21 Stabilization, Remediation, and Integrated Management of Metal-Contaminated Ecosystems by Grasses (Poaceae)

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CONTENTS

21.1 INTRODUCTION

Heavy metals are being released into the environment by anthropogenic activities, primarily associated with industrial processes, manufacturing, and disposal of industrial and domestic refuse and waste materials [1]. Mining activities produce a large quantity of waste materials (such as tailings), which frequently contain excessive concentrations of heavy metals. These mining activities and waste materials create pollution and land degradation without vegetation coverage. Phytoremediation of metalliferous mine tailings is necessary for long-term stability of the land surface or removal of toxic metals. Soils and water contaminated with heavy metals pose a major environmental and human health problem that needs an effective and affordable technological solution. Phytostabilization is an important subset of phytoremediation technology in which plants stabilize the pollutants in soils, thus rendering them harmless [2].

Metal-tolerant grasses are preferred for phytostabilization, and heavy metal hyperaccumulators are the best choice for phytoextraction. Plants that can adapt to wetland conditions are useful for phytofiltration. The success of reclamation schemes depends upon the choice of plant species and their methods of [3,4]. There are some important considerations when selecting plants for phytostabilization. Plants should be tolerant of the soil metal levels as well as the other inherent site conditions (e.g., soil pH, salinity, soil structure, water content, lack of major nutrients, and organic materials). Plants chosen for phytostabilization should also be poor translocators of metal contaminants to above-ground plant tissues that could be consumed by humans or animals. Additionally, the plants must grow quickly to establish ground cover, have dense rooting systems and canopies.

The heavy metal acquisition in the members of Poaceae (grasses) is by strategy II, which is much simpler than strategy I. Roots of grasses release to soil specific chelating substances called phytosiderophores, which are mainly derivatives of mugineic acid synthesized from nicotianamine — a nonprotein aminoacid. They extract and chelate ferric ions from soil sorption complex; the whole complex with oxidized Fe ions is taken up by plant. Ferric ions release and their immediate reduction takes place inside root cells. Strategy II is more effective and more resistant to unfavorable environmental factors than strategy I is because two important steps (Fe release from chelate and Fe reduction) are shifted from the rhizosphere into homeostatic conditions inside living root cells. The tolerance of monocots to heavy metals is generally higher than that of dicots and requires rather a long exposure time to heavy metals.

The tolerance of *Agrostis tenuis* Sibth. populations growing in mining areas was investigated by exposing tillers to different concentrations of metals in nutrient solutions. An index of tolerance was calculated by comparing root length with specimens grown in solutions without trace metals [5]. Species of *Agrostis* (Poaceae) have different mechanisms of tolerance to trace metals. Relationships between different species and metal complexation processes in roots and plant tissues have been characterized [6–18] (Table 21.1).

21.2 VETIVER GRASS FOR PHYTOSTABILIZATION OF METALLIFEROUS ECOSYSTEMS

The vetiver grass (*Vetiveria zizanioides L.*) has rather unique morphological and physiological characteristics. It has the ability to control erosion and sedimentation, to withstand extreme soil and climatic variations, and to tolerate elevated heavy metal concentrations in water and soil [107,108]. Vetiver is sterile and noninvasive; it does not compete with native vegetation and, as a nurse plant, it fosters the voluntary return of native plants. Most importantly, however, vetiver is proven technology: its effectiveness as an environmental protection tool has been demonstrated around the world and in some parts of the U.S. [109]. Due to its unique character, vetiver grass has been widely known for its effectiveness in erosion and sediment control [110,111].

The most conspicuous characteristics of vetiver grass include its fast growth, large biomass, strong root system, and high level of metal tolerance; therefore, it is an important candidate for stabilization of metal-contaminated soils. Results from glasshouse studies show that, when adequately supplied with nitrogen and phosphorus fertilizers, vetiver can grow in soils with very high levels of acidity, aluminum, and manganese. Vetiver growth was not affected and no obvious symptoms were observed when soil pH was as low as 3.3 and the extractable manganese reached 578 mg kg^{-1}, and plant manganese was as high as 890 mg kg^{-1}. Bermuda grass (*Cynodon dactylon*), which has been recommended as a suitable species for acid mine rehabilitation, has 314 mg kg^{-1} of manganese in plant tops when growing in mine wastes containing 106 mg kg^{-1} of manganese [112]. Vetiver also produced excellent growth at a very high level of soil aluminum saturation percentage (68%), but it did not survive an aluminum saturation level of 90% at soil pH of 2.0. The toxic level of aluminum for vetiver would be between 68 and 90% [113]. It also has been observed that vetiver thrives well on highly acidic soil with aluminum saturation percentage as high as 87%.

In Australia, *V. zizanioides* has been successfully used to stabilize mining overburden and highly saline, sodic, magnesic, and alkaline (pH 9.5) tailings of coal mines, as well as highly acidic (pH 2.7) arsenic tailings of gold mines [114]. In China, it has been demonstrated that *V. zizanioides* is one of the best choices for revegetation of Pb/Zn mine tailings due to its high metal tolerance [115,116]; furthermore, this grass can also be used for phytoextraction because of its large biomass.

TABLE 21.1
Adaptive Physiological Investigations of Metal Toxicity and Tolerance Using Grasses

Grasses' interaction with metals and associated functions	Ref.
Agrostis capillaries	
Phosphate uptake and transport in L.: effects of nontoxic levels of aluminum and the significance of P and Al speciation	19
Cd–thiolate protein	14
Quantification of metalothionein in small root samples exposed to Cd	9
Isolation and partial purification of Cd-binding protein from roots	10
The amount of Cd associated with Cd-binding protein in roots	11
HPLC characterization of Cd-binding protein	15
Relationship between tolerance to heavy metals	7
A. gigantea	
Differential tolerance of three cultivars to Cd, Cu, Pb, Ni, and Zn	16
Agrostis tenuis	
Pb- and Zn-tolerant populations	6
Combined tolerance to Cu, Zn, and Pb	5
Subcellular distribution of Zn and Cu in the roots of metal-tolerant clones	17
ATPase from the roots: effect of pH, Mg, Zn, and Cu	18
Agrostis tenuis and *A. capillaris* L.	
Relationship between tolerance to heavy metals	7
***Agrostis* sp.**	
Heavy metal-tolerant populations and other grasses	8
Agrostis tennis	
Evolutionary processes in copper-tolerant populations	20
Agrostis stolonifera	
The rapid evolution of copper tolerance	21
The uptake of copper and its effect upon respiratory processes of roots of copper-tolerant and -nontolerant clones	22
Populations resistant to lead and zinc toxicity	6
Heavy metal tolerance in populations of sibth. and other grasses	23
Combined tolerance to copper, zinc, and lead	5
Variability between and within *Agrostis tenuis* populations regarding heavy metal tolerance	24
Selective adaptation to copper in population studies	25
Occurence of metalothioenein in roots tolerant to Cu	12
Avena sativa	
Oxidative damage by excess of Cu	26
Growth and some photosynthetic characteristics of field grown under copper and lead stress	27
Deschampsia cespitosa	
Metal cotolerance	28
Multiple and cotolerance to metals: adaptation, preadaptation and "cost"	29
Role of metallothionein like proteins in Cu tolerance	30
Festuca rubra	
Selective adaptation to copper in population studies	25

TABLE 21.1
Adaptive Physiological Investigations of Metal Toxicity and Tolerance Using Grasses (continued)

Grasses' interaction with metals and associated functions	Ref.
Holcus lanatus	
Environment-induced Cd tolerance	31
Induction and loss of metal tolerance to Pb, Zn, and Cd factorial combination	32
Tolerance to lead, zinc, and cadmium in factorial combination	33
Cadmium tolerance and toxicity, oxygen radical process, and molecular damage in cadmium-tolerant and cadmium-sensitive clones	34
Interactive effects of Cd, Pb, and Zn on root growth of two metal-tolerant genotypes	35
Hordeum vulgare	
Accumulation of cadmium, molybdenum, nickel, and zinc by roots and leaves	36
Compartmentalization and transport of zinc in barley primary leaves is the basic mechanism of zinc tolerance	37
Zinc stress induces changes in apoplasmic protein content and polypeptide composition of barley primary leaves	37
Comparison of Cd-, Mo-,Ni-, and Zn-stress is partially related to the loss of preferential extraplasmic compartmentalization	38
Critical levels of 20 potentially toxic elements	39
Comparative analysis of element composition of roots and leaves of seedlings grown in the presence of Cd, MO, Ni, and Zn	36
Effects of aluminum on the growth and distribution of calcium in roots of an aluminum-sensitive cultivar of barley	40
Oryza sativa	
Isolation of Cd-binding protein from Cd-treated plant	41
Chemical form of Cd and other trace metals	42
Limiting steps on photosynthesis Cu-treated plants	43
Cd content in rice and its daily intake in various nations	44
Cd content in rice and rice field soils in China, Indonesia, and Japan with difference to soil type and daily intake from rice	45
Cd-induced peroxidase activity and isozymes	46
Cd and Ni effects on mineral nutrition and interaction with ABA and GA	47
Heavy metalñhormone interactions in rice plants: effects on growth, net photosynthesis, and carbohydrate distribution	48
Sorghum bicolor	
Detailed characterization of heat shock protein synthesis and induced thermotolerance in seedlings	49
Heat shock proteins in ethanol, sodium arsenite, sodium malonate, and the development of thermotolerance	50
Arsenate-inducible heat shock proteins	50
Triticum aestivum	
Physiological and ultrstructural effects of cadmium	51
Aluminum effects on photosynthesis and elemental uptake in an aluminum-tolerant and -nontolerant wheat cultivars	52
The effect of crop rotations and tillage practices on cadmium concentration in grains	53
Physiological and ultrastructural effects of cadmium in wheat leaves	54

TABLE 21.1
Adaptive Physiological Investigations of Metal Toxicity and Tolerance Using Grasses (continued)

Grasses' interaction with metals and associated functions	Ref.
Citrate reverses the inhibition of wheat root growth caused by aluminum; water relations of wheat cultivars grown with cadmium	55
Differential transport of Zn, Mn, and sucrose along the longitudinal axis of developing grains	56
Transport of zinc and manganese to developing wheat grains	57
Uptake and distribution of ^{65}Zn and ^{54}Mn in wheat grown at sufficient and deficient levels of Zn and Mn, I. during vegetative growth.	59
Uptake and distribution of ^{65}Zn and ^{54}Mn in wheat grown at sufficient and deficient levels of Zn and Mn, II. during grain development	59
Induction of microsomal membrane proteins in roots of aluminum-resistant cultivars of L. under conditions of aluminum stress	60
Induction of microsomal membrane proteins in roots of an Al-resistant cultivars under conditions of Al stress	60
Residual Cd in soil and accumulation in grain	61
Differential aluminum tolerance in a high yielding, early maturing wheat	62
Effect of Cu on active and posssibe Rb influx in roots	63
Influence of Se on uptake and toxicity of Cu and Cd	64
Chemical form of Cd and other heavy metals	42
Mn effects on photosynthesis and chlorophyll	65
Mn produces organic acids in Mn-tolerant and Mn-sensitive cultivars	66
Mn tolerance	67
Chlorophyll and leaf growth as a measure of Mn tolerance	68
Heat shock and protection against metal toxicity	69
A protein similar to pathogenesis-related (PR) proteins is elicited by metal toxicity in roots	70
Chemical form of cadmium and other heavy metals in rice and wheat plants	42
Effect of heavy metals on isoperoxidases of wheat	71
Influence of toxic metals on the multiple forms of esterases	72
Influence of selenium on uptake and toxicity of copper and cadmium in wheat	64
Aluminum induces rapid changes in cytosolic pH and free calcium and potassium concentrations in root protoplasts	73
Excess manganese effect on photosynthetic rate and concentration of chlorophyll grown in solution culture	65
Effects of excess manganese on production of organic acids in Mn-tolerant and Mn-sensitive cultivars of *Triticum aestivum* (wheat)	66
Chlorophyll content and leaf elongation rate as a measure of managanese tolerance	67
Pedigree analysis of manganese tolerance in Canadian spring wheat	68
Influence of aluminum on photosynthetic function in phosphate-deficient	74
Increased nonphotochemical quenching in leaves of aluminum-stressed plants is due to Al $^{3+}$-induced elemental loss	75
Metal content, growth responses, and some photosynthetic measurements on field-cultivated in wheat growing on ore bodies enriched in Cu	76
Citrate reverses the inhibition of root growth caused by Al	55
Crop rotation and tillage practices on Cd accumulationin grain	53
Distribution of Cd, Cu, and Zn in fruit	77
Mechanisms of Al tolerance — role in nitrogen nutrition and differential pH	78–81
Al tolerance is independent of rhizosphere pH	82, 83

TABLE 21.1
Adaptive Physiological Investigations of Metal Toxicity and Tolerance Using Grasses (continued)

Grasses' interaction with metal and associated function	Ref.
Differential uptake and toxicity of ionic and chelated Cu cultivars	78, 79
Kinetics of Al uptake by excised roots ofAl-tolerant and Al-sensitive cultivars	84, 85
Interactive effects of Cd, Cu, Mn, Ni, and Zn on root growth in solution culture	86
Effects of biological inhibitors on kinetics of Al uptake by excised roots and purified cell wall material of Al-tolerant and Al-sensitive cultivars	87
Vetiveria ziznioides	
Integrated management of metal-contaminated environment	88
Zea mays	
Measurements of Pb, Cu, and Cd bindings with mucilage exudates	89
Compartmentalization of cadmium, copper, lead, and zinc in seedlings of maize and induction of metallothionein	90
Silicon amelioration of aluminum toxicity	91
Inhibition of photosynthesis by lead	92
Changes in photosynthesis and transpiration of excised leaves by Cd and Pb	93, 94
The effect of heavy metals on plants, II net changes in photosynthesis and transpiration by Pb, Cd, Ni, and Tl	95
Differing sensitivity of photosynthesis and transpiration to Pb contamination	93
Effect of pH on Cd distribution in maize inbred lines	96, 97
PC concentration and binding state of Cd in roots of maize genotypes differing in shoot and root Cd partitioning	96
Cd distribution in maize inbred lines and evaluation of structural physiological characteristics	97
Zn and Cd in corn plants	98
X-ray microanalytical study and distribution of Cd in roots	99
Compartmentalization of Cd, Cu, Pb, and Zn in seedlings and MT induction	90
Localization of Pb	100
Mobilization of Cd and other trace metals from soil by root exudates	101, 102
Metal-binding properties of high molecular weight soluble exudates	89
Measurements of Pb, Cu, and Cd bindings with mucilage exudates from roots	103
Regulation of assimilatory sulfate reduction by Cd	10
Quantification of metalothionein in small root samples exposed to Cd	104
Cd binding proteins in roots	105
Changes in seedling glutathione exposed to Cd	11, 106

Recent research also suggests that *V. zizanioides* has higher tolerance to acid mine drainage (AMD) from a Pb/Zn mine, and wetland microcosms planted with this grass can effectively adjust pH and remove $SO4^{2-}$, Cu, Cd, Pb, Zn, and Mn from AMD [117]. All of these demonstrate that *V. zizanioides* has great potential in phytoremediation of heavy metal-contaminated soils and water, and an integrated vetiver technique can be developed for remediation of metal pollution, especially in mining areas.

Vetiver grass is tolerant to a wide variety of environmentally adverse conditions and tolerates elevated concentrations of trace elements; its stems are stiff and erect, forming dense hedges [109]. Acid sulfate soils (ASS) are highly erodible and difficult to stabilize and rehabilitate due to extremely acidic conditions, with pH between 3 and 4. Eroded sediment and leachate from ASS are also extremely acidic. The leachate from ASS has led to disease and death of fish in several coastal zones of eastern Australia. Vetiver has been successfully used to stabilize and rehabilitate highly erodible ASS on the coastal plain in tropical Australia, where actual soil pH is around 3.5 and oxidized pH is as low as 2.8 [118].

A series of glasshouse trials was carried out to determine the tolerance of vetiver to high soil levels of heavy metals. A literature search indicated that most vascular plants are highly sensitive to heavy metal toxicity and most plants were also reported to have very low threshold levels for arsenic, cadmium, chromium, copper, and nickel in the soil. Table 21.3 shows results that demonstrate that vetiver is highly tolerant to these heavy metals. Research on wastewater treatment in Thailand found that vetiver grass could absorb substantial quantities of Pb, Hg, and Cd in waste water [119].

21.3 VETIVER IN COMBINATION WITH GREEN MANURE LEGUMES ON LEAD/ZINC MINE TAILINGS

Shu and Xia [120] conducted a field trial to compare growth performance, metal accumulation of vetiver (*Vetiveria zizanioides*), and two legume species (*Sesbania rostrata* and *Sesbania sesban*) grown on tailings amended with domestic refuse and/or fertilizer. The lead (Pb)/zinc (Zn) tailings in China contained high concentrations of heavy metals (total Pb, Zn, Cu, and Cd concentrations of 4164, 4377, 35, and 32 mg kg^{-1}, respectively), and low contents of major nutrient elements (N, P, and K) and organic matter. It was revealed that domestic refuse alone and the combination of domestic refuse and artificial fertilizer significantly improved the survival rates and growth of *V. zizanioides* and two *Sesbania* species, especially the combination.

However, artificial fertilizer alone did not improve the survival rate and growth performance of the plants grown on tailings. Roots of these species accumulated similar levels of heavy metals, but the shoots of two *Sesbania* species accumulated higher (three- to fourfold) concentrations of Pb, Zn, Cu, and Cd than shoots of *V. zizanioides* did. Most of the heavy metals in *V. zizanioides* were accumulated in roots, and the translocation of metals from roots to shoots was restricted. Intercropping of *V. zizanioides* and *S. rostrata* did not show any beneficial effect on individual plant species in terms of height, biomass, survival rate, and metal accumulation, possibly due to the rather short experimental period of 5 months.

One developing alternative remediation technique for metal-contaminated sites is phytostabilization, also called “in-place inactivation” or “phytorestoration.” It is a type of phytoremediation technique that involves stabilizing heavy metals with plants in contaminated soils. To be a potentially cost-effective remediation technique, plants selected must be able to tolerate high concentrations of heavy metals and to stabilize heavy metals in soils by roots of plants with some organic or inorganic amendments, such as domestic refuse, fertilizer, and others. Revegetation of mining wastes is one of the longest practiced and well documented approaches for stabilization of heavy metals in mining wastes [3].

Shu and Hanping conducted field experiments to assess the role of vetiver grass in phytostabilization of metal-contaminated sites at Guangdong Province, South China. They compared the growth of four grasses (*Vetiveria zizanioides*, *Paspalum notatum*, *Cynodon dactylon*, and *Imperata cylindraca* var. *major*) on Lechang Pb/Zn mine tailings with different amendments, for screening the most useful grass and the most effective measure for revegetation of tailings. They also investigated the abilities of heavy metal accumulation in the four tested plants for assessing their different roles in phytoremediation.

They observed that the concentrations of Pb, Zn, and Cu in shoots and roots of *V. zizanioides* were significantly less than those of the other three species and the shoot/root metal concentration quotients (MS/MR for Pb, Zn, and Cu) in *V. zizanioides* were also lower than those of other three species, which indicated that *V. zizanioides* was an excluder of heavy metals. First, roots of the species accumulated low levels of metals by avoiding or restricting uptake. Second, shoots of the species accumulated much lower concentrations of metals by restricting transport. In general, metal tolerance and metal uptake were functionally related; exclusion was one of the basic strategies of metal uptake by tolerant species [121]. Judging from the metal contents in plant tissues, *V. zizanioides* was more suitable for phytostabilization of toxic mined lands than *P. notatum* and *C. dactylon*, which accumulated relatively high levels of metals in their shoots and roots. It was also noted that *I. cylindrica* accumulated lower amounts of Pb, Zn, and Cu than *C. dactylon* and *P. notatum* did and could also be considered for phytostabilization of tailings.

21.4 TOLERANCE OF VETIVER GRASS TO SUBMERGENCE

These grasses are ideal for biological or ecological measures protecting or stabilizing inner slopes of rivers, reservoirs, and lakes, because plants established on the inner slopes are almost all drowned by the elevated water level in the rainy season. Thus, the key is to screen out plant species strongly tolerant to submergence in order to stabilize and vegetate the "wet" slopes effectively (Table 21.2).

The selected eight grasses were all excellent plant species for soil and water conservation in southern China. The common features of the eight species are high erosion control and high resistance to adverse conditions. They all have been applied widely in erosion control and slope stabilization in southern regions of China. Among them, vetiver is the sole high-stalk type; the other seven species are procumbent types. These plants were raised in pots first and then put into a cement tank filled with water to investigate their tolerance to complete submergence. In an experiment conducted for 3 years, Xia et al. [122] have observed that vetiver and Bermuda grass could tolerate the longest time of submergence — at least up to 100 days — and probably more than that.

21.5 REHABILITATION OF GOLD MINE TAILINGS IN AUSTRALIA

The environment of fresh and old gold tailings is highly hostile to plant growth. The fresh tailings are typically alkaline (pH = 8 to 9); low in plant nutrients; and very high in free sulphate (830 mg kg^{-1}), sodium, and total sulfur (1 to 4%). Vetiver established and grew very well on these tailings without fertilizers, but growth was improved by the application of 500 kg $ha^{-1}$of di-ammonium phosphate. Due to high sulfur content, old gold tailings are often extremely acidic (pH = 2.5 to 3.5), high in heavy metals, and low in plant nutrients. Revegetation of these tailings is very difficult and often very expensive and the bare soil surface is highly erodible. These tailings are often the source of above-ground and underground contaminants to the local environment. When vetiver was

TABLE 21.2
A Comparative Study on the Tolerance of Eight Grasses to Submergence

Scientific name of the grass	Common name of the grass	Duration of submergence
Vetiveria zizanioides Nash	Vetiver grass	100
Cynodon dactylon Pars	Bermuda grass	100
Paspalum notatum Flugge	Bahia grass	70
Axonopus compressus Beauv	Carpet grass	30–40
Chrysopogon aciculatus Trin	Aciculate chrysopogon	25–30
Paspalum conjugatum Bergius	Sour paspalum	25–30
Stenotaphrum secundatum Kuntze	St. Augustine	20–30
Eremochloa ophiuroides Hack.	Common centipede grass	10

Source: Xia et al., *Proc. 3rd Int. Vetiver Conf.*, Guangzhou, China, 532.

adequately supplied with nitrogen and phosphorus fertilizers, excellent growth was obtained on sites with pH ranging from 2.7 to 3.6, high in sulphate (0.37 to 0.85%), total sulfur (1.31 to 3.75%), and low in plant nutrients. Liming was not needed on sites with higher pH (3.5), but the addition of 20 t ha^{-1} of agricultural lime significantly improved vetiver growth on sites with pH of 2.7.

21.6 REHABILITATION OF MINE TAILINGS IN SOUTH AFRICA AND CHINA

Rehabilitation trials conducted by De Beers on slime dams at several sites have found that vetiver possess the necessary attributes for self-sustainable growth on kimberlite spoils. Vetiver grew vigorously on the alkaline kimberlite and contained runoff, arrested erosion, and created an ideal microhabitat for the establishment of indigenous grass species [123]. Vetiver has also been used successfully in the rehabilitation diamond mines at Premier and Koffiefonteine and slime dams at the Anglo–American platinum mine at Rastenburg, and the Velkom, President Brand gold mine (Tantum, pers. com.). In China, vetiver produced biomass more than twice that of *Paspalum notatum, Cynodondactylon,* and *Imperata cylindrica* in the rehabilitation of the Lechang Pb and Zn mine; tailings there contain very high levels of heavy metals (Pb at 3231 mg kg^{-1}, Zn at 3418 mg kg^{-1}, Cu at 174 mg kg^{-1}, and Cd at 22 mg Kg^{-1}) [124].

Vetiver grass has also been successfully used to rehabilitate coal mine tailings that were saline and highly sodic, with high levels of soluble sulfur, magnesium, and calcium and extremely low nitrogen and phosphorus levels [125]. Bentonite tailings are extremely erodible because they are highly sodic (with exchangeable sodium percentage (ESP) values ranging from 35 to 48%), high in sulphate, and extremely low in plant nutrients. Vetiver established readily on these tailings and effectively controlled erosion, conserved moisture, and improved seedbed conditions for the establishment of indigenous species. Residue of bauxite processing known as red mud is highly caustic, with pH levels as high as 12. Vetiver established successfully on the red mud when its pH was raised to 9.0.

Vetiver has been used very successfully for this purpose in Australia to contain leachate runoff from landfills. For this application, high-density planting is required at strategic locations such as the toes of major slopes of highly contaminated areas.

The vetiver system is proven technology; its effectiveness as an environmental protection tool has been demonstrated around the world. It is a very cost-effective, environmentally friendly, and practical phytoremedial tool for the control and attenuation of heavy metal pollution when appropriately applied.

Jarvis and Leung [126] observed *Chamaecytisus palmensis* plants growing in hydroponic culture exposed to Pb(NO3)2, with and without the addition of the chelating agents H-EDTA and EDTA, for 1 week. The unchelated lead accumulated predominantly in root tissue; lead chelated with H-EDTA or EDTA was taken up principally by the shoots. With transmission electron microscopy, ultrastructural observations were carried out on ultrathin sections derived from lead-treated *C. palmensis* tissues. Unchelated lead was found in cell walls, bacteroids and mitochondria in root nodule tissue, and in middle lamellae and intercellular spaces in root tissues. In roots, chelated lead was found in mitochondria, and in shoot tissues it was found in chloroplasts, pit membranes, and plasmodesmata.

21.7 EXPRESSION OF STRESS PROTEINS BY THE MEMBERS OF POACEAE

The relationship between some plant hormones and heavy metal stress in *Oryza sativa*; trace element stress and expressionof signalling/metabolic pathways in some Poaceae are detailed in Tables 21.3 and 21.4 respectively.

TABLE 21.3
Relationships between Some Plant Hormones and Heavy Metal Toxicity in *Oryza sativa*

Phytohormone	Function	Ref.
GA_3	Partially reverses the effect of Cd or Ni	48
ABA	Enhances plant growth inhibition by Cd or Ni; does not affect the influence of Al on growth	48; 130

TABLE 21.4
Trace Element Induced Stress and Expression of Signaling/Metabolic Pathways in Some Poaceae

Signaling/metabolic function	Metal	Plant	Ref.
$[Ca^{2+}]_c$ concentration (Ca-dependent signaling):			
Increased			
	Al	*Hordeum vulgare* *Triticum aestivum*	40
Decreased	Al	*Triticum aestivum*	73
Expression of stress-responsive gene:			
Induced	Cu, Al, Cd, Co	*Hordeum vulgare*	131
Not induced	Cd, Cu, Zn, Al		
Ethylene content increased	Cd, Cu,Zn, Al	*Triticum aestivum*	132
Induction of Octadecenoic pathway	Fe^{2+}	*Oryza sativa*	70
	Cu	*Oryza sativa*	133

Cd induced 70-kDa protein in roots of maize (*Zea mays* L.), which was precipitated by hsp70 antibodies. *In vitro* phosphorylation assay showed that the hsc70 (heat shock proteins induced by factors other than heat were termed “hsp cognates” [hsc]) is a phosphoprotein. Phosphoamino acid analysis of immunoprecipitated hsc70 by paper chromatography showed that serine and tyrosine are phosphorylated in control seedlings. Serine (but not tyrosine) is phosphorylated in Cd-treated seedlings in spite of increase in hsc70 amount [127]. This could possibly be due to inhibition of a tyrosine kinase.

This change in hsp70 cognate phosphorylation might be playing the role of a chaperone. Possibly, the hsc70 in maize might be acting to limit and rescue the damage to proteins caused by environmental stress like other chaperones [127]. Cd induced a number of stress proteins ranging in molecular weight from 10 to 70 kDa. In *Oryza sativa*, Cd-induced proteins are 70-, 42-, 40-, 26-, 23-, 15-, and 11-kDa proteins [128]. Similar results were also noticed in Cd-treated maize [127–129].

Barley and maize seedlings exhibited retardation in shoot and root growth after exposure to Cu, Cd, and Pb. The Zn ions practically did not influence these characteristics. The total protein content of barley and maize roots declined with an increase in trace metal concentration [134,135]. Total glutathione content was decreased when exposed to Cd (more so in roots than in shoots). Robinson and Jackson [136] suggested the involvement of glutathione in the biosynthesis of PCs. Rauser and Curvetto [13] have isolated Cu-binding complexes from *Agrostis gigantea*. In oat (*Avena sativa*) roots, Cd transport from cytosol to vacuole across the tonoplast is demonstrated through Cd^{2+}/H^+ antiport activity [137]. Compartmentalization, complexation, and transport are the primary and basic mechanisms involved in Zn tolerance in barley (*Hordeum vulgare*) [37]. In wheat, transport of Rb and Sr to the ear in mature excised shoots was affected by temperature and stem length and also, perhaps, the ligands in the xylem [138].

Barley (*Hordeum vulgare* L.) was used as a bioassay system to determine the mobility of heavy metals in biosolids applied to the surface of soil columns with or without plants. Three weeks after barley was planted, all columns were irrigated with the disodium salt of the chelating agent, EDTA (ethylenediamine tetraacetic acid). Drainage water, soil, and plants were analyzed for heavy metals (Cd, Cu, Fe, Mn, Ni, Pb, and Zn). Total concentrations of the heavy metals in all columns at the end of the experiment generally were lower in the topsoil with EDTA than in that without EDTA. The chelate increased concentrations of heavy metals in shoots.

With or without plants, the EDTA mobilized Cd, Fe, Mn, Ni, Pb, and Zn, which leached to drainage water. Drainage water from columns without EDTA had concentrations of these heavy metals below detection limits. Only Cu did not leach in the presence of EDTA. Even though roots retarded the movement of Cd, Fe, Mn, Ni, Pb, and Zn through the EDTA-treated soil, the drainage water from columns with EDTA had concentrations of Cd, Fe, Mn, and Pb that exceeded drinking water standards by 1.3, 500, 620, and 8.6 times, respectively. Because the chelate rendered Cd, Fe, Mn, Ni, Pb, and Zn mobile, it is suggested that the theory for leaching of soluble salts, put forward by Nielsen and associates in 1965, could be applied to control movement of the heavy metals for maximum uptake during chelate-assisted phytoremediation using grasses [139] (Figure 21.1).

Chen et al. [140] used a modified glass bead compartment cultivation system in which glass beads were used in the hyphal compartment but replaced by coarse river sand in the compartments for host plant roots and mycorrhizal hyphae. Arbuscular mycorrhizal (AM) associations were established using maize (*Zea mays* L.) and two AM fungi, *Glomus mosseae* and *G. versiforme*. When the standard and modified cultivation systems were compared, the new method yielded much more fungal tissue in the hyphal compartment. Using *G. versiforme* as the fungal symbiont, up to 30 mg of fungal dry matter (DM) was recovered from the hyphal compartment of mycorrhizal maize and about 6 mg from red clover.

Multielement analysis was conducted on samples of host plant roots and shoots and on harvested fungal biomass. Concentrations of P, Cu, and Zn were much higher in the fungal biomass than in the roots or shoots of the host plants but fungal concentrations of K, Ca, Mg, Fe, and Mn were similar to or lower than those in the plants. Nutrient concentrations were also significantly different between the two AM fungi; these may be related to differences in their proportions of extraradical mycelium to spores. The high affinity of the fungal mycelium for Zn was very striking and is discussed in relation to the potential use of arbuscular mycorrhiza in the phytoremediation of Zn-polluted soils.

Vetiver grass is a tall perennial tussock grass from Asia that has been used in a variety of soil conservation applications in that region. Interest in this grass outside Asia is increasing, but its application is handicapped by a lack of quantitative knowledge of its flow-retarding and sediment-trapping capability [88]. Vetiver grass hedge is being considered for control of soil erosion on a cultivated flood plain of low slope subject to overland flow [88].

Rengel [142] for the first time reported the coexistence of traits for tolerance to Zn toxicity and Zn deficiency in a single plant genotype (i.e. *Holcus lanatus*). Genotypes tolerant to zinc (Zn) toxicity accumulated Zn in their roots better than Zn-sensitive genotypes, even in Zn-deficient soil. *Holcus lanatus* L. ecotypes differing in tolerance to Zn toxicity were grown in Zn-deficient Laffer soil, which was amended with Zn to create a range of conditions from Zn deficiency to Zn toxicity. Increasing Zn additions to the soil, up to the sufficiency level, improved growth of all ecotypes. At toxic levels of added Zn, the Zn-sensitive ecotype suffered a greater decrease in growth than the Zn-tolerant ecotypes. All ecotypes accumulated more Zn in roots than in shoots, with root concentrations exceeding 8g Zn kg^{-1} dry weight in extreme cases.

When grown in Zn-deficient or Zn-sufficient soil (up to 0.5mg Zn kg^{-1} soil added), ecotypes tolerant to Zn toxicity took up more Zn, grew better, and had greater root and shoot Zn concentration than the control (Zn-sensitive ecotype). Zn-tolerant ecotypes transported more Zn, copper (Cu), and iron (Fe) from roots to shoots in comparison with the Zn-sensitive ecotype. The average Zn uptake rate from Zn-deficient soil (no Zn added) was greater in the Zn-tolerant ecotypes than in the Zn-sensitive ecotype. In conclusion, ecotypes of *H. lanatus* tolerant to Zn toxicity also tolerate

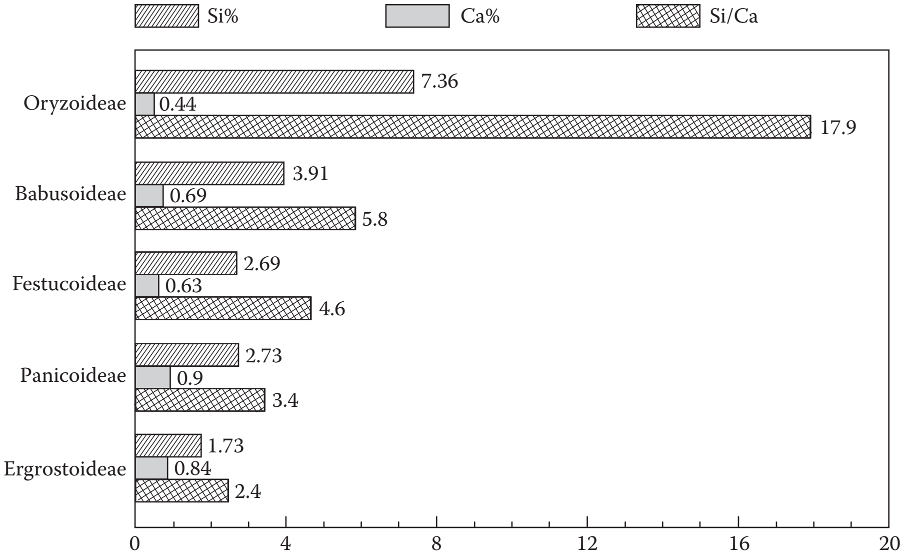

FIGURE 21.1 Ranking of silicon, calcium percentage, and Si/Ca ratio in the subfamilies of Poaceae.

Zn deficiency better than the Zn-sensitive ecotype because of their greater capacity for taking up Zn from Zn-deficient soil.

Zinc tolerance in *Setaria italica* L. *in vitro* was achieved by Samantaray et al. [142] through callus culture derived from leaf base and mesocotyl explants on Murashige and Skoog's medium, supplemented with 0.5 mg/l kinetin and 3.0 mg/l 2,4-D + 0.24 m*M* zinc. Tolerant calli showed vigorous growth in medium containing 0.24 m*M* zinc compared to the non-tolerant calli. Biochemical studies on the basis of activities of peroxidase and catalase as well as estimation of protein and chlorophyll were more in tolerant calli than non-tolerant ones. The accumulation of zinc in the callus was increased significantly with the increase in zinc concentrations in the medium. Plant regeneration via somatic embryogenesis was achieved in tolerant and non-tolerant calli on MS medium containing 1.0 mg/l BA, 1.0 mg/l Kn, and 0.5 mg/l 2,4-D. The somatic embryo-derived plantlets were tested in MS liquid medium with 0.24 m*M* zinc for selection of tolerant clones. This study may help in the selection and characterization of metal-tolerant lines of *Setaria italica* for breeding programs [142].

Using a pot experiment, Chen et al. [143] investigated the uptake of cadmium (Cd) by wetland rice and wheat grown in a red soil contaminated with Cd. The phytoremediation of heavy metal-contaminated soil with vetiver grass was also studied in a field plot experiment. Results showed that chemical amendment with calcium carbonate (CC), steel sludge (SS), and furnace slag (FS) decreased Cd uptake in wetland rice and wheat by 23 to 95% compared with the unamended control. Among the three amendments, FS was the most efficient at suppressing Cd uptake by the plants, probably due to its higher content of available silicon (Si). The concentrations of zinc (Zn), lead (Pb), and Cd in the shoots of vetiver grass were 42 to 67%, 500 to 1200% and 120 to 260% higher in contaminated plots than in control, respectively. Cadmium accumulation by vetiver shoots was 218 g Cd/ha at a soil Cd concentration of 0.33 mg Cd/kg. These results suggest that heavy metal-contaminated soil could be remediated with a combination of chemical treatments using rapidly growing grasses.

The U.S. introduced automobile exhaust catalysts in 1974, followed later by Japan and Europe. This resulted in enormous emissions of platinum (Pt) into the environment, far greater than had been expected. For Pt speciation *Lolium multiflorum* (forage plant) was found to be a reliable bioindicator, in particular for heavy metals [144]. It was shown that Pt was bound to a protein in

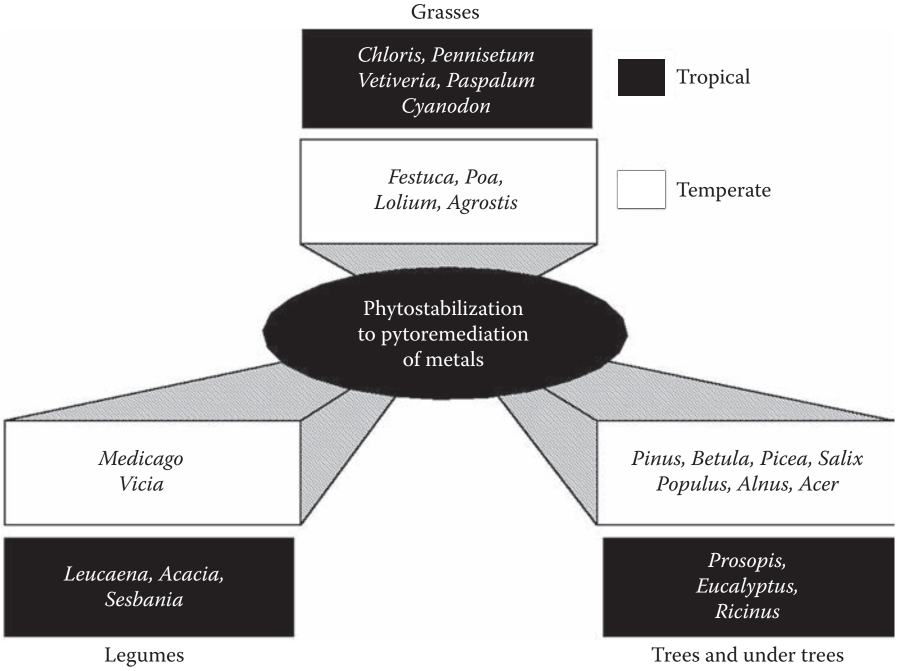

FIGURE 21.2 A community of grass, legume, and tree-based phytoremediation systems would be advantageous.

the high molecular mass fraction in this grass species (160 to 200 kDa) [145]. In Pt-treated cultures, about 90% of the total Pt was observed in a fraction of low molecular mass (<10 kDa) [145]. The Pt-binding ligands could be identified only tentatively; some Pt coeluted with phytochelatin fractions and some with polygalacturonic acids [146].

21.8 SIGNIFICANCE OF SILICON-ACCUMULATING GRASSES FOR INTEGRATED MANAGEMENT AND REMEDIATION OF METALLIFEROUS SOILS

Silica is the second most abundant element in the Earth's crust, so it would be useful for amelioration of aluminum (Al) toxicity in acidic soils. Ranking of silicon accumulation in the subfamilies of Poaceae is shown in Figure 21.2. Subsoil acidification is a serious global environmental concern. Acid soils occupy nearly 30% (3.95 ha) of the arable land area in tropical and temperate belts. In addition to the natural processes, farming and management practices such as high use of nitrogen fertilizers, removal of cations by harvested crops, and leaching and runoff of cations resulted in acidification of soils. In many industrialized areas, the atmospheric deposition of sulfur and nitrogen compounds is a major source of proton influx to soils. More than the low pH of the soils, the major problem associated with acid soils is the toxicity of Al and manganese and the deficiency of phosphorus, calcium, magnesium, and potassium.

In addition to these nutritional factors, the acid soils are also characterized by low water-holding capacity due to compaction of soils. Apart from mineral toxicities, soil acidification is also known to change the species spectrum of the forest soils by changing the microbial activity of the soils. Acidification is also known to reduce the degradation of soil organic matter and alters cation and nutrient flow in the ecosystem. In general, most of the acid soils have low exchangeable bases (e.g., Ca, Mg, and K), mainly because of the low cation exchange capacity (CEC) of the soils. The oxides of Al and Fe in wet acidic soils fix large fractions of the phosphate, making it unavailable to plants

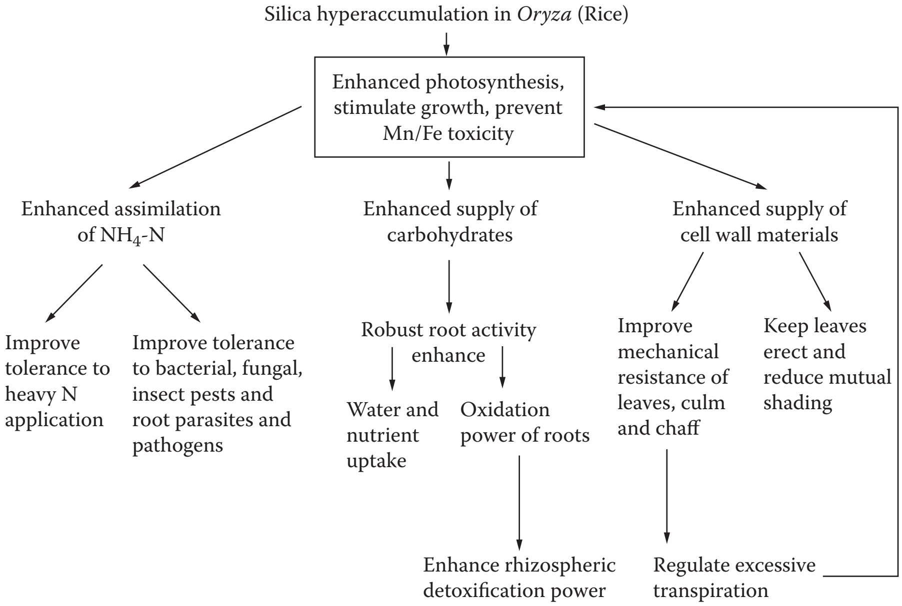

FIGURE 21.3 Silica hyperaccumulation in rice and its functions.

and leading to lower crop yields. Therefore, Al toxicity is the major agronomic problem in acid soils and it has been reported that silica had an ameliorative function [147] (Figure 21.3).

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