

Terrestrial Higher Plants which Hyper-accumulate Metallic Elements – A Review of their Distribution, Ecology and Phytochemistry

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ABSTRACT

This paper reviews the plant geography, ecology, metal tolerance and phytochemistry of terrestrial higher plants which are able to accumulate metallic elements in their dry matter to an exceptional degree. The review is limited to the elements Co, Cu, Cr, Pb, Mn, Ni and Zn. Hyperaccumulators of Co, Cu, Cr, Pb and Ni are here defined as plants containing over 1000 µg/g (ppm) of any of these elements in the dry matter; for Mn and Zn, the criterion is 10,000 µg/g (1%). A unifying feature of hyperaccumulating plants is their general restriction to mineralized soils and specific rock types. Lists of hyperaccumulating species are presented for the elements considered. These suggest that the phenomenon is widespread throughout the plant kingdom. For example, 145 hyperaccumulators of nickel are reported: these are distributed among 6 superorders, 17 orders, and 22 families and include herbs, shrubs and trees from both the temperate and tropical zones. Although some phylogenetic relationships emerge, the evolutionary significance of metal hyperaccumulation remains obscure. Phytochemical studies however suggest that hyperaccumulation is closely linked to the mechanism of metal tolerance involved in the successful colonization of metalliferous and otherwise phytotoxic soils. The potentialities of hyperaccumulating plants in biorecovery and soil detoxification are indicated.

INTRODUCTION

It is probably true to say that the concentrations of all elements in soils will be reflected to some extent in the dry matter of plants which grow in them.

Accumulation of heavy metals is not therefore *per se* of particular interest unless the plant/soil ratio remains relatively constant, in which case the plants can be used for biogeochemical prospecting (Brooks, 1983), or unless the accumulations are inordinately high. In the latter case, considerable interest can be engendered among plant scientists because this high uptake (*hyperaccumulation*) implies extremely high tolerance to specific elements which are often present at concentrations in the substrate which would normally be considered to be phytotoxic (Peterson, 1971, 1975, 1983; Baker, 1981).

Accumulator plants are usually indigenous to a particular type of soil or parent rock and may prove to be valuable geobotanical indicators for mineral deposits. A further potential use of such accumulator plants is in indicating anthropogenic pollution, since these plants are often some of the few species capable of colonizing ground contaminated with high concentrations of heavy metals, and in so doing can outline the areal extent of pollution. The fact that element concentrations in hyperaccumulating species can often exceed 1% in the dried plant material makes it possible to carry out phytochemical studies involving milligram quantities of organo-metallic complexes, hence facilitating characterization. Consequently, there have been several detailed studies on the phytochemistry of plants that accumulate metals. These have been carried out in an attempt to understand the underlying mechanisms of metal tolerance involved (e.g. Farago *et al.*, 1975; Kelly *et al.*, 1975; Lee *et al.*, 1977b, 1978; Kersten *et al.*, 1980; Morrison *et al.*, 1981).

There exists therefore, a considerable body of information in the literature on metal accumulation by plants, ranging from ecological and biogeochemical studies, to detailed phytochemical aspects. It is the purpose of this review to bring together such diverse information in order to reveal the potentialities of terrestrial higher plants as accumulators of metallic elements. Within the plant kingdom, it is likely that there will be at least one taxon capable of concentrating any one element to an extraordinary degree. We have however confined this review to the following metals: Co, Cr, Cu, Mn, Ni, Pb and Zn, since these have been studied to the greatest extent.

To establish the degree to which accumulation of specific elements by plants has actually occurred, it is appropriate to present data for the mean abundances of the element in various geological environments such as the earth's crust, soils, rocks of igneous, sedimentary and ultramafic type, and in vegetation over non-mineralized sites.

Each of the elements listed above (except Co and Cu) will be considered separately below, but it will first be necessary to define terminology. Brooks *et al.* (1977a) first used the term *hyperaccumulators* to describe plants which contain >1000 µg/g (0.1%) Ni in their dried tissues. This concentration

threshold is an order of magnitude higher than the Ni levels in "normal" plants, even when the latter grow in nickeliferous substrates such as soils derived from ultramafic rocks. Jaffré (1980) has refined the nomenclature still further by using the terms *hypermanganésophores* and *hypernickelophores* to describe plants containing $>10,000 \mu\text{g/g}$ ($>1\%$) of Mn and Ni respectively. The threshold of $>1000 \mu\text{g/g}$ used to define hyperaccumulators of Ni (*sensu* Brooks *et al.*, 1977a) will not necessarily apply in the case of other elements. For example, the background concentration of Zn in plants (Brooks *et al.*, 1983) is around $100 \mu\text{g/g}$. Concentrations of Zn exceeding $1000 \mu\text{g/g}$ are not at all uncommon, and only values about 1% should be considered as exceptional. At the other extreme, since natural concentrations of gold in plants are around $0.001 \mu\text{g/g}$ (Boyle, 1979), a concentration of $1 \mu\text{g/g}$ would certainly indicate hyperaccumulation of this metal.

COBALT AND COPPER

Introduction

Hyperaccumulators of Co and Cu will be considered together because these plants are usually both found in the same area of southcentral Africa in the Shaban Copper Arc (Zaire) and in the Zambian Copperbelt. A map of these important mining districts is given in Figure 1. The metallophytes of the region have been described by Brooks and Malaisse (1985). Such hyperaccumulators have been found nowhere else in the world, not even over cobalt deposits.

Copper is an essential plant micronutrient, being a component of the protein structure of a range of enzymes involved in electron transport and redox reactions in mitochondria, chloroplasts, cell walls and the cytoplasm of plant cells. Other copper proteins play important roles in carbohydrate and nitrogen metabolism, and lignification of cell walls (Marschner, 1986). Copper deficiency symptoms are well-characterized and include stunted growth, distortion and bleaching of young leaves, and necrosis of shoot tips. Because Cu is an essential element for plant nutrition, the copper content of most plants tends to be internally rather than externally regulated (Timperley *et al.*, 1970), so that concentrations in plants tend to be relatively constant (around $10 \mu\text{g/g}$ – see Table 1) and relatively independent of the nature of the substrate. Indeed most plants have Cu concentrations well below those of the soil in which they grow (see Table 1) and it is only for very high concentrations in the substrate that the exclusion mechanism breaks down. At this point concentrations of Cu in the plant increase very greatly over a small range of increasing concentrations in the soil until the point is

TABLE I

Mean elemental concentrations ($\mu\text{g/g}$) in rocks, soils and vegetation

	Co	Cr	Cu	Pb	Mn	Ni	Zn
Earth's crust	25	100	55	13	950	75	70
Granite	3	20	13	48	195	1	45
Basalt	47	114	110	8	1280	76	86
Ultramafic rocks	150	1600	10	1	1620	2000	50
Soils (non-ultramafic)	10	60	20	10	850	40	50
Soils (ultramafic)	250	2500	20	10	1000	2500	40
Vegetation (non-ultramafic)	1	1	10	10	80	2	100
Vegetation (ultramafic) ^a	10	10	10	10	100	80	100

^aexcluding hyperaccumulators.

Source: Brooks (1987) and authors' own unpublished data.

rapidly reached where the plant can no longer tolerate the hostile edaphic conditions.

Concentrations of Co in plant tissue (normally in the range 0.03–1 $\mu\text{g/g}$ – see Table I) tend to reflect the composition of the soil to a greater degree since this element is not normally considered to be essential for plant nutrition, although it may have beneficial effects. There is now good evidence that cobalt is essential for the growth of legumes under symbiotic conditions and in other nitrogen-fixing systems (Bollard, 1983; Marschner, 1986).

When Brooks *et al.* (1977a) defined hyperaccumulators of Ni, the level of 1000 $\mu\text{g/g}$ included in the definition was not an arbitrary choice since hyperaccumulators of Ni have concentrations an order of magnitude greater than those of "normal" plants even when growing over ultramafic rocks. In the case of Co and Cu, the difference is less obvious. However Brooks *et al.* (1980) used abundance data for Cu and Co in 400 specimens of numerous species of Shaban plants growing over or near mineralization and plotted the data as cumulative frequency curves which showed identical cut-off points for Co, Cu and Ni at 1000 $\mu\text{g/g}$.

Brooks *et al.* (1980, 1987) have reported hyperaccumulation of Cu and Co by 43 Shaban taxa as shown in Table 2.

Hyperaccumulators of cobalt

Table 2 lists 26 hyperaccumulators of Co including nine which have the same property towards Cu. *Haumaniastrum robertii* is perhaps the most unusual

TABLE 2
Hyperaccumulators of copper and cobalt (> 1000 µg/g)

Species	Concentrations (µg/g)		Reference
	Copper	Cobalt	
<i>Aeollanthus biformifolius</i> De Wild. - LAMIAC.	3920	2820	9
<i>A. saxatilis</i> Duvign. et Plancke	-	1000	3
<i>Alectra sessiliflora</i> (Vahl) O.K.			
var. <i>sessiliflora</i> - SCROPHULARIAC.	-	2782	4
var. <i>senegalensis</i> (Benth.) Hepper	1590	-	3
<i>A. welwitschii</i> Hemsl.	-	1561	4
<i>Anisopappus davyi</i> S. Moore - ASTERAC.	2889	2650	1
<i>A. hoffmanianus</i> Hutch.	1065	-	7
<i>Acolepis metallorum</i> Duvign. et Léon. - CYPERAC.	1200	-	2
<i>Becium aureoviride</i> Duvign.			
subsp. <i>lupotoense</i> Duvign. - LAMIAC.	1135	-	4
<i>Buchnera henriquesii</i> Engl. - SCROPHULARIAC.	3520	2435	3,4
<i>Bulbostylis mucronata</i> (Steud.) Cl. - CYPERAC.	7783	2130	3,4
<i>Celosia trigyna</i> L. - AMARANTHAC.	2051	-	4
<i>Commelina zigzag</i> Duvign. et Dewit. - COMMELINAC.	1210	-	1
<i>Crassula alba</i> Forsk. - CRASSULAC.	-	1712	4
<i>C. vaginata</i> Eckl. et Zeyh.	-	1405	1
<i>Crotalaria cobalticola</i> Duvign. - FABAC.	-	3010	3
<i>Cyanotis longifolia</i> Benth. - COMMELINAC.	-	4200	1
<i>Eragrostis boehmii</i> Hack. - POACEAE	2800	-	5
<i>Gutenbergia cupricola</i> Duvign. - ASTERAC.	5095	2309	4
<i>Haumaniastrum homblei</i> (De Wild.) Duvign. et Denacyer - LAMIAC.	-	2633	1,4
<i>H. katangense</i> (S. Moore) Duvign. et Plancke	8356	2240	3,4,7
<i>H. robertii</i> (Robyns) Duvign. et Plancke	2070	10200	2,4,10
<i>H. rosulatum</i> (De Wild.) Duvign.	1089	-	4
<i>Hibiscus rhodanthus</i> Gürke - MALVAC.	-	1527	4
<i>Icomum tuberculatum</i> De Wild. - LAMIAC.	-	1429	4
<i>Ipomoea alpina</i> Rendle - CONVULVULAC.	12300	-	1
<i>Lindernia dumblonii</i> Duvign. - SCROPHULARIAC.	-	1113	4,5
<i>L. perennis</i> Duvign.	9322	2300	4,5
<i>Monadenium cupricola</i> Malaisse - EUPHORBIAC.	-	1234	4
<i>Pandiaka metallorum</i> Duvign. et Van Bockst. - AMARANTHAC.	6260	2139	1,4
<i>Rendlia cupricola</i> Duvign. - POACEAE	1560	-	6
<i>Silene cobalticola</i> Duvign. et Plancke - CARYOPHYLLAC.	1660	-	2
<i>Sopubia dregeana</i> Benth. - SCROPHULARIAC.	-	1767	4,5
<i>S. metallorum</i> Duvign.	-	1742	4
<i>S. neptunii</i> Duvign. et Van Bockst.	-	2476	4
<i>Striga hermontheca</i> Benth.	1105	-	4
<i>Triumfetta digitata</i> (Oliv.) Sprague et Hutch. - TILIAC.	1060	-	3
<i>T. welwitschii</i> Mast. var. <i>descampii</i>	-	2201	4
<i>Vernonia petersii</i> Oliv. et Hiern. - ASTERAC.	1555	-	4,8
<i>Vigna dolomitica</i> Wilczek - FABAC.	3000	-	5
<i>Xerophyta retinervis</i> Bak. var. <i>retinervis</i> - VELLOZIAC.	-	1520	4

1 - Malaisse *et al.* (1979); 2 - Duvigneaud and Denacyer de Smet (1963); 3 - Brooks *et al.* (1980); 4 - Brooks *et al.* (1987); 5 - Malaisse and Grégoire (1978); 6 - Brooks and Malaisse (1985); 7 - Brooks *et al.* (1982); 8 - De Plaen *et al.* (1982); 9 - Brooks *et al.* (1978); 10 - Brooks (1977).

of the cobaltophytes since it can contain up to 1.02% Co (a factor of 10,000 above the content in plants not growing over mineralized ground) in the dried leaves (Brooks, 1977) and can accumulate up to 0.2% Cu. This taxon was referred to as a "copper flower" by Duvigneaud and Denaeyer-De Smet (1963) and is confined to metalliferous ground. Brooks (1977) has suggested that its distribution may be controlled by Co rather than Cu. In pot trials on *H. robertii*, Morrison *et al.* (1979) showed that this taxon tends to restrict Cu uptake much more than it does that of Co, apparently confirming the conclusions of Brooks (1977). Moreover, correlation analysis of the data from the above pot trials also showed a very highly significant relationship ($P < 0.001$) between the Co content of *H. robertii* and that of the soil. This indicates the possibility of using this species in biogeochemical prospecting for Co, but not for Cu for which there was a non-significant relationship for the plant-soil data. It should also be noted that the closely-related *H. katangense* is also a hyperaccumulator of Co. Though tolerant of Cu mineralization (this species is another "copper flower"), it does not accumulate Cu to any significant degree.

The first of the Co hyperaccumulators to be discovered was *Crotalaria cobalticola*. Duvigneaud (1959) reported 354 $\mu\text{g/g}$ Co in this species. Although this value was well below the threshold of 1000 $\mu\text{g/g}$, Brooks *et al.* (1980) have reported 970–3010 $\mu\text{g/g}$ (mean 2010 $\mu\text{g/g}$) in other specimens. We have also identified (unpublished work) a population of *C. cobalticola* growing away from mineralization and which contained negligible concentrations of both Cu and Co. It seems that *C. cobalticola* is the only species of this genus which can hyperaccumulate either Cu or Co. In a study of 284 of the 438 known species of African *Crotalaria* (including all those known to be found in Shaba), Brooks *et al.* (1977b) found that excepting *C. cobalticola*, maximum values never exceeded 336 $\mu\text{g/g}$ Co (in *C. cornetii* Taub. et Dewèvre) and 705 $\mu\text{g/g}$ Cu (in *C. peschiana* Duvign. et Timp.).

Crassula vaginata though a hyperaccumulator of Co, is not confined to Cu/Co deposits. It is common on the non-mineralized high plateaux (Biano, Kibara, Kundelungu and Marungu). It is also found on rocky cupriferous steppe at Fungurume and Kwatebala where it is a local indicator.

Hyperaccumulators of copper

Table 2 also lists 24 hyperaccumulators of Cu. Of these perhaps the most unusual is *Aeollanthus biformifolius* which also hyperaccumulates Co. This species was shown by Malaisse *et al.* (1978) to contain as much as 13,700 $\mu\text{g/g}$ (1.37%) in the whole plant (dry weight). This is factor of 1000 higher than the content in plants not growing over mineralization. Most of the Cu was in the corms with lesser (though very high) contents in the aerial parts of the

plants. The same authors noted a seasonal variation in the Cu content of individual specimens. Concentrations in specific organs were lowest at the beginning of the rainy season and then increased rapidly with the development of the new corm which is fully mature three months later. The Cu concentration in the corms of this species is easily the highest ever recorded for any vascular plant. Only bryophytes can contain Cu concentrations of this order. It appears that *A. biformifolius* is the only member of this family which can accumulate Cu to any significant degree. Brooks *et al.* (1978) determined Cu and Co in 49 species of African *Aeollanthus* including all those found in Shaba. The highest value found for species other than *A. biformifolius* was 28 µg/g in *A. elongatum* De Wild.

The first workers to present data indicating hyperaccumulation of Cu were Duvigneaud and Denaeyer-De Smet (1963) who reported values of 1200, 1660, and 1960 µg/g (dry weight) for: *Ascolepis metallorum*, *Silene cobalticola*, and *Haumaniastrum robertii* respectively. It is noteworthy that the list of hyperaccumulators of Cu does not include any of the genus *Becium* which encompasses the well-known Cu indicator *B. homblei* (De Wild.) Duvign. et Plancke. In unpublished work we have analyzed herbarium specimens of all members of the *Becium* genus found in Shaba Province but have not found appreciable uptake of Cu or Co in any of the constituent species.

Metal tolerance of hyperaccumulators of cobalt and copper

As far as is known, only two laboratory studies expressly linked to plant tolerance have been carried out on Shaban plants. Morrison *et al.* (1979) carried out pot trials on plants of *Haumaniastrum katangense*, *H. robertii* and *Aeollanthus biformifolius*. These Shaban metallophytes were grown in peat/pumice mixtures (1:1) with varying amounts of Cu or Co ranging from 0–10,000 µg/g (0–1%) added as metal salts. The tolerance of each species was determined and for *H. robertii* corresponded to a maximum Co concentration of 4000 µg/g in the mixture. *A. biformifolius* was able to tolerate up to 9000 µg/g Cu. Discontinuities in the plant vs. soil concentration curves for each element indicated an exclusion mechanism operating for all three species at intermediate metal concentrations in the soils. All three species would grow in soils containing only traces of Cu or Co and this indicated that uptake of heavy metals was not linked to a physiological need for either metal. The plant – soil correlation for Co concentrations ($P < 0.001$) was sufficiently good for all three species for them to have some promise for biogeochemical prospecting. Experimental data are shown in Figure 2.

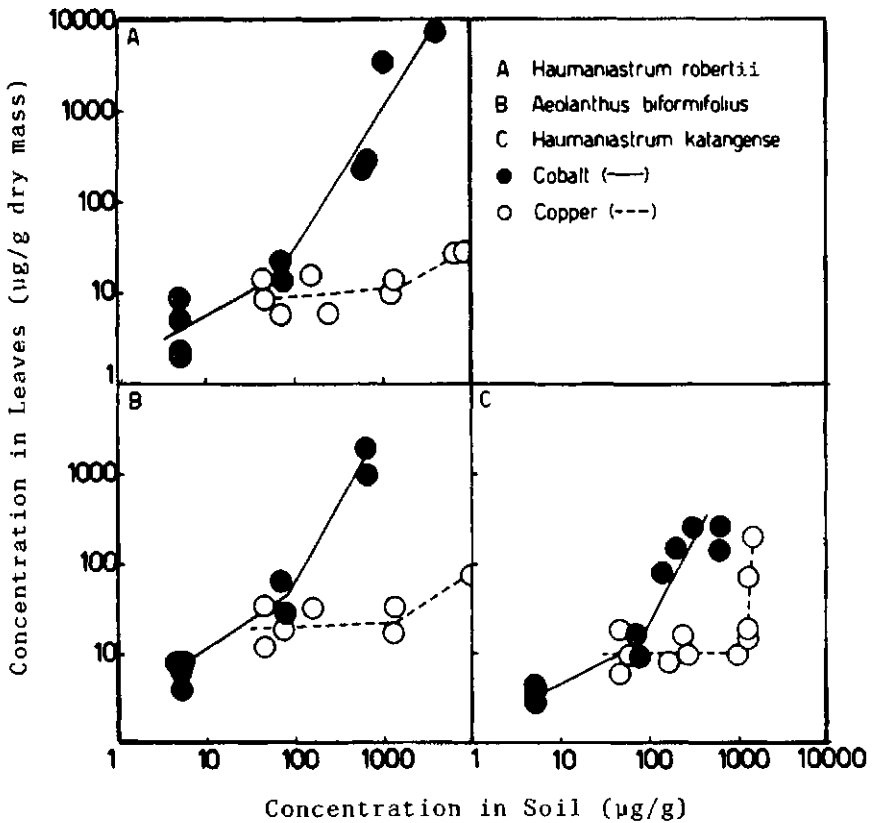


FIGURE 2 Copper and cobalt concentrations in three plant species expressed as a function of the corresponding concentrations in the soil. Source: Morrison *et al.* (1979).

A survey of Cu and Co tolerance by an ecophyletic series of Shaban taxa within the genus *Silene* was carried out by Baker *et al.* (1983). Plants studied were the widespread and presumably non-tolerant *Silene burchelli* Otth. var. *angustifolia* Sond. (A), a more tolerant ecotype of the former (B), and the metallophyte *S. cobalticola* (C). Some of the experimental data are shown in Figures 3 and 4.

In Figure 3, the effect of Cu and/or Co in the substrate upon plant performance (heights and yields) is demonstrated. In this comparative experimental study, there was a steady gradation of tolerance from the non-tolerant precursor *Silene burchelli* var. *angustifolia* (B), via the more tolerant ecotype (E) to the tolerant metallophyte *S. cobalticola* (C).

A pattern similar to the above was found in metal uptake by these taxa expressed as a function of the concentration of the element in the substrate (Figure 4).

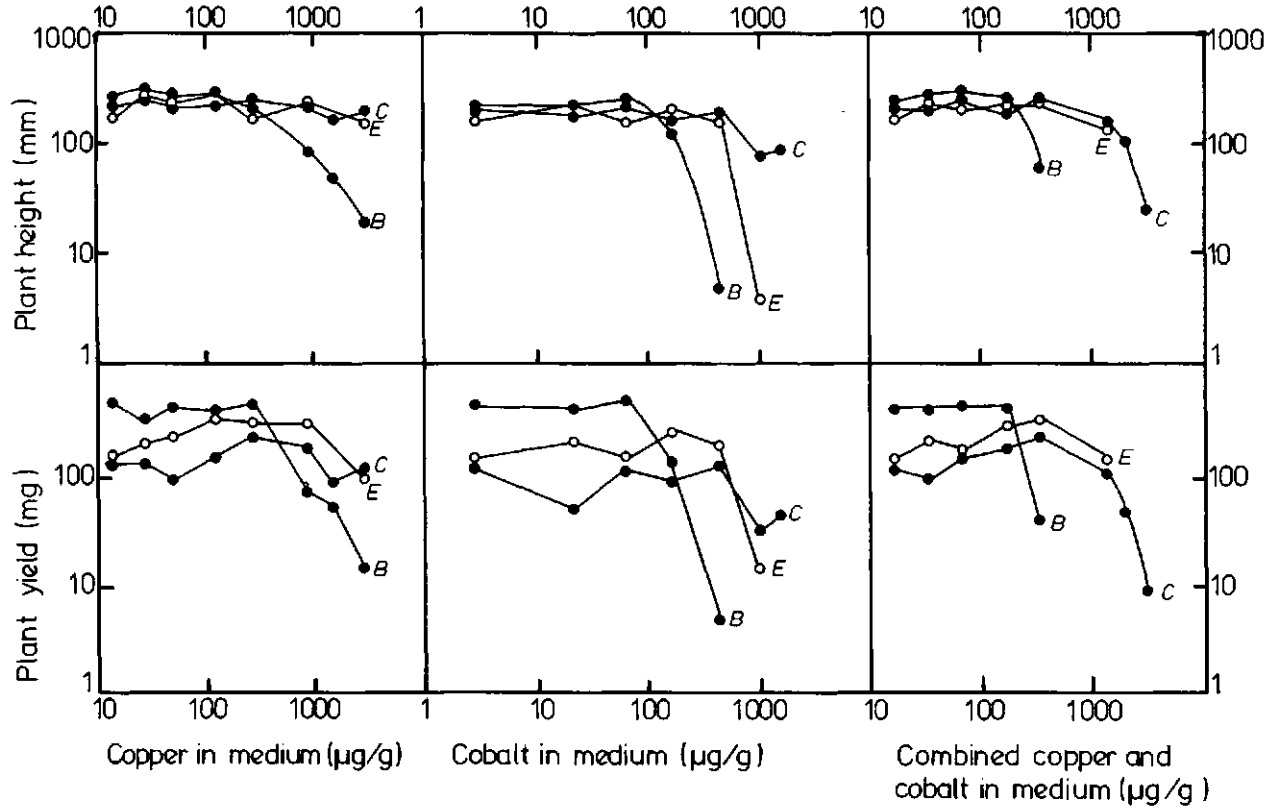


FIGURE 3 Plots showing mean plant heights and yields for three *Silene* taxa grown for 8 weeks in substrates containing graduated concentrations of copper and cobalt. B – *Silene burchelli* var *angustifolia*; E – Ecotype of above; C – *S. cobalticola*. Source: Baker *et al.* (1983).

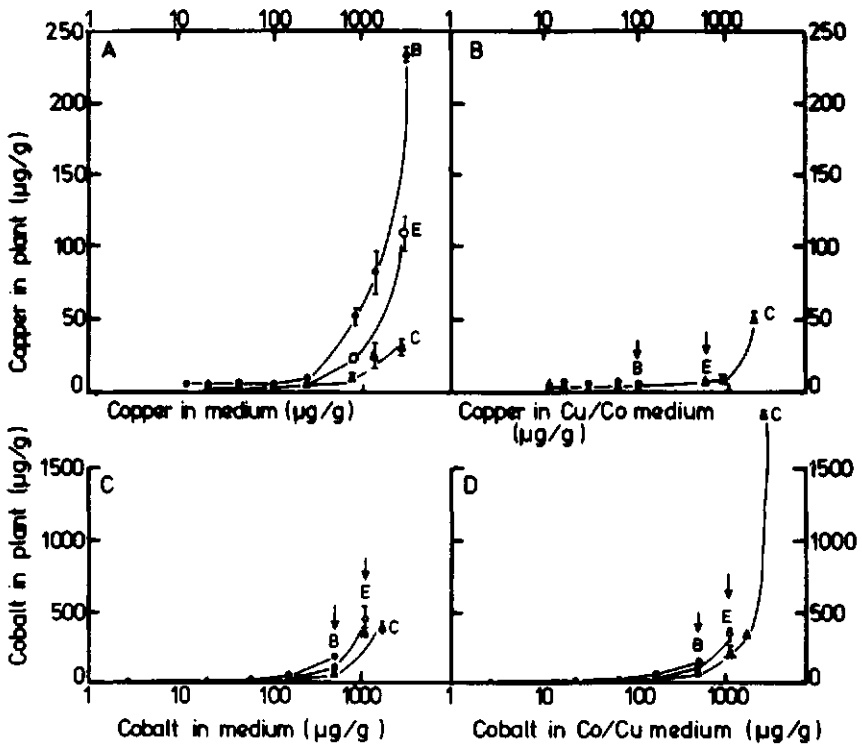


FIGURE 4 Plots showing mean copper and cobalt concentrations ($\mu\text{g/g}$ dry weight) in the shoots of three *Silene* taxa after 8 weeks' growth in substrates containing graduated concentrations of copper and cobalt. The vertical arrows indicate limits of tolerance. B - *Silene burchelli* var. *angustifolia*; E - Ecotype of above; C - *S. cobalticola*. Source: Baker *et al.* (1983).

The degree of germination of seeds in the presence of toxic metals is to some extent a measure of tolerance of that particular taxon to the element concerned. Figure 5 shows the results of germination tests carried out on two of the above ecophyletic series in which seeds were placed in Petri dishes containing filter papers soaked in either distilled water or solutions of either Co or Cu ($50 \mu\text{g/ml}$ of either element). These germination tests clearly demonstrated the greater tolerance of *Silene cobalticola* to both Cu and Co compared with the precursor *S. burchelli* var. *angustifolia*. Plant performance and yield also confirmed the sequence C > E > B in relation to tolerance to Cu and/or Co by the above series.

Germination tests were carried out by Morrison (1980) on the two hyperaccumulators *Haumaniastrum robertii* and *Aeollanthus biformifolius*. The former hyperaccumulates Co and the latter Cu. In tests on *H. robertii*

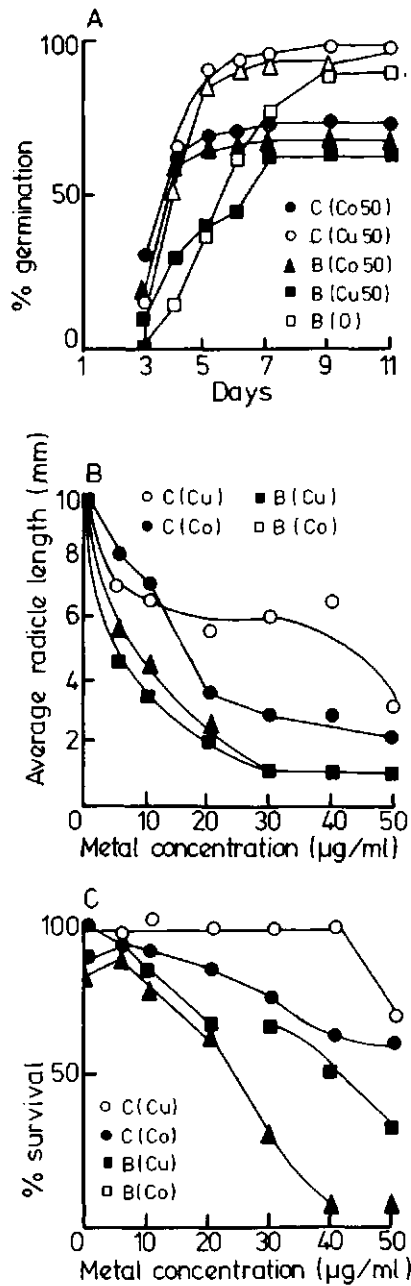


FIGURE 5. Plots showing percentage germination of two taxa in the presence of solutions of copper and cobalt (50 µg/g) and in distilled water controls. C - *Silene cobalticola*, B - *Silene burchellii* var. *angustifolia*. Source: Baker *et al.* (1983)

using the procedure used above, it was observed that germination in the control (distilled water) did not differ from germination in the presence of 0.1% Cu or Co. Germination in combined solutions of both metals was however lower. Germination occurred in Co solutions up to 1% but there was none in Cu solutions exceeding 0.1%. These findings tend to reinforce the belief that this species is a 'cobalt flower' rather than a 'copper flower'. *Aeollanthus biformifolius* readily germinated in solutions containing low concentrations of Cu or Co or Cu + Co (0.1%). This species did however have an overall higher tolerance to Cu than did *H. robertii* since it would germinate (albeit only 15%) in 5% Cu solutions. Its tolerance to Co was however lower than that of the *Haumaniastrum*. Once again a pattern emerged of a plant showing a greater tolerance to an element which it hyperaccumulates.

Though the question of plant evolution is not seriously addressed in this review, it may be appropriate to give a short rider on the subject. Plants found on mineral deposits may be said to be *facultative* or *obligate* metallophytes. *Facultative* taxa will grow quite well off mineralized ground and clearly do not have a specific physiological requirement for this particular set of edaphic conditions. Although most of the metallophytes of southcentral Africa are clearly *facultative*, several taxa are confined to mineralized ground and are clearly *obligate*. In spite of this however, all obligates tested in the laboratory invariably grew quite well in "normal" soils (e.g. *Haumaniastrum robertii* and *H. katangense*). These plants are however very sensitive to fungal attack and it is probably this factor as well as inability to withstand interspecific competition which restricts them to mineralized substrates. They are nevertheless still obligates even though this "obligatory" property may be linked to low populations of fungi or other competing species.

Phytochemical studies on hyperaccumulators of cobalt and copper

The metallophytes of southcentral Africa are in the main useful subjects for phytochemical studies. This is because many of these taxa have elemental concentrations which are so high that milligram amounts of purified metal complexes can be isolated and characterized.

The same factors which have discouraged tolerance studies on plants from southcentral Africa (i.e. paucity of local institutions and practical problems in obtaining field material) are also applicable to phytochemical studies on the nature and function of organo-metallic complexes and other associated problems. Perhaps for this reason there are only a few studies which have so far been carried out in this area of research. Several experiments were

carried out by Morrison (1980) and Morrison *et al.* (1981) on metallophytes from Shaba. The taxa studied were: *Aeollanthus biformifolius*, *Buchnera henriquesii*, *Faroa chalcophila* R. Taylor, *Haumaniastrum robertii* and *Silene cobalticola*. Three of these are of particular interest since *H. robertii* is a hyperaccumulator of Co and Cu and *A. biformifolius* is a hyperaccumulator of Cu (see Table 2 for both taxa). In addition, *S. cobalticola* is the most extreme metallophyte of an ecophyletic sequence of taxa which shows a gradual evolutionary trend from the probable precursor *Silene burchelli* var. *angustifolia* (Baker *et al.*, 1983).

The distribution of heavy metals in plant tissue extracts of the above five taxa has been reported by Morrison *et al.* (1981). The data are shown in Table 3 from which several distinct patterns emerge. In all cases, by far the greatest proportion (usually 85–90%) of the heavy metals was found in only three fractions (B, C and E). These fractions all comprised predominantly polar compounds. Fraction B contained water-soluble polar compounds of relatively low molar mass. In all cases, extraction of Cu in this fraction was lower than that of Co. This was particularly evident in *Haumaniastrum robertii*. In previous studies (Kelly *et al.*, 1975) it had been found that most of the Ni (65–94%) in Ni hyperaccumulators was readily extractable into

TABLE 3

Percentage of heavy metals in various fractions of extracts of leaves of metallophytes from Shaba Province, Zaïre.

Fraction	a		b		c		d			e	
	Cu	Co	Cu	Co	Cu	Co	Cu	Co	Mn	Cu	Co
A	0.8	0.5	0.1	0.1	1.4	1.2	1.7	0.2	0.8	2.5	0.5
B	11.3	33.8	9.1	10.3	16.6	20.4	12.6	42.9	21.7	22.2	34.9
C	47.8	31.7	59.3	36.3	54.6	48.9	39.9	39.7	58.7	38.8	53.4
D	4.2	1.8	4.0	1.5	0.8	0.9	3.0	1.6	1.8	1.9	1.2
E	26.0	22.0	20.7	39.6	17.4	21.3	18.3	9.9	11.6	23.2	9.0
F	1.2	0.9	0.6	0.7	0.2	0.2	2.5	0.7	0.8	0.5	0.1
G	1.8	8.5	2.0	7.0	3.6	4.4	7.1	4.5	4.0	5.4	0.7
H	6.9	0.9	4.3	4.7	5.3	2.7	14.9	0.5	0.8	5.6	0.4

a - *Aeollanthus biformifolius*; b - *Buchnera henriquesii*; c - *Faroa chalcophila*; d - *Haumaniastrum robertii*; e - *Silene cobalticola*; A - ethanol extract (neutral small molecules); B - water extract (water-soluble low MW polar compounds); C - 0.2M hydrochloric acid (acid-soluble polar compounds); D - precipitate from C with acetone (proteins and pectates); E - residue dissolved in 0.5M perchloric acid (lignin, cellulose etc.); F - precipitate from E with acetone (nucleic acids); G - residue dissolved in 2M sodium hydroxide (proteins and polysaccharides); H - final residue (cellulose, lignins and insoluble material on cell walls). After Morrison *et al.* (1981).

deionized water. This compares with a maximum of 42.9% Co in *H. robertii* and 22.2% Cu in *Silene cobalticola*. The more powerful extracting ability of 0.2M hydrochloric acid (fraction C) resulted in a significantly increased Co extraction and usually a greater degree of extraction of Cu relative to Co. Treatment with perchloric acid (fraction E) gave further significant amounts of Cu and Co. It appeared therefore that most of the Co and Cu in these plants were bound to organic ligands and that these complexes were all somewhat polar in character. In contrast to Ni hyperaccumulators (Lee *et al.*, 1977b, 1978) where the metal is bound simply to citric and/or malic acids, there was no simple and consistent pattern for the Shaban plants.

The presence of Co in plants raises the question of whether it is bound in a cobaltamin complex such as vitamin B-12. This however is unlikely as such a complex should be extractable into fraction A (95% ethanol) which invariably contained insignificant concentrations of Co. It should also be mentioned that non-accumulating plants fed with Co usually have a high degree of extraction of this element into fraction A (Bowen *et al.*, 1962). This extractability is usually of the order of 30-60% and contrasts very strongly with that of the hyperaccumulators. The same is true, though to a lesser extent, for Cu. The wide distribution of Cu and Co in at least three fractions leads to the conclusion that these metals may not be bound to a single ligand.

The spatial distribution of Co, Mn, Ca and K in leaves of *Haumaniastrum robertii* was determined by Morrison *et al.* (1981) using a proton microprobe. Photographs of raster scans across a leaf of *H. robertii* (Figure 6) showed several regions of elevated Co and Ca concentrations in which there was a corresponding deficiency of K. In Figure 6 the scan (*ca.* 100 μm resolution) showed that the distribution of Ca, Co and Mn was remarkably uniform with variation of not more than 10% from the mean. Potassium showed rather more variation (20%). The anomalous region of the leaf however showed a much higher concentration of Mn than the remainder of the leaf and there was a five-fold increase in the Co concentration. At the centre of the anomaly there was a substantial decrease in the K concentration. Surrounding the Co and Mn anomaly was a region with elevated Ca, although this was slightly less marked at the centre where the Co and Mn concentrations were highest.

It is known (Al-Rais *et al.*, 1971) that Ca oxalate crystals are often found in plant material and it is possible that this study on *H. robertii* reveals that a significant part of the Co is linked to a complex insoluble in water but soluble in dilute hydrochloric acid. Alternatively, it is possible that Co (II) replaces Ca (II) in the oxalate crystals. This could account for the presence of 30-50% of the Co in fraction C (see Table 3 above) which is an extract with 0.2M hydrochloric acid. Further support is provided by the observation that

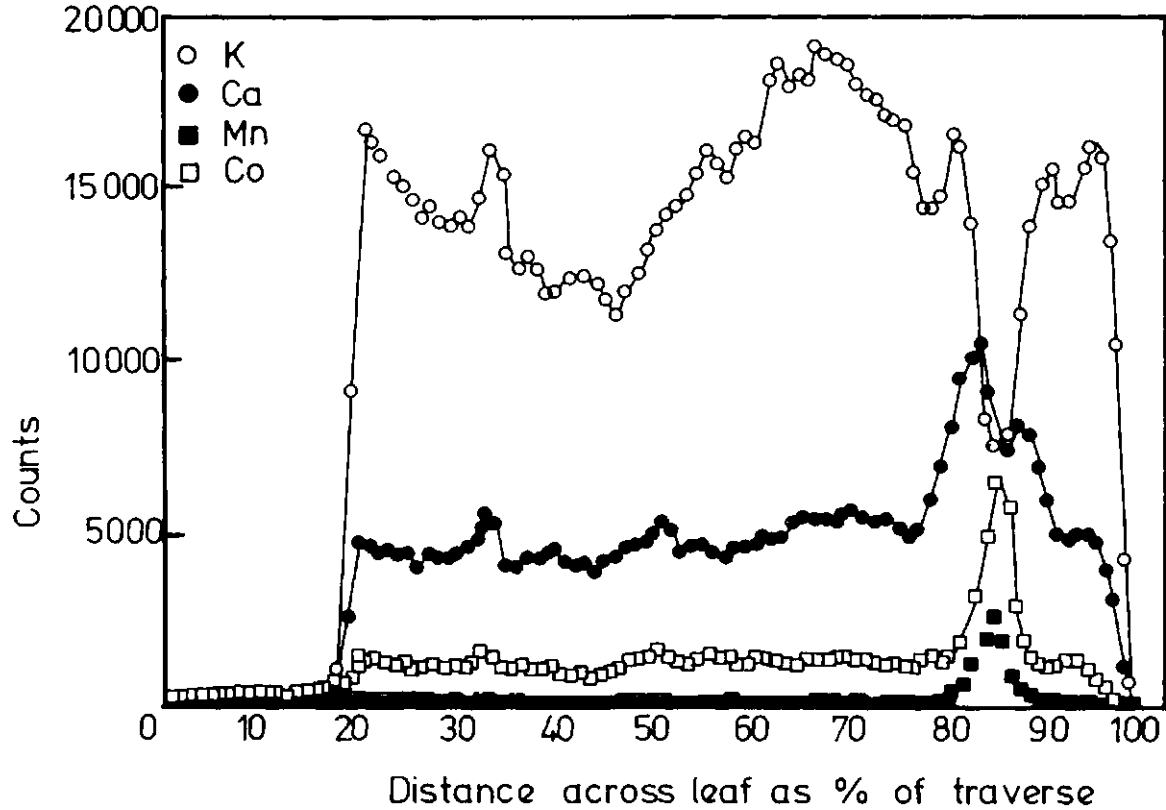


FIGURE 6 Proton microprobe scan of leaf of *Haumaniastrum robertii* showing spatial distribution of potassium, calcium, cobalt and manganese. The region occupied by the leaf is between 18 to 99% of total distance traversed and covers 6.75 mm. Source: Morrison *et al.* (1981).

the permanganate titration of an aliquot of a fraction containing 17 μmol of Co obtained from *H. robertii* indicated the presence of 18 μmol of oxalate.

Following the proton probe studies, Morrison *et al.* (1981) separated the water-soluble complex from *Haumaniastrum robertii* which contained 1.14% Co with a molecular weight of about 5200 per mole of Co. It was therefore clearly not proteinaceous in nature. Electrophoresis was carried out on the Co complex and the results gave a somewhat complicated pattern. The dominant peak was an anionic peak whereas the cationic fraction appeared to be composed of three overlapping peaks. When the material was removed and analyzed by gas-liquid chromatography after methylation of the complex, two distinct peaks were obtained which have not yet been identified. The field is therefore open for further work on the nature of complexes in this hyperaccumulator of Co.

CHROMIUM

Background concentrations of chromium in plant tissues are generally well below 1 $\mu\text{g/g}$ (Table 1), but do vary according to plant species and organ sampled. Chromium is not considered to be an essential element but there are several reports of the apparent stimulation of plant growth by low concentrations of Cr in solution culture (0.05–0.1 $\mu\text{g/ml}$) or when added to soil, but these remain unexplained (Bollard, 1983). The toxic properties of Cr can be demonstrated by solution culture at concentrations as low as 1–2 $\mu\text{g/ml}$.

Outside southcentral Africa there are no recorded cases of inordinate accumulation of Cr among vascular plants, despite Cr contents 60 and 2500 $\mu\text{g/g}$ in non-ultramafic and ultramafic soils respectively (Table 1). The reluctance of these plants to accumulate Cr is so great that the Cr concentration of plant material is often used (Ti has a similar use) to assess the degree to which a plant sample has been contaminated from the surrounding soil. However, Wild (1974) has reported extraordinary concentrations of Cr in leaves of *Dicoma niccolifera* Wild (estimated at 1500 $\mu\text{g/g}$ dry weight), and *Sutera fodina* Wild (est. 2400 $\mu\text{g/g}$). These plants were found near the Noro chrome mine on the Great Dyke, a large ultramafic intrusion which bisects Zimbabwe in a N-S direction. Brooks and Yang (1984) have analyzed the same species for Cr and other elements and have found concentrations of this element at least 20 times lower than those reported above. It may be therefore that the samples from the Noro mine had been contaminated by wind-blown ore.

LEAD

Lead is neither an essential nor beneficial element in plant nutrition and is generally present at background concentrations in plant tissues (10 µg/g – see Table 1). Accumulation, let alone hyperaccumulation of Pb by plants is an exceedingly rare event due to the readiness with which this element can be precipitated as the insoluble sulphate in the rhizosphere, hence minimizing potential uptake and transport to the aerial parts of the plants. Among the few records of hyperaccumulation of Pb (here defined as > 1000 µg/g) are a report by Linstow (1924) of 1300 µg/g of this element in *Armeria maritima* var. *halleri* from Germany, a value of 1044 µg/g reported by Cole *et al.* (1968) for a single specimen of *Polycarphaeae synandra* from Northern Australia, and a concentration of up to 2740 µg/g in *Thlaspi alpestre* growing over Pb mines in England (Shimwell and Laurie, 1972). The latter report gives only a range of 100–2740 µg/g Pb and does not state how many individuals had values exceeding 1000 µg/g.

Reeves and Brooks (1983a) discovered very high Pb levels in two brassicaceous plants (*Alyssum wulfenianum* Schlecht. and *Thlaspi rotundifolium* subsp. *cepaefolium*) from mine tailings and gravels centred on Cave del Predil in Northern Italy.

Data for a number of hyperaccumulators of Pb are given in Table 4. The Pb concentration in the *Thlaspi* subspecies is easily the highest recorded for any flowering plant. The existence of a non-tolerant taxon *Thlaspi rotundifolium* (L.) Gaud. growing in the vicinity of subsp. *cepaefolium* leads to the conclusion that colonization of the mine wastes is probably a neo-endemic process.

TABLE 4

Hyperaccumulators of lead (> 1000 µg/g)

Species	Location	Lead content (µg/g)	Ref.
<i>Armeria maritima</i> (Mill.) Willd. var. <i>halleri</i> (Wallr.) Rothm. – PLUMBAGINACEAE	Harz Mts	1600	2
<i>Polycarphaeae synandra</i> F. Muell. – CARYOPHYLLACEAE	Australia	1044	3
<i>Thlaspi alpestre</i> L. – BRASSICACEAE	Derbyshire	2740	4
<i>T. rotundifolium</i> (L.) Gaudin subsp. <i>cepaefolium</i> (Wulf.) Rouy et Fouc.	Central Europe	8200	1

1 – Reeves and Brooks (1983a); 2 – Linstow (1924); 3 – Cole *et al.* (1968); 4 – Shimwell and Laurie (1972).

MANGANESE

Manganese is an essential micronutrient and an activator of a number of enzymes, particularly decarboxylases and dehydrogenases of the tricarboxylic cycle. The specific requirement for Mn as a mineral nutrient is thought to be related to its tightly bound form in metalloproteins where it may act as a structural constituent, an active binding site, or like Fe, as a redox system (Marschner, 1986). The critical concentration of Mn in plant foliage below which deficiency symptoms can be detected, is 10–20 $\mu\text{g/g}$ on a dry weight basis. The mean Mn concentration of leaves is of the order of 80 $\mu\text{g/g}$ (range 20–500 $\mu\text{g/g}$ – see also Table 1) in “normal” vegetation. Foliar concentrations below 20 $\mu\text{g/g}$ are often an indication of Mn deficiency, whereas at about 1500 $\mu\text{g/g}$, symptoms of toxicity usually start to appear. Despite this, there are several taxa which have a Mn content about 10,000 $\mu\text{g/g}$ (1%) in dried tissue. These are the *hypermanganésophores* of Jaffré (1980). We consider that hyperaccumulation of this element according to our own system should refer to levels of 10,000 $\mu\text{g/g}$ compared with the 1000 $\mu\text{g/g}$ thresholds for Cu, Co and Ni.

Jaffré (1980) has listed seven New Caledonian taxa with Mn concentrations exceeding 1% in leaves. These are shown in Table 5. He also reported (Jaffré, 1977, 1979) up to 3.2% Mn in dried leaves of *Maytenus bureauvianus* growing on basic hypermagnesian soils and 1.2% in *Alyxia rubricaulis* on weathered acid soils. The respective Mn concentrations in the soils were 1.5% and 0.25%. Jaffré (1979) has also reported even higher Mn concentrations in a number of Proteaceae including a massive 4.07% in *Macadamia neurophylla*.

TABLE 5

Hyperaccumulators of manganese (> 10,000 $\mu\text{g/g}$)

Species	Manganese concn (%)	Ref.
<i>Alyxia rubricaulis</i> (R. Br.) Guill. – APOCYNAC.	1.15	1,2
<i>Beaupreopsis paniculata</i> (Brongn. et Gris.) Viot – PROTEAC.	1.20	3
<i>Eugenia clusioides</i> Brongn. et Gris. – MYRTAC.	1.09	3
<i>Garcinia amplexicaulis</i> Vieill. – CLUSIAC.	1.05	3
<i>Macadamia angustifolia</i> Viot – PROTEAC.	1.16	3
<i>M. neurophylla</i> (Guill.) Viot	5.18	4
<i>Maytenus bureauvianus</i> (Loes.) Loes. – CELASTRAC.	3.38	1,3
<i>M. sebertiana</i> (Loes.) Loes.	2.25	3

1 – Jaffré (1977); 2 – Brooks *et al.* (1981b); 3 – Jaffré (1980); 4 – Jaffré (1979).

The presence of such high Mn concentrations in several New Caledonian genera, raises the question as to whether other hyperaccumulators of this element are to be found within them and also raises the possibility of using Mn levels as a guide to species identification.

So far, hyperaccumulators of Mn have only been found in New Caledonia, though Duvigneaud (1959) has reported *Crotalaria florida* Welw. ex Bak. var. *congolensis* (Bak. f.) Wilczek growing on Mn ores in Zaïre with 16.5% Mn in the soil. This plant has not yet been analyzed.

NICKEL

Introduction

Until fairly recently, Ni was thought to play no essential or beneficial role in plant nutrition and metabolism. Whereas there is still no unequivocal evidence for its essentiality, it has been shown that it will stimulate the growth of higher plants. It has also been shown to be the metallic component of the enzyme urease, which catalyses the hydrolysis of urea in the jack bean (*Canavalia ensiformis* L.) (Marschner, 1986). The Ni content of plants is generally in the range 0.1–1 µg/g when growing on "normal" soils (Bollard, 1983; Brooks, 1983). In plants growing on serpentine soils, concentrations may be 100–1000 times greater.

Nearly 40 years ago, Minguzzi and Vergnano (1948) reported the unusually high accumulation of Ni by the serpentine-endemic crucifer *Alyssum bertolonii*. Dried leaves of this plant contained over 1% Ni, a concentration perhaps 100 times greater than had ever been reported before for any flowering plant and a factor of 5000 above the Ni content of plants growing on non-nickeliferous soils. A few years later, Doksoopulo (1961) reported over 1% Ni in leaves of *A. murale*.

In the early 1970s a third hyperaccumulator of Ni, *Hybanthus floribundus* was found in Western Australia by Severne and Brooks (1972) and Cole (1973). This species contained 9800 µg/g (0.98%) Ni in its leaf dry matter. Shortly afterwards, Jaffré and Schmid (1974) discovered an equally high Ni concentration in *Geissois pruinosa*, *Homalium guillainii* and *Hybanthus austrocaledonicus* growing over ultramafic soils in New Caledonia.

The discovery of two species of *Hybanthus* with very high tissue Ni concentrations led Brooks *et al.* (1977a) to investigate the Ni content of as many species as possible from this genus as well as in *Homalium*, which also contained a species exhibiting this very high degree of accumulation of Ni. To carry out this work, these authors used herbarium material for their chemical analysis.

Hyperaccumulators of nickel

Brooks *et al.* (1977a) determined Ni in about 2000 specimens of most of the constituent species of the genera *Hybanthus* and *Homalium* from all parts of the tropical and warm-temperate world corresponding with a sampling density of about one specimen per 2000 km². Analyses had been performed by atomic absorption spectrometry on acid solutions of plant ash obtained by igniting the material at 500°C in a muffle furnace before dissolution. In view of much debate in the past on the respective merits of "dry" and "wet" ashing, we should state that as a result of our own extensive analyses (over 20,000 specimens during the past decade), we have found no significant losses on ignition of any of the elements reviewed here, with the possible exception of lead which we prefer to determine after wet ashing with a nitric/perchloric acid mixture.

The above *Hybanthus-Homalium* survey resulted in the reidentification of all previously known hyperaccumulators of Ni and in the discovery of five additional taxa possessing this character. From the collection localities of these plants, it was possible to pinpoint many of the world's major serpentine occurrences. The principle was obviously applicable to other genera with significant numbers of serpentine-tolerant species. Table 6 lists the 145 hyperaccumulators of Ni which have been discovered so far. The list is not likely to become much larger in the course of time because most of the genera and species likely to contain hyperaccumulators have already been examined. Figure 7 places hyperaccumulators of Ni in their proper perspective in which the Ni content of dried leaves of many hyperaccumulators is equal to, or exceeds, that of the ultramafic rocks on which the soils developed. The Ni content of the ash of these plants greatly exceeds that of the rocks.

The distribution of hyperaccumulators of nickel

Alyssum

The geographical distribution of hyperaccumulators of Ni is shown in Figure 8. It is apparent that all of the Ni plants so far discovered have been found in one or more of seven distinct regions: 1 – New Caledonia, 2 – Western Australia, 3 – southern Europe and Asia Minor, 4 – The Malay Archipelago, 5 – Cuba, 6 – western United States, 7 – Zimbabwe (Great Dyke). It will also be noted that hyperaccumulators are never found over previously-glaciated terrain, presumably because the 10,000 years since the last glaciation does

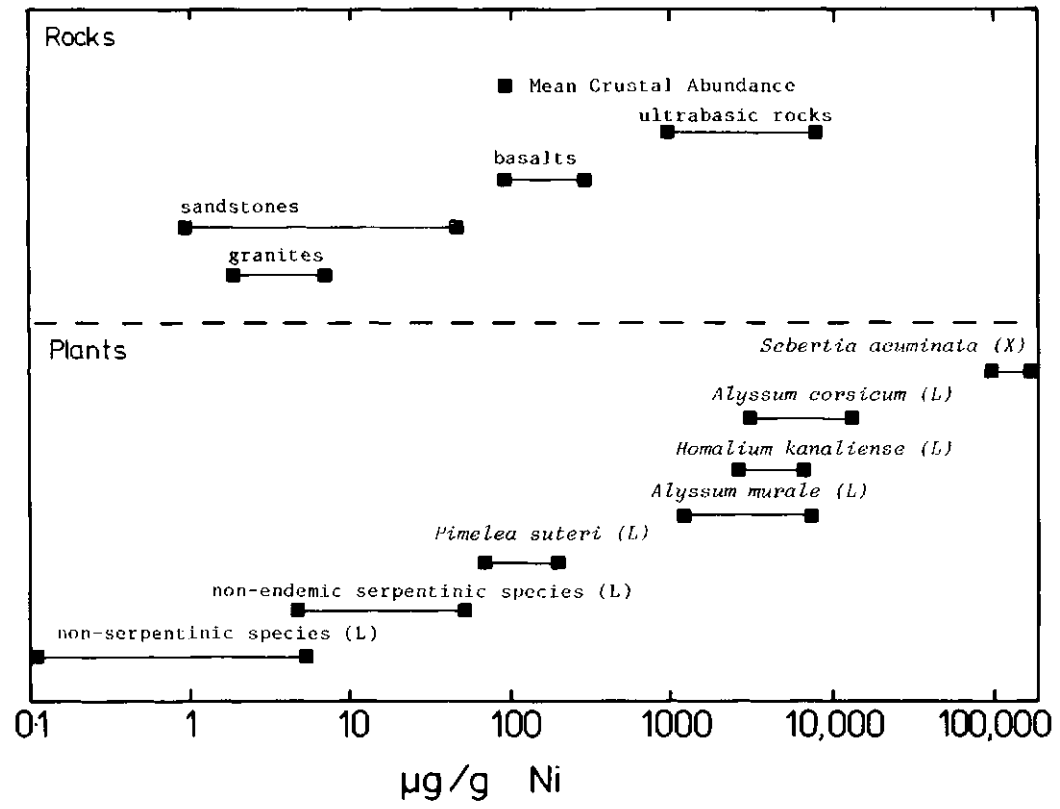


FIGURE 7 Range of values for nickel concentrations in plants and various rock types (L = leaves, X = sap). Source: Brooks (1987).

TABLE 6

Hyperaccumulators of nickel (> 1000 µg/g)

No.	Genus	Species	Location	Max Ni (µg/g)	References
1	<i>Agatea</i> (Violac.)	<i>deplanchei</i> Brongn. et Gris.	New Caledonia	2500	36
2	<i>Alyssum</i>	<i>akamasicum</i> Burt	Cyprus	9090	1
3	(Brassicaceae)	<i>alpestre</i> L.	S. Europe	4480	2, 3
4		<i>anatolicum</i> Hausskn. ex Nyar.	Anatolia	8170	1
5		<i>argenteum</i> All.	Italy	29400	1, 3, 4, 13
6		<i>bertolonii</i> Desv.	Italy	13400	2, 3, 5
7		subsp. <i>scutarinum</i> Nyar.	Italy	10200	13
8		<i>callichroum</i> Boiss. et Bühse	Anatolia	10900	1
9		<i>caricum</i> T.R. Dudley et Huber-Morath	Anatolia	16500	1, 13
10		<i>cassium</i> Boiss.	Anatolia	20000	1
11		<i>chondrogynum</i> Burt	Cyprus	16300	1
12		<i>cicilicum</i> Boiss. et Balansa	Anatolia	13500	1
13		<i>condensatum</i> Boiss. et Hausskn.	Syria, Iraq	4990	1, 13
14		<i>constellatum</i> Boiss.	Anatolia	18100	1
15		<i>corsicum</i> Duby	Anatolia, Corsica	13500	1
16		<i>crenulatum</i> Boiss.	Anatolia	10400	1
17		<i>cypricum</i> Nyar.	Cyprus	23600	1
18		<i>davisianum</i> T.R. Dudley	Anatolia	19600	1
19		<i>discolor</i> T.R. Dudley et Huber-Morath	Anatolia	11700	1
20		<i>dubertrenii</i> Gombault	Anatolia	16500	1
21		<i>eriophyllum</i> Boiss. et Hausskn.	Anatolia	11500	1
22		<i>euboicum</i> Halácsy	Euboea	4550	2, 13
23		<i>fallacinum</i> Hausskn.	Crete	3960	2
24		<i>floribundum</i> Boiss. et Balansa	Anatolia	7700	1
25		<i>giosnanum</i> Nyar.	Anatolia	7390	1
26		<i>heldreichii</i> Hausskn.	Greece	12500	2
27		<i>hubermorathi</i> T.R. Dudley	Anatolia	13500	1
28		<i>janchenii</i> Nyar.	Albania	9610	1
29		<i>lesbiacum</i> (Candargy) Rech. f.	Lesvos	22400	1
30		<i>malactitanum</i> T.R. Dudley	Spain	10000	6
31		<i>markgrafii</i> O.E. Schulz	Albania	13700	1, 13
32		<i>masmenaeum</i> Boiss.	Anatolia	24300	1
33		<i>murale</i> Waldst. et Kit.	Balkans	7080	1, 2, 7

34		<i>obovatum</i> (C.A. Meyer)	Russia	4590	1
35		<i>oxycarpum</i> Boiss. et Balansa	Anatolia	7290	1
36		<i>petarioides</i> Boiss. subsp. <i>virgatiforme</i>	Anatolia	7600	1, 13
37		<i>penjwinensis</i> T.R. Dudley	Iraq	7860	1
38		<i>pinifolium</i> (Nyar.) T.R. Dudley	Anatolia	21100	1, 13
39		<i>pinodasilvae</i> T.R. Dudley	Portugal	9000	2, 8, 9
40		<i>perocarpum</i> T.R. Dudley	Anatolia	22200	1, 13
41		<i>robertianum</i> Bernard ex Gren. et Godr.	Corsica	12500	1
42		<i>samariferum</i> Boiss. et Hausskn.	Samar	18900	1, 13
43		<i>singarensis</i> Boiss. et Hausskn.	Iraq	1280	1
44		<i>smolikanum</i> Nyar.	Greece	6600	2
45		<i>syriacum</i> Nyar.	Syria	10200	1
46		<i>tenium</i> Halácsy	Tinos	3420	2
47		<i>trapeziforme</i> Waldst. et Kit.	Anatolia	11900	1
48		<i>troadii</i> Boiss.	Cyprus	17100	1, 13
49		<i>virgatum</i> Nyar.	Anatolia	6230	1
50	<i>Argophyllum</i> (Escaloniae.)	<i>grunowii</i> Zahlbr.	New Caledonia	1375	36
51		<i>laxum</i> Schlecht.		1900	36
52	<i>Blepharis</i> (Acanthaceae)	<i>acuminata</i> Oberm.	Zimbabwe	2000	10
53	<i>Bormuelleria</i> (Brassicaceae)	<i>baldacci</i> (Degen) Heywood subsp. <i>baldacci</i>	Greece	21300	13
54		subsp. <i>markgrafii</i> (Schulz) T.R. Dudley	Albania	27300	13
55		subsp. <i>rechingeri</i> Greuter	Greece	12000	13
56		<i>glabrescens</i> (Boiss. et Bal.) Cullen et Dudley	Anatolia	19200	13
57		<i>tymphaea</i> (Hausskn.) Hausskn.	Greece	31200	13
58		X <i>petri</i>	Greece	11400	13
59	<i>Buxus</i> (Buxaceae)	<i>flaviramea</i> (Britton) Howard	Cuba	4500	11
60	<i>Cardamine</i> (Brassicaceae)	<i>resedifolia</i> L.	Italy	1050	4, 34
61	<i>Casearia</i> (Flacourtiaceae)	<i>silvana</i> Schlecht.	New Caledonia	1490	12
62	<i>Chrysanthemum</i> (Asteraceae)	<i>alpinum</i> L.	Italy	3200	34

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63	<i>Cleidion</i> (Euphorbiac.)	<i>lasiophyllum</i> Pax et Hoffman	New Caledonia	9900	36
64	<i>Dicoma</i>	<i>niccolifera</i> Wild	Zimbabwe	1000	38
65	<i>Geissois</i> (Cunoniaceae)	<i>hirsuta</i> Brongn. et Gris.	New Caledonia	4000	14
66		<i>intermedia</i> Vicill. ex Pampan		22900	14
67		<i>magnifica</i> Bak. f.		3250	14
68		<i>montana</i> Vicill. ex Brongn. et Gris.		5740	14
69		<i>pruinosa</i> Brongn. et Gris.		34000	14, 15
70		<i>racemosa</i> Labill.	1000	14	
71		<i>trifoliolata</i> Guill.	6250	14	
72	<i>Homalium</i> (Flacourtiac.)	<i>australcaledonicum</i>	New Caledonia	1805	12, 16
73		Seemann <i>deplanchei</i> (Vicill.) Warburg		1850	12, 16
74		<i>francii</i> Guill.		14500	12, 16
75		<i>guillainii</i> Vicill. Briq.		11700	12, 15, 16, 36
76		<i>kanaliense</i> Vicill. Briq.		9420	16-18
77		<i>mathieuanum</i> Vicill. Briq.		1694	12, 16
78		<i>rubrocostatum</i> Sleumer		1157	12, 16
79	<i>Hybanthus</i> (Violaceae)	<i>australcaledonicus</i> Schinz et Guill.	New Caledonia	25500	15, 18, 19, 36
80		<i>caledonicus</i> (Turcz.) Cretz. <i>floribundus</i> (Lindl.) F. Muell.	W. Australia	17500	16, 17, 36
81		subsp. <i>adpressus</i> Bennett		3000	20
82		subsp. <i>curvifolius</i> Bennett		9000	20
83		subsp. <i>floribundus</i>		9800	20-22
84	<i>Lasiochlamys</i> (Flacourtiaceae)	<i>peltata</i> Sleumer	New Caledonia	1000	12
85	<i>Leucocroton</i> (Euphorbiaceae)	<i>flavicans</i> Muell.	Cuba	7700	11
85	<i>Linaria</i> (Scrophularia- ceae)	<i>alpina</i> L.	Italy	1990	34
87	<i>Luzula</i> (Juncaceae)	<i>lutea</i> (All.) DC.	Italy	2050	34
88	<i>Merremia</i> (Asclepiadaceae)	<i>xanthophylla</i> Hall. f.	Zimbabwe	1400	10
89	<i>Minuartia</i> (Caryophyllac.)	<i>laricifolia</i> Schinz et Thell.	Italy	1910	34
90	<i>Myristica</i> (Myristicaceae)	<i>laurifolia</i> Spruce ex DC. var. <i>bifurcata</i>	Obi (Indon.)	1100	23
91	<i>Noccaea</i> (Brassicaceae)	<i>aptera</i> (Velen.) F.K. Meyer	Jugoslavia	13600	24
92		<i>boetica</i> F.K. Meyer	Greece	23400	24

93		<i>firmiensis</i> F.K. Meyer	Greece	16200	24
94		<i>tymphaea</i> F.K. Meyer	Greece	11800	24
95	<i>Oncotheca</i> (Oncothecac.)	<i>balansae</i> Baill.	New Caledonia	2500	36
96	<i>Pancheria</i> (Cunoniaceae)	<i>engleriana</i> Schlecht.	New Caledonia	6300	36
97	<i>Pearsonia</i> (Fabaceae)	<i>metallifera</i> Wild	Zimbabwe	10000	35
98	<i>Peltaria</i> (Brassicaceae)	<i>emarginata</i> Boiss. Hausskn.	Greece	34400	25
99		<i>dumulosa</i> Post	Asia Minor	18900	25
100	<i>Phyllanthus</i>	<i>aeneus</i> Baill.	New Caledonia	2100	26
101	(Euphorbiac.)	<i>balansaeanus</i> Guill.		1820	26
102		<i>cataractarum</i> Muell.		1450	26
103		<i>chrysanthus</i> Baill.		1180	26
104		<i>induratus</i> S. Moore		1480	26
105		<i>kanalensis</i> Baill.		1090	26
106		<i>maytenifolius</i> S. Moore		1420	26
107		<i>ngoyensis</i> Schlecht.		9550	26
108		<i>peltatus</i> Guill.		2830	26
109		<i>serpentinus</i> S. Moore		38100	26
110	<i>Planchonella</i> (Sapotaceae)	<i>oxyedra</i> Dubard	Indonesia	19600	23
111	<i>Psychotria</i> (Rubiaceae)	<i>douarrei</i> (Beauv.) Dän.	New Caledonia	47500	15, 19, 33, 36
112	<i>Rhus</i> (Anacardiaceae)	<i>wildii</i> R. & A. Fernandez	Zimbabwe	1600	10
113	<i>Rinorea</i> (Violaceae)	<i>bengalensis</i> (Wall.) O.K.	S.E. Asia	17500	27
114		<i>javanica</i> (Bl.) O.K.	Borneo	2170	28
115	<i>Saxifraga</i> (Saxifragac.)	<i>aizoon</i> Jacq.	Italy	3840	34
116		<i>exarata</i> Vill.	Italy	2970	34
117	<i>Sebernia</i> (Sapotaceae)	<i>acuminata</i> Pierre ex Baill.	New Caledonia	17750	29, 36
118	<i>Stachys</i> (Lamiaceae)	<i>recta</i> L.	Italy	2600	30
119	<i>Streptanthus</i> (Brassicaceae)	<i>polygaloides</i> Gray	W. USA	14800	31
120	<i>Thlaspi</i>	<i>alpestre</i> L.	Central Europe	4000	24
121	(Brassicaceae)	subsp. <i>virens</i> (Jord.) Hook. f.	France	4100	24
122		<i>alpinum</i> Crantz subsp. <i>sylvium</i> (Gaud.) Clapham	Central Europe	31000	24
123		<i>bulbosum</i> Spruner ex Boiss.	Greece	2000	24
124		<i>epirotum</i> Halácsy	Greece	3000	24
125		<i>goesingense</i> Halácsy	Austria	12000	24
126		<i>graecum</i> Jordan <i>montanum</i> L.	Greece	12000	24
127		var. <i>californicum</i>	W. USA	7940	32
128		var. <i>montanum</i>	W. USA	5530	32
129		var. <i>siskiyouense</i>	W. USA	11200	32
130		<i>ochroleucum</i> Boiss. ex Helder.	Greece	4000	24

131	<i>roundifolium</i> (L.) Gaudin	Central Europe	18300	24
132	var. <i>corymbosum</i> (Gay) Gaudin	Central Europe	2000	24
133	<i>Trichospermum kjellbergii</i> Burret (Tiliaceae)	Celebes	1600	23
134	<i>Trifolium pallescens</i> Schreber (Fabaceae)	Italy	2000	24
135	<i>Xylosma boulindae</i> Sleumer	New Caledonia	1930	12
136	(Flacourtiac.) <i>confusum</i> Guill.		1630	12
137	<i>dothioense</i> Guill.		1780	12
138	<i>kaalense</i> Sleumer		1900	12
139	<i>molestrium</i> Sleumer		1140	12
140	<i>pancheri</i> Guill.		1130	12
141	<i>peltatum</i> (Sleumer) Lescot		1000	36
142	<i>pininsulare</i> Guill.		1280	12
143	<i>serpentinum</i> Sleumer		1490	12
144	<i>tuberculatum</i> Sleumer		1600	12
145	<i>vincentii</i> Guill.		3750	12

1 – Brooks *et al.* (1979); 2 – Brooks and Radford (1978); 3 – Vergnano Gambi *et al.* (1979); 4 – Vergnano Gambi and Gabbrielli (1979); 5 – Minguzzi and Vergnano (1948); 6 – Brooks *et al.* (1981a); 7 – Doksopulo (1961); 8 – Menezes de Sequeira (1969); 9 – Dudley (1986b); 10 – Brooks and Yang (1984); 11 – Berzain Iturralde (1981); 12 – Jaffré *et al.* (1979b); 13 – Reeves *et al.* (1983a); 14 – Jaffré *et al.* (1979a); 15 – Jaffré and Schmid (1974); 16 – Brooks *et al.* (1977a); 17 – Brooks *et al.* (1974); 18 – Lec *et al.* (1977a); 19 – Kelly *et al.* (1975); 20 – Severne (1972); 21 – Severne and Brooks (1972); 22 – Cole (1973); 23 – Wither and Brooks (1977); 24 – Reeves and Brooks (1983b); 25 – Reeves *et al.* (1980); 26 – Kersten *et al.* (1979); 27 – Brooks and Wither (1977); 28 – Brooks *et al.* (1977b); 29 – Jaffré *et al.* (1976); 30 – Lisanti (1952); 31 – Reeves *et al.* (1981); 32 – Reeves *et al.* (1983b); 33 – Baker *et al.* (1985); 34 – Vergnano Gambi and Gabrielli (1981); 35 – Wild (1970); 36 – Jaffré (1980); 37 – Dudley (1986a); 38 – Proctor *et al.* (1980).

not represent a sufficiently long period for the character of hyperaccumulation to evolve.

The genus with the greatest number of hyperaccumulators of Ni (see Table 6) is *Alyssum* (Dudley, 1964) which contains 48 species of Ni plant (Brooks *et al.*, 1979). The distribution of hyperaccumulating *Alyssum* species is unusual. They are confined to ultramafic substrates in southern Europe and Asia Minor stretching from Portugal in the west to the Iraq/Turkey/Iran border areas in the east. Anatolia is the site of their maximum multiplicity and diversity. Figure 9 shows the distribution according to *vilayets* (provinces) of Anatolian *Alyssum* from section Odontarrhena (which contains all the hyperaccumulating species of this genus). The figure classifies the plants into four categories depending on their Ni concentration: > 10000 µg/g, 1000–10000 µg/g, 100–999 µg/g, and < 100 µg/g. The locations of the plants correspond exactly with the areas of ultramafic rocks in Anatolia. There is hardly a single serpentine outcrop in the territory,

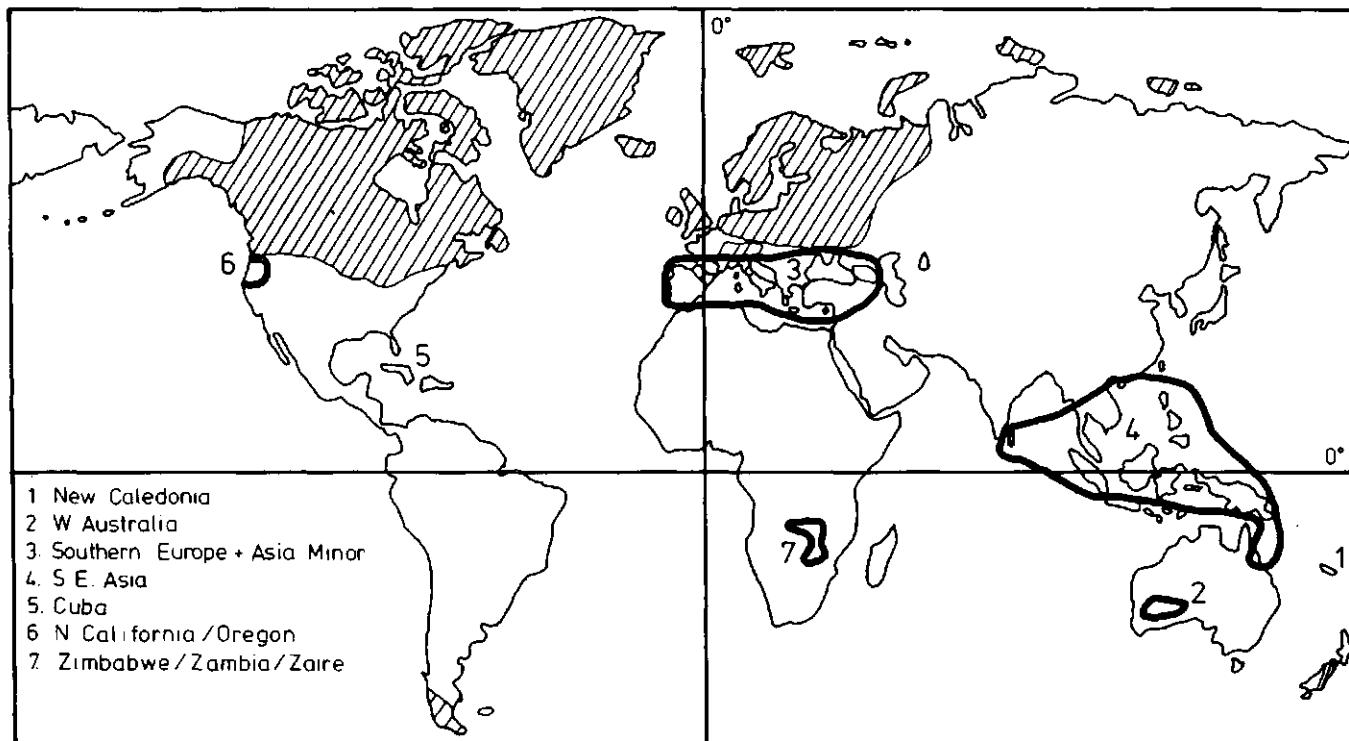


FIGURE 8 Worldwide distribution of hyperaccumulators of nickel. The shaded areas indicate the limits of the ice sheets during the last glaciation. Source: Brooks (1983).

however small, which does not support at least one distinctive hyperaccumulator of Ni of the genus *Alyssum*. Although species of *Alyssum* extend through central Asia and even across the Bering Strait to Alaska and the Yukon, few hyperaccumulators are found east of Turkey, despite the fact that there are plenty of potential serpentine soils in Asia and northwestern North America. Only *A. penjwinensis* (northwestern Iran) and *A. singarense* (northeastern Iraq) have the ability to hyperaccumulate Ni anywhere to the east of Turkey.

It has been proposed by Brooks *et al.* (1979) that the magnitude of the areal distribution of a Ni plant is inversely related to its Ni concentration. For example, of the 18 species of *Alyssum* in section *Odontarrhena* containing over 10000 $\mu\text{g/g}$ Ni in their leaf dry matter, only one, *A. constellatum*, has a wide distribution, and even that is confined to eastern Turkey and northern Iraq. *Alyssum* species containing 1000–5000 $\mu\text{g/g}$ Ni have a much wider dissemination. Examples of this are *A. alpestre* and *A. obovatum*. There is therefore a relationship in *Alyssum* species between diversity, proliferation and endemism on the one hand, and extraordinarily high concentrations of Ni on the other.

Multiplicity and diversity of species recognized by morphological discontinuities, together with a high level of endemism, have often been associated with ancient floras. A third characteristic may now be added: hyperaccumulation of Ni. This cannot however be regarded as a universal characteristic of ancient floras because ultramafic rocks have always been rare and their presence is obviously a prerequisite for extreme Ni uptake.

It would seem that hyperaccumulation of Ni, like endemism, is an evolutionary adaptation typical of many ancient floras. By their extraordinary ability to accumulate massive concentrations of normally phytotoxic Ni, some genera such as *Alyssum* have adjusted genetically to survive in very hostile edaphic conditions. The development of this physiological tolerance may perhaps be a survival or defence strategy against competition from other species. Certainly within section *Odontarrhena* of *Alyssum*, there is no question that the hyperaccumulators of Ni have been enormously successful. This is illustrated by several taxa that occur on serpentine outcrops as extensive or nearly pure populations with an almost total absence of any other competing species. Examples of such "weedy" colonizers and "superadaptors" are *A. murale* which occurs throughout the Balkans and particularly in the Pindus mountains of Greece, and *A. corsicum* and *A. cyprium* throughout western Anatolia.

Thlaspi

Like *Alyssum*, the genus *Thlaspi* contains a number of hyperaccumulators of Ni and tends to occupy the same ecological niches in central and southern

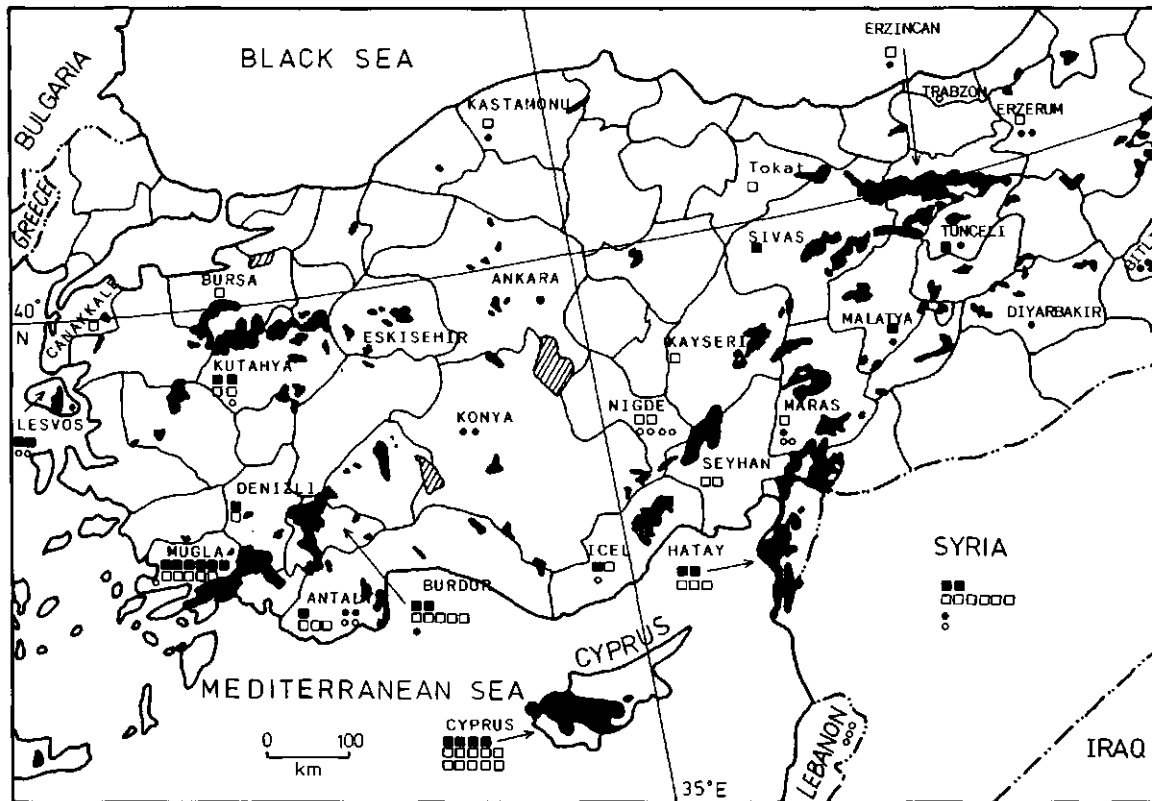


FIGURE 9 Ultramafic rocks (solid black) and the distribution of *Alyssum* hyperaccumulators in Anatolia and the eastern Mediterranean. Solid squares = $>10000 \mu\text{g/g}$, open squares = $1000\text{--}10000 \mu\text{g/g}$, solid circles = $100\text{--}999 \mu\text{g/g}$, open circles = $<100 \mu\text{g/g}$. Source: Brooks *et al.* 1979.

Europe. Unlike *Alyssum* however, *Thlaspi* Ni plants are not represented in Anatolia. Reeves and Brooks (1983b) have shown that several of the southern European taxa of this genus can hyperaccumulate Ni. They found at least 12 species with this character. The centre of maximum diversity and multiplicity appears to be in Greece (Figure 10) where there are seven taxa which may be classified as Ni plants. Other hyperaccumulators of Ni in the genus *Thlaspi* are to be found in the Switzerland/Italy/France alpine border area, in southcentral France, and in Austria and Yugoslavia. American varieties of *T. montanum* have also been found to hyperaccumulate Ni and are discussed below. It will be noted from Table 6 that several Ni plants are classified as *Noccaea* but had been assigned to *Thlaspi* until the genus was revised by Meyer (1973). Reeves and Brooks (1983b) showed that 38 out of 54 taxa studied were also able to hyperaccumulate Zn.

The nickel plants of New Caledonia

An example of the clear relationship between Ni hyperaccumulation, endemism and multiplicity of taxa is shown by the flora of New Caledonia. Nearly 50 hyperaccumulators of Ni from 14 genera and eight different families have been reported from this Pacific island. These New Caledonian Ni plants belong to the following genera and families: *Agatea* (Violaceae), *Argophyllum* (Escalloniaceae), *Casearia* (Flacourtiaceae), *Cleidion* (Euphorbiaceae), *Geissois* (Cunoniaceae), *Homalium* (Flacourtiaceae), *Hybanthus* (Violaceae), *Lasiochlamys* (Flacourtiaceae), *Oncotheca* (Oncothecaceae), *Pancheria* (Cunoniaceae), *Phyllanthus* (Euphorbiaceae), *Psychotria* (Rubiaceae), *Sebertia* (Sapotaceae), and *Xylosma* (Flacourtiaceae). Among the above genera, the largest number of hyperaccumulators is found in *Homalium*. Out of the 16 New Caledonian species recognized by Sleumer (1974), seven possess this unusual accumulatory character. Not only are these seven taxa endemic to New Caledonia, but they are predominantly restricted to ultramafic substrates.

The nickel plants of Southeast Asia

Several hyperaccumulators of Ni have been reported from Southeast Asia. Brooks and Wither (1977) found hyperaccumulation of Ni by *Rinorea bengalensis* which grows throughout the region and extends from Sri Lanka to Queensland. Although not all specimens of the herbarium material analyzed contained $> 1000 \mu\text{g/g}$ Ni, it was clear that such concentrations were attained or exceeded whenever the plant was growing on ultramafic

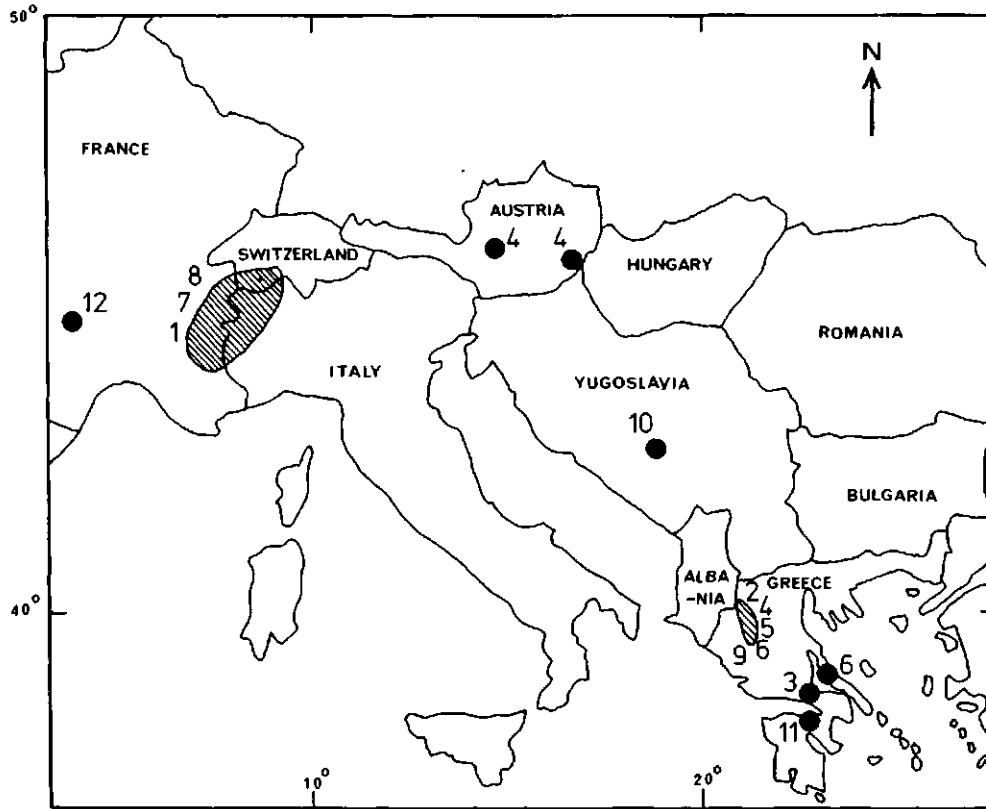


FIGURE 10 Distribution of *Thlaspi* throughout southern and central Europe. 1 - *T. alpestre*, 2 - *T. graecum*, 3 - *T. bulbosum*, 4 - *T. goesingense*, 5 - *T. epirotum*, 6 - *T. ochroleucum*, 7 - *T. alpestre* subsp. *sylvium*, 8 - *T. roundifolium*, 9 - *Nocca tymphaea*, 10 - *N. aptera*, 11 - *N. boetica*, 12 - *N. firmiensis*. Source: Reeves and Brooks (1983b).

rocks. Brooks and Wither used the Ni content of the herbarium specimens to identify previously undiscovered areas of ultramafic rocks from Indonesian New Guinea. In the analogous investigation of herbarium material, Wither and Brooks (1977) identified hyperaccumulation of Ni by *Planchonella oxyedra* and *Trichospermum kjellbergii* and used the information to detect previously-unrecorded ultramafic rocks from the Moluccas.

Nickel plants from other regions

Only brief mention will be made of hyperaccumulators from other parts of the world. Perhaps the most important of the remaining areas is the Great Dyke in Zimbabwe. Wild (1970) reported the presence of the hyperaccumulator *Pearsonia metallifera* and later, Brooks and Yang (1984) found a further three Ni plants (*Blepharis acuminata*, *Merremia xanthophylla* and *Rhus wildii*) from this area.

Apart from two species in Cuba (*Buxus flaviramea* and *Leucocroton flavicans*) reported by Berazain Iturralde (1981) and three from Western Australia (*Hybanthus floribundus* var. *floribundus*, var. *adpressus* and var. *curvifolia*) reported by Severne (1972, 1973), Severne and Brooks (1972) and Cole (1973), the only other hyperaccumulators of Ni found elsewhere in the world are from the western United States. In this region, Reeves *et al.* (1981, 1983b) discovered hyperaccumulation of Ni by *Strepanthus polygaloides* and by three varieties of *Thlaspi montanum* (var. *californicum*, var. *montanum* and var. *siskiyouense*). The evolution of serpentine-tolerant forms of *Thlaspi montanum* is another example of neoendemism. The existence of forms intermediate between *T. montanum* and var. *siskiyouense* has been observed at Waldo, Oregon by Holmgren (1971). A taxon in this intermediate category contained 12850 µg/g (1.28%) Ni.

The phytochemistry of hyperaccumulators of nickel

Inorganic constituents

The first investigation of the chemical composition of hyperaccumulators of Ni was that of Minguizzi and Vergnano (1948) who determined Si, Fe, Mg, Ca and Ni in various organs of *Alyssum bertolonii*, a Ni plant from Tuscany. These workers suggested that the plant is able to compensate for increased Ni levels by increased uptake of Ca.

Morrison (1980) carried out a thorough investigation of the Ni content of various plant parts of *Alyssum heldreichii*. His findings are summarized in

Table 7. It will be seen that the greatest accumulation of Ni occurred in the leaf material and the least in the roots. The lower stem and middle stem zones had lower Ni levels than the upper stems and lower lateral stems. This is of some significance because the latter two plant parts were green whereas the former were brown and woody. It would seem that Ni can be preferentially accumulated in photosynthetic tissues rather than in non-photosynthetic material. Similar observations were made by Minguzzi and Vergnano (1948) and Vergnano Gambi *et al.* (1977) using *A. bertolonii*. The same workers concluded that the degree of accumulation of Ni was related to the length of the growing period rather than to the Ni content (total or exchangeable) of the soil.

Several studies have been carried out to determine the relationship between hyperaccumulation of Ni and the uptake of other trace elements. In a study on Ni and 15 other elements in endemic plants of the Great Dyke in Zimbabwe, Brooks and Yang (1984) found that the Ni concentration in leaf material was correlated positively only with other elements such as Co, Cr and Mn but was not related to any of the plant nutrient elements. A similar study by Yang *et al.* (1985) on serpentine-endemic *Flacourtiaceae* from New Caledonia showed an almost identical pattern: Ni in leaves correlated positively only with Co, Cr and Mn as well as with Na and Zn.

From the above studies, it does not seem that Ni is able to affect the nutrient balance of serpentinophytes to any marked degree in spite of its hyperaccumulation by some taxa.

TABLE 7

The distribution of nickel among various organs of *Alyssum heldreichii*

Organ	% of total wt	Ni ($\mu\text{g/g}$) in dry organ	% of total Ni
Lower roots	11.9	4330	4.5
Upper roots	9.4	9150	7.6
Lower stems	10.0	7190	6.4
Middle stems	5.4	9660	4.6
Upper stems	3.4	16740	5.0
Lower lateral stems	10.0	17060	15.1
Lower lateral leaves	27.2	12150	29.2
Midstem leaves	12.9	11890	13.5
Upper stem leaves	7.6	14070	9.5
Apical buds	2.2	23400	4.6
Seeds		1880	

Source: Morrison (1980).

Organic constituents

Jaffré *et al.* (1976) determined that the sap of *Sebertia acuminata* (sève bleue), a serpentine-endemic tree from New Caledonia, contained an inordinately high Ni concentration (11.2% Ni) in its blue-green latex (hence the French common name for the tree). The 11.2% Ni contained in the fresh latex represents by far the highest Ni concentration recorded for any living material. Lee *et al.* (1977b) isolated and characterized the Ni compound in this material and in leaves of other hyperaccumulators such as *Homalium francii*, *H. guillainii*, *H. kanaliense*, *Hybanthus austrocaledonicus* and *H. caledonicus*. Experiments using high-voltage paper electrophoresis showed that at pH 6.5, an aqueous extract of *Sebertia acuminata* gave peaks corresponding to $\text{Ni}(\text{H}_2\text{O})_6^{2+}$ and a negatively-charged 2:1 citrato-Ni complex. An extract of *Homalium guillainii* showed only the presence of the citrato complex. The identity of these peaks was further confirmed by a combination of gas-liquid chromatography and mass spectrometry. The counter-cation to the Ni citrato complexes was a mixture of $\text{Ni}(\text{H}_2\text{O})_6^{2+}$ and hydrated Ca^{2+} and Mg^{2+} ions in the case of the *Homalium* and *Hybanthus* plants but was only the aquo complex of Ni in the latex of *S. acuminata*.

The relationship between Ni and citric acid was further investigated by Lee *et al.* (1978) who found both constituents in mature leaves of 15 New Caledonian hyperaccumulators and in two *Alyssum* species as well as in the Zimbabwean Ni plant *Pearsonia metallifera*. There was a close association between the two variables. The same workers also found traces of malic and malonic acids in the extracts, though these were minimal in the latex of *S. acuminata*.

Further work on the composition of Ni complexes was performed by Kersten *et al.* (1980) using both gel and ion-exchange chromatography, as well as high performance liquid chromatography (HPLC), and a combination of gas-liquid chromatography and mass spectrometry. In their work on *Psychotria douarrei*, these workers showed that the Ni was present mainly as a negatively-charged malate complex balanced by the cationic Ni aquo complex. In contrast, the hyperaccumulator *Phyllanthus serpentinus* had its Ni bound as 42% citrate and 40% malate.

Nickel complexes in *Alyssum bertolonii* have been isolated by Pelosi *et al.* (1974) using gel chromatography to separate soluble Ni complexes. They deduced that the Ni was bound mainly to an organic acid. Later, Pelosi *et al.* (1976) purified a Ni complex from the same species, again using gel chromatography. The purified product was examined by a mixture of gas-liquid chromatography and mass spectrometry and was found to contain a mixture of malic and malonic acids. The association of Ni with these acids in *A. bertolonii* and in *A. pintoasilvae* was further investigated by Pancaro *et*

al. (1978a, b). These workers used as a control, specimens of *A. bertolonii* grown in ordinary non-serpentine garden soil. They found that Ni in leaves of plants grown on serpentine soil was mainly associated with malic and malonic acids present in an approximately 1:1 mole ratio (ca. 200 $\mu\text{mol/g}$ dry mass). Control samples of *A. bertolonii* obtained from plants grown on ordinary garden soil and therefore low in Ni ($< 40 \mu\text{g/g}$), contained malic and malonic acid concentrations an order of magnitude lower. High levels of malic acid (120 $\mu\text{mol/g}$) related to high Ni concentrations (166 $\mu\text{mol/g}$) were found in the leaves of *A. pintodasilvae*, though in this case the level of malonic acid was very low. Experiments on purified extracts from *A. bertolonii* confirmed the involvement of the organic acids in the Ni metabolism of the leaf tissues which contained 1400 $\mu\text{g/mol/g}$ of malonic acid and 800 $\mu\text{g/mol/g}$ of malic acid. In the seeds of this plant, Ni was bound mainly to malic acid (300 $\mu\text{mol/g}$).

From the above work, it is possible to draw the conclusion that Ni is bound primarily to malic and malonic acids in *Alyssum* and to citric acid in many other hyperaccumulators such as *Sebertia acuminata*, *Homalium*, and *Hybanthus*. Although there is some evidence for an association between citric acid and Ni in *Alyssum*, it seems that this is subsidiary to that involving the other two organic acids. It should not be assumed that Ni in hyperaccumulators is bound only to citric, malic and malonic acids. Morrison (1980) studied the organic constituents of several *Alyssum* species and found many organic constituents other than these three organic acids. Many of these were derivatives of one or more of these acids such as the trimethyl esters of citric and homocitric acid. The latter parent acid has also been identified in the Zimbabwean *Pearsonia metallifera* by Stockley (1980).

Phytochemical studies on hyperaccumulators of Ni such as *Hybanthus floribundus* have also been performed by Farago and Mahmoud (1984), and Farago *et al.* (1980). These studies showed that Ni in leaves and to some extent in woody parts is associated with pectic carbohydrates.

The phytochemistry of Zimbabwean serpentine-tolerant plants has also been investigated by Ernst (1972). He studied *Indigofera setiflora* Bak. and *Dicoma niccolifera* from the Great Dyke area. The latter species contains up to 700 $\mu\text{g/g}$ Ni in dried leaves and does not quite qualify for hyperaccumulator status. It has however been included for the sake of completeness. The same is true of *I. setiflora* (415 $\mu\text{g/g}$ Ni).

Ernst found 73 $\mu\text{g/ml}$ of Ni in the cell sap of *D. niccolifera* and also carried out sequential extraction of root material from this species and in leaves of *I. setiflora*. The proportion of Ni extractable with water from leaves of both species closely followed the Ni content of the cell sap. It appeared therefore that much of the water-soluble fraction was located within the leaf vacuole system. As reported by Ernst (1972), about 75% of the Ni in leaves of

I. setiflora was relatively tightly bound to the structural plant material and could only be removed by solvents with a high exchange capacity such as sodium chloride and citric acid. Ernst concluded that an appreciable proportion of the Ni in the residue was bound to the cell walls. He suggested that fixation at these sites was a mechanism whereby the Ni could be detoxified by storage and could be removed from the plant at leaf fall. When Ni levels in the substrate were high, these storage sites became saturated and the Ni burden in the cells was characterized by an increased water-soluble fraction.

Perennial organs of *I. setiflora* such as roots present an entirely different problem compared with leaves. In the latter case, leaf fall can lead to removal of toxic metals, whereas in roots the same mechanism cannot be operative. In roots the capacity to tolerate heavy metals depends on the capacity to render the metals either soluble or insoluble and to retain the insoluble fraction while permitting the soluble fraction to translocate to the leaves for subsequent removal at leaf fall. The data of Ernst (1972) showed that for roots of *Dicoma niccolifera* (Table 8), residual Ni is much more abundant in the root cortex than in the woody material. In general the older roots are predominant sites for the inactivation of heavy metals accumulated by these plants. The Ni in the wood was characterized by a high percentage of water-soluble and easily-exchangeable forms.

The phytochemistry of the Zimbabwean hyperaccumulator *Pearsonia metallifera* was investigated by Stockley (1980) using a sequential extraction system (Bowen *et al.*, 1962 as modified by Lee, 1977). Over 75% of the total

TABLE 8

Solubility of heavy metals ($\mu\text{g/g}$ of original dry material) in extracts of root cortex and root xylem of *Dicoma niccolifera* and *Indigofera setiflora* from Zimbabwe

Element	Plant	Tissue	a	b	c	d	e	f	g
Chromium	<i>Dicoma</i>	Cortex	-	-	4.6	4.7	9.2	91.0	109
		Xylem	-	-	10.8	2.7	2.7	14.0	30
Copper	<i>Indigofera</i>	Cortex	7.5	5.5	4.7	11.0	26.0	36.0	91
		Xylem	0.8	1.9	1.8	3.8	1.7	2.0	12
Nickel	<i>Dicoma</i>	Cortex	-	108.0	32.0	108.0	99.0	164.0	510
		Xylem	70.1	51.0	32.0	48.0	32.0	6.4	238
Zinc	<i>Indigofera</i>	Cortex	14.0	8.0	42.0	52.0	19.0	55.0	190
		Xylem	2.3	6.1	7.8	4.6	1.5	3.1	25

a - butanol; b - water; c - sodium chloride solution; d - citric acid solution; e - hydrochloric acid; f - residue; g - total.

After Ernst (1972).

Ni was extractable with water. The aqueous fractions were subjected to gel chromatography to separate a green crystalline material which contained most of the Ni. The extract was methylated and passed through a gas-liquid chromatographic column coupled to a mass spectrometer. Two peaks were obtained of which one was the trimethyl ester of citric acid. The other appeared to be the trimethyl ester of 3-hydroxy-3-carboxylhexanedioic acid. Stockley (1980) also found malonic acid in the crude extract of the plant material and proposed that this causes the tricarboxylic acid cycle (Krebs Cycle) to be inhibited at the step involving conversion of succinate to fumarate. Because of this, the conversion of oxaloacetate to citrate is controlled by the amount of malate produced from back-to-back condensation of acetate units, and by the small residual conversion of succinate to fumarate. The phytochemistry of *Pearsonia metallifera* is obviously much more complicated than that of other hyperaccumulators such as *Sebertia acuminata*.

A process of sequential extraction was also used by Brooks *et al.* (1981) to study Ni in *Alyssum serpyllifolium* Desf. and its close relatives *A. pintodasilvae* and *A. malacitanum*. They found that more than half of the Ni was soluble in water and dilute acid showing that it was present as polar complexes. They also found an association between Ni and citric, malic and malonic acids (cf. Pelosi *et al.*, 1974, 1976).

General discussion

Although the 145 hyperaccumulators of Ni in Table 6 are distributed among 6 superorders, 17 orders, 22 families and 38 genera (Table 9), it is remarkable that 117 of the taxa are in superorder Dilleniidae (using the system of Cronquist, 1981). Moreover, 95 of these are found in the two families Brassicaceae and Flacourtiaceae. The concentration of Ni plants in such a small number of families presupposes some sort of evolutionary factor perhaps linked to families with a ready ability to produce organic acids capable of complexing with Ni. In contrast with the above findings, hyperaccumulators of Co and Cu (Brooks *et al.*, 1980) are found predominantly in the Asteridae, a superorder poorly represented in Ni plants. Hyperaccumulation of a particular element by plants is clearly a very selective event.

A great deal of research still remains to be done on hyperaccumulators of Ni. It is suggested that fruitful avenues of research on these interesting plants should be centred on mechanisms of Ni uptake. Such work may well lead to progress in colonizing serpentine areas with suitable species and cultivars of crop plants and help to raise the standard of living of many Third

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TABLE 9

Taxonomic classification of hyperaccumulators of nickel (using system of Cronquist, 1981)

Class	Subclass	Order	Family	Genus	No.	
Magnoliopsida	Magnoliidac	Magnoliales	Myristicaceac	<i>Myristica</i>	1	
	Caryophyllidac	Caryophyllales	Caryophyllaceac	<i>Minuartia</i>	1	
	Dilleniidac	Theales	Oncothecaceac	<i>Oncotheca</i>	1	
		Malvales	Tiliaceac	<i>Trichospermum</i>	1	
		Violales	Flacourtiaceac	<i>Casearia</i>	1	
				<i>Homalium</i>	7	
				<i>Lasiochlamys</i>	1	
				<i>Xylosma</i>	11	
			Violaceac	<i>Agatea</i>	1	
				<i>Hybanthus</i>	5	
				<i>Rinorea</i>	2	
			Capparales	Brassicaceac	<i>Alyssum</i>	48
					<i>Bornmuellera</i>	6
					<i>Cardamine</i>	1
					<i>Noccaea</i>	4
					<i>Peltaria</i>	2
					<i>Streptanthus</i>	1
					<i>Thlaspi</i>	13
			Ebenales	Sapotaceac	<i>Planchonella</i>	1
					<i>Sebertia</i>	1
		Rosidac	Rosales	Cunoniaceac	<i>Geissois</i>	7
					<i>Pancheria</i>	1
				Grossulariaceac	<i>Argophyllum</i>	2
				Saxifragaceac	<i>Saxifraga</i>	2
			Fabales	Fabaceac	<i>Pearsonia</i>	1
					<i>Trifolium</i>	1
			Euphorbiales	Buxaceac	<i>Buxus</i>	1
				Euphorbiaceac	<i>Cleidion</i>	1
					<i>Leucocroton</i>	1
					<i>Phyllanthus</i>	10
		Sapindales	Anacardiaceac	<i>Rhus</i>	1	
	Asteridac	Gentianales	Asclepiadaceac	<i>Merremia</i>	1	
		Lamiales	Lamiaceac	<i>Stachys</i>	1	
		Scrophulariales	Scrophulariaceac	<i>Linaria</i>	1	
			Acanthaceac	<i>Blepharis</i>	1	
		Rubiales	Rubiaceac	<i>Psychotria</i>	1	
		Asterales	Asteraceac	<i>Chrysanthemum</i>	1	
				<i>Dicoma</i>	1	
Liliopsida	Commelinidac	Juncales	Juncaceac	<i>Luzula</i>	1	
TOTAL					145	

World countries where so much potentially-arable land is unexploited because of the presence of serpentine and lateritic soils.

ZINC

Zinc has long been known as an essential element in plant nutrition. It acts either as a metal component of enzymes (such as alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural or regulatory cofactor of a large number of enzymes (Marschner, 1986). It is thus important in both carbohydrate metabolism and protein synthesis. Zinc is readily taken up by plants from soil and is translocated in either free ionic form or as simple organic acid complexes to the shoots, where it may accumulate to varying degrees. The mean concentration of Zn in plant leaves is around 100 $\mu\text{g/g}$ (Table 1).

For well over 100 years it has been recognized that certain Zn-rich soils in Western Germany and Eastern Belgium are characterized by the presence of an unusual plant community: the so-called Zn flora or "Galmei" flora. Two of the best known species among this community are *Viola calaminaria* and *Thlaspi calaminare*. A notable feature of the latter is its unusual ability to hyperaccumulate both Ni and Zn (see above). Baumann (1885) first reported Zn concentrations of around 1% in dried leaves of *T. calaminare*. Early observations on hyperaccumulation of Zn by this species were made by Linstow (1924), and later by Ernst (1967).

Since the mean Zn concentration in plant leaves is around 80 $\mu\text{g/g}$ (Brooks *et al.* 1983), values of up to 1000 $\mu\text{g/g}$ in non-mineralized ground are not at all unusual. We conclude on the basis of our experience that a threshold of 10,000 $\mu\text{g/g}$ (1%) in dried plant tissue should be considered as hyperaccumulation of Zn.

Table 10 lists a number of hyperaccumulators of Zn. Since there have been no widespread surveys for this element (unlike the case of Co, Cu and Ni), the list is far from complete. Most of the taxa in Table 10 are from the genus *Thlaspi* following the work of Reeves and Brooks (1983b) who determined Zn in most of the European and American species of this genus.

CONCLUSIONS

Hyperaccumulation of metallic elements as reviewed above, is a phenomenon which is likely to attract continuing and increasing attention from a wide range of plant scientists in such differing disciplines as ecology, biogeography, mineral exploration, evolutionary biology, taxonomy, physiology

TABLE 10

Hyperaccumulators of zinc (> 10,000 µg/g (1%))

Species	Location	Max concn %	Ref.
<i>Cardaminopsis halleri</i> (L.) Hayek - BRASSICAC.	Germany	1.36	1
<i>Haumaniastrum katangense</i> (S. Moore) Duvign. et Plancke - LAMIACEAE	Zaire	1.98	2
<i>Noccaea eburneosa</i> F.K. Meyer - BRASSICACEAE	Switzerland	1.05	3
<i>Thlaspi alpestre</i> L. - BRASSICACEAE	Derbyshire, UK	2.50	3, 4
<i>T. brachypetalum</i> Jordan	France	2.00	3
<i>T. bulbosum</i> Spruner ex Boiss.	Greece	1.05	3
<i>T. calaminare</i> (Lej.) Lej. et Court.	Germany	3.96	3
<i>T. caerulescens</i> J. et C. Presl	Germany, Belg.	2.73	3, 5
<i>T. cepaeifolium</i> (Wulfen) Koch	Italy	2.10	3, 6
<i>T. limosellifolium</i> Reuter	France	1.10	3
<i>T. praecox</i> Wulfen	Bulgaria	2.10	3
<i>T. stenopterum</i> Boiss. et Reuter	Spain	1.60	3
<i>T. tatraense</i> Zapal.	Czechoslovakia	2.70	3
<i>Viola calaminaria</i> Lcj. - VIOLACEAE	Germany	1.00	7

1 - Ernst (1968); 2 - Brooks (unpub.); 3 - Reeves and Brooks (1983b); 4 - Shimwell and Laurie (1972); 5 - Denaeyer-De Smet and Duvigneaud (1974); 6 - Reeves and Brooks (1983a); 7 - Linstow (1924).

and phytochemistry. We have used as examples, studies relating to common elements; these include essential, "beneficial" and non-essential trace elements in biological systems. Our review has revealed the diversity of species possessing extreme powers of accumulation for the elements concerned. Metal hyperaccumulation has evolved in a wide range of apparently unrelated taxa, although as in the case of nickel, some phylogenetic relationships at the family level can be detected. The information available at present is clearly fragmentary even in the case of common elements such as copper and zinc. As further basic work proceeds, it is clear that the lists of hyperaccumulating species will be extended and will no doubt include plants able to accumulate other less common metallic elements such as the platinum group elements.

Metal hyperaccumulating species occur both in the tropics and temperate zones and include herbs, shrubs and trees. The one unifying feature of these disparate taxa and growth forms other than their unique physiological properties, is their restricted field distribution and high degree of endemism. The most fruitful areas in the quest for other hyperaccumulating species will be sites of isolated and ancient natural mineralization.

The potential industrial and commercial exploitation of metal hyper-accumulating plants has not been explored in this review; our aim has been merely to present a background of information against which these possibilities can be considered. We envisage two distinct areas for future applied research into the use of hyperaccumulators for biorecovery. Firstly, there is the possibility of "metal mining" using extreme hyperaccumulators established either naturally or in cultivation on low-grade or otherwise uneconomic metal-enriched soils. Such substrates could include the wastes from the metalliferous mining industry, or soils which have become enriched with metals as a result of the dumping of other industrial wastes. In this context, the nickel hyperaccumulating tree from New Caledonia, *Sebertia acuminata*, could have theoretical potential in the tropics for biorecovery of nickel from mineral resources with too low a nickel content to merit conventional extraction techniques. The tree could be grown as a plantation crop and either harvested and ashed to concentrate nickel or alternatively, its latex (> 11% Ni) could be tapped continuously as in a rubber plantation. This approach will not however be economic in the case of nickel because of the low world price for this metal. If however a hyperaccumulator of any of the noble metals could be discovered, the prospects for biorecovery would be very exciting.

A second possibility for the use of hyperaccumulators in biorecovery lies in the detoxification of metal-contaminated soils by continuous cropping over a period of years. Experiments are already under way in the United States in which a crop of the Zaïrean Cu/Co hyperaccumulator *Haumanias-trum katangense* is being grown and continually cropped over soil contaminated with radioactive cobalt. Schemes of this nature could provide a viable alternative to expensive restoration options where short-term pressure on land use is not intense.

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