

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/284669331>

Metal hyperaccumulator plants: a review of the ecology and physiology of a biological resource for phytoremediation of metal-polluted soils

Article · January 2000

CITATIONS

1,266

READS

8,618

4 authors, including:



Alan J M Baker

The University of Queensland

347 PUBLICATIONS 38,656 CITATIONS

[SEE PROFILE](#)



Roger D. Reeves

None (retired)

231 PUBLICATIONS 21,211 CITATIONS

[SEE PROFILE](#)



J.A.C. Smith

University of Oxford

180 PUBLICATIONS 13,563 CITATIONS

[SEE PROFILE](#)

5 Metal Hyperaccumulator Plants: A Review of the Ecology and Physiology of a Biological Resource for Phytoremediation of Metal-Polluted Soils

*Alan J. M. Baker, S. P. McGrath, Roger D. Reeves,
and J. A. C. Smith*

CONTENTS

Plant Strategies Toward Metals	86
What is Metal Hyperaccumulation?.....	86
Geographical Distribution of Metal Hyperaccumulators.....	88
Hyperaccumulation of Metals by Different Taxa	90
Nickel.....	90
Zinc	91
Cadmium.....	92
Lead.....	92
Copper	93
Cobalt	93
Manganese.....	94
Evidence for Multimetal Accumulation	94
Metal Tolerance and Metal "Requirement" of Hyperaccumulator Plants.....	94
Relationship Between Metal Accumulation and Tolerance	94
Metal Detoxification and Sequestration	95
Oxygen Donor Ligands.....	96
Sulfur Donor Ligands	97
Nitrogen Donor Ligands	98
Metal "Requirement" of Hyperaccumulator Plants	99
Use of Hyperaccumulator Plants in Phytoremediation.....	100
Acknowledgments.....	101
References.....	101

PLANT STRATEGIES TOWARD METALS

All plants take up metals to varying degrees from the substrates in which they are rooted. The concentrations in plant parts depend both on intrinsic (genetic) and extrinsic (environmental) factors and vary greatly for different species and for different metals. Baker (1981) proposed two basic strategies by which higher plants can tolerate the presence of large amounts of metals in their environment: (1) exclusion, whereby transport of metals is restricted, and low, relatively constant metal concentrations are maintained in the shoot over a wide range of soil concentrations; and (2) accumulation, whereby metals are accumulated in nontoxic form in the upper plant parts at both high and low soil concentrations. He suggested that accumulators can be characterized by a leaf:root metal concentration ratio of >1 because of the tendency to translocate metals from root to shoot, whereas in excluders the ratio is <1 . An intermediate response is that of indicator plants, in which shoot metal concentrations reflect those in the soil. Whilst exclusion is more characteristic of species with both metal-tolerant and nontolerant genotypes, accumulators are commonly species restricted to metalliferous soils.

WHAT IS METAL HYPERACCUMULATION?

Peterson (1971) defined metal accumulation in two ways: (1) accumulation of an element within an organism to concentrations greater than those found in the growth medium and (2) possession of greater quantities of an element than is usual for that organism. These definitions create some problems for the interpretation of metal-accumulation patterns in plants. Only in laboratory studies using nutrient solutions is it possible to know the metal concentration of a precisely defined growth medium, while in soil it is necessary to base the discussion on total metal concentration, or one of a variety of measures of "available" metal concentration. For some metal-accumulating plant species, extreme accumulation of a metal is a normal, not an abnormal, feature of those species growing in their natural habitats.

Responses by plant species to exposure to metalliferous soils can range from phytotoxicity to survival by exclusion, with only small elevations of metal concentration (relative to the same species on nonmetalliferous soil) to survival with accumulated metal constituting a significant percentage of the plant dry matter. In the past 2 decades a number of plant species endemic to metalliferous soils have been reported to be capable of accumulating exceptional concentrations of metals such as nickel, zinc, copper, cobalt, and lead in their above-ground parts (Baker and Brooks, 1989). Such concentrations are far in excess of those normally considered to be phytotoxic. Brooks et al. (1977a) used the term *hyperaccumulators* to describe plants with Ni concentrations $>1000 \mu\text{g/g}$ (0.1%) in their dried leaves, which is at least an order of magnitude greater than Ni concentrations present in nonaccumulator plants found on nickeliferous soils. Reeves (1992) further elaborated on this definition, including only those species which accumulated such concentrations when growing in their natural habitats. This criterion was also considered appropriate to specify hyperaccumulation of copper (Brooks et al., 1980) and lead (Reeves and

Brooks, 1983a), while for zinc a threshold of 10,000 $\mu\text{g/g}$ (1.0 %) in dried plant material was suggested (Reeves and Brooks, 1983a) because of greater background concentrations of this metal (Table 5.1). Metal hyperaccumulation by terrestrial vascular plants was reviewed by Baker and Brooks (1989), and nickel hyperaccumulation has been discussed in detail by Reeves (1992).

TABLE 5.1

Numbers of Metal Hyperaccumulator Plants

Metal	Criterion (% in Leaf Dry Matter)	No. of Taxa	No. of Families
Cadmium	> 0.01	1	1
Cobalt	> 0.1	28	11
Copper	> 0.1	37	15
Lead	> 0.1	14	6
Manganese	> 1.0	9	5
Nickel	> 0.1	317	37
Zinc	> 1.0	11	5

Recent studies have further extended the range of species known to be capable of metal hyperaccumulation. This is an extreme evolutionary response to the presence of high metal concentrations in the soil and is not a common characteristic of terrestrial higher plants. Taxonomically, the metal hyperaccumulators identified to date account for less than 0.2% of all angiosperms. Hyperaccumulator species typically maintain high tissue metal concentrations across a wide range of soil metal concentrations, in agreement with the accumulator strategy proposed by Baker (1981). This raises important questions as to the nature of the (presumably) highly efficient metal-uptake mechanism at low soil metal concentrations, and of the processes responsible for transport and sequestration of metal in nontoxic form at higher soil concentrations. Metal concentrations in leaves, and often stems, of hyperaccumulators greatly exceed those of roots and other storage organs (Rascio, 1977; Hajar, 1987; Homer et al., 1991). Thus the roots, which are the perennial organs of many of these plants, are protected from very high metal levels, while there is the possibility of eliminating a considerable amount of accumulated metals through leaf fall (Vergnano Gambi et al., 1982; Schlegel et al., 1991). Many of the known hyperaccumulators are biennial or short-lived perennial herbs, or are shrubs or small trees.

Little is known of the mechanisms of transport of metals in hyperaccumulator plants. In view of the limited analyses of woody tissues available, it is unclear what roles xylem and phloem transport play in hyperaccumulation in leaves. The importance of nonvascular transport through laticifers, for example, is also obscure. The extent to which secondary xylem tissues become a long-term repository for accumulated metals is not known. Indeed, detailed metal budgets for individual plants

are rare in the literature (see Baker et al., 1992). A research priority is therefore to gain basic information on the dynamics of metal movements, and on the sinks and their capacities in woody species capable of metal hyperaccumulation.

GEOGRAPHICAL DISTRIBUTION OF METAL HYPERACCUMULATORS

The high degree of endemism to metal-rich soils shown by hyperaccumulating taxa has been related to their survival. Such species do not compete well with nontolerant species under normal soil conditions, perhaps reflecting the metabolic "cost" of hyperaccumulation, or conceivably an elevated requirement for a particular metal compared with nonaccumulator species (Macnair and Baker, 1994; Macnair et al., Chapter 13). However, on metalliferous outcrops or on anthropogenically metal-contaminated substrata, hyperaccumulators are able to survive the strong selective pressures exerted upon other species and can become dominants, sometimes existing as nearly pure populations. Brooks et al. (1979) have drawn attention to a definite relationship in *Alyssum* species between endemism, species diversity, and proliferation on the one hand, and nickel hyperaccumulation on the other. Such a relationship is also apparent in the serpentine floras of both New Caledonia and Cuba, where an unusually large number of serpentine-endemic hyperaccumulators from several genera can be concentrated in one locality (Brooks et al., 1979; Reeves et al., 1996).

Serpentine (ultramafic) soils are generally infertile, with low NPK levels and a characteristically low Ca:Mg ratio, as well as having potentially phytotoxic concentrations of nickel (and often of chromium and cobalt), all of which have been shown to be important edaphic controlling factors (Yang et al., 1985; Proctor and Nagy, 1992; Robinson et al., 1997). The nickel-accumulating *Alyssum* species seem to be particularly well adapted to the low soil Ca:Mg ratio, still being able to acquire remarkably high Ca concentrations in their leaf tissues (Reeves et al., 1997). Studies with solution cultures have shown that the levels of Ca that are beneficial to the growth of a serpentine race of a nonaccumulator (*Silene italica*) actually depress both growth and nickel uptake by *A. bertolonii* (Gabbrielli et al., 1990).

The success of many metallophytes in overcoming the adverse edaphic conditions is such that they can exist relatively free from competition. Furthermore, the high metal concentrations of the native soils of hyperaccumulators can repress fungal growth and thus protect these plants from fungal diseases, to which they are susceptible when grown on nonmetalliferous soils (Morrison et al., 1979). Defense against fungal pathogens has been suggested as one of the most plausible reasons for the evolution of metal hyperaccumulation (Reeves et al., 1981; Boyd and Martens, 1992), at least for plants of families (such as the Brassicaceae) which are known to be very sensitive to fungal diseases. Experimental data for *Thlaspi caerulescens* and *Alyssum murale* (Hussain, 1994) have confirmed a protective role of zinc and nickel accumulated in the root cortex of seedlings in resistance against infection by the damping-off fungus, *Pythium ultimum*. Boyd and Martens (1992) and Pollard and Baker (1997) also present data to suggest that hyperaccumulated metals in leaves may play a role in defense against insect herbivory.

Two phytogeographical aspects of hyperaccumulator distribution have been noted. Serpentine endemism generally, and the occurrence of nickel hyperaccumulators in particular, are both much more marked in tropical to warm temperate parts of the world, i.e., those regions that have not suffered extensively from the effects of the Pleistocene glaciations (Reeves et al., 1983) (Figure 5.1). Within the tropics there are also marked differences in the incidence of serpentine endemism and of hyperaccumulation, related to the time of continuous availability of the metalliferous soil (Reeves et al., 1996).

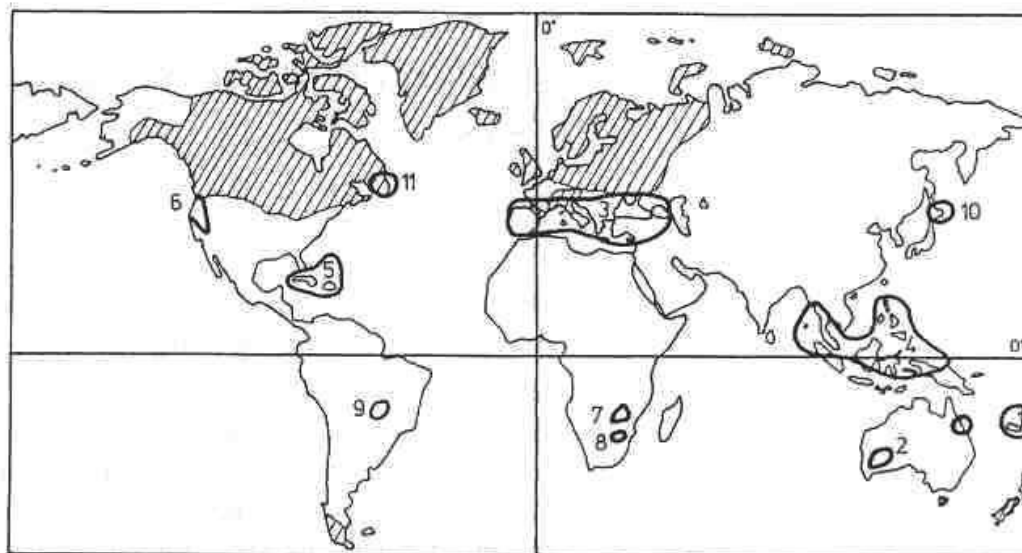


FIGURE 5.1 Global distribution of nickel hyperaccumulation. (Adapted from Brooks, R.R. *Biological Methods for Prospecting for Minerals*. John Wiley & Sons, New York, 1983.) Hatched areas indicate the limits of the ice sheets during the last glaciation. Areas and number of hyperaccumulators: 1. New Caledonia (50), 2. Australia (5), 3. S. Europe/Asia Minor (90), 4. S.E. Asia (11), 5. Cuba (128) and Dominican Republic (1), 6. U.S. (Pacific N.W. and California) (5), 7. Zimbabwe (5), 8. S. Africa (Transvaal) (4), 9. Brazil (Goiás) (11), 10. Japan (Hokkaido) (1), 11. Canada (Newfoundland) (4).

It is debated whether the endemism represented by the restriction of metal-tolerant species to such soils should be regarded as paleo-endemism or neo-endemism (Antonovics et al., 1971; Baker, 1987). In the former, originally widespread populations have become confined to severe environments through competition from other species. Neo-endemics are regarded as having developed from nontolerant precursors which have colonized metalliferous areas by the process of natural selection; in this case, more widely occurring, closely related species are often found in the surrounding area. For taxa now found exclusively on mine spoil, the neo-endemic hypothesis is favored, e.g., the metallophyte *Alyssum wulfenianum* may have the neighboring, related *A. ovirense* as precursor (Reeves and Brooks 1983a), and there are widespread species of *Alyssum*, *Thlaspi*, *Dichapetalum*, and *Brackenridgea*, for example, for which metal-accumulating taxa are recognized at subspecific rank. In genera such as *Alyssum*, *Thlaspi*, *Phyllanthus*, *Buxus*, and *Xylosma*, containing many metal-accumulating and nonaccumulating taxa, there is scope for the study of the geographic distribution and morphology to shed light on evolutionary relationships

(Morrison, 1980; Reeves et al., 1999). Phylogenetic questions may also be resolved using the techniques of molecular systematics, which have revealed, for example, that metal hyperaccumulation within *Thlaspi sensu lato* is restricted to a lineage consisting of *Noccaea* and *Raparia*, but does not occur in *Thlaspi sensu stricto* (Mummenhoff and Koch, 1994; Mummenhoff et al., 1997).

Whichever factors have promoted the evolution and survival of hyperaccumulating taxa, it is clear that their tolerance of stressed metalliferous environments, coupled with their exceptional ability to accumulate large quantities of metals, could be exploited in a practical way for the detoxification of metal-contaminated soils.

HYPERACCUMULATION OF METALS BY DIFFERENT TAXA

NICKEL

With the definitions set out earlier in this chapter, there are many more hyperaccumulators of nickel than of any other metal (Table 5.1). This is partly owing to the relative importance, on a global scale, of soils derived from ultramafic rocks, typically containing 0.1 to 1.0% Ni, but may also be because most effort has been expended on this group. Surface exposures of ultramafic rocks occur as continuous or narrowly separated edaphic "islands" of significant area for evolutionary purposes, especially in countries such as Cuba, Brazil (Goiás), U.S. (Pacific Northwest), Canada (Newfoundland), Italy, Greece and the Balkans, Turkey, Japan, Indonesia, the Philippines, New Caledonia, Australia (Western Australia, Queensland), South Africa (eastern Transvaal) and Zimbabwe.

At least 317 nickel-hyperaccumulating taxa are now known. They fall largely into two groups: (1) within the tropical genera and species of families such as the Violaceae and Flacourtiaceae (Order Violales), and the Buxaceae and Euphorbiaceae (Order Euphorbiales) and (2) among genera of the Brassicaceae (Order Capparales) of the northern temperate zone. In the Brassicaceae, nickel hyperaccumulators show a remarkable concentration in the genus *Alyssum* L., which contains some 48 taxa with 0.1 to 3.0% Ni, confined to Section *Odontarrhena* (Minguzzi and Vergnano, 1948; Brooks and Radford, 1978; Brooks et al., 1979; Vergnano Gambi et al., 1979). The geographical distribution of *Alyssum* hyperaccumulators is strongly correlated with the occurrence of nickel-rich serpentines and other ultramafic rocks of southern Europe, the eastern Mediterranean, and Asia Minor (Brooks et al., 1979); the genus shows a high degree of serpentine endemism. The genus *Thlaspi* L. (Brassicaceae) also contains a significant number of species with the ability to accumulate nickel to levels of 0.1 to 3.0% on a dry matter basis (Reeves and Brooks, 1983b); some of these are also known to be hyperaccumulators of zinc. These species have a similar distribution to *Alyssum* in central and southern Europe (although *T. caerulescens* extends more widely into northern Europe, especially on zinc-rich soils), reaching maximum diversity in Greece.

New Caledonia possesses some of the world's largest areas of ultramafic soils and is noted for its nickel flora (Figure 5.1). About 50 hyperaccumulators of nickel from 14 genera and 8 families have been identified as endemic to this island (Brooks

et al., 1974, 1977a; Jaffré et al., 1976, 1979). Notable genera include *Homalium* Jacq. (Flacourtiaceae) and *Phyllanthus* L. (Euphorbiaceae).

Metal hyperaccumulators have been recognized on the basis of concentrations of metals in leaf dry matter. Little information is available on the accumulation of metals in other organs and tissues, although such analyses are needed to provide a more complete picture of metal-allocation patterns within the plant (Ernst, 1995). The majority of nickel hyperaccumulators from New Caledonia (Baker and Brooks, 1989), Indonesia (Wither and Brooks, 1977), the Philippines (Baker et al., 1992), and Cuba (Reeves et al., 1999) are trees or shrubs. The most remarkable is perhaps the New Caledonian tree *Sebertia acuminata* (Sapotaceae), which can grow to a height of >10 m and which produces a blue-green latex containing nearly 26% nickel on a dry-weight basis (or 11% on a wet-weight basis) (Jaffré et al., 1976). The woody tissues contain about 0.17% Ni compared with about 1.2% in the leaf dry matter. Similar concentrations (about 0.2%) have been reported in the wood of *Psychotria douarrei* (Rubiaceae) and *Hybanthus floribundus* (Violaceae) from New Caledonia and Western Australia, respectively. Baker et al. (1992) have described nickel hyperaccumulators from Palawan (Philippines) including *Phyllanthus* "palawanensis" (Euphorbiaceae), *Dichapetalum gelonioides* ssp. *tuberculatum* (Dichapetalaceae), *Walsura monophylla* (Meliaceae), and *Brackenridgea palustris* ssp. *foxworthyi* (Ochnaceae). The first-mentioned has woody stems that produce a jade-colored sap on cutting, and phloem tissues with >9% Ni (dry-weight basis). Analysis of herbarium specimens of taxa closely related to the Palawan plants has revealed more nickel hyperaccumulators as well as three zinc hyperaccumulators (subspecies of *D. gelonioides*). Leaves of one specimen of *D. gelonioides* ssp. *tuberculatum* from West Sumatra were found to contain 3% Zn even though the soils of the area are not thought to be metalliferous according to site notes on the herbarium sheet (Baker et al., 1992). Analysis of woody tissues has not been possible for these herbarium samples.

Morrey et al. (1992) and Anderson et al. (1997) have reported very high (up to 3.7%) Ni and elevated Cr concentrations in the tall, productive herb *Berkheya coddii* (Asteraceae) from the ultramafics of northeastern Transvaal, South Africa. This (and related) species may have a high phytoremediation potential, in view of its strong hyperaccumulation, relatively high biomass production, and its ability to grow in dense stands on nickeliferous soils. Similar potential is seen in the many hyperaccumulators in *Buxus*, *Leucocroton*, *Phyllanthus*, and *Euphorbia* from Cuba (Reeves et al., 1999).

ZINC

The range of hyperaccumulators of zinc (>1.0% dry weight) is less extensive than for nickel, possibly because of the less frequent occurrence of large exposures of zinc-rich soil and the higher threshold set in defining hyperaccumulation. It appears thus far to be limited to a few genera such as *Thlaspi* and *Cardaminopsis* in the Brassicaceae and several species in other families. Early detailed investigations of the "Galmei" (calamine) flora of western Germany and eastern Belgium by Baumann (1885) and others showed that Zn concentrations in the leaves of *Thlaspi calaminare*

and *Viola calaminaria* can reach 3.5 and 1.0%, respectively. The former species, together with others regarded for many years as part of the *Thlaspi alpestre* complex, is now properly referred to as *T. caerulescens* (Ingrouille and Smirnoff, 1986). Several other *Thlaspi* species from lead/zinc-mineralized soils have been reported with up to 2.0% Zn (Reeves and Brooks, 1983a). The distribution of *T. caerulescens* in Britain and Belgium is strongly linked to lead/zinc mines (Shimwell and Laurie, 1972; Ingrouille and Smirnoff, 1986; Baker and Proctor, 1990; Baker et al., 1994) and other industrially polluted areas such as the vicinity of metal smelters (Denaeyer-De Smet and Duvigneaud, 1974). *Cardaminopsis halleri* (Brassicaceae) has also been reported as a hyperaccumulator of zinc (Ernst, 1968; Macnair et al., Chapter 13).

CADMIUM

Most plants growing over lead/zinc-mineralized soils show elevated cadmium concentrations in the range 10 to 100 $\mu\text{g/g}$. A concentration of $>100 \mu\text{g/g}$ (0.01%) Cd in plant dry matter is exceptional and could be a suitable criterion for the recognition of hyperaccumulation. *Thlaspi caerulescens* has been demonstrated to accumulate Cd (occasionally) in leaf dry matter to 1000 $\mu\text{g/g}$; values above 100 $\mu\text{g/g}$ generally accompany extreme zinc accumulation from mine or smelter waste.

LEAD

Hyperaccumulation of lead is particularly rare (Table 5.1). The low solubility of most lead compounds in circum-neutral media, and the ready precipitation of lead by sulfate and phosphate at the root systems may partly explain this. Despite this, *Thlaspi rotundifolium* ssp. *cepaefolium* from a lead/zinc mining area of Cave del Predil (northern Italy) has been found with lead up to 8200 $\mu\text{g/g}$ of dry weight (Reeves and Brooks, 1983a). *Alyssum wulfenianum* Schlecht. from the same location also contained remarkably high Pb concentrations, reaching 860 $\mu\text{g/g}$ in leaf dry matter. A concentration of up to 2740 $\mu\text{g/g}$ Pb was also reported in *T. caerulescens* colonizing a lead mine district in the Pennines, England (Shimwell and Laurie, 1972). A recent report of lead hyperaccumulation in the grass *Arrhenatherum elatius* (Deram and Petit, 1997) growing on calamine soils in France is of great interest as there have been no convincing reports of the phenomenon in the Poaceae. Barry and Clark (1978) recorded shoot Pb values of 130 to 11,750 $\mu\text{g/g}$ in *Festuca ovina*, and Williams et al. (1977) found a mean shoot Pb value of 13,488 $\mu\text{g/g}$ in pasture species growing on mining waste in the U.K. The rather erratic nature of Pb values found in this kind of environment raises the question of the relative importance of entry via the root system and direct deposition on or into the leaves (e.g., from smelter emissions and wind-blown soil particles). Careful study of this point is still required. Nevertheless, high average Pb concentrations can be achieved by uptake through the root system: Baker et al. (1994) found that seedlings of *T. caerulescens* exposed to a nutrient solution containing 20 $\mu\text{g/ml}$ Pb contained 4500 to 7000 $\mu\text{g/g}$ Pb after 21 days (roots 29,000 $\mu\text{g/g}$; shoots 280 $\mu\text{g/g}$). Even higher whole-plant concentrations can also be achieved by exposing nontolerant species to high levels of soluble

lead for short periods, even though the plants may not survive the treatment. For example, addition of synthetic chelates such as EDTA (ethylenediaminetetraacetic acid) to the root medium can dramatically increase lead uptake and accumulation in *Zea mays* and *Pisum sativum*, giving shoot concentrations in excess of 1% of dry weight (Huang and Cunningham, 1996; Huang et al., 1997). Similarly, shoot concentrations of 1.5% lead by dry weight have been observed in *Brassica juncea* growing in contaminated soil amended with EDTA (Blaylock et al., 1997). These results are especially notable because they indicate that, in the presence of appropriate ligands, it is possible for lead to remain in soluble form for transport through the root system and translocation to the above-ground parts of the plant.

COPPER

Most of the hyperaccumulators of copper discovered to date are confined to Shaba Province in Zaïre (now the Democratic Republic of Congo) and the Copper Belt of northwest Zambia (Brooks et al., 1980). The copper mineralization is dispersed over some 22,000 km² and has provided a large area for the evolution of a multiplicity of species with the ability to accumulate and/or tolerate high Cu levels in the soil in virtual isolation, as the metal acts as a barrier to competing species. Elsewhere, copper-mineralized areas that have been subjected to detailed vegetation study cover only a few hectares, which probably explains why the known hyperaccumulators of copper are concentrated here. Some 24 hyperaccumulators of copper, several of which also hyperaccumulate cobalt, have been reported from Africa, occurring principally in advanced families such as Lamiaceae and Scrophulariaceae (Brooks and Malaisse, 1985). Particularly elevated Cu concentrations (up to 13,700 µg/g) have been found in *Aeollanthus biformifolius* De Wild. (Lamiaceae), a dwarf perennial herb which also hyperaccumulates cobalt, endemic to the southern part of the Shaban Copper Arc (Malaisse et al., 1978). Beyond Africa, there have been a few reports of high Cu concentrations in species such as *Minuartia verna* (Caryophyllaceae; 1070 µg/g) in Germany (Ernst, 1974) and *Millotia myosotidifolia* (Asteraceae; 2400 µg/g) in South Australia (Blissett, 1966). Confirmation of these reports would be helpful to ascertain whether any of these plant analyses may have been affected by dust containing nearly pure secondary copper minerals, a problem that must be kept in mind when biogeochemical work is done over copper-rich rocks and soils.

COBALT

Baker and Brooks (1989) list 26 hyperaccumulators (>1000 µg/g) of cobalt, 9 of which also hyperaccumulate copper. All are species of the vegetation of the Shaban Copper Arc, Zaïre. The majority are low-growing herbs in a range of families including Lamiaceae, Scrophulariaceae, Asteraceae, and Fabaceae. The highest concentration reported to date is 10,200 µg/g Co in *Haumaniastrum robertii* (Robyns) Duvign. et Plancke (Lamiaceae). Abnormal uptake of cobalt, occasionally exceeding 100 µg/g, has been recorded for the North American tree *Nyssa sylvatica* (Nyssaceae) from soils not known to be metalliferous (Brooks et al., 1977b).

MANGANESE

Eight hyperaccumulators of manganese were listed by Baker and Brooks in 1989. The short list remains the basis of the summary figures presented in Table 5.1 and the criterion for recognition of hyperaccumulator status is still realistically set at 10,000 $\mu\text{g/g}$ (1.0%) Mn in aerial plant dry matter. To date there has been little interest in pursuing the possible exploitation of these plants from five unrelated families in phytoremediation, as manganese has not been recognized as a metal of major environmental or economic concern.

EVIDENCE FOR MULTIMETAL ACCUMULATION

Just as cotolerances have been shown to exist, it is evident from the above that the ability to accumulate unusually high levels of more than one heavy metal (coaccumulation) is present in several species, e.g., *Aeollanthus biformifolius* (Cu, Co), *Thlaspi rotundifolium* ssp. *cepaefolium* (Pb, Zn), *T. caerulescens* (Zn, Cd, Pb). Homer et al. (1991) studied a number of *Alyssum* species in pot trials and demonstrated the ability of nickel hyperaccumulators to behave in the same way toward cobalt when this was made available, and suggested a similar mechanism of uptake for the two metals. Similarly, co-accumulation of Ni, Zn, Co, and Mn by the hyperaccumulator *Thlaspi goesingense* was attributed by Reeves and Baker (1984) to the existence of a nonspecific system of metal detoxification. Baker et al. (1994) reported that *T. caerulescens* seedlings, exposed for 21 days to nutrient solutions containing any of a variety of heavy metals at low concentration (0.5 to 25 $\mu\text{g/ml}$), were capable of taking up most of them to high total plant concentrations (>1000 $\mu\text{g/g}$). They distinguished between metals that were readily translocated to the shoot (e.g., Zn, Cd, Ni, Co, Mn) and those that became predominantly fixed at high concentrations in the root system (e.g., Pb, Fe, Al, Cu). The latter group could be found with concentrations of $>10,000$ $\mu\text{g/g}$ in the root, but generally <600 $\mu\text{g/g}$ in the shoot.

In contrast, McGrath et al. (Chapter 6) showed that differences in zinc and cadmium uptake occurred between two populations of *T. caerulescens* grown in the same soil. Both hyperaccumulated zinc but only one accumulated cadmium, suggesting strong specificity in the uptake mechanisms for the two metals.

METAL TOLERANCE AND METAL "REQUIREMENT" OF HYPERACCUMULATOR PLANTS

RELATIONSHIP BETWEEN METAL ACCUMULATION AND TOLERANCE

The precise relationship between metal accumulation and tolerance has not yet been resolved. Because hyperaccumulator plants are usually restricted in their natural distribution to metalliferous soils, it is possible to regard the hyperaccumulation trait as one form of tolerance mechanism. Some workers have proposed that there is no correlation between metal hyperaccumulation and tolerance, i.e., that they may be independent characteristics (Ingrouille and Smirnof, 1986; Baker and Walker, 1990;

Baker et al., 1994; Lloyd-Thomas, 1995), while others state that accumulators possess a high degree of tolerance (Mathys, 1977; Reeves and Brooks, 1983b) or "hypertolerance" (Chaney et al., 1997). Screening experiments for zinc, lead, cadmium, and nickel accumulation by *T. caerulescens* populations using solution culture (Lloyd-Thomas, 1995; Chaney et al., Chapter 7) have revealed significant differences between calamine, serpentine, and alpine (nonmetallophyte) populations but no direct relationship to the metal status of the parent soils. The relationship between tolerance and accumulation may be difficult to test directly because (1) it is difficult to find populations which lack either tolerance or hyperaccumulation and (2) "tolerance" can be defined in a number of different ways. Mutagenesis of hyperaccumulator species could be one way to test for a causal relationship between hyperaccumulation and tolerance, but a mechanistic understanding of the origins of metal tolerance at the physiological and biochemical levels is also required.

The response of different plant species to particular metals varies markedly. Species from the same geographical location have evolved contrasting tolerance mechanisms toward the same metals, e.g., *Silene italica* L. and *Alyssum bertolonii* Desv. are both found on serpentine soils and are, respectively, an excluder and accumulator of nickel (Gabbrielli et al., 1990). Indeed, in most habitats with metal-rich soils it is found that hyperaccumulator species are in a minority, suggesting that metal exclusion is the more widespread strategy taxonomically.

Given that true hyperaccumulator plants are normally restricted to metalliferous soils, it is axiomatic that they must be metal tolerant, at least in the general sense of being able to grow and complete their life cycle on metal-rich soils. But it is difficult from these ecological associations alone to make inferences about tolerance mechanisms. Formally, it will not be possible to claim a causal relationship between hyperaccumulation and tolerance until the genetic basis of metal hyperaccumulation is understood. For example, the close association between nickel hyperaccumulators and ultramafic soils does not prove that this results from the nickel tolerance mechanism per se. These plants might simply be particularly well adapted to some other peculiarity of the soils, such as their nutrient imbalance (high Mg:Ca ratio, etc.). By the same token, species may be excluded from ultramafic soils because they cannot tolerate such nutrient imbalances, rather than because of their sensitivity to nickel. Nevertheless, in the largest single genus of hyperaccumulator plants, *Alyssum*, laboratory experiments have shown that the nickel-hyperaccumulator species are much more nickel tolerant than the nonaccumulator species (Homer et al., 1991; Krämer et al., 1996). Further experiments in this direction are clearly needed with other genera, but the hypothesis of an underlying, mechanistic association between metal hyperaccumulation and tolerance still seems perfectly plausible.

METAL DETOXIFICATION AND SEQUESTRATION

In hyperaccumulator plants, metal concentrations can exceed 1% of plant dry weight without any adverse effect on growth. This implies the existence of mechanisms for metal detoxification within the plant, most likely involving chelation of the metal cation by ligands and/or sequestration of metals away from sites of metabolism in the cytoplasm, notably into the vacuole or cell wall. Other possible adaptive

responses include activation of alternative metabolic pathways less sensitive to metal ions, modification of enzyme structure, or alteration of membrane permeability by structural reorganization or compositional changes (Ernst et al., 1992). As yet, there is no evidence that these latter mechanisms of cellular metal tolerance are important in hyperaccumulator plants, but tolerance might be the result of a number of these processes acting collectively (Baker, 1987).

Recent analytical studies have begun to provide information on the localization of metals within hyperaccumulator plants. In the roots of plants from a population of *T. caerulescens* able to accumulate zinc and cadmium, x-ray microanalysis demonstrated that zinc accumulated principally in the vacuoles of epidermal and subepidermal cells, with smaller amounts stored in cell walls, while the apoplast was the main storage site of cadmium, with some cadmium being stored in vacuoles (Vázquez et al., 1992). Perhaps of even greater interest in hyperaccumulator plants are the sites of metal localization in the shoot, since bulk metal concentrations typically exceed those in the root, and the metal has to be translocated through the entire plant in soluble form to arrive at its final destination. In shoots of *T. caerulescens*, the highest zinc concentrations again appeared to be in the vacuoles of epidermal and subepidermal cells (Vázquez et al., 1994). This type of tissue distribution of metals may prove to be characteristic of hyperaccumulator plants, as nickel appears to be preferentially accumulated in epidermal cells of the nickel hyperaccumulators *Hybanthus floribundus* (Severne, 1974), *Senecio coronatus* (Mesjasz-Przybylowicz et al., 1994), and *Berkheya zeyheri* ssp. *rehmanii* var. *rogersiana* (Mesjasz-Przybylowicz et al., 1995).

Because it is clear that most of the metal taken up by hyperaccumulator plants must be chelated if acute metal toxicity is to be avoided, much interest has focused on identifying the intracellular ligands involved. We shall consider these in terms of the characteristic electron donor centers in the different classes of ligand.

Oxygen Donor Ligands

The feasibility of the involvement of organic acids in metal detoxification has long been recognized. Carboxylic acid anions are abundant in the cells of terrestrial plants and form complexes with divalent and trivalent metal ions of reasonably high stability. In particular, carboxylates such as malate, aconitate, malonate, oxalate, tartrate, citrate, and isocitrate are commonly the major charge-balancing anions present in the cell vacuoles of photosynthetic tissues, and they have been shown to chelate high concentrations of calcium and magnesium (Smith et al., 1996).

Analysis of metal-rich extracts from several nickel hyperaccumulators from New Caledonia has shown that nickel is predominantly bound to citrate (Lee et al., 1977, 1978) and the amount of citrate produced is strongly correlated with the accumulated nickel. Citrate also forms a 1:1 complex with aluminium in leaves of the aluminium hyperaccumulator *Hydrangea macrophylla* (Ma et al., 1997). In some other nickel-hyperaccumulating species from New Caledonia (Kersten et al., 1980), the Philippines (Homer et al., 1991), South Africa (Anderson et al., 1997), and particularly in the Mediterranean *Alyssum* species such as *A. bertolonii* (Pelosi et al., 1976), *A. serpyllifolium* subspecies (Brooks et al., 1981), and *A. troodii* (Homer et al., 1995),

malate has consistently appeared as the major ligand associated with the nickel in aqueous extracts of leaf material. Malate also extracts with nickel from the root cortex, xylem fluid, and leaf sap of *A. bertolonii* (Gabbrielli et al., 1997).

Mathys (1977) found that malic acid levels were correlated with the degree of resistance to zinc, with far greater concentrations present in zinc-tolerant ecotypes. Similarly, the synthesis of mustard oils by *T. caerulescens* and of oxalate by *Silene vulgaris* was significantly greater in the Zn-resistant populations. On the basis of these findings, Mathys proposed a mechanism for zinc tolerance, whereby Zn^{2+} ions are bound by malate upon uptake into the cytoplasm, and the malate then serves as a carrier to transport the Zn^{2+} ions to the vacuole. The Zn^{2+} ions are then complexed by a terminal acceptor, possibly a sulfur-containing mustard oil in *T. caerulescens* and oxalate in *S. cucubalus*, and the released malate is able to return to the cytoplasm where it is ready to transport more Zn^{2+} ions. However, the molar ratio of S:Zn in *T. caerulescens* has been shown to be only 0.4 (Shen et al., 1997), and most of the total S would in fact be associated with protein in the plant. This makes it unlikely that mustard oils are responsible for increased tolerance and hyperaccumulation of zinc in this species.

Although the carboxylates are undoubtedly quantitatively important ligands for metal chelation in the vacuole, they tend to be present constitutively in the shoots of terrestrial plants and do not seem to account for either the metal specificity or species specificity of hyperaccumulation (Woolhouse, 1983; Ernst et al., 1992; Harmens et al., 1994). Even though the concentrations of ligands such as malate and citrate can be higher in metal-treated plants (e.g., Mathys, 1977; Thurman and Rankin, 1982; Godbold et al., 1984), this may be a general metabolic response that serves to maintain charge balance by organic acid synthesis (Osmond, 1976), rather than a specific one that accounts for tolerance toward a particular metal. This was the conclusion reached by Thurman and Rankin (1982) based on their experiments with *Deschampsia cespitosa*, which failed to show reduced metal tolerance when grown under conditions that decreased the amounts of citrate produced. Also, Qureshi et al. (1986) did not observe any correlation between malate accumulation and zinc tolerance in cell cultures of tolerant and nontolerant ecotypes of *Anthoxanthum odoratum*. The quantitative importance of the vacuole as a final repository for metals and the effectiveness of the vacuolar carboxylates in metal chelation are not in doubt, as the detailed studies of Krotz et al. (1989) and Wang et al. (1991, 1992) emphasize. Current evidence, however, suggests that metal-induced organic acid synthesis is unlikely to account for the specificity of the hyperaccumulation phenomenon.

Sulfur Donor Ligands

The sulfur donor atom in organic ligands is a considerably better electron donor than oxygen and leads to complexes of very high stability with first-row transition metals (Fraústo da Silva and Williams, 1991). Two classes of sulfur-containing ligand have been identified in plants that may play an important role in metal tolerance — metallothioneins and phytochelatins. The metallothioneins are the small cysteine-rich proteins known as metallothioneins, which are subdivided into three classes

(Robinson et al., 1993). In fungi and mammals, metallothioneins are known to be able to function in metal detoxification (Hamer, 1986; Tohayama et al., 1995), but their role in plants has been controversial (Robinson et al., 1993; Zenk, 1996). It is only recently that the protein products have been identified and purified (Murphy et al., 1997), but there is convincing evidence that expression of the *MT2a* gene correlates with copper tolerance in different ecotypes of *Arabidopsis thaliana* (Murphy and Taiz, 1995). Since the metallothioneins are evidently encoded in plants by multi-gene families (Zhou and Goldsbrough, 1995; Murphy et al., 1997), further studies of their expression patterns should provide valuable information on the involvement of specific genes in metal homeostasis.

Phytochelatin is a low-molecular-weight, cysteine-rich peptide (Rauser, 1995; Zenk, 1996) now designated class III metallothionein, as they can be regarded generically as nontranslationally synthesized metal-thiolate polypeptides (Robinson et al., 1993). They are synthesized by representatives of the whole plant kingdom upon exposure to heavy metals (Grill et al., 1987), and they are especially produced by plants growing in metal-enriched ecosystems (Grill et al., 1988). Phytochelatin is believed to be functionally analogous to the metallothioneins produced by animals and fungi (Tomsett and Thurman, 1988; Robinson et al., 1993) and consequently to be involved in cellular homeostasis of metal ions. They have the ability to bind a wide range of metals and it has been suggested by some researchers (Jackson et al., 1987; Salt et al., 1989) that phytochelatin is directly involved in heavy metal tolerance. However, there is considerable evidence to contradict this view. Metal induction of phytochelatin has been observed in both metal-resistant and metal-sensitive plants (Schultz and Hutchinson, 1988; Verkleij et al., 1991; Harmens et al., 1993). Furthermore, buthionine sulfoximine (BSO), an inhibitor of phytochelatin synthesis, has been shown not to decrease zinc tolerance (Reese and Wagner, 1987; Davies et al., 1991), while sulfur deficiency was seen to have no effect on copper tolerance of *Deschampsia cespitosa* (Schultz and Hutchinson, 1988). It is therefore questionable what exact role phytochelatin plays in cellular metal-tolerance mechanisms.

There is some evidence for the involvement of phytochelatin in cadmium tolerance, but it has been argued that they are principally involved in detoxification rather than being the basis of genetically determined cadmium tolerance (Verkleij et al., 1991; de Knecht et al., 1994). This interpretation is supported by the phenotype of the *cad1* mutants of *Arabidopsis*, which have reduced phytochelatin levels and are cadmium-sensitive, but which are only slightly affected in their tolerance toward copper and zinc (Howden et al., 1995). Even if phytochelatin deficiency leads to hypersensitivity toward a particular metal, it does not follow that unusual accumulation of this metal will be related to phytochelatin production. More work is needed to establish whether this class of ligand plays any role in metal homeostasis or tolerance in the true hyperaccumulator plants.

Nitrogen Donor Ligands

Organic ligands containing nitrogen donor centers also form complexes of high stability with first-row transition metals, but with thermodynamic stability constants

intermediate between those of the oxygen and sulfur donor ligands (Fraústo da Silva and Williams, 1991). In a prescient publication, Still and Williams (1980) postulated that the selectivity of the metal uptake and translocation process in nickel hyperaccumulators could be explained by chelation involving a ligand containing two nitrogen and one oxygen donor centers. This class of ligand has received relatively little attention, though Homer et al. (1995) observed in a study of bulk leaf extracts of four nickel hyperaccumulators that total amino acids changed only slightly over a wide range of nickel contents.

Earlier studies with crop plants indicated that amino acids, together with carboxylic acids, could play a significant role in metal chelation in the xylem (White et al., 1981a,b; Cataldo et al., 1988). Because root-to-shoot transfer of metals must be extremely effective in hyperaccumulator plants, Krämer et al. (1996) investigated the relationship between metal transport and xylem sap composition in species of *Alyssum* exposed to different nickel concentrations. This revealed a striking linear correlation between the concentration of nickel and histidine in the xylem of three hyperaccumulator species (*A. lesbiacum*, *A. murale*, and *A. bertolonii*). Over a range of nontoxic nickel concentrations, histidine was the only amino acid or carboxylic acid to show a significant response to metal treatment. Chemically, this is a striking vindication of the proposal of Still and Williams (1980), and in fact, at the prevailing xylem pH values, the effective stability constant for the nickel-histidine complex is higher than for any other amino acid or organic acid. Furthermore, it was shown that supplying exogenous histidine to the nonaccumulator *A. montanum*, either as a foliar spray or in the root medium, considerably increased the nickel tolerance of this species and greatly increased nickel flux through the xylem (Krämer et al., 1996). These results with *Alyssum* suggest that histidine is involved both in the mechanism of nickel tolerance and in the effective translocation of nickel to the shoot that characterizes these hyperaccumulator plants. Because nickel is complexed mainly with carboxylic acids in the shoot, the primary role of histidine may be to chelate the nickel taken up by the root cells and then to facilitate export of nickel to the shoot in the xylem. In this way, there may be a direct mechanistic link between the nickel hyperaccumulation and tolerance mechanisms, at least in the genus *Alyssum*. Further work will be required to ascertain whether metal-induced production of histidine (or perhaps other nitrogen-containing ligands) is found in other groups of nickel hyperaccumulators, or indeed in plants that hyperaccumulate other metals.

METAL "REQUIREMENT" OF HYPERACCUMULATOR PLANTS

When transplanted to soil containing only traces of the metals that they usually accumulate, hyperaccumulators display normal growth (Morrison et al., 1979; Reeves and Baker, 1984). This could indicate that there is no direct physiological requirement for elevated tissue metal concentrations and thus for their geographical distribution on mineralized or metal-enriched soils. However, when grown for long periods on some potting composts, *T. caerulescens* can become zinc deficient (McGrath, 1998). In solution culture they do show a greater requirement for some metals than nonaccumulators (Li et al., 1995; Chaney et al., 1997; Shen et al., 1997). For example, the critical solution concentrations which resulted in zinc deficiency

in *T. caerulescens* grown in buffered media ($<10 \mu\text{M}$) were at least five orders of magnitude higher than those for most plant species (McGrath, 1998). Even very zinc-deficient plants grown with an external concentration of 0.1 nM (10^{-10} M) Zn, contained 300 to $500 \mu\text{g/g}$ Zn in the shoots, which is approximately 20 times the tissue concentration for the threshold of deficiency found in other plants. The reason for this high requirement may be related to the operation of strong constitutive mechanisms for sequestration and tolerance of zinc (Lloyd-Thomas, 1995). Similar stimulatory responses have been shown in the nickel hyperaccumulator *A. lesbiacum*, which when grown in solution culture (modified 0.1-strength Hoagland solution) had a growth optimum of $30 \mu\text{M}$ Ni (Krämer et al., 1996).

USE OF HYPERACCUMULATOR PLANTS IN PHYTOREMEDIATION

It has been recognized for more than 16 years that plant uptake by hyperaccumulator plants could be exploited as a biological cleanup technique for various polluted rooting media including soils, composted materials, effluents, and drainage waters. The possibility of their use in phytomining has also been demonstrated (Nicks and Chambers, 1995). However, before phytoextraction of soils is possible on a large scale, a number of important issues must be addressed. First, metal hyperaccumulator plants are relatively rare, often occurring in remote areas geographically and being of very restricted distribution in areas often threatened by devastation from mining activities. Population sizes can be extremely small. There is thus an urgent need to collect these materials, bring them into cultivation and establish a germplasm facility for large-scale production for future research and development and trials work. Secondly, the potential exploitation of metal uptake into plant biomass as a means of soil decontamination is clearly limited by plant productivity. Many of the European hyperaccumulator plants are of small biomass, although considerable natural variation exists within populations (Lloyd-Thomas, 1995; Chaney et al., 1997). In view of their infertile native habitats (and likely nutritional adaptations to these edaphic conditions), it is surprising that plants such as *Thlaspi* and *Alyssum* spp. are responsive to nutrient additions to the soil, and thus their growth potential can be enhanced by soil fertilization. Selection trials are needed to identify the fastest growing (largest potential biomass and greatest nutrient responses) and most strongly metal-accumulating genotypes. However, such a combination may not be possible and a tradeoff between extreme hyperaccumulation and lower biomass (or vice versa) may be acceptable. Selection could also identify the individuals with the deepest and most extensive and efficient root systems, and those of greatest resistance to disease. Breeding experiments are required to incorporate all these desirable properties into one plant. Future work could involve genetic engineering to further improve metal uptake characteristics, if the genes for metal accumulation can be identified and manipulated. The possibility would then exist to transfer genes for metal hyperaccumulation into a very productive (but inedible), sterile host plant. Excellent opportunities also exist through protoplast fusion techniques.

Further geobotanical exploration of metalliferous environments is needed, both in the tropics and temperate zones, to identify additional plants with potential for phytoremediation of contaminated soils. Our knowledge of hyperaccumulator plants is still fragmentary, and it is clear from the recent major discoveries in Cuba, Australia, and Brazil within the past decade that further hyperaccumulating taxa await discovery and/or recognition. The list presented in Table 5.1 will certainly prove to be incomplete, as new hyperaccumulator plants are regularly being reported. Increasing systematic effort in screening plant materials for these characteristics will most certainly reveal new hyperaccumulator plants and new potentials both for phytoremediation and biorecovery of metals.

ACKNOWLEDGMENTS

The authors wish to record their particular gratitude to the following organizations for their past and ongoing support for research into hyperaccumulator plants: U.K. Natural Environment Research Council (NERC), Royal Society of London, British Ecological Society, U.S. National Geographic Society, U.S. Army Corps of Engineers, and E.I. Du Pont de Nemours & Co, Inc.

REFERENCES

- Anderson, T.R., A.W. Howes, K. Slatter, and M.F. Dutton. Studies on the nickel hyperaccumulator, *Berkheya coddii*, in *Écologie des Milieux sur Roches Ultramafiques et sur Sols Metallifères*. Jaffré, T., Reeves, R.D., and Becquer, T., Eds., Documents Scientifiques et Techniques, ORSTOM, Nouméa, New Caledonia, 261-266, 1997.
- Antonovics, J., A.D. Bradshaw, and R.G. Turner. Heavy metal tolerance in plants. *Adv. Ecol. Res.* 7, 1-85, 1971.
- Baker, A.J.M. Accumulators and excluders — strategies in the response of plants to heavy metals. *J. Plant Nutr.* 3, 643-654, 1981.
- Baker, A.J.M. Metal tolerance. *New Phytol.* 106, 93-111, 1987.
- Baker A.J.M. and R.R. Brooks. Terrestrial higher plants which hyperaccumulate metallic elements — a review of their distribution, ecology and phytochemistry. *Biorecovery* 1, 81-126, 1989.
- Baker, A.J.M. and J. Proctor. The influence of cadmium, copper, lead and zinc on the distribution and evolution of metallophytes in the British Isles. *Plant Sys. Evol.* 173, 91-108, 1990.
- Baker, A.J.M. and P.L. Walker. Ecophysiology of metal uptake by tolerant plants, in *Heavy Metal Tolerance in Plants: Evolutionary Aspects*, Shaw, A.J., Ed., CRC Press, Boca Raton, FL, 155-177, 1990.
- Baker, A.J.M., J. Proctor, M.M.J. van Balgooy, and R.D. Reeves. Hyperaccumulation of nickel by the flora of the ultramafics of Palawan, Republic of the Philippines, in *The Vegetation of Ultramafic (Serpentine) Soils*, Baker, A.J.M., Proctor, J., and Reeves, R.D., Eds., Intercept Ltd, Andover, Hants., U.K., 291-304, 1992.
- Baker, A.J.M., R.D. Reeves, and A.S.M. Hajar. Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl (Brassicaceae). *New Phytol.* 127, 61-68, 1994.

- Barry, S.A.S. and S.C. Clark. Problems of interpreting the relationship between the amounts of lead and zinc in plants and soil on metalliferous wastes. *New Phytol.* 81, 773-783, 1978.
- Baumann, A. Das Verhalten von Zinksalzen gegen Pflanzen und im Boden. *Landwirtsch. Vers. Stn.* 31, 1-53, 1885.
- Blaylock, M.J., D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley, and I. Raskin. Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. *Environ. Sci. Technol.* 31, 860-865, 1997.
- Blissett, A.H. Copper-tolerant plants from the Upakaringa Copper Mine, Williamstown. *Quarterly Geological Notes, Geological Survey of South Australia*, 18, 1-4, 1966.
- Boyd, R.S. and S.N. Martens. The raison d'être for metal hyperaccumulation in plants, in *The Vegetation of Ultramafic (Serpentine) Soils*, Baker, A.J.M., Proctor, J., and Reeves, R.D., Eds., Intercept Ltd, Andover, Hants., U.K., 279-289, 1992.
- Brooks, R.R. *Biological Methods for Prospecting for Minerals*. John Wiley & Sons, New York, 1983.
- Brooks, R.R., J. Lee, and T. Jaffré. Some New Zealand and New Caledonian plant accumulators of nickel. *J. Ecol.* 62, 493-499, 1974.
- Brooks, R.R., J. Lee, R.D. Reeves, and T. Jaffré. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. *J. Geochem. Explor.* 7, 49-77, 1977a.
- Brooks, R.R., J.A. McCleave, and E.K. Schofield. Cobalt and nickel uptake by the Nyssaceae. *Taxon* 26, 197-201, 1977b.
- Brooks, R.R. and F. Malaisse. *The Heavy Metal-Tolerant Flora of Southcentral Africa*. Balkema, Rotterdam, 1985.
- Brooks, R.R., R.S. Morrison, R.D. Reeves, T.R. Dudley, and Y. Akman. Hyperaccumulation of nickel by *Alyssum* Linnaeus (Cruciferae). *Proc. R. Soc. London, Ser. B.* 203, 387-403, 1979.
- Brooks, R.R. and C.C. Radford. Nickel accumulation by European species of the genus *Alyssum*. *Proc. R. Soc. London, Ser. B.* 200, 217-224, 1978.
- Brooks, R.R., R.D. Reeves, R.S. Morrison, and F. Malaisse. Hyperaccumulation of copper and cobalt — a review. *Bull. Soc. Bot. Belg.* 113, 166-172, 1980.
- Brooks, R.R., S. Shaw, and A. Asensi Marfil. The chemical form and physiological function of nickel in some Iberian *Alyssum* species. *Physiol. Plant.* 51, 167-170, 1981.
- Cataldo, D.A., K.M. McFadden, T.R. Garland, and R.E. Wildung. Organic constituents and complexation of nickel (II), iron (III), cadmium (II), and plutonium (IV) in soybean xylem exudates. *Plant Physiol.* 86, 734-739, 1988.
- Chaney, R.L., M. Malik, Y.M. Li, S.L. Brown, J.S. Angle, and A.J.M. Baker. Phytoremediation of soil metals. *Curr. Opin. Biotechnol.* 8, 279-284, 1997.
- Davies, K.L., M.S. Davies, and D. Francis. The influence of an inhibitor of phytochelatin synthesis on root growth and root meristematic activity in *Festuca rubra* L. in response to zinc. *New Phytol.* 118, 565-570, 1991.
- de Knecht, J.A., M. van Dillen, P.L.M. Koevoets, H. Schat, J.A.C. Verkleij, and W.H.O. Ernst. Phytochelatins in cadmium-sensitive and cadmium-tolerant *Silene vulgaris*. Chain length distribution and sulfide incorporation. *Plant Physiol.* 104, 255-261, 1994.
- Denaeyer-De Smet, S. and P. Duvigneaud. Accumulation de métaux lourds dans divers écosystèmes terrestres pollués par les retombées d'origine industrielle. *Bull. Soc. Bot. Belg.* 107, 147-156, 1974.
- Deram, A. and D. Petit. Ecology of bioaccumulation in *Arrhenatherum elatius* L. (Poaceae) populations — applications of phytoremediation of zinc, lead and cadmium contaminated soils. *J. Exp. Bot.* 48, Suppl., 98, 1997.

- Ernst, W.H.O. Das Violetum calaminariae westfalicum, eine Schwermetallpflanzengesellschaft bei Blankenrode in Westfalen. *Mitt. Florist. Soz. Arbeitsgem.* 13, 263-268, 1968.
- Ernst, W.H.O. *Schwermetallvegetation der Erde*. Fischer, Stuttgart, 1974.
- Ernst, W.H.O. Sampling of plant material for chemical analysis. *Sci. Total Environ.* 176, 15-24, 1995.
- Ernst, W.H.O., J.A.C. Verkleij, and H. Schat. Metal tolerance in plants. *Acta Bot. Neerl.* 41, 229-248, 1992.
- Fraústo da Silva, J.J.R. and R.J.P. Williams. *The Biological Chemistry of the Elements: The Inorganic Chemistry of Life*. Clarendon Press, Oxford, 1991.
- Gabbrielli, R., T. Pandolfini, O. Vergnano, and M.R. Palandri. Comparison of two serpentine species with different nickel tolerance strategies. *Plant Soil* 122, 271-277, 1990.
- Gabbrielli, R., P. Gremigni, L. Bonzi Morassi, T. Pandolfini, and P. Medeghini. Some aspects of Ni tolerance in *Alyssum bertolonii* Desv.: strategies of metal distribution and accumulation, in *Écologie des Milieux sur Roches Ultramafiques et sur Sols Metallifères*. Jaffré, T., Reeves, R.D., and Becquer, T., Eds., Documents Scientifiques et Techniques. ORSTOM, Nouméa, New Caledonia, 225-227, 1997.
- Godbold, D.L., W.J. Horst, J.C. Collins, D.A. Thurman, and H. Marschner. Accumulation of zinc and organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitosa*. *J. Plant Physiol.* 116, 59-69, 1984.
- Grill, E., E.-L. Winnacker, and M.H. Zenk. Phytochelatins, a class of heavy metal binding peptides from plants, are functionally analogous to metallothioneins. *Proc. Nat. Acad. Sci. USA.* 84, 439-443, 1987.
- Grill, E., E.-L. Winnacker, and M.H. Zenk. Occurrence of heavy metal binding phytochelatins in plants growing in a mining refuse area. *Experientia* 44, 539-540, 1988.
- Hajar, A.S.M. Comparative ecology of *Minuartia verna* (L.) Hiern and *Thlaspi alpestre* L. in the Southern Pennines, with special reference to heavy metal tolerance. Ph.D. thesis, University of Sheffield, U.K., 1987.
- Hamer, D.A. Metallothionein. *Annu. Rev. Biochem.* 55, 913-951, 1986.
- Harmens, H., P.R. Hartog, W.M. ten Bookum, and J.A.C. Verkleij. Increased zinc tolerance in *Silene vulgaris* (Moench) Garcke is not due to increased production of phytochelatins. *Plant Physiol.* 103, 1305-1309, 1993.
- Harmens, H., P.L.M. Koevoets, J.A.C. Verkleij, and W.H.O. Ernst. The role of low molecular weight organic acids in the mechanism of increased zinc tolerance in *Silene vulgaris* (Moench) Garcke. *New Phytol.* 126, 615-621, 1994.
- Homer, F.A., R.S. Morrison, R.R. Brooks, J. Clemens, and R.D. Reeves. Comparative studies of nickel, cobalt and copper uptake by some nickel hyperaccumulators of the genus *Alyssum*. *Plant Soil.* 138, 195-205, 1991.
- Homer, F.A., R.D. Reeves, and R.R. Brooks. The possible involvement of aminoacids in nickel chelation in some nickel-accumulating plants. *Curr. Top. Phytochem.* 14, 31-37, 1995.
- Howden, R., P.B. Goldsbrough, C.R. Andersen, and C.S. Cobbett. Cadmium-sensitive, cad1 mutants of *Arabidopsis thaliana* are phytochelatin deficient. *Plant Physiol.* 107, 1059-1066, 1995.
- Huang, J.W. and S.D. Cunningham. Lead phytoextraction — species variation in lead uptake and translocation. *New Phytol.* 134, 75-84, 1996.
- Huang, J.W.W., J.J. Chen, W.R. Berti, and S.D. Cunningham. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.* 31, 800-805, 1997.

- Hussain, A. Susceptibility of metal-accumulating plants to damping off by *Pythium ultimum*. Master's thesis, University of Sheffield, U.K., 1994.
- Ingrouille, M.J. and N. Smirnoff. *Thlaspi caerulescens* J. & C. Presl (*T. alpestre* L.) in Britain. *New Phytol.* 102, 219-233, 1986.
- Jackson, P.J., C.J. Unkefer, J.A. Doolen, K. Watt, and N.J. Robinson. Poly(γ -glutamylcysteiny)glycine: Its role in cadmium resistance in plant cells. *Proc. Nat. Acad. Sci. USA.* 84, 6619-6623, 1987.
- Jaffré, T., R.R. Brooks, J. Lee, and R.D. Reeves. *Sebertia acuminata*: a hyperaccumulator of nickel from New Caledonia. *Science* 193, 579-580, 1976.
- Jaffré, T., W.J. Kersten, R.R. Brooks, and R.D. Reeves. Nickel uptake by the Flacourtiaceae of New Caledonia. *Proc. R. Soc. London, Ser. B.* 205, 385-394, 1979.
- Kersten, W.J., R.R. Brooks, R.D. Reeves, and T. Jaffré. Nature of nickel complexes in *Psychotria douarrei* and other nickel-accumulating plants. *Phytochemistry.* 19, 1963-1965, 1980.
- Krämer, U., J.D. Cotter-Howells, J.M. Charnock, A.J.M. Baker, and J.A.C. Smith. Free histidine as a metal chelator in plants that accumulate nickel. *Nature* 379, 635-639, 1996.
- Krotz, R.M., B.P. Evangelou, and G.J. Wagner. Relationships between Cd, Zn, Cd-peptide and organic acid in tobacco suspension cells. *Plant Physiol.* 91, 780-787, 1989.
- Lee, J., R.D. Reeves, R.R. Brooks, and T. Jaffré. Isolation and identification of a citrate-complex of nickel from nickel-accumulating plants. *Phytochemistry.* 16, 1503-1505, 1977.
- Lee, J., R.D. Reeves, R.R. Brooks, and T. Jaffré. The relation between nickel and citric acid in some nickel-accumulating plants. *Phytochemistry* 17, 1033-1035, 1978.
- Li, Y.M., R.L. Chaney, F.A. Homer, J.S. Angle, and A.J.M. Baker. *Thlaspi caerulescens* requires over 10^3 higher Zn^{2+} activity than other plant species. *Agron. Abstr.* 1995, 261, 1995.
- Lloyd-Thomas, D.H. Heavy metal hyperaccumulation by *Thlaspi caerulescens* J. & C. Presl. Ph.D. thesis, University of Sheffield, U.K., 1995.
- Ma, J.F., S. Hiradate, K. Nomoto, T. Iwashita, and H. Matsumoto. Internal detoxification of Al in *Hydrangea*. Identification of Al form in the leaves. *Plant Physiol.* 113, 1033-1039, 1997.
- Macnair, M.R. and A.J.M. Baker. Metal-tolerant plants: an evolutionary perspective, in *Plants and the Chemical Elements*, Farago, M.E., Ed., VCH, Weinheim, 67-85, 1994.
- Malaisse, F., J. Grégoire, R.R. Brooks, R.S. Morrison, and R.D. Reeves. *Aeolanthus biformifolius* De Wild.: a hyperaccumulator of copper from Zaire. *Science.* 199, 887-888, 1978.
- Mathys, W. The role of malate, oxalate and mustard oil glucosides in the evolution of zinc-resistance in herbage plants. *Physiol. Plant.* 40, 130-136, 1977.
- McGrath, S.P. Phytoextraction for soil remediation, in *Plants that Hyperaccumulate Heavy Metals*, Brooks, R.R., Ed., CAB International, Wallingford, 1998, 261.
- Mesjasz-Przybylowicz, J., K. Balkwill, W.J. Przybylowicz, and H.J. Annegarn. Proton microprobe and x-ray fluorescence investigations in serpentine flora from South Africa. *Nucl. Instrum. Methods Physics Res.* B89, 208-212, 1994.
- Mesjasz-Przybylowicz, J., K. Balkwill, W.J. Przybylowicz, H.J. Annegarn, and D.B.K. Rama. Similarity in nickel distribution in leaf tissue of two distantly related hyperaccumulating species, in *The Biodiversity of African Plants*, van der Maesen, L.J.G., van der Burgt, X.M., and van Medenbach de Roy, J.M., Eds., Kluwer Academic Publishers, Dordrecht, 331-335, 1995.

- Minguzzi, C. and O. Vergnano. Il contenuto di nichel nelle ceneri di *Alyssum bertolonii* Desv. *Atti Soc. Toscana Sci. Nat., Ser. A.* 55, 49-77, 1948.
- Morrey, D.R., K. Balkwill, M.-J. Balkwill, and S. Williamson. A review of some studies of the serpentine flora of Southern Africa, in *The Vegetation of Ultramafic (Serpentine) Soils*, Baker, A.J.M., Proctor, J., and Reeves, R.D., Eds., Intercept Ltd, Andover, Hants., U.K., 147-157, 1992.
- Morrison, R.S. Aspects of the accumulation of cobalt, copper and nickel by plants. Ph.D. thesis, Massey University, NZ, 1980.
- Morrison, R.S., R.R. Brooks, R.D. Reeves, and F. Malaisse. Copper and cobalt uptake by metallophytes from Zaire. *Plant Soil* 53, 535-539, 1979.
- Mullins, M., K. Hardwick, and D.A. Thurman. Heavy metal location by analytical electron microscopy in conventionally fixed and freeze-substituted roots of metal tolerant and non-tolerant ecotypes. *Proc. Int. Conf. Heavy Metals Environ.* Athens, CEP Consultants, Edinburgh, 43-45, 1985.
- Mummenhoff, K. and M. Koch. Chloroplast DNA restriction site variation and phylogenetic relationships in the genus *Thlaspi sensu lato* (Brassicaceae). *System. Bot.* 19, 73-88, 1994.
- Mummenhoff, K., A. Franzke, and M. Koch. Molecular phylogenetics of *Thlaspi* s.l. (Brassicaceae) based on chloroplast DNA restriction site variation and sequences of the internal transcribed spacers of nuclear ribosomal DNA. *Can. J. Bot.* 75, 469-482, 1997.
- Murphy, A.S. and L. Taiz. Comparison of metallothionein gene expression and nonprotein thiols in ten *Arabidopsis* ecotypes. Correlation with copper tolerance. *Plant Physiol.* 109, 1-10, 1995.
- Murphy, A.S., J. Zhou, P.B. Goldsbrough, and L. Taiz. Purification and immunological identification of metallothioneins 1 and 2 from *Arabidopsis thaliana*. *Plant Physiol.* 113, 1293-1301, 1997.
- Nicks, L.J. and M.F. Chambers. Farming for metals? *Min. Environ. Manage.* September 1995, 15-18, 1995.
- Osmond, C.B. Ion absorption and carbon metabolism in cells of higher plants, in *Encyclopedia of Plant Physiology (New Series)*, vol. 2A, Lüttge, U. and Pitman, M.G., Eds., Springer-Verlag, Berlin, 347-372, 1976.
- Pelosi, P., R. Fiorentini, and C. Galoppini. On the nature of nickel compounds in *Alyssum bertolonii* Desv.-II. *Agric. Biol. Chem.* 40, 1641-1642, 1976.
- Peterson, P.J. Unusual accumulation of elements by plants and animals. *Sci. Progr., Oxford* 59, 505-526, 1971.
- Pollard, A.J. and A.J.M. Baker. Deterrence of herbivory by zinc hyperaccumulation in *Thlaspi caerulescens* (Brassicaceae). *New Phytol.* 135, 655-658, 1997.
- Proctor, J. and L. Nagy. Ultramafic rocks and their vegetation: an overview, in *The Vegetation of Ultramafic (Serpentine) Soils*, Baker, A.J.M., Proctor, J., and Reeves, R.D., Eds., Intercept Ltd., Andover, Hants., U.K., 469-494, 1992.
- Qureshi, J.A., K. Hardwick, and H.A. Collin. Malic acid production in callus cultures of zinc and lead tolerant and non-tolerant *Anthoxanthum odoratum*. *J. Plant Physiol.* 122, 477-479, 1986.
- Rascio, N. Metal accumulation by some plants growing on zinc mine deposits. *Oikos.* 29, 250-253, 1977.
- Rausser, W.E. Phytochelatins and related peptides. *Plant Physiol.* 109, 1141-1149, 1995.
- Reese, R.N. and G.J. Wagner. Effects of buthionine sulfoximine on Cd-binding peptide levels in suspension-cultured tobacco cells treated with Cd, Zn, or Cu. *Plant Physiol.* 84, 574-577, 1987.

- Reeves, R.D. Hyperaccumulation of nickel by serpentine plants, in *The Vegetation of Ultramafic (Serpentine) Soils*, Baker, A.J.M., Proctor, J., and Reeves, R.D., Eds., Intercept Ltd., Andover, Hants., U.K., 253-277, 1992.
- Reeves, R.D., R.R. Brooks, and R.M. Macfarlane. Nickel uptake by Californian *Streptanthus* and *Caulanthus* with particular reference to the hyperaccumulator *S. polygaloides* Gray (Brassicaceae). *Am. J. Bot.* 68, 708-712, 1981.
- Reeves, R.D., R.R. Brooks, and T.R. Dudley. Uptake of nickel by species of *Alyssum*, *Bornmuellera* and other genera of Old World Tribus Alysseae. *Taxon.* 32, 184-192, 1983.
- Reeves, R.D. and R.R. Brooks. Hyperaccumulation of lead and zinc by two metallophytes from mining areas of Central Europe. *Environ. Pollut. Ser. A.* 31, 277-285, 1983a.
- Reeves, R.D. and R.R. Brooks. European species of *Thlaspi* L. (Cruciferae) as indicators of nickel and zinc. *J. Geochem. Explor.* 18, 275-283, 1983b.
- Reeves, R.D. and A.J.M. Baker. Studies on metal uptake by plants from serpentine and non-serpentine populations of *Thlaspi goesingense* Halácsy (Cruciferae). *New Phytol.* 98, 191-204, 1984.
- Reeves, R.D., A.J.M. Baker, A. Borhidi, and R. Berazaín. Nickel-accumulating plants from the ancient serpentine soils of Cuba. *New Phytol.* 133, 217-224, 1996.
- Reeves, R.D., A.J.M. Baker, and A. Kelepertsis. The distribution and biogeochemistry of some serpentine plants of Greece, in *Écologie des Milieux sur Roches Ultramafiques et sur Sols Metallifères*. Jaffré, T., Reeves, R.D., and Becquer, T., Eds., Documents Scientifiques et Techniques, ORSTOM, Nouméa, New Caledonia, 205-207, 1997.
- Reeves, R.D., A.J.M. Baker, A. Borhidi, and R. Berazaín. Nickel hyperaccumulation in the serpentine flora of Cuba. *Ann. Bot.* 83, 29-38, 1999.
- Robinson, B.H., R.R. Brooks, J.H. Kirkman, P.E.H. Gregg, and H. Varela Alvarez. Edaphic influences on a New Zealand ultramafic ("serpentine") flora: a statistical approach. *Plant Soil.* 188, 11-20, 1997.
- Robinson, N.J., A.M. Tommey, C. Kuske, and P.J. Jackson. Plant metallothioneins. *Biochem. J.* 295, 1-10, 1993.
- Salt, D.E., D.A. Thurman, A.B. Tomsett, and A.K. Sewell. Copper phytochelatins of *Mimulus guttatus*. *Proc. R. Soc. London, Ser. B* 236, 79-89, 1989.
- Schlegel, H.G., J.-P. Cosson, and A.J.M. Baker. Nickel-hyperaccumulating plants provide a niche for nickel-resistant bacteria. *Bot. Acta* 104, 18-25, 1991.
- Schultz, C.L. and T.C. Hutchinson. Evidence against a key role for methallothionein-like protein in the copper tolerance mechanism of *Deschampsia caespitosa* (L.) Beauv. *New Phytol.* 110, 163-171, 1988.
- Severne, B.C. Nickel accumulation by *Hybanthus floribundus*. *Nature.* 248, 807-808, 1974.
- Shen, Z.G., F.J. Zhao, and S.P. McGrath. Uptake and transport of zinc in the hyperaccumulator *Thlaspi caerulescens* and the non-hyperaccumulator *Thlaspi ochroleucum*. *Plant Cell Environ.* 20, 898-906, 1997.
- Shimwell, D.W. and A.E. Laurie. Lead and zinc contamination of vegetation in the Southern Pennines. *Environ. Pollut.* 3, 291-301, 1972.
- Smith, J.A.C., J. Ingram, M.S. Tsiantis, B.J. Barkla, D.M. Bartholomew, M. Bettey, O. Pantoja, and A.J. Pennington. Transport across the vacuolar membrane in CAM plants, in *Crassulacean Acid Metabolism: Biochemistry, Ecophysiology and Evolution*, Winter, K. and J.A.C. Smith, Eds., Springer-Verlag, Berlin, 53-71, 1996.
- Still, E.R. and R.J.P. Williams. Potential methods for selective accumulation of nickel (II) ions by plants. *J. Inorg. Biochem.* 13, 35-40, 1980.
- Thurman, D.A. and J.L. Rankin. The role of organic acids in zinc tolerance in *Deschampsia caespitosa*. *New Phytol.* 91, 629-635, 1982.

- Tohayama, H., M. Inouhe, M. Joho, and T. Murayama. Production of metallothionein in copper and cadmium resistant strains of *Saccharomyces cerevisiae*. *J. Ind. Microbiol.* 14, 126-131, 1995.
- Tomsett, A.B. and D.A. Thurman. Molecular biology of metal tolerances of plants. *Plant Cell Environ.* 11, 383-394, 1988.
- Vázquez, M.D., J. Barceló, Ch. Poschenreider, J. Mádico, P. Hatton, A.J.M. Baker, and G.H. Cope. Localization of zinc and cadmium in *Thlaspi caerulescens* (Brassicaceae), a metallophyte that can hyperaccumulate both metals. *J. Plant Physiol.* 140, 350-355, 1992.
- Vázquez, M.D., Ch. Poschenrieder, J. Barceló, A.J.M. Baker, P. Hatton, and G.H. Cope. Compartmentation of zinc in roots and leaves of the zinc hyperaccumulator *Thlaspi caerulescens* J & C Presl. *Bot. Acta.* 107, 243-250, 1994.
- Vergnano Gambi, O., R.R. Brooks, and C.C. Radford. L'accumulo di nichel nelle specie italiane del genere *Alyssum*. *Webbia.* 33, 269-277, 1979.
- Vergnano Gambi, O., R. Gabbriellini, and L. Pancaro. Nickel, chromium and cobalt in plants from Italian serpentine areas. *Acta Oecol. Plant.* 3, 291-306, 1982.
- Verkleij, J.A.C., P.C. Lolkema, A.L. De Neeling, and H. Harmens. Heavy metal resistance in higher plants: biochemical and genetic aspects, in *Ecological Responses to Environmental Stress*, Rozema, J., and Verkleij, J.A.C., Eds., Kluwer, Amsterdam, 8-19, 1991.
- Wang, J., B.P. Evangelou, M.T. Nielsen, and G.J. Wagner. Computer-simulated evaluation of possible mechanisms for quenching heavy metal ion activity in plant vacuoles. I. Cadmium. *Plant Physiol.* 97, 1154-1160, 1991.
- Wang, J., B.P. Evangelou, M.T. Nielsen, and G.J. Wagner. Computer-simulated evaluation of possible mechanisms for sequestering metal ion activity in plant vacuoles. II. Zinc. *Plant Physiol.* 99, 621-626, 1992.
- White, M.C., A.M. Decker, and R.L. Chaney. Metal complexation in xylem fluid. I. Chemical composition of tomato and soybean stem exudate. *Plant Physiol.* 67, 292-300, 1981a.
- White, M.C., F.D. Baker, A.M. Decker, and R.L. Chaney. Metal complexation in xylem fluid. II. Theoretical equilibrium model and computational computer program. *Plant Physiol.* 67, 301-310, 1981b.
- Williams, S.T., T. McNeilly, and E.M.H. Wellington. The decomposition of vegetation growing on metal mine waste. *Soil Biol. Biochem.* 9, 271-275, 1977.
- Wither, E.D. and R.R. Brooks. Hyperaccumulation of nickel by some plants of Southeast Asia. *J. Geochem. Explor.* 8, 579-583, 1977.
- Woolhouse, H.W. Toxicity and tolerance in the responses of plants to metals, in *Encyclopedia of Plant Physiology (New Series)*, vol. 12C, Lange, O.L., Osmond, C. B., Nobel, P.S., and Ziegler, H., Eds., Springer-Verlag, Berlin, 245-300, 1983.
- Yang, X.H., R.R. Brooks, T. Jaffré, and J. Lee. Elemental levels and relationships in the Flacourtiaceae of New Caledonia and their significance for the evaluation of the "serpentine problem." *Plant Soil.* 87, 281-291, 1985.
- Zenk, M.H. Heavy-metal detoxification in higher plants. A review. *Gene.* 1179, 21-30, 1996.
- Zhou, J. and P.B. Goldsbrough. Structure, organization and expression of the metallothionein gene family in *Arabidopsis*. *Mol. Gen. Genet.* 248, 318-328, 1995.