T. caerulescens* and *A. halleri* are also the two best known Cd hyperaccumulators (Kupper *et al.*, 2000; Dahmani-Muller *et al.*, 2000; Bert *et al.*, 2002). The first As hyperaccumulator

plant was recently discovered - the fern species *Pteris vittata* (Ma *et al.*, 2001). Since the discovery of *P. vittata* a number of other ferns from the same order, Pteridales, have been shown to be As hyperaccumulators. These include *Pityrogramma calamelanos* from the Hemionitidaceae family (Visottiviseth *et al.*, 2002) and *Pteris cretica*, *Pteris longifolia* and *Pteris umbrosa* from the Pteridaceae family (Zhao *et al.*, 2002).

1.2 OBJECTIVES

Given that phytoextraction of contaminants from soils is a relatively new, albeit promising technology, there are still a number of aspects of phytoextraction that are poorly understood. This project aimed to investigate some of these poorly understood aspects of metal/metalloid hyperaccumulation, specifically for As, Cd and Zn which are common contaminants in agricultural and industrial soils in the UK. In addition, an important aspect of any remediation technology is to assess whether it is an economically sustainable process. Therefore the practicality and economic feasibility of using hyperaccumulators to remediate contaminated soils were reviewed. Accordingly, the project comprised three objectives:

1. To assess the effectiveness of the ferns *Pteris vittata* and *Pteris cretica* to hyperaccumulate As from contaminated soils.
2. Field test the phytoextraction potential of *Thlaspi caerulescens* and *Arabidopsis halleri* for extracting Cd and Zn from soils previously contaminated with varying amounts of heavy metals.
3. Review the practical and economic feasibility of using hyperaccumulators to remediate As, Cd and Zn contaminated soil by undertaking a desk study using data obtained from the field trials.

Note that Pteris cretica was tested as an addition to the work originally proposed in this project.

1.3 ORGANISATION OF REPORT

The report is organised into three sections which address each of the three objectives. Chapter 2 comprises the results of a field trial and five complementary laboratory experiments investigating As uptake by the hyperaccumulators *Pteris vittata* and *Pteris cretica*. Chapter 3 presents the results of a field trial investigating Cd and Zn uptake by the hyperaccumulators *Thlaspi caerulescens* and *Arabidopsis halleri*. Chapter 4 is a discussion of the practical and economic feasibility of using hyperaccumulators to remediate As, Cd and Zn contaminated soil.

2. HYPERACCUMULATION OF ARSENIC BY *PTERIS VITTATA* AND *PTERIS CRETICA*

2.1 BACKGROUND

Arsenic (As) is a non-essential metalloid that at high concentrations is toxic to plants and animals. In the UK, particularly Southwest England, large areas of soil are considered contaminated with As, either geogenically or as a result of various anthropogenic activities such as mining and smelting. Cleaning up contaminated land can be a difficult and very expensive task. However, recent studies have shown that phytoextraction using hyperaccumulators may offer an efficient, low-cost and environmental-friendly approach to this problem (McGrath *et al.*, 2002). With the recent discovery of the first As hyperaccumulating plant species *P. vittata* (Ma *et al.*, 2001), there is now an opportunity to use these types of plants to remediate As contaminated soils. *Pteris vittata* has a remarkable ability to hyperaccumulate As, with studies showing shoot concentrations up to 23,000 mg kg^{-1} (Ma *et al.*, 2001). However, given that the phenomenon of As hyperaccumulation is a relatively new discovery, many of the mechanisms involved in this process such as uptake, translocation and tolerance are largely unknown. It is therefore vital that these mechanisms are understood in order to develop a strategy for using phytoextraction as a tool to remediate As contaminated soils. Consequently objective one of this project comprised two different kinds of work (i) a field study and (ii) several complementary laboratory studies.

i. The field study involved an evaluation of the ability of two As hyperaccumulators, *P. vittata* and *P. cretica* to extract As from contaminated soils.

ii. To complement the field study five laboratory experiments were undertaken investigating different aspects of As accumulation in *P. vittata* and *P. cretica.*
   - An assessment of As uptake by *P. vittata* in soils contaminated with different amounts and sources of As;
   - Measurement of the rate of uptake and tolerance of As in *P. vittata* and *P. cretica*;
   - An evaluation of the effect of soil amendments on As uptake in *P. vittata*;
   - Investigation of the response of *P. vittata* roots to 'hot spots' of As in soils;
   - An investigation of the role of mycorrhizal fungi in As uptake in *P. vittata* and *P. cretica.*

2.2 FIELD STUDY – ARSENIC UPTAKE BY *P. VITTATA* AND *P. CRETICA*

2.2.1 INTRODUCTION

Pteris vittata has been shown to be very effective in accumulating large amounts of As in both hydroponic and pot trial experiments (Tu *et al.*, 2002; Ma *et al.*, 2001). However the feasibility of using *P. vittata* for As phytoextraction in soils has as yet not been tested in the field. Furthermore, *P. vittata* is a mesophytic sub-tropical plant that prefers sunny alkaline sites (Jones, 1987) and does not grow naturally in England. It is therefore vital to ascertain whether *P. vittata* can grow under British conditions. Recently, another As hyperaccumulating fern, *P. cretica* was discovered (Zhao *et al.*, 2002), which unlike *P. vittata,* is naturalised in England. The discovery of *P. cretica* provided an excellent opportunity to compare As uptake in a naturalised Pteris species with that of the introduced *P. vittata*.

The aims of the field trial were therefore to compare As uptake in *P. vittata* and *P. cretica* and to evaluate their potential for remediation of an As contaminated soil and determine whether *P. vittata* can grow under British climatic conditions.

2.2.2 MATERIAL AND METHODS

2.2.2.1 Experimental Design

A field trial was undertaken near Camborne in Cornwall, England. The trial consisted of *P. vittata* and *P. cretica* grown in two separate trial plots (Figures 2.1 and 2.2).

Figure 2.1: General design of the field trial site at Camborne, Cornwall

Figure 2.2: Detailed design of the field trial site at Camborne, Cornwall

For *P. vittata*, a 4 x 4m plot was used that was further divided into four subplots i.e. 1 – 4 (Figure 2.2). For *P. cretica*, one 1.5 x 1.75 m plot was used (2.6 m^2) due to the limited amount of material available of this newly discovered hyperaccumulator, i.e. plot 5 (Figure 2.2). Prior to planting, topsoil samples (0-20 cm) were taken and lime and fertiliser were applied by hand to each of the trial plots. Lime was applied at a single rate equivalent to 12 t ha^{-2} and N at 100 kg ha^{-2} as nitrochalk. After fertiliser application, to suppress weeds, black matting was laid onto the surface of each plot and holes were cut into the matting to allow plants to be transplanted (Plate 2.1). Sporelings (130 day old) were transplanted out at a density of 25 per subplot of *P. vittata* and 15 seedlings per plot of *P. cretica*. After transplanting, plots were watered with 20 L m^{-2} of local tap water. Pests were controlled during the trial by applying a slug killer around the outside border of the plots.

Plate 2.1: View of *Pteris vittata* planted out in the field trial and the black matting to suppress weeds

2.2.2.2 Soil Analysis

Basic physiochemical characteristics such as soil pH, organic matter, texture, total nutrient concentrations, and heavy metal and As content were determined. Soils also underwent a sequential chemical fractionation procedure (Table 2.1). This produces five operationally defined As fractions corresponding respectively to (F1) non-specifically sorbed As, (F2) specifically-sorbed As, (F3) As adsorbed to amorphous and poorly-crystalline hydrous oxides of iron (Fe) and aluminium (Al), (F4) As adsorbed to well-crystallised hydroxide ions of Fe and Al, and (F5) As strongly bound to the residual phases (Wenzel, *et al.*, 2001).

Table 2.1: Sequential fractionation procedure

Step	Nominal Fraction	Extractant
F1	Non-specifically	$0.05\ M\ (NH_4)_2SO_4$
F2	Specifically sorbed	$0.05\ M\ NH_4H_2PO_4$
F3	Amorphous and poorly crystalline Fe and Al hydrous oxides	$0.2\ M\ (COONH_4)_2.H_2O$
F4	Crystalline Fe and Al hydrous oxides	$0.2\ M\ (COONH_4)_2.H_2O + 0.1\ M\ C_6H_8O_6$
F5	Residual	Aqua regia

2.2.2.3 Plant Sampling

Plants were harvested three times during the course of the field trial. The first harvest was made in July 2002, the second in August 2002 and a third was taken in October 2003. The first harvest consisted of taking the longest frond from each fern. The fronds sampled from each subplot were combined into one composite sample, hence there were four samples of the fronds of *P. vittata* and one sample of *P. cretica* from each trial plot. In the second harvest, again the longest frond from each fern was sampled. In addition, the remaining expanded fronds from each plot were sampled and analysed separately. This left only croziers (young shoots) that were then allowed to grow again over the winter period. However, *P. vittata* did not survive the winter and consequently the roots of the dead plants were removed and new ferns transplanted as described above. *Pteris cretica* on the other hand did re-grow after overwintering. All of the fronds of *P. vittata* and *P. cretica* were harvested in October 2003 (harvest three).

2.2.2.4 Plant Analysis

After sampling, fronds were washed in deionised water, dried and the dry weight of the biomass was measured. Sub-samples (0.5 g) of finely ground tissue were digested with a mixture of HNO_3 and $HClO_4$ (Zhao *et al.*, 1994). The concentrations of As and other elements in the digests were determined using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Fisons-ARL Accuris, Ecublens, Switzerland).

2.2.3 RESULTS AND DISCUSSION

2.2.3.1 Soils

The soil at the trial site was a slightly acidic, clay loam with a moderate organic matter content (Table 2.2). The total soil As concentration was 471 mg kg^{-1}, which is substantially higher than the soil guideline value (SGV) of 20 mg As kg^{-1} for the land uses of allotments or residential uses with or without plant uptake according to the Contaminated Land Exposure Assessment (CLEA) model (Department of Environment, Food and Rural Affairs and Environment Agency, 2002). Copper (Cu), lead (Pb), Cd and Zn were also present in amounts above background for UK soils, however Pb and Cd concentrations were below the current SGV's for all land uses according to the CLEA model.

Table 2.2: Physical and chemical soil properties at the trial site
The results are means $\pm$ standard errors (n = 3)

Soil Property	
Soil pH$_{(water)}$	6.2 $\pm$ 0.1
Organic matter (%)	4.1 $\pm$ 0.2
Particle size analysis (%)	
< 2 µm	22.9 $\pm$ 0.1
2 µm – 63 µm	44.3 $\pm$ 0.6
63 µm – 2 mm	32.9 $\pm$ 0.5
Total contents (mg kg^{-1})	
N	3035 $\pm$ 45
K	3239 $\pm$ 165
S	482 $\pm$ 32
As	471 $\pm$ 27
Cd	0.9 $\pm$ 0.06
Cu	370 $\pm$ 20
Fe	48,000 $\pm$ 2541
Pb	147 $\pm$ 78
Zn	371 $\pm$ 14

The concentrations of As in the fractions determined using sequential extraction are given in Table 2.3. While total As analysis provides no information regarding the potential bioavailability or mobility of arsenic in contaminated soil, sequential extraction analysis partitions soil arsenic into a range of fractions from highly mobile to recalcitrant. Results indicate that As was mostly associated with amorphous (c. 59 %) and crystalline (c. 21 %) hydrous oxide fractions. The most available form of soil As, non-specifically sorbed, was the smallest fraction (0.4 %). This indicates that while this soil is considered highly contaminated, the bioavailable fraction is relatively small.

Table 2.3: Arsenic concentrations (mg kg^{-1}) in the different fractions
The results are means $\pm$ standard errors (n = 3)

Step	Nominal Fraction	(mg As kg^{-1})	(% of Total)
F1	Non-specifically sorbed	1.7 $\pm$ 0.7	0.4
F2	Specifically sorbed	57 $\pm$ 4	11.9
F3	Amorphous and poorly crystalline Fe and Al hydrous oxides	282 $\pm$ 15	58.8
F4	Crystalline Fe and Al hydrous oxides	100 $\pm$ 5	20.8
F5	Residual	39 $\pm$ 7	8.1

2.2.3.2 Plant Numbers

At the time of transplanting, the young fronds were green and healthy. However, by the time of the first harvest, *P. vittata* appeared yellow and a number of seedlings were dead i.e. 11,

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7, 12, and 11 plants in subplots 1 to 4 respectively. By the second harvest, no further plants had died, the plants had grown significantly and fronds were again green and healthy (Plate 2.2).   This suggests that damage to the plants during transportation before transplanting was the reason for the high plant mortality.   At the third harvest, after transplanting new seedlings in the spring, the number of dead seedlings on the *P. vittata* plot was 3, 1, 2, 0 for subplots 1 to 4 respectively, significantly better than the previous planting.

Plate 2.2: View a *P. vittata* before the second harvest

2.2.3.3   Plant Biomass

Table 2.4 shows the dry biomass yield for *P. vittata* and *P. cretica*. For *P. vittata*, the dry biomass for the longest frond was 3-fold higher in harvest two than harvest one, which clearly reflects the longer growth period, i.e. 77 days at harvest one and 124 days at harvest two. The total biomass yield was 2-fold higher in harvest three than harvest two. This is a result of the higher plant survival rate for harvest three compared to harvest two, as discussed above.

Biomass for the longest frond for *P. cretica* was higher in harvest two than in harvest one, and again this reflects the longer plant growth period. In contrast to the result for *P. vittata* however, the total biomass yield for *P. cretica* was more than four times higher at harvest two compared to harvest three.

Unlike *P. vittata, P. cretica* survived the winter, and it would appear that this may have had an effect on subsequent yield, although other factors such as temperature and moisture availability cannot be dismissed as playing a role in affecting the recorded yield differences between the two fern species.

Table 2.4: Mean dry biomass yield for *P. vittata* and *P. cretica* fronds (t ha^{-1} DM)
The results are means $\pm$ standard errors (n = 4)

	Longest Frond	Remaining biomass	Total biomass
P. vittata			
Harvest 1 (2002)	0.023 ($\pm$ 0.001)	n.d.	n.d.
Harvest 2 (2002)	0.071 ($\pm$ 0.005)	0.416 ($\pm$ 0.031)	0.487 ($\pm$ 0.036)
Harvest 3 (2003)	n.d.	n.d.	1.032 ($\pm$ 0.040)
P. cretica			
Harvest 1 (2002)	0.0051	n.d.	n.d.
Harvest 2 (2002)	0.0073	0.252	0.260
Harvest 3 (2003)	n.d.	n.d.	0.056

n.d. not determined

2.2.3.4   Plant Arsenic Concentrations

Total elemental concentrations in the fronds of *P. vittata* are given in Table 2.5. Arsenic concentrations were in excess of 4000 mg kg^{-1} in the longest fronds in both harvest one and two and in the remaining biomass for harvest two, while slightly but not significantly lower for

harvest three. Of particular note, there was no difference in As concentration in the longest fronds for harvests one and two, despite a 3-fold difference in biomass (Table 2.4). Likewise, there was no significant difference in As concentrations in the total biomass for harvests two and three, despite a 2-fold difference in biomass.

Bioconcentration values (the leaf:soil As ratio) for *P. vittata* were of the order of 9, clearly confirming the extraordinary ability of *P. vittata* to hyperaccumulate As. This value is at the lower end of the range of those previously reported (i.e. 7 - 22) (Ma *et al.*, 2001; Zhao *et al.*, 2002; Cao *et al.*, 2003; Tu and Ma, 2003). However as discussed in the next section, bioconcentration values can be strongly affected by the form in which As is applied to the soil, i.e. soluble As salt versus aged geogenic As, as well as the experimental design e.g. hydroponic, pot trial or field trial. In contrast to As, none of the other elements measured displayed enhanced uptake by *P. vittata.*

Table 2.5: Elemental concentrations (mg kg^{-1} DM) in the fronds of *P. vittata*

The results are means $\pm$ standard errors (n = 4)

		As	Cd	Cu	Zn	Fe	P	K	S
Harvest 1 (2002)	Longest fronds	4156 $\pm$ 276	< 4	12.8 $\pm$ 1.1	56 $\pm$ 39	240 $\pm$ 36	2965 $\pm$ 172	23,924 $\pm$ 961	2089 $\pm$ 202
Harvest 2 (2002)	Longest fronds	4244 $\pm$ 909	< 4	11.6 $\pm$ 2.0	45 $\pm$ 9	208 $\pm$ 68	3462 $\pm$ 679	21,517 $\pm$ 2500	2342 $\pm$ 466
	Remaining biomass	4371 $\pm$ 905	< 4	10.4 $\pm$ 1.9	31 $\pm$ 7	179 $\pm$ 42	2887 $\pm$ 425	19,668 $\pm$ 4058	2073 $\pm$ 466
Harvest 3 (2003)	Total biomass	3795 $\pm$ 301	< 4	9.71 $\pm$ 0.5	33.5 $\pm$ 15	133.7 $\pm$ 14	3615 $\pm$ 158	21,790 $\pm$ 806	1903 $\pm$ 115

Total elemental concentrations in the fronds of *P. cretica* are given in Table 2.6. Arsenic concentrations were lower than those found for *P. vittata,* but nonetheless are still in excess of 2000 mg kg^{-1} in the longest fronds for both harvest one and two. The concentration patterns are similar to those shown by *P. vittata*, in which there was no difference in As concentration in the longest fronds for harvests one and two, despite a difference in biomass between harvests (Table 2.4). Likewise for As concentrations in the total biomass, there appeared to be no difference in As concentration for harvests two and three, despite a greater than 4-fold yield difference. This suggests that "growth dilution" effects are not important for either *P. cretica* or *P. vittata*. Interestingly, the smaller yield for harvest three when plants were allowed to grow over the winter period compared to plants that were transplanted in the spring, was the opposite to what was found for the Cd and Zn hyperaccumulator *T. caerulescens* (Lombi *et al.* unpublished work). *Thalspi caerulescens* plants that were allowed to grow over the winter in contaminated soil showed a large increase in biomass (5.6-fold increase) and Zn concentration (1.6-fold increase). It would appear that being an alpine plant, *T. caerulescens* is more tolerant to cold conditions than *P. cretica*.

Table 2.6: Elemental concentrations (mg kg^{-1} DM) in the fronds of *P. cretica*

		As	Cd	Cu	Zn	Fe	P	K	S
Harvest 1 (2002)	Longest fronds	2366	< 4	111	56	236	3866	29,540	1789
Harvest 2 (2002)	Longest fronds	2227	< 4	15.6	50	271	3736	29,472	1921
	Remaining biomass	1322	< 4	10.3	319	319	3900	23,119	1784
Harvest 3 (2003)	Total biomass	1548	< 4	9.6	47	249	3834	29,325	2060

Like *P. vittata, P. cretica* also showed large soil to frond bioconcentration factors, with values ranging between 3-5. Although these are lower than for *P. vittata,* the values are greater than 1, which is the lower limit for the definition of a hyperaccumulator species (McGrath *et al.*, 2002).

2.2.3.5 Plant Arsenic Uptake

The total amounts of As extracted by *P. vittata* for harvests two and three were calculated to be 2.1 and 3.9 kg ha^{-1} respectively. In comparison, the amounts of As extracted by *P. cretica* were 0.35 and 0.09 kg ha^{-1} respectively. Assuming the ferns were extracting As to a soil depth of 25 cm; (which is likely given that even deeper rooting plants extract nutrients and contaminants mainly from the topsoil, which is typically 25 cm on cultivated land) and the soil has a bulk density of 1 t m^{-3}, we can estimate the proportion of As removed from the soil by two harvests of either *P. vittata* or *P. cretica*. In this way, we calculated that *P. vittata* removed 0.51 % of the total soil As burden compared to 0.038 % for *P. cretica*. These values are substantially lower than those found by Tu *et al.* (2002), who showed that *P. vittata* extracted 26 % of the total soil As content in a pot trial experiment using a moderately As contaminated soil and 8.3 % by Cao *et al.* (2003) in another pot trial using a P-amended chromated-copper-arsenate contaminated soil.

The relatively low amount of As removed by the plants in this study can be explained by two factors; the low biomass of the fern species and the small amount of 'available' As in the study soil. The efficiency of phytoextraction is greatly affected by the size of the biomass yield and the bioconcentration factor. Given that clearly both *P. vittata* and *P. cretica* had high concentrations of As in their fronds (i.e. up to 4000 mg kg^{-1}), increasing the plant biomass will increase the amount of As extracted from the soil. That is assuming of course that increasing biomass does not result in a dilution of As concentration, which clearly was not the case in the present study. Hence, low biomass, which was only 0.5 – 1 t ha^{-1}, could perhaps most easily be improved by increasing plant density. In addition, other routine agronomic practices such as irrigation, fertilisers, frost cloths, weed and pest control may also help maximise biomass production and therefore As uptake from soils, although these factors are yet to be assessed in the field.

Regardless of the amount of biomass, and despite the high As concentrations in the fronds, the frond-to-soil As concentration ratios observed in this field trial were lower than values found in previous experiments (Cao *et al.*, 2003; Tu and Ma, 2003). The amount of total As that was in 'available' forms in our field trial soil was very low compared to most of these other reported studies, with less than 1 % of the total soil As content in the non-specifically sorbed fraction. However, several previous studies (Zhao *et al.*, 2002; Cao *et al.*, 2003; Tu and Ma, 2003) added soluble As salts to uncontaminated soils or composts, which are likely to result in a much higher proportion of As in 'available' forms as compared to aged soils which are low in bioavailable As, as was the case in this field trial. For example, Cao *et al.* (2003) showed that the proportion of As in the water soluble and exchangeable fraction was much higher in a salt amended soil (~30 %) than in a copper-chromate-arsenate contaminated soil (~5 %). Clearly, bioavailability is an important factor that needs to be taken into account when considering the using hyperaccumulators to remediate contaminated soil.

2.2.4 CONCLUSIONS

The results obtained from the field trial demonstrate that the As hyperaccumulators *P. vittata* and *P. cretica* can be grown in field in the climatic conditions of southwest England. The biomass of both species was low, with biomass generally higher for *P. vittata* compared to *P. cretica*. Both ferns efficiently transferred As from the soil to their fronds, however on average, As concentrations in *P. vittata* were twice as high as those of *P. cretica*. The findings in the present study would indicate that the time required for remediation of an As contaminated soil will depend on a number of variables including the size of the 'available' As fraction in soil, plant biomass production and performance of the hyperaccumulating plant.

2.3 AN ASSESSMENT OF ARSENIC UPTAKE BY *P. VITTATA* IN SOILS CONTAMINATED WITH DIFFERENT AMOUNTS AND SOURCES OF ARSENIC

2.3.1 INTRODUCTION

The hyperaccumulator *P. vittata,* has been shown to be able to extract large amounts of As from soils. In a pot study, Tu *et al*. (2002) showed that up to 26 % of the 50 mg As kg^{-1} added to the soil was taken up by *P. vittata*. However, the phytoremediation potential of *P. vittata* has not been tested in soils naturally contaminated with As, which are likely to have a much lower As bioavailability than soils amended with soluble As. In fact, in the same study by Tu *et al*. (2002), As uptake by *P. vittata* was much lower when soil was amended with insoluble As compounds such as $FeAsO_4$ and $AlAsO_4$. Furthermore, the presence of other metals at high concentrations in some naturally contaminated soils may affect plant growth and As uptake.

The aim of this experiment was therefore to test the efficiency of *P. vittata* to extract As from soils contaminated in the field by different amounts and forms of As, along with other heavy metals such as Cu and Zn.

2.3.2 MATERIALS AND METHODS

2.3.2.1 Soils

Five As contaminated topsoils (0-20cm) were collected from the Camborne area in Cornwall. The five soils were from Grenville, Bissoe, Roscroggan, Brea Adit and Tresevean. The As contamination was derived from both geogenic sources i.e. underlying bedrock and various anthropogenic sources i.e. atmospheric deposition from smelters and mining activity. Soil samples were analysed for basic physiochemical characteristics such as soil pH, organic matter content, texture, total metal and As content. In addition, soils also underwent chemical fractionation using a sequential extraction procedure to estimate the relative proportions of available As in the soils, as outlined in Table 2.1.

2.3.2.2 Pot Experiment

Spores of *P. vittata* were germinated on a general-purpose compost. At the 3-4 frond stage, for each of five soils, an individual sporeling was transplanted into a pot containing approximately 1 kg of soil. Each soil was replicated four times. Pots were arranged randomly on a bench inside a greenhouse with day/night temperatures of 28/20°C (16/8h), and a minimum light intensity of 350 µmol m^{-2} s^{-1}. Sodium nitrate and K_2SO_4 were added on days 7, 36, 89 and 118 to supply 125 mg N kg^{-1}, 63 mg S kg^{-1} and 156 mg K kg^{-1}, respectively. Fronds were harvested twice, at 82 and 198 days after transplanting and roots were collected at the end of the pot experiment. After sampling, fronds and roots were washed in deionised water, dried, and the dry biomass measured. Sub-samples (0.5 g) of finely ground tissue were digested with a mixture of HNO_3 and $HClO_4$ and the concentrations of As and other elements in the digest were determined using ICP-AES.

2.3.3 RESULTS AND DISCUSSION

2.3.3.1 Soils

The Grenville, Bissoe and Roscroggan soils are clay loams, the Brea Adit soil is a sandy loam and the Tresevean soil is a silty clay loam (Table 2.7). All five soils were acidic, containing no free calcium carbonate. Arsenic concentrations in the five soils ranged from 67 to 4550 mg kg^{-1}. These concentrations are substantially higher than the soil guideline value of 20 mg As kg^{-1} for the land uses of allotments or residential uses with or without plant uptake according to the Contaminated Land Exposure Assessment (CLEA) model, indicating potentially significant risk to human health and a requirement for further investigation and/or remediation (Department of Environment, Food and Rural Affairs and Environment Agency,

2002), although some allowance may be made for increased local "background" values. The Tresevean soil was not only highly contaminated with As, but also with Cu, Pb and Zn (Table 2.7), with Pb concentrations exceeding SGV's for all land uses according to the CLEA model.

Table 2.7: Physical and chemical properties of the soils used in the pot experiment

Soil	Grenville	Brea Adit	Bissoe	Roscroggan	Tresevean
pH	5.2	5.6	5.0	6.1	5.2
O.M. (%)	11.58	4.00	7.25	4.13	7.58
Particle size analysis (%)					
< 2 µm	12.2	11.9	20.5	22.9	22.7
2 µm-63 µm	46.3	32.3	48.9	44.3	68.3
63 µm-2000 µm	41.6	54.8	30.6	32.9	8.9
Total concentration (mg kg^{-1})					
As	67	357	417	475	4550
Cu	197	451	176	370	5500
Pb	61	86	229	147	3903
Zn	199	158	200	370	1242
Mn	341	482	835	1134	1221
N	4040	2600	5980	3030	3890
P	490	583	1031	891	1318
Olsen P	12	8	17	28	55

2.3.3.2   Arsenic Fractionation

Despite differences in pH, organic matter, texture and total As concentration, the distributions of As in the five sequentially extracted fractions were similar for all five soils (Figure 2.3).   Arsenic extracted during the first step (non-specifically sorbed As) was negligible. Nearly 10 % of the total As content was extracted during the second step, which corresponds to the specifically-sorbed As fraction. Most As was extracted during the third (60 %) and to a lesser extent the fourth steps (20 %) of the sequential extraction procedure. The residual fraction (F5) accounted for approximately 10 % of total As in the soils.   The results indicate that As in the five soils was adsorbed mainly to hydrous oxides of Fe and Al which is in agreement with previous studies showing a close association of As to iron oxides (Matera *et al*., 2003; McLaren *et al*., 1998), particularly amorphous iron oxyhydroxides (Bowell, 1994).

Copper, Pb and Zn concentrations were also measured in the first step of the sequential extraction procedure, which corresponds to the exchangeable metal fraction, which is the most readily available to plants (Table 2.8). Large amounts of Cu and Zn were extracted particularly from the Tresevean soil, with much less extracted from the other four soils, while extractable Pb concentrations were small in all soils.
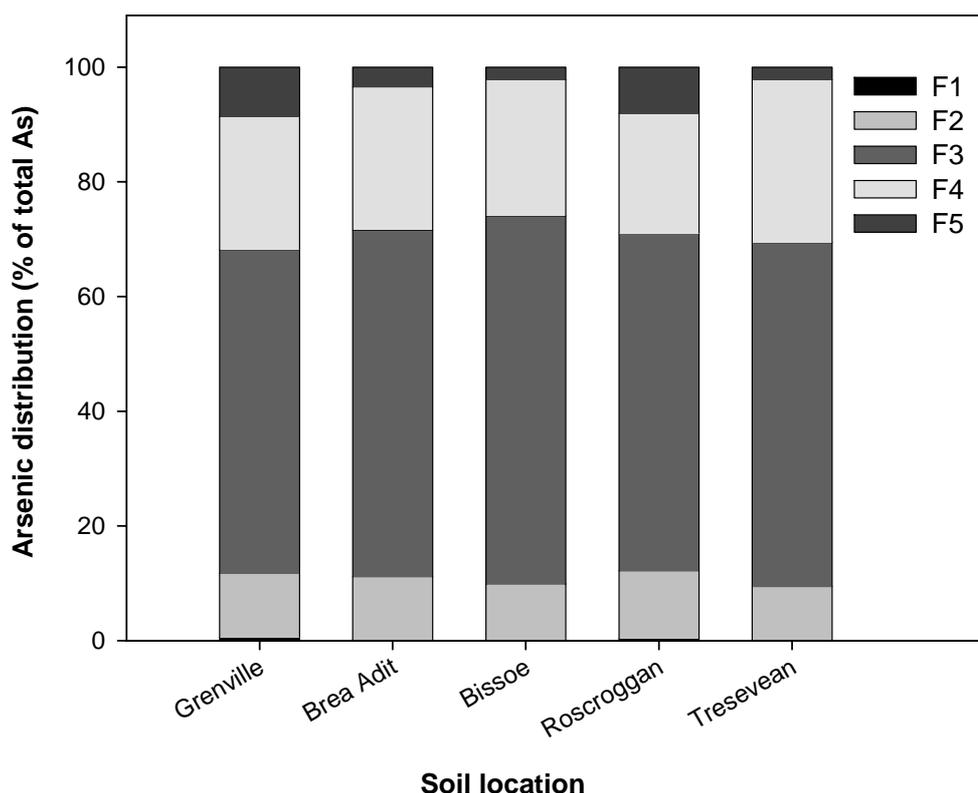
Figure 2.3: Arsenic distribution (% of total As) in the Grenville, Brea Adit, Bissoe, Roscroggan and Tresevean soils using the sequential extraction

Table 2.8: Copper, Pb and Zn concentrations (mg kg^{-1}) extracted by the first step of the sequential extraction procedure in the five soils used in the pot experiment

Extractable metal (mg kg^{-1})	Soil				
	Grenville	Brea Adit	Bissoe	Roscroggan	Tresevean
Cu	7.7 ± 3.0	1.3 ± 0.5	2.5 ± 0.8	2.9 ± 1.3	346 ± 55
Pb	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3	0.0 ± 0.0	3.1 ± 1.5
Zn	4.7 ± 1.5	6.3 ± 1.6	15.6 ± 1.7	2.7 ± 1.4	238 ± 8

2.3.3.3   Plant Biomass Yield

Pteris vittata grew well on the Bissoe, Brea Adit and Grenville soils, less well on the Roscroggan soil and very poorly on the soil from Tresevean. Chlorosis and necrosis were apparent on the fronds of *P. vittata* grown on the Tresevean soil, which produced a small mass of fronds and no roots could be collected at the end of the experiment (Table 2.9). The dry weights of *P. vittata* fronds were similar for the two harvests on the Bissoe and Brea Adit soils, but were much higher in the second than the first harvest on the Grenville and Roscroggan soils (Table 2.9).

Table 2.9: Dry biomass yields (g DM pot^{-1}) and metal concentrations (mg kg^{-1}) in the fronds and the roots of *P. vittata*

		Soils				
		Brea Adit	**Roscroggan**	**Bissoe**	**Grenville**	**Tresevean**
Biomass	Fronds 1st h.	1.8b	0.3c	4.0a	1.2b	0.2c
	Fronds 2nd h.	2.2bc	1.9c	4.1a	2.9b	0.1d
	roots	3.4ab	2.9bc	4.3a	2.2c	-
As	Fronds 1st h.	3600a	3510a	575b	269b	362b
	Fronds 2nd h.	2044a	1797b	389c	84d	182cd
	roots	307a	285a	79b	33c	-
Cu	Fronds 1st h.	7.1b	7.0b	9.8b	8.6b	62a
	Fronds 2nd h.	4.6b	5.1b	7.3b	6.4b	81a
	roots	461a	238b	102c	88c	-
Pb	Fronds 1st h.	<3	<3	<3	<3	<3
	Fronds 2nd h.	<3	<3	<3	<3	<3
	roots	43a	47a	17b	48a	-
Zn	Fronds 1st h.	35b	28b	69b	28b	908a
	Fronds 2nd h.	38b	18b	57b	31b	6093a
	roots	167a	207a	177a	90b	-

(Figures followed by different letters in the same rows are statistically significantly different from each other)

2.3.3.4 Arsenic Concentrations

Arsenic concentration in the fronds of *P. vittata* ranged from 84 to 3600 mg kg^{-1} DM (Table 2.9). The variation in frond As concentration was not correlated with total soil As. The bioconcentration factor was 0.04-0.08 for the most contaminated Tresevean soil, but much higher (0.9-10) for the other four soils, with the highest bioconcentration factor measured in the plants growing on the Brea Adit soil in the first harvest (~10). It is notable that the As concentration in the roots of *P. vittata* was 2.5- to 12.3-fold less than the As concentration in the fronds, and this is one of the typical traits of hyperaccumulator plants (McGrath *et al.*, 2002).

2.3.3.5 Plant Arsenic Uptake

The amount of As removed by two harvests of *P. vittata* ranged from 0.9 to 3.1 % of the total content of As in the Grenville, Brea Adit, Bissoe and Roscroggan soils. In contrast, a negligible amount of As was taken up from the Tresevean soil, accounting for only 0.002 % of the total soil As content. The amount of As removed by two harvests of *P. vittata* was 2.7-14 fold greater than the amount of As present in the F1 fraction (non-specifically sorbed As) in the Brea Adit, Bissoe and Roscroggan soils, but was similar to the F1 fraction in the Grenville soil. In these four soils, frond As uptake accounted for 5.3 – 26.8 % of the As present in the F1 and F2 fractions combined (non-specifically and specifically sorbed As). In the highly contaminated Tresevean soil, frond As uptake represented only 1.8 % and 0.02 % of the As present in F1 alone and in F1 and F2 combined, respectively.

Copper concentrations in the fronds of *P. vittata* were <10 mg kg^{-1} for the plants growing on the Grenville, Brea Adit, Bissoe and Roscroggan soils, but were much higher on the Tresevean soil (60-80 mg kg^{-1}). Similarly, Zn concentrations in the fronds were much higher on the Tresevean soil (908-6093 mg kg^{-1}) than in plants growing on other four soils (<70 mg kg^{-1}). For all soils, Pb concentrations in the fronds were less than 3 mg kg^{-1}. In contrast to what was observed for As, concentrations of Cu, Zn and Pb were substantially higher in the roots than in the fronds of *P. vittata* (Table 2.9).

The results confirm that *P. vittata* can hyperaccumulate As from soils low in 'bioavailable As'. The bioaccumulation factors ranged from 0.04 to 10, which are similar to values found for *P. vittata* in the field trial. However, like the results in the field trial, bioaccumulation values are lower than those previously reported (Zhao *et al.*, 2002; Cao *et al.*, 2003; Tu and Ma, 2003).

The disparity can be explained by two factors. Firstly as already discussed in the previous section, the source of the As can affect the availability of As in the soil. The As in the soils used in this study was from naturally contaminated and aged sites with the proportion non-specifically sorbed As, accounting for less than 1 % of the total As in all five soils. This compares to other studies where As has been added to soil in the form of soluble salts which is clearly more 'available' for plant uptake. Secondly, co-contamination may affect plant growth and As uptake: *Pteris vittata* grew poorly on the Tresevean soil, probably as a result of Zn and Cu toxicity. The concentrations of Zn and Cu in the plants from the Tresevean soil were well above the toxicity thresholds of 100-500 mg Zn kg^{-1} and 20 mg Cu kg^{-1}, respectively, reported for a wide range of plant species (Kabata-Pendias and Pendias, 1992). Phytotoxicity of Zn and Cu not only decreased plant growth, but also As uptake, resulting in a negligible phytoextraction of As from the Tresevean soil. It is clear that contamination of soils with multiple metals or metalloids, particularly at high concentrations, presents a difficult challenge for successful phytoremediation.

2.3.4   CONCLUSIONS

Pteris vittata was able to hyperaccumulate As from naturally contaminated soils. However, the bioaccumulation factor varied between soils with a bioaccumulation factor > 10 met in only one out of the five soils tested. Co-contamination of Zn and Cu at high concentrations greatly affected plant growth and As uptake. The percentage of soil As removed by *P. vittata* ranged from 0.9 to 3.1 %.

2.4 MEASUREMENT OF THE RATE OF UPTAKE AND TOLERANCE TO ARSENIC OF *P. VITTATA* AND *P. CRETICA*

2.4.1 INTRODUCTION

Interestingly, not all members of the Pteris genus are As hyperaccumulators. Meharg (2003) for example reported that *P. dentata* (also known as *P. straminea*) and *P. tremula* did not accumulate As in their fronds. It is not known whether this is due to a lack of internal tolerance, a lower rate of As uptake, or low root to frond transport of As compared to known hyperaccumulators. These differences in As uptake and tolerance within the Pteris genus are not only important in terms of the evolution of As hyperaccumulators, but may also provide an insight into the mechanisms of As uptake at physiological and molecular levels.

Hence, the aim of this experiment was to investigate the mechanisms of As hyperaccumulation by comparing As uptake and tolerance in the As hyperaccumulator *P. vittata* and the non-As hyperaccumulator *P. tremula*.

2.4.2 MATERIALS AND METHODS

2.4.2.1 Kinetics of Arsenic Uptake

The kinetics of As influx were studied using a solution depletion experiment for *P. vittata* and *P. tremula*. Seedlings of *P. vittata* and *P. tremula* were pre-cultured hydroponically for three weeks in a modified Hoagland nutrient solution. The roots of intact plants were then removed and rinsed with deionised water and transferred into a vessel that contained a pre-treatment solution of 0.5 mM $CaCl_2$ and 5 mM 2-morpholinoethanesulphonic acid (MES) with the pH maintained at 6.0. After 12 h, the pre-treatment solution was replaced with an uptake solution that contained 5 µM arsenate (Na_2HAsO_4), together with 0.5 mM $CaCl_2$ and 5 mM MES adjusted to pH 6, that was continuously aerated. After 0, 15, and 30 mins and then at 30 min intervals thereafter up to 8 h, a 0.3 mL aliquot of uptake solution was removed for the determination of As and replaced with 0.3 mL of deionised water. Water losses through transpiration were compensated by the addition of deionised water at hourly intervals and the temperature of the solution was maintained at 25ºC ± 0.5ºC throughout the experiment. After 8 h, roots and shoots were separated, rinsed with deionised water, dried and dry weights recorded. The concentration of As in the uptake solution was determined by hydride generation atomic absorption spectrometer (HGAAS). Root and shoots were digested in a mixture of HNO_3 and $HClO_4$ and As in digests measured by ICP-AES.

2.4.2.2 Arsenic Tolerance

A pot experiment was set up to evaluate the effect of increasing soil As concentrations on the tolerance of As in *P. vittata* and *P. tremula*. Spores of *P. vittata* and *P. tremula* were germinated on general-purpose compost. At the 3-4 frond stage, one sporeling was transferred into each pot each containing 1 kg of air-dried compost. The compost had previously been amended with either 0, 12.5, 25, 50, 100, 250 and 500 mg As kg^{-1} as Na_2HAsO_4. Each treatment was replicated eight times i.e. four pots for growing plants and the other four pots without plants for extracting pore water using soil moisture samplers (Knight *et al.*, 1998) for the determination of soil solution As concentration. Pots were arranged randomly on a bench inside a greenhouse with day/night temperatures of 28/20°C (16/8h), and a minimum light intensity of 350 µmol m^{-2} s^{-1}. Fronds were harvested after 35 days of growth from all treatments while roots were collected from the 12.5 and 25 mg As kg^{-1} treatments only. Roots and shoots were rinsed with deionised water, dried and the dry weights recorded. Root and shoots were digested in a mixture of HNO_3 and $HClO_4$ and As in digests measured by ICP-AES.

2.4.3 RESULTS AND DISCUSSION

2.4.3.1 Kinetics of Arsenic Uptake

The depletion of As in the uptake solution was monitored over 8 h, and reflects the uptake of As by plant roots. Average root fresh weights were comparable in *P. tremula* (3.68 ± 0.51 g) and *P. vittata* (3.73 ± 0.49 g), whereas frond fresh weight was higher for *P. tremula* (4.2 ± 1.1 g) than for *P. vittata* (2.8 ± 0.5 g). The concentration of As in the uptake solution decreased for both *P. vittata* (5 to 2.2 µM) and *P. tremula* (5 to 3.9 µM) (Figure 2.4).
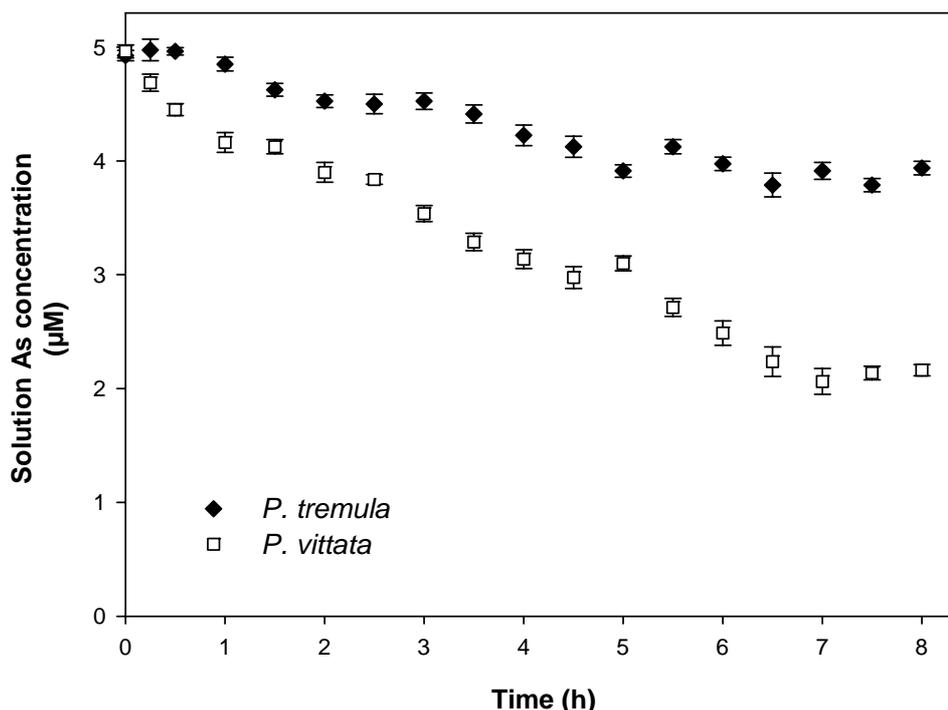
Figure 2.4: Depletion curves of arsenate (µM) in the uptake solution by *P. vittata* and *P. tremula.* The results are means ± standard errors (n = 8).

Cumulative As uptake was approximately linear in the first 7 h for both *P. vittata* and *P. tremula* (Figure 2.5). This decrease in As concentration in solution as a function of time was used to calculate the relative cumulative uptake of As for both species. The rate of As uptake from solution was twice as fast for *P. vittata* (36.2 nmol g^{-1} root FW h^{-1}) compared to *P. tremula* (16.4 nmol g^{-1} root FW h^{-1}). The large difference in cumulative uptake of As between species indicates differences in their maximum influx velocities, which implies a higher density of transporters for As on the plasma membranes of root cells in *P. vittata* compared to *P. tremula*. Similar results have been found for the Zn hyperaccumulator *T. caerulescens*, which had a 4.5-fold higher maximum influx velocity than the non-hyperacumulator *T. arvense* (Lasat *et al.*, 1996).
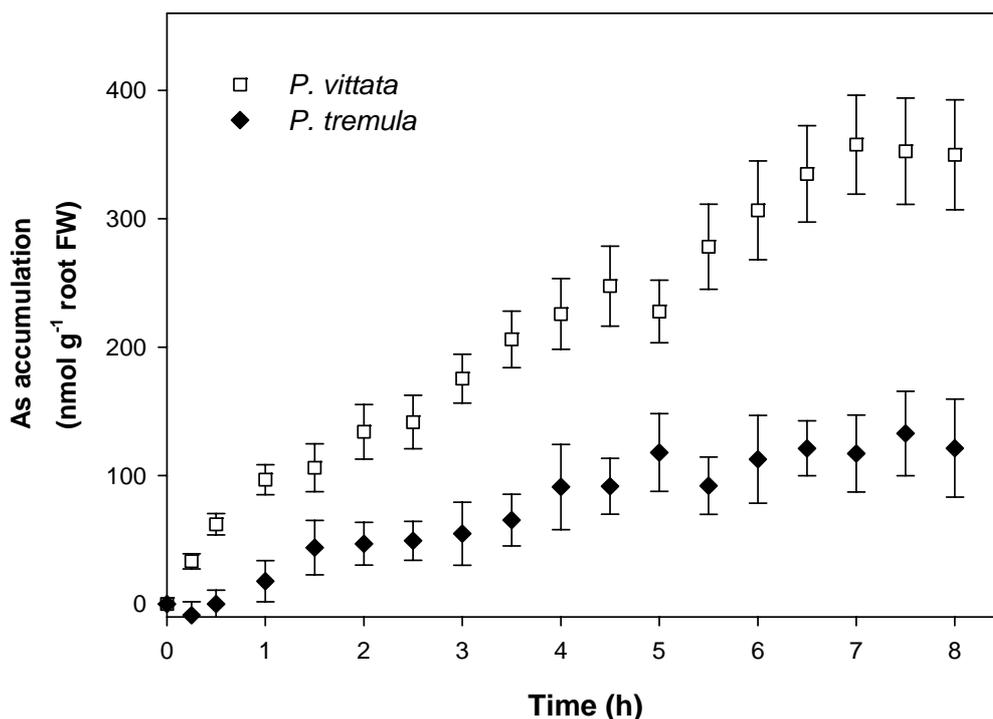
Figure 2.5: Cumulative uptake of arsenate by *P. vittata* and *P. tremula*, expressed on a fresh root weight basis. The results are means $\pm$ standard errors (n = 8).

Following the 8 h uptake experiment, As concentrations were determined in the roots and fronds of both species. There was no difference in the As concentration in the roots and fronds of *P. vittata* (Figure 2.6). In contrast, As concentrations in the fronds of *P. tremula* were only 3 % of that in the roots. On average, 76 % of the total As taken up by *P. vittata* was translocated to the fronds, compared to only 9 % for *P. tremula*. This indicates that not only was *P. vittata* very effective in absorbing As from solution, compared to *P. tremula* it was also very efficient in transporting As from the root to its fronds. The efficient root to shoot translocation of As is a typical characteristic of hyperaccumulators.
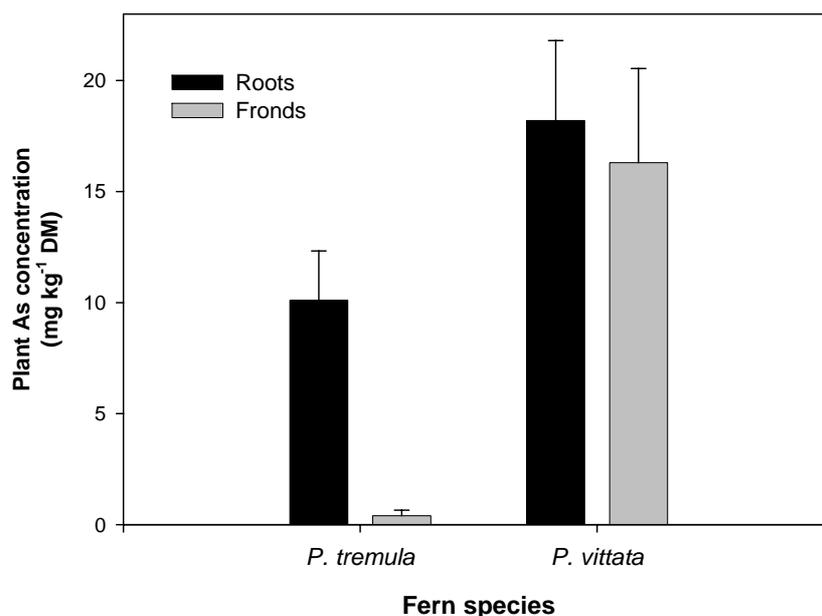
Figure 2.6: Arsenic concentrations (mg kg^{-1} DM) in the roots and fronds of *P. tremula* and *P. vittata* and after 8 h. The results are means $\pm$ standard errors (n = 8)

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2.4.3.2   Arsenic Tolerance Pot Experiment

2.4.3.2.1   Pore water

Pore water As concentrations increased with the amount of As added to the compost, ranging from 11 µM at day 35 of the incubation in the 12.5 mg kg^{-1} treatment, up to 126 µM at day 8 of the incubation in the 500 mg kg^{-1} treatment (Figure 2.7). In addition, As concentrations in solution decreased linearly with increasing incubation time.
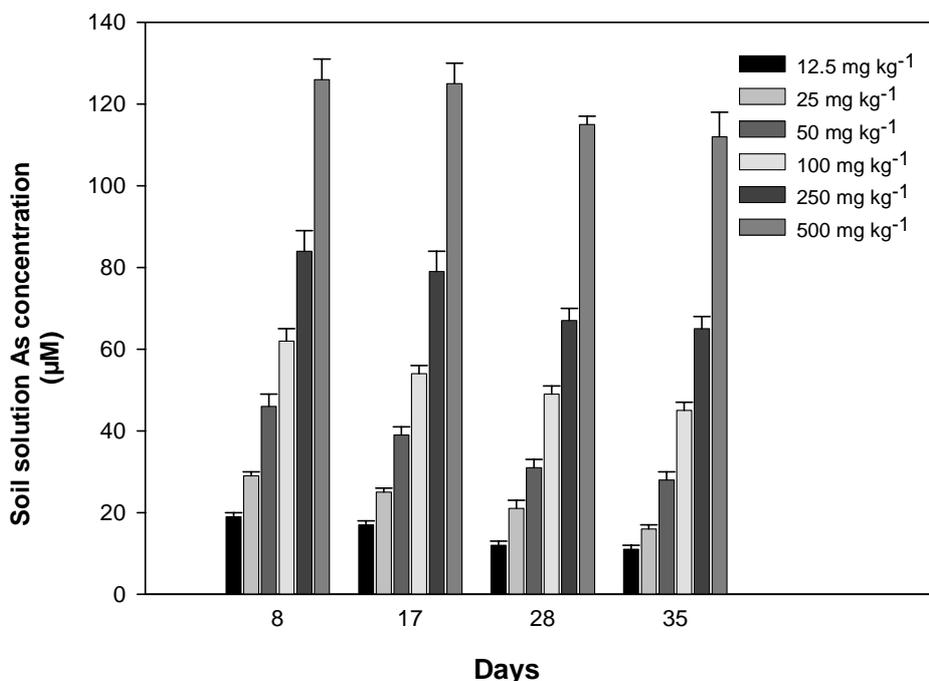
Figure 2.7: Arsenic concentration (µM) in the pore waters extracted from the unplanted pots treated with different concentrations of arsenate. The results are means ± standard errors (n = 4).

2.4.3.2.2   Plant biomass yield

In the control treatment, *P. tremula* produced 73 % more biomass than *P. vittata* (Figure 2.8), and the fronds were generally green and healthy. However with increasing compost As concentrations, the biomass of *P. tremula* decreased from 10.3 g kg^{-1} in the control treatment, to 3.6 g kg^{-1} in the 100 mg kg^{-1} treatment. Furthermore, phytotoxicity symptoms such as chlorosis and necrosis were observed at 100 mg As kg^{-1} and total mortality occurred 250 mg As kg^{-1}. In contrast, concentrations of As in the compost up to 500 mg As kg^{-1} had no significant effect on *P. vittata* biomass which showed healthy fronds and no phytotoxicity symptoms.
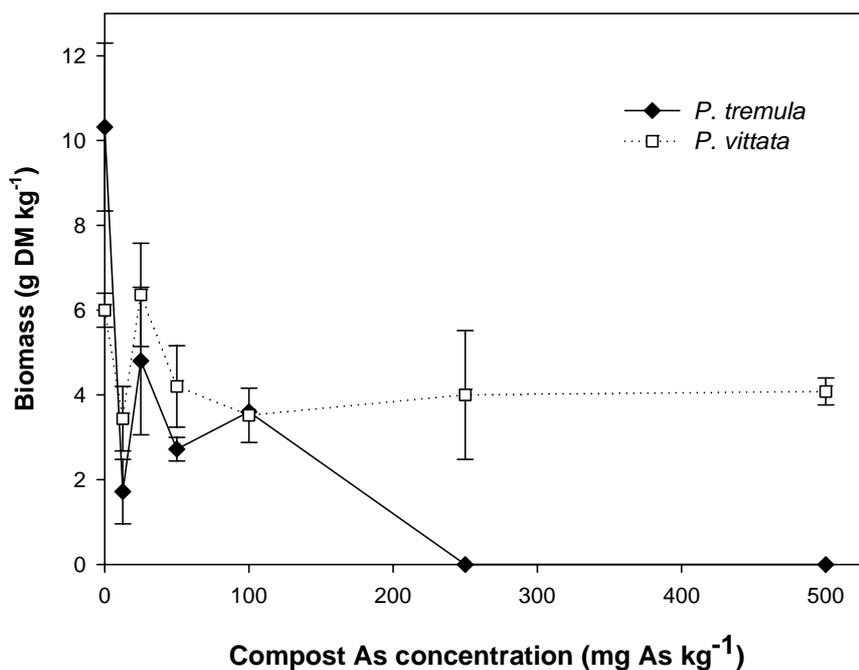
Figure 2.8: The effect of compost As concentration on biomass yield in *P. tremula* and *P. vittata*. The results are means ± standard errors (n = 4)

2.4.3.2.3   Plant Arsenic concentrations

Arsenic concentrations in the fronds of *P. tremula* averaged 60 mg kg^{-1} in treatments up to 100 mg kg^{-1} (Figure 2.9). Furthermore, the bioconcentration ratio decreased from 4.3 in the 12.5 mg kg^{-1} treatment to 0.9 in the 100 mg kg^{-1} treatment. In contrast, As concentration in *P. vittata* fronds increased linearly with increasing As concentrations, reaching 2500 mg kg^{-1} in the 500 mg kg^{-1} treatment, with the bioconcentration ratio for *P. vittata* ranging from 5 to 10.
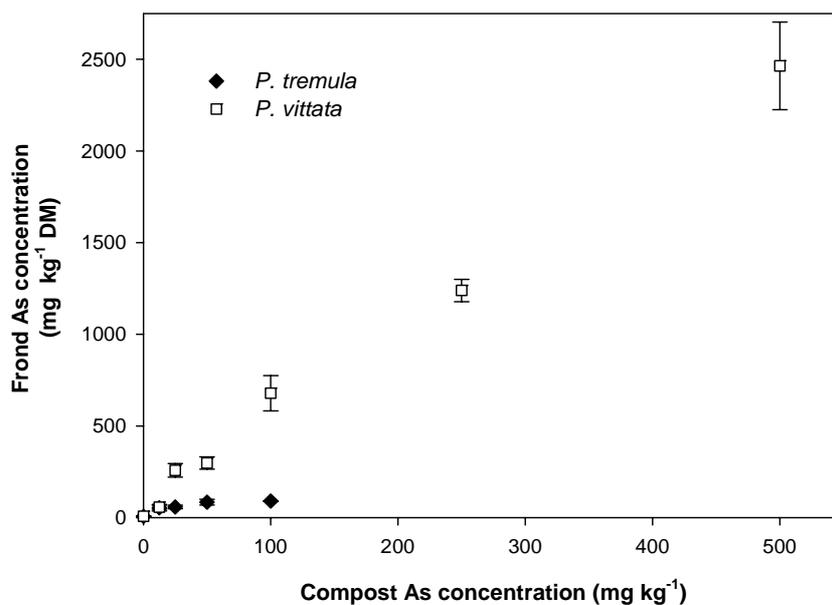
Figure 2.9: Effect of As additions on the concentrations of As in the fronds of *P. vittata* and *P. tremula* in the pot experiment. The results are means ± standard errors (n = 4, except that only one *P. tremula* plant survived in the 100 mg As kg^{-1} treatment)

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These findings indicate that not only can *P. vittata* accumulate significantly greater amounts of As than *P. tremula*, it also has a markedly greater tolerance to As, showing no signs of toxicity or decreased biomass with increasing compost As concentrations up to 500 mg kg^{-1}. It is this tolerance which may allow *P. vittata* to grow and thrive on As contaminated soils and hyperaccumulate As compared to non-accumulators. In contrast, *P. tremula* had a toxicity threshold of < 100 mg kg^{-1}, which clearly would prevent it from growing in even moderately As contaminated soils.

2.4.4 CONCLUSIONS

In the As depletion experiment, it was shown that *P. vittata* took up As twice as fast as the non-hyperaccumulator *P. tremula*. In addition, *P. vittata* was more efficient at transporting As from roots to fronds than *P. tremula*. In the pot experiment, it was found that *P. vittata* was more tolerant of compost As concentrations than *P. tremula*. Compost As concentrations up to 500 mg kg^{-1} had no effect on biomass of *P. vittata* compared to *P. tremula*, which did not survive beyond an As concentration of 100 mg kg^{-1}. The As hyperaccumulator *P. vittata* possesses three key traits which are characteristic of all hyperaccumulators: efficient root uptake, efficient root to shoot translocation and hyper-tolerance to As concentrations.

2.5 AN EVALUATION OF THE EFFECT OF SOIL AMENDMENTS ON ARSENIC UPTAKE IN *P. VITTATA*

2.5.1 INTRODUCTION

Effective phytoextraction of As from soils requires adequate As concentrations to be maintained in soil solution throughout the period of plant growth. The concentration or activity of As in soil solution is strongly controlled by sorption-desorption processes in soils. In turn, these processes are affected by the amounts and types of sorption components in soils such as hydroxides of Fe and Al, Mn oxides, clay minerals and soil properties such as pH, redox potential and the presence of competing oxyanions such as phosphate. Therefore, any soil property or soil amendment which affects sorption-desorption of As is likely to influence soil solution As concentrations, and hence the efficiency of As phytoextraction in soils.

Two soil factors that may have an effect on As availability in soils are soil pH and the addition of phosphate. Arsenic sorption onto soils or soil constituents, such as Al and Fe hydroxides and clay minerals, is strongly influenced by pH, with sorption of arsenate generally decreasing with increasing pH (Xu *et al.*, 1998; Smith *et al.*, 1999). Therefore, increasing pH by liming could increase the availability of As and consequently enhance As uptake by plants.

Another important factor affecting As uptake is the interaction between phosphate and arsenate in terms of their competition for sorption sites in soil and absorption by roots. Arsenate and phosphate are chemical analogues. The addition of phosphate to soils has been shown to result in an increase in desorption of As from the soil solid phase into soil solution (Peryea, 1991). However, it has also been shown that *P. vittata* takes up arsenate via the phosphate transport system (Wang *et al.*, 2002). Therefore, increased phosphate concentration in soil solution may be expected to decrease arsenate uptake. Wang *et al.* (2002) and Tu and Ma, (2003) both showed in hydroponic experiments that increasing phosphate supply markedly decreased As uptake in plants. It is not clear, however, whether the addition of phosphate fertiliser to soil will result in an increase in As uptake by *P. vittata* from naturally As contaminated soils.

The aim of this experiment is to determine whether the addition of lime and phosphate could increase the uptake of As by *P. vittata* grown on an As contaminated soil.

2.5.2 MATERIALS AND METHODS

2.5.2.1 Soil

An arsenic contaminated topsoil (0 - 20 cm) was sampled from Rosewarne, near Camborne. Details of the soil are given in Table 2.2. Soil was air-dried and sieved to 2 mm prior to analysis.

2.5.2.2 Experimental Design

Four treatments were used in a pot experiment: (i) control; (ii) addition of lime; (iii) addition of phosphate; and (iv) addition of lime and phosphate. Lime was added as $CaCO_3$ at a rate of 4.6 g kg^{-1} soil, which equates to 12 t ha^{-1}. Phosphate was added as NaH_2PO_4 at a rate of 50 mg P kg^{-1} soil, equivalent to an application of 130 kg P ha^{-1}, which is 3-4 fold higher than common agricultural practice. Lime and phosphate were added to the soil one week before transplanting. One *P. vittata* plant (12-16 fronds) was transplanted into each pot and each treatment was replicated four times. In addition, for comparison, another fern species, *P. cretica* was planted only in the control treatment. Pots were randomly arranged in a glasshouse with day/night temperatures of 28/20°C (16/8h), and a minimum light intensity of 350 µmol m^{-2} s^{-1} and plants were watered with deionised water when necessary. Ammonium nitrate fertiliser was added to give 125 mg N kg^{-1} soil on days 8, 43 and 100. The

same amount of N, with potassium and sulphur (as K_2SO_4) to supply 63 mg S kg^{-1} and 156 mg K kg^{-1}, were added on days 110, 160 and 190. Four harvests were made during the course of the pot experiment, at 48, 82, 185 and 343 days after transplanting. Fronds were cut at approximately 1 cm above the soil surface, and at the fourth harvest, plant roots were also sampled. After harvesting, fronds and roots were washed in tap water and rinsed in deionised water, dried and dry weights recorded. Plant samples were ground to <0.5 mm, and digested with a mixture of HNO_3 and $HClO_4$ and the concentrations of As and other metals in the digests were determined by ICP-AES.

2.5.2.3 Incubation

A separate incubation experiment was set up in parallel to investigate the effects of phosphate and lime additions on the solubility of As in the soil. Phosphate and lime were added to 100 g soil separately to mimic the treatments described above. The moisture content was raised to 100 % of water holding capacity, and the soil was kept inside a centrifugation tube and left to equilibrate for one week in the dark at room temperature before extraction. Soil solution was extracted by centrifuging soil at 3000 *g* for 10 mins and solutions filtered through a 0.45µm filter membrane. The concentrations of As in pore water samples were determined using HGAAS.

2.5.3 RESULTS AND DISCUSSION

2.5.3.1 Soil

The soil used in the pot experiment had a total As content of 361 mg kg^{-1} and a solution concentration of 1.6 ± 0.2 µM. After one week of reaction, the addition of phosphate (50 mg P kg^{-1} soil) increased the As concentration in the soil pore water to 2.6 ± 0.1 µM, whereas the addition of lime ($CaCO_3$) decreased it to 0.72 ± 0.08 µM, possibly as a result of the formation of $Ca_3(AsO_4)_2$. The pore water pH of the control soil was 6.57 ± 0.08 and this decreased slightly to 6.27 ± 0.02 after the addition of phosphate, but increased to 7.36 ± 0.06 after liming.

2.5.3.2 Plant Biomass Yield

Pteris cretica produced smaller yields than *P. vittata* (Figure 2.10), which is consistent with results found in the field trial comparing these two fern species. Yields for both *P. vittata* and *P. cretica* were significantly higher in the first harvest compared to subsequent harvests, with the fourth harvest producing the smallest yields. The large biomass in the first harvest is likely related in part to the fact that plants were pre-grown before transplanting. In addition it may be that frequent cutting of fronds could have decreased the vigour of the rhizomes to re-grow, resulting in a decrease in frond biomass with cutting. The application of either lime or phosphate and the combination of both amendments had no significant effect on yield. Plant root yields for both species, although more so for *P. vittata*, were found to comprise a significant proportion of the total biomass in both species.
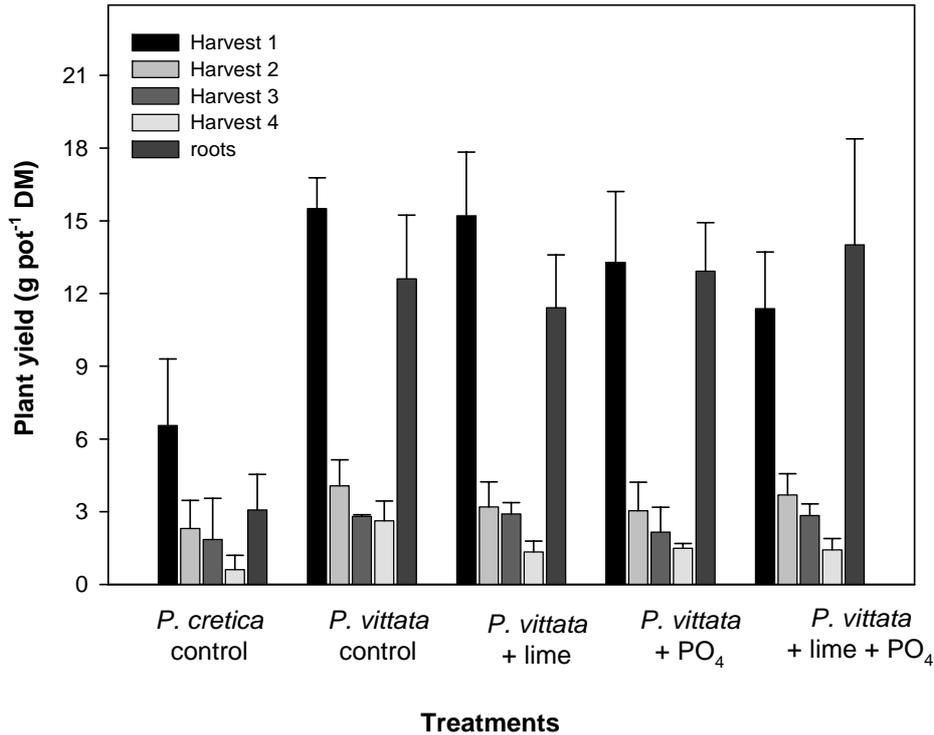
Figure 2.10: Average dry weight yields (g pot^{-1} DM) of *P. cretica* and *P. vittata* in four successive harvests. The results are means ± standard errors (n = 4)

2.5.3.3 Plant Arsenic Concentrations

Arsenic concentrations in the fronds and roots of *P. cretica* and *P. vittata* are given in Figure 2.11. Concentrations in the fronds of *P. vittata* ranged from 246 (±61) to 1675 (±233) mg kg^{-1} and between 219 (±117) to 973 (±256) mg kg^{-1} for *P. cretica*. By comparison, As concentrations in the roots were lower than frond concentrations with values for *P. vittata* ranging from 178 (±47) to 269 (±51) mg kg^{-1} and 171 (±35) for *P. cretica*. Arsenic concentrations also varied between species, with on average across all four harvests, concentrations 1.5 times higher in *P. vittata* than *P. cretica.* Similar to biomass yield, the differences in As concentrations between species are consistent with the results found in the field trial when comparing these two ferns. There were also differences in As concentrations between harvests. Arsenic concentrations were significantly lower in harvest one compared to the subsequent harvests. This may reflect the significantly higher biomass of harvest one, but more likely the fact that the plants were pre-cultured in an As free compost medium prior to transplanting into pots. There were no significant differences in As concentrations between treatments.

Average bioconcentration factors for *P. vittata* ranged between 0.9 for harvest one to 4.5 for harvest three, while for *P. cretica* factors were 0.6 to 2.7 respectively. Surprisingly these values are lower than those found in the field experiment, where values up to 9 were recorded. Given that the plants only had a small amount of soil in which to grow, this may have contributed to the relatively low As uptake.
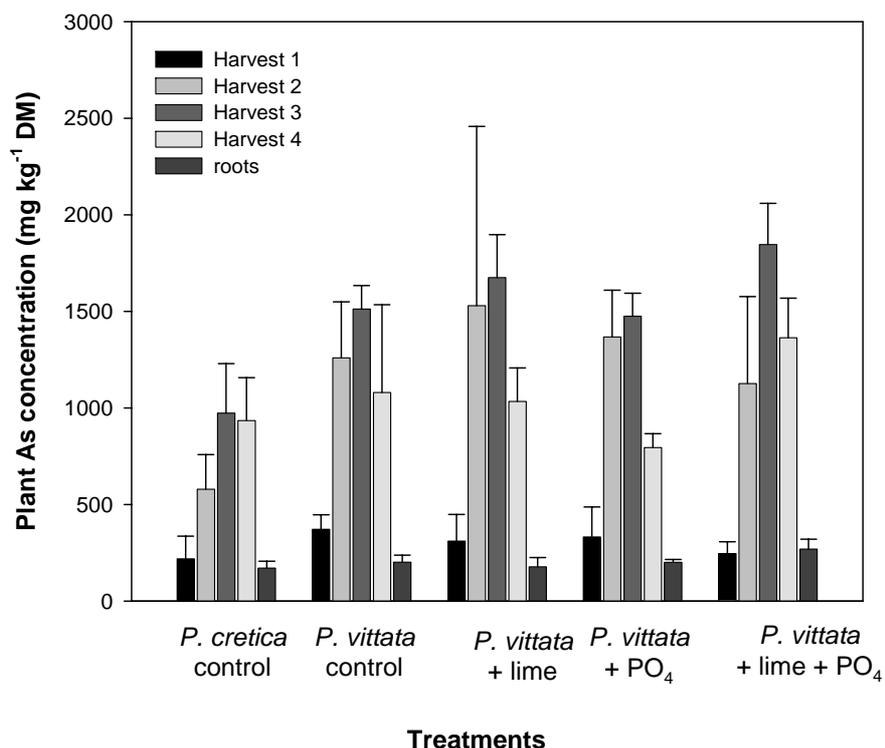
Figure 2.11: Arsenic concentrations (mg kg^{-1} DM) in fronds and roots for *P. cretica* and *P. vittata* in four successive harvests. The results are means ± standard errors (n = 4)

2.5.3.4   Plant Arsenic Uptake

Arsenic uptake for *P. cretica* and *P. vittata* for each harvest in addition to total As uptake are given Figure 2.12. The amount of As extracted by *P. vittata* in the four harvests ranged between 15 to 21 mg As pot^{-1}, accounting for 4-7 % of soil total As content. Again, phosphate and lime treatments had no significant effect on the amount of As taken up by *P. vittata*. The total mass of As extracted by *P. cretica* was lower, 6 mg pot^{-1}, accounting for 1.9 % of the total As content in the soil.

Interestingly, neither liming nor addition of phosphate increased the removal of As by *P. vittata,* this is despite an increase in the As concentration in the soil pore water after phosphate addition. An increase of arsenate in soil pore water following the addition of phosphate was expected, because phosphate can desorb sorbed arsenate from the soil solid phase into soil solution (Peryea, 1998). However, because phosphate also competes with arsenate for the same transport systems in the roots of *P. vittata* (Wang *et al.*, 2002), addition of phosphate to soil did not lead to a higher uptake of As. These findings contrast with those of Cao *et al.* (2003), who showed that As concentration in the fronds of *P. vittata* was increased by 265 % when 15 g kg^{-1} phosphate rock was added to a chromated-copper-arsenate contaminated sandy soil. The rate of P added in the present study was significantly lower, although already 3-4 fold higher than commonly used in agriculture and in a water soluble form. Also, the soil used by Cao *et al.* (2003) had a much higher proportion of plant available As than the soil used in the present study, in which non-specifically sorbed As accounted for less than 1 % of the total As content.
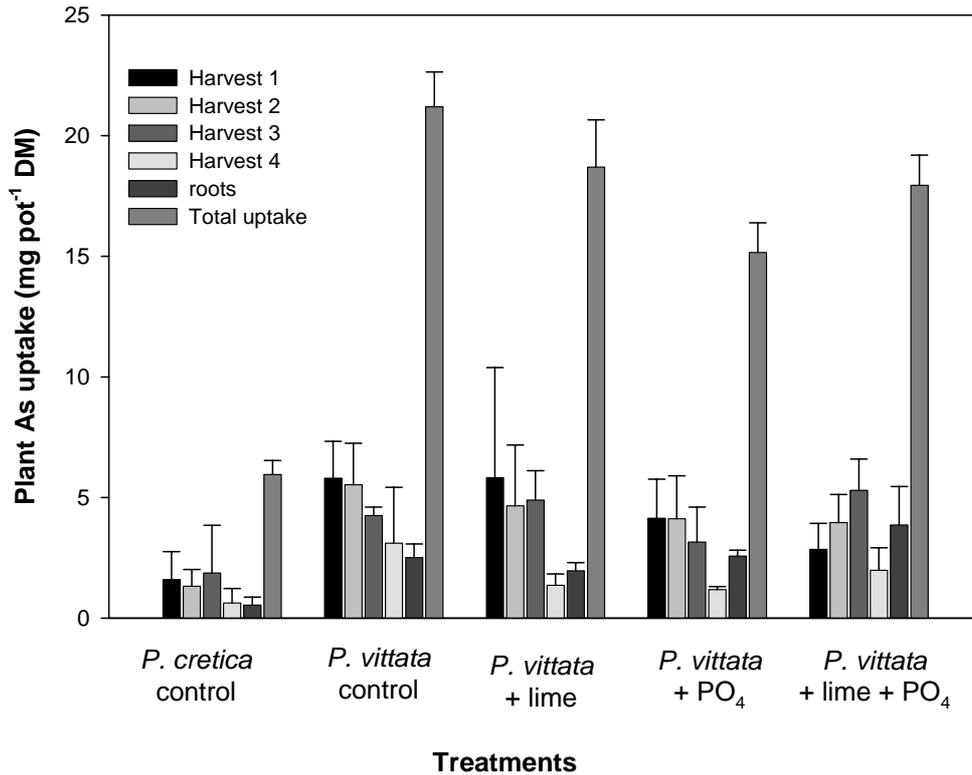
Figure 2.12: Arsenic uptake (mg pot^{-1} DM) in fronds and roots for *P. cretica* and *P. vittata* in four successive harvests. The results are means $\pm$ standard errors (n = 4)

2.5.4   CONCLUSIONS

On the soil used with pH 6.2, the application of either lime or phosphate and the combination of both amendments had no significant effect on yield. The percentage of soil As removed by *P. vittata* ranged from 4 to 7 %. Liming or the addition of phosphate at a rate of 50 mg P kg^{-1} soil had no significant effect on As uptake by *P. vittata*. The effect of P on increasing soluble As concentration in soil pore water was probably reduced by the competition between P and As during the root uptake processes.

2.6 INVESTIGATION OF THE RESPONSE OF *P. VITTATA* ROOTS TO HOT SPOTS OF ARSENIC IN SOILS

2.6.1 INTRODUCTION

Two recent studies (Schwartz *et al.*, 1999; Whiting *et al.*, 2000) provided insight into the rooting pattern of the Zn and Cd hyperaccumulator *T. caerulescens*. These studies showed that the roots of this species predominantly colonised Zn or Cd rich zones embedded within an uncontaminated agricultural soil. These results suggested that the roots of *T. caerulescens* were able to sense and actively forage in Zn or Cd-rich patches in soil. Because of the usual micro-heterogeneity of metal concentrations in soil, it has been hypothesised that the positive response of rooting pattern of this hyperaccumulator may be one important reason for its hyperaccumulation ability compared to non-hyperaccumulators. However, there is currently no information available on the rooting pattern for the As hyperaccumulator *P.vittata*.

The aim of this experiment was therefore to investigate the root distribution of *P. vittata* in the presence of As patches in soil.

2.6.2 MATERIALS AND METHODS

2.6.2.1 Soil

A silty clay loam soil with a low available P status was sampled from Rothamsted Experimental Station.

2.6.2.2 Pot Experiment Design

Three treatments were used in the pot experiment; (a) control (b) heterogeneous As and (c) homogeneous As. One kg pots were divided into quarters using plastic partitions (Figure 2.13). In the control treatment, equal proportions of un-amended soil were placed into each quarter. In the heterogeneous As treatment, soil amended with the equivalent of either or 900 or 100 mg As kg^{-1} was placed into opposite quarters while un-amended soil were placed in the remaining quarters. In the homogenous As treatment, soil amended with the equivalent of 250 mg As kg^{-1} (Na_2HAsO_4) was placed into each quarter. Following the application of the different amendments, the plastic partition was removed and one *P. vittata* sporeling was transplanted into the centre of each pot at the intersection of the amended soil. Treatments were replicated six times. Potassium, nitrogen (N) and sulphur (S) fertiliser was added at a rate of 126, 100, and 100 mg kg^{-1} to each pot at the beginning of the trial. Pots were arranged randomly in a glasshouse with day/night temperatures of 28/20°C (16/8h), and a minimum light intensity of 350 µmol m^{-2} s^{-1} and plants watered with deionised water when necessary. Ferns were grown for 63 days after which time fronds were cut at approximately one cm above the soil surface and root distributions and average root diameters were determined separately for each quarter of each treatment. After harvesting, fronds and roots were rinsed in deionised water, dried and dry weights recorded. Plant samples were ground to <0.5 mm, and digested with a mixture of HNO_3 and $HClO_4$ and As concentrations in the digest determined by HGAAS.
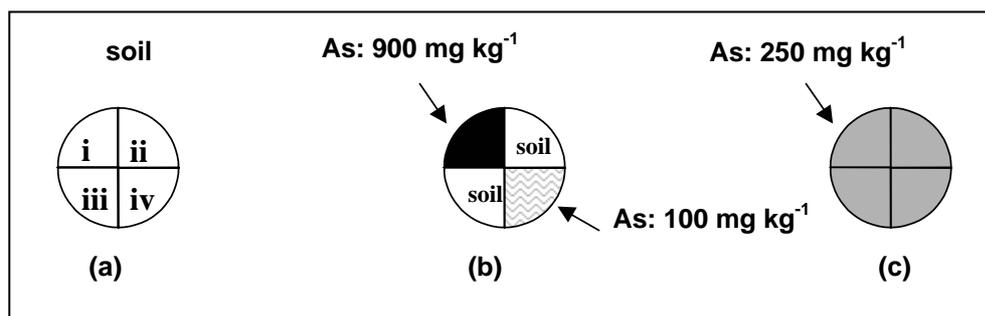
Figure 2.13: Experimental design: (a) control soil, (b) As heterogeneous and (c) As homogeneous treatment.

2.6.3   RESULTS AND DISCUSSION

2.6.3.1   Plant Biomass Yield and Arsenic Uptake

The highest biomass yield was recorded for the control treatment, and there were no significant yield differences in the two other treatments (Table 2.10). The fronds of *P. vittata* grown on all treatments were slightly yellow. This chlorosis was possibly related to the low P availability in this soil which had an Olsen P value of only 3 mg kg^{-1}. Furthermore, necrosis was observed on the surface of the fronds of *P. vittata* grown on the As homogeneous treatment. Despite this apparent toxicity in the As homogeneous treatment however, it did not appear to have any significant effect on biomass. Not surprisingly, As concentrations and As uptake in *P. vittata* were significantly higher for the As treatments compared to the control soil (Table 2.10).

Table 2.10: Biomass yield (g pot^{-1} DM), As concentration (mg kg^{-1} DM) and As uptake (µg pot^{-1}) of *P. vittata* fronds grown on different treatments

	Control	Homogeneous As treatment	Heterogeneous As treatment
Frond yields (g pot^{-1} DM)	1.1a	0.5b	0.7b
As concentration (mg kg^{-1} DM)	16.8b	811a	1290a
As uptake (µg pot^{-1})	19.2b	465a	1027a

(Figures followed by different letters in the same rows are statistically significantly different from each other)

2.6.3.2   Root Yield and Distribution

There were no significant differences in root yields for *P. vittata* between treatments (Table 2.11). Furthermore, roots appeared to be randomly distributed in the pots, independent of As patches in the soil. This observation was confirmed by the results for root yield (Table 2.11) where there were no significant differences between treatments. The results indicate that *P. vittata* does not appear to actively forage As, as has been shown for the Zn and Cd hyperaccumulator *T. caerulescens* (Haines, 2002; Schwartz *et al.*, 1999; Whiting *et al.*, 2000).

Table 2.11: Root yields (g pot^{-1} DM) and diameter (mm) of *P. vittata* after 63 days of growth in the pot quarters i, ii, iii and iv (see Figure 2.13). Note no significant differences were observed between treatments

		Control	Homogeneous As treatment	Heterogeneous As treatment
Root yields (g pot^{-1} DM)	Total	0.28	0.39	0.14
	i	0.06	0.08	0.05
	ii	0.04	0.07	0.02
	iii	0.05	0.09	0.03
	iv	0.13	0.15	0.04
Diameter (mm)				
	i	0.40	0.35	0.42
	ii	0.43	0.35	0.40
	iii	0.45	0.37	0.41
	iv	0.41	0.36	0.40

2.6.4   CONCLUSIONS

The root distribution of the As hyperaccumulator *P. vittata* was not significantly affected by the presence of As patches in soil and it would appear it has no ability to forage for this element. Nevertheless, even though *P. vittata* does not forage for As, its roots do not appear to actively avoid As patches in soil, which in itself is a positive trait for the successful application of this fern for phytoremediation.

2.7 INVESTIGATION OF THE ROLE OF MYCORRHIZAL FUNGI IN ARSENIC UPTAKE IN *P. VITTATA* AND *P. CRETICA*

2.7.1 INTRODUCTION

Mycorrhizae are symbiotic fungi that are present in most soils. They attach themselves onto the roots of plants and by doing so increase root surface area several hundred-fold, thereby helping the host plant to absorb more water and nutrients while at the same time the host plant provides it with food. The ability of mycorrhizal fungi to enhance plant uptake of nutrients has been demonstrated for P (Hattingh *et al.*, 1973), Cu (Li *et al.*, 1991) and Zn (Burkert and Robson, 1994). In contrast, mycorrhizae can also decrease metal concentrations in leaves and enhance plant resistance to metals. For example, it has been shown that the presence of *Glomus mossae* restricted the transfer of Cd to the leaves of clover as a result of fungal immobilization of Cd (Joner and Leyval, 1997). Moreover, two Arbuscular-mycorrhiza (AM), *Glomus caledonium* and *Glomus mossae*, isolated from an arsenic-contaminated mine-spoil conferred enhanced resistance to the grass *Holcus lanatus* (Meharg and Hartley Whitaker, 2002).

The aim of this work was to firstly determine whether mycorrhizal fungi colonise the roots of *Pteris vittata* and *Pteris cretica* growing on an As contaminated soil. Secondly, if an infection is observed, to determine what effect these fungi may have on As uptake in these fern species. Consequently, this experiment comprised two parts (i) an evaluation of mycorrhizal fungi colonisation of *P. vittata* and *P. cretica* growing in the field and (ii) a complementary pot experiment.

2.7.2 PART 1 - EVALUATION OF MYCORRHIZAL FUNGI COLONISATION

2.7.2.1 Materials and Methods

Roots were taken from *P. vittata* and *P. cretica* plants growing in the field trial in Cornwall (refer section 2.2.2.1 for details). Roots were sampled using an auger from individual plants within each sub-plot, which were then combined to provide one composite sample. Hence four samples were obtained for *P. vittata* and three samples for *P. cretica*. To determine the extent of fungi infection, root samples were cleaned and stained using the method of Phillips and Hayman (1970). Root samples were put into a glass vial containing 15 ml of 10 % (v/v) KOH and heated for 2 hours at 90°C. Roots were then washed with KOH and immersed in an alkaline solution of H_2O_2 (20 mL volume) for 30 minutes at room temperature, then rinsed with de-ionised water to remove the H_2O_2. After rinsing, roots were then immersed in an acid solution of 2 % (v/v) HCl and heated at 90°C for 5 minutes. Staining was performed by boiling the samples at 90°C for 4 minutes in a 0.05 % blue trypan solution. The percentage of root infected by mycorrhizal fungi was calculated as described by Giovannetti and Mosse (1980). One centimetre sections of root were randomly selected from stained samples and mounted onto a microscope slide in groups of 10. The length of the cortex that was infected was measured for each of the 10 root samples, and values averaged and expressed as a percentage.

2.7.2.2 Results and Discussion

Arbuscules and vesicles of mycorrhizal fungi were present in the cortex root of *P. cretica* (Plate 2.3a), whereas only fragments of fungi and arbuscules were present in *P. vittata* (Plate 2.3b). Arbuscules are small tree like structures formed by mycorrhizal fungi inside a cell within the plant root. They are the places where the plant and the fungus exchange food and nutrients with each other, formed by repeated branching of a hypha when it enters a cell. Vesicles on the other hand are compartment type structures in cells formed inside the plants' roots that are thought to store cellular products as such as nutrients. Arbuscular-mycorrhiza (AM) are the most common type of mycorrhizal fungi and can be found in most families of Angiosperms, as well as Gymnosperms, Pteridophytes and Bryophytes.

Arbuscular-mycorrhiza hyphae are restricted to the cortical region of the roots and never penetrate the vascular portion of the stem or roots (stele).
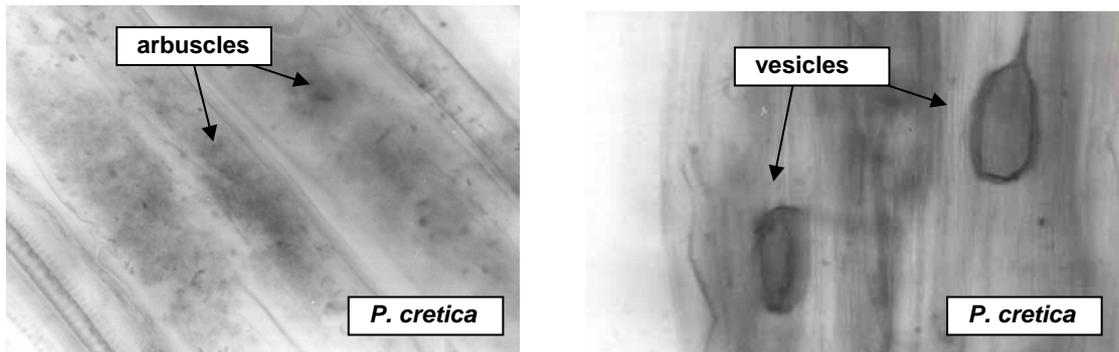
Plate 2.3a: View of arbuscules and vesicles in roots of *P. cretica*
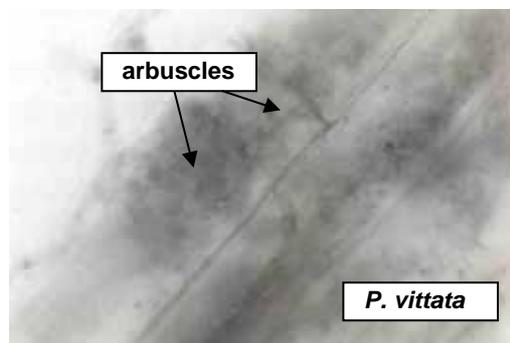
Plate 2.3b: View of arbuscules in roots of *P. vittata*

Most of the *P. vittata* root samples collected from the field plots were not colonised by AM, with on average only 3.1 (± 9.6) root infection. In contrast, 47 % of *P. cretica* root samples had more than 35 % of their cortex infected by AM, with a mean value of root infection of 27.2 % (± 34.7).

Soil fertility and notably P availability affect the extent of mycorrhizal infection in soils. The Olsen P concentration of the soil was 28 mg kg^{-1}, which is high and may have limited the development of mycorrhizal fungi. In fact, analysis of plant samples showed that *P. cretica* contained nearly 1000 mg kg^{-1} more P in its fronds than *P. vittata*. This higher P requirement for *P. cretica* may explain the higher proportion of roots infected by AM compared to *P. vittata*.

2.7.2.3   Conclusions

The extent of mycorrhizal infection observed in *P. cretica* differed from that of *P. vittata*. On average, 27 % of *P. cretica* roots contain mycorrhizal fungi, whereas no significant infection was observed for *P. vittata*.

2.7.3 PART 2 – POT EXPERIMENT

Given the results from the field trial revealed that AM fungi could infect the roots of *P. cretica*, a pot experiment was set-up to evaluate the affect of AM fungi on As uptake in *P. cretica* and *P. vittata*.

2.7.3.1 Material and Methods

Soil sampled from the Cornwall trial site was sterilised using gamma radiation. Approximately 400 g of sterilised soil was mixed with the same amount of sand and this was inoculated with either 20 g of fresh AM fungi inoculated soil that had originated from the field trial site in Cornwall (+ inoculum treatment) or inoculated with 20 g of fresh inoculated soil that had been autoclaved (- inoculum treatment). *Pteris vittata* and *P. cretica* sporelings were transplanted into pots and each treatment was replicated six times. Pots were arranged randomly on a bench inside a greenhouse with day/night temperatures of 28/20°C (16/8h), and a minimum light intensity of 350 μmol m^{-2} s^{-1} and plants grown for five months. After five months, shoots were harvested and rinsed with deionised water, dried and dry weights recorded. Ground plant tissue was digested in a mixture of HNO_3 and $HCLO_4$ and As in digests measured by ICP-AES.

2.7.3.2 Results and Discussion

2.7.3.2.1 Plant biomass yield

There was no significant difference in biomass yield between *P. vittata* and *P. cretica* (Figure 2.14). Furthermore, there was no significant difference in yield between treatments for either species. This suggests that while mycorrhizae may result in a several hundred-fold increase in the surface area of roots and help the host plant to absorb more water and nutrients, in this experiment it did not appear to make a significant difference to biomass production.
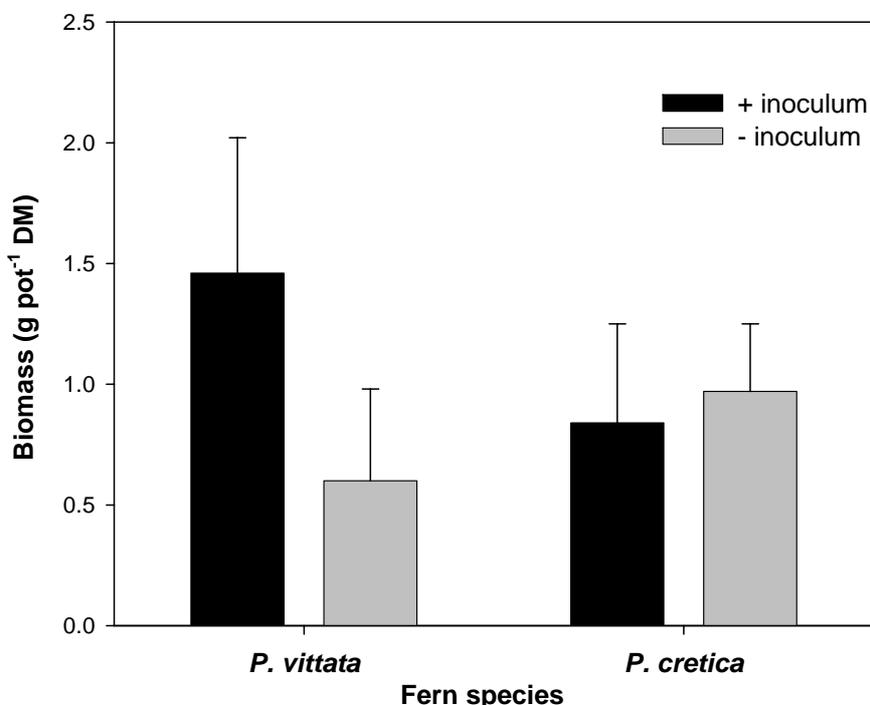
Figure 2.14: The effect of inoculum application on biomass yield (g pot^{-1} DM) of *P. vittata* and *P. cretica*. The results are means ± standard errors (n = 6).

2.7.3.2.2   Plant arsenic concentration

Arsenic concentrations in the fronds of *P. vittata*, as has been previously reported, were significantly higher than those found in *P. cretica* (Figure 2.15). Arsenic concentrations ranged between 115 (± 34) to 180 (± 27) mg kg^{-1} in *P. cretica* and between 283 (± 45) to 365 (± 33) mg kg^{-1} in *P. vittata*. Interestingly, there were no significant differences in As uptake between treatments for either species. This is despite the observation made in the field trial that showed *P. cretica* was infected with AM fungi. So while it was postulated that a possible reason for the lower As uptake in *P. cretica* in the field trial was the higher infection of *P. cretica* roots with AM fungi, this now seems unlikely given the findings in the pot trial which showed inoculum had no effect on As uptake.
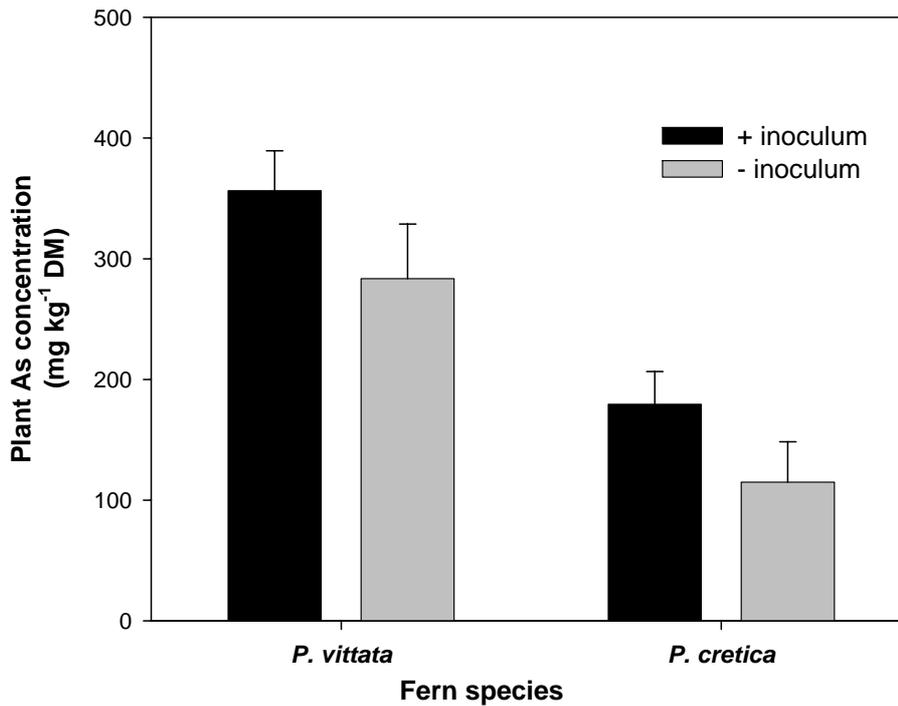
Figure 2.15: The effect of inoculum application on As concentrations (mg kg^{-1} DM) for *P. vittata* and *P. cretica*. The results are means ± standard errors (n = 6)

2.7.3.3   Conclusions

Inoculation of two Pteris fern species, *P. vittata* and *P. cretica* with AM fungi had no effect on either biomass yield or plant As concentrations.

3. HYPERACCUMULATION OF CADMIUM AND ZINC BY *THLASPI CAERULESCENS* AND *ARABIDOPSIS HALLERI*

3.1 INTRODUCTION

There are a large number of sites scattered around the UK that have soils co-contaminated with Zn and Cd as a result of a variety of past anthropogenic activities such as mining and land application of sewage sludge. While Zn is an essential nutrient for living organisms, at elevated concentrations in soils it can result in phytotoxicity or adversely affect microorganisms. Cadmium on the other hand is a non-essential, biotoxic heavy metal, the biggest risk of which is transfer from soil to food crops via the food chain.

The traditional method of dealing with this legacy of Cd and Zn contamination would have been to excavate the soil and dump it into landfills. However, this is considered not to be a sustainable approach to remediation of contaminated soils. As discussed in the introduction of this report, more environmentally sustainable, *in situ* approaches for cleaning up metal contaminated soils have been proposed such as phytoextraction using hyperaccumulators.

In the Brassicaceae family, several hyperaccumulators have been identified. Among them are *Thlaspi caerulescens* and *Arabidopsis halleri*, both able to hyperaccumulate Cd and Zn. *Thlaspi caerulescens*, for example, has been shown to accumulate > 1000 mg Cd kg^{-1} and 10,000 mg Zn kg^{-1} (Zhao *et al.*, 2003), while *Arabidopsis halleri* has been shown to accumulate > 100 mg Cd kg^{-1} and > 20,000 mg Zn kg^{-1} (Dahmani-Muller *et al.*, 2000). Whilst the phytoextraction potential of *T. caerulescens* has been known for some years, there have been very few attempts to quantify the ability of this hyperaccumulator to extract Cd and Zn from soils under field conditions (e.g. Brown *et al.*, 1995; Kayser *et al.*, 2000; Hammer and Keller, 2003) and there are no known field studies using *A. halleri*.

The aim of objective two is to field test the phytoextraction potential of *T. caerulescens* and *A. halleri* for extracting Cd and Zn from soils previously contaminated with varying amounts heavy metals.

3.2 MATERIAL AND METHODS

3.2.1 FIELD SITE

A field trial was conducted on the Woburn Market Garden Experimental site, located in Bedfordshire, England. At Woburn, different rates of sewage sludge, farmyard manure and composts were applied to land annually between 1942 and 1961, after which time application ceased. The application of varying amounts of sludge, manure and compost has resulted in the accumulation of heavy metals to different concentrations in soils on the experimental plots. A full description of the original treatments is given by McGrath (1984).

3.2.2 EXPERIMENTAL DESIGN

Nine 4 m^2 plots were selected that had a range of different total metal contents. Each plot was further subdivided into four 1 m^2 subplots. In May 2003, two-month old *T. caerulescens* (Ganges ecotype) and *A. halleri* seedlings were transplanted into opposite quarters of each plot at a density of 25 plants m^{-2} per subplot (Figure 3.1). Plants were grown for three months and their aboveground parts were harvested in August 03 (crop 1). In October 03, all plots were then planted exclusively with two-month old *T. caerulescens* seedlings (Ganges ecotype) at a density of 25 plants m^{-2} per subplot. These plants flowered in April 04, so flowers and stems were harvested and plants grown for a further two months

before aboveground plants were harvested in June 04 (crop 2). Fertiliser was applied to each plot by hand before planting at a rate of 150 kg N ha^{-1}, 75 kg P_2O_5 ha^{-1} and 75 K_2O ha^{-1}. Throughout the experiment, the plots were watered and weeded when necessary.
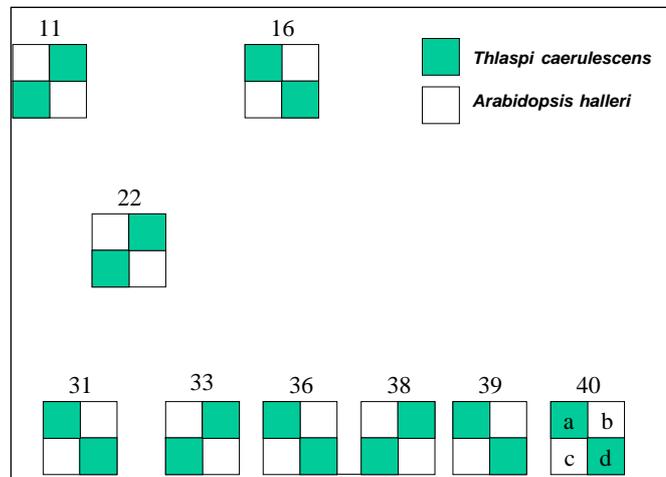
Figure 3.1: Experimental layout of the 9 plots of *T. caerulescens* and *A. halleri* grown at Woburn Market Garden

3.2.3 PLANT ANALYSIS

The number of plants per subplot was recorded and plants harvested by cutting them at the soil surface. Plants were rinsed with deionised water, dried and dry weights recorded before being ground to <0.5 mm for analysis. Sub-samples of ground plant tissue were digested with a mixture of HNO_3 and $HClO_4$ and the concentrations of Cd, Zn and other elements in the digest determined using ICP-AES.

3.2.4 TERRASEED® TECHNOLOGY

In addition to planting seedlings directly into field plots, Terraseed® technology was trialled. Terraseed® is a semi-permeable, degradable seed mat in which seeds along with fungicides and fertilisers are laminated within the mat which is then mechanically laid out onto the soil surface for germination. Upon germination, roots grow through the lower layer of the seed mat and shoots penetrate through upper perforations.

It is recognised that the larger the biomass of a hyperaccumulator plant, the greater its potential for extraction of heavy metals from a contaminated soil. However, competition for water, nutrients and light by weeds limits biomass production. Terraseed® was therefore trialled to determine if it could increase hyperaccumulator biomass production in a metal contaminated soil by overcoming these limitations. It was envisaged that Terraseed® potentially could facilitate a rapid establishment and growth of hyperaccumulator plants by maintaining adequate soil moisture and importantly reducing competition from competing weeds species after germination. However, in practice, the mat did not keep in contact with the soil surface and the seeds dried out and did not grow. The matting was removed and the plots were established by transplanting as described above.

3.3 RESULTS AND DISCUSSION

3.3.1 SOILS

Cadmium and Zn concentrations in soils varied from background in plot 11, to concentrations of both Cd and Zn in plots 40, 16 and 39 that are in excess of the limits set by the Commission of the European Communities Directive for sludge-treated agricultural

soils (CEC, 1986) (Table 3.1). Other soil properties such as soil pH and organic carbon, which are likely to affect metal availability in soils, were essentially the same across all nine plots (data not shown).

Table 3.1: Soil Cd and Zn heavy concentrations in the Woburn Market Garden plots

Plot Number	Cd	Zn
	(mg kg^{-1})	
11	D.L.	83
31	0.3	107
36	1.7	150
38	3.3	185
22	4.0	217
33	3.3	239
40	5.6	333
16	4.5	354
39	8.1	366

D.L. detection limit; CEC Limit Cd 3 mg kg^{-1}; Zn 300 mg kg^{-1}

3.3.2   PLANT BIOMASS YIELD

Average biomass yields for *A. halleri* and *T. caerulescens* from individual plots are given in Table 3.2. Yields for crop 1 ranged from 0.002 to 0.25 t ha^{-1} for *A. halleri* and from 0.44 to 1.31 t ha^{-1} for *T. caerulescens*. The total yield for *T. caerulescens* in crop 2 (i.e. flowers and stems + remaining biomass) was similar to crop 1, with values from 0.29 to 1.44 t ha^{-1}.

There are no other published data on biomass yields for *A. halleri* in field trials. However for comparison, in a pot trial study, yields of between 0.35 – 2.1 t ha^{-1} were estimated for *A. halleri* (Dahmani-Muller *et al.*, 2000). Biomass yields for *T. caerulescens* in field trials appear to be quite variable. For example, McGrath *et al.* (2000) previously found annual yields of between 4 to 5 t DM ha^{-1} at the same field trial site used in the present study. This compares to values ranging between 0.5 t ha^{-1} to 1.8 t ha^{-1} found in several field trials in Switzerland (Kayser *et al.*, 2000; Hammer and Keller, 2003), that are more in line with results found in the present investigation.

Table 3.2: Dry biomass *(*t ha^{-1} DM) for *A. halleri* and *T. caerulescens* in crop 1 and crop 2
The results are means ± standard errors (n = 2 in crop 1 and n = 4 in crop 2)

Plot Number	Crop 1		Crop 2		
	A. halleri	*T. caerulescens*	*T. caerulescens*		
			Stems & flowers	Remaining biomass	Total biomass
11	0.04 (0.02)	0.60 (0.07)	0.04 (0.01)	0.19 (0.03)	0.29 (0.03)
31	0.04 (0.05)	0.74 (0.13)	0.11 (0.03)	0.48 (0.01)	0.60 (0.06)
36	0.08 (0.02)	1.13 (0.09)	0.14 (0.04)	0.61 (0.14)	0.75 (0.07)
38	0.09 (0.05)	1.30 (0.67)	0.14 (0.14)	0.88 (0.04)	1.06 (0.05)
22	0.002 (0.00)	0.44 (0.09)	0.08 (0.02)	0.52 (0.21)	0.60 (0.11)
33	0.06 (0.05)	1.17 (0.06)	0.07 (0.02)	0.53 (0.15)	0.60 (0.08)
40	0.06 (0.02)	1.07 (0.10)	0.22 (0.01)	1.22 (0.53)	1.44 (0.26)
16	0.16 (0.10)	1.22 (0.18)	0.10 (0.08)	0.53 (0.30)	0.63 (0.19)
39	0.25 (0.06)	1.31 (0.09)	0.21 (0.06)	1.11 (0.35)	1.32 (0.20)

Interestingly, there was no significant difference in the average yield across all plots for *T. caerulescens* for crop 1 (1.00 ± 0.11 t ha^{-1}) and crop 2 (0.81 ±0.13 t ha^{-1}). This is despite the fact that in crop 1, plants were grown for three months compared to nine months for crop 2. This finding is contrary to what has been previously found for *T. caerulescens,* where plants that were allowed to grow over the winter showed a significant increase in biomass compared to plants planted in the spring (McGrath *et al.* in press). The similarity in yields, despite differences in the length of growth may in part be explained by differences in the

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morphology of *T. caerulescens* harvested in the two crops. Plants harvested in crop 1 were generally stocky and leafy with a rosette habit, compared to plants harvested in crop 2 which were spindly with smaller leaves. *Thlaspi caerulescens* being a perennial plant had flowered in early spring, producing long racemes with many flowers that were removed in the hope that the plants would again bulk up with vegetative material later in the growing season. However this did not happen and by the time of the final harvest, plants were generally elongated and on the verge of producing winged fruit (silicula) which clearly contain little biomass.

3.3.3 PLANT CADMIUM AND ZINC CONCENTRATIONS

Cadmium and Zn concentrations in the aboveground parts of *A. halleri* and *T. caerulescens* are given in Table 3.3. Cadmium concentrations in crop 1 ranged from 5.8 to 17.4 mg kg^{-1} for *A. halleri* and from 39 to 520 mg kg^{-1} for *T. caerulescens*. In crop 2, Cd concentrations in the stems and flowers of *T. caerulescens* ranged from 100 to 1011 mg kg^{-1}, while Cd concentrations in the remaining biomass were significantly lower, ranging from 18 to 152 mg kg^{-1}. As has been found in several previous studies (Lombi *et al.*, 2000; Zhao *et al.*, 2003) there were significant positive relationships between soil and *T. caerulescens* Cd concentrations (Figure 3.2). However there was no relationship between soil and *A. halleri* Cd concentrations.

Compared to Cd, Zn concentrations were significantly higher in both *A. halleri* and *T. caerulescens.* Zinc concentrations in crop 1 ranged from 1509 to 2869 mg kg^{-1} for *A. halleri* and from 2016 to 3143 mg kg^{-1} for *T. caerulescens* (Table 3.3). In crop 2, Zn concentrations in the stems and flowers of *T. caerulescens* ranged from 3799 to 5246 mg kg^{-1}, while Zn concentrations like for Cd, were lower in the remaining biomass ranging from 1147 to 1759 mg kg^{-1}. As has been previously found (Lombi *et al.*, 2000), Zn was accumulated across a wide range of soil metal concentrations in both *A. halleri* and *T. caerulescens* (Figure 3.3).

Table 3.3: Cadmium and Zn concentrations (mg kg^{-1}) in *A. halleri* and *T. caerulescens* in crop 1 and 2. The results are means $\pm$ standard errors (n = 2 in crop 1 and n = 4 in crop 2).

Plot Number	Crop 1		Crop 2	
	A. halleri	*T. caerulescens*	*T. caerulescens* Stems & flowers	*T. caerulescens* Remaining biomass
Cadmium				
11	5.8 (1.7)	39 (23)	100 (21)	18 (2)
31	9.3 (6.6)	91 (18)	204 (50)	26 (6)
36	13.1 (2.1)	161 (34)	369 (53)	40 (7)
38	17.4 (4.7)	269 (3)	812 (136)	110 (14)
22	13.9	216 (17)	493 (85)	78 (14)
33	16.8 (3.2)	206 (110)	740 (100)	76 (13)
40	11.5 (3.3)	317 (44)	763 (100)	90 (6)
16	17.0 (2.8)	264 (45)	825 (116)	107 (13)
39	16.6 (3.0)	520 (117)	1011 (141)	152 (27)
Zinc				
11	1884 (566)	2042 (725)	4688 (607)	1434 (175)
31	1511 (1353)	3110 (279)	4338 (549)	1171 (136)
36	1509 (571)	2521 (168)	4810 (126)	1147 (92)
38	2360 (1054)	2521 (180)	4127 (104)	1430 (38)
22	2539 (90)	2016	4282 (139)	1206 (131)
33	2869 (169)	2751 (665)	5246 (274)	1372 (104)
40	1574 (126)	3074 (105)	4859 (261)	1608 (84)
16	2590 (324)	2538 (341)	4508 (143)	1759 (90)
39	2503 (697)	3143 (249)	3799 (300)	1476 (79)
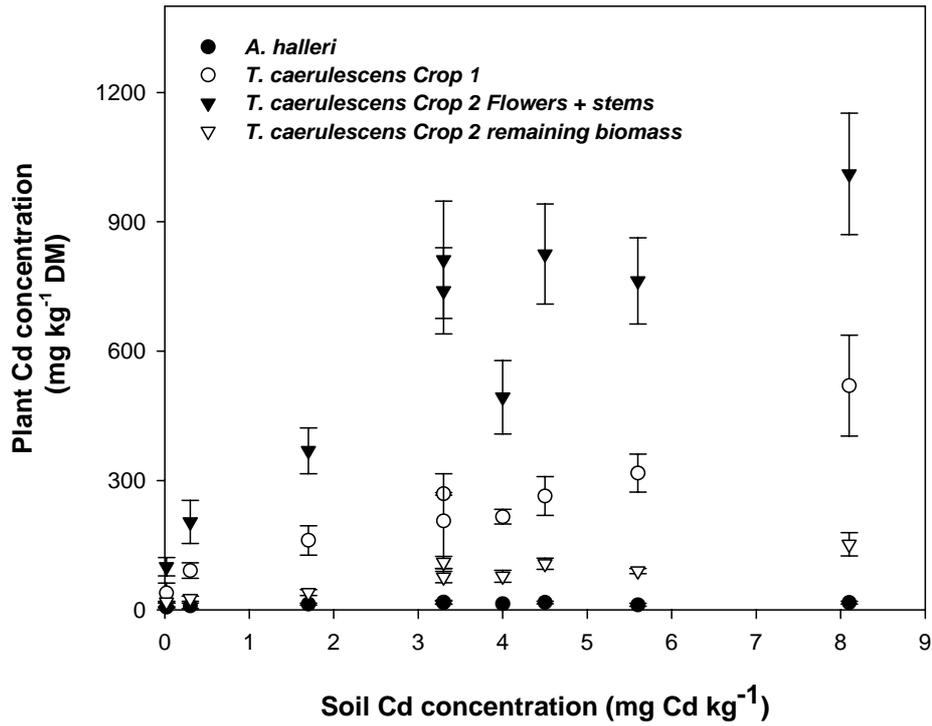
Figure 3.2: The relationship between soil Cd and *A. halleri* or *T. caerulescens* Cd concentration. The results are means ± standard errors.
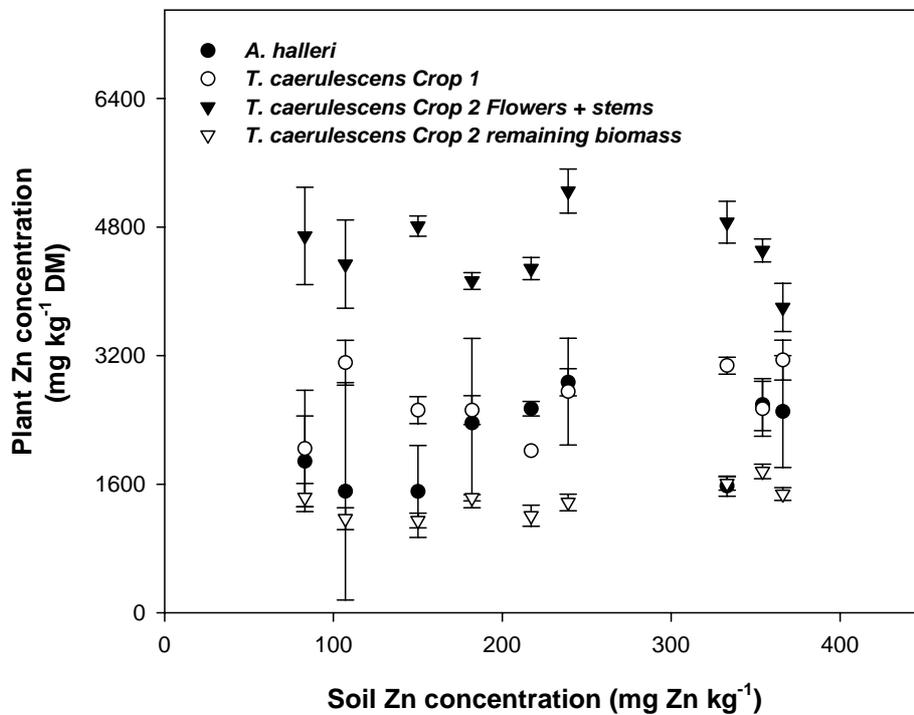
Figure 3.3: The relationship between soil Zn and *A. halleri* or *T. caerulescens* Zn concentration. The results are means ± standard errors.

As has been reported in previous studies (Lombi *et al*., 2000), the *T. caerulescens* ecotype Ganges that was used in the field trial, had a high efficiency to take up and accumulate both Cd and Zn. For example, the mean bioconcentration factor in crop 1 for all plots where Cd or Zn exceeded limits set by the Commission of the European Communities Directive for sludge-treated agricultural soils (CEC, 1986) was 63 (±4) for Cd and 202 (±35) for Zn. Even though Cd and Zn uptake in *A. halleri* was less than for *T. caerulescens*, there was still significant translocation of these metals from the soil to the leaves. The mean bioconcentration factor was 3.6 (±0.6) for Cd and 6.3 (±0.8) for Zn. While these are significantly less than for *T. caerulescens,* they are greater than values of 3.5 and 3.0 found for Cd and Zn by Dahmani-Muller *et al*. (2000) for *A. halleri* growing on smelter-contaminated soil in northern France.

3.3.4   CADMIUM AND ZINC UPTAKE

The amount of Cd and Zn extracted from the field plots depended upon the plant species and which cropping (Table 3.4). For example, *A. halleri* extracted between 0.00003 to 0.042 kg Cd ha^{-1} and 0.01 to 0.63 kg Zn ha^{-1}. This compares to between 0.02 to 0.68 kg Cd ha^{-1} and 0.88 to 4.1 kg Zn ha^{-1} for *T. caerulescens* in crop 1 and 0.01 to 0.38 kg Cd ha^{-1} and 0.46 to 3.03 kg Zn ha^{-1} in crop 2.

Comparing the amount of Cd and Zn extracted from soils using hyperaccumulators such as *T. caerulescens* between studies is difficult given the importance that soil type, source of contamination and environmental factors play in affecting metal uptake. Nevertheless, McGrath *et al*. (2000) previously calculated the maximum potential removal of Zn for *T. caerulescens* grown at Woburn to be 25-50 kg ha^{-1}. These values were based on the metal uptake and biomass of the largest two rows of the plants observed in the field experiment. This value is similar to that found by Hammer and Keller (2003) who calculated a value of 20 kg ha^{-1} in an acidic, Zn contaminated soil (i.e. 1158 mg Zn kg^{-1}). However in the same study, another soil with a Zn concentration more in line with the present study (i.e. 673 mg Zn kg^{-1}), had an uptake value of 3.7 kg ha^{-1}. Furthermore, Hammer and Keller (2003) calculated extraction values for Cd of 0.13 and 0.54 kg ha^{-1} which like for Zn are of the same order of magnitude as was found in the present investigation.

Table 3.4: Cadmium and Zn uptake (kg ha^{-1}) in *A. halleri* and *T. caerulescens* in crop 1 and crop 2

Plot Number	Crop 1				Crop 2	
	A. halleri		*T. caerulescens*		*T. caerulescens*	
	Cd	Zn	Cd	Zn	Cd	Zn
11	0.0002	0.08	0.02	1.23	0.01	0.46
31	0.0004	0.06	0.07	2.29	0.04	1.04
36	0.0011	0.13	0.18	2.85	0.08	1.37
38	0.0015	0.21	0.35	3.28	0.25	1.84
22	0.0000	0.01	0.09	0.88	0.08	0.97
33	0.0010	0.17	0.24	3.21	0.09	1.09
40	0.0007	0.10	0.34	3.28	0.28	3.03
16	0.0027	0.40	0.32	3.09	0.14	1.38
39	0.0042	0.63	0.68	4.12	0.38	2.44
mean	0.0013	0.20	0.26	2.69	0.14	1.5

Assuming plants were extracting Cd and Zn to a soil depth of 20 cm; and the soil has a bulk density of 1.3 t m^{-3}, the proportion of Cd and Zn removed from the soil by either *T. caerulescens* or *A. halleri* can be estimated. It was calculated that in two crops, for all plots where Cd or Zn exceeded limits set by the Commission of the European Communities Directive for sludge-treated agricultural soils (CEC, 1986), *T. caerulescens* removed between 1.7 to 5.0 % (mean of 3.9 %) of the total soil Cd content and 0.5 to 0.7 % (mean 0.6 %) of the soil Zn. In comparison, a single crop of *A. halleri* removed between 0.0001 to 0.023 % (mean 0.010 %) of the total soil Cd content and 0.012 to 0.066 % (mean 0.040 %) of the soil Zn. These values compare well to extraction rates for *T. caerulescens* calculated

from a field trial by Hammer and Keller (2003): the proportion of total soil Cd and Zn that was removed by *T. caerulescens* across three harvests were between 1.8 – 8.0 % for Cd and 0.0005 to 0.7 % for Zn. There are no reports for extraction rates for *A. halleri* in field trials.

The field study has demonstrated that *T. caerulescens* can extract significant quantities of Cd and to a lesser extent Zn from a metal contaminated soil. However the ability of *A. halleri* to extract Cd and Zn is appreciably less than *T. caerulescens* due to a low biomass and relatively low Cd uptake. Bioaccumulation factors for both Cd and Zn were generally high, however one of the other important drivers of successful phytoextraction, plant biomass, was relatively low compared to what has been previously found on this field site. The climatic conditions experienced during the first crop were abnormally hot and dry which likely had a large impact on yield production. In addition, growing plants over the winter did not appear to be as successful as previously found, resulting in decreased biomass yields. It would appear that if biomass production can be improved, which may involve installation of an irrigation scheme to maintain adequate moisture and shading during hot weather, phytoextraction of Zn and in particular Cd using *T. caerulescens* at this site appears to be a practical and relatively low input remediation option.

3.4   CONCLUSIONS

This study demonstrated that both *T. caerulescens* and *A. halleri* were able to hyperaccumulate Zn from a contaminated soil, however only *T. caerulescens* was found to hyperaccumulate Cd. However, *T. 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*. As a proportion of the total metal concentration, *T. caerulescens* could more easily extract Cd than Zn. Even with a low biomass, phytoextraction of Cd from moderately contaminated soils was found to be feasible.

4. COST AND FEASIBILITY OF PHYTOEXTRACTION

4.1 INTRODUCTION

Phytoextraction is viewed as a possible option for the remediation of soils moderately contaminated with heavy metals and metalloids. However, if phytoextraction is ever to be commercially implemented, there are several factors that need to be satisfied. It firstly must meet the requirements of environmental legislation. For example, phytoextraction must demonstrate that hyperaccumulator plants can reduce contaminants moving offsite from an affected zone, be it from the reduction of dust particles or perhaps leaching losses through the soil profile and thereby minimise the risk contaminants may then pose to receptors such as humans. Secondly, successful phytoextraction must be able to deal with the disposal of a small amount of contaminated crop material. In response there have been a number of different methods proposed for disposing of contaminated crops after phytoextraction. These include pre-treatment processes which essentially reduce biomass volume such as composting, compaction and pyrolysis, through to final disposal processes such as incineration, ashing and direct disposal into landfills (Sas-Nowosielska *et al.*, 2004). Finally, phytoextraction of a contaminated site will only be considered if it is economically attractive, which in itself is reliant on a number of factors. These include comparing the cost of phytoextraction to an alternative method of remediation such as excavation and dumping, capping etc. It must also take into account the cost of doing nothing, which is mainly due to legislation, loss of income from the land and damage to reputation or goodwill. In addition, another aspect of phytoextraction that may have a significant economic impact on the overall benefit of phytoextraction is the feasibility and cost associated with recovering the contaminant from the plant post harvest. Obviously if the contaminant can be recovered from the harvested plant it then could be sold to offset the cost of the phytoextraction operation. This has been undertaken for metals such as Ni (Brooks *et al.*, 2001).

The aim of this section is therefore to review some of the costs of using phytoextraction to remediate As, Cd and Zn contaminated soil.

4.2 PRODUCTION OF BIOMASS

The first and most obvious cost associated with phytoextraction is biomass production. The typical components required for the production of biomass and their associated costs are given in Table 4.1. The components for the production of a hyperaccumulator crop are essentially the same as those for any 'normal' crop and are based on best agronomic practice.

Table 4.1: The costs of producing biomass for phytoextraction

Production of biomass (ha)	£ ha^{-1}
Cost of ploughing	60
Cost of cultivation	25
Cost of planting	30
Cost of fertiliser and application	20
Cost of pesticide and application	30
Cost of harvesting	80
Total production cost	**£245**

In light of the absence of reliable information on the costs associated with growing hyperaccumulators, the costs for each part of the production process have been estimated based on the costs for growing a crop of spring wheat from information available in the Farm Management Pocketbook (Nix, 2003). Costs include the hire of labour and equipment such

as tractors for ploughing, cultivation, spraying etc as well as the costs of materials such as fertiliser, sprays etc which would for all intents and purposes be the same for a crop of wheat as for a crop of *T. caerulescens*. It was estimated that the costs of growing a single crop of a hyperaccumulator to be of the order of £245 per ha.

4.3 BIOMASS PRE-TREATMENT AND CONTAMINANT RECOVERY

Post harvest, the biomass collected during phytoextraction needs to be reduced in volume for disposal or alternatively to be processed in order to recover the extracted contaminant. As previously noted, there have been several different pre-treatment and treatment techniques proposed for the disposal of contaminated biomass after phytoextraction.

Composting and compaction have both been suggested as techniques for pre-treating metal-rich biomass for disposal (Blaylock and Huang, 2000). While both techniques reduce the volume of biomass, composting is very time consuming (i.e. 2-3 months) adding to the final cost of processing. Moreover, both composting and compaction can result in the production of leachate with high contaminant concentrations. This leachate needs to be collected and treated, further adding to processing costs. Furthermore, there is currently little information on the composition and concentrations of metals in leachate as a result of composting and compaction of hyperaccumulator plants and there are no production-scale processes for recovering metals from the leachate.

Final treatment techniques such as ashing and liquid extraction have been proposed, not only for reducing biomass, but also as potential methods for recovering metals from biomass. Hetland *et al.* (2001) for example showed in a laboratory study that co-firing Pb contaminated plant material with coal reduced plant biomass by 90 % and apportioned the Pb into the resulting ash. However, currently there are no data on this application beyond laboratory testing. Liquid extraction involves leaching metals from biomass with a chelating agent. Hetland *et al.* (2001) showed that 98.5 % of the Pb in contaminated biomass could be recovered by two extractions with ethylenediamine tetra-acetic acid (EDTA) buffered at pH 4.5 and at a 1:5 molar ratio. However, as for extracting metals from leachate, there is currently no production-scale technology available for the recovery of metals from the EDTA.

In terms of techniques that look to be the most practical and cost-effective for biomass disposal and/or metal recovery, incineration and pyrolysis appear to be the most promising.

4.3.1 INCINERATION

The most favourable option for the recovery of metal/metalloids in contaminated biomass is incineration. Biomass can be incinerated in a rotary kiln, with temperatures up to 1000 °C that can destroy organic matter releasing metal/metalloid contaminants. The liberated contaminants are entrained within the resulting slag or volatilised as gas which can then be recovered by scrubbers. The main forms of metallic compounds in this waste slag are oxides, carbonates, chlorides and sulphates (Chen *et al.*, 1997; Zhang *et al.*, 2001; Belevi and Moench, 2000). It is proposed that the metal or metalloid within the slag can then be recovered by further smelting. The estimated costs associated with processing biomass using incineration are between £120 – 150 t[-1] (Sas-Nowosielska *et al.*, 2004).

4.3.2 PYROLYSIS

Pyrolysis is a technique that has been proposed for the treatment of municipal waste that thermally decomposes material under anaerobic conditions. There is no emission to the air and the process is completely hermetic, with the final products being pyrolytic gas, pyrolytic oil and ash/char. Whereas incineration fully converts the input waste into energy and ash, pyrolysis deliberately limits the conversion so that combustion does not take place directly. It is envisaged that potentially, the biomass produced during phytoextraction can be processed by pyrolysis, with the metal/metalloid that is released from the biomass being concentrated and complexed within ash/char. Any element that is incidentally volatilised

during pyrolysis will be released into the gas stream, however, the process can be tailored so these elements can be re-captured later (David Sweeting personal comm.).

To avoid the high costs associated with the installation and operation of a pyrolysis operation, it is envisaged that the biomass produced during phytoextraction will be able to be processed along with municipal wastes. This also has the added benefit of reducing the overall moisture content of the biomass which is required to be less than 30 % for the pyrolysis operation. In reality, given the infrequency of biomass produced during phytoextraction from field sites (at most three times per annum), it may be possible to buy capacity from a merchant when required, which would further reduce processing costs.

There is generally a shortage of hard data on the true capital and operating costs for 'real-world' applications of the pyrolysis operation. Many projects using pyrolysis are supported by subsidies. However, Sas-Nowosielska *et al.* (2004) estimated costs associated with processing biomass to be between £15 – 20 t^{-1}. While costs from a fully commercial UK based pyrolysis operation were estimated at £50 t^{-1} (David Sweeting personal comm.). In addition to these figures, there are also the costs associated with extracting the metals from the coke residue, which are difficult to estimate. For the purposes of this exercise, smelting costs were estimated at £400 t^{-1}.

4.4   COST OF RECOVERING CONTAMINANT

Table 4.2 gives a simple model for the cost of phytoextraction and the potential recovery of As from biomass based on data obtained from the field trial given in Chapter 2. It uses costs of biomass production as outlined in Table 4.1 and estimated costs of extracting As by either incineration or pyrolysis, followed by smelting to recover the metal.

From the results of the field trial, the average biomass yield for *P.vittata* was 0.76 t ha^{-1} and the As concentration in the harvested biomass was 4000 mg kg^{-1}. Results indicate the cost of phytoextraction (i.e. growing the crop and processing the biomass to recover the metal) from the field site at Camborne in Cornwall to be approximately £313 ha^{-1} using pyrolysis and £378 ha^{-1} using incineration. Furthermore, assuming a total recovery of As from the harvested biomass, at the current market value of As, it could only be expected to recover approximately £2 ha^{-1} of the costs of phytoextraction.

Table 4.2: Estimated costs per annum of processing and recovering As from *P. vittata* from the field site at Camborne

	(cost £)
Cost of producing biomass ha^{-1}	245
Biomass (t ha^{-1} DM)	0.76
As concentration (mg kg^{-1})	4000
Total As uptake (kg)	3.0
Cost of processing biomass	
Incineration of DM @ £135 t^{-1}	103
Pyrolysis of DM @ £50 t^{-1}	38
Smelting cost £400 t^{-1}	30
(assuming 10 % of original dry biomass)	
Total cost of recovering As ha^{-1} - incineration	£378
Total cost of recovering As ha^{-1} - pyrolysis	£313
As recovery	(Assume 100 %)
Market price of As £ kg^{-1}	£0.55
Value of As extracted £ ha^{-1}	£1.67

A similar exercise was undertaken for Cd and Zn extracted by *T. caerulescens* at the Woburn field trial given in Chapter 3 (Tables 4.3 and 4.4). The biomass and metal

concentrations were averaged across all the nine plots for harvest one. Like for As, assuming a total recovery of Cd or Zn from the harvested biomass, at the current market value of these metals, it could only be expected to recover approximately £0.13 and £3 per ha respectively of the costs of phytoextraction.

Table 4.3: Estimated costs per annum of processing and recovering Cd from
T. caerulescens from the field site at Woburn

	(cost £)
Cost of producing biomass ha^{-1}	245
Biomass (t ha^{-1} DM)	1.0
Cd concentration (mg kg^{-1})	230
Total Cd uptake (kg)	0.23
Cost of processing biomass	
Incineration of DM @ £135 t^{-1}	135
Pyrolysis of DM @ £50 t^{-1}	50
Smelting cost £400 t^{-1}	40
(assuming 10 % of original dry biomass)	
Total cost of recovering Cd ha^{-1} - incineration	£420
Total cost of recovering Cd ha^{-1} - pyrolysis	£335
Cd recovery	(Assume 100 %)
Market price of Cd £ kg^{-1}	£0.50
Value of Cd extracted £ ha^{-1}	£0.13

Table 4.4: Estimated costs per annum of processing and recovering Zn from
T. caerulescens from the field site at Woburn

	(cost £)
Cost of producing biomass ha^{-1}	245
Biomass (t ha^{-1} DM)	1.0
Zn concentration (mg kg^{-1})	2635
Total Zn uptake (kg)	2.64
Cost of processing biomass	
Incineration of DM @ £135 t^{-1}	135
Pyrolysis of DM @ £50 t^{-1}	50
Smelting cost £400 t^{-1}	40
(assuming 10 % of original dry biomass)	
Total cost of recovering Zn ha^{-1} - incineration	£420
Total cost of recovering Zn ha^{-1} - pyrolysis	£335
Zn recovery	(Assume 100 %)
Market price of Zn £ kg^{-1}	£1.10
Value of Zn extracted £ ha^{-1}	£2.90

4.5   COST OF DISPOSAL

Clearly, recovering heavy metals/metalloids such as Cd, Zn and As from plant material is at present not economically viable. As a result, after harvest and reduction of the volume of plant material by incineration or pyrolysis, this waste will need to be disposed of in a monitored repository such as a landfill site. The costs associated with landfill disposal of plant material containing elevated concentrations of heavy metals/metalloids that are considered hazardous are difficult to approximate. However, conservative estimates from industry indicate disposal costs of between £100-150 per tonne of pre-treated plant material

(Hugh Stewart pers comm.). For example, this would mean that disposal of the harvested plant material would add an additional £10-15 ha^{-1} to the cost of the phytoremediation process, assuming a 1 t ha^{-1} dry matter yield and that the residue of the plant material which is disposed is only 10 % of the original dry matter.

To allow comparison with other remediation technologies currently in use, an estimated cost of using phytoremediation to remediate a heavy metal/metalloid contaminated site was made using data on the cost of producing biomass, the cost of pre-treating the dry biomass by incineration and also the cost of landfilling the incinerated waste (Table 4.5). Calculations assumed that 1 tonne of dry matter was harvested from 1 ha of land, and remediation was made to a soil depth of 0.25 m with a soil bulk density of 1 t m^{-3}. Three scenarios are presented for the estimated costs of phytoremediation using 1, 10 or 100 harvests. Calculations indicate that if up to 100 harvests are required to remediate a contaminated site, it would cost approximately £16 ha^{-1}. This relatively low figure is partially attributed to the fact that phytoremediation is a bioconcentration process, whereby large amounts of heavy metals/metalloid from a large mass of contaminated soil are efficiently concentrated into a relatively small final mass which appears to be relatively inexpensive to dispose into landfill.

Table 4.5: Cost (£ t^{-1} soil) of phytoremediation

Cost of biomass production ha^{-1}	£245
Pre-treatment cost of biomass by incineration t^{-1}	£135
Cost of landfill t^{-1}	£15
(assuming residue of the plant material was only 10 % of the original DM)	
Total cost of production and disposal ha^{-1}	**£395**
1 ha of land 2500 t	
(assuming soil depth 0.25 m and soil bulk density of 1 t m^{-3})	
Cost of 1 harvest by phytoremediation (£ t^{-1} soil)	£0.16 t^{-1} soil
Cost of 10 harvests by phytoremediation (£ t^{-1} soil)	£1.6 t^{-1} soil
Cost of 100 harvests by phytoremediation (£ t^{-1} soil)	£16 t^{-1} soil

As it was assumed for illustrative purposes that 1 t soil = 1 m^3 in Table 4.5, the costs associated with phytoremediation can be compared with those of other *in situ* remediation techniques currently being used in the field (Nathanail, 2000) (Table 4.6). Clearly, the costs associated with phytoremediation are often significantly lower than some of the engineering remediation options currently in practice. However, the practicability depends on the time taken to reach acceptable concentrations.

Table 4.6: Indicative remediation costs for some inorganic contaminants in the UK

Remediation technology	Indicative unit price
Encapsulation	£40 - £120 m^{-2}
Engineering capping	£15 - £30 m^{-2}
Excavation and disposal to landfill	£50 m^{-3}
Vitrification	£40 t^{-1}
Soil washing	£30 - £35 t^{-1}
Solidification/stabilisation	£30 - £60 m^{-3}

4.6   CONCLUSIONS

The costs associated with growing hyperaccumulator plants are similar to normal crops. However, the biomass produced after harvest requires specialist treatment prior to disposal, or alternatively to recover the extracted contaminant. Incineration and pyrolysis appear to be the most promising techniques for the recovery of metal extracted from soil using hyperaccumulators. Using biomass yields and contaminant concentrations in plants from the field trials carried out for the current project, results would indicate that there is little value in trying to recover the contaminant from the biomass for purely economic reasons.

5. 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 soils 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 soils 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 an 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.
- 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 had 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 metals/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 < 20cm 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 metals/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 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.

GLOSSARY OF TERMS

Arbuscles
Tree-shaped outgrowths of fungus which can grow within plant cells of a host plant.

Bioconcentration factor
The ratio of plant shoot to soil metal concentration.

Geogenic
Derived from geological i.e. natural sources.

Hermetic
Completely sealed and airtight.

Hydroponics
A technique for growing plants in water containing dissolved nutrients.

Hyphae
The fine, branching filaments which make up the body of a multicellular fungus.

Mesophytic
Being or growing in or adapted to a moderately moist environment.

Mycorrhiza
The symbiotic association between fungus hyphae and plant roots.

Necrosis
The localised death of living cells within a plant.

Olsen P
A measurement of plant available phosphorus in soil. It involves the extraction of soil with 0.5M sodium bicarbonate at pH 8.5.

Phytotoxic
Being poisonous to plants.

Racemes
An elongated cluster of flowers along the main stem of a plant in which the flowers at the base open first.

Stele
The cylindrical, central, vascular portion of the stem and roots within a vascular plant.

Symbiotic
The living together of different species of organisms with benefit to each of the partners.

Translocation
The transport of dissolved material within a plant.

Vesicle
A small, enclosed compartment within a cell which stores, transports or digests cellular products and wastes.

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APPENDIX A: Photos

Plate A1: View of *Pteris vittata*

Plate A2: View of *Pteris cretica*

Plate A3: View of plots prior to harvest. *Pteris cretica* in foreground.

Plate A4: View of plots after harvest. *Pteris vittata* in foreground.