25 Metal-Tolerant Plants: Biodiversity Prospecting for Phytoremediation Technology

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CONTENTS

25.1 INTRODUCTION

Metals, radionuclides and other inorganic contaminants are among the most prevalent forms of environmental contaminants, and their remediation in soils and sediments is rather a difficult task [1]. Sources of anthropogenic metal contamination include smelting of metalliferous ore, electroplating, gas exhaust, energy and fuel production, the application of fertilizers and municipal sludges to land, and industrial manufacturing [1,3]. Heavy metal contamination of the biosphere has increased sharply since 1900 [4] and poses major environmental and human health problems worldwide [5]. Unlike many organic contaminants, most metals and radionuclides cannot be eliminated from the environment by chemical or biological transformation [6,7]. Although it may be possible to reduce the toxicity of certain metals by influencing their speciation, they do not degrade and are persistent in the environment [8]. The various conventional remediation technologies used to clean heavy metal-polluted environments are soil *in situ* vitrification, soil incineration, excavation and landfill, soil washing, soil flushing, solidification, and stabilization electrokinetic systems. Each conventional remediation technology has specific benefits and limitations.

All compartments of the biosphere are polluted by a variety of inorganic and organic pollutants as a result of anthropogenic activities and alter the normal biogeochemical cycling. A variety of biological resources have been employed widely in developed and developing nations for cleanup of the metal-polluted sites. These technologies have gained considerable momentum in the last decade and are currently in the process of commercialization [9–19]. The U.S. Environmental Protection Agency's remediation program included phytoremediation of metals and radionuclides as a thrust area during the year 2000.

Plants that hyperaccumulate metals have tremendous potential for application in remediation of metals in the environment. This approach is emerging as an innovative tool with greater potential

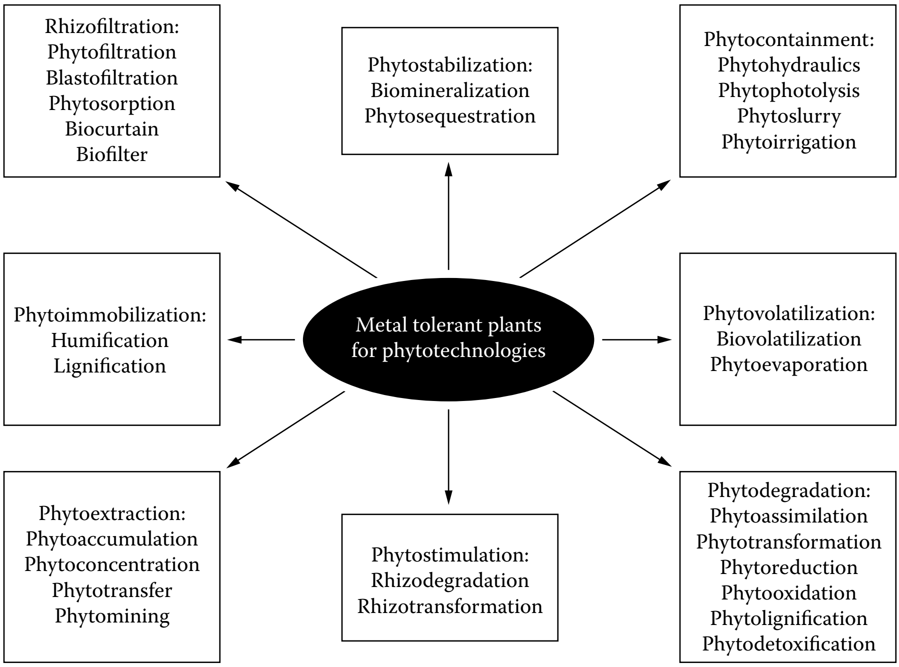

FIGURE 25.1 Various phytotechnologies in which metal-tolerant plants play a key role.

for achieving sustainable development and also to decontaminate metal-polluted air, soil, and water, and for other environmental restoration applications through rhizosphere biotechnology [20] (Figure 25.1 and Figure 25.2). Metal hyperaccumulating plants are thus not only useful in phytoremediation, but also play a significant role in biogeochemical prospecting; they have implications on human health through the food chain and possibly exhibit elemental allelopathy (metallic compounds leached through plant parts of the hyperaccumulator would suppress the growth of other plants growing in the neighborhood) and resistance against fungal pathogens (Figure 25.3) [21]. In order to be realistic about the phytoremediation, focused studies on factors regulating phytoremediation are necessary (Figure 25.4).

The term phytoremediation ("phyto" means plant, and the Latin suffix "remedium" means to clean or restore) actually refers to a diverse collection of plant-based technologies that use naturally occurring or genetically engineered plants for cleaning contaminated environments [1,22]. The primary motivation behind the development of phytoremediative technologies is the potential for low-cost remediation [5].

Although the term "phytoremediation" is a relatively recent invention, the act of phytoremediation is an age-old practice [1]. Research using semiaquatic plants for treating radionuclide-contaminated waters existed in Russia at the dawn of the nuclear era [24,25]. Some plants that grow on metalliferous soils have developed the ability to accumulate massive amounts of indigenous metals in their tissues without exhibiting symptoms of toxicity such plants are termed as "metallophytes" and are of considerable significance for *in situ* decontamination of toxic metals [28,29]. Baker and Brooks [26] and Chaney [29] first suggested using these "hyperaccumulators" for the phytoremediation of metal-polluted sites. However, hyperaccumulators were later believed to have limited potential in this area because of their small size and slow growth, which limit the speed of metal removal [30]. By definition, a hyperaccumulator must accumulate at least 100 μg g^{-1} (0.01%

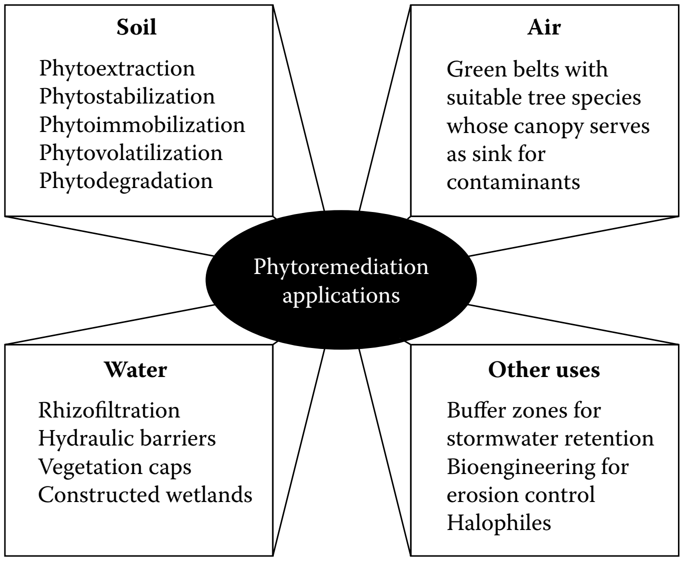

FIGURE 25.2 The important phytoremediation technologies applied are rhizofiltration, phytostabilization, phytovolatilization, and phytoextraction. The term phytoremediation ("phyto" means plant and the Latin suffix "remedium" means to clean or restore) actually refers to a diverse collection of plant-based technologies that use naturally occurring or genetically engineered plants for cleaning contaminated environments. One of the primary objectives behind the development of phytoremediation technologies is its potential for application at a low cost. Although the term phytoremediation is of a relatively recent origin, the practice is not.

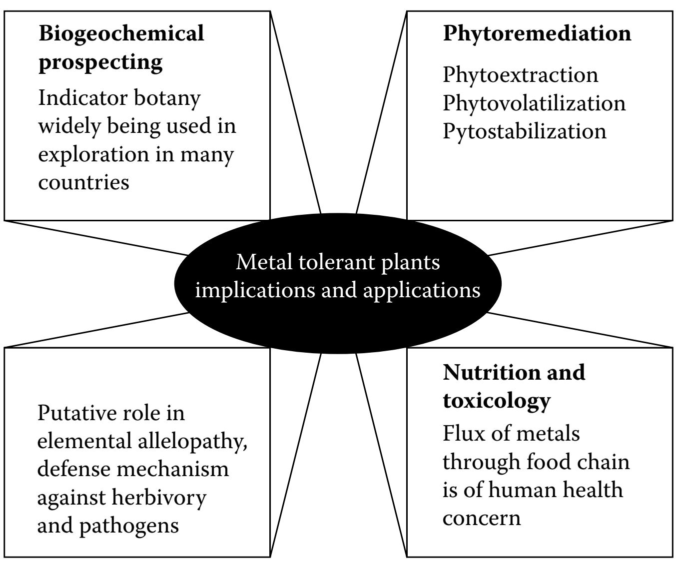

FIGURE 25.3 Metal hyperaccumulators were later believed to have limited potential in the area of phytoremediation, owing to their slow growth and low biomass production, which limit the speed of metal removal. By definition, a hyperaccumulator must accumulate at least 100 μg g^{-1} (0.01% dry wt.); Cd, As, and some other trace metals, 1000 μg g^{-1} (0.1 dry wt.); Co, Cu, Cr, Ni, and Pb and 10,000 μg g^{-1} (1% dry wt.); Mn and Ni. Plants that hyperaccumulate metals have other applications and implications. The most important applications are phytoremediation and biogeochemical prospecting. The other implications are elemental allelopathy and nutrition and toxicology, which are human health-related subjects.

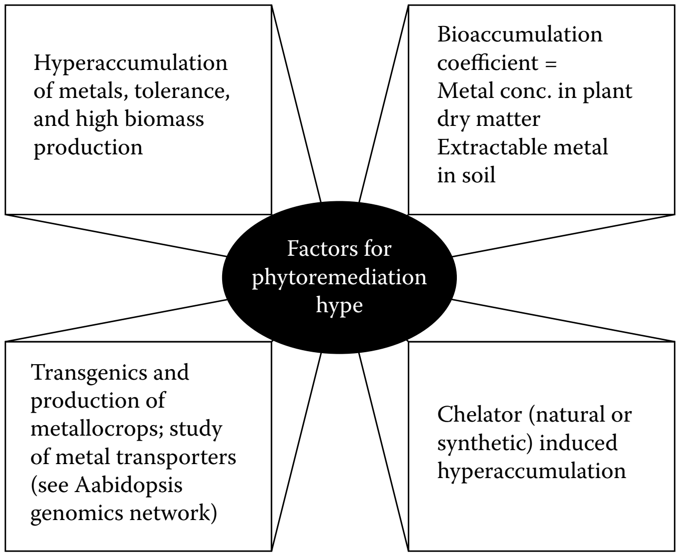

FIGURE 25.4 Several factors would accelerate phytoremediation technology. The prime is genetic engineering and production of transgenics having tolerance and metal accumulation ability for use in phytoremediation, facilitating the factors that would influence the metal bioaccumulation coefficient; in turn, this will depend upon heavy metal availability in the soil, absorption, transport and sequestration, etc., as well as development of low-cost technologies for chelate-induced hyperaccumulation.

dry wt.) of Cd, As, and some other trace metals; 1000 μg g^{-1} (0.1% dry wt.) of Co, Cu, Cr, Ni, and Pb; and 10,000 μg g^{-1} (1% dry wt.) of Mn and Ni [19,31].

The following technical terms are connected with phytoremediaton of metals in the environment:

- *Phytoremediation:* use of plants to remediate contaminated soil, water, and air
- *Phytoaccumulation:* the uptake and concentration of contaminants (metals or organics) within the roots or above-ground portion of plants
- *Phytoaccumulation coefficient:* metal concentration in plant dry matter/extractable metal in soil
- *Phytoextraction*: the use of plants at waste sites to accumulate metals into the harvestable, above-ground portion of the plant and, thus, to decontaminate soils
- *Phytoextraction coefficient*: the ratio of extractable metal concentration in the plant tissues (g metal/g dry weight tissue) to the soil concentration of the metal (g metal/g dry weight soil)
- *Phytomining:* use of plants to extract inorganic substances from mine ore
- *Phytostabilization*: plants tolerant to the element in question used to reduce the mobility of elements and thus stabilized in the substrate or roots
- *Phytovolatilization:* the uptake and transpiration of a contaminant by a plant, with release of the contaminant or a modified form of the contaminant to the atmosphere from the plant
- *Rhizofiltration/Phytofiltration*: process in which roots or whole plants of element-accumulating plants absorb the element from polluted effluents and are later harvested to diminish the metals in the effluents
- *Rhizosecretion:* a subset of molecular farming, designed to produce and secrete

25.2 BIODIVERSITY PROSPECTING FOR PHYTOREMEDIATION OF METALS IN THE ENVIRONMENT

Biodiversity prospecting" offers several opportunities, of which the most important is to save as much as possible of the world's immense variety of ecosystems. Biodiversity prospecting would lead to the discovery of a wild plant that could clean polluted environments of the world. This subject is in its infancy, with a great hope of commercial hype. The desire to capitalize on this new idea needs to provide strong incentives for conserving nature [32].

Potentially toxic trace elements are increasing in all compartments of the biosphere, including air, water, and soil, as a result of anthropogenic processes. For example, the metal concentration in river water and sediments has been increased several thousand-fold by effluents from industrial and mining wastes [33]. Aquatic plants in freshwater, marine, and estuarine systems act as receptacles for several metals [34–39]. Published literature indicates that an array of bioresources (biodiversity) has been tested in field and laboratory (Table 25.1). Remediation programs relying on these materials may be successful [40–42,51] (Figure 25.5).

The most successful monitoring methods for metals in the environment are based on gene-based and protein-based bacterial heavy metal biosensors [42]. Mosses, liverworts, and ferns are also capable of growing on metal-enriched substrates. These plants possess anatomical and physiological characteristics enabling them to occupy unique ecological niches in natural metalliferous and manmade environments. For example, groups of specialized bryophytes are found on Cu-enriched substrates — so-called "copper mosses" — and come from widely separated taxonomic groups. Other bryophytes are associated with lead- and zinc-enriched substrates. However, the information about bryophytes growing on serpentine soils is rather scanty. Pteridophytes (ferns) are associated with serpentine substrates in various parts of the world. Brake fern (*Pteris vittata*), a fast growing plant, is reported to tolerate soils contaminated with as much as 1500 ppm arsenic and its fronds concentrate the toxic metal to 22,630 ppm in 6 weeks [43]. Among angiosperms, about 450 metal hyperaccumulators have been identified, which would serve as a reservoir for biotechnological application [44] (Figure 25.6).

25.3 METAL-TOLERANT PLANTS FOR PHYTOREMEDIATION

Mine reclamation and biogeochemical prospecting depend upon the correct selection of plant species and sampling. The selection of heavy metal-tolerant species is a reliable tool to achieve success in phytoremediation. Table 25.2 shows 163 plant taxa belonging to 45 families found to be metal tolerant and capable of growing on elevated concentrations of toxic metals. The use of metal-tolerant species and their metal indicator and accumulation is a function of immense use for biogeochemical prospecting [45–47].

Brassicaceae had the highest number of taxa, i.e., 11 genera and 87 species that are established for hyperaccumulation of metals (Figure 25.7). In Brassicaceae, Ni hyperaccumulation is reported in seven genera and 72 species [48,49], and Zn in three genera and 20 species (Figure 25.8 and Figure 25.9). Different genera of Brassicaceae are known to accumulate metals (Figure 25.10).

Considerable progress had been achieved recently in unraveling the genetic secrets of metal-eating plants. Genes responsible for metal hyperaccumulation in plant tissues have been identified and cloned [50]. These findings are expected to identify new nonconventional crops, *metallocrops*, that can decontaminate metals in the environment [51–53]. The fundamental aspects of microbe/plant stress responses to different doses of metals coupled with breakthrough research innovations in biotechnology would successfully provide answers such as how to apply the biodiversity for advancing phytoremediation technology

TABLE 25.1
Biodiversity Exhibiting Resistance to Metals and with Potential to Clean Up Toxic Metals in all Three Compartments of the Environment (Atmosphere, Hydrosphere, and Lithosphere)

Bacteria

Acinetobacter
Agrobacterium
Alcaligenes eutrophus
A. faecalis
Arthrobacter sp.
Bacillus spp
Citribacter freundii
Comamonas sp.
Desulfobulbus spp.
Desulfomicrobium
Desulfovibrio spp.
Enterobacter colacae
Leptospirillum sp.
Pseudomonas aeruginosa
P. putida
P. syringae
Photobacterium phosphoreum
Ralstonia eutropha
R. metallidurans
Salmonella typhimurium
S. aureus
Thiobacillus ferrooxidans

Mycorrhizae

Albatrellus ovinus
Amanita muscaria
Chantharellus tubaeformis
C. ciliarius
Cortinarius sp.
Dermocybe sp.
Glomus mosseae
Gomphidius sp.
Hebeloma sp.
Hydnum sp.
Hymenoscyphus ericae
Laccaria laccata
Leccinum spp.
Oidiodendron maius
Paxillus involutus
Pisolithus tinctorius
Russula sp.
Scleroderma sp.
Suillus bovinus
S. luteus
Thelephora terrestris

Freshwater algae

Achanthes microcephala
A. minutissima

Freshwater algae

Anabaena cylindrica
A. doliolum
A. inaequalis
A. lutea
Anacystis nidulans
Ankistridesmus falcatus
Aphanocapsa sp.
Asterococcus sp
Chlamydomonas acidophila
C. ampla
C. bacilus
C. pyrenoidisa
C. reinhardtii
C. subglobosus
C. vulgaris
Chamaesiphon minutus
Chara corallina
Chlamydocapsa bacillus
Chlamydocapsa cf. petrify
Chlorella fusca var vacuolata
Cladophora glomerata
Cosmarium sp.
Cyanidium caldarium
Dictyococcus sp.
Dunaliella bioculata
Eisenia bicylis
Euglena gracilis
E. mutabilis
Eunotia exigua
Gleocapsa turfosa
Gleochrysis acoricola
Gleococcus
Gleocystis gigas
Gomphenema
Hormidium rivulare
Hypnomonas
Klebsormidium klebsii
K. rivulare
Microspora pachyderma
M. stagnorum
M. stagnosum
M. strictissimum
M. tumidula
M. willeana
M. floccosa
Microthamnion kutzingianum
Mougeotia

TABLE 25.1
Biodiversity Exhibiting Resistance to Metals and with Potential to Clean Up Toxic Metals in all Three Compartments of the Environment (Atmosphere, Hydrosphere, and Lithosphere) (continued)

Freshwater algae	Bryophytes
Navicula	*Ditrichum cornubicum*
Nitchia palea	*D. plumbicola*
Nostoc calcicola	*Funaria hygrometrica*
Oedogonium sp.	*Grimmia atrata*
O. nephrocytioides	*Gymnocolea acutiloba*
Oocystis elliptica	*Mielichhoferia macrocarpa*
O. lacustris	*M. elongata*
Oscillatoria	*M. nitida*
Phormidium foveolarum	*M. mieilichhoferi*
P. luridum	*Pholia nutans*
Pinnularia acoricola	*P. andalusica*
Plectonema	*P. nutans*
Plerococcus	*Pottia sp.*
Pseudoanabaena catenata	*Scopelophila cataractae*
Pseudococcomyxa adhaerens	*S. ligulata*
Scenedesmusobliqus	*S. cataractae*
S. quadricauda	*S. cataractae*
S. subspicatus	*Scapania undulata*
Scenedesmus acutiformis	**Pteridophytes**
Schizothrix sp.	*Asplenium adiantum-nigrum*
Selenastrum capricornutum	*A. cuneifolium*
Snechococcus sp.	*A. hybrida*
Spartina maritima	*A. presolanense*
Spirogyra sp.	*A. ruta-muraria*
Spirulina platensis	*A. septiontrionale*
Stegeoclonium sp.	*A. trichomanes*
Stegioclonium sp.	*A. viride*
Stichococcus sp.	*Ceratopteris cornuta*
Stichococcus bacillaris	*Cehaloziella calyculata*
Stigeoclonium tenue	*Cheilanthes hirta*
Surirella angustata	*C. inaequalis var. lanopetiolata*
Synechocystis aquatilis	*C. inaequalis var. inaequalis*
Synedra filiformis	*C. hirta Mohria lepigera*
Bryophytes	*Nardia scalaris*
Barbula recurvirostrata	*Nothalaena marantae*
B. acuminatum	*Oligotrichum hercynicum*
B. philonotula	*Ophiglossum lancifolium*
Bryum argenteum	*Pellea calomelanos*
B. rubens	*Pteris vittata*
Campylopus bequartii	**Lichens**
Cephalozia bicuspidata	*Bryoria fuscescens*
Cephaloziella hampeana	*Diploschistes muscorum*
C. masalongi	*Flavoparmelia*
C. nicholsonii	*Baltimorensis*
C. rubella	*Hypogyminia physodes*
C. stellulifera	Lobaria pulmonaria
C. integerrima	*Parmelia caperata*

TABLE 25.1
Biodiversity Exhibiting Resistance to Metals and with Potential to Clean Up Toxic Metals in all Three Compartments of the Environment (Atmosphere, Hydrosphere, and Lithosphere) (continued)

Lichens	**Angiosperms**
Peltigera canina	*A. giosnanum*
Ramalina duriaei	*A. huber-morathii*
R. farinacea	*A. janchenii*
R. fastigata	*A. malacitanum*
Gymnosperms	*A. markgrafii*
Abies chamaecyparis	*A. masmenaeum*
Chamaecyparis	*A. murale*
Cryptomeria	*A. obovatum*
Gingko	*A. oxycarpum*
Juniperus	*A. penjwinensis*
Larix picea	*A. pinifolium*
Pinus ponderosa	*A. pintodasilave*
Pseudotsuga	*A. pterocarpum*
Taxodium	*A. robertianum*
Angiosperms	*A. samariferum*
Acer saccharinum	*A. serpyllifolium*
Aeollanthus biformifolius	*A. singarense*
Agrostis capillaris	*A. smolikanum*
A. gigantea	*A. stolonifera*
A. tenuis	*A. syriacum*
Alyssum heldreichii	*A. tenium*
A. lesbiacum	*A. trapeziforme*
A. perenne	*A. troodii*
A. akamasicum	*A. virgatum*
A. alpestre	*A. wulfenianum*
A. americanum	*A. montanum*
A. anatolicum	*A. serpyllifolium subsp. malacinatum*
A. argenteum	*Amaranthus retroflexus*
A. bertlonii	*Anthoxanthum odoratum*
A. bertolonii subsp. scutarinum	*Arabidopsis halleri*
A. callichroum	*A. thaliana*
A. carcium	*Arabis stricta*
A. cassium	*Armeria maritima subsp. elongata*
A. chondrogynum	*Arrhenatherum pratensis*
A. cilicium	*Astragalus racemosus*
A. condensatum	*Avenella flexuosa*
A. constellatum	*Berkheya coddi*
A. corsicum	*Betula papyrifera*
A. crenulatum	*Bornmuellera glabrescens*
A. cypricum	*B. tymphea*
A. davisianum	*B. baldaccii subsp. baldacci*
A. discolor	*B. baldaccii subsp. markgrafii*
A. dubertretii	*B. baldaccii baldaccii subsp.*
A. eriophyllum	*Brassica nigra*
A. euboeum	*B. pendula*
A. fallacinum	*B. pubescens*
A. floribundum	*B. rapa*

TABLE 25.1
Biodiversity Exhibiting Resistance to Metals and with Potential to Clean Up Toxic Metals in all Three Compartments of the Environment (Atmosphere, Hydrosphere, and Lithosphere) (continued)

Angiosperms	Angiosperms
	Mimulus guttatus
B. campestris	*Minuartia hirsuta*
B. hordeaceus	*Nardus stricta*
B. japonica	*Noccaea aptera*
B. juncea	*N. boeotica*
B. napus	*N. eburneosa*
B. narinosa	*N. firmiensis*
B. pekinensis	*N. tymphaea*
B. ramosus	*Pelargonium*
Brachypodium chinensis	*Peltaria dumulosa*
Brachypodium sylvaticum	*P. emarginata*
Calystegia sepium	*Podophyllum peltatum*
Cardamine resedifolia	*Polygonum cuspidatum*
Cardminopsis halleri	*Populus tremula*
Carex echinata	*Pseudosempervium aucheri*
Chrysanthemum morifolium	*Quercus rubra*
Cochlearia aucheri	*Q. ilex*
C. pyrenaica	*Ranunculus baudotti*
C. sempervium	*Rauvolfia serpentina*
Colocasia esculenta	*Ricinus communis*
Cynodon dactylon	*Rumex hydrolapathum*
Danthonia decumbens	*Salix viminalis*
D. linkii	*Sebertia acuminata*
Datura innoxia	*Senecio cornatus*
Deschampsia caespitosa	*Silene cucubalus*
Echinochloa colona	*S. compacta*
Epilobium hirsutum	*S. italica*
Eriophorum angustiflolium	*Solanum nigrum*
Eschscholtzia californica	*Sorghum sudanense subsp. halleri*
Fagopyrum esculentum	*S. sudanens subsp. maritima*
Fagus sylvatica	*Stanleya sp.*
Festuca rubra	*Streptanthus polygaloides*
Fraxinus angustifolia	*Thlaspi alpestre subsp. virens*
Gossypium hirsutum	*T. arvense*
Haumaniastrum katangense	*T. brachypetalum*
Helianthus annuus	*T. bulbosum*
Holcus lanatus	*T. bulbosum*
Hordelymus europaeus	*T. caerulescens*
Hybanthus floribundus	*T. calaminare*
Hydrangea	*T. cepaefolium*
Hydrocotyle umbellata	*T. cepaeifolium subsp.*
Limnobium stoloniferum	*T. cypricum*
Lolium multiflorum	*T. elegans*
L. perenne	*T. epirotum*
Macadamia neurophylla	*T. goesingense*
Medicago sativa	*T. graecum*
Melilotus officinalis	*T. idahoense*

TABLE 25.1
Biodiversity Exhibiting Resistance to Metals and with Potential to Clean Up Toxic Metals in all Three Compartments of the Environment (Atmosphere, Hydrosphere, and Lithosphere) (continued)

Angiosperms	**Aquatic macrophytes**
T. japonicum	*Lemna minor*
T. jaubertii	*L. trisulca*
T. kovatsii	*Myriophyllum spicatum*
T. liliaceum	*Najas sp.*
T. limosellifolium	*Phragmites australis*
T. magallanicum	*Potamogeton pectinatus*
T. montanum	*P. perfoliatum*
T. montanum var.montanum	*Ruppia sp.*
T. ochroleucum	**Tree crops**
T. oxyceras	*Acer pseudoplatanus*
T. parvifolium	*Betula alleghanensis*
T. praecox	*B. pendula*
T. repens	*B. tauschii*
T. rotundifolium	*Cryptomeria japonica*
T. rotundifolium subsp. cepaefolium	*Eucalyptus camaldulensis*
T. rotundifolium var. corymbosum	*Fagus japonica*
T. stenocarpum	*F. sylvatica*
T. sylvium	*Liriodendron tulipifera*
T. tatraense	*P. deltoides x P. nigra*
T. tymphaeum	*P. maximowiczii*
T. violascens	*P. nigra*
Thinopyrum bessarabicum	*P. taeda*
Trifolium pratense	*P. trichocarpa x P. deltoides*
Viola calaminaria	*Picea abies*
Viola arvensis	*Pinus strobus*
Aquatic macrophytes	*Populus alba*
Arenicola christata	*Prunus virginiana*
A. marina	*Salix arenaria*
Carex sp.	*S. burjatica cv. aquatica*
Ceratophyllum demersum	*S. x caprea*
Glyceria fluitans	*S. viminalis*
Hydrilla verticillata	*S. triandra*
Ipomea aquatica	*S. dasyclados*

Notes: Several of the listed organisms are used for laboratory and field experiments. The results obtained are found to be useful to advance the knowledge of bioremediation and metal monitoring in the environment. (The list is not exhaustive).

Sources: Bargagli, R., *Trace Elements in Terrestrial Plants — an Ecophysiological Approach to Biomonitoring and Biorecovery*, Springer–Verlag, Heidelberg, 1998, 324 pp; Markert, B., *Plants as Biomonitors: Indicators for Heavy Metals in the Terrestrial Environment*, 1993, VCH Publishers; Prasad, M.N.V., in Leeson, A. et al. (Eds.), *Proc. 6th Int.* in Situ *On-Site Bioremediation Symp*., Battelle Press, Columbus, OH, 2001.

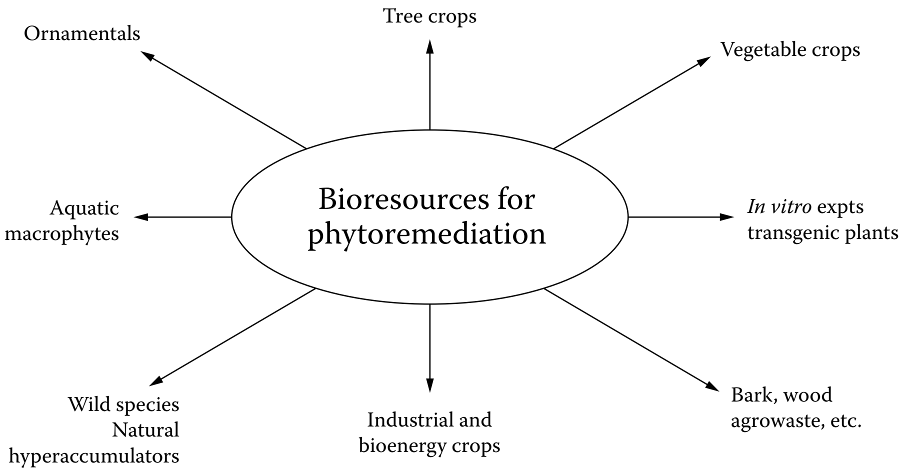

FIGURE 25.5 Biodiversity prospecting for phytoremediation of metals in the environment. Please see the cited references for additional information.

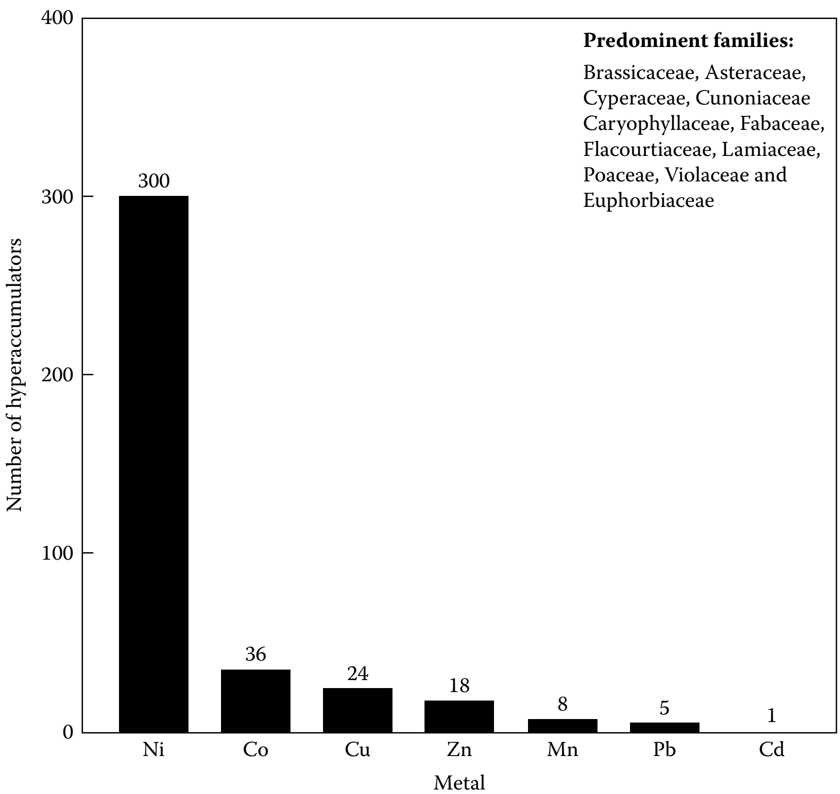

FIGURE 25.6 Taxa of various angiospermous families that hyperaccumulate metals. The families dominating the metal accumulators and hyperaccumulators are Asteraceae, Brassicaceae, Caryophyllaceae, Cyperaceae, Cunoniaceae, Fabaceae, Flacourtiaceae, Lamiaceae, Poaceae, Violaceae, and Euphorbiaceae.

TABLE 25.2
Vascular Plants Growing on Mine Refuse in Portugal

Apiaceae

Daucus crinitus H. Desp.
D. carota L. var. *maritimus* (Lam.) Steud.
Eryngium campestre L.

E. tenue Lam.
Foeniculum vulgare Miller subsp. *piperitum* (Ucria) Big.
Oenanthe crocata L.
Pimpinella villosa Schousb.
Seseli peixotianum Samp.

Aristolochiaceae

Aristolochia longa L.

Aspleniaceae

Asplenium adiantum-nigrum L. var. *corunnense* Christ

Asteraceae

Carlina corymbosa L. subsp. *corymbosa*
Crepis capillaris (L.) Wallr.
Dittrichia viscosa (L.) W. Greuter
Filago lutescens Jordan subsp. *lutescens*
Helichrysum stoechas (L.) Moench.
Hieracium peleteranum Mérat subsp.*ligericum* Zalm
Hispidella hispanica Lam.
Hypochaeris radicata L.
L. viminea (L.) J. & C. Presl. subsp. *viminea*
L. virosa L.
Lactuca viminea (L.) J. & C. Presl
Lapsana communis L. subsp. *communis*
Leontodon taraxacoides (Vill.) Mérat subsp.*longirostris* Finch & P.D. Sell
Logfia gallica (L.) Cosson & Germ.

L. minima (Sm.) Dumort.
Santolina semidentata Hoffmans & Link
Senecio gallicus Vill.
Tolpis barbata (L.) Gaertner

Boraginaceae

Anchusa arvensis (L.) Bieb. subsp. *arvensis*
Echium lusitanicum L. subsp. *lusitanicum*
E. plantagineum L.

Brassicaceae

Alyssum serpyllifolium Desf. subsp. *lusitanicum* Dudley & Pinto da Silva
Lepidium heterophyllum Bentham

Erysimum linifolium (Pers.) Gay subsp. *linifolium*

Campanulaceae

Campanula rapunculus L.
Jasione crispa (Pourret) Samp. subsp. *serpentinitica* P. Silva

Caprifoliaceae

Lonicera periclymenum L. subsp. *periclymenum*
Sambucus nigra L.

Caryophyllaceae

Agrostemma githago L.
Arenaria montana L. subsp. *montana*
A. querioides Pourret ex Willk. subsp. *fontequeri* (P. Silva) R. Afonso
Dianthus laricifolius Boiss. & Reuter subsp. *marizii (*Samp.*)* Franco
Ortegia hispanica Loefl.
*Petrorhagia nantevillii (*Burn.*)* P.W. Ball & Heywood
Saponaria officinalis L.
Silene scabriflora Brot. subsp. *scabriflora*
S. coutinhoi Rothm. & Pinto da Silva
Spergula pentandra L.
S. purpurea (Pers.) G. Don. fil.

Chenopodiaceae

Chenopodium album L. subsp. *album*

Cistaceae

Cistus ladanifer L.
C. salvifolius L.
Tuberaria guttata (L.) Fourr.

Clusiaceae

Hypericum perforatum L.

Convolvulaceae

Convolvulus arvensis L. subsp. *arvensis*

Crassulaceae

Sedum arenarium Brot.
S. forsteranum Sm.
S. tenuifolium Strob.

Dioscoreaceae

Tamus communis L.

Elatinaceae

Elatine macropoda Guss.

Euphorbiaceae

Euphorbia falcata L.

Fagaceae

Castanea sativa Miller
Quercus faginea Lam. subsp. faginea
Q. ilex L. subsp. *ballota* (Desf.) Samp.
Q. pyrenaica Willd.

Gentianaceae

Centaurium erythraea Rafin subsp. *majus* (Hoffmans & Link) Meldéris

Geraniaceae

Geranium purpureum Vill.

Haloragaceae

Myriophyllum alterniflorum DC.

Hypolepidaceae

Pteridium aquilinum (L.) Kuhn subsp. *aquilinum*

TABLE 25.2
Vascular Plants Growing on Mine Refuse in Portugal (continued)

Lamiaceae

Clinopodium vulgare L.
Dorycnium pentaphyllum Scop. subsp. *transmontanum* Franco
Lavandula stoechas L. subsp. *pedunculata* (Miller) Samp. & Rozeira
L. stoechas L. subsp. *sampaiana* Rozeira
L. stoechas L. subsp. *stoechas*
Mentha pulegium L.
M. spicata L.
M. suaveolens Ehrh.
Origanum virens Hoffmanns & Link
Phlomis lychnitis L.
Prunella vulgaris L. subsp. *vulgaris*
Salvia verbenaca L.
Teucrium scorodonia L. subsp. *scorodonia*
Thymus mastichina L.

Fabaceae

A. stoechas L.
Acacia dealbata Link
Adenocarpus complicatus (L.) J. Gay
Anthyllis lotoides L.
C. multiflorus (L. Hér.) Sweet
C. striatus (Hill.) Rothm.
Cytisus grandiflorus (Brot.) DC.
G. polyanthos Willk. subsp. *hystrix* (Lange) Franco
Genista triacanthos Brot.
Lotus tenuis Willd.
L. uliginosus Schkuhr.
Lotus corniculatus L. var. *corniculatus*
O. spinosa L. subsp. *antiquorum* (L.) Arcangeli
Ononis cintrana Brot.
Ornithopus compressus L.
Phagnalon saxatile (L.) Cass.
Pisum sativum L. subsp. *elatius* (Bieb.) Ascherson & Graebner
Pterospartum tridentatum L.
Trifolium arvense L. var. *arvense*
T. glomeratum L.
T. repens L. subsp. *repens*
T. campestre Schreber
Vicia sativa L. subsp. *nigra* (L.) Ehrh.
V. laxiflora Brot.

Liliaceae

Allium vineale L.
A. sphaerocephalos L. subsp. *sphaerocephalos*

Lythraceae

Lythrum hyssopifolia L.

Malvaceae

Malva sylvestris L.

Oleaceae

Fraxinus angustifolia Vahl

Ongagraceae

Epilobium tetragonum L. subsp. *tetragonum*

Orchidaceae

Serapias lingua L.

Papaveraceae

Papaver rhoeas L.

Pinaceae

Pinus pinaster Aiton

Plantaginaceae

Plantago lanceolata L.
P. radicata Hoffmans & Link subsp. *radicata*

Plumbaginaceae

Armeria langei Boiss. subsp. *langei*

Poaceae

Aegilops triuncialis L.
Agrostis curtisii Kerguélen
Arrhenatherum elatius (L.) J. & C. Presl.
Avena sterilis L.
Briza maxima L.
Bromus hordeaceus L. subsp. hordeaceus
Festuca pseudotricophylla Patzke
Holcus lanatus L.
Melica ciliata L. subsp. *ciliata*
Phleum pratense L. subsp. *bertolonii* (DC.) Bornm.
Sanguisorba minor Scop. subsp. *magnolii* (Spach) Coutinho
Setariopsis verticillata Samp.
Trisetaria ovata (Cav.) Paunero

Polygonaceae

Polygonum arenastrum Boreau
P. minus Hudson
Rumex pulcher L.
R. crispus L.
R. acetosella L. subsp. *angiocarpus* (Murb.) Murb.
R. induratus Boiss. & Reuter

Portulacaceae

Portulaca oleraceae L. subsp. Oleraceae

Primulaceae

Anagalis monelli L. var. *linifolia* (L.) Lange

Resedaceae

Reseda virgata Boiss. & Reuter

Rosaceae

Agrimonia procera Wallr.
Crataegus monogyna Jacq.
C. monogyna Jacq. subsp. *brevispina* (G. Kunze) Franco
Filipendula vulgaris Moench
Potentilla erecta (L.) Rauschel

TABLE 25.2
Vascular Plants Growing on Mine Refuse in Portugal (continued)

Rosaceae

Rosa canina L.
Rubus caesius L.
R. ulmifolius Schott
Sanguisorba verrucosa (Link) Ces.

Rubiaceae

Asperula aristata L. fil. subsp. scabra (J. & C. Presl) Nyman
Galium palustre L.

Salicaceae

Salix salvifolia Brot.
S. triandra L.

Scrophulariaceae

Anarrhinum bellidifolium (L.) Willd.
Digitalis purpurea L. subsp. *purpurea*
Linaria aeruginea (Gouan) Cav.
L. spartea (L.) Willd. subsp. *virgatula* (Brot.) Franco
Odontites tenuifolia (Pers.) G. Don fil.
Scrophularia auriculata
Verbascum virgatum Stokes
Digitalis thapsi L.

Thymelaeaceae

Daphne gnidium L.

Valerianaceae

Centranthus calcitrapae (L.) Dufresne subsp. *calcitrapae*

Sources: Bargagli, R., *Trace Elements in Terrestrial Plants — an Ecophysiological Approach to Biomonitoring and Biorecovery*, Springer–Verlag, Heidelberg, 1998, 324 pp.; Markert, B., *Plants as Biomonitors: Indicators for Heavy Metals in the Terrestrial Environment*. 1993. Vch Publishers; Prasad, M.N.V., in A. Leeson et al. (Eds.), *Phytoremediation, Wetlands, Sediments*, 6(5), 165–172, *Proc. 6th Int.* in Situ *On-Site Bioremediation Symp*., Battelle Press, Columbus, OH, 2001.

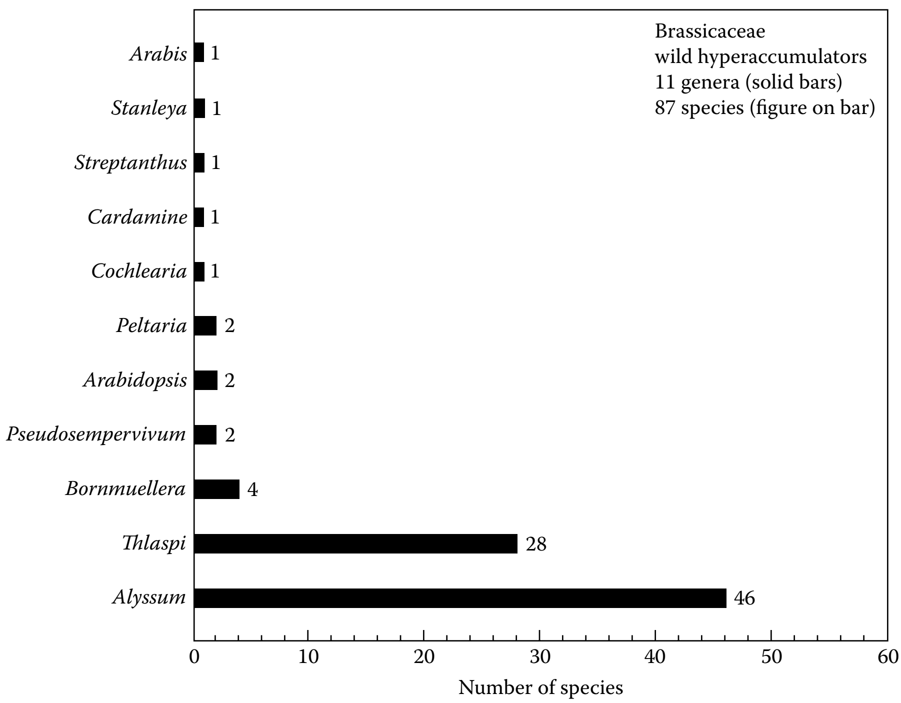

FIGURE 25.7 Among wild Brassicaceae, 11 genera and 87 species are known to hyperaccumulate metals.

25.4 ORNAMENTALS

Nerium oleander leaves collected from urban areas of Portugal accumulated lead up to 78 μg/g dry weight in leaves and are suitable for monitoring lead in air [59]. Canna x generalis is an

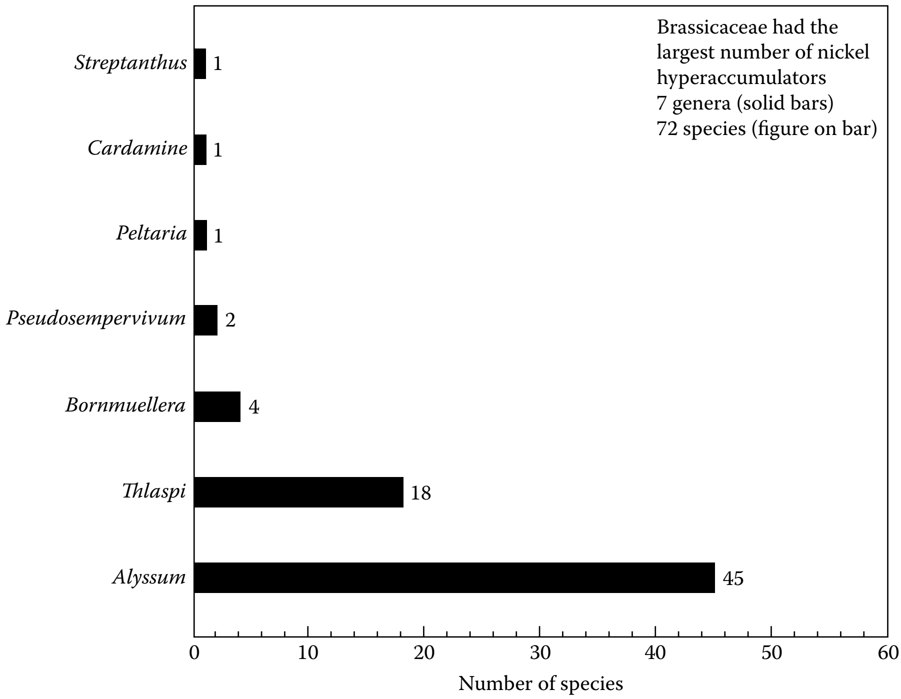

FIGURE 25.8 Brassicaceae has the largest amount of nickel (seven genera and 72 species).

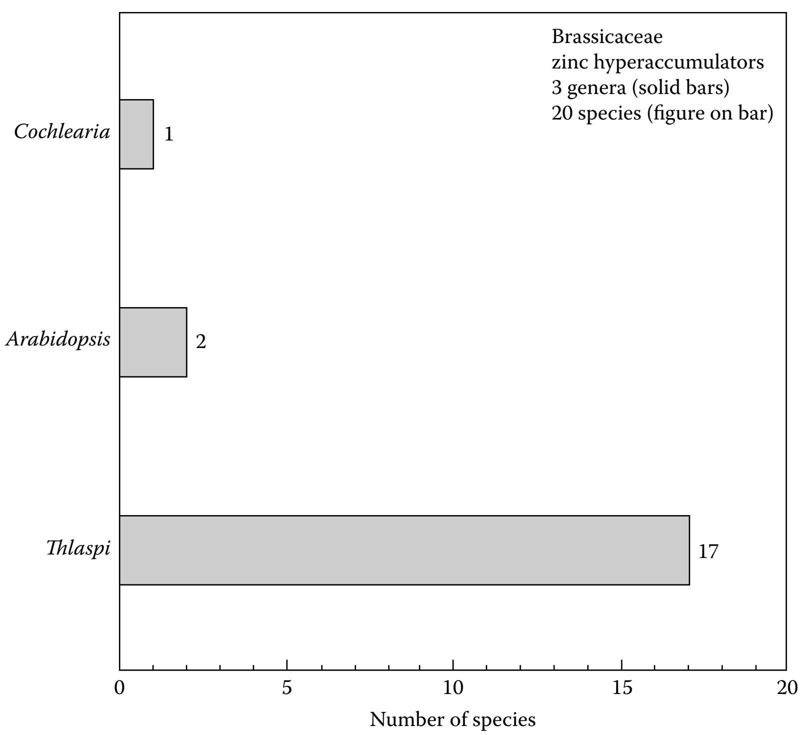

FIGURE 25.9 Brassicaceae has the largest number of zinc hyperacccumulators (three genera and 20 species).

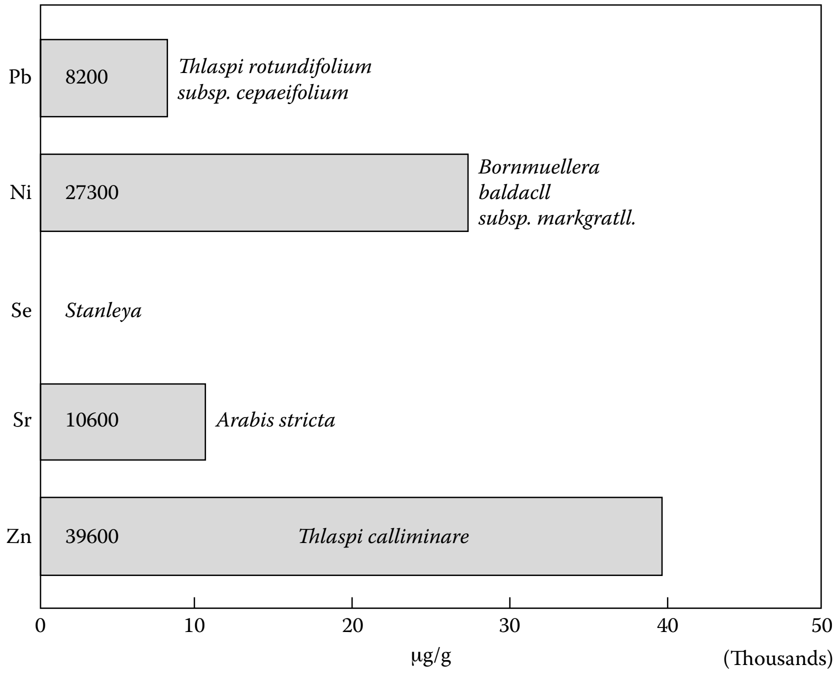

FIGURE 25.10 Selected examples of Brassicaeae that hyperaccumulate lead, nickel, strontium, and zinc.

important ornamental cultivated in urban landscapes. Hydroponic cultures of this plant treated with lead for 1 month suggest that it is suitable for phytoextraction of lead because the plant produces an appreciable quantity of biomass [60]. Pelargonium sp. "Frensham" (scented geranium) was identified as one of the most efficient metal hyperaccumulator plants [61]. In a greenhouse study, young cuttings of scented geranium grown in artificial soil and fed different metal solutions were capable of taking up large amounts of three major heavy metal contaminants (e.g., Pb, Cd, and Ni) in a relatively short time. These plants were capable of extracting from the feeding solution and stocking in their roots amounts of lead, cadmium, and nickel equivalent to 9, 2.7, and 1.9% of their dry weight material, respectively.

With an average root mass of 0.5 to 1.0 g in dry weight, scented geranium cuttings could extract 90 mg of Pb, 27 mg of Cd, and 19 mg of Ni from the feeding solution in 14 days. If these rates of uptake could be maintained under field conditions, scented geranium should be able to clean up heavily contaminated sites in less than 10 years. (Growth and uptake in nutrient solution are extremely different to that in soil, and scientific studies indicate the hydroponic culture is not indicative of a real-world situation, due to ion competition, root impedance, and the fact that plants do not grow root hairs when they are grown in solution.) For example, a phytoremediation lead cleanup program consisting of 16 successive croppings of scented geranium planted at a density of 100 plants m^{-2} over the summer could easily remove up to 72 g of lead m^{-2} yr^{-1}. In the authors' estimates, scented geranium would extract 1000 to 5000 kg of lead per ha^{-1} yr^{-1}. Thus, these reported figures are close to the estimations of metal removal rates of 200 to 1000 kg ha^{-1} yr^{-1} for plants capable of accumulating 1.0 to 2.0% metal [6].

Thus, if scented geranium is planted in soil where the lead contamination is 1000 µg kg^{-1} of soil, which is the acceptable limit for the province of Ontario (Canada), it can clean up the soil completely in 8 years. Scented geranium also has the ability to survive on soils containing one or more metal contaminants (individually or in combination) and on soils contaminated with a mixture of metal and hydrocarbons (up to three metal–hydrocarbon-contaminated soils) > 3% total hydrocarbon in combination with several metal contaminants.

25.5 METAL TOLERANT PLANTS AND CHELATORS MIGHT PROMOTE PHYTOREMEDIATION TECHNOLOGY

Use of soil amendments such as synthetics (ammonium thiocyanate) and natural zeolites has yielded promising results [62–66]. EDTA, NTA, citrate, oxalate, malate, succinate, tartrate, phthalate, salicylate, acetate, etc. have been used as chelators for rapid mobility and uptake of metals from contaminated soils by plants. Use of synthetic chelators significantly increased Pb and Cd uptake and translocation from roots to shoots, facilitating phytoextraction of the metals from low-grade ores. Synthetic cross-linked polyacrylates, hydrogels have protected plant roots from heavy metal toxicity and prevented the entry of toxic metals into roots. Application of low-cost synthetics and natural zeolites on a large scale are applied to the soil through irrigation at specific stages of plant growth; this might be beneficial to accelerate metal accumulation [67].

A major factor influencing the efficiency of phytoextraction is the ability of plants to absorb large quantities of metal in a short period of time. Hyperaccumulators accumulate appreciable quantities of metal in their tissue regardless of the concentration of metal in the soil [68], as long as the metal in question is present. This property is unlike moderate accumulators now used for phytoextraction in which the quantity of absorbed metal is a reflection of the concentration in the soil. Although the total soil metal content may be high, it is the fraction that is readily available in the soil solution that determines the efficiency of metal absorption by plant roots. To enhance the speed and quantity of metal removal by plants, some researchers advocate the use of various chemicals for increasing the quantity of available metal for plant uptake.

Chemicals suggested for this purpose include various acidifying agents [6,20,69], fertilizer salts [71,72], and chelating materials [67,73,74]. These chemicals increase the amount of bioavailable metal in the soil solution by liberating or displacing metal from the solid phase of the soil or by making precipitated metal species more soluble. Research in this area has been moderately successful, but the wisdom of liberating large quantities of toxic metal into soil water is questionable.

Soil pH is a major factor influencing the availability of elements in the soil for plant uptake [75]. Under acidic conditions, H^+ ions displace metal cations from the cation exchange complex (CEC) of soil components and cause metals to be released from sesquioxides and variably charged clays to which they have been chemisorbed (i.e., specific adsorption) [76]. The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption. Many metal cations are more soluble and available in the soil solution at low pH (below 5.5), including Cd, Cu, Hg, Ni, Pb, and Zn [2,76]. It is suggested that the phytoextraction process is enhanced when metal availability to plant roots is facilitated through the addition of acidifying agents to the soil [2,24,70].

Possible amendments for acidification include NH_4-containing fertilizers, organic and inorganic acids, and elemental S. Trelease and Trelease [77] indicated that plant roots acidify hydroponic solutions in response to NH_4 nutrition and cause solutions to become more alkaline in response to NO_3 nutrition. Metal availability in the soil can be manipulated by the proper ratio of NO_3 to NH_4 used for plant fertilization by the effect of these N sources on soil pH, but no phytoremediation research has been conducted on this topic to date.

The acidification of soil with elemental S is a common agronomic practice that can be used to mobilize metal cations in soil. Brown et al. [69] acidified a Cd- and Zn-contaminated soil with elemental S and observed that accumulation of these metals by plants was greater than when the amendment was not used. Acidifying agents are also used to increase the availability of radioactive elements in the soil for plant uptake. Huang et al. [70] reported that the addition of citric acid increases U accumulation in Indian mustard (*B. juncea*) tissues more than nitric or sulfuric acid, although all acids decrease soil pH by the same amount. These authors speculated that citric acid chelates the soil U, thereby enhancing its solubility and availability in the soil solution. The addition of citric acid causes a 1000-fold increase of U in the shoots of *B. juncea* compared to accumulation

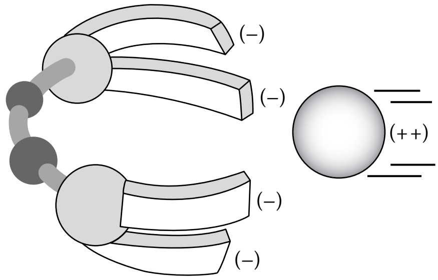

FIGURE 25.11 Citric acid is a naturally occurring chelating agent. The chelation process is water activated. EDTA, NTA, citrate, oxalate, malate, succinate, tartrate, phthalate, salicylate, acetate, etc. have been used for chelate-induced hyperaccumulation. Synthetic soil amendments such as ammonium thiocyanate and natural zeolites have yielded promising results in inducing hyperaccumulation of metals.

in the control (no citric acid addition) (Figure 25.11). Despite the promise of some acidifying agents for use in phytoextraction, little research is reported on this subject.

25.6 CONCLUSIONS

Implementing phytoremediation with the use of biodiversity has certain limitations [6,78,79]. To a considerable extent, these include potential contamination of the vegetation and food chain and the often extreme difficulty in establishing and maintaining vegetation on contaminated sites (e.g., mine tailings with high levels of residual metals). For metal contaminants, plants show the potential for phytoextraction (uptake and recovery of contaminants into above-ground biomass) [62,80,81], filtering metals from water onto root systems (rhizofiltration), or stabilizing waste sites by erosion control and evapotranspiration of large quantities of water (phytostabilization) [82,83].

After the plants have been allowed to grow for some time, they are harvested and then incinerated or composted to recycle the metals. This procedure may be repeated as necessary to bring soil contaminant levels down to allowable limits. If plants are incinerated, the ash must be disposed of in a hazardous waste landfill. Finally, phytoremediation in some countries has limited acceptance by the local government and takes a long time to mitigate the contaminant. Metal hyperaccumulators are generally slow growing with a small biomass and shallow root systems. Plant biomass must be harvested and removed, followed by proper disposal. Plants experience stress due to prevailing high concentrations of metals.

One of the main advantages of phytoextraction is that the plant biomass containing the extracted contaminant can be a resource. For example, biomass that contains selenium (Se), an essential nutrient, has been transported to areas that are deficient in Se and used for animal feed [84,85]; metal hyperaccumulators are of special significance in biogeochemical prospecting of minerals.

Rhizofiltration has the advantage that terrestrial or aquatic plants are used for this purpose. Although terrestrial plants require support, such as a floating platform, they generally remove more contaminants than aquatic plants do. This system can be *in situ* (floating rafts on ponds) or *ex situ* (an engineered tank system). An *ex situ* system can be placed anywhere because the treatment does not need to be at the original location of contamination [86–88].

Rhizofiltration has the following disadvantages:

- The pH of the influent solution may need to be adjusted continually to obtain optimum metal uptake.
- The chemical speciation and interaction of all species in the influent must be understood for proper application.
- A well engineered system is required to control influent concentration and flow rate.

- Plants (especially terrestrial plants) may need to be grown in a greenhouse or nursery and then placed in the rhizofiltration system.
- Periodic harvesting and plant disposal are required.
- Metal immobilization and uptake results from laboratory and greenhouse studies might not be achievable in the field.

In phytovolatilization, the advantages are that contaminants could be transformed to less toxic forms, such as elemental mercury and dimethyl selenite gas, and that contaminants' or metabolites released to the atmosphere might be subject to more effective or rapid natural degradation processes, such as photodegradation [89].

A disadvantage of phytovolatilization is that the contaminant (such as Se) might be released into the atmosphere [84,85,89]. Therefore, adequate planning is needed for phytoremediation-based systems integrated with the environment, e.g., green belts (invaluable ecological niches, particularly in urban industrial areas) and constructed wetlands in which *Eichhornia crassipes* (water hyacinth), *Hydrocotyle umbellata* (pennywort), *Lemma minor* (duckweed), and *Azolla pinnata* (water velvet) are maintained and managed; these can take up Pb, Cu, Cd, Fe, and Hg from aqueous solutions [90].

Nicotianamine (NA), a plant nonproteinogenic amino acid, is an efficient complexing agent for Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, and other divalent transition metals. Genetic manipulation of genes involved in the biosynthesis of metal-sequestering compounds and introduction to desirable plant species might attract phytoremediation strategies. It is very advantageous to use commonly cultivated crops such as *Brassica juncea, Armoracia rusticana*, and *Helianthus annuus*, which are reported to accumulate many toxic metals [91]. Plants amenable to genetic manipulation and *in vitro* culture play a significant role in the success of phytoremediation; however, socioecological and regulatory acceptance using genetically modified organisms and their field trials make up a challenging task in the coming days (Figure 25.12 and Figure 25.13).

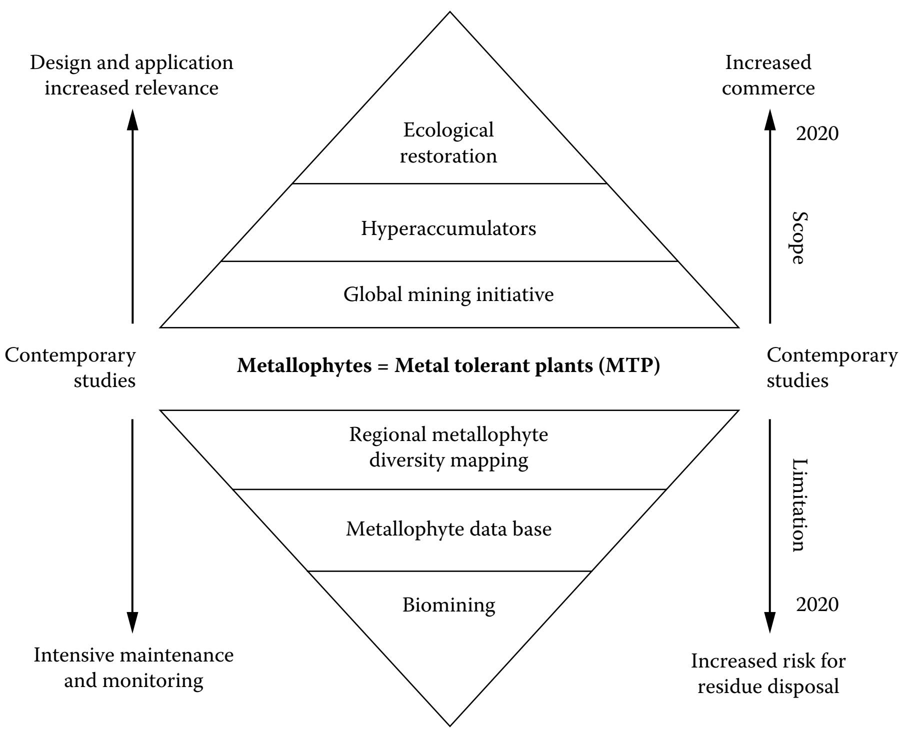

FIGURE 25.12 Advantages and limitations of using metallophytes in phytotechnologies.

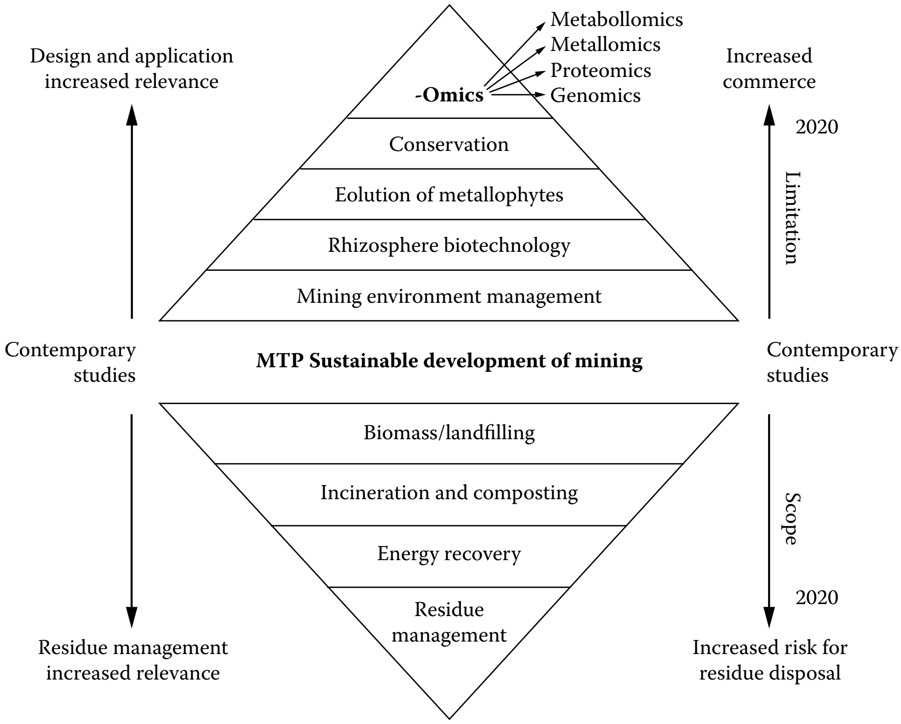

FIGURE 25.13 To move ahead in phytotechnologies, the existing gaps in knowledge must be thoroughly investigated with the available modern tools.

ACKNOWLEDGMENTS

The authors thank Prof. Graciela Munoz, editor in chief, *Electronic Journal of Biotechnology* for according permission to reproduce large portions of text from a previously published article in the online electronic journal http://www.ejbiotechnology.info/content/vol6/issue3/index.html.

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