# Practical Aspects of Phytoextraction

A thesis presented in partial fulfilment of the requirements for the degree of

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Christopher William Noel Anderson

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Palmerston North
New Zealand

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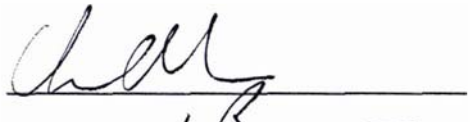
Signed (Candidate):  Date: 18/2/00

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# Abstract

Phytoextraction for heavy metals is an emerging technology that has potential application for the remediation of many contaminated sites around the world. The technology has similar application to the mining of low-grade ore bodies. Several practical aspects of the technology are addressed in this thesis.

Natural and induced-uptake phytoextraction trials have been conducted on two contaminated substrates: an area of industrial pollution in northern France, where base metals are present in an oxide and carbonate mineral phase, and an area of mine tailings in New Zealand, where base metals are present in a sulphide or sulphate mineral phase. The uptake response of several hyperaccumulator and non-accumulator plant species is described. Geochemical models are then presented that explain the observed metal uptake as a function of the predominant chemical form of metal present in the soil. Natural uptake is dependent upon the form of metal. It appears that the relative efficacy of various hyperaccumulator species to accumulate metals is also dependent upon site-specific geochemistry. The efficacy of chelating agents, in particular EDTA, to induce uptake is similarly dependent upon the chemical form of metals in the soil.

A field trial for cadmium phytoextraction was conducted on an area of pastoral land contaminated with this metal due to the application of cadmium to soil through superphosphate fertilisation. Natural uptake at this site by the hyperaccumulator species *Thlaspi caerulescens* could remove the equivalent of 17 years of annual cadmium application in one harvest. The chelating agent EDTA (ethylenediaminetetraacetic acid) did not induce significant uptake by the non-accumulator *Brassica* species. Instead, the action of this chemical was to redistribute 14% of the cadmium initially present in the 0-5 cm soil depth to the 5-10 cm depth, and to leach approximately 4% of the cadmium initially present at the site to below 10 cm in the soil profile, as shown by mass balance calculations. Phytoextraction effected by *T.caerulescens* is proposed as a management tool for cadmium in the pastoral environment.

Phytoextraction for nickel has been investigated at a field site in the central North Island of New Zealand. Hyperaccumulation was effected by two *Alyssum* species and by *Berkheya coddii*. However, the biomass of the harvested plant material was below that reported in the literature. The conclusion from this trial is that substrate modification of ultramafic soil may be necessary before phytoextraction for nickel could be implemented.

A significant obstacle hindering the practical application of phytoextraction in some environments, is the paucity of hyperaccumulator species that are native to some parts of the world. Western Australia has many sites that may benefit from phytoextraction for nickel. However, only one hyperaccumulator species is native to this region, *Hybanthus floribundus*, a species that has in the past been difficult to germinate from seed. This thesis describes a successful approach to germination, involving the use of one-year-old seeds, treated with 'Regen 2000 smoke water' and germinated under dark conditions, that may overcome this practical aspect (a limitation) of phytoextraction technology.

The most recent advance of induced phytoextraction technology has been the thioligand-induced uptake of gold by plants. The initial discovery and the geochemical rationale behind the induced uptake of gold is described. The maximum gold uptake presented is accumulation of 57 mg/kg dry weight gold by *Brassica juncea* and it is proposed that this level of uptake could make the phytomining of gold from tailings areas an economic proposition.

The conclusion of this thesis is that potential for the implementation of phytoextraction is large. Globally, the technology could offer an environmentally and economically friendly alternative to the traditional decontamination of metals from some sites. There is also potential for the phytomining of metals from low-grade ores. The social implications of phytoextraction technology in third-world countries could also be large. Phytoextraction for gold, for example, from auriferous tailings in Africa and South America, has the potential to improve both the environment and the standard of living of the local communities who live off contaminated land.

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I say a big thank you to all the staff and students, past and present, who have supported me through my years in the Soil Science Department. Five years ago I swore I was never going to complete Honours let alone a PhD! The value of the advice, encouragement and assistance that goes with being part of a good department cannot be measured.

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# Chapter 1 - Introduction and Overview of the Current Study

Botanical exploration has identified many plant species that naturally accumulate very high concentrations of elements, including the group commonly referred to as 'heavy metals'. The enormous potential application for the use of some of these species to extract metals from soil (phytoextraction) is being slowly realised.

Phytoextraction is a relatively new scientific term, and came about due to pioneering research conducted during the late 1970s that was led by scientists from New Zealand (e.g. Brooks *et al.* 1974, 1977a, 1977b, 1979). The 1990s saw an explosion in the interest surrounding phytoextraction due to growing concerns over 'heavy metal' contamination of soils around the world. A large volume of scientific literature has appeared during this timeframe, but the technology has not yet advanced to the point where large-scale implementation of working operations has ensued. This statement may be somewhat generalistic as examples of small-scale operations exist, one such example is discussed in Chapter 12, but is generally true. Implementation has been slower than some would have expected.

The aim of the present study was to investigate the potential for phytoextraction in several different environments, and to examine various practical aspects of its implementation as they became apparent; practical aspects that may hinder or promote development of the technology. Research was conducted in parallel for two different groupings of metal and is described in two separate sections. Section A describes the phytoextraction of the metals cadmium, lead and zinc. Section B describes the phytoextraction of the metals nickel and gold.

Chapter 1 serves as an overview of the study, and over the next several pages, the reasoning behind the various practical aspects of phytoextraction studied and described by this thesis are presented.

*Chapter 2 - Phytoextraction: a general introduction*

Before any practical aspects of phytoextraction can be described, the discussion must be prefaced with a review of the relevant literature that defines the current state of phytoextraction technology. Chapter 2 reviews the development of phytoextraction: the reason why studies into phytoextraction have been persued, the discovery of the plants that could effect the necessary concentrations of metal uptake, the reason why these plants accumulate metal, and the practical application of these plants to a contaminated environment.

## Section A - Phytoextraction of cadmium, lead and zinc: observed and modelled uptake

*Chapter 3 - Trials on contaminated substrates*

Two sites contaminated with the metals Cd, Pb and Zn were investigated for this study, two sites that differ in the chemical form of metal that contaminates each environment. Phytoextraction trials conducted on substrates from each site are described in Chapter 3. Phytoextraction is not proposed as a viable method of remediation at either of the described locations, due to the high metal loadings. However, each does allow for the testing of uptake mechanisms and techniques. The metal response of several hyperaccumulator and non-accumulator species to both natural and induced uptake is described.

*Chapters 4, 5 and 6 - Geochemical models for lead, cadmium and zinc uptake*

The results obtained from the trials described in Chapter 3 were surprising when compared to the evidence for Cd, Pb and Zn accumulation found in the literature. To further examine the possible reasons for discrepancies that existed between observed and literature reported uptake, experiments were designed to test the effect of different chemical forms of metal present in a soil on both metal bioavailability and subsequent plant uptake. The mixing of Pb, Cd and Zn mineral salts with a commercial seed-raising

mix generated artificially contaminated soils. Chapters 4, 5 and 6 present, in turn, geochemical models for Cd, Pb and Zn uptake that explain the uptake of each metal as a function of the chemical form of that metal in a contaminated soil.

*Chapter 7 - An integrated geochemical model for Cd, Pb and Zn uptake*

In chapter 7, the results from the previous three experiments are integrated to generate a geochemical model that explains bioavailability and plant uptake of each of these metals as a function of the chemical form, and thus the source of metal contamination, in the soil. This model is then used to explain the metal uptake patterns observed for experiments conducted on the natural substrates of Chapter 3.

*Chapter 8 - Phytoremediation: a possible management solution for New Zealand pastoral soils*

Chapter 8 describes a phytoextraction field trial conducted on an area of agricultural land contaminated with cadmium. The report of this trial is prefaced with a review of the literature describing the problem of cadmium accumulation in New Zealand pastoral soils. McGrath (1998) has previously suggested that pastoral land in Australia, lightly contaminated with cadmium or zinc, could be remediated using hyperaccumulator species. The aim of the trial described in Chapter 8 was to test this theory on a New Zealand site. The results show that natural hyperaccumulation by *Thlaspi caerulescens* effected phytoremediation. Mass balance calculations have been used to show that EDTA application to pastoral land may not induce increased metal uptake, but leach metals down the soil profile.

The results for cadmium uptake described for Chapter 8 are subsequently related to the geochemical model of Chapter 7 in the conclusion to Section A.

**Section B - Phytoextraction for nickel and gold**

*Chapter 9 - A New Zealand field trial for nickel phytoextraction*

Chapter 9 describes a field trial where the phytoextraction potential of several known nickel hyperaccumulators was tested on an area of nickeliferous soil. The aim of this trial was to ascertain the efficacy of nickel phytoextraction, for both phytoremediation and phytomining, in an environment foreign to the hyperaccumulator species used. No ultramafic flora was endemic to this site. Nickel phytoextraction has been proposed for areas of ultramafic soil based upon data generated from controlled pot experiments. However, little work has translated this potential to the field.

*Chapter 10 - Hybanthus floribundus, a native Australian nickel hyperaccumulator*

At the outset of this research programme, one of the aims of the thesis was to test the phytoextraction potential of several nickel hyperaccumulator species on mine tailings in Western Australia. This part of the project was limited by the requirement that only native Australian species could be used, narrowing the choice of hyperaccumulators to one, *Hybanthus floribundus*. However, this species has in the past proved difficult to germinate from seed. The requirement that only native species may be used hampers the implementation of phytoextraction technology in some environments. Chapter 10 addresses this limitation and describes the approaches that I used to overcome the difficulties of seed germination by *H.floribundus*.

*Chapter 11 - Phytoextraction for gold*

Phytoextraction for gold is the most recent advance in the technology of induced phytoextraction. Chapter 11 is prefaced with a review of the literature relevant to the geochemical mobility of gold, before a detailed description of the induced uptake of this metal is presented. My discovery that plants could be induced to accumulate gold was serendipitous: cadmium, lead and zinc induced-uptake experiments on auriferous rock generated unexpectedly high uptake results for this precious metal. The geochemical focus of this aspect of phytoextraction technology is described in Chapter 11.

*Chapter 12 - Practical scenarios for nickel and gold phytoextraction*

Chapter 12 discusses several scenarios where the practical implementation of phytoextraction for nickel and gold may prove viable. Of all the metals for which phytoextraction is possible, nickel and gold hold the greatest promise for phytomining due to a combination of value, and in the case of nickel, known hyperaccumulator species of high biomass. However, sites where phytoextraction of nickel may prove viable are less well documented in the literature than those for Cd, Pb and Zn. The potential for gold phytoextraction has never previously been described.

**Section C: Conclusion**

*Chapter 13 - Practical aspects of phytoextraction: a general conclusion*

Conclusions from the previous 12 chapters are integrated in Chapter 13, and used to review the practical aspects of phytoextraction described by this thesis.

The range of phytoextraction applications described by the 13 chapters of this thesis are broad, but reflect the diverse range of environments in which phytoextraction may prove viable.

**A note on concentration, content and uptake**

Debate surrounds the correct use of the three words concentration, content and uptake. The definition of concentration is simple and in this thesis is expressed as the mass of metal (mg) per unit dry weight (kg) of plant. Content is the total mass of metal (mg) in the plant and is a function of both the metal concentration and the mass (biomass) of the plant. Uptake, however, does not specifically refer to either concentration or content and may be represented by either word. In this thesis I have chosen to express heavy metal uptake by plants using the concentration of metal accumulated by the plant, and hence in this thesis uptake refers directly to metal concentration rather than metal content.

# Chapter 2 - Phytoextraction: a General Introduction

## 2.1 Contamination vs pollution; sources of heavy metal in soil

'Heavy metal' is a loose and ill-defined term used to describe a group of metals that are generally associated with pollution and toxicity (Alloway, 1990). The list includes all the alkali metals, alkaline earth metals and aluminium, as well as the 'metalloids' arsenic, antimony and selenium (Striet and Stumm, 1993).

Heavy metals exist naturally, in all parts of the environment, but the concentration of metal observed varies dramatically. Soils are the weathering products of rocks, and thus the metal loading of a soil will be a function of the parent bedrock. Table 1.1 illustrates this by comparing several heavy metals and the range of these metals that can be found 'naturally' in soil. Soils at the low end of the range are formed from parent bedrock devoid of metal, while anomalous values at the high end of the range owe their origin to metalliferous bedrock. For example, a high nickel concentration can be due to soil development from serpentine rock (see Chapter 10), while a high cadmium concentration can be due to soil development from marine black shales (Peterson and Alloway, 1979) or from sulphide ores (Nriagu, 1978a).

**Table 2.1**. The relative abundance of a selection of biologically significant trace elements.

| Element | Estimated crustal abundance (mg/kg) | Global mean soil concentration (mg/kg) | Range in non-polluted soils mg/kg) | EU limit for soils* |
|---|---|---|---|---|
| Arsenic | 1.8 | 6 | 0.1-40 | - |
| Cadmium | 0.2 | 0.06 | 0.01-30 | 3 |
| Cobalt | 25 | 8 | 1-40 | - |
| Copper | 55 | 20 | 2-300 | 140 |
| Lead | 12.7 | 10 | 2-200 | 300 |
| Nickel | 75 | 40 | 10-1000 | 75 |
| Zinc | 70 | 50 | 10-300 | 300 |

After Peterson and Alloway (1979).
* Maximum concentration allowed in agricultural soils receiving sewage sludge. From CEC (1986), *Official Journal of the European Community* No L181, 6-12 as cited by McGrath *et al.* (2000).

Anthropogenic sources of contamination have dramatically exacerbated the heavy metal loading of soils over recent history. The sources for this metal loading are varied (Fig. 2.1). The temporal increase of metal in the environment is particularly well highlighted by analysing chronological layers of ice from the polar ice caps. The concentration of lead in layers of ice sampled from Greenland and dated at 800 BC was 0.0005 μg/kg (ppb). This rose to 0.2 μg/kg for ice dated from 1965 (Murozumi *et al.*, 1969).

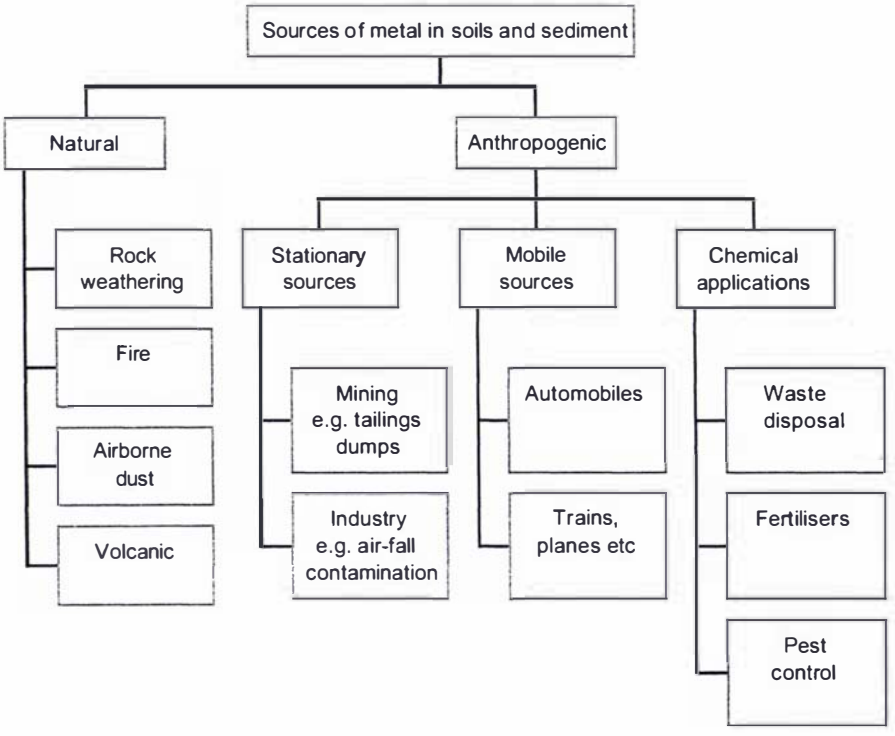


**Figure 2.1**. Sources of metal in soils and sediment. After Nriagu (1978a).

The presence of heavy metals in soil does not necessarily represent a problem to the flora and fauna supported by that land. The two terms pollution and contamination are in this sense often confused. Where metal is present in soil at a concentration above average background (Table 2.1) then that land can be considered as contaminated. Such contamination may be either natural or anthropogenic in origin, although it must be mentioned that no fixed 'threshold criterion' for contamination can be quantified due to the variability of metal concentrations in soils around the world. Where the metal concentration negatively affects the health of flora and fauna living off this land, then the land can be considered as polluted. The two terms should be used carefully and

should not be interchanged. Polluted land is contaminated land, however contamination does not necessarily constitute pollution.

Thousands of sites exist worldwide that can be considered polluted, and as such, means are actively sought for their effective decontamination. A 1993 report tabled by the United States Environmental Protection Agency estimated that more than 300 billion dollars would be needed to remediate 1 235 continental USA 'Superfund' sites contaminated by human activities (USEPA, 1993). Traditional remediation techniques involve, for the most part, excavation and either leaching or disposal of the bulk material. These traditional techniques are costly and energy intensive, and generally simply relocate the mode and occurrence of the metal problem to a more contained site.

Many new and emerging plant-based techniques for the removal and management of heavy metals are beginning to appear. The *phytoextraction* (plant extraction) technologies of *phytoremediation* and *phytomining* have captured public and scientific interest, as they potentially offer a 'green' and environmentally friendly alternative to the traditional decontamination and management of some contaminated sites.

## 2.2 Phytoextraction: phytoremediation and phytomining

During the 1980s, a practical application for plants that accumulate very high levels of heavy metal was recognised, and phytoremediation became defined as the *in situ* remediation of an area of land contaminated with heavy metals using hyperaccumulator plants (Chaney, 1983; Baker and Brooks, 1989). During each growing cycle, these plants accumulate metals in their aerial parts. Harvesting and burning the foliage concentrates the polluting metal into a small volume of ash, called a 'bio-ore', that can be safely disposed of as landfill at a contained site (Kumar *et al.*, 1995). Phytomining is a more recent advance on this phytoremediation technology. Here the target metal is of sufficient economic value to warrant recovery of the metal from the plant ash. Phytoextraction is a term that describes both phytoremediation and phytomining - the use of plants to remove metals from either an area of contaminated land or a low-grade ore body.

The concept of phytoextraction is thus relatively simply to define (Fig. 2.2) and involves 3 steps:

1. growth of plants that can accumulate metals (natural or induced hyperaccumulation) from an area of metalliferous land,
2. harvesting and burning of the metal-rich biomass, and
3. smelting (phytomining) or disposal (phytoremediation) of the ash.

There is a significant financial incentive for the successful use of phytoextraction. Cunningham and Berti (2000) modelled a reduction of the soil-lead concentration at a US contaminated site from 0.14% to 0.04% at a cost of $279 thousand/ha for phytoextraction but almost $2.5 million/ha for conventional technology. Clearly, if these figures are true, phytoextraction may represent a cost-effective option relative to traditional means of soil decontamination. Phytoremediation is well established as a commercial enterprise, offered by various small phytotechnology companies for a limited range of metals, and is regarded by many as a potential growth market in the field of biotechnology. Phytomining, however, has yet to be proven on a large scale.

## 2.3 Natural hyperaccumulation

The ability of some plant species to accumulate inordinately high levels of one or more heavy metals was first reported by Baumann (1885) for zinc accumulation by *Thlaspi calaminare* growing near Aachen, Germany. The first quantitative record for any other metal was made in Italy by Minguzzi and Vergnano (1948), with regard to nickel accumulation by the small perennial shrub *Alyssum bertolonii*. Minguzzi and Vergnano reported a nickel content of 0.79% (7 900 mg/kg) in the dry leaves of plants growing in soil containing only 0.42% of this metal.

It was not until the late 1970s, however, that the 'ability' of certain plants to actively accumulate metals was formally named. Brooks *et al.* (1977a) used the term *hyperaccumulation* to describe this unusual plant character. At the time, the threshold concentration was set at 1 000 mg/kg (0.1%) dry weight (DW) for most metals, with the

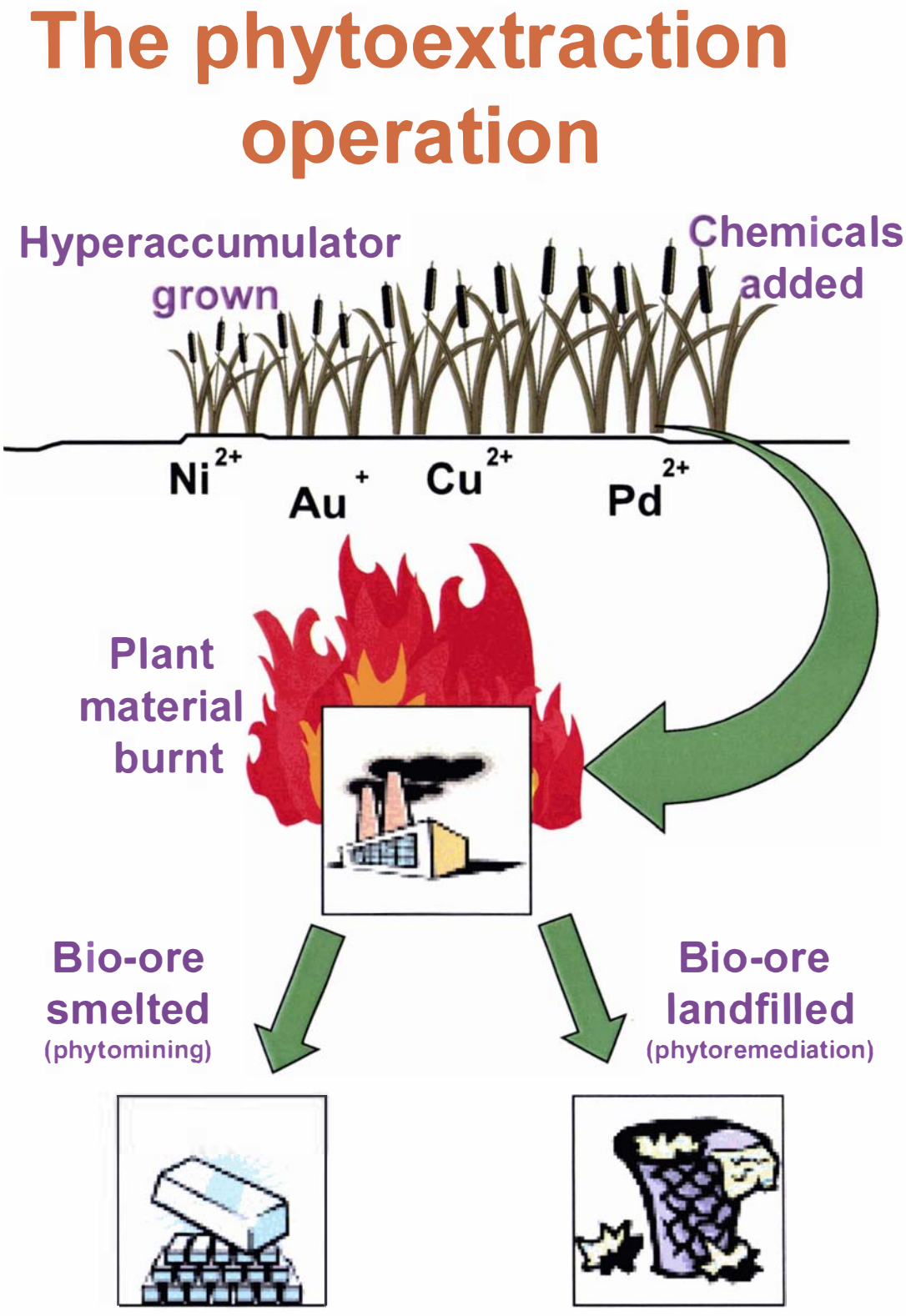


**Figure 2.2.** Diagrammatic representation of the phytoextraction operation.

exception of zinc, for which the threshold was set at 10 000 mg/kg (1%). The modern and more accepted threshold criterion is accumulation 100 times greater than in non-accumulator plants growing in the same environment (Table 2.2). To date there are more than 400 known hyperaccumulator species, of which three-quarters are hyperaccumulators of nickel. The first plant species to be given formal hyperaccumulation status was *Alyssum bertolonii*. It is important to note that the list of species reported in Table 2.2 is in no way restrictive and many more species and metals will undoubtedly be added in the future. Rather, the list reflects the current focus of research into the trait of hyperaccumulation.

**Table 2.2**. Normal elemental concentration, hyperaccumulation criterion concentration and number of known representative hyperaccumulator species.

| Metal | Normal range (mg/kg DW) | Criterion (mg/kg DW) | No. of species |
|---|---|---|---|
| Cadmium | 0.03 – 20 | 100 | 2 |
| Cobalt | 0.05 – 50 | 1 000 | 26 |
| Copper | 1 – 100 | 1 000 | 24 |
| Manganese | 5 – 2000 | 10 000 | 8 |
| Nickel | 0.2 – 100 | 1 000 | 315 |
| Selenium | 0.01 – 10 | 1 000 | 19 |
| Thallium | 0 – 0.1 | 500 | 2 |
| Zinc | 5 – 2000 | 10 000 | 18 |

Source: Baker *et al.* (2000), Brooks (1998), Leblanc *et al.* (1999) and Reeves *et al.* (1995).

The following paragraphs discuss in more detail the reported evidence and importance of hyperaccumulation for the metals presented in Table 2.2.

*Hyperaccumulation of cadmium*

A review of the literature yields only one species that can hyperaccumulate cadmium, the herbaceous biennial species *Thlaspi caerulescens* (Fig 2.3), which can accumulate up to 1 000 mg/kg cadmium DW (Baker *et al.*, 2000). In this thesis the herbaceous metallophyte *Cardaminopsis halleri* is also reported as a hyperaccumulator of cadmium, based upon field observations in northern France (Chapter 3). Near the city of Lille, *Cardaminopsis halleri* is part of a metalliferous floral association indicative of land contaminated by industrial activity. Hyperaccumulation for *C.halleri* is contrary to the observations of McGrath (1998), but is agreement with a paper currently in press (Dahmani-Muller *et al.*, 2000).

**Figure 2.3**. *Thlaspi caerulescens*, a hyperaccumulator of cadmium and zinc growing on base-metal mine tailings, Massey University, New Zealand.

*Hyperaccumulation of copper and cobalt*

Some degree of uncertainty surrounds the hyperaccumulation of copper and cobalt. Brooks and Malaisse (1985) surveyed the Shaba Provence in Zäire (now the Democratic Republic of Congo) and reported 24 hyperaccumulator species of copper and 26 hyperaccumulator species of cobalt (9 species accumulated both metals). However, in subsequent studies these species raised from seed have all failed to hyperaccumulate either metal (e.g. Bennett, 1998), and very little evidence for copper and cobalt hyperaccumulation has been observed outside the Democratic Republic of Congo. Reports of high copper accumulation by *Millota myosotidifolia* (2 400 mg/kg - Blissett, 1996) and by *Mimarta verna* (1 070 mg/kg - Ernst, 1974) are exceptional. Confirmation of the African hyperaccumulation would be desirable but difficult due to the political climate in central Africa.

*Hyperaccumulation of lead*

Debate surrounds the existence of natural hyperaccumulators of lead. Many authors report species that can accumulate large levels of this metal (e.g. Reeves and Brooks, 1983), however, all such reports are based upon field-collected samples where the chance of contamination by an adjacent metal smelter or other airborne sources of lead was high. Replication of this field hyperaccumulation for lead has not been reported under controlled laboratory or greenhouse conditions. For this reason, reported hyperaccumulators of lead are not included in Table 2.2.

*Hyperaccumulation of manganese*

Eight hyperaccumulators of manganese are reported in Table 2.2, a number which is probably in no way indicative of the number of species in nature that hyperaccumulate this metal. Little attention has focussed on manganese, as little environmental or economic emphasis attracts the exploitation of manganese hyperaccumulators for phytoextraction. However, my own correspondence with mining companies in South East Asia has identified waste rock and tailings areas where high concentrations of manganese inhibit the revegetation of these substrates with the native, non-tolerant species, perhaps suggesting a reason for the further study of this metal.

*Hyperaccumulation of nickel*

Considerably more species of hyperaccumulator are known for nickel than for any other metal. This is perhaps due to two reasons. Firstly, the ultramafic soils on which nickel hyperaccumulators are found have a very specific 'ultramafic' flora, and secondly, because of a very effective field test for nickel hyperaccumulation that involves the chemical dimethylglyoxime (DMG). Nickel forms a diagnostic red colour with DMG at concentrations above 1 000 mg/kg DW in the plant (the hyperaccumulation criterion), a property that allows rapid field screening of large numbers of plant species. Ultramafic soils are characterised as being very rich in Cr, Co, Fe, Mg and Ni, and deficient in the essential nutrients Ca, Mo, N, P and K (Brooks, 1987). Ultramafic floras have thus adapted and evolved to withstand harsh environmental conditions. Brooks *et al.* (1995)

reported 188 hyperaccumulators of nickel, although with the recent discovery of additional species in Cuba (Reeves *et al.*, 1996) the total is now 315 (Table 2.2).

*Hyperaccumulation of selenium*

Nineteen hyperaccumulators of selenium have been reviewed by Brooks (1998). With the exception of two species native to Queensland, Australia, all selenium hyperaccumulator species are endemic to seleniferous soils of the USA. An exciting new advancement in the phytoextraction for this metal is the subsequent volatilisation of selenium metal to dimethyselenide vapour by some hyperaccumulator species. Loss of the metal burden to the atmosphere would overcome the problem of disposal of a metal-rich biomass.

*Hyperaccumulation of thalium*

Discovery of plant species that can accumulate inordinately high concentrations of thallium is a relatively recent event (Leblanc *et al.*, 1997). Hyperacumulation of thalium has been defined by Leblanc *et al.* (1999) as accumulation greater than 500 mg/kg DW. The authors of the 1999 study reported a maximum metal content of 0.28% metal in the dry leaves of *Iberis intermedia* growing on mine tailings in the south of France. Thallium is a rare metal in the environment, but is very toxic. Concentrations of 20 mg/kg DW in vegetables and 40 mg/kg for *Brassica napus* have been reported for crops growing in France (Tremel, 1996; Tremel and Mench, 1997; Tremel *et al.* 1997). Concern is mounting over the presence of such high concentrations of thallium in the food chain. Thallium is also a relatively valuable metal (ca. US$300/kg) and this combination of value and toxicity make it attractive for phytoextraction studies. LaCoste *et al.* (1999) discussed the phytoremediation potential of *Iberis* species, while Anderson *et al.* (2000) have discussed the potential for the phytomining of this metal.

*Hyperaccumulation of zinc*

The metal that has attracted the second largest focus of attention for phytoextraction studies (after nickel) is zinc, a phytotoxic metal that will inhibit crop yields before harmful effects are manifest upon the food chain (Shen *et al.*, 1997; Ebbs and Kochian,

1998; McGrath, 1998; Zhao *et al.*, 1998). Sixteen species are known that hyperaccumulate zinc, and two, in particular, have been studied in detail: *Thlaspi caerulescens* and *Cardaminopsis halleri*, species that also hyperaccumulate cadmium. Zinc hyperaccumulator species are in all cases species of low biomass, a factor which limits their effectiveness for phytoextraction. However, this is compensated for, to some degree, by the high concentrations of zinc accumulated (up to 3.5% for *T.caerulescens*). McGrath *et al.* (1993) and McGrath (1998) discussed the phytoextraction potential of the two hyperaccumulator species mentioned here for the remediation of zinc and cadmium concentrations elevated in European pastoral soils as a result of the application of sewage sludge to land. These authors concluded that phytoextraction of zinc (and cadmium) could be entirely feasible using these two species, but only for relatively low levels of soil contamination (e.g. 500 mg/kg zinc and 5 mg/kg cadmium - the European limits for these metals in soil are 300 and 3 mg/kg respectively).

## 2.4 Induced hyperaccumulation

Uptake of metals that are not 'naturally' accumulated by any recognised plant species can still be effected using phytoextraction technology. If a chemical amendment added to soil 'targets' certain insoluble metals, these metals can be 'induced' into the soil solution. Plants often accumulate this soluble metal complex passively through transpiration - induced hyperaccumulation, although the exact mechanisms for induced-metal uptake are poorly understood and a matter of some debate.

The first successful report of induced-hyperaccumulation technology was for lead (Huang and Cunningham, 1996). *Zea mays* (maize) growing on contaminated soil was induced to accumulate over 1% lead in its dry-weight foliage through amendment of the soil with a protonated form of ethylenediaminetetraacetic acid (EDTA). Subsequent studies showed that increased uptake of the metals Cd, Cu, Ni and Zn as well as lead by *Brassica juncea* could be induced using EDTA (Blaylock *et al.*, 1997; Huang *et al.*, 1997). Conflicting reports, however, show that for some plant species, metal combinations, EDTA may actually effect a decrease in metal uptake by the plant. Robinson *et al.* (1997b, 1999a) showed that EDTA reduced the uptake of cobalt and nickel by the hyperaccumulator species *Berkheya coddii*.

EDTA is a well-known chelating ligand and has been used in agriculture since the 1960s as a commercial soil amendment to improve micronutrient availability to plants (Li and Shuman, 1996). In particular Mn-EDTA and $Na_2Zn$-EDTA are used as effective fertilisers (Kabata-Pendias and Pendias, 1984). There are, however, inherent problems surrounding the widespread use of EDTA to increase the uptake of less-desirable metals. The chemical is biodegraded in soil, but relatively slowly (Means *et al.*, 1980). Persistence in the soil, in combination with its efficacy for complexing a wide range of metals, may lead to the leaching of soluble metal complexes into an underlying water table and the subsequent contamination of groundwater. Indeed the principle of leaching for soil remediation was reported by Kobayashi *et al.* (1974 - as cited by Kabata-Pendias and Pendias, 1984) who decreased the cadmium concentration of a surface soil from 28 mg/kg to 14 mg/kg by successive treatment of the soil with EDTA. Tejowulun and Hendershot (1998) reported a similar but more contemporary example. This aspect, or 'environmental concern' regarding the secondary effects of EDTA in soil, hampers the practical application of EDTA-induced phytoextraction.

The only report in the literature of a field trial to test EDTA-induced phytoextraction for lead was conducted by the company 'Phytotech' (now known as Edenspace) in the USA. Blaylock (2000) reports effective soil decontamination of lead-contaminated soil, using a combination of EDTA and *Brassica juncea*, based upon the change in soil lead concentrations observed in soil cores. However, the author does not support his conclusion with figures for the metal concentration within the plants, and more importantly, does not calculate soil mass balances to account for all of the 'remediated' metal. Blaylock's report does not counter the criticism that EDTA may have leached lead to below the soil sampling depth, thus allowing for the appearance of soil decontamination.

Another aspect of induced phytoextraction that is poorly addressed in the literature is the economic cost involved - the chelates used are expensive. In a recent exercise, Rufus Chaney (pers. commn to R.R.Brooks, 2000) calculated that an application rate of 10 mmol EDTA per kg soil (3.52 g/kg) would require 7 040 kg of chelate per hectare. At a unit cost of $4.30 a kg (quoted by Dow Chemicals) the cost of EDTA-induced phytoextraction of lead would be US$30 272 a hectare. An application rate of 3.52 g/kg

has been reported by the Phytotech group as the rate necessary to effectively induce lead uptake.

EDTA is not the only chemical that has been used to effect induced-metal uptake. Huang *et al.* (1997) reported the use of EGTA (ethylenebis[oxyethylenetrinitrilo] tetraacetic acid), DTPA (diethylenetrinotrilopentaacetic acid) and HEDTA (N-(2-hydroxyethyl)ethylenetriacetic acid – a protonated form of EDTA) as well EDTA to effect lead uptake, but EDTA was the most effective. Blaylock *et al.* (1997) included CDTA (*trans*-1,2-cyclohexylenedinitrilotetraacetic acid) and citric acid in their studies, while Cooper *et al.* (1999) report the use of NTA (nitrilotriacetic acid).

Inherent problems notwithstanding, induced hyperaccumulation remains a potentially powerful tool. Natural hyperaccumulator species are generally specific for only one or two metals and in many cases are species of low biomass. Hyperaccumulators of the 2 metals zinc and cadmium, for example, have a biomass of approximately 2 t/ha (*Thlaspi caerulescens* and *Cardaminopsis halleri*). If a high biomass species (e.g. maize with 30 t/ha) could be induced to hyperaccumulate the same concentration of these two metals from a contaminated site, then metal uptake would be increased by a factor of 15. Induced hyperaccumulation also has the potential to effect the phytoextraction of metals for which no natural hyperaccumulators have been recognised. The most recent example of induced hyperaccumulation was that reported for gold (Anderson *et al.*, 1998; Chapter 11), but work is also focussing on induced uptake for the platinum group metals and mercury.

## 2.5 The distribution of hyperaccumulators and reason for the phenomenon

Some metals are essential for living processes and deficiency can lead to poor growth or even death. However, excessive metal accumulation by living tissues is always toxic (Ensley *et al.*, 1997). In contrast, hyperaccumulator species appear to have the unique ability to tolerate a higher concentration of metal than is necessary to perform metabolic functions.

All known hyperaccumulator species occur naturally on metalliferous soils. Robinson (1997) summarised 4 types of soil environment where hyperaccumulation has been observed:

1. serpentine - distributed throughout the world, rich in Cr, Mn and Ni,
2. base-metal (calamine) - distributed throughout the world, rich in Cd, Pb, Zn and Tl,
3. copper-cobalt - found only in the Democratic Republic of Congo, rich in Co and Cu, and
4. seleniferous soils - found only in the USA and Australia and rich in Se.

The presence of a metalliferous soil does not necessarily predicate the presence of hyperaccumulator species. Hyperaccumulators have only been discovered on metalliferous soils present in tropical and temperate regions (Fig. 2.4). This worldwide species distribution indicates that evolution has played an important role in the development of the trait, and that this evolution has occurred over a relatively long timescale. Quaternary glaciations have cleared all record of hyperaccumulator species that may have existed in environments outside contemporary tropical and temperate regions. Hyperaccumulation has subsequently not re-evolved in these recently (10 000 years before present) glaciated environments (Brooks, 1987).

Why plants have evolved the hyperaccumulation trait is poorly understood. Boyd and Martins (1992) summarised a number of possible explanations for hyperaccumulation:

1. tolerance or disposal of metal from the plant,
2. drought resistance,
3. interference with neighbouring plants,
4. inadvertent uptake, and
5. defence against herbivores and/or soil pathogens.

Of these hypotheses, perhaps the most notable is the interference hypothesis. Baker and Brooks (1989) suggested that the hyperaccumulation of nickel might be a 'survival or defence strategy against competition from other plant species'. Boyd (1998) further

described this hypothesis as 'elemental allelopathy'. Metal accumulated by a species, sequested in leaves and then returned to the ground through leaf abscission, will poison the surrounding soil to non-tolerant species. Boyd and Jaffré (1999) reported a higher nickel concentration in the leaf litter and soil beneath the canopy of the New Caledonian nickel hyperaccumulator tree *Sebertia acuminata* than for non-accumulator species, and proposed that this was elemental allelopathy in action. Support for the defence hypothesis was reported by Pollard and Baker (1997), who found that the high foliar-zinc concentration of *Thlaspi caerulescens* acted as a natural deterrent to herbivores. No advancement beyond the theory stage has yet been reported for the possible explanations of drought resistance and inadvertant uptake.

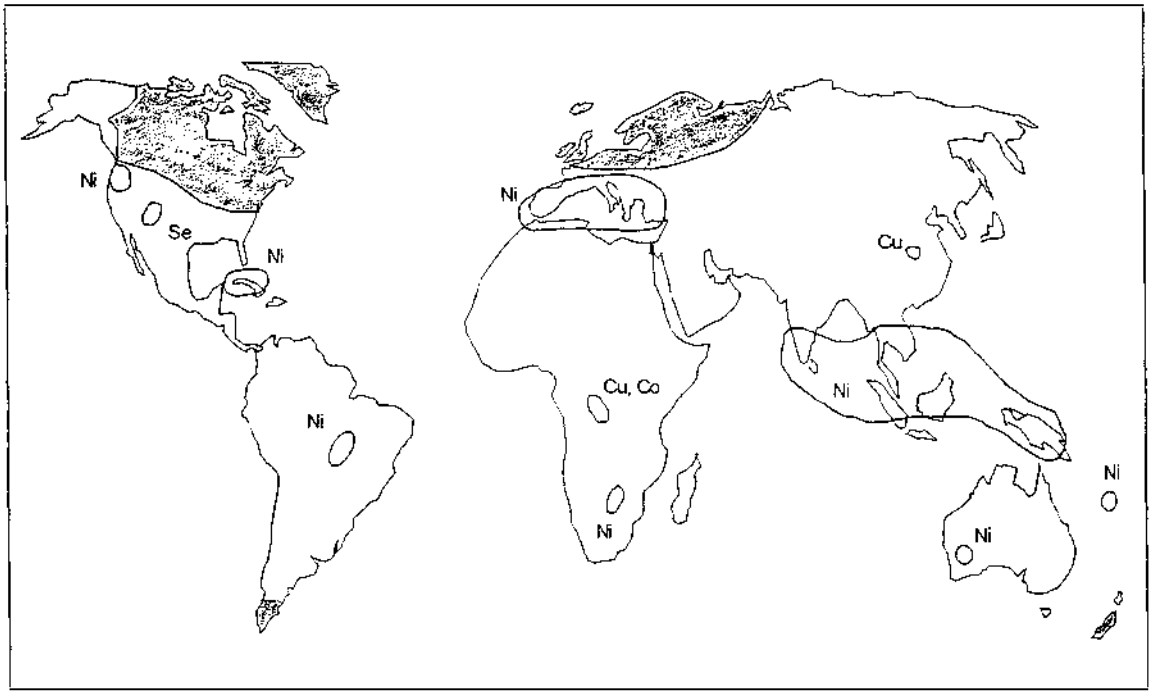


**Figure 2.4.** Map showing the locations of the majority of hyperaccumulators and associated metalliferous soils. Shaded areas indicate the extent of world-wide glaciation at the end of the last Ice Age. After Brooks (1987).

The link between metal tolerance and accumulation in hyperaccumulator species is unclear. Baker *et al.* (1994) and Chaney *et al.* (1997) have suggested that the two are inextricably linked and that hyperaccumulation necessarily requires tolerance. However Macnair *et al.* (1999) showed genetic segregation of tolerance and hyperaccumulation for zinc, exhibited by the F2 generation of *Cardaminop\$is halleri* crossed with

*C.petraea*. Macnair (pers. commn. 1999) has gone further to suggest that the criterion for hyperaccumulation should be re-assessed in light of this new evidence for the independence of tolerance and hyperaccumulation. Macnair suggests that hyperaccumulation should be better defined than simply accumulation 100 times greater than that for non-accumulator plants growing in the same environment. What is clear is that the reason for hyperaccumulation remains speculative. Further research needs to be conducted in this controversial area.

## 2.6 The mechanisms for hyperaccumulation

For uptake to occur, metals need to be solubilised in the rhizosphere and then moved across the root cell plasma membrane for subsequent transport into the xylem (Robinson, 1997). Species that naturally hyperaccumulate metals presumably excrete metal-binding compounds into the rhizosphere to effect this process of accumulation (Raskin *et al.*, 1994). These compounds have been termed phytochelatins and metallothioneins (Grill *et al.*, 1987).

Hyperaccumulation is an active uptake process, as opposed to the passive uptake mechanism true for accumulator (indicator) species. Hence, while the concentration of metal in an accumulator species is a linear function of metal in the soil, the concentration of metal in a hyperaccumulator species is not. Species that exude compounds into the rhizosphere that act to immobilise metals through precipitation are excluders. Induced hyperaccumulation can be considered a passive uptake process - phytochelatins and metallothioneins are artificially created in the rhizosphere through the addition of soil amendments such as EDTA.

Baker (1981) presented a series of theoretical curves to explain behaviour of the three metal-response mechanisms described above (Figure 2.5). The hyperaccumulator curve shows physiological control of metal uptake; the plant has the ability to regulate and block metal uptake above a certain concentration, while the excluder curve shows the opposite physiological response, blocking of metal uptake below a certain concentration, but no control over metal uptake at higher concentration.

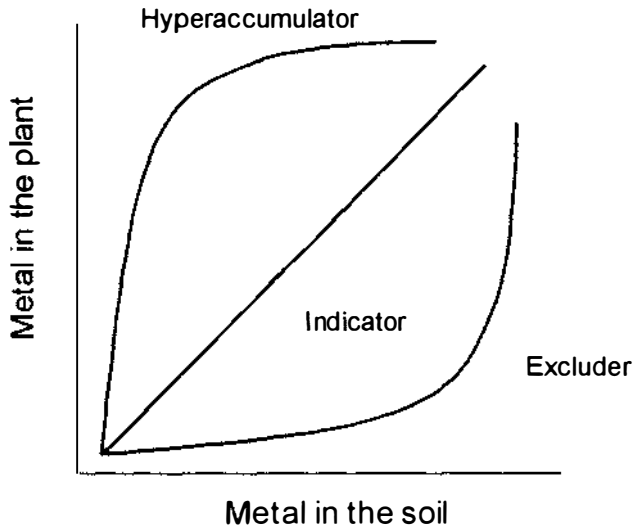


**Figure 2.5**. Theoretical uptake response of plants to heavy metal in soils. After Baker (1981).

Increased knowledge of the mechanisms and reasons behind hyperaccumulation has shown these curves to be unrealistic. McGrath *et al.* (2000) presented a set of three new curves, although as early as 1997 McGrath had disputed the curves of Baker (Fig. 2.6). Baker's curves give the impression that indicator and excluder species will grow in the same soils as hyperaccumulator species, and similarly, that hyperaccumulator species will grow in soils with low metal concentrations populated by indicator and excluder species. McGrath's curves clearly show the shift of thinking with regard to hyperaccumulation and tolerance, as they show the 'death' of indicator and excluder species at a much lower metal concentration than hyperaccumulator species. McGrath's curves also show that hyperaccumulators will not grow in soils of low metal concentration. In support of this change is evidence showing that *Thlaspi caerulescens* has a high physiological requirement for zinc, precluding its growth in soils with a low zinc concentration (McGrath *et al.*, 1997). Evidence presented in this thesis contradicts this theory (Chapter 8).

The work of Robinson *et al.* (1997a,b) showed that McGrath's curves could be further modified (Fig. 2.7). Research into the response of the nickel accumulating species *Alyssum bertolonii* and *Berkheya coddii* to increasing levels of soil nickel indeed showed hyperaccumulator behaviour consistent with McGrath's curves. But Robinson *et al.* (1997a,b) noticed that the curve of the hyperaccumulator species eventually reached

another point of inflection. Presumably this point represents the onset of phytotoxic levels of metal in soil solution, a subsequent breakdown of the physiological mechanisms controlling metal uptake and therefore a flood of nickel into the plant effecting necrosis.

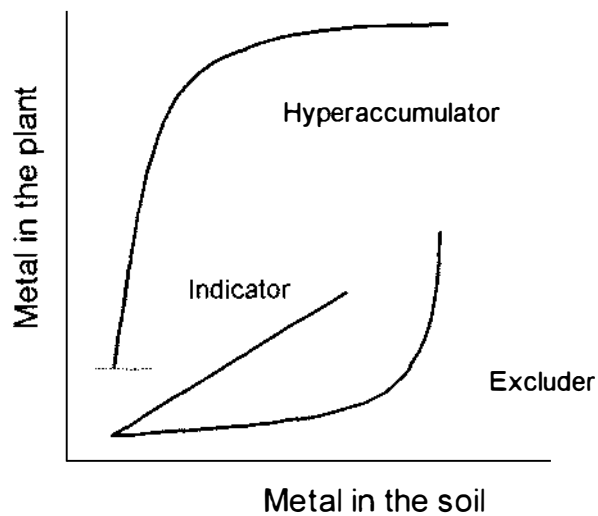


**Figure 2.6**. Theoretical uptake response of plants to heavy metals in soil. After McGrath (2000)

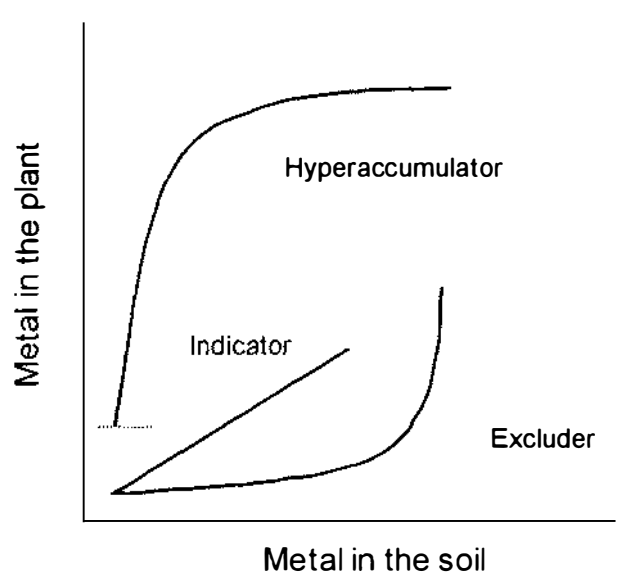


**Figure 2.7**. Modified theoretical uptake response of plants to metals in the soil.

Hyperaccumulator species are not only peculiar for their ability to accumulate metals within living tissues, but also for their ability to transport metals from roots to shoots.

Many authors have studied the biochemistry of fluid extracts sampled from hyperaccumulator species, particularly from nickel-accumulating plants. Lee *et al.* (1977) found nickel to be complexed with citric acid in the sap of many New Caledonian hyperaccumulators. Pancaro *et al.* (1978) showed the nickel in *Alyssum bertolonii* to be complexed with a 1:1 molar ratio of malic and malonic acids. Stockley (1980) deduced that the biochemistry of the Zimbabwean nickel hyperacumulator *Personia metallifera* was more complicated than that of other species, although nickel still appeared to be associated with carboxylic acids. Homer (1991) further confirmed the association of nickel with citric and malic (carboxylic) acids. Recent work (Homer *et al.*, 1997; Krämer *et al.*, 1996) has shown that nickel is also complexed with amino acids within the xylem. The limited studies on zinc hyperaccumulator species suggest an association of this metal with citric acid (Godbold *et al.*, 1988). The picture beginning to emerge is that amino acid complexes are involved in the transport of metal within a plant, but that carboxylic acids are involved with metal storage (Brooks, 1998).

The question that is now posed is 'what happens to the metal complex?' as metals need to be sequested in a non-toxic form. Zinc accumulated by the hyperaccumulator species *Thlaspi caerulescens* is stored within the vacuoles of epidermal and subepidermal cells (Vázquez *et al.*, 1992), while nickel in the South Africa hyperaccumulator *Senecio coronatus*, is stored in the epidermal regions of this species leaves, stems and roots (Mesjasz-Przybyłowicz *et al.*, 1994). The tissues where metals have been found to be sequested are physiologically inert. That is to say, storage in these regions would allow a plant's critical biological machinery to operate in relative isolation of toxins (Boyd, 1998).

A mechanism for nickel hyperaccumulation was proposed by Morrison (1980) based on the work of Still and Williams (1980). The first component of this model is a 'selector' (S) molecule, confined to the root membrane. Presumably the selector exudes phytochelatins and metallothiones into the rhizosphere that selectively bind metals in a soluble form, and thus facilitate their uptake (S-Ni). The selector-metal complex is then passed through the root membrane to the inner surface, where a ternary complex is formed with a 'transport' ligand (S-Ni-T). A breakdown of this ternary complex frees the transport-metal complex to travel through the xylem, and allows the selector ligand to pass back through the root membrane. Metal is exchanged between the transport

ligand and an acceptor ligand at the boundary of the vacuole. This forms a terminal acceptor-metal (A-Ni) complex which is sequested within the physiologically inert vacuole, effectively detoxifying the metal. The transport ligand is then free to flow back through the xylem and the process is repeated.

## 2.7 Bioavailability of metals in the soil

The key limitation to metal uptake is solubility in the rhizosphere, since metals must be soluble to some degree in the soil solution for uptake to ensue. As has been discussed, solubility can be a natural function of the soil (passive uptake), effected by the plant (active uptake), or can be artificially induced (passive uptake).

The fraction of soil-metal that is in a readily available form to both plants and animals is termed *bioavailable*. Plant availability infers solubility of metals in the soil solution; animal availability infers solubility of metals in the gastrointestinal tract of animals (Thornton, 1999). Heavy metals can enter the food chain through accumulation by plants or through the direct ingestion of soil and/or dust by animals.

The 'toxicity' of soil at contaminated sites is thus dependent upon the bioavailability of the associated metals. Soil physical factors such as water availability, air availability and soil strength indirectly control metal uptake through constraining plant growth (Robinson, 1997). Soil chemical factors such as pH, nutrient status, and toxin concentrations (Ernst, 1996), affect metal uptake more directly. Likewise, the specific action of individual plant species may also affect metal uptake through the exudation of chemicals into the rhizosphere. This is not only true for hyperaccumulator species, but also for accumulator and non-accumulator species that can adjust the conditions of the rhizosphere. For example, many plant species can change the rhizosphere pH by as much as two units (Marschner, 1995).

The only definitive way to assess the potential for metal availability in soil is to grow plants *in situ* and analyse these for accumulated metal. However, this method is time-consuming. Many chemical-extractant solutions have been used to estimate the bioavailability of metals in soil. Solutions have included; distilled water (Sing and

Narwal, 1984), 0.5M $CaCl_2$ (Whitten and Ritchie, 1991), 1M $NH_4OAc$ (Haq *et al.*, 1980; Ernst, 1996), 1M $NH_4NO_3$ (McGrath *et al.*, 1997), and EDTA and DTPA (Haq *et al.*, 1980). EDTA and DTPA show chelate properties in the soil, while the other extractants are used to model natural plant exudates that increase metal solubility in the rhizosphere.

Robinson (1997) compared the efficacy of several chemical extractants to estimate the bioavailable fraction of metal for a Cd, Pb and Zn contaminated soil from northern France. The author of this study showed that ammonium acetate (1M) (Ernst, 1996) gave reproducible results that could be mathematically related to bioavailability using extractant concentration and pH, and was thus a useful extractant to compare the bioavailability of metals between soils. Subsequently, ammonium acetate has been adopted as a Massey University standard measure for plant-available metal in soil, and as such is used in this study. There is considerable debate between research groups as to the usefulness of the various chemical extractant systems to estimate bioavailability. However, there is as yet no strong data to show that there is a better extractant to use than ammonium acetate.

Modelling of plant-available metal is a useful tool, because, if accurate, such modelling will predict the potential danger of metal loadings in soil. If metal is not bioavailable to either plants or animals then it is of reduced concern. However, if bioavailable, this metal should be removed, by phytoextraction or more traditional techniques.

# SECTION A - PHYTOEXTRACTION OF CADMIUM, LEAD AND ZINC: OBSERVED AND MODELLED UPTAKE

Publications arising from section A:

Anderson, C.W.N., Brooks, R.R., Stewart, R.B. and Simcock, R., 2000. Phytoremediation: a possible management solution for New Zealand pastoral soils. *Australian Journal of Experimental Agriculture*, in prep.

Anderson, C., Deram, A., Petit, D., Brooks, R., Stewart, R. and Simcock, R., 1999. Induced hyperaccumulation: metal movement and problems. In *Proceedings of Extended Abstracts: 5th International Conference on the Biogeochemistry of trace Elements* (Eds W.W.Wenzel, D.C.Adriano, B.Alloway, H.E.Doner, C.Keller, N.W.Lepp, M.Mench, R.Naidu, G.M.Pierzynski) pp 122-123 (July 11-15, 1999, Vienna, Austria).

Anderson, C., Deram, A., Petit, D., Brooks, R., Stewart, R. and Simcock, R., 2000. Induced hyperaccumulation: is lead uptake a function of mineral-phase geochemistry? In *Symposium Volume ICOBTE 1999: Bioavailability, Fluxes and Transfer of Trace Elements in Soils and Soil Components* (Ed I.K.Iskander) in press (CRC press: Florida).

Hyperaccumulation of heavy metals and the associated technology of phytoextraction may be an exciting area of environmental science, but a literature review on the subject reveals that little or no attention has been paid to the potential importance of the chemical form of metal present in the soil at a contaminated site. The chemical form of metal is a function of the original source of this metal contamination. Nriagu (1978a), in a review of lead in the atmosphere, summarised the composition of particulate lead forms in aerosols originating from different sources of pollution (Table A.1).

**Table A.1**. Composition of lead aerosols originating from different sources of pollution.

| Source | Composition of particulates |
|---|---|
| Mining activities | PbS, $PbCO_3$, $PbSO_4$, $Pb_5(PO_4)_3Cl$, $PbO_x$ |
| Base-metal smelting and refining | Pb, $PbO_x$, $PbSO_4$,, $PbCO_3$, |
| Coal-fired power generation | surface complex in fly ash, $PbO_x$, $PbSO_4$, $Pb(NO_3)_2$ |
| Cement manufacture | $PbCO_3$, $Pb_5(PO_4)_3Cl$ |
| Fertiliser manufacture | $Pb_5(PO_4)_3Cl$, $PbO_x$, $PbCO_3$ |

Note. Ordering of lead forms indicates decreasing abundance. Only simple lead salts are presented. Source: Nriagu (1978a).

Using electron microprobe techniques, Tee Haar and Bayard (1971) determined the following relative abundance of metal salts found in airborne lead particles at a rural site (Table A.2). The data of these authors support contamination of the environment

with lead from urban sources, i.e., the metal possibly has its origin from metal refining and from fertiliser/cement manufacture. The relatively minor bromine-lead compounds described in Table A.2 can be attributed to the exhaust of petrol-powered motor engines.

**Table A.2**. Relative abundance of the chemical forms of airborne lead collected from an urban site.

| Metal form | % |
|---|---|
| $PbCO_3$ | 30 |
| $(PbO)_2PbCO_3$ | 28 |
| PbO | 21 |
| $PbCl_2$ | 5.4 |
| $PbOPbSO_4$ | 5.0 |
| Pb(OH)Cl | 4.0 |
| $PbSO_4$ | 3.2 |
| PbBrCl | 1.6 |
| $(PbO)_2PbCl_2$ | 1.5 |
| $(PbO)_2PbBrCl$ | 1.0 |
| $PbBr_2$ | 0.1 |

Chemical fractionation methods are commonly used to understand the distribution of metals between various soil components. The bioavailability of metal in soil is strongly related to its 'chemical form'. There are many extraction methods used, all modifications on several standard schemes, two examples being the methods of Shuman (1985) and Tessier, *et al.* (1979). The basis of a fractionation method is to use sequentially stronger 'extracting agents' to extract metals from the soil. A generalised description of these fractions is as follows (after Gommy, 1997):

1. the exchangeable fraction (weakly stable organic-metal complexes);
2. acid-soluble fraction (carbonates);
3. the reducible fraction (oxides);
4. oxidisable fraction (sulphides, sulphates and some organic-metal complexes); and
5. the residual fraction (silicate-bound minerals).

While it is known that the chemical fractionation of soil metals affects bioavailability and thus metal uptake, the idea that different forms of metal may be present at different sites appears not to have been integrated into models for phytoextraction. Little is

known about the specific effect of the form of metal present at a site on metal uptake, both natural, and more particularly, induced.

Chapter 3 presents data collected from field and pot trials conducted on two contaminated substrates typical of environments where anthropogenic metal loading to the soil is of concern. Cadmium, lead and zinc contaminate these environments and occur in different chemical forms. Several species, both hyperaccumulator and non-accumulator, have been trialled for phytoextraction, and the metal response of these species to hyperaccumulation (natural and induced) is described.

To explain the observed findings, geochemical models for Pb, Cd and Zn uptake are presented in Chapters 4, 5 and 6. The aim of generating these models was to explain variations in metal uptake exhibited by *Brassica juncea*, *Cardaminopsis halleri* and *Thlaspi caerulescens* as a function of the chemical form of metal present in the substrate. In an attempt to predict the plant-availability of metals in each experimental soil, ammonium acetate (1M, pH 7) was used as an extractant based upon the conclusions of Robinson (1997). The weak salt ammonium acetate is often used in sequential extraction schemes to extract the readily exchangeable fraction of soil metal (e.g. Almås *et al.*, 1999).

An integrated geochemical model is presented in chapter 7 that highlights the importance of the chemical form of metal present in the soil on the following practical aspects of phytoextraction for the metals Cd, Pb and Zn:

1. the choice of plant species to be used for natural hyperaccumulation,
2. the choice of chemical agent to used for induced hyperaccumulation,
3. the choice of plant species to be used to maximise induced phytoextraction, and
4. the value of ammonium acetate (1M) as an extractant to model plant-available metal.

An additional aim of this section of work was to quantify the effect that different metal salts, used to generate artificially contaminated soils for phytoextraction studies, would have on metal uptake. For example, Blaylock *et al.* (1997) reported the use of carbonate

salts to generate contaminated soils. Epstein *et al.* (1999) and Robinson *et al.* (1997a,b) reported the use of nitrate salts for this purpose while Li and Shuman (1996) used nitrate and chloride salts. Equilibration times for soils used in such experiments also vary dramatically, from days to months. How the use of these different metal salts will effect uptake (natural and induced) is poorly addressed in the literature. Two questions that can be raised are: 'How will relative uptake between experiments compare?' and 'Do these artificial soils accurately model natural contamination?'

In Chapter 8, a phytoextraction field trial conducted on an area of agricultural land is described. This trial represents a test case for the model generated in the previous chapters. This third substrate is pastoral land contaminated by the heavy metal cadmium, and represents an environment where phytoextraction could potentially be very successful.

The results from the field trial in Chapter 8 are integrated with the geochemical model of Chapter 7 in the conclusion to Section A.

# Chapter 3 - Trials on Contaminated Substrates

## 3.1 Introduction

Environmental concentrations of Cd, Pb and Zn have increased exponentially since about AD 1750, a date that heralded the onset of the industrial revolution in Europe (Nriagu, 1978b). The metals Cd, Pb and Zn are all chalcophilic (have an affinity for sulphur) and are thus geochemically similar. These three metals often occur naturally together in the rock ores from which they are mined. Human toxicological conditions arising from heavy metal exposure are not new. Some archaeologists even believe that lead poisoning contributed to the fall of the Roman Empire (Nriagu, 1978b). Improved understanding of human physiology has lead to greater concerns over the presence of these metals in the environment, and hence technology to minimise and reduce potential problems is actively sought.

Ingestion of large doses of cadmium can cause acute gastrointestinal disturbances; an aqueous cadmium concentration of 15 mg/L will induce vomiting (Lauwreys, 1977). Longer-term exposure to lower doses of cadmium can impair the functions of the kidneys, liver and the lungs. There is also evidence that cadmium can contribute to hypertension in humans and that the metal may be carcinogenic (Lauwreys, 1979). Toxicological affects induced by lead poisoning include detrimental affects on the nervous system, renal system and gastrointenstinal tract, cardiovascular system, reproductive system and the endocrine system. Lead has also been implicated as a carcinogen and a mutagen (Newland and Dunn, 1982). Zinc is slightly different to cadmium and lead in that it is an essential element to many plant and animal metabolic functions (Ainscough and Brodie, 1976; Marschner, 1995; Williams, 1973). However, at levels greater than trace concentration, similar toxicological problems can be expected (Knight *et al.*, 1997).

Two contaminated sites have been studied in detail for this chapter, pastoral land in northern France and mine tailings in New Zealand. Both sites are heavily contaminated. Metal pollution of farmland surrounding the field site in France has lead to increased deformities in the local human population (A.Deram, pers. commn. 1998), while the

metal leachate from the mine tailings in New Zealand has polluted local waterways. I do not propose that either of these sites could realistically be remediated by phytoextraction utilising the current state of technology. The metal loadings at each site would necessitate hundreds of years of cropping. However, heavily contaminated land does provide an ideal 'natural laboratory' to test the mechanisms and techniques for uptake, because the metal-uptake response of plants to natural and induced hyperaccumulation can easily be measured.

## 3.2 Field trials in northern France

During the Northern Hemisphere summer of 1998, I initiated a phytoextraction field trial on an area of land in northern France. This trial was run in collaboration with the University of Lille on land that has been contaminated by industrial activity near the French town of Auby in the province 'Nord Pas de Calais' (Table 3.1; Fig. 3.1). The surface profile (0-10 cm) of the Auby soil is peaty and extremely high in organic matter (C=34%, N=0.6%). The high heavy metal concentration has inhibited the microbial breakdown of plant material (Labrune and Douay, 1997). Perdix *et al.* (1997) described the use of chemical fractionation methods to determine the distribution and form of the heavy metals Cd, Cu, Pb and Zn in industrially polluted soils from northern France. They showed that the principal chemical forms of metal present in soils surrounding the Auby region are heavy metal oxides and carbonates.

**Table 3.1**. Selected geochemical properties of the Auby soil used in this study.

| **Total metal** | **Concentration** | | |
|---|---|---|---|
| Cd (mg/kg) | 300 | Plant-available Cd (mg/L) | 7.2 |
| Pb (%) | 1.0 | Plant-available Pb (mg/L) | 74 |
| Zn (%) | 4.0 | Plant-available Zn (mg/L) | 450 |
| pH | 6.0 | | |

Note – total metal concentrations determined by extraction with HCl (5M). Plant-available metal concentrations determined by extraction with $NH_4OAc$ (1M, pH 7) at a 1:10 soil:liquid ratio. Soil pH was determined in water, at a 1:2.5 substrate:liquid ratio.

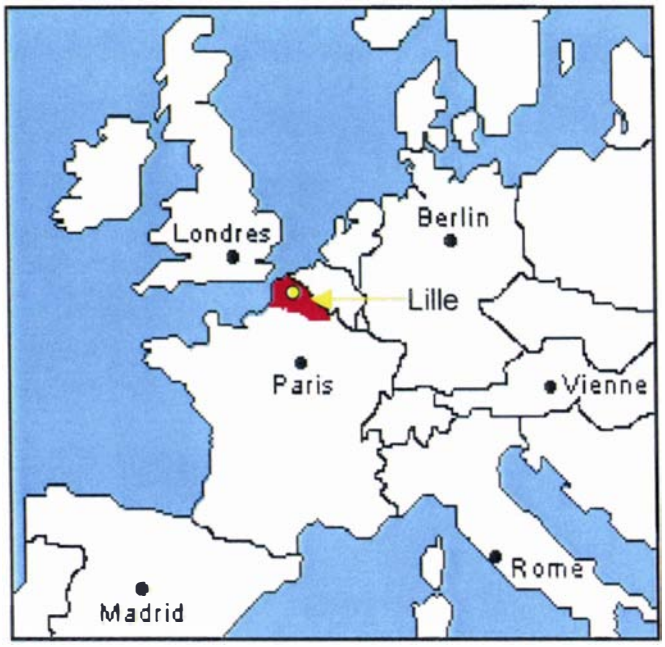


**Figure 3.1**. Map of Central Europe. The red shaded region of France is the Nord Pas de Calais.

**Figure 3.2**. Field-trial site at Auby, northern France. EDTA solution is being applied to plots marked by stakes, by a colleague from the University of Lille (Annabelle Deram).

Two areas, each measuring five square metres, were selected at the Auby site and divided into 9 individual one square metre plots (Fig. 3.2). The natural vegetation in each of these plots was a mixture of the zinc hyperaccumulator *Cardaminopsis halleri*, the metal tolerant grasses *Arrhenatherum elatius* and *Agrostis tenius*, and the metal tolerant herb *Silene humilis*. In each area, three replicate plots were treated with solutions of EDTA (disodium salt) or citric acid at an application rate of 0.5 g per kg of soil (75 g/m$^2$ surface area to 15cm depth). Vegetation was sampled each week for four weeks, dried, and digested in a mixture of concentrated nitric and perchloric acids (3:1 ratio). The solution was subsequently analysed by flame atomic absorption spectroscopy[1] (FAAS) to determine the effect of the chemical amendments on either inducing Cd, Pb and Zn uptake, or on increasing the already apparent level of uptake (Table 3.2).

**Table 3.2.** Metal concentration (DW) in field plants 21 days after EDTA treatment. Mean (±sd).

| | ***Arrhenatherum elatius*** | | | ***Cardaminopsis halleri*** | | |
|---|---|---|---|---|---|---|
| | Cd (mg/kg) | Pb (mg/kg) | Zn (mg/kg) | Cd (mg/kg) | Pb (mg/kg) | Zn (%) |
| Control | 7.05 (2.76) | 17.10 (4.23) | 609.5 (292.2) | 167.6 (36.4) | 50.0 (7.4) | 2.47 (0.81) |
| Citric acid | 7.03 (6.77) | 16.68 (1.85) | 733.0 (191.4) | 123.4 (7.4) | 49.7 (10.3) | 1.64 (0.23) |
| EDTA | 6.90 (1.30) | 17 60 (1.30) | 712.0 (222.3) | 174.0 (24.3) | 78.2 (29.7) | 2.47 (0.51) |
| Sig | ns | ns | ns | ns | ns | ns |

Note – significance refers to differences between the treatments and control (ANOVA $p<0.05$).

In addition to plant samples, soil cores to a depth of 0.6 m were sampled each week during the trial to monitor the possible movement of heavy metals down the soil profile. These cores were divided into 5 cm segments and ground using a porcelain mortar and pestle. Subsamples were extracted with hydrochloric acid (5 M - 1:10 soil:liquid ratio) and the filtrate was analysed by FAAS.

Cadmium and zinc were hyperaccumulated by *Cardaminopsis halleri* but there were no significant differences between the treatments for each plant species (Table 3.2). The addition of soil chemical amendments did not improve the uptake of Cd, Pb and Zn in either the hyperaccumulating or non-hyperaccumulating species studied. Similarly, no significant differences were observed between soil profiles (Appendix 7). Natural variation across each area was very large, showing the pollution at the site to be very

[1] Instrument operating parameters for all FAAS analyses in this thesis are reported in Appendix 1.

heterogeneous. This natural variation may have masked any induced differences in metal distribution within the soil profile.

## 3.3 Trials on tailings from the Tui base-metal mine

The Tui base metal mine, located on the flanks of Mount Te Aroha in the Coromandel district of the northern part of the North Island, New Zealand (Fig. 3.3, 3.4), has had a long history of exploration and aborted attempts at active production. The most recent period of activity occurred between 1967 and 1974 with the extraction and processing of up to 100 tons of ore a day, yielding up to 10 tons of a Pb-Cu-Zn concentrate containing minor amounts of Cd-Ag-Au (Cochrane, 1969). The cessation of mining activities in 1974 left a tailings damn containing 100 000 $m^2$ of tailings with high levels of heavy metals (Morrell *et al.*, 1996). The tailings continue to weather (oxidise) producing acid mine drainage. The principle metal-bearing minerals present in the Tui ore are: galena (PbS), sphalerite (ZnS) and chalcopyrite ($CuFeS_2$) (Morrell, 1998). Less-abundant cadmium minerals are the sulphides greenockite and hawleyite (Cochrane, 1969; Courtney *et al.*, 1990). Lead in the Tui mine tailings has been oxidised *in situ* to an anglesite ($PbSO_4$) mineral phase. The primary zinc phase in the tailings is sphalerite and, although not identified, it seems likely that cadmium sulphide remains the primary mineral phase of cadmium in the tailings, due to the high solubility of the respective zinc and cadmium oxidised sulphates.

There are several reports on the environmental impact of the Tui mine (Tay, 1980; Ward *et al.*, 1976; Ward *et al.*, 1977) and revegetation trials on this substrate have been ongoing at Massey University for a number of years (e.g. Morrell, 1998). More recently, however, hyperaccumulation has been trialled as a potential answer to the problem of heavy metal pollution at this particular location. The Tui mine tailings represent one of the few 'real-life' contaminated environments in New Zealand with a substrate concentration of heavy metals high enough to be of interest for phytoextraction studies (Table 3.3).

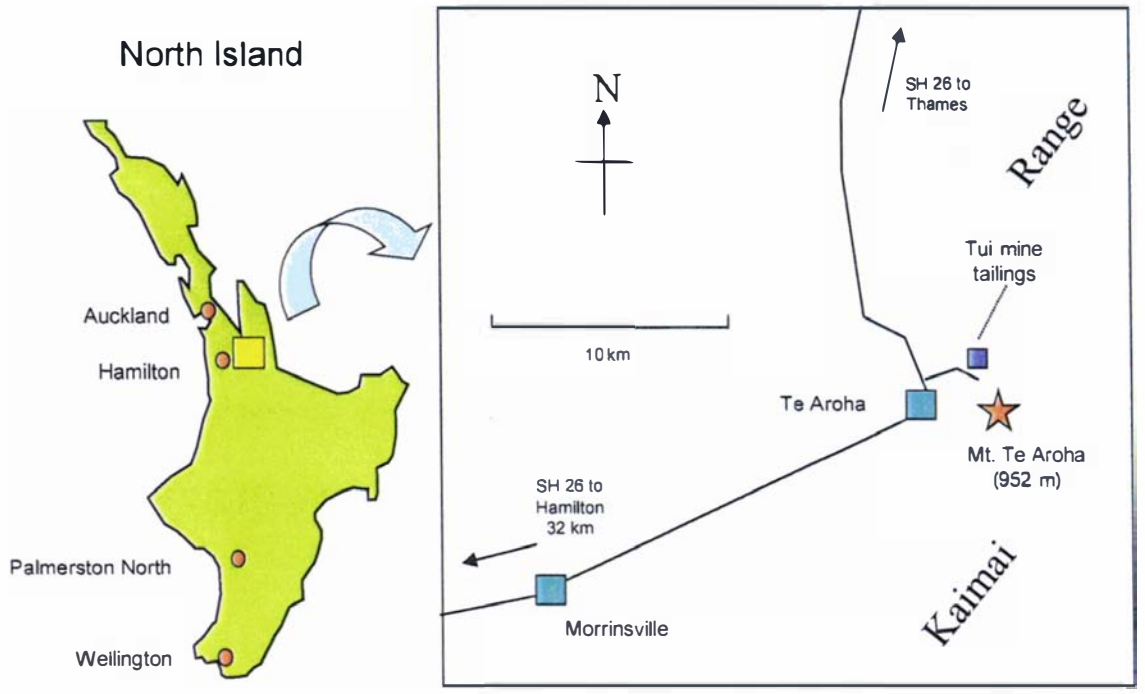


**Figure 3.3**. Map of the North Island of New Zealand showing in detail the location of the Tui mine tailings.

**Figure 3.4**. The Tui mine tailings (centre of the photograph) on the flanks of Mt. Te Aroha, looking northeast across the rich farmland of the Bay of Plenty (background). The barren ground in the foreground was the site of the ore separation plant.

Several trials have been conducted on the Tui tailings, using both accumulator and non-accumulator species. Several chemicals have been used in an attempt to induce the uptake of metals into these plants. The following sections present and summarise the data from these experiments.

**Table 3.3.** Selected geochemical properties of the Tui tailings material used in this study.

| Total metal | Concentration | | |
|---|---|---|---|
| Cd (mg/kg) | 26 | Plant-available Cd | 9.4 ng/L |
| Pb (%) | 1.15 | Plant-available Pb | 264.4 mg/L |
| Zn (%) | 0.54 | Plant-available Zn | 1.3 mg/L |
| pH | 3.5 | | |

Note – total metal concentrations determined by digestion with *aqua regia*. Plant-available metal concentrations determined through extraction with $NH_4OAc$ (1M, pH 7) at a 10:1 soil liquid ratio. Substrate pH was determined in water, at a 1:2.5 soil:liquid ratio.

*Experimental Design*

For each greenhouse experiment, the species used was planted in replicate pots (250 mL) containing a 1:1 mix of Tui tailings limed with 'agricultural lime' at a rate of 2.5% w/w (ca. 50 t/ha), and sieved (<2mm) pumice. Addition of pumice to the tailings was necessary to improve drainage of the substrate. Addition of lime to the tailings raised the pH from 3.5 to 7.5. None of the plant species used would grow at pH 3.5. The substrate was fertilised with Osmocote 'slow-release' fertilisers at a rate recommended by the manufacturer. During the growing cycle, pot positions were randomly changed on a periodic basis to equalise light exposure. The ambient temperature of the greenhouse was set at 15-25°C with no humidity control. Overhead watering was carried out each day with a hand-held hose.

Once established in the substrate (approximately 6 weeks), replicate numbers of each plant species for induced-uptake experiments were treated with a solution of the chemical agent used. After a treatment period of 10 days the aerial portion of each plant was harvested. For natural-uptake experiments the aerial portion of replicate plants was harvested after approximately 6 weeks. Dried (constant weight) subsamples were digested in concentrated nitric acid, diluted with deionised water, and the resultant solution was analysed by FAAS. Each data set was tested for normality. Where the data

were normally distributed (all experiments reported in Sections 3.4, 3,5 and 3.6), ANOVA was used for statistical analysis.

Replicate numbers varied for each experiment, but are reported with the results for each individual trial.

The ligands and chelating agents used in these trials were EDTA, DTPA, thiocyanate and thiosulphate. EDTA and DTPA have been used successfully by many authors for induced phytoextraction studies (see Chapter 2.4). The aim of their use in this study was to determine the relative efficacy of these chelating agents on metals from the Tui tailings substrate. Thiocyanate and thiosulphate, however, have not been used for induced phytoextraction studies in the past. Each is a ligand that naturally occurs within some geochemical environments and each has been implicated in the geochemical mobility of metals. A literature review of their formation can be found in Chapter 11.

## 3.4 Hyperaccumulation trials using *Berkheya coddii*

The high biomass nickel hyperaccumulator *Berkheya coddii* was grown in the Tui substrate and subsequently treated with EDTA to induce uptake of heavy metals. Only EDTA was used to induce uptake in this experiment, although 3 concentrations of chemical were used. EDTA induced a highly significant increase in lead uptake by *Berkheya coddii,* although there was no difference between the three treatment levels used (Fig. 3.5). EDTA did not induce an increase in cadmium uptake by the plants relative to the control treatment

## 3.5 Hyperaccumulation trials using *Cardaminopsis halleri*

A similar trial to that conducted for *Berkheya coddii* was conducted using the known cadmium and zinc hyperaccumulator species *Cardaminopsis halleri* (Table 3.4). In this trial a single application rate of four chemicals was used.

**Table 3.4.** Natural and induced-accumulation of cadmium, lead, and zinc by *Cardaminopsis halleri* growing on Tui mine tailings. Mean (±sd).

| Treatment | n | Cadmium (mg/kg) | Lead (mg/kg) | Zinc (mg/kg) |
|---|---|---|---|---|
| control | 6 | 4.17 (0.3) | 185 (100) | 210 ( 75) |
| EDTA (2 g/kg) | 6 | 4.94 (0.8) | 1105 (550) ** | 290 (140) |
| DTPA (2 g/kg) | 7 | 4.94 (2.6) | 640 (280) * | 260 (110) |
| thiosulphate (1 g/kg) | 6 | 3.65 (0.9) | 210 ( 50) | 250 (100) |
| thiocyanate (0.25 g/kg) | 7 | 3.93 (1.2) | 260 (110) | 255 ( 50) |

** = very highly significantly different to control ($p<0.001$), and * = significantly different from control ($p<0.05$) as determined by ANOVA.

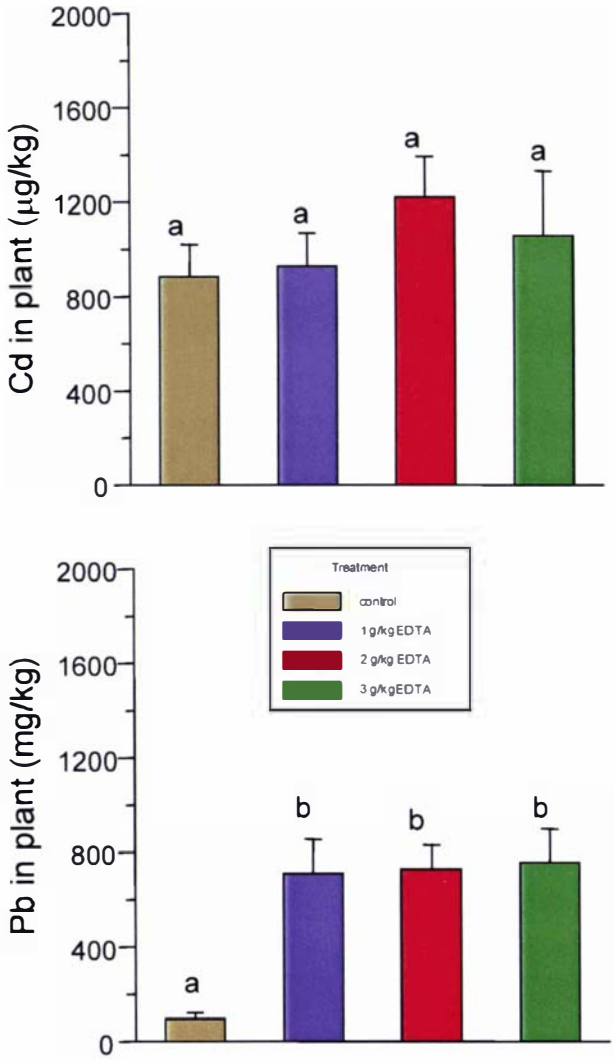


**Figure 3.5.** EDTA-Induced uptake of cadmium and lead by *Berkheya coddii* growing on Tui mine tailings (mean + SE, n=5). Means with the same letter are not significantly different (ANOVA $p>0.05$).

EDTA effected a significant increase in lead uptake (Table 3.4). DTPA also increased the level of lead accumulation, although this increase was not as significant as that for EDTA. The other chemicals used; ammonium thiocyanate and sodium thiosulphate, did not result in increased lead accumulation. With respect to cadmium and zinc, the 4 chemicals did not induce an increase in metal uptake; there were no significant differences among the 5 treatments for either metal. The final concentration of cadmium and zinc observed in *Cardaminopsis hallei* was surprising as it indicated this species, a known cadmium and zinc hyperaccumulator, did not hyperaccumulate these metals from the Tui tailings.

## 3.6 Hyperaccumulation trials using other species

The hyperaccumulator species *Thlaspi caerulescens* and non-accumulator species *Brassica juncea* were also tested for their natural and induced-uptake potential from Tui tailings. The results follow the same pattern as for other species: 1) increased uptake of lead through treatment with EDTA, but no significant differences between treatment levels, and 2) no EDTA-induced effect on the cadmium and zinc concentration accumulated by these plants. *Thlaspi caerulescens*, a known hyperaccumulator of cadmium and zinc, like *Cardaminopsis halleri*, failed to hyperaccumulate these metals from the Tui tailings by natural or induced means. Ammonium thiocyanate added to the Tui tailings similarly failed to induce the uptake of Cd, Pb and Zn by *B.juncea* (Fig. 3.6).

## 3.7 Problems with metal uptake

Sections 3.2 – 3.6 have outlined trials conducted on two different substrates that are typical of two different polluted environments. They both represent areas that could benefit from phytoremediation, using either natural or induced hyperaccumulation.

Results from induced hyperaccumulation experiments on these substrates have, however, been somewhat surprising. Enhanced accumulation of lead was observed in all species tested on the Tui mine tailings once EDTA was applied to the substrate. However, increased uptake of cadmium and zinc was never apparent. No induced

uptake of any metal was observed in any of the species tested during the Auby field trial. The concentration levels observed in both of these environments fell short of the lead and cadmium values observed in the literature, for plants after treatment with EDTA (Huang and Cunningham, 1994; Blaylock *et al.*, 1997).

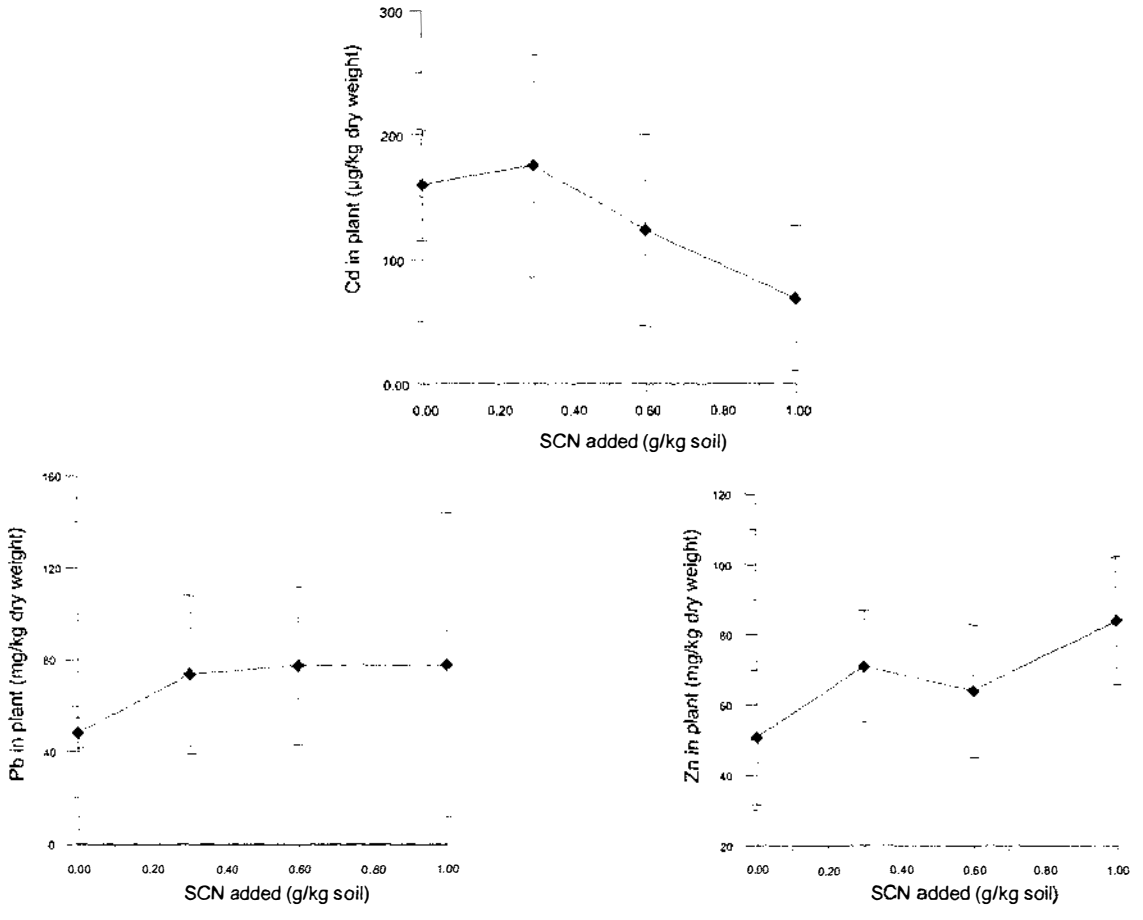


**Figure 3.6**. Thiocyanate-induced uptake (SCN) of Cd, Pb and Zn by *Brassica juncea* from Tui mine tailings (mean ± SE). n (control) = 3, n (treatments) = 7.

Both of the sites can be characterised as being contaminated with heavy metals that exist in different chemical forms (Table 3.5). Another site is introduced in this table to illustrate a third possible environment where heavy metal contamination may be encountered. The site is an area of pastoral land adjacent to a superphosphate storage shed, contaminated with cadmium due to the high levels of this heavy metal found in some phosphatic fertilisers. The results from a field trial conducted at this site are presented in Chapter 8.

**Table 3.5.** The dominant chemical form of metal present at 3 contaminated sites.

| Location | Dominant chemical form of the contaminating metal | Source of Contamination |
|---|---|---|
| Auby – northern France | Oxide, carbonate | Industrial air-fall |
| Tui mine tailings - New Zealand | Sulphide, sulphate | Mine tailings |
| Wairarapa – New Zealand | Phosphate, carbonate | Superphosphate fertiliser |

A question raised by the summation presented in Table 3.5 is - what effect do these different metal forms have on a) the bioavailability of metals to the plant under conditions of natural uptake, and b) the bioavailability of metals to the plant under conditions of induced uptake?

To answer and model this question, a series of greenhouse pot trials were carried out in conjunction with the 1998/1999 field trials, in which plants were grown in a commercial seed-raising mixture spiked with cadmium, lead and zinc salts of different mineral phases. The results were used to develop uptake models for each metal. Each model is discussed in turn through the next 3 chapters.

*Terminology for Chapters 4, 5 and 6*

The term 'metal/mineral phase' is used in Chapters 4, 5 and 6 to describe the chemical form of metal that was added to each soil, to model heavy metal contamination that could originate from different sources. The phrase is used in this sense to describe the 'primary metal phase' that may exist in soils, and not the secondary phases that may exist due to the release of metals from the contaminant's mineral lattice.

Natural uptake refers to metal uptake effected by the plant, as opposed to induced uptake effected by chemicals added to the soil.

# Chapter 4 - Geochemical Model for Lead Uptake

## 4.1 Introduction

To model the geochemical conditions prevalent in the Auby soil, commercial seed-raising mix was used as a base substrate to create an artificial, lead-contaminated 'soil' (C=20%, N=0.6%). A substrate lead concentration of 1% was used. To induce hyperaccumulation, a high concentration of acetic acid, citric acid or EDTA was added to each relevant pot. A high concentration was used to ensure that the chemical-inducing agent was present in the soil at an excess concentration, thus minimising the chance that differential rates of chemical degradation (function of the half life of the chemicals used) was a factor controlling metal uptake. The aim of this experiment was to determine the affect of the chemical form of lead on induced and natural lead uptake. I realise that the rate of chemical-inducing agent may have negatively affected plant health and thus metal uptake, however, this factor is independent of chemical form and was not considered in this experiment. Leaching of metal out of the various pots was similarly not considered.

## 4.2 Experimental Design

Six mineral salts were chosen to represent the initial form of lead contamination that could occur in a wide range of environments: carbonate, nitrate, oxide, phosphate, sulphate and sulphide. The soluble nitrate salt was chosen to model lead, after dissolution, as part of the soil organic phase, i.e., after a period of days no lead would remain in the soil as part of the original crystalline phase. Each salt (as a solid) was added to commercial potting-mix to give a final soil-lead concentration of 1% (w/w). Pots (250 mL – 280 in total) were planted, in equal numbers, with either the non-accumulator species *Brassica juncea* or the hyperaccumulator species *Thlaspi caerulescens*. A control substrate was used, where the two species were planted in pots containing potting-mix with no added lead. During the growing cycle, pot positions were randomly changed on a periodic basis to equalise light exposure. The ambient temperature of the

greenhouse was set at 15-25°C with no humidity control. Overhead watering was carried out each day with a hand-held hose.

After approximately 10 weeks growth, five replicates of each plant species, for each metal phase, were treated with one of 2 g/kg[2] EDTA (disodium salt), 2 g/kg citric acid, 2 g/kg acetic acid or water as a control. All treatments were applied as a solution (20 mL) and were randomly allocated to replicate pots. Two weeks after treatment, the above-ground portions were harvested[3], dried at 60°C to constant weight and subsamples digested in concentrated nitric acid before analysis by FAAS. As replicate specimens of *Brassica juncea* had reached different stages of maturity, and thus showed different weight ratios of stems, leaves and flowers, only the leaves of this species were analysed to minimise the variation in results that could be attributed to an uneven distribution of organs for an individual plant.

Substrate samples were 'cored' from the control pots of each metal phase at the time of plant harvest, ground using a porcelain mortar and pestle, and subsamples digested in *aqua regia* to give the total-metal concentration for each prepared 'soil'. Ammonium acetate (1M, pH 7) was used to estimate the concentration of plant-available lead (Ernst, 1996; Robinson, 1997) for each mineral phase, by overnight shaking at a soil:liquid ratio of 1:10. In each case, analysis of the filtrate was performed using FAAS. Measurement of the soil pH was conducted in water, using a 1:2.5 soil:liquid ratio.

All data were tested for normality, and analysed using ANOVA due to the observed normal distribution.

## 4.3 Results: *Brassica juncea.*

For each metal phase, EDTA caused a significant increase in the concentration of lead accumulated by the plant (Fig. 4.1). There was no significant difference between control

[2] g/kg is grams of chemical applied per kilogram fresh (moist) weight potting-mix
[3] Plant health at the time of harvesting is reported in Appendix 2

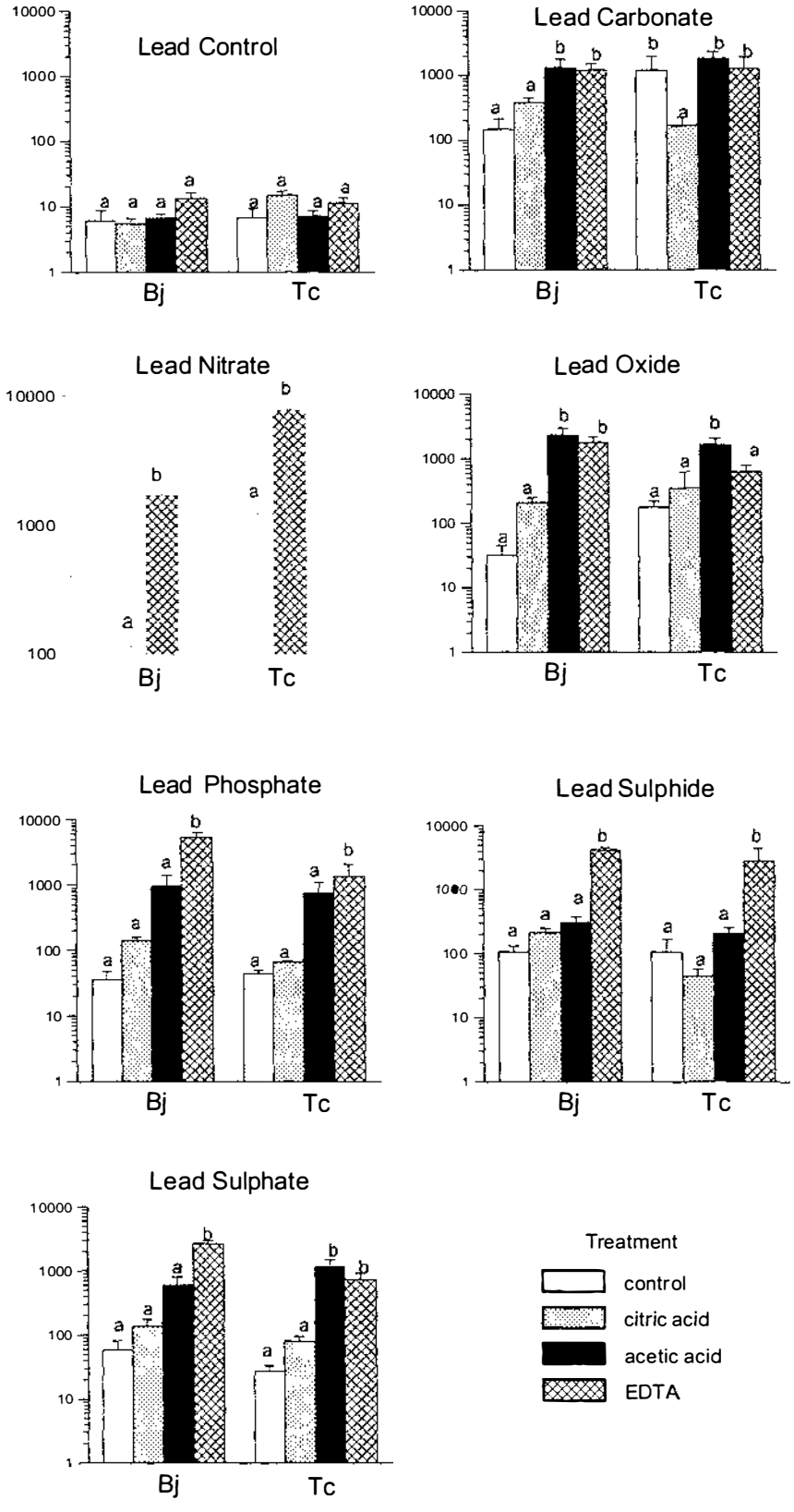


**Figure 4.1** Natural uptake, and acetic acid-, citric acid- and EDTA-induced uptake of lead by *Brassica juncea* (Bj) and *Thlaspi caerulescens* (Tc) growing on artificial 1% lead soils of different metal phases (mean + SE, n=5). Means for the same species and phase, with the same letter, are not significantly different (ANOVA $p>0.05$).

and citric acid treatments for each lead salt. In the case of the carbonate and oxide salts, acetic acid also caused a significant increase in the plant lead concentration.

The results clearly show that the relative efficacy of EDTA to induce lead hyperaccumulation is dependent upon the chemical form of the metal present in the soil (Fig. 4.2). The suitability of these forms to EDTA-induced hyperaccumulation can be written as follows:

control (a) < carbonate (bc) ~ nitrate (cd) = oxide (cd) ~ sulphate (d) < sulphide (e) < phosphate (f)[4]

This ordering is different from that of the plant-available lead concentration in the soil, estimated by extraction with ammonium acetate (1M, pH 7):

control (a) < sulphide (b) ~ sulphate (bc) ~ phosphate (c) < carbonate (d) = nitrate (d) = oxide (d)

The difference is attributable to the chelation effect of EDTA on lead, i.e., the effect of EDTA to induce uptake is independent of the plant-available or 'soluble' concentration of lead in the soil. Perhaps the most interesting point to note is the efficacy for EDTA-induced uptake from a sulphide lead form. Lead associated with the sulphide salt had the lowest plant-available metal concentration of the soils. However, EDTA-induced uptake was very high, more so than for the soluble nitrate form.

## 4.4 Results: *Thlaspi caerulescens*

A metal uptake pattern is less obvious for *Thlaspi caerulescens* (Fig. 4.1). With respect to the lead sulphate, sulphide and phosphate salts, EDTA caused a significant increase in lead uptake relative to the control treatment. In the case of the sulphate salt, acetic acid

[4] Letters refer to statistical differences between the mean values presented in Figure 4.1 and Appendix 7. Means with the same letter are not statistically different, ANOVA $p>0.05$.

also caused a significant increase in lead uptake. For each of these three salts, citric acid caused no significant increase in lead uptake relative to the control treatment.

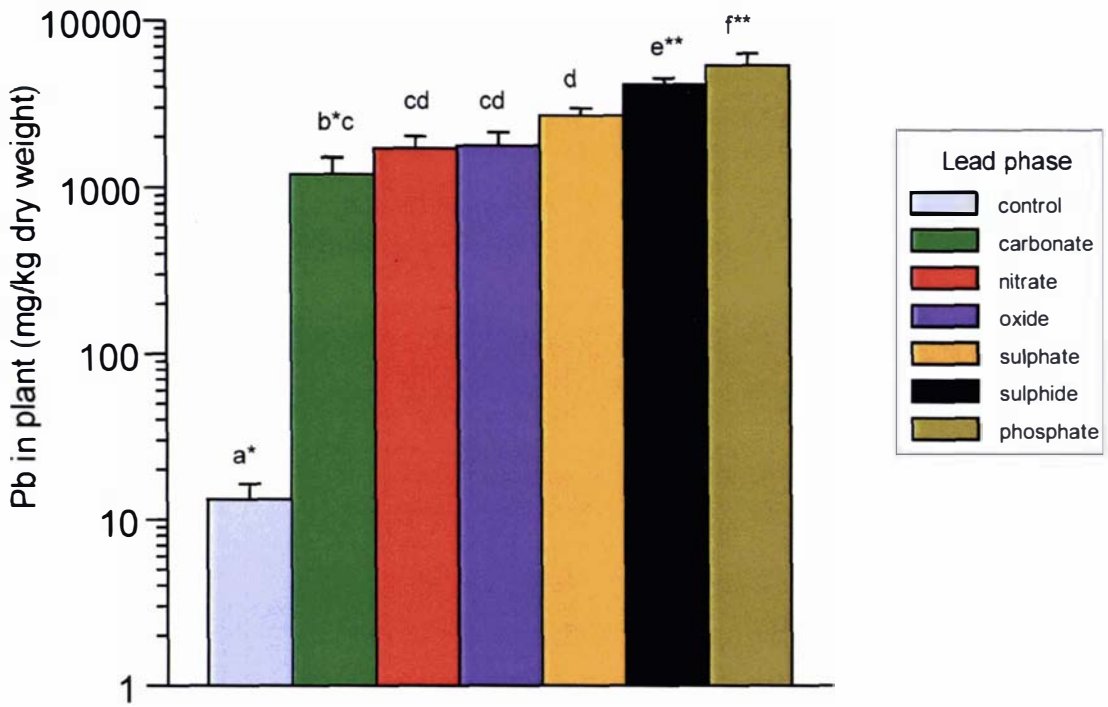


**Figure 4.2**. Summary: efficacy of EDTA-induced lead uptake by *Brassica juncea* as a function of metal phase. Means with the same letter are not significantly different (ANOVA p>0.05). Note *p B>A = 0.087 ** p F>E = 0.069.

In the case of lead carbonate, acetic acid and EDTA did not cause a significant increase in metal uptake relative to the control. However, citric acid caused a significant decrease in plant lead. The mean lead concentration for control plants grown on the carbonate contaminated soil was 1 175 mg/kg. Natural hyperaccumulation was observed for *Thlaspi caerulescens* in this particular model environment. Hyperaccumulation was similarly observed for lead added as a nitrate salt (mean value of 1 220 mg/kg). After treatment with EDTA, the increase in lead uptake was significant and represented the greatest increase in metal concentration observed in this experiment.

The only treatment to cause a significant increase in lead uptake from the oxide salt was acetic acid. For the oxide form of lead the increase in uptake due to EDTA was not significant.

## 4.5 Results: total soil lead

Digestion by *aqua regia*, and subsequent analysis of subsamples of substrate taken from every control treatment soil (n=5), showed the final lead concentration of each metal phase to agree with the 'designed' concentration of 1%. Soil pH was independent of mineral salt used (Table 4.1).

**Table 4.1**. Total soil lead and pH for the control treatment soils of each metal phase. Mean values with the same letter are not significantly different (ANOVA p>0.05).

| Lead phase | pH | Total Lead (%) |
|---|---|---|
| Control | 4.4 | 2.42 (mg/kg) ± 0.88 (a) |
| Sulphide | 4.3 | 0.81 ± 0.28 (b) |
| Oxide | 4.8 | 0.92 ± 0.24 (b) |
| Sulphate | 4.2 | 1.20 ± 0.22 (c) |
| Carbonate | 4.4 | 1.21 ± 0.10 (c) |
| Phosphate | 4.3 | 1.30 ± 0.20 (c) |
| Nitrate | 4.5 | 1.30 ± 0.20 (c) |

## 4.6 Results: plant-available lead

Under the geochemical conditions of this study, the relative ordering of the plant-available lead concentration, modelled by ammonium acetate (Table 4.2), confirmed that bioavailability of lead was strongly dependent upon the chemical form of lead contamination present in the soil.

**Table 4.2.** Plant-available (ammonium acetate) lead for the control treatment soils of each metal phase. Mean lead concentrations with the same letter are not statistically different (ANOVA p>0.05).

| Lead phase | $NH_4OAc$-extractable lead (mg/L) |
|---|---|
| Control | 0.28 ± 0.2 (a) |
| Sulphide | 33.1 ± 5.4 (b) |
| Sulphate | 42.7 ± 4.1 (bc) |
| Phosphate | 55.2 ± 9.5 (c) |
| Carbonate | 92.6 ± 34.5 (d) |
| Nitrate | 106.6 ± 33.1 (d) |
| Oxide | 115.5 ± 26.4 (d) |

## 4.7 Discussion - a model for lead uptake

Except for the high lead concentration observed in *Thlaspi caerulescens* from the carbonate and nitrate-contaminated soils, natural uptake for both plant species used was never more than 100 mg/kg. This was not modelled by ammonium acetate (Table 4.2), which predicted that significant metal accumulation could be expected by both *Brassica juncea* and *T.caerulescens*, due to the high values for plant-available metal[5]. Hyperaccumulation of lead by *B.juncea* is an induced phenomenon that is highly dependent upon the chemical form of lead present in the soil. In the absence of soil applied chelates *Brassica juncea* may 'exclude' lead uptake, but natural hyperaccumulation of lead by *T.caerulescens* appears to be realistic under some geochemical conditions. Ammonium acetate did not predict this hyperaccumulation.

Phytoextraction of lead is maximised from each mineral phase through EDTA-induced hyperaccumulation by the plant species *Brassica juncea.*

The best choice of chemical inducing agent for an environment contaminated with lead present as a phosphate or sulphide phase is, based on the results of this experiment, EDTA. The individual plant species, lead phase combination plots (Fig. 4.1) show that for an environment contaminated with lead as an oxide or carbonate phase, acetic acid would be a better choice, as its relative efficacy compared with EDTA in each of these cases is nominally greater. It is interesting to note that EDTA-induced hyperaccumulation by *Brassica juncea* from the nitrate phase was relatively low. The nitrate salt is completely soluble, and hence, after dissolution, was likely to be present in the substrate as an organic phase or in equilibrium with the soil solution. Soluble metal salts have often be used to model polluted soils in pot trials. This result suggests that data obtained from such experiments may be misleading, and may underestimate the ability of EDTA to induce hyperaccumulation in *B.juncea* relative to the less soluble forms of lead in soil.

[5] The assumption true for a good extractant is that the concentration of plant-available metal will be directly proportional to the concentration of metal accumulated by the plant.

The concentration of lead accumulated once EDTA is applied appears to be species-independent. The final metal concentrations of both *Brassica juncea* and *Thlaspi caerulescens* once hyperaccumulation was induced were very similar. This is in agreement with trials on the Tui mine tailings, where all species used could be induced to accumulate a similar concentration of lead. An exception to this observation is the EDTA-induced uptake by *T.caerulescens* of lead added to the soil as a nitrate salt. The comparison of induced lead uptake between the two plant species is interesting, and may involve some physiological response of *T.caerulescens* to the lead-organic phase - EDTA interaction. The nature of this response will not be speculated on here. It is important to note, however, that again, pot trials conducted where lead is added as a soluble metal salt may be misleading and overestimate the induced metal uptake potential of *T.caerulescens* from contaminated soils. Where the metal-chelate complex effects no physiological response by the plant, the technology of induced hyperaccumulation for lead may well be equally effective for all plant species.

## 4.8 Conclusion

Uptake of lead by both *Brassica juncea* and *Thlaspi caerulescens* is maximised by induced hyperaccumulation. The choice of chemical to be used to effect this uptake is dependent upon the chemical form of the lead that initially contaminated the soil. Natural hyperaccumulation of lead was not observed for *B.juncea* but may be true for *T.caerulescens* for some forms of lead in the environment.

Ammonium acetate did not effectively model lead uptake. The high values of plant-available lead did not translate to metal uptake. Hyperaccumulation of lead by *Thlaspi caerulescens* was not reflected by anomalies in the plant-available metal concentration.

# Chapter 5 - Geochemical Model for Cadmium Uptake

## 5.1 Introduction

To model the geochemical conditions prevalent in the Auby soil, commercial seed-raising mix was used as a base substrate to create an artificial, cadmium-contaminated 'soil' (C=20%, N=0.6%). A soil cadmium concentration of 200 mg/kg (0.02%) was used. To induce hyperaccumulation a high concentration of citric acid or EDTA (disodium salt) was added to each relevant pot. A high concentration was used to ensure that the chemical-inducing agent was present in the soil at an excess concentration, thus minimising the chance that differential rates of chemical-degradation (a function of the half life of the chemicals used) was a factor controlling metal uptake. The aim of this experiment was to determine the affect of metal phase on induced and natural cadmium uptake. I realise that the rate of chemical-inducing agent may have negatively affected plant health and thus metal uptake, however, this factor is independent of mineral phase and was not considered in this experiment. Leaching of metal out of the various pots was similarly not considered.

## 5.2 Experimental design

Five mineral salts were chosen to represent the initial form of cadmium contamination that could occur in a wide range of environments: carbonate, nitrate, oxide, phosphate and sulphide. The soluble nitrate salt was chosen to model cadmium, after dissolution, as part of the soil-organic phase, i.e., after a period of days no cadmium would remain as part of the original crystalline phase. Each salt (as a solid) was added to commercial potting-mix to give a final soil-metal concentration of 200 mg/kg (w/v). Pots (250 mL – 315 in total) were planted, in equal number, with the non-accumulator species *Brassica juncea*, or the hyperaccumulator species *Cardaminopsis halleri* or *Thlaspi caerulescens*. A control substrate was used, where the three species were planted in pots containing potting-mix with no added cadmium. During the growing cycle, pot positions were randomly changed on a periodic basis to equalise light exposure. The ambient

temperature of the greenhouse was set at 15-25°C with no humidity control. Overhead watering was carried out each day with a hand-held house.

After approximately 10 weeks growth, 5 replicates of each plant species, for each cadmium phase, were treated with one of 2 g/kg EDTA (disodium salt), 2 g/kg citric acid or water as a control. All treatments were applied as a solution (20 mL) and were randomly allocated to replicate pots. Two weeks after treatment, the above-ground biomass was harvested[6], dried at 60°C to constant weight and subsamples were digested in concentrated nitric acid before analysis by FAAS. As replicate specimens of *Brassica juncea* had reached different stages of maturity, and thus showed different weight ratios of stems, leaves and flowers, only the leaves of this species were analysed to minimise the variation in results that could be attributed to an uneven distribution of organs for an individual plant.

Substrate samples were 'cored' from the control pots of every metal phase at the time of plant harvest, ground using a porcelain mortar and pestle, and subsamples digested in concentrated nitric acid to give the total metal concentration for each of the prepared soils. Ammonium acetate (1M, pH 7) was used to estimate the concentration of plant-available cadmium (Ernst, 1996) for each metal phase by overnight shaking at a soil:liquid ratio of 1:10. In each case, analysis of the filtrate was performed using FAAS. Measurement of the soil pH was conducted in water, using a 1:2.5 soil:liquid ratio.

Al data were tested for normality and analysed using ANOVA due to the observed normal distribution.

## 5.3 Results: *Brassica juncea*

With the exception of the carbonate salt, EDTA caused a significant increase in cadmium uptake from every metal phase (Fig. 5.1). There was no significant difference between control and citric acid treatments. The concentration of metal accumulated through natural uptake was a function of the chemical form of metal in the soil.

[6] Plant health at time of harvesting is reported in Appendix 2.

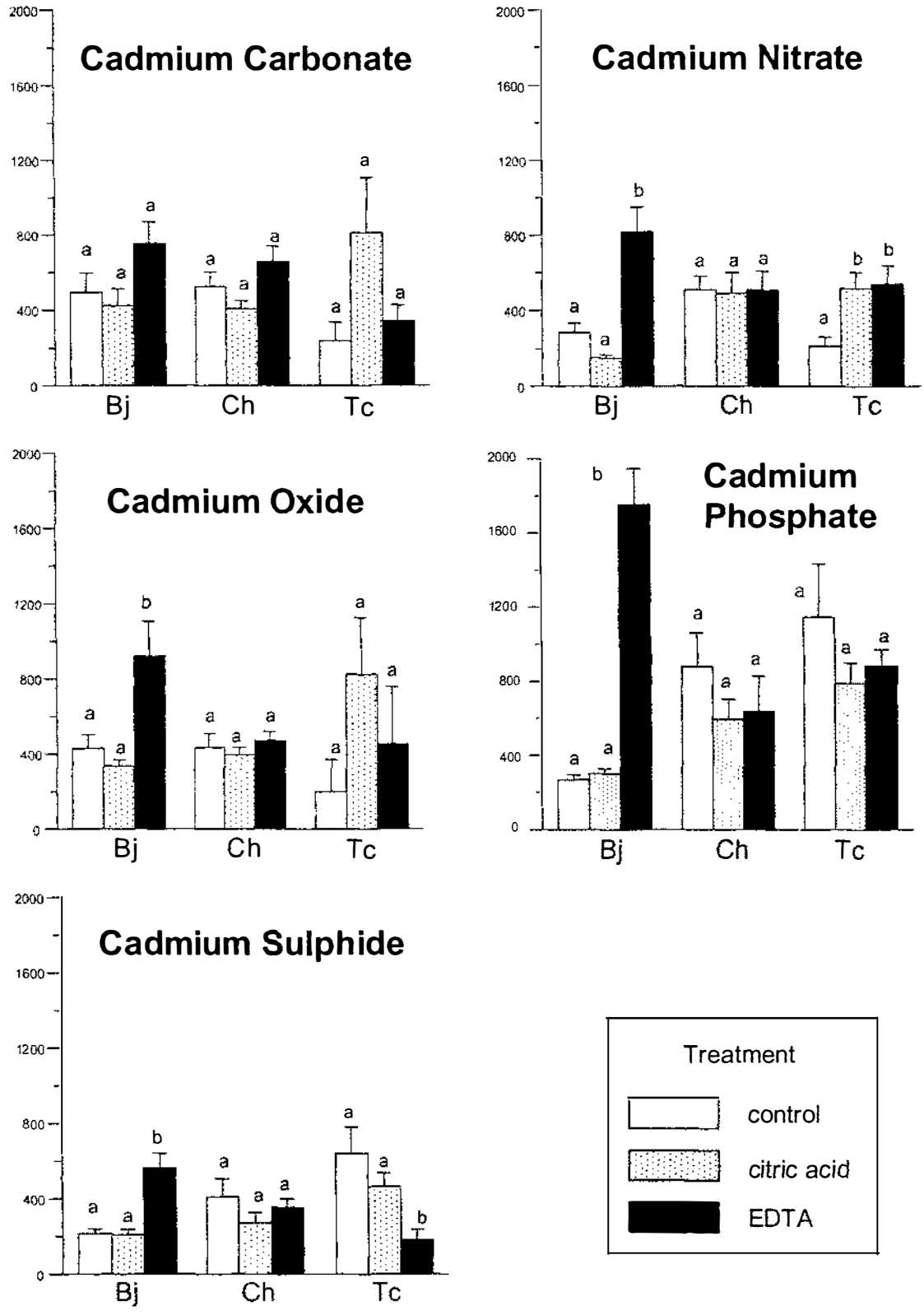


**Figure 5.1**. Natural uptake and EDTA- and citric acid-induced uptake of cadmium by *Brassica juncea* (Bj), *Cardaminopsis halleri* (Ch) and *Thlaspi caerulescens* (Tc) growing on artificial 200 mg/kg cadmium soils, of different metal phases (mean + SE, n=5). Means for the same species and phase, with the same letter, are not significantly different (ANOVA $p>0.05$).

The relative suitability of the 5 salts to natural uptake can be ordered as:

sulphide (a) ~ phosphate (ab) ~ nitrate (ab) ~ oxide (bc) ~ carbonate (c)[1]

This is different from the ordering of plant-available cadmium, estimated by extraction of each control soil with ammonium acetate (1M, pH 7):

sulphide (a) = nitrate (a) = oxide (a) < phosphate (b) = carbonate (b)

Summarising the EDTA treatment response of cadmium uptake by *Brassica juncea* (Fig. 5.2), allows comparison of the relative efficacy of EDTA to induce uptake from each metal phase.

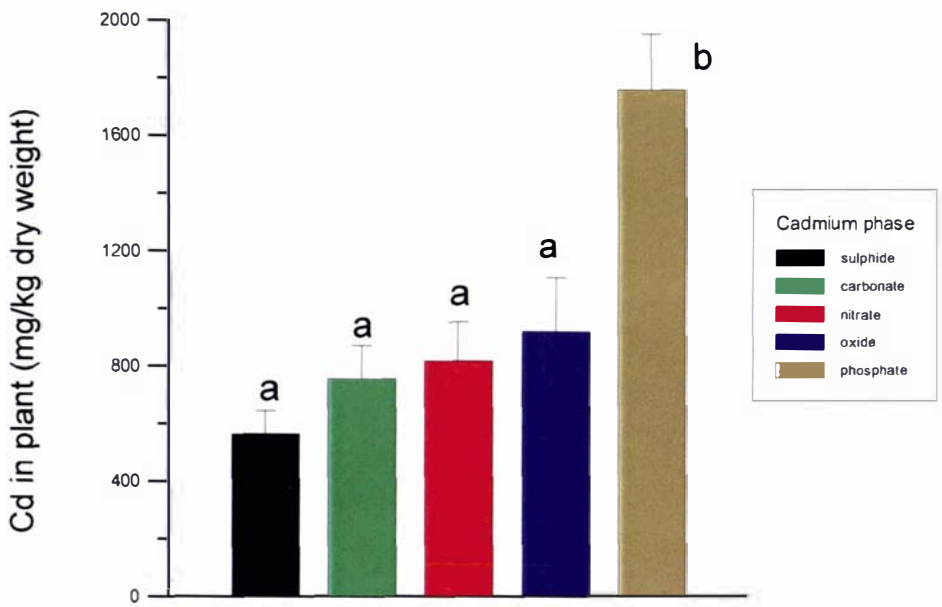


**Figure 5.2**. Summary: efficacy of EDTA-induced cadmium uptake by *Brassica juncea* as a function of the metal phase.

The ordering of induced uptake can be written as:

sulphide (a) = carbonate (a) = nitrate (a) = oxide (a) < phosphate (b)

[1] Letters refer to statistical differences between the mean values presented in and Appendix 6. Means with the same letter are not statistically different, ANOVA $p>0.05$.

EDTA has a similar effect on each of the tested metal salts, with the exception of cadmium phosphate.

## 5.4 Results: *Cardaminopsis halleri*

For every metal phase there was no difference in cadmium uptake between the three treatments (Fig. 5.1). Neither citric acid nor EDTA influenced the cadmium uptake potential of *Cardaminopsis halleri* under the geochemical conditions of this study. Summarising natural uptake from each of the 5 cadmium salts (Fig. 5.3) allows comparison of the importance of the chemical form of cadmium on this species ability to effect natural hyperaccumulation.

The ordering of natural uptake can be written as:

sulphide (a) = oxide (a) = nitrate (a) = carbonate (a) < phosphate (b)

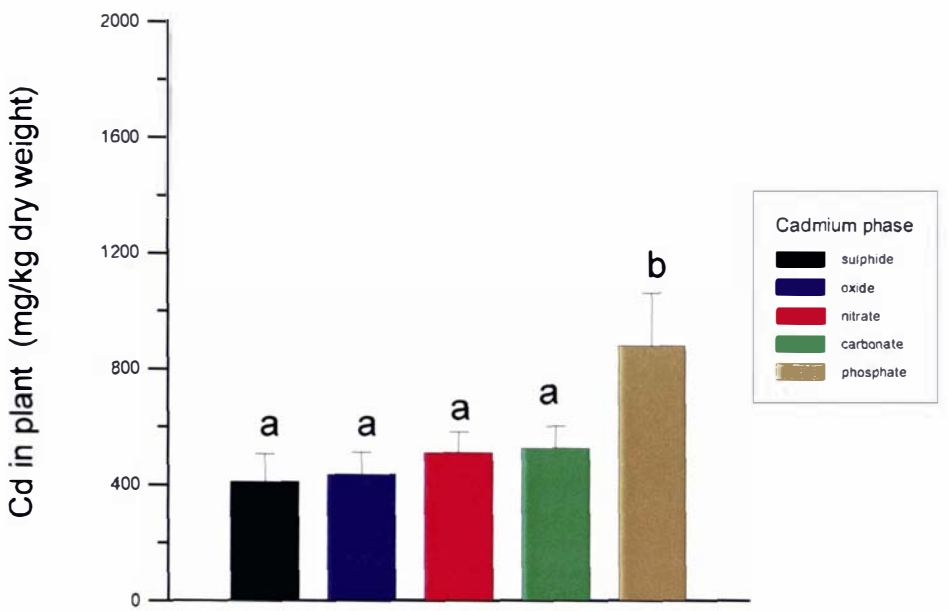


**Figure 5.3** Summary: efficacy of natural cadmium uptake by *Cardaminopsis halleri* as a function of the metal phase.

With the exception of the phosphate salt, natural uptake is the same from each cadmium phase. This observation is inconsistent with the ordering of plant-available cadmium (ammonium acetate extractable), estimated from the control soils of each cadmium salt:

sulphide (a) = carbonate (a) = nitrate (a) < oxide (b) < phosphate (c)

## 5.5 Results: *Thlaspi caerulescens*

The cadmium uptake response of *Thlaspi caerulescens*, effected by the three treatments on each metal phase, followed a less simple pattern than for the other two plant species (Fig. 5.1). For the carbonate, oxide and phosphate phases there was no significant difference among the three treatments. Replicate uptake values for each treatment on these three phases were highly variable; outliers were not removed from the data set before statistical analysis. For the nitrate phase, both citric acid and EDTA caused a significant increase in cadmium uptake. However, for the sulphide phase, EDTA caused a significant decrease in cadmium uptake. There was no significant citric acid-induced effect on uptake from the sulphide phase.

The natural cadmium uptake potential of *Thlaspi caerulescens* from each mineral phase (Fig. 5.4) can be written as follows:

oxide (a) = nitrate (a) = carbonate (a) < sulphide (b) ~ phosphate (b)

Again, natural uptake from the phosphate phase was the highest of all the salts. For *Thlaspi caerulescens*, natural uptake from the sulphide phase was also significantly greater than from the remaining three salts.

The ordering of natural uptake for *Thlaspi caerulescens* is very different from the ordering of plant-available (ammonium acetate) cadmium estimated from the control soils of each mineral phase:

sulphide (a) < oxide (b) ~ carbonate (bc) ~ nitrate (c) < phosphate (d)

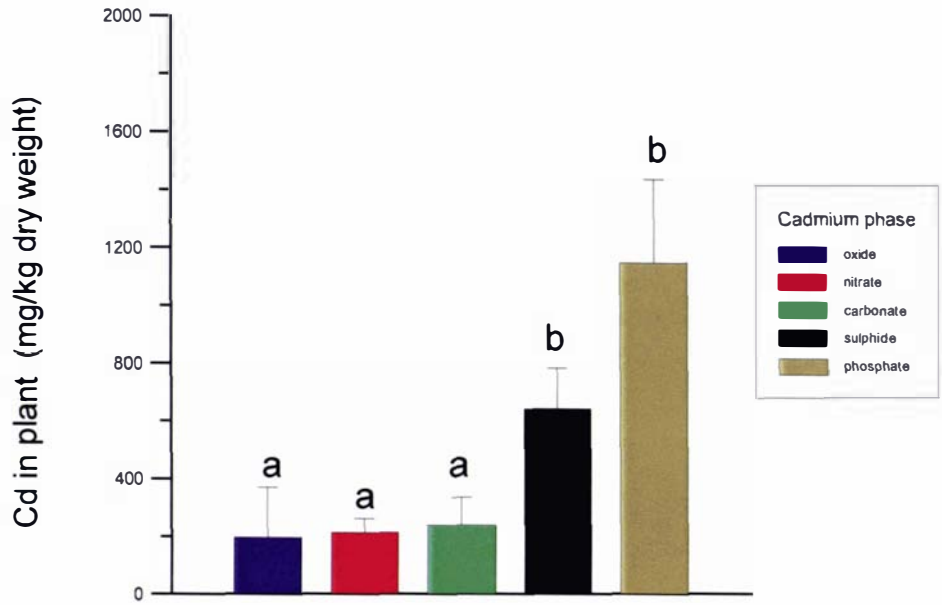


**Figure 5.4**. Summary: efficacy of natural cadmium uptake by *Thlaspi caerulescens* as a function of the metal phase.

## 5.6 Results: total soil cadmium

Digestion by concentrated nitric acid and subsequent analysis of subsamples of substrate taken from each of the control treatment pots, showed significant variation in the final cadmium concentration for each phase (Table 5.1). The five metal salts did not affect the pH of the soil.

**Table 5.1**. Total soil cadmium and pH for the control treatment soils of each mineral phase. Mean values with the same letter are not statistically different (ANOVA p>0.05). Mean (±sd).

| Cadmium phase | pH | Total cadmium (mg/kg) |
|---|---|---|
| Control | 4.9 | Note |
| Sulphide | 4.9 | 180 ± 20 (a) |
| Carbonate | 4.8 | 220 ± 80 (b,c) |
| Nitrate | 4.8 | 240 ± 15 (c) |
| Oxide | 4.8 | 240 ± 35 (c) |
| Phosphate | 4.9 | 485 ± 50 (d) |

Note. The total cadmium concentration of the control soil was below detection limits by FAAS and is not reported in this table.

*Effect of metal concentration on uptake*

The concentration of 200 mg/kg cadmium was chosen for this experiment to model the level of cadmium observed in Auby field soils, and to ensure that cadmium was present

in the soil to excess concentration (i.e. excess metal relative to the potential for uptake). To test the effect of metal concentration on plant uptake, two cadmium phosphate soils were prepared; a soil with a final concentration of 485 mg/kg cadmium (Table 5.1) and a soil prepared to have a final concentration of 200 mg/kg cadmium. For the control plants of each species, the concentration of cadmium accumulated by the plant was the same for both soil concentrations. By ensuring that soil cadmium was present at an excess level, differences in the total cadmium concentration between each phase did not affect plant uptake. The important factor was the mineral phase itself, i.e., uptake was independent of the total soil-metal concentration.

## 5.7 Results: plant-available cadmium

The ordering of plant-available cadmium, reported for each plant species, was based upon values determined from each of the control treatment soils for each species. These values do not account for differences in metal concentration between each set of soils, and are therefore only relevant to their respective plants. To accurately compare the bioavailablility of each mineral phase, and hence the potential solubility of cadmium in the rhizosphere as a function of cadmium geochemistry, these data for plant-available metal must be corrected for total concentrations (Table 5.2). Reporting the percentage of the total metal in the soil that is plant-available has made this correction.

**Table 5.2**. Percentage of the total cadmium that is plant-available (1M ammonium acetate) from each metal phase using a 1:10 soil:liquid ratio. Mean cadmium values with the same letter are not statistically different (ANOVA p>0.05).

| Cadmium phase | % extractable cadmium |
|---|---|
| Control | Note |
| Sulphide | 27.7 ± 2.6 (a) |
| Nitrate | 32.4 ± 1.0 (ab) |
| Phosphate | 34.3 ± 2.2 (b) |
| Oxide | 38.1 ± 10.1 (b) |
| Carbonate | 44.5 ± 15.0 (c) |

Note. The plant-available cadmium concentration of the control soil was below detection limits by FAAS and is not reported in this table.

Under the geochemical conditions of this study, the concentration of plant-available cadmium, estimated by ammonium acetate, confirms that bioavailability of cadmium is strongly dependent upon mineral phase.

## 5.8 Discussion - a model for cadmium uptake

Increased uptake of cadmium can be induced in the non-accumulator species *Brassica juncea* using the chemical EDTA. With the exception of a phosphate salt, a two- to three-fold increase in cadmium uptake was apparent. Uptake from cadmium phosphate was increased approximately five-fold. Induced uptake is dependent upon the phase of the metal present.

In this experiment, natural uptake by *Brassica juncea* from each mineral phase was above the cadmium hyperaccumulation threshold. This was a surprising result, but the plants, while showing symptoms of metal stress, were all alive and relatively healthy (Fig. 5.5; Appendix 2). *Brassica juncea* appears to have the ability to uptake high levels of cadmium from soils with a geochemistry similar to that of the experimental substrate used in this study, a finding that is agreement with Blaylock *et al.* (1997). These authors found that *B.juncea* could 'naturally' accumulate 220 mg/kg cadmium DW when grown on a carbonate-contaminated soil (artificial) with a substrate cadmium concentration of 100 mg/kg.

Cadmium itself rarely inhibits plant growth (McGrath, 1998). However, cadmium uptake occurs naturally, contemporaneously with zinc which is considerably more phytotoxic. In the absence of zinc, it is possible that non-accumulator species may be able to 'hyperaccumulate' cadmium. In light of this conclusion, the question of 'what is hyperaccumulation?' can be raised. *Brassica juncea* should not be regarded as a hyperaccumulator species, as no evidence indicates that this species has evolved the perculiar trait to allow it to survive in metal-rich environments, although it may be an indicator species. The issue that the criteria for hyperaccumulation should be re-examined has been raised (M.Macnair, pers. commn. 1999 - Chapter 1.5), and evidence such as this supports such a re-examination.

Based upon the results of this experiment, there is no strong evidence that citric acid- or EDTA-induced uptake to a higher concentration of cadmium is possible for the hyperaccumulator species *Cardaminopsis halleri* and *Thlaspi caerulescnes*. This is in contradiction to the findings in Chapter 4 where EDTA-induced uptake of lead was apparent for *T.caerulescens*. The natural uptake potential for each of these species

varied between each of the different mineral phase. *Thlaspi caerulescens* showed poor uptake from the oxide, nitrate and sulphide phases relative to *C.halleri*, but greater relative uptake from the carbonate and phosphate phases. The relationship between this uptake and the plant-available cadmium concentration is discussed below.


**Figure 5.5**. *Brassica juncea* growing on an artificial, carbonate phase, cadmium-contaminated soil shortly before harvesting. The EDTA treated specimen shows signs of chlorosis, however, the control-treatment specimen to the left shows no sign of metal stress.

*Relationship between ammonium acetate and the metal phase*

## 1. *Brassica juncea*

The ordering of natural uptake from each metal phase, effected by *Brassica juncea*, was not the same as that modelled using ammonium acetate. This can be presented graphically (Fig. 5.6).

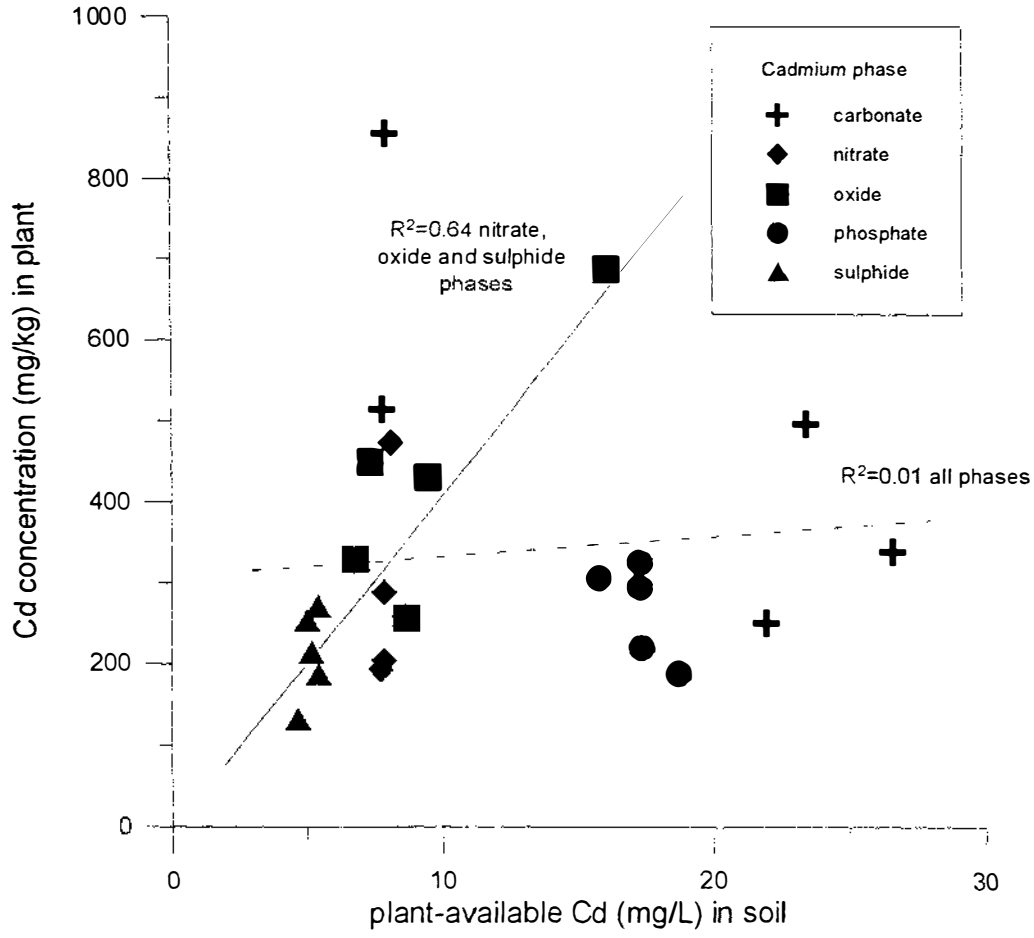


**Figure 5.6.** Plot of the cadmium concentration in *Brassica juncea* as a function of the plant-available cadmium concentration in the soil for each metal phase.

The sulphide, oxide and nitrate phases appear to plot on a linear relationship ($R^2$=0.64 – Fig. 5.6). However, the phosphate and carbonate phases behave differently. The regression coefficient for all phases modelled together is very low ($R^2$=0.01). This suggests that ammonium acetate may not model plant-available cadmium equally well for each metal phase, an observation that could explain the discrepancy between modelled uptake and the actual ordering of accumulated metal in the plant as a function of metal phase.

## 2. *Cardaminopsis halleri*

EDTA and citric acid did not affect the cadmium uptake of *Cardaminopsis halleri* in this experiment. The data show that these chemicals neither increase nor decrease the concentration of accumulated metal. The discrepancy between the observed ranking of metal uptake (natural) from each mineral phase and the modelled potential for uptake using ammonium acetate, can again be depicted graphically (Fig 5.7).

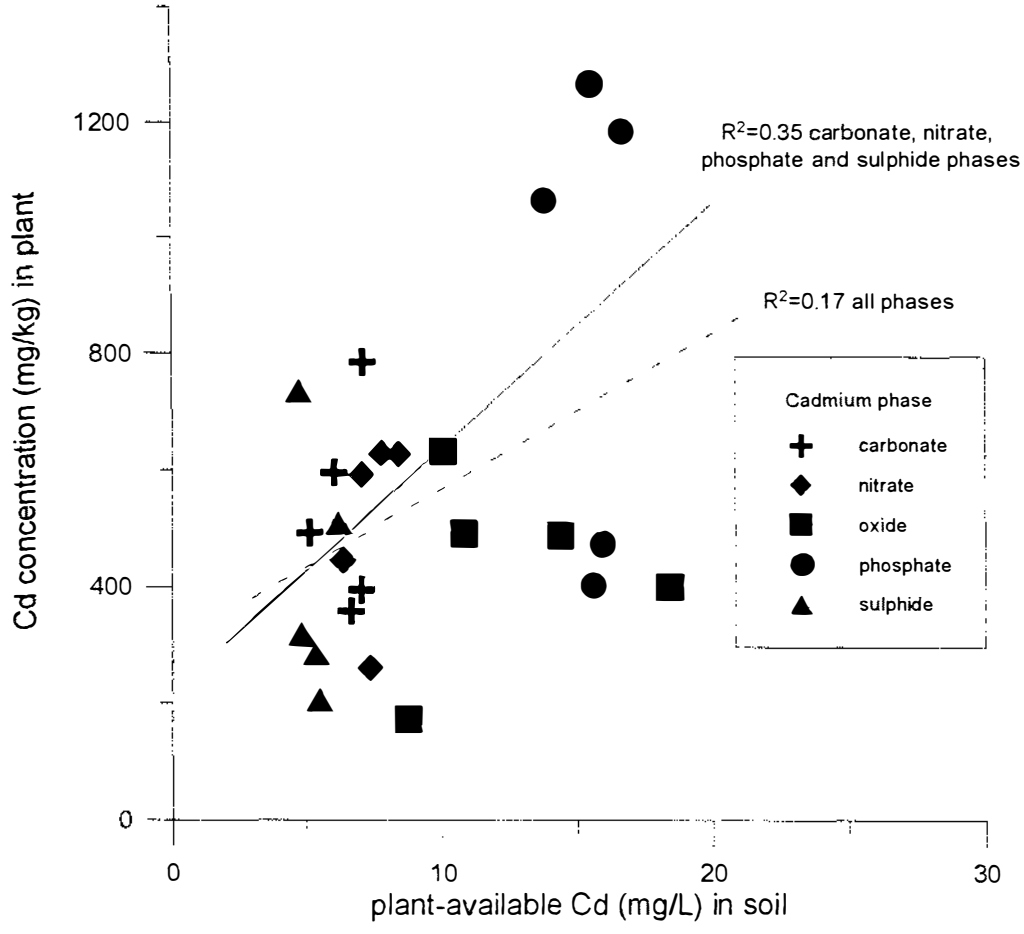


**Figure 5.7.** Plot of the cadmium concentration in *Cardaminopsis halleri* as a function of the plant-available cadmium concentration in the soil for each metal phase.

The sulphide, nitrate, carbonate and phosphate phases all appear to plot roughly on a linear relationship ($R^2$=0.35 – Fig. 5.7). For *Cardaminopsis halleri*, the oxide phase behaves differently and effects a drop in the regression coefficient of the data set when analysed with the other 4 phases. This result supports that assertion that ammonium acetate as an extractant does not accurately model plant-available cadmium equally well for every mineral phase.

### 3. *Thlaspi caerulescens*

*Thlaspi caerulescens* responded differently to EDTA and citric acid treatment when compared with *Cardaminopsis halleri*. There was a significant increase in cadmium uptake from the organic (nitrate) phase using both of these chemicals. From the sulphide phase, EDTA caused a significant decrease in plant cadmium. Graphical representation of predicted verses modelled natural cadmium uptake clearly shows this discrepancy in uptake from the sulphide phase (Fig 5.8).

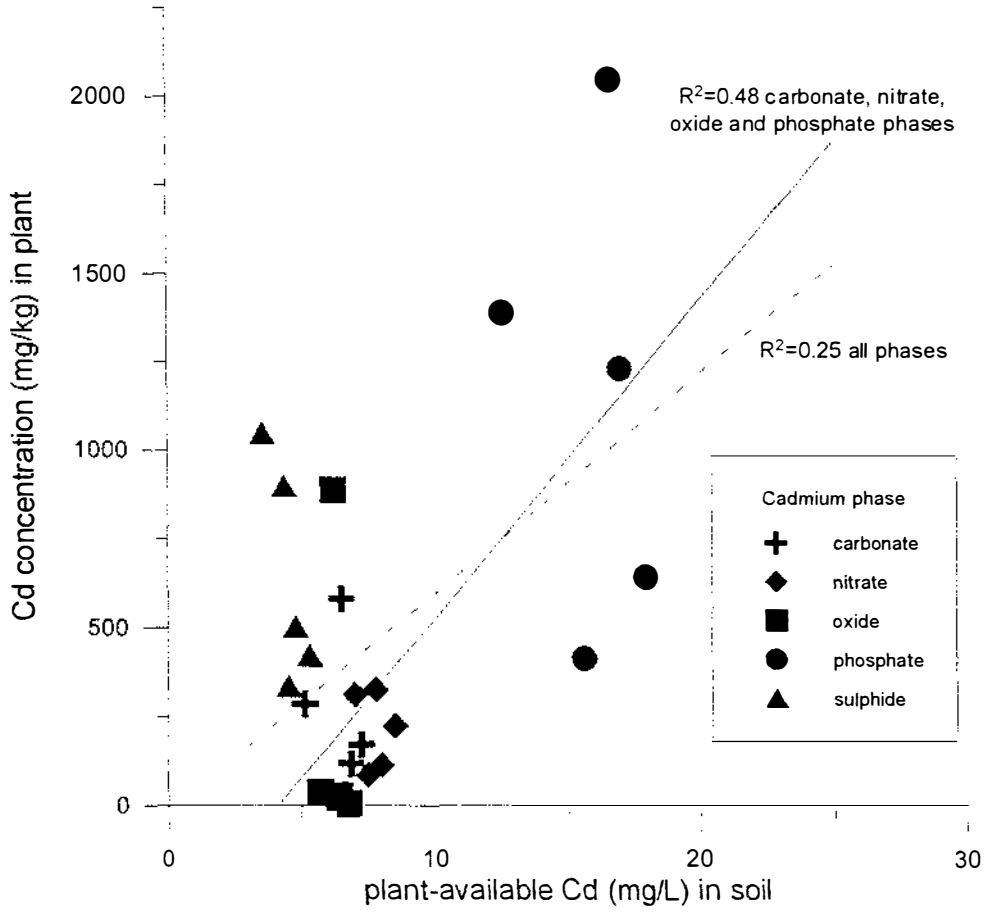


**Figure 5.8.** Plot of the cadmium concentration in *Thlaspi caerulescens* as a function of the plant-available cadmium concentration in the soil for each metal phase.

The carbonate, nitrate, oxide and phosphate phases all appear to plot on a linear relationship ($R^2$=0.48 – Fig. 5.8). The sulphide phase plots independent of the other points and when analysed with the other 4 phases, effects a drop in the value of the regression coefficient for the data set. Again the explanation for this discrepancy could lie in the relative efficacy of ammonium acetate as an extractant for the various mineral salts as was shown true for *Brassica juncea* and *Cardaminopsis halleri*.

## 5.9 Conclusion

Natural and induced uptake of cadmium by the non-accumulator species *Brassica juncea* is dependent upon the metal phase of cadmium present in the soil, a function of the source of the original metal contamination. An increase in uptake was observed for all phases except the carbonate phase using EDTA. The phosphate phase had a significantly greater 'amenability' to induced uptake. Citric acid had no effect on any phase. The phytoextraction potential of *B.juncea* under the geochemical conditions of this study is maximised using EDTA.

Induced hyperaccumulation for cadmium is apparently ineffective using the hyperaccumulating species *Thlaspi caerulescens* and *Cardaminopsis halleri*. The uptake potential of *T.caerulescens* was increased from the nitrate (organic) phase using both citric acid and EDTA. However, uptake by *C.halleri* was not increased for any phase using either chemical. The uptake mechanisms of these species appear different. *Thlaspi caerulescens* has a greater potential for metal uptake from the phosphate and sulphide phases, while *C.halleri* has a greater potential for uptake from the carbonate, oxide and nitrate phases. The reason for this difference can only be speculated on here as it is beyond the scope of this study. However, the chemicals actively exuded by each species may in fact be different, and more suited to 'dissolving' metals from different minerals.

The use of ammonium acetate as an extractant to estimate the plant-available fraction of cadmium in soil also appears to be dependent upon the mineral phase. If each phase was equally modelled by ammonium acetate, then a single curve describing the relationship would be expected. This is not the case when several phases are modelled in the same graph. Further work should be conducted to clarify the relationship between mineral phase and species-specific modelled plant availability. It appears that ammonium acetate may not be a suitable extractant to estimate plant-available cadmium for all sources of contamination, or for all plant species.

# Chapter 6 - Geochemical Model for Zinc Uptake

## 6.1 Introduction

To model the geochemical conditions prevalent in the Auby soil, commercial seed-raising mix was used as a base substrate to create an artificial, zinc-contaminated 'soil' (C=20%, N=0.6%). A substrate zinc concentration of 0.2% was used. To induce hyperaccumulation a high concentration of EDTA (disodium salt) was added to each relevant pot. A high concentration was used to ensure that the chemical-inducing agent was present in the soil at an excess concentration, thus minimising the chance that differential rates of chemical degradation (a function of the half life of EDTA) was a factor controlling metal uptake. The aim of this experiment was to determine the affect of mineral phase on induced and natural zinc uptake. I realise that the rate of EDTA applied may have negatively affected plant health and thus metal uptake, however, this factor was independent of mineral phase and was considered in this experiment. Leaching of metal out of the various pots was similarly not considered.

## 6.2 Experimental design

Five metal salts were chosen to represent zinc pollution that could occur in a wide range of environments: carbonate, oxide, phosphate, sulphate and sulphide. The soluble sulphate salt was chosen to model zinc, after dissolution, as part of the soil organic phase, i.e., after a period of days no zinc would remain in the soil as a crystalline phase. Each salt (as a solid) was added to commercial potting mix to give a final soil-metal concentration of 2 000 mg/kg (w/v). Pots (250 mL – 180 in total) were planted, in equal numbers, with either the non-accumulator species *Brassica juncea*, or the hyperaccumulating species *Cardaminopsis halleri* or *Thlaspi caerulescens*. A control substrate was used, where the three species were planted in pots containing potting-mix with no added zinc. During the growing cycle, pot positions were randomly changed on a periodic basis to equalise light exposure. The ambient temperature of the greenhouse was set at 15-25°C with no humidity control. Overhead watering was carried out daily using a hand-held house.

After approximately 10 weeks growth, 5 replicates of each plant species, for each mineral phase, were treated with either 2 g/kg EDTA or water as a control. All treatments were applied as a solution (20 mL) and were randomly allocated to replicate pots. Two weeks after treatment, the above-ground biomass was harvested[8], dried at 60°C to constant weight and subsamples were digested in concentrated nitric acid before analysis by FAAS.

Substrate samples were 'cored' from the control pots of every metal phase at the time of plant harvest, ground using a porcelain mortar and pestle, and subsamples were extracted with hydrochloric acid (5M) to give the total metal concentration for each of the prepared soils. Ammonium acetate (1M, pH 7) was used to model the concentration of plant-available zinc (Ernst, 1996; Robinson, 1997) for each metal phase by overnight shaking at a soil:liquid ratio of 1:10. In each case, analysis of the filtrate was performed using FAAS. Measurements of soil pH were performed in water, using a 1:2.5 soil:liquid ratio.

Data were tested for normality, and analysed using ANOVA due to the observed normal distribution.

## 6.3 Results: *Cardaminopsis halleri*

Zinc uptake was increased from the phosphate phase using EDTA, but decreased from the oxide phase relative to the control (Fig. 6.1). From the carbonate, sulphate and sulphide phases, no change in plant zinc was apparent after treatment. Summation of the natural uptake response of *Cardaminopsis halleri* to each of the 5 salts illustrates the importance of the chemical form of zinc present in the soil on this species ability to naturally accumulate zinc (Fig. 6.2). The highest metal uptake observed in this experiment was for EDTA-induced uptake by *C.halleri* from the carbonate phase (2.8%).

[8] Plant health at time of harvesting is reported in Appendix 2.

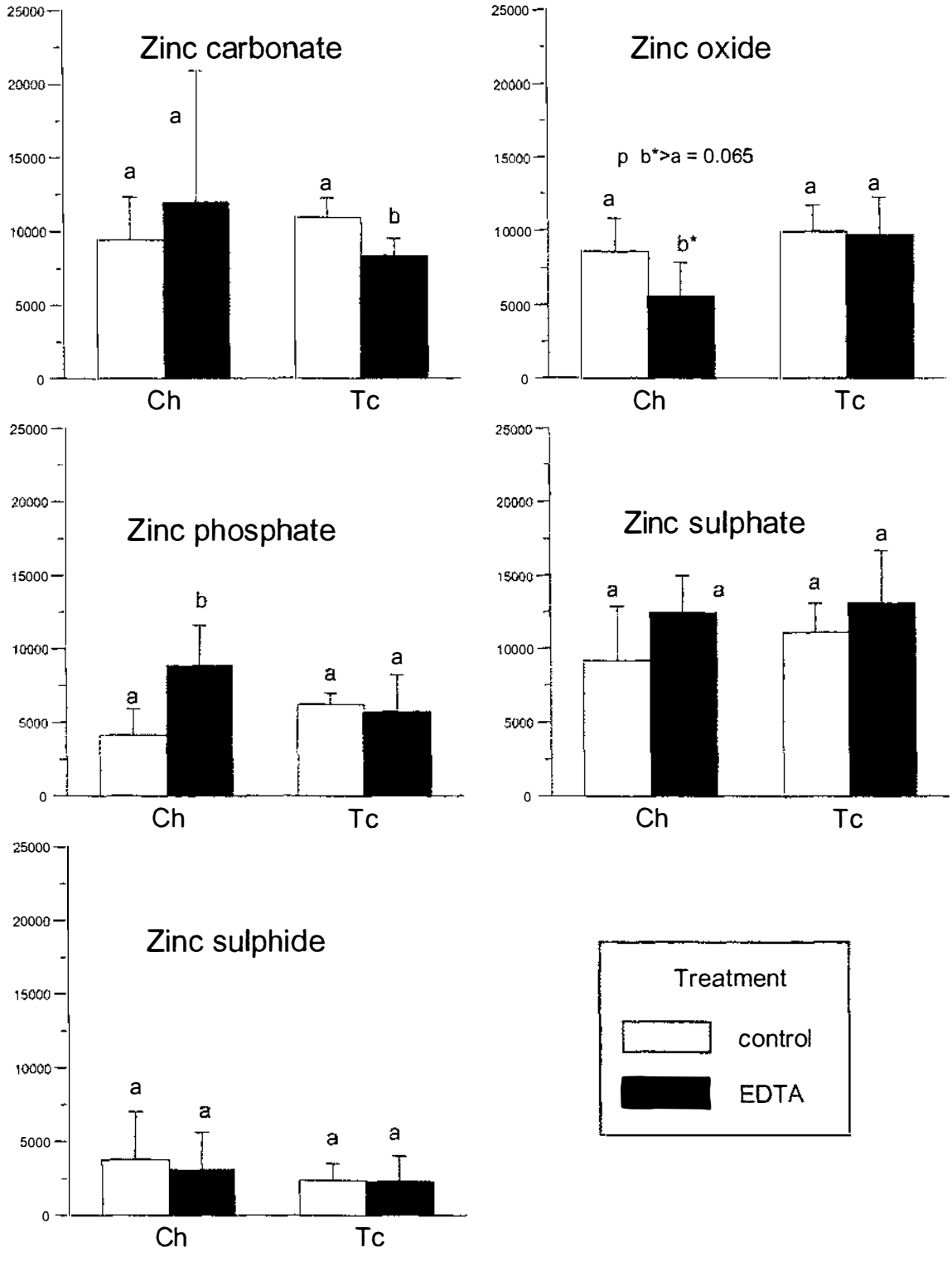


**Figure 6.1.** Natural uptake and EDTA-induced uptake of zinc by *Cardaminopsis halleri* (Ch) and *Thlaspi caerulescens* (Tc) growing on artificial 0.2% Zn soils of different metal phases (mean + SE, n=5). Means for the same species and phase, with the same letter, are not significantly different (ANOVA p>0.05).

However, mean EDTA-induced uptake by this species from the carbonate salt was not significantly different to mean uptake for the control treatment, due to the anomalously high standard-error-bar for this species, treatment combination (Fig. 6.1).

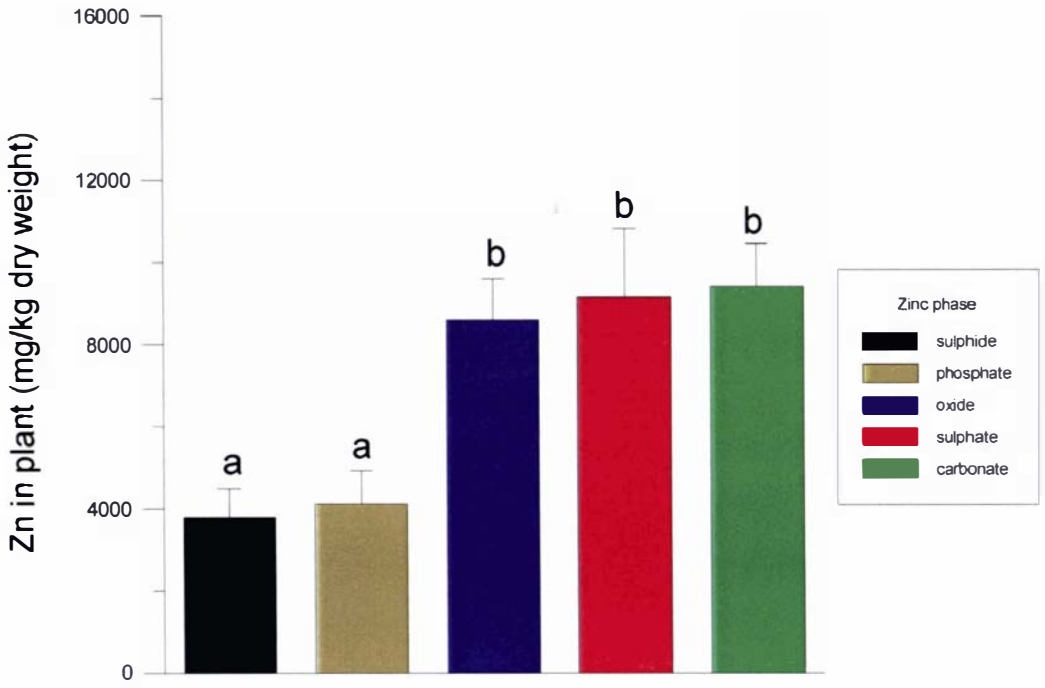


**Figure 6.2**. Summary: efficacy of natural zinc uptake by *Cardaminopsis halleri* as a function of metal phase.

The ordering of natural uptake can be written as:

sulphide (a) = phosphate (a) < oxide (b) = sulphate (b) = carbonate (b)

This is different to the ordering of plant-available zinc for this species, estimated by extraction with ammonium acetate (1M, pH 7):

sulphide (a) << phosphate (b) < carbonate (c) < oxide (d) = sulphate (d)

There appears to be a large discrepancy between modelled and observed zinc uptake from the sulphide phase.

## 6.4 Results: *Thlaspi caerulescens*

For all zinc phases except the carbonate phase, treatment with EDTA effected no change in the zinc uptake of *Thlaspi caerulescens* relative to the control. EDTA caused a significant decrease in zinc uptake from the carbonate salt (Fig. 6.1). Summary of the natural response of *T.caerulescens* to each zinc salt allows qualification of the importance of mineral phase on natural uptake (Fig. 6.3).

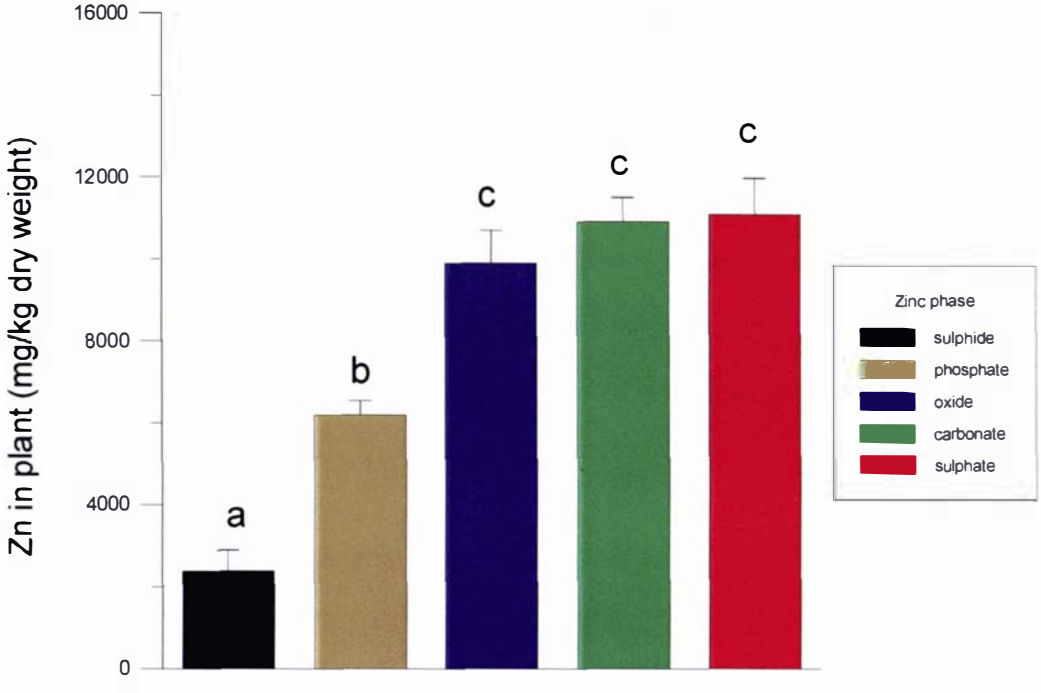


**Figure 6.3**. Summary: efficacy of natural zinc uptake by *Thlaspi caerulescens* as a function of metal phase.

The ordering of natural uptake can be written as:

sulphide (a) < phosphate (b) < oxide (c) = carbonate (c) = sulphate (c)

The above is similar to the ordering of natural uptake for *Cardaminopsis halleri.* However, the ordering of uptake for *Thlaspi caerulescens* is more similar to the ordering of plant-available zinc for this species, estimated using ammonium acetate, than was true for *C.halleri.*

sulphide (a) << phosphate (b) < carbonate (c) = oxide (c) < sulphate (d)

## 6.5 Results: total soil zinc

Subsamples of substrate taken from each of the control treatment pots were extracted with hydrochloric acid (5M) at a soil to acid ratio of 1:10. Analysis of the filtrate showed reasonable agreement of total zinc with the 'designed' concentration of 2 000 mg/kg, although significant differences in zinc concentration between phases were apparent (Table 6.1). The pH of the substrate was independent of mineral phase.

**Table 6.1**. Total soil zinc and pH for the control treatment soils of each metal phase. Mean zinc values with the same letter are not significantly difference (ANOVA p>0.05). Mean (±sd).

| Zinc phase | pH | Total zinc (mg/kg) |
|---|---|---|
| Carbonate | 5.2 | 2355 ± 176 (a) |
| Sulphate | 4.9 | 2517 ± 214 (ab) |
| Oxide | 5.3 | 2563 ± 190 (b) |
| Phosphate | 5.2 | 2769 ± 174 (c) |
| Sulphide | 5.3 | 3039 ± 196 (d) |
| Control | 5.2 | Note |

Note. The total zinc concentration of the control-phase soil was below detection limits using FAAS and is not reported in this table.

## 6.6 Results: plant-available zinc

The ordering of plant-available zinc, reported for each of the two hyperaccumulator species, was based upon values determined from each of the control treatment soils for each species. These values do not account for differences in metal concentration between each set of soils, and are therefore only relevant to their respective plants. To accurately compare the bioavailablility of each metal phase, and hence the potential solublility of zinc in the rhizosphere as a function of zinc geochemistry, these data for plant-available metal must be corrected for total concentrations (Table 6.2). Reporting the percentage of the total metal in the soil that is plant-available has made this correction

Under the geochemical conditions of this study, the percentage concentration of plant-available zinc, estimated by ammonium acetate, confirms that bioavailability of zinc is strongly dependent upon mineral phase.

**Table 6.2**. Percentage zinc that is plant-available (ammonium acetate) from each metal phase. Mean values with the same letter are not statistically different (ANOVA p>0.05). Mean (±sd).

| Zinc phase | % extractable zinc |
|---|---|
| Sulphide | 0.7 ± 0.1 (a) |
| Phosphate | 18.0 ± 1.6 (b) |
| Carbonate | 27.2 ± 3.0 (c) |
| Oxide | 27.8 ± 1.7 (c) |
| Sulphate | 31.0 ± 3.5 (d) |

Note. The total and plant-available zinc concentrations of the control-phase soil were below detection limits by FAA and are not reported in this table.

## 6.7 Discussion - a model for zinc uptake

Of the three metals studied in Section A, zinc is the most phytotoxic. The non-accumulating and non-tolerant species *Brassica juncea* would not grow in any of the zinc soils of this study, including the sulphide soil with a very low plant-available metal concentration (2.2 mg/L). This statement explains why no results for uptake by *B.juncea* have been presented. It appears that the phytotoxicity of zinc would preclude the use of non-accumulator species for phytoextraction, except where the concentration of zinc in the soil was very low.

There was no increase of zinc uptake by *Thlaspi caerulescens* in response to EDTA treatment for any metal phase. However, EDTA caused a decrease in the plant zinc concentration taken up by this species growing on the carbonate phase soil. For *Cardaminopsis halleri*, EDTA effected no response in zinc uptake from the carbonate, sulphate and sulphide phases. EDTA caused an increase in zinc uptake from the phosphate phase, but a decrease in plant zinc from the oxide phase.

Based upon the results of this experiment, there is no strong evidence that EDTA-induced uptake of zinc to a higher concentration, is possible using the hyperaccumulator species *Cardaminopsis halleri* and *Thlaspi caerulescnes*. This contradicts the findings in Chapter 4 where EDTA-induced uptake of lead was apparent for *T.caerulescens*, but is in agreement with the findings of Chapter 5, where EDTA-induced uptake of cadmium, to a higher concentration relative to natural uptake, was not observed for either hyperaccumulator species. The natural uptake potential for each of these two species varied between each of the different mineral phases. The relationship between this uptake and the plant-available zinc concentration is discussed below.

*Relationship between ammonium acetate and the metal phase*

## 1. *Thlaspi caerulescens*

Natural uptake from each salt agrees well with the predicted ordering of uptake, estimated using ammonium acetate, as shown in Figure 6.4. Each phase plots on a linear relationship ($R^2$=0.82), indicating that for *T.caerulescens*, ammonium acetate models plant uptake of zinc equally well from each of the 5 metal phases.

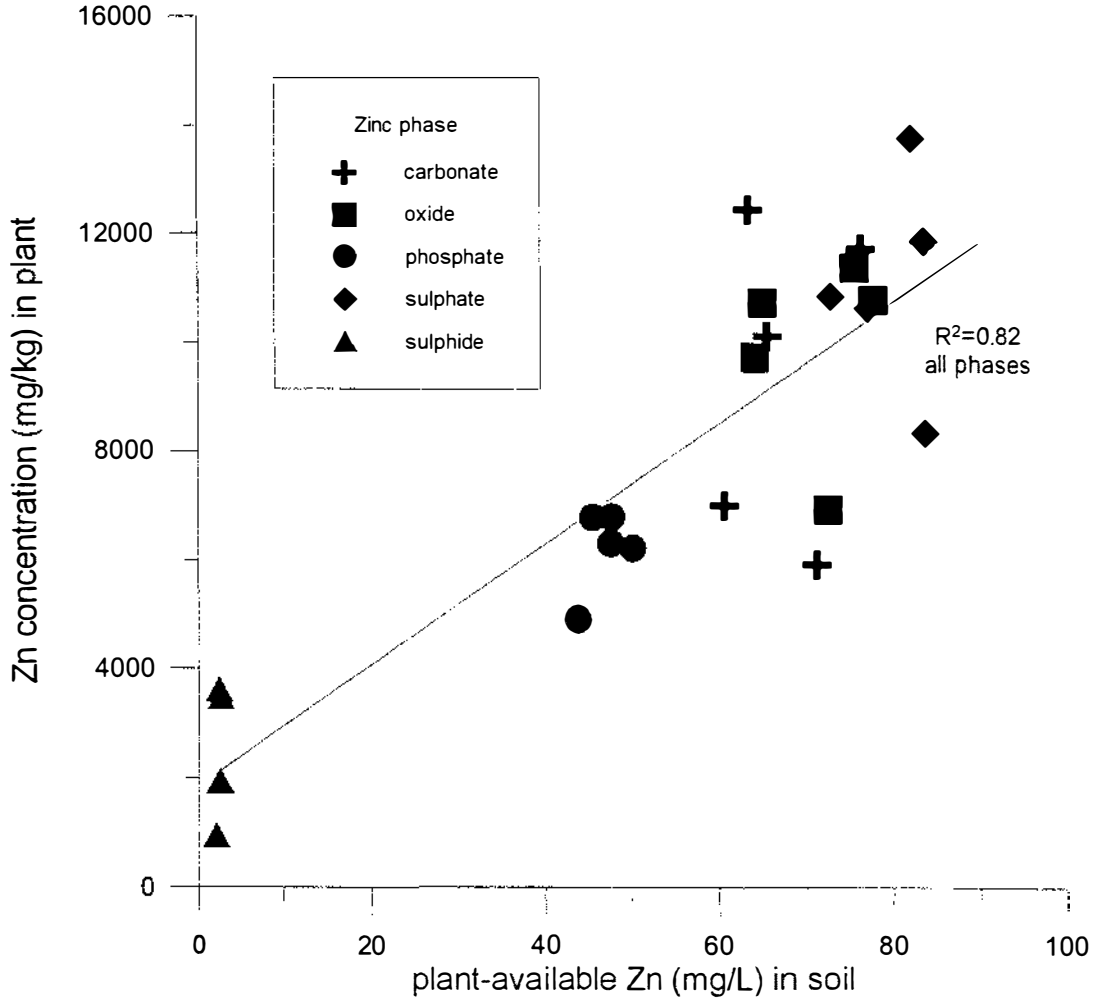


**Figure 6.4**. Plot of the zinc concentration of *Thlaspi caerulescens* as a function of the plant-available zinc concentration in the soil for each metal phase.

## 2. *Cardaminopsis halleri*

The ordering of natural uptake from each metal salt by *Cardaminopsis halleri* disagrees with the predicted ordering, estimated using ammonium acetate. Graphical presentation of this discrepancy (Fig. 6.5) shows no clear trend. It is not possible to fit an accurate linear model to this data set ($R^2$=0.30), indicating that the efficacy of ammonium acetate to estimate plant-available zinc for *C.halleri* is not equal for each metal phase.

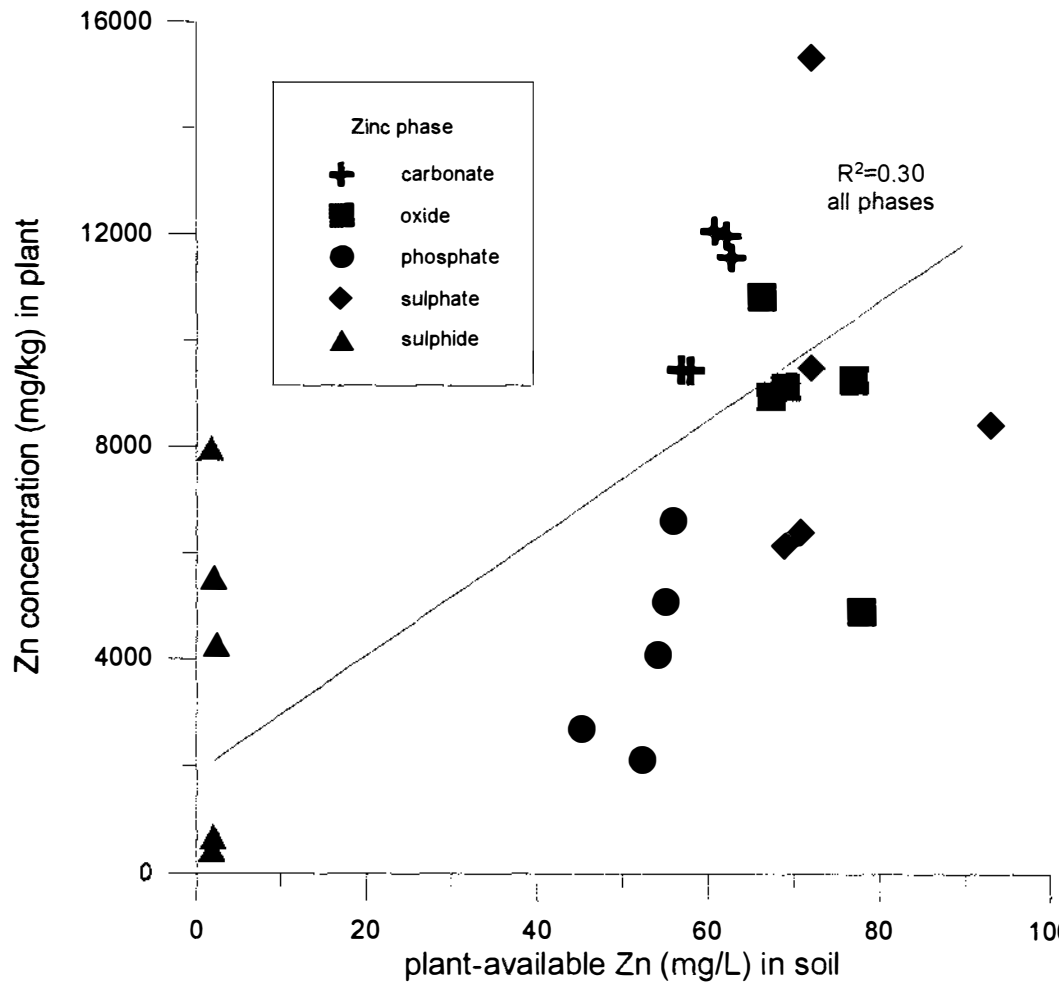


**Figure 6.5**. Plot of the zinc concentration of *Cardaminopsis halleri* as a function of the plant-available zinc concentration in the soil for each metal phase.

Comparison of Figure 6.4 with 6.5 shows a noticeable difference in the point distribution of replicates for each metal phase. This is particularly true for the carbonate, phosphate and sulphide phases. This difference between the two plots further indicates that the relative efficacy of ammonium acetate as an extractant to

estimate plant-available metal is plant-species specific. The difference in relative efficacy could perhaps be related to the uptake mechanisms exhibited by each species affecting the concentration of plant-available metal. If the uptake mechanisms were the same then both graphs should have a similar point distribution for soils of the same mineral phase.

## 6.8 Conclusion

Modelling the response of *Brassica juncea* to zinc present as different geochemical phases was not possible in this study. The poor growth of this species on each of the experimental soils is indicative of a very low zinc tolerance. The inhibition of plant growth observed from the sulphide soil was surprising as the ammonium acetate zinc concentration of this soil was low (2.2 mg/L). Due to the lack of data, it is not possible to assess the suitability of ammonium acetate as an extractant to model plant uptake of zinc for this species. Hence, the efficacy of this extractant to predict the response of *B.juncea* to zinc can not be quantified.

Induced uptake of zinc was only observed in *Cardaminopsis halleri* from the phosphate phase. The increase in mean uptake was by a factor of two. In general, the action of EDTA was not of sufficient merit to justify the practical use of this chemical to maximise the zinc phytoextraction potential of *C.halleri* due to the costs (economic and environmental) that may be involved. EDTA did not increase uptake by *Thlaspi caerulescens.* Again, for this species, natural uptake affords the greatest potential for phytoextraction. The zinc uptake mechanisms, as for cadmium, appear different for each species. Except for the sulphide phase, *T.caerulescens* shows greater uptake potential, and clearly differentiates between the sulphide and phosphate phases. Neither species differentiates between the oxide, carbonate and organic (sulphate) phases. *Cardaminopsis halleri* does not differentiate between the sulphide and phosphate phases.

The difference in point distribution between the plots for modelled plant-available zinc, and the discrepancy observed in metal uptake from the sulphide phase, support the conclusion that the mechanisms of uptake for the two hyperaccumulator species are in

some way different. If these mechanisms were the same, the plotted point distribution of modelled versus observed natural uptake would be similar, inferring that the ordering of natural uptake from each phase was equal.

The results of this experiment show that ammonium acetate accurately models zinc uptake by *Thlaspi caerulescens*, regardless of mineral phase, by virtue of the linear association of each individual phase. However, uptake of zinc by *Cardaminopsis halleri* is not accurately modelled by ammonium acetate. In particular, a large discrepancy exists between the modelled and actual metal uptake from the sulphide phase. The plant-available concentration of zinc from the sulphide phase is 20 times less that from the phosphate phase, although *C.halleri* accumulates zinc equally well from each phase. This discrepancy for zinc appears to be a function of plant-species specific uptake mechanisms, not metal-phase geochemistry.

# Chapter 7 - An Integrated Geochemical Model for Cadmium, Lead and Zinc Uptake

The aim of the research reported in the previous 3 chapters was to determine the importance of mineral-phase geochemistry on several practical aspects of phytoextraction, and to use the associated models to explain the findings of pot and field trials conducted on naturally occurring substrates.

## 7.1 Practical aspects of phytoextraction for cadmium, lead and zinc

1 - *the choice of plant species to be used for the natural hyperaccumulation of Cd, Pb and Zn*

On soils where 200 mg/kg cadmium was present, *Brassica juncea* grew well, except where the metal was added to the substrate as a soluble (nitrate) salt. This species hyperaccumulated cadmium (accumulation greater than 100 mg/kg DW) from every mineral phase, but the relative degree of accumulation was dependent upon the mineral phase as follows:

sulphide (a) ~ phosphate (ab) ~ nitrate (ab) ~ oxide (bc) ~ carbonate (c)

Natural hyperaccumulation of cadmium by *Cardaminopsis halleri* was equal for each tested form of metal, except from the phosphate phase where uptake was significantly higher. Natural uptake of cadmium by *Thlaspi caerulescens* was higher from the sulphide and phosphate phases than from the oxide, nitrate and carbonate phases while *Cardaminopsis halleri* showed greater uptake from the oxide, nitrate and carbonate phases. The biomass of *C.halleri* and *T.caerulescens* is very similar, however, *C.halleri* grows best in a cool, wet, shaded environment, whereas *T.caerulescens* shows superior growth in a hot, dry, open environment (A.Deram, pers. commn. 1999). The site-specific 'physical' environment would hence also determine the species with the greater phytoextraction potential. If the uptake of cadmium observed in this experiment for *B.juncea* could be realised in the field, then this species would offer by far the greatest

uptake potential due its superior biomass (10 times greater than that for the two hyperaccumulator species). Accumulation of cadmium by *B.juncea* from the substrates of Chapter 5 was for some forms of metal greater than that for the hyperaccumulator species.

*Brassica juncea* grew well on soils containing 1% lead where this metal was not added to the substrate as a soluble form, but failed to 'naturally' accumulate significant levels of metal from any mineral phase. *Thlaspi caerulescens* hyperaccumulated lead from the carbonate and nitrate phases, but not from the remaining salts, where the level of uptake was equal to that of *B.juncea.* Growth of the latter species was only inhibited by soil lead for the nitrate salt. If natural hyperaccumulation of lead is to be effected, then of the species tested, only *T.caerulescens* can be used. However, natural hyperaccumulation by *T.caerulescens* in Chapter 4 was only apparent for lead added to the substrate as a carbonate or nitrate form and thus will not be observed for all lead 'environments'.

*Brassica juncea* failed to grow on soils containing approximately 2 000 mg/kg zinc, even where the plant-available metal concentration was very low (sulphide phase 2.2 mg/kg). Natural uptake of zinc by *Cardaminopsis halleri* was significantly greater from the oxide, sulphate (soluble) and carbonate phases than from the sulphide and phosphate phases. Natural uptake of zinc by *Thlaspi caerulescens* was also greater from the oxide, carbonate and sulphate phases relative to the sulphide and phosphate phases. For zinc contaminated soils, *B.juncea* shows little potential for phytoextraction (natural), but the potential of the two hyperccumulating species is equal. Choice of *C.halleri* or *T.caerulescens* would be made solely upon the basis of greater biomass production, a function of the environment in which the plant was growing. Both species grew equally well at the soil zinc concentration used for this study.

2 - *the choice of chemical agent to be used for the induced hyperaccumulation of Cd, Pb, and Zn*

Induced uptake of cadmium was only effected using EDTA. With the exception of the action of citric acid on organic-phase cadmium, no increased uptake was induced using this chemical. Acetic acid was not tested as part of the cadmium experiment.

EDTA induced a significant increase in lead uptake by *Brassica juncea* and *Thlaspi caerulescens* from each mineral phase. Acetic acid was equally effective in its ability to induce uptake from the carbonate and oxide phases, and from the sulphate phase for *T.caerulescens*. Citric acid did not induce an increase in uptake from any phase. EDTA was the most effective chemical agent for induced uptake of lead from the organic, phosphate, sulphide and sulphate phases. Acetic acid may be a better choice for the carbonate and oxide phases.

EDTA induced a significant increase in zinc uptake by *Cardaminopsis halleri* from the phosphate phase. The effect of EDTA on the zinc uptake of *Brassica juncea* could not be tested due to necrosis of these plants in each of the zinc substrates of the experiment.

3 - *the choice of species to be used to maximise the induced phytoextraction of Cd, Pb and Zn*

The advantage of induced hyperaccumulation is that species of high biomass may be employed. The uptake models of Chapters 4, 5 and 6 show that where the target metal of the operation is not subject to active uptake mechanisms, induced hyperaccumulation appears to be species independent. It appears, however, that induced uptake of a metal that is being hyperaccumulated by a plant species, to a higher concentration, is not possible. For example, neither *Brassica juncea* nor *Thlaspi caerulescens* can be regarded as lead hyperaccumulators, and as a result, EDTA-induced hyperaccumulation was possible. Induced uptake of cadmium was possible using the non-accumulator species *B.juncea*, but not for the known hyperaccumulator species *Cardaminopsis halleri* and *T.caerulescens*. Induced uptake of zinc was not possible for either hyperaccumulator species. These observations are in agreement with those of Robinson *et al.* (1997b) who showed that addition of EDTA to a nickeliferous substrate on which the nickel hyperaccumulator *Berkheya coddii* was growing, decreased the concentration of nickel in the plant. The authors of this study suggested that chemical agents added to the rhizosphere of a hyperaccumulator species may compete with that species active uptake mechanism, causing metal to diffuse out and down its concentration gradient into the plant root.

Induced phytoextraction therefore appears to be maximised by using a plant species with the greatest growth potential that exhibits no active uptake mechanism for the target metal. The only proviso is that the site-specific geochemical environment must facilitate uptake of the soluble metal-complex. Metal extraction is directly proportional to biomass in such a scenario.

4 - *the value of ammonium acetate (1M) as an extractant to model plant-available metal*

Ammonium acetate may accurately model plant-available metals, and hence the potential for plant uptake, but not equally well for each mineral phase. The relative, modelled efficacy for these same phases also varies between plant species.

Natural uptake of cadmium by *Brassica juncea* from an organic, sulphide or sulphide phase can be equally modelled by ammonium acetate ($R^2$=0.64 – Fig. 5.5). The carbonate and phosphate phase, however, are not modelled by the same linear relationship: the regression coefficient for a linear model to describe all phases of cadmium was very low ($R^2$=0.01 – Fig. 5.5). Ammonium acetate appears to model cadmium uptake by *Cardaminopsis halleri* more closely, with all phases except the oxide phase plotting on the same linear model ($R^2$=0.35 – Fig. 5.6), although it must be mentioned that the regression coefficient for this model is low. Uptake by *Thlaspi caerulescens* appears to be equally well modelled for all phases except the sulphide phase ($R^2$=0.48 – Fig. 5.7).

Natural uptake of zinc by *Thlaspi caerulescens* appears to be very well modelled by ammonium acetate: all phases plotted on a linear relationship ($R^2$=0.82 – Fig. 6.4). However, no accurate linear model can be plotted for uptake of zinc by *Cardaminopsis halleri* ($R^2$=0.30, Fig. 6.5). Variation in the point distribution between the two zinc plots illustrates that the mechanism of zinc uptake for *Cardaminopsis halleri* and *Thlaspi caerulescens* could in fact be different. If the mechanisms were the same, very similar plots would be expected.

Ammonium acetate does not accurately model bioavailability of lead from any mineral phase. The highest plant-available metal concentrations extracted from any of the Cd, Pb

and Zn soils were observed for the lead-contaminated soils (Table 7.1). Natural lead uptake was only high, however, for *Thlaspi caerulescens* growing on the carbonate and nitrate phase soils. The reason for these two uptake anomalies is not clear. The associated soils do not have unusually high values of plant-available metal. It appears that lead is actively excluded by each of the tested plant species, with the exception of the two *T.caerulescens* phase combinations summarised here. Ammonium acetate does not model this unusual hyperaccumulation. Further work should be conducted to examine this anomaly. If the reason why *T.caerulescens* hyperaccumulated lead from these two metal forms but not the other four could be understood, then we may be able to form a better description of the mechanism behind lead uptake and exclusion.

**Table 7.1**. Summary of the mean plant-available metal concentrations (ammonium acetate) extracted from each metal phase.

| Mineral phase | Cadmium (mg/L) | Lead (mg/L) | Zinc (mg/L) |
|---|---|---|---|
| Carbonate | 10 | 93 | 64 |
| Nitrate | 8 | 107 | - |
| Oxide | 10 | 112 | 71 |
| Phosphate | 16 | 55 | 48 |
| Sulphide | 5 | 33 | 22 |
| Sulphate | - | 43 | 78 |

Caution should be used when comparing modelled data for plant-available metal if these data compare several sites or areas where the contaminating metal form is different. It may not be possible to fit a curve to a combined phase data set, and thus generate a model to predict the metal-uptake potential of an individual species, when that metal does not have the same initial geochemistry in the soil. Similar caution should be observed when using a predicted model for one species, to estimate the response of another species to plant-available soil metal.

## 7.2 Application of the geochemical model to results from pot and field trials

*Auby - northern France*

Trials conducted at the Auby site in northern France, where no further accumulation was induced in either hyperaccumulator or non-hyperaccumulator species, may be explained

by the geochemical model summarised in the previous section (7.1). Cadmium, lead and zinc are present as carbonate and oxide phase minerals, in an environment rich in organic material similar to the substrate used for Chapters 4, 5 and 6. The pH of the Auby soil is also similar to that modelled by the greenhouse trial soils.

EDTA has been shown to be relatively ineffective at inducing lead uptake from both the carbonate and oxide phases, relative to other sources of contamination (Fig. 4.1). It appears, at the Auby site, that acetic acid may have been a better chemical to use. Acetic acid is a naturally occurring organic acid and has very limited toxicity - it seems likely that fewer environmental problems would be associated with the use of this chemical than have been encountered with the use of EDTA. It would be interesting to test this theory, and attempt to induce the hyperaccumulation of lead in the field using acetic acid. Colleagues at the University of Lille intend to carry out this trial during the European summer of 2000.

The geochemical model of Section 7.1 predicts that cadmium and zinc will be hyperaccumulated by *Cardaminopsis halleri* from a carbonate or oxide mineral phase, but that induced hyperaccumulation of these metals to a higher concentration will not be possible. The field data supports this conclusion. EDTA-induced uptake by the non-accumulator species *Brassica juncea* of cadmium present in the soil as a carbonate phase was not significant. This observation may explain why no increase in cadmium uptake was observed in the non-accumulator species *Arrhenatherum elatius*, growing at Auby after EDTA had been applied to the site (Chapter 3.2). There is a major discrepancy between modelled and field data for natural cadmium uptake by non-accumulator species; cadmium uptake by *Brassica juncea* was shown to be high from every mineral phase (Fig. 5.1). However, field uptake by *A.elatius* was low. Perhaps the presence of zinc in the soil solution at the Auby site inhibited field uptake of cadmium by this species. Alternatively, *A.elatius* could be an excluder species, while *B.juncea* could be an indicator species.

*Tui Mine tailings - New Zealand*

The data obtained from trials conducted on material from the Tui mine tailings, do not fit so well with the geochemical model of Section 7.1. Cadmium, lead and zinc within the Tui tailings exist predominantly as a sulphate or sulphide mineral phase.

Increased uptake of lead by all trialled species was induced using EDTA. This observation supports a species independence for lead hyperaccuumulation. However, the levels of subsequent uptake were lower than those predicted by the geochemical model. The mean concentration of lead accumulated by *Berkheya coddii* after treatment was less than 1 000 mg/kg (0.1%). The model predicts that uptake closer to 4 000 mg/kg could be expected. Deram *et al.* (2000) showed that addition of lime to the Tui mine tailings dramatically decreased the induced uptake of lead by *Arrhenatherum elatius* above an addition rate of 0.125% lime. The tailings from which the data set for this thesis was derived were limed to 2.5%. The difference in pH could account for the discrepancy of the data set with the predicted model. Stability and subsequent uptake of a metal-EDTA complex may have a pH dependency.

The effect of pH on the EDTA-induced uptake of Cd, Pb and Zn will be discussed further after Chapter 8 in the conclusion to Section A.

The geochemical model predicts that increased uptake of cadmium and zinc by the hyperaccumulator species *Cardaminopsis halleri* and *Thlaspi caerulescens* will not be induced using EDTA. This is observed for data from the Tui substrate. However, the model predicts that increased uptake of cadmium by *Brassica juncea* can be expected using EDTA. Induced uptake by this species was not observed in the Tui trials. As for lead, pH dependency of the metal-EDTA complex may preclude uptake.

The geochemical model predicts that natural hyperaccumulation of cadmium and zinc should be observed for *Cardaminopsis halleri* and *Thlaspi caerulescens* growing on a sulphide mineral phase. The model similarly predicts that significant natural uptake of cadmium should be observed for *Brassica juncea* growing on a sulphide mineral phase. However, natural uptake by all of these species growing on the Tui tailings was low.

The increase in pH (from 3.5 to 7.5), through liming the tailings substrate, could once again explain this anomalously low cadmium and zinc uptake, although McGrath *et al.* (1997) stated that, in their experience, the active zinc-uptake mechanism of *Thlaspi caerulescens* was independent of rhizosphere pH, a statement that contradicts the theory of Brown *et al.* (1994) who reported that pH was the most important variable controlling metal uptake by *T.caerulescens*. The model of this thesis does show, however, that natural uptake of zinc by both *C.halleri* and *T.caerulescens* is significantly lower from the sulphide salt than from other metal forms. Cadmium uptake from the sulphide phase should be significant for *T.caerulescens*. The anomaly between modelled and observed natural uptake of cadmium by *B.juncea* may also be explained, at least in part, through the presence of zinc in the soil solution. The presence of zinc may have excluded uptake of both metals by the non-accumulator species. Exactly why cadmium uptake did not occur for each of these three species remains a topic for further research.

## 7.3 Conclusion

The primary aim of generating the geochemical model described in this chapter was to examine how different chemical forms of metal, representing different sources of metal contamination, would affect the natural and induced uptake of Cd, Pb and Zn. In particular, the aim of this section of work was to explain the metal uptake patterns observed in phytoextraction data sets from the Auby site and for pot trials conducted on material from the Tui mine tailings.

The discussion of the previous chapters has shown that the form of metal present in soil does affect metal uptake and how an understanding of this effect can begin to explain metal uptake patterns observed on natural substrates. This is thinking that should now be integrated into phytoextraction trials.

Four important and sound conclusions applicable to phytoextraction that have arisen from this research are:

1. Hyperaccumulation cannot be induced in a species that is already actively accumulating the target metal.
2. The relative efficacy of chemical extractants to model the plant-available metal concentration of a soil may have a degree of plant species and metal form specificity. This is true for ammonium acetate.
3. Hyperaccumulation of cadmium is not necessarily an active-uptake phenomenon. Non-accumulator plants, for example *Brassica juncea*, may have the ability to passively take up this metal to a hyperaccumulation concentration when zinc is not in the soil solution.
4. The chemical form of a metal at a contaminated site should influence the choice of plant species and chemical solubilising agent to be used to effect phytoextraction.

An additional aim of this research was to quantify the effect of different chemical forms of metal on controlled 'artificial substrate' experiments. In particular, two questions were presented earlier: 1 - 'How does relative uptake between experiments compare?' and 2 - 'Do these artificial soils accurately model natural contamination?'

The answer to these questions is not a simple one. My belief is that soluble, nitrate salts, the most commonly used salts, are not the correct choice of metal form to use. However, nitrate salts do not consistently over- or underestimate the potential for phytoextraction. This is perhaps best illustrated by the lead model of Chapter 4. *Thlaspi caerulescens* hyperaccumulated lead from the nitrate salt, an unexpected result that is perhaps not representative of this specie's natural response to lead in the field. EDTA-induced lead uptake by this species was exceptionally high, and as discussed in the Chapter 4 perhaps an overestimate of this species response to the lead-EDTA complex in the field. However, the opposite was true for *Brassica juncea.* EDTA-induced uptake by this species of lead added as a nitrate salt was exceptionally low, relative to the other chemical forms of lead used and is perhaps an underestimate this time, of *B.juncea's* response to the lead-EDTA complex in the field.

To answer these questions I would have to conclude that 1 - relative uptake between experiments is not particularly good, and 2 - that lead nitrate does not accurately model

lead contamination in soil. My suggestion in response to these conclusions would be double edged. Firstly, the idea of a standard, artificially contaminated soil should be introduced, a soil with a 'recipe' using the same metal salt, a similar base substrate and pH etc that is equilibrated under the same conditions for the same period of time. This soil, as well as a standard extractant to model the plant-available metal in the soil, would allow good continuity and comparability between the results of different research groups. The 'phytoextraction community' is still relatively small, and hence this suggestion would be relatively simple to implement.

Secondly, I would suggest that for a more applied trial, where the aim was to test the phytoextraction potential that a plant species may have in a particular environment, the chemical form of metal, as well as the pH and base substrate used, should replicate as close as possible that specific environment in any artificial substrate used.

**Concluding remark**

Artificially contaminated soils are very useful, and have a definite place in phytoextraction experiments. Research groups often do not have access to large amounts of a range of naturally contaminated substrates. This is particularly true for Massey University by virtue of the relatively low number of contaminated sites in this country. Thus artificially contaminated soils are the best choice of substrate to use. The results presented in the current study show that the lack of standardisation, true for artificial soil used by different groups, may negatively affect the quality and usefulness of generated data. Suggestions to overcome such problems have been presented.

Further discussion on the soil/root reaction and interactions for chelated metals can be found in the following literature: DeKock and Mitchell, 1957; Crowdy and Tanton, 1970; Tanton and Crowdy, 1971; Elgawhary *et al.*, 1970; Laurie *et al.*, 1991; Laurie and Manthey, 1994; Wallace *et al.*, 1977; Sommers and Lindsay, 1979; Taylor and Foy, 1985; McLaughlin *et al.*, 1997.

# Chapter 8 - Phytoremediation: a possible management solution for New Zealand pastoral soils

Chapter 8 describes a field trial conducted on an area of agricultural land, contaminated with cadmium, where the feasibility of phytoremediation using both natural and induced hyperaccumulation was studied. McGrath (1998) has previously suggested that phytoextraction of cadmium from lightly contaminated agricultural land could be a viable application of the technology. The aim of the trial was therefor to examine the effectiveness of phytoremediation as a viable management tool for cadmium contamination in the New Zealand pastoral environment.

## 8.1 Introduction and literature review of the issue of cadmium in soils

Perhaps the best example of a detailed contemporary study into the presence of cadmium in the environment and the issues that surround cadmium contamination, with particular reference to New Zealand pastoral land, is Zanders (1998). Several other recent and important reviews also cover the subject (Bramley, 1990; Roberts *et al.*, 1994; McLaughlin *et al.*, 1996; Tiller *et al.*, 1997) while two detailed texts are Webb (1979) and Nriagu (1980). The more important aspects of cadmium contamination with particular reference to the New Zealand pastoral environment are covered in this section.

Cadmium is a biotoxic heavy metal that accumulates in the liver and kidneys of animals, potentially causing damage to the health of these organs. The metal has no known biological function. Cadmium occurs naturally at very low concentrations in all parts of the environment. Roberts *et al.* (1993, 1994) reported a mean background concentration for 86 'pristine' New Zealand soils (0 - 7.5 cm) of 0.20 mg/kg, although historic seabird breeding sites can be 'naturally' elevated above this concentration (e.g. 0.49 mg/kg at a site in North Canterbury – Hawke *et al.*, 1998). Anthropogenic sources of pollution increase cadmium concentrations above natural background levels.

*Sources of cadmium contamination*

Global input of cadmium to soil has been reported as between 5.6 and 38 thousand tonnes per annum (Nriagu and Pacyna, 1988), with major contributing factors identified as industrial atmospheric fallout, fly ash and urban refuse. Minor inputs on a global scale are identified as sewage sludge and phosphatic fertilisers. However, the level of contribution true for each of these factors varies from country to country, and for the New Zealand environment superphosphate fertiliser applied to agricultural land is the major source of anthropogenic cadmium.

*Phosphatic fertilisers*

For much of the past century, phosphatic fertilisers used in New Zealand agriculture have been derived from phosphorite, a guano-based, oceanic sedimentary rock. Cadmium is an integral component of such rocks. The metal is concentrated in the faeces of seabirds through the ocean food chain and then substituted isomorphously for calcium in the phosphorite crystalline mineral lattice (Cook and Freney, 1988). Nauru, as well as Ocean and Christmas Island, have, until recently, been the major sources of New Zealand's phosphate rock. The cadmium concentrations of rock from these islands range between 38 and 134 mg/kg (Bramley, 1990). As stocks of these island phosphorites have become depleted, phosphate has been sourced from North American, Middle Eastern and African countries where the cadmium concentrations are considerably lower (<40 mg/kg).

*Cadmium in agricultural land*

To sustain long term agriculture, superphosphate fertiliser is currently applied to New Zealand agricultural land at rates up to 60 kg P/ha/yr (Roberts and Morton, 1998). The highest loadings occur for land subject to dairy farming. Hill-country land used for sheep farming receives significantly lower rates of application, approximately 15 kg P/ha/yr (Gillingham *et al.*, 1998). Superphosphate fertiliser has a P concentration of 9%. Loganathan *et al.* (1995, 1997) reported an annual cadmium application rate of up to 14 g/ha to agricultural land, associated with a phosphorus application rate of 38 kg/ha/yr. This figure assumes the use of low cadmium fertilisers. The annual cadmium

application rate used for the purpose of this thesis is 10 g/ha (Fergusson and Stewart, 1992) a figure reported by these authors to indicate the average annual application rate of cadmium to the whole of New Zealand.

Cadmium is immobile in soil under conditions that support good pasture growth, and is generally retained in the upper 12 cm of the soil profile (Loganathan and Hedley, 1997) by sorption on soil colloids (Gray *et al.*, 1999). A mean value of 0.44 mg/kg cadmium has been reported for soils (0-7.5 cm) sampled from 312 fertilised sites throughout New Zealand (Roberts *et al.*, 1993). 'Hot-spots' exist where soil cadmium concentrations are considerably higher. These include land subject to very intensive fertilisation (some dairy farms), land near top-dressing strips, and land near superphosphate fertiliser sheds. Cadmium concentrations in these environments can exceed the New Zealand Ministry of Health maximum guideline of 3 mg/kg of soil.

Cadmium is a cumulative toxin, and once concentrated in the liver and kidneys of animals, is not excreted. The cadmium concentration in the kidneys of grazing animals is therefore strongly correlated with age (Roberts *et al.*, 1994). The most important pathway of cadmium from soil into animals is probably direct ingestion of cadmium associated with soil particles; animals are known to ingest large quantities of soil while grazing, especially in times of drought (Healy, 1967). Approximately 20 % of the kidneys of slaughted grazing animals of all ages (22-28% ovine and 14-20% bovine), tested in New Zealand between the years 1988 and 1991, had cadmium levels above 1 mg/kg; the maximum permissible concentration set by the New Zealand Department of Health (Roberts *et al.*, 1994). Hence, the New Zealand Meat Industry condemns the kidneys of slaughtered animals over 2.5 years of age.

## 8.2 Materials and methods

*Experimental Design*

An area of cadmium-contaminated land was identified adjacent to a superphosphate fertiliser shed, at the end of an airstrip on a farm near Blairlogie, 24 km east of the Wairarapa town of Masterton (Fig. 8.1). The land adjacent to fertiliser storage sheds and

near the ends of top-dressing airstrips are traditional hot-spots for cadmium contamination as they receive high loadings of superphosphate. An initial soil survey showed concentrations of cadmium in the surface (0-5 cm) soil horizon ranging between 1 and 6 mg/kg, however, a cadmium gradient was apparent at the site. Concentrations of cadmium increased away from the fenceline and away from the fertiliser shed. Mean pH across the trial site was 6.3.

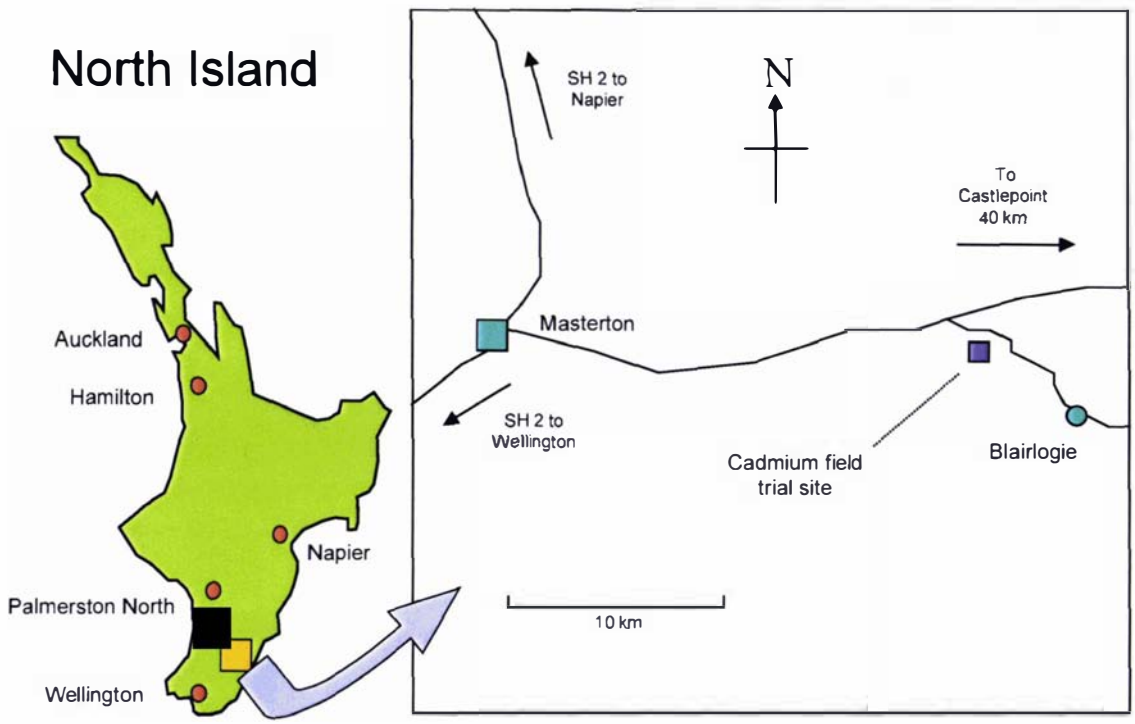


**Figure 8.1**. Map of the North Island of New Zealand, showing in detail the location of the cadmium phytoextraction field-trial site.

Twenty, 1 $m^2$ plots were marked out during November 1998 (Fig. 8.2). The design of the trial was such that the effects of the cadmium gradient could be removed from the final data set, by averaging results for metal uptake across the plot area. Each of these plots was further divided into four quadrats before soil at the 4 corners of each quadrat was sampled to 10 cm depth. Ten plots were seeded with the high-biomass, non-accumulating species, *Brassica juncea* (Indian mustard) and *Brassica napus* (rape). The remaining ten plots were seeded with the known cadmium and zinc hyperaccumulating species *Thlaspi caerulescens* and *Cardaminopsis halleri*. Two quadrats for each of the species were seeded in each plot (Fig. 8.3, 8.4).

Once the *Brassica* species had reached maturity (approx. 3 months), the above-ground biomass of one quadrat of each of the 4 species, from each plot, was harvested. A solution of the chelating agent EDTA (disodium salt) was then applied to the entire trial area at a rate of 0.5 g of chemical per kg of soil, to an effective depth of 20 cm. After three weeks, the remaining biomass was harvested and soil to 10 cm depth was sampled from within each quadrat.

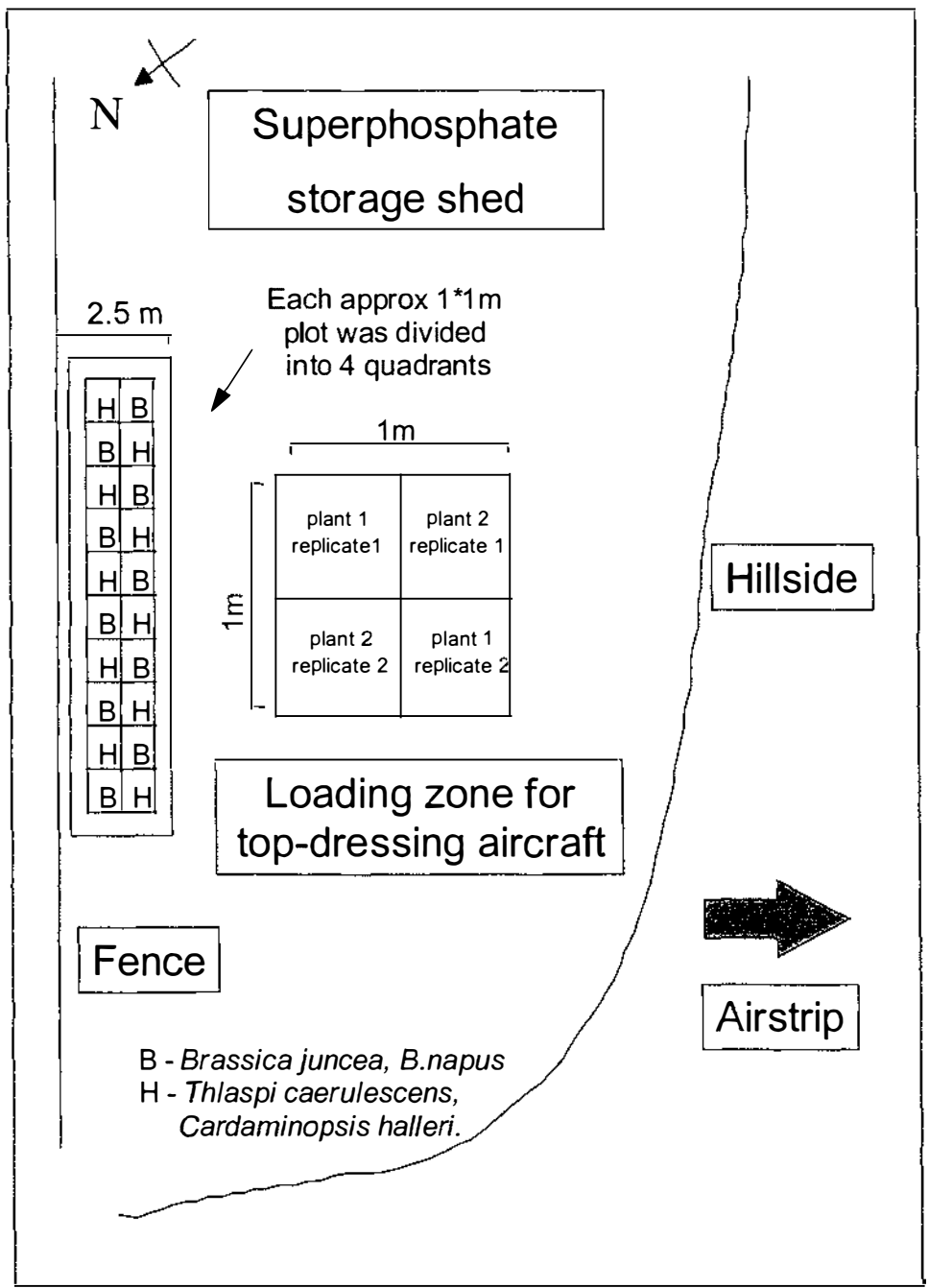


**Figure 8.2.** Schematic plan of the experimental-trial set up.

**Figure 8.3**. View of two *Brassica* species in flower, growing at the Wairarapa trial site shortly before treatment with EDTA.

**Figure 8.4**. View of *Thlapsi caerulescens* (C) and *Cardaminopsis halleri* (H) growing at the Wairarapa trial site.

*Analysis of plant samples*

The biomass of *Brassica juncea* and *B.napus* harvested from each *Brassica* quadrat was weighed and ground. Subsamples (200 mg) were subsequently digested in concentrated nitric acid (10 mL), diluted to final volume with deionised water and analysed for cadmium using graphite furnace atomic absorption spectroscopy (GFAAS)[9]. Samples of the hyperaccumulator species *Cardaminopsis halleri* and *Thlaspi caerulescens* were digested in concentrated nitric acid before analysis by flame atomic absorption spectroscopy (FAAS) for cadmium and zinc. The entire biomass of each of these latter species was digested and analysed due to the low weights of material harvested.

*Analysis of soil samples*

Several analytical techniques exist for the digestion of soil before analysis by atomic absorption spectroscopy. Zanders (1998) found that digestion by a 3:1 mixture of concentrated hydrochloric and nitric acids (*aqua regia*) gave a good approximation for total cadmium. Other authors have used more dilute acid systems to 'digest' soil for total metal analysis (e.g. 4M $HNO_3$ – Taylor and Percival, 1992). In general, more acid digests are likely to dissolve soil metals more readily than dilute mixtures, although, concentrated acid digests are more time consuming and less reproducible than dilute acid 'extractions'. This is because a greater number of samples can be treated in the same batch, at the same time, using extraction methods.

The aim of determining the soil cadmium concentration at the site was two-fold: 1) to estimate the total metal loading of the soil that would be available for plant uptake, and 2) to monitor the changes in soil cadmium concentration as a function of the trial. The larger the number of soil samples collected over the sample area, the better the integrity of the final result. Therefore, an extraction method was devised which used dilute nitric acid (1M) as an extractant to give a 'total' soil cadmium concentration.

[9] GFAAS instrument operating parameters are presented in Appendix 2.

The method developed was as follows. Ground soil (2 g) was weighed into 50 mL polypropylene extraction tubes, before nitric acid (10 mL of 1M) was added. The tubes were subsequently shaken overnight end-over-end. The extracts were filtered through Whatman #42 filter paper and analysed by FAAS. This ratio of soil to extractant was found to give a final concentration in the solution that could be detected by FAAS.

To compare the relative efficacy of the above extraction method to a concentrated acid digest, 31 soil samples randomly selected from both the 0-5 and 5-10 cm soil depths, were digested using concentrated nitric acid. Dry samples (2 g) were weighed into 50 mL conical flasks and concentrated acid (10 mL) was added. The flasks were allowed to stand overnight before digestion on a hotplate to almost dryness (the soil appeared moist, but no residual liquid remained within the flask). After cooling, the soil residue was resuspended in 10 mL of 1M nitric acid before filtration and analysis by FAAS.

Regression of the two sets of results yielded a very good linear relationship over the range of expected soil cadmium concentrations, $R^2$=0.93 (Fig. 8.5). The 1M extraction gave a slightly higher total result. This could be due to volatilisation of a small percentage of the total cadmium during the digestion procedure, or through partial absorption of the extractant solution by dry soil particles.

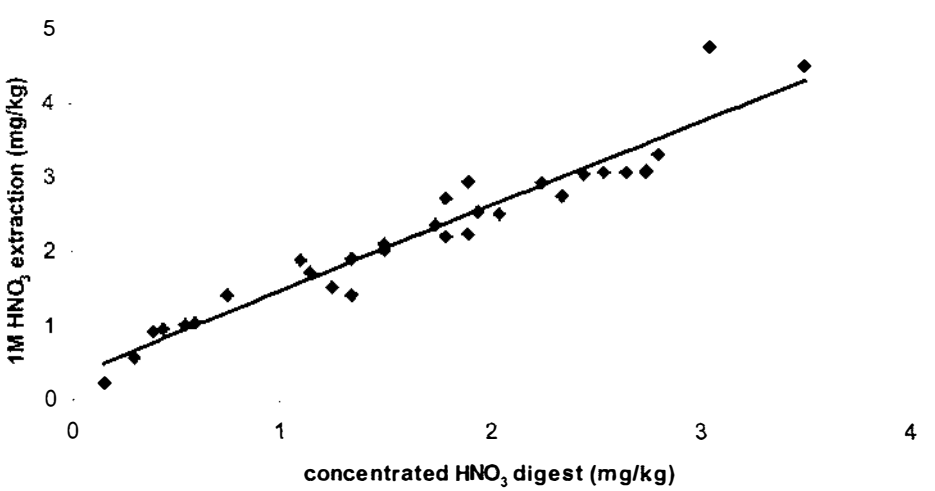


**Figure 8.5**. Comparison of two methods to determine the cadmium concentration of soil. ($R^2$ = 0.93).

**Adopted method**

Based upon the results from the comparison of dilute versus concentrated acid 'extraction' systems, the 1M nitric acid system was adopted for soil analysis.

## 8.3 Results

High levels of cadmium were accumulated (naturally) by the two hyperaccumulator species. *Thlaspi caerulescens* contained significantly more metal than *Cardaminopsis halleri.* Neither of the two *Brassica* species accumulated high levels of cadmium, although *B.juncea* accumulated significantly more metal than *B.napus* (Table 8.1).

**Table 8.1**. Summary of the metal concentrations found in the harvested plant material, values are mean ± sd and (range). Significance compares between and within species differences (ANOVA p>0.05).

| | Before EDTA (mg/kg) | After EDTA (mg/kg) | Significance |
|---|---|---|---|
| ***B.juncea*** | 0.67±0.28 (0.28-1.08) n=10 | 0.62±0.25 (0.18-0.92) n=10 | ns |
| ***B.napus*** | 0.34±0.15 (0.23-0.67) n=10 | 0.55±0.23 (0.18-0.98) n=10 | s |
| **Significance** | s | ns | |
| ***T.caerulescens*** | 84.0±55.2 (27.4-193.4) n=10 | 57.1±44.5 (7.1-137.0) n=10 | ns |
| ***C.halleri*** | 27.0±10.6 (17.6-45.4) n=8 | 5.18±1.16 (4.2-7.10) n=4 | s |
| **Significance** | s | s | |

Application of EDTA to the trial area significantly increased the cadmium uptake by *Brassica napus* but not by *B.juncea.* EDTA significantly decreased the level of cadmium uptake for *Cardaminopsis halleri.* EDTA did not adversely affect the health of the *Brassica* species. There was no significant change in biomass between the before and after treatment harvested material. However, the same cannot be said for the hyperaccumulator species. Application of EDTA to the site effected necrosis in *C.halleri* and *Thlaspi caerulescens.* This was an unexpected factor, and forced harvesting of the remaining hyperaccumulator species at the time of after-treatment harvesting of the *Brassica* species.

Total-soil cadmium concentrations were analysed using the Golden Software graphics package, Surfer, and statistically 'kriged' to generate profile plots of soil cadmium concentrations before and after treatment of the trial area with EDTA, for both the 0-5 cm and 5-10 cm soil depths (Fig. 8.6, 8.7).

There is a clear difference in the concentration and distribution of cadmium between the before-and-after trial 0-5 cm cadmium contour plots (Fig. 8.6). Before the trial, the highest cadmium concentrations could be observed in the corner of the plot area away from the shed, on the airstrip side of the hill (cadmium concentration greater than 5 mg/kg). This is where aircraft have in the past parked to receive a reload of superphosphate material. The affect of the trial was to reduce soil metal concentrations across the plot area to between 3 and 5 mg/kg, a 15% reduction in the mean metal concentration. There was, however, a 12% increase in the mean cadmium concentration of the plot area effected by the trial for the 5-10 cm soil depth (Fig. 8.7).

## 8.4 Discussion

*Plant metal uptake*

Equivalent biomass figures per hectare for the *Brassica* species were calculated by extrapolating the harvested weight recorded from each quadrat to a one-hectare area (Table 8.2). *Cardaminopsis halleri* and *Thlaspi caerulescens* were harvested well before maturity and thus realistic biomass figures cannot be extrapolated from the weights of this trial. The value of 2 t/ha for *C.halleri* has been determined from field observations in northern France. The biomass value of *T.caerulescens* (2 t/ha) is that reported by McGrath *et al.* (1993) and is for field growth in the United Kingdom under similar climatic conditions to those experienced in New Zealand. This biomass figure for *T.caerulescens* is conservative; some researchers believe a biomass for this species of up to 6 t/ha is sustainable (C.Schwartz, pers. commn. 1999)

Based upon the weight of *Thlaspi* harvested at the end of the trial, a biomass of 10 kg/ha for each of the hyperaccumulator species would have been apparent.

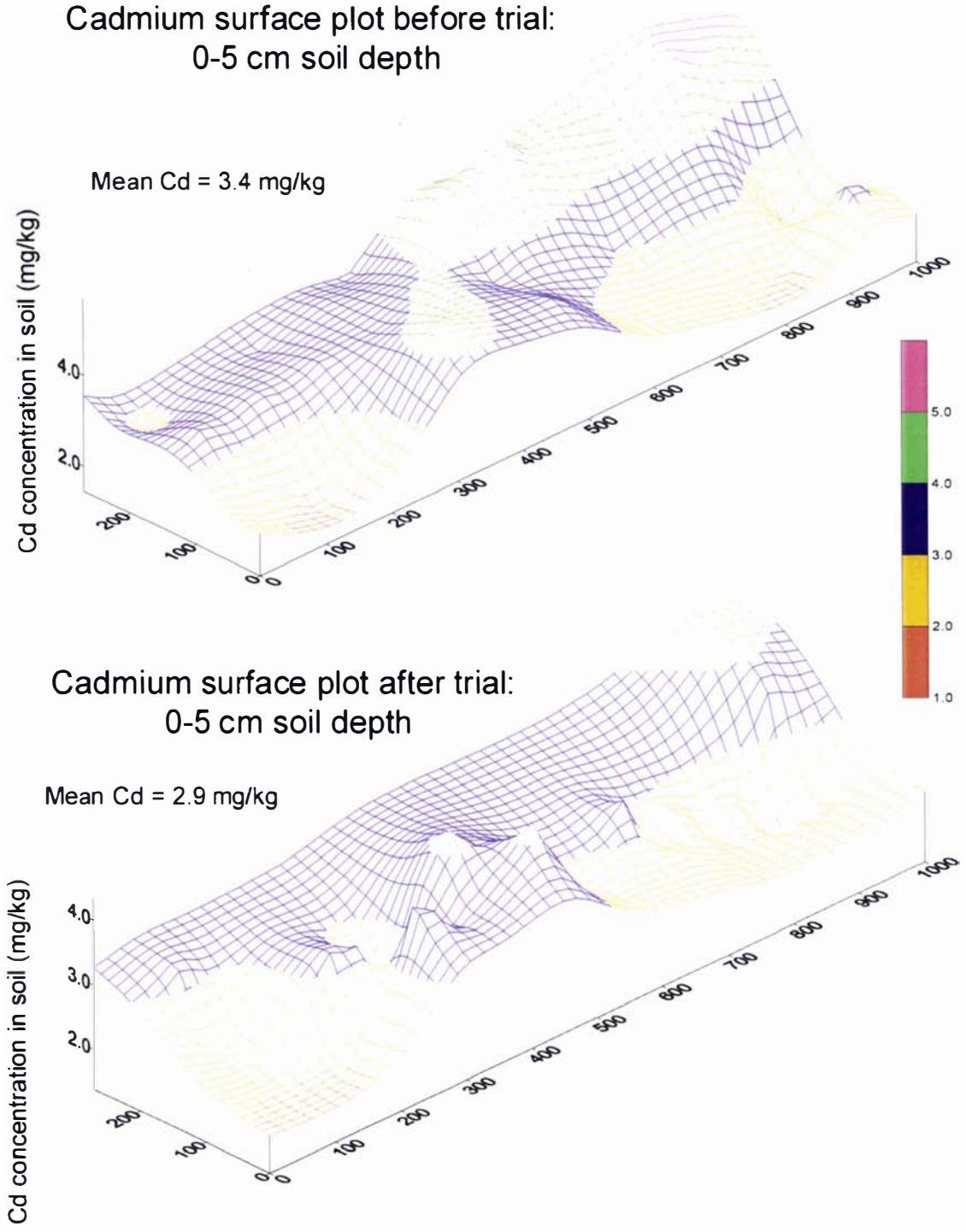


**Figure 8.6.** Surface plot (0-5 cm) showing the change in soil cadmium concentration effected by the trial.

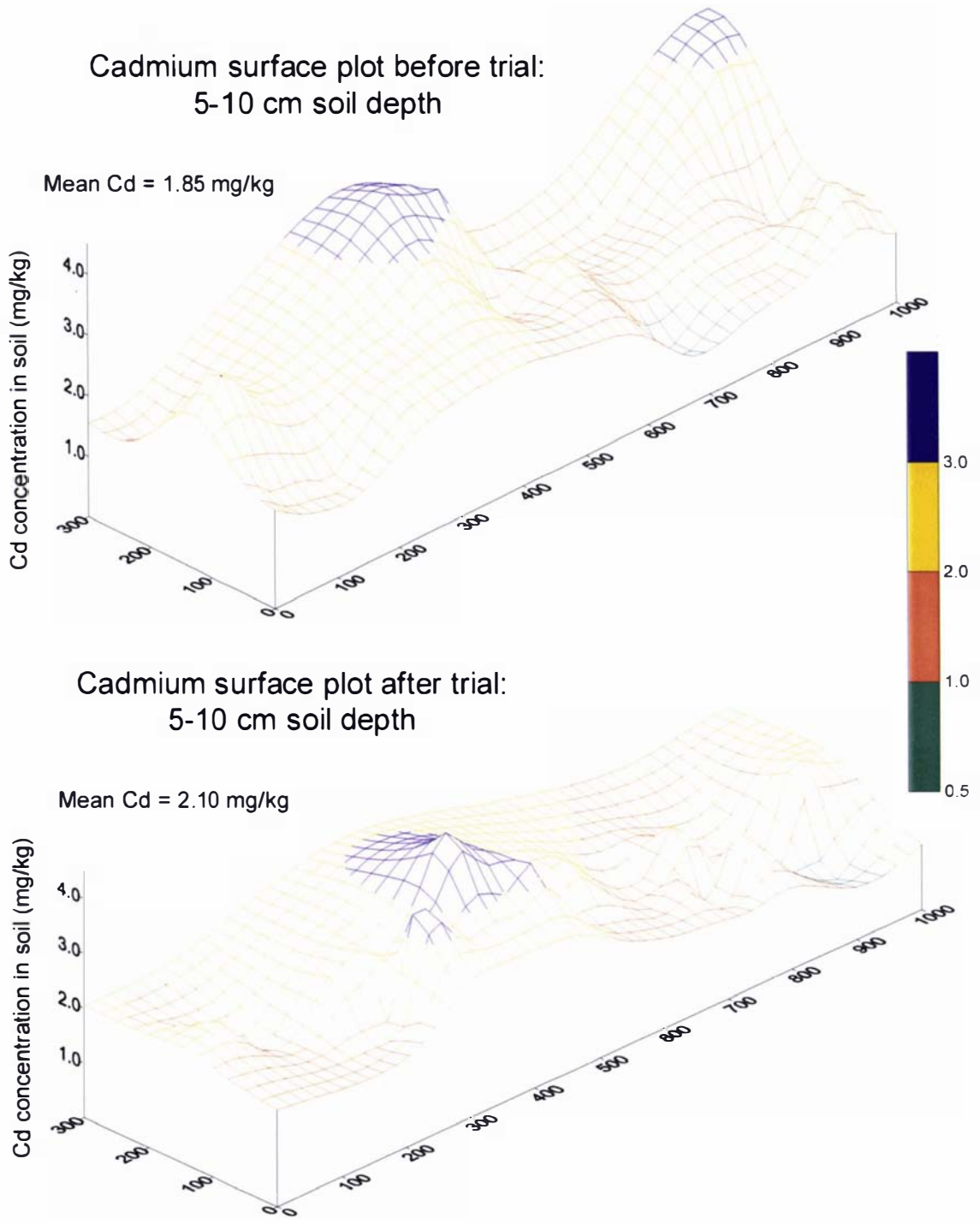


**Figure 8.7.** Surface plot (5-10 cm) showing the change in soil cadmium concentration effected by the trial.

**Table 8.2**. Biomass and extrapolated uptake figures over a one-hectare area for the plant species used in this trial.

| | Biomass (t/ha) | Natural uptake (g Cd/ha) | Induced uptake (g Cd/ha) |
|---|---|---|---|
| *B.juncea* | 15 | 10 | 9 |
| *B.napus* | 7 | 2 | 4 |
| *T.caerulescens* | 2 | 168 | 114 |
| *C.halleri* | 2 | 54 | 10 |

Using these biomass figures, the annual cadmium removal potential a crop of each of these plants may offer can be predicted (Table 8.2). *Thlaspi caerulescens* has the greatest natural potential for uptake, up to 170 g of cadmium per hectare per crop. This is equivalent to 17 years of annual cadmium application to the soil removed in one growing season. The *Brassica* species could accumulate only a limited amount of metal, approximately one year of annual cadmium application in each growing season, even after the application of EDTA. The annual application rate of cadmium to soils used for these calculations is 10 g/ha/yr (Fergusson and Stewart, 1992).

*Effect of soil cadmium concentration on metal uptake*

To investigate the importance of the total-soil cadmium concentration on metal uptake exhibited by *Thlaspi caerulescens*, the four values for soil cadmium at the corner of each plot determined from soil samples collected at the start of the trial were averaged, to give a figure for the mean cadmium concentration within each plot. The plant cadmium concentration of *T.caerulescens* was then plotted as a function of these values for soil metal (Fig. 8.8).

The regression coefficient for the data set is very low ($R^2$=0.001), thus the cadmium uptake potential of *Thlaspi caerulescens* is independent of the cadmium concentration in the soil.

*Cadmium redistribution within the soil*

The average soil-metal concentration across the plot area before and after the trial was calculated to quantify the change in the soil cadmium balance effected by this

experiment (Table 8.3). Soil concentrations were used to calculate the total amount of cadmium within the plot area (Table 8.4), based on each 5 cm unit of soil having a mass of 900 kg over the plot area (20 $m^2$), and a bulk density (dry) of 1.2 $g/cm^3$.

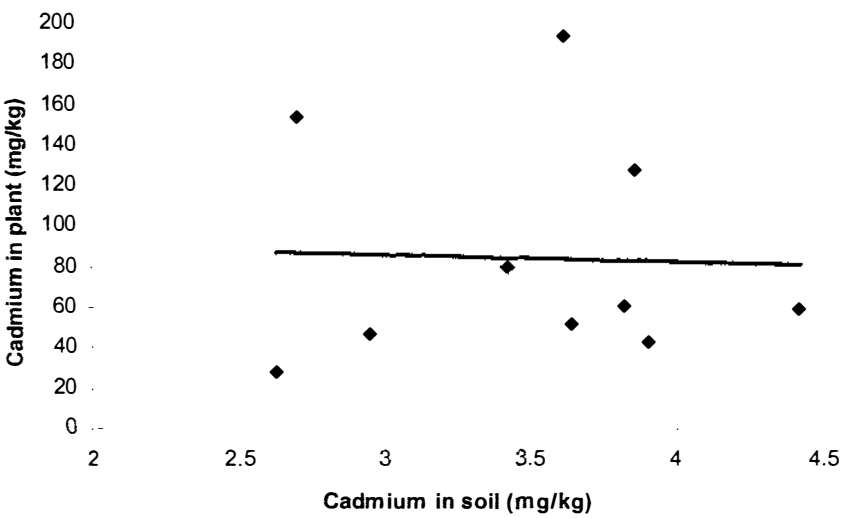


**Figure 8.8**. Plot of the cadmium concentration in *Thalaspi caerulescens* as a function of the cadmium concentration in the soil.

**Table 8.3.** Average soil-cadmium concentrations across the plot area.

| **Soil depth** | **Cd before trial (mg/kg)** | **Cd after trial (mg/kg)** | **Cd change (mg/kg)** |
|---|---|---|---|
| 0-5 cm | 3.40 | 2.93 | - 0.47 (14 %) |
| 5-10 cm | 1.85 | 2.10 | + 0.25 (12%) |
| | **Cadmium loss or gain from 0-10 cm soil profile** | | 0.23 mg/kg loss |

**Table 8.4.** Average total-soil cadmium levels for the plot area.

| **Soil depth** | **Cd before trial (mg)** | **Cd after trial (mg)** | **Cd change (mg)** |
|---|---|---|---|
| 0-5 cm | 3670 | 3190 | - 480 (14%) |
| 5-10 cm | 2000 | 2270 | + 270 (12%) |
| | **Cadmium loss or gain from 0-10 cm soil profile** | | 210 mg net loss |

*Soil cadmium mass balance calculations*

Considering the maximum possible biomass of 2 t/ha for *Thlaspi caerulescens*, and an average metal concentration within the plant of 84 mg/kg, 168 g of cadmium removed per hectare equates to a decrease in the soil cadmium concentration of only 0.08 mg/kg. The *Thlaspi* plants of this trial had a biomass only a fraction of 2 t/ha (10 kg/ha) due to harvesting well before maturity, and thus changes in soil cadmium would be difficult to detect in this experiment. Harvesting before maturity was unfortunate, but necessary due to necrosis of the hyperaccumulator species apparent after the application of EDTA. It was initially envisaged that these species would remain at the site until flowering. The large differences between across-plot cadmium distribution for the 0-5 and 5-10 cm soil profiles can therefore be attributed to EDTA redistribution of metal within the soil profile.

Total plant-metal uptake across the plot area was 6.92 mg cadmium for the non-accumulator *Brassica* species, and approx. 0.40 mg of cadmium for the hyperaccumulator species. Table 4 shows a net loss of 210 mg cadmium from the 0-10 cm soil depth, but only 7.3 mg of this cadmium loss can be accounted for by plant uptake. Assuming all of this 7.3 mg of cadmium was removed from the top 5 cm of soil, then more than 470 mg of metal was leached from this depth. Redistribution of 270 mg of this cadmium can be accounted for in the 5-10 cm depth. The remaining 200 mg of cadmium, or 4 % of the cadmium initially present within the top 10 cm of the trial area, was leached to below 10 cm soil depth.

*Zinc accumulation by the hyperaccumulator species*

Zinc was accumulated by the hyperaccumulator species *Thlaspi caerulescens* and *Cardaminopsis halleri*, but was not 'hyperaccumulated' (Fig. 8.9). Mean accumulation by *T.caerulescens* was 610 mg/kg (170 mg/kg – 1 900 mg/kg range) before treatment of the site with EDTA, although no significant change in the foliar-zinc concentration was observed as a result of EDTA treatment. Similarly there was no significant difference in zinc accumulation between the two species. Both species are known hyperaccumulators of zinc.

McGrath (1998) stated that zinc hyperaccumulation by *Thlaspi caerulescens* is a 'physiological requirement' and that the minimum shoot concentration necessary for growth was in the order of 1 000 mg/kg. However, the low level of accumulation by *T.caerulescens* in this trial was within the 'normal-range' of zinc accumulation by non-accumulator species. The plants were very healthy (until treatment with EDTA) and successfully hyperaccumulated cadmium. The evidence from the Wairarapa trial is in contadiction to the work of McGrath, and suggests that yet again our understanding of the mechanisms underlying hyperaccumulation is relatively poor.

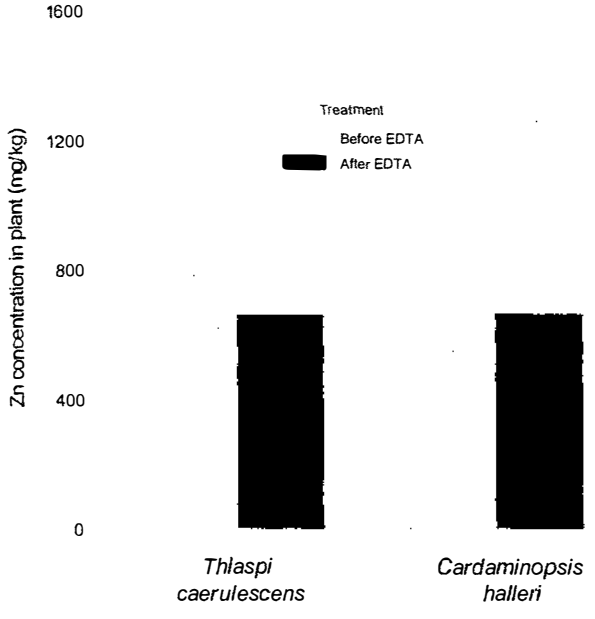


**Figure 8.9**. Zinc accumulation by *T.caerulescens* and *C.halleri* from the Wairarapa trial site.

The observation that *Thlaspi caerulescens* did not hyperaccumulate zinc is an important one. A concern sometimes voiced with regard to phytoremediation for pastoral cadmium, is that an operation using hyperaccumulator species would also remove trace concentrations of zinc from the soil, leading to a soil deficiency in the concentration of this important trace element. The results from this trial suggest that this is not an important concern.

## 8.5 Summary – a practical application for phytoextraction

Phytoremediation has been proposed as a solution for areas where anthropogenic pollution has contaminated natural environments. The example illustrated in this chapter is of elevated cadmium levels in New Zealand pastoral soils subjected to superphosphate fertilisation. A key drawback of phytoremediation technology is the long time frame over which decontamination will occur. In this trial a 2 t/ha crop of *Thlaspi caerulescens* would only decrease soil metal levels by approximately 0.08 mg/kg in each growing season.

An alternative option is to view this technology as a management tool for areas with low levels of soil contamination. If cadmium is applied to soil at a rate of 10 g/ha/yr, then natural hyperaccumulation effected by a crop of *Thlaspi caerulescens*, which in this trial could remove up to 168 g of Cd/ha in one season (assuming a biomass of 2 t/ha is achieved), would remove the equivalent of 17 years of annual fertiliser application. *Thlaspi caerulescens* in this scenario could be planted periodically in a crop rotation cycle, to manage effectively an increasing cadmium load to the soil, and thus maintain cadmium at an arbitrarily set baseline level. At the trial site *T.caerulescens* performed better than *Cardaminopsis halleri*. This was due to both superior cadmium uptake potential and the preference of this species for an open and dry soil environment, typified by the Wairarapa, in contrast to the sheltered and moist environment favoured by *Cardaminopsis halleri* (A.Deram, pers. commn., 1999).

There was no relationship at this site between total soil cadmium and total plant cadmium. Total metal uptake for hyperaccumulator species appears to be independent of the metal concentration in the soil. It seems likely that *Thlapsi caerulescens* will hyperaccumulate cadmium equally well from areas of land that have a lower metal loading than the 'hot-spot' used in this trial, although this theory has yet to be tested. It also appears that *Thlaspi caerulescens* will not significantly and adversely decrease the zinc concentration of the surface soil, to the extent where zinc fertilisation would be necessary subsequent to removal of cadmium. This species did not hyperaccumulate zinc.

Hyperaccumulator species do not respond well to vegetative competition. If an area of land were to be managed in this way, an application of herbicide before seeding would be necessary. Once the crop reached maturity, harvesting would yield a low-volume of material, concentrated in cadmium, that could be disposed of or recycled. Growth of other species in subsequent seasons would effectively outcompete the foreign hyperaccumulator plants; the potential of hyperaccumulator species to become 'weeds' is therefor low.

Induced hyperaccumulation, effected by the chelating agent EDTA, did not result in high levels of cadmium uptake by the non-accumulating *Brassica* species. The amount of metal 'naturally' removed by the hyperaccumulator species remained higher. In this trial, the effect of EDTA was to leach approximately 4% of the cadmium initially present in the surface soil (0-10 cm depth) to below 10 cm. EDTA did successfully lower the cadmium concentration of the surface soil and this in itself was a useful result. Soil leaching of cadmium using chelating agents has been suggested as a solution for soils with high cadmium surface concentrations. However, it is likely that other more important trace metals (e.g. Co, Cu, and Zn) will also have been complexed and thus leached down the soil profile, leading to a deficiency in the nutrient balance of the surface soil (Tejowulan and Hendershot, 1998).

## 8.6 Conclusion

The loading of cadmium to agricultural land will remain a problem to both New Zealand and worldwide agriculture, as long as the heavy metal remains in phosphatic fertilisers. Removal of this cadmium is expensive, and although desirable, may not be feasible. Phytoremediation using hyperaccumulator species, in particular the species *Thlaspi caerulescens*, may be an effective management tool for cadmium in the New Zealand pastoral environment. The equivalent of 17 years of annual cadmium application to agricultural land could be removed in one season assuming an attainable biomass of 2 t/ha. Using this technology, the cadmium concentration of New Zealand pastoral soils could be maintained at the current level which is safe for intensive agriculture, while still allowing for annual application of superphosphate fertiliser.

A chapter summary is presented as Figure 8.10.

Overleaf:

**Figure 8.10**. Phytoremediation of cadmium from New Zealand soils: a poster summary of a practical application.

# PHYTOREMEDIATION OF CADMIUM FROM NEW ZEALAND SOILS

Chris Anderson, Robert Brooks, Bob Stewart and Robyn Simcock*
Soil and Earth Sciences, Massey University, Palmerston North
*Landcare Research Ltd., Palmerston North

## Introduction

- In New Zealand heavy metal contamination can occur in pastoral land due to the association of Cd with superphosphate fertiliser.
- Phytoremediation is a developing technology that uses plants to remove metals from an area of contaminated land.
- These plants take-up very high levels of metal, either naturally or with the assistance of chemicals applied to soil (eg EDTA).

## Objective

- To test the efficacy of both natural and induced hyperaccumulation to remove Cd from a contaminated field site.

## Materials and Method

- A small site contaminated with Cd (1-6 mg/kg), was identified on a farm 24 km SE of Masterton. The site was adjacent to a superphosphate fertiliser shed and loading zone for aerial top-dressing planes.
- Soil (0-15 cm) was sampled at regular spacing over the area before treatment
- A 2 by 10 metre area was divided into 20 plots, and each plot divided into 4 quadrats (Fig. 1). In November 1998 quadrats were seeded with one of two non-accumulating species, *Brassica juncea* and *Brassica napus* (Fig. 2) or two Cd hyperaccumulating species, *Cardaminopsis halleri* and *Thlaspi caerulescens*.
- In February 1999, biomass from half of all quadrats was harvested. EDTA was then applied at the equivalent rate of 1 t/ha to the trial area to induce additional Cd uptake.
- After 3 weeks the remaining biomass was harvested and soil sampled.
- Plant and soil samples were analysed for total Cd metal concentrations.

Figure 1 Plot layout. Wooden pegs mark out the plots divided into 4 quadrats. Small plants are fast growing *Brassica juncea* and *B.napus*.

Figure 2 Mature *B.juncea* and *B.napus* after two months of growth.

## Results and Discussion

- The two hyperaccumulating species took up high concentrations of Cd (Table 1). *T.caerulescens* had a significantly higher concentration of Cd than *C.halleri*.
- Non-hyperaccumulating species showed limited Cd uptake both before and after EDTA treatment. *B.juncea* contained a significantly higher Cd concentration than *B.napus*.
- After the trial, Cd was either removed or mobilised deeper than 5cm within the soil profile (Fig. 3).

Table 1: Summary of the Cd concentrations in the harvested biomass. Uptake values are mean and standard deviation.

| | Natural uptake (mg/kg) | EDTA induced uptake (mg/kg) | Biomass (t/ha) | Natural uptake (g Cd/ha) |
|---|---|---|---|---|
| *B.juncea* | 0.7 ± 0.3 (A) | 0.6 ± 0.3 (A) | 15 | 10 |
| *B.napus* | 0.3 ± 0.2 (A) | 0.6 ± 0.2 (B) | 7 | 2 |
| *T.caerulescens* | 84 ± 55 (A) | 57 ± 45 (A) | 2* | 168 |
| *C.halleri* | 27 ± 11 (A) | 5 ± 1 (B) | 2* | 54 |

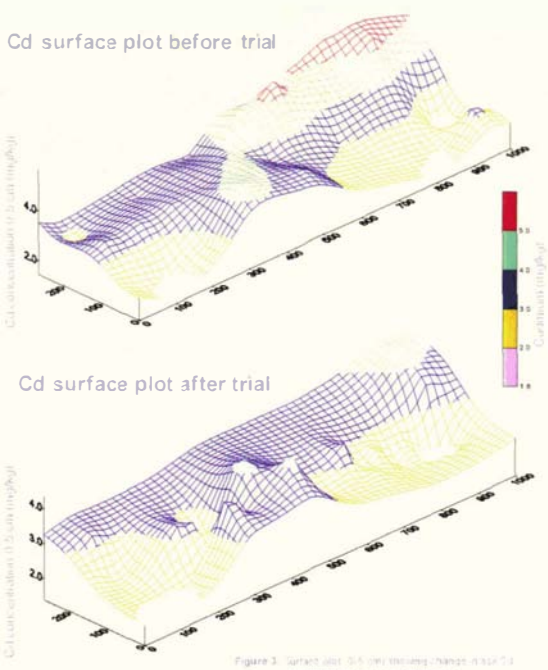


Figure 3 Surface plot of Cd concentration (mg/kg) showing changes in soil Cd concentration as a result of the trial.

- Fergusson and Stewart (1992) report a Cd application rate of 10 g/ha/yr can be expected on a well managed farm.
- In this trial *B.juncea* had a mean biomass of 15 t/ha and would remove about 10 g of Cd per hectare.
- *T.caerulescens* has a biomass of about 2 t/ha (Baker *et al.*, 1994). A crop of this hyperaccumulator could remove up to 168 g of Cd/ha.

## Conclusions

- The hyperaccumulating species *T.caerulescens* removed the equivalent of more than 15 years of accumulated Cd in three months.
- Phytoremediation could prove a useful management tool to reduce Cd contamination in pastoral soils.
- There is little point in inducing hyperaccumulation for Cd in plants of higher biomass, due to the associated costs and limited increase in uptake.

## References

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Fergusson, J.E., and Stewart, C., (1992) The transport of air-borne trace elements copper, lead, cadmium, zinc and manganese from a city into rural areas *Science of the Total Environment, 121* 247-269

## Acknowledgments

The authors gratefully acknowledge Mr Don Adams of Blairlogie, Wairarapa for providing a field trial-site and for his assistance in running the trial
We also gratefully acknowledge AGMARDT of New Zealand for the award of a doctoral scholarship to support the senior author

# CONCLUSION TO SECTION A

The integrated geochemical model for Cd, Pb and Zn presented in Chapter 7 described many of the results observed for metal uptake from the Tui and Auby contaminated substrates. The results from the Wairarapa field trial described in Chapter 8 present a test of this model in a third environment.

**Hyperaccumulator species**

Neither *Thlaspi caerulescens* nor *Cardaminopsis halleri* hyperaccumulated cadmium from the Wairarapa field site (using the criterion concentration of 100 mg/kg), although uptake was very high. The relative uptake efficacy of these two species from cadmium present in the soil as phosphate salt was in agreement with the cadmium-uptake model. Mean cadmium uptake by *T.caerulescens* was greater than that for *C.halleri*, and for both species, EDTA decreased the foliar-cadmium concentration. Uptake of zinc by these species from the Wairarapa site was in similar agreement with the zinc uptake model. Hyperaccumulation was not effected by either species from the zinc phosphate soil of Chapter 6, although induced-uptake of zinc to a higher concentration by *C.halleri* could have been expected.

The plant-available (ammonium acetate) concentration of cadmium for the Wiararapa site was not reported in chapter 8, however, it is worth mentioning here. The mean concentration of cadmium across the plot area was 3.1 ng/mL which is lower than the concentration of plant available cadmium from the Tui substrate of Chapter 3 (9.5 ng/mL). Yet uptake was higher from the Wairarapa substrate. The plot of the cadmium concentration in *Thlaspi caerulescens* as a function of the plant-available cadmium concentration in the soil, presented in Chapter 5 (Fig. 5.8), showed anomalous uptake from the sulphide phase relative to the other forms of soil cadmium. Clearly, uptake of cadmium from the sulphide metal phase is not the same as uptake from a phosphate metal phase, and this inequality is not modelled by ammonium acetate.

**Non-accumulator species**

The geochemical model for cadmium uptake predicts that EDTA could effect a large increase in metal uptake from the phosphate phase by the non-accumulator species *Brassica juncea.* However, no such increase in uptake was observed for this species in the Wairarapa trial, although the cadmium concentration in *B.napus* was doubled. The soil mass balance presented in Chapter 8 shows clearly that EDTA induced a large increase in soluble cadmium that was subsequently leached below 5 cm soil depth. The question is 'why was this soluble cadmium not taken up by the plant?'

Blaylock *et al.* (1997) showed a strong pH dependency for EDTA-induced lead uptake. At pH 5.0, lead-contaminated soil (600 mg/kg) supported EDTA-induced uptake to almost 4 000 mg/kg by *Brassica juncea.* This uptake decreased to approximately 500 mg/kg for a pH of 7.0. The authors of this study justified the decrease in uptake through retention of lead within the root cell wall. EDTA in combination with low pH effectively prevents cell-wall retention of lead, freeing the metal for transport in the xylem to the shoots. Subsequent necrosis of these shoots shows that the non-accumulator plant has no mechanism to sequester the metal in a non-toxic form, i.e., it is not an accumulator species.

Root samples were never analysed for the experiments described in Section A and thus this hypothesis cannot be tested. The design of experiments described in the previous chapters did not account for root uptake; the plant organs of interest were the aerial portions, representing biomass that could be easily harvested for a phytoextraction operation. However, if cadmium behaves similarly to lead, sequestering of metal in the roots of the *Brassica* species could help account for the discrepancy between modelled and observed data. Sequestering of cadmium in the roots may also account for some of the cadmium lost below 10 cm depth for the Wairarapa soil profile, although the conclusion that cadmium was leached as a function of EDTA treatment remains true. Any roots sampled with the soil were separated at the time of grinding.

The above conclusion may also be valid for the poor EDTA-induced uptake results from the Tui tailings, and to some extent from the Auby soil. However, pH dependency does

not explain why the high concentrations of plant-available lead modelled by ammonium acetate did not translate into high natural uptake. Active exclusion of lead uptake appears to be true for 'natural (non-induced) uptake'. Could an active exclusion mechanism for a metal-EDTA complex be manifest at high pH? Maybe steric factors preclude the uptake of a bulky metal-chelate complex at this same high pH. Garvan (1964) showed the following pH-dependency of the EDTA chelate:

$$H_4EDTA \overset{OH^-}{\underset{H^+}{\leftrightarrow}} (H_3EDTA)^- \overset{OH^-}{\underset{H^+}{\leftrightarrow}} (H_2EDTA)^{2-} \overset{OH^-}{\underset{H^+}{\leftrightarrow}} (HEDTA)^{3-} \overset{OH^-}{\underset{H^+}{\leftrightarrow}} (EDTA)^{4-}$$

pH 2.3 pH 4.5 pH 8.1 pH 12.4

Presumably as the shape (conformation) of the chelate changes with the protonation or deprotonation of binding sites (a function of pH), the size of any chelate-metal complex will also change. Blaylock *et al.* (1997) and Vassil *et al.* (1998) suggested that the entire metal-EDTA complex is accumulated by a plant and transported to the shoots. Another theory is that the metal-EDTA complex is dissociated at the soil-root interface and the metal is subsequently taken up as a free-metal ion. If the first of these two theories is correct, then steric factors should be an important aspect of uptake.

Section A of this thesis has shown there is a complex interaction between geochemistry and species-specific plant uptake (natural and induced). At constant pH, the mineral phase strongly influences both the ammonium acetate concentration of plant-available metal in the soil and the concentration of metal in the plant. The relationship between these two variables is not equal for every environment. I am unsure how variation of pH will, in practice, affect the geochemical uptake model of this study but the effect of pH remains an area for future research.

The assumption, for example, that plant-available lead, estimated by ammonium acetate, can be removed from a contaminated soil using EDTA-induced hyperaccumulation, can be a dangerous one. Without specific knowledge of the soil geochemistry, the choice of plant species, the choice of extractant to model plant-available metal, and the choice of chemical to induce metal uptake may lead to less than optimal results for phytoextraction.

# SECTION B - PHYTOEXTRACTION FOR NICKEL AND GOLD

Publications arising from Section B:

Anderson, C.W.N., Brooks, R.R., Stewart, R.B. and Simcock, R., 1998. Harvesting a crop of gold in plants. *Nature* **395**: 553-554.

Anderson, C.W.N., Brooks, R.R., Stewart, R.B. and Simcock, R., 1999. Gold uptake by plants. *Gold Bulletin* **32**: 48-51, 58.

Anderson, C., Brooks, R., Stewart, R., Simcock, R. and Robinson B., 1999. The phytoremediation and phytomining of heavy metals. In *Proceedings, PACRIM'99 Congress* (Ed G.Weber) pp 127-135 (Australasian Institute of Mining and Metallurgy, Victoria).

Anderson, C.W.N., Brooks, R.R., Chiarucci, A., LaCoste,C.J., Leblanc, M., Robinson, B.H., Simcock, R. and Stewart, R., 2000. Phytomining for thallium, nickel and gold. *Journal of Geochemical Exploration*, in press.

Anderson, C.W.N., Brooks, R.R., Stewart, R.B. and Simcock, R., 2000. Seed germination in *Hybanthus floribundus* F. Muell. *Australian Journal of Botany*, in press.

Brooks, R., Anderson, C., Stewart, R. and Robinson, B., 1999. Phytomining: growing a crop of metal. *Biologist* **46**: 201-205.

Phytoextraction for the metals nickel and gold, unlike that for cadmium, lead and zinc, has potential for both phytoremediation and phytomining. In fact, it is hard to conceive a scenario where gold would exist as a contaminant necessitating site remediation. Fewer detailed studies have been published on the phytoextraction of nickel than for the metals covered in section A, perhaps because of the economic sensitivity inherent to research into nickel uptake. Nickel phytoextraction technology has been patented for uptake effected by *Alyssum* species (Chaney *et al.*, 1998), and the patentees believe they are close to commercial success (J.S.Angle, pers commn. 1999).

There are two reasons why phytoextraction for nickel holds such great potential. Firstly, more than 300 species are known to hyperaccumulate this metal (Chapter 1), and secondly, some of these species yield a very large annual biomass. *Berkheya coddii*, a nickel hyperaccumulator native to South Africa, for example, has a maximum known metal concentration of over 1% (DW) and a maximum possible biomass of 22 t/ha/year.

Gold is a very recent addition to the list of metals for which phytoextraction is possible, and is effected by induced hyperaccumulation. This thesis reports the first detailed study of such precious metal uptake.

Chapter 8 presents data from a field trial conducted on an area of 'serpentine soil' located in the central North Island of New Zealand. While phytoremediation of this site is not necessary, and any phytomining operation would be of such small scale that economic return seems unlikely, the area is a test case to examine the potential for phytoremediation in an environment foreign to the several hyperaccumulator species used.

Chapter 9 describes a major constraint imposed on the successful implementation of phytoextraction technology. Phytoextraction for nickel holds great potential in many parts of Western Australia, however, only native species may be used. This excludes the use of *Alyssum bertolonii* and *Berkheya coddii*. Instead, the only nickel hyperaccumulator native to Western Australia, *Hybanthus floribundus*, must be used. Until the study described in Chapter 9 this species had proven difficult to germinate from seed.

Phytoextraction of gold is described in Chapter 10, the mechanism for induced uptake and the geochemical rational behind this phenomenon.

Finally, Chapter 11 presents various practical scenarios that illustrate environments where phytoextraction for nickel and/or gold may prove environmentally and/or economically viable.

# Chapter 9 - A New Zealand Field Trial for Nickel Phytoextraction

The purpose of the project described in this chapter, was to field test the viability of both phytoremediation and phytomining on an area of 'quasi' serpentine soil, the terraced, 'wet-serp' byproduct material of a serpentine rock quarry operation located near Piopio, southwest of Te Kuiti, central North Island, New Zealand (Fig 9.1). This substrate represents a foreign environment for the hyperaccumulator species that were used. The concept of phytoremediation is well accepted, but the hard question of 'will the technology be field effective in many different metal-rich environments', has not been well addressed. If phytoextraction could be successfully effected in this environment, then the practical future of the technology for nickel could be better assured. Chapter 8 has not addressed the potential importance of specific nickel mineral phases on plant uptake. As there is little reason to induce the hyperaccumulation of nickel due to the large number of high-biomass nickel hyperaccumulator species, such a study is less relevant for this metal than for lead, zinc and cadmium.

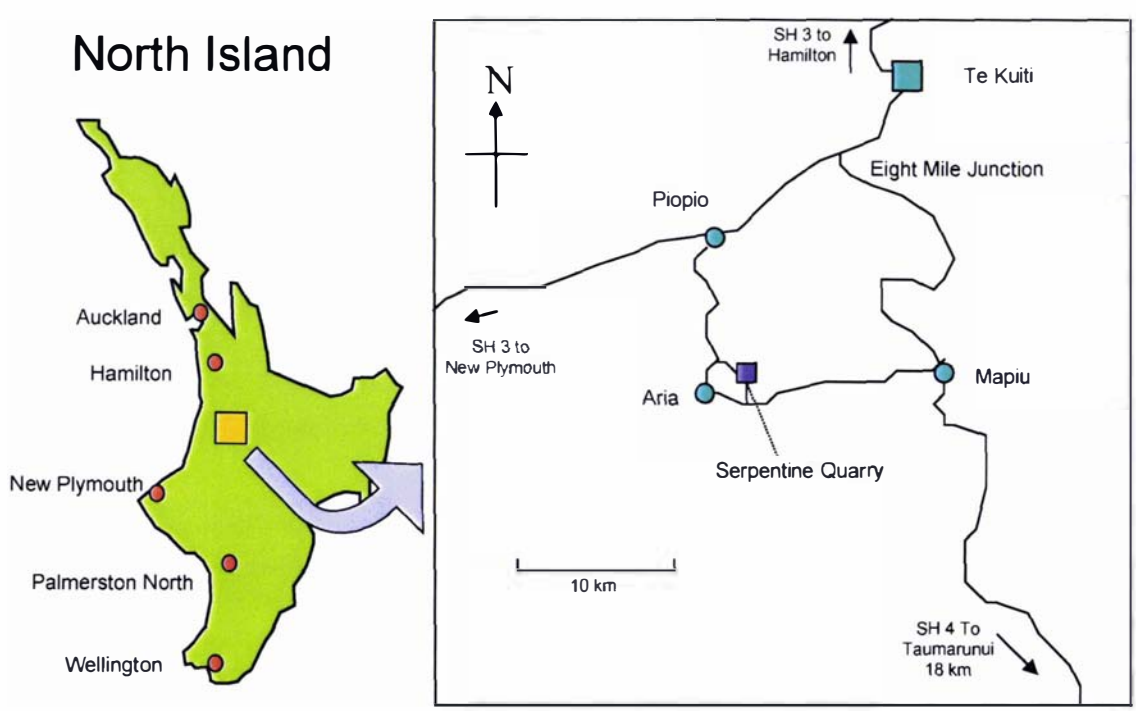


**Figure 9.1**. Map of the North Island of New Zealand showing in detail the location of the nickel phytoextraction trial site.

## 9.1 Introduction

Nickel is a heavy metal with the potential to have an adverse affect on life. While the metal is an essential trace ingredient in many of the living processes of higher plants (Marschner, 1995), at higher concentration (10 – 47 mg/kg), plant species can suffer from chlorosis, stunted root growth or necrosis (Uren, 1992; Krämer *et al.*, 1997). Excessive exposure to nickel compounds can result in damage to the lungs, brain, liver, kidneys, adrenal glands, spleen and pancreas of mammals. In extreme cases, ingestion of nickel compounds has been implicated in effecting fatal cardiac arrest (Knight *et al.*, 1997).

Nickel, like most other heavy metals, is a cumulative poison and thus low levels of anthropogenic contamination over a time frame of many years can result in a serious toxicity problem. Such anthropogenic contamination occurs in pastoral land to which sewage sludge has been applied. Nickel contamination of European soils above the EU maximum permissible guideline level of 75 mg/kg is common (McGrath and Smith, 1990).

Areas of anthropogenic nickel contamination are not as widespread as those for the more common heavy metals Cd, Pb and Zn. Except for the contamination described above, hot-spots for nickel are generally associated with the mining and processing of nickel ores. Nicks and Chambers, (1994, 1995, 1998) and Robinson *et al.* (1997a,b) have studied the nickel uptake potential of various plant species to assess their potential for phytoremediation and phytomining. The work of these two research groups is reviewed here.

*The initial study of Nicks and Chambers*

A pioneering study to examine the feasibility of applied nickel phytoextraction, was commissioned by the US Bureau of Mines (Reno, Nevada) in 1993. The trial used a natural stand of the nickel hyperaccumulator *Streptanthus polygaloides* that was growing on ultramafic soil at Red Hills, California (Nicks and Cambers, 1994, 1995, 1998). After harvesting, the plants contained, under optimal agronomic conditions, a

mean nickel concentration of 5 300 mg/kg (0.53 %) DW while the soil contained only 0.35 % nickel. The maximum known nickel concentration of this particular species is 1.48 % (Reeves, *et al*., 1981).

Nicks and Chambers (1998) proposed that a net return of US$513/ha to the grower could be achieved, assuming the following:

- selective breeding of the genotypes to yield a nickel concentration of 1 % and biomass of 10 t/ha DW,
- a world nickel price of US$7.65/kg (the price at the time of the trial – although the price now, Feb 2000, is closer to US$10/kg),
- the harnessing of 25 % of the energy produced from combustion of the biomass to produce electricity that could be sold to a local power grid, and
- a return to the grower of 50 % of the gross metal yield plus the value of the energy yield.

The possible metal recovery from a 10 t/ha crop with a nickel content of 1 % is 100 kg. Incineration of this biomass would yield approximately 500 kg/ha of bio-ore containing 20 % nickel.

*Subsequent studies*

Subsequent to the *Streptanthus polygaloides* investigation, Robinson *et al* (1997a) conducted experiments in Italy, using the hyperaccumulator *Alyssum bertolonii*, endemic to the ultramafic flora of the Tuscan Hills. This species can sustain, after moderate fertilisation, a biomass of 12 t/ha/yr with a mean nickel concentration of 0.8% dry matter. With these metal concentration and biomass figures, a nickel yield of 96 kg/ha can be realised, comparable to the yield of *S.polygaloides* in the above California project.

Trials have also been conducted using the South African nickel hyperaccumulator *Berkheya coddii* (Robinson *et al*, 1997b). This plant in the wild has a maximum reported nickel content 1.7% (Morrey *et al*, 1992). Based on New Zealand plot trials,

Robinson *et al.* (1997b) reported a sustainable biomass for *B.coddii*, after moderate fertilisation, of up to 22 t/ha/yr, and a conservative mean nickel concentration of 0.5% dry weight. Using these figures, a crop of *B.coddii* would remove 110 kg of nickel in each hectare, worth $1 100 at a current world nickel price of approx US$10.00/kg. Taking into account the energy derived from combustion and a return of 50% of the nickel value to the grower, each crop would be worth approximately US$660 a hectare.

*The Ultramafic Belt of New Zealand*

The basement rock for part of the New Zealand continental landmass is an 'ultramafic' igneous rock enriched in iron, nickel and magnesium relative to sodium, potassium and silica. The most common product resulting from the alteration of ultramafic rocks by heat and pressure in a hydrous environment is serpentine, and the name given the subsequent altered rock type is serpentinite (Gage, 1980).

Ultramafic rocks occur with a surficial exposure in Northwest Nelson for approximately 150 km north of the Alpine Fault, and then again south of the fault for another 185 km in Otago and Southland. Dextral movement on the alpine fault has displaced these two segments of what was once a continuous ultramafic assemblage some 500 km relative to each other. Gravity anomalies apparent on either side of the contemporary ultramafic exposure suggests that it extends hundreds of kilometres further, in both directions, which would explain the otherwise anomalous presence of serpentine rock in the North Island Tertiary sediments at Piopio and North Cape. At these two sites it appears that a slice of ultramafic rock has been fault-emplaced into the younger strata from the ultramafic belt constituting the present day basement (Gage, 1980).

*Geology of the Piopio serpentine exposure*

The surface exposure of serpentine rocks at Piopio is the result of serpentine basement intrusion up the plane of the Waipa fault. This fault separates the shallow, shelf-facies Mesozoic sediments from the deeper, marginal-facies Mesozoic strata. The intrusion of serpentinite has tipped the proximal Oligocene limestone to a near vertical orientation.

At least two periods of intrusion can be recognised due to the fact that the limestone contains serpentinite clasts (Kear 1960, 1967).

Serpentinite rock is quarried at the Piopio site, crushed, and added to superphosphate fertiliser as a 'flowing agent' for aerial topdressing. Low-grade serpentinite material, mixed with the surrounding limestone and sandstone/siltstone sediments (papa) not suitable for crushing, is stockpiled as 'wet-serp' on terraces adjacent to the quarry (Fig. 9.2).

**Figure 9.2**. North facing perspective of the Piopio serpentine quarry. Serpentinite rock is the dark-coloured material, running through the centre of the photograph, fault emplaced adjacent to the pale-coloured limestone. The wet-serp terraces lie to the left of this photograph.

## 9.2 Materials and Methods

*Design of the experimental area*

An 11 metre square area on the top terrace of the 'wet serp' stockpile was bulldozed in September 1997 and fenced to keep feral sheep, goats and rabbits out of the trial. Large

rubble clasts of limestone and serpentinite were removed by hand. The area was divided into 5 blocks, each of 2 metres by 10 metres, and each containing 20 individual 1 $m^2$ plots (Fig. 9.3).

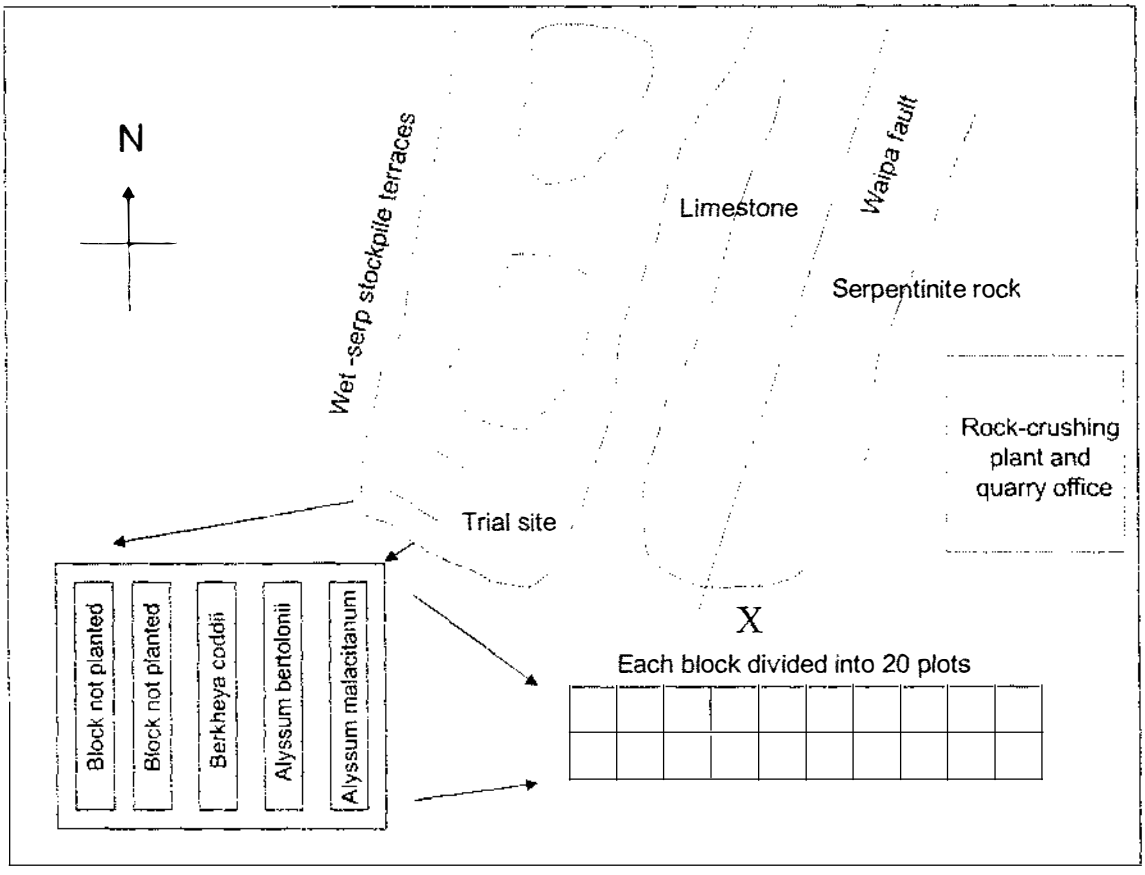


**Figure 9.3**. Schematic plan of the Piopio experimental trial set up. X marks the location where the photograph in Figure 9.2 was taken from.

One block was planted with seedlings of each of *Alyssum bertolonii, Alyssum malacitanum* and *Berkheya coddii* (Fig. 9.4). Due to problems of seed germination for an Australian hyperaccumulator species that was to be used for this trial (Chapter 10), the remaining 2 blocks were not planted during the trial, but have subsequently been planted with *B.coddii* (1999). Each plot was planted with 5 individual plants. Two replicates of 10 fertiliser treatments were applied within each block: $N_0P_0$, $N_0P_1$, $N_0P_2$, $N_1P_0$, $N_1P_1$, $N_1P_2$, $N_2P_0$, $N_2P_1$, $N_2P_2$ and S. Nitrogen was applied in the form of calcium ammonium nitrate (27%N) where $N_1$ represents an application rate of 50 kg N/ha. Phosphorus was applied in the form of superphosphate (9% effective P) where $P_1$ represents an application rate of 20 kg P/ha. Sulphur was applied as elemental 'flowers

of sulphur' at a rate of 40 kg/ha. Fertiliser was first applied to each block on the 16th September 1997. This application was repeated on 14th March 1998. A final application rate, double the concentration of that described above, was applied to the *Berkheya coddii* block only on 26th January 1999.

**Figure 9.4**. Photograph of the Piopio trial site before harvest of *B.coddii* in May 1999, looking towards the northeastern corner of the site. Wooden pegs separate each plot. The block in the foreground is newly planted *B.coddii*. The block in the middle of the photograph shows the *B.coddii* plants analysed for this chapter.

A small amount of the Piopio wet-serp was collected and brought back to Massey University. Two outside plots were filled with this material, each with dimensions of 1 by 1 by 0.15 metres. The first plot contained 'pure' wet-serp, the second a 1:1 mixture of wet-serp and sieved bark. Both plots were planted with *Berkheya coddii*.

*Analytical procedure*

Plant samples were collected, rinsed in deionised water and dried at 60°C to constant weight. *Alyssum* samples (ca. 200 mg) were weighed directly into borosilicate test tubes, ashed at 550°C overnight and the residue taken up in hot hydrochloric acid (10

mL of 2M) before analysis by FAAS. *Berkheya coddii* was first ground before subsamples (ca. 200 mg) were treated using the same procedure.

Ground subsamples of *Berkheya coddii* were analysed for carbon and nitrogen using a Leco CNS furnace. Phosphorus was analysed using the Kjeldahl digest method of Blakemore *et al.* (1987). A soil:water suspension ratio of 1:2.5 was used to measure pH.

Three replicate cores (0-8cm) were sampled from each of the *Berkheya coddii* plots at the conclusion of the trial. These cores were then mixed to generate a composite sample from each plot, air dried and sieved with a nylon sieve (<500µm). Sieved subsamples (1g) were digested in *aqua regia* before analysis by FAAS. Plant-available nickel was estimated by extraction with ammonium acetate (1M) at a soil:liquid ratio of 1:10 (Table 9.1).

**Table 9.1**. Piopio wet-serp geochemical properties

| | | | | | |
|---|---|---|---|---|---|
| Mg (%) | 23 | Zn (mg/kg) | 48 | Cr (mg/kg) | 910 |
| Fe (%) | 4.8 | Cu (mg/kg) | 12 | Ni (mg/kg) | 1880 |
| Mn (mg/kg) | 850 | Co (mg/kg) | 96 | Ext-Ni (ng/L) | 2.2 |
| C(%) | 0.47 | N (%) | 0.02 | pH | 8.4 |

Notes (1) metal analyses performed by FAAS
(2) Ext-Ni is extraction by 1M $NH_4OAc$ at a 10:1 liquid soil ratio
(3) C and N analyses performed by Leco furnace.

## 9.3 Results

*Alyssum malacitanum*

This species did not grow well in the wet-serp environment and did not appear to increase in size from the initial planting. Samples collected during the trial showed an average nickel concentration of approximately 1 400 mg/kg across the block. However, due to the poor growth, no harvest was made at the end of trial.

*Alyssum bertolonii*

Samples of *Alyssum bertolonii* were harvested from each of the plots in January 1999 and analysed for nickel (Table 9.2; Fig. 9.5). The mean concentration of plant nickel across the 20 plots was 4 160 mg/kg.

**Table 9.2**. Mean *Alyssum bertolonii* nickel concentration for each fertiliser treatment (n=2).

| Fertiliser treatment | Mean nickel in plant (mg/kg) |
|---|---|
| $N_0P_0$ | 4 130 (± 360) |
| $N_0P_1$ | 2 980 (± 500) |
| $N_0P_2$ | 4 660 (± 640) |
| $N_1P_0$ | 3 580 (± 900) |
| $N_1P_1$ | 5 095 (± 350) |
| $N_1P_2$ | 4 900 (± 1 600) |
| $N_2P_0$ | 2 690 (note) |
| $N_2P_1$ | 4 220 (±1 010) |
| $N_2P_2$ | 4 630 (± 290) |
| S | 4 730 (± 990) |

Note: only one plot was harvested for the $N_2P_0$ treatment.

**Figure 9.5**. Flowering specimen of *Alyssum bertolonii* growing at the Piopio trial site. Photograph taken December 1999.

Application of N did not significantly change the nickel concentration in *Alyssum bertolonii*. However, there was evidence for a significant increase in plant nickel with increasing application of P (Fig. 9.6). There was no significant evidence for any interactive effect of N and P fertiliser on the nickel concentration in the plant. Application of sulphur did not result in higher metal uptake at the application rate used.

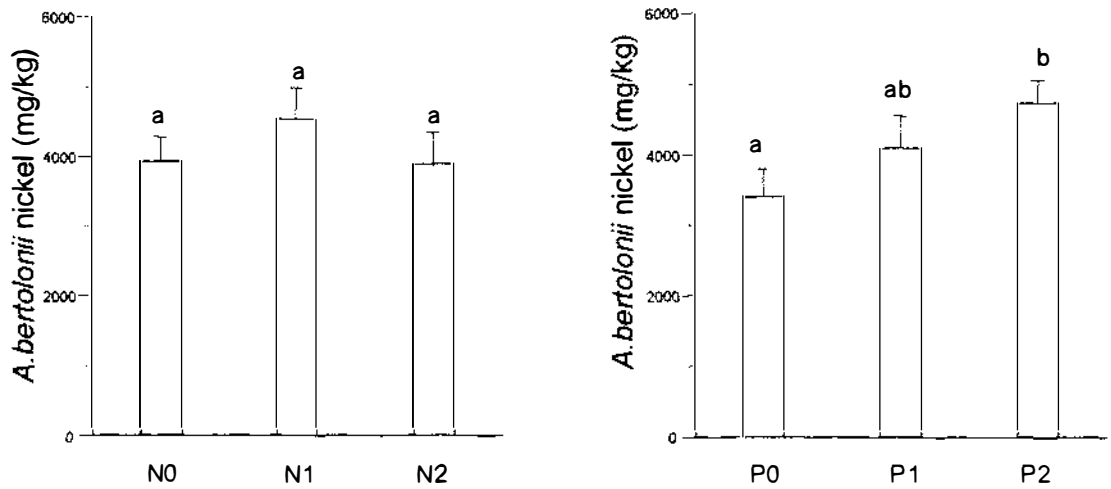


**Figure 9.6**. Nickel concentration in *Alyssum bertolonii* as a function of the soil fertiliser treatments. Error bars represent standard error. Means with the same letter are not significantly different (ANOVA p>0.05).

*Berkheya coddii*

The above-ground biomass of *Berkheya coddii* was harvested from each of the plots in May 1999 (Fig. 9.7), weighed and analysed for nickel (Table 9.3), cobalt, nitrogen, carbon and phosphorus. The mean concentration of plant nickel across the 20 plots was 1 400 mg/kg.

There was a significant decrease in the nickel concentration in *Berkheya coddii* with addition of N fertiliser, but no significant change in nickel through the addition of P (Fig. 9.8). Again, there was no significant evidence for any interactive N and P fertiliser affect on the plant nickel concentration. Application of sulphur to the plots did not result in higher metal uptake.

**Table 9.3**. Mean *Berkheya coddii* nickel concentration for each fertiliser treatment.

| Fertiliser treatment | mean nickel in plant (mg/kg) |
|---|---|
| $N_0P_0$ | 2 210 (± 95) |
| $N_0P_1$ | 1 200 (± 150) |
| $N_0P_2$ | 1 780 (± 730) |
| $N_1P_0$ | 1 135 (± 4) |
| $N_1P_1$ | 1 520 (± 650) |
| $N_1P_2$ | 900 (± 220) |
| $N_2P_0$ | 1 305 (± 120) |
| $N_2P_1$ | 1 205 (± 115) |
| $N_2P_2$ | 810 (± 250) |
| S | 1 590 (± 175) |

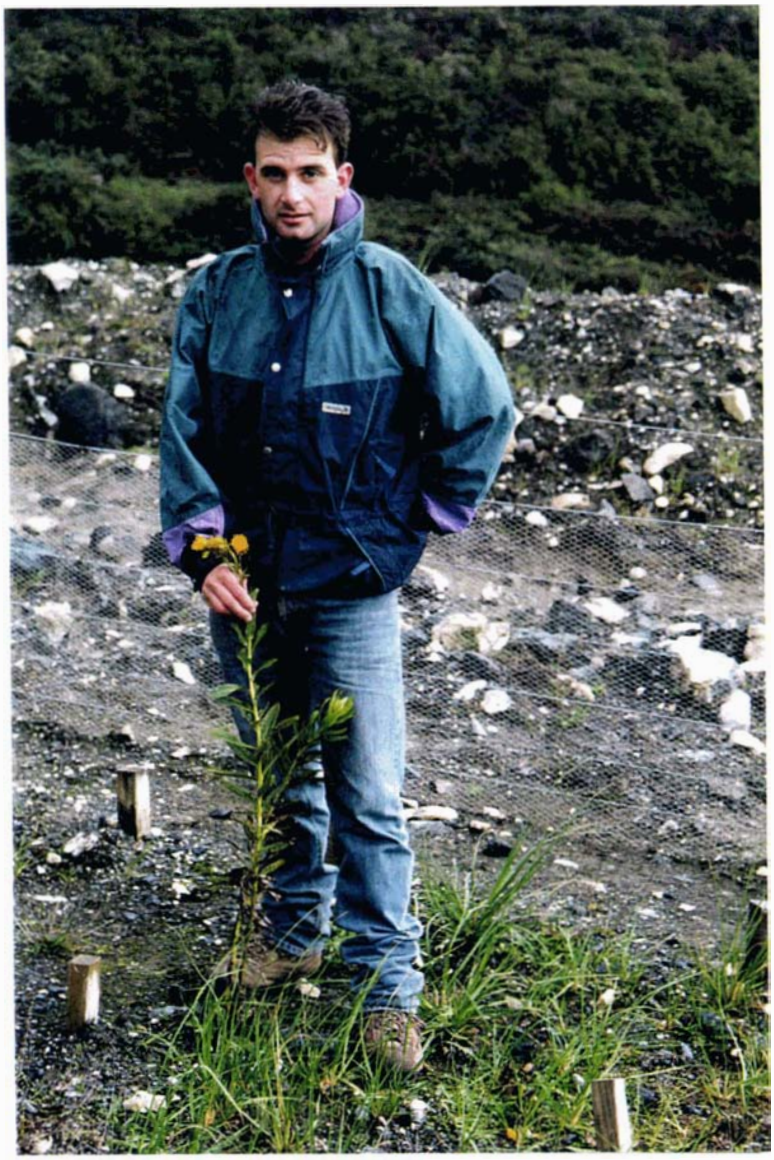

**Figure 9.7**. The author with a flowering stem of *Berkheya coddii*, growing at the Piopio site. Photograph taken May 1999.

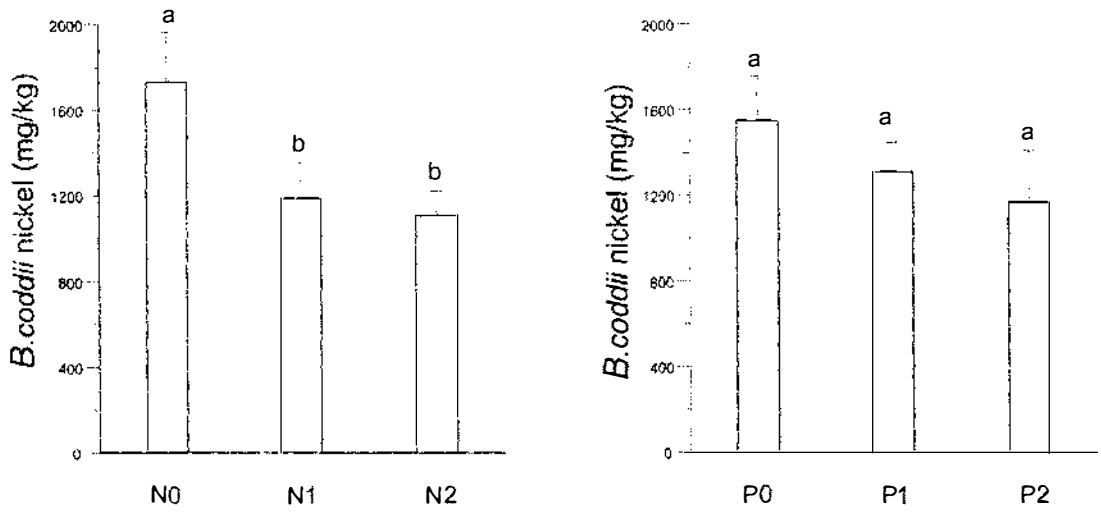


**Figure 9.8.** Nickel concentration in *Berkheya coddii* as a function of the soil fertiliser treatments. Error bars represent standard error. Means with the same letter are not significantly different (ANOVA $p>0.05$).

To further elucidate any possible relationships between plant-nickel concentration and plant nutrient status, samples of *Berkheya coddii* were analysed for C, N and P. The plot of nickel as a function of nitrogen (Fig. 9.9) shows no relationship between these elements within the plant ($R^2=0.03$). These data do not support a decrease in plant nickel with increasing application of N fertiliser. Similarly, there was no intraplant relationship between nickel and phosphorus ($R^2=0.009$) and nickel and carbon ($R^2=0.007$).

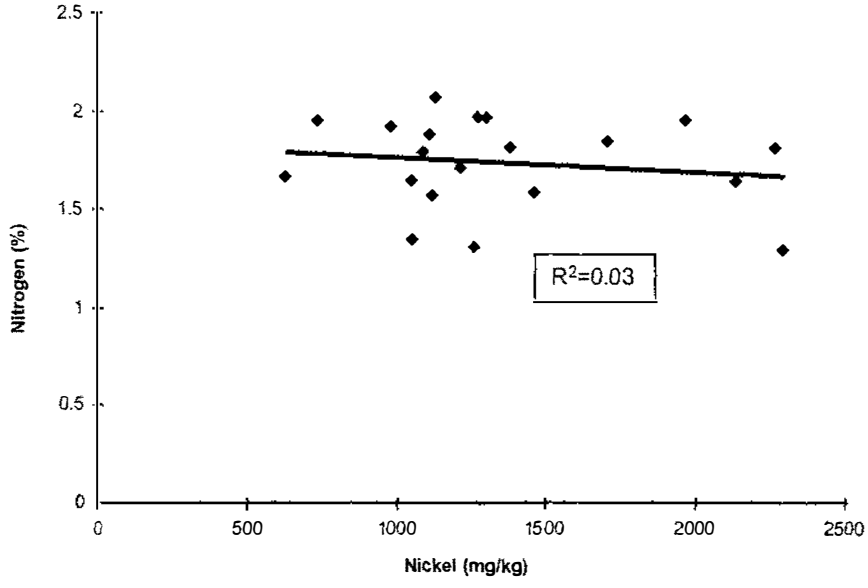


**Figure 9.9.** Nickel concentration in *Berkheya coddii* as a function of the plant nitrogen concentration.

## Cobalt

Samples of *Berkheya coddii* prepared for nickel analysis were also analysed for cobalt. The mean concentration of plant cobalt across the block was 110 mg/kg. Cobalt was correlated with nickel (Fig. 9.10) but was not, however, hyperaccumulated. The hyperaccumulation criterion for cobalt is accumulation greater than 1 000 mg/kg DW, and the range in 'normal' plants is 0.05 – 50 mg/kg.

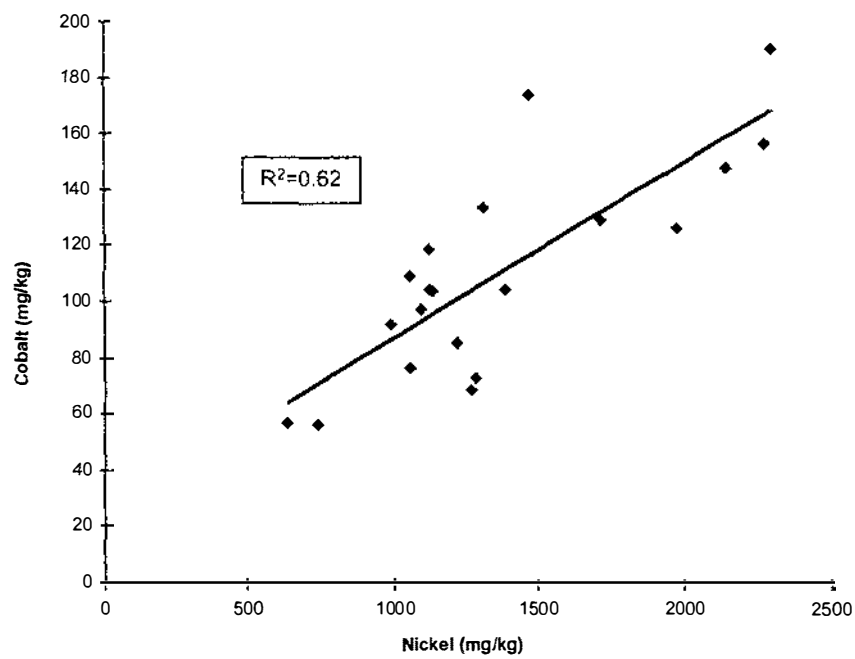


**Figure 9.10**. Plot of the cobalt concentration in *Berkheya coddii* as a function of the nickel concentration.

### *Native species*

Several weed species have naturally colonised parts of the wet-serp terraces. Samples of these were collected from within the trial area and analysed for nickel uptake. The highest concentration of nickel observed in any of these species was 53 mg/kg dry weight in a thistle plant.

*Soil samples*

Analysis of the substrate samples from each of the *Berkheya coddii* plots showed no significant difference in soil nickel concentration relative to control samples collected outside the plot area.

## 9.4 Discussion

The three plant species used in this trial all failed to realise the high biomass yields reported by Robinson 1997 and Robinson *et al.* (1997a,b). Biomass calculations were not made for the *Alyssum* species, although individual *Berkheya coddii* plants were weighed. The total dry mass of *B.coddii* plant material harvested from the plot area was 480 g, and represents an average mass of 20 g per individual plant. Robinson *et al.* (1997b) reported a fertilised biomass for individual *B.coddii* plants of 125 g (22 t/ha[10]) and an increase in biomass with increasing fertilisation. There was no evidence in this field trial of increased biomass for the plots with higher fertiliser loadings.

The metal concentration of the harvested plants was also relatively low. Robinson *et al.* (1997a) reported mean nickel concentrations of up to 0.77% for *Alyssum bertolonii* grown during a field trial in Tuscany, Italy (this species natural environment). *Berkheya coddii* is known to contain up to 1.7% nickel in its leaves in the wild (Morrey *et al.*, 1992), although a more conservative mean nickel concentration of 0.5% was reported by Robinson *et al.* (1997b).

*Reasons for the poor species performance*

The carbon and nutrient status of the Piopio substrate is very low: 0.47% carbon and 0.02 % nitrogen. This low nutrient status could explain the poor performance of control plants grown in plots where no fertiliser was added, but may not explain the low metal concentrations and low biomass in general.

[10] Planting density for the trial described in this thesis was 5 plants/$m^2$. The planting density of *Berkheya coddii* for Robinson (1997b) was 16 plants/$m^2$.

The lack of positive correlation for both the plant-nickel concentration and the harvested biomass with increasing fertiliser application was somewhat surprising. Robinson *et al.* (1997b) showed a positive response of both of these variables with N fertiliser application. This evidence is in agreement with the observations of Krämer *et al.* (1996), who showed an increase in the intraplant production of the amino acid histidine in response to plant-available nickel.

Histidine acts to bind nickel and thus render the metal safe for intracellular storage (Chapter 2). Krämer *et al.* (1996) suggested that the concentration of histidine in a plant (quantified by N%) could be directly proportional to the concentration of nickel accumulated by the aerial herbage. The negative response of plant nickel to the soil-applied fertiliser for *Berkheya coddii* in this trial (Fig. 9.3), not supported by the intraplant nitrogen - nickel correlation (Fig 9.4), while significant, can be attributed to random variation across the block.

The lack of organic material in the soil would have limited the number of 'capture sites' for N and P as these elements were released from the fertiliser granules. As a result, the residence time of the fertiliser in the rhizosphere would have been short. The form of N fertiliser used (calcium ammonium nitrate) is very water-soluble. Lack of nutrients and poor soil physical conditions could explain why no relationship existed between biomass and the plant nickel concentration with soil-applied fertiliser. All *Berkheya coddii* plants showed nitrogen and phosphorus deficiencies.

Similarly, the lack of any effect of sulphur application on plant-nickel concentration could be attributed to the leaching of this element out of the rhizosphere before uptake (assuming the sulphur was released in the rhizosphere through biodegradation). Sulphur will lower the pH of soil, making metals more bioavailable (Robinson, 1997), but is also an integral component of many phytochelatins. An increased intraplant sulphur concentration through sulphur fertilisation could conceivably be correlated with an increased nickel concentration.

Increasing the organic matter in the soil would lengthen the residence time of nutrients in the rhizosphere, but organic matter also binds metals into an insoluble form. Mean

metal uptake for *B.coddii* grown in outside plots of Piopio material at Massey was 1700 mg/kg from the 100% wet-serp, but only 515 mg/kg from the serp/bark mixture. Both of these plots were fertilised with slow-release Osmocote fertiliser. There is clearly a trade off between organic material and metal bioavailability and hence uptake.

Physical factors could have also contributed to the poor results yielded by the trial. The Piopio substrate has poor drainage due to compaction evident across the site. Compaction may have limited the rooting depth of individual plants. Several whole *Berkheya coddii* plants were excavated from the site, and it was noted that the root system of each of these was less prolific than that observed for specimens excavated from the outside plots at Massey University. Presumably, the potential for metal uptake is related to the root mass of an individual plant.

Climatic conditions could have similarly affected plant growth. The summers of 97/98 and 98/99 saw low rainfall at the site and near drought conditions. The *Alyssum* species used have evolved to withstand dry conditions, however, the natural climate of *Berkheya coddii* in South Africa is characterised by a relatively higher rainfall.

## 9.5 Conclusion

The Piopio trial showed that nickel pytoextraction could successfully be carried out in a hostile environment. The *Alyssum* species and *Berkheya coddii* all hyperaccumulated nickel, although the biomass production and metal accumulation were lower than could have been expected. Poor performance and yield in these factors explains why there was no significant decrease in soil nickel concentration effected by the trial.

Nickel was removed from the site. Considering only the *Berkheya coddii* plants, a biomass of 480 g dry weight with an average metal concentration of 0.14% equates to 0.67 g of metal removed from this block. However, with greater fertiliser loading and substrate amendments to improve the soil physical conditions and texture, factors that would increase the biomass of individual plants, as well as an increased

hyperaccumulator species planting density, this figure could be significantly increased. Knowledge of these limitations and parameters are essential to ensure the successful implementation of phytoextraction technology.

The trial has highlighted very clearly that extrapolation of pot and greenhouse trial data to the field can not easily be made. Physical conditions in the field are often not ideal for plant growth, a statement that is particularly true for ultramafic soils. Hyperaccumulator species grow well in their natural environment, where they have evolved to the prevalent environmental conditions. However, these same species may not 'perform' so well in a foreign environment.

# Chapter 10 - *Hybanthus floribundus*, a Native Australian Nickel Hyperaccumulator

Hyperaccumulator species have a limited worldwide distribution (see Chapter 2). This poses a serious constraint on the practical application of phytoextraction, for countries/sites where the use of exotic plant species is discouraged. Induced hyperaccumulation may allow the use of native non-accumulator species, but, as was discussed in section A, the technology has yet to advance to a level where induced hyperaccumulation can be guaranteed to work for all metal/plant combinations.

Australia has many environments that may benefit from the use of hyperaccumulation technology. Decades of mining for base and precious metals has left overburden dumps and tailings dams contaminated with anthropogenic sources of metal. Since October 1997, part of my research has investigated the potential for the use of nickel hyperaccumulators to remediate areas of nickel contamination on the mining lease of the Western Mining Corporation Ltd., near Kambalda, Western Australia. Three nickel hyperaccumulators exist in Australia, and one of these, *Hybanthus floribundus*, is native to the goldfields of Western Australia. Due to limitations on the use of exotic species in Australia, attention focused on the use of this particular plant. However, *H.floribundus* had never been germinated from seed with any substantial degree success (no more than 2%).

This chapter outlines my approach to overcome the inherent problems of germination associated with *Hybanthus floribundus*, problems that limit the practical application of this species for phytoextraction.

## 10.1 Introduction

*Hybanthus floribundus*

*Hybanthus floribundus* (Fig. 10.1) is a small woody shrub found throughout southern Australia. Its most common occurrence is in the south of Western Australia and southeast of South Australia. Its distribution has been described by Bennett (1969) who

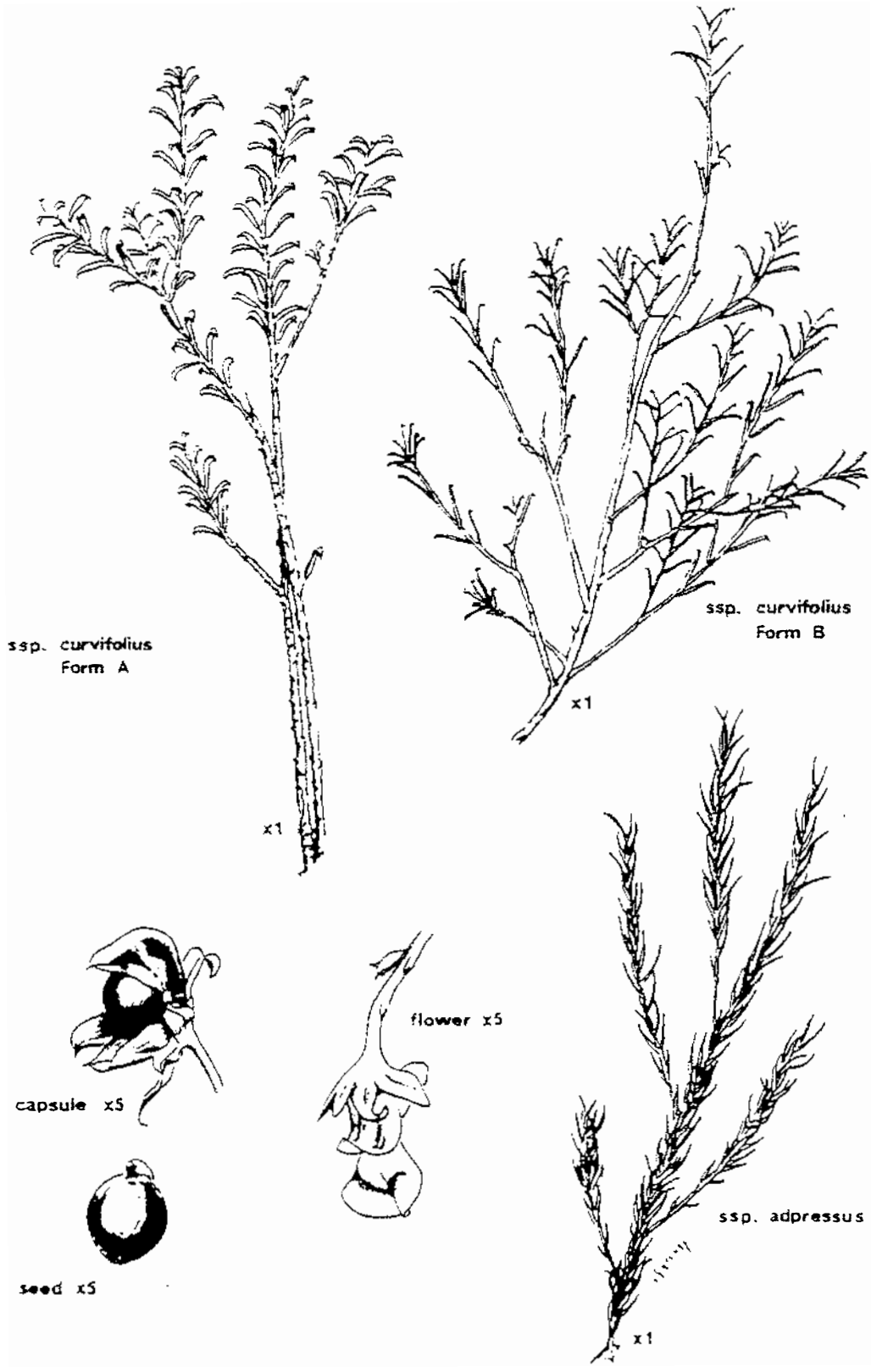


**Figure 10.1**. *Hybanthus floribundus*, F. Muell.

recognised several subspecies. These include subspecies *floribundus, curvifolius* and *adpressus,* found mainly in the Eastern Goldfields region of Western Australia and extending from Leonora in the north to Esperance in the south.

The present-day distribution of *Hybanthus floribundus* and its subspecies is considered to be the relic of a much wider distribution that occurred before the onset of aridity in Australia. *Hybanthus* occurs in the warm-temperate zone where annual rainfall exceeds 250 mm and winter and summer isotherms are 12°C and 25°C respectively. Phytosociologically, this plant has been assigned by Ernst (1974), to the Hybanthion floribundii alliance. Together with *Grevillea acuaria*, it is a character species of this alliance.

Severne and Brooks (1972) and Cole (1973) were the first to report nickel hyperaccumulation exhibited by *Hybanthus floribundus.* This was only the third species reported as a hyperaccumulator of this metal. Even today, only two other hyperaccumulators of nickel is known from Australia (*Stackhousia tryonii*, Batianoff *et al.*, 1990; *Pimelia leptospermoides*, S.Bidwell, pers. commn. 1999).

Nickel concentrations found in the various subspecies of *Hybanthus floribundus* are high; >1% dry mass in some individuals (Table 10.1). This is unusual because the soils of Western Australia typically contain low nickel concentrations (ca. 1000 mg/kg) relative to the usual 5000 mg/kg found in ultramafic ('serpentine') soils. The plant/soil nickel concentration quotient is often >10 and highlights the remarkable hyperaccumulating ability of this species. A plant/soil metal concentration quotient of 10 is 100 times greater than that for non-accumulator plants growing in the same environment (criterion of hyperaccumulation).

Severne (1972) concluded that *Hybanthus floribundus* is confined to laterised ultramafic outcrops and creek beds, but Cole (1973) proposed that this species is confined to soils derived from highly nickeliferous rocks although it does not necessarily indicate a sulphide orebody. It is interesting that the surficial expression of the first nickel orebody discovered on the Western Mining Corporation Ltd. lease in Kambalda, was expressed by the growth of *H.floribundus* (R.Brooks, pers. commn. 1997).

**Table 10.1.** Mean nickel concentrations (mg/kg DW) of species and subspecies of *Hybanthus* together with their locations in Western Australia. Values for the associated soils are also given.

| Species | Location | Ni in plant | Ni in soil | Plant/soil Ni |
|---|---|---|---|---|
| *H.floribundus* | | | | |
| Subsp. *Curvifolius* | Marshall Pool | 7030 | 800 | 8.8 |
| Form A | Kurrajong | 3100 | 900 | 3.4 |
| Form B | Kambalda | 3000 | 900 | 3.3 |
| | Southern Cross | 4510 | 1400 | 3.2 |
| | Widgiemooltha | 6010 | 2000 | 3.0 |
| | Spargoville | 740 | 970 | 0.8 |
| Subsp. *Floribundus* | Lake King | 260 | 50 | 5.3 |
| | Southern Cross | 1020 | 50 | 20 |
| | Widgiemooltha | 12 200 | 1000 | 12 |
| | Dordie Rocks | 13 800 | 1000 | 14 |
| Subsp. *Adpressus* | Ravensthorpe | 1270 | 130 | 9.5 |
| *H. epacroides* | | | | |
| Subsp. *Bilobus* | Scadden | 200 | 10 | 20 |

Source: Cole (1973) and Severne (1972).

The practical application of phytoremediation or phytomining for nickel in Western Australia has been hampered by the requirement that only native species can be used. *Hybanthus floribundus* is therefore the only acceptable hyperaccumulator. There has been little or no success over recent years in inducing *Hybanthus* seeds to germinate. Plants have been raised either by tissue culture (S. Bidwell, pers. commn. 1999), or by striking cuttings.

*Importance of 'fire' to promote seed germination*

Seeds have evolved dormancy strategies that prevent germination from occurring until conditions exist that are likely to promote growth (Goodwin *et al.*, 1995). In parts of Australia, Africa, California and Mexico fire has played an important role in the evolution of native flora. It appears that a fire event in these environments may trigger seed germination (Gill and Groves, 1981). Two factors are involved with fire: heat and smoke. The positive effect of heat on overcoming seed dormancy mechanisms has been recognised for some time (Beadle, 1940; Boughton, 1970), however other 'fire products' are now receiving greater attention.

Dixon *et al.* (1995) tested the effect of greenhouse exposure to cool smoke on the germination of 94 species of Australian natives. For 45 of these species exposure to

smoke significantly enhanced germination, and at least 23 of these had been totally unamenable to conventional methods of seed propagation. *Hybanthus floribundus* was included with 7 species for which germination was not significantly improved relative to the control (ca. 2% germination rate).

Smoke was generated by Dixon *et al.* (1995) through the controlled burning of *Banksia-Eucalyptus* woodland material in a large drum. This smoke was pumped through a water-cooled pipe before passage into a fumigation tent housing seed trays of the species being used. Commercially, smoke is available in the form of 'smoke water'. The 'Kings Park smoke research group' has been instrumental in developing this technology to assist with the germination of Australian native species. In addition to Kings Park smoke water, 'Regen 2000 Smokemaster', a commercial smoke water produced by Tecnica Pty Ltd., Melbourne, was used as part of this study. Sales material accompanying this product reports that the genus *Hybanthus* is responsive to Regen 2000 under nursery conditions, although no specific species is mentioned.

## 10.3 Methods and Materials

The following experiments were carried out to increase the germination rate of *Hybanthus floribundus*. Few experimental details are given for methods that were not successful. After treatment, seeds in tests 1-4 were placed in moistened germination blotters enclosed in Petri dishes sealed with `Parafilm'. The seeds for experiments 1-6 were ca. 6 months old. Those for experiment 7 were ca. one-year- old.

1 - Boiling seeds for periods of up to 10 sec.
2 - Scarification by placing seeds in a sealed round tin coated with sand paper and rotating the tin in an end-over-end shaker for periods of up to 7 days.
3 - Treating seeds with smoke generated from burning Australian wattle leaves.
4 - Treating seeds with smoke water supplied by the Perth Botanical Garden.
5 - Direct planting into ultramafic soil.

6 - Placing seeds on a 'Grant' Temperature Gradient Plate covered with damp K22 'Kimpac' sheets covered with three germination blotters. The temperature gradient was maintained from 7 to 33°C over a 43-day period.

7 - Placing about 35 one-year-old seeds on moistened germination blotters in Petri dishes covered with waterproof film. There were 8 different conditions in this final experiment (Table 10.2). Germination was studied over a period of 35 days and the temperature was maintained in the range of 15°C - 21°C during this period. Scarification was carried as in 2 above. For the water treatment, seeds (scarified and non-scarified) were soaked in either deionised water, or a 10 % solution of 'Regen 2000 Smokemaster' for 24 hours. Seeds were subsequently dried and used in the germination trials as per the manufacturer directions.

**Table 10.2**. End of experiment results of germination test 7 on seeds of *Hybanthus floribundus*

| Smoke $H_2O$ | RO $H_2O$* | Scarified | Light | Dark | N | %Germinated |
|---|---|---|---|---|---|---|
| Yes | No | No | No | Yes | 38 | 36.8 |
| No | Yes | No | No | Yes | 35 | 14.3 |
| Yes | No | No | Yes | No | 37 | 10.8 |
| No | Yes | No | Yes | No | 33 | 3.0 |
| Yes | No | Yes | No | Yes | 33 | 3.0 |
| No | Yes | Yes | No | Yes | 34 | 2.9 |
| Yes | No | Yes | Yes | No | 35 | 0 |
| No | Yes | Yes | Yes | No | 37 | 0 |

* RO $H_2O$ is water purified by reverse osmosis.

Experiments 1-6 had no positive results. The results from experiment 7 are discussed in the following section.

## 10.4 Results and Discussion

Optimum germination occurred for the aged, smoke water (Regen 2000) treated seeds, germinated under dark conditions (Table 10.2). The germination rate of 37% was the highest recorded for this species. The use of one-year-old seeds appears to be of paramount importance, as the previous smoke water experiment conducted on younger seeds (expt. 4) was not successful. This suggests that seeds of *Hybanthus floribundus* may need to ripen within the seed coat after leaving the plant (E.Bennett, pers. commn.

1997). Scarification appeared to hinder germination, perhaps because of tissue damage resulting from the long period of abrasion. It appears that although darkness and smoke water are individually helpful in promoting germination, together they have a synergistic effect. Even when smoke water was absent and the seeds were exposed to light, there was still a small degree of germination (3%).

Subsequent to the successful results for experiment 7, the optimal-germination conditions were replicated to confirm these mechanisms for enhanced seed germination. Sixty, one-year-old seeds were soaked in a 10% solution of 'Regen 2000 smokemaster' for 24 hours, dried and placed on filter paper moistened with deionised water. Seeds were then place in the dark with a constant temperature range of 15°C - 21°C in Petri dishes covered by parafilm (n=2). The germinate number was recorded over 45 days (Fig. 9.2). Of the sixty seeds used, 30 had germinated after 6 weeks, with replicate germination rates of 56% and 47% (Fig. 10.2).

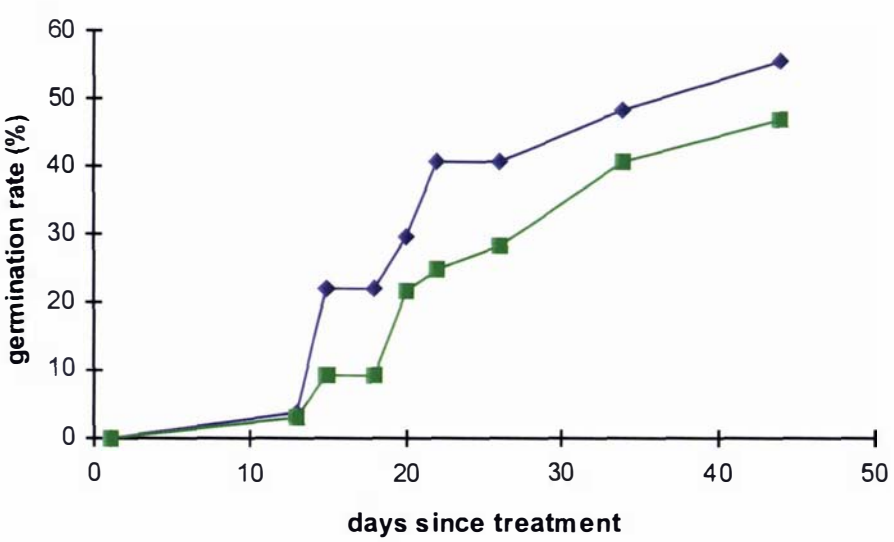


**Figure 10.2.** Germination experiment for *Hybanthus floribundus* showing a final germination rate of 56% for replicate 1 (diamonds) and 47% for replicate 2 (squares).

## 10.5 Conclusion

This study highlights the importance of seed age, as well as the potential for the use of smoke or smoke water in combination with dark conditions to induce the germination of *Hybanthus floribundus.* The importance of smoke has been shown previously for many other hard-to-germinate Australian species (Read 1997; Dixon *et al.*, 1995). Dark conditions in combination with the apparent necessity of embryo ripening within the seed coat are of paramount importance; it seems likely that *Hybanthus* seeds must be buried in the soil for germination to ensue. I am not sure to what extent the necessity of fire is practical for natural in-field germination of the species. I can find no recent record of a fire event in the WA Goldfields, even though natural populations of this species do exist. Scarification had no effect on germination, it is therefore probable that breaking of the seed coating is not one of the variables involved in germination of this species.

The successful germination technique presented in this chapter overcomes the practical limitations hindering the use of *Hybanthus floribundus* for the phytoremediation and phytomining of nickel-contaminated soil; the plant has an appreciable biomass of around 10 t/ha. Even if neither of these possibilities are exploited, the plant should have potential for rehabilitation of mine dumps and tailings because of its high tolerance to adverse edaphic conditions. At many locations in the Eastern Goldfields Region it is the only species able to colonise such mineralised sites. Field trials have not yet been conducted using *Hybanthus floribundus*, due to the initial unforeseen difficulties in seed germination hindering project advancement. However, implementation of such trials is now possible.

# Chapter 11 - Phytoextraction for Gold

Phytoextraction for gold is the most recent advance in the growing technology of induced hyperaccumulation. Due to the extremely low solubility of gold in soil solution, no known plant will naturally accumulate the high levels of this metal necessary to successfully effect phytoextraction. Induced hyperaccumulation had been proven viable for lead, so the challenge was to extend this work to other metals. Gold seemed a very attractive target, as the significant uptake of gold by plants has long been the 'philosopher's stone' of some scientists.

This chapter reviews and outlines the background to gold mobility within soil, and hence the potential for uptake by plants. The initial experiments are then described, through which the ability to induce accumulation of gold was discovered. Finally, the current direction of research is presented, describing some of the inherent problems associated with this technology, and my approach to a better understanding of them.

## 11.1 Introduction and review of the solution geochemistry of gold

*Solution geochemistry of gold*

The solution geochemistry of metal-ligand complexes can be described and predicted, to some degree, by the hard-soft acid-base theory of Pearson (1963). Hard acids are defined as small, slightly polarisable metal ions (e.g. $Al^{3+}$, $Ti^{4+}$, and $Co^{3+}$) that preferentially complex with ligands through 'hard' binding sites such as P and O sites (e.g. $H_2EDTA^{2-}$ and $PO_4^{3-}$); hard bases. Soft acids are larger and more easily polarisable metal ions (e.g. $Au^{+}$, $Pd^{+}$ and $Hg^{+}$) that preferentially complex with ligands through 'soft' binding sites such as S and N sites (e.g. $SCN^{-}$ and $CN^{-}$); soft bases[11].

Using Pearson's hard-soft acid-base theory, the metal gold is classed as a soft acid in its cationic form, and hence in solution will have an affinity for soft bases. This theory has

[11] A more detailed description of this theory and a more complete list of hard and soft acids and bases can be found in most inorganic chemistry textbooks.

led to the attribution of some degree of the geochemical mobility of gold as thioligand complexes; the sulphur atoms and overall electronic structure of these ligands imparting soft base chemistry. Bowell *et al.* (1993) speculated that thiosulphate and thiocyanate ligands could be responsible for the mobility of gold within a weathering terrain in Ghana, although this was only modelled evidence to implicate thioligands in gold mobility. Kucha *et al.* (1995) presented direct physical evidence for the deposition and transport of gold by thiosulphate in the Veitsch magnesite deposit, Austria.

Gold mobility occurs in geological domains called weathering terrains. Examples of these terrains can be found in Western Australia, West Africa and South America, but the geochemical characteristics of the associated weathering fluids are poorly understood. A better understanding of the mobility of metals under weathering conditions is recognised by the minerals industry as being important. Such understanding will lead to the more effective use of 'pathfinder elements' (elements geologically associated with gold that are more abundant and hence more easily detected e.g. As), and 'geochemical haloes' (zones of metal enrichment that indicate the presence of an orebody) for defining and delineating orebodies. Likewise, at an environmental level, understanding the geochemical mobility of residual metals within a previously mined laterite terrain will effect maximisation of phytoremediation technology in the choice of plant needed to take up metals of interest. The geochemical mobility of metals and the biogeochemical pathways of plant metal cycling are inextricably linked in such a fashion.

*Economic mineralisation within a weathering profile*

Laterisation is a chemical weathering process that breaks down the primary fabric of the host bedrock. The resultant weathering profile has several component features. The surface expression of a complete laterite is a ferruginous zone, enriched in iron. Below the ferruginous zone is a clay-rich body of weathered rock called a mottled zone which grades into fresh, unweathered bedrock. The bedrock/mottled zone interface is termed a 'saprolite' while the term 'laterite' is used to describe the upper parts of the profile. Approximately 65% of the World's nickel deposits are hosted in lateritic weathering profiles. Of this total, 38% can be found in countries of the Pacific Rim.

In a discussion of the geochemistry of gold in lateritic terrains, Gray *et al.* (1992) described two regions that constitute a complete lateritic weathering profile. Economic mineralisation associated with the upper, laterite, zone is termed a 'lateritic supergene deposit'. Economic mineralisation associated with the saprolite zone is termed a 'saprolitic supergene deposit'. Supergene enrichment is defined as the secondary mobilisation and deposition of metals and enrichment through reprecipitation of any part of an orebody by the movement of geochemical fluids.

The process of laterisation only occurs in humid, tropical, environments, characterised by a high water-flux rate. Much of Western Australia is today subject to more arid environmental conditions and the associated contemporary weathering is an overprint of the laterisation that occurred during the tropical conditions prevalent at various times since the Mesozoic Era (Mann, 1984). The geological setting of Western Australia within a stable intracratonic basin, and associated low sedimentation rates, has led to the preservation of economic mineralisation within these relatively old lateritic profiles.

*Geochemical mobility of gold*

Over the past 50 years, many authors have presented evidence based on experiments conducted under laboratory conditions, to try to explain the movement of gold within a weathering profile. It seems likely that three sets of chemical species can be considered important, each having a domain dependent upon depth, pH and Eh (redox potential), where the individual species concerned can be considered as the primary species responsible for gold mobility.

1. organic acids and inorganic components generated through the degradation of organic material within the surface layers of the weathering profile,
2. free thiosulphate created through the weathering of mineral sulphides, and
3. halogen species, in particular the $Cl^-$ ion, derived from the movement of often very saline waters through a weathering profile.

## 1. Degradation of organic material

### Humic acids

Many authors have implicated humic acids in the supergene movement of gold (Baker, 1973; Baker, 1978; Mann, 1984). Wood (1995) goes further to attribute the potential mobility of gold as well as Pt, Pd, U, V, to individual organic acid components of the humic macromolecule. However, very little work has been carried out to determine directly the extractability of gold from an ore substrate by either humic acid or its more simple acid components. Baker (1986) described a limited study on the effect of 500 mg/L humic substance solutions, showing significant levels of gold in solution after a 50-day extraction relative to $H_20$ equilibrated with atmospheric $CO_2$. Baker also reported the extractability of a range of other metals of economic importance from the mineral phase by both humic acids and the more simple organic constituent compounds. However, he did not report the extractability of gold by these organic acids. There is no report in the literature of physical evidence for the plant uptake of gold-humic or gold-organic acid compounds. This is somewhat surprising, given the implication of these compounds in the biogeochemical pathway of gold, and represents a fruitful area for future work.

### Cyanogenic species

Cyanide complexes have attracted much attention as solubilising agents for gold, due to the natural presence of these compounds in the environment. Natural cyanides exist due to the hydrolysis of cyanogenetic glycosides (Lakin *et al.* 1974), and thiocyanates can be created through similar biochemical pathways. The dissolution of gold by cyanide and thiocyanate can be written as:

$$4Au + 8CN^- + O_2 + 2H_2O = 4[Au(CN)_2]^- + 4OH^-$$

$$2Au + 8[CNS]^- + O_2 + 2H_2O = 2[Au(CNS)_4]^- + 4OH^-$$

Boyle (1979) reported the various gold cyanide and thiocyanate complexes to be stable in mildly acid, neutral and alkaline conditions. This may be true for the gold cyanide

complex, but this present study shows that more acidic conditions may be necessary to form stable, gold thiocyanate complexes.

## 2. Genesis of free thiosulphate

Goldharber (1983) stated that metastable[12] sulphur oxyanions accumulate as intermediates in the pathway of pyrite oxidation over the pH range 6 to 9:

$$FeS_2 \text{ (pyrite)} + 1.5O_2 \rightarrow Fe^{2+} + S_2O_3^{2-}$$

One would assume that other mineral sulphides, such as chalcopyrite, would also release sulphur oxyanions under similar conditions. Goldharber goes further to state that these metastable species show a systematic pH dependence, with a more oxidised assemblage detected at lower pH.

The transient nature of the $S_2O_3^{2-}$ species under weakly acidic conditions was shown by Lyons and Nickless (1968), who claimed that thiosulphate was readily oxidised to tetrathionate by weak oxidising agents:

$$2S_2O_3^{2-} + 2H^+ + \tfrac{1}{2}O_2 \rightarrow S_4O_6^{2-} + H_2O$$

Under more strongly acid conditions, Davis (1958) observed disproportionation to elemental sulphur and bisulphite:

$$S_2O_3^{2-} + H^+ \rightarrow HSO_3^- + S^o$$

Goldharber inferred that the thiosulphate species was metastable under alkaline conditions as an intermediate in the sulphur oxidation pathway, along which further oxidation was somewhat arrested. However, Rolla and Chakrabarti (1982) showed that thiosulphate is eventually oxidised to sulphate by dissolved oxygen in an alkaline medium:

[12] Metastable ions are unstable on thermodynamic grounds but may exist in solution for long periods of time due to their kinetic inertness.

$$S_2O_3^{2-} + 2O_2 + H_2O \rightarrow 2SO_4^{2-} + 2H^+$$

This oxidative pathway is characterised by an induction period, the duration of which increases with increasing pH.

Given the above equations, one would assume that gold mobility as a $S_2O_3^{2-}$ complex would be unlikely. This is due to the highly acid nature of supergene weathering fluids derived from the oxidation of ferrous iron (ferrolysis) within a lateritic profile (Webster, 1986). Instead we would expect the acid conditions to favour mobility in the form of $Cl^-$ ion complexes, and/or humic acid complexes.

However, if during laterisation sufficient carbonate is present, then the weathering pyrite will be buffered, leading to the release of thiosulphate in a neutral to moderately alkaline environment:

$$2Au + 4S_2O_3^{2-} + \tfrac{1}{2}O_2 + 2H^+ \rightarrow 2Au(S_2O_3)_2^{3-} + H_2O$$

Mann (1984) calculated that 400 - 800 g of $CaCO_3$ would be sufficient for every 240g $FeS_2$ to maintain a pH at which $S_2O_3^{2-}$ would remain in solution.

This then, could justify the existence of supergene thiosulphate within a lateritic profile. Lintern *et al.*, (1996) illustrated the relationship in gold values between the soil substrate and overlying vegetation for three goldfields in southern Western Australia which are characterised as having high levels of pedogenic carbonate.

### 3. Chloride ion complexes

Gold chloride complexes are important in specific environments where the geochemical conditions are acidic (pH<4.0), very saline (Cl>35 000 mg/L[13]) and highly oxidising (Eh>0.8 V):

$$2Au + 4Cl^- + \tfrac{1}{2}O_2 + 2H^+ \rightarrow 2AuCl_2^- + H_2O$$

[13] This is approximately twice the salinity of seawater.

Under these conditions, Gray *et al.* (1992) theorised that gold concentrations in solution could reach 200 μg/L (ppb). Such high values have never been observed in nature. These conditions are, however, realistic for arid environments such as much of Western Australia, but are not associated with tropical, humid environments undergoing laterisation.

*The biogeochemical pathway of gold*

Since the turn of the 20th century, there have been many reports of gold accumulation by plants, in particular trees (Lungwitz, 1900). These reports have, however, often been discredited over time. Reliable evidence has come to light in the later half of the century, in particular due to the development of biogeochemical prospecting, an exploration technology that uses gold levels in plants to delineate underlying auriferous mineralisation. Work conducted in Canada showed that common conifers could accumulate up to 20 μg/kg gold dry weight over such mineralisation (Warren and Delavault, 1950). Two reviews during the 1980s have listed over 150 references on this subject (Erdman and Olson, 1985; Brooks, 1992). In a more recent review, Dunn (1995) reported a background level of gold in plants of only 0.2 μg/kg dry weight, although values up to 100 μg/kg could possibly be believed.

Shacklette *et al.* (1970) carried out a series of experiments in which they grew 2 species of *Impatiens* hydroponically in gold solutions prepared with several anionic species (Table 11.1). Included among these species were the thioligands thiosulphate and thiocyanate. This experiment showed that rooted plants and cuttings with their roots excised, could take up gold from solution. Clearly plants, or maybe only certain plants, can and will take up gold once the metal is in solution.

The key point that limits plant uptake of gold is the limited solubility of this metal in solution. All relevant literature that describes the geochemical mobility of gold stresses this point. There are no known hyperaccumulators or gold and hence plant uptake is an induced phenomenon. My research to replicate and increase the naturally apparent levels of induced uptake necessary to effect phytoextraction, has focussed on the same chelates and ligands that perform this function in nature.

**Table 11.1.** Gold concentrations (mg/kg dry weight) in *Impatiens balsamina* and *I.holstii* immersed for 48 hours in gold solutions of different anionic composition.

| Gold salt | conc. (mg/L) | PH | Rooted *I.balsamina* | Non-rooted *I.balsamina* | Rooted *I.holstii* | Non-rooted *I.holstii* |
|---|---|---|---|---|---|---|
| Cyanide | 29 | 11.0 | nd | 32 | 160 | 320 |
| | | 7.7 | nd | 32 | 1.7 | 4.2 |
| | | 6.5 | 78 | 260 | 180 | 130 |
| Chloride | 5.7 | 6.2 | 7.5 | 7.5 | 1.0 | 7 |
| Bromide | 29 | 6.2 | 39 | 160 | 28 | 55 |
| Iodide | 29 | 6.0 | 2.7 | 45 | 64 | 33 |
| Thiosulphate | 0.1 | 6.2 | 0.9 | <0.4 | <1 | <0.8 |
| Thiocyanate | 2.2 | 6.2 | 3.3 | 28 | 1.4 | 6.6 |

Source: Shacklette *et al.*, (1970). nd signifies no reported data.

## 11.2 Analytical methodology

Gold is generally present in geological samples at low concentration and hence graphite furnace atomic absorption spectroscopy must be employed (GFAAS). Interference of the absorption signal by iron is a common problem. Clarity of the signal is enhanced through selective extraction of a gold chloride complex into an organic phase. The solvent of choice for this research was methylisobutylketone (MIBK), after Brooks and Naidu (1985). At low molality hydrochloric acid (ca. 2M), log $K_d$ for gold in the organic phase is ca. 6, while iron is only very slightly adsorbed from the aqueous phase (Kraus and Nelson, 1956). Cook (1998) established after exhaustive testing, the precision of this method using a GBC 3000 GFAAS instrument at Massey University.

*Analysis of substrate samples*

**Total gold**

The bulk geochemistry of an auriferous substrate dictates the method that must be employed for the sample-digestion procedure. The method of choice for this research was digestion in *aqua regia,* giving a pseudo-total gold value. For some samples, however, this procedure would not remove gold from the substrate and an additional step of hydrofluoric acid (HF) digestion needed to be added.

Samples that are oxidising, or samples that have completely oxidised are amenable to the psuedo-total, *aqua regia*, digestion procedure. Samples that are reduced are not amenable, as unweathered sulphide minerals within a silicate lattice may occlude the gold. This lattice needs to be destroyed with HF before the gold can be dissolved by *aqua regia.* Only one sample of this study, ore material from the Macraes Gold Mine in central Otago, New Zealand, needed to be treated in this way.

Subsamples of sieved or crushed bulk sample were digested in the relevant acid mixture through heating on a hotplate to almost dryness. The residual liquid was subsequently diluted with hydrochloric acid (2M), filtered and adjusted to a final volume with deionised water. Aliquots were then extracted with MIBK and the organic phase analysed by GFAAS[14].

**Extractable gold**

The key variable for successful plant uptake of gold is dissolution of the metal within the soil solution. As a potential screening tool to assess the suitability of a substrate for induced solubility, a thiocyanate extraction system has been developed as part of this research. Thiocyanate was chosen due to the apparent efficacy of this ligand to induce the uptake of gold (Shacklette *et al.*, 1970).

Crushed or sieved auriferous substrate (2g) was weighed into a 50 mL polypropylene centrifuge tube. Ammonium thiocyanate solution (20 mL of 0.2 g/L) was added and the tube rotated in an end-over-end shaker for approximately 20 hours. The solution was subsequently filtered and analysed by GFAAS. For some samples, the gold concentration in solution was high and clean enough to allow direct analysis of the aqueous phase. Where this was carried out, the results of the respective MIBK organic phase agreed within 5%. An equal volume of hydrochloric acid (2M) was used to acidify the filtrate before extraction with MIBK.

A similar extraction is also possible using thiosulphate solution. An inherent problem with the use of thiosulphate is precipitation of elemental sulphur following acidification

[14] The GFAAS operating parameters for gold analysis are presented in Appendix 1.

of the solution[15]. Native sulphur will subsequently occlude any gold in solution, leading to low or no recovery of metal in the final MIBK analyte. Where the aqueous concentration of gold is high, this is not a problem as a 0.2% solution of ammonium thiosulphate gives a clear GFAAS gold signal. However, it must be noted that only ammonium thiosulphate can be used for direct aqueous analysis. The sodium salt of this ligand gives a large interference pattern. Where the gold concentration of the extractant solution is low, and thus not detectable in the aqueous phase, sulphur must be removed before analysis may proceed. The following experimental procedure was thus developed:

**1** 10:1 solution to substrate extraction ratio overnight in an end-over-end shaker,
**2** filter solution through Whatman No 44 filter paper into a 100 mL glass flask,
**3** add 5 mL concentrated $HNO_3$ to precipitate native sulphur,
**4** heat the solution on a hotplate. As the liquid boils the precipitated sulphur will form a small globule,
**5** add dropwise sufficient liquid bromine to destroy the elemental sulphur,
**6** evaporate the solution to near dryness. Care must be taken not to plate any gold on to the glass flask through heating to total dryness,
**7** digest the residue in 10 mL *aqua regia* to a final volume of ca. 2 mL,
**8** dilute the acid to 10 mL with distilled water,
**9** extract the final aqueous phase into MIBK (2 mL) and analyse by GFAAS.

This method is time consuming and a value for the percentage recovery of gold into the organic phase was not determined. However, the method has still been used as part of this research project to estimate the thiosulphate-extractable gold concentration of ore material from the Macraes mine in central Otago.

*Analysis of plant samples*

Again the method of Brooks and Naidu (1985) was employed. Dried subsamples of plant material were ashed at 550°C overnight and the ash digested in *aqua regia* to near

[15] Gold will only extract into MIBK as an acidic chloro complex.

dryness. The concentrated solution was diluted to 10 mL with hydrochloric acid (2M) and extracted into MIBK before analysis by GFAAS.

A single *Brassica juncea* plant induced to accumulate gold from an artificial ore substrate (see section 10.3) was finely ground and mixed with gold-free *Brassica juncea* material to provide a test herbage-standard. Subsamples of this standard were analysed to examine alternative methods for the dissolution of gold from dry plant material (Table 11.2).

**Table 11.2.** Digestion methodology test for replicate (3) subsamples of an adopted herbage standard. Mean gold concentrations with the same letter are not significantly different (ANOVA $p>0.05$).

| Method | Mean gold concentration (µg/kg dry weight) |
|---|---|
| 1. aqua regia digestion of dry material | 717 (a) |
| 2. 6 hrs at 550°C, aqua regia ash | 639 (a,b) |
| 3. 6 hrs at 550°C, 2M HCl to hot ash | 629 (a,b) |
| 4. 20 hrs at 550°C, aqua regia ash | 529 (b) |

This comparison shows that missing the ashing step gives a higher concentration of gold in the plant. This is in agreement with the often-quoted theory that some gold may be lost on ashing (Hall *et al.*, 1991). The observation that ashing for 20 hours results in less gold in the plant than does ashing for 6 hours, further supports this theory. The most important observation is that digestion of the plant ash in *aqua regia* is not necessary. The quickest, cleanest and most accurate method appears to be ashing for a short period of time, followed by dissolution of the ash in hydrochloric acid (2M). Unfortunately, this experiment was conducted at the end of the section on gold work, and thus all analytical results in this thesis are based on a relatively long ashing time (ca. 20 hrs), followed by digestion in *aqua regia*. Further proving of this observation, for a range of plant species, will be the focus of future research.

*Contamination*

Contamination of laboratory equipment and analytical samples is a problem with geochemical analyses. Fortunately, the levels of gold in plants and soils of this study were above natural background. All glassware and non-disposable plasticware was

washed in dilute *aqua regia* and dried thoroughly before each new analysis, to minimise chances of sample carry-over between analyses. Blank samples were carried through with every analytical procedure.

## 11.3 Induced uptake of gold

*Initial discovery*

The initial discovery (October 1997) that plants could be induced to accumulate gold was serendipitous. Replicates of *Brassica juncea* seedlings had been transferred into a mixture of sieved Waihi gold ore material[16]. The aim of this experiment was to induce the uptake of the metals Cd, Pb and Zn from this particular substrate, one of few at my disposal at that time with sufficiently high levels of these metals to be of interest. Results from the common chelating agent EDTA were as predicted; induced accumulation of lead, but little change in the uptake of cadmium and zinc. However, in this particular experiment, I also decided to test the efficacy of ammonium thiocyanate and sodium thiosulphate as chelating agents to induce the uptake of heavy metals. The results for Cd, Pb and Zn were disappointing. There was no increase in the uptake of these metals by *Brassica juncea* through the addition of these chemicals. In an attempt to salvage something from this experiment, I decided to analyse the plants for gold, the theory being that this was an auriferous substrate and that thiocompounds were known to dissolve gold in geological terrains. The work of Shacklette *et al.*, (1970) had shown that plants would take up gold thiocomplexes from solution.

Subsamples of plant material were prepared for analysis and the gold concentrations in *Brassica juncea* determined (Table 11.3).

[16] Wahi material was sieved to 4 grain-size fractions. Experimental substrate was a 1:1 x,y mixture of $x<300$ μm and 710 μm$<y<$1000 μm. Gold concentration of this mixture was 3 mg/kg, pH = 4.8.

**Table 11.3**. Induced uptake of gold by *Brassica juncea* growing on Waihi gold ore. Concentrations are mean and (range).

| Chemical used | Treatment level | Replicates | Mean Au in plant (µg/kg) |
|---|---|---|---|
| Thiosulphate[1] | 0.5 g/kg | 4 | 20 ( 1.3 – 55.9) |
| Thiosulphate[1] | 1.0 g/kg | 4 | 405 ( 100 – 860) |
| Thiocyanate[2] | 0.5 g/kg | 4 | 10 800 ( 900 – 19 300) |
| Thiocyanate[2] | 1.0 g/kg | 4 | 4 900 ( 2 200 - 9 300) |
| Water | Control | 4 | 5.1 ( < 0.3 – 17.4) |

Notes. 1 - after a treatment period of 10 days plants were alive and healthy.
2 – after 5 days, plants at 1.0 g/kg treatment were dead, and after 10 days at 0.5 g/kg.

Both thiosulphate and thiocyanate induced uptake of gold in this experiment. The increase in uptake due to the solubilising effect of thiocyanate was by a factor of over 2000. In fact, the levels of gold in these samples contaminated the graphite furnace to such an extent that gold analyses could not be performed in the following month. Over this time, gold was gradually flushed out of the instrument.

The higher values of gold reported in Table 11.3 for the lower applied amount of thiocyanate could be explained through the more rapid rate of death of the plants treated with 1 g/kg thiocyanate. It seems likely that the longer the plant is alive, the higher the final achievable concentration of gold in the plant.

## Hyperaccumulation of gold

As the commonly accepted criterion for hyperaccumulation of a metal is accumulation by two orders of magnitude higher than in normal plants (Brooks *et al.*, 1977a), Anderson *et al.* (1998) defined hyperaccumulation of gold, as a result of this experiment, as accumulation above 1 mg/kg dry weight.

### *Subsequent work*

Attempts to replicate these initial results for thiocyanate-induced uptake met with varying degrees of success. The fact that it was possible to induce uptake of gold using thiocyanate was proved for a number of plant/substrate combinations (Table 11.4). However, using fresh Waihi ore, I was unable to reproduce the very high uptake results

achieved in the initial experiment (20 mg/kg). No experiments conducted immediately after the initial discovery could successfully replicate the conditions under which significant gold uptake was induced using thiosulphate. A summary outlining the experiments conducted for which no significant concentration of gold was detected in the plant is presented in Appendix 3.

**Table 11.4.** Selected summary of thiocyanate ($SCN^-$)-induced uptake of gold for various plant/substrate combinations.

| Species | Substrate | Treatment (g/kg SCN) | Replicates | Au uptake (µg/kg) |
|---|---|---|---|---|
| Chicory | Tui tailings (0.5 mg/kg Au) | 0.64 | 5 | 70-1 190 |
| *Impatiens* sp. | Waihi ore (3 mg/kg Au) | 0.20 | 1 | 3 090 |
| *Arrhenatherum elatius* | Waihi ore (3 mg/kg Au) | 0.50 | 4 | 70-1 430 |

Notes: 1 Tui talings were unlimed. 2 Waihi ore was from the original batch used for Table 11.3.

## Discussion

The initial discovery of gold uptake was made for *Brassica juncea* plants growing in gold ore collected from the Waihi mine in December 1996. Subsequent experiments (Appendix 3) used Waihi ore collected late in 1997. This second batch of material was from a different part of the ore body, with a lower total gold concentration (1 mg/kg) and had a higher pH than the original material (5.1 cf. 4.8). Residual Waihi 1996 material was mixed with the Waihi 1997 ore.

As my understanding of the possible mechanisms behind induced-gold uptake developed, I began to envisage possible explanations for the lack of uptake apparent for each experiment. To examine the possible affect of pH on uptake, a more detailed experiment was thus designed.

Waihi gold ore from the 1997 batch was mixed with lime at the following rates, the pH of the resultant admixture is given in brackets: 0 % (5.1), 0.5% (6.4), 1.0% (7.3), 1.5% (7.3), 2.0% (7.3). Three replicates for each lime rate were subsequently treated with either thiocyanate (1 g/kg), thiosulphate (2 g/kg), a mixture of these two rates of thiocyanate and thiosulphate, or water as a control. Aerial portions of these plants were

harvested after 10 days, ashed and analysed by GFAAS. Where it was possible to recover and wash root samples, root samples were also analysed for gold. Thiosulphate and thiocyanate extractions were carried out on subsamples for each lime rate to estimate the thiosulphate and thiocyanate-induced gold concentration of each substrate. The results are summarised in Appendix 4, but again showed very poor uptake for all treatment and lime rate combinations. Mean uptake for the aerial portions was never above 100 μg/kg, although some of the root analyses showed evidence for hyperaccumulation (greater than 1 000 μg/kg). However, the poor replication for root results suggested contamination from the auriferous substrate in which they were grown. It is important to note for the discussion in section 11.4 that the thiocyanate-extractable gold concentration of the Waihi 1997 material was low (<30 μg/kg).

This series of experiments failed time and again to replicate the results from the original serendipitous discovery. The question was 'why?'

*Artificial gold experiment*

To overcome the problem of the non-homogeneous distribution of gold in both ore and mine wastes, as well as metal toxicity and pH conditions that inhibit plant growth, an artificial, finely disseminated, gold ore was created. The plant chosen for use in this medium was *Brassica juncea.* The initial Waihi experiment had established that this plant could be induced to accumulate gold with thiocyanate. To make the artificial ore, gold chloride solution (100 mL of 50 mg/L) was slowly dripped into 2 kg of finely sieved (<200 μm) silica sand. This sand was subsequently heated to 100°C overnight to evaporate the liquid leaving a finely disseminated 50 mg/kg colloidal 'gold ore.' Further dilution with sand produced a final 5 mg/kg gold ore (pH 7.0).

*Brassica juncea* was grown in pots (250 mL) containing this artificial ore. After approximately 3 weeks of growth, the plants were treated with ammonium thiocyanate solution at application levels of 0, 0.08, 0.16, 0.32, and 0.62 g/kg dry substrate weight (n=12). Seven days after application, the aerial portion of each plant was harvested and analysed by standard methodology. The seven-day treatment period was chosen arbitrarily based on data from the Waihi experiment. Figure 11.1 is a plot of gold

content of the plant (mg/kg dry weight) as a function of the treatment level. The data show induced accumulation of gold for all thiocyanate treatment levels. The highest individual value obtained was 57.3 mg/kg gold. However, the values overall were very variable, showing perhaps non-homogeneous gold distribution even in this artificial medium, or necrosis of the plants due to the higher amendment levels. This second factor could have led to differentials in the time for which replicates remained viable.

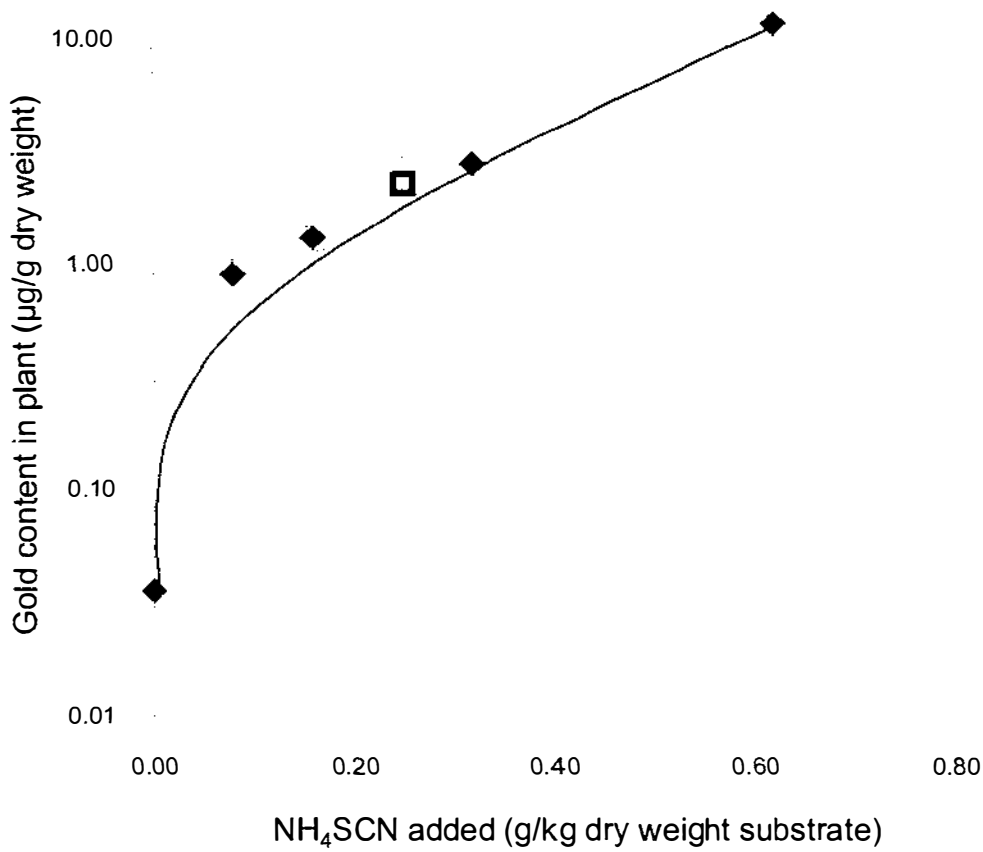


**Figure 11.1.** Thiocyanate-induced uptake of gold by *Brassica juncea* (Indian mustard) from finely disseminated (diamond) and native (open square) 5 mg/kg synthetic gold ores (mean±SE).

To test that the treatment was not dissolving gold salts residual from the ore genesis, finely powdered gold (44 µm) was mixed with sand to create a medium containing 5 mg/kg elemental gold (pH 7.0). *Brassica juncea* was grown in this 'ore' before treatment with ammonium thiocyanate at an application rate of 0.25 g/kg (n=8). The mean uptake value for this native gold experiment agreed very well with the colloidal gold experiment (Fig. 11.1), hence the action of the chemical amendment was to

solubilise the metallic gold present. The results from this artificial ore experiment were also in agreement with results from the original Waihi experiment.

I was thus able to prove the induced hyperaccumulation of gold observed for *Brassica juncea* growing on the original Waihi (1996) substrate. The following section explains why I believe induced uptake was possible for some substrate/treatment combinations, but not others.

## 11.4 Model for induced uptake of gold

The key variable necessary for the successful induced uptake of gold by plants is formation of a stable, soluble, gold-ligand complex within the soil solution. This variable can be described as induced solubility. If such a complex is formed, it seems likely that a plant will have little choice but to act as a pump and accumulate the metal during transpiration. Indeed this appears to be the principle behind the technology of induced hyperaccumulation.

Gold will only form stable and soluble complexes for a narrow range of pH and redox (Eh) parameters: parameters that change as a function of the specific ligand employed. I believe that it is possible to predict the potential suitability of a substrate to induced solubility through a simple extraction.

*Thiocyanate-induced solubility*

Within an ore or tailings body, several different geochemical conditions may be apparent. A good example of this is the Tui Mine tailings (Chapter 2 - Fig. 2.4). Morrell (1998) studied the revegetation potential of this tailings area, and thus collected substrate samples for greenhouse trials (designated JWM tailings). Samples used for the present research were collected in December 1997 (designated 12/97 tailings). The upper zone of the Tui tailings (0-50 cm) is an oxidised/oxidising material, sandy in texture and yellow in colour. Below this zone is unoxidised or anoxic material, with a clay texture and dark-blue colour. The tailings storage facility (TSF) has a reasonably homogeneous gold concentration, however, thiocyanate-extractable gold concentrations,

determined through extraction with thiocyanate solution, varied considerably (Table 11.5).

**Table 11.5**. Comparison of Tui tailings samples collected from three locations within the TSF illustrating the broad range of SCN-extractable gold values that can be observed.

| Tailings source | Total gold (µg/kg) | Extractable gold (µg/kg) | pH |
|---|---|---|---|
| JWM | 459 | 115 | 3.45 |
| Anoxic 12/97 | 508 | 3.78 | 3.51 |
| Oxidising 12/97 | 434 | 11.4 | 3.35 |

The value of extractable gold for the JWM tailings is very high. This particular substrate could have very good potential for phytoextraction. Put in perspective, through comparison with substrates for which uptake trials have been conducted, the logic of this potential can be realised (Table 11.6). Tui JWM has a thiocyanate-extractable gold concentration nearly twice that of the Waihi material on which *Brassica juncea* was induced to take up almost 20 mg/kg gold (Table 10.3). The extractable gold concentration for the artificial gold ore of Figure 11.1 is also given here.

**Table 11.6**. Comparison of the total and extractable gold concentrations for Tui JWM with selected substrates on which plant trials have been conducted.

| Tailings source | Total Au (µg/kg) | Extractable Au (µg/kg) | pH |
|---|---|---|---|
| Artificial ore | 5000 | 461 | 7.0 |
| Macraes ore | 4630 | 27.7 | 8.4 |
| Tui JWM | 459 | 115 | 2.8 |
| Waihi ore | 3000 | 61.6 | 4.8 |

Unfortunately, I have not been able to find exactly where on the TSF the Tui WJM samples were collected from, as this information was never recorded. Only small samples were available and thus no plants could be grown to test the plant-uptake potential. It would be interesting as a follow-up project, to survey the surface profile of the Tui TSF, collect samples in a grid pattern and determine the thiocyanate-extractable gold concentration at each location. These data would generate a spatial model of induced solubility, and hence induced-uptake potential. In this way, the specific location of the Tui WJM substrate could be identified, and the appropriate plant trial undertaken.

**Explanation for the variability in thiocyanate induced solubility**

Thiocyanate-induced solubility is independent of the total metal concentration of a substrate. The natural auriferous material with the highest total gold concentration of Table 11.6 had the lowest concentration of extractable gold. This was gold ore from the Macraes mine, an unoxidised, high pH, sulphide ore. In contrast, the substrate with the lowest total gold concentration had the highest extractable gold concentration - Tui WJM material, oxidising tailings with low pH.

Thiocyanate can form naturally in soil solution as a result of biochemical pathways. Bowell *et al.*, (1993) implicated thiocyanate as a potential agent for the mobility of gold in a tropical rainforest weathering profile located at the Ashanti mine, Ghana. They modelled the stability fields of the gold thiocyanate complex (Fig. 11.2). These stability fields are plotted within a pH-Eh diagram and show that a stable, soluble, gold thiocyanate complex will only form under acidic and oxidising geochemical conditions. Mine tailings commonly exhibit these geochemical parameters. As sulphide rock weathers, acid is released into solution due to the oxidation of sulphide to sulphate. Hence, during this oxidation phase, the pH is low and the Eh high: geochemical conditions that would support the formation of a gold-thiocyanate complex. An Eh-pH diagram shows the optimal 'theoretical' geochemical conditions that support a given metal-ligand complex, but does not preclude the formation of this same complex outside the plotted parameters.

Figure 11.2 could explain variations in the induced solubility concentration of gold observed in Table 11.6. Tui WJM, the natural substrate with the highest concentration of extractable gold, has the lowest pH and presumably the highest Eh of the 4 substrates. Hence, this substrate shows geochemical characteristics that are most likely to support the formation of a soluble and stable gold-thiocyanate complex. In contrast, the Macraes ore with a high pH is by definition not oxidising and the Eh of this substrate is likely to be very close to zero. These geochemical conditions do not support a gold-thiocyanate complex (Fig. 11.2).

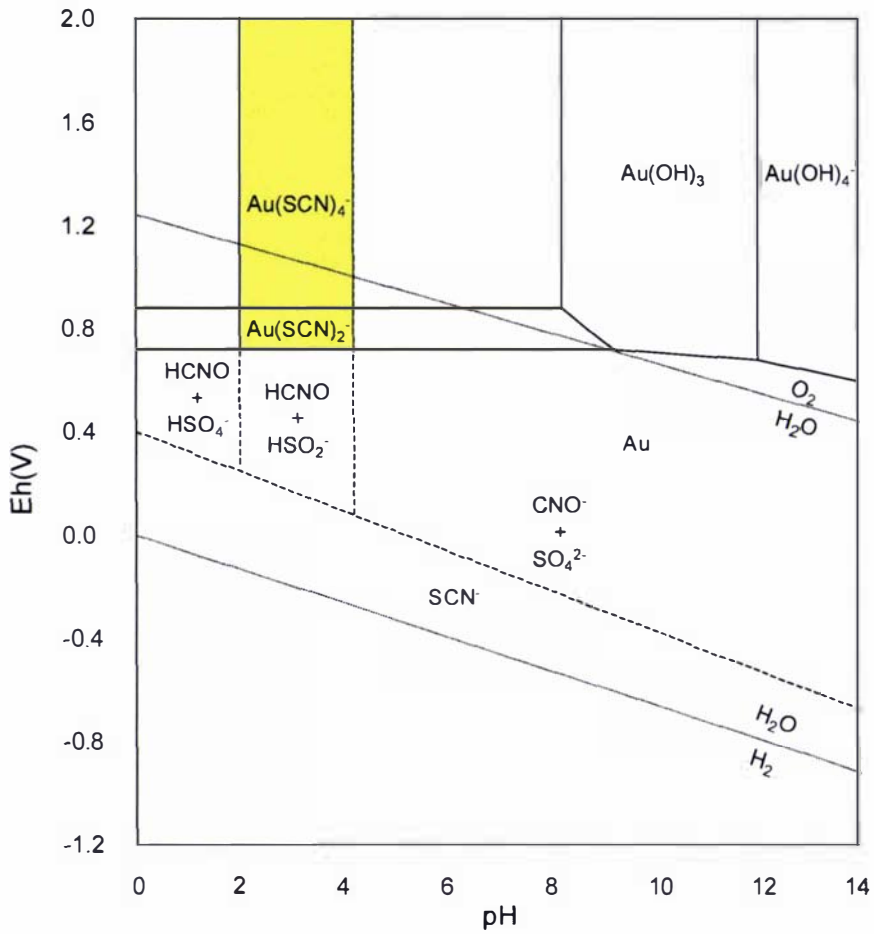


**Figure 11.2.** Eh-pH diagram of the Au-SCN-$H_2O$ system at 25°C and 1 bar pressure. The yellow-shaded area highlights the geochemical parameters under which the Au-SCN complex will be most stable. Redrawn from Bowell *et al.* (1993).

This model is not without its flaws. I am unable to explain why Tui 12/97 has such a low thiocyanate-extractable gold concentration. This sample has a low pH, but perhaps its Eh is lower than Tui JWM. Redox potential has not been measured on any of these samples.

**Limitations of the geochemical conditions that support thiocyanate induced solubility**

No plants of high biomass will grow well at low pH and thus it would not be practical to phytomine an area of auriferous material with a pH of less that 4, regardless of the thiocyanate-induced solubility concentration. The pH of the substrate would need to be raised to support plant growth.

Using mine tailings collected from the Paris gold mine near Kambalda, Western Australia, it is possible to show that there is a decrease in thiocyanate-soluble gold with increasing substrate pH (Fig. 11.3). For the Paris tailings, it appears the maximum concentration of extractable gold occurs after a small adjustment of pH. Incremental addition of lime past this optimal pH decreases the extractable gold concentration. Trials on this substrate have only just begun and research into this material does not constitute a part of this thesis. However, the aim in studying the Paris tailings is to identify a combination of geochemical conditions that will support both plant growth and the formation of a stable gold-thiocyanate complex. It is interesting to note that the Paris tailings show the greatest potential for thiocyanate-induced uptake of any natural substrate thus far discovered. The concentration of SCN-extractable gold is approximately 1 000 μg/kg. This represents 100% of the total gold (*aqua regia).*

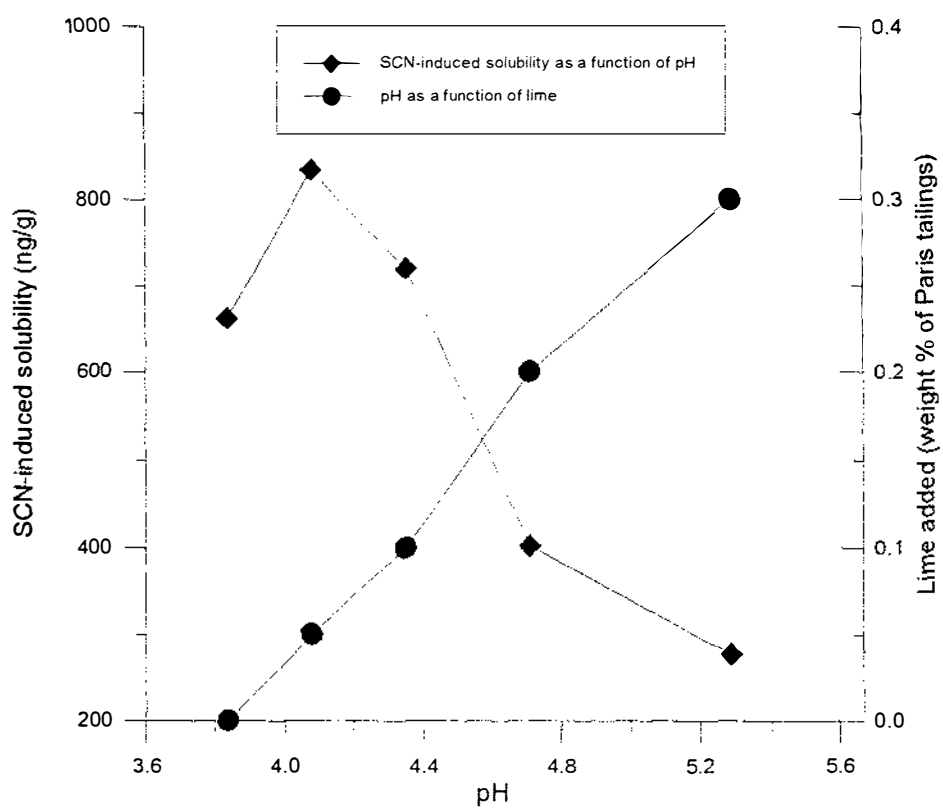


**Figure 11.3.** Effect of increasing pH, through the addition of incremental amounts of lime, on the thiocyanate-induced solubilty of gold.

A possible approach to phytomine the Paris tailings would be to apply excess lime to the substrate, bringing the pH to the optimal growth conditions of the plant species being

used. Neutralisation of this lime through the oxidative release of acid sometime after the plants were established would begin to lower the pH of the substrate. Monitoring this pH drop would establish the time at which optimal conditions of thiocyanate-induced solubility were realised and hence dictate the time at which thiocyanate was to be applied. Alternatively, once the maximum biomass of the plant was realised, acidified thiocyanate could be applied, theoretically reducing the pH at the time of gold dissolution. These theories have yet to be tested exhaustively although acidification of the substrate was the aim of applying citric acid to plants growing on Macraes gold ore (Appendix 3).

This model is likely to be an over simplification of thiocyanate-induced solubility. It certainly does not explain the high concentration of induced solubility for the artificial ore used in this research. Occlusion of gold by sulphur and competition for thiocyanate ligand binding sites are likely to be important factors for the natural substrates. The geochemical system of the artificial ore was free from these variables.

*Thiosulphate-induced solubility*

Gold ore from the Macraes mine in central Otago has a very low thiocyanate-induced gold solubility. However, the high pH of this substrate makes the ore amenable to thiosulphate-induced solubility (Fig 11.4). Bowell *et al.* (1993) modelled the stability fields of the gold thiosulphate system (Fig. 11.5). This pH, Eh diagram shows that formation of the gold-thiosulphate complex would be expected under a range of alkaline to moderately acid conditions. Figure 11.5 does not, however, preclude the formation of a gold-thiosulphate complex outside the indicated stability region.

Investigations into the use of thiosulphate to induce uptake of gold are less advanced than those for thiocyanate. Trials have only been conducted on ore from the Macraes mine, the Waihi mine and tailings from the Tui Mine (Table 11.3; Appendix 3,4). Unfortunately, the high concentration of thiosulphate-induced gold for the former substrate has not translated to plant uptake. Thiosulphate-induced solubility extractions have been carried out for limed Waihi gold ore (Appendix 4), but the concentration of soluble gold detected has proven low.

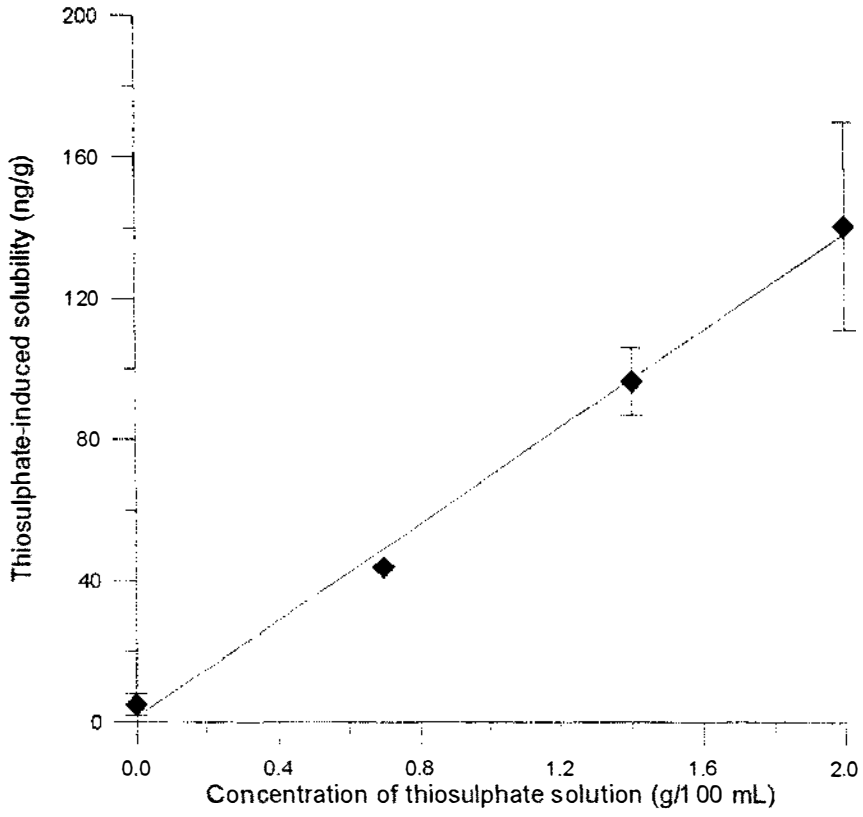


**Figure 11.4.** Thiosulphate-induced solubility of gold. Concentration of gold in solution at pH=8.4 is a linear function of the concentration of the extractant solution over this extractant range. The linear relationship can be described by the equation Y=68.4X + 1.34.

## Constraints on the uptake of a gold thiosulphate complex

Many plants exude acid from their root hairs. That is to say, they have the ability to regulate rhizospheric acidity, to a certain extent, to attain/maintain geochemical conditions that are in equilibrium with their own optimal growth parameters. At high pH, for example, trace micronutrients are relatively insoluble. If acid exuded into the rhizosphere in response to low concentrations of trace elements changed the pH by as little as one unit, then this could cause degradation of the $S_2O_3^{2-}$ ligand and the precipitation of elemental sulphur. The gold would subsequently be 'deactivated' through sulphur occlusion. Marscher (1995) shows that such a pH change is realistic for at least some species (Table 11.7).

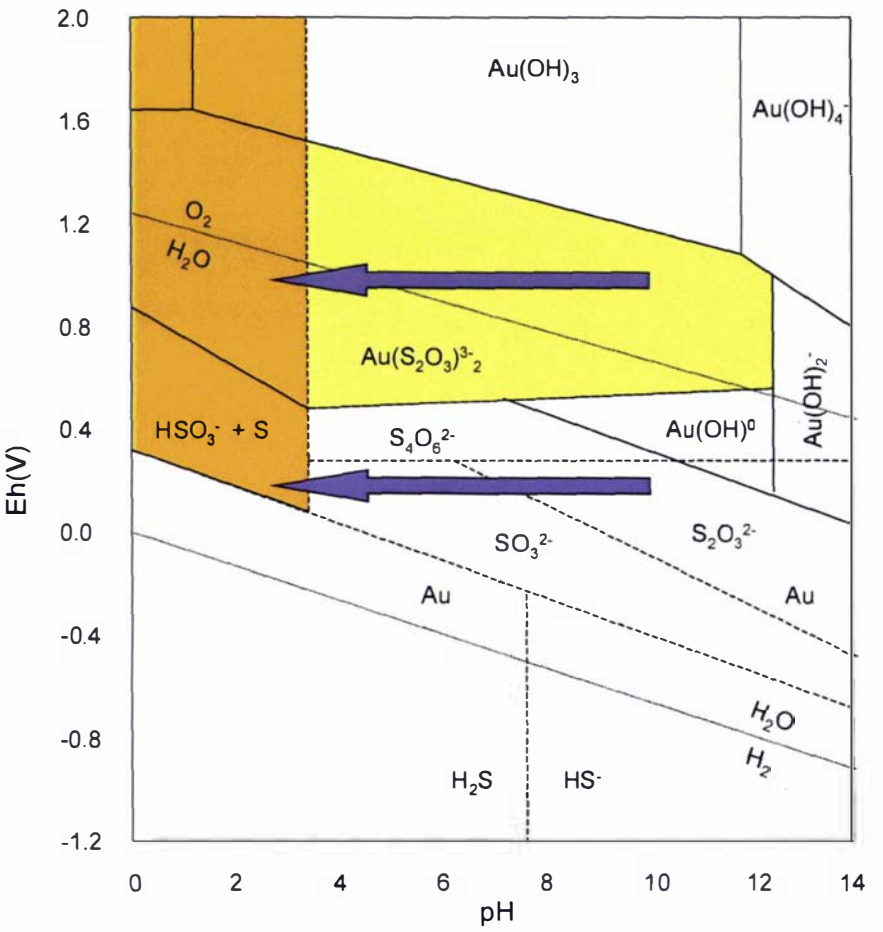


**Figure 11.5**. Eh-pH diagram of the Au-S-$H_2O$ system at 25°C and 1 bar pressure. The yellow-shaded area highlights the geochemical parameters under which the Au-$S_2O_3$ complex will be most stable. The orange-shaded area highlights the conditions under which elemental sulphur will precipitate. The blue arrows trace the degradation pathway of thiosulphate to elemental sulphur. Redrawn from Bowell *et al.* (1993).

**Table 11.7.** pH and micronutrient concentrations of bulk and rhizosphere soil of white lupin (*Lupinus albus*) grown in a phosphorus deficient soil.

| Property | Bulk soil | Rhizosphere soil |
|---|---|---|
| pH | 7.5 | 4.8 |
| Bioavailable metal μmol.kg)[1] | | |
| Iron | 34 | 251 |
| Manganese | 44 | 222 |
| Zinc | 2.8 | 16.8 |

1 –bioavailable metal concentration as measured by a DTPA extraction
Source: Marschner (1995)

Acid exudation could explain the disappointing results for experiments to date, obtained from studies of *Brassica juncea* growing on Macraes gold ore.

Application of thiosulphate solution could indeed have complexed a certain amount of gold, which then began to move through the ore substrate in the greenhouse pot. Upon coming into contact with the plant root hairs a pH drop in the local substrate environment could have been encountered, resulting in precipitation of sulphur and occlusion of gold. This theory is diagrammatically represented by the blue arrows drawn on Figure 11. 5.

For some substrates, thiosulphate would be a more suitable choice for induced gold solubility. Many auriferous substrates exist which have a high pH and an Eh near zero due to oxidation that has either not yet been initiated (Macraes ore) or has finished. Alternatively, such substrates may be relatively barren of sulphide minerals. As for thiocyanate, some degree of substrate amendment may be necessary to achieve the geochemical conditions at which optimal uptake would be expected. Lowering the pH to where plant-acid exudation does not occur, yet formation of a thiosulphate-gold complex is still facilitated, seems likely to support the optimal level of thiosulphate-induced uptake of gold. Alternatively, if a plant that does not exude acids could be identified, then this could be a possible solution. The liming rate would then be solely dependent on this plant's growth habit.

Another approach could be to satisfy a plant's nutrient requirements with soluble fertiliser, directly negating the need for acid extrusion. This may, however, create a secondary problem. An oversupply of cations in solution promotes exudation of $H^+$ ions, while an oversupply of anions in solution promotes $OH^-$ exudation (in the form of $HCO_3^-$ or dissociated organic anions). This exudation is essential to retain a charge balance within the plant (Marschner, 1995) and may again complicate the uptake of an $Au\text{-}S_2O_3$ complex.

*Application of the model to induced-uptake results from Waihi gold ore*

The induced-uptake model presented in this section can be used to explain, relatively simply, why thiocyanate-induced uptake by *Brassica juncea* growing on the initial Waihi 1996 substrate was high, but why subsequent studies on fresh Waihi 1997 material yielded low uptake values (section 11.3). The original Waihi ore was more acidic and

presumably had a higher Eh relative to the Waihi 1997 material, and hence the inherent geochemical conditions readily facilitated thiocyanate-induced solubility. This statement is supported by the concentrations of thiocyanate-soluble gold determined from each substrate, 62 μg/kg compared with <30 μg/kg for the 1996 and 1997 batches respectively. The pH was high enough in the original substrate to support plant growth, but low enough to support thiocyanate-induced gold solubility. Increasing the pH through liming the substrate in subsequent experiments probably inhibited the formation of the gold-thiocyanate complex.

Gold uptake by *Brassica juncea* from the Waihi ore was also induced using thiosulphate, but the concentration (< 1 mg/kg) was significantly lower than that for thiocyanate (Table 11.3). While Figure 11.5 shows that a gold-thiosulphate complex could be formed under the acid conditions apparent for the Waihi ore, I believe that it is only under more neutral conditions (e.g. Macraes ore) that the concentration of thiosulphate-induced gold will be maximised, such that phytoextraction of gold is effected. Hence, the induced-uptake model presented in this chapter for thiosulphate has less relevance to the Waihi gold ore relative to the thiocyanate model. It is possible that the geochemical parameters of the Waihi 1996 material promoted limited thiosulphate-induced solubility of gold and that these same parameters negated the necessity of acid exudation by *B.juncea*, thereby effecting a small degree of accumulation. The geochemical parameters apparent for the Waihi 1997 material (with and without lime) may have promoted acid exudation, and hence may have precluded gold uptake. However, the thiosulphate-induced concentration of gold extracted from the Waihi 1997 material was low (again < 30 μg/kg).

As I have pointed out, studies into the phytoextraction of gold are very young. I am, as yet, unaware of any other research group that has been able to replicate my findings. Much research remains to be conducted to develop the uptake model I have presented in this chapter, especially with regard to thiosulphate-induced gold solubility. However, I believe the geochemical approach is the correct one to follow. Induced uptake of gold will only ensue if a stable, aqueous complex is formed in the soil solution, under geochemical conditions that will promote uptake.

## 11.5 Conclusion: the choice of thiocyanate or thiosulphate

At face value the interpretation of the data presented in this chapter makes the choice appear clear. To induce the solubility and hence uptake of gold, choose thiocyanate for substrates with low pH and choose thiosulphate for substrates with high pH. However, at low pH (<3.5) plants will not grow, and at high pH it appears acid exudation will occur. In both cases plant uptake of gold will not ensue. Some degree of substrate modification will be necessary: either liming of acid substrates to promote plant growth with subsequent thiocyanate or thiosulphate treatment; or acidification to lower the pH, followed by thiocyanate or thiosulphate treatment. In all cases, further research is necessary to identify the optimum geochemical parameters for induced gold solubility and thus the optimal parameters for plant uptake. Simple extractions have the potential to screen and model large numbers of geological samples, providing data for this identification.

The geochemical model presented in this chapter needs to be further developed with future research. In particular, an important parameter has been missed from the analytical testing of each substrate, the redox potential. Data for Eh needs to be integrated into the model to further define and prove the theories I have presented. The economic potential of the phytoextraction for gold will be examined in the next chapter. However, even if the technology does not prove economically viable, the scientific principles underlying gold uptake should remain a fruitful area for future research.

# Chapter 12 - Practical Scenarios for Nickel and Gold Phytoextraction

## 12.1 Introduction

The preceding chapters of Section B have described the approach of my research into phytoextraction technology with reference to the metals nickel and gold. Phytoextraction for nickel has proved viable at the small-scale pilot stage, but extensive field trials are necessary to implement and test the viability of nickel phytoremediation and phytomining in a natural setting. The technology is less advanced for gold. To date only basic pot trials have been conducted.

Sites potentially suitable for the phytoextraction of Cd, Pb and Zn are well documented. However, sites where phytoextraction for nickel and gold may prove viable are perhaps less obvious. This chapter considers and discusses several scenarios where phytoextraction technology for nickel and gold may be practical, with particular reference to the mining industry.

## 12.2 Nickel

Over the past four years the world nickel price has dropped from approx US$7.60 a kg (1996) to a low of approx US$4.00. At the time of writing this chapter (Feb 2000), the price has recovered to over US$9.50 a kg. However, world supply is predicted to increase with the commissioning of new plants that can economically process low-grade laterite ores. There will, however, still be regions of ultramafic soil that have nickel levels too low for conventional processing. Likewise, industry-related pollution in the form of tailings, stockpiled low-grade ore, waste rock and historic nickel-salt spillages found on some mining leases, can be of concern. In such cases, soil-nickel concentrations can be toxic to the native flora and thus inhibit successful revegetation. Some of these areas could be good candidates for phytoremediation and phytomining.

*Phytoremediation scenario*

The mining industry must minimise the degradation of land through all stages of an operation, but conventional open-pit mining is and has been the only way to recover nickel economically from some ore bodies. Not only does the mining phase have a huge impact at the time, but residual tailings, waste rock, and low-grade ore deemed not suitable for processing can have a geochemistry that is different from the original surface material to which plants and fauna have adapted. In most regions of the Pacific Rim it is desirable to rehabilitate a decommissioned mine site to a similar ecological condition to that which existed before mining. Past mining activities, however, have in some cases changed the environment to the extent where it can be unrealistic to expect effective rehabilitation with native flora and fauna. It must be pointed out that during a modern operation, topsoil is stripped before mining and replaced during site rehabilitation to bury any 'foreign' material, minimising the chance of post-mining disturbance.

Hyperaccumulator plants that have evolved to grow on ultramafic soils offer an alternative to non-hyperaccumulator native flora. These hyperaccumulator plants could represent an intermediate stage in rehabilitating a mine site. Over a number of years of successive cropping, these plants could modify the soil geochemistry, lowering the level of plant-available nickel that is toxic to non-hyperaccumulator flora and improve the soil texture of an environment that is not ideally suited to indigenous plants. If the plants used are a perennial species, then regrowth from the base after harvesting would continue each year for the duration of the project. For example, trial plots of *Berkheya coddii* at Massey University are now in their 4$^{th}$ year. The success rate of subsequent, post-colonisation revegetation with the native, pre-mining flora could in this way be considerable enhanced. Any metal recovered during the phytoremediation operation could help cover rehabilitation costs.

**Example of a working operation**

A good example of where phytoremediation technology is being successfully used is in Rustenburg, South Africa, at the site of refining operations for the Anglo-American

Platinum Corporation (AMPLATS). The mining company is recovering nickel, platinum and several other metals from the Merensky Reef and the land near the refining operations has been contaminated with nickel from a number of sources.

In 1996 AMPLATS commissioned a project to investigate the feasibility of using *Berkheya coddii* to phytoremediate this area of contaminated land. The project has continued and the 1997/98 crop was harvested in March of 1998. The collected biomass was incinerated to produce a bio-ore and smelted. The crude metal was then refined and cast into small nickel ingots. In carrying out this process, the Amplats team was the first in the world to show that the metal from a hyperaccumulator crop could effectively be recovered in a relatively pure form.

A more likely scenario for such an operation would be to feed the dry biomass directly into the metal smelter. AMPLATS have done this in subsequent seasons. In this way, the resulting bio-ore was incorporated into the bulk metal ore, and the contaminating metal from outside the refinery turned into a 'valuable' product. Such an operation could be a viable management tool for the remediation of localised areas of industrial anthropogenic pollution.

Extended field trials examining the long term benefits of this scenario are still in their infancy, and to date no conclusive evidence can be offered to show that phytoremediation does permanently reduce levels of soil metal. It may seem intuitively obvious that phytoremediation will permanently reduce soil metal concentrations, but some sceptics of the technology will not be convinced until they see a site effectively remediated. Bioavailability extractions have shown a drop in the pool of plant-available zinc from experiment media effected by *Thlaspi carerulescens* (McGrath, 1998), but long-term soil monitoring of field sites is necessary to prove this aspect of pytoextraction more fully.

Models have been proposed, based upon biomass and nickel uptake data from pot and small-scale field trials, to show the time frame over which remediation results could be

expected. Table 12.1 illustrates this for the species *Berkheya coddii*, and shows the number of crops needed to reduce the nickel concentration in soil to the European Union level of 75 mg/kg that is considered safe for agriculture (McGrath and Smith, 1990). Clearly, the time frame shown in this table dictates that phytoremediation could only be a viable proposition for mildly contaminated soils. If the time frame for realistic phytoremediation is too great, then traditional land remediation techniques may represent a better option.

**Table 12.1.** Number of crops of *Berkheya coddii* required to reduce the nickel contamination in soil to the European Union guideline of 75 mg/kg.

| Initial nickel in soil (mg/kg) | Content after one year | Number of crops needed to decontaminate |
|---|---|---|
| 2 000 | 1 932 | 34 |
| 1 500 | 1 435 | 26 |
| 1 000 | 939 | 18 |
| 750 | 691 | 14 |
| 500 | 445 | 10 |
| 250 | 200 | 4 |
| 100 | 59 | 2 |

Assumptions: 1 - biomass of 22 t/ha,
2 - only half the total nickel is extractable,
3 - nickel content of the plant, and hence its extractive power, is a function of the extractable nickel content of the soil.
Source: Robinson *et al.* (1997b).

*Phytomining scenario*

Eleven nickel hyperaccumulators have been recognised in Brazil. Brooks (1998) proposed that the land of peasant farmers who attempt to grow crops of soybean on ultramafic soils of the Goiás State, would be better suited to supporting crops of nickel hyperaccumulators. Each farmer may grow only a few hectares of hyperaccumulator plants but, spread over a large area with many farmers, the total biomass could be sufficient to maintain a central incineration plant. The resultant bio-ore could then be sold to a nickel smelter. In this way the farmers would have funds to purchase foodcrops for themselves and their livestock, leading to a better standard of living than is currently the case. An analogous scenario could be envisioned in South East Asia where there are many areas of ultramafic soil. To date, 12 hyperaccumulator plants have been discovered in this region (Brooks, 1998).

A phytomining operation such as the above could be a non-destructive and environmentally friendly mining operation. Sustainable over a relatively long time frame with little capital outlay, the operation would utilise mineralised land that is not suited to current agronomic practice. More importantly, the operation would integrate peasant communities who live off these ultramafic soils with the mining process. Today's operating conditions are very much focussed on the political, social and environmental impacts of a mining operation and hence a positive public perception of the industry is of paramount importance.

*Environmental concerns*

Problems would be encountered in attempting to introduce exotic species into many parts of the world. Historical evidence shows that introduced plants can become weeds and displace indigenous species, the justification as to why in Western Australia I was unable to use *Alyssum* species and *Berkheya coddii*. Hyperaccumulator plants, however, have evolved to occupy only a very specific environmental niche where inter-species competition is minimised. Ultramafic hyperaccumulator floras are constrained to areas of elevated soil nickel, as it is only in this type of environment that hyperaccumulator species have a competitive advantage over non-hyperaccumulator floras.

Management of the plant resource would minimise the potential for negative effects on the wider environment. The use of sterile species would be a possible solution to any concerns; any seeds escaping beyond the subject area would not germinate. Alternatively harvesting the crop before the onset of flowering would also achieve the same aim (assuming seed production was the plants only means of propagation). Once a plant begins to flower there is minimal increase in biomass and hence this stage in a plant's life cycle would signal the optimal time for harvesting.

In a situation where the successful regrowth of native plant species is initially inhibited by a combination of phytotoxic soil metals levels and low nutrient status, a phytoextraction operation would result in the indigenous flora competitively superseding the exotic species once soil nickel levels began to diminish. This could effectively rehabilitate the site to a setting similar to that existing before soil nickel

levels became artificially elevated. As the metal concentration in the substrate diminishes over time, the environment would become suitable for an increasingly greater and diverse range of species.

*Sustainability of a nickel phytomining operation*

The level of nickel that can potentially be removed from a site and the sustainability of a phytomining operation, is dependent upon the geochemistry of the underlying substrate. Robinson *et al.* (1999b) used a series of sequential extractions in an attempt to monitor the changes in plant-available nickel over time, for a number of different ultramafic soils. Figure 12.1 shows the nickel yields in kg/ha of successive crops of a hyperaccumulator growing over these ultramafic soils, assuming an initial dry yield of 100 kg/ha with 50 mg nickel removed from each kg of soil. Location and geochemical details of the substrates used to construct this figure are presented in Appendix 5.

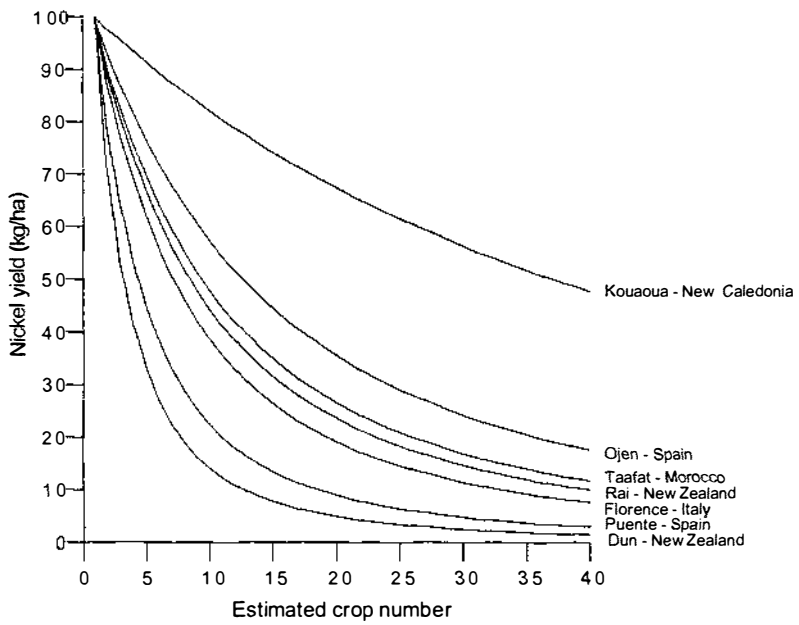


**Figure 12.1**. Nickel yields (kg/ha) of successive crops of a theoretical hyperaccumulator plant growing over various ultramafic soils. From Robinson *et al.* (2000b).

The sustainability model shown in Figure 12.1 is a simplistic representation of a natural environment, but does show that a phytomining operation could be sustainable over a reasonable time frame. The nickel yield in successive crops would be expected to drop

as the pool of plant-available nickel is diminished. However, once a minimum desirable yield is reached, ploughing to transfer nickel-rich subsoil to the surface could provide a substrate amenable to subsequent harvests. It seems likely that unless the nickel is firmly bound within the silicate lattice, an equilibrium could be envisaged, where the pool of available nickel is replenished directly from metal-rich minerals as this nickel is removed through plant uptake (Robinson *et al*, 1999b). When the metal source for this replenishment becomes exhausted, the concentration of plant-available nickel in the soil solution will drop, leaving only the silicate-bound metal that is not available for uptake.

The success of a phytomining operation is not governed by a 'timeframe' as is a phytoremediation operation. Phytomining, by definition, would be carried out on a site where the soil nickel levels pose no danger to health. In a phytomining scenario, the economic attractiveness of an operation would be proportional to its lifespan. Initially there would be a large capital outlay to establish the project if no incineration and/or ore-processing plant was available. However, if viable, subsequent years would see a profit return for little further expenditure. It is this conceptual difference that clearly distinguishes between phytoremediation and phytomining.

## 12.3 Gold

Gold is not a toxic metal, and thus the phytoextraction of gold *per se* using induced hyperaccumulation would only be a phytomining exercise.

*Phytomining scenario*

Auriferous tailings usually contain residual gold in low concentrations. As extraction technologies have advanced over the years, the concentration that remains within the tailings has decreased from several thousands to several hundreds of ppb. This residual gold from both old and new tailings could be extracted using induced hyperaccumulation if the substrate were amenable to plant growth. Phytomining could also be used to extract the gold present in low-grade ores, stockpiled due to an unfavourable combination of metal yield and world gold price.

## Economics of a crop of gold

The feasibility of a gold phytomining operation using induced hyperaccumulation has been examined, assuming a crop with a biomass of 20 t/ha per annum (Fig. 12.2). Such an operation is strongly dependent upon the world market price of this metal. At a unit cost of US$3/kg, the cost per hectare of thiocyanate applied to a depth of 15 cm at a rate 0.64 g/kg dry weight of substrate (the highest application rate of Fig 11.1) would be US$3627. At the current world price of approx US$300 an ounce, a gold concentration in the plants of around 17 mg/kg would be necessary to cover the cost of the chemical. Concentrations of this order were achieved in several experiments where *Brassica juncea* was grown both on artificial ore, and on ore material from the Waihi gold mine in New Zealand (Chapter 11).

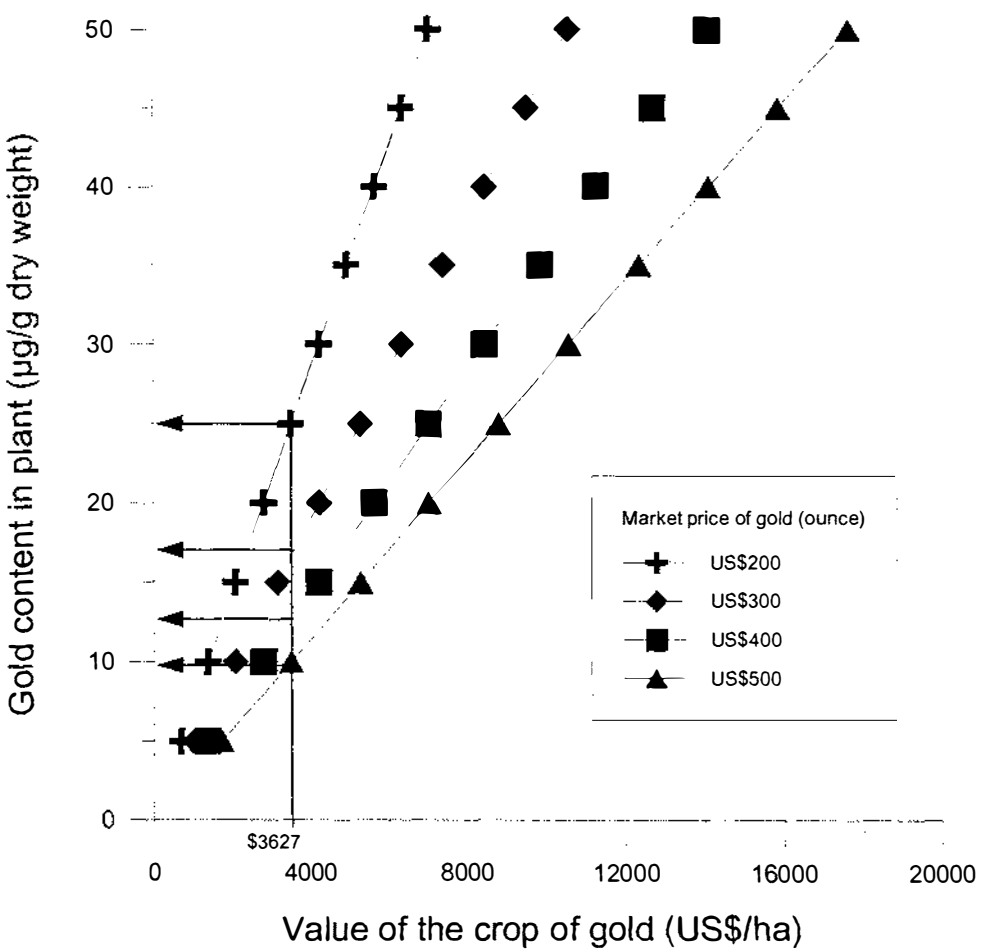


**Figure 12.2.** The possible economic value of a phytomined crop of gold as a function of the concentration in a plant with a biomass of 20 t/ha. The break-even point to recoup the cost of the reagent applied at a rate of 0.64 g/kg is shown by the line cutting the x-axis at $3627.

Figure 12.2 indicates that gold phytomining might be feasible, although induced hyperaccumulation for precious metals has yet to be tested in the field.

The choice of plant species to be used in an operation would be made according to the specific environment. It appears that most plants will take up metals that are in solution, providing the geochemical conditions are correct. The best choice would be a species, either exotic or native, that is hardy, withstanding extremes of temperature, water stress, acidity and salinity (where these factors were important), has a high biomass, and is fast growing. The area to be mined would first be amended to attain geochemical conditions that will effect optimal phytoextraction and plant growth, and then planted or seeded with the chosen species. The plants would be grown until the maximum biomass were realised. The area would subsequently be irrigated with the chosen solubilising chemical to induce hyperaccumulation. Once metal and/or chemical stress in the crop inhibits transpiration (metal uptake occurs during transpiration), the plants would be harvested, the biomass incinerated and the metal recovered.

*Environmental concerns*

When thiocyanate or other chemicals are applied to induce hyperaccumulation, other metals can also be made soluble and available for plant uptake. For some substrates thio-ligands are relatively selective for gold (Tui tailings, see Fig. 12.5). However, in other substrates these same thio-ligands are less selective (Paris tailings). It seems likely that this 'cocktail of metals', as well as any inherent toxicity of the solubilising chemical itself, is responsible for the stress apparent in plants after hyperaccumulation has been induced. It is for toxicity reasons that it would never be a viable proposition to induce the hyperaccumulation of gold using sodium cyanide. It must be stressed, however, that thiocyanates themselves, are only very slightly toxic (Van Hoek, 1995).

Table 12.2 compares the indicative toxicity of the cyanide, thiocyanate and thiosulphate salts of sodium with that of sodium chloride. It is interesting to note that sodium thiocyante is only 4 times more toxic to rats than common salt, while sodium thiosulphate has a toxicity very similar to that of salt.

**Table 12.2**. Comparative toxicity of 4 sodium salts that can solubilise gold and may be used for induced hyperaccumulation.

| Chemical | Criteria | Toxicity |
|---|---|---|
| NaCl | $LD_{50}$ oral in rats | 3000 mg/kg[1] |
| NaCN | $LD_{50}$ oral in rats | 6.4 mg/kg[1] |
| NaSCN | $LD_{50}$ oral in rats | 764 mg/kg[1] |
| $Na_2S_2O_3$ | $LD_{50}$ iv in rabbits | 4000 mg/kg[2] |

Source – 1 Merck catalogue 1999/2000, 2 – Merck Index (8th edition) 1968.

The rate and volume of chemical application would have to be carefully controlled. Excess levels of a solubilising agent could lead to the loss of metals out of the rhizosphere zone for plant-metal uptake. This would occur if the amount of chemical applied, and hence the concentration of all metals introduced into solution, was above the level of metal in soil solution that could be effectively taken up by plants. Consideration would need to be given to climatic factors such as humidity, temperature and rainfall that would dictate the evapotranspiration rate of the plant crop and the degree of dissolution of chemical in soil. All of these factors would be important in minimising the degree of residual leachate that could potentially pollute adjacent water catchments.

In the case of thiocyanate, the residence time of the chemical in the environment is relatively short, and the biodegradation pathways to ammonia, bicarbonate and sulfate have been well-studied (Hung and Pavlostathis, 1997). It is not known, however, to what extent different substrates will affect these biodegradation pathways. It has been suggested that the use of transgenic plants expressing a bacterial thiocyanate-degrading system might be possible (Anderson *et al.*, 1999). Further research needs to be conducted to ensure that the application of chemicals during an induced hyperaccumulation operation will not create secondary environmental problems.

The choice of the plant species to be used could also be of environmental concern. Unlike a natural hyperaccumulation operation, the plants used for induced hyperaccumulation do not have to be exotic species. A native plant that best suits the requisite features of high and rapid biomass production would be the ideal choice, minimising the risk of the uncontrollable colonisation of an unwanted exotic species in any one area.

*Practical Scenario 1 - gold tailings*

The Paris gold tailings of Western Australia (Fig. 12.3) have been discussed previously. Based on the results of the diagnostic tool for induced solubility described in this thesis, they offer very good potential for gold phytoextraction. 100% of the gold is soluble in a weak solution of thiocyanate. The TSF covers an area 50 by 50 metres square (one quarter of a hectare) and is 6.6m high. It would be entirely feasible to lower the elevation and expand the lateral dimensions to one hectare. The tailings on average have a gold grade of 1.88 mg/kg but also have a copper concentration of 3 600 mg/kg. This high copper concentration precludes the use of conventional technology to reprocess the material. The pH of the TSF is 3.8.

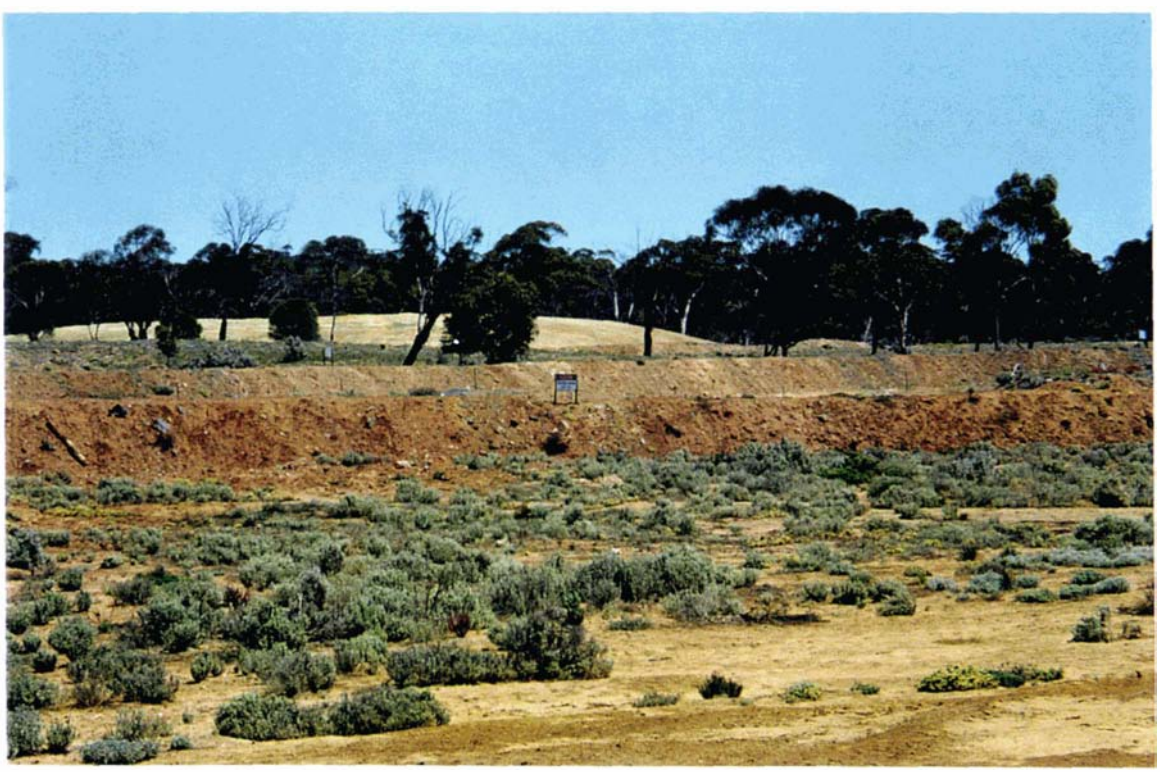

**Figure 12.3**. Mine tailngs at the now closed Paris gold mine, on the mining lease of the Western Mining Company Ltd., near Kambalda, Western Australia. The pale mound in the background of this photo is the TSF with the highest thiocyanate-extractable gold concentration discovered to date.

Some degree of substrate modification will be necessary before plants will grow in this material - the low pH is not only inherently hostile, but also promotes the leaching of phytotoxic levels of copper into solution. Trials are underway to identify the minimum

pH at which plants will grow in this material. It may be that this minimum pH will still support the formation of an Au-SCN complex but inhibit the leaching of copper. Alternatively, thiosulphate may be a better choice; at pH 7.0 the thiosulphate-extractable gold concentration is very high (F.Msuya, unpublished data). Use of thiosulphate, however, would necessitate identification of a plant that did not exude acid. Initial indications are that *Iberis intermedia* may be a suitable candidate (S.Keeling, unpublished data).

If future data suggest that geochemical parameters are attainable that support both plant growth and the formation of a stable gold-ligand complex, then a field trial should follow. The success of such a trial over a one-hectare area would offer some insight into the potential future commercial success for gold phytoextraction.

Another geographical area rich in gold tailings is South Africa. In particular, the city of Johannesburg appears to be 'built on gold tailings.' I have tested samples from one such Johannesburg tailings facility (Fig. 12.4). The thiocyanate-extractable gold is low. However the potential for thiosulphate-induced gold uptake appears good. Again, further testing is necessary, but the Rotary Club of South Africa has expressed an interest in instigating phytomining of these tailings, as a way of employing and subsequently improving the living standards of the local communities.

*Practical scenario 2 - artisanal mining*

A significant portion of the tropical rainforest of the Amazon basin has been polluted with mercury due to the mining activities of artisanal gold miners. The underlying lateritic soils are rich in low-grade gold. Artisanal mining is a low-cost mining operation, and uses very simple, informal and unregulated technology that relies on the formation of an Au-Hg amalgam to recover the metal. The process is, however, very inefficient and leads to a waste of the gold resource. The associated hazards to human and environmental health are significant due to the large amounts of mercury lost to the environment, the siltation of drainage systems and the destruction of forest and landscape. Conservative figures estimate that a total of approximately 5 million people

are involved with artisanal mining throughout Asia, Sub-Sahara Africa and South and Central America (H.Dahlberg *et al.*, pers commn. 1999).

**Figure 12.4**. The author, Robert Brooks, and a local security guard surveying gold-mine tailings in the suburb of Germinston, Johannesburg, South Africa.

The solution geochemistry of mercury is similar to that of gold. This metal can also be made soluble with thioligands: with thiocyanate at low pH and thiosulphate at higher pH. The reasoning behind this is similar to that for the formation of gold complexes.

It has been suggested that induced phytoextraction could remove both mercury and gold together from such a contaminated environment. The key target metal for the operation would be mercury. However, any gold recovered would pay some of the costs of the operation. The attraction of such a scenario is that the local communities could be involved or employed to carry out the phytoextraction process. Such an operation would be a mixture of both phytoremediation (mercury) and phytomining (gold). With the help of colleagues in the USA, Canada and Brazil this scenario may eventuate into a working trial.

*Summary - growing a crop of gold*

Figure 12.5 illustrates and summarises the complete cycle involved in growing a crop of gold, from identification of a suitable site to processing of the final ore. Each of the relevant steps of the cycle have been described in this section, with the exception of step 5, processing the bio-ore to yield the final product. Very little work on this step has been carried out to date.

A small experimental test run, based on a total mass of 100 g dry weight of *Brassica juncea* material containing an average of 7 mg/kg gold, was processed according to the cycle depicted in step 5 of Figure 12.5. This test run was filmed by a BBC film crew, and screened as a segment on the BBC show 'Tomorrow's World' in England on the 14th April 1999. On a large scale, the illustrated recovery process would never be economic. The chemical used to oxidise, and thus precipitate, the gold out of the organic phase was sodium borohydride, which is more costly than the gold recovered. However, this experiment showed that the gold in the plants was real, and could be recovered in a pure form. Future work needs to concentrate on devising an efficient and cost-effective process to recover the accumulated gold (and any other metals), from the plant material.

Overleaf:

**Figure 12.5**. Growing a Crop of Gold: summary poster describing the steps involved with the phytoextraction for gold.

# GROWING A CROP OF GOLD

Chris Anderson, Robert Brooks, Bob Stewart, Fletcher Msuya and Hutham Sabti

Soil and Earth Sciences, Institute of Natural Resources, Massey University, Palmerston North

## Step 1 - selection of a suitable site

- Many thousands of hectares of gold-bearing rock exist throughout Asia, the Americas, Africa and Australasia (Fig. 1).
- The gold content of this rock ranges from 0.1 to more than 2 g/ton, but is often unsuitable for conventional mining.

Figure 1 - mine tailings in Western Australia containing gold that may be suitable to plant uptake.

## Step 2 - growing a crop of plants

- The selected area is seeded with a suitable plant species, or seedlings planted directly on site (Fig. 2).
- A suitable plant species is one that has a high biomass, rapid growth rate and is tolerant to extremes of salinity, aridity and heavy metal toxicity.

Figure 2 - a crop of the high biomass plant *Berkheya coddii*, native to South Africa.

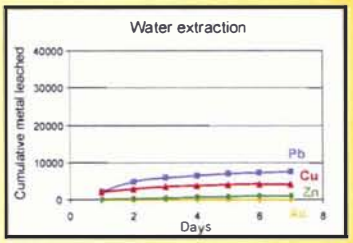


Figure 3a - column experiment where acid mine tailings was leached with water for 7 days (Cu, Zn and Pb - µg, Au - ng).

## Step 3 - induced hyperaccumulation

- Once the crop has reached its maximum biomass, a chemical is applied to the site to make the gold soluble.
- Experiments at Massey University have used ammonium thiocyanate (SCN) and ammonium thiosulphate, both of which complex gold under different conditions.
- We have shown that ammonium thiocyanate is specific in solubilising gold (Fig. 3a,b).
- There is little risk of other dangerous heavy metals being leached into the groundwater.

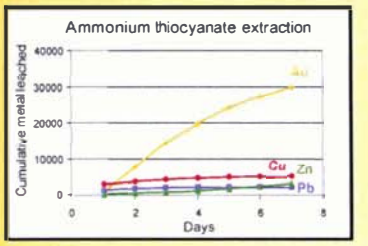


Figure 3b - column experiment where acid mine tailings was leached with SCN for 7 days (Cu, Zn and Pb - µg, Au - ng).

## Step 4 - uptake

- Once the gold is soluble, plants act as a pump and hyperaccumulate gold inside living tissues (Fig. 4).
- All organs of a plant accumulate gold - roots, shoots and leaves.

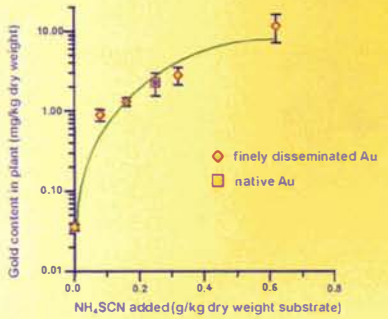


Figure 4 - induced uptake of Au by *Brassica juncea* from two types of artificial ore. After Anderson *et al*., 1998.

## Step 5 - harvesting and processing

- Eventually the crop will begin to die. This may take as little as a week, or as long as a month depending on the chemical used and concentration applied.
- At this point the biomass is collected and the gold processed (Fig. 5).

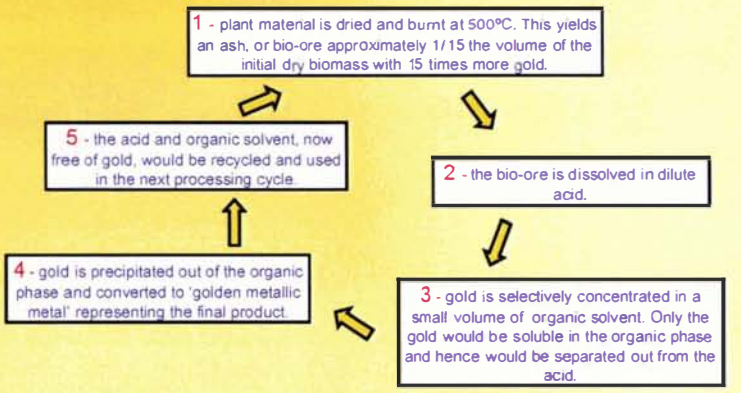


Figure 5 - a possible processing cycle for the recovery of gold from plants.

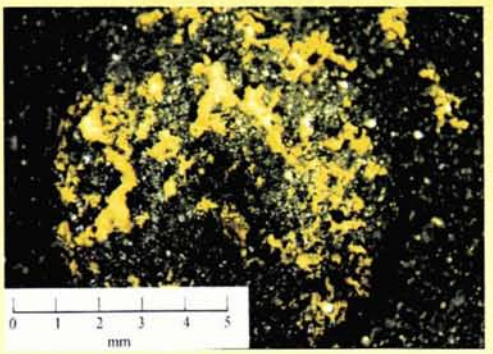


Figure 6 - the final product: gold recovered from plants.

## Step 6 - the final product

- Figure 6 shows the amount of gold processed from 100 g of dried *Brassica juncea* plant material.
- There is only 0.7 mg of gold shown here, but when considered over a larger scale like a hectare, several hundred grams of gold could be recovered.
- Assuming not all the gold initially present at the site was removed in the operation, the cycle could continue, and the phytomining process repeated.

**Reference** Anderson C W N, Brooks R R, Stewart R B and Simcock R (1998) Harvesting a crop of gold in plants. *Nature* 395: 553-554.

# SECTION C: CONCLUSION

# Chapter 13 - Practical Aspects of Phytoextraction: a General Conclusion

## 13.1 Conclusions from this research

A general theme of this thesis has been the function of geochemistry on plant uptake, and how this factor affects phytoextraction. This geochemical focus on phytoextraction has been deliberate, because it represents an important part of the technology that has been poorly studied in the past. Several general conclusions can be drawn from the findings of this thesis; conclusions based on the broad range of environments and associated metals that constituted this study.

1 *Natural uptake of metals is dependent upon geochemistry.*

To say that a hyperaccumulator of a metal will 'hyperaccumulate' that metal when grown on contaminated soil containing that metal, is incorrect. Chapter 2 illustrates why. Both *Cardaminopsis halleri* and *Thlaspi caerulescens*, known hyperaccumulators of cadmium, failed to hyperaccumulate this metal from Tui mine tailings that contained 26 mg/kg cadmium. Yet in Chapter 7, *T.caerulescens* accumulated close to 100 mg/kg cadmium from a substrate containing less than 5 mg/kg metal. The difference between the two sites was geochemical. Uptake (or lack of it) in Chapter 2 was from a sulphide metal phase in the substrate, but uptake in Chapter 7 was from metal contamination as a phosphate form.

The data presented in Section A also show that certain hyperaccumulator plants may be better suited to specific geochemical environments than others. The greatest cadmium-uptake potential from the phases used in this study was shown by *Cardaminopsis halleri* for carbonate, oxide and nitrate (organic) phase contamination, but by *Thlaspi caerulescens* from the phosphate and sulphide phases.

2 *The modelling of plant-available metal is dependent upon geochemistry.*

Modelling of the readily-soluble metal concentration in soil is a useful tool. Metal in soil is not necessarily bad, but is a danger to the health of plants and/or animals when that metal loading is bioavailable. The efficacy of ammonium acetate to model this fraction of a soil's metal loading appears to be dependent upon site-specific geochemistry. The data presented in chapters 4, 5 and 6 show that the relationship between plant-available metal and metal accumulated by a plant varies between different chemical forms of metal that may be present in the soil. Caution should be observed when an extractant is used to model plant-available metal if no consideration is given to the geochemistry of the test substrate. Under some geochemical conditions, ammonium acetate, and presumably other extractants as well, may not accurately model the plant-available fraction of soil metal.

3 *The potential for induced hyperaccumulation is large.*

Discovery that plants could be induced to accumulate gold has excited significant interest within the scientific community and especially among the general public, more so than any other aspect of phytoextraction. Yet gold uptake is nothing special. It is simply an advancement of the discovery that EDTA could be used to induce lead uptake. The key to induced uptake is again geochemistry, and that poses the question of 'why stop there?' Mercury has a similar geochemistry to gold, as do the platinum group metals. Current research is focussing on these metals. My belief is that induced uptake of any metal can be effected, if first, a suitable ligand can be found to induce solubility, and second, if rhizosphere conditions that facilitate uptake are promoted. This may be through species selection or amendment of the substrate.

4 *The limitations of phytoextraction must be realised.*

An important aspect for the practical development of phytoextraction technology is the establishment of achievable objectives. Hyperaccumulation will not be a viable answer to metal contamination in all environments. This may simply be a function of a high level of metal contamination (e.g. Auby). It may be due to environmental conditions that

preclude plant growth (some areas of Western Australia), or it may be due to site-specific geochemical conditions precluding economic levels of metal uptake (e.g. Piopio nickel trial). It must also be remembered that the presence of a given metal is not necessarily undesirable environmentally. This point is particularly pertinent to induced hyperaccumulation. Removal of lead from a site effected by EDTA treatment may be an admirable goal, but if subsequent to lead removal an underlying water table is polluted with soluble metal, or if trace nutrients are leached out of the soil, then the secondary environmental cost would be too high. Lead may be better left in the soil, or alternatively, removed using conventional technology. Care must be observed in the development of induced hyperaccumulation technology for gold. The public's perception of thiocyanate is generally bad, due in part to the similarity of the names 'cyanide' and 'thiocyanate' even though the latter is over 100 times less toxic than cyanide. The potential danger of these chemicals in the environment needs to be addressed.

5 *Problems inherent in the practical development of phytoextraction technology must be overcome.*

A key problem illustrated by this research is the limitation imposed on which hyperaccumulator species may be used in certain environments. In Western Australia there is no legal constraint on species use, so the easy option in Australia would be to ignore public opinion and use *Alyssum* species that could grow well in the Australian climate. However, in the interest of favourable public perception, mining companies stipulate that only native species may be used for revegetation. The problem of seed germination in *Hybanthus floribundus*, existent during the early stages of this research project, appears now to have been solved by my research. This potentially facilitates the use of this species for phytoextraction. In environments where no suitable native species exist, exotics will have to be used if a phytoextraction operation is to be implemented. Approaches to minimise potential environmental harm in such a scenario were presented in Chapter 10.2.

## 13.2 Future research

The practical future of phytoextraction appears encouraging, but ongoing research is necessary to ensure a smooth transition from modelled to working systems.

Implementation of laboratory results to the field environment is necessary for the 'real-life' potential of this technology to be realised.

Continuing geochemical research into the importance of mineral phase as a controlling factor of species-specific bioavailability is essential. This will allow for extractant and plant species choices that optimise modelled and physical uptake, specific for a wide range of metals and environment.

Development of induced hyperaccumulation should continue actively. There are practical scenarios where removal of metals cannot be effected using other means. The list of metals for which induced hyperaccumulation is practical should increase dramatically over the next few years. The potential for the economic recovery of precious metals using induced phytoextraction is particularly encouraging.

The search for new natural-hyperaccumulator species should continue in many parts of the world that remain unexplored. As demand for the implementation of phytoextraction technology increases, the need for native hyperaccumulator species will also increase. Much of South East Asia holds promise for new discoveries.

Better knowledge of the physiological mechanisms behind plant uptake is necessary, mechanisms both inside and outside root systems. The role of soil bacteria and fungi in hyperaccumulation remains poorly understood.

## 13.3 Concluding remarks

The potential for phytoextraction around the globe is enormous. Phytoremediation and phytomining are technologically simple processes. They are relatively inexpensive and could return large environmental and economic profits. In wealthy countries

implementation should be a matter of time. The example of cadmium contamination in New Zealand pastoral soils, described in Chapter 7, is one such environment where phytoremediation could be very close to practical implementation. To the mining industry, phytoextraction represents a potential useful tool. Various Australasian mining companies have already expressed interest in implementing the technology on various mine sites.

Benefits from phytoextraction could be far greater for less affluent parts of the globe. Many third-world countries have large expanses of land contaminated by industrial activity, and the local communities that live on, or make a living from this land, represent a large and able workforce that could benefit from the technology. If profit could be made from 'phytomined' metal, this would be advantageous. However, the key aim of nickel, gold or other precious-metal return from the land would be to create a self-sustaining operation, worked by indigenous communities, to effect improved environmental conditions and a rise of living standards. This scenario is one of both phytoremediation and phytomining.

The scope for future research into phytoextraction is as diverse as it is large. Phytoextraction is one of the areas of science that does not fall under one discipline. Botanical, physiological, agronomic and geochemical research approaches (to name but are few) are necessary to unlock the secrets of both natural and induced-metal uptake by plants.

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# Appendices

## Appendix 1a: Methods for atomic absorption spectroscopy

| Element | Lamp current (mA) | Wavelength (nm) | Slit Width (nm) |
|---|---|---|---|
| Cadmium (Cd) | 3.0 | 228.8 | 0.5 |
| Cobalt (Co) | 6.0 | 240.7 | 0.2 |
| Gold (Au) | 5.0 | 242.8 | 0.5 |
| Copper (Cu) | 3.0 | 324.7 | 0.5 |
| Lead (Pb) | 5.0 | 217.0 | 1.0 |
| Zinc (Zn) | 5.0 | 213.9 | 0.5 |

*Programme used for the determination of cadmium using GFAAS.*

For graphite furnace and phosphoric acid (1000 mg/kg) as a modifier:

| Step number | Final temp (°C) | Ramp time (s) | Hold time (s) | Gas type | Signal read |
|---|---|---|---|---|---|
| 1 | 20 | 1.0 | 0.0 | $N_2$ | |
| 2 | 95 | 20.0 | 20.0 | $N_2$ | |
| 3 | 500 | 15.0 | 10.0 | $N_2$ | |
| 4 | 1500 | 1.0 | 3.0 | None | |
| 5 | 2000 | 1.0 | 2.0 | None | Peak height |

Multiple loadings were dried to step number 2.

*Programme used for the determination of gold using GFAAS.*

For graphite furnace and MIBK extract:

| Step number | Final temp (°C) | Ramp time (s) | Hold time (s) | Gas type | Signal read |
|---|---|---|---|---|---|
| 1 | 70 | 5.0 | 2.0 | $N_2$ | |
| 2 | 120 | 6.0 | 10.0 | $N_2$ | |
| 3 | 180 | 5.0 | 3.0 | $N_2$ | |
| 4 | 300 | 5.0 | 15.0 | $N_2$ | |
| 5 | 2500 | 1.1 | 2.0 | None | Peak height |

Multiple loadings were dried to step number 2.

For graphite furnace and aqueous extraction analyte:

| Step number | Final temp (°C) | Ramp time (s) | Hold time (s) | Gas type | Signal read |
|---|---|---|---|---|---|
| 1 | 70 | 5.0 | 2.0 | $N_2$ | |
| 2 | 120 | 6.0 | 10.0 | $N_2$ | |
| 3 | 180 | 5.0 | 3.0 | $N_2$ | |
| 4 | 500 | 5.0 | 15.0 | $N_2$ | |
| 5 | 2500 | 1.1 | 2.0 | None | Peak height |

Multiple loadings were dried to step number 2.

## Appendix 1b: Physical characteristics of experimental substrates

Auby Soil

The pedological characteristics of contaminated soils of the Auby region have been described in Perdix *et al.* (1997). A summary is as follows:

| Depth | Horizon | Texture | Pb | Zn | Cd | Cu | Fe | Ca |
|---|---|---|---|---|---|---|---|---|
| 0-8 cm | A1ca | A1-A1s | 1030 | 995 | 19 | 44 | 45640 | 22100 |
| 10-25 cm | A2ca | A1-A1s | 830 | 750 | 15 | 37 | 46550 | 21900 |
| 35-45 cm | ACcag | AL | 18 | 75 | 3 | 9 | 50450 | 22650 |
| 55-80 cm | IIC2cag | L-LA | <17 | 35 | <2 | 5 | 23700 | 58800 |

Notes: 1. Metal concentrations are in mg/kg.
2. Soils described are classified as colluviosol redoxisol.
3. The characteristics summarised here are for field samples collected 1 km NE of the metal smelter. Auby soils on which field experiments for this thesis were conducted were located 100 m NE of the smelter, and hence show significantly greater contamination.

Further details specific to the Auby site for this thesis can be found in Appendix 7.

Commercial Seed Raising Mix

The commercial seed raising mix used in Chapters 4, 5 and 6 was 'Liddle Wonder' mix with the following composition:

2/3 peat w/w
1/3 pumice w/w
per $m^3$ 1 kg nutricote or osmocote 5-6 month release fertiliser
3 kg agricultural lime
2 kg dolomite
1 kg superphosphate
1$m^3$ of mixture weighs 600 kg.

Wairarapa Soil

The soil at the Wairarapa field site has been mapped and appears on the National Water and Soil Conservation Organisation - New Zealand Land Resource Inventory Worksheet number 158 (Masterton).

The specific soil present has been described by Heine (1975) as Pirinoa hill soil (Atua silt loam, hill soil; soil set number 29H), a moderately leached intergrade between yellow-grey earths and yellow-brown earths of low P and Ca nutrient status.

Further chemical characteristics of the soil may be found in Appendix 7.

Reference Heine, J.C. 1975. Interim report on soils of Wairarapa Valley, New Zealand. Restricted Soil Bureau Internal Report, N.Z. Soil Bureau Record 40.

## Appendix 2: Plant health at the time of harvest for soils contaminated with Cd, Pb and Zn (Chapters 3 – 5).

Lead experiment

1 - *Brassica juncea*

| Lead salt | Control | Acetic acid | Citric acid | EDTA |
|---|---|---|---|---|
| Control | No signs of chlorosis | Treatment caused necrosis | No change in plant health relative to control | No change in plant health relative to control |
| Carbonate | No sign of chlorosis | Treatment caused necrosis | No change in plant health relative to control | Chlorosis caused by treatment, but plants remained healthy and turgid |
| Nitrate | Very poor growth – stunted and unhealthy plants | Treatment caused necrosis | Treatment effected necrosis | Treatment effected necrosis |
| Oxide | No sign of chlorosis | Treatment caused necrosis | No change in plant health relative to control | Chlorosis caused by treatment, but plants remained healthy and turgid |
| Phosphate | No sign of chlorosis | Treatment caused necrosis | No change in plant health relative to control | Chlorosis caused by treatment, but plants remained healthy and turgid |
| Sulphate | No sign of chlorosis | Treatment caused necrosis | No change in plant health relative to control | Chlorosis caused by treatment, but plants remained healthy and turgid |
| Sulphide | No sign of chlorosis | Treatment caused necrosis | No change in plant health relative to control | Chlorosis caused by treatment, but plants remained healthy and turgid |

2. *Thlaspi caerulescens.*

Plants grew poorly in the nitrate salt. Treatment of the nitrate phase soil with acetic acid, citric acid or EDTA caused necrosis.

For all remaining mineral phases the plants grew well, with no apparent sign of chlorosis. Acetic acid treatment caused necrosis of the plants but there was no visible change in plant health through treatment with citric acid or EDTA.

Cadmium experiment

1. *Brassica juncea*

| Cadmium salt | Control | Citric acid | EDTA |
|---|---|---|---|
| Control | No sign of chlorosis | No change in plant health relative to the control | No change in plant health relative to the control |
| Carbonate | No sign of chlorosis | No change in plant health relative to the control | Treatment caused some leaf abscission and a general increase of chlorosis |
| Nitrate | Visible chlorosis of the leaves, but plants growing | No change in plant health relative to the control | Treatment caused necrosis of the plants |
| Oxide | No sign of chlorosis | No change in plant health relative to the control | Treatment caused an increase in chlorosis but no leaf abscission |
| Phosphate | Visible chlorosis of the leaves slower growth relative to the other metal phase soils | No change in plant health relative to the control | Treatment caused 50% leaf abscission and a significant general increase of chlorosis |
| Sulphate | No sign of chlorosis | No change in plant health relative to the control | No change in plant health relative to the control |

2. *Cardaminopsis halleri*

This species grew well in all the soils for this experiment prior to treatment. There were no visible signs of chlorosis on the foliage. Citric acid caused no change in plant health. EDTA caused minor chlorosis and a loss of turgid pressure for plants growing on the carbonate and oxide phase soils.

3. *Thlaspi caerulescens*

This species grew well in all the soils for this experiment prior to treatment. There were no visible signs of chlorosis on the foliage. Citric acid effected no change in plant health. EDTA caused minor chlorosis and a loss of turgid pressure for plants growing on the sulphate phase soil.

Zinc experiment

*Brassica juncea* failed to grow in any of the zinc soils of this experiment. *Cardaminopsis halleri* and *Thlaspi caerulescens* showed signs of chlorosis and stunted growth on the sulphate phase soil. EDTA treatment caused necrosis of these plants. For the remaining phases, growth was unchanged by the form of cadmium present in the soil relative to the control both before and after EDTA treatment.

## Appendix 3: Unsuccesful induced-gold uptake experiments described in Chapter 11.

| Plant species used | Substrate | Treatment | Notes |
|---|---|---|---|
| Chicory | Waihi ore, 1998 batch - 2.5% lime | SCN - 0, 0.3, 0.6, 1.0 g/kg | SCN treatment caused necrosis |
| | | $S_2O_3$ - 0, 0.3, 0.6, 1.0 g/kg | Plants remained healthy |
| | | EDTA - 0, 0.3, 0.6, 1,0 g/kg | Plants remained healthy |
| *Brassica juncea* | Macraes ore | SCN - 0, 0.2, 0.5, 0.8, 1.0 g/kg | SCN treatment caused necrosis |
| | | $S_2O_3$ - 0, 0.2, 0.5, 0.8, 1.0 g/kg | plant remained healthy |
| *Brassica juncea* | Macraes ore | SCN - 0.1 g/kg with 2g or 4g citric acid | citric acid treatement caused necrosis |
| | | $S_2O_3$ - 0.1 g/kg with 2g or 4g citric acid | citric acid treatment caused necrosis |
| *Cardaminopsis halleri* | Tui talings - 2.5 % lime | SCN - 0.25 g/kg | SCN treatment caused necrosis |
| | | $S_2O_3$ - 1 g/kg | plant remained healthy |
| | | EDTA - 2 g/kg | plant remained healthy |
| | | DTPA - 2 g/kg | plant remained healthy |

## Appendix 4: Induced-gold uptake experiment for *Brassica juncea* growing on limed Waihi ore, discussed in Chapter 11

| Lime rate (pH) | Treatment | Gold in aerial parts (µg/kg) | Gold in roots (µg/kg) | Induced solubility (µg/kg) |
|---|---|---|---|---|
| 0 (5.1) | control | 35 | | |
| | SCN | 70 | 1180 | 29 |
| | $S_2O_3$ | Nd | | 27 |
| | SCN/ $S_2O_3$ | Nd | | |
| 0.5 (6.4) | control | <5 | | |
| | SCN | 25 | | 9 |
| | $S_2O_3$ | 47 | | 14 |
| | SCN/ $S_2O_3$ | 30 | 567 | |
| 1.0 (7.3) | control | 18 | | |
| | SCN | 12 | | 34 |
| | $S_2O_3$ | 19 | | 26 |
| | SCN/ $S_2O_3$ | 45 | 324 | |
| 1.5 (7.3) | control | 16 | | |
| | SCN | 21 | | 18 |
| | $S_2O_3$ | 28 | | 13 |
| | SCN/ $S_2O_3$ | 15 | 331 | |
| 2.0 (7.3) | control | 27 | | |
| | SCN | 88 | | 16 |
| | $S_2O_3$ | 57 | 172 | 13 |
| | SCN/ $S_2O_3$ | 78 | 1460 | |

Note: nd signifies that the plants for this treatment died before application.

The assistance of Anthony Kirk is gratefully acknowledged in the preparation of this data set.

## Appendix 5: Data used to construct Figure 11.1.

| Country | Location | Principal minerals | Ni content (mg/kg) | pH |
|---|---|---|---|---|
| New Zealand | Dun Mountain | Quartz, goethite | 2244 | 6.7 |
| New Zealand | Rai Valley | Antigorite, goethite, quartz | 2109 | 7.5 |
| New Caledonia | Kouaoua (Mea Mine) | Antigorite, goethite, quartz | 17 208 | 7.2 |
| Spain | Ojén | Antigorite, quartz, olivine | 1800 | 7.8 |
| Spain | Puente Basadre | Antigorite, quartz, talc | 2100 | 6.3 |
| Italy | Firenze (Tuscany) | Antigorite, quartz | 1609 | 6.9 |
| Morocco | Taafat | Antigorite, chlorite,olivine | 1700 | 7.5 |

Source Robinson *et al.* (1999).

## Appendix 6: Plant references

| | |
|---|---|
| *Agrostis tenius* | L. |
| *Alyssum bertolonii* | Desv. |
| *Alyssum malacitanum* | T. Dudley |
| *Arrhenatherum elatius* | L. |
| *Berkheya coddii* | Roessl. |
| *Brassica juncea* | (L.) Czern. |
| *Brassica napus* | L. |
| *Cardaminopsis halleri* | (L). Hayek |
| *Cardaminopsis petrea* | L. |
| *Grevillea acuaria* | F. Muell. Ex Benth. |
| *Hybanthus epacroides* subsp. *bilobus* | Bennett |
| *Hybanthus floribundus*<br>subsp. *adpressus*<br>subsp. *curvifolius*<br>subsp. *floribundus* | (Lindl.) F. Muell.<br>Bennett<br>Bennett<br>Bennett |
| *Iberis intermedia* | Guersent |
| *Impatiens balsamina* | L. |
| *Impatiens holstii* | Engler & Warb. |
| *Lupinus albus* | L. |
| *Millota myosotidifolia* | F. Muell. |
| *Minuarta verna* | (L.) Hiern. |
| *Personia metallifera* | Wild |
| *Pimelia leptospermoides* | F. Muell. |
| *Sebertia accumuinata* | Pierre ex Baillon |
| *Senico coronatus* | (Thunb.) Harv. |
| *Silene humilis* | L. |
| *Stackhousia tryonii* | Bailey |
| *Streptanthus polygaloides* | Gray |
| *Thlaspi caerulescens* | J.C. & R.Presl. |
| *Zea mays* | L. |

**Appendix 7**: the complete summary data set for this thesis is saved on the accompanying disk, or (if the disk is not attached) can be obtained from:

Soil and Earth Sciences
Institute of Natural Resources
Massey University
Palmerston North
New Zealand