Chapter fifteen:

The Potential Use of Hyperaccumulators and Other Plants for Phytomining

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Introduction

The first suggestion that hyperaccumulator plants could be used for "metal mining" was made by Baker and Brooks (1989), but another eight years were to pass until Nicks and Chambers (1995 - see also Chapter 14) demonstrated the economic feasibility of growing a crop of nickel by use of the nickel hyperaccumulator *Streptanthus polygaloides* in California. They showed that the potential value of a crop of nickel was about the same as that of a crop of wheat provided that use could be made of some of the energy used in combustion of the dry material to produce the *bio-ore* containing about 15% nickel metal.

The benchmark paper by Nicks and Chambers (1995) was followed two years later by a study by Robinson *et al.* (1997) in Italy using the well known hyperaccumulator *Alyssum bertolonii*, the first plant of this type to be identified (Minguzzi and Vergnano, 1948). Salient details of this latter research are given below.

The term *phytomining* has been used to describe this emerging technology which at present is very far from being established commercially. Nevertheless, some new developments described below, may well serve to make the technique a viable proposition in the next decade. These include the following:

1 - discovery of hyperaccumulators with a large biomass;

2 - discovery of fertiliser amendments that can increase the biomass of hyperaccumulators without affecting metal uptake to any significant degree;

3 - utilisation of chelates that would permit higher metal uptake by hyperaccumulators or by plants of high biomass that do not usually hyperaccumulate the target metals;

4 - selection for plant breeding, of individual wild hyperaccumulators with

greater than average metal contents or biomass;

5 - use of biotechnology to introduce *hyperaccumulation genes* into non-accumulators of high biomass.

It should be emphasized that all of the above strategies are equally applicable to phytoextraction for remediation of polluted sites and that current research is mainly directed to this latter purpose. The essential difference between phytoremediation and phytomining is that economic considerations are paramount for the latter procedure, whereas they are of lesser importance in phytoremediation.

Potential Strategies for Enhanced Phytomining

Use of hyperaccumulators of high biomass

It is obvious that it is preferable to use hyperaccumulators with high biomass rather than those of lower biomass, provided that there is not the trade-off of a lower metal content. Unfortunately, most of the well known hyper-accumulators of heavy metals have a low biomass. This is especially true of plants such as *Thlaspi caerulescens*, that can contain over 3% zinc. Field trials with this plant over a soil containing 381 μg/g zinc showed that it contained about 6000 μg/g dry weight of this element (McGrath *et al.*, 1993). In these trials, the fertilised plant biomass was around 5.1 t/ha. Several of our field trials have shown that fertilising crops of many hyperaccumulators with NPK amendments usually increases the biomass by 300%.

Table 15.1 is a rough estimate of expected biomasses and potential metal yield of fertilised and unfertilised crops of selected hyperaccumulators of various metals. Though not an accumulator, *Zea mays* has been included for comparison since it affords one of the highest biomasses of any annual plant species.

Table 15.1. Unfertilised (A) and fertilised (B) biomasses (t/ha) and highest % metal content (C) of selected plants species. The final column shows the potential metal yield in kg/ha.

Plant species	Target	A	B	C	kg/ha
Zea mays	None	20.0	30.0	-	-
Berkheya coddii	Ni	12.0	24.0	0.60	144
Homalium kanaliense	Ni	12.0	36.0	*0.056	20
Alyssum tenium	Ni	7.7	23.0	0.34	78
A.lesbiacum	Ni	5.0	15.0	1.00	150
A.murale	Ni	4.6	13.8	0.71	98
A.bertolonii	Ni	3.0	9.0	1.34	121
Haumaniastrum katangense	Co/Cu	2.5	7.5	0.22	17
Thlaspi caerulescens	Zn/Cd	1.7	5.1	3.00	153
Cardaminopsis halleri	Zn	0.9	2.6	0.40	10

*Though leaves contain typically 0.6% nickel, the whole plant contains far less. Data based on own studies, McGrath *et al.* (1993), and Brooks *et al.* (1979).

The biomass of the plant must be related to its metal content in assessing its suitability for phytoremediation or phytomining. The final column of Table 15.1 shows (in kg/ha) the product of the biomass (t/ha) and the metal content (%) of the plant. This highest expected metal yield is more important than the actual biomass. It would appear from the table that the plants with the highest biomass are in descending order: *Homalium kanaliense, Zea mays, Berkheya coddii, Alyssum tenium, A.lesbiacum, A.murale, A.bertolonii, Haumaniastrum katangense, Thlaspi caerulescens* and *Cardaminopsis halleri*. In terms of expected metal yield (kg/ha) the order is somewhat different and, again in descending order, follows the sequence: *Thlaspi caerulescens, Alyssum lesbiacum, Berkheya coddii, A.bertolonii, A.murale, A.tenium, Homalium katangense, Haumaniastrum katangense* and *Cardaminopsis halleri*. It must be remembered that the essential difference between phytoremediation and phytomining is that in the latter technology, the value of the metal crop is of paramount importance. Whereas *Thlaspi caerulescens* would appear to extract the greatest mass of metal among the plants listed in Table 1.2 (Chapter 1), the commercial value of this metal (zinc) is so low that the plant could never be used for biomining. On the other hand, the Zaïrean copper/cobalt accumulator, *Haumaniastrum katangense* with a yield of only 17 kg/ha of each of copper and cobalt might well be able to provide an economic crop because of the current high price of cobalt ($48,000/t). This question will be addressed in a later subsection of this chapter.

Fertiliser amendments as a means of increasing biomass and metal yields

There has already been some discussion in Chapters 10 and 11 of the use of fertilisers to increase the biomass of selected hyperaccumulator plants. There will therefore be only limited discussion of the subject in this chapter. Table 15.2 summarises the increases of biomass achieved in various experiments with hyperaccumulator plants.

Table 15.2. Increases of hyperaccumulator plant biomass achieved by various fertiliser amendments.

Plant species	Fertiliser amendment	% increase	Reference
Alyssum bertolonii	N + P	760	Chapter 11
	Calcium	51	Robinson *et al.* (1997)
	Nitrogen	130	Robinson *et al.* (1997)
	Phosphorus	101	Robinson *et al.* (1997)
	N + P	189	Robinson *et al.* (1997)
	N + P + K	308	Robinson *et al.* (1997)
	As above + calcium	294	Robinson *et al.* (1997)
Streptanthus polygaloides	Nitrogen & phosphorus	153	Chapter 11
Thlaspi caerulescens	Nitrogen	195	Chapter 11

be achieved by fertilisation of the substrate. The experiments carried out by Robinson *et al.* (1997) were performed on naturally occurring populations of *Alyssum bertolonii* in Italy, whereas the other experiments were carried out as pot trials.

In the Italian work there was a highly significant positive correlation ($0.01 > P > 0.001$) between nickel uptake in the reproductive structures of *A. bertolonii* and the extractable nickel content of the associated soils (Fig.15.1). This indicates that addition of fertilisers that increase this available nickel content should increase the nickel content of the plants.

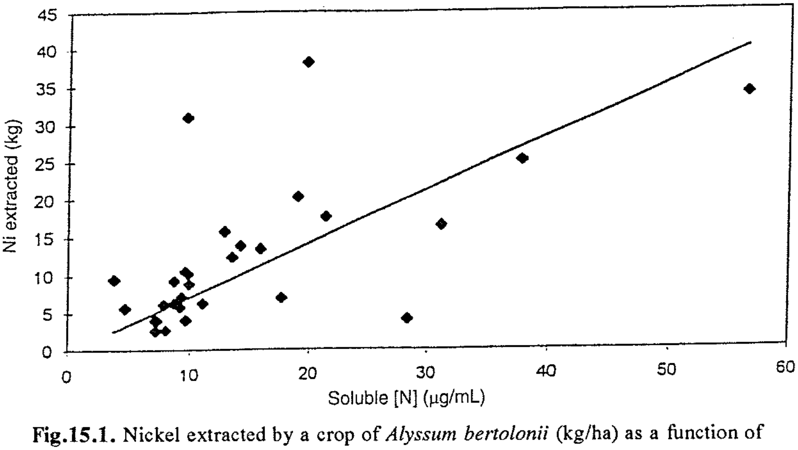


Fig.15.1. Nickel extracted by a crop of *Alyssum bertolonii* (kg/ha) as a function of the available nickel content of the soil as determined by ammonium acetate extraction at pH 7. Source: Robinson *et al.* (1997).

Table 15.2 shows that extractability of nickel 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 fertiliser. Robinson *et al.* (1996) 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 nickel "crop" is desired. There was a similar reduction in magnesium availability when calcium was used in fertilisers. Paradoxically, addition of $CaCO_3$, though reducing nickel and magnesium 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 nickel.

With the addition of fertilisers, the maximum annual biomass increase (ABI) in this Italian study, was about 300% (Table 15.2). The highest individual increase (130%) was with nitrogen alone, and the highest combined increase (308%) was with N+P+K.

The nickel content of the Italian field specimens of *Alyssum bertolonii* remained fairly constant in the range 0.54-0.77% for all fertiliser amendments

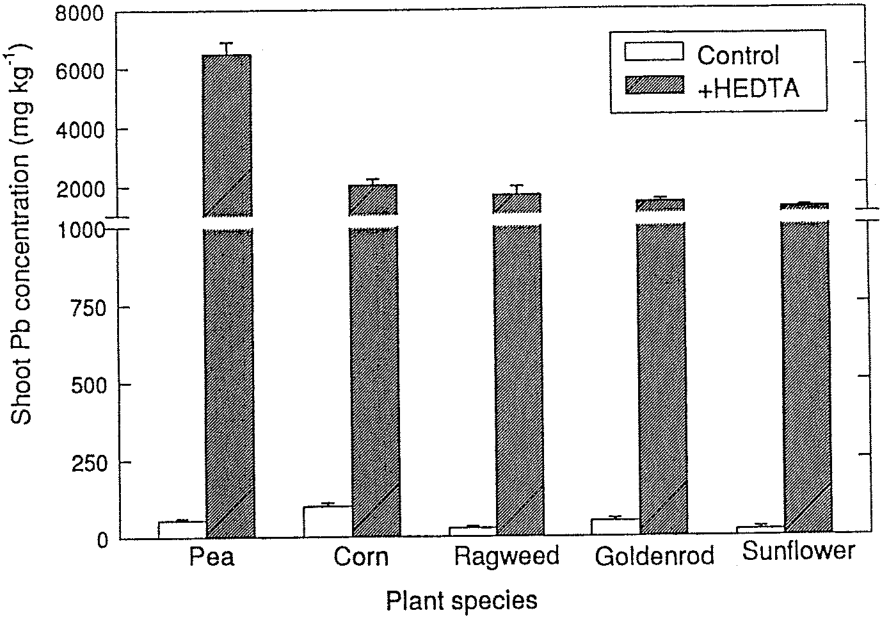


Fig.15.2. Lead accumulation in shoots of five plant species grown in lead-contaminated soil (2500 μg/g) after addition of 0.5 g/kg of HEDTA. Source: Huang *et al.* (1997).

except in the treatment with four fertiliser elements including calcium. It therefore appears that there is no appreciable decrease of nickel concentration with increase in biomass except when the pH is increased by addition of calcium. In other words there is no trade-off in reduced nickel concentration to offset the gain in biomass. Similar findings were made in work described in Chapter 11 (Figs 11.5 and 11.6).

The use of chelates in soils to increase elemental uptake by plants

The use of chelates in soils to increase metal uptake by plants represents perhaps the greatest step forward in the technology of phytoremediation. Early experiments by Norvell (1972), Wallace *et al.* (1977), Checkai *et al.* (1987) and Sadiq and Hussain (1993) showed that synthetic chelating agents could be used to increase uptake of various cations by plants. More recently, Huang and Cunningham (1996) and Huang *et al.* (1997) have shown that complexing of lead with EDTA renders this element much more available to large-biomass non-accumulators such as *Zea mays* so that a metal content of 1% (dry weight) can be obtained in the plant material. Harvesting of these plants permits a reduction of the lead content of the contaminated soil so that a satisfactory level can be reached in a small number of sequential crops.

concerns the accumulation of anthropogenic lead in soils throughout the world. Lead is normally very immobile to plants as it can precipitate at roots systems as the highly insoluble phosphate or sulphate and does not readily penetrate from roots to the stems.

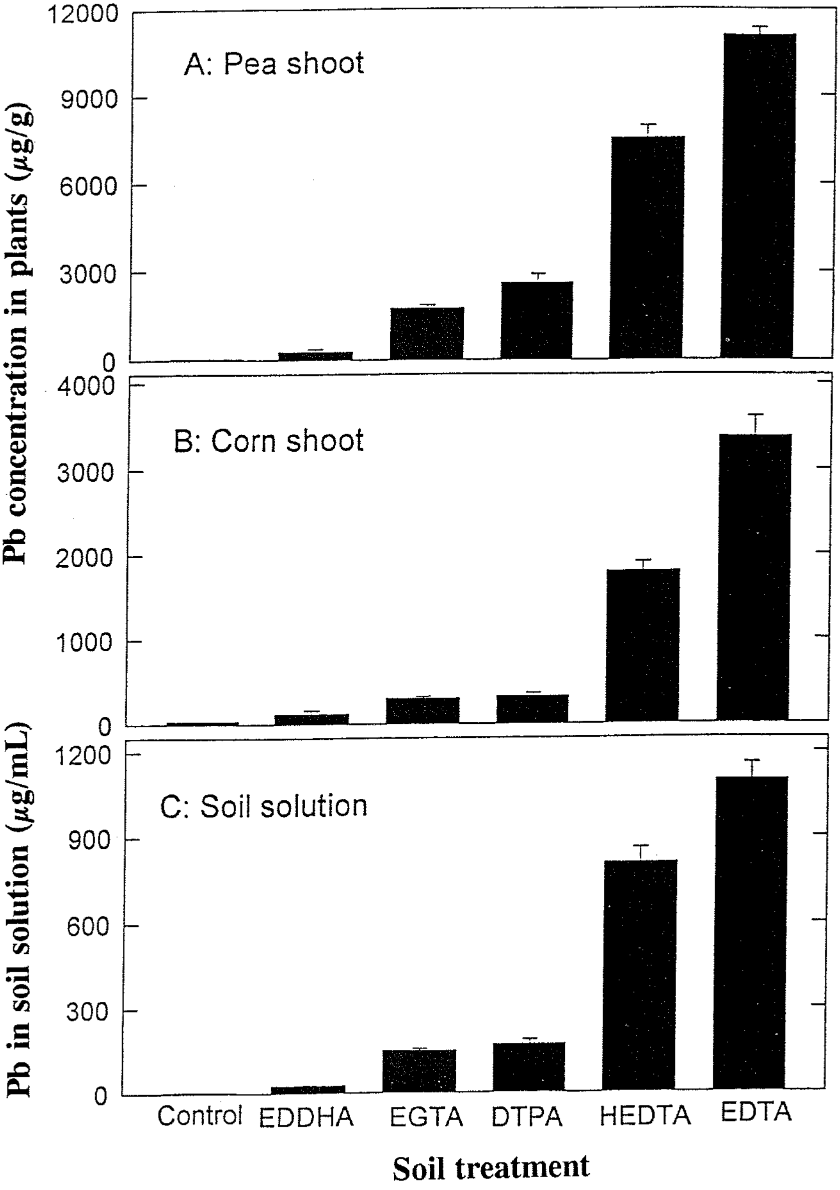


Fig.15.3. Lead uptake (mg/L = μg/g) by pea and corn plants treated for one week with 0.5 g of various complexing agents to 1 kg of soil. Values for the soil solution are also shown. Source: Huang *et al.* (1997).

However, Marten and Hammond (1966) found that ethylenediaminetetraacetic acid (EDTA) applied to lead-contaminated soils, increased the lead content of bromegrass from 5 to 35 μg/g.

Huang *et al.* (1997) have reported experiments in which EDTA (as the trisodium salt Na_3HEDTA) was added to lead-contaminated soils (2500 μg/g) at the rate of 0, 0.5, 1.0 and 2.0 g/kg. Within 24 hours the soluble lead content of the soil solution increased from almost zero to about 4000 μg/mL. Lead accumulation in shoots of five plant species grown in the same soil and with the addition of 0.5 g/kg of EDTA, is shown in Fig.15.2. It will be observed that the lead content of pea (*Pisum sativum*) reached over 6000 μg/g lead whereas that of corn (*Zea mays*) was only 2000 μg/g. However, the biomass of corn being at least ten times that of pea, shows that the former would be much more effective in removing lead from the soil. The same is true for the high-biomass sunflower (*Helianthus annuus*) with well over 1000 μg/g of the same element.

The effect of different complexing agents on the uptake of lead by pea and corn shoots is shown in Fig.15.3. It is clear that EDTA (trisodium salt) is by far the most efficient of the five chelates used, followed by HEDTA (N-(2-hydroxyethyl)ethylenediaminetetraacetic acid, DTPA (diethylenetriaminepentaacetic acid), EGTA (ethylenebis(oxyethylenenitrilo)tetraacetic acid), and EDDHA. EDTA has the further advantage of being the least expensive of the chelating agents (about $40/kg).

The above discussion has centred around complexing of lead in contaminated soils since this element is one of the most ubiquitous in sites polluted with heavy metals. The beneficial application of the technique to phytoremediation cannot be doubted, whereas for phytomining, the thrust of this chapter, the benefits are negligible because the low world price of lead (ca. $800/t) would militate against its economic recovery by growing plants. Nevertheless, the principles of chelate-assisted metal uptake by plants are equally applicable to other more valuable elements such as nickel, cobalt, or even gold. As far as we know, no other studies have yet been published in which chelates have been used to increase metal uptake by hyperaccumulators of these more expensive metals. Details of our experiments with chelating agents are given below.

Selection by plant breeding of specific cultivars and wild strains of hyperaccumulators

Among small-biomass hyperaccumulating plants such as *Streptanthus polygaloides* (Ni) and *Thlaspi caerulescens* (Zn/Cd) there is often a large difference of biomass and metal uptake among individual plants. In Chapter 14, field observations of *Streptanthus polygaloides* showed that it was not uncommon to find two plants growing less than 20 cm apart to differ in biomass by a factor of two to three. These variations made estimates of potential crop yields difficult but it is important to note that they also indicated a rich variety of genotypes in the wild application of selective breeding techniques to develop a high-yielding strain.

In our studies on *Thlaspi caerulescens* growing over zinc/lead mine wastes at Les Avinières near Montpellier in southern France, we have observed a very wide range of biomass ranging from 1 to 64 g. This extremely variable species is also a good candidate for selection of high-biomass strains by plant breeding.

Kumar *et al.* (1995) have reported studies in which they tested 106 different cultivars of Indian mustard (*Brassica juncea*) for their ability to accumulate lead in roots and transport this element to the shoots. Some of their data are shown in

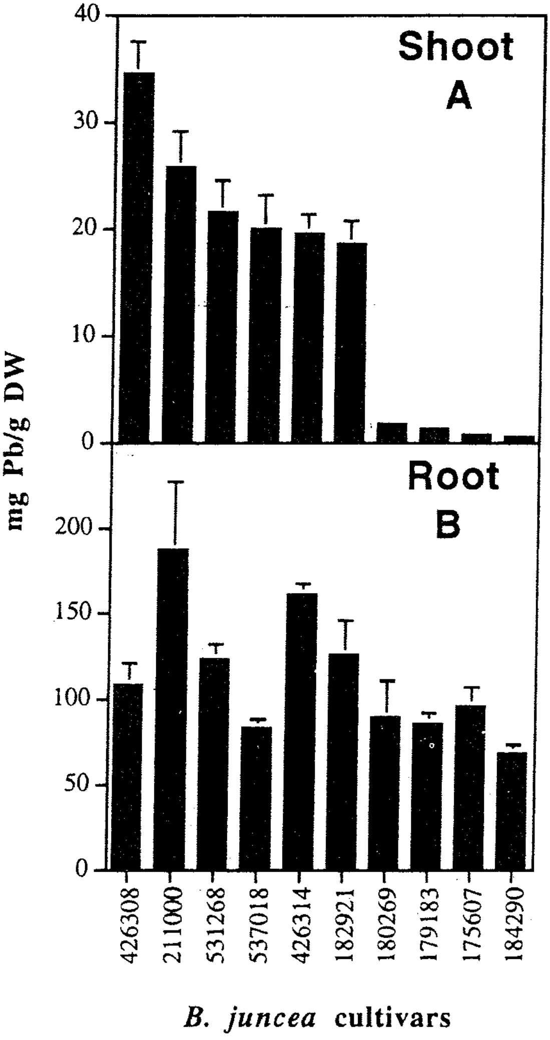


Fig.15.4. Lead content of shoots (a) and roots (B) of *Brassica juncea* cultivars grown for 14 days in a sand/Perlite mixture containing 625 μg/g lead. Source: Kumar *et al.* (1995).

Fig.15.4. The most efficient cultivar (No.426308) contained 35 μg/g lead in the dry shoots compared with only 0.4 μg/g for the least efficient (No.184290).

The above observations clearly show the wide range of biomass and metal uptake that can be expected in wild strains and cultivars of many plant species, both hyperaccumulators and non-accumulators.

Use of biotechnology to introduce "hyperaccumulation" genes into plants of high biomass

The main problem in phytomining and phytoremediation technologies is that there are few plants that combine high biomass with a high degree of accumulation of the target heavy metals. In some cases, notably in Cuba and New Caledonia, there are indeed plants of high biomass such as the New Caledonian *Hybanthus austrocaledonicus* and *Sebertia acuminata*. These trees are however difficult to grow and have a slow rate of growth.

There is clearly a need for species that combine the extraordinarily high uptake of metals by low-biomass plants such as *Thlaspi caerulescens* with high-biomass fast-growing plants such as *Brassica juncea*. This problem has been discussed by Kumar *et al.* (1995) and by Cunningham and Ow (1996) who have suggested that the answer to the problem lies in genetic engineering to transfer the "hyperaccumulation genes" from plants of low biomass to fast-growing high-biomass plants such as *Brassica juncea* or *Helianthus annuus*. Such experiments are still in their infancy but encouraging results have been obtained by experiments in which scientists have cloned the gene for a vacuolar membrane transport pump that facilitates sequestering of the peptide-Cd complex (Ortiz *et al.*, 1995). Hyperproduction of this protein in the fission yeast *Schizosaccharomyces pombe* enhances tolerance to, and accumulation of, cadmium. Ow (1993) has suggested that hyperexpression of this yeast protein may yield similar results in a higher plan.

Cropping Sustainability of Phytomining Operations

Robinson *et al.* (1997) have examined the question of whether growing a crop of nickel will entail quick removal of the soluble fraction of this element from the soil in the way that a conventional crop will quickly remove plant nutrients. It would be scarcely feasible to grow a crop on an annual basis for a decade in order to answer this question. An alternative approach is the method of sequential extraction that we have developed in our laboratories. Using cumulative extraction of nickel by KH phthalate at pH 2, 4 and 6 shows that:

$$t_e = t - tc/(x+c)$$

Where: t_e = cumulative extracted concentration of nickel, t = concentration of potentially available nickel, x = number of extractions, and c = a constant extraction. The cumulative extractions approach a limiting value where fewer extractions are needed at lower pH values.

In experiments carried out on ultramafic rocks of the Murlo area of Tuscany Italy (Fig.15.5), the limiting value was found to be 768 μg/g nickel in the soil (approximately the amount of nickel removed in a single extraction with 0.1M HCl). If we assume that removal of up to 30% of this limiting value would be acceptable economically, a simple calculation shows how many crops could be sustained by the site. Assuming that the soil is being phytomined to a depth of 0.15 m, the volume of a hectare of soil to this depth would be 1500 m^3. For a density of 1.3, the mass would be 1950 t. For a crop producing 72 kg nickel/ha, the concentration of removable nickel would be 72,000/1950 g/t = 40 μg/g for a single crop. The number of potential croppings before the soil was exhausted would therefore be 30% of 768 divided by 40: i.e. 230/40 = 5.8 croppings.

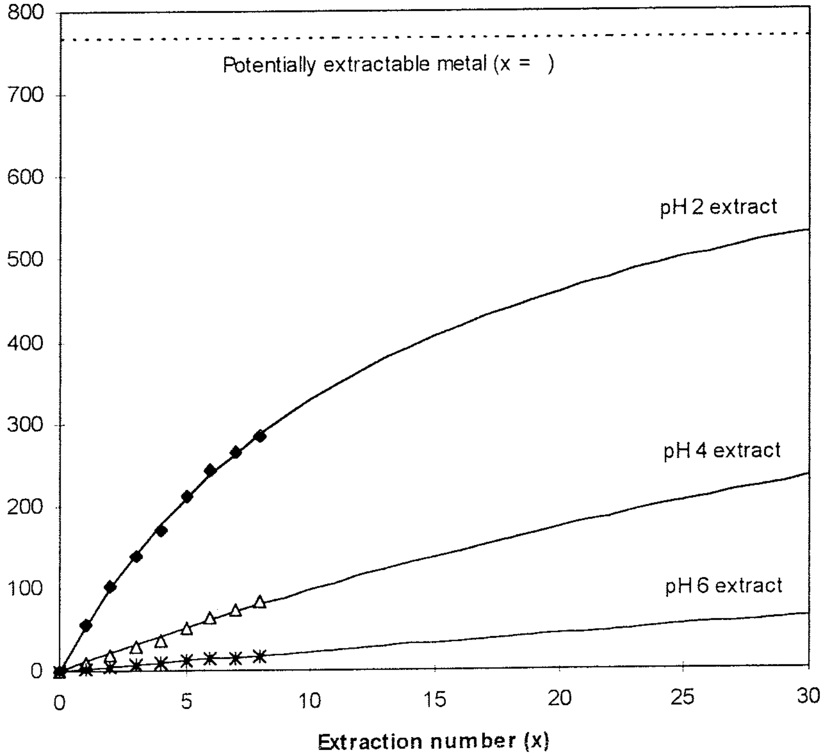


Fig.15.5. Sequential extraction of ultramafic soils from Murlo, Italy using potassium hydrogen phthalate buffers at pH values of 2,4 and 6.

After each of the sequential extracts, the equilibrium of soluble to total nickel restores the soluble fraction to almost its original value otherwise it would be quickly depleted. The procedure obviously cannot reproduce the exact field conditions but will certainly err on the conservative side since, in the laboratory, we are looking at an equilibrium recovery time of only as long as it takes to replace the extractant with a fresh supply compared with a period of 12 months under field conditions.

The reality is therefore that under field conditions, nearly 6 croppings would keep within the guidelines of 30% removal of available nickel. An undeniable advantage of using a perennial with a life of about 10 years is that resowing would not be needed. After harvesting, the plant would regenerate and would be reinforced by seedlings from the crop itself.

When the pool of available nickel is reduced by 30%, ploughing to bring fresh soil to the surface would be followed by resowing. The latter might even not be necessary if sufficient seed from the current crop were already distributed in the soil.

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 any plant for phytomining, the same is not true for the wider subject of phytoremediation. Whereas phytomining is limited by the need to produce a commercially viable metal crop, this is not the case for phytoremediation.

Table 15.3. 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	30
Au	13,600,000	36.8	7.4	3.7	2.5	1.8	1.2
Pd	4,464,000	112.0	22.4	11.2	7.5	5.6	3.7
Ag	183,000	2732	546	273	182	137	91
Co	48,000	10,417	2083	1042	694	521	347
Tl	15,000	33,333	6667	3333	2222	1667	1111
Ni	7485	66,800	13,360	6680	4453	3340	2227
Sn	6200	80,650	16,129	8065	5376	4032	2688
Cd	3750	133,333	26,667	13,333	8889	6667	4444
Cu	1961	254,970	50,994	25,497	16,998	12,749	5666
Mn	1700	294,120	58,824	29,412	19,608	14,706	9804
Zn	1007	496,520	99,305	49,652	33,102	24,826	16,551
Pb	817	612,000	122,400	61,200	40,800	30,600	20,400

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

Table 15.3 shows the elemental content (µg/g in dry matter) that would be required in plants with fertilised biomasses of 1-30 t/ha to give a gross financial for phytomining with plants of a 10 t/ha biomass. Hyperaccumulators with 1% (10,000 μg/g) cobalt are known from Zaïre (Brooks and Malaisse, 1985) and many *Alyssum* species can have nickel contents well in excess of 1% (Brooks *et al.*, 1979). There are no records of high-biomass plants (i.e. around 30 t/ha) whose noble metal contents exceed the values shown in Table 15.3 and neither are there any for cadmium, copper, lead, tin or zinc. It must be emphasized however, that many hyperaccumulators of nickel will also accumulate the much more valuable cobalt. Similarly, cobalt hyperaccumulators often hyperaccumulate copper as well. Though copper is not valuable enough for phytomining in its own right, it can add value to a crop of cobalt. Consider the copper/cobalt hyperaccumulator *Haumaniastrum katangense* from Zaïre. It typically contains 0.2% (dry weight) of both elements. Assuming a fertilised biomass of 7.5 t/ha, a hectare of mineralised soil could provide 0.015 tonne of cobalt worth $720 plus the same weight of copper worth $29. The total of $749/ha would be easily economic.

The above problem can be addressed to some extent by use of hyperaccumulators of higher biomass combined with a sufficiently high metal content. Table 15.4 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. For example, using the data of Jaffré *et al.* (1976), it can be calculated that a mature specimen of the New Caledonian tree *Sebertia acuminata* (see Fig.3.6) contains a total of about 40 kg of nickel. A crop of these trees planted at the rate of 2000/ha would produce 8 tonnes of nickel worth about $60,000 at today's prices. Assuming that this tree would have taken 40 years to mature, the annual yield is then only $7500 after 40 years and much less in a shorter time frame when the tree was initially only a sapling.

The reproductive matter of *Alyssum bertolonii* after fertilising, has a biomass

Table 15.4. 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
Thallium	3.33	Zinc	49.6
Nickel	6.68	Lead	61.2

After: Robinson *et al.* (1997)

of about 13.5 t/ha. This value is in the middle to low part of the potential economic range. The South African *Berkheya coddii* (Morrey *et al.*, 1992) has an unfertilised biomass of about 12 t/ha but our field trials in New Zealand have shown that a fertilised biomass of 22 t/ha can be achieved (Robinson *et al.*, 1998). Together with its high nickel content of over 1%, it is probably one of the best candidates to extend the outermost limits of phytomining for nickel. Experiments with both of these hyperaccumulators are described below.

Examination of the Potential of Phytomining by Pot Trials and Field Tests

Experiments with *Alyssum bertolonii*

Introduction

The work described in this subsection has already been reported by Robinson *et al.* (1997). Therefore, only the salient details will be reported here. The study was based on the principles established by Nicks and Chambers (1995 - see also Chapter 14) and as far as we know, represents only the second report ever published on the potential of phytomining based on field work under natural conditions and using native plant species rather than exotic taxa. The work was carried out in Tuscany, Italy, using populations of *Alyssum bertolonii* growing under natural conditions over the ultramafic (serpentine) soils of the region. The aims of the experiments were to assess: the approximate yield of nickel per hectare; the relation between the nickel content of the plant and the available nickel status of the soil; the effect of plant age and size on the nickel content of the plant; the effect of fertilisers on biomass and nickel content of the plants; the reduction of nickel availability in the soil after successive croppings.

The test areas were located on Monte Pelato (350 m) in Livorno Province and near the village of Murlo (350 m) south of Siena, Italy (Fig.15.6). 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 to 7.4 on serpentinite and 6.8 to 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 the Tuscan ultramafic outcrops. It encompasses all the serpentine-endemic plants including of course, *Alyssum bertolonii* 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 and Murlo. Both sites can be classified bioclimatically as Mediterranean pluvio-seasonal oceanic.

For Murlo the mean annual temperature is 13.8°C ranging from 5.8° in

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.

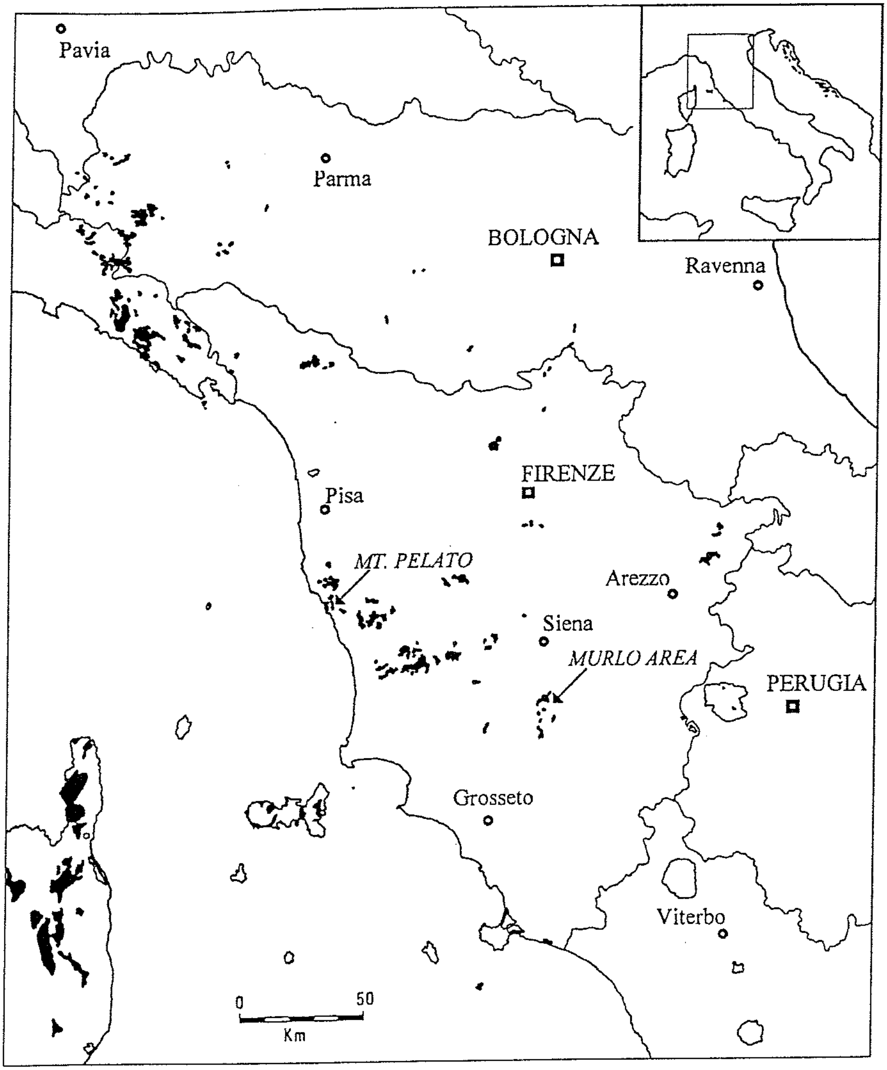


Fig.15.6. Map of north-central Italy showing areas of ultramafic rocks (dark) and sites of the study. Source: Robinson *et al.* (1997).

Brief description of experiments

At Monte Murlo, 35 random quadrats (1 m × 1 m) 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-quadrat 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 quadrats encompassed natural populations 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 10 g/m^2, 3 - ammonium nitrate at 10 g/m^2, 4 - sodium dihydrogen phosphate + ammonium nitrate each at 10 g/m^2, 5 - sodium dihydrogen phosphate + ammonium nitrate + potassium chloride each at 10 g/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 quadrat at the end of the two-year period and a soil sample (0-15 cm depth and weighing 100 g) was also taken.

Subsamples (0.5 g) of the vegetative and reproductive parts of the plants were removed and placed in small borosilicate test tubes. These were ashed overnight at 500°C and 10 mL of hot 2M HCl added to each tube to dissolve the ash. The solutions were analysed for trace elements using flame atomic absorption spectrometry.

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 of a proposed pilot project for biomining

Earlier in this chapter, it has been shown that fertilisation of the natural populations of *Alyssum bertolonii* was able to increase the biomass of this plant by a factor of three (Table 15.2) without concomitant reduction in the nickel concentration in the fertilised plant.

We have calculated the biomass of a fertilised crop of *Alyssum bertolonii* 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 fertilised biomass of about 9.0 t/ha. It appears that this factor was indeed too conservative because in of 3.4 and 13.0 t/ha for unfertilised and fertilised plots respectively. This is in very good agreement with the Italian findings on natural populations of *Alyssum* and suggests that our estimate of 9 t/ha for the biomass is on the conservative side.

From our experience in the field at Murlo, we envisage 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 fertiliser 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 - burn the crop in some type of incinerator and collect the bio-ore which will have a nickel content of about 11%. With application of N+K+P fertiliser, the yield of upper reproductive tissue should be at least 9.0 t/ha. There would be 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

Nicks and Chambers (1995) have proposed that commercial exploitation of the annual Californian hyperaccumulator *Streptanthus polygaloides* would produce about 100 kg/ha of nickel after moderate application of fertilisers. Our own calculation with *Alyssum bertolonii* have arrived at a conservative value of 72 (more probably 108) kg of nickel/ha containing 0.08% nickel worth $539 at the present world price. The value of the accompanying cobalt from the 80 μg/g of this element in dry plant tissue (0.72 kg/ha) is about $35, making a total of $574 for the entire crop.

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 $287, 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 nickel would be the same as for wheat. It must be remembered however, that native plants growing in their own natural environment should require less fertiliser and irrigation than a crop of wheat. There is also the fact that the *Alyssum bertolonii* crop is perennial and will not require resowing the following year.

The yield of nickel 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 nickel (see above). 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 (Huang and Cunningham, 1996; Blaylock *et al.*, 1997; Huang *et al.*, 1997). 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.

Our chelation experiments with *Alyssum bertolonii* are only at an early stage, but there is no evidence of plant death after adding EDTA. It may well be that plants already adapted to high metal uptake (unlike *Brassica*) will tolerate both EDTA and the increase in yield of the target metal to which it is already adapted. We do not believe that EDTA in itself is phytotoxic. It is only the phytotoxic metals that cause plant death. Residual EDTA in the soil might also be a problem. There is nevertheless scope for controlled trials with EDTA or other complexing agents.

From our own experiments with *Alyssum*, a second crop seems to be quite feasible. We obtained 13.0 t/ha of biomass for plants harvested in December 1996 (Southern Hemisphere early summer). Three months later (early autumn) a second crop estimated at 5 t/ha emerged from the stubble.

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 $793 t/ha. If half of this sum were recovered by the company after making allowance for capital costs, fertilisers etc, the net return of $396 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 as mentioned above. This would not only increase nickel 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.

Experiments with *Berkheya coddii*

Introduction

a height of about 2 m. In its natural state it is confined to ultramafic outcrops in the Eastern Transvaal, near Barberton. The nickel content of *B. coddii* has been investigated by various workers (Morrey *et al.*, 1989,1992; Howes 1991) who have shown that it is a hyperaccumulator of nickel containing up to 1.7% of this element in the dry mass.

Berkheya coddii begins to grow at the onset of the rainy season in late winter and finishes its cycle at the end of March. It is a perennial that dies back after

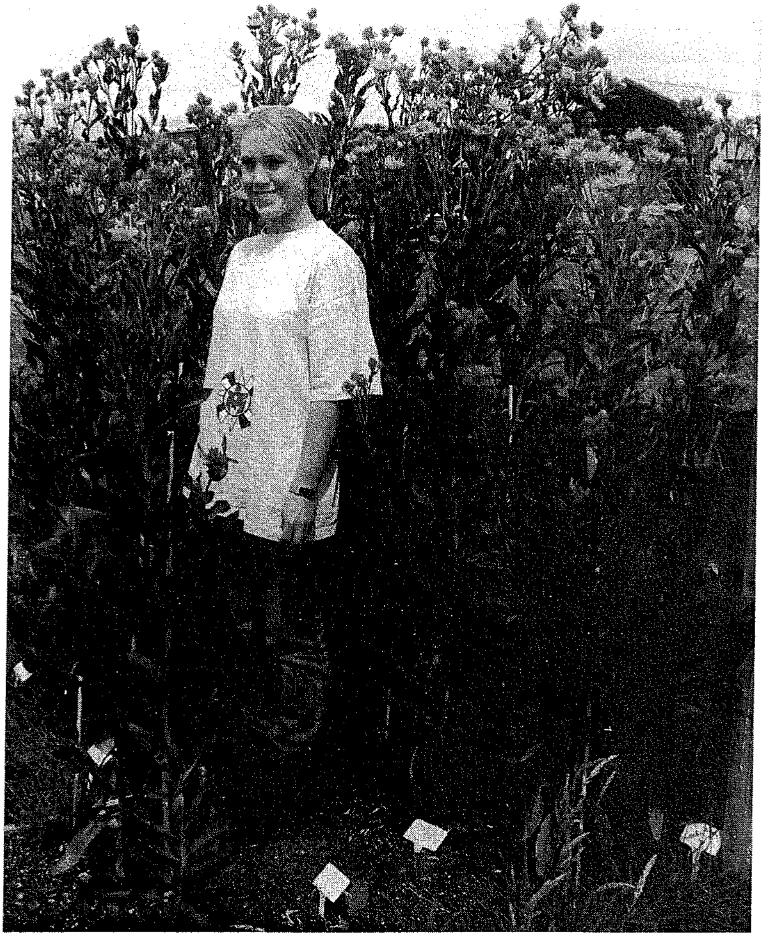


Fig.15.7. Stand of *Berkheya coddii* growing in late summer (March) in an artificial serpentine soil (550 μg/g nickel) in experimental plots in New Zealand.

flowering and re-emerges at the start of the next rainy season.

Experiments were carried out at this university with both pot trials and growth in outside plots (Robinson *et al.*, 1998). The aims of the experiments were to determine the following:

1 - the biomass achievable in outside plots using an artificial "serpentine soil";

2 - effect of fertiliser addition on nickel uptake;

3 - the effect of excision on the nickel content of new growth;

4 - the limiting nickel content that could be achieved under controlled conditions;

5 - calculation of the probable nickel yield of plants grown over serpentine soils in various parts of the world;

6 - the potential of the species for phytoremediation as well as phytomining.

The achievable biomass of Berkheya coddii *under controlled outside conditions*

After one year's growth from seed, the total above-ground dry biomass of plants in two 1 m × 1 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.15.7. This biomass was achieved with moderate addition of Osmocote slow-release fertilisers. However field observations indicate that the plant attains this height in its natural habitat. Poorer soils may however need fertiliser addition for optimal production. 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 by Robinson *et al.* (1997) to have the potential of being able to provide an economic crop of nickel.

The effect of fertiliser on nickel uptake by Berkheya coddii

Chemical analysis of leaves collected in from plants subjected to various fertiliser amendments (N and P) showed no statistically significant relationship between the nickel content and phosphorus amendment when the nitrogen content was constant. If however the experimental samples were considered as three separate populations with treatments of 0, 100 and 200 μg/g N added to a 3:1 bark:crushed serpentine mixture, the mean nickel content rose from 2300 μg/g d.w. for zero addition of N, to 3250 for the 100 μg/g N treatment and to 4200 μg/g for the 200 μg/g N amendments.

The effect of leaf excision on the nickel content of fresh Berkheya *shoots*

Plants that were excised at ground level rapidly grew new foliage. This new growth had a much higher nickel concentration than the original plant (Fig.15.8). The difference is on average over three times greater in the optimum range of

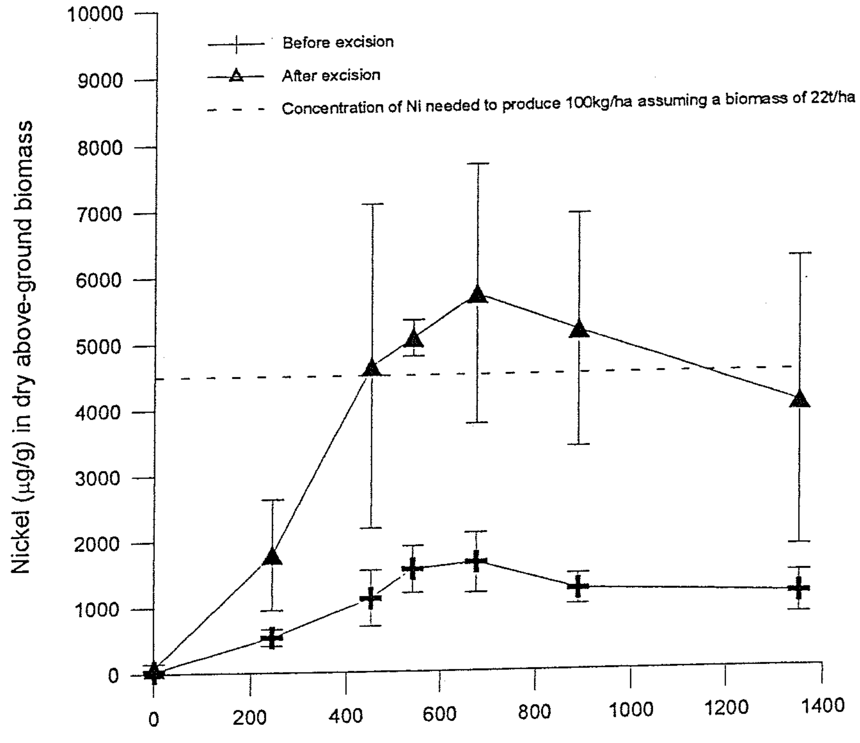


Fig.15.8. The effect of excision on the enhancement of the nickel content of fresh shoots of *Berkeya coddii.*

(1996) for the nickel hyperaccumulator *Alyssum pintodasilvae.*

Enhanced nickel uptake after excision could be due to two factors. The plant may be removing more nickel from the soil, or it may simply be translocating existing nickel in the plant to the new growth structures. The higher nickel 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 nickel from the soil, it may be possible to induce increased nickel uptake by removal of the apical meristem. The new growth could be harvested as another high nickel 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 the total available nickel in the substrate

The results of experiments in which three-month-old whole plants of *B.coddii* were grown in standard seed mix containing incremental concentrations of available nickel (0-10,000 μg/g) are shown in Fig.15.9.

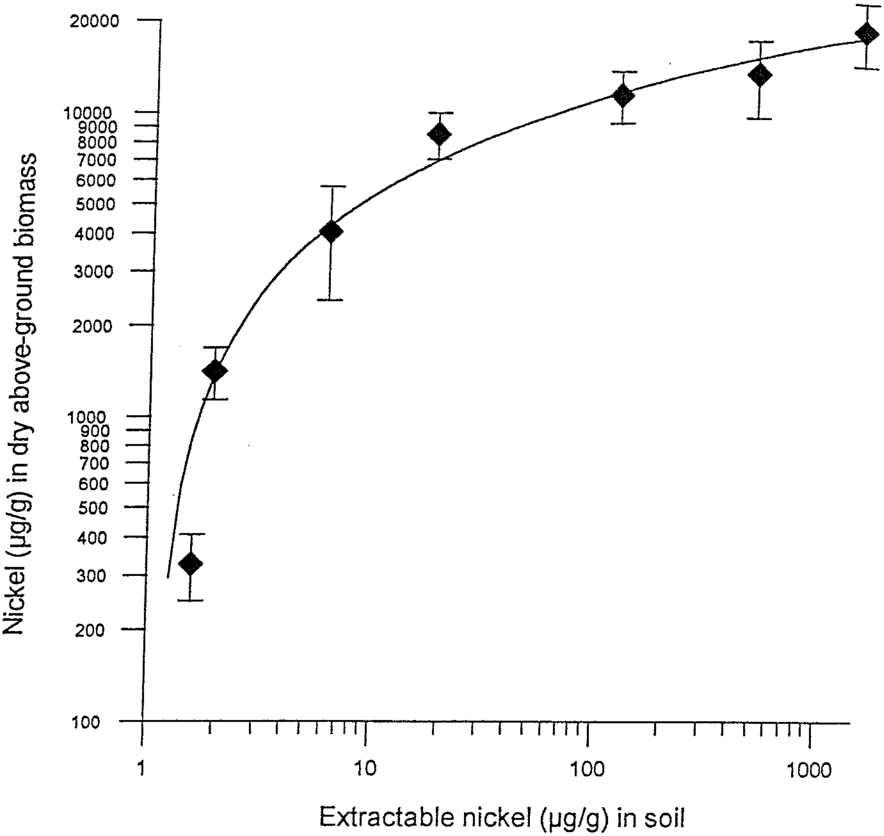


Fig.15.9. Results of pot trials showing the relationship between nickel in *Berkheya coddii* and the available nickel in the substrate.

The plants would not grow in substrates containing more than 3333 μg/g available nickel and the highest level of just over 10,000 μg/g nickel 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 our experimental value of 1% in pot trials.

Prediction of the probable nickel yield of Berkheya coddii *grown over serpentine soils throughout the world*

From Fig.15.9 and analogous experiments in which the ammonium acetate worldwide, it was observed that there was a linear relationship between the nickel content of *Berkheya coddii* and the extractable fraction of this element in the soil. The nickel content of *B. coddii* growing on a nickeliferous soil can therefore be predicted by the extractable nickel as determined by use of ammonium acetate solutions. Obviously there are other factors involved in nickel uptake such as the pH of the soil, nutrient availability, and the concentration of other heavy metals, however the predictions should give a rough guide to a soil's suitability for phytomining or phytoremediation. Predictions of crop yields for nickel are shown in Table 15.5.

Table 15.5. 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	2.30	460
California	Red Hills (Chinese Camp)	2	26.3	1.93	386
New Zealand	Coppermine Saddle	6	19.3	1.40	280
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

A - extractable soil nickel (µg/g), B - estimated nickel content of plant (%), C - estimated nickel yield (kg/ha).
Assumptions: 1 - biomass of 22 t/ha, 2 - nickel yield of the plant, and hence its extractive power, is a function of the extractable nickel content of the soil as determined from Fig. 15.9 and other experiments.

Phytomining by use of Berkheya coddii

It is possible that economic crops of nickel could be phytomined from sites (Table 15.5) 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.

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 or other suitable territories.

It is important to mention, however, 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 our 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 our outside plots in New Zealand 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 outcompeting 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.

Phytoremediation of nickel-contaminated soils by use of Berkheya coddii

The combination of high biomass and high nickel 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 nickel are less numerous than those contaminated with lead and zinc. There is however a need for some degree of remediation of several sites throughout the world where pollution from nickel is a problem. McGrath and Smith (1990) have reviewed the problem of nickel pollution of the environment. Apart from the obvious local pollution from smelters, a significant problem arises from addition of nickel to pastures via sewage sludges. At Beaumont Leys (UK) for example, the nickel content of surface soils at a sewage farm was found to be as high as 385 μg/g.

European Community guidelines for nickel 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 nickel that could potentially be removed annually from contaminated pastures using a crop of this species. Using the data in Fig. 15.9, an estimate can be made for the probable nickel content of a *Berkheya* crop growing over polluted soils. It must be remembered, however, that the experiments portrayed in Fig. 15.9 were carried out with substrates containing nickel as the totally soluble nitrate, though a high proportion of this element 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 nickel might not be known in advance, we have adopted a conservative approach that assumes that 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 nickel burden of soils down to the EU level of 75 μg/g is summarised in Table 15.6. For moderate nickel contamination (100 μg/g) two crops would be guidelines. Even at 250 μg/g (few polluted sites would exceed this value) only 4 crops of *Berkheya coddii* would be needed.

Table 15.6. 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)	Ni content after one year (μg/g)	Number of crops to decontaminate
10,000	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 nickel is extractable, 3 - nickel content of the plant, and hence its extractive power, is a function of the nickel content of the soil.

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

Some Philosophical Observations on the Feasibility or Desirability of Phytomining

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

1. In the case of phytomining for nickel, would conservationists allow a large area of serpentine soil to be colonised by commercial crops of an exotic or native hyperaccumulator?

To answer this question, it must be appreciated that there is a difference in the acceptability of exotics compared with local hyperaccumulators. To take the example of Italy, it would be technically feasible to carry out phytomining by use of the serpentine-endemic *Alyssum bertolonii*. 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 their very nature, mineralised soils are extremely hostile for unadapted plant life and are almost never used for food production.

3. *What will happen to the price of metals in the future?*

This question is hard to answer. Some metals such as nickel have been constantly rising in price for the past few years, but there is no guarantee that their price will not one day collapse. Nickel is 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. *Where are the most likely sites where phytomining might one day be used?*

The technique would be most applicable in areas where there are large areas of subeconomic metal reserves. Unless some way can be found to discover plants that hyperaccumulate the noble metals, we are essentially looking at nickel and cobalt as potential targets for phytomining. Only in central Africa (Zaïre and Zambia) as well as possibly Canada and Russia are there significant areas of low-grade cobalt ores. Nickel is an entirely different matter because of the widespread occurrence of ultramafic soils throughout the world. Table 15.7 lists some areas of the world where major subeconomic deposits of nickel or cobalt are to be found and suggests potential hyperaccumulators that could be used for phytomining. Forest species are not included in the listing as they would not lend themselves to cultivation in unshaded areas.

Table 15.7. Potential regions suitable for phytomining by use of local hyperaccumulator plants.

Element	Region	Suitable species for phytomining
Cobalt/copper	Zaïre/Zambia	*Haumaniastrum katangense, H.robertii*
Nickel	Australia	*Hybanthus floribundus, Stackhousia tryonii*
	Brazil	Numerous local endemic plants (Brooks *et al.* (1992).
	California/Oregon	*Streptanthus polygaloides*
	Cuba	Numerous plants of the *Phyllanthus* and *Leucocroton* genera
	New Caledonia	*Geissois pruinosa* and several others
	South Africa/Zimbabwe	*Berkheya coddii*
	Turkey/Italy/Greece	*Alyssum bertolonii, A.lesbiacum*

5. *How hardy are hyperaccumulators and how resistant to insect attack or disease, i.e. could a crop be wiped out in a single year by such agencies?*

Hyperaccumulators of nickel and other elements have a strong protection against predator attack because of their high nickel content. Boyd and Martens (1992) have carried out experiments on nickel hyperaccumulators to illustrate this *bertolonii* and *Berkheya coddii* we have not observed any tendency to disease in either plant. *A. bertolonii* is an exceptionally hardy plant that will withstand extremes of temperature. Our tests in New Zealand have shown that it will even grow over asbestos tailings where no other dicotyledonous plant will survive. Although *Berkeya coddii* grows in a warm part of South Africa, it will readily overwinter in New Zealand where ground frosts occur in winter.

6. 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 phytomining plant. This question is a moral one and hopefully some solution will be found to satisfy all interested parties.

7. What is the potential of obtaining dual crops of two different metals?

There is clearly a possibility of obtaining crops of two different elements whenever a given species of hyperaccumulator has the ability to accumulate two elements. The most obvious possibilities (see also above) are nickel and cobalt from "nickel plants" growing over ultramafic soils that usually contain around 100 μg/g cobalt. Initial experiments with EDTA chelation indicate that there might be a dramatic increase of the cobalt content of some plants after such treatment even if there is no great increase in the nickel content. The other obvious possibility is concomitant uptake of copper when growing a crop of cobalt using some of the Zaïrean dual hyperaccumulators such as *Haumaniastrum katangense*. The copper is far less valuable than the cobalt, but might just tip the scales to make a subeconomic crop viable.

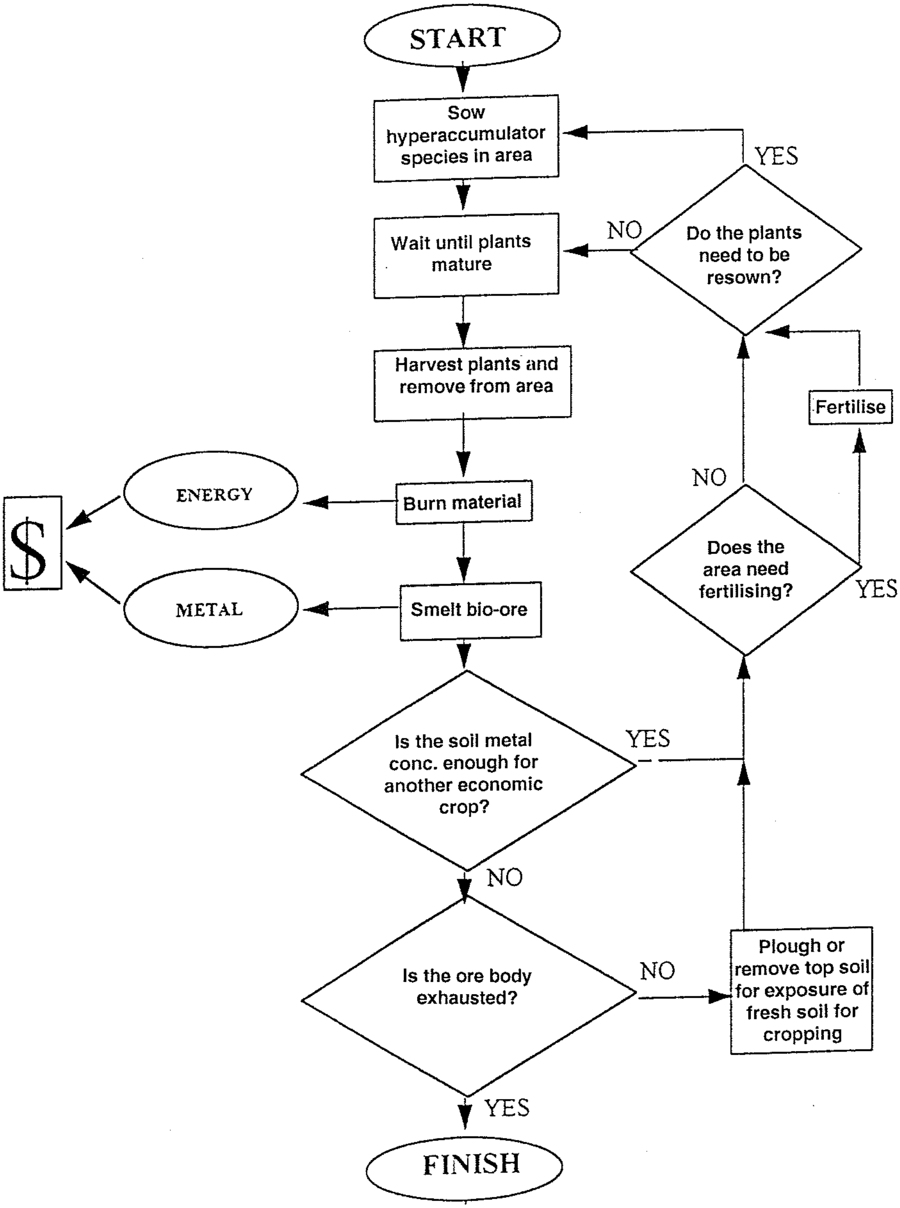
A Model of a Possible Economic Phytomining System

A model of a possible economic phytomining system is shown in Fig.15.10. The system differentiates between annual and perennial crops and encompasses the questions of fertilising and soil exhaustion. Probably the success or failure of a project will depend on whether or not some of the energy of combustion of raw material can be recovered. In tropical regions of the earth, it should be possible to have crops maturing in each month of the year so that the incineration plant could be kept busy yearlong.

If phytomining proceeds beyond the theoretical and pilot plant stages there are two possible scenarios that might be envisaged. The first of these presupposes a commercial project on a very large scale involving a few square kilometres of ultramafic soils or low-grade nickel mineralization.

The second scenario, perhaps the more likely, could involve phytomining being farmed out to smallholders throughout the region in which a peasant farmer might grow a few hectares of plant material and have it collected for processing at a nearby facility preferably close to a large city where industrial waste could also be used as feedstock for the incineration plant which in turn could supply steam for producing local supplies of electricity. A country such as Brazil that has large areas of sub-economic nickel mineralisation and ultramafic soils might be an obvious site of the small-farmer scenario.

I have myself seen in Brazil (Goias State) several fields of failed soyabean



crops where peasant farmers had sought to grow these crops over ultramafic soils. The surrounding natural vegetation was in contrast quite luxuriant and included several nickel hyperaccumulators (Brooks *et al.* 1992) that would have grown quite well as an alternative to failed soyabean crops.

The future of phytomining remains unknown at present, but who would have thought 20 years ago that the Brazilians could have grown their own motor fuel (alcohol) from sugar cane. To grow a crop of a metal such as nickel is only an extension of that idea. Only time will tell whether it is merely a flight of fancy or an idea whose time has come.

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