DEVELOPING ARSENIC PHYTOREMEDIATION TECHNOLOGY FOR NEW ZEALAND

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1 ABSTRACT

A survey was carried out on the arsenic concentration of terrestrial and aquatic vegetation in the Taupo Volcanic Zone (TVZ), North Island, New Zealand. Arsenic concentrations were also determined in the ambient waters, sediments and soils. All of the 16 aquatic species tested had average arsenic concentrations greater than 28 μg As g^{-1} on a dry matter basis. Some specimens of 3 species had arsenic concentrations greater than 1000 μg g^{-1}, the threshold for arsenic hyperaccumulation. By contrast, none of the terrestrial vegetation had average arsenic concentrations greater than 2 μg As g^{-1} on a dry matter basis, and most species tested were below detection limits (0.5 μg g^{-1}).

Potentially, aquatic As-hyperaccumulators could be used as a low-cost method of removing arsenic from contaminated drinking water in developing countries. Aquatic plants may also be used as bio-indicators of arsenic contamination in waterways where levels are variable or are unable to be measured using available methods.

Beside the survey of TVZ vegetation, a greenhouse experiment involving five species of native ferns and one specie of aquatic plant was carried at HortResearch site, Palmerston North, New Zealand. The plants were grown during three months in pots containing soil contaminated by different concentrations of arsenic, then harvested and analysed.

Although some hyperaccumulator ferns species have been recently identified, this was not the case of the species chosen for this study. None of the tested species accumulated arsenic, and most of the samples had concentrations lower than the detection limit. Not even the aquatic plant, known for its ability to take up arsenic from water, accumulated arsenic during this experiment.

2 RÉSUMÉ

Une étude concernant la concentration en arsenic de plantes terrestres et aquatiques a été menée dans la zone volcanique de Taupo, Île du Nord, Nouvelle-Zélande. De même, les niveaux d'arsenic des eaux, sols et sédiments en cette zone ont été déterminés.

Toutes les 16 espèces de plantes aquatiques qui ont été testées présentaient une concentration moyenne en arsenic supérieure à 28 μg As g^{-1} poids sec. Certains individus de trois espèces se situaient au-dessus de la limite de définition d'un hyperaccumulateur, c'est-à-dire 1000 μg g^{-1}. Au contraire, aucune plante terrestre n'a accumulé As à des concentrations supérieures à 2 μg As g^{-1}, et la plupart des espèces étaient en dessous de la limite de détection (0.5 μg g^{-1}).

Au vu des résultats obtenus lors de cette étude, les plantes aquatiques hyperaccumulatrices d'arsenic présentent un potentiel intéressant pour leur utilisation en tant que méthode de décontamination d'eau potable peu coûteuse, et donc particulièrement adaptée aux pays en voie de développement. De plus, les plantes aquatiques peuvent aussi jouer le rôle de bio-indicateurs de contamination par l'arsenic dans des cours d'eau ayant un débit variable ou pour lesquels des analyses ne peuvent pas être effectuées avec les méthodes traditionnelles.

À côté de l'étude sur la végétation des zones volcaniques, un essais sous serre avec cinq espèces de fougères et une plante aquatique a été conduit à HortResearch, Palmerston North, Nouvelle-Zélande. Les plantes ont été cultivées pendant trois mois dans des pots contenant de la terre contaminée par différentes concentrations d'arsenic, et par la suite fauchées et analysées.

Bien que des études récentes aient mené à l'identification de quelques fougères hyperaccumulatrices d'arsenic, ceci n'a pas été le cas pour l'essai mené dans le cadre de cette étude. Aucune des cinq espèces de fougères n'a accumulé de l'arsenic dans ses tissus, et la plupart des échantillons contenaient une quantité d'arsenic inférieure à la limite de détection. La plante aquatique testée dans cette étude n'a pas accumulé d'arsenic non plus, même si elle est reconnue comme hyperaccumulatrice d'arsenic lorsqu'elle pousse en milieux aquatique.

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5 INTRODUCTION

This chapter presents the theoretical basis on which this study is founded. The problem of arsenic pollution and a general introduction to phytoremediation techniques are presented in the first two sections. Further on, I focus on phytoextraction techniques and on hyperaccumulators. Finally, the present knowledge about As hyperaccumulation and phytoremediation forming the background for this study is presented.

5.1 Arsenic and the problem of arsenic pollution

This section presents general information on arsenic (As), its sources in the natural environment, its toxicity and effects on human health.

The word arsenic comes from the greek *arsenikon*, meaning "yellow orpiment". Arsenic (As) is a semi-metallic (metalloid) element (atomic number 33, atomic mass 74.9) belonging to group V. Arsenic occurs in two solid allotropes: a metallic modification (gray arsenic) and a non-metallic one (yellow arsenic). The element itself as well as its compounds are poisonous.

The principal oxidation states of As are +3 and +5; the –3 state is represented by the poisonous compound AsH_3 (arsine). In the +3 state, arsenic forms arsenious oxide (As_2O_3), commonly called "white arsenic"; in the +5 state, the principal compounds of As are arsenic acid and its derivatives, the arsenates. Salts of arsenic acid, especially lead arsenate and calcium arsenate, are much used as insecticides (Sienko and Plane 1961).
Arsenic is found as the free element occasionally, but it usually occurs as a major constituent in more than 200 minerals, including arsenides, sulphides, oxides, arsenates and arsenites. The most common minerals are realgar (As_4S_4), orpiment (As_2S_3), arsenolite (As_2O_3), and iron metals such as arsenopyrite (FeAsS). The abundance of As in Earth's crust, has been estimated at 1.5 to 2 $\mu g\ g^{-1}$ (= $mg\ kg^{-1}$ = ppm) (Ure and Berrow 1982), although baseline concentrations of As in soils are slightly higher, between 5 and 10 $\mu g\ g^{-1}$: (Boyle and Jonasson 1973) quoted an average value of 7.2 $\mu g\ g^{-1}$.

Arsenic is a non-essential element in plants and animals, but it has an affinity for biological material, and can be detected in trace amounts in virtually all living organisms. At low concentrations As is tolerated by most organisms. As the concentration increases, the element becomes toxic and eventually lethal. Arsenic forms kinetically stable bonds to sulphur and carbon in organic molecules. The reaction of As^{III} with sulfhydryl groups of cystine in proteins causing enzyme inactivation may be the primary mode of arsenic toxicity (Ferguson and Gavis, 1972).

Humans have used As compounds as insecticides, vermicides, fungicides, herbicides, and for homicidal purposes, both covertly (food laced with arsenious oxide) and overtly (the war gas lewisite). At present As is only used in some herbicides, the other roles being filled by more effective agents (Penrose, 1974).

5.1.1 Arsenic in the environment

Numerous natural sources and human activities introduce arsenic in soil, food and drinking water, and As contamination is widespread. Figure 1 shows the forms of arsenic that are pertinent to living systems.

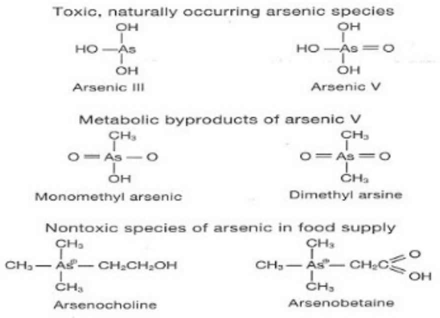


Figure 1: Biologically significant arsenic species

The most abundant forms of arsenic in the environment are the inorganic As^{V} and As^{III} species, and only the organic species monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA) can be found in detectable concentrations (Takamatsu *et al.* 1982, Tlustoš *et al.* 2002). The form in which As is present in the environment influences its chemical behaviour and its toxicity. Generally, As^{III} is more mobile and more toxic than As^{V}, and inorganic arsenicals are more toxic than organic arsenicals (Fowler 1977, Adriano 2001).

The primary natural sources are geothermal volcanic activity (Figure 2) and leaching from As-rich rocks. Human activities include the timber treatment industry, which treats wood with CCA (copper, chromium, arsenic) compounds to prevent decomposition, sheep dipping (Figure 3), mining and sewage sludge application to agricultural land.

Figure 2: Geothermal activity (Craters of the Moon, NZ)

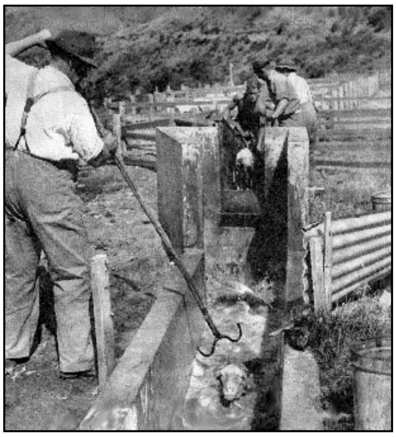


Figure 3: Sheep-dipping

5.1.2 Arsenic in soils

In soils, As has a high affinity for oxide surfaces, and its behaviour is most commonly controlled by surface complexation reactions on oxides/hydroxides of Al, Mn and especially Fe (Inskeep *et al.* 2002). Since sorbing oxides/hydroxides are generally concentrated in the clay size fraction, these smaller textural fractions contains higher sorbed and total amounts of As (Lombi *et al.* 2000); hence, fine textured As-contaminated soils are less toxic then coarse textured ones (Jacobs *et al.* 1970).

Soil pH plays a role in As mobility on soils, and the two forms of arsenic show opposite reactions to changes in acidity: As^{V} solubility increases upon pH increase within pH-ranges commonly found in soils (pH 3-8), whereas As^{III} tends to follow the opposite pattern (Manning and Goldberg 1997, Smith *et al.* 1999, Tyler and Olsson 2001, Raven *et al.* 1998, Jain and Loeppert 2000).

Redox potential influences speciation and solubility of As. In aerobic conditions (i.e. high redox potential), inorganic As is mostly present as As^{V} in soil solution, which is desorbed from adsorption sites upon pH increase: in flooded soils, the main form is As^{III}, due to the low redox potential of this environment. Under reduced conditions, As solubility is increased due to the dissolution of Fe and Mn oxides/hydroxides (Masschelyn *et al.* 1991, Marin *et al.* 1993, McGeehan and Naylor 1994).

Finally, redox potential and pH are two important parameters controlling the forms and behaviour of As in solution; reduction of As^{V} to As^{III} results in enhanced bioavailability and toxicity to plants (Carbonell-Barachina *et al.* 1999, Marin *et al.* 1993).

Some other geochemical processes can lead to an increase of As concentration in groundwater. Carbonate ions form stable complexes with As^{III} and As^{V} in solution; this will lower the activity of free As ions in solution and will tend to increase the solubility of any As minerals and cause the desorption of As from oxides.
Reduction of Fe^{3+} leads to the (partial) dissolution of Fe^{III} oxides and the release of Fe^{2+}. This will lead to the co-release of both adsorbed solutes (including As) and structural (solid solution) components.
Anything that leads to an increase in the concentration of strongly sorbed anions such as phosphate, silicate and bicarbonate could lead to the desorption of As^{III} and As^{V} through competitive sorption reactions (BGS).

Arsenic mobility and bioavailability in soils can also be influenced and modified by bacteria or by adding soil amendments.
Metal accumulation by plants can be significantly enhanced with the application of metal chelates to the soil (see section 5.3.3), but in this case great care must be taken to avoid a contamination of the soil and groundwater by the complexing agent and the solubilised metal.
Only a few studies have been done on the use of microorganism to improve the plant-metal uptake from soils, but it is possible to enhance the phytoextraction efficiency by populating the rhizosphere with selected microorganisms (Garbisu and Alkorta, 2001).

5.1.3 Arsenic in aquatic systems

The background level of As in the aquatic environment is 1.7 ng g^{-1} (Boyle and Jonasson, 1973). Ground waters tend to have higher As concentrations than surface waters. The As contents of rocks in an area has a large bearing on the As concentrations in the waters and sediments of nearby lakes and rivers.

There are some organisms in aquatic environments that accumulate or change the form of As, including several species of bacteria that reduce As^{V} to the more toxic As^{III}. Some aquatic macrophytes accumulate the element and may act as a sink for arsenic in the aquatic environment. Arsenic in the aquatic environment may be transported to the terrestrial and marine environments and vice-versa by a number of mechanisms. These include flooding, leaching / runoff, irrigation, biotic transport, and flowing into the sea.

5.1.4 Arsenic and human health

Arsenic poses a threat to human health, since it is toxic and carcinogenic. The consumption of contaminated water or food can result in cancer, hyperkeratosis (Figure 4), effects on the nervous system, heart and circulatory systems (ATSDR 2000). For this reason, WHO (World Health Organisation) set a guideline value for As in drinking water at 10 $\mu g\ l^{-1}$ and at 2 $\mu g\ g^{-1}$ on a fresh weight basis in food. In New Zealand, the maximum acceptable value (MAV) for arsenic is also 10 $\mu g\ l^{-1}$ (DWSNZ 2000); the standard states that this is the maximum concentration of As that will not cause any significant health risk to a 70 kg consumer over a lifetime consumption of 2 l of water per day.

However, in some regions as West Bengal (India) and Bangladesh, drinking water often contains more than 50 $\mu g\ l^{-1}$ (Figure 5). This is not only a problem if the water is drunk, but also if it is used for cooking (for example, rice can absorb As from the cooking water) or for the irrigation (*arum*, a popular vegetable, showed some high As concentrations) (Bae *et al.* 2002; Meharg and Rahman 2002).
As more contaminated drinking water is found, there is increasing concern about arsenic pollution worldwide.

Figure 4 : Deformity due to As toxicity

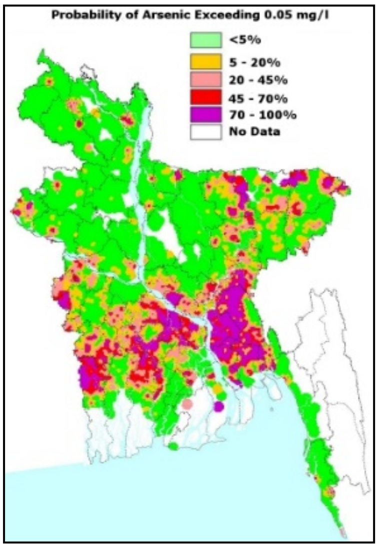


Figure 5: Probability of As concentration > 50 $\mu g\ l^{-1}$ in Bangladesh

Arsenic is a trace element that comes to the environment and enters the food chains naturally and as a result of human activity. It is toxic, and its presence in drinking water and in food poses a treat to human health. Arsenic behaviour in soils and groundwater, and thus its bioavailability for plants, is controlled by some key factors as pH, redox potential and complexation.

5.2 Phytoremediation

Phytoremediation is a potential solution to worldwide arsenic contamination. This section presents a definition and discussion of this burgeoning new technology.

Phytoremediation is the use of plants to improve degraded environments. Research in this field has grown significantly in the last 10 years, as this technique can provide a low-cost way to decontaminate polluted soils and water.

In phytoremediation plants are exploited as bio-pumps that use the sun’s energy to remove water and contaminants from the soil to the above-ground portions or to immobilise pollutants, and return some of the products of photosynthesis back into the root-zone (Figure 6).

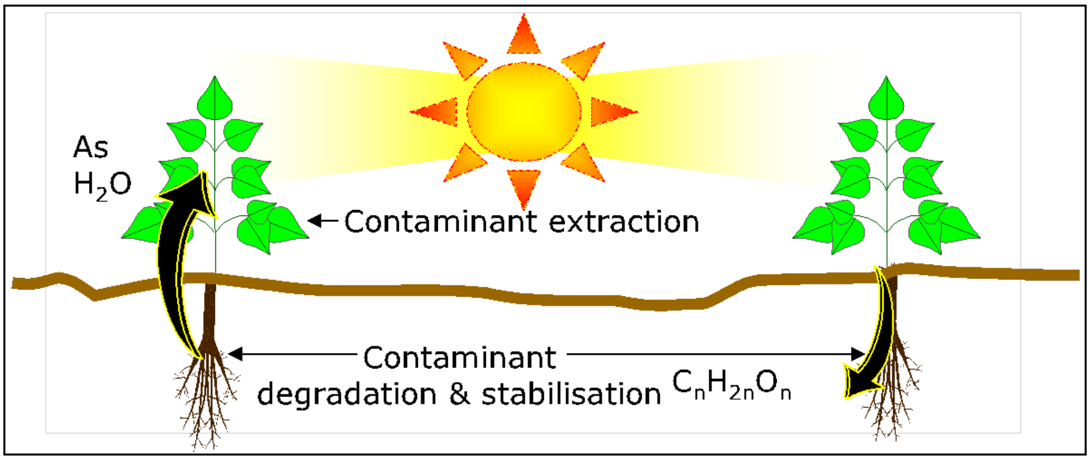


Figure 6: General diagram of phytoremediation

Phytoremediation can be broadly categorised by the way plants are being used.
Phytostabilisation and *hydraulic control* are used to immobilise contaminants by reducing leaching, controlling erosion, creating an aerobic environment in the root-zone, and adding organic matter to the substrate that binds the contaminant. By reducing its mobility, the contaminant becomes less ‘bio-available’ and less likely to enter food-chains.

Phytodegradation, *phytostimulation*, *phytodetoxification* and *rhizodegradation* define the use of plants in association to soil microbiota, to degrade contaminants in the root-zone. Plant-assisted bioremediation is mostly used for the treatment of organic pollutants.

Phytoextraction describes the use of plants to remove trace elements from soils. Phytoextraction can be used to decontaminate soils and waters. This is the focus of the present study and is described in detail below (section 5.3.5). For completeness, however, I include a definition of *phytomining*, a subset of phytoextraction, in this section.

Phytomining was first described by (Nicks and Chambers 1995). It describes the use of plants for economic gain in the mining industry. This operation generates revenue by extracting saleable metals from otherwise sub-economic ore bodies (Robinson *et al.* 1997a, 1997b). No commercial phytomining operations have yet been conducted, although an American company, Viridian Environmental, has patents on the phytomining process. (US patent N. 5711784 & 5944872).

5.2.1 Advantages and limitations of phytoremediation

Phytoremediation has several advantages over other remediation and metal extraction technologies. First, and foremost, is the low cost of phytoremediation, which is, in essence, not dissimilar to normal agricultural cropping practices. Competing technologies such as soil removal, capping and ex situ cleansing can cost around $US 1 M/ha, as compared to an estimated US$ 60,000 - 100,000/ha for phytoremediation (Salt *et al.* 1995a). Other benefits of phytoremediation include the ultimate fertility of the cleansed site, the high public appeal of 'green' technology, and the possibility of producing secondary products that offset the cost of the operation or even produce a small profit.

(Huang *et al.* 1991) and (Pulford 1995) suggested that phytoremediation could be combined with conventional silviculture, as long as the growth of the trees was unimpeded by the soil contaminant. An elevated concentration of contaminants in the wood of the trees is unimportant for human health. Vegetation could also be combusted to produce electricity in a bio-energy operation (Nicks and Chambers 1994). If a metal hyperaccumulator is used, and the metal is of sufficient value, then the metal could be smelted from the plant-ash and resold. Plants that accumulate essential trace elements such as Zn, Co, and B may be used as an organic mineral supplement to crops, livestock or even humans.

Basic plant physiology, nonetheless, limits the scope of phytoremediation. Only surface contamination can be removed or degraded, and the clean-up is restricted to areas that are amenable to plant growth. Most importantly, it may take a long time for site remediation to be effective. Phytoremediation can only be used if it meets environmental regulation during the operation as well as at its end point.

Plants may provide an exposure pathway for the soil contaminants to enter the food chain if the plants are consumed (Tibazarwa *et al.* 2001). This will be particularly relevant if plants that are genetically modified to accumulate heavy metals cross-pollinate with crop species. Care has to be taken to avoid such scenarios that could stifle innovation by adding fuel to the anti genetic engineering lobby (Watanabe 2001).

Plants can be used in different ways to decontaminate soils polluted by trace elements or organic pollutants. Compared to traditional techniques, phytoremediation is cheaper and less invasive for the environment. However, the application of these techniques is still limited by plant physiology. Some ecological and health implications have also been highlighted.

5.3 Hyperaccumulators and phytoextraction for remediation

Hyperaccumulator plants extract inordinate amounts of one or more elements in their above-ground tissues. Phytoextraction describes the use of these plants to remove contaminants from soils and water.

5.3.1 Hyperaccumulators

All plants remove trace elements from their surroundings. Some of these elements are essential for the plant to complete its life cycle. Others may be taken up incidentally, and at high concentrations may have a deleterious effect on the plants' growth.

Some plants take up extraordinary quantities of metals, far more than are required for plant growth. These plants may even accumulate metals that are non-essential and often toxic to plants. The first discovery of one of these plants was made by (Baumann 1885) when he reported that the small herbaceous biennial *Thlaspi calaminare*, found near Aachen, Germany, had a foliar zinc concentration of around 1% on a dry weight basis. This concentration is about two orders of magnitude higher than the mean concentration of zinc in plants (Table 1). The unusual uptake, however, could partially be accounted for by the very high concentration of zinc in the substrate

The second, and even more extraordinary of these discoveries came when (Minguzzi and Vergnano, 1948) observed that a small herbaceous perennial, *Alyssum bertolonii*, from the Impruneta region near Florence, Italy contained inordinate concentrations of nickel. They reported a concentration of 7900 µg g^{-1} nickel on a dry weight basis. This was extraordinary not only because it was over two orders of magnitude higher than plants growing on the same or similar soil (Table 1.) but also because the metal concentration in the plant was actually greater than that of the soil (4900 µg g^{-1}).

The phenomenon of plants accumulating inordinate concentrations of heavy metals was termed hyperaccumulation by (Brooks *et al.* 1977). In this benchmark paper, the minimum concentration of metal a plant needed to contain to be termed a hyperaccumulator of nickel was set at 1000 µg g^{-1} (0.1%) on a dry weight basis. In the case of zinc, the level was later set at 10,000 µg g^{-1} (1%) (Reeves and Brooks, 1983). These values were used because they represent a concentration about ten times greater than the highest levels found in 'ordinary' non-hyperaccumulator plants. (Reeves *et al.* 1995) considered that the minimum level for cadmium hyperaccumulation should be lowered to 100 µg g^{-1} and that the limit for manganese be raised to 10,000 µg g^{-1} to be consistent (Robinson, 1997).

Table 1. Normal elemental concentrations in plants and lower limit for hyperaccumulation. (After Reeves et al., 1995).

Element	Normal Range (µg/g).	Lower limit for hyperaccumulation (µg/g).
Arsenic*	0.01 – 5	1000
Cadmium	0.03 - 20	100
Cobalt	0.05 - 50	1000
Copper	1 – 100	1000
Manganese	5 – 2000	10,000
Nickel	0.2 - 100	1000
Selenium	0.01 - 10	100
Thallium**	0 - 0.1	1000*
Zinc	5 – 2000	10,000

*Ma *et al.* (2001)
**Leblanc *et al.* (1997)

At present there are about 400 species of known terrestrial plants that hyperaccumulate one or more of several heavy metals. There has been considerable work done on nickel, an element found in many metalliferous soils, consequently nickel hyperaccumulators make up the majority of known hyperaccumulator species. In contrast there has been very little work done on thallium, the single study (Leblanc *et al.*, 1997) finding two hyperaccumulators.

5.3.2 Distribution of hyperaccumulators

All known hyperaccumulator species occur naturally on soils or in waters that contain elevated concentrations of the metal they accumulate, though some will accumulate other metals not normally found in the substrate upon which they are found. An example of this is *Thlaspi caerulescens*, which is found naturally with very high zinc and cadmium concentrations. The plant will also hyperaccumulate nickel, however, if grown on a nickeliferous soil (Baker *et al.*, 1994).

Nearly all hyperaccumulator plants are found on metalliferous soils in temperate and tropical regions. An explanation for this distribution was proposed by (Brooks, 1987) who suggested that the time required for the evolution of hyperaccumulator species was longer than the time elapsed since the last Ice Age. Hyperaccumulators usually grow poorly on non-metalliferous soils, and are out-competed by non-metallophyte species. Since metalliferous soils occur in small disjunct locations, hyperaccumulators in areas further from the equator had no refugia when the areas were glaciated during the Ice Ages. Warm temperate and equatorial regions were not glaciated during this period, so metalliferous soils have had a longer time to evolve an endemic flora. The occurrence of hyperaccumulators is largely restricted to metalliferous soils that often support an endemic flora, distinct from surrounding soils.

5.3.3 The process of hyperaccumulation

The hyperaccumulation of metals involves uptake of the soluble metal species by the root system, translocation to the aerial parts, and storage in a non-phytotoxic form in the aerial portions. (Chaney 1997) suggested that this process necessarily requires tolerance to high concentrations of heavy metals, but (Baker *et al.* 1994), considered that the hyperaccumulation event is independent of tolerance mechanisms.

The first stage in metal uptake, is the solubilisation and sequestration of the metal ions and their transport across the root cell plasma membrane into the symplast for subsequent passage into the xylem. Solubilisation may involve acidification of the rhizosphere, reduction of the metal ions present, and the excretion of metal-binding compounds (Raskin *et al.*, 1994). There has been much work on compounds secreted by root systems, which bind to metal ions in the soil solution. The generic name for these compounds is phytosiderophores, and they have been classified into two types: phytochelatins and metallothiones. These compounds may either prevent the metal from crossing the plasma membranes (exclusion) or actively promote it (hyperaccumulation). Recent work has shown that it is possible to induce the hyperaccumulation of lead in non-metallophytes by the addition of the chelating agent EDTA (Huang and Cunningham, 1996; and Blaylock *et al.*, 1997). The addition of this chelating agent not only solubilises lead in the soil (Li and Shuman, 1996) but also allows its passage into the xylem of the root and subsequent translocation to the aerial portions. The plants die soon after the application of the chelating agent, but not before the lead concentration in the aerial portions has reached up to 2% on a dry matter basis. The fact that these plants die indicates that in natural hyperaccumulator plants there must be a detoxification mechanism so that the accumulated heavy metals do not kill the plant. The addition of EDTA to plants growing in non-metalliferous soils, and their survival thereon, indicates that the compound itself is non-toxic. Further discussion on induced hyperaccumulation is done in section 5.3.5.

It is widely accepted that heavy metals within plants need to be in some organic complex to reduce toxicity. Uncomplexed metal ions are more likely to result in enzyme inhibition and free radical production. The metal complex within the plant necessarily has to be of a low molecular weight as metal concentrations can sometimes exceed 3% on a dry matter basis. (Lee *et al.* 1978) found that nickel in New Caledonian hyperaccumulators was present in a citric acid complex. Studies by (Pancaro *et al.* 1978) showed that nickel in *Alyssum bertolonii* was complexed by malate. In contrast, (Krämer *et al.* 1996) demonstrated that free histidine stimulated the nickel chelation in some European species of *Alyssum*. Zinc may be transported into cell vacuoles as a malate complex (Ernst, 1992). It is possible that the metals may have different forms during translocation and storage. Further work needs to be done to elucidate the forms of heavy metal complexes within plants. It seems that citrate and malate may be involved in many of these complexes.

The phytotoxicity of complexed heavy metals can be further reduced by compartmentalisation and storage in some areas of the plant. Most of the zinc in the metallophyte *Thlaspi caerulescens* is found within the vacuoles of epidermal and subepidermal cells (Vázquez *et al.*, 1994). Similarly, the greatest concentration of nickel in some South African hyperaccumulator species is in the epidermal regions of the leaves (Mesjaez-Przybylowicz *et al.* 1994). Storage in the vacuole may reduce interactions between metal and the cells' metabolic processes. Localisation of the metals in epidermal tissues may have less impact than in more metabolically active tissues such as the mesophyll. Epidermal tissues are also the first to be encountered by pathogens and predators. The toxic effects of heavy metals in these tissues may have a protective function. This hypothesis is supported by the observation that the leaves (usually the first organs to be attacked by herbivores) usually contain the highest concentration of metal.

5.3.4 Raison d'être for hyperaccumulators

Most hyperaccumulators have a range restricted to metalliferous soils. Although many species are able to grow on 'normal' soils, they are not found there under natural conditions. This implies that either there is a significant metabolic cost for hyperaccumulation and/or that the presence of a high foliar metal concentration confers some advantage to the plant, without which it cannot survive in a competitive environment. Why then does hyperaccumulation occur? (Boyd and Martens 1992) advanced five hypotheses to account for this process:

1 - tolerance to, or disposal of, the element from the plant;
2 - a drought-resistance strategy;
3 - a means of avoiding competition from less metal-tolerant plants;
4 - inadvertent uptake of heavy metals;
5 - defence against herbivores or pathogens.

(Martens and Boyd 1994), showed that insect herbivores preferentially avoided grazing the nickel hyperaccumulator *Streptanthus polygaloides*. They attributed this to the toxic effects of nickel, and suggested that hyperaccumulation conferred a competitive advantage due to reduced grazing. This theory was further supported by the findings of (Pollard and Baker 1997), who found that herbivores were deterred by high foliar zinc concentrations in *Thlaspi caerulescens*. Although there is mounting evidence that the fifth hypothesis is a factor in some species, little or no work has been conducted investigating the other four.

5.3.5 Phytoextraction for phytoremediation

Phytoextraction describes the use of plants to remove metals and other contaminants from soils. This technology involves the repeated cropping of plants until the soils' contaminant concentrations have reached acceptable levels. After each cropping, the plant biomass is removed from the area and may be burned to reduce its volume whereupon it can be stored in an appropriate area, such as a contained landfill, that does not pose a risk to the environment (Figure 7).

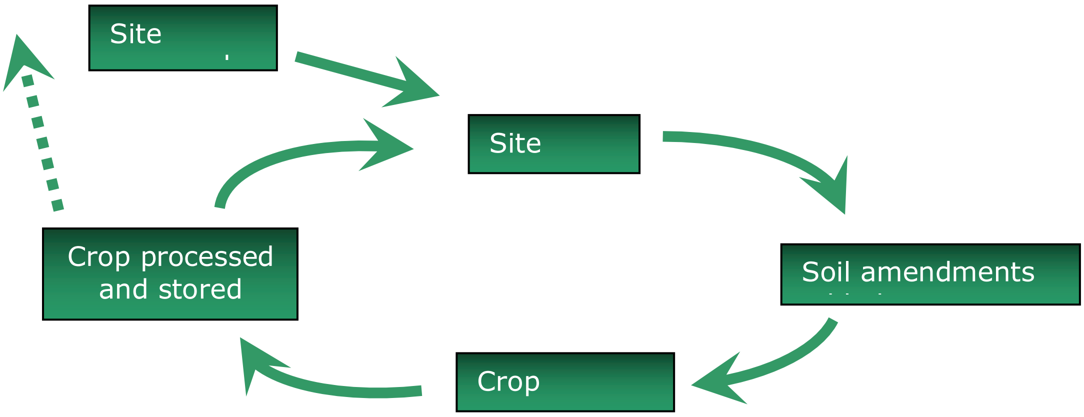


Figure 7: Simplified phytoremediation scheme

Phytoextraction relies on plants that translocate inordinate amounts of one or more contaminants into their above ground biomass, such as the hyperaccumulator plants that are described above.

The mass of metal that can be removed by a single crop sets a practical limit on the speed of any phytoextraction operation. While hyperaccumulator plants can achieve a high metal concentration in their biomass, their biomass production is usually inferior to non-hyperaccumulator plants.

For some common metals, such as Pb, there are no reliable reports of any hyperaccumulator species. A possible solution is the use of induced hyperaccumulation. Non-hyperaccumulator plants can be made to take up metals such as Pb, or even Au, by the addition of solubilising agents to the substrate (Huang and Cunningham, 1996; Blaylock *et al.* 1997; Anderson *et al.* 1998). Such additions increase the mobility of the metal in the soil, allowing it to be taken up more easily by the plant. At the same time, however, there is the possibility that some of the metals might leach through the soil profile, possibly entering groundwater (Lombi *et al.* 2001). Physical soil processes such as preferential flow may exacerbate metal leaching (Bundt *et al.* 2000), and these soil amendments may also persist in the environment creating additional and unforeseen problems. Environmental concerns may limit the use of induced hyperaccumulation to hydraulically isolated treatment sites where the connection to receiving waters has been "broken" (Robinson *et al.*, 2003).

More promising work is being conducted where high biomass plants are being genetically altered to extract larger amounts of metal from soils (Rugh *et al.* 1998), or where the potential biomass of smaller varieties of hyperaccumulator plants is being improved (Ow *et al.* 1998). (Dhankher *et al.* 2002) demonstrated that *Arabidopsis thaliana* could be engineered to accumulate As by inserting two bacterial genes that imparted tolerance and the ability to translocate arsenic to the aerial portions. The soil's microbiota plays a crucial role in plant – metal tolerance and uptake (Whiting *et al.* 2001) and can be manipulated to enhance plant uptake (Nie *et al.* 2002).

Phytoremediation also can also be applied to contaminated water. Rhizofiltration is a process that consists in using plant-roots to extract pollutants from water (Dushenkov *et al.* 1995); in this case, metals only need to be absorbed by plant roots, that can easily be removed from a hydroponic system.

The objective in any phytoextraction operation is to remove the maximum quantity of metal from the soil in the minimum of time. This implies using a species with a high biomass production and a high metal content. The plant used should be able to perform under the local conditions. Temperature, competition and intolerance to pests or pathogens may also prohibit the growth of plants out of their natural range. Plants to be used over many hectares should be easily propagated, preferably by seed that does not require pretreatment. A profuse root system maximises plant-soil contact and therefore the ability to extract large quantities of trace-elements. Harvesting should be possible using existing agronomic techniques. Care should be taken to avoid the plant itself from becoming a weed. Finally, the use of the plant for secondary products (wood, metal), may offset the cost of phytoextraction (Garbisu and Alkorta 2001).

Phytoextraction is based on the use of hyperaccumulator plants. These plants can accumulate large amounts of toxic elements by combining several processes, but the reason for doing this is still unknown. The possibility to achieve phytoremediation using non-hyperaccumulator plants, or to increase the efficiency of the existing hyperaccumulators, was the aim of several projects conducted over the last few years, and represents an important challenge also for the future of this technology.

5.4 Arsenic hyperaccumulation, the foundation for As phytoremediation

In order to develop an arsenic phytoextraction technology, As hyperaccumulators must be identified. The present knowledge about arsenic phytoremediation is presented in this section.

Although As is not an essential element for plants, some species have been found that hyperaccumulate this toxic element in their tissues. Some aquatic macrophytes growing in the Taupo Volcanic Zone (TVZ), North Island, New Zealand have been reported to contain high concentrations of arsenic (Reay, 1972; Liddle, 1982). (Robinson *et al.* 1995) reported >1000 μg g^{-1} (ppm) arsenic (dry weight) in samples of *Egeria densa* and *Ceratophyllum demersum* growing in the Waikato River system. A survey of watercress (*Rorippa nasturtium*) growing in the region revealed concentrations as high as 1766 μg g^{-1} in the dried leaves (Robinson *et al.*, 2003). This is of concern to human health because watercress is consumed as a vegetable. On a fresh-weight basis, nearly all the samples tested were above 2 μg g^{-1}, the World Health Organisation (WHO) limit for arsenic in foodstuffs (Brandsetter *et al.*, 2000). Plant-accumulation of arsenic may also facilitate the entry of this toxic element into the food chain.

(Ma *et al.* 2001) reported As concentrations in the Brake fern, *Pteris vittata*, of up to 2.2% on a dry matter basis: this is the first terrestrial species reported to take-up arsenic to concentrations greater than 1000 μg g^{-1}, the threshold for plant to be considered a 'hyperaccumulator' (Brooks *et al.*, 1977). A survey of Thailand's vegetation lead to the identification of another hyperaccumulator fern, *Pityrogramma calomelanos*, which accumulated up to 8000 μg g^{-1}. In the same study, an analysis of *Pteris vittata* showed an As concentration of 6000 μg g^{-1} (Visoottiviseth *et al.* 2002). Another study carried on different ferns species highlighted the fact that three ferns of the *Pteris* genus hyperaccumulate As. (Zhao *et al.* 2002) found three new *Pteris* hyperaccumulators: *P. cretica*, *P. longifolia* and *P. umbrosa* reached 6200-7600 μg g-1 on a dry weight basis.

Arsenic uptake by plants is thought to be associated with the phosphate uptake mechanism, where presumably arsenate is taken up as a phosphate analogue (Khattak *et al.*, 1991; Meharg and Macnair, 1991; Pickering *et al.*, 2000). (Dhankher *et al.* 2002) demonstrated the importance of arsenate reductase and γ-glutamylcysteine synthetase in the tolerance and accumulation of arsenic.

Many of the physical process or As accumulation are still not understood: however, in aquatic plants it is has been shown that they accumulate trace elements by absorption followed by passive or active transport across membranes (Forstner and Wittman 1981, Smies 1983).

Arsenic is not essential for plants, but some species can hyperaccumulate this element. Previous research identified some aquatic and terrestrial species able to take up As from soil or water. Further research focuses on the search for new hyperaccumulators, but also on the understanding of the mechanisms involved in this process.

5.5 Basis for study and aims

New Zealand has strict bio-security laws that proscribe the importation of exotic species, and for this reason the use of *Pteris vittata* for field decontamination projects cannot be proposed without a rigorous and lengthy risk assessment. Anyway, New Zealand has a very rich fern flora, with more than 50 endemic species, including relatives of *Pteris*. New Zealand also has geothermal areas that have naturally elevated levels of As in soils and water. The possibility exists, therefore, that some of New Zealands native fern flora may also hyperaccumulate As. Five aquatic species have also been found to hyperaccumulate arsenic in these geothermal areas.

The aims of this study, therefore were to determine:

a) The arsenic uptake by 5 different species of native ferns grown in soils with three different concentrations of arsenic in a green-house experiment.

b) The arsenic concentration in a range of previously untested aquatic macrophytes and terrestrial plants grown in the TVZ, and relate the concentration of arsenic in the plants to the concentration of arsenic in water, soils and sediments.

6 MATERIALS AND METHODS

In this chapter, the sampling and the analysis methods are explained for the two parts of the study. Section 6.1 presents the greenhouse trial conducted with ferns; section 6.2 is about the survey of TVZ vegetation.

6.1 Greenhouse trial

6.1.1 Preparation of soil and ferns growing

Shade house experiments were performed at HortResearch, Palmerston North, New Zealand (40.2 °S, 175.4 °E). Five species of native ferns (*Asplenium bulbiferum*, *Blechnum discolor*, *Histiopteris incisa*, *Pneumatopteris penningera* and *Polystichum vestitum*) as well as watercress (*Rorippa nasturtium*) were grown in 15 l buckets with varying As additions as described in Table 1. The species involved in this experiment are described in appendix A.

The soil used was Manawatu silt loam, with a pH of 4.4, total organic carbon 6.3% and an exchange capacity of 13.4 cmol(+) kg-1 Soils were prepared by adding varying amounts of a 1% As solution to ca 15 kg of soil and mixed using a concrete mixer (Figure 8). The arsenic was added as As_2O_3 dissolved in 1M KOH.

Soils were fertilised with Nitrosol^{TM} liquid fertiliser at a rate recommended by the manufacturer. Planting occurred at the end of October 2002, some six weeks after preparation of the pots. This delay was to allow time for the added As to come to equilibrium with the soil. The pots were protected from the sun with a shade cloth (a black mesh) above the plants (Figure 9). Plants were watered at least every two days, according to evaporative demand. Pots were arranged in a randomised block design within the shade house. Samples were taken for chemical analysis in mid-January, 2003, 10 weeks after planting.

Figure 8: Soil preparation

Figure 9: Pots layout in the greenhouse

In January 2003, about 10 weeks after plantation, ferns and watercress samples were collected. Sample preparation and As determination are detailed on section 6.2.3.

6.2 Arsenic concentration in plants from TVZ

6.2.1 Study area

The Taupo Volcanic Zone (TVZ) covers an area of 6000 square kilometres in the central North Island, New Zealand. It extends from White Island south-west to Mount Ruapehu in a long narrow belt (Figure 10). Appendix C gives more details about all the country.

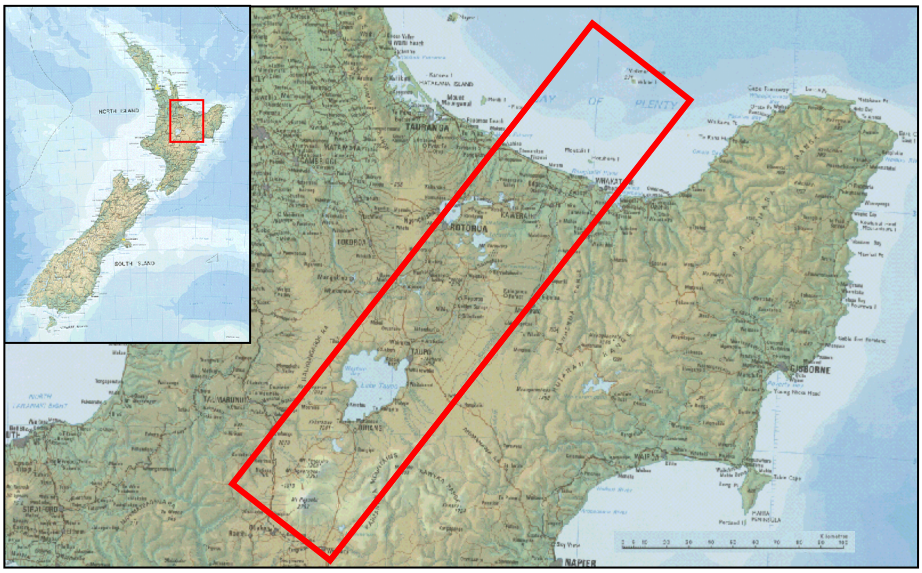


Figure 10: The Taupo Volcanic Zone (adapted from Land Information New Zealand)

The area features five active volcanoes, from north to south these are: White Island, Mt Tarawera, Mt Tongariro, Mt Ngauruhoe and Mt Ruapehu. The area is rich in hydrothermal activity, abounding in hot springs, geysers and mud pools. Such areas occur in specific fields of geothermal activity.

The Taupo Volcanic Zone contains over 32 natural lakes, including Lake Taupo the largest lake in New Zealand, and Lake Rotorua. New Zealand's longest river, the Waikato, flows out of Lake Taupo, through several geothermal fields and north westward to the Tasman sea. Seven hydro-electric power dams have been built to exploit the 366 m fall to the sea (Robinson 1994).

Geothermal activity in the Taupo Volcanic Zone (TVZ), North Island, has resulted in elevated As concentrations in some waterways and associated lands (Liddle 1982). This As is derived from geothermal activity to some extent exacerbated by commercial exploitation of geothermal power (Figure 11) (Robinson *et al.* 1998).

Figure 11: Ohaaki geothermal power station (Broadlands, NZ)

On the basis of what was discussed so far, known sources of arsenic pollution in the TVZ are; (1) As arising from naturally occurring geothermal activity (Smith, 1985), (2) geothermal bores that release As-rich water into the aquatic biosphere, these include the geothermal power stations, (3) runoff of As-based pesticides (Hill, 1975), (4) Arsenic from timber treatment sites such as the pulp and paper mill at Kinleith, and (5) As added to lakes to control weeds; e.g. sodium arsenate added to Lake Rotorua (Robinson 1994).

6.2.2 Sample collection and pre-treatment

Plant samples were taken at several sites (Figure 12) within the TVZ in November, 2002. Plants growing in soils as well as plants growing in water were taken. Only leaves and stems were taken for analyses. It is well known that leaves usually have greater metal concentrations than stems (Brooks, 1998). The goal of the present, study however, is to survey the vegetation for plants that could be used for the phytoextraction of As. Here we are not interested in the distribution of arsenic in the plant, rather the total amount of As in the above-ground portions (leaves + stems). Furthermore, separating leaves and stems would increase the time and cost of the study unnecessarily.

Samples were also taken from Pukaka Stream and Spring Creek near Blenheim, South Island, New Zealand (Figure 12, B zone). Pukaka Stream has been reported with to have arsenic concentrations up to 8 µg l^{-1} during times of low-flow due to arsenic rich rocks near its source. Spring Creek was used as a control, and has no known arsenic contamination.

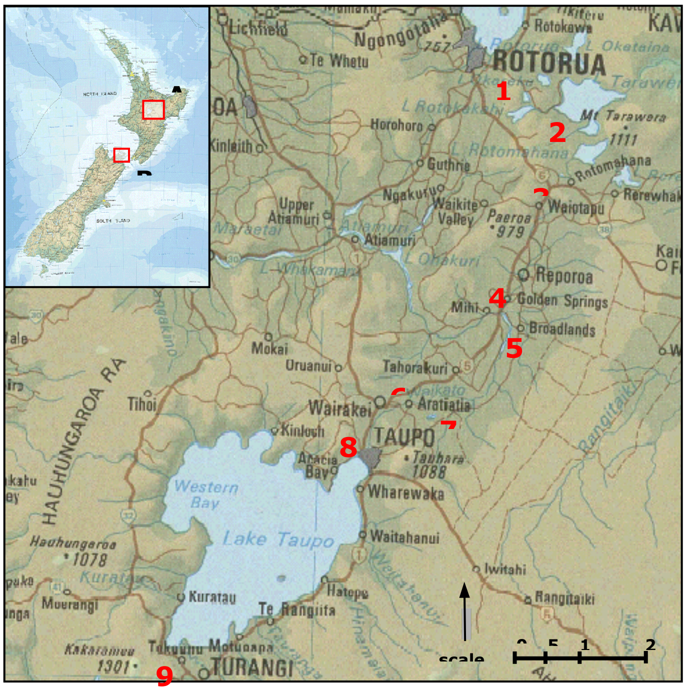


Figure 12: Sampling sites in the TVZ (adapted from Land Information New Zealand)

Legend of the sampling sites:

A	Taupo Volcanic Zone
1	Whakarewarewa Thermal Village
2	Rainbow
2	Kerosene Creek
3	Waiotapu Wonderland
4	Waikato River
5	Broadlands
6	Lake Aratiatia
7	Lake Rotokawa
8	Craters of the Moon
8	Tokaanu
B	Blenheim region

Leaves were collected from the terrestrial plants, while the whole specimens of the aquatic plants were taken (Figure 13). Water, sediment, and soil samples were also collected from the aforementioned sites, and stored in 100 ml polythene bottles, respectively in sealed plastic bags. Plant material was washed thoroughly using tap water and placed in plastic bags until return to the laboratory, where further washing in distilled water was carried out; the washed samples were stored in paper bags for drying.
The different species collected in the TVZ are described in appendix B.

6.2.3 Sample preparation and As determination

Plant material, sediments and soils were placed in a drying cabinet at 80ºC until a constant weight was reached. Plant material was ground using a mortar and pestle. Sediment and soil samples were sieved to <1 mm size using a nylon sieve and stored in sealed plastic bags; later on, they were digested as follows.
Between 0.15 and 0.2 g of ground plant material, or 0.5 g of sieved sediment and soil from each sample was accurately weighed into 50 ml Erlenmeyer flasks. 10 ml of concentrated nitric acid (69%) were added to each flask and the mixtures heated and evaporated on a heating block until a final volume of ca. 3 ml was reached (Figure 14). The samples were then diluted to 10 ml using distilled water and stored in polythene containers.

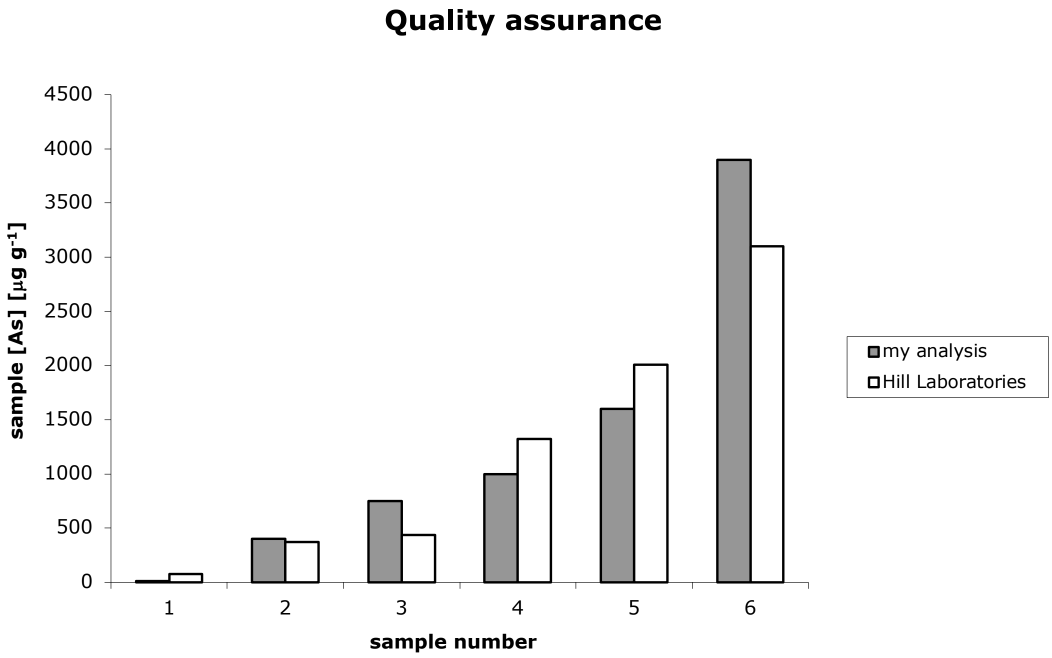
Figure 13: Sample collection

Figure 14: Flasks on heating block

*Figure 15**:** GFAAS*

All digested samples of plant material, sediment, and waters were analysed for arsenic at Massey University, Palmerston North, using a GBC 909 AA graphite furnace atomic absorption spectrometer (Figure 15). This method has the advantage to be much more sensitive than flame atomic absorption spectrometry (typically 100 times), because the ground-state atoms remain longer in the graphite tube during reading: the residence time is of seconds compared to microseconds (Brooks 1998). More sensitive methods such as hydride generation atomic adsorption and inductively coupled plasma mass spectroscopy were unavailable and in any case are very expensive. The limit of detection was about 10 µg l^{-1} for waters and 500 µg kg^{-1} for plant, sediment and soil samples.

Six samples were sent to R.J. Hill Laboratories, Hamilton (NZ), for quality assurance using the more sensitive technique of hydride generation atomic absorption spectroscopy. Graph 1 shows a comparison of the results.



Graph 1 : Results for quality assurance

7 RESULTS AND DISCUSSION

In this chapter, the results of the analysis are presented and discussed in relation to the results found in the literature. The next section is about the green house trial involving ferns and watercress. Section 7.2 focuses on the survey of the vegetation growing in the TVZ.

7.1 Greenhouse trial

In this section, the results obtained for the analysis of ferns and watercress from the greenhouse trial are presented. Some hypothesis are made about the reasons that could have prevented the ferns and the watercress to accumulate As.

All the ferns grew in all the treatments and there were no signs of As toxicity. I did not measure the final biomass of the plants because they will be used for further studies at HortResearch**.**

The As concentrations in the soils and ferns from the greenhouse experiment are shown in Table 1. The raw data are given in appendix D.
None of the fern species or the watercress had foliar As concentrations greater than 0.5 μg g^{-1} dry matter (d.m.), my detection limit for arsenic, even at the highest soil concentration of 105 μg g^{-1}.

Table 1: Summary of the results obtained with the greenhouse trial

Species	**Soil [As] (μg g^{-1})**	**Plant [As] (μg g^{-1})**
Asplenium bulbiferum	55	< 0.5
	105	< 0.5
Blechnum discolor	55	< 0.5
	105	< 0.5
Histeropteris incisa	55	< 0.5
	105	< 0.5
Pneumatopteris penningera	55	< 0.5
	105	< 0.5
Polystichum vestitum	55	< 0.5
	105	< 0.5
Rorippa nasturtium	55	< 0.5
	105	< 0.5

The concentration of arsenic of the all the soils in the pots was measured before and after the experiment, to verify the presence of the contaminant and any possibly leaching. The arsenic concentrations among each treatment were consistent, and a comparison between the initial and the final concentrations showed that there had been no detectable leaching of As out of the pots.

None of the ferns tested has potential for As phytoextraction because they failed to accumulate significant amounts of arsenic from the soil. Watercress (*Rorippa nasturtium*), which is known to accumulate As in aqueous media, did not show the same characteristic when grown in the soil, where all the plants were below detection limits for arsenic.

The low plant uptake in the green-house experiment may be explained by low As bioavailability in the soil. Arsenic bioavailability in soils is a key factor for the uptake of this element by plants. Even if the concentration of As in the soils were high, it is possible that the element was strongly bound to the soil particles, and thus relatively unavailable for plant uptake. The relatively acid soil pH (measured at 4.4 in my experiments), that ferns favour (Metcalf, 1993), may render As less available than at higher pHs (Manning and Goldberg, 1997; Smith *et al.* 1999; Tyler and Olsson, 2001; Raven *et al.* 1998). Arsenic bioavailability may be further reduced by As binding to clay particles and organic matter in the soil.

When comparing my experiments with those of (Ma *et al.* 2001), who showed *Pteris vittata* could hyperaccumulate As from a soil containing less As than my highest treatment, it is clear that the ferns trailed in my experiment have no specialised As uptake mechanism. The arsenic hyperaccumulator, *Pteris vittata*, has the ability to solubilise As in the root zone, then translocate it to the aerial tissues. This mechanism must overcome the relatively low bioavailability of arsenic in the soil.

HortResearch will periodically analyse the ferns used in these experiments to determine if plant development results in increased As uptake. In December 2003, a soil amendment such as a chelating agent could be added to determine if As uptake can be induced.

None of the five species of ferns used for the greenhouse trial accumulated As. The same result was found for the watercress, although this species is known to hyperaccumulate As when growing in aqueous media. Arsenic was presumably bioavailable for the plants, so this doesn't appear as a key factor for the results I obtained. Probably the species I choose have no specialised as uptake mechanism.

7.2 Survey of New Zealand vegetation in the TVZ

All the plants, soils and water collected in the TVZ have been analysed as explained in section 6.2. Data have been treated to extrapolate some conclusions on the basis of the analysis results.

Section 7.2.1 summarises the results obtained for all the samples collected in the TVZ. The arsenic accumulation by terrestrial plants is discussed in the next section. Section 7.2.2 focuses on the aquatic plants, that showed the best potential for As hyperaccumulation; some advises on the use of these plants are given in sections 7.2.4 and 7.2.5, and the ecological implications of this methods are discussed in the last two sections.

7.2.1 Summary of the results

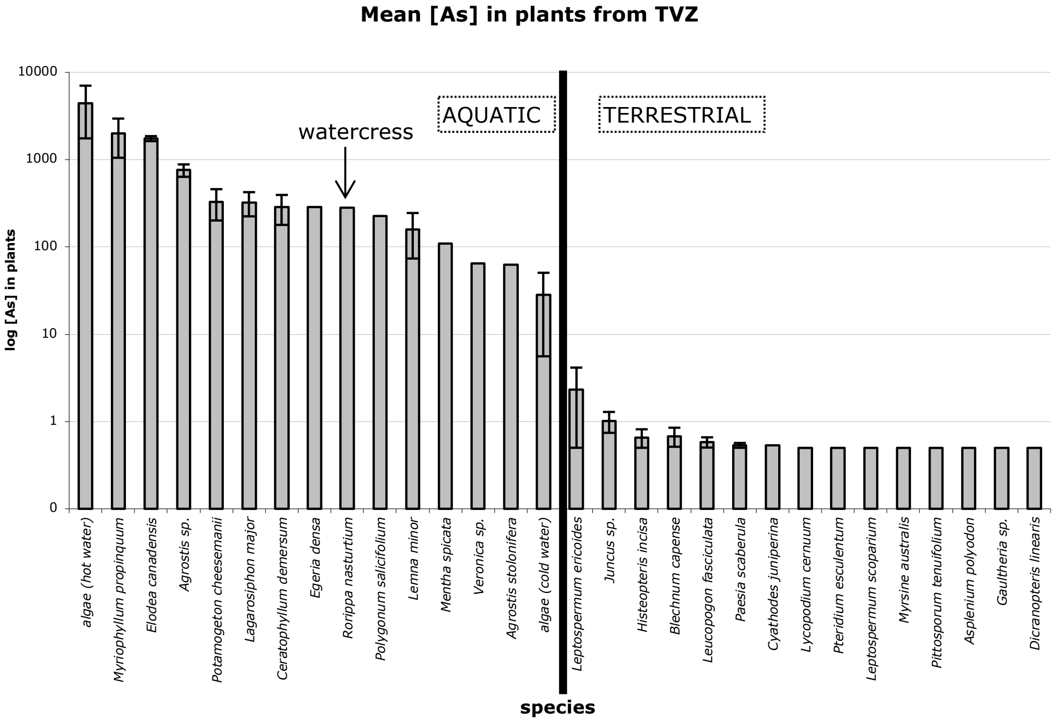
Table 2 shows the average arsenic concentrations in the soils, sediments, waters, as well as aquatic and terrestrial plants from the TVZ.

Table 2: Average arsenic concentrations in samples from the Taupo Volcanic Zone

Sample type	**No. of samples**	**[As] μg g^{-1}**
Soils	4	50 (23 – 89)
Terrestrial plants	51	1 (<0.5 – 11)
Waters	25	0.056 (<0.01 – 0.358)
Sediments	10	895 (35 – 4470)
Aquatic plants	43	775 (0.5 – 9632)

Table 2 clearly shows that soils sediments and waters from the TVZ have arsenic concentrations far above the background level for arsenic in the environment (Boyle and Jonasson 1973). The average arsenic concentration in the water samples was over five times higher than the New Zealand Drinking Water Standard (0.01 μg l^{-1}). There was a clear demarcation between terrestrial plants, that accumulated negligible concentrations of As, and aquatic species that had arsenic concentrations several times higher than the ambient soils or waters whence they were taken.

Graph 2 shows the mean arsenic concentrations in all the plants tested from the TVZ. All the results are reported in appendix E.



Graph 2: Mean [As] and standard error of the mean of all the plants collected in TVZ

The graph clearly displays the difference on As accumulation between aquatic and terrestrial plants. Aquatic plants, grouped on the left hand side, had As concentrations up to 4000 µg g^{-1} on a dry matter basis (d.m.). By contrast, terrestrial plants, on the right hand side, showed much lower As concentrations. The graph has been plotted using a logarithmic scale, to allow variation in all the results to be visible. Standard errors of the mean (= standard deviation / square root n) are often very high, due to the environmental heterogeneity such as pH, temperature and ambient arsenic concentration.

All the aquatic plants tested accumulated As to concentrations greater than 5 µg g^{-1}on a dry matter basis, and none of the terrestrial plants tested had arsenic concentrations surpassing 11 µg g^{-1}. Most of the terrestrial plants tested were below the detection limit for arsenic (0.5 µg g^{-1}), even when growing in soil containing up to 89 µg g^{-1}As.

The difference in the behaviour between aquatic and terrestrial plants was observed by (Outridge and Noller 1991) in their review of hyperaccumulation of elements by aquatic plants. However, they did not provide an explanation of this phenomenon, and several factors may concur simultaneously to give this result (see sections 5.1.2, 5.3.3, and 5.4). For instance, in terrestrial systems, the solubilisation of As in the rhizosphere is necessary to allow the plant roots to take up this element and transport it in the aerial parts of the plant. This is not the case when the plant grows in an aqueous medium (Brooks 1998).

7.2.2 Arsenic accumulation by terrestrial plants from the tvz

None of the terrestrial plants tested accumulated more than 11 μg g^{-1} As, even though the average soil As concentration was over 50 μg g^{-1}. Unlike our green house experiments, surface contamination of the plants by dust cannot be ruled out, even though they were washed thoroughly before analysis. Therefore the true As concentrations in the terrestrial plants may be even lower than I have reported.

There have been no previous studies reporting on As accumulation by terrestrial the plants I tested in the TVZ, and therefore I cannot compare my results with those of other studies. The results clearly indicate, however, that none of the species tested have potential for As phytoextraction.

As discussed above, As bioavailability in soil plays a key role in plant-uptake and may explain the low plant As concentrations that I found. The pH of the soils tested ranged from 2.9 to 5.6, indicating that at least some of the soils would have a pH favourable for arsenic solubilisation.

7.2.3 Arsenic accumulation by aquatic plants from the tvz

Sixteen different species of aquatic plants were tested, and all of them gave higher results than the terrestrial plants. On average, a species of *algae* growing in the water of Tokaanu had the highest concentration of As: more than 4000 μg g^{-1} on a dry matter basis (range 1252 – 9632 μg g^{-1}.) Two other species were found to accumulate more than 1000 μg g^{-1} As on average, *Myriophyllum propinquum* (mean 2000, range 974 - 3900 μg g^{-1}) and *Elodea canadensis* (mean 1743, range 1628 - 1857 μg g^{-1}). There have been no previous reports of arsenic hyperaccumulation by these two species. Nine other species that have also not been reported in the literature as arsenic accumulators, were found to have arsenic concentrations between 6 μg g^{-1} and 800 μg g^{-1} on average.

Previous studies of aquatic plants from the TVZ (Liddle 1982; Robinson *et al.* 1995; Robinson *et al.* 1998) focused on four species: *Ceratophyllum demersum*, *Egeria densa*, *Lagarosiphon major* and *Rorippa nasturtium*. These previous studies reported average arsenic concentrations in *C. demersum, E. densa*, *L. major* and *R. nasturtium*, were 400, 500, 300, 400 μg g^{-1} respectively. Although sampled at different locations, the results in my study were in good agreement, being 300, 300, 500, and 300 μg g^{-1} respectively.

Our study also determined arsenic levels in 12 species that had not previously been analysed. I found that *M. propinquum*, *E. canadensis* and a hot water *algae* from Tokaanu all accumulated arsenic to levels greater that 1000 μg g^{-1} d.m., the threshold for arsenic hyperaccumulation. Additionally, *Agrostis sp*., *Potamogeton cheesemanii*, *Polygonium salicifolium*, *Lemna minor*, and *Mentha spicata* all accumulated more than 100 μg g^{-1} d.m. Although not technically hyperaccumulators, arsenic hyperaccumulation by these species may be environmentally significant because they represent a food source for animals and, in the case of *Mentha spicata*, for humans.

Unlike the terrestrial plants tested, that may have shown elevated As concentrations due to dust contamination, the As concentrations found in the aquatic plants is due to plant-uptake rather than contamination by waters or sediments. This is because the As concentration in the plants was higher than the environments where they were growing (see next section). Therefore any waters or sediment contamination of plant material would result in an apparent lower plant–As concentration.

Watercress, a plant that grows in both the aquatic and terrestrial environment, only accumulated arsenic in the former. This implies that watercress has no specialised mechanism for translocating As to the above-ground tissues. It also raises the question as to whether As is absorbed or adsorbed. Arsenic may simply bind to the cell surfaces of aquatic plants, indicating that there is no specialised As-uptake mechanism. However, in such a scenario, the plants would still need to have a tolerance mechanism for As.

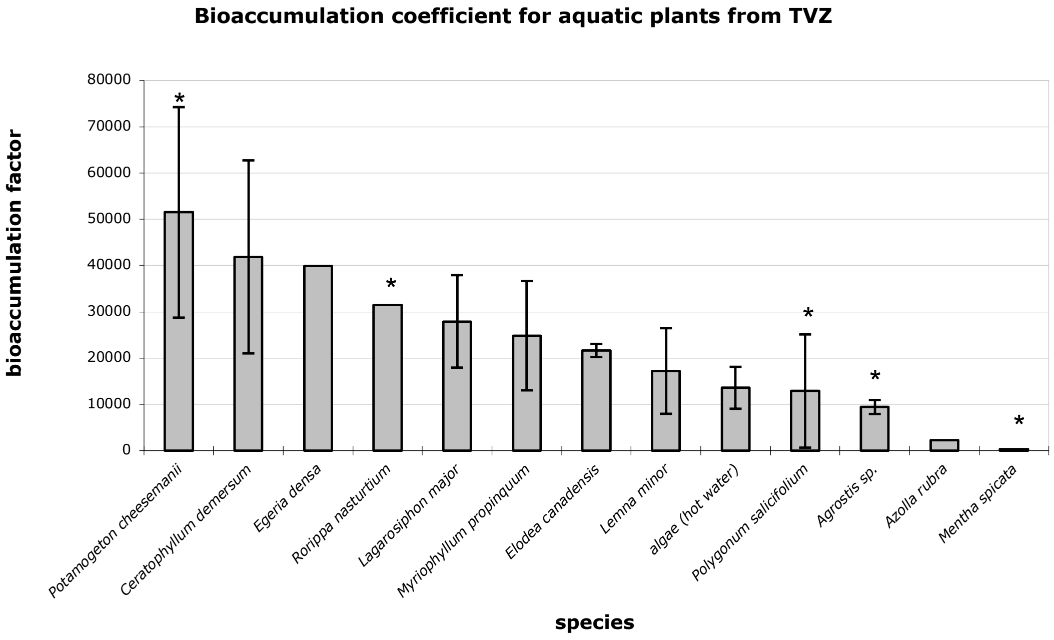
7.2.4 Bioaccumulation coefficient

The concentration of As in the plant doesn’t tell everything about its efficiency in extracting the element from soil or water, because the substrate arsenic concentration partially determines plant-uptake. It is therefore necessary to compare the concentration of As in the plant to the concentration found in the environment in which the plant is growing. It seems logical that a plant growing in a medium rich in As will contain a higher amount of this element in its tissues, but for the phytoextraction one interesting characteristic of the plants is their ability to extract As even if this is only found in very small amounts in the substrate. The *bioaccumulation coefficient* is defined as the ratio of the concentration of As in the plant and the concentration of As in the medium the plant is growing in (Robinson *et al.,* 2000):

$$\text{bioaccumulation coefficient} = \frac{[\text{As}]\,\text{plant}}{[\text{As}]\,\text{environment}}$$

The average bioaccumulation coefficients for the aquatic plants tested in my experiment are shown in Graph 3. The raw data about As concentration is soils, sediments and water, as well as the bioaccumulation coefficient are given in appendix F. The results presented here show only the plant/water bioaccumulation coefficient. Some of the aquatic plants tested (marked with an ‘*’) were rooted to sediments that had an As concentration that was many times the level of the water, but still less than the plant concentration (see appendix F). There is no evidence of a strong correlation between the fact that the plant is rooted or free-floating, and its ability to take up As. Of the plants having a bioaccumulation coefficient higher than 20’000, only two are rooted, against five free-floating weeds.

The bioaccumulation coefficients for the aquatic plants in this study are higher than any previously reported for terrestrial species, but are comparable to those calculated using previous studies on aquatic plants (Reay, 1972; Liddle, 1982; Robinson *et al.,* 1995; Robinson *et al.,* 2003).



*Graph 3: Bioaccumulation coefficient and standard error for aquatic plants from TVZ (note: * = rooted plants)*

The ranking of the bioaccumulation coefficients is different from the results for the total As concentration in the plants: the first 5 positions of the bioaccumulation coefficient ranking are occupied by plants ranked between 5 and 9 for total As concentration. Plants such as *M. propinquum, E. canadensis* and the hot-water *algae* may have accumulated high concentrations of As because they were growing in very polluted water, not only because they are particularly efficient in metal uptake. On the contrary, plants that didn't accumulate high total concentrations of As may be more useful for phytoextraction, as they may be more efficient on metal uptake. Insufficient samples of each species were taken to determine the effect of ambient arsenic concentration on the bioaccumulation coefficient. Studies on terrestrial plants have shown that bioaccumulation coefficients tend to decrease as the ambient concentration increases (Robinson *et al.,* 2000). It is also not possible to categorise these plants as "hyperaccumulators, indicators or excluders" according to the responses proposed by (Baker *et al.* 1984). Previous work on *Ceratophyllum demersum* shows that this plant behaves as an "indicator". This is the subject of the next section.

7.2.5 Aquatic macrophytes for the bio-indication of As

The high bioaccumulation coefficients of the aquatic plants tested may make them good candidates for the bioindication of As contamination in aquatic systems. A bio-indicator is an organism or a set of organisms whose biological response towards different environmental factors gives information about the present condition and/or the evolution of an ecosystem.

In the case of aquatic plants, the pertinent biological response is high bioaccumulation coefficient. The arsenic concentration in the aquatic plants is important for two reasons.

The first is that As is difficult and expensive to measure at low concentrations. Sophisticated techniques such as Hydride Generation Atomic Absorption Spectroscopy and Inductively Coupled Plasma Mass Spectroscopy are beyond the reach of villagers who live in developing countries. By contrast, concentrations greater than 500 μg g^{-1} can be easily measured using a low-cost colorimetric test. Low-cost testing kits could, for example, be provided to determine the As concentration in aquatic weeds that grow in water used for the village. If the test were positive then the water should not be used for drinking, cooking or irrigation. Even in developed countries, where expensive techniques are available, analyses of aquatic plants may provide information on very low concentrations in the water at the pg ml^{-1} (parts per trillion) level. This information could be useful for biogeochemical surveys.

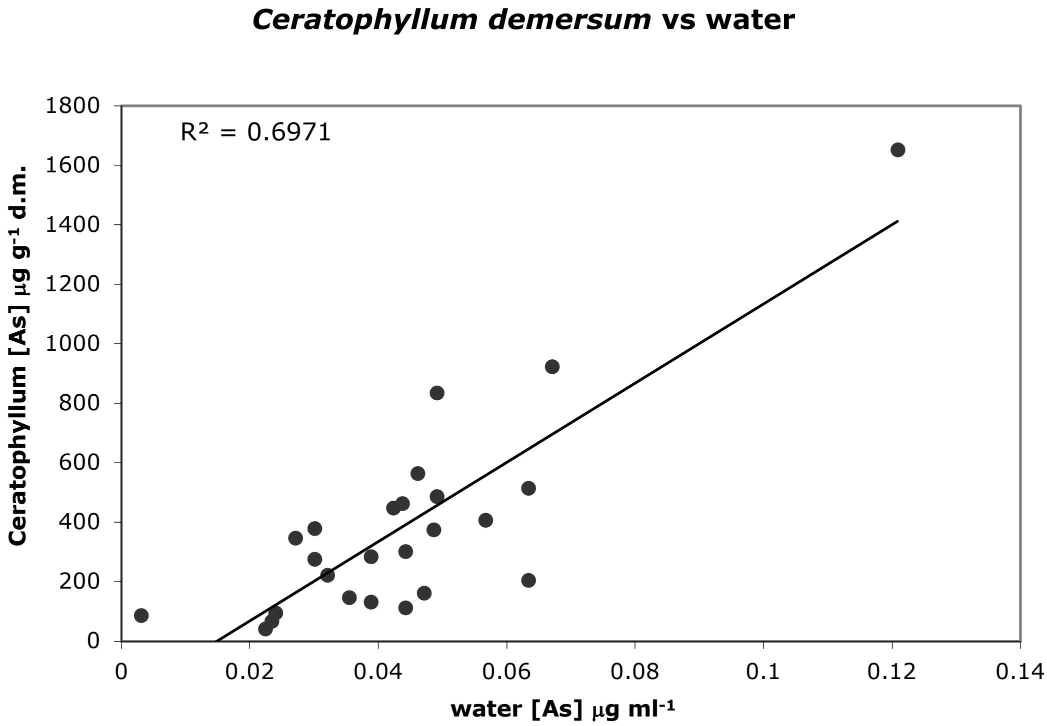
The second reason for using aquatic macrophytes as bioindicators is that they provide information on the history of arsenic in the water, rather than a 'snapshot' at the time of sampling. Arsenic levels in water can vary due to rainfall, evaporation, or sporadic contamination, for example a chemical spill. Analysing aquatic macrophytes may elucidate any historic As contamination, even if current levels in water are within acceptable limits.

An example of using aquatic macrophytes in this role is given by plants collected near Blenheim, South Island, New Zealand. Analysis carried on water samples collected from Pukaka Stream and Spring Creek showed As concentrations lower than our detection limit of 5 ng g^{-1}, although Pukaka Stream has been reported to have As concentrations up to 8 ng g^{-1} during low-flow times. Analysis of *E. canadensis* collected in Pukaka Stream showed an average arsenic concentration of 2.5 μg g^{-1} on a dry matter basis, a result that indicates the presence of elevated As levels in the water, if not at the time of sampling, then certainly in the past. *E. canadensis* taken from Spring Creek had <0.5 μg g^{-1} As indicating the absence of significant quantities of arsenic.

Generally, bio-indicators should be easily identified, abundant and widely distributed (for a better comparison between different sites); a large size, limited mobility and a have long life cycle are other important characteristic. Aquatic macrophytes such as the hornwort, *Ceratophyllum demersum* fill all of these criteria, with the possible exception of mobility. This plant is free floating and may migrate downstream in times of flood.

Arsenic concentration data for *Ceratophyllum demersum* and ambient water was reprocessed from a previous study (Robinson *et al.,* 1995) to show the relationship between plant As concentration and that of the ambient water (Graph 4). Raw data are on appendix G. There was a highly significant correlation (r^2=0.70, P<0.001) indicating that the As concentration of the plants could be used to predict the As concentration of the river water. Moreover, the plants (average [As] 412 μg g^{-1}) contained around ten times the As concentration of the sediments (average [As] 46 μg g^{-1}) that, in turn, had over 1000 times the As concentration of the ambient water (average [As] 0.042 μg g^{-1}). In this case, *Ceratophyllum demersum* provides an excellent bio-indicator for As.

Other factors, such as the pH, water temperature and nutrient availability will doubtless affect As accumulation. Further work needs to be carried out on these effects to determine how well these bioindicators would perform under various scenarios. This study and earlier studies analysed whole specimens of *C. demersum* rather than stems and leaves. Leaves and stems could well have different arsenic concentrations and hence different bioaccumulation coefficients.



7.2.6 Aquatic macrophytes for the phytoremediation of As contaminated water

The high bioaccumulation factor of aquatic plants may make them useful for the phytoextraction of As from contaminated drinking water. The possibility of using plants to remove As has been discussed by (Brooks and Robinson 1998). More generally, the use of aquatic plants to remove pollutants from the water has been outlined by (Wolverton 1975), (Wolverton and McDonald, 1975), and (Wolverton *et al.* 1975).

Two methods have been suggested for using plants for the phytoremediation of contaminated water (Brooks 1998).

The first involves the use of free-floating plants growing in monospecific pond cultures. Plants accumulate As until an equilibrium state is reached, and are then harvested. There are several problems related to this method. The main problem is the disposal of the harvested matter enriched with the contaminant; a secondary problem relative to monospecific cultures is the risk of developing some plants disease that can destroy the whole culture.

The second method is set up using rooted plants growing in trickling bed filters. In this case it is hypothesised that metal uptake is caused by rhizosphere microorganisms with just a small contribution of plants roots (Brooks, 1998).

To give an idea of the suitability of aquatic plants for the phytoremediation of contaminated waters, here an example calculation is made, using *Myriophyllum propinquum* from Tokaanu. This species was found to accumulate 2000 μg g^{-1} As on a dry matter basis, from water containing 80 μg l^{-1} arsenic. My experiments indicate that 1 kg of plant material (d.m.) grown in a water containing 80 μg l^{-1} As should contain 2000 μg of As. Thus 1 kg of plant material could halve the concentration in 50,000 l of water that had an initial concentration of 80 μg l^{-1}.

Aquatic plants could, therefore, provide a very efficient means for removing As from contaminated drinking water. Plants would be grown in ponds containing contaminated water, then simply removed and placed in an area where they do not pose a risk to the environment, such as a sealed landfill. Very simple low-cost technology such as this could potentially save lives in developing countries that cannot afford expensive water purification systems.

Much work needs to be done, however, to determine the feasibility of aquatic arsenic phytoremediation. I have made several assumptions in my calculations, that may not apply in differing scenarios.
Firstly, I tested *Myriophyllum propinquum* growing in flowing water containing 80 μg g^{-1}. The bioaccumulation factor may be much less if it is grown in still water. Another problem relating to this is that my calculations assume that the final plant-water concentration ratio is 2000:0.004, which is twice the observed ration of 2000:0.008.
Secondly, the biomass production of these plants is unknown. The growth rate is critical in determining the time needed to remove arsenic from the water. Anecdotal evidence indicates that the biomass production is rapid, because these plants are weeds and choke waterways. Nevertheless, experiments need to be done to quantify the biomass production in various scenarios.
Thirdly, As accumulation by *Myriophyllum propinquum* is doubtless affected by other environmental parameters such as water temperature and nutrient availability. It is conceivable that plant uptake could be improved by water amendments.

Even if my calculations overestimate the efficiency of phytoextraction by a factor of 100, and 1 kg of plant material could be used to treat only 500 l of drinking water, then phytoextraction could still be a cost-effective technology, when comparing it to other water treatment techniques such as distillation and reverse osmosis.

7.2.7 Ecological and health implications

Arsenic accumulation by aquatic macrophytes may facilitate the entry of this poisonous element into the food chain. Plants are the primary producers of most food chains, and are consumed by herbivores and omnivores. Humans may be affected directly, if plants such as watercress and mint are consumed, or indirectly when humans consume species that have high arsenic levels due to contamination of the food-chain. Much of the Waikato River is surrounded by farmland and stock may occasionally have access to aquatic weeds that have been left on the banks after a flood. Fishing is also popular in the region. Presumably the fish eat plants and animals that have high arsenic concentrations. However, a previous study (Robinson *et al.*, 1995), showed that arsenic levels in fish were below the WHO limit for arsenic in foodstuffs.

In a lake or river system, the amount of plant-bound As at any one time may be a significant portion of the total amount of arsenic in the river. Therefore if, for example, drought or pesticides kill the plants, then there may be a large pulse of arsenic released into the water as the plants decay. The desorption of arsenic by these plants has not been tested.

7.2.8 Genetic resource

The aquatic plants analysed in this study have the capacity to take up and tolerate high concentrations of an element that is toxic to most organisms. The mechanisms of this uptake and tolerance are unknown, however, they may involve some genetically controlled enzymatic systems (Dhankher *et al.*, 2002). The possibility exists, therefore, that these plants could be ‘mined’ for genetic material that could be used to induce terrestrial plants to accumulate As. Again, further research needs to be done to determine if As accumulation is genetically controlled or simply related to the normal metabolic processes of aquatic plants.

All the aquatic plants accumulate arsenic, whereas none of the terrestrial species do. On the basis of these results, I can suggest some uses for aquatic hyperaccumulators. These can work as bio-indicators of As in waterways, or be used for the phytoremediation of As contaminated waters. Aquatic hyperaccumulators may provide a genetic resource to improve the accumulation potential of terrestrial plants. However, the presence in the environment of this plants rich in As can have some ecological and health implications, related to the accidental release of As or to the entry of this poisonous element into food chains.

8 SUMMARISING CONCLUSION

There is a clear distinction between aquatic and terrestrial plants regarding their ability to accumulate arsenic. Obviously the reported terrestrial arsenic hyperaccumulators have specialised mechanisms that allow them to solubilise, take-up and store As in a non-toxic form. Arsenic accumulation is widespread among aquatic plants, and this study identified three new arsenic hyperaccumulators. More species will undoubtedly be found as further surveying is conducted. The question as to how aquatic macrophytes accumulate arsenic could be partially resolved by conducting microanalyses on the tissues to determine if As is present inside the cells or simply bound to the cell walls.

Arsenic accumulation by aquatic macrophytes may make them valuable tools for the bioindication and phytoremediation of arsenic. Additionally the ecological impacts of arsenic accumulation are likely to be profound. Accidental As release and its entry in food chains can be harmful to human and animal health.

The five species of ferns tested in the greenhouse trial did not accumulate any arsenic. However, new potential species of ferns that hyperaccumulate arsenic probably exist in New Zealand. This country has very rich fern flora, with more than 50 endemic species. Some of this species have been found growing in soils with natural high As concentrations due to geothermal activity.

This study has produced more questions than answers regarding As hyperaccumulation. However, some preferential directions for future developments are well defined.

The development of an effective aquatic phytoremediation system could save millions of lives in countries such as Bangladesh or India, where drinking water with high as levels poses a treat to human health.

Further studies of the mechanism of As accumulation by aquatic plants may enable us to induce terrestrial plants to take up arsenic.

HortResearch is going to continue research into the possibility of As phytoextraction.

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10 APPENDIX

APPENDIX A: List of plants used for the greenhouse trial

APPENDIX B: List pf plants collected in the TVZ

APPENDIX C: Facts about New Zealand

APPENDIX D: Results of the greenhouse experiment

APPENDIX E: Results of the survey of the vegetation in the TVZ

APPENDIX F: Bioaccumulation factor and concentration of As in soil&water

APPENDIX G: Results of previous study on *Ceratophyllum demersum*