The phytoextraction of heavy metals from metalliferous soils

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Abstract

This study concerns the investigation of the potential use of plants to remove heavy metals, in particular nickel, zinc and cadmium, from soils. The study is divided into three sections, bioavailability, phytomining, and phytoremediation.

The effect of various extractants on the solubility of heavy metals in some soils was tested. It was found that the solubility of the metal invariably increased as the pH of the extractant decreased, and as the concentration of the extractant increased. These relationships could be predicted mathematically. It was concluded that a more concentrated, neutral, non-chelating extractant such as 1M ammonium acetate, is the most suitable for estimating bioavailability.

An investigation into the addition of some compounds to soils to increase metal solubility showed that EDTA and citric acid should maximise solubility while keeping soil parameters (pH, osmotic potential, nutrient availability) within the growth range of the plant. These compounds are also relatively inexpensive. The effect of bioavailable elements on a New Zealand serpentine flora was investigated with regard to the soil's potential for phytoextraction. It was found that nickel was the only element that would be suitable for phytoextraction from these soils. The relative infertility of the serpentines was attributed primarily to the toxic effects of magnesium and/or nickel. Removing nickel from the soil by phytoextraction may enable the soil to be used as a magnesium-rich fertiliser.

The potential of the hyperaccumulator plants *Alyssum bertolonii* and *Berkheya coddii* for nickel phytoextraction was studied. With the addition of fertilisers, the plants could remove respectively in excess of 72 kg and 100 kg of nickel per hectare per annum. Were the metal to be sold, and energy from the plant's incineration utilised, the net profit per hectare would be greater than that of a crop of wheat. Both of these plants re-grew after harvesting without the need to re-sow. In the case of *B. coddii*, the nickel concentration in the regrowth was more than twice that of the original crop. Phytomining for nickel is a viable proposition provided the operation is carried out over a large area. The nickel concentration in the plants was logarithmically related to the nickel extracted by a 1M ammonium acetate extract. Theoretical nickel yields from various soils could be predicted by performing a 1M ammonium acetate extraction on the soils. About half of the soils tested from various locations around the world contained enough soluble nickel to be economically phytomined. *Alyssum bertolonii* and *B. coddii* also have the potential to remove nickel from polluted soils. The addition of EDTA and citric acid to the soil in which the plants grew actually decreased the nickel concentration in the plants despite increasing the nickel solubility in the soil. The economics of phytomining are closely linked to the value of the metal. Cobalt and possibly even the noble metals could be economically phytomined at low concentrations in plants: a fertile area for future research.

Sequential extractions were used to model the effect of successive hyperaccumulator crops on the bioavailability of nickel in ultramafic soils from around the world. The nickel concentration in all of the soils tested, decreased in a regular manner and could be predicted mathematically. Assuming an initial nickel crop of 100 kg/ha, the number of nickel crops above 70 kg/ha that could be obtained was calculated for each soil. The number of economic nickel crops varied between 3 and 18 before the soil would have to have been modified to increase nickel bioavailability.

The possibility of removing zinc and cadmium using *Thlaspi caerulescens* showed that the plant has a potential use for removing cadmium from weakly polluted soils. The low bioaccumulation factor for zinc accumulation means that this element will never be removed in a reasonable time span. The concentration of both zinc and cadmium in the plant could be predicted by the extractable fraction in the soil as estimated by using 1M ammonium acetate as an extractant.

It was concluded that phytomining and phytoremediation are feasible possibilities under certain conditions. (1) Metals necessarily have to be slightly soluble in the soil before they can be phytoextracted. Metal solubility may be improved by the addition of chemicals such as chelating agents. (2) Due to relatively low yields, phytomining will only be viable for more-valuable elements where the concentrations in soils are too low for conventional mining. (3) Phytoremediation will be most effective on weakly polluted soils. (4) The economics of phytoextraction favour its use over large areas. The amount of metal able to be extracted from an area can be predicted by performing an extraction with 1M ammonium acetate.

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Chapter 1
General Introduction

Phytoextraction is the removal of metals from soil using plants. It is a novel concept that could augment existing capabilities for mining and soil decontamination. The basic concept is summarised in Fig. 1., and involves.

1 - Growing and harvesting of plants on soils containing modest concentrations of some heavy metal(s),
2 - burning of the plants,
3 - smelting or storing of the ash.

The process relies on the ability of a plant crop to remove and concentrate heavy metals from metalliferous soils. The term ‘heavy metal’ is loose and ill defined. It is widely understood, however, as referring to all metals excluding the alkali metals, alkaline earth metals, and aluminium (Striet and Stumm, 1993). The metalloids arsenic, antimony and selenium are often included.

Phytoextraction is used to describe two types of operation, *phytomining* and *phytoremediation*. Phytomining is the phytoextraction of metals for commercial gain. Although it has never been tested industrially, it could be used instead of conventional mining techniques in some instances. It also has the potential to be used to mine areas that are unable to be exploited using conventional techniques. Phytoremediation by way of phytoextraction, is the decontamination of polluted soils by using plants to remove polluting metals. World wide there are thousands of hectares contaminated with heavy metals. In the United States alone, there are over 1000 “Superfund” sites that will need to be remediated in the near future. Cleaning these soils using conventional methods is, in many cases, too expensive or simply not possible for technical reasons. Phytoremediation has the potential to decontaminate economically many of these polluted areas. Unlike phytomining, phytoremediation is emerging as a proven technology that is starting to be used in commercial operations.

The basis for any phytoextraction operation is the plants used. They belong to a group of plants that have some very peculiar properties. These are known as *hyperaccumulators*

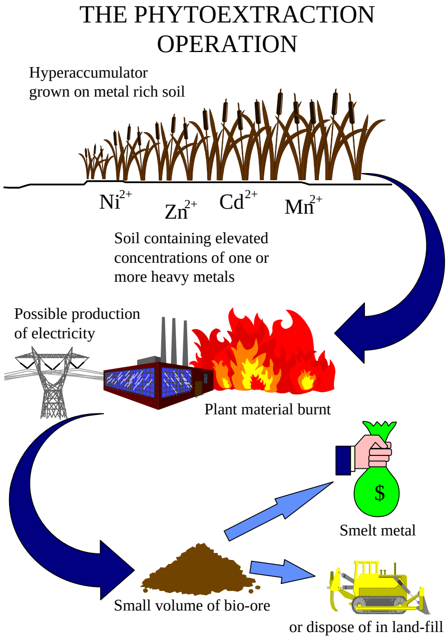

Figure 1

Hyperaccumulators

All plants remove metals from their surroundings. Some of these metals are essential for the plant to complete its life cycle. Other metals may be taken up incidentally, and at high concentrations may have a deleterious effect on the plants’ growth. Brooks (1983) divided the elements into essential and non-essential, depending whether the plant can complete its life cycle without them. Plant responses to essential elements can be divided into three categories according to increasing elemental concentrations:

(1) deficiency, (2) optimal, and (3) toxicity. Non-essential elements are tolerated by plants at low concentrations and may be toxic at higher concentrations.

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

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

Fig. 2. Sève bleue, “blue sap”, the forest tree Sebertia acuminata from New Caledonia. The dry sap contains 26% nickel.

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

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

*Leblanc *et al.* (1997)

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

At present, there are about 400 species of known terrestrial plants that hyperaccumulate one or more of several heavy metals (there are also several aquatic species that are not listed). Table 2 shows the number of species that have been found to hyperaccumulate each metal. This table is by no means a representation of the total number of hyperaccumulators for each element in existence, as species are being continuously discovered. There has been considerable work done on nickel, an element found in many metalliferous soils, consequently nickel hyperaccumulators make up the majority of known hyperaccumulator species. In contrast, there has been very little work done on thallium, the single study (Leblanc *et al.*, 1997) finding a single hyperaccumulator.

Table 2. Numbers of known plant hyperaccumulators and the families in which they are most often found (after Brooks, 1997).

Element	Number of species	Families
Cadmium	1	Brassicaceae
Cobalt	26	Lamiaceae, Scrophulariaceae
Copper	24	Cyperaceae, Lamiaceae, Poaceae, Scrophulariaceae
Manganese	11	Apocynaceae, Cunoniaceae, Proteaceae
Nickel	290	Brassicaceae, Cunoniaceae, Euphorbiaceae, Flacourtiaceae, Violaceae
Selenium	19	Fabaceae
Thallium	1	Brassicaceae
Zinc	16	Brassicaceae, Violaceae

Distribution of hyperaccumulators

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

Table 3. Types of soil upon which most hyperaccumulator species are found.

	Serpentine	Base-metal	Copper-cobalt	Seleniferous
Heavy metals contained in elevated concentrations	Ni, Cr, Co, Mn, Fe, V, Ti (Cu)	Zn, Cd, Pb, Ba, Tl, As	Co, Cu	Se
Location	worldwide	worldwide	former Zaïre	United States
Typical pH	7.5	< 5.0	5.0 - 7.0	>8.0
Plant nutrients	poor	poor	poor	Poor
Other properties	very high Mg:Ca ratio, sandy and prone to erosion.	sandy, metals often present as sulphides		

Metalliferous soils supporting hyperaccumulators may be divided into four categories, and are summarised in Table 3. Most known hyperaccumulators accumulate nickel, and occur mainly on ultramafic (serpentine) soils. Ultramafic soils contain elevated concentrations of magnesium and the first transition series metals (V, Ti, Cr, Mn, Co, Ni, Fe), which has been one reason for the study of these metals in plants. Fig. 4 shows the locations of the more important bodies of metalliferous soils, and the distribution of known hyperaccumulator plants. Nearly all hyperaccumulator plants are found on metalliferous soils in temperate and tropical regions. An explanation for this distribution was proposed by (Brooks, 1987) who suggested that the time required for the evolution of hyperaccumulator species was longer than the time elapsed since the last Ice Age. Hyperaccumulators usually grow poorly on non-metalliferous soils, and are out-competed by non-metallophyte species. Since metalliferous soils occur in small disjunct locations, hyperaccumulators in areas further from the equator had no refugia when the areas were glaciated during the Ice Ages. Warm temperate and equatorial regions were not glaciated during this period, so metalliferous soils have had a longer time to evolve an endemic flora. The occurrence of hyperaccumulators is largely restricted to metalliferous soils (such as in Fig. 3) that often support an endemic flora, distinct from surrounding soils.

Fig. 3. Cardaminopsis halleri a small herbaceous metallophyte and hyperaccumulator of zinc growing near the town of Auby, Northern France. The soil is polluted with lead, zinc and cadmium from a nearby metal smelter.

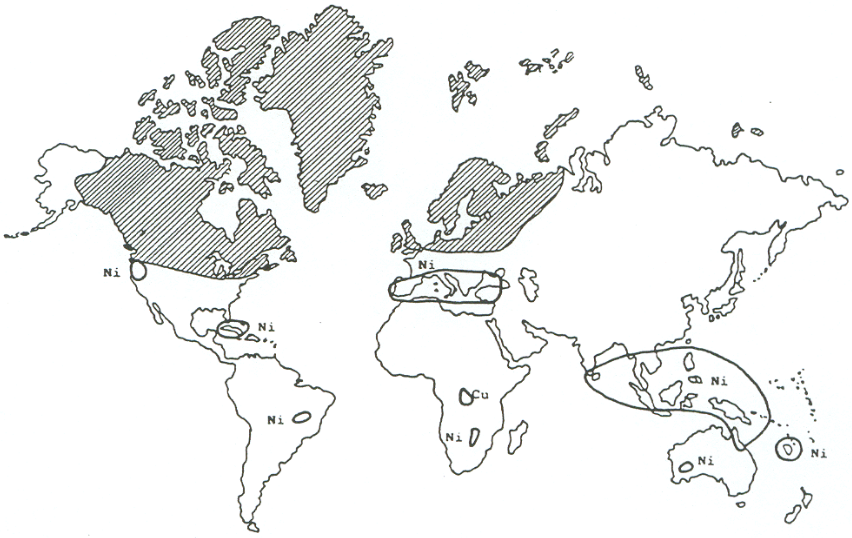

Fig. 4. A map showing the locations of the majority of hyperaccumulators and associated metalliferous soils. The shaded portions indicate areas that were under ice during the last glaciation, and have a notable paucity of hyperaccumulator species.

The process of hyperaccumulation

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

The first stage in metal uptake is the solubilisation and sequestration of the metal ions and their transport across the root cell plasma membrane into the symplast for subsequent passage into the xylem. Solubilisation may involve acidification of the rhizosphere, reduction of the metal ions present, and the excretion of metal-binding compounds (Raskin *et al.*, 1994). There has been much work on compounds secreted by root systems that bind to metal ions in the soil solution. The generic name for these compounds is *phytosiderophores*, and they have been classified into two types: *phytochelatins* and *metallothiones*. For a further discussion of their properties, see Chapter 2. These compounds may either prevent the metal from crossing the plasma membranes (exclusion) or actively promote it (hyperaccumulation).

Recent work has shown that it is possible to induce the hyperaccumulation of lead in non-metallophytes by the addition of the chelating agent EDTA (Huang and Cunningham, 1996; and Blaylock *et al.*, 1997). The addition of this chelating agent not only solubilises lead in the soil (Li and Shuman, 1996) but also allows its passage into the xylem of the root and subsequent translocation to the aerial portions. The plants die soon after the application of the chelating agent, but not before the lead concentration in the aerial portions has reached up to 2% on a dry matter basis. The fact that these plants die indicates that in natural hyperaccumulator plants there must be a detoxification mechanism so that the accumulated heavy metals do not kill the plant. The addition of EDTA to plants growing in non-metalliferous soils indicates that the compound itself is non-toxic.

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

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

Raison d'être for hyperaccumulators

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

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

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

Other uses of hyperaccumulators

Besides the extraction of heavy metals from soil, hyperaccumulator plants have been put to several other uses. Hyperaccumulators generally only occur on soils with an elevated concentration of the metal they accumulate. These soils may indicate the presence of an underlying ore-body, which could be mined. The plants thus find a use in mineral exploration, a process called *geobotanical prospecting*. The genus *Astragalus* contains several species that hyperaccumulate selenium, which is geochemically associated with uranium. The distribution of *Astragalus* species was used by scientists at the U.S. Geochemical Survey to find areas of uranium mineralisation (Cannon, 1960). The presence of *Becium homblei* from Central Africa was used to delineate copper deposits in the Zambian Copper Belt (Plaen *et al.*, 1982).

Phytoarchaeology is the process whereby the presence of plants has been used to investigate some archaeological event (Brooks and Johannes, 1990). Copper that was smelted in Central Africa by ancient artisans has left areas of localised copper pollution near the ancient smelters. Archaeologists search for the presence of copper hyperaccumulators such as *Haumaniastrum katangense* and *H. robertii,* then dig to find ancient artefacts (Plaen *et al.*, 1982). Ancient trade routes between Anatolia and Corsica (at the town of Bastia) were identified by a colony of *Alyssum corsicum* growing over the ultramafics at Bastia. The seeds had been brought along with cargoes of wheat, showing the existence of ancient Venetian trade routes (Brooks, 1997).

Dushenkov *et al.* (1995), introduced the concept of *rhizofiltration*. In this process, the roots of terrestrial plants are used to extract toxic metals or radionuclides from polluted aquatic systems. Rhizofiltration is seen as an improvement over *aquatic phytoremediation* (using aquatic plants to extract metals from polluted water) because of the superior biomass production of the terrestrial plants.

Some toxic elements can be converted to gaseous compounds and removed from polluted soils via plants, a process that might be termed *phytovolatilisation.* Zayed *et al.* (1995) investigated the phytovolatilisation of selenium, a common pollutant of industrial sites.

Hyperaccumulators necessarily have to be tolerant of high concentrations of heavy metals in the soil. For this reason they may find a use in *revegetation* and /or *phytostabilisation* of toxic soil, such as might be found on abandoned mines or around metal smelters. Phytostabilisation may also involve plants changing the chemical form of a pollutant into a less toxic state. James (1996) discussed the relative inertness of chromium in the chromic (+3) oxidation state as opposed to the chromate (+6) state. A reduction of chromate to chromic could be effected by chemical or biological methods. Rugh *et al.* (1996) Showed that genetically modified *Arabidopsis thaliana* could reduce toxic mercuric ions into relatively inert metallic mercury.

Selection of hyperaccumulators for phytoextraction

The objective in any phytoextraction operation is to remove the maximum quantity of metal from the soil in the minimum of time. This implies using a hyperaccumulator with a high biomass production and a high metal content. Large trees such as *Sebertia acuminata* from New Caledonia (Fig. 2) that has a mean nickel content of 0.25% and a biomass of many tonnes, could remove hundreds of kilograms of nickel per hectare. The tree requires decades to grow however, so the rate of nickel extraction is comparatively low. Similarly, small prostrate herbs are unlikely to remove enough metal to make the phytoextraction worthwhile. Fast growing shrubby or herbaceous plants provide the maximum biomass production, and should extract the greatest amount of metal provided they have a sufficient metal concentration. The hyperaccumulator used should be able to perform under the local conditions. Xerophytic plants are unlikely to flourish in a high rainfall environment. Temperature, competition and intolerance to pests or pathogens may also prohibit the growth of plants out of their natural range. *S. acuminata* will only grow in a forest environment, requires a high annual temperature, and high rainfall. Even if a means were found to augment the biomass production of this tree, it could not be grown in the conditions found in most phytoextraction operations. Plants to be used over many hectares should be easily propagated, preferably by seed that does not require pre-treatment.

It is possible that there are numerous hyperaccumulators with the desired properties for phytoextraction that have yet to be discovered. It may also be possible to modify known plants to these specifications (see Introductions of Sections 2 and 3).

Table 4. Plants investigated in this study

Name	*Alyssum bertolonii*	*Berkheya coddii*	*Thlaspi caerulescens*
Natural range	Central Italy	South Africa	Europe
Climate	Mediterranean	Warm temperate	Variable from Mediterranean to cold-temperate.
Growth habit	Perennial woody shrub	Perennial herbaceous	Biennial herbaceous
Propagation	Seed	Seed	Seed
Metals accumulated	Ni	Ni	Zn, Cd, Ni
Metal Content	Up to 3%	Up to 2%	Up to 3% Zn, 0.1% Cd

Table 4 shows the plants used to investigate phytoextraction in this study. The plants were chosen because they had many or most of the properties mentioned above. Another plant initially tested but abandoned because it was unsuitable, was the nickel hyperaccumulator *Streptanthus polygaloides* from California USA. Although easily grown, under local conditions (both indoors and in outside plots) the biomass production was less than the other nickel accumulators tested.

Aims of the study:

The broad aim of this study was to determine the feasibility of the phytoextraction of nickel, zinc and cadmium using the species in Table 4. There were three parts of the investigation:

1- the bioavailability of heavy metals in soils and how their uptake might be improved chemically. The solubility of metals in the soil is of crucial importance to their phytoextraction,
2 - the possibility of phytomining nickel, and
3 - the phytoremediation of zinc-cadmium-and nickel-contaminated soils.

It was planed to relate the chemical properties of the soil to the plants' metal uptake, and be able to predict the phytoextraction performance of these plants when grown on a variety of soils.

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SECTION A
THE BIOAVAILABILITY OF HEAVY METALS IN SOILS

The first requirement of any phytoextraction operation is that the metals in the soil are bioavailable to plants. Both abiotic and biotic factors are required for the uptake of metals by plants. The primary requirement is that the metal is soluble in the soil solution. The soluble fraction has to be in a form where it can be taken up by plants. The soluble metal complex in the soil has to be able to cross the plasma membranes in plant roots before it can be translocated through the xylem to the aerial parts. This requirement necessarily limits the size of the complex. The uptake of the soluble metals is sensitive to many other abiotic factors such as pH, soil water content, temperature, light, and wind. Biotically the plant may secrete chemicals that inhibit or promote uptake of metals by altering their ability to traverse the plasma membrane.

Two aspects of metal bioavailability are of interest regarding phytoextraction:

1 - Determination of metal solubility. This is necessary to determine if the metal could be hyperaccumulated. If the solubility of the metal in the soil can be related to plant uptake, then future metal harvests on a soil may be predicted by performing a soil extraction experiment.
2 - The enhancement of the solubility of metals in the soil.

This may be needed before phytoextraction can commence, or to improve metal yields after several hyperaccumulator croppings.

Chapter 2 is concerned with chemical methods for the determination of metal solubility in the soil, and ways that this solubility might be increased. It is accepted that solubility is not equivalent to bioavailability. Chapter 3 investigates the effect of elements in ultramafic soils (which are the leading candidates for phytomining) on the associated flora. The aims are to determine which elements control the flora, and which may be extracted using plants.

Chapter 2

Chemical methods for the determination and enhancement of heavy metal bioavailability in soils.

Abstract

Extractions were performed on two metalliferous soils, a zinc-, cadmium-, and lead-contaminated soil from Auby, Northern France, and an ultramafic soil from the Rai Valley, South Island, New Zealand. The effect of extractant concentration and pH on extractability of elements from these soils was determined, as was the effect of adding amendments to the soil to increase metal solubility. It was found that for all metals in all soils, the amount extracted was proportional to the concentration and pH of the extractant. All metals except lead with potassium chloride as an extractant increased their solubility logarithmically as the extractant concentration increased. The solubility of all metals increased exponentially as the pH of the extractant decreased. Equations can be used to relate metals extracted by different extractants. The complex and dynamic nature of plant - soil interactions implies that no single extractant can be used to predict bioavailability. It was shown that 1M ammonium acetate gave reproducible results that could be mathematically related to bioavailability using equations based on extractant concentration and pH. The addition of small amounts of citric acid or EDTA significantly increases the solubilities of heavy metals in the soil and may be used to augment metal crops in phytoextraction operations.

Introduction

Elevated concentrations of heavy metals can occur naturally in serpentine or calamine soils, or can be deposited in soils by the actions of humans. In both cases, the presence of heavy metals may be undesirable because of their potential toxicity to plant and animal life. Alternatively, the soil and/or its underlying strata may represent a potential source for the commercial extraction of these metals.

Determination of the solubility of heavy metals in a soil is necessary before decisions as to soil management can be made. There is an important difference between the total concentration of a metal in the soil, and the fraction that interacts with the biota, i.e. the soluble fraction. The total concentration of a metal in the soil may give no indication of the soil's toxicity or potential to be used in a phytoextraction operation. Silicate-bound metal will have extremely low solubility even if the metal has a very high total concentration. Such a metal will not be toxic to the soil biota, nor is it plant extractable.

From a practical perspective, the total concentration of a metal in a soil can be divided into three fractions (Fig. 5).

1 - available; the metal that is available to an organism at any given time,
2 - potentially available; the metal that becomes available once the available fraction has been removed, and
3 - unavailable; the metal that is chemically bound to an organic or silicate matrix, or present as an insoluble salt.

These three fractions are determined by the chemical and physical properties of the soil. Normal physiological processes remove water and some dissolved elements. *Pisum sativum* (pea) plants have the ability to reduce iron (III) and copper (II), which increases uptake (Welch *et al.*, 1993). In some species, the rhizosphere may be acidified by up to 2 pH units (Salisbury and Ross, 1978), while others excrete heavy-metal-binding agents. These used to be termed *phytosiderophores* but have since been classified as phytochelatins and metallothioneins (Grill *et al.*, 1987). These compounds are short-chained proteins. Phytochelatins have the general formula of $(\gamma\text{-Glu-Cys})_n\text{-Gly}$. A general formula of metallothionenes has yet to be derived, but it is known that Cys is a large component (Goldsbrough, 1996). There also appear to be other compounds employed by plants in heavy metal uptake. Lee *et al.* (1978) found citric acid to be the agent involved in nickel complexing in New Caledonian hyperaccumulator species. It was also noted that this compound was not associated with nickel in European hyperaccumulators of the *Alyssum* genus. The nickel chelator for these plants was demonstrated to be free histidine (Krämer *et al.*, 1996). Due to the large variety of compounds associated with heavy metals in different species, it is obvious that the bioavailability of heavy metals is to some degree species dependent.

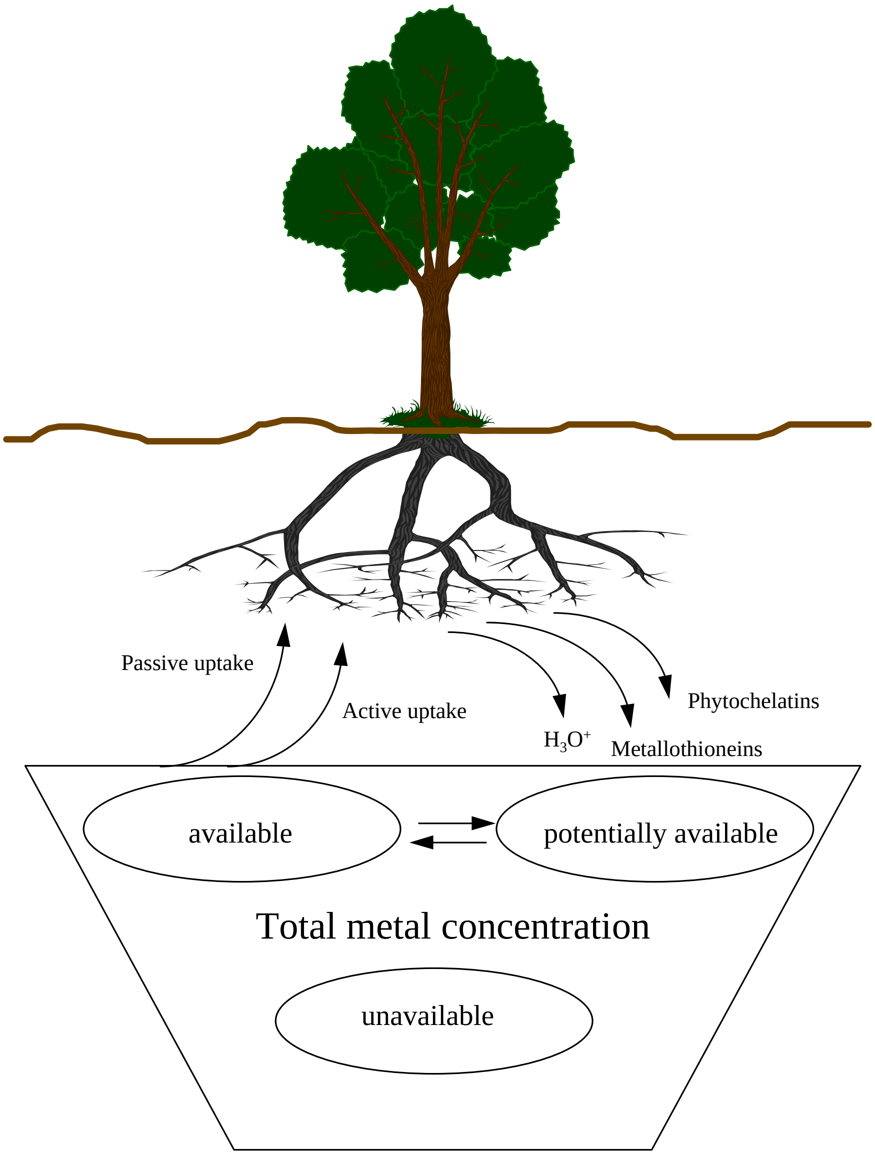

Fig. 5. Plant availability of heavy metals in the rhizosphere

Regarding the potential of soils for phytoextraction, metal solubility and bioavailability are of crucial importance. A plant cannot extract a metal from soil if the metal is not at least slightly soluble. Other factors need to be considered which may affect plant uptake. The physical characteristics of the soil such as particle size, water-holding capacity and density profoundly affect plant growth. Chemical factors such as pH, nutrient availability, and other toxins present and the binding agents excreted by the plant are equally important (Ernst, 1996). It is difficult to model all these variables as they depend not only on the plant species but also on the edaphic conditions at the time, which may have considerable variation. The best estimation that can be made in the laboratory is using the assumption that the bioavailable fraction may be estimated by the soluble fraction.

There are several methods currently employed to investigate the bioavailability of heavy metals in soils. An estimation can be made by analysing plants that grow on the soil. The uptake response of a plant to the presence of heavy metals in the soil can be exclusion or indication or hyperaccumulation (see Fig. 6). The metal concentrations can vary widely between species and unless the metal-uptake characteristics for the analysed species are known, it is difficult to interpret the results. Another disadvantage with this method is that it is slow. It is necessary to wait until the plants are mature before the measurement can be made. Finally, it requires enough soil for the plant to mature in and may have to be conducted *in situ*.

Hinkley (1979) measured the concentrations of metals in very small volumes of soil solution, a similar technique was used by Proctor *et al.* (1981) to measure metal concentrations in solutions extracted from serpentine soils at field capacity.

Most bioavailability estimations are made by agitating the soil with an extracting agent, then measuring the metal concentration in the filtered solution. The extracting agent is chosen to model the chemical environment to which soil organisms are exposed.

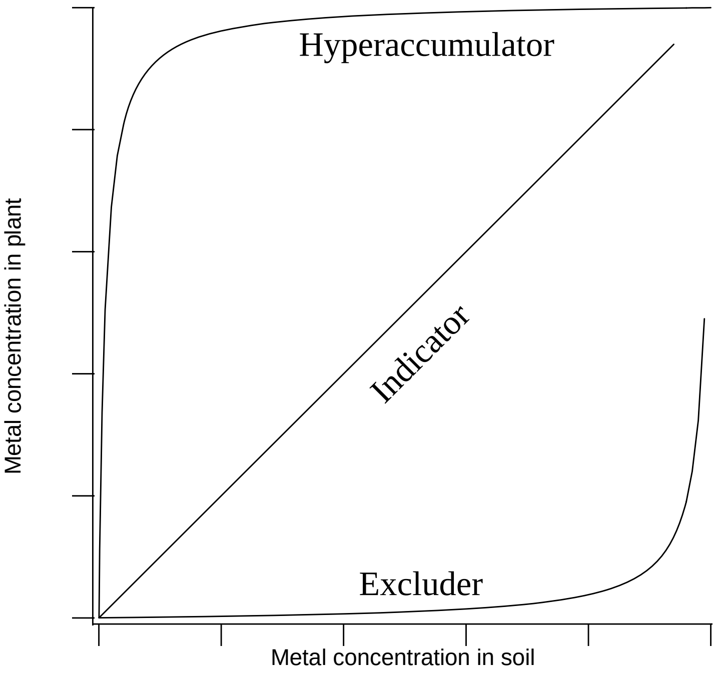

Fig. 6. The possible uptake responses of plants to heavy metals in soils.

Several solutions have been employed; distilled water (Sing and Narwal, 1984), 0.5M $CaCl_2$ (Whitten and Ritchie, 1991) 1M NH_4OAc (Haq *et al.*, 1980), 1M NH_4NO_3 (McGrath *et al.*, 1997), 1 M HOAc, 0.1M $NaNO_3$ (Gupter and Allen, 1983) EDTA, and DPTA (Haq *et al.*, 1980). EDTA and DPTA are chelating agents used to approximate the actions of phytochelatins. For a given soil, there is a large difference in the metals extracted using the above solutions. Nitric acid and chelating agents extract a large proportion of the total metal present, while dilute salts and distilled water often extract over an order of magnitude less.

The large variation in the metal concentration removed by different extractants means that results obtained by different methods are not comparable. It would thus be desirable to quantify the effects of different extracting agents.

Together with the merits of each extractant for modelling the chemical environment to which the plant is exposed, there are practical considerations that need to be addressed. The extractant should be low-cost, stable, easily prepared, non-toxic and extract enough metal so that analysis can be done on existing equipment. In the latter category, there is an important difference in choosing an extractant to estimate bioavailability of metals in a soil that could be used for phytoextraction, and one to measure these metals in the trace amounts found in the majority of soils. Since metal concentrations in soils where phytoextraction could be used, contain concentrations of heavy metals many times that of ‘normal’ soils, the levels that are extracted are usually easily detectable on standard atomic absorption spectrometric equipment.

Table 5. Elemental concentrations (%) in a typical serpentine soil of the Nelson region

Al	1.53	Ca	0.262	Co	0.054	Cl	0.013	Cr	0.785
Cu	0.010	Fe	23.42	K	0.017	Mg	15.92	Mn	0.278
Na	0.009	Ni	0.609	O	41.5	P	0.034	S	0.052
Si	15.7	Ti	0.013	V	0.002	Zn	0.014	Total	100.2

Notes: (1) analyses performed by X-ray fluorescence spectrometry; (2) soil from Serpentine Rd between Rai Valley and Nelson.

A study into the effects of extractants on measuring soil metal bioavailability may also have ramifications for the addition of these extractants to soils to enhance the metal harvest in a phytoextraction operation. Li and Shuman (1996) showed that EDTA additions to heavy-metal-contaminated soils significantly increased the solubility of nickel and zinc, but not cadmium. The metal content in a crop of plants could be increased if more metal was available to the plant. This was strikingly demonstrated by Huang and Cunningham (1996) and Blaylock *et al.* (1997) where a crop of *Zea mays* was grown on a lead-contaminated soil. When mature, the plants contained only trace amounts of lead but two weeks after the addition of EDTA (1g /kg of soil) the lead concentrations in the plants were elevated to around 2% on a dry weight basis. The plants rapidly died due to lead poisoning, but this was not important since the lead was already above ground-level and the crop could be harvested thus removing the lead. A company in the United States, *Phytotech*, undertakes the clean up of lead-contaminated soils by techniques similar to this.

This Chapter is focuses on two soils, a serpentine soil from the Nelson region, and a base-metal polluted soil from Northern France. Soils from the ophiolitic belt near Nelson are composed principally of the minerals antigorite and magnetite with lesser amounts of quartz and talc. A typical soil was chosen for these experiments. Its chemical composition is shown in Table 5. Chemically this soil is typical of many serpentine soils. It has high concentrations of the first series transition metals, very high levels of magnesium relative to calcium, and a high pH. The soil supports a characteristic flora and vegetation, distinct from adjacent areas (see Chapter 3).

Table 6. Concentrations (µg/g) of some heavy metals in soil from Auby.

Element	Concentration
Cadmium (total)	360
Lead (total)	6209
Zinc (total)	40,416
Zinc EDTA extraction	10,972

Soils near the town of Auby in northern France contain high levels of zinc, cadmium, and lead (Table 6) due to aerial pollution from a nearby base metal smelter. The severe toxicity of the soil is indicated by the high organic matter content (presumably due to the poisoning of soil bacteria that are then not available to break down the humus) and the presence of the metallophytes *Armeria maritima* and *Cardaminopsis hallerii*. A previous study found that the concentration of zinc extracted from this soil using an EDTA solution was 10,971 µg/g. Experiments show that the metallophyte *Thlaspi caerulescens* grows well on this soil, but will not even germinate in 1000 µg/g zinc (one tenth of what was extracted by the EDTA) indicating that the EDTA extraction grossly overestimates the bioavailability of zinc in this soil.

The aims of this study were:

1 - to investigate the effects of pH, composition and concentration of extractants on the amounts of heavy metals extracted from the two metalliferous soils,
2 - to determine the most suitable extractant for future experiments on phytoextraction,
3 - to find compounds for soil amendments to enhance the bioavailability of heavy metals.

Materials and Methods

Approximately 50 kg of soil from a polluted site near the town of Auby, Northern France and from the Rai Saddle, South Island, New Zealand were collected. The soils were homogenised using a spade and concrete mixer. Three lots of ca 400g of soil were taken and placed in an oven at 75^o C until a constant weight was obtained. The weights were recorded and the percentage water in the soil calculated. Soil was sieved using a 2 mm sieve. The soil was left un-ground, as grinding would have broken down some of the matrices and thus increase the solubility of some metals.

Extractions on the Auby soil were carried out using the following extractants: distilled water, 0.1M HCl, 0.5M $CaCl_2$, 0.125, 0.25, 0.5, 1, 2, and 4M solutions of KCl and NH_4OAc. Solutions (0.1 M) of KH Phthalate buffered with NaOH to produce final soil pHs of 7, 6.4, 6, 5.7, 5.4, 5.2, and 5.0 were also used.

Extractions of the serpentine soil were made using the following reagents: 0.5M CaCl2, 1M NH_40Ac (pH 7) EDTA, citric acid, NH_4citrate, tartaric acid, oxalic acid, urea, and KSCN at concentrations of 0.5% and 2% w/v. Other extracts were made at pH 1,2,3,4,5,6,7 and 8 using buffered solutions of potassium hydrogen phthalate.

For each extraction, 0.2 g of soil was weighed into a 150 mL container and agitated overnight at 75 rpm. Two replicates were prepared. Elemental determinations were carried out using flame atomic absorption spectroscopy, solutions containing >1% of solutes were diluted to produce a final solution of less than 1% w/v.

Results and Discussion

Table 7. Amounts (µg/g) of zinc, cadmium and lead extracted from Auby soil using different extractants.

Extractant	Zn	Cd	Pb
distilled water	240	1	4
0.5M $CaCl_2$	5414	60	156
1 M KCl	2974	86	73
1 M NH_4OAc	4528	72	736
0.1 M HCl	13,500	90	80

The solubility of heavy metals using different extractants

The amounts of heavy metals extracted from the Auby and Rai valley soils are given in Tables 7 and 8, respectively. The results show that regardless of the extractant used, zinc is by far the most available heavy metal in the soil from Auby, with the extractable concentrations of cadmium and lead over 50 times lower. Considering the total concentrations of these elements (Table 6), the solubility of lead is disproportionately low which accounts for its low uptake by the plants growing on this soil. The Rai valley soil contained soluble nickel, manganese, iron and to a lesser extent cobalt. The concentration of soluble chromium was below detection limits (1 µg/g) in all but the most acid extractants. There was considerable variation between extractants. The variation may have been caused by the pH, composition or concentration of the extractant. Results in Table 8 indicate that in the serpentine soil, more concentrated extractant solutions extract greater concentrations of heavy metals.

Table 8 Amounts (µg/g) of heavy metals extracted from the Rai Valley serpentine soil using a variety of extractants.

Extractant	pH	Ni	Mn	Fe	Co	Cr
distilled water	7.0	2.2	0.5	2	<0.1	<1
0.5M $CaCl_2$		6.9	1	27	1.7	<1
1M NH_4OAc	7.0	4.3	1	38	1	<1
0.1M $NaNO_3$	6.8	2.2	1	21	<0.1	<1
0.5% EDTA	5.2	151	475	415	71	3.2
2% EDTA	4.8	167	508	557	87	6.8
0.5% citric acid	3.1	143	296	830	36	12
2% citric acid	2.5	226	400	1612	57	24.5
0.5% NH_4citrate	7.2	47	49	107	5	3.1
2% NH_4citrate	6.8	70	104	213	13	5.0
0.5% tartaric acid	2.9	133	192	591	26	8.7
2% tartaric acid	2.3	258	326	1823	50	26.7
0.5% oxalic acid	3.6	399	437	7045	94	29.5
2% oxalic acid	1.7	590	409	19659	55	69.9
2% urea	8.0	15	1.6	142	0.3	2.8
0.5% KSCN	7.0	3.0	0.7	1.8	0.3	1.6
2% KSCN	7.0	7.5	1.3	6.9	1.6	1.8

Effect of extractant concentration on the heavy metal extractability in Auby soil.

Extractions using KCl and NH_4OAc solutions at different concentrations were carried out. These extractants were used because changing their concentration did not alter the pH of the solution (c.f. $CaCl_2$). The results show that the amounts of zinc and cadmium increase logarithmically with increasing extractant concentration (Figs. 7 and 8).

Lead behaves similarly to zinc and cadmium with the NH_4OAc solution, but is insoluble in KCl solutions below 1M. The results indicate that the amount of heavy metals extracted using different concentrations of the same extractant may be predicted mathematically, provided that the pH remains constant. Thus, the amount of zinc extracted using NH_4OAc from the Auby soil may be predicted by

$$[Zn] = C_1 - \frac{(C_1 * k)}{([E] + k)}$$

Where: C_1 is the [Zn] (µg/g) as [E] approaches infinity
k is a constant dependent on soil properties
[E] is the concentration of the extractant solution.

In the Auby soil, C_1 was 8220 µg/g and k was 0.766. Gupter and Alan (1993) suggested that dilute extractants, such as 0.1M $NaNO_3$ give better estimations of metal bioavailability than more concentrated extractants because the natural concentration of solutes in the soil approximates to this concentration.

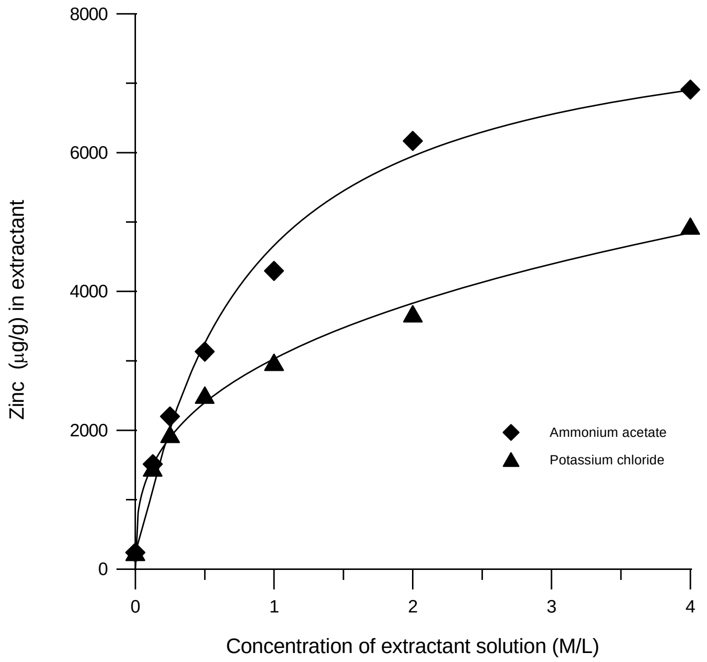

Fig. 7. Amount of zinc extracted from Auby soil using ammonium acetate and potassium chloride at different concentrations

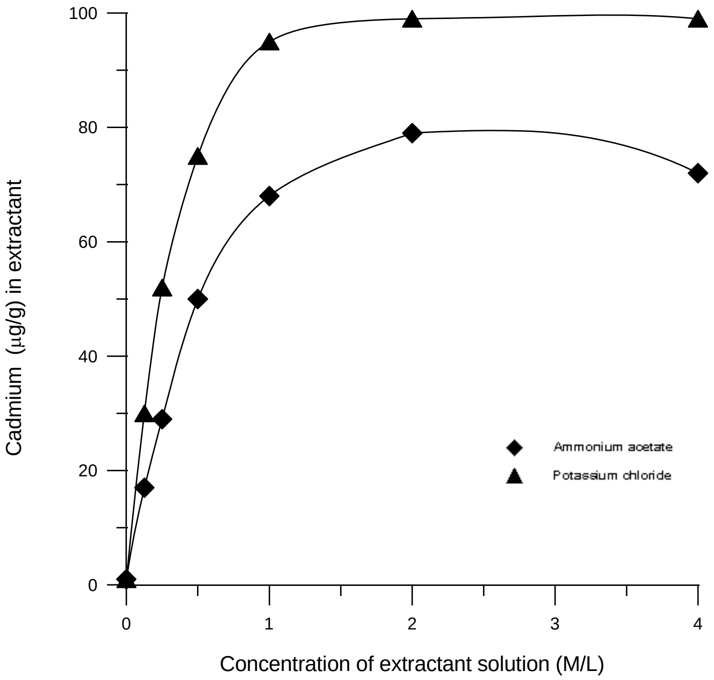

Fig. 8. Amount of cadmium extracted from Auby soil using ammonium acetate and potassium chloride at different concentrations.

The water content (and hence the concentration of dissolved solutes) of soils may vary greatly over a given period. When the soil is at field capacity, dissolved solutes will be diluted by excess water. The opposite will be true under drought conditions. Thus, the bioavailability of the heavy metals in the soil will also change over time. If the condition of the soil solution is known, then the solubility of heavy metals in an extractant solution of the same concentration may be calculated from the above equation.

A more concentrated solution such as 1M NH_4OAc will overestimate the metal solubility in a soil. The concentration extracted will be proportional, however, to the true metal solubility and this may be calculated using the above equation. Small differences in extractant concentration will have a much smaller effect on the amount of metal extracted if the extractant is more concentrated. Small differences in extractant concentrations will have much less effect if the concentrations are around 1M as opposed to

0.1M. Extractions will thus be more comparable and more repeatable. Another advantage to using a more concentrated extractant is that because more metal is extracted, it is easier to measure.

Effect of extractant pH on metal extractability

It is well documented that the pH of the rhizosphere of some plants is often up to 2 pH units below the surrounding soil, thus an indication as to the effect of pH on metal solubility would be useful in predicting metal bioavailability. This experiment was conducted using 0.1M KH phthalate buffered with NaOH. This extractant was chosen because the pH could be easily controlled without greatly altering the composition and concentration of the extractant, and because there are no chelating effects. The results show that in the Auby soil, extractable zinc, cadmium, and lead increase exponentially as the pH is lowered (Fig. 10 and 11). The concentrations of heavy metals in the Rai valley soil behaved in similarly (Fig. 17 Chapter 3).

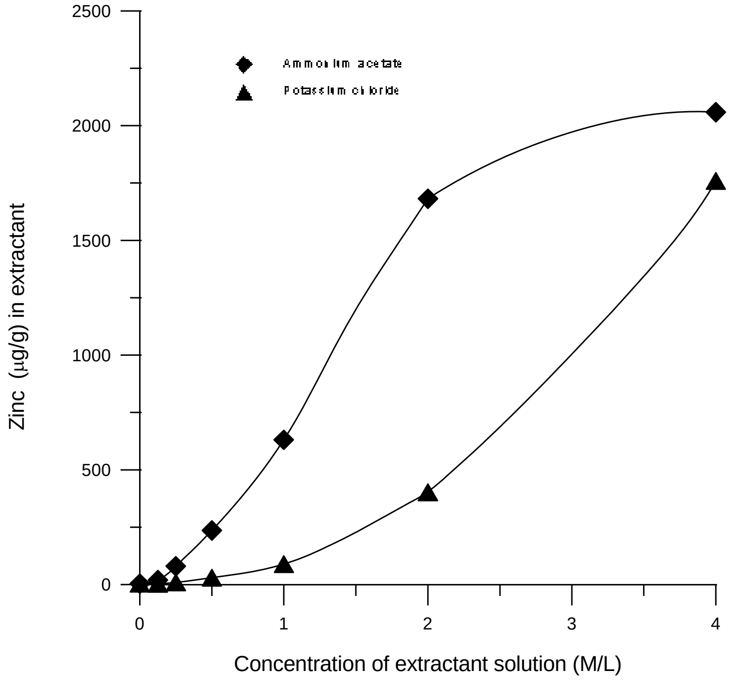

Fig. 9. Amounts of cadmium extracted from Auby soil using ammonium acetate and potassium chloride at different concentrations

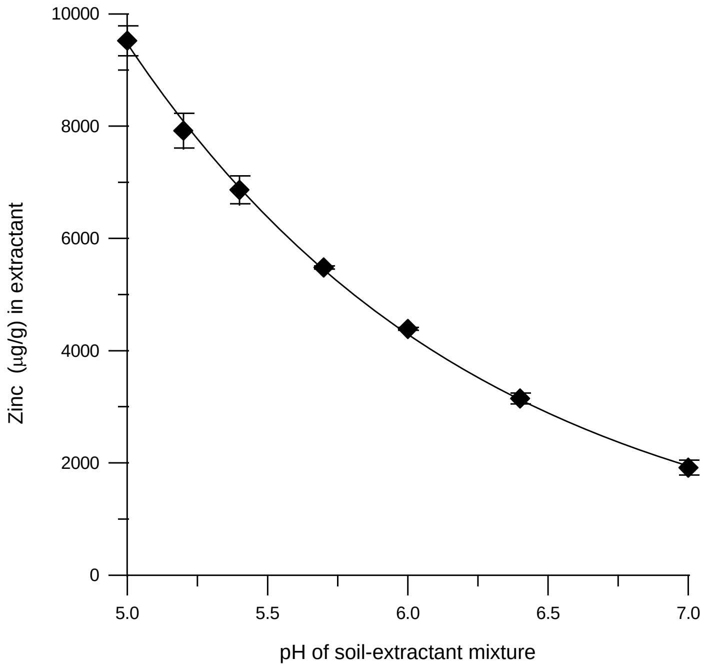

Fig. 10. Amount of zinc extracted at different pH values from Auby soil.

Chromium, though present in large total concentrations was below detection limits in all extractants with a pH greater than 3. It is thus unlikely that this metal is available to plants in the neutral to alkali conditions of serpentine soils. Since the pH of the rhizosphere is species, and probably, age-dependent, it is impossible to state a single value for all plants. It is possible however to predict the concentration *range* of heavy metals that all plants are exposed to in a given soil. From a given extraction value at pH 7, the amount of extractable zinc at lower pHs can be calculated by;

$[Zn] = M + k_1M(k_2-P)^2$ where: M is the [Zn] (µg/g) extracted at pH 7
P is the pH of the extractant solution.
k_1 and k_2 are constants in this case 0.747 and 7.42 respectively.

As with the concentration of an extractant solution, the metals extracted at different extractant pH values can be predicted using equations similar to the above. Acid extractants have the advantage of extracting more metal, making elemental determination easier, but at lower pH values small differences in extractant pH values give large differences in the amount of metal extracted. Using an extractant with a pH of 7 gives an approximation to the pH of most ultramafic soils, and many other metalliferous soils. Variation in soil and/or rhizosphere pH could be calculated mathematically.

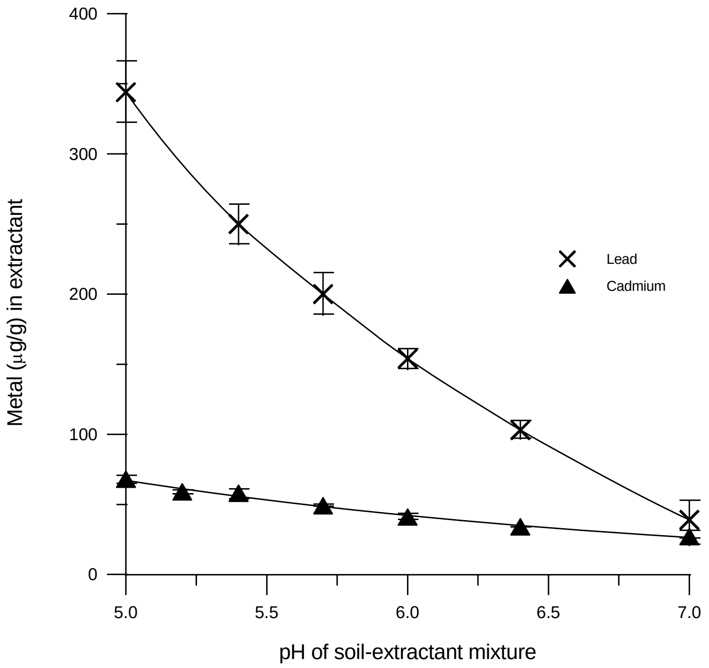

Fig. 11. Amount of lead and cadmium extracted at different pH values from Auby soil.

Enhancement of heavy metal solubility in serpentine soils.

The lead content of *Zea mays* may be increased by adding EDTA to the soil (Huang and Cunningham, 1996; Blaylock *et al.*, 1997). The metal yields of other hyperaccumulator crops may be increased by soil amendments. When adding an agent to the soil to increase metal uptake, several factors must be considered:

1 - The amendment must be cheap, particularly in phytomining operations. The cost of adding chemicals to the soil will offset the net profits of an operation.
2 - The amendment must be conducive to and not inhibit plant growth.
3 - The compound should be "environmentally friendly" i.e. non-toxic to other life forms, and should be rapidly broken down in the soil.
4 - the compound must significantly increase the metal's availability to plants.

Extractants with a high concentration and low pH solubilise large amounts of heavy metals in soils. Increasing the concentration of solutes in the soil or decreasing its pH may increase metal uptake by hyperaccumulator plants. There are limits to the degree to which this can be done. Plants can only survive in a relatively narrow pH range, pH 4.5 being the lower limit for most species (Salisbury and Ross, 1978), and high soil solute concentrations will make water uptake osmotically unfavourable. In addition, a large amount of compound would need to be added to change these soil properties, thus increasing costs.

Apart from acidification and increasing solute concentration, chelating agents may also be added to soils. Table 8 shows the concentrations of heavy metals extracted by various chelating agents. The effect of the chelating agent has to be balanced against the effect of pH. Lowering the pH of the soil to 2 will make plant growth unlikely. Thus, the results obtained for citric, oxalic and tartaric acids give no indication as to their potential value as chelating agents. These acids may, however, be added in much smaller quantities to acidify the soil, thus increasing metal solubility. Ammonium citrate and EDTA extracted significantly more metal than the simple salts, at pH values within the growth range of most plants (>4.5) indicating that there is a strong chelating effect occurring. In the case of ammonium citrate, the chelating effect is due to the citrate, as other ammonium salts (eg NH_4OAc) do not exhibit the same effect. Thus, small amounts of citric acid added to soils may greatly increase metal solubility. The cost of citric acid (US$ 20 per kilo) is also relatively low (cf EDTA @US$ 40 per kilo). Future work needs to be conducted to verify that the addition of these agents actually increases plant metal uptake, and if so, to investigate the economic feasibility of their use.

Conclusions

The amount of metal extracted varied by over an order of magnitude depending on soil type and the extractant used, indicating that comparisons of bioavailability using different extractants are of little value. The amount of metal extracted was dependent on both the concentration and the pH of the extractant. At low concentrations, such as occur naturally in soils, the amount of metal extracted at a given pH did not vary much between extractants and could be mathematically predicted. A similar calculation could also be used to predict the effect of changing pH. Under natural conditions, the water content (and hence the solute concentration) and pH of the soil vary depending on climatic conditions and plant species present. This implies that no single value can be given to express the bioavailability of metals in the soil, but a bioavailability range can be predicted using information about soil solutes and rhizosphere acidification.

A non-chelating, extractant at pH 7 such as 1M NH_4OAc will give reproducible results that are proportional to the bioavailability of the heavy metals present. Thus, different soils can be compared. If future work can relate the metal concentration in a hyperaccumulator plant to the extractable metal in the soil, then a potential metal harvest from a metalliferous area could be predicted by performing a simple extraction. This will assist decision making for both phytoremediation and phytomining.

More work could be done repeating these experiments on other soils, to develop a more useful model for predicting the bioavailability of heavy metals. Sequential extractions, and experiments to determine the effect of chelating agents excreted by some plants, would yield useful data on the potential for removal of heavy metals from a soil by phytoextractive operations.

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Chapter 3
The influence of plant-available elements in soils upon the vegetation over ultramafic ("serpentine") rocks in New Zealand

Publications arising from chapter:

Robinson, B.H.,Brooks, R.R., Kirkman, J.H., Gregg P.E.H., and Gremigni, P., 1996. Plant-available elements in soils and their influence on the vegetation over ultramafic (serpentine) rocks in New Zealand. J.R. Soc. N.Z. 26, 455-466.

Robinson, B.H.,Brooks, R.R., Kirkman, J.H., Gregg P.E.H., and Varlela Alvarez, H., 1997. Edaphic influences on a New Zealand ultramafic ("serpentine") flora: a statistical approach. Pl. Soil. 188, 11-20.

Abstract

Ultramafic ("serpentine") soils from the Nelson Region, New Zealand contain low total levels of calcium, potassium, and phosphorus and high total levels of chromium, cobalt, iron, magnesium, manganese and nickel. The plant-available fraction, however, as measured by extraction procedures, differs considerably from the total elemental abundances in the soils. The extractability of individual elements bears little or no relation to their total abundance in the soil. Only extractable manganese and iron could be predicted by their total concentration. Extraction experiments indicated that chromium and cobalt have low solubility in the pH range to which serpentine plants are exposed and therefore are unlikely to be toxic. Soil analyses across a sedimentary/ultramafic soil boundary indicated that only higher levels of extractable nickel and/or magnesium seem likely to account for the observed vegetation change. The pH of soils under beech forest was significantly lower than that under serpentine vegetation and was probably caused by humic decay of forest litter. Nickel availability increases with decreasing pH. This effect is not limiting on nickel-poor sedimentary soils, but the increased nickel availability at lower pH on serpentine soils may prohibit forest colonisation of this ultramafic environment. This hypothesis is supported by the observation that isolated *Nothofagus* and *Pinus radiata* have colonised humus-free ultramafics at Hackett Creek and the Cobb Asbestos Mine. Using principal components analysis with a mutual plot of the first two principal components, involving the total elemental concentrations in the soils, it was possible to divide the soils into 6 virtually non-overlapping fields, each of which represented a specific vegetation community. Component 1 was essentially an "ultramafic plot" with heavy loadings from the elements chromium, cobalt, iron, magnesium, manganese, and nickel. Component 2 was a "non ultramafic" plot with heavy loadings from aluminium, copper and zinc. For elements extracted from the soils at pH 5.9, discrimination was somewhat poorer but confirmed the great importance of magnesium and nickel as controlling elements for the serpentine vegetation.

Introduction

Ultramafic ("serpentine") rocks are formed from magma rich in olivine and pyroxene minerals. When exposed at the surface of the earth due to tectonic movement, these rocks weather to give a variety of soils known collectively as ultramafic or serpentine (Brooks 1987). The term "serpentine" is a misnomer since it refers to a mineral rather than a rock type, but the term as applied to "serpentine rocks" or "serpentine floras" is so widespread that it has defied all attempts to replace it. Ultramafic rocks occur at several sites in New Zealand such as in Northland, Nelson Province and Otago.
Characteristics that typify serpentine soils (Brooks 1987) are:

1 - they contain predominantly ferromagnesian minerals, the silica and magnesium content decreasing as the soils are weathered,
2 - they contain relatively large amounts of nickel, chromium, manganese and cobalt,
3 - they have relatively low concentrations of nitrogen, phosphorus and potassium,
4 - they have a high magnesium/calcium quotient,
5 - they have a low organic matter content,
6 - they are sandy, relatively homogeneous, and generally shallow with typically 150 mm of soil.

Ultramafic areas nearly always support a characteristic vegetation, distinct from adjacent areas. Fig. 12 shows some typical species. The vegetation boundary between ultramafic and non-ultramafic soils is often sharp, occurring over only a few metres. The Dun Mountain Belt is a particularly good example of this sharp differentiation (Fig.13).

Fig. 12. Dun Mountain vegetation. From front to back : *Melicytus alpina, Chionochloa pallens, Dracophyllum uniflorum, Gentiana corymbifera, Phormium colensoi.*

There is much debate concerning why serpentine areas have such a distinctive stunted flora. The nature of the so-called "serpentine factor" (Krause 1958, Kinzel 1982) has been sought by looking for consistent chemical and physical characteristics of soils that either individually or collectively allow for the development of "serpentine floras" throughout the world (Brooks 1987, Kinzel 1982). Suggested factors influencing the development of "serpentine floras" are:

1 - the toxic effects of nickel, chromium, cobalt, and manganese,
2 - the toxic effect of magnesium at very high concentrations,
3 - the very high magnesium/calcium quotient causing inhibition of calcium uptake,
4 - the low soil concentrations of available plant-essential macronutrients,
5 - the unfavourable physical nature of the soil (sandy, shallow and susceptible to erosion - Jørgensen 1974), and
6 - a combination of some or all of the above.

The large variation in chemical composition of ultramafic soils from one geographic area to the next suggests that a single underlying "serpentine factor" common to all areas is unlikely.

Many previous studies of plant/soil relationships in serpentine floras have focussed on the *total* metal content of the soils rather than the *plant-available* fraction. The work presented below follows the logical approach that the availabilities of soil constituents rather than their totalities are more relevant when considering possible edaphic factors that control a serpentine flora. The objectives of this study are to:

1 - present data on the chemical composition of ultramafic soils from the Nelson area,
2 - compare total elemental concentrations with extracts simulating the plant-available fraction,
3 - to examine changes in the total and extractable fractions across an ecotone,
4 - to relate soil composition to the composition and physiognomy of the overlying vegetation using principal components analyses.

Fig.13. Vegetation boundary between ultramafic (scrub) and sedimentary/ultramafic (forested) soils on the south slope of Dun Mountain.

The Study Area

Geology

The Dun Mountain Ophiolitic ("Mineral") Belt in Nelson Province was the type locality for dunite ($FeMgSiO_4$) first described by Hochstetter at Dun Mountain early in the 19th Century (Bell *et al.,* 1911).

The belt (Fig.14) extends southwards from d'Urville Island through Saddle Hill, and the Dun Mountain, to Red Hill in the Wairau Valley, where it is truncated by the alpine fault. The Dun Mountain Massif, is situated some 13 km east of Nelson and rises to an altitude of 1129 m. The ultramafic rocks intrude the older surrounding sediments and volcanic rocks. The main rock types are dunite and harzburgite with a little pyroxene and they have a concentric serpentinisation at the contacts with sediments and other ultramafic rock types.

Chrome and copper (mined at the turn of the century) are associated with a prominent fault near Wooded Peak. The surrounding rocks are predominantly spilites interspersed in a Permian sedimentary sequence composed of greywackes, argillites and calcareous rocks (Lauder 1965).

Other mining activities were carried out at the United and Champion Mines in the Mineral Belt inland from Richmond along the United and Champion Creeks respectively. The primary ore was a mixture of pyrrhotite and chalcopyrite (Bell *et al*., 1911) and extensive cupriferous mine waste is still to be found at the abandoned United Mine site.

The Cobb Valley asbestos mine (Fig.15) is located in the Upper Takaka Valley. The deposits were mined from 1949 to 1963 (Williams 1965). All the ultramafic rocks are hydrothermally altered and the peridotitic differentiate has been completely serpentinised to chrysotile, bastite, and to an antigorite-like serpentine.

Vegetation and soils

The vegetation of the Dun Mountain Belt has been described by Lee (1980;1992) who recognised several distinct communities:

1 - Mixed beech forest dominated by *Nothofagus fusca* with associated *N. menziesii*, *N. solandri* var. *cliffortioides,* and *Podocarpus hallii* reaching a canopy height of 23 m on sedimentary rocks of Wells's Peak.

2 - Transition forest between ultramafics and sedimentaries consisting of a low tree assemblage of variable composition depending on the predominance of one or more parent materials in the soil. The canopy is dominated by *Nothofagus solandri* var. *cliffortioides*.

3 - *Leptospermum scoparium* shrubland forms a large part of the serpentine vegetation and is host to endemics such as *Pimelea suteri* and *Myosotis monroi*. The nearly endemic *Notothlaspi australe* is often found in damper places. The small gentian *Gentiana corymbifera* is also very common and is often the only coloniser of serpentine scree. Larger shrubs include *Hebe odora* and *Cassinia vauvilliersii.*

4 - *Chionochloa* tussock grassland typical of the open vegetation of the dunites and peridotites of the Dun Mountain Massif increases in importance with altitude.

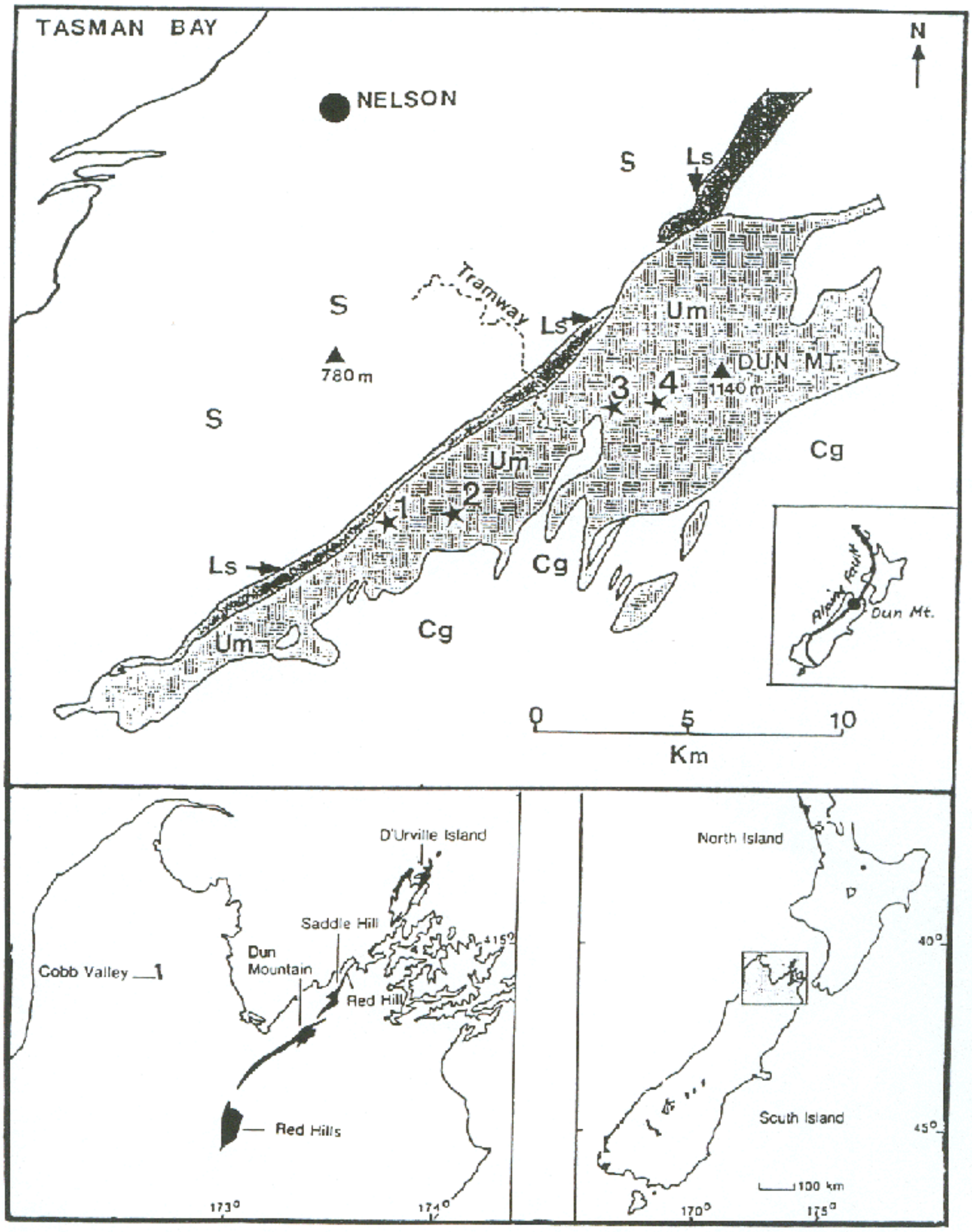

Fig.14. Map of the Dun Mountain Ophiolitic Belt. Cg - Patuki Melange of a conglomerate of sedimentary, volcanic and ultramafic rocks. Ls - Wooded Peak Limestone consisting of poorly bedded grey limestone with lenses of grey siltstone. S - Greville Formation of interbedded laminated grey sandstone, siltstone and mudstone. Um - Layered dunite, harzburgite and pyroxenite with widespread serpentinisation.

The vegetation of the Cobb asbestos mine tailings is exceedingly sparse. On the slopes, it is confined to *Colobanthus strictus* and a dwarf tussock grass (*Poa* sp.). In the flat areas below the tailings, there is an occasional *Nothofagus* that has succeeded in colonising this exceedingly hostile environment (Fig.15).

Fig.15 Individual specimen of *Nothofagus solandri* colonising a patch of humus-free serpentine soil below asbestos tips at the Cobb Valley asbestos mine.

The soils of the Dun Mountain Ophiolitic Belt are quite variable but were originally classified as "Dun Steepland" (Chittenden *et al*., 1966). A soil profile in the Lee Valley under stunted manuka and native grasses (15° slope) was described as follows:

1 - 2.5 cm - dark brown sandy loam, friable, moderately developed fine soft crumb structure.
2 - 12 cm - dark brown silt loam with rock fragments, friable, strongly developed medium nutty structure.
3 - 22 cm - dark olive-brown silt loam with many rock fragments, friable, moderately developed medium granular structure.
4 - Weathered serpentinite rock.

Elsewhere the soil cover consists of little more than weathered serpentine scree with little or no organic matter. The modern nomenclature for this serpentine soil type is *magnesic mafic brown* (Anon, 1992)

Methods

Sampling and sample sites

Visits were made to Dun Mountain, United and Champion copper mines, Hackett chromite mine and Cobb Valley asbestos mine and to the ultramafic terrain surrounding these areas (Fig. 14). Soils were collected from each site, samples being taken from the material immediately under the layer of humus and surface litter (which was usually sparse). It might be argued that the surface organic layer should have been sampled rather than the lower inorganic fraction. In most cases, however, there was virtually no surface organic material. In the non-ultramafic forested areas, there was indeed a shallow organic layer, but it would have been inappropriate to compare this organic layer with the remainder of the soils where such a layer was absent. Moreover, in the forested areas, the plant roots were located deep in the lower horizons and had little contact with the surface material. These samples were composites of approximately 5 different samples

At Coppermine saddle near the Dun Mountain, a transect was defined across a beech forest/serpentine scrub ecotone over a soil boundary. The position of the transect was determined by selection of a site with flat topography free of down-slope movement of material and where a sharp vegetation boundary was observed. From X-ray diffraction studies, it was established that the beech forest overlies a sedimentary/ultramafic melange dominated by talc and quartz. The scrub covers a typical ultramafic assemblage of antigorite and magnetite. The transect was 30 m in length, and extended 15 m into each soil type. Along the transect, soil samples were collected every 2 m and represented a composite of three individual samples taken on a parallel line at right angles to the direction of the transect. The samples were taken immediately below the forest litter at depths ranging from about 20 cm in the forest to 10 cm over the open ultramafic terrain..

Vegetation mapping

Plant mapping was carried out on the plant species occurring over the various geological units as shown below. See Table 9 for species lists. The mapping procedure consisted of dividing the terrain into 10m x 10m quadrates and counting the number of plant species within each quadrate.

(A) Gabbroic rocks adjacent to the Cobb Valley talc-magnesite workings carry a low forest dominated by species such as *Nothofagus menziesii* and *Weinmannia racemosa*. There is a relatively sharp transition to the adjacent *Leptospermum* shrubland over serpentinite.

(B) The low transition forest between the ultramafics and the sedimentary/ultramafic melange consists of a low tree assemblage of variable composition depending on the predominance of one or more parent materials in the soil. The canopy is dominated by *Nothofagus solandri* var. *cliffortioides*.

(C) *Chionochloa* tussock grassland is very typical of the open vegetation of the dunites and peridotites of the Dun Mountain Massif and increases in importance with altitude.

(D) The vegetation of the Hackett Creek chrome mine closely resembles that of the *Leptospermum scoparium* shrubland (F) of the Dun Mt. Saddle except that the individual plants are more stunted. The only endemic plant found in this community is *Pimelea suteri*.

(E) *Leptospermum scoparium* shrubland forms a large part of the serpentine vegetation and is host to endemics such as *Pimelea suteri* and *Myosotis monroi*. The partially endemic *Notothlaspi australe* is often found in damper places. The small gentian *Gentiana corymbifera* is also very common and is often the only coloniser of serpentine scree. Larger shrubs include *Hebe odora* and *Cassinia vauvilliersii.*

(F) The talc- and quartz-magnesite tailings of the Cobb asbestos mine are an environment extremely hostile to vegetation. On the slopes of these tailings, the only species are *Colobanthus strictus* and *Poa picta.* In the more level parts of these tailings are to be found, *Hebe odora* and the ubiquitous *Leptospermum scoparium*.

Table 9. Floristic composition of plant communities in the Dun Mountain Ophiolitic Belt

Community	Geology	Dominant species and relative abundance
A - Cobb Valley beech	Gabbro	*Nothofagus menziesii* (5), *Weinmannia racemosa* (3), *Pseudopanax crassifolius* (1), *Leucopogon fasciulatum* (1).
B - Dun Saddle beech	Sedimentary /ultramafic melange	*Nothofagus solandri* var. *cliffortioides* (5), *Phyllocladus trichomanoides* (3), *Podocarpus hallii* (2), *Blechnum proceru* (3).
C - Cobb valley kanuka scrub forest	Sedimentary to ultramafic	Sedimentary: *Nothofagus solandri var. cliffortoides* (5). Ultramafic: *Kunzea ericoides* (4), *Cyathodes juniperina* (2), *Pseudopanax crassifolius*
D - Dun Mt. *Chionochloa* grassland	Dunite	*Chionochloa diffracta* (5), *Dracophyllum uniflorum* (3), *Leptospermum scoparium* (3), *Phormium cookianum* (2), *Gentiana corymbifera* (2), *Melicytus alpina* (2), *Notothlaspi australe* (2), *Euphrasia monroi* (1), *Hebe odora* (2), *Cassinia vauvilliersii* (2).
E - Hackett Creek serpentinite	Serpentinite + chrome	*Leptospermum scoparium* (2), *Pteridium aquilinum* (2), *Cyathodes juniperina* (1), *Melicytus alpina* (2), *Phormium cookianum* (2), *Gentiana corymbifera* (3), *Cassinia vauvilliersii* (1).
F - Cobb asbestos tailings	Talc-magnesite	*Colobanthus strictus* (2), *Poa picta* (2), *Hebe odora* (1), *Leptospermum scoparium* (1), *Coria arborea* (1).

Methods of sample treatment

All soils were sieved to -70 mesh (0.2 mm) size and 0.5 g subsamples were weighed into polypropylene cups. Twelve mL of 1:1 HF/HNO_3 was added to each cup and the solutions were evaporated to dryness on a water bath. Then 12 mL of 2M HCl was added to each cup and the solutions warmed to redissolve the material. The chemical elements in solution were determined by flame atomic absorption spectrometry (FAAS). To measure the pH of the soils, 2.5 mL of water was added to 1 g of sieved soil and the mixture left overnight before measurement with a pH meter. A total elemental analysis by X-ray fluorescence spectrometry was also performed.

The question of which extractant should be used to simulate the plant-available fraction of metals in soils has received a great deal of attention without any real consensus. The pH of the soil in the rhizosphere of plants is often more acidic than the bulk soil, two pH units lower being typical (Salisbury & Ross 1978). The pH values of the soil samples taken in this survey ranged from 4.4 under forest litter to 7.7 under serpentine scrub (the majority of the samples). It was decided that extractants at pH 4.6, 5.9 and 7.0 would simulate conditions that the plants are exposed to on all these soils. The pH value of 5.9 was about 2 units below the pH of serpentine soils.

There was a further rationale for selection of pH 5.9 as the one most likely to simulate uptake of elements from the soils. An experiment was carried out in which the serpentine-endemic Italian crucifer *Alyssum bertolonii* was grown for three months in serpentine soil from the Dun Mountain complex. This soil contained 6090 μg/g Ni (Table 5, Chapter 2). The plants had been sown in a tray containing 3.46 kg of soil and extracted 0.019 g of nickel. This represented an extraction of 5.5 μg/g of this metal. The rhizosphere of plants is usually in contact with only 0.4-2.8 % by volume) of the surrounding soil (Barber 1984). If soil interception were the only factor, the total available nickel in the soil should therefore be in the range 196-1375 μg/g. This figure is however far too high because it does not take into account mass flow and diffusion (Barber 1984) that together can amount to ten times the root interception factor. If this combined figure is taken (i.e. 4-28%), the plant experiments indicate a probable 20-138 μg/g extractable nickel with a mean of 79 μg/g. The experimental value here, using KH phthalate at pH 5.9, was 84 μg/g Ni.

Table 10. Extractants used to measure available elemental concentrations in soils

pH	Extractant	pH	Extractant
1.0	0.2M HCl	2.3	KH phthalate/HCl
3.2	KH phthalate/HCl	4.2	KH phthalate/NaOH
5.2	KH phthalate/NaOH	5.3	1M NH_4NO_3
5.9	0.05M $CaCl_2$	6.2	KH phthalate/NaOH
6.3	0.1M $NaNO_3$	7.0	1M NH_4OAc
8.5	H_3BO_3/KCl	9.0	H_3BO_3/KCl

Note: the natural pH of the soil was 7.5

The extractable fraction of soil elements was determined by adding 10 mL of extractant to 0.5 g of soil. The mixtures were shaken overnight and the supernatant decanted. The extracts were analysed by FAAS. Extractions were performed on a bulk quantity of serpentine soil collected from near the United Mine. The extractants used are summarised in Table 10.

It was decided to use NH_4OAc and buffered KH Phthalate to investigate the extractable elements from the other soils. This was because:

1 - The pH could be easily controlled,
2 - The metal concentrations in solution could be determined without prior centrifuging,
3 - The extractant did not contain calcium or magnesium which were target elements.

Extracts were made of all the soil samples using NH_4OAc at pH 7, KH phthalate/NaOH at pH 5.9 and KH phthalate/NaOH at pH 4.6.

Statistical treatment

Principal components analysis [PCA] (SAS Institute, 1987) was used to relate soil composition to the floristic composition of vegetation overlying the ultramafic substrate. The procedure involves finding one or more new variables (principal components) that condense as much as possible of the original data into a smaller set of uncorrelated variables. The variation in the first principal component accounts for as much as possible of the total variance in the original data set. The second principal component, at right angles to the first, expresses the next highest possible variance, and so forth. The main aim of PCA is to simplify the data. All data were transformed logarithmically because they represented *lognormal*, rather than *normal* distributions.

Results and Discussion

Elemental concentrations in serpentine soils

The total chemical composition of the soils examined (Table 5 Chapter 2; Table 11) is consistent with the general chemical properties of a serpentine soil described in the introduction. The salient characteristics of the soils are the very high magnesium/calcium quotients, and the high concentrations of elements of the iron family such as chromium, cobalt, manganese, and nickel. The essential plant nutrients such as potassium and phosphorous also have very low concentrations in these soils. The molybdenum concentrations were below the detection limit (1 μg/g).

The solubility of some elements in serpentine soils

The toxicity of an element to a plant depends on its availability in the soil rather than on its total concentration. Figures 16 and 17 show the extractability at different pH levels, of some elements in the same soil described in Table 12. These figures show that elements that make up a large proportion of the soil do not necessarily form a similarly large proportion of the available elements. Magnesium is the most available metal in this soil, followed in decreasing order by iron, calcium, nickel, manganese, cobalt, copper, zinc and chromium. Magnesium, nickel and manganese were available at concentrations above those shown to be toxic to most plants (Proctor, 1970; Robertson, 1985). The availability of all transition metals increased exponentially as the extractant became more acid.

Figure 16 shows that there is a large excess of extractable magnesium over calcium, of the order of 20:1. This is very different to non-serpentine soils where the ratio is typically less than 1:1 (Brooks 1987). The magnesium/calcium quotient decreased from pH 1 to pH 4 then increased from pH 4 to pH 7. As might have been expected, the extractabilities of Ni, Mn, Co, Cr and Zn increased markedly with lower pH values (Fig. 17).

Table 11. Total elemental concentrations (μg/g except where otherwise stated) in serpentine soils

Element	AA (n=10)	B (n=10)	C (n=5)	D (n=5)	E (n=12)	F (n=5)
pH	4.9	4.9	6.9	6.6	7.4	7.7
Al (%)						
g.m.	0.93	4.10	0.53	0.10	0.22	0.05
s.d.r.	0.45-1.94	2.51-6.69	0.30-0.95	0.06-0.16	0.06-0.78	0.04-0.08
Ca (%)						
g.m.	1.60	0.16	0.15	0.39	0.58	0.01
s.d.r.	1.10-2.33	0.06-0.39	0.09-0.22	0.24-0.65	0.16-2.11	0.009-0.018
Co						
g.m.	35	33	217	214	176	121
s.d.r.	29-43	23-48	173-272	180-254	112-278	105-138
Cr						
g.m.	373	146	887	936	1889	532
s.d.r.	247-565	43-495	790-996	660-1328	1170-3048	470-603
Cu						
g.m.	10	35	57	10	34	10
s.d.r.	5-20	19-65	45-73	6-16	22-53	6-17
Fe (%)						
g.m.	2.2	4.0	22.3	7.1	11.5	5.1
s.d.r.	1.7-2.7	3.0-5.0	18.3-27.1	5.2-9.6	.2-18.3	4.0-6.1
K						
g.m.	247	302	1681	694	1078	757
s.d.r.	183-333	218-418	1614-1751	282-1707	608-1911	600-955
Mg (%)						
g.m.	9.6	4.0	7.2	19.2	12.4	19.0
s.d.r.	7.3-12.5	2.3-5.2	5.5-9.2	17.2-21.5	8.8-17.5	17.7-21.0
Mn						
g.m.	474	592	1801	1565	2318	382
s.d.r.	368-612	426-823	1421-2282	1250-1960	1335-4014	287-509
Ni						
g.m.	221	164	57	2418	1478	2674
s.d.r.	173-281	55-484	45-73	2210-2646	774-2823	2449-2919
Zn						
g.m.	14	46	106	25	67	14
s.d.r.	10-20	30-71	88-129	16-37	53-84	11-17

g.m. = geometric mean, s.d.r. = standard deviation range

A - Cobb Valley beech forest, B - Dun Saddle beech forest, C - Cobb Valley Kanuka Scrub, D - Dun Mt grassland, E - Serpentine scrub over Hackett Creek chromite mine, F - Cobb asbestos mine.

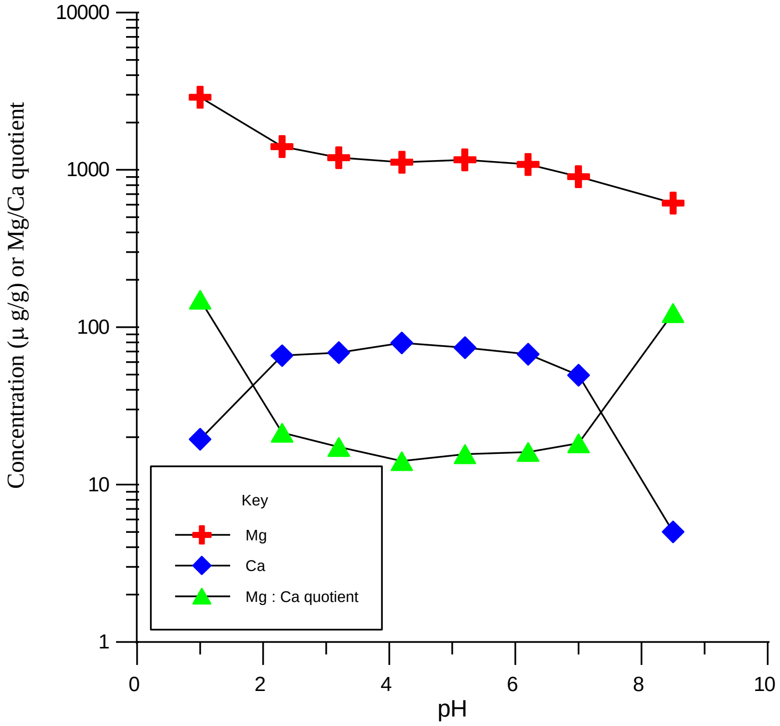

Fig.16 Extractability of calcium and magnesium from a serpentine soil at various pH values.

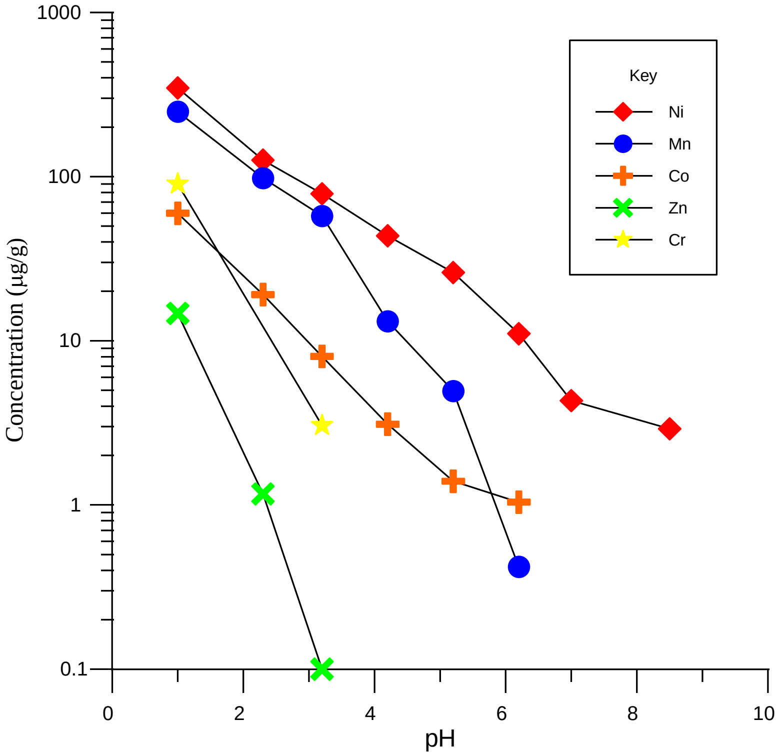

Fig.17. Extractability of chromium, cobalt, manganese, nickel and zinc from a serpentine soil at various pH values.

Elemental variation within serpentine soils and between serpentine and non-serpentine soils

The chemical compositions of all the soils tested were similar to that of a typical serpentine soil, with the exception of soils from the United Mine, which were high in copper and soils from United Valley and Cobb Mine that were low in chromium (Table 12). The growth of stress-tolerant plants such as *Cyathodes juniperina*, *Leptospermum scoparium* and *Pteridium aquilinum* on the waste material from the United Mine is strong evidence of its infertility.

The very low concentrations of extractable chromium, as well as the presence of very stunted serpentine vegetation in the United Valley and Cobb Asbestos Mine, which have soils low in chromium, indicate that chromium is not important in controlling vegetation on ultramafic areas. Serpentine plants, without exception, contain only trace quantities of this element (Brooks 1987). Similarly, the recorded levels of extractable copper, zinc and cobalt were below 0.1 µg/g even using the most acidic extractant (pH 4.6), indicating that these elements are unlikely to be toxic to plants.

The concentration of extractable iron was very low compared with its total concentration in the soil. In many cases it was lower than extractable nickel. At pH 7, the concentration of extractable iron in all the soils was below the detection limit (0.1 µg/g), indicating that plants growing over iron-rich ultramafic soils can actually suffer from an iron deficiency because of the exceeding low availability of iron to plants at pH 7.

The concentrations of total soil nickel, chromium, manganese and cobalt all showed significant increases with distance into the ultramafics across an ecotone near the Dun saddle (Fig. 6). Abundance data for these and other elements are shown statistically in Table 13, which gives the level of significance (n=15) for *t*-tests based on the differences in the geometric means of the two populations. Apart from four elements shown in Fig. 18, magnesium and total iron also show a significant increase. There was no significant increase for calcium, copper, phosphate and zinc, and a highly significant decrease in aluminium with distance across the transect. The same was true for extractable iron at pH 4.6 and 5.9. The latter can easily be explained from the effect of pH already shown in Table 12. In this transect the pH ranged from 4.4 under the *Nothofagus* forest to 7.2 under the serpentine scrub.

Table 12. Mean elemental content (total and extractable) of some ultramafic soils taken from around the Nelson region. All values are in µg/g unless otherwise indicated.

	Dun Mountain	Dun Saddle	Cobb Mine	United Mine	United Valley
	(n=5)	(n=3)	(n=5)	(n=8)	(n=2)
pH	6.9	7.4	7.7	6.0	7.5
Al	6036	1001	576	2931	697
Ca	1553	11316	226	2946	963
Ca*	457	279	46	318	123
Ca**	203	142	14.9	99	143
Ca***	197	147	18.7	-	372
Co	222	190	121	112	391
Cr	892	3151	536	456	589
Cu	59	29	11.0	3101	107
Cu*	-	-	-	90	-
Cu**	-	-	-	314	-
Fe(%)	22.6	13.0	4.9	21.5	29
Fe**	24.7	1.3	5.1	14.0	8.4
Fe***	61.2	70	24	-	61
K	1682	1735	773	1423	4831
Mg(%)	7.3	18.0	19.1	11.5	16.7
Mg*	784	854	322	666	1520
Mg**	903	1005	408	765	1874
Mg***	825	1099	574	-	1977
Mn	1844	1708	395	624	3126
Mn*	6.9	2.4	0.6	3.7	5.4
Mn**	26	12.3	3.1	8.9	18.2
Mn***	61	30	120	-	65
Ni	2490	1505	2682	827	4713
Ni*	11.7	7.4	2.5	14.0	19.5
Ni**	27	28	7.6	38	42
Ni***	57	72	25	-	113
Zn	108	55	14.0	56	76

*extracted with NH_4OAc at pH 7.0; **extracted with KH phthalate at pH 5.9;
***extracted with KH phthalate at pH 4.6.

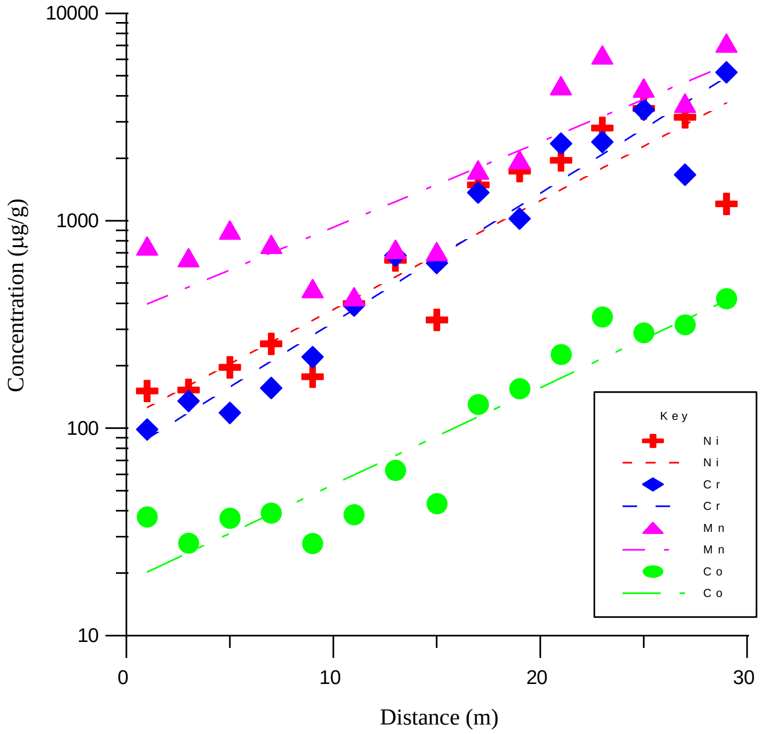

Fig.18 Total abundances of chromium, cobalt, manganese and nickel in an ecotone across a sedimentary/ultramafic soil boundary.

Elemental effects on the vegetation

The results indicate that the toxic effects of chromium, cobalt and manganese are unlikely to be responsible for the vegetation change across the above ecotone either because they are available in extremely low concentrations, or they are not significantly greater in soil under the serpentine vegetation. The lack of a significant change in the concentrations of the plant nutrient phosphate, and the significant increase of available calcium across the ecotone indicates that the vegetation on serpentine soil is not controlled by any deficiency of these elements. Surprisingly, the abundance of potassium was less under the forest than in the ultramafic soil even though both values were low compared with "normal" soils (Table 13). From Table 13 the most significant edaphic factors correlated with the distribution of the serpentine vegetation are an excess of available nickel and magnesium and/or an iron deficiency.

The concentration of extractable nickel increases significantly at lower pH values as is clearly shown in Fig. 19. When the beech species colonise the ecotone, humic acids generate a lower soil pH and increase the availability of nickel. This prevents further progress across this ecotone, implying that serpentine soils are unfavourable for forest communities rather than individual species. This observation was endorsed by the presence of a specimen of *Nothofagus solandri* (Fig. 14) growing below the asbestos mine tip at the Cobb Mine. This individual plant will presumably continue to grow at the site until sufficient humus has accumulated to lower the pH to a level at which the extractable nickel will be toxic to the plant. A further piece of evidence to support this theory is afforded by the observation that in the ultramafics of the Hackett Creek area, individual, albeit stunted, specimens of *Pinus radiata* are able to survive, but only singly, and never as a group or clump. Exactly the same pattern has been observed by R.R. Brooks (pers. commn.) over the ultramafics of southeastern Spain where isolated specimens of *Pinus pinaster* are able to survive in an environment as equally hostile edaphically as in the Dun Mountain Ophiolitic Belt.

It might be argued that serpentine scrub also produces an acidic litter. However, the production of this organic matter is far lower than under forest, and it is therefore likely that the rate of production will be more than counterbalanced by the oxidation of the organic matter in the more open environment of serpentine vegetation. The above theory should be tested by growing individual forest species in serpentine soil where there is no litter to lower the pH.

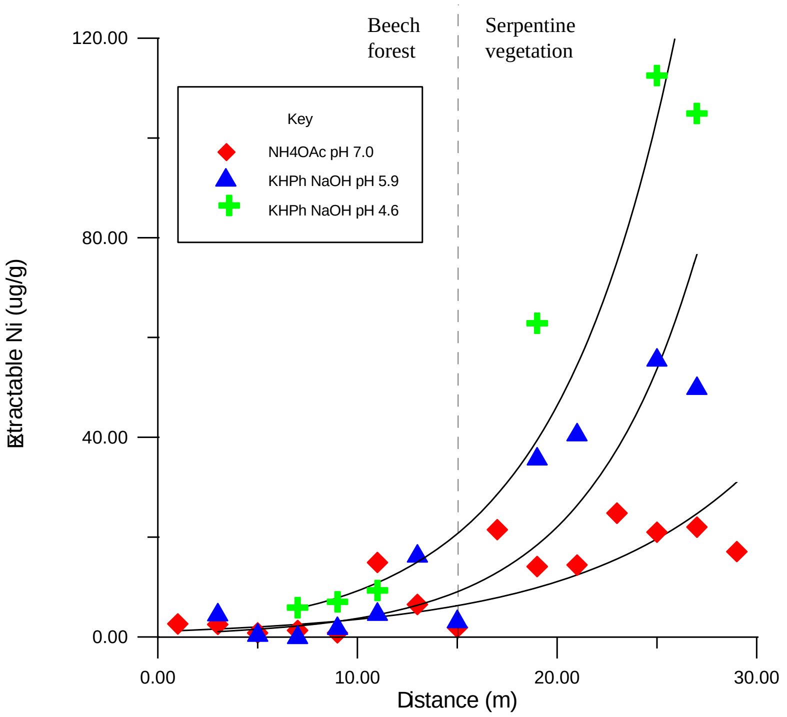

Fig.7 Extractability of nickel at different pH values across an ecotone over sedimentary conglomerate / ultramafic soil boundary.

Table 13 - Statistical analysis (*t* -tests) of elemental concentrations (µg/g unless otherwise stated) in soils as a function of distance across an ecotone over a sedimentary/ultramafic soil boundary

Variable	Sedimentary mean	Ultramafic mean.	p	Significance
pH	4.9	6.6	<0.001	S**
Al	4.6%	0.23%	<0.001	S**
Ca	2500	3000	0.87	NS
Ca^	450	1190	0.012	S
Ca^^	160	420	0.043	S
Ca^^^	120	340	0.21	NS
Co	39	268	<0.001	S**
Cr	302	2490	<0.001	S**
Cu	43	33	0.68	NS
Fe	4.0%	17.3%	<0.001	S**
Fe^^	549	9.2	0.027	S
Fe^^^	285	74	0.007	S**
K	308	1509	<0.001	S**
Mg	4.2%	12.1%	<0.001	S**
Mg^	429	1755	<0.001	S**
Mg^^	370	1942	<0.001	S**
Mg^^^	274	1875	0.0015	S*
Mn	678	4250	<0.001	S**
Mn^	38	18	0.11	NS
Mn^^	64	33	0.175	NS
Mn^^^	1.69	1.74	0.0017	S*
Ni	288	2259	<0.001	S**
Ni^	3.9	19.3	<0.001	S**
Ni^^	4.8	53.6	<0.001	S**
Ni^^^	7.4	93	<0.001	S**
PO_4	0.6	0.8	0.55	NS
Zn	55	91	0.047	S

Notes: S** = very highly significant (P<0.001), S* =highly significant (0.01>P>0.001) S = significant (0.01<P<0.05), NS = not significant (P>0.05).
^extracted with NH_4OAc at pH 7.0, ^^extracted with KH phthalate/NaOH at pH 5.9, ^^^extracted with KH phthalate/NaOH at pH 4.6.

Correlation analysis

Correlation analysis of the total concentrations (transformed logarithmically because distributions were lognormal) of elements in the soils is shown in Table 15. Aluminium is negatively correlated with all other elements except calcium, copper and zinc. This reflects the low proportion of clays in the soils tested. Copper and zinc are typical chalcophile elements usually associated with sulphide mineralisation, which was virtually absent in all samples. Elements associated with serpentine soils (Co, Cr, Fe, Mg, and Mn) were invariably mutually correlated.

In the case of elements extracted from soils at pH 5.9 (Table 14), there were no significant correlations since the geochemical associations of the total elemental concentrations were not reflected in the extracts. It will be noted that the extractabilities of only 5 of the original 11 elements has been reported because the concentrations of the other 6 (poorly extractable) were below the limit of detection (usually < 1 μg/g) of the analytical method.

Table 14 Concentrations (μg/g) of elements extractable from serpentine soils at pH 5.9

Element	A (n=10)	B (n=7)	C (n=4)	D (n=5)	E (n=7)	F (n=3)
Ca						
g.m.	80	138	198	131	274	14.6
s.d.r.	39-165	68-280	160-246	99-172	134-558	11.4-18.7
Fe						
g.m.	66	48	23	7.5	1.0	4.7
s.d.r.	30-145	29-80	15-23	3.7-15	0.03-25	2.7-8.1
Mg						
g.m.	70	278	900	1622	1589	407
s.d.r.	33-147	127-608	825-981	1304-2018	1110-2275	376-440
Mn						
g.m.	32	50	24	12	21	2.9
s.d.r.	16-62	23-106	16-36	7.6-18	9.5-44	1.7-4.8
Ni						
g.m.	1.2	2.8	27	26	42	6.7
s.d.r.	0.18-7.7	0.83-9.3	25-29	20-35	27-66	3.7-12

Symbols as in Table 11

Table 15 Correlation analyses of total elemental concentrations in soils (n=50)

	Al	Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Ni
Ca	NS									
Co	-S**	NS								
Cr	-S**	NS	S**							
Cu	S	NS	NS	NS						
Fe	-S**	NS	S**	S**	S*					
K	-S**	-S	S**	S**	S	S**				
Mg	-S**	NS	S**	S**	-S*	S	S*			
Mn	-S	NS	S**	S**	S*	S**	S**	NS		
Ni	-S**	-S	S**	S**	NS	S**	S**	S**	S**	
Zn	NS	NS	S**	S	S**	S**	S**	NS	S**	S

S** = very highly significant (P<0.001), S* = highly significant (01>P>0.001), S = significant (0.05>P>0.01), NS = not significant (P>0.05). Negative sign implies inverse relationship.

Principal components analysis (PCA)

Table 16 shows eigenvectors and cumulative eigenvalues for logarithmically transformed total elemental concentrations in the soils. The first three principal components that altogether accounted for 90.1% of the total variance of the system. A plot of component 2 vs. component 1 is given in Fig.20. From the plot, it is clear that the soils of the 6 plant communities are almost completely separated from each other with very little overlap of the fields. Plots of other combinations of the first three components were not as successful at separating the fields and are not discussed further. Other statistical techniques exist (e.g. discriminant analysis) which would maximise separation of plant communities, but PCA is sufficient in this case.

From Table 16 it can be noted that the most important positive eigenvector coefficients in component 1 are those involving chromium, cobalt, iron, manganese, nickel and potassium in total soil analyses.

It is appropriate at this stage to explain in greater detail the significance of these eigenvectors. The magnitude of the coefficients in these vectors and their sign, determine where a given plant community will appear in the plot. In Table 16, there are negative coefficients in the first principal component. These are for aluminium and calcium. There are positive values for chromium to zinc. This implies that in a plot of Component 2 vs. Component 1 (Fig.20), groupings along the right-hand side of the *x*-axis will generally correspond to low concentrations of aluminium and calcium, and high concentrations of the other elements (i.e. a strongly ultramafic grouping) in the soils. Thus community E (Hackett Creek) is a typical ultramafic grouping. To the left of the x-axis are communities A (Cobb Valley beech forest) and B (Dun Saddle beech forest) whose sedimentary substrates are high in aluminium and calcium but low in the other (ultramafic) elements.

Table 16. Eigenvectors and eigenvalues of the first three principal components of the soil system

Variable	Component 1	Component 2	Component 3
	TOTAL ELEMENTAL CONTENT IN SOILS		
Eigenvectors			
Aluminium	-0.30	0.37	0.11
Calcium	-0.08	-0.02	0.85
Chromium	0.34	-0.12	0.36
Cobalt	0.39	-0.03	-0.04
Copper	0.08	0.53	-0.01
Iron	0.37	0.20	-0.04
Magnesium	0.25	-0.45	0.11
Manganese	0.33	0.21	0.24
Nickel	0.37	-0.14	-0.13
Potassium	0.36	0.09	-0.17
Zinc	0.21	0.50	0.06
Eigenvalues	6.00	2.64	1.27
Cumulative percentage	54.56	78.56	90.12
	ELEMENTAL CONTENT OF EXTRACTS AT pH 5.9		
Eigenvectors			
Calcium	0.23	0.70	-0.03
Iron	-0.45	0.01	0.87
Magnesium	0.60	0.03	0.30
Manganese	-0.21	0.71	0.02
Nickel	0.58	-0.03	0.38
Eigenvalues	2.22	1.56	0.71
Cumulative percentage	44.44	75.72	89.99

Further discrimination of the vegetation communities is shown in Fig.20 where at the bottom of the *y*-axis (principal component 2) the Cobb Asbestos Mine community (F) grows over soils rich in the "ultramafic" elements chromium, cobalt, magnesium and nickel (large negative coefficients) and where at the top of the plot, the positive eigenvector coefficients indicate elevated levels of the other elements aluminium, copper, iron, manganese, potassium and zinc in the soils.

The correlation coefficients of Table 15 are further evidence of the inverse relationship between aluminium and the typical "ultramafic" magnesium and heavy metals. The respective contributions of components 1 and 2 to the total variance of the system are 54.56% and 24.00% The third component, a further 11.56% of the variance, is weighted very heavily to "non-ultramafic" calcium (eigenvector coefficient of 0.85 in principal component 3).

When PCA was applied to a number of extractable metals (calcium, iron, magnesium, manganese and nickel), the cumulative percentage of the first three components was 89.99%. Considering ultramafic and non-ultramafic soils as separate populations, the spatial separation of the fields was better than in the case of the whole-element analyses (Fig.21) but there was some overlap of the individual fields. The major loadings of the eigenvector coefficients for component 1 were from nickel and magnesium (0.58 and 0.60 respectively) though there was a surprisingly high negative loading for iron (-0.45). Component 2 was dominated by calcium (0.70) and manganese (0.71). Even after making allowance for the smaller number of elements used for the extracts (poor detection limits rendered impracticable the determination of the other elements), it is clear that PCA using extractable fractions of the elemental content of the soils would also allow for a satisfactory discrimination of the different groups of soils and the vegetation communities that they supported.

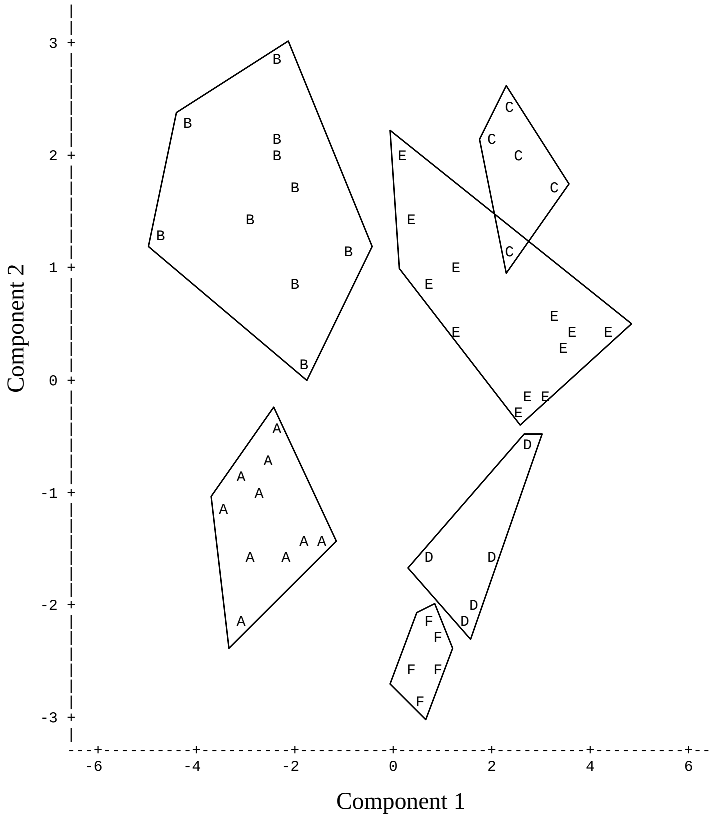

Fig. 20 Plot of component 2 vs. component 1 for logarithmically transformed total elemental concentrations in soils. Symbols as in Table 9.

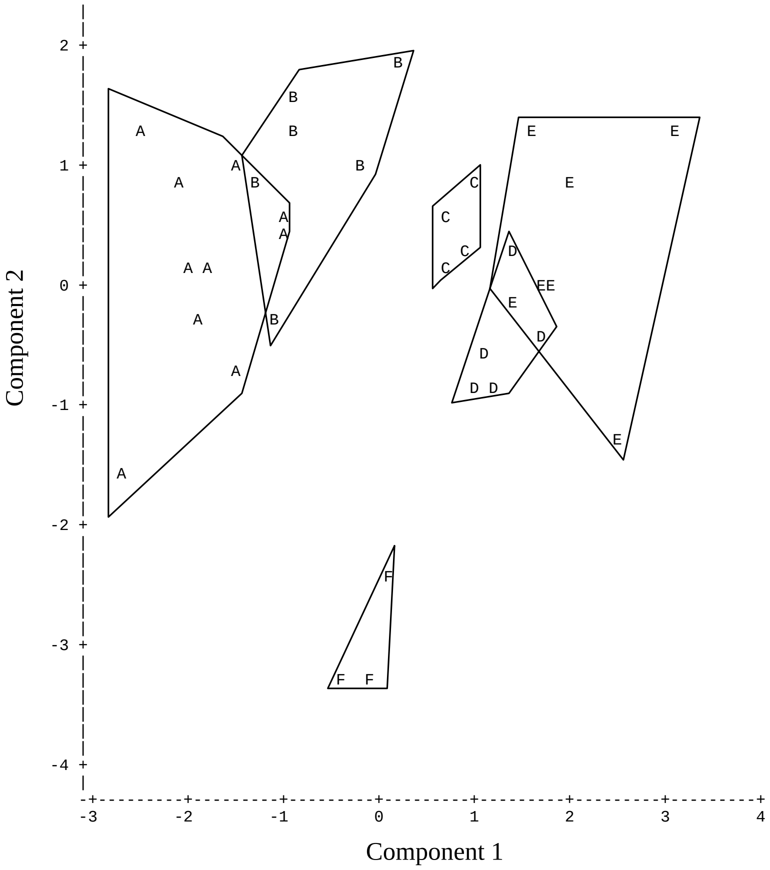

Fig.21. Plot of component 2 vs. component 1 for logarithmically transformed extractable concentrations of 5 elements in soils. Symbols as in Table 9.

It was concluded that the vegetation groupings are strongly dependent on the chemical composition of the soils. Since both magnesium and nickel have a dominant role (high eigenvalues) in the major PCA component of both the total and extractable elemental concentrations in soils, these two elements are clearly important in defining the character of the overlying vegetation. This role is likely to be more important than in the case of elements of lower mobility such as chromium.

One problem that has to be addressed in making conclusions from this study, is the degree to which other "serpentine factors" such as lack of nutrients and drainage control the nature of the overlying flora. Such factors are dependent, however, on the chemical composition of the soils or the rocks from which they are derived. They are adjuncts to the edaphic factors rather than separate causative effects and cannot be considered in isolation.

It is proposed that PCA applied to the elemental content of soils has proved to be a useful tool to establish perhaps the most important of the causes of the plant communities found over soils of the Dun Mountain Ophiolitic Belt and its immediate surroundings. In this example, edaphic factors such as the elemental content of the soil have predominant influence on the development of vegetation. The findings may not represent a final solution of the "serpentine problem" but they have highlighted the fact that it is likely that the solution to the problem will be found in these edaphic factors.

Summarising conclusion

The infertility of the ultramafic soils tested is primarily attributed to the toxic effects of nickel and/or magnesium. The situation is worsened by the relatively high solubilities of these two elements that increase significantly across an ecotone going from non-serpentine to serpentine soils. At present it is not possible to distinguish whether nickel or magnesium is the more important factor. This may be resolved in the future if the soils are to be used for nickel phytomining; Several nickel hyperaccumulator crops will deplete the nickel in the soil, without greatly reducing the levels of magnesium. Assuming the soil is fertilised so that plant nutrients are not depleted, the fertility of a relatively nickel-free soil may be tested on non serpentine-tolerant species. If these species are able to survive then the toxicity of nickel would be likely to be the primary factor controlling the vegetation.

Although not primarily responsible for the occurrence of the serpentine vegetation in New Zealand, the lack of plant nutrients and the erosive and xeric nature of the soils also contribute to their infertility.

It is to be noted that the results of these experiments are only applicable to the ultramafic soils of the Nelson Region, New Zealand. The flora of other serpentine soils may be controlled by different factors. This is more likely to be the case in the laterised serpentine soils found in tropical and subtropical regions, such as New Caledonia and North Cape, New Zealand. Separate studies will need to be conducted on these soils to determine which factor or factors are the most important in controlling their vegetation.

Implications for phytoremediation and phytomining

Of the heavy metals found in the soils tested, only nickel, iron, manganese and possibly cobalt are soluble at sufficient concentrations to be taken up in significant quantities by plants. Of these metals, iron and manganese are of insufficient value to be economically extracted and in any case do not contribute appreciably to the toxicity of the soil. The relative solubility of the essential plant nutrient cobalt is comparatively low, and though it is taken up by serpentine plants to concentrations of up to 100 μg/g (cf. chromium, which is never taken up to any significant degree, Brooks, 1987), there are no reported hyperaccumulator plants of this element that grow naturally on serpentine soils. The only known cobalt hyperaccumulators occur on the non-ultramafic copper-cobalt soils of former Zäire (Reeves *et al.*, 1995). It is not known if these plants are able to survive on serpentine soils, and if they could still hyperaccumulate cobalt. Nickel is relatively soluble, and has sufficient economic value to be potentially extracted commercially from serpentine soils if a hyperaccumulator plant can be found with sufficient biomass. Most of the 400 known species of nickel hyperaccumulator occur naturally on serpentine soils, and thus are tolerant to the soils' inherent infertility.

Although the New Zealand ultramafic regions do not support any native species of hyperaccumulator plant, glasshouse experiments have shown that several exotic species will grow in these soils (unpublished data).

The relatively low concentrations of plant nutrients in serpentine soils imply that yields of hyperaccumulator crops may be substantially increased after fertilisation.

If excess nickel is the primary cause of serpentine infertility, the fertility of serpentine soils may be improved by phytoremediation. This operation could be cost effective as the nickel could be sold after incineration of plant material. Serpentine soils however, have several other properties that make them relatively infertile, and these are unlikely to be improved by phytoremediation. Serpentine soils are often valued for their natural vegetation and this has to be balanced against any potential gains of phytoremediation and/or phytomining.

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SECTION B
THE PHYTOMINING OF NICKEL FROM SERPENTINE SOILS.

One of the first potential uses of hyperaccumulator plants that comes to mind to an individual who has been newly introduced to the subject is “Could the metal in these plants be harvested”. Despite this obvious conclusion, very little work has been done in the field of phytomining. This is because it has always been assumed that the metal concentration in the plants, and their biomass production would always be too low to exploit commercially the metals they accumulate. In any case, there are existing techniques that enable the efficient extraction of vast quantities of metal from the earth. There is no world-wide shortage of metals, so why bother investigating a new and dubious technique? In 1995 Nicks and Chambers published the first work on this subject. They showed that a small herb found on the Californian serpentines, *Streptanthus polygaloides* had the potential to extract 100kg / ha of nickel. The value of this metal was at the time $US 720. If only half of this value is returned to the farmer, the net profit ($US 360) is greater than the net return on a crop of wheat ($US 333). The authors suggested that the combustion of the plant material (10t / ha) would provide 175GJ of energy worth $3 per GJ. Even a low 25% recovery of this energy would add $131/ha, further augmenting the profitability of the operation. It would seem that phytomining might be economically feasible after all, if other factors are favourable.

A generalised diagram of a phytomining operation is shown in Fig. 22. As with any phytoextraction operation, the process is centred around plants capable of hyperaccumulating the metals that are to be mined. Returns would come from the sale of the metals and from energy derived from the combustion of the plant material. The economics of phytomining rely overridingly on the price of the metal. Metal yields per hectare by plants could never exceed a theoretical maximum of 900 kg/Ha. For yields of this order of magnitude, the crop would have to contain 3% metal (above the highest reported metal concentration in a hyperaccumulator Brooks, 1998) and have a biomass production of 30t/ha/yr (equivalent to *Zea mays*). At present there are no known hyperaccumulators that combine high biomass yields and a high metal concentration. This limit of production means that cheaper metals such as iron, manganese, and lead will never be phytomined economically (however they may help recoup the cost of a phytoremediation operation see Sec. 3). As the price of the metal increases however, the required biomass production and metal content of the crop decrease exponentially (Fig. 24). For costly metals such as gold, the amount required per hectare to yield $US 500 is only 37 g. This quantity could be achieved with a plant metal content as low as 1 μg/g. This blurs the definition of a hyperaccumulator. It might be argued that in the case of phytomining a hyperaccumulator is a plant which as the ability to return an economic crop of metal. There have been no reports of plants that accumulate noble metals to any degree. It may however be possible to induce the uptake of noble metals by the addition of complexing agents to the soil. Anthony and Williams (1994) showed that the addition of thiosulphate to soil significantly increased the solubility of some platinum group metals. Mehra *et al.* (1996) showed the presence of specific silver binding phytochelatins in some yeasts and plants. Future genetic modification of plants and/or soil amendments may allow the hyperaccumulation of silver, gold and the platinum group elements.

The most economically valuable metal which plants have been reported to hyperaccumulate is cobalt. Cobalt hyperaccumulators are found exclusively on the copper-cobalt soils of Zaïre. Serpentine soils represent the majority of low-grade cobalt ore-bodies, and it is unknown if these plants will even grow, much less hyperaccumulate cobalt, on such soils. Work done at Massey University, showed that *Haumaniastrum katangense,* and *H. robertii,* two copper-cobalt accumulators from Zaïre, do not accumulate cobalt or copper on serpentine soil (Homer, 1991). The next most valuable metal that can be hyperaccumulated is nickel. This metal has probably the best chance of being phytomined economically. There is a plethora of known nickel hyperaccumulators, and large expanses of sub-economic ore bodies or serpentine soils upon which operations may be conducted.

So why bother to phytomine? Why not use conventional techniques that might have greater profit margins? The first response to these questions is that phytomining allows the exploitation of ore-bodies that contain too little metal to be economically extracted and smelted by conventional techniques. The soil upon which Nicks and Chambers conducted their experiment contained 0.5% Ni. This is well below the minimum concentration needed to commercially smelt nickel (3%). *Streptanthus. polygalodies* contains around 1% nickel in its dry biomass. When burnt, the resulting bio-ore contains 15% nickel, more than enough to be economically smelted. Moreover associated pollutants such as sulphur and iron that are often found in nickel ores are virtually absent from bio-ore, which principally consists of potassium and calcium carbonates, and nickel oxide. Nickel can be smelted from crops containing as little as 0.2% of the metal in the dry biomass, and some plants can attain this concentration from soils with less than 0.05% nickel. Phytomining could therefore allow the exploitation of many ore bodies that are unusable using current techniques, and may allow the continuation of mining on existing mines that have become exhausted of nickel-rich ore. There will be a greater need to exploit such areas in the future, as current mines become exhausted and there is a greater world-wide demand for the metal.

PHYTOMINING MODEL

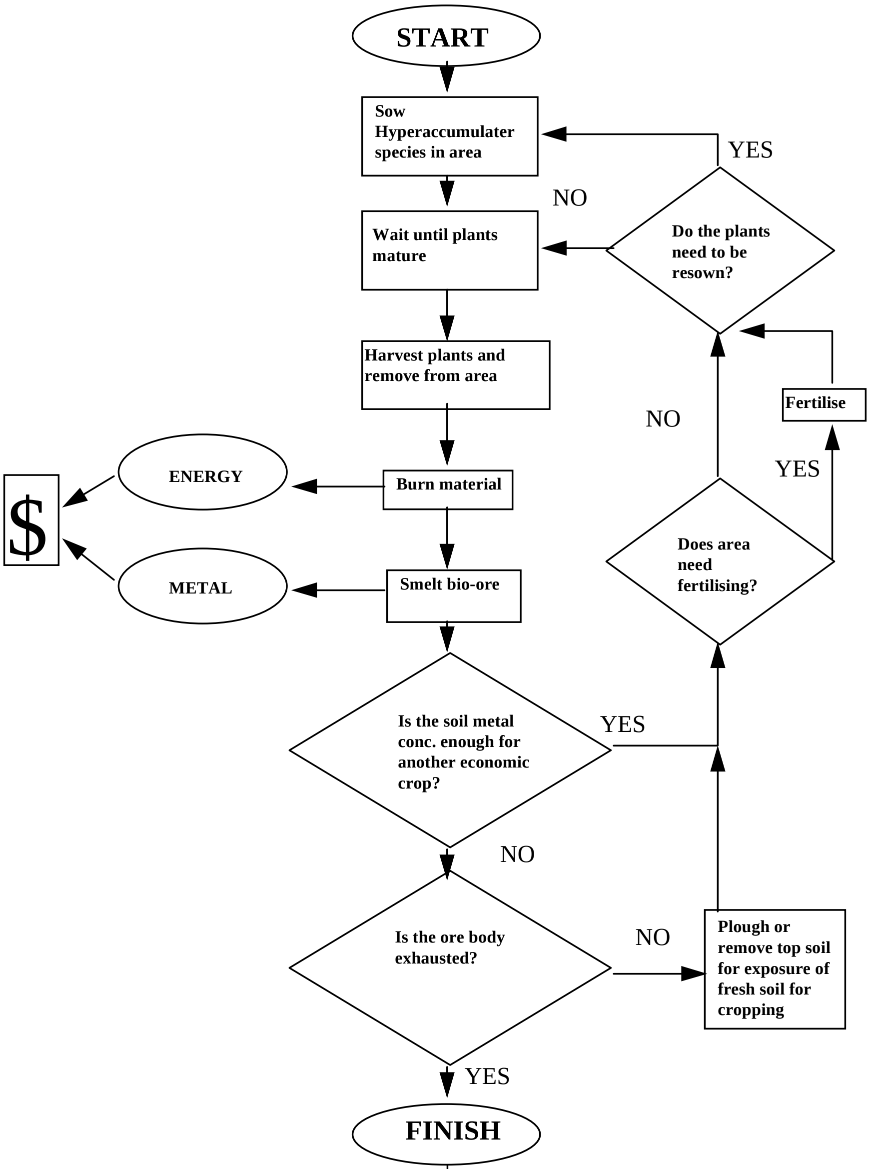

Fig. 22 Flow diagram of phytomining operation

Phytomining would be very different from conventional mining in its environmental impact (Fig. 23). Sites necessarily have to be vegetated (using hyperaccumulators) to be phytomined, this removes many problems associated with conventional mining techniques. Erosion, wind-borne dust laden with heavy metals, and cave-ins will not affect any phytomining operation. At the end of the operation there will be no revegetation problem, as the hyperaccumulator plant will remain on the exhausted area. There will also be negative effects however. To be economic, a phytomining operation will require many hectares, as opposed to a more localised conventional operation. This large area will have to be completely denuded of its natural vegetation to make way for the hyperaccumulator species. When the operation has finished, these species may be difficult to remove, becoming a localised weed. As with any mining operation, the conservation value of the land upon which it is to be undertaken will need to be carefully assessed.

Fig. 23. The effects on the landscape of open-cast nickel mining. Thio, New Caledonia.

The three following chapters investigate the possibility of phytomining nickel from serpentine soils using two different nickel hyperaccumulators. The broad aims of each chapter are to determine the economic feasibility of the phytomining operation, both on the plants natural ranges and in other locations throughout the world. The third chapter investigates the sustainability of nickel phytomining on serpentine soils i.e. how many economic croppings can be sustained from a soil before it needs to be modified.

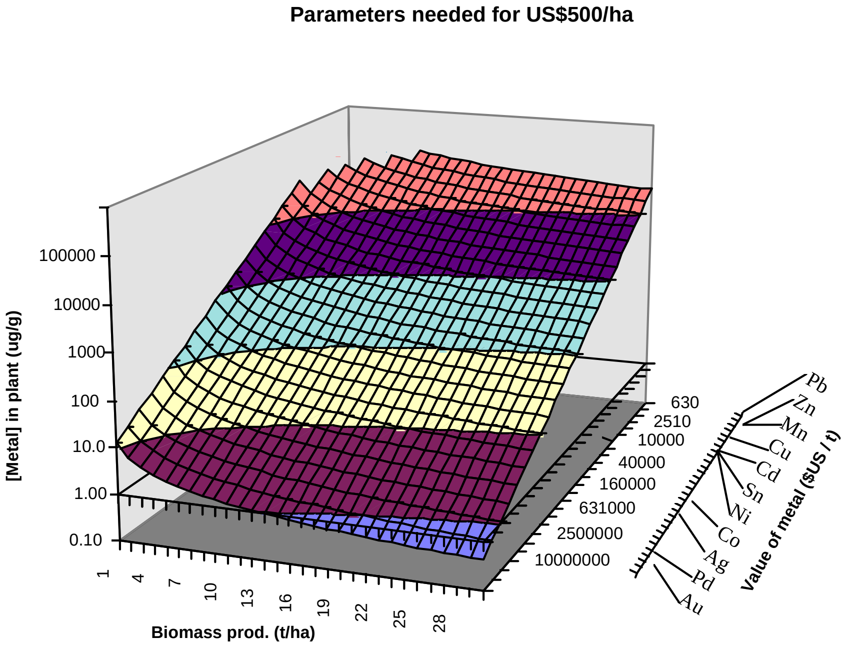

Fig. 24.

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Chapter 4

The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel

Publication arising from chapter:

Robinson, B.H., Chiarucci, A., Brooks, R.R., Petit, D., Kirkman, J.H., Gregg, P.E.H., and De Dominicis, V., 1997. The nickel hyperaccumulator plant *Alyssum bertolonii* as a potential agent for phytoremediation and phytomining of nickel. J. Geochem. Explor. 59, 75-86.

Abstract

Experiments were carried out in Italy on the potential use of the hyperaccumulator *Alyssum bertolonii* in phytomining of ultramafic soils for Ni. *In situ* experimental plots at Murlo, Tuscany were fertilized with various regimes during a two-year period. The best fertilizer treatment (N+K+P) gave a threefold increase of the biomass of reproductive matter to 9.0 t/ha without dilution of the unfertilised Ni content. A Ni content of 0.8% in dry matter (11% in ash), would give a Ni yield of 72 kg/ha without need of re-sowing for a further crop. There was no correlation between the age of a plant and its Ni content. A proposed model for phytomining involves harvesting the crop after 12 months and burning the material to produce a sulphur-free bio-ore with about 11% Ni. Utilising the energy of combustion is also discussed. It is considered that *Alyssum bertolonii* or other *Alyssum* species might be used for phytomining throughout the Mediterranean area including Anatolia, as well as in Western Australia and the western United States. The economic limits of phytomining are proposed and at current world prices, the technique would only be feasible for Ni and Co with plants of at least the same biomass as *Alyssum*. Plants of higher biomass and similar Ni uptake potential could extend the limits to other elements.

Introduction

Alyssum bertolonii Desv. (Brassicaceae) is a most unusual plant. It was first reported by Caesalpino (1583) who described an "alyson" that appeared to be confined to nickel-rich serpentines near Florence, Italy. Its presence over all Tuscan ultramafic outcrops was recorded by several authors (see Pichi Sermolli (1948). In the late 1940s, Minguzzi and Vergnano (1948) discovered that this plant had an extraordinarily high Ni content of about 10,000 μg/g (ppm)[1%] in dried matter that translated to well over 10% of this element in the ash.

Since the discovery of the world's first "nickel plant”, several others were identified by Brooks *et al.*, (1977) who named them "hyperaccumulators of Ni" in a paper that appeared in *Journal of Geochemical Exploration*. They set the threshold of hyperaccumulation at 1000 μg/g (0.1%) in the case of Ni. Since this early work, the total of hyperaccumulators of Ni has now exceeded 400 (Baker and Brooks, 1989; Brooks, 1987; Jaffré, 1980; Reeves *et al*., 1996). There are also hyperaccumulators of Cu and Co (Brooks *et al*., 1980) and of Zn (Baumann, 1885; Reeves and Brooks, 1983). The hyperaccumulation threshold level for Zn has been set at 10,000 μg/g (Reeves and Brooks, 1983).

Hyperaccumulation of Ni has also been recorded in *Streptanthus polygaloides* from California by Reeves *et al*. (1981) who reported up to 14,800 μg/g (1.48%) in the dried matter of this plant. This is the only truly native nickel hyperaccumulator so far discovered in North America.

Over a decade was to pass until it was realised that the hyperaccumulator plants might be used for removal of pollutants from soil (McGrath *et al*.,1993). A group at Rutgers University is also actively engaged in exploring the commercial potential of *phytoremediation* (removal of pollutants from soils by use of plants) and has formed a company for this purpose.

The potential for using hyperaccumulators as a means of "growing a commercial crop of nickel" represents a quantum leap in the possible extension of phytoremediation to this new technology and is due to the pioneering work of L. Nicks and M.F. Chambers at the US Bureau of Mines, Reno, Nevada (Nicks and Chambers, 1994, 1995). Their work was based on the principle that it might be possible to use plants to extract Ni from low-grade ores that would otherwise not be economic. Low-grade nickel ores cover large areas of the Earth's surface, particularly in Western Australia, Italy, Brazil, Canada, Russia and many other countries and territories.

Fig. 25. *Alyssum bertolonii* a hyperaccumulator of nickel containing 1% of this element.

Nicks and Chambers grew the Californian hyperaccumulator *Streptanthus polygaloides* in various soil mixtures and found that there was a five-fold increase of biomass when N,K,P fertilizer was added. Over an area of serpentine soils near Chinese Camp in California, they found that a natural crop of this plant was capable of producing up to 100 kg/ha of Ni (worth $550/ha at the prices at that time). If a large-scale industry could be developed with continuous incineration of the crop, an additional $219/ha from the energy of combustion would be available. They concluded that the return to a farmer growing a "crop of nickel" (i.e. half the gross yield) would be roughly comparable, or superior to, that obtained for a crop of wheat.

The work reported below is based on the principles established by Nicks and Chambers (1994) and appears to represent only the second report ever published on the potential of phytomining based on field work under natural conditions using native plant species rather than exotic taxa. The work was carried out in Tuscany, Italy, using stands of *Alyssum bertolonii* growing under natural conditions over the ultramafic (serpentine) soils of the region.

The aims of the experiments were to assess:
1 - the approximate yield of Ni per hectare,
2 - the relation between the Ni content of the plant and the available Ni status of the soil,
3 - the effect of plant age and size on the Ni content of the plant,
4 - the effect of fertilisers on biomass and Ni content of the plants,
5 - the reduction of Ni availability in the soil after successive croppings.

Site description

The test areas were located on Mont Pelato (350 m) in Livorno Province and near the village of Murlo (350 m) south of Siena, Italy (Fig.26). The rocks are composed of lherzolitic serpentinites emplaced in gabbro and basalt. The soils are often skeletal with a low water-holding capacity (Vergnano Gambi, 1992). The pH ranges from 6.6-7.4 on serpentinite and 6.8-7.0 on gabbro.

Arrigoni *et al.* (1983) distinguished a specific vegetation community on the screes and debris. It is known as the *Armerio-Alyssetum bertolonii* vegetation type and is spread over all of the Tuscan ultramafic outcrops. It encompasses all the serpentine-endemic plants including of course, *Alyssum bertolonii* (Fig.25) itself (Chiarucci *et al.*, 1995). This species and its community are absent over the gabbro and basalt.

Climatic data are available for both Monte Pelato (Cozzi, 1996) and Murlo (Barazzuoli unpub. data). Both sites can be classified bioclimatically as Mediterranean pluvio-seasonal oceanic (Rivas Martinez, 1996).

Fig. 26. Map of north-central Italy showing areas of ultramafic rocks and sites of this study.

For Murlo the mean annual temperature is 13.8°C ranging from 5.8° in January to 22.7° in July. The total annual rainfall is 893 mm ranging from 37 mm in July to 129 mm in November. For Monte Pelato the mean annual temperature is 12.6°C ranging from 4.3° in January to 21.7° in July/August. The total annual rainfall is 978 mm ranging from 21 mm in July to 137 mm in November.

Materials and methods

Forty plants of *Alyssum bertolonii* were selected over serpentines of the Monte Pelato area of Livorno Province, Italy. The crown and stem diameters of each plant were measured along with the biomass and Ni content of the reproductive and vegetative structures. The ages were determined by ring counting. Soil (ca. 100 g) was collected below each plant and the soluble fraction of various elements was determined by extraction with ammonium acetate at pH 7.

Nickel was added as the nitrate to a standard commercial seed compost to give total concentrations of 0, 14, 41, 123, 370, 1111, 3333, and 10000 μg/g. The organic components in the seed compost absorbed some of the nickel, reducing the soluble fractions in the soil (as measured by a 1M ammonium acetate extract) to 1.6, 1.9, 6.1, 18.2, 120.8, 492.1, and 1500.0 μg/g. Plants were grown in 500 mL pots for 6 months then excised at ground level and analysed.

At Monte Murlo, 35 random quadrates (1m x 1m) were selected in Spring 1994 on a gently sloping hillside. In each plot the presence of all vascular plants was recorded and their coverage estimated by the points-quadrate method which estimates the ground cover of a given plant by the relative interceptions of the plant canopy with regularly spaced point observations which, in this case, were made on a square grid with 5 cm spacing of each point. These quadrates encompassed natural stands of *Alyssum bertolonii* that were treated with the following fertiliser regimes in Autumn (October) of the same year: 1 - calcium carbonate at 100 g/m^2, 2 - sodium dihydrogen phosphate at 10g/m^2, 3 - ammonium nitrate at 10 g/m^2, 4 - sodium dihydrogen phosphate + ammonium nitrate each at $10g/m^2$, 5 - sodium dihydrogen phosphate + ammonum nitrate + potassium chloride each at $10g/m^2$, and 6 - calcium carbonate + sodium dihydrogen phosphate + ammonium nitrate + potassium chloride each at the loadings shown above. There were five replicates of each treatment including five controls.

The fertiliser treatment was repeated one year later in October 1995. The increase in biomass of *Alyssum bertolonii* was noted by measuring the increase in cover (the relationship between cover and biomass having been previously established by experiments in which plants were harvested, dried and weighed at the end of each year for a period of two years). The plants were harvested from each quadrate at the end of the two-year period and a soil sample (0-15 cm depth and weighing 100 g) was also taken.

Measurement of plant-available trace elements in the soils was performed by placing 2 g of sieved (2 mm) soil samples in polythene containers and adding 20 mL of 1M ammonium acetate. The containers were shaken overnight and the filtered liquid phase was analysed by plasma emission (ICP) spectrometry.

Measurement of pH in soils before and after fertilisation experiments was performed by preparing a 1:2.5 soil/water suspension in which the readings were made.

In statistical tests on the biomass of *Alyssum*, the total biomass was transformed logarithmically and submitted to analysis of variance (ANOVA). Statistically significant differences at the 5% level ($P<0.05$) were determined by the LSD test.

Results and discussion

Natural levels of nickel in *Alyssum bertolonii*

Natural concentration levels of Ni in 40 specimens of *Alyssum bertolonii* from Monte Pelato are given in Table 17. The mean value of 0.70% for dried tissue of reproductive and vegetative structures is almost exactly the same as the 0.71% recorded for the 15 specimens analysed by Brooks and Radford (1978). The latter reported a maximum value of 1.35% compared with the somewhat higher maximum of 1.65% in this study. In absolute terms, mean Ni concentrations in plant ash were on average over 60 times that of the soil and in some cases could exceed the soil content by a factor of over 160.

Table 17. Nickel concentrations (%) in soil and in *Alyssum bertolonii* (N=40) from Monte Pelato

Material	Dry material	Plant ash	Plant ash/soil
PLANT			
Reproductive tissue mean	0.72	10.24	64
Reproductive tissue range	0.3-1.48	4.33-21.12	27-132
Vegetative tissue mean	0.69	9.81	61
Vegetative tissue range	0.23-1.83	3.32-26.11	21-163
SOIL	0.16		

Relationship between soil nickel content and nickel concentration in *Alyssum bertolonii*

Fig. 27 shows the nickel concentration in *A. bertolonii* grown in soils with varying nickel concentrations. The relationship is logarithmic between 1.6 and 120 μg/g. In soils with nickel concentrations above this value, the nickel concentration in the plant increased exponentially. This may be explained by the saturation of the plant's nickel uptake control system. Plants in these higher concentrations showed reduced growth and chlorosis relative to the other plants, indicating nickel toxicity. The results indicate that the maximum concentration of nickel obtainable in *A. bertolonii* is around 1.5%, before biomass production is impaired.

Relationship between age and nickel content of *Alyssum bertolonii*

Statistical analysis of the data indicated that there was no strong correlation between the age of the plant and its Ni content ($P>0.05$). This implies that Ni crops could be harvested from all plants up to the maximum age recorded (11 years) without there being any differences in yield associated with varying ages of the plants.

Effect of fertilisers on biomass increase and nickel content of *Alyssum bertolonii*

In the experimental quadrates at Murlo, there was a highly significant positive correlation ($0.01>P>0.001$) between Ni uptake in the reproductive structures of *A. bertolonii* and the extractable Ni content of the associated soils (Fig.28 - because of overlap only 28 of 35 points are shown). This indicates that addition of fertilisers that increase this available Ni content should increase the Ni content of the plants. Table 18 shows the effect of 6 fertiliser regimes on the extractability at pH 7 of various heavy metals from the soils. Extractability of Ni was virtually unaffected by the nature of the treatment except in the case of $CaCO_3$ where the extractability of the former was halved. A similar result was obtained with the N+K+P+Ca fertilizer. Chapters 2 and 3 have shown that the availability of trace elements in serpentine soils decreases exponentially as the pH is raised. This highlights the importance of avoiding $CaCO_3$ if a Ni "crop" is desired. There was a similar reduction in Mg availability when Ca was used in fertilizers. Paradoxically, addition of $CaCO_3$, though reducing Ni and Mg availability, has the effect of rendering the soil more fertile for non-serpentinic plants by increasing the Ca/Mg quotient from ca. 0.5 to 3.0. This increase would clearly improve a crop such as wheat or barley but reduce a "crop" of Ni.

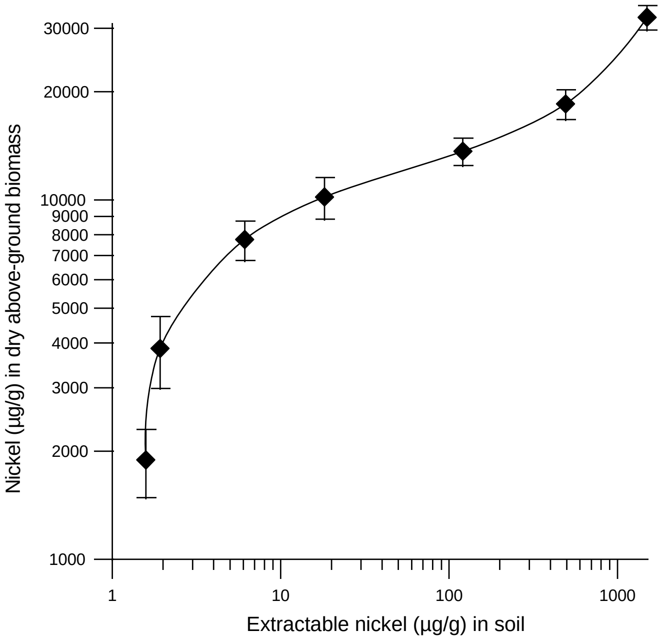

Fig. 27. Nickel uptake by Alyssum bertolonii vs ammonium acetate extractable nickel in soil

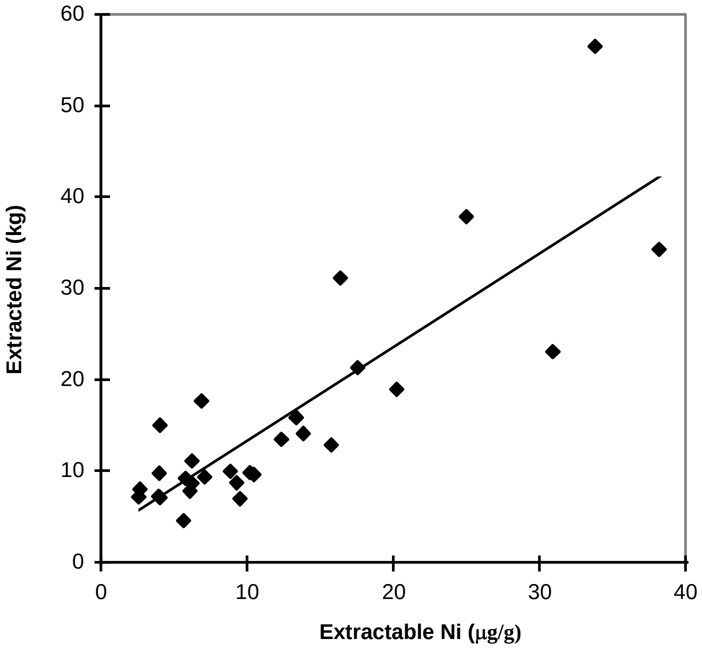

Figure 28. Ni extracted by a crop of Alyssum bertolonii (kg/ha) as a function of the available Ni content of the soil as determined by ammonium acetate extraction at pH 7.

With the addition of fertilizers, the maximum annual biomass increase (ABI) was about 300% (Table 19). The highest individual increase (129%) was with N alone, and the highest combined increase (308%) was with N+P+K. Table 19 shows the product of ABI and Ni content. It appears that there is no appreciable decrease of Ni concentration with increase in ABI. In other words there is no trade-off in reduced Ni concentration to offset the gain in biomass. The biomass of a fertilized crop of *Alyssum bertolonii* was calculated by multiplying the unfertilised yield (4.5 t/ha) by a factor of two rather than three in order to err on the conservative side. This gives a fertilized biomass of about 9.0 t/ha. In experimental 1 m^2 serpentine plots at Massey University yields of 3.4 and 13.0 t/ha for unfertilised and fertilised plots respectively were obtained. This is in very good agreement with the Italian findings on natural stands of *Alyssum*. This also compares with the 23.0 t/ha obtained by McGrath *et al.* (1993) for fertilised plots of *Alyssum tenium*.

Table 18. Mean (N=5) extractability at pH 7 of trace elements (µg/g in original soil) in serpentine soil from Murlo.

Treatment	Ni	Co	Cr	Ca	Mg	Ca/Mg
Control	4.43	0.095	0.048	837	1555	0.54
Ca	2.23	0.072	0.034	3801	1274	2.98
N	4.51	0.082	0.026	790	1702	0.46
P	3.53	0.084	0.036	817	1918	0.42
N+P	3.64	0.088	0.012	822	1855	0.44
N+K+P	4.37	0.072	0.026	959	1808	0.53
N+K+P+Ca	2.39	0.090	0.012	3718	1148	3.23

Table 19. Percentage annual biomass increase (ABI) and elemental composition of *Alyssum bertolonii* given various fertilizer treatments at Murlo. All values given as % on a dry matter basis. The pH of the soils after treatment are shown in the final column.

Treatment	ABI	Ni	K	Na	Ca	Mg	NiB	pH
Control	*21.6ab	0.77	1.19	0.13	1.66	0.74	16.6	7.37a
Ca	51.3abc	0.68	1.20	0.16	1.65	0.46	34.9	7.94e
N	129.7abc	0.54	1.09	0.13	1.01	0.43	68.7	7.50bc
P	100.8a	0.61	1.39	0.16	0.81	0.41	61.4	7.41ab
N+P	188.6abd	0.76	1.60	0.19	1.10	0.49	143.3	6.93cd
N+P+K	308.4cd	0.76	1.41	0.17	0.99	0.40	234.4	6.86d
N+P+K+Ca	294.2bc	0.53	1.18	0.15	0.89	0.25	155.0	7.75e

NiB - product of nickel concentration and biomass increase.

*This value is the normal annual biomass increase without use of fertilizers.

The biomass and pH data were subjected to ANOVA and statistically significant differences at the 5% level ($P<0.05$) were determined by the SNK test and indicated as letters after the ABI data. Groups having the same letters were not statistically different.

A proposed model for phytomining for nickel on a pilot scale using *Alyssum bertolonii*

From experience in the field at Murlo, it was envisaged that a pilot project might involve the following programme:

1 - select a suitable site where the topography would permit harvesting of the crop, ploughing, and fertilizer addition to the serpentine soil,
2 - seed the site directly or plant out seedlings at a rate of approximately 16 plants per m^2 (160,000 per ha),
3 - after a period of 12 months, harvest the reproductive structures with a harvester set to collect all vegetation above 10 cm from the ground,
4 - incinerate the crop in some type of incinerator and collect the bio-ore, which will have a Ni content of about 11%.

With application of N+K+P fertilizer, the yield of upper reproductive tissue should be about 9.0 t/ha. There would no problem in producing the seedlings as *Alyssum bertolonii* grows very quickly and produces a large quantity of viable seed that germinates in a few days. It must be emphasized that the first crop could not be taken during the first season in order to allow the plants to grow large enough in the second season for sustainable cropping

The economics of commercial exploitation of *Alyssum bertolonii* for phytomining or phytoremediation

Nicks and Chambers (1995) have proposed that commercial exploitation of the annual Californian hyperaccumulator *Streptanthus polygaloides* would produce about 100 kg/ha of Ni after moderate application of fertilizers. Calculations with *Alyssum bertolonii* gave a conservative value of 72 kg Ni/ha containing 0.08% Ni worth $539 at the present world price of Ni. However this Italian plant is a perennial with a life of about 10 years that might be extended with annual removal of the crowns. If only half of this sum represented a net return to the "phytominer", the value of the crop would be $269, a little lower than the net return of a hectare of wheat ($309). This of course presupposes that the costs associated with farming a crop of Ni would be the same as for wheat. It must be remembered however that native plants growing in their own natural environment should require less fertilizer and irrigation than a crop of wheat. There is also the fact that the *Alyssum bertolonii* crop is perennial and will not require re-sowing the following year.

The yield of Ni could be increased by removing some of the vegetative tissue of the plant along with the reproductive material. Another approach might be to add a complexing agent to the soil in order to increase the availability of Ni. Such a procedure would not be without risk. In experiments carried out in the United States, a crop of *Brassica juncea* has been grown in lead-contaminated soil and EDTA added to the soil once the plants became well established. At this stage the formerly immobile lead is complexed and taken up by the plant which then starts to die because of the phytotoxic nature of this element. The plants were harvested at this stage to phytoremediate the polluted soil. If such a procedure were carried out with *Alyssum bertolonii*, there would be a danger of death of the plant so that it could not be reharvested in the following growing season. Residual EDTA in the soil might also be a problem. There is nevertheless scope for controlled trials with EDTA or other complexing agents.

Another cost-effective strategy that might be adopted would be to recover some of the energy released during incineration of the biomass (17,500 kJ/kg for cellulose material). To quote Nicks and Chambers (1995), if only 25% of this energy were recovered, an additional $219/ha could be recovered making a gross return of $758/ha. If half of this sum were recovered by the company after making allowance for capital costs, fertilizers etc, the net return of $379 would be well above the net return of $309 from a crop of wheat obtained by American farmers in the period 1993/1994.

An obvious problem with the use of an incinerator to produce steam for power generation is that the crop harvesting would occur over a fairly short space of time and therefore the power plant should be situated near an urban area where domestic waste might be used as a feedstock to keep the plant going the rest of the year. There is also the possibility of two crops a year that would not only increase Ni yield but would give more work to a nearby incineration plant.

Although it must be clearly stated that the economics of *A. bertolonii* for phytomining are at the lower range of economic viability, the same is not true if instead this plant is used for phytoremediation of soils polluted with nickel. The costs of conventional methods of remediation such as removal and replacement of polluted soil and storage of the toxic material ($1,000,000/ha according to Salt *et al*., 1995), are so great, that a "green" method that would also permit recouping some of the costs by sale of an environmentally friendly "bio-ore" will clearly be of economic benefit.

In contemplating the possibility of phytomining in the future, a number of questions need to answered:

1 - Would conservationists allow a large area of serpentine soil to be colonised by commercial crops of A. bertolonii? To answer this question, it must be appreciated that in Italy at least, the species is endemic to serpentine soils and there would be no question of introducing an exotic species. In the worst case scenario the *Alyssum* would merely be replacing other serpentine-tolerant plants. Nevertheless, it would be unreasonable to expect Italian conservationists to allow unrestricted use of serpentine environments for phytomining unless such use were confined to degraded land such as in the vicinity of former mines. The most likely sites where phytomining could be allowed, either in Italy or elsewhere, would be as a "green" alternative to opencast mining. If *Alyssum bertolonii* were to be used for phytoremediation of soils polluted by nickel as a result of industrial activity, a very different situation would arise, a situation where the blessing of conservationists might be expected.

2 - Would phytomining involve using land that might have been used for agriculture? The answer here is very clear. By its very nature, ultramafic soils are extremely hostile for unadapted plant life and are almost never used for food production.

3 - What will happen to the price of Ni in the future? This question is hard to answer. The metal has been constantly rising in price for the past few years, but there is no guarantee that its price will not collapse. It is however a metal whose price is relatively stable unlike metals such as tin. In any case, phytomining will be just another mining technique no more susceptible than others to fluctuating world prices.

4 - Are there any other Italian hyperaccumulators that might usefully be used? Alyssum argenteum Burtt. occurs over ultramafic outcrops to the northwest of Italy and has an even higher biomass than the closely related *A. bertolonii*. It might be equally useful for phytomining, though this will have to be tested by experimentation.

5 - Could Alyssum bertolonii be used for phytomining in other parts of the world? This plant is only one of 48 species of *Alyssum* reported by Brooks *et al*. (1979) as being hyperaccumlators of nickel. These plants are found in most parts of southern Europe where there are extensive areas of serpentine rocks. The centre of diversity of these plants is in Anatolia, Turkey, where there are large areas of ultramafic rocks (see Fig.29). Most of the Turkish *Alyssum* species have similar biomass and metal accumulation as *A. bertolonii*. There is no reason why phytomining could not be carried out in that country using the Italian plant or local *Alyssum species.* Throughout the Balkans there are also extensive outcrops of ultramafic rocks that might be colonised by other high biomass/high Ni uptake species such as *A. murale*. Other areas that might be considered for phytomining are the extensive outcrops of nickel-rich serpentine soils in Western Australia, California and Oregon where climatic conditions are similar to those of the Mediterranean area.

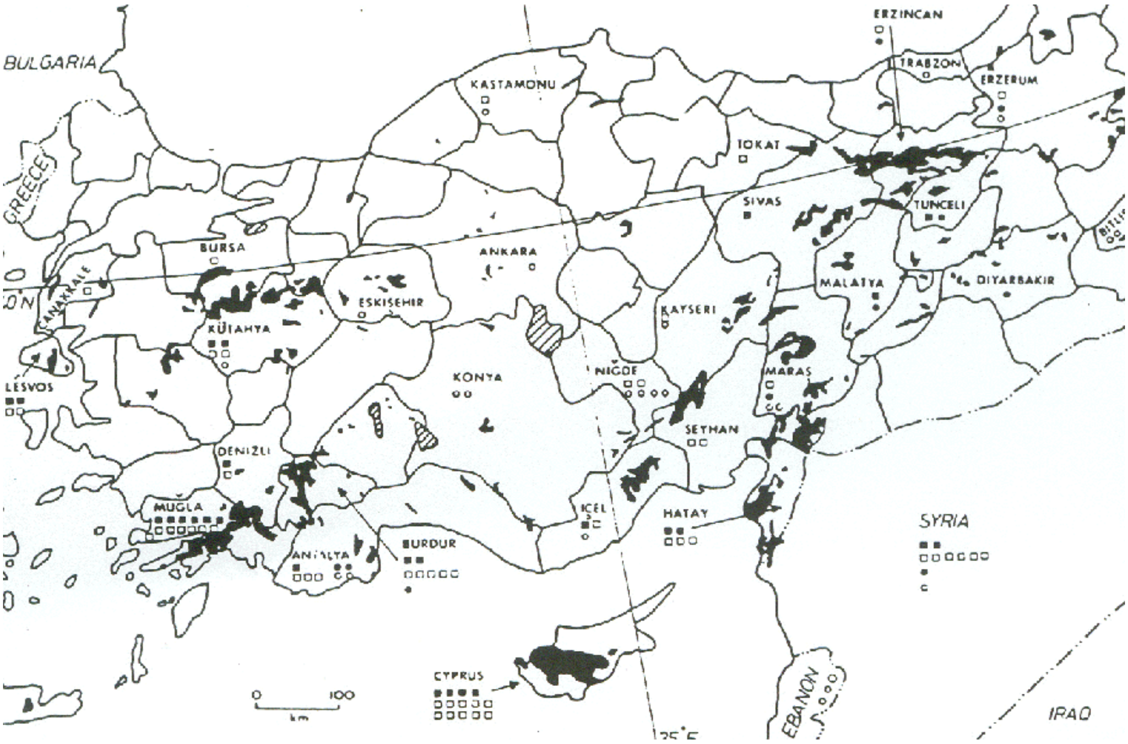

Fig. 29. Map of Anatolia, Turkey, showing location of ultramafic rocks and the distribution of Turkish *Alyssum* species. The Key for the latter is as follows (all Ni concentrations in dry material): solid squares - >1% Ni, open squares - 0.1-1.0% Ni, solid circles - 0.01-0.1% Ni, open circles - <0.01% Ni. Source: Brooks *et al.* (1979).

6 - How hardy is A. bertolonii and resistant to insect attack or disease, i.e. could a crop be wiped out in a single year by such agencies? Hyperaccumulators of Ni and other elements have a strong protection against predator attack because of their high Ni content. Boyd and Martens (1994) have carried out experiments on nickel hyperaccumulators to illustrate this protection. In the course of extensive trials on the above plant no tendency to disease was observed. *A. bertolonii* is an exceptionally hardy plant that will withstand extremes of temperature. Tests in New Zealand have shown that it will even grow over asbestos tailings where no other dicotyledonous plant will survive.

7 - How environmentally acceptable would phytomining be? By its very nature phytomining will be seen as "green" by many critics of the mining industry. There will be others, however, who will decry the suggestion of bulldozing away other native plants to make way for large crops of *A. bertolonii*. This question is a moral one and hopefully some solution will be found to satisfy all interested parties.

The economic limits of phytomining

Although there are clearly economic limits in terms of biomass production and metal content in respect of the potential use of *A. bertolonii* or other plants of similar biomass for phytomining, the same is not true for the wider subject of phytoremediation. This new technology is now being developed (Salt *et al.*, 1995) in which hyperaccumulators are proposed as a means of removing many pollutants from soils using this essentially "green" and inexpensive technique, of which phytomining is merely an extension. Whereas phytomining is limited by the need to produce a commercially viable metal crop, this is not the case for phytoremediation where *A. bertolonii* should also have a wide application.

Table 20 shows the elemental content (μg/g in dry matter) that would be required in a plant with the same biomass as *A. bertolonii* in order to give the same gross financial return as that indicated by experiments for the Murlo area. So far, only Co and Ni appear to be suitable candidates for phytomining with plants of this size of biomass. Hyperaccumulators with 1% (10,000 μg/g) Co are known from the former Zaïre (Brooks and Malaisse, 1985) and many *Alyssum* species can have Ni contents well exceeding 1% (Brooks *et al.*, 1979). There are no records of plants whose noble metal contents exceed the values shown in Table 20 and neither are there any for Sn, Cd, Cu, Zn or Pb.

The above problem can be addressed to some extent by use of hyperaccumulators of higher biomass combined with a sufficiently high metal content. Table 21 shows the biomass needed to give a gross return of \$500/ha assuming that the plant contains 1% (dry weight) of the target metal. The table shows that values range from 0.0037 t/ha for gold and platinum to 61 t/ha for lead. For annual crops the biomass range is up to about 30 t/ha (maize) with a value of about 5 t/ha for hay. It is not likely that an unfertilised hyperaccumulator annual crop will be found with a biomass exceeding that of maize although there are several large trees that can hyperaccumulate metals. The reproductive matter of *Alyssum bertolonii* after fertilizing, has a biomass of about 13.5 t/ha. This value is in the middle to low part of the potential economic range.

Table 20. Concentration of trace metals (in dry matter) that would be needed in a plant with the same biomass as fertilized (N+K+P) *Alyssum bertolonii* (9.0 t/h) to provide a crop with a gross value of $500/ha

Metal	Price/tonne in $US*	%	μg/g
Gold or platinum	13,600,000	0.0004	4
Palladium	4,464,000	0.00124	12
Silver	183,000	0.0304	304
Cobalt	48,000	0.12	1,157
Nickel	7485	0.74	7,422
Tin	6200	0.90	8,960
Cadmium	3750	1.48	14,815
Copper	1961	2.83	28,330
Manganese	1700	3.27	32,679
Zinc	1007	5.52	55,169
Lead	817	6.80	67,999

*September 1996 prices

Table 21. Biomass (t/ha) of a hypothetical hyperaccumulator containing 1% (dry weight) of a given metal that would be required to give a crop with a gross metal value of $500/ha

Metal	Biomass	Metal	Biomass
Gold or platinum	0.0037	Tin	8.06
Palladium	0.011	Cadmium	13.3
Silver	0.27	Copper	25.5
Cobalt	1.04	Manganese	29.3
Nickel	6.68	Zinc	49.6
		Lead	61.2

General conclusions and final comments

It is concluded that the experiments have demonstrated that the Italian serpentine-endemic shrub *Alyssum bertolonii* has a potential for phytomining for Ni in Italy and elsewhere, particularly in the Mediterranean area. The potential limits for phytomining as a realistic commercial possibility have been set. These limits are set by the biomass production and natural Ni content of the plant. It has been shown that adding fertiliser to the plants can result in a dramatic increase of biomass without corresponding loss of nickel concentration in the tissue.

A clear differentiation must be made between phytomining and phytoremediation (green remediation) where commercial yields of metals are not required. In this latter technique many elements may be removed from polluted soils using a wide variety of plant extractors including *Alyssum bertolonii*. Another approach to phytomining could be the use of biotechnology to introduce hyperaccumulator genes into other plants of high annual biomass production. Such an approach is being investigated in several research centres, principally in the United States.

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Chapter 5

The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytoremediation and phytomining

Publication arising from chapter:

Robinson,B.H.,Brooks,R.R.,Howes,A.W.,Kirkman,J.H. and Gregg,P.E.H.1997b. The potential of the high-biomass nickel hyperaccumulator *Berkheya coddii* for phytormediation and phytomining. J. Geochem. Explor. 60, 115-126.

Abstract

Pot trials and tests in outside plots were carried out on the South African Ni hyperaccumulator plant *Berkheya coddii* in order to establish its potential for phytoremediation of contaminated soils and for phytomining of Ni. Outside trial plots showed that a dry biomass of 22 t/ha could be achieved after moderate fertilisation. Pot trials with varying soil amendments with nitrogen and phosphorus fertilisers showed enhanced uptake of Ni with increasing nitrogen addition, though there was no reaction to phosphorus. The Ni content of the plant was directly related to the ammonium acetate extractable fraction of Ni in a wide range of natural and artificial substrates. Excision of shoots induced a dramatic increase in the Ni content in the new growth (5500 μg/g compared with 1800 μg/g Ni). When plants were grown in pots with Ni added to the substrate (0-1%), the Ni content of the plants rose to a maximum value of about 1% dry mass. The data from this last experiment were used to calculate the probable Ni yield (kg/ha) of plants grown in nickel-rich soils in different parts of the world. It was calculated that moderately contaminated soils (100 μg/g Ni) could be remediated with only two crops of *Berkheya coddii*. The potential of this species for phytomining has also been evaluated and it is proposed that a yield of 100 kg/ha of Ni should be achievable at many sites worldwide.

Fig. 30. Flowers and buds of Berkheya coddii

Introduction

Berkheya coddii is an asteraceous perennial plant that typically grows to a height of 1.5 metres. It is found naturally only on serpentine soils in southern Africa. *Berkheya coddii* belongs to a group of very unusual plants termed *hyperaccumulators* (Brooks *et al.*, 1977). Hyperaccumulator plants accumulate heavy metals from the soil into the aerial parts to concentrations in excess of a threshold of 1000 μg/g (ppm) set by Brooks *et al.* (1977) for dry biomass. In the case of Zn, the threshold has been set at 10000 μg/g. There are known hyperaccumulator plants for As, Cd, Co, Cu, Mn, Ni, Pb, Se and Zn, of the order of 700 species in all, of which more than half are Ni hyperaccumulators. *Berkheya coddii* falls into this latter category, with foliar Ni concentrations reported in excess of 11,600 μg/g (1.16%) dry mass (Morrey *et al.*, 1992).

A potential practical use for hyperaccumulators was first suggested by Chaney (1983) and later by McGrath *et al.* (1993) who carried out the first systematic field trials and showed that such plants might be used to remove metals from polluted soils.

Results in Chapter 4 showed that the Ni hyperaccumulator *Alyssum bertolonii* from central Italy could potentially be used to phytomine Ni commercially, though the operation was nearing the low end of economic feasibility. It was found that the Ni content of the plants was correlated positively with the ammonium acetate extractable fraction of Ni in the soil.

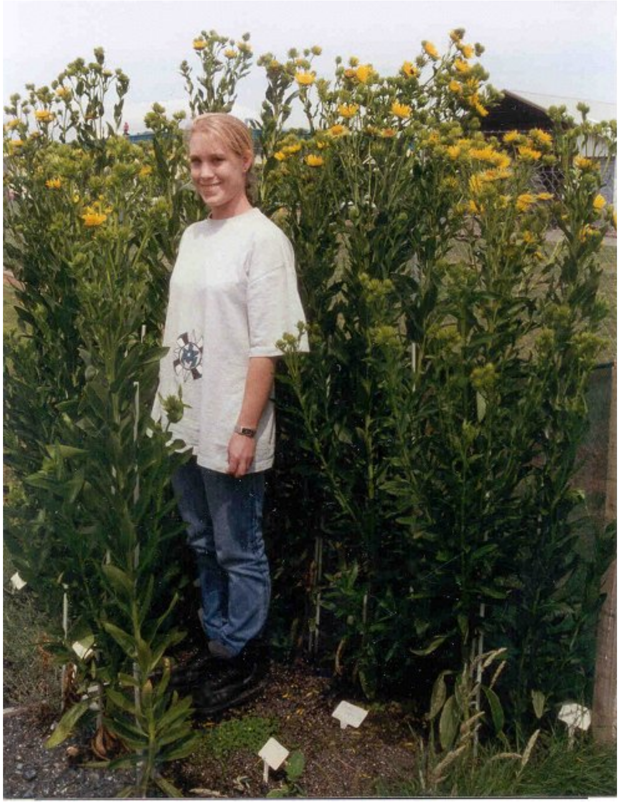

Figure 31. Experimental out-door stand of Berkheya coddii with a biomass of 22 t/ha and height of about 1.8 m.

It has been proposed that adding a compound to the soil that solubilises non-available heavy metals would increase metal yields in the plant. Evidence for this comes from Blaylock *et al.* (1997) who showed that Indian mustard (*Brassica juncea*) plants accumulated Pb to a level of 1.5% (dry mass) in the aerial parts one week after addition of EDTA to the soil. The plant rapidly died, but this is not important for a phytoextraction operation as dead tissue can be harvested and burnt as easily as live material. The disadvantage with this strategy is the cost involved in adding such compounds and the risks that they may pose to the environment. So far, the problem with most potential phytoextraction operations has been that the hyperaccumulator plants that would be used, often have a low biomass production. If recourse is made to high-biomass plants, the advantage is offset by a lower degree of metal accumulation compared with hyperaccumulators. One strategy for increasing metal yields at present is, as mentioned above, to try to induce high biomass non-accumulator plants to accumulate a higher concentration of metal by adding chelating agents to the soil, or in the longer term by inducing the plant to produce its own complexing agents, by introducing genes from other hyperaccumulating species.

It is clearly desirable on economic grounds to discover high-biomass hyperaccumulators for phytoremediation/phytomining operations. Initial study of *Berkheya coddii* (Fig. 30) in its natural environment by R.R. Brooks (pers commn) showed that this plant certainly combines a high biomass (individual plants averaged 300 g d.w. at a site in Barberton, South Africa) with a high Ni content (up to 1.7% Ni in dried leaves). This plant has been grown experimentally in New Zealand for two years during which the effect of climate, shoot excision, soil amendments (including chelating agents) and varying Ni contents in the substrate were included as part of this study. These studies were performed both in plant growth units and outside under field conditions and had the purpose of establishing the suitability of this species for phytomining and/or phytoremediation.

Materials and Methods

Environmental control and growth media

All experiments, except the biomass estimation, were conducted in glasshouses at the Plant Growth Unit, Massey University, New Zealand. The temperature was controlled year-round within the limits of 15°-25°C. Plants were watered daily. The biomass production experiments were conducted out-of-doors in experimental plots at Massey University during a 12-month period. Plants were also grown outside for 24 months in order to study their resistance to frost during the winter.

In Palmerston North mean maximum and minimum temperatures for July (winter) are 12.1° and 4.0°C. For January (summer) the values are 22.3° and 13.1°C. In the winter a rare ground frost could be as cold as -5°C.

Various growth media (Table 22) were used. These included a serpentine soil from Red Hills in California, and a serpentine soil from South Island, New Zealand. Other growth media were artificial "serpentine soils" prepared from additions of a serpentine mineral ("Te Kuiti Serpentine") to either garden soil or finely sieved (<2.0 mm) bark.

Fertilisation was carried out by addition of "Osmocote" slow-release fertilisers at the rates recommended by the manufacturers.

Estimation of annual biomass production

Two adjacent 1x1 m plots, containing a 3:1 mixture of bark: crushed serpentine rock to a depth of 20 cm (see Table 22) and fertilised with "Osmocote", were each planted with 16 seedlings. The boundaries of these plots were surrounded with 1 m high shade cloth. This was added to reduce edge effects: i.e. extra peripheral light and the effects of wind. One year after planting, the plants were excised approximately 5 cm above ground level. The plants were dried and the weight of the stems, leaves and flowers from each plant were recorded and their Ni concentrations determined.

Table 22. Elemental concentrations (total) and pH of natural and artificial growth media used in experiments.

Growth medium	Mg(%)	Fe(%)	Ni(μg/g)	Cr(μg/g)	Co(μg/g)	pH
Bark*	0.0001	0.001	<1	<1	<1	6.2
Serpentine mineral (Te Kuiti)	23.0	4.8	1880	910	96	9.2
Bark:serpentine (v/v)**						
10:1	3.83	0.8	313	152	16	7.0
5:1	6.57	1.4	537	260	27	7.4
4:1	7.67	1.6	626	303	32	7.4
3:1	9.20	1.9	752	364	38	7.7
2:1	11.5	2.4	940	455	48	8.1
1:1	15.3	3.2	1253	606	64	8.3
Bark:serpentine:gravel	6.57	1.4	537	260	27	7.9
Bark:californian serpentine soil 1:1 (v/v)**	10.7	6.5	2226	1027	116	6.5
Californian serpentine soil(Chinese Camp)	16.1	9.7	3340	1540	174	6.5
South Island serpentine rubble(Rai Valley)	15.9	23.4	6090	7850	540	7.6
South African serpentine soil(Barberton)	5.26	16.1	3000	6200	-	6.7

NB All artificial growth media had been amended with "Osmocote" slow-release fertilisers.

*The bark contained 10 μg/g N and 2 μg/g P. **Mixtures prepared with fresh bark on a volume basis. On a weight basis, the dry bark had half the mass of the fresh material.

Effect of fertiliser additions on nickel uptake

The effect of nitrogen and phosphorus on Ni uptake was studied by growing specimens of *Berkheya coddii* in 500 mL plastic pots for a period of 20 weeks in a 3:1 bark: serpentine mineral mixture (see Table 22) to which was added incremental amounts of nitrogen and phosphorus from calcium ammonium nitrate and superphosphate fertiliser. There were a total of 9 different amendments with replicates of 5 plants within each group. The amendments were as follows: N_0P_0, N_0P_1, N_0P_2, N_1P_0, N_1P_1, N_1P_2, N_2P_0, N_2P_1 and N_2P_2; where N_0-N_2 were 0, 100 and 200 μg/g nitrogen as N, and P_0-P_2 were 0, 50 and 100 μg/g phosphorus as P. The leaves of the plants were excised after 20 weeks and analysed for Ni.

Effect on soil nickel concentration, soil nickel solubility and excision on nickel uptake

Nickel was added as the nitrate to a standard commercial seed compost to give concentrations of 0, 14, 41, 123, 370, 1111, 3333, and 10000 μg/g.

In a further experiment, mixtures were also prepared with crushed serpentine rock: bark in the following ratios: 1:1, 1:2, 1:3, 1:4, 1:5, 1:10 and pure bark. Californian serpentine soil (from Red Hills near Chinese Camp), and a 1:1 mixture of bark/Californian serpentine were also used. Five replicates were prepared for each treatment. Plants were grown in 500 mL pots for 6 months then excised at ground level and analysed. The re-growth of the plants in the same mixtures were re-sampled and re-analysed after two months.

Effect of citric acid and EDTA on plant metal uptake

Forty plants were grown in pots containing 500 g of a 3:1 mixture of bark: crushed serpentine rock (see Table 22) for a period of 5 months. The plants were divided into five groups of eight and the following treatments prepared for each specimen: control, 5 g citric acid, 10 g citric acid, 2 g EDTA, and 2 g EDTA and 5g citric acid. Citric acid was added as a 20% solution, and the EDTA added as a 5% solution of the trisodium salt. Leaf samples were taken after the first and third weeks after treatment, and the entire plant harvested one month after treatment.

Determination of the nickel content of the plants

Plant material was placed in a drying cabinet at 70°C until a constant weight was reached. Approximately 0.1 g samples of plant material were weighed accurately into 15 mL borosilicate test tubes. The samples were ashed overnight at 500°C. Five mL volumes of warm (80°C) 2*M* HCl was added to each, and the samples were mixed and shaken to dissolve the ash. Nickel in the solutions was determined by flame atomic absorption spectroscopy (FAAS).

Determination of the solubility of nickel in the soil

Soils were dried at 70°C. Soil samples (2 g) were weighed accurately into 50 mL centrifuge tubes and 20 mL of 1M ammonium acetate (pH 7.0) was then added to each. This extractant was used so that the results could be compared with those of other studies (e.g. Chapter 4) that had shown that the ammonium acetate extract could be used to predict the nickel content of a plant grown in the extracted soil. The tubes were agitated for 24 hours and the mixtures filtered. Nickel was determined in these extracts by graphite furnace atomic absorption spectroscopy (GFAAS).

Results and Discussion

Table 23. Fertilised biomass, mean metal content and extraction potential of various hyperaccumulators of metals.

Species	Fertilised biomass/ha	Mean metal content (µg/g)	Metal extracted (kg/ha)
Homalium kanaliense (Ni)	36	*556	20
Zea mays (Ni)	30	1	0.03
Alyssum tenium (Ni)	23	3391	78
Berkheya coddii (Ni)	21.4	7880	168
Alyssum lesbiacum (Ni)	15	10000	150
Alyssum murale (Ni)	13.8	7101	98
Alyssum bertolonii (Ni)	9	7500	68
Haumaniastrum katangense (Co/Cu)	7.5	2266	17
Thlaspi caerulescens (Zn)	5.1	30000	153
Cardaminopsis hallerii (Zn)	2.6	3846	10

*Leaves have 7000 µg/g Ni but the stems have much less; hence the low extraction potential.

The biomass production of *Berkheya coddii*

After one year's growth from seed, the total above-ground dry biomass of plants in the two 1x1 m plots were 2.08 and 2.20 kg respectively. This translates to a mean biomass production of 21.4 t/ha/annum. Plants were on average 180 cm tall and are shown in Fig.31. This biomass was achieved with moderate addition of Osmocote slow-release fertilisers. Field observations indicate, however, that the plant attains this height in its natural habitat. Poorer soils may need fertiliser addition for optimal production. Table 23 compares the biomass production of *B. coddii* with some other plant species, both non- and hyper-accumulating. The value of 21.4 t/ha is among the highest reported for any natural hyperaccumulator species, and over twice as high as the biomass production of *Alyssum bertolonii* (9 t/ha/yr) that has been shown in Chapter 4 to have the potential of being able to provide an economic crop of nickel.

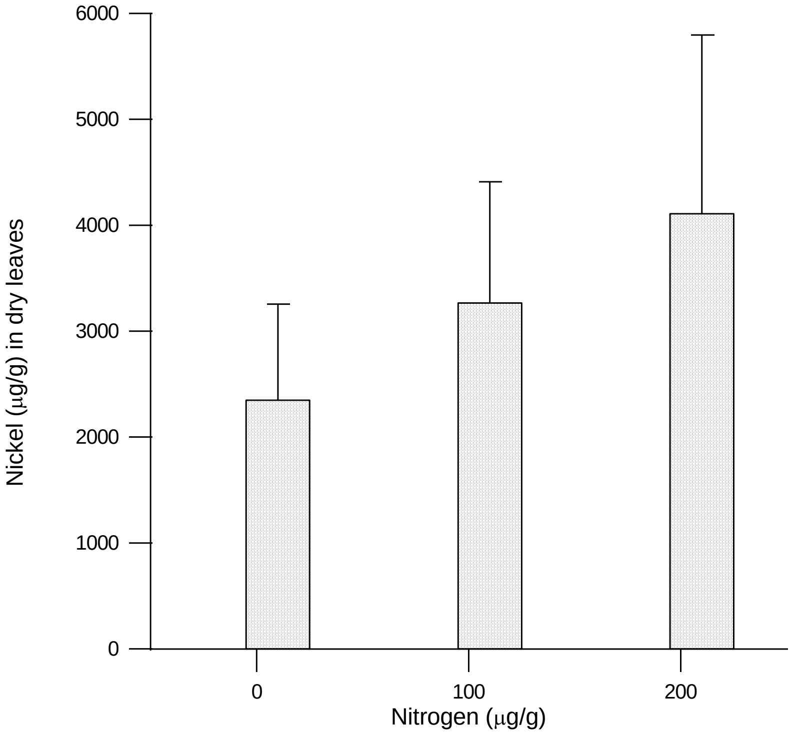

Fig. 32. Effect of nitrogen fertiliser on the nickel concentration of Berkheya coddii.

Effect of fertiliser amendments on nickel uptake by *Berkheya coddii*

Chemical analysis of leaves collected in these experiments showed no statistically significant relationship between the nickel content of the plants and the phosphorus content of the medium when the nitrogen content was constant. If, however, the experimental samples were considered as three separate populations with treatments N_0, N_1 and N_2 (i.e. ignoring the P treatments), there was a significant increase of Ni content with increasing addition of N (Fig.32). The mean Ni content rose from 2300 µg/g d.w. for zero addition of N, to 3250 for the N_1 treatment and to 4200 µg/g for the N_2 amendments (see also Fig. 32).

The nickel concentration of *Berkheya coddii* in relation to soil nickel solubility

Nickel concentrations in the plants grown in various substrates as detailed in Table 22, ranged from 31 to 7880 µg/g (Fig.33). The values represent the whole of the aerial parts of the plants as calculated from analyses of leaves, flowers and stems (percentages being 49, 19, and 32 by weight respectively).

No significant correlation was found between the total metal content of the soil and that of the plants. This was almost certainly due to the fact that it is the soluble Ni fraction in the soil that determines plant uptake (see Chapter 4). This hypothesis is supported by the fact that there was a very highly significant ($P<0.001$) positive correlation between the metal content of the plant and the soluble Ni concentration as determined by extraction into a 1*M* ammonium acetate solution (Fig.33). This concurs with results obtained in Chapter 4 where the Ni content of *Alyssum bertolonii* had a significant positive correlation with the extractable Ni concentration in the supporting soil. These results indicate that the concentration of extractable Ni in a soil is a better indicator of the probable metal content of the plant than is the total concentration of Ni in the soil.

Prediction of the probable nickel content of *Berkheya coddii* grown on various serpentine soils

From Fig.33 it will be observed that there is a linear relationship between the Ni content of *Berkheya coddii* and the extractable fraction of this element in the soil. The Ni content of *B. coddii* growing on a nickeliferous soil can therefore be predicted by the extractable Ni as determined by use of ammonium acetate solutions. Obviously there are other factors involved in Ni uptake such as the pH of the soil, nutrient availability, and the concentration of other heavy metals, but the predictions should give a rough guide to a soil's suitability for phytomining or phytoremediation.

It is important to mention other considerations that would need to be addressed before *B. coddii* were to be introduced to an area intended for phytomining or phytoremediation. All the plants in these experiments, as well as plants growing naturally in South Africa were not under water stress. It has yet to be determined how *B. coddii* would tolerate xeric conditions. The plants in the outside plots withstood ground frosts of up to -5°C, though growth will undoubtedly suffer in very cold climates.

The question arises as to whether *B. coddii* could potentially become a weed. The rapid growth rate, and the production of large quantities of wind-borne seeds could in theory make the species invasive of surrounding areas, thus out-competing native vegetation. Even though this plant is entirely confined to ultramafic environments in South Africa where the limiting factor may indeed be lack of competition from non-serpentine plants, it should not be assumed that there is no risk of its becoming a weed in other environments and this question should therefore be addressed in future field trials in other countries.

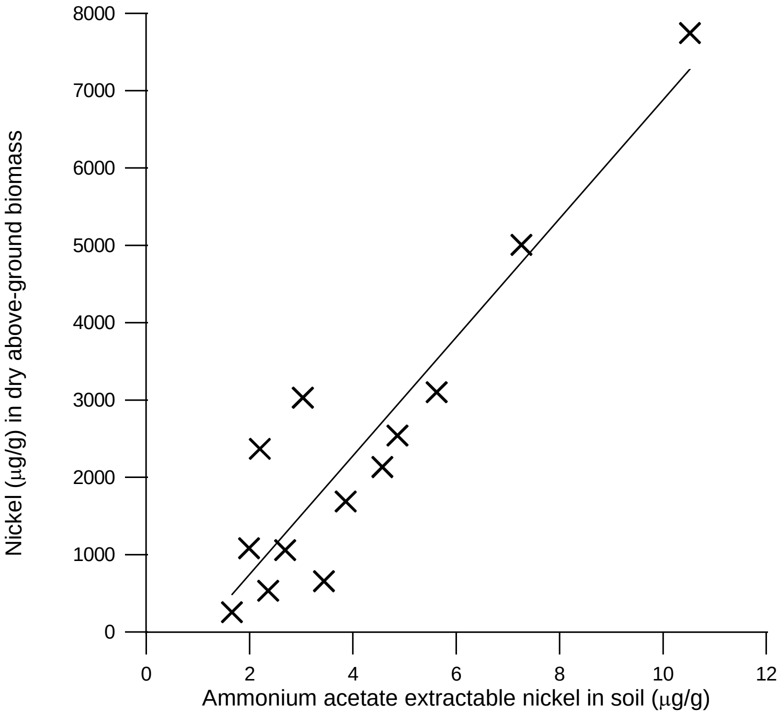

Fig. 33. Nickel concentrations in plants vs the ammonium acetate extractable nickel in the soil.

The effect of excision

Plants that were excised at ground level rapidly grew new foliage. This new growth had a much higher Ni concentration than the original plant (Fig.34). The difference is on average over three times greater in the optimum range of 600-1000 μg/g in the soil. The same behaviour has been noted by Varennes *et al.* (1996) for the Ni hyperaccumulator *Alyssum pintodasilvae*.

Enhanced Ni uptake after excision could be due to two factors. The plant may be removing more Ni from the soil, or it may be translocating existing Ni in the plant to the new growth structures. The higher Ni in the new growth would be advantageous to the plant if it inhibited its predation by folivores (Boyd and Martens, 1992). Were the plant to be extracting more Ni from the soil, it may be possible to induce increased Ni uptake by removal of the apical meristem for example, or the addition of Ni binding compounds to the soil. This could be tested by measuring the change in the Ni concentration of the regrowth over time. No significant decrease in Ni would indicate increased uptake of the metal by the plant. Whichever mechanism is responsible for the increase in Ni, the new growth could be harvested as another high Ni crop a few months after the original cropping, or the plant may be cropped once a year, removing the need to resow the plants.

Nickel in plants as a function of total nickel in the substrate

The results of experiments in which two-month-old whole plants of *B. coddii* were grown in standard seed mix containing incremental concentrations of Ni (0-10,000 μg/g) are shown in Fig.35. The plants would not grow in substrates containing more than 3333 μg/g available Ni and the highest level of just over 10,000 μg/g

Ni in the plants grown in pot trials is probably a limiting value. Although under natural conditions the plant can have up to 17,000 μg/g (1.7%) of this element in its dry leaves, the whole plant has a nickel content around 0.8% and this is of the same order as the experimental value of 1% in pot trials.

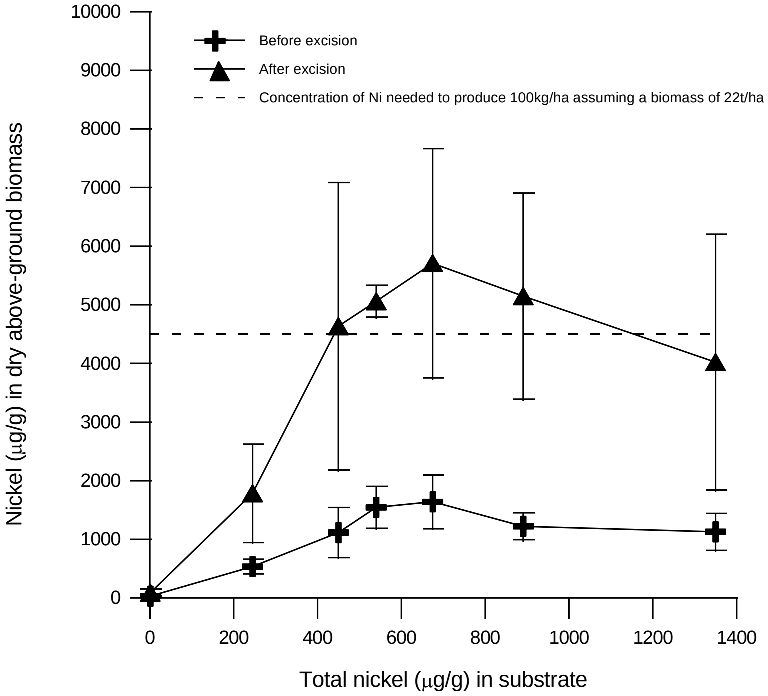

Fig. 34. The effect of excision on enhancement of the nickel content of fresh shoots of Berkheya coddii.

The effect of chelating agent addition on the metal uptake of *Berkheya coddii*

Compounds to be added to augment metal crops in a phytoextraction operation necessarily have to be cheap and relatively non-toxic as large amounts may need to be added. Citric acid was used in these experiments to lower the pH of the soil. This increase of acidity has been shown in Chapters 2 and 3 to increase metal solubility. Citric acid costs around $20 US per kg and is rapidly broken down in the soil. EDTA has been shown by Blaylock *et al.* (1997) and by Huang and Cunningham (1996) to induce hyperaccumulation of Pb in crop plants such as *Zea mays* and *Brassica juncea* that do not usually hyperaccumulate this element. EDTA is a well-known chelating agent that bonds with many metals including Ni. It costs around $40 US per kilogram, and is degraded in the natural environment in a few months (Means *et al*., 1980).

The relationship shown in Fig.33 implies that the Ni content of the plant might be increased by increasing the availability of Ni in the soil. Citric acid and EDTA (trisodium salt) were added to plants in an attempt to augment their uptake of Ni and other metals. Table 24 shows the effect of these additions.

Table 24. Effect of various treatments on metal uptake (μg/g d.m.) by *Berkheya coddii*

Treatment (n=8)	Ni	Co	Cr
Control	3480	38	0.9
5g citric acid	1612*	121*	1.0
10g citric acid	770*	149*	7.9**
2g EDTA	1559*	29	1.1
2g EDTA + 5g citric acid	1306*	44	1.7

*Very highly significant difference from the control (P<0.001) as determined by *t*-test.
**Significant difference from control (P=0.037) as determined by *t*-test.
All other values showed no significant differences from the control.

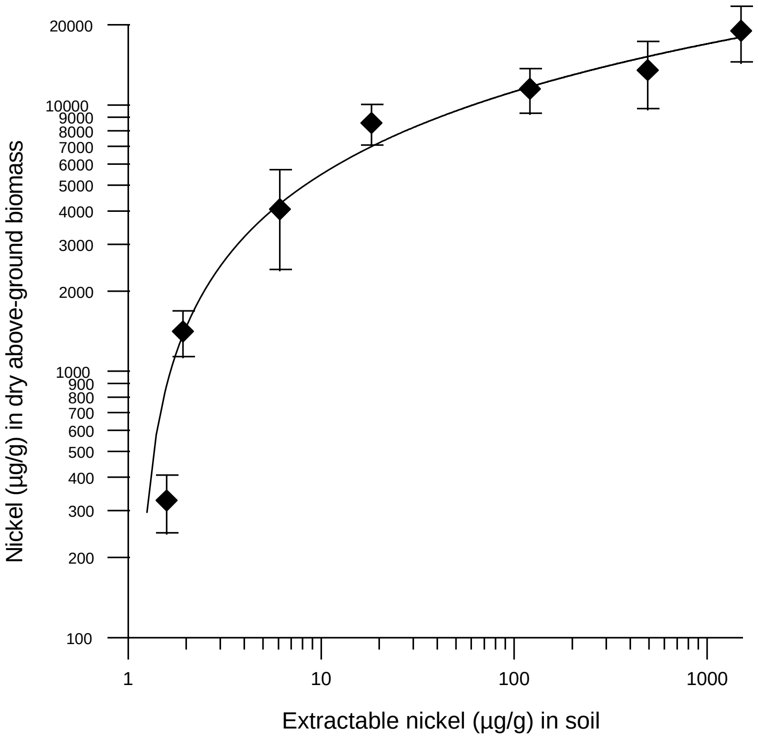

Fig. 35. Results of pot trials showing the relationship between nickel in *Berkheya coddii* and the available (ammonium acetate extractable) nickel content of the substrate.

Surprisingly, the addition of both citric acid and EDTA caused a highly significant decrease ($P<0.001$) of Ni content in all relative to the control. This was in spite of an increase in the extractable Ni content of the soils. There was no noticeable reduction in the biomass yield. A possible explanation for reduced Ni is that the compounds added to the soil compete with the plant's own nickel-binding agents causing the Ni to diffuse out down its concentration gradient. This is consistent with an active Ni uptake system, which can be contrasted to the passive uptake of Pb by *Zea mays* (Huang and Cunningham, 1996). In the latter system, the addition of EDTA solubilises large quantities of Pb from the soil, which then diffuses down its concentration gradient into the plant root, or is taken up by mass flow. More evidence for this comes from the fact that addition of citric acid caused a significant increase in concentrations of other elements not normally accumulated by *B. coddii*. The uptake of the other elements upon the addition of citric acid, though significant, are not sufficient to make their phytoextraction an economic means of increasing the value of a metal crop. Unlike in the case of Pb, there is thus no advantage in adding citric acid or EDTA to the soil, if Ni extraction is the aim of the operation.

Discussion of the Potential Role of Berkheya coddii in Phytoremediation and Phytomining

Phytoremediation of nickel-contaminated soils

The combination of high biomass and high Ni content of *Berkheya coddii*, together with its ease of propagation and culture as well as its tolerance of cool climatic conditions, should render this species a suitable agent for phytoremediation. Sites highly polluted with Ni are less numerous than those contaminated with Pb and Zn. There is however a need for some degree of remediation of several sites throughout the world where pollution from Ni is a problem. McGrath and Smith (1990) have reviewed the problem of Ni pollution of the environment. Apart from the obvious local pollution from smelters, a significant problem arises from addition of Ni to pastures via sewage sludges. At Beaumont Leys (UK) for example, the Ni content of surface soils at a sewage farm was found to be as high as 385 µg/g.

European Community guidelines for Ni in pastures receiving sewage sludge have been set at a maximum level of 75 µg/g where background levels are around 25 µg/g for UK (McGrath and Smith, 1990). Assuming a biomass of 22 t/ha for *Berkheya coddii* and a soil depth of 15 cm and density of 1.3, it is possible to calculate the amount of Ni that could potentially be removed annually from contaminated pastures using a crop of this species. Using the data from Fig.35, an estimate can be made for the probable Ni content of a *Berkheya* crop growing over polluted soils. It must be remembered that the experiments portrayed in Fig.35

were carried out with substrates containing Ni as the soluble nitrate, though a high proportion of this Ni would have been absorbed by complexing with the organic matter of the substrate. In applying these data to a hypothetical situation involving a contaminated soil in which the availability of the Ni might not be known in advance, a conservative approach has been adopted that assumes only half of the metal burden of the soil would be available to the plants.

The number of annual crops of *Berkheya coddii* that would be required to reduce the Ni burden of soils down to the EU level of 75 µg/g is summarised in Table 25. For moderate Ni contamination (100 µg/g) two crops would be sufficient to reduce the metal content to well below the 75 µg/g of the EU guidelines. Even at 250 µg/g (few polluted sites would exceed this value) only 4 crops of *Berkheya coddii* would be needed.

Table 25. Number of annual crops of *Berkheya coddii* required to reduce nickel contamination in soils to the EU guideline of 75 µg/g.

Initial Ni in soil (µg/g)	Content after one year	Number of crops to decontaminate
10000	9918	138
5000	4925	74
2000	1932	34
1500	1435	26
1000	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 Ni is extractable, 3 - Ni content of the plant, and hence its extractive power, is a function of the Ni content of the soil as determined from Fig.5.

Current EU guidelines (CEC, 1986) permit an annual addition of only 3 kg/ha Ni when sewage sludge is used as fertiliser for pastures and cropping. One crop of *Berkheya* (Table 25) would remove the equivalent of 24 years of annual fertiliser additions assuming only half of the Ni is extractable.

Phytomining for nickel

The figure of 100 kg/ha quoted by Nicks and Chambers (1995) as the minimum amount of Ni per hectare in an economic phytomining operation would require a minimum plant Ni content of about 4500 µg/g in *Berkheya coddii* assuming a biomass yield of 22 t/ha as found in these experiments. The experiments and field observations in South Africa indicate that this limit should be easy to reach

At the highest recorded concentration of 7880 µg/g, a 1 ha crop of *Berkheya coddii* would remove 168 kg of Ni. This combined with the energy derived from the combustion of the plant material ($288) translates to $1260 + $288 = $1548 per hectare. If half of this sum could be returned to the producer ($774), this is about twice the value of a wheat crop.

Some caution must be applied in extrapolating the results of pot trials and limited field trials to large-scale metal farming.. It has not been possible to achieve experimentally the 7880 µg/g Ni found in wild plants thus a more conservative level of 5000 µg/g Ni seems more realistic. This could provide 110 kg/ha Ni worth $792 at today's prices. Adding the energy "profit" and assuming a 50% return to the operator, the value of the crop then becomes $540, still well above that of a wheat crop.

Berkheya coddii has several advantages over other phytomining candidates:

1 - its biomass production is superior to that reported of any other hyperaccumulator except perhaps *Alyssum lesbiacum* (McGrath *et al*., 1993) and is not at the expense of Ni content,

2 - the plant is a perennial that can be harvested and regrown the following year without need of resowing,

3 - preliminary observations indicate that the Ni content of regrowth tissue is significantly higher than in the original first-year's growth,

4 - *Berkheya coddii* is an exceptionally hardy plant that will tolerate cool climatic conditions including frost. It is easy to grow from seed but does not propagate from cuttings,

5 - although the plant is tolerant of the relatively mild New Zealand winter, this would probably not be the case for North America. However, it could conceivably be grown as an annual crop in North America during say the March-September period,

6 - *Berkheya coddii* produces seed readily for future crops and the flowers are easily fertilised by local honey bees and bumble bees, though in South Africa they appear to be fertilised by a local species of flying beetle,

7 - finally, *Berkheya* appears to be resistant to insect attack and soil pathogens. Very few failures were encountered in pot trials even when growing plants in non-serpentinic substrates where soil pathogens and fungi might be expected to flourish.

The first requirement for successful phytomining is that there should be a terrain with extensive areas of subeconomic Ni mineralisation (often lateritic). The Ni yield of such phytomining operations would be governed by a number of factors such as climate and the degree of plant-availability of the Ni. This latter factor is of supreme importance as established from the above experimentation. Ni extractability (using ammonium acetate) of eleven serpentine soils from throughout the world has been determined. Relating this extractability to the projected Ni content of *Berkheya coddii* (see Fig.33), the probable Ni yield of crops of this species grown in various parts of the world has been calculated as is shown in Table 26. It is possible that economic crops of Ni could be phytomined from those sites with >98 kg Ni/ha projected yields from *B. coddii,* provided of course that other factors were favourable, not the least of which would be a sufficiently large area for economic metal farming. Although some of the soils show extractable Ni levels well in excess of the maximum of 10.5 μg/g shown in Fig.33, it was chosen not to extrapolate beyond this level since as is shown in Fig.5, Ni levels in *Berkheya* tend to level off at just over 1% in dry matter.

Table 26. Predicted nickel yields for crops of *Berkheya coddii* grown on nickel-rich soils throughout the world.

Country/State	Location	N	A	B	C
New Caledonia	Plaine des Lacs	1	30.8	1.00*	200*
California	Red Hills (Chinese Camp)	2	26.3	1.00*	200*
New Zealand	Coppermine Saddle	6	19.3	1.00*	200*
Italy	Monte Pelato	40	14.4	1.00*	200*
New Zealand	Dun Mountain	5	11.7	0.82	164
South Africa	Barberton	2	10.5	0.73	146
Italy	Monte Murlo	76	7.46	0.49	98
Argentina	Vitali Quarry, Cordoba	1	3.40	0.18	39
Morocco	Taafat	1	2.91	0.14	31
New Zealand	Cobb asbestos mine	5	2.46	0.11	24
Portugal	Bragança	1	1.63	0.05	10

*It is assumed that there is a limiting value of 1% Ni in *B. coddii* irrespective of the extractable Ni in the soil. N - number of soil samples tested, A - extractable soil nickel (%), B - estimated nickel content of plant (%), C - estimated nickel yield (kg/ha). Assumptions: 1 - biomass of 22 t/ha, 2 - Ni content of the plant, and hence its extractive power, is a function of the Ni content of the soil as determined from Fig.5.

The most obvious regions on earth where metal farming with *Berkheya* might be possible are the ultramafics of California/Oregon, Central Brazil in Goiás State, New Caledonia, Anatolia, and Western Australia. The next step in the development process would be the establishment of a pilot scheme in one of these territories.

Phytomining for other metals

The practical limits of phytomining have been discussed in Chapter 4. The main variables that control the economic feasibility of phytomining are: metal price, plant biomass, and the metal content of the plant. These variables are highlighted in Table 27. The metal values extend from $13,600,000/t for Au to $817/t for Pb. A plant with a biomass of 20 t/ha such as *Berkheya* would need to contain 1.8 μg/g Au or 4.08% Pb at the two extremes. To achieve either of these extremes would require some type of substrate modification such as EDTA addition, as natural concentrations of these two elements do not usually exceed 0.1 μg/g for gold and 50 μg/g for Pb. It is true that the Pb content of *Zea mays* with a biomass of 30 t/ha, can be raised to close to the limit of 2.04% by addition of EDTA to the substrate (Blaylock *et al*., 1997), however, the cost of the EDTA alone would exceed the value of the Pb yielded from the soil.

To be realistic, It may be assumed that phytomining, if realised as a commercial enterprise, will be confined to metals such as Co and Ni that lie in the price range of $7500 to $48,000 per tonne and which are known be susceptible to plant hyperaccumulators.

Finally, there is the possibility of simultaneous extraction of two metals with the same plant species since some plants (e.g. the Co/Cu hyperaccumulators of the former Zaïre in Central Africa) can accumulate two metals of which the lesser priced of the two could contribute to the financial return, though neither might be economically viable in its own right.

Table 27. Metal concentrations (μg/g d.m.) in vegetation required to provide a total $US500/ha return* on hyperaccumulator crops with varying biomass.

		Biomass production (t/ha)						
Metal	($US/t)	1	5	10	15	20	25	30
Au	3,600,000	36.8	7.4	3.7	2.5	1.8	1.5	1.2

Pd	4,464,000	112.0	22.4	11.2	7.5	5.6	4.5	3.7
Ag	183,000	2732	546	273	182	137	109	91.0
Co	48,000	10417	2083	1042	694	521	417	347
Tl	15,000	33333	6667	3333	2222	1667	1333	1111
Ni	7,485	66800	13360	6680	4453	3340	2672	2227
Sn	6,200	80650	16129	8065	5376	4032	3226	2688
Cd	3,750	133333	26667	13333	8889	6667	5333	4444
Cu	1,961	254970	50994	25497	16998	12749	10199	8499
Mn	1,700	294120	58824	29412	19608	14706	11765	9804
Zn	1,007	496520	99305	49652	33102	24826	19861	16551
Pb	817	612000	122400	61200	40800	30600	24480	20400

*Excluding any profit from sale of the energy of biomass incineration.

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Chapter 6
The nickel phytoextraction potential of some ultramafic soils as determined by sequential extraction

Publication arising from chapter:

Robinson,B.H.,Brooks,R.R.,Gregg,P.E.H. and Kirkman,J.H. 1999 The nickel phytoextraction potential of some ultramafic soils as determined by sequential extraction. Geoderma. 87, 293-304.

Abstract

Sequential extractions were used to model the effect of successive crops of Ni hyperaccumulator plants on the Ni availability of several ultramafic soils. All soils behaved in a similar manner. It was found that between 13% and 80% of the total Ni in the soil was potentially available to plants. The remainder could not be removed even after a large number of theoretical hyperaccumulator harvests. This necessarily limits the soils' potential for phytoremediation and phytomining. The concentration of soluble Ni after several harvests could be predicted mathematically. As it has been shown that the Ni concentration in hyperaccumulator plants is related to the soluble Ni fraction in the soil, an estimation was made of how many economic harvests of Ni could be obtained from each soil. It was found that between 3 (Dun Mountain, New Zealand) and 18 (Kouaou, New Caledonia) economic Ni harvests could be obtained before the soil would need to be amended. Sequential extractions may be used to determine the bioavailability of other heavy metals in soils that are to be phytoremediated or phytomined.

Introduction

Commercial mining of Ni is usually performed from ores that have a high concentration of the target metal. To be economically viable, such operations require ore bodies with a relatively high (ca. 3% in New Caledonia) Ni content. Ore bodies of this type occur only in small localised areas, and in some cases are becoming exhausted. There are, however, much larger areas of low grade ores, which, using the present techniques, are not economical to exploit. Most of these ore bodies are associated with ultramafic (*serpentine*) outcrops. Such outcrops are scattered throughout the world and usually support a characteristic flora. In serpentine-derived soils, Ni is usually present at concentrations between 0.1 and 0.7% This is well below the minimum Ni content (3%) required for extraction by conventional mining.

Chapters 4 and 5 introduced the concept of nickel phytoextraction, using hyperaccumulator plants to remove economic quantities of nickel from ore-bodies with a nickel concentration too low for conventional mining. Central to this process, is the nature of the soil on which the hyperaccumulator plants are to be grown. Chapters 4 and 5 show that the metal concentration in the plant is proportional to the plant-available metal concentration in the soil. It was noted that a high total concentration did not necessarily indicate a high concentration of soluble metal. Predictions can be made of the metal concentration in plants by measuring the soluble Ni content.

In the process of phytomining, the aim should be to grow several successive crops on each soil. The question arises how these crops would affect the bioavailability of Ni, and whether subsequent crops would contain economically viable Ni concentrations. In the best case (for the phytominer) the available Ni in the soil will remain relatively constant in a scenario where an equilibrium situation between soluble and insoluble metal allows for full or partial replacement of the soluble fraction removed by the plant until all the metal in the soil has been removed. In a worst-case scenario, most of the metal would be matrix-bound, and once the initial metal crop had been obtained, no further crops could be grown without soil amendment.

Amendment may include acidification, which was shown in Chapters 2 and 3 to increase the availability of Ni in ultramafic soils. Another possibility is the addition of chelating agents such as EDTA although this was found to decrease the Ni uptake of *Berkheya coddii* despite increasing the soluble Ni (Chapter 5). This was attributed to competition from the plant's own Ni uptake system. It is yet to be established if this is the case for all Ni hyperaccumulators. When profitable crops can no longer be grown, the topsoil will have to be removed or ploughed to bring fresh material to the surface. If this nickel-free topsoil were to be removed, however, it could still have a use as a nickel-free magnesium additive to fertilisers. Addition of serpentine to superphosphate fertilisers was first carried out in New Zealand during World War II when magnesium fertilisers were scarce (During 1984). An ever-present problem with such admixtures has been the unwanted accumulation of Ni in the treated pastures.

An important factor influencing the number of Ni crops that could be removed from a soil will be the percentage of Ni that is silicate bound or present in an insoluble form. Such metal will not be removed by plants, no matter how many crops are grown on the soil. This necessarily limits the effectiveness of any phytoextraction operation. It might be argued that, in the case of phytoremediation, the metal that will remain insoluble will not affect the biota and therefore does not need to be removed.

The aim of this study was to investigate how many profitable crops of Ni could be taken from some ultramafic soils before they would need to be amended. It would be impractical to test each soil by growing successive crops of hyperaccumulators (a process that would take many years). Therefore, it was decided to use a method of sequential extractions to model the behaviour of the soil.

Materials and methods

Soils studied

The soils used in these experiments and some of their properties are given in Table 28. They are comprised of minerals typically associated with serpentine soils, antigorite being common to all except for the soils from Dun Mountain New Zealand. With the exception of the Kouaoua soil, all the soils used in these experiments have nickel concentrations of around 2000 μg/g. The concentrations of Ni are all elevated compared to "normal" non-serpentine soils, which have an average Ni concentration of around 30 μg/g (Brooks, 1983). The Ni concentrations in all the ultramafic soils are all well below the minimum value of 3% (30,000 μg/g) necessary for commercial mining.

Table 28.Origin, principal minerals, nickel content (μg/g dry weight) and pH of soils used in the experiments.

Country	Location	Principal minerals	Ni content	pH
New Zealand	Dun Mountain	quartz, goethite	2244	6.7
New Zealand	Rai Valley	antigorite, magnetite	2109	7.5
New Caledonia	Kouaoua (Mea mine)	antigorite, goethite, quartz	1,7208	7.2
Spain	Ojén	antigorite, quartz, olivine	1800	7.8
Spain	Puente Basadre	antigorite, quartz, talc	2100	6.3
Italy	Tuscany (Firenze)	antigorite, quartz	1609	6.9
Morocco	Taafat	antigorite, chlorite, olivine	1700	7.5

Time taken for extractions to reach equilibrium

The time taken for an extraction system to reach equilibrium was of crucial importance to these experiments. Obviously there will be a decrease in the amount of metal removed if the soil/extractant mixture has not had time to reach equilibrium. A common practice is to allow 24 hours for equilibrium to be reached. To determine the time needed for Ni extractions to reach equilibrium the following experiment was conducted on serpentine soil from the Rai Valley, South Island, New Zealand. One gram samples of soil were weighed into 20 mL centrifuge tubes and 10 mL of potassium hydrogen phthalate buffered to pH 2 was added to each. Samples were placed on an end-over-end agitator. Samples were removed, centrifuged (3000 rpm for 5 mins) and filtered after 5, 10, 15, 30, 45, 60, 135, 240, 360, 960, and 1455 minutes. The Ni concentrations were determined by flame atomic absorption spectrometry. The results are shown in Fig.36. The curve indicates that after 24 h the samples were close to equilibrium. Leaving the samples for longer periods of time would increase the effect of abrasion on the soil particles, thus giving an artificially high Ni reading. It was assumed that the time taken to reach equilibrium for the extractants at higher pH would be less, as these extractants remove less Ni.

Experimental procedure

Soils were sieved to <0.25 mm. Three lots of 1 g of each soil were weighed accurately into 40 mL centrifuge tubes. For each soil, 10 mL of potassium hydrogen phthalate buffered with NaOH to pH values 2, 4 and 6 was added. This extractant was used because the pH could be easily controlled without greatly changing the concentration or chemical composition of the extractant. The three pH values were chosen to extract different amounts of nickel in a single extraction with an extractant of similar chemical composition and concentration. More acid extractants remove larger amounts of metal (Chapter 2), this metal may be available to plants at a higher pH due to the actions of natural metal binding agents. The extractants were not intended to model the pH of the rhizosphere. Tubes were placed on an end-over-end agitator for 24 h. Each tube was then removed, centrifuged and the top 8 mL of extractant carefully removed using an auto-pipette. This fraction was stored for analysis. Fresh extractant (8 mL) was added and the samples replaced on the agitator. This process was repeated eight times. It will be noted that at each step only 8 mL and not the total of 10 mL was removed. This was meant to simulate the situation where under field conditions the equilibrium between soluble and insoluble metal would tend to replace some of the available metal removed by an annual crop of a selected plant.

Extractions were also carried out with 0.1M and 1M HCl in an attempt to obtain an estimate of the potentially available (i.e. non silicate-bound) metal fraction. Solutions were analysed using flame atomic absorption spectrometry.

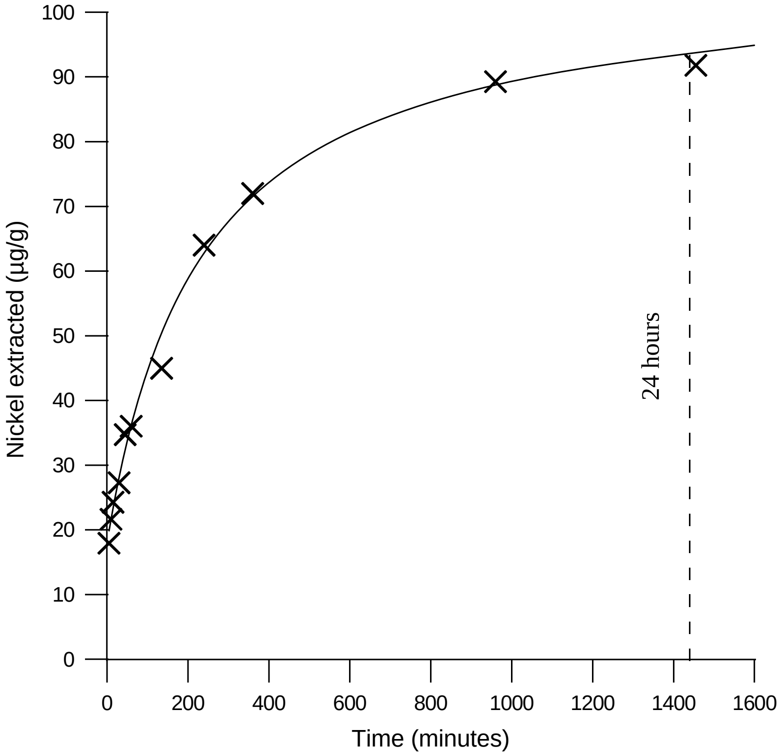

Fig.36. Rate of extraction of nickel at pH 2 from a New Zealand (Rai Valley) ultramafic soil.

Results and discussion

Table 29. Potentially available nickel (µg/g or percentage of total) in ultramafic soils as determined by various extractants or extrapolation from Fig.37.

Location of soil	A	B	C	D
Dun Mountain (NZ)	298 (13%)	231	1040	205
Rai Valley (NZ)	925 (44%)	490	1725	373
Kouaou (New Caledonia)	4383 (25%)	2093	6531	1760
Ojén (Spain)	1432 (80%)	483	1454	305
Puente Basadre (Spain)	432 (20%)	244	962	199
Tuscany (Firenze)	767 (48%)	286	1084	273
Taafat (Morocco)	1039 (61%)	403	1227	292

A - Calculated by extrapolation from Fig.37, B - extracted with 0.2 M HCl, C - extracted with 1 M HCl, D - sum of 8 cumulative extractions at pH 2.

Determination of the potentially available nickel fraction of the soils

Table 29 shows the amounts of Ni extracted by using 0.1M and 1M HCl. The 1M acid removed about 5 times the amount of the 0.1M extractant. This could be accounted for if the 0.1M extractant does not remove all the potentially available Ni, or if the 1M acid removes some of the silicate-bound Ni. The total Ni extracted after 8 extractions at pH 2 was only slightly less than that removed by the 0.1M HCl extractant. If the rate of decrease in subsequent extractions follows the trend of the first 8, then the total Ni extracted after about 10 extractions will be greater than that extracted by 0.1M HCl. This indicates that a stronger extractant is needed to estimate the potentially available Ni.

The cumulative quantity (average of 7 samples) of Ni as a percentage of the total amount extracted by 1M HCl (assumed to reflect the total non-silicate Ni) vs. the number of extractions is shown in Fig. 37. The pH 2 extractant removed the most Ni, followed by the pH 4 and 6 extractants. All three curves followed the general formula:

$$M_c = TX / (X + [T/M_1] - 1) \text{..........(1)}$$

where M_c is the cumulative total percentage of the available metal removed, M_1 is the percentage of extractable Ni removed by the initial extract, T is the potentially available Ni percentage and X is the extraction number. Fitting curves to each of these lines gives a value of T of 39% for all three extraction curves. The fact that all three curves independently give the same result for the total potentially available Ni is good evidence that this value is close to being the true value, which could also be estimated by using an HCl extractant between 0.1 and 1M for the soils tested. It should be emphasised that the pH values 4 and 6 shown in these curves, represent the more likely extractive behaviour of plants under field conditions because the pH at plant rhizospheres is within this range (Trolove *et al.*, 1996).

For the 7 soils tested, the calculated potentially available Ni was in the range 13-80% of the total Ni content of the soil (Table 29) showing that the total Ni concentration gives no indication of the fraction that could be removed in a phytoextraction operation. This has implications for both phytoremediation and phytomining. Heavy metals that are unavailable to plants may still be toxic if the soil itself is ingested. Wind-borne dust consumed on foodstuffs or inhaled may prove toxic if the metals are liberated in the body by stomach acids etc. The Ni in soils that are to be phytomined needs to be available to plants as well as be present in large total concentrations.

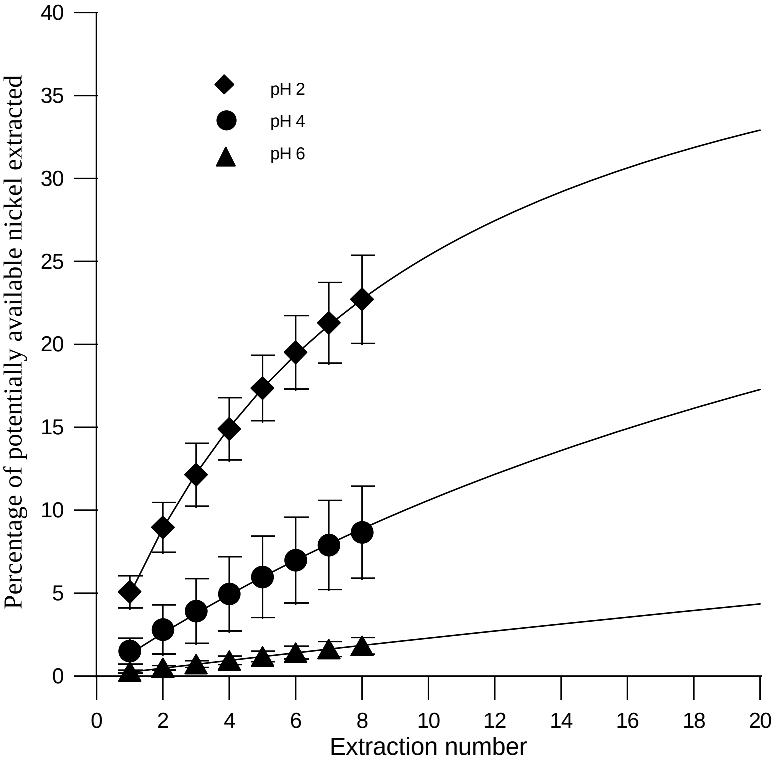

Fig.37. The potentially available nickel content of various ultramafic soils (averages) as determined by sequential extractions at 3 pH values.

Decrease in soluble nickel after successive croppings

Since the Ni concentration in a hyperaccumulator plant is correlated with the soluble Ni concentration in the soil (see Chapters 4 & 5, Figs. 27, 28, 33, and 35), the decreasing soil Ni solubility after several extractions will theoretically affect the Ni yield of the crop. These extractions, however, may not model all the biotic and abiotic processes that result in nickel uptake over long periods of time. The decrease in nickel concentration of the solutions vs. extraction number is shown in Fig. 38.

Equations of the plots follow the formula:

$$M_e = T([T/M_1]-1)/\{(X+[T/M_1]-1)([T/M_1]-2)\}.............(2)$$

where M_e is the percentage of the potential extractable metal contained in a given sequential extraction. All other symbols are as equation (1) above.

It must be noted that equation (2) is not directly dependent on pH since it is based on M_1 the percentage of the potentially available metal removed by the first extraction.

Under field conditions, the number of economic nickel harvests (>70 kg/ha and assuming an initial harvest of 100 kg/ha) can be calculated from equation (2) and the results are shown in Fig.38. Before depletion to the limit of 70 kg/ha, the number of crops ranges from a high of about 20 (Kouaou) to a low of about 3 for Dun Mountain soils. The higher number of theoretical croppings that could be taken from the Kouaoua soil may be expected because is total nickel concentration is nearly an order of magnitude higher than the other soils used in these experiments. These calculations assume a starting point of 100 kg/ha and it must be remembered that nickel yields well in excess of this figure could to be realised. The value of 100 kg/ha is based on a soil depth of 15 cm and density of 1.33 (typical for ultramafic soils). One ha of soil would therefore weigh 2000 t and a crop with a yield of 100 kg/ha of Ni would remove 50 µg/g of Ni from the soil. Once the lower economic limit of 70 kg/ha has been reached, ploughing to transfer subsoil to the surface should provide sufficient fresh material for more harvests.

The data in Fig.39 show that there is a large difference between different soils in their ability to be phytomined or phytoremediated. The soil from Kouaou (New Caledonia) could sustain seven times the number of croppings as the soil from Dun Mountain (New Zealand).

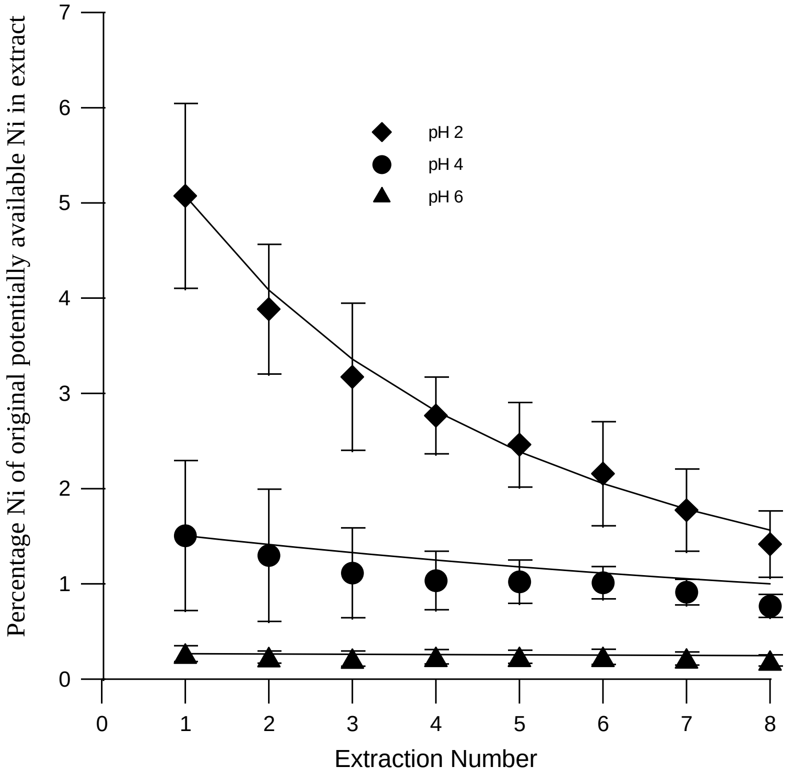

Fig.38. Percentage of the original available nickel content of ultramafic soils as a function of the number of crops of a hyperaccumulator plant.

Concluding discussion

It is to be emphasised that the data are only an approximation of the field situation. Factors such as climate, weathering and mass flow of Ni, and the effect of plant phytochelatins have not been considered here. These factors might increase the availability of Ni in these soils. The results should therefore give a conservative estimate of the Ni availability after several hyperaccumulator crops have been grown. The ability of most soils to sustain several theoretical Ni crops indicates that phytomining could be a viable economic proposition on these soils.

Field trials will ultimately be necessary to determine the Ni availability of the soils after several harvests. If the results of field trials indicate that the sequential extractions accurately model the field situation, then similar extraction methods could be used to determine the potential of a soil for phytoextraction without the need for growing experimental crops, which can be a costly and time-consuming process.

The sequential extraction method may also be used to investigate the behaviour of other metals such as lead, zinc and cadmium in polluted soils. These metals are contaminants of many soils near industrial sites. Many such sites are too large to be cleaned economically using conventional methods, so that phytoremediation might be the only viable decontamination method.

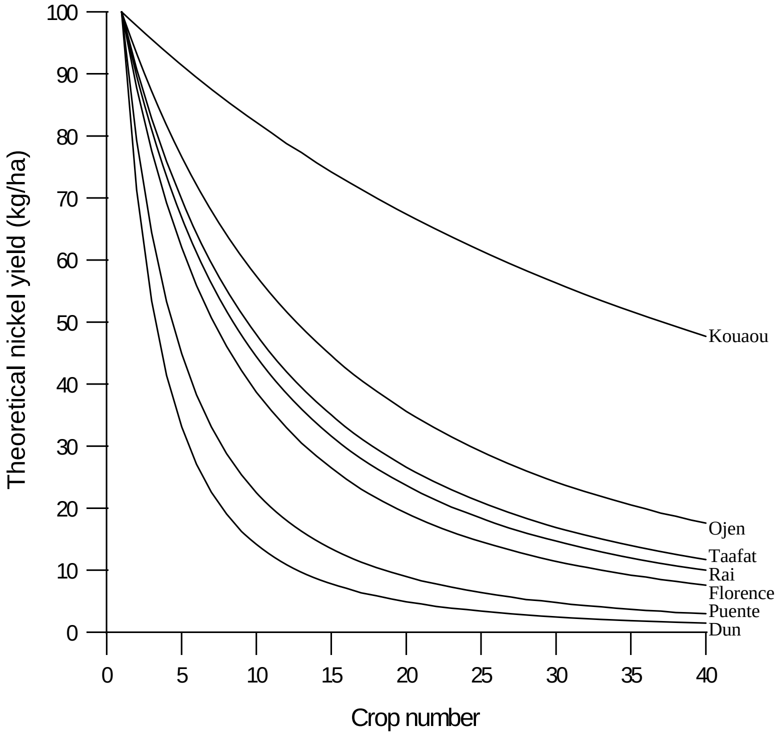

Fig.39. Nickel yields (kg/ha) of successive crops of a theoretical hyperaccumulator plant growing over various ultramafic soils. The curves are based on a yield of 100 kg/ha for the initial crop that is assumed to remove 50 mg/g of the available nickel.

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SECTION C
THE PHYTOEXTRACTION OF ZINC AND CADMIUM FROM CONTAMINATED SOILS.

Many soils throughout the world have undesirably high concentrations of heavy metals. These elevated concentrations usually arise anthropogenically due to aerial pollution from metal smelters (Fig. 40) and other industrial activity, or by proximity to metal mines (Fig. 41). Elevated concentrations of cadmium can occur on farmland fertilised with superphosphate derived from "phosphate rock" which is high in cadmium.

Fig. 40. A base metal smelter near the town of Auby, Northern France
Surrounding soils contain up to 4% zinc and 1% lead

Conventional treatments for these soils fall into four general categories: Removal, covering, cleansing, and inerting. Removal of the polluted material involves a large transportation cost, the polluted material still has to be "stored" somewhere where it will not cause a problem. Polluted sites may be covered with fresh topsoil, or concrete, isolation of the polluted area may also be achieved by forming a hydraulic isolation curtain (Cunningham and Berti, 1995). Covering with topsoil allows vegetation growth, but may not be a long-term solution due to erosion and leaching. Covering with an inorganic material such as concrete makes the site unsuitable for any agricultural use. Acids may be used to leach heavy metals from the soil. This may be done *in situ* or the soil may be removed, cleansed, then replaced. In situ treatment is cheaper because no earth-moving operation is required, but this procedure may lead to contamination of the local water ways causing other problems. Inerting is the process whereby heavy metals are chemically altered to a state where their effect on the environment is minimised. Chromium in the +6 oxidation state is very toxic, however when reduced to the +3 state it is very inert, and causes few environmental problems (James, 1996). Ma *et al.* (1995) demonstrated that phosphate rock addition to lead-contaminated soils could immobilise this toxic element. These methods are relatively cheap, but can only be used on a limited number of metals (Cr and Pb). Inerting may also be achieved by absorption or entrapment of a pollutant into a stable matrix such as cement (Cunningham and Berti, 1993). Most of these conventional soil-remediating procedures destroy the biotic components of the soil (Hinchman and Negri, 1996).

The conventional procedures listed above cost between $US 100,000 and $US 1,000,000 per hectare. The expected cost for the clean-up of heavy metal polluted sites over the next 30 years in the USA alone is $US 750 billion (Russel *et al.*, 1991). Because of the large area of some of these sites, there is at present no practical method for their decontamination.

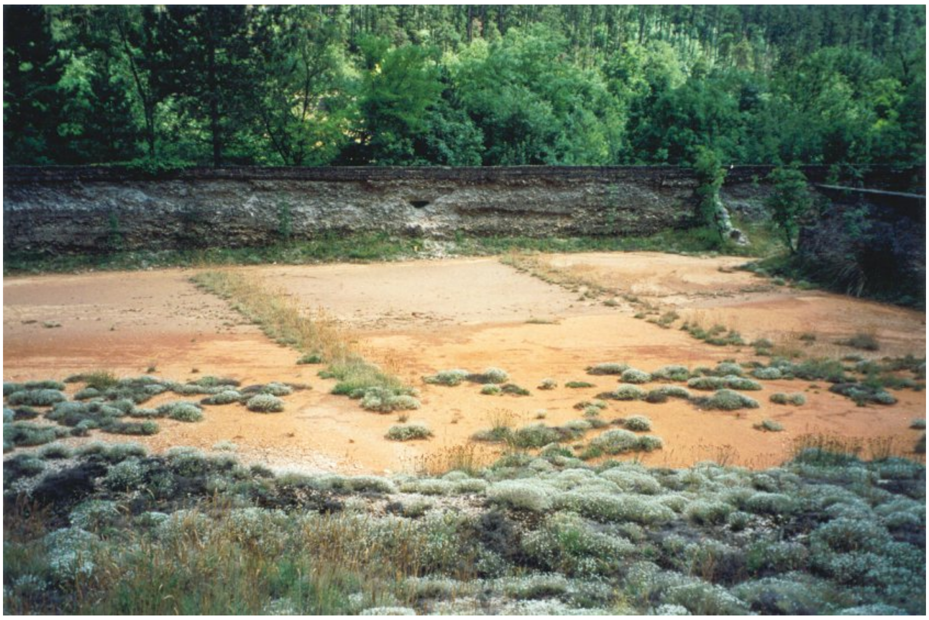
Fig. 41. *Minuartia verna* growing on soils polluted from a nearby lead mine. St Laurent le Minier, Southern France.

Chaney (1983) suggested that hyperaccumulator plants may be used to remove heavy metals from polluted soils *in situ*. A polluted area would be planted with a hyperaccumulator species, which, when mature, would be harvested and burnt, producing a low-volume bio-ore. This material, containing several percent heavy metal, could then be stored in a ‘safe area’ or smelted, and the metal recovered and sold, thus recouping some of the costs of the operation. The procedure could be repeated several times until the soil’s heavy metal content had reduced to acceptable levels. Fig. 42 show a flow-chart of the theoretical operation. The cost of phytoremediation is estimated to be $US 60,000 - 100,000 per hectare (Salt *et al.*, 1995), around half that of the cheapest conventional operation. This is because the operation in its most basic form is simply growing and harvesting a crop of plants. Hyperaccumulators are less likely to be damaged from pests such as insects and soil pathogens due to the toxic effect of their high metal content (Boyd and Martins, 1995). Hyperaccumulator plants generally have a low biomass production. There are two schools of thought as to the type of plants best suited to this operation. The first is the use of conventional hyperaccumulator - “hypertolerator” plants such as *Thlaspi caerulescens* with a low biomass. This approach was favoured by Chaney *et al.* (1997). Work in this area is focused upon increasing the biomass of these plants, by fertiliser addition, selection of larger ecotypes, and gene manipulation. The second school focuses upon plants with a high biomass that are able to withstand - but not necessarily accumulate large concentrations of heavy metals (Salt *et al.*, 1995). It is argued that the lower metal concentration in the plant is offset by the much greater biomass. Work in this field has centred upon increasing the plant’s metal content.

Hinchman and Negri (1996) suggested that using “water poor” plants i.e. plants which occur in wet areas and have high transpiration rates could uptake metals by mass flow from a metalliferous soil. It would, however, be necessary to keep the environment continuously moist.

Phytoremediation of heavy metals may be achieved while growing a crop of trees (Pulford *et al.*, 1995), the long time necessary for the soils remediation being unimportant if economic crops of trees can be grown unimpeded. The augmentation of metal uptake was striking in the case of lead where chelate addition to the soil induces plant uptake to a maximum of 2% on a dry matter basis (Huang and Cunningham, 1996; Blaylock *et al.*, 1997). It had previously been thought (Cunningham and Berti 1993) that this metal was a poor candidate for phytoremediation due to its immobility in the soil. Other possible methods of increasing plant metal uptake are use of fertilisers, and gene manipulation (Raskin, 1996). It is hoped that if the genes responsible for the hyperaccumulation phenomena are identified, they might be introduced into non-hyperaccumulator plants with a high biomass.

PHYTOREMEDIATION MODEL

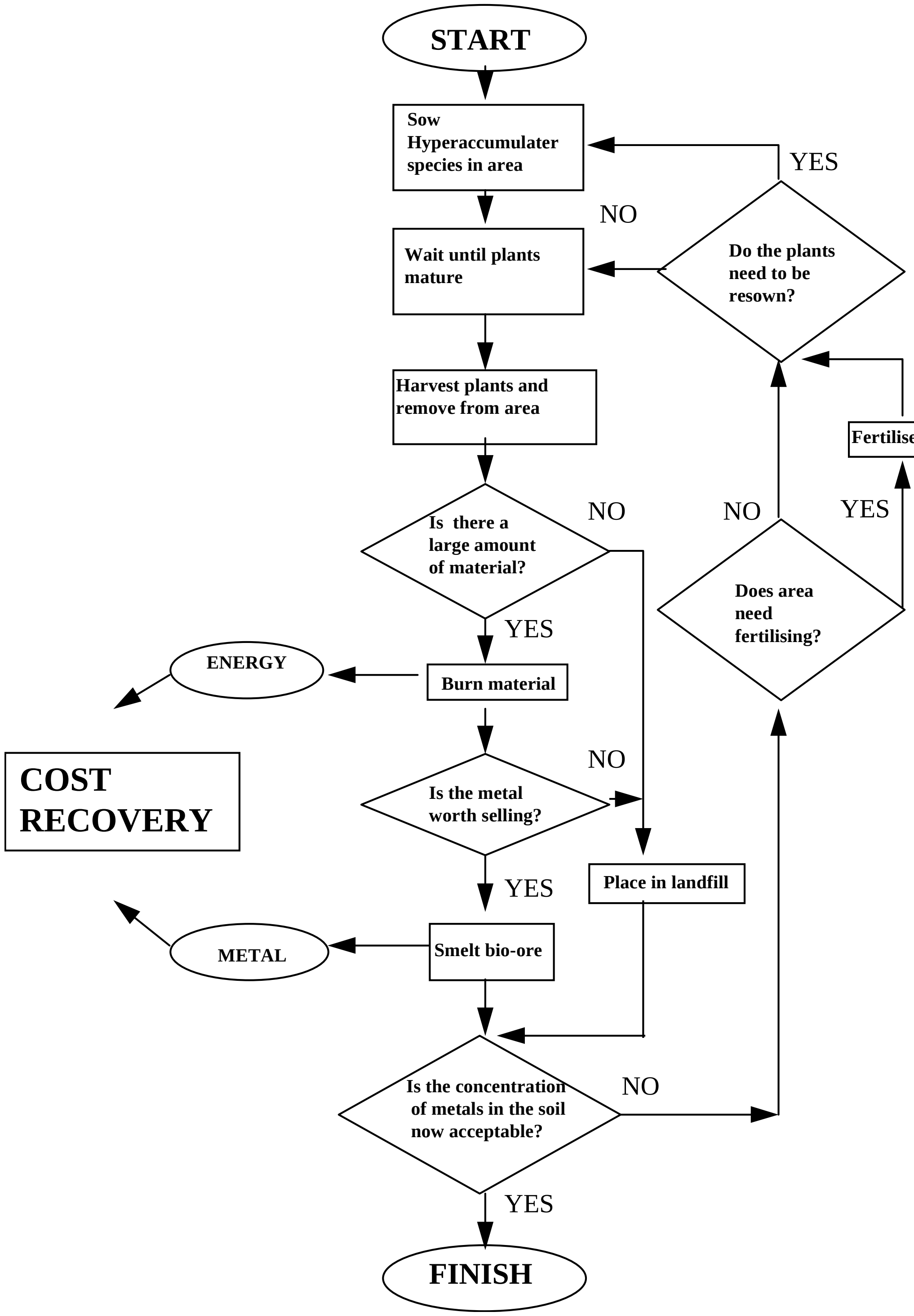

Fig. 42. Flow diagram of phytoremediation operation

There are several inherent limitations to phytoremediation. The main one is that plants can only extract a limited amount of metal per hectare per annum, only a few hundred kilograms at the absolute maximum. This factor places a limit on the amount of pollution that can be removed in a “reasonable” time frame. Soil containing several percent heavy metals, such as occur in Northern France, would take millennia to clean up using phytoremediation. The technology is thus necessarily limited to weakly polluted soils, with levels of contamination no greater than four or five times the acceptable limit. The economics of phytoremediation favour large areas, rather than small ones. The reasons for this are that the relative cost saving of using phytoremediation rather than conventional techniques is greater for large areas. The larger the crop grown, the smaller the costs of phytoremediation per hectare, moreover large quantities of plant material can be burnt economically and the metal sold to help in recovering the cost of the operation. Another limitation to phytoremediation is that the area upon which it is to take place must be amenable to the growth of the hyperaccumulator used in the operation. Climatic conditions must favour the crop's growth, and this involves remediating an area with a plant that naturally occurs in a similar climate. Any retardation of the plants growth due to maladaption to the climate, will not only slow the rate of metal extraction from the soil, but may also allow weeds to establish. Edaphic factors such as particle size, pH, and presence of other toxins must not prohibit plant growth. The addition of lime, fertilisers or other soil amendments may be necessary before the operation can take place.

The next chapter investigates the potential of the small zinc - cadmium hyperaccumulator plant *Thlaspi caerulescens* to remediate some soils contaminated with these elements. Specific aims were to determine the time needed to remediate soils with various metal loadings, and to measure the effect of soil-metal solubility on plant uptake of metals.

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Chapter 7

The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils

Publication arising from chapter:

Robinson,B.H.,Leblanc,M.,Petit,D.,Brooks,R.R.,Kirkman,J.H., and Gregg,P.E.H. 1998 The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils. Plant and Soil. 203, 47-56.

Abstract

Uptake of cadmium, zinc, lead and manganese by the hyperaccumulator *Thlaspi caerulescens* was studied by pot trials in plant growth units and in populations of wild plants growing over lead/zinc base-metal mine wastes at Les Malines in the south of France. The pot trials utilised metal-contaminated soils from Auby in the Lille area. A direct comparison of the behaviour of *Thlaspi* in these two types of environment was possible because of the fortuitous similarity of the pH of their soils. Zinc and cadmium concentrations in wild plants averaged 1.16% and 0.16% (dry weight) respectively. The unfertilised biomass of the plants was 2.6 t/ha, though this could at least be doubled by addition of nitrogen fertiliser. A single fertilised crop with the above metal content could remove 60 kg of zinc and 8.4 kg cadmium per hectare. Experiments with pot-grown and wild plants showed that metal concentrations (dry weight basis) were up to 1% zinc (4% Zn in the soil) and just over 0.1% cadmium (0.02% Cd in the soil). The metal content of the plants was correlated strongly with the plant-available fraction in the soils as measured by extraction with ammonium acetate and was inversely correlated with pH. Bioaccumulation factors (plant/soil metal concentration quotients) were in general higher for cadmium than for zinc except at low metal concentrations in the soil. There was a tendency for these factors to increase with decreasing metal concentrations in the soil. It is proposed that phytoremediation using *Thlaspi caerulescens* would be entirely feasible for low levels of cadmium where only a single crop would be needed to halve a cadmium content of 10 μg/g in the soil. It will never be possible to remediate elevated zinc concentrations within an economic time frame (<10 years) because of the lower bioaccumulation factor for this element coupled with the much higher zinc content of the soils.

Introduction

The inordinately high uptake of zinc by *Thlaspi calaminare* (Brassicaceae) was first described by Baumann (1885) who found that this plant accumulated over 1% zinc in dry tissue. This represents the first record of a plant hyperaccumulator of a heavy metal. Reeves and Brooks (1983) studied uptake of zinc by numerous *Thlaspi* L species and found several that were able to hyperaccumulate both zinc and nickel. The threshold of hyperaccumulation for zinc was set at 10,000 μg/g (1%) in dry matter.

The most important consideration in phytoremediation is that the plant should translocate metals from the soil to the aerial parts allowing a significant quantity of metal to be removed from the soil with each crop. In some cases, there are plants (such as *Thlaspi* L.) that can hyperaccumulate two or more elements at the same time and there is also the possibility of growing a mixed crop of two or more species to improve the versatility of the phytoremediation method.

Thlaspi caerulescens is a biennial herbaceous plant that is known to have a very high foliar zinc content (up to 3% dry weight - Baker and Brooks, 1989). It will also hyperaccumulate cadmium and nickel (>0.1% d.w. in each case). It occurs frequently on mineralised soils, particularly those with a high zinc content.

Fig. 43. La papeterie, Saint Laurent le Minier. At the rear of the village are old mine tailings rich in zinc, cadmium, lead and thallium.

Except for studies by McGrath *et al*. (1993) and Brown *et al*. (1994), most experiments with *Thlaspi caerulescens* (e.g. Vázquez *et al*. 1992; Brown *et al*. 1995; Pollard and Baker, 1996) have used hydroponic experiments to test the behaviour of this species with respect to zinc and cadmium. Hydroponic experiments are perhaps favoured probably because it is very easy to control the conditions so that the data are inherently more reproducible than where soil mixtures are used. Experiments using soils rather than solutions, however, approximate more closely to field and natural conditions where the effect of soil buffering capacity influences nutrient availability to plants..

The aims of this present study were to investigate the phytoextractive potential of *T.caerulescens* to remediate metal-contaminated soils. Specific aims were to:

1 - determine what metals might be extracted using *T.caerulescens*,
2 - establish how much metal could be extracted per hectare per annum,
3 - calculate the number of annual crops that might be needed to decontaminate soils with specific metal concentrations.

The above programme involved a combination of both pot trials and studies on wild plants. Although the pot trials were with substrates contaminated from smelter emissions and the field trials were on wild plants growing over mine tailings, it was possible to make a valid comparison between the two media because they both had the same pH. Metal uptake by *Thlaspi* is influenced more by pH than the chemical composition of the substrate as has been shown by Brown *et al.*. (1994).

Materials and Methods

Site description

Samples were collected from sites around St Laurent le Minier, Southern France (Figs. 43 and 44). The region, known as Les Malines, is the location of one of the largest base metal mines in Europe that has been exploited since Roman times and only ceased operations about 5 years ago. The mineralisation consists of Zn/Pb sulphides and oxides associated with barite. Although operations have now ceased, an attempt is being made to revegetate and ameliorate the enormous area of mine waste that now surrounds the little town of St Laurent le Minier.

The mine tips contain typically several percent of both lead and zinc with associated thallium and cadmium.

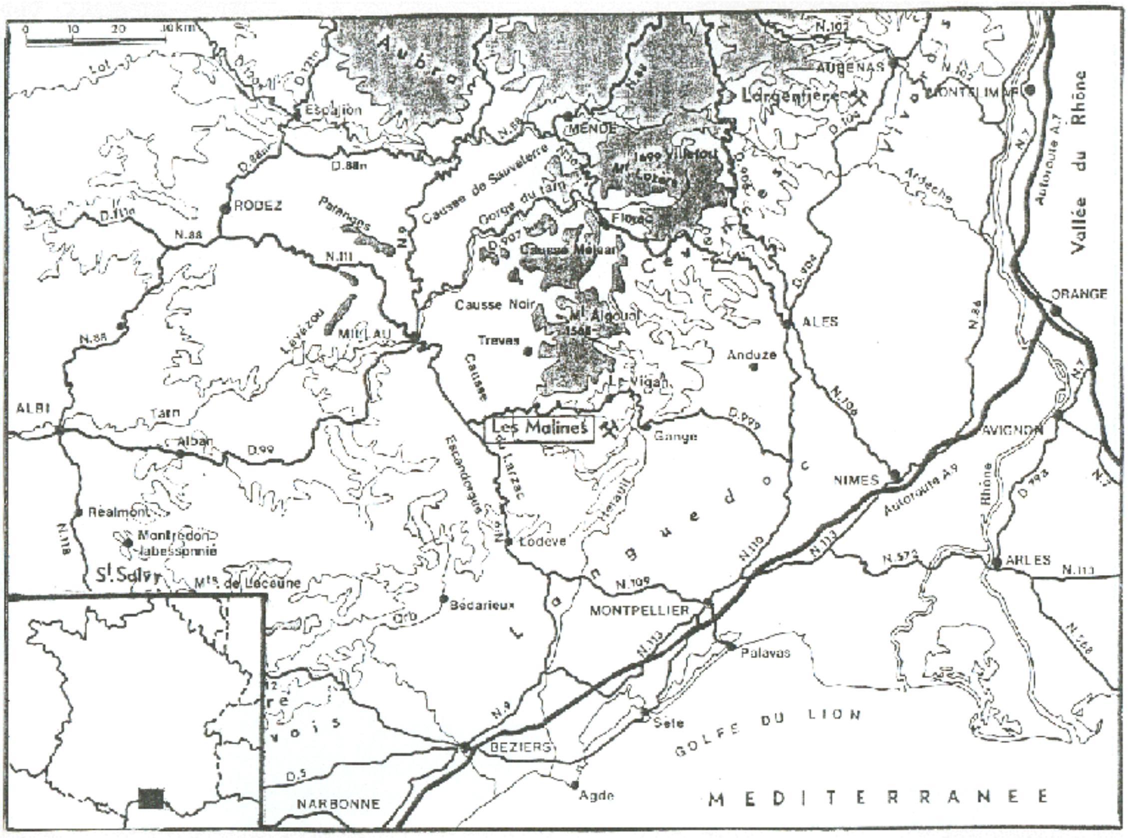

Fig. 44. Location map of the Les Malines base metal mining area of southern France. Dark areas are land over 1000 m altitude.

The pH of the tailings is typically 7.3 (mean of 60 samples) with a range of 6.4-7.7. The surprisingly high pH is due to the presence of calcareous material in the material.

The mine waste has been colonised by a typical base metal flora dominated by *Minuartia verna* and *Thlaspi caerulescens* with associated *Iberis intermedia* and *Armeria maritima*. Figure 45 shows a view of the base-metal plant community in this region.

Sample collection

Sixty plants were taken at various sites within Les Malines. The soils in which the plants were growing were also sampled. The diameter of each plant was measured and the dried plant weighed. From these values an estimation of the biomass production per hectare could be made. This was done by calculating how many plants could cover one hectare assuming 70% ground cover. This value was then multiplied by the dry weight of the plant to give the theoretical yield per hectare.

Experiments performed in plant growth units

Contaminated soil from the town of Auby, Northern France which contains 4% zinc, 1% lead and 300 µg/g cadmium, was mixed with soil from the university of Lille (which contains negligible amounts of these elements) in the following proportions (Auby: Lille): 1:0, 1:1, 1:3, 1:7, 1:15, 0:1. The pH of both soils was 7.0. Plants (n=10) were grown in pots (250 mL) containing each of the six mixtures. The experiments were performed in an unheated plant growth unit and the growing period was May-November 1996. The plants were harvested and analysed as described below.

Preliminary sample treatment

Plants were rinsed thoroughly in distilled water and dried at 50°C. Approximately 0.2 g of material from each plant was accurately weighed into a set of 20 mL boiling tubes. Concentrated nitric acid (10 mL) was added to each tube and the mixtures heated on a heating block until a final volume of ca. 3 mL was reached. The samples were then diluted to 50 mL using distilled water and stored in polythene containers.

Fig. 45. View of the metal-tolerant plant communities (mainly Thlaspi caerulescens) over the base-metal mine waste of the Les Malines mining area near St Laurent le Minier, France.

Soil digestion

Soil samples were dried at 50°C and sieved to <2 mm size using a nylon sieve. About 0.2 g quantities of sieved soil were ground using a mortar and pestle and then accurately weighed into boiling tubes. Ten mL of concentrated nitric acid was then added and the mixtures boiled until a final volume of 3 mL was reached. A further 10 mL of concentrated hydrochloric acid was then added and the mixtures again evaporated to 3 mL. After filtration, the solutions were diluted to 100 mL with distilled water.

Estimation of the pH and plant-available elemental fractions in the soils and mine waste

Approximately 5 g samples of sieved soil were weighed accurately into 150 mL polythene containers. Then 50 mL of 1*M* ammonium acetate was then added to each container. Samples were gently agitated (75 rpm) for 24 h, filtered, and stored in polythene containers. Ammonium acetate was chosen for the experiments because of its well proven use as a measure of the plant-available fraction of soils.

The pH measurements were made by shaking 4 g samples of soils or mine waste with 10 mL of distilled water for a period of 1 h. After being allowed to settle for 24 h, the samples were again shaken for a few minutes and the pH measured after an appropriate settling period.

Chemical analysis

Chemical analyses on the plant and soil solutions were performed using a GBC 904 atomic absorption spectrometer. The following elements were quantified: Cd, Mn, Pb and Zn.

Results and discussion

Accumulation of metals by wild specimens of *Thlaspi caerulescens*

The metal content of wild plants and associated soils collected from St Laurent le Minier is shown in Table 30. Since the data were lognormally distributed, the data (all as % except where otherwise stated) are shown as geometric means with the corresponding standard deviation ranges.

All plant specimens contained elevated concentrations of cadmium, lead manganese and zinc relative to "normal" plants growing in non-mineralised soils (see Table 30), for which respective concentrations of these four elements are of the order of 0.5, 5, 10 and 20 μg/g in the dry plant material. From Table 30 it will be noted that the mean elemental contents of cadmium, lead, manganese and zinc in one-year-old plants were respectively 3236, 169, 6.1 and 581 times higher than for other plants growing in non-mineralised soils. For two-year-old plants the respective values were 1054, 44, 1.6 and 262. The higher relative metal concentrations in the one-year-old plants may be due to the higher rate of metabolism of younger plants and the onset of senescence in the older plants, as might be expected in a biennial species. The higher metal concentrations in younger plants could also be due to their greater proximity to the ground and hence greater risk of contamination from wind-borne soil. If all the lead content of the plants is assumed to be due to contamination, however, and knowing the Cd/Pb and Zn/Pb quotients in the soils (0.01 and 2.30 respectively) corrected concentration values can be obtained for cadmium and zinc in *Thlaspi* that do not differ greatly from the original total metal burden (see Table 30). Because the cadmium content of the plants was considerably higher than that of the soil, the effect of contamination from wind-blown dust would have been to reduce the original metal content of the plants rather than to increase it.

Table 30 also shows the "corrected" metal concentrations in the plants, assuming that the entire lead burden is derived from wind-blown contamination. It will be seen that there is accumulation of all of the heavy metals concerned.

Table 30. Mean (geometric) elemental concentrations (μg/g in dry matter) in wild populations of *Thlaspi caerulescens* growing over base-metal mine wastes at St Laurent le Minier, near Montpellier, southern France. Values in parentheses are the standard deviation range.

Material	N	Zn	Cd	Pb	Mn
Soil (geom. mean)	60	38010	163	16531	688
(std. dev. range)	60	9907-157939	45-997	3074-67427	144-4493
Normal* plants	-	20	0.5	5	10
One-year *Thlaspi* plants (geom. mean)	40	11627	1618	844	61
(std. dev. range)	40	5463-27385	378-3689	95-4318	10-515
(corrected values**)	40	10207	1695	-	59
Two-year *Thlaspi* plants (geom. mean)	20	5242	527	219	16
(std. dev. range)	20	1137-29238	107-2413	6-2143	3-152
(corrected values**)	20	4866	532	-	7

*Mean elemental concentrations to be expected in vegetation not growing over mineralisation. **Corrected values assuming that the entire lead content is due to contamination. **Corrected values assuming that all of the lead content is derived from wind-borne contamination.

Metal accumulation by *T. caerulescens* under controlled conditions

The results of experiments with *T. caerulescens* raised in plant growth units at the University of Lille are shown in Figs. 46 and 47. In the case of zinc (Fig.46) there was a gradual increase of the metal concentration in the plants to about 1% d.w. as the zinc concentration in the soil increased to 4.0% thus giving a bioaccumulation factor (concentration of the metal in dried plants divided by the soil content of the same element) of 0.25. A similar observation can be made for cadmium (Fig.47) where there appeared to be a limiting value approached 0.2% (2000 μg/g) for soils containing 400 μg/g cadmium. This limiting value represents a bioaccumulation factor (BF) of 5.0.

Bioaccumulation factors for zinc and cadmium as a function of metal content of the soil for the plant-growth-unit experiments are shown in Figs. 48 and 49. Inspection of these two plots allows the following observations to be made:

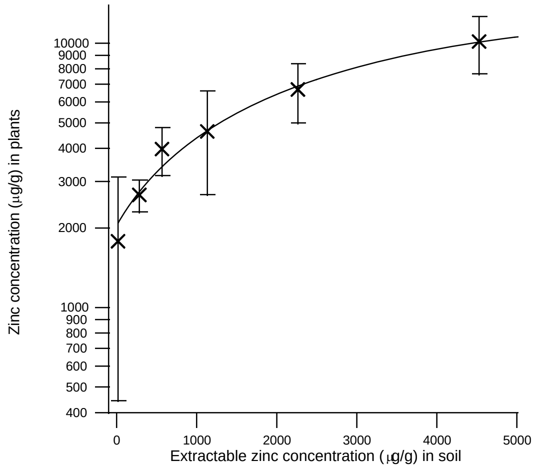

Fig. 46. The zinc content of specimens of Thlaspi caerulescens as a function of the extractable (1M ammonium acetate) zinc content of the soil.

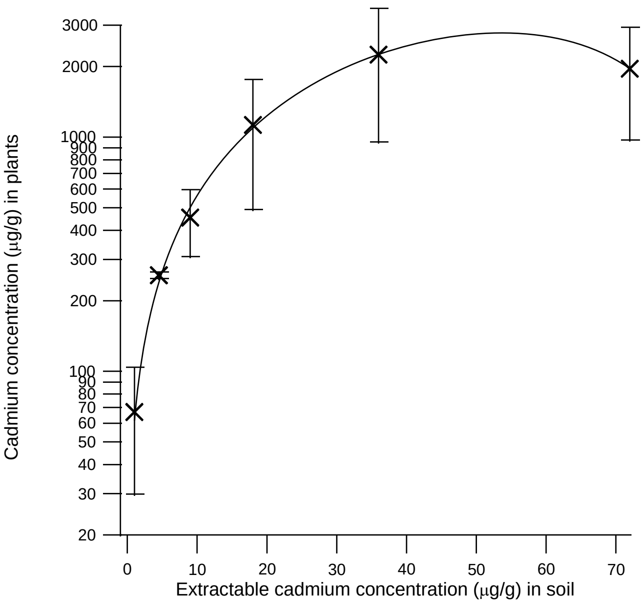

Fig. 47. The cadmium content of specimens of Thlaspi caerulescens as a function of the extractable (1M ammonium acetate) cadmium content of the soils.

1 - in the case of zinc (Fig.48), bioaccumulation factors decreased with increasing metal content in the soil. BF values ranged from 104 (0.0017% Zn) to 0.25 (4% Zn). For cadmium (Fig.49) the BF values ranged from 67 (1 µg/g Cd) to 5.4 (360 µg/g Cd). It follows therefore, that if *Thlaspi caerulescens* were to be used for phytoremediation of cadmium and zinc in contaminated soils, the efficiency of removal would be inversely related to the degree of contamination of the substrate, since BF values are higher at these lower concentrations of the two metals.

2 - as indicated by the high error bars (i.e. standard deviations) of the data points in the two figures, there was a great deal of variability in individual metal contents. For example, the cadmium content of the plants varied from 1000-4000 µg/g for 200 µg/g cadmium in the soil. In the case of zinc, the corresponding figures were 2500-7500 µg/g for 1% of this metal in the soils. This large variation was encountered despite the plants being closely controlled and having been planted in homogeneous soil mixtures.

The high degree of variability of metal levels in *T. caerulescens* found in our study has been mirrored in hydroponic experiments by Pollard and Baker (1996) who reported a range of 1.8-3.3% zinc in foliar material. McGrath *et al.* (1993) reported a range of zinc concentrations from 0.25-0.66% in plants for a metal content of just under 300 µg/g in the soil. These and the present experiments, highlight the extreme genetic variability of *Thlaspi caerulescens* that could lead to successful selection of individuals with inordinately high uptake of heavy metals and their propagation by plant breeding for use in phytoremediation.

Biomass production and potential metal yields of *Thlaspi caerulescens* under natural conditions

Our field observations and measurements on natural populations of *Thlaspi caerulescens* growing over base-metal waste near St Laurent le Minier, southern France, have shown that unfertilised plants have an annual biomass production of 2.6 t/ha. This is very low compared with maize (30 t/ha) or even the unfertilised biomass of the nickel hyperaccumulator *Alyssum bertolonii* at 4.5 t/ha (Chapter 4). Taking the mean zinc content of the plants of 1.16% (Table 30) and cadmium content of 0.16%, it can be calculated that a crop of this same biomass could remove 30.2 kg/ha of zinc and 4.2 kg/ha of cadmium.

Bennett *et al.* (1998) have shown that fertilising crops of *T. caerulescens* grown in base-metal mine tailings from the Tui Mine, Te Aroha, New Zealand, can increase the biomass by a factor of three without appreciable reduction in the zinc or cadmium concentrations. If a conservative approach is adopted and use a factor of two, a biomass production of 5.2 t/ha would yield 60 kg/ha of zinc and 8.4 kg/ha of cadmium. This is in reasonable agreement with the findings of McGrath *et al.* (1993) who in their benchmark paper, reported 30.1 kg/ha for zinc extracted by moderately fertilised plants from soils containing only 444 µg/g. In relative terms, the data of McGrath *et al.* (1993) indicate a much higher uptake than found by us. As mentioned above, bioaccumulation factors are much higher for lower metal concentrations in the substrate so that the two sets of data are not in conflict.

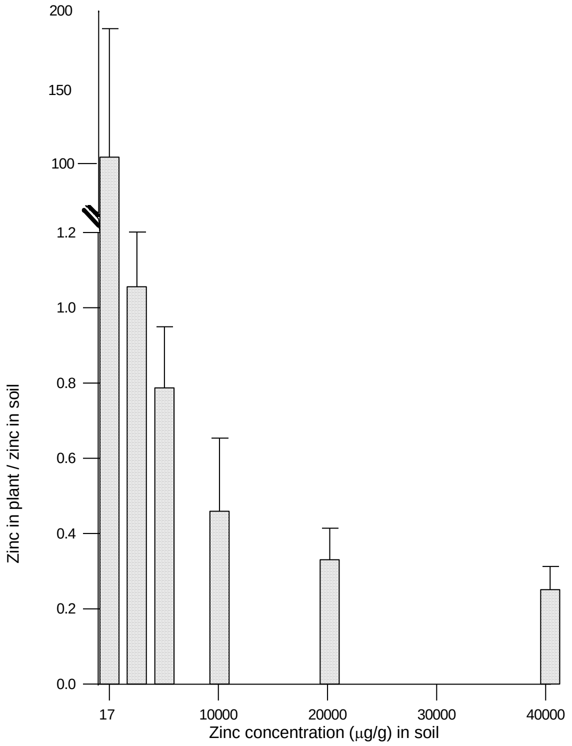

Fig. 48. Bioaccumulation coefficients (plant/soil concentration quotients) for zinc in Thlaspi caerulescens as a function of the zinc content of the soils.

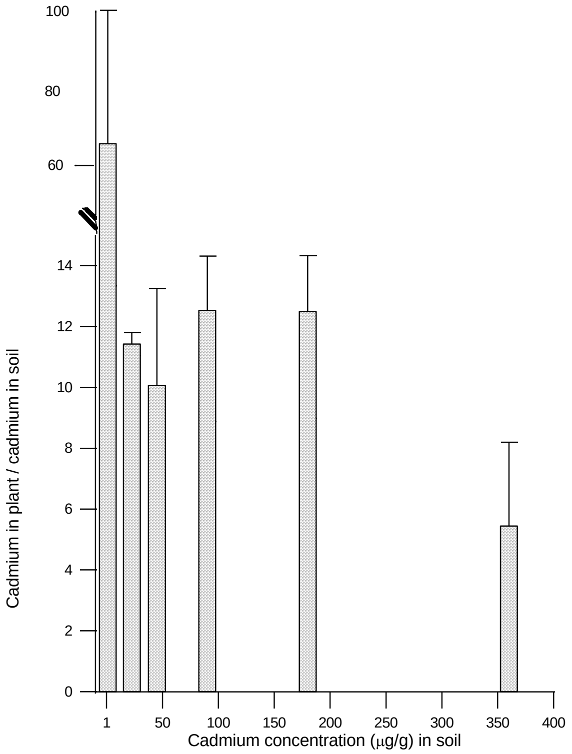

Figure 49. Bioaccumulation coefficients (plant/soil concentration quotients) for cadmium in Thlaspi caerulescens as a function of the cadmium content of the soils.

The relationship between the metal content of wild plants (Les Malines) and pH and the "available" and total elemental concentrations in the soils or mine tailings

Table 31 shows a matrix of correlations based on lognormally distributed abundance data for elemental concentrations in soils and one-year- and two-year-old wild plants from mine tailings at Les Malines. Considering the soils alone, all three elements tested (Cd, Pb and Zn) had concentrations (total and/or extractable) that were significantly correlated with each other except for extractable cadmium vs. total zinc and total lead. This is despite the fact that total cadmium/zinc relationship (r=0.95) is by far the strongest in the set. The difference in extractability of two of the elements (11.6% for cadmium and 5.7% for zinc) may explain why bioaccumulation factors for cadmium were in general higher than those or zinc except at very low concentrations of either element in the soil.

It is well known (e.g. Brown *et al*. 1994) that the pH of the soil is one of the most important factors governing elemental accumulation by plants. It will be noted from Table 31, that pH was inversely correlated with all elements in soils and with the same three metals in two-year-old plants. The pH vs. soil inverse correlations for extractable metals merely highlight the well-known fact that solubility of most elements in soils decreases when the pH is raised. It would appear however that only the two-year-old plants showed an inverse correlation between pH and elemental abundances in their tissues. The lack of similar inverse correlations for the one-year-old plants may be related to their juvenile nature, presumably it is only when the plants are fully mature that the pH of the soil has its maximum effect on metal uptake. It is clear that metal yields will be able to be increased by addition of acidifying agents such as humus or acid fertilisers such as ammonium sulphate to the soils.

Despite the overriding effect of pH on metal uptake, it must be realised that the pH range of the Les Malines tailings was only 6.4-7.7 with a mean of 7.3. This mean was very close to that of the Auby soils (7.0) and allows for a certain degree of comparison between the two types of soil as well as allowing for meaningful observations on plant/soil relationships in the Les Malines region.

The concentrations of zinc and cadmium in both one-year- and two-year-old wild plants (Table 31) showed a highly or very highly significant correlation with the extractable metal content of the supporting soils. For plants raised in plant growth units, the correlation was less strong but nevertheless significant (0.05>P>0.01).

Table 31. Correlation matrix for concentrations of total and extractable elements in soils (n=60) and their corresponding values in one-year-old (n=20) and two-year-old (n=40) plants.

	aCd	aPb	aZn	bCd	bPb	bZn	cCd	cPb	cZn	dCd	dPb	dZn
aPb	S*											
aZn	S*	S**										
bCd	S*	S**	S**									
bPb	NS	S**	S**	S**								
bZn	NS	S**	S**	S**	S**							
cCd	S**	NS	NS	NS	NS	NS						
cPb	NS	S*	S*	S*	S*	NS	NS	NS				
cZn	S*	S*	S*	S**	NS	NS	NS	S**				
dCd	S*	NS	NS	NS	NS	NS	S**	NS	NS			
dPb	NS	S**	NS	NS	S*	NS	NS	NS	NS	NS		
dZn	NS	NS	S**	S*	NS	S*	NS	S**	S**	NS	NS	
pH	-S*	-S**	-S*	-S*	-S*	-S*	NS	NS	NS	-S*	-S*	-S*

a - extractable element in soil, b - total element in soil, c - one-year plants, d - two-year plants.
S** - very highly significant (P<0.001), S* - highly significant (0.001<P<0.01), NS - not significant (P>0.01).
NB - negative symbols imply an inverse relationship.

In the case of extractable lead in soils, there was a very highly significant plant/soil correlation for 2-year-old plants wild plants, and a highly significant correlation for 1-year-old specimens. For total lead in soils there was no plant/soil correlation with lead in one-year-old plants but a highly significant relationship for two-year-old plants. This again highlights the different behaviour between young and mature plants as already mentioned above.

Figures 50 and 51 show the relationship between zinc and cadmium concentrations in *Thlaspi* plants as a function of the corresponding concentrations in ammonium acetate extracts of the supporting soils. These plots do not differentiate between young and mature plants and for both elements show a trend towards limiting values of just over 1000 μg/g cadmium and 10,000 μg/g (1%) zinc. The two plots are similar in shape to the plant/soil relationships for Auby soils (Figs. 46 and 47) and appear to show a similar behaviour for both metals bioaccumulated either from Auby soils contaminated by smelter emissions or from mine waste at Les Malines. This similarity of behaviour is probably due to the fortuitous similarity of the pH of the two environments. The two sites would not have been comparable otherwise.

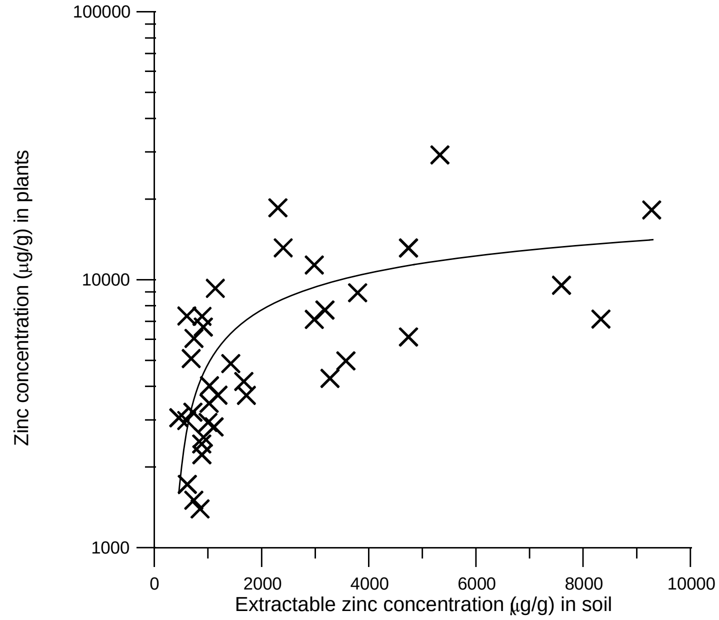

Fig. 50. Uptake of zinc by specimens of Thlaspi caerulescens as a function of the extractable (1M ammonium acetate) zinc content of the soil.

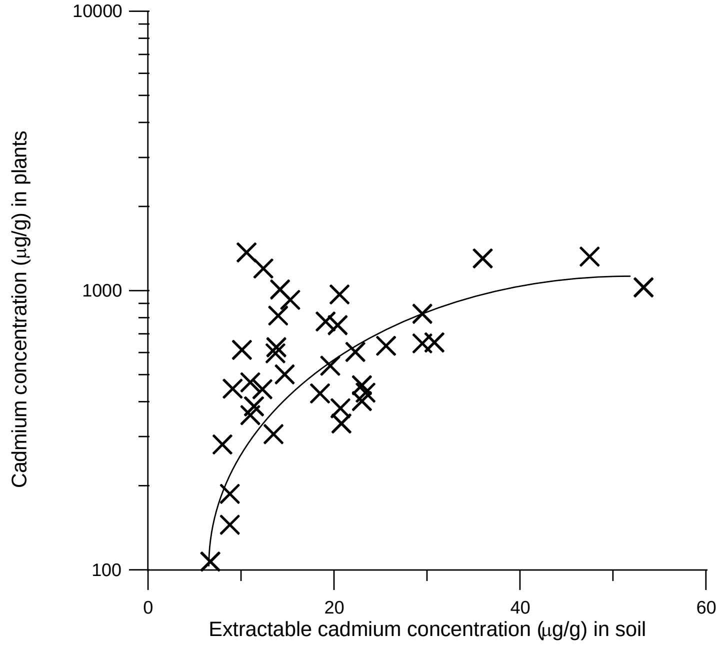

Fig. 51. Uptake of cadmium by specimens of Thlaspi caerulescens as a function of the extractable (1M ammonium acetate) cadmium content of the soil.

The true potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils

For the successful practical application of phytoremediation a number of variables must be considered. Among the most important of these is the product of biomass and metal content of the plant material. This has been shown by Bennett *et al.* 1998 in experiments with *Thlaspi caerulescens* growing over base-metal mine waste. The product of biomass (weight of individual plants) and the zinc concentration in the plants rose from 22 μg for unfertilised specimens to 497 μg for plants fertilised with 50 μg/g nitrogen as calcium ammonium nitrate. The second variable to be considered is the time span (i.e. number of annual crops) needed to achieve the desired degree of remediation of the soil. In making these calculations, it cannot be assumed that all of the target element is plant-available. Adopting the conservative approach that only half of the total metal content of the soils will be available to plants, it has been calculated the total number of annual croppings of *T. caerulescens* needed to remove half of the metal burden of contaminated soils down to a depth of 15 cm assuming that the soil has a density of 1.3. The plant is assumed to have a fertilised biomass of 5.2 t/ha and to contain 1.16% zinc and 0.16% cadmium. These data are shown in Table 32

Some of the values in Table 32 are of course extraordinarily high and have only been included for the sake of completeness. Any period of annual cropping exceeding 10 years would obviously be totally uneconomic. This implies that only contaminated soils with <500 μg/g zinc could be remediated to half this value within the limits of 10 years (actual time 8.13 years). This is somewhat lower than the 13 croppings suggested by McGrath *et al*. (1993) to reduce a soil content of 444 μg/g zinc to 300 μg/g but can be accounted for by the higher biomass reported here for fertilised plants. It is obvious that it will never be economically feasible to remediate mine tips or heavily contaminated soils by phytoremediation if zinc is the target metal.

In the case of cadmium, the problem is different. Much lower concentrations of cadmium than of zinc, are to be expected in contaminated soils and the mean bioaccumulation factor for *Thlaspi caerulescens* is much higher for cadmium than zinc, except where the concentrations of both elements are <1 μg/g in the supporting soil. From Table 32, a burden of 500 μg/g cadmium (hardly to be expected under natural conditions) would require just under 60 crops to be reduced to half this value but 20 μg/g could be reduced to 10 μg/g in just over 2 years and if the soil contained 10 μg/g cadmium, a single crop would reduce this to nearly one half after only one year.

There is at present much concern worldwide about the steady build-up of cadmium in pastures from application of sewage sludge or superphosphate dressings. Levels as high as 7 μg/g have been recorded in pastures (Taylor and Percival, 1994) and it would seem that growing a crop of *Thlaspi caerulescens* might be an efficacious and cost-effective option in specific cases. A few words of caution must be expressed here.

Table 32. Total number of annual croppings required to remove half of the metal burden of contaminated soils using fertilised *Thlaspi caerulescens* plants with a biomass of 5.2 t/ha and containing 1.16% zinc and 0.16% cadmium in dry matter. The soil is assumed to have a density of 1.3 and to be penetrated by plant roots to a depth of 15 cm.

Initial metal content (μg/g)	Mass of metal (kg)	Zinc	Cadmium
100,000 (10%)	150,000	1625	11606
20,000 (2%)	30,000	325	2320
10,000 (1%)	15,000	163	1160
2000 (0.2%)	3000	33	231
1500 (0.15%)	2250	24	174
1000 (0.1%)	1500	16	116
500 (0.05%)	750	8.1	59
200 (0.02%)	300	3.3	23
100 (0.01%)	150	1.6	12
20 (0.002%)	30	0.33	2.3
10 (0.001%)	15	0.17	1.2
2 (0.0002%)	3	0.04	0.23

In suggesting that *T. caerulescens* might be used for phytoremediation of low levels of cadmium (i.e. <2 μg/g) in soils, it is assumed that the bioaccumulation factors will be as high as in the case of the higher concentrations used in these experiments. It is also assumed that competition from weeds (never a problem in highly mineralised soils) will not be an insurmountable problem.

Because of the much higher phytotoxicity of cadmium with respect to zinc, there is even the possibility of removing the former selectively from mine tailings in order to make the substrate more amenable to revegetation with other species.

A new and burgeoning technology in phytoremediation of contaminated soils involves the use of complexing agents such as EDTA in order to increase the solubility of metals. So far the emphasis has been on complexing of lead (Huang and Cunningham 1996) but there is no reason why the same procedure should not be used to increase cadmium or zinc uptake by *Thlaspi* or other plant accumulators. It is probable that saturation has already been reached by *Thlaspi* for zinc, but there should be some scope to increase the cadmium uptake by this plant. If this could be achieved, the use of *Thlaspi caerulescens,* at least for phytoremediation of cadmium contamination, will become an entirely feasible proposition.

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Chapter 8
Concluding summary

Plants have the potential to be used for the extraction of heavy metals from soils in some instances. Hyperaccumulator plants have potential use for both phytomining and phytoremediation, but several conditions have to be met before the operations can be viable. The conclusions drawn from findings of this study are:

(1) *The heavy metal in the soil must be soluble to some degree before it can be taken up by plants.*
This fact precludes the phytomining of the valuable metal chromium from serpentine soils. Although the total concentration of this element in such soils is high, its solubility is very low and consequently it is not taken up by plants. Compounds may, however, be added to the soil to increase an element's solubility. All the heavy metals investigated in this study became exponentially more soluble as the soil was acidified. Acidification may be used to increase plant uptake, provided that the pH remains in the plant's growth range. A small decrease in soil pH can greatly increase metal solubility. Chelating agents may also be added to solubilise metals. Further work needs to be done to elucidate the relationship between metal solubility and metal uptake. Although previous studies have shown that EDTA addition to soils increases lead solubility and induces lead hyperaccumulation, EDTA addition to serpentine soils decreases the nickel content of hyperaccumulators, despite increasing the solubility of the metals.

(2) *The metal concentration in plants is proportional to the extractability of the metal in the soil.*
All the hyperaccumulators tested, showed a significant correlation between the metal concentration in the plant and the ammonium acetate extractable metal in the soil. In all cases the metal concentration in the plant increased logarithmically as the soil soluble metal fraction increased. This allows a prediction of the metal removed per hectare by performing a chemical extraction on the soil. Despite this relationship, artificially increasing nickel solubility by the addition of chelating agents actually caused a decrease in plant nickel content of some hyperaccumulators of nickel.

(3) *The solubility of nickel in ultramafic soils decreases logarithmically after successive removals by extraction.*
Ultramafic soils are able to support several theoretical crops of nickel before the solubility of nickel in the soil drops to levels that are too low for hyperaccumulators to extract economic quantities. Field trials will ultimately be necessary to determine if sequential extractions accurately model the behaviour of nickel in the soil. There is a need to determine the solubility of other metals upon successive extractions, in both natural and anthropogenic metalliferous soils. The phytoremediation of some metals may not be feasible if their solubility drops to levels where plants can no longer hyperaccumulate them.

(4) *The plant needs to extract a significant proportion of the metal if the operation is to be viable.*
In the case of phytomining, the amount of metal extracted required for the success of the operation is dependant on the price of the metal. The maximum theoretical metal yield per hectare per annum is around 500 kg, achievable only under optimal conditions. It will therefore never be economic to phytomine metals of low economic value.
Plants used in phytoremediation operations should be able to remove enough metal so that the soil-metal concentration will fall below acceptable limits in a reasonable time span. This is proportional to the initial soil-metal concentration. Phytoremediation may only be used on weakly contaminated soils.

(5) *Phytoextraction is more viable over large areas.*
Factors which make phytoremediation favourable over conventional techniques i.e. cost, apply more so when the area is large. This is determined by economics of scale of growing a large crop of plants. Large areas also have the possibility of producing plant material for combustion and metal reclamation, both of which offset the costs of the operation. In the case of phytomining this is the single most important factor, indicating that these operations can only be successfully carried out over large areas.

The future of phytoextraction

At the present time, phytoextraction has the possibility to be economically and effectively utilised under certain conditions, but there is capacity for improvement. This would expand the range of conditions in which phytoextraction could be used. All the potential advances centre around increasing the metal extracted per hectare by plants. There are several paths by which this might be achieved:

(1) The discovery of new hyperaccumulators.

The current list of hyperaccumulators may not be complete. Large areas of vegetation throughout the world have not been analysed, and many species have been analysed for only a few elements. Phytoextraction will be improved by the discovery of high-biomass, high metal content plants. Hyperaccumulators of precious or semi-precious metals may be discovered. This is not as ludicrous as might first appear. Serpentine soils often contain elevated concentrations of the platinum metals. Some sulphide deposits and geothermal areas, are relatively rich in gold and silver. Gold might be economically extracted from plants with a foliar concentration as low as 1 μg/g.

(2) Selection of plants with a high metal - high biomass genotypes.

There is some evidence that populations of hyperaccumulators vary with respect to metal content and biomass production. There is probably also genetic variation between individuals. Selection of individuals with genetic coding for high metal content, high biomass production, and superior tolerance to soil heavy metal content will not only augment metal crops, but may also provide genetic material capable of being introduced into other species.

(3) Genetic manipulation to create superior plants.

Work is currently being conducted at the University of Lille, to identify gene(s) responsible for the hyperaccumulation phenomenon. The isolation of this genetic material may allow the genetic manipulation of high biomass plants such as *Zea mays* to produce a plant that will extract large quantities of metal from soils. Alternatively, the biomass production of an existing hyperaccumulator may be improved. A good candidate for this would be *Thlaspi caerulescens* which can hyperaccumulate nickel, zinc and cadmium but has only a small biomass production.

(4) Addition of soil amendments.

The chemical inducement of lead hyperaccumulation is now well known. The addition of chemicals to the soil to induce the hyperaccumulation of other metals will be a fertile area of research. The addition of thiosulphate to soils solubilises gold and palladium. Experiments with *Berkheya coddii* have shown that solubilising an element does not necessarily increase its uptake by the plant. More work needs to be conducted on how the plant chemically sequesters metals from soil solution.

A major factor in soil amendments is the cost of the compounds. This is particularly important in the field of phytomining where anything added to the soil is subtracted from profit.

(5) The study of the biota associated with the rhizosphere

The role of soil bacteria and fungi in the hyperaccumulation phenomena is unknown. These organisms may play an important role in the solubilising, sequestering, and transportation of metals into the plant.

Final comments

Phytoextraction has potential as an efficient method for removing metals from soils under some circumstances. It will remain a fertile area for research due to demands to remediate the vast world-wide heavy-metal-contaminated areas, and to extract metals from low-grade ore bodies as existing mining techniques exhaust the remaining high-grade deposits. The next step in these studies will be larger-scale field trials *in situ* on contaminated sites and low-grade ore bodies.

Appendix: References for plant names

NAME	REFERENCE
Alyssum bertolonii	Desv.
Alyssum corsicum	Duby
Alyssum lesbiacum	(Candargy) Rech. f.
Alyssum murale	Waldst & Kit
Alyssum tenium	Halacsy
Arabidopsis thaliana	
Armeria maritime	(Miller) Willd.
Astragalus spp.	L.
Becium homblei	(De Wild.) Duvign. & Plancke
Berkheya coddii	
Blechnum procerum	
Brassica juncea	
Cardaminopsis halleri	
Cassinia vauvilliersii	(Decne.) Hook. f.
Caulanthus spp.	G. Don
Chionochloa diffracta.	
Colobanthus strictus	
Corira arborea	
Cyathodes juniperina	(J.R. & G. Forst.) Druce
Dracophyllum uniflorum	Bergg.
Euphrasia monroi	Hook. f.
Gentiana corymbifera	T. Kirk
Haumaniastrum katangense	
Haumaniastrum robertii	(Robyns) Duvign. & Plancke
Hebe odora	(Hook.) ckn.
Iberis intermedia	
Kunzea ericoides	(A. Rich.) J. Thompson
Leptospermum scoparium	Forster et Forster f.
Leucopogon fasciulatum	#Hook. f.
Melicytus alpina	
Minuartia verna	(L.) Hiern.
Myosotis monroi	Chessem.
Nothofagus fusca	(Hook. f.) Oerst.
Nothofagus menziesii	(Hook. f.) Oerst.
Nothofagus solandri	(Hook. f.) Oerst.
Nothofagus solandri var cliffortoides	(Hook. f.) Poole
Notothlaspi australe	Hook. f.
Phormium cookianum	
Phyllocladus trichomanoides	D. Don
Pimelea suteri	Kirk
Pinus pinaster	Aiton
Pinus radiata	D. Don
Pisum sativum	L.
Poa picta	
Podocarpus hallii	Kirk-
Pseudopanax crassifolius	(Sol. ex A. Cunn.) C. koch-
Pteridium aquilinum	(L.) Kühn.-
Sebertia acuminata	Pierre ex Baillon-
Streptanthus polygaloides	Gray
Thlaspi caerulescens	J.C. & R. Presl.
Thlaspi calaminare	(Lej.) Lej. & Court.
Thlaspi ochroleucum	Boiss. & Heldr.
Trifolium spp.	L.
Zea mays	L.-