27 Plants That Accumulate and/or Exclude Toxic Trace Elements Play an Important Role in Phytoremediation

M.N.V. Prasad

CONTENTS

27.1 INTRODUCTION

Global industrialization has resulted in the release of large amounts of potentially toxic trace elements into the biosphere — notably, arsenic, cadmium, lead, mercury, and nickel. Cleaning up most of these soils is necessary to minimize the entry of potentially toxic elements into the food chain. Phytoremediation is an environmental cleanup strategy in which green plants are employed to remove or contain environmentally toxic contaminants, or render them harmless [1]. This strategy is rapidly expanding, highlighting the uses of plants beyond food fiber and fuel.

It is estimated that cleanup of toxic metal using conventional technologies will cost at least $200 billion in the U.S. [4]. The sources of metallic contaminants and pollutants are listed in Table 27.1. Lead is one of the most frequently encountered heavy metals in polluted environments. For example, the primary sources of Pb include mining and smelting of metalliferous ores, burning of leaded gasoline, disposal of municipal sewage, and industrial wastes enriched in Pb, as well as use

TABLE 27.1
Sources of Trace Elements

Contaminant	Major source
Aluminum (Al)	Paper coating pretreatment sludge and drinking sludge
Arsenic (As)	Production of pesticides and veterinary pharmaceuticals, and wood preservatives
Cadmium (Cd)	Cd–Ni battery production, pigments for plastics and enamels, fumicides, and electroplating and metal coatings
Chromium (Cr)	Corrosion inhibitor, dyeing and tanning industries, plating operations, alloys, antiseptics, defoliants, and photographic emulsions
Cobalt (Co)	Steel and alloy production, paint and varnish drying agent, and pigment and glass manufacturing
Copper (Cu)	Textile mills, cosmetic manufacturing, and hardboard production sludge
Lead (Pb)	Battery industry, fuel additives, manufacturing of ammunition, caulking compounds, solders, pigments, paints, herbicides, and insecticides
Mercury (Hg)	Electrical apparatus manufacture, electrolytic production of Cl and caustic soda, pharmaceuticals, paints, plastics, paper products, batteries, pesticides, and burning of coal and oil
Nickel (Ni)	Production of stainless steel, alloys, storage batteries, spark plugs, magnets, and machinery
Selenium (Se)	Coal power plant fly ash
Silver (Ag)	Photographic, electroplating, and mirror industries
Tin (Son)	Can production
Zinc (Zn)	Brass and bronze alloy production, galvanized metal production, pesticides, and ink

Note: Sources of trace elements as soil contaminants have been elaborately detailed in Thangavel, P. and Subbhuraam, C.V., *Proc. Indian Natl. Sci. Acad*., 70, 109, 2004; and Ross, S.M., Ed., *Toxic Metals in Soil Plant Systems*. John Wiley & Sons, Chichester, U.K., 1994, 469.

of Pb-based paint [5]. The threat that heavy metals pose to human and animal health is aggravated by their long-term persistence in the environment. For instance, Pb, one of the more persistent metals, was estimated to have a soil retention time of 150 to 5000 years [6]. Also, the average biological half-life of cadmium has been estimated to be about 18 years [7].

Phytoremediation is an emerging low-cost technology that utilizes plants to remove, transform, or stabilize contaminants located in water, sediments, or soils. Vegetation growing on toxic trace element-contaminated sites is expected to evolve a mechanism of tolerance to withstand the inadequate environment. Therefore, a well adapted flora, tolerant to edaphic climax conditions, is a prerequisite for successful phytoremediation [8,9].

Plants termed phytoremediators are capable of absorbing large amounts of heavy metals from the soil and accumulating these metals in plant tissues [10,11]. Different biochemical studies of heavy metal transport in plants have been conducted [12]. Plants that accumulate and exclude toxic trace elements can play an equally important role in phytoremediation technology. Plants capable of growing on soils contaminated with toxic metals and accumulating extraordinarily high levels of them are shown in Table 27.2. To date, over 450 different hyperaccumulator species have been identified [8]. No one knows why some plants accumulate metals instead of keeping them out. Thus, it is possible to extract and recycle the metals from plants [13].

The ideal phytoextractor should:

- Grow rapidly
- Produce high amount of biomass
- Tolerate and accumulate high concentrations of toxic metals
- Contain substances that deter herbivores from feeding, thus preventing the heavy metal transfer to the food chain

TABLE 27.2
Plants That Hyperaccumulate Trace Elements in Their Tissues

Trace element	Accumulation in plant tissues (mg/g DW)	Number of taxa	Number. of families	Examples
Cd	>0.1	1	1	*Thlaspi caerulescens*
Pb	>1	14	6	*Minuartia verna*
Co	>1	28	11	*Aeollanthus biformifolius*
Cu	>1	37	15	*Aeollanthus biformifolius*
Ni	>1	317	37	*Alyssum bertolonii, Berkheya coddi*
Mn	>10	9	5	*Macadamia neurophylla*
Zn	>10	11	5	*Sedum alfredii Thlaspi caerulescens*
As	>22	2	1	*Pteris vittata, Pityrogramma calomelanos*

Sources: Francesconi, K. et al., *Sci Total Environ.*, 284, 27, 2002; Brooks, R.R., Ed., *Plants That Hyperaccumulate Heavy Metals and Their Role in Phytoremediation, Microbiology, Archeology, Mineral Exploration and Phytomining*. CAB International, New York, 1998; Prasad, M.N.V., Ed., *Heavy Metal Stress in Plants: from Molecules to Ecosystems*. Springer–Verlag, Heidelberg, 2004, 462, xiv; and Hossner, L.R. et al., Amarillo National Resource Center for Plutonium. Amarillo, TX, 1998.

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 increased several thousand-fold by effluents from industrial and mining wastes [14]. Published literature indicates that an array of bioresources (biodiversity) have been tested in the field and laboratory. Remediation programs relying on these materials may be successful [15–17].

The most successful monitoring methods for metals in the environment are based on bacterial heavy metal biosensors, namely, gene-based biosensors and protein-based biosensors [18]. 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. *Pteris vittata* (brake fern), a fast growing Pteridophytes (fern) 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 [19]. The fern possesses three key features that are typical of metal/metalloid hyperaccumulator plants: an efficient root uptake, an efficient root-to-shoot translocation, and a much enhanced tolerance to As inside plant cells. After the discovery of this first As hyperaccumulator, several other fern species, including *Pityrogramma calomelanos, Pteris cretica, Pteris longifolia,* and *Pteris umbrosa* [20,21] have recently been added to the list of As hyperaccumulators. The hyperaccumulation trait of these ferns may be potentially exploitable in phytoremediation of As-contaminated soils.

27.2 METAL HYPERACCUMULATORS FOR PHYTOREMEDIATION HYPE

Metal accumulation in higher plants is a complex phenomenon involving (1) transport of metals across the plasma membrane of root cells; (2) xylem loading and translocation; and (3) detoxification and sequestration of metals at the whole plant and cellular levels [22]. Among angiosperms, about 400 metal hyperaccumulators have been identified that would serve as a reservoir for biotechnological application [17,23]. 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 27.3 shows plant taxa belonging to 45 families are found to be metal tolerant and are 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 [26,27]. Brassicaceae had the highest number of taxa (i.e., 11 genera and 87 species that are established for hyperaccumulation of metals). In Brassicace, Ni hyperaccumulation is reported in seven genera and 72 species [28,29] and Zn in three genera and 20 species [17].

The choice of phytoremediation strategy strongly depends on the risk presented by different metal-polluted soils. For this purpose, instead of chemical analyses, several authors recommend use of plant-based bioassays for risk evaluation. Obviously, metal phytoextraction is not the best approach for industrially polluted soils, where phytostabilization is more appropriate due to a high pollution level suppressing plant growth and productivity. Furthermore, the so-called treatability studies should be conducted before the implementation of phytoextraction technology for the evaluation of the site suitability.

Also, the success of the phytoremediation depends on the nature of the target metal. For example, due to different degrees of soil pollution and solubility, the cases of Cd and Pb are completely different. In many slightly Cd-contaminated agricultural soils, the phytoextraction approach should be more successful than on Pb-polluted soils, which need significant effort to immobilize Pb and to extract much higher metal content. Some crops produce biomass with an added value. For example, crops for industrial products, chemicals, biodiesel and other aromatic compounds.

Certain areas, e.g., rhizosphere biotechnology (and its associated microbes, including mycorrhizae) deserve a much more exhaustive treatment because this is where trace metals come in contact with plant roots in unsterilized field soil conditions. Sources of heavy metal contaminants in soils (Table 27.1) include [30]:

- Metalliferous mining and smelting sites
- Metallurgical industries
- Sewage sludge applications
- Warfare and military training areas or shooting ranges
- Waste disposal sites
- Agrochemicals
- Electronic industries

Once deposited on the soil, certain metals such as Pb and Cr may be virtually permanent [31].

27.3 MECHANISMS OF METAL UPTAKE BY PLANTS

Following mobilization in the rhizosphere, which is controlled by the soil chemistry, the metals must be taken up by the root cells. Transport proteins and intracellular high-affinity binding sites mediate the uptake of metals across the plasma membrane [32]. Several studies have shown that metal hyperaccumulation of Zn and Cd by *T. caerulescens* involves enhanced metal uptake by the roots [33,34]. Several Zn transporter genes have been cloned recently from *T. caerulescens;* these belong to the ZIP (Zn-regulated transporter/Fe-regulated transporter-like proteins) family [35]. These genes, named *ZNTl* and *ZNT2,* are highly expressed in the roots of *T. caerulescens,* but their expression is not responsive to the Zn status of the plant. Through functional complementation in

TABLE 27.3
Plants That Accumulate Trace Elements

Species	Max. Conc. mg/kg	Ref.
Al		
Hydrangea	3,000	47
Miconia acinodendron	66,100	48
As		
Pterir cretica	3,030	21
P. umbrosa	7,600	21
Pityrogramma calomelanos	8,350	20
Pteris vittata L.	22,630	19
Au		
Brassica juncea	57	49
Cd		
Thlaspi caerulescens	2,130	50
Co		
Hibiscus rhodanthus	1,527	51
Cyanotis longifolia	4,197	52
Haumaniastrum robertii	10,232	51
Cr		
Leptospermum scoparium	20,000	53
Dicoma niccolifera	30,000	54
Sutera fodina	48,000	54
Cu		
Ergrostis recemosa	2,800	55
Vigna dolomitica	3,000	55
Pandiaka metallorum Haumanisatrum	6,270	51
katangense	9,222	51
Ipomea alpina Aeollanthus subcaulis	12,300	1
	13,700	56
Hg		
Lemna minor	25,800	57
Mn		
Eugenia clusioides	10,880	58
Macadamia angustifolia	11,500	58
Alyxia rubricaulis	14,000	58
Maytenus pancheriana	16,370	58
M. sebertiana	22,500	58
M. bureaviana	33,750	58
M. neurophylla	55,200	1
Ni		
Cardamine redisifolia	1,050	59
Alyssum singarense	1,280	60
Thlaspi bulbosum	2,000	50
T. japonicum	2,440	61
T. epirotum	3,000	50
Pseudosempervivum sempervium	3,140	62

TABLE 27.3
Plants That Accumulate Trace Elements (continued)

Species	Max. Conc. mg/kg	Ref.
Ni		
Alyssum tenium	3,420	62
A. fallacinum	3,960	50
Thlaspi ochroleucum	4,000	62
Alyssum alpestre	4,480	62
A. euboeum	4,550	63
A. obovatum	4,590	60
A. condensatum	4,900	60
Thlaspi cypricum	5,120	63
Thlaspi montanum var. montanum	5,530	64
Alyssum virgatum	6,230	60
A. smolikanum	6,600	62
A. murale	7,080	62
A. oxycarpum	7,290	60
A. giosnanum	7,390	60
A. peltarioides subsp.	7,600	60
virgatiforme	7,700	63
A. floribundum	7,860	60
A. penjwinensis	8,170	60
A. anatolicum	9,090	60
A. akamasicum	10,000	60
A. serpylifolium	10,000	65
A. bertolonii subsp.scutarinum	10,200	63
A. syriacum	10,400	60
A. crenulatum	10,900	60
A. callichroum	11,400	60
Bornmulleria sp.	11,500	63
Alyssum eriophyllum	11,700	60
A. discolor	11,800	60
Thlaspi tymphaeum	11,900	50
Alyssum trapeziforme	12,000	66
Thlaspi goesingense	12,400	46
T. graecum	12,500	60
Alyssum heldreichii	12,500	63
A. robertianum	13,400	62
A. bertolonii	13,500	50
A. cilicium	13,500	60
A. huber-morathii	13,600	66
Thlaspi kovatsii	13,600	60
Alyssum markgrafii	13,700	67
Streptanthus polygaloides	14,800	60
Thlaspi caerulescens	16,200	60
Alyssum chondrogynum	16,300	60
A. dubertretii	16,500	60
A. carcium	16,500	61
A. troodii	17,100	60
Pseudosempervium aucheri	17,600	50
Alyssum constellatum	18,100	60
Thlaspi rotundifolium var. corymbosum	—	—
corymbosum	18,300	60

TABLE 27.3
Plants That Accumulate Trace Elements (continued)

Species	Max. Conc. mg/kg	Ref.
Ni		
Peltaria dumulosa	18,900	63
Alyssum samariferum	18,900	68
Bornmuellaria glabrescens	19,200	63
Alyssum davisianum	19,600	60
Alyssum cassium	20,000	60
Thlaspi elegans	20,800	61
T. rotundifolium var.corymbosum	18,300	50
A. samariferum	18,900	60
A. pinifolium	21,100	63
Bornmuellera baldaccii	21,300	60
Alyssum pterocarpum	22,200	60
A. lesbiacum	24,000	60
A. cypricum	23,600	60
A. masmenaeum	24,300	61
Thlaspi jaubertii	26,900	50
T. caerulescens	27,300	60
Alyssum argenteum	29,400	63
Thlaspi sylvium	31,000	50
Bornmuellaria tymphea	31,200	63
Peltaria emarginata	34,400	68
Thlaspi oxyceras	35,600	61
Pb		
Polycarpaea synandra	1,044	69
Acer pseudoplatanus	1,955	70
Thlaspi alpestre	2,740	71
T. rotundifolium	8,200	1
Agrostis tenuis	13,490	72
Minuartia verna	20,000	70
Se		
Acacia cana	1,121	73
Atriplex confertifolia Machaeranthera	1,734	74
glabriuscula	1,800	74
Neptunia amplexicaulis Astragalus	4,334	73
bisulcatus	8,840	74
Astragalus racemosus	14,900	75
Lecythis ollaria	18,200	76
Zn		
Thlaspi idahoense	1,150	64
T. caerulescens	1,400	50
Cochlearia pyrenaica	1,680	77
Thlaspi violascens	2,700	61
T. montanum	3,000	64
T. ochroleucum	3,000	50
T. parvifolium	3,090	61, 64
T. liaceum	3,520	61
T. magellanicum	3,890	50
T. bulbosum	10,500	50

TABLE 27.3
Plants That Accumulate Trace Elements (continued)

Species	Max. Conc. mg/kg	Ref.
Zn		
T. praecox	11,000	61
Arabidopsis thaliana	11,000	50
Thlaspi stenocarpum	16,000	50
T. rotundifolium subsp.cepaeifolium	21,000	50
T. rotundifolium	21,000	78
Thlaspi taraense	25,000	50
Rumex acetosa	26,700	70
Thlaspi alpestre	30,000	50
Arabidopsis halleri	39,600	79
Thlaspi calaminare	39,600	50
Thlaspi caerulescens	51,600	80

Note: The tabulation is based on ascending order of metal accumulation for a given element.

Sources: Thangavel, P. and Subbhuraam, C.V., *Proc. Indian Natl. Sci. Acad*., 70, 109, 2004; Prasad, M.N.V., Ed., *Heavy Metal Stress in Plants: from Molecules to Ecosystems*. Springer–Verlag, Heidelberg, 2004, 462, xiv; and Palmer, C.E. et al., *Int. J. Phytoremed.,* 3, 245, 2001.

yeast, it was shown that *ZNT*[1] mediates high-affinity uptake of Zn^{2+} and low-affinity uptake of Cd^{2+} [36]. Specific alterations in Zn-responsive elements, such as transcriptional activators, may play an important role in Zn hyperaccumulation in *T. caerulescens* [36]. However, increased uptake of Cd by *T. caerulescens* cannot be explained by the Zn transport pathway, but may be related to an enhanced expression of the *IRT*[1] gene, which is essential for Fe uptake [22]. The *IRT1* gene was shown to be able to mediate high-affinity uptake of Cd^{2+} in *A. thaliana* [37,38].

Several classes of proteins have been implicated in transport in plants. These include the metal P-type ATPases that are involved in overall ion homeostasis and tolerance in plants, natural resistance-associated macrophage protein (NRAMP) proteins, and cation diffusion facilitator family proteins [39]. CPx type ATPases have been identified in a wide range of organisms and have been implicated in the transport of potentially toxic metals like Cu, Cd, and Pb across cell membranes [39]. These transporters use ATP to pump a variety of substrates across cell membranes.

Arabidopsis P-type ATPase was the first CPx ATPase reported in plants [40]. Most CPx type ATPases identified so far have been implicated in Cu transport. The physiological role of the metal transporters in higher plants is not clearly known. Because *Arabidopsis* CPx ATPases transport different substrates. They may be present in the membranes and function as efflux pumps. They may also be present at various intracellular membranes and be responsible for the compartmentalization of metals, e.g., sequestration in the vacuoles, golgi, or endoplasmic reticulum.

Because cellular levels of metals must be carefully controlled, transporters represent good candidate for their regulation. How they may be regulated at the transcriptional level or at the translational level as was observed in bacteria and yeast [39] mentioned earlier. In higher plants, three *Nramp* homologues have been identified in rice [41].

In *Arabidopsis,* two genes showing similarity to *Nramps* have also been identified [42]. Initial results suggest that *Arabidopsis Nramp* homologues encode functional metal transporters [43]. Northern analysis indicates that the rice *Nramp* gene *OsNrampl* is primarily expressed in the roots, *OsNramp2* in the leaves, and *OsNramp3* in both tissues of rice [41]. This distinct pattern of expression could mean that they are regulated differently and have distinct functions in different tissues or that they transport distinct but related ions in different parts of the plant.

CDF proteins have been primarily implicated in the transport of Zn, Co, and Cd in bacteria and some eukaryotes. Certain members of CDF have been implicated in Cu or Cd transport. A related Zn transporter (*ZAT1*) from *Arabidopsis* was reported by Van der Zaal et al. [44]. This *ZATl* transporter may have a role in Zn sequestration in plants. Enhanced Zn resistance was observed in transgenic plants overexpressing *ZAT1,* expressed constitutively throughout the plant. High Zn exposure of these plants led to increased Zn content in the roots. Zinc transporter (ZIP) proteins have been found to be involved in Zn and Fe uptake [45].

In order to enhance metal uptake, the number of uptake sites could be increased, the specificity of the uptake proteins could be altered, and sequestration capacity could be enhanced by increasing the number of intracellular high-affinity binding sites or the rates of transport across organelles. A comprehensive understanding of the metal transport processes in plants is essential for formulating effective strategies to develop genetically engineered plants that can accumulate specific metals.

27.4 PHYTOMASS OF ACCUMULATORS/HYPERACCUMULATORS OF METALS IS A VALUABLE RESOURCE FOR PHYTOEXTRACTION

Plants are selected according to the application and the contaminants of concern. In temperate climates, phreatophytes (e.g., hybrid poplar, willow, cottonwood, aspen) are often selected because of fast growth, a deep rooting ability down to the level of groundwater, large transpiration rates, and the fact that they are natives of most of the countries. The idea of using plants to remove metals from soils came from the discovery of different wild plants, often endemic to naturally mineralized soils that accumulate high concentrations of metals in their foliage [81] (Figure 27.1).

Ideal attributes for metal accumulators are [2]:

- No geographical preference and fast growth
- High bioproductivity
- Capable of producing multiple use products
- Robust and profuse root system
- Ability for metal hyperaccumulation
- High metal tolerance
- Rapid transport to harvestable plant parts
- Capable of accumulating multiple metals with stable properties
- Resistance to disease and pests

Metal hyperaccumulators usually have an antiherbivory function to minimize human health risks through the trophic chain.

27.5 ACCUMULATION OF METALS BY PLANTS

27.5.1 Root Uptake

Roots can reduce soil-bound metal ions by specific plasma membrane bound metal reductases. Plant roots can solubilize heavy metals by acidifying their soil environment with protons extruded from the roots. All of these processes could also be preformed by mycorrhizal fungi or root-colonizing bacteria. Solubilized metal ions can enter the roots via extracellular (apoplastic) or intracellular

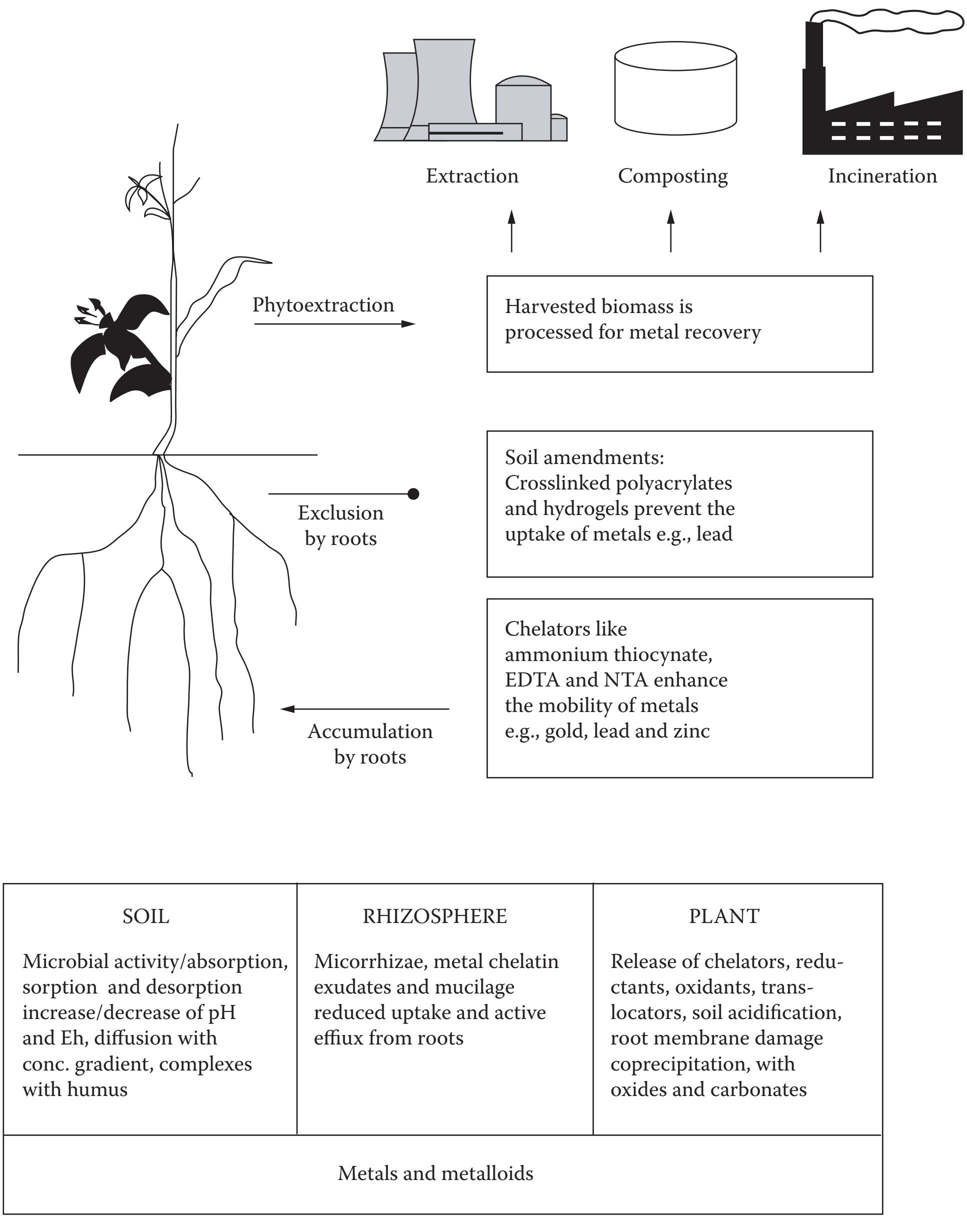

FIGURE 27.1 Phytoextraction consists of (1) planting a species that tends to accumulate and store, transpire, or degrade the target contaminant; (2) letting the crop grow; and (3) harvesting it. Cofiring of phytomass or incineration and composting are concentration methods. Liquid extraction is another separation method. Chelates and soil-amending agents enhance the phytoextraction. The soil–rhizosphere–plant continuum needs critical study for successful phytoremediation [24].

(symplastic) pathways. Nonessential heavy metals may effectively compete for the same transmembrane carriers used by essential heavy metals.

27.5.2 Transport within Plants

Once in the root, metal ions can be stored or exported to the shoot. Metal transport likely occurs in the xylem, but metals may redistribute in the shoot via the phloem. For metals to enter the xylem,

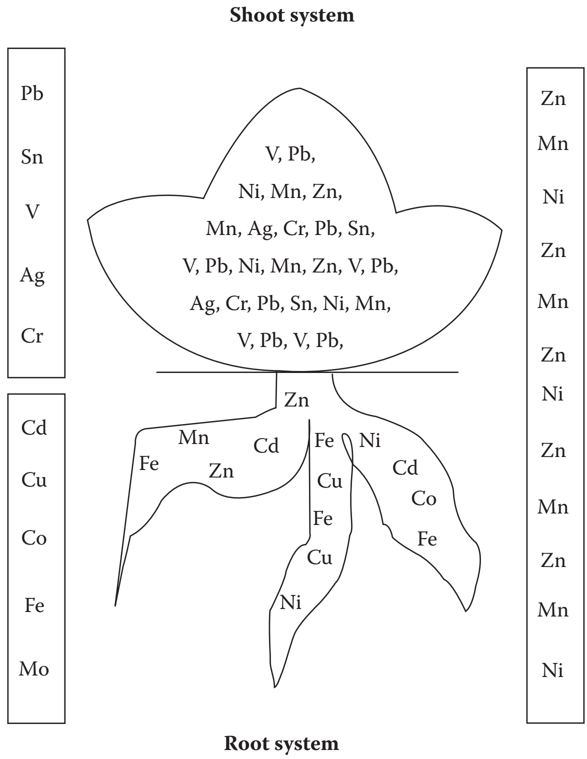

FIGURE 27.2 A generalized pattern of partitioning of metals in the root and shoot system. Silver, chromium, lead, tin, and vanadium accumulate more in shoot (stems and leaves) compared to roots and rhizomes. Cadmium, cobalt, copper, iron, and molybdenum accumulate more in roots and rhizomes than in shoot (stems and leaves). Nickel, manganese, and zinc are distributed more or less uniformly in root and shoot of the plant [82].

they must cross the casparian strip symplastically (intracellular) and this may be the rate-limiting step in metal translocation to the shoot.

27.5.3 Multiple Metal Accumulation

Siedlecka [82] divided the metal-accumulating plants into three categories based on metal accumulation in plant parts (Figure 27.2):

- Accumulate more in roots/rhizomes: Cd, Co, Cu, Fe, and Mo in beetroot, carrot, radish, Jerusalem artichoke, and potato
- Accumulate more in shoots (stems/leaves): Ag, Cr, Pb, Sn, and V in cabbage, cauliflower, tomato, rice, barley, oats, wheat, corn, pigeon pea, chick pea, soybean, peanut, broccoli, lettuce, spinach, and amaranthus
- More or less uniform distribution in roots/shoots: Mn, Ni, and Zn in bush bean, broad bean, mung bean, and cucumber

Soils contaminated with multiple heavy metals can present a difficult challenge for phytoextraction. Although some hyperaccumulators appear to be capable of accumulating elevated concentrations of several heavy metals simultaneously, there is still considerable specificity in metal hyperaccumulation [83]. However, phytoextraction using nontolerant cultivars of *Brassica* sp. is unlikely to succeed in soils contaminated with higher concentrations of Cu, Cd, and Zn, which are usually much more bioavailable, and thus more phytotoxic, than Pb [84]. Generally, monocotyledon species are usually more tolerant to metals than dicotyledon species [85].

The distribution of metals in a contaminated soil is never uniform and, in most agricultural soils, the highest concentrations are usually found near the soil surface. Urban and industrial soils are usually more heterogenous with high metal concentration "hot spots" occurring at depth [86]. Kramer et al. [87] found that, depending on plant species and metal considered, the rates of Ni uptake and root-to-shoot translocation were the same in the Ni hyperaccumulator *Thlaspi goesingense* and the nonaccumulator *Thlaspi arvense*, as long as both species were unaffected by Ni toxicity. Multiple metal hyperaccumulation is particularly advantageous for phytoremediation because soils are often contaminated with various metals such as Cu, Cd, and Zn [88]. Baker et al. [83] reported that, apart from Zn hyperaccumulation, five British populations of *T. caerulescens* also had exceptionally high uptakes of Cd, Co, Mn, and Ni. They suggested that common mechanisms of uptake and translocation existed for several metals in this species.

It is generally accepted that, under natural conditions, a majority of plants have mycorrhizae [89], which have been shown to reduce or enhance metal uptake by plants [85]. Many hyperaccumulators belong to the family Brassicaceae and do not have mycorrhizal associations. It is therefore unlikely that mycorrhizal fungi are directly involved in the enhanced acquisition of metals by Brassicaceous hyperaccumulator plants. In addition, arbuscular mycorrhizal fungi are known to colonize ferns [90], suggesting a possible role of mycorrhizal associations in the recently reported As hyperaccumulation [19]. The effect of mycorrhizal associations on metal root uptake is not clear and appears to be metal and plant specific [91].

The concept of using hyperaccumulator plants to take up and remove heavy metals from contaminated soils was first proposed by Chaney [92]. However, it was not until the early 1990s that field experiments were carried out to test the potential of phytoextraction of metals with hyperaccumulator plants [10]. Hyperaccumulators take up a large quantity of toxic metals through their roots and transport them to the stems or leaves. The word "hyperaccumulator" was coined by the late R.R. Brooks [93] and has been defined as metal accumulation exceeding a threshold value of shoot metal concentration of 1% (Zn, Mn), 0.1% (Ni, Co, Cu, Pb), or 0.1% (Cd) of the dry weight shoot biomass [94].

Compared to nonaccumulator plants, metal concentrations in hyperaccumulator plants are one to three orders of magnitude higher. Apart from these rather arbitrary criteria, hyperaccumulator plants usually have a shoot/root metal concentration ratio greater than one; nonhyperaccumulator plants generally have higher metal concentrations in roots than in shoots [95]. The bioaccumulation factor (shoot/soil concentration ratio) is more important than shoot concentration per se when one considers the potential of phytoextraction for a given species. In metal excluder species, the bioaccumulation factor is typically less than one, and in metal accumulator species, the factor is often greater than one [96].

Several studies indicate the extraction of the various heavy metals (Se, B, Zn, Cd, As, Cu, Co, Ni, Hg, and Cr) used in different industrial processes and other anthropogenic activities from contaminated soils or mine drainage areas using selected hyperaccumulator species [97]. The remediation of other elements (Al, Cs, Sr, and U) from soils by hyperaccumulator crops has not been documented, but is expected to be possible if creative research is applied [98]. The rapidly growing nonaccumulator plants could be engineered so that they achieve some of the properties of hyperaccumulators. Al content of green gram leaves was greater than 1000 mg/kg; therefore, *Vigna radiata* is an Al hyperaccumulator per the definition [99].

Phytoextraction using forestry species in a forestation program is predicted to be a financially attractive option. Trees are potentially the lowest cost plant type used for phytoremediation. Trees have the most massive root systems of all plants; these penetrate the soil for several meters, farther than most herbaceous plants. In some tree species, above-ground biomass can be harvested, and trees will resprout without disturbance of the site. This coppicing shoot system would be valuable if periodic removal of pollutants sequestrated in plant tissue were desirable, as in the case of heavy metals bound to wood often used in chemically enhanced phytoextraction [102]. However, other plant species such as maize and pea (*Pisum sativum* L.) have also been used [103]. The high biomass crop plants, such as Indian mustard, corn, and sunflower, could accumulate significant amounts of Pb when induced through the addition of metal chelates. Simultaneous accumulation of several metals (Pb, Cd, Cu, Ni, Zn) by Indian mustard plants after applying metal chelates has been reported [104]. Metal accumulation efficiency suggested the possibility of using the introduced *Prosopis juliflora* as a metalophyte for the biorecovery of metals from contaminated industrial sites [2,100].

27.6 STRATEGIES FOR ENHANCED UPTAKE OF TRACE ELEMENTS TO FACILITATE PHYTOEXTRACTION

27.6.1 CHELATE-ASSISTED OR CHEMICALLY INDUCED PHYTOEXTRACTION

This strategy of phytoextraction is based on the fact that the application of metal chelates to the soil significantly enhances metal accumulation by plants. The literature to date reports a number of chelates that have been used for chelate-induced hyperaccumulation. These include EDTA, CDTA (*trans*-1,2-diaminocyclohexane-N,N,N′,N′-tetraacetic acid), DTPA (diethylene triaminepentaacetic acid), EGTA [ethyleneglycol-bis(β-aminoethyl ether),N,N,N′,N-tetraacetic acid], EDDHA [ethylenediaminedi (o-hydroxyphenylacetic acid)], HEDTA (N-hydroxyethyl enediaminetriacetic acid), HEIDA [N-(2-hydroxyethyl)iminodiacetic acid], and NTA (nitrilo-triacetic acid) [101].

For chemically enhanced phytoextraction, establishment of a high biomass crop is required before chelate application. *Brassica* sp. are to be directly related to the affinity of the applied chelate for the metal [105]. Therefore, it can be concluded that, for efficient phytoextraction to occur, synthetic chelates having a high affinity for the metal of interest should be used: EDTA for lead, EGTA for cadmium [104], possibly citrate for uranium [106], etc. Also, adding ammonium thiocyanate to the substrate [49] showed that *Brassica juncea* can be induced to accumulate up to 57 mg/kg gold. The mechanisms involved in metal-chelate induced plant uptake and translocation of metals are not well understood. Chemically induced phytoextraction has been described as a two-step process in which plants first accumulate metals in their roots and then, by application of an inducing agent, enhanced transfer of the metals to the shoots occurs [105,107]. This transfer is due to disrupting the plant metabolism that regulates the transport of metal to the shoots. Lombi et al. [84] reported that the application of EDTA alone increases metal mobility in soil and accumulation in roots, but does not substantially increase the transfer of metals to shoots.

Apart from the addition of synthetic chelates, plants secrete to the rhizosphere natural metal-chelating molecules to mobilize soil-bound metals. Thus far, only phytosiderophores, iron-chelating compounds, have been studied in detail. Some of these phytosiderophores include mugeneic and deoxymugeneic acids from barley and corn, and avenic acid from oats [108]. It is also possible that metal-chelating proteins, perhaps related to metalothioneins or phytochelatins, may act as phytosiderophores [81].

Chelate-assisted phytoextraction in field conditions is likely to increase the risk of adverse environmental effects such as ground water pollution due to leaching of metal-laden seepage during extended periods after chelate application. Wenzel et al. [109] hypothesize that free protonated EDTA enters the roots, subsequently forming metal complexes that enhance metal transport to shoots. However, the study of Vassil et al. [110] was conducted in hydroponic conditions, whereas EDTA in soil is expected to form complexes with Ca and other metals. Greman et al. [111] reported ethylenediaminedisuccinate (EDDS) as a promising new chelate for enhanced, environmentally safe phytoextraction of Pb-contaminated soils. It caused only minor leaching of Pb and was significantly less toxic to plants and soil microbes. To avoid possible chelate-metal movement into ground water, the amount, time, and method of chelate application should be carefully controlled.

Due to the severe limitations of chelate-assisted phytoextraction, further efforts should focus on natural, continuous technologies using high biomass perennial plants such as willows or poplar.

27.6.2 Rhizosphere-Assisted Processes for Metal Accumulation and Exclusion

27.6.2.1 Bioavailability of Metals in Soils

Heavy metal accumulation in soils is highly dependant on the availability of metals for plant uptake. Soils consist of a heterogeneous mixture of different minerals (primary minerals, clay minerals, and hydrous oxides of Al, Fe, and Mn); organic and organo–mineral substances, and other solid components. The binding mechanisms for heavy metals are therefore complex and vary with the composition of the soil, soil acidity, and redox conditions. Heavy metal behavior (e.g., mobility, bioavailability) depends upon several factors (Table 27.2), which can be classified as [31]:

- Geochemical characteristics of a metal
- Plant capacity to take up a metal
- Soil chemical equilibria
- Climatic and other environmental variables
- Agricultural or remedial soil management

Generally, the solubility of metal fractions is in the order [112]:

exchangeable > carbonate specifically adsorbed > Fe–Mn oxide > organic–sulfide > residual

Furthermore, only a fraction of soil metal is readily available (bioavailable) for plant uptake. The bulk of soil metal is commonly found as insoluble compounds unavailable for transport into roots. With the exception of Hg, metal uptake into roots occurs from the aqueous phase. In soil, easily mobile metals such as Zn and Cd occur primarily as soluble or exchangeable, readily bioavailable forms. Cu and Mo predominate inorganically bound and exchangeable fractions. Slightly mobile metals such as Ni and Cr are mainly bound in silicates (residual fraction). Soluble, exchangeable, and chelated species of trace elements are the most mobile in soils and govern their migration and phytoavailability [31]. Others, such as Pb, occur as insoluble precipitates (phosphates, carbonates, and hydroxyoxides), which are largely unavailable for plant uptake [113]. Binding and immobilization within the soil matrix can significantly restrict the potential for metal phytoextraction.

Despite the adverse effect on metal root uptake, soil inactivation with chemical amendments has been proposed as a temporary solution for the remediation of metal-contaminated soils, especially for Pb. Also, the effect of soil amendment on bioavailability is metal specific. Increased mobility of metals can be stimulated by plant roots; this includes changes in pH, reducing capacity, the amount and composition of exudates [114], and use of chelating agents. Soil amendments can increase or decrease biological availability of the contaminant for plant uptake. Bioavailability and metal uptake can often be increased by lowering soil pH, adding chelating agents, using appropriate fertilizers (containing ammonium), altering soil ion composition, soil microorganisms, phytosiderophores, and root exudates [2].

- *Soil pH.* The lower soil pH increases concentration of heavy metals in solution via decreasing their adsorption. Soil pH was adjusted using HNO_3 and $CaCo_3$ to provide a range of pH before planting. Acidified treatments were leached to remove excess nitrate before fertilizers were added. Chaney et al. [97] pointed out that, because soil pH is known to affect plant uptake of most heavy metals from soils, studies needed to be conducted to evaluate the independent effect of soil pH and soil metal concentration on hyperaccumulator yield and metal uptake.

- *Chelate amendments.* Chelate additions (EDTA, HEDTA, DTPA, EGTA, EDDHA, NTA, citrate, and hydroxylamine) are commonly used in soil washing technologies because they cause metal desorption from clay minerals and dissolution of certain precipitates such as Fe and Mn oxides. Artificial chelates, such as EDTA, have been tested to enhance metal phytoavailability and subsequent uptake and translocation in shoots. Two strategies have been proposed regarding the mode of chelate application. The chelates may be added at once a few days before harvest [103] or gradually during the growth period [115]. The type of chelate and its time of application are important considerations. Pierzynski and Schwab [116] investigated the effect of chemical amendments on the potential for phytoextraction of several toxic metals including Cd, Pb, and Zn. They showed that addition of limestone, cattle manure, and poultry litter to soil significantly reduced Zn bioavailability. Experiments indicate that biosurfactants have the potential to enhance metal bioavailability in contaminated soil and sediments [117]. EDTA-amended soils increased Pb availability. Therefore, chelator application might pose a risk to the environment [118]. If the metal availability could be locally improved by increasing reductase activity or the amount of chelating agents — e.g., phytosiderophores [119] — without harmful effects on the environment, hyperaccumulators might be used safely for phytoremediation.
- *Soil fertilizers.* Fertilization with N, P, and K more than doubled annual biomass production without reducing the shoot Ni concentration. This suggested that soil fertility management will be important for commercial phytoextraction [120].
- *Competition for sorption sites.* Using the competition of metal ions in solution for sorption sites may also be a useful tool. For example, addition of phosphate to soil may help to extract Cr, Se, and As on exchange sites by binding to the sites, thereby increasing bioavailability.
- *Soil microorganisms.* The soil microbes have been documented to catalyse redox reactions leading to changes in metal mobility in soils and propensity for uptake into roots. For example, chemolithotrophic bacteria have been shown to enhance environmental mobility of metal contaminants via soil acidification or, in contrast, to decrease their solubility due to precipitation as sulfides [121]. Several strains of *Bacillus* and *Pseudomonas* increased the total amount of Cd accumulated by *Brassica juncea* seedlings [122]. Furthermore, soil microorganisms have been shown to exude organic compounds, which stimulate bioavailability and facilitate root absorption of a variety of metal ions, including Fe^{2+} [123], Mn^{2+} [124], and possibly Cd^{2+} [122]. The microbial activity is stimulated by adding carbon substrates such as agricultural wastes [125], water, and nutrients. Growth of crops also provides these materials to the soil microbiota due to standard farming practices and the process of C loss from roots, called “rhizodeposition.” It is interesting that rhizodeposition increases after clipping plants [126]; this could partly explain the enhanced Se removal after cutting treatments, plus the enhanced biomass production.
- *Phytosiderophores.* Plants possess highly specialized mechanisms to stimulate metal bioavailability in the rhizosphere and to enhance uptake into roots [127]. Thus, gramineaceous species (grass sp.) have been documented to exude a class of organic compounds termed siderophores (mugineic and avenic acids) capable of enhancing the availability of soil Fe for uptake into roots [128].
- *Root exudates.* It is well established that roots of many plant species release specific metal-chelating or reducing compounds into the rhizosphere to mobilize Fe and, possibly, Zn [85]. For Zn/Cd/Cu/Pb hyperaccumulators, there are no studies on the role of root exudates in metal accumulation to date.

27.6.2.2 Exclusion of Trace Elements to Foster Phytostabilization

The bioavailability of metal ions depends on their solubility in the soil solution, i.e., their general solubility and the stage of equilibrium between the metal cation in its bound form and the free soluble cation. Because the concentration of heavy metal cations in forest soils is usually so low that the solubility behavior of the metal salts will govern their concentration in the soil solution, the dominating factor for the bioavailability of heavy metal cations in soils is their adsorption to soil structures.

The capacity of a given soil to bind given heavy metals depends on the amount and nature of binding sites in the soil structures and the pH of the soil solution. Generally, it can be stated that the lower the pH value is, the more soluble are the metal cations, and the more binding sites that are available in a given soil, the lower will be the solubility of the heavy metals. In the case of cadmium ions, the increase in solubility with decreasing pH values starts at a pH of 6.5. In the case of lead and mercury ions, it starts at a pH value of 4; ions of arsenic, chrome, nickel, and copper start to dissolve at pH values between these two extremes [129]. Thus, the pH value of the soil solution in principle is one of the main factors governing the solubility of heavy metal cations in the soil solution; its influence on plants under heavy metal stress is well established [130–138]. Unfortunately, the acid deposition prevalent all over Europe during recent decades has increased the mobility of heavy metals considerably [139,140].

Thus, increasing the pH could be a measure to reduce the bioavailability of heavy metals. This has been shown by Walendzik [141] for spruce in the Western Sudety Mountains. Liming, however, may not always be a good solution because it may increase the rate of nitrogen mineralization and thus aggravate the NO_3 load in the groundwater [142–144]. The approach of using waste materials such as fuel ash [145] or sewage sludge [146] to improve the growth of trees on mine spoils may not always be successful, as the authors cited previously have shown. Better results using municipal sewage sludge for establishing sagebrush vegetation on copper mine spoils were reported by Sabey et al. [147]. The simple addition of inorganic fertilizer may not work at all [146].

Another way to decrease the bioavailability of heavy metals is to increase the binding sites for heavy metal ions in the soil, e.g., by amendment with humic substances or zeolites [148] or expanded clay and porous ceramic material [149]. When organic substances are added to the soil, it is very important to work with water-insoluble material, which is not available for rapid degradation by microorganisms [150]. The authors found that an addition of hay to a soil contaminated with heavy metals increased the solubility of Cu, Cd, and Zn, but this effect was not observed for Pb. Amendment of the soil with peat had the opposite effect.

Hüttermann and coworkers applied cross-linked polyacrylates, hydrogels, to metal-contaminated soils. When such a compound (Stockosorb K400) was applied to hydrocultures of Scots pine (*Pinus sylvestris*), which contained 1 μM of Pb, two effects were observed: (1) the hydrogel increased the nutrient efficiency of the plants; and (2) the detrimental effect of the heavy metal was completely remediated. Determination of the heavy metal content of the roots revealed that the uptake of the lead was greatly inhibited by the hydrogel. Analysis of the fine roots of 3-year-old spruce grown for one vegetation period in lead-contaminated soil, with and without amendments with the hydrogel, showed that the amendment of the soil with the cross-linked acrylate did indeed prevent the uptake of the lead into the stele of the fine roots. The hydrogel acts as a protective gel that inhibits the entrance of the heavy metal into the plant root [151].

27.6.2.3 Metal Exclusion by Organic Acids

Organic acids are natural products of root exudates, microbial secretions, and plant and animal residue decomposition in soils [152] (Figure 27.3 and Figure 27.4). These biomolecules have been implicated for altering the bioavailabilities and phytoremediation efficiencies of heavy metals in soils. Some researchers showed that amendment of contaminated soils with organic acids reduced

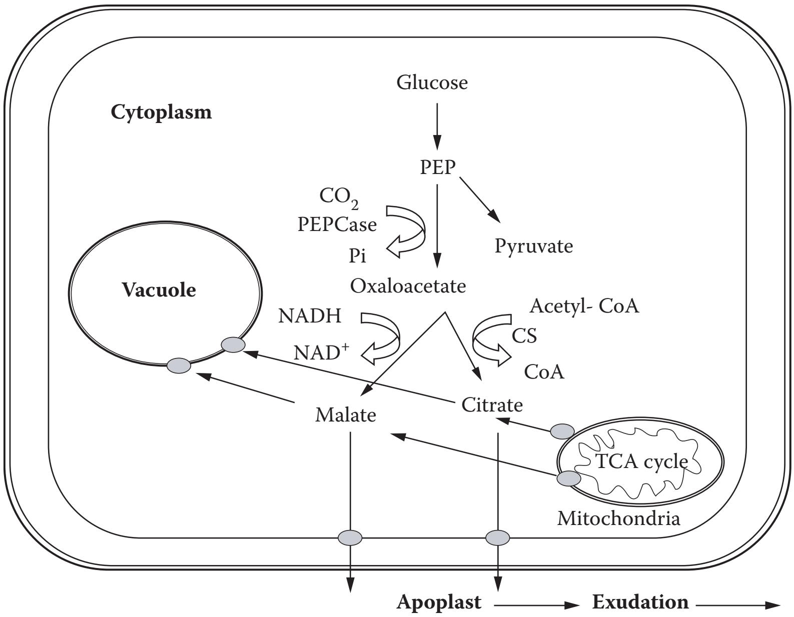

FIGURE 27.3 A comprehensive model to explain the availability of substrates required for the biosynthesis and exudation of organics acids, namely, citrate and malate. These organic acids are present in continuous exchange between mitochondria and the cytosol. Organic acids can be accumulated in the vacuole or excreted into the apoplast by specific carrier proteins, transported towards phloem, and directed to roots for exudation. Plants that exclude toxic trace metals would be the best for photostabilization.

the bioavailability of heavy metals [153]. In contrast, Huang et al. [103] investigated the effect of organic acids amendment of uranium-contaminated soils and found that citric acid significantly increased metal availability and enhanced uranium accumulation many folds in the shoots of selected plants. The contradictory results may be tightly related to the concentration of heavy metal in soil solution and may sequentially be the results of desorption behavior of heavy metal from this soil.

In plants, organic acids may be implicated in detoxification, transport, and compartmentalization of heavy metals. Organic acids are low molecular weight compounds containing carbon, hydrogen, and oxygen and are characterized by one or more carboxylic groups. The number and the dissociation properties of the carboxylic groups determine the negative charges carried by the molecules: the number of metal cations that can be bound in solution or the number of anions that can be displaced from the soil matrix [39]. The most stable ligand–meta1 complexes have the highest number of carboxyl groups available for binding metal cations. Metal complexes with citrate (tricarboxylate) are more stable than those with ma1ate^{2-}, oxalate^{2-}, or malonate^{2-} (dicarboxylate) and acetate (monocarboxylate) [154].

In several plant species, organic acids participate in the metal exclusion mechanism as metal chelators excreted by the root apex outside the plant and in metal hyperaccumulation as metal chelators inside the plant, with various degrees of metal retention within root and shoot [155,156]. The total concentration of organic acids in the root is generally about 10 to 20 m*M*, but may vary depending on the degree of cation–anion imbalance because organic acids often provide the negative charges that balance excess cations [157]. Within the plant cell, organic acids are mainly synthesized in mitochondria through the tricarboxylic acid cycle, but the site of preferential storage is the vacuole. Usually, root vacuoles contain two- to tenfold higher concentrations of malate and citrate than cytosol (5 m*M*) [157] and organometallic chelates can be found in the cell wall, cytoplasm,

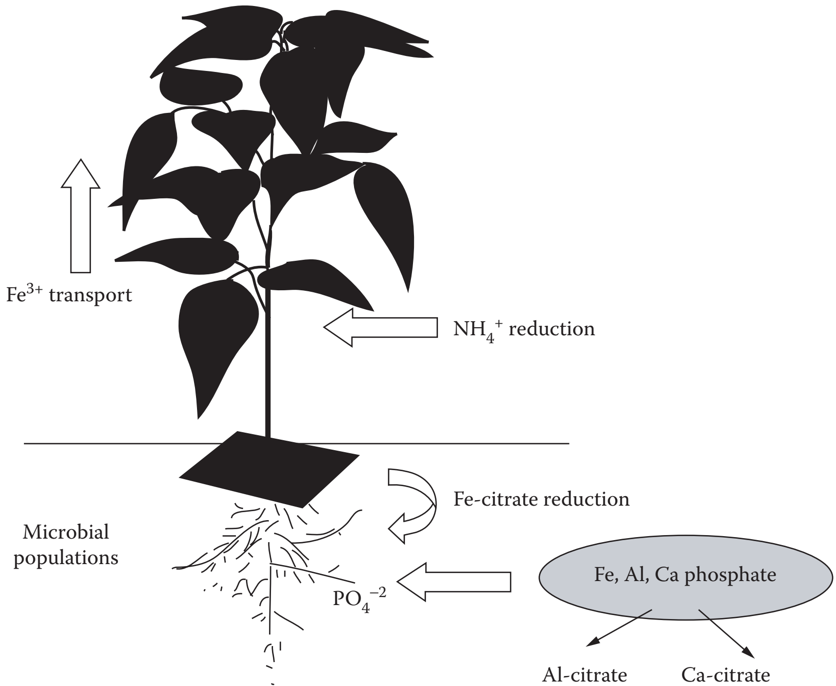

FIGURE 27.4 The ubiquity of organic acids mediating the response of plants to soil stress. Organic acids are strong cation chelators, which act in important adaptive processes in the rhizosphere, such as P and Fe acquisition, Al tolerance, NH4 uptake and reduction, and microbial attraction.

and vacuoles. The composition of root exudates varies greatly, depending on environment, plant species, and age [157–159].

Researchers have found that plant roots exude a variety of organic compounds. Root exudates contain components that play important roles in nutrient solubilization (e.g., organic acids, phyto-siderophores, and phenolics), restricting the passage of toxic metals across the root (e.g., citrate, malate, small peptides) and attracting beneficial microorganisms (e.g., phenolics, organic acids and sugars). Often, the excretion of these organic molecules increases in response to soil stress.

27.7 ORGANIC ACIDS PLAY AN IMPORTANT ROLE IN ADAPTIVE PHYSIOLOGY

Krotsky et al. [160] showed that a sorghum cultivar efficiently colonized by N2 fixing free-living bacteria released more malic, fumaric, and succinic acid than a less active cultivar [160,161]. Rennie [161] reported that the addition of sugars and organic acids to maize inoculated with N2 fixing *Azospirillum brasilense* promoted the incorporation of atmosphere-derived nitrogen in the plant. Succinate and malate stimulated nitrogen fixation more than sucrose [162]. More recently, it was found that aluminum-tolerant wheat cultivars that produce high concentrations of low molecular dicarboxylic acids had higher associative nitrogen fixation rates than nontolerant cultivars did [163].

The adequacy of organic acids as carbon and energy sources has been demonstrated with studies on other microbial species. Some *Campylobacter* sp. isolated from roots of *Spartiana alterniflora* were found to metabolize amino and organic acids efficiently [164]. In legume–*Rhizobium* symbiosis, the preferred substrate taken up by the bacteroids from the host is malate, which may be oxidized to oxaloacetate by malate dehydrogenase or may be converted to acetyl CoA by the malic enzyme and pyruvate dehydrogenase. Further oxidation of acetyl CoA in the tricarboxylic acid cycle can generate the large amount of energy required by the nitrogenase reaction.

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