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

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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 2-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 unfertilized 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 resowing for a further crop. There was no correlation between the age of a plant and its Ni content. The long-term cropping sustainability of the soils was simulated by sequential extractions with KH phthalate solutions at pH 2, 4 and 6 that showed a limiting available Ni content of 768 μg/g. Thus just over seven croppings at pH 6 in the rhizosphere would reduce the available Ni pool by 30%. 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 uptake potential as for Ni, could extend the limits to other elements.

Keywords: *Alyssum bertolonii*; phytomining; bio-ore; Tuscany

1. 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 in the vicinity of 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 which translated to well over 10% of this element in the ash.

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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'. 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 (Jaffré, 1980; Brooks, 1987; Baker and Brooks, 1989; 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* Gray 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

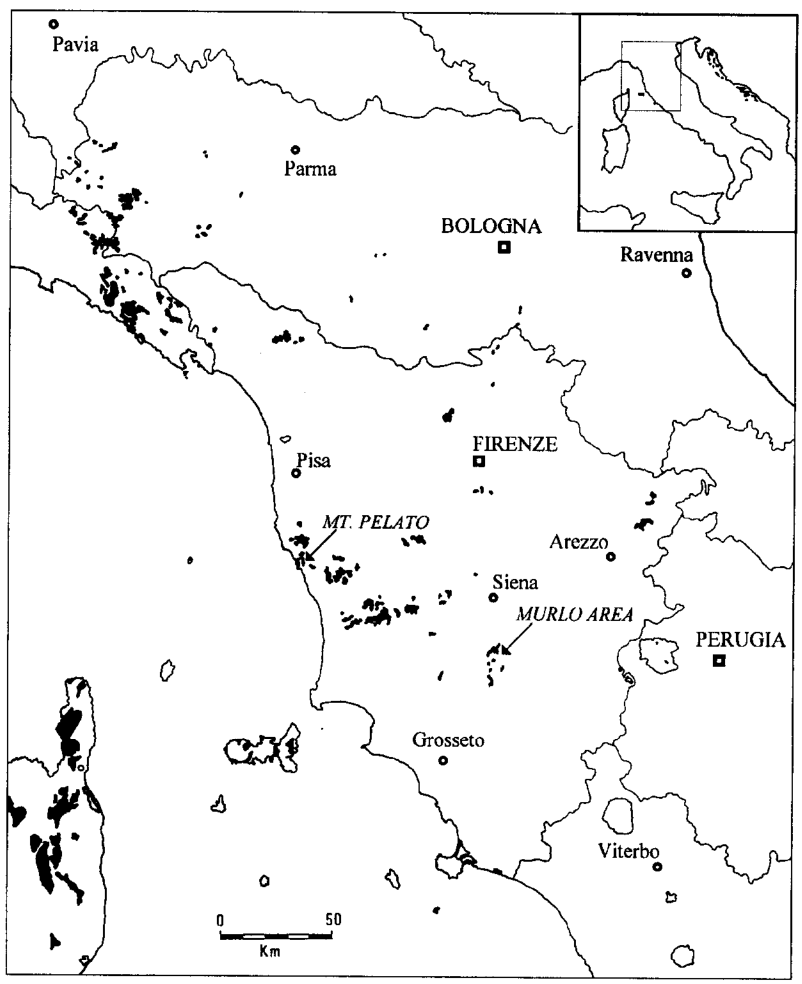

Fig. 1. Map of north-central Italy showing areas of ultramafic rocks and sites of this study.

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 U.S. 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.

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 that we report below is based on the principles established by Nicks and Chambers (1994) 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: (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 fertilizers on biomass and Ni content of the plants; (5) the reduction of Ni availability in the soil after successive croppings.

2. Site description

The test areas were located on Mont Pelato (350 m) in Livorno Province and near the village of

Fig. 2. *Alyssum bertolonii* a hyperaccumulator of nickel containing 1% of this element.

Murlo (350 m) south of Siena, Italy (Fig. 1). 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 from 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 of the Tuscan ultramafic outcrops. It encompasses all the serpentine-endemic plants including of course, *Alyssum bertolonii* (Fig. 2) 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. Both sites can be classified bioclimatically as Mediterranean pluvio-seasonal oceanic (Rivas Martinez, 1996).

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.

3. 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.

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 stands of *Alyssum bertolonii* that were treated with the following fertilizer 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 fertilizer 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 2 years). The plants were harvested from each quadrat at the end of the 2-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 1 *M* 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 order to simulate removal of plant-available nickel by successive crops, sequential extractions of the soils were made at pH 2, 4, and 6 using potassium hydrogen phthalate buffered with HCl (pH 2) and NaOH (pH 4 and 6). This extractant was used because there are no chelating effects and the extractant does not contain Ca or Mg (of interest in this study). These extractions were carried out using 0.7 g soil and 7 ml of extractant contained in 40 ml centrifuge tubes. The tubes were shaken for 24 h and centrifuged at 9000 rpm for 5 min. A portion of the extractant (5 ml) was removed and replaced with a further 5 ml of extractant. The procedure was repeated for eight successive extractions.

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 2 *M* 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.

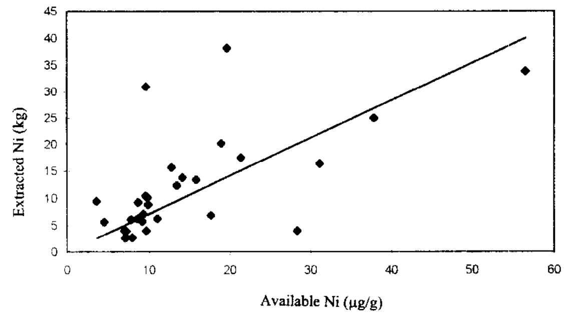

Fig. 3. Ni extracted by a crop of *Alyssum bertolonii* (kg/ha) as a function of the available Ni content of the soil as determined by ammonium acetate extraction at pH 7.

4. Results and discussion

4.1. 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 1. 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 our somewhat higher maximum of 1.65%.

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 this soil content by a factor of over 160.

Table 1
Nickel concentrations (%) in soil and in *Alyssum bertolonii* ($N = 40$) from Monte Pelato

Material	Dry	Ash	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		

4.2. 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.

4.3. Effect of fertilizers on biomass increase and nickel content of Alyssum bertolonii

In the experimental quadrats 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. 3—because of overlap only 28 of 35 points appear to be shown). This indicates that addition of fertilizers that increase this available Ni content should increase the Ni content of the plants. Table 2 shows the effect of six fertilizer 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. 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 impor-

Table 2
Mean ($N = 5$) extractability at pH 7 of trace elements ($\mu g/g$ 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

tance 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.

With the addition of fertilizers, the maximum annual biomass increase (ABI) was about 300% (Table 3). The highest individual increase (129%) was with N alone, and the highest combined increase (308%) was with N + P + K. Table 3 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. We have calculated the biomass of a fertilized crop of *Alyssum bertolonii* by multiplying the unfertilized 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 here at Massey University we obtained yields 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 stands of *Alyssum*. This also compares with the 23.0 t/ha obtained by McGrath et al. (1993) for fertilised plots of *Alyssum tenium*.

4.4. Cropping sustainability of nickel uptake by *Alyssum bertolonii*

A question which readily comes to mind is 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 Ni by KH phthalate at pH 2, 4 and 6 shows that:

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

where: t_e = cumulative extracted concentration of Ni; t = concentration of potentially available Ni; x = number of extractions; and c = a constant dependent on the type of soil and the amount of Ni removed in a single extraction. The cumulative ex-

Table 3
Percentage annual biomass increase (ABI) and elemental composition of *Alyssum bertolonii* given various fertilizer treatments at Murlo

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

All values given as % on a dry matter basis. The pH of the soils after treatment are shown in the final column. NiB = product of nickel content and biomass. 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.

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

tractions approach a limiting value where fewer extractions are needed at lower pH values. The limiting value was found to be 768 μg/g Ni in the Murlo soil (approximately the amount of Ni removed in a single extraction with 0.1 *M* 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 1.5, the mass would be 2250 t. For a crop producing 72 kg Ni/ha, the concentration of removable Ni would be 72,000/2250 g/t = 32 μg/g for a single crop. The number of potential croppings before the soil was exhausted would therefore be 30% of 768 divided by 32, i.e., 230/32 = 7.2 croppings.

After each of the sequential extracts, the equilibrium of soluble : total Ni 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, as many as seven 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 Ni 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.

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

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 fertilizer addition to the serpentine soil; (2) seed the site directly or plant out seedlings at a rate of approximately sixteen 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 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 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.

4.6. 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. Our own calculations with *Alyssum bertolonii* have arrived at 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 resowing 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 one day 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

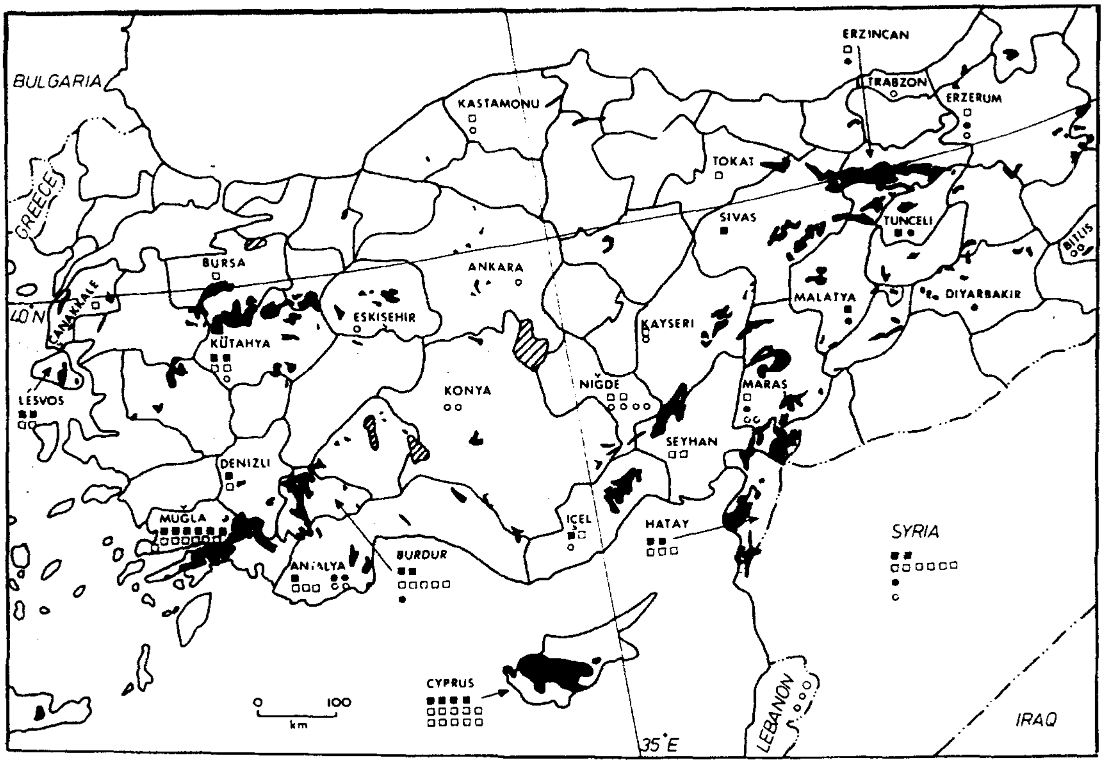

Fig. 4. Map of Anatolia, Turkey, showing location of ultramafic rocks and the distribution of Turkish *Alyssum* species. The Key for the latter is as follows (all Ni concentrations in dry material): ■, > 1% Ni; □, 0.1–1.0% Ni; ●, 0.01–0.1% Ni; ○, < 0.01% Ni. Source: Brooks et al. (1979).

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. 4). 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 which 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.

(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 (1992) have carried out experiments on nickel hyperaccumulators to illustrate this protection. In the course of extensive trials on the above plant we have not observed any tendency to disease. *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.

(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.

4.7. 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 burgeoning 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 4 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 which our experiments have suggested 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 Zaire (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 4 and neither are there any for Sn, Cd, Cu, Zn or Pb.

Table 4
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 [a]	%	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	7,485	0.74	7,422
Tin	6,200	0.90	8,960
Cadmium	3,750	1.48	14,815
Copper	1,961	2.83	28,330
Manganese	1,700	3.27	32,679
Zinc	1,007	5.52	55,169
Lead	817	6.80	67,999

[a] September 1996 prices.

Table 5
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
Gold or platinum	0.0037
Palladium	0.011
Silver	0.27
Cobalt	1.04
Nickel	6.68
Tin	8.06
Cadmium	13.3
Copper	25.5
Manganese	29.3
Zinc	49.6
Lead	61.2

The above problem can be addressed to some extent by use of hyperaccumulators of higher biomass combined with a sufficiently high metal content. Table 5 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 unfertilized 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. The South African *Berkheya coddii* (Morrey et al., 1992) has an unfertilized biomass of about 12 t/ha, a value that should be able to be increased considerably by fertilizing. Together with its high Ni content of over 1%, it is probably the best candidate to extend the outermost limits of phytomining for Ni.

5. General conclusions and final comments

It is concluded that our 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. We believe that we have set the potential limits for phytomining as a realistic commercial possibility. These limits are set by the biomass production and natural Ni content of the plant. We have also shown that adding fertilizer 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 hyperaccumulatory genes into other plants of high annual biomass production. Such an approach is being investigated in several research centres, principally in the United States.

Although our data do not represent an intensive evaluation of the potential of commercial phytomining, we believe that we have pointed the way to future research into the use of hyperaccumulator plants in order to exploit the world's vast reserves of 'subeconomic' deposits of metals such as cobalt, and nickel.

It is difficult in the enthusiasm of describing a new technique not to be accused of overstating the case, but who in the 1950s would have ever believed that Brazil would one day be able to grow a crop of motor fuel by producing alcohol from sugar cane for this purpose? The potential of phytomining is but a corollary to this.

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