28 Phytoremediation of Trace Element Contaminated Soil with Cereal Crops: Role of Fertilizers and Bacteria on Bioavailability

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CONTENTS

28.1 INTRODUCTION

The selection of appropriate plant species is a cornerstone of successful application of phytoremediation methods and probably one of the most important factors affecting the extent of metal removal from contaminated soils. As a general rule, native plant species are preferred to exotic plants that can affect the harmony of the ecosystem [1]. The optimum metal phytoextraction plants should be able to accumulate and tolerate rather large amounts of toxic metals. Combined with a rapid growth rate and the potential to produce large biomass in the field, this can help to remove more metals per planting. Thus, the main goal is to find species able to accumulate large amounts of metals in harvestable plant parts without harmful consequences for the plants.

In the last two decades, the development of phytoremediation was mainly focused on the use of plants as hyperaccumulators [2–7]. Although the metal-accumulating plants have a good potential, so far there is no clear evidence that the plants may be actually used for commercial remediation of contaminated soils. Most hyperaccumulators are relatively small and grow slowly and often can accumulate only one metal; however, in many cases, metal pollution of soils is a multielement problem. Therefore, one of the alternative ways is to identify plants with a large biomass production capable of mobilizing several metals in the rhizosphere, assimilating the metals, and tolerating high metal contents in their tissues.

Recently, phytoremediation has been modified to include certain native grasses as potential candidates [8,9]. Plant species with large biomass are easily available around the world and, though they cannot be classified as hyperaccumulators, these plants can compensate the lower metal concentrations in their aboveground parts by greater biomass production and higher transpiration rate. These factors are critical in proving the successful field applicability of phytoremediation techniques. Comparing a large mass of soil with a mass of the plants to be used for soil cleaning, it becomes apparent that only large biomass species able to take up great amounts of metals may be used for phytoremediation of metal-contaminated soils.

Moreover, as a "soft" technique, phytoextraction may be successfully used probably only for remediation of slightly, and sometimes moderately, contaminated soils. In this case, an ability of certain plant species with high root and shoot biomass to affect chemical situation in the rhizosphere (by producing specific organic exudates capable of transferring metals to more soluble and more available to plant forms) may be expected to result in actual metal removal from contaminated soils. The attempt to use phytoextraction for cleaning highly contaminated soils is probably the main problem of phytoremediation philosophy. Each technique has certain limits where it may be the most suitable. It seems that phytoextraction procedures may be successfully applied mainly for remediation of slightly contaminated soils. Furthermore, even in this case we will have to "help" plants: first, by enhancing plant yields; second, by stimulating an increased uptake and translocation of several metals; and, last, by improving the ability of plants to tolerate high contents of metals in their tissues.

To identify the plant species best adapted for site-specific conditions (different levels and types of metal contamination, physicochemical parameters of soil), it is necessary to screen various natural and cultural species and evaluate these species in controlled greenhouse and field conditions. This will help to increase the number of potential candidate plants by expanding the type, range, and adaptability of the species perspective for metal phytoextraction and phytoaccumulation.

28.2 APPLICATION OF CEREAL CROPS IN PHYTOREMEDIATION STUDIES

The most important group of cultivated plants is cereal crops, which include eight genuses: wheat, rice, rye, oats, barley, corn (maize), sorghum, and some of the millets. Wheat, oats, barley, and rye can grow under relatively cold conditions (10 to 12°C). Rice, maize, and sorghum are warm-season crops; rice is unique in that it can germinate and thrive in water. Proso millet is a short-season crop grown on a small acreage in the areas of spring-sown grains [10].

In spite of wide diversity, cereal crops have many common morphological characters. The cereals have fibrous roots and the main root biomass is located in the arable layer. The depths of root penetration differ among different cereal crops: more than 100 cm for wheat, 120 to 200 cm for rye, and up to 200 cm for corn. Notice that plant species with fibrous roots and relatively large proportion of fine roots seem to have additional advantages for increased nutrient and metal uptakes.

- Wheat is planted on more acreage than any other crop is. It is grown most effectively in grassland climates, which have the appropriate level of rainfall and include a cold season. Wheat is classified in several ways, based on botany of the plant. The literature describes 18 species of wheat, but only two are of commercial importance: durum wheat and bread wheat. These two types of wheat are grown extensively throughout the world and account for 90% of world production [11].
- Rice is one of the most important crop plants and feeds more people than any other plant species on Earth. Cultivated rice belongs to two species, *Oryza sativa* and *O. glaberrima*. Of the two, *O. sativa* is by far the more widely utilized. A big difference between rice and other cereal crops is that this species often grows in water and thus the conditions of metal uptake can differ significantly between rice and other cultural plants (water-soluble metals may be more easily taken by plants) [10].
- Barley occurs in many types, but the most commonly cultivated type is *Hordeum vulgare* L. The best soils for growing barley are loam and clay loam. This plant is the most tolerant of cereal species to alkaline soils (pH 6 to 8) and the least tolerant of acid soils (below pH 5) of the cereal species [12]. As a cultivated crop, oats came after wheat and barley.
- Oat (*Avena*) is a grass; several species exist, though taxonomists do not agree on all the classifications. Of the cultivated species *Avena sativa* (common white oats) is the most important [13].
- Corn, or maize (*Zea mays* L.), was originally produced in the Western Hemisphere by Indians and was then carried to Europe by the early explorers. Today, it is cultivated in many temperate climates [10].
- Rye (*Secale cerealeis* L.) is the second most widely used cereal (after wheat) for bread making, though its gross production is less than 1/15 that of wheat. Rye can be grown on relatively poor soils and it is able to survive more severe winters than most grains.
- Forage sorghum (*Sorghum vulgare*, *S. bicolor* L.) is an important cereal grass native to Africa that ranks fourth after rice, corn, and wheat in terms of the importance for human nutrition. It is especially used as grass in summer because of its great resistance to heat and drought [10].
- Proso millet (*Panicum miliaceum* L.) is a member of the tribe Paniceae of the Panicoideae subfamily of grasses. This tribe of grasses originated in the tropics and most of these crops are grown in the hot, semiarid regions of the world. Proso millet is a warm-season grass and is usually capable of producing seed anywhere from 60 to 100 days after planting. Because of its relatively short growing season, it has a low moisture requirement and is capable of producing food or feed where other grain crops would fail. Proso millet is often planted as an emergency catch crop for situations in which other crops have failed, been hailed out, or were never planted due to unfavorable conditions. Proso is versatile in that it can be successfully grown on many soil types and is probably better adapted than most crops to "poor" land, such as land with soils having low water-holding capacity and low fertility.

28.3 METAL UPTAKE BY CEREAL CROPS

At present, not much of the literature concerns application of cereal crops for phytoremediation of contaminated soils, though experimental data on metal uptake by different cereals may be found in biological and analytical journals. Recent experimental studies showed that certain cereal genotypes are able to tolerate high concentrations of several toxic metals and thus can provide a good basis for successful phytoremediation of metal-contaminated soils [14–20]. Different species, and indeed different cultivars, regulate metal uptake at the soil–root and root–shoot interfaces to varying degrees. Among other cereals, rice and wheat are prospectively the best plants for soil phytoremediation.

Wheat cultivars vary considerably in their ability to grow and yield well in contaminated soils. An ability of wheat to accumulate different metals has been demonstrated in many publications [21–25]. According to recent literature data, different cultivars of wheat may differ in the ability to take up heavy metals [26–28]. Moreover, the experimental data may be very contradictory. It was reported [29] that whole-plant Cd accumulation and Cd translocation to shoots is greater in the bread wheat cultivar than in the durum cultivar. On the other hand, Li et al. [30] found that many durum wheat cultivars accumulate two to three times as much Cd in grain as does bread wheat. Greger and Löfstedt [27] showed that different Cd accumulation in wheat grains grown in nutrient solution was related to variations in the translocation from root to shoot and to the Cd concentration in shoot, flag leaf, and grain coats, but not to the uptake of Cd by roots.

Comparison of hyperaccumulator species *Thlaspi caerulescens* and wheat *Triticum aestevum* showed that root exudates of wheat generally are able to mobilize more metals from soil as compared to hyperaccumulators [31]. Considering that shoot and root biomass of wheat is significantly higher than that of hyperaccumulators, wheat may be a promising plant species for the aims of soil phytoremediation. It is also important to note here that an increase in the degree of metal accumulation in wheat may be accompanied by activation of antioxidant enzymes. It was found that bioaccumulation of metals capable of inducing the antioxidant protection may be fixed in the next generation of wheat seedlings [32].

The author's recent experimental data on wheat *Triticum aestevum* type Umanka indicates that this type can uptake and tolerate large amounts of different metals in its tissues [33]. As an example, Table 28.1 shows concentrations of Th in different parts of wheat seedlings grown in soil artificially contaminated with Th.

It is important that metal content increase not only in roots, but also in leaves of the wheat seedlings. This means that Th was actually transferred from soil to plant. In the course of the short-term (7 days), vegetation test concentration of Th in the contaminated soil decreased ~1.7 times (Figure 28.1). Moreover, Th bioaccumulation had not affected biomass of the plants. After 7 days, lengths of leaves in the experiment and in the control were the same. This indicates that Th was not toxic for the wheat seedlings. However, a rather short-term Th exposure was used for the experiment. The physiological and biochemical consequences of longer radiolitic and chemical Th stress would not appear in the further stages of the plant growth which cannot be excluded.

Rice can accumulate high amounts of different metals, including Cd, Co, Cu, Cr, Ni, Pb, and Zn [34–36]. Successful examples of application of rice to remove Cd from contaminated soils have been reported [37]. Data on using rice to remove Se from drainage water are also available [38].

TABLE 28.1
Mean Concentrations of Th[a] in Wheat Seedlings and in Soil where Plants Were Grown and Ratio of Th Content in Plants Grown in Th-Enriched Soil to That in Control

	Control	Experiment (+Th)	Ratio experiment/control
Leaves	0.10 ± 0.03	1.45 ± 1.25[a]	18.6
Roots	0.71 ± 0.61	43.9 ± 4.6[b]	69.2
Soil	7.0 ± 2.9	39.1 ± 11.6[a]	

[a] mg kg^{-1}.

[b] Differences between control and experiment are significant at $P < 0.01$.

[c] Differences between control and experiment are significant at $P < 0.001$.

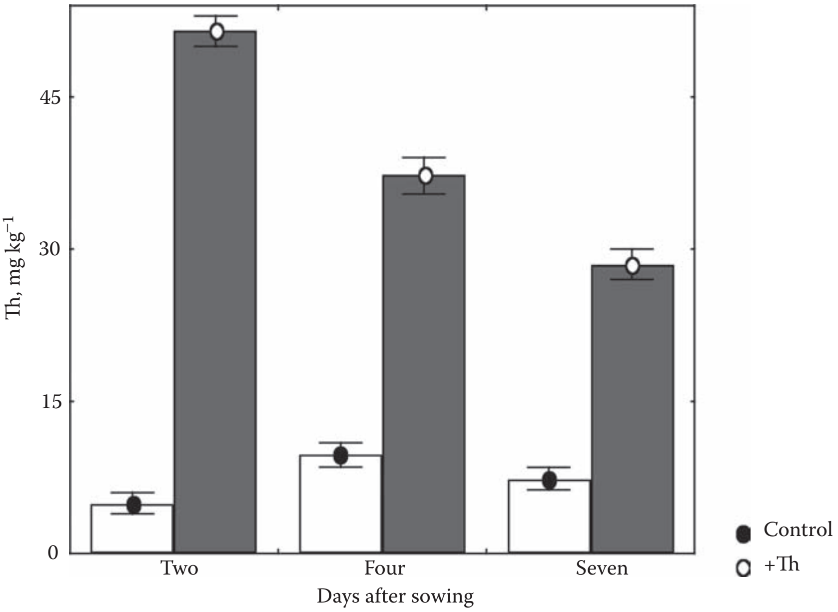

FIGURE 28.1 Variations in Th concentration in clean (control) and artificially contaminated with Th soil within 2, 4, and 7 days after adding Th to the experimental soil.

Application of maize in phytoremediation studies is described in numerous reports in the literature [39–41]. Maize can grow quickly, has high biomass, and has been found to accumulate different metals in its shoots and roots [42]. For example, Kalisova–Spirochova et al. [43] compared maize and sunflower grown in highly contaminated soil (Zn: 75,000 mg kg^{-1}; Pb: 16,000 mg kg^{-1}). The highest values of accumulation of Zn and Pb were found in roots and in leaves of corn (whole plant: 1158 mg kg^{-1} for Zn and 500 mg kg^{-1} for Pb). Sunflower showed considerably lower accumulation ability (whole plant: 47 mg kg^{-1} for Zn and 9 mg kg^{-1} for Pb).

Keltjens and van Beusichem [44] compared an uptake of Cu and Cd by wheat (*Triticum aestevum*) and maize (*Zea mays*) grown in nutrient solution. They found that, at identical external Cd levels, maize accumulated significantly more Cd in the shoots than wheat did, making maize a more pronounced “shoot Cd accumulator” than wheat. Unfortunately, similar metal uptake by these plants when they grow in soil cannot be predicted. In this case, quite opposite effects may be observed; these may be explained by differences that can arise when comparing experiments on metal uptake by plants grown in soil and culture solutions.

Much less information is available on uptake of metals by other cereal crops. Madrid and Kirkham [45] studied an uptake of Cd, Fe, Mn, Ni, and Pb by barley (*Hordeum vulgare* L.) grown in animal-waste lagoon soil. They found that amendment of the soil with EDTA resulted in an increase of uptake of Fe, Mn, Ni, and Pb. Luo and Rimmer [46] studied multielement toxicity at the early stages of barley growth. It was shown that growth of barley was controlled by the amount of plant-available Zn, which depended on the amounts of added Zn and added Cu. The effect of the added Cu was to increase the toxicity of the added Zn. Adams et al. [47] compared metal uptake by wheat and barley and found that, when it was grown under comparable soil conditions, barley could take up much lower amounts of Cd than wheat could.

Cobb et al. [48] compared three plant species (oats, radish, and lettuce) grown in soil contaminated with Cd, Cr, Ni, and Pb. They found that oats were the most tolerant compared to other plant species and were able to accumulate rather high amounts of Cd and Ni.

Knox et al. [49] studied an uptake of Cd by rye, maize, and oats grown in soils with different levels of Cd contamination. It was found that an increase of Cd content in soil up to 20 mg kg^{-1}

resulted in a significant increase of Cd level in all the plant species: oats accumulated 112 mg kg^{-1} Cd in their leaves, and concentrations of Cd in leaves of rye and maize were 43 and 41 mg kg^{-1}, respectively. When concentration of Cd was increased up to 40 mg kg^{-1}, Cd contents in leaves were 80, 98, and 198 mg kg^{-1} in rye, maize, and oats, respectively. Rye was found to be able to accumulate more Cu and Zn in its shoots compared to wheat and oats grown in the same soils [50].

Schumann and Sumner [51] performed experiments with sorghum grown in silty loam soil amended and non-amended with by-products. It was shown that As concentrations in sorghum leaves were linearly correlated ($R^2 = 0.895$) to total As in soil. An application of coal combustion by-products (fly ash) for crop fertilization led to a significant (four times) increase of As content in leaves that finally reached the level of 120 mg kg^{-1}. Estevez Alvarez et al. [19] studied the uptake of Cr, Cd, Ni, Pb, and Zn by sorghum and toxicity of the metals for this species. They observed a remarkable effect of Zn and Cr on the growth of the plant. Even insignificant increases of Zn and Cr concentrations in soil resulted in a death of sorghum. On the other hand, Cu, Ni, and Pb did not affect the plant yield.

The author's experimental data on oats and barley indicate that these plant species may be used for metal phytoextraction in slightly and moderately contaminated soils [15,16]. Vegetation tests showed that growth of oats and barley in different soils contaminated with various metals and metalloids resulted in a decrease of metal concentrations in soil solutions. As an example, Figure 28.2 illustrates variations in Cu and Zn contents in soil samples treated with 1 *M* ammonium acetate, pH 4.8 (this soil extraction can characterize sorbed and loosely bound metals) [52]. It is important to note that, after soil cultivation, total amounts of several elements in the soils also decreased (Table 28.2).

Behavior of As in the urban soils was the most interesting. During a 1-month vegetation test, As content in slightly contaminated soils cultivated with oats decreased up to the level of As in the clean soil (Table 28.2). Concentration of As in moderately contaminated soil also decreased significantly. In this case, such an effect was observed as a result of cultivation of soil with both oats and barley.

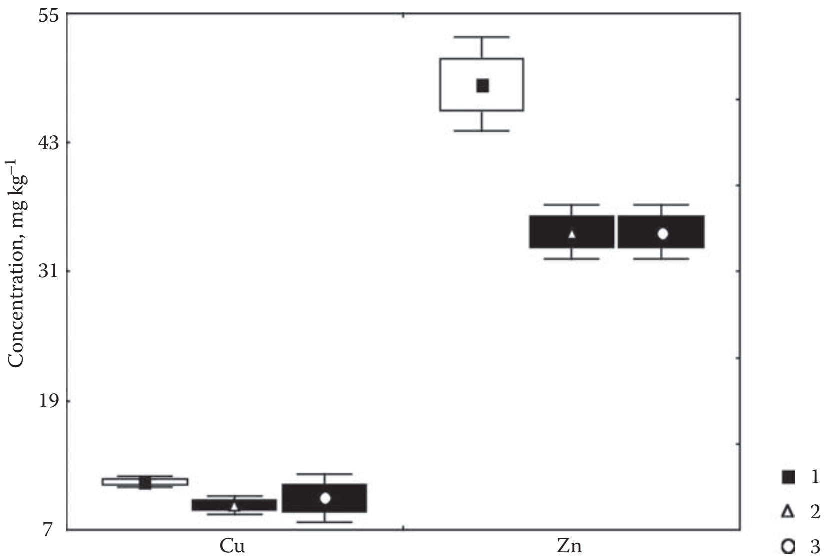

FIGURE 28.2 Concentrations of Cu and Zn in soil leachates before (1) and after growth of oats (2) and barley (3) in the soil.

TABLE 28.2
Total Amounts of Elements[a] in Soils Taken from Different Sites in St. Petersburg before (A) and after (B) Growth of Oats and Barley in Clean (1), Slightly Contaminated (2), and Moderately Contaminated (3) Soils

			Cr	Fe (%)	Co	Zn	As
A		1	20.1 ± 2.8[c]	1.29 ± 0.16	2.98 ± 0.38[b]	94.7 ± 11.3[c]	1.94 ± 0.21[c]
		2	27.7 ± 2.9[c]	1.57 ± 0.11	8.61 ± 0.47[b]	176 ± 19	2.66 ± 0.65[c]
		3	126 ± 13	1.57 ± 0.11	5.33 ± 0.61	245 ± 25	9.92 ± 1.04
B	Oats	1	26.3 ± 4.9	1.53 ± 0.16	3.85 ± 0.61	120 ± 17	2.11 ± 0.67
		2	27.7 ± 6.5	1.95 ± 1.22	3.8 ± 0.8[d]	140 ± 31	1.79 ± 0.45[d]
		3	103 ± 31	1.85 ± 0.45	5.65 ± 0.91	208 ± 59	6.47 ± 0.79[d]
	Barley	1	26.4 ± 4.1	1.38 ± 0.31	3.85 ± 0.63	124 ± 25	2.18 ± 0.68
		2	26.7 ± 6.8	1.31 ± 0.24	3.66 ± 0.86[d]	148 ± 51	2.17 ± 1.22
		3	116 ± 47	2.2 ± 0.5	6.21 ± 1.43	221 ± 70	6.85 ± 2.01[d]

[a] mg kg^{-1}.

[b] Differences between concentration of the elements in soils taken from site 1 and site 3 and soils taken from site 2 and site 3 are significant at $P < 0.01$.

[c] Differences between concentration of the elements in soils taken from site 1 and site 3 and soils taken from site 2 and site 3 are significant at $P < 0.001$.

[d] Differences between concentrations of the elements in cultivated and non-cultivated soils are significant at $P < 0.001$

28.3.1 PHYTOTOXICITY OF SOME ULTRATRACE METALS

Ideally, screening of plant species for phytoremediation purposes should involve a broad range of trace metals and metalloids. The list of elements commonly considered as pollutants is rather short and usually includes Al, As, Cd, Cr, Cu, Hg, Ni, Pb, Se, Zn, and some radionuclides. Meanwhile, many other trace elements may also be toxic if their concentrations in soil exceed ordinary concentrations typical for the soil.

To complete the life cycle, plants must acquire not only macronutrients (N, P, K, S, Ca, and Mg), but also many trace elements [53]. Unfortunately, little or no attention has been paid to the majority of the 90 elements that occur in soil and in different plant species, though it may be assumed that these elements are also involved in specific physiological activities and their biological significance is presently unknown. Until the present time, few investigations have been conducted on phytotoxicity of various trace metals not included in the short list mentioned earlier.

During the last few years, much attention has been paid to toxic effects of the rare earth elements (REE) widely used in agricultural practice in China [54–56]. It was shown that plants can take up rather large amounts of REE and this can affect concentrations of main nutrients in the plants. The experiments performed by the author on Eu effects on plant nutrition showed that germination of wheat seeds in the medium enriched with Eu and growth of wheat seedlings in the soil to which a small amount of Eu was added may result in significant variations in concentrations of macronutrients in the plants. Figure 28.3 shows variations in K content in roots and leaves of 7-day-old wheat seedlings germinated in medium to which 20 μg kg^{-1} Eu was added. Although concentration of K in roots remained unchanged, K content in leaves decreased significantly compared to that in the control plants. Such a decrease of K in generative plant parts may have harmful consequences for future development of the plant.

Many other ultratrace metals — for example, exotic elements such as Sc — were never considered as pollutants. However, even insignificant (1.5 times) increase of Sc content in soil may result in Sc accumulation in plants growing in the soil (concentration of Sc in roots can increase up to 20 times compared to Sc content in the roots of the control plants) and significant variations

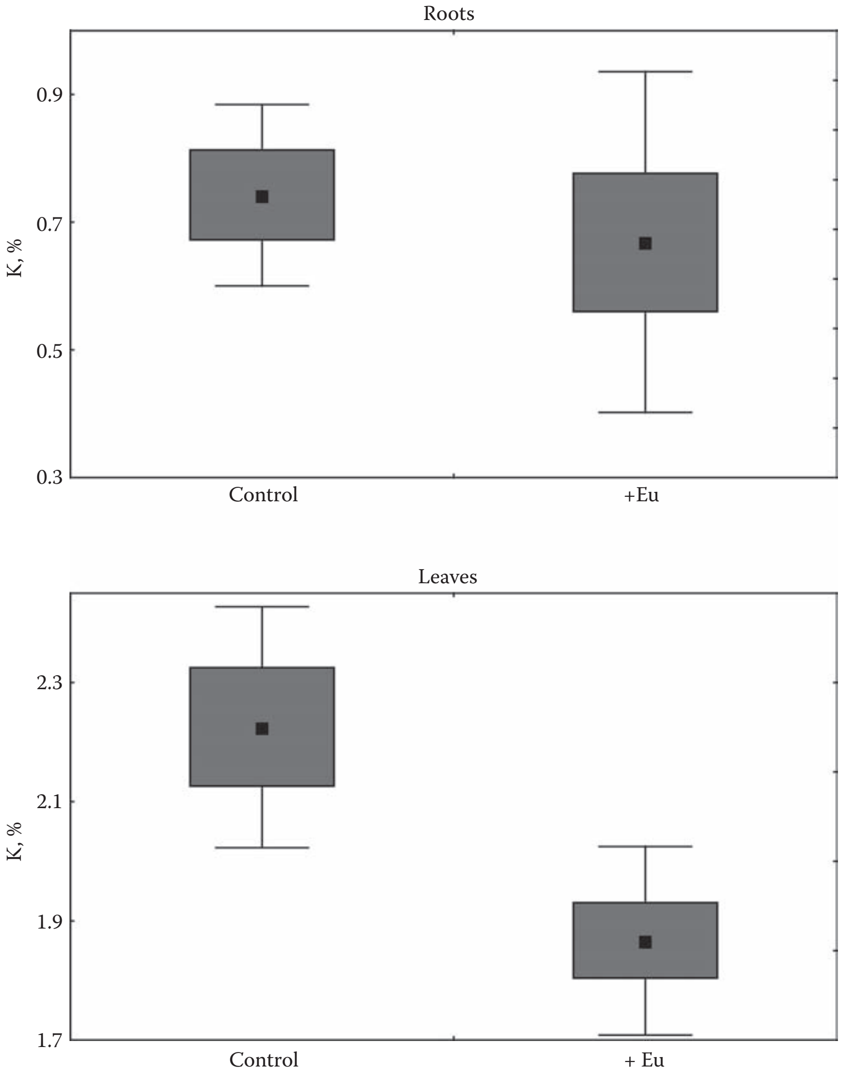

FIGURE 28.3 Variations in K content in roots and leaves of the wheat seedlings grown in the control soil and in the soil where a small amount of Eu was added.

in concentrations of main nutrients in the plants [57]. In particular, an increase of Sc concentration in the plants led to a decrease of K in seeds and increased Na, Ca, and Cs contents in leaves. It is interesting that negative effects of Sc were found to be higher than phytotoxic effects of such radioactive element as Th. At first glance, this seems strange because, in the case of radionuclides, effects of chemical and radiolitic toxicity have been combined. However, vegetation tests performed by the author showed that an increase of Th content in soil can cause only minor variations in elemental composition of wheat seedlings (concentration of Sb in leaves and concentrations of Hf and Cr in roots of the plants grown in Th-contaminated soil became higher ($P < 0.01$) than in the control plants grown in ordinary soil) [33].

Plants have evolved highly specific mechanisms to take up, translocate, and store different elements. The uptake mechanism is selective; plants preferentially acquire some ions over others [1]. For example, mean Au content in soil is very low: 10^{-7}% [58]. It may be assumed that rather high ($n_* 10^{-6}$ to $n_* 10^{-5}$% of dry weight) amounts of Au found in different plant species [59,60] may be attributable to the demand of the plants for these amounts of Au to complete certain biochemical processes.

28.4 METAL DISTRIBUTION BETWEEN ROOTS AND UPPER PLANT PARTS

Uptake of metals by root cells, the point of entry into living tissues, is a step of major importance for the process of metal phytoextraction. However, for phytoextraction to occur, metals must also be transported from the roots to the upper plant parts. It is quite possible that a plant species exhibiting a significant metal accumulation into the roots may have a limited capacity for phytoextraction. For example, many publications have reported that concentrations of trace metals in roots may be several times higher than in shoots [61,62]. Kim et al. [36] found that Pb content in roots may increase with increased concentrations of Pb^{2+} applied to soil, but Pb content in shoots will remain unchanged. In addition, the mechanisms of metal translocation from root to shoot may be different for different metals.

As an example, Table 28.3 shows concentrations of several elements in different parts of three cereal crops (oats, barley, and wheat) grown simultaneously on the same soil. Concentrations of all the elements (except calcium in barley) were higher in roots than in leaves. It is also important to remember that only a part of the total amount of ions associated with the root is absorbed into cells. A significant ion fraction is just physically adsorbed at the extracellular negatively charged sites of the root cell walls [1]. The cell wall-bound fraction cannot be translocated to the shoots and, therefore, cannot be removed by harvesting shoot biomass (phytoextraction).

Binding to the cell wall is not the only plant mechanism responsible for metal immobilization into roots and subsequent inhibition of ion translocation to the shoots. Metals can also be complexed and sequestered in cellular structures (e.g., vacuoles), thus becoming unavailable for translocation to the shoot [63]. In addition, some plants, so-called excluders, possess specialized mechanisms to restrict metal uptake by roots. Movement of metal-containing sap from root to shoot is primarily controlled by two processes: root pressure and leaf transpiration. Following translocation to leaves, part of metals can be reabsorbed from the sap into leaf cells. However, certain amounts of metals may evaporate during transpiration together with water (the water will carry dissolved mineral salts). As has been reported, elemental composition of transpiration solutions correlates well with elemental composition of soil water [64].

TABLE 28.3
Concentrations of Elements[a] in Roots and Leaves of Wheat, Barley, and Oats

Element	Wheat		Barley		Oats	
	Roots	Leaves	Roots	Leaves	Roots	Leaves
Ca	0.30 ± 0.23	0.17 ± 0.10	0.50 ± 0.17	0.50 ± 0.21	0.70 ± 0.34	0.34 ± 0.10
Cr	9.4 ± 1.4	0.82 ± 0.22	10.7 ± 11.2	2.0 ± 0.6	8.9 ± 3.0	1.8 ± 0.4
Fe	686 ± 199	162 ± 30	1319 ± 1373	113 ± 24	1576 ± 683	132 ± 29
Co	0.29 ± 0.08	0.04±0.01	1.1 ± 0.8	0.07 ± 0.02	2.0 ± 1.2	0.07 ± 0.02
Zn	235 ± 90	61.2 ± 11.8	200 ± 80	136 ± 160	165 ± 38	55.0 ± 9.0
As	0.64 ± 0.31	<0.1	0.62 ± 0.30	0.22 ± 0.24	0.79 ± 0.60	0.12 ± 0.06
Sb	0.12 ± 0.07	0.07 ± 0.03	0.17 ± 0.08	0.03 ± 0.01	0.27 ± 0.10	0.05 ± 0.01
Sm	0.16 ± 0.03	0.005 ± 0.002	0.25 ± 0.22	0.03 ± 0.01	0.21 ± 0.02	0.03 ± 0.01
Th	0.56 ± 0.23	0.10 ± 0.03	0.49 ± 0.47	0.10 ± 0.12	0.51 ± 0.13	0.05 ± 0.02
U	0.41 ± 0.05	<0.04	0.30 ± 0.18	0.05 ± 0.03	0.48 ± 0.17	0.05 ± 0.02

[a] mg kg^{-1}.

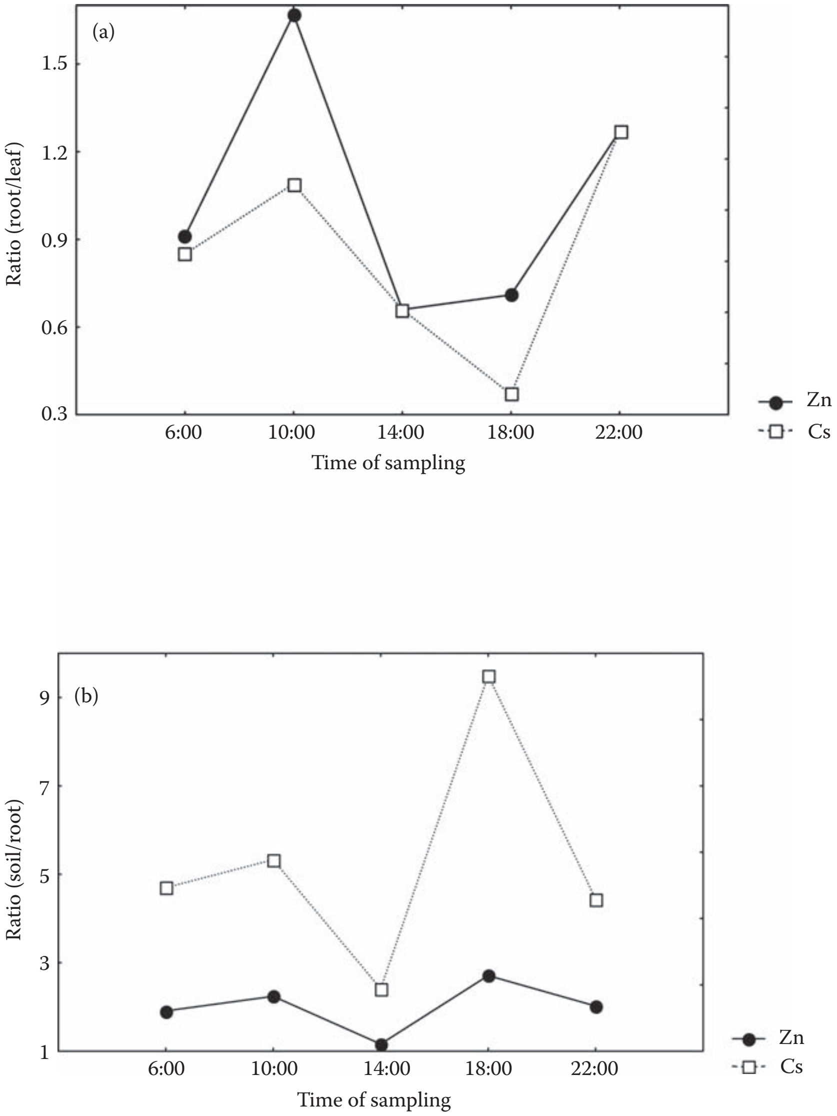

FIGURE 28.4 Daily dynamics of ratio of Cs and Zn concentrations in roots to those in leaves (a) and ratio of Cs and Zn concentrations in soil to those in roots (b) of wheatgrass.

A certain part of metals may also return back to soil. This process is especially active during first phases of plant growth [65]. Figure 28.4 illustrates daily dynamics of ratios of Zn and Cs concentrations in roots to those in leaves of wheatgrass and ratios of Zn and Cs concentrations in roots to those in the soil where the plants grow. During the day, the variations in metal concentrations are quite significant and very similar for different elements.

28.4.1 EFFECTS OF SOIL CHARACTERISTICS, WEATHER CONDITIONS, AND PLANT PHYSIOLOGICAL ACTIVITY ON METAL UPTAKE

Physicochemical characteristics of contaminated soils are also important for the selection of the remediating plants. Implementation of phytoremediation methods in real environmental conditions requires a careful preliminary assessment of each site and, first of all, detailed analysis of soil matrix. Each site is unique with regard to its chemical and physical characteristics, such as temperature, pH, moisture content, soil texture, nutrients available for microbes and plants, etc. The process of metal phytoextraction is very sensitive to many of these variables.

In particular, it is necessary to take into account a high variability of soil metal contents within plots and between years. The variability between plots may result from microtopographic relief of the area. For example, extreme care is required in the interpretation of phytoremediation experiments in a the case where the experimental site is situated on slightly sloping territory. Under these circumstances, significant variations in concentrations of main cations such as Ca, K, Na, and trace metals along the soil profile that are caused by quite natural reasons can be expected.

Sunlight for photosynthesis, water, and nutrient supply, suitable temperature, and humidity are essential constituents of plant growth [66]. Differences between years may be rather significant because of variations in climatic conditions [67]. This can include plant dry matter yields, nutrient uptake, and distribution of nutrients between different plant parts [50]. As a result, the rates of metal uptake by the same plant species grown in the same place can vary between years depending only on climatic conditions during the vegetation seasons.

Figure 28.5 and Figure 28.6 illustrate distribution of Cr, Ni, and Pb in different parts of wheat (*Triticum aestevum*) grown in 2002 and 2003 in loam soil and in loam sand soil. The two years differed significantly in temperature and mean summer rainfall. The vegetation experiments showed significant variations in concentrations of different elements in the plants. In a more wet and warm season (2002), uptake of metals by the plants was lower compared to that in a more dry and cold season (2003). Additionally, the metal uptake depended on soil texture (it is known that water content and soil texture are generally among the main factors controlling metal uptake [1]). Finally, biomass production, an important parameter of soil phytoremediation, is also determined by climatic conditions during vegetation season [68].

Numerous experiments have been conducted to estimate the adsorption or solubility of trace elements as a function of soil pH and its effect on metal uptake by plants. It was shown that, in general, metal solubility in soil decreases as pH increases [69–71]. However, it is necessary to take into account that plants can survive and produce sufficient biomass only at certain ranges of soil pH: 5.0 to 8.0. Metal phytotoxicity increases with decreasing pH [72] and may result in inhibition of plant growth. An increased metal availability associated with the low soil pH may also be detrimental to rhizosphere microorganisms [40]. For example, Angle and Heckman [73] reported that a change of pH from 6.2 to 5.6 in soil treated with metal-contaminated sewage sludge resulted in a dramatic decline of AM colonization in plant roots. However, it was also reported [67,74] that, even under minimal pH changes, significant variations in metal uptake can often be observed. Therefore, soil pH alone cannot explain these changes in metal bioaccumulation.

Seed germination, planting, water irrigation and fertilization, and time of harvesting are important factors responsible for successful implementation of the phytoremediation method. Many efforts are required to find an optimal combination of all these parameters to provide the most efficient metal phytoextraction. For example, environmental conditions during seed germination and planting have a considerable influence on future plant development, including an ability of the plants to take up nutrients and metals [75]. With respect to cereal crops, the examples of wheat [25], barley, and oats [16] showed that younger plants can take up and translocate higher amounts of metals than older plants can. Laboratory tests clearly indicate that chlorophyll, protein, and many chemical elements in cereal grasses reach their peak concentrations in the period just prior to the jointing stage of the green plant. Although this period lasts for only a few days, cereal grasses should be harvested precisely during this stage of the plant development. These observations were also supported by data on other plant species [76].

Exudation of organic compounds by roots is one of the most important factors influencing the ion solubility and metal uptake through their indirect effects on microbial activity, rhizosphere physical properties, and root growth dynamics and directly through acidification, chelation, precipitation, and oxidation/reduction reactions in the rhizosphere [53,77]. The components of plant

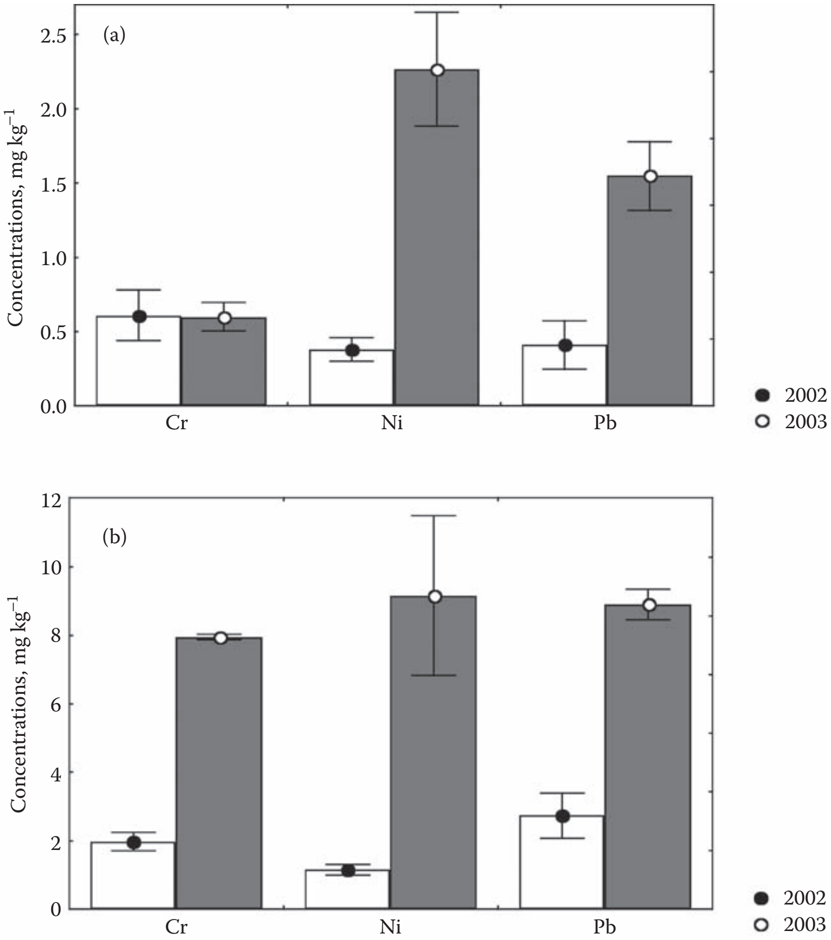

FIGURE 28.5 Concentrations of Cr, Ni, and Pb in leaves (a) and roots (b) of the wheat grown in loam soil in 2002 and 2003.

root exudates are various and complex; they serve as a source of carbon substrate for microbial growth and also contain chemical molecules that promote chemotaxis of soil microbes to the rhizosphere [78]. In addition, root exudates control the nutrient and metal uptake. The root-derived exudates can improve the availability of nutrients in a case when they were released in response to a particular nutrient deficiency.

It was also shown that plants under stress tend to produce large amounts of secondary metabolites, which may influence the availability of nutrients and metals in the rhizosphere [79]. The metal-induced exudation of organic acids might be a common response in plants. It should be remembered that root exudates are species specific and also may vary when the same plant species is growing in different soils [80]. For example, in response to Cu stress, rye can exude two times more organic acids from roots than triticale and six times more than maize [81].

28.4.2 IDENTIFICATION OF SOIL AMENDMENTS CAPABLE OF ENHANCING PLANT YIELD AND METAL PHYTOEXTRACTION

The mobilization of metal contaminants in soil and in plant is an important factor influencing the success of phytoremediation. Many metals have low solubility in soils because of adsorption of

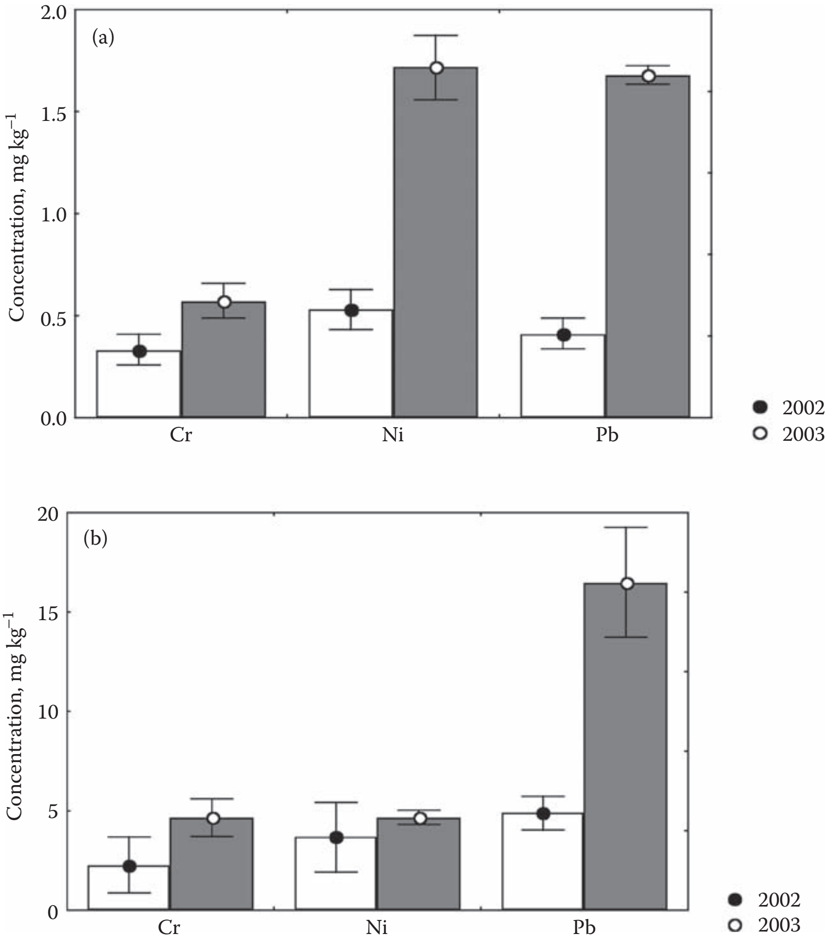

FIGURE 28.6 Concentrations of Cr, Ni, and Pb in leaves (a) and roots (b) of the wheat grown in loam sandy soil in 2002 and 2003.

the metals to soil particles. For example, most clay particles are covered with a thin layer of hydrous Fe, Mn, and Al oxides. These selective sites can maintain metal activity in the soil solution at low levels [82]. Mobility of metals in soil may also be reduced because of metal precipitation as insoluble phosphates, carbonates, and (hydr)oxides [83]. In addition, plants usually do not translocate metals efficiently from roots to shoots.

Commercial fertilizers not only play an important role in the improvement of soil fertility and the increase of crop production, but can also enhance the uptake of metals by plants through improvement of plant growth and root density (in general, the more biomass the plants have, the more metals can be accumulated because the metal uptake is a function of the overall biomass). Organic and inorganic fertilizers can provide a potent means of stimulating the root and shoot biomass. In addition, fertilizers can effectively and specifically increase solubility and, therefore, bioaccumulation of metals because metal bioavailability may often be increased by lowering soil pH and altering soil ion composition. Therefore, phytoremediation effects may be enhanced using the agronomic approaches, and success of the manipulation measures will depend significantly on the agronomic practices applied at the site.

It should be remembered, however, that consequences observed as a result of the soil fertilization may be rather different. The effects will depend on

- Type of soil
- Climatic conditions
- Type and amount of applied fertilizer
- Species of plants growing in the soil
- Character of microbiological processes

In fact, options for increasing agricultural productivity are limited. At present, the phytoremediation practice has several commonly accepted agricultural approaches. They include application of organic materials, sewage sludge, chelates, and inorganic agents such as nitrogen, potassium, calcium, etc.

28.4.3 SEWAGE SLUDGE

Land application of sewage sludge — the solid portion that remains after wastewater treatment — provides a means of disposing unwanted waste products as well as returning valuable nutrients and organic matter to the land [25]. During the past several decades, sludge has been applied in increasing amounts to agricultural lands. Sewage sludge might be an effective and cheap alternative to commercial fertilizers because it usually has high contents of nutrients and organic matter. However, the rather high level of numerous trace metals is the most significant impediment of wider land application of sludge and can negate the benefits of the manipulation measure.

It was assumed that metals in sewage sludge are generally organically bound and therefore less available for plant uptake than more mobile metal salt impurities found in commercial fertilizers [84,85]. However, this conclusion was not supported by experimental data. For example, it was reported that increasing sewage sludge doses induced a linear increase in Cu, Ni, and Zn concentrations in soil solutions [86]. Speir et al. [71] showed that New Plymouth sewage sludge contained 3 to 10 mg kg^{-1} Cd, 180 to 320 mg kg^{-1} Cr, 400 to 570 mg kg^{-1} Cu, 190 to 270 mg kg^{-1} Ni, 64 to 130 mg kg^{-1} Pb, and 1620 to 2400 mg kg^{-1} Zn. Moreover, pH of the tested light-textured sandy soil was markedly reduced after sludge application (up to pH 4 in some samples), presumably as a result of breakdown of the unstable organic matter, nitrification of the NH_4^+–N, and sulphide oxidation. As a consequence, soil solution concentrations of Cu, Ni, and, especially, Zn increased significantly.

Also, sludge is an imbalanced source of plant nutrients. Approximate N:P:K ratios for sewage sludge are 2.5:1.0:0.9 [51]. This ratio implies that P will be in excess for most field and forage crops when they are fertilized with the sludge to supply the total crop N requirement because, for example, recommended N:P:K for maize is 7.5:1.0:4.4, and 9.0:1.0:4.7 for grain sorghum. Finally, the benefits of the sludge application may be reduced with time because of mineralization of the sludge organic fraction [86].

28.4.4 CHELATES

The use of synthetic chelates has been shown to stimulate the potential for Pb accumulation in plants dramatically. These compounds prevent Pb precipitation and keep the metal as soluble chelate–lead complexes available for uptake by roots and transport within a plant. It was reported that an addition of EDTA (ethylene-diamine-tetraacetic acid) at a rate of 10 mmol kg^{-1} soil stimulated Pb accumulation in shoots of maize up to 1.6% and simultaneously altered root/shoot partitioning in a wide variety of crop plants [87]. However, certain differences in Pb accumulation levels between different plant species under equivalent added chelate [88] may be present.

Chemically assisted phytoextraction has also been applied for phytoremediation of soils contaminated with other metals. Anderson et al. [89] reported their finding that plants could be induced to hyperaccumulate Au using thiocyanate and thiosulphate. Uranium concentrations may be strongly increased when citric acid is applied [90]. Thayalakumara et al. [91] showed that EDTA application increased the leaf Cu concentration of grass from 30 to 300 mg kg^{-1}. They found that more Cu was accumulated in the leaves when EDTA was applied in numerous small doses than in just one large dose. The authors also found that, as a result of EDTA applications, about 100 times more Cu was leached than was taken up by grasses. This indicates that a strategy for managing leaching losses must be a part of the EDTA-enhanced phytoremediation procedures.

Chemically assisted phytoextraction can work well when the metal to be extracted has initially a very low bioavailability, and thus is not phytotoxic, allowing the establishment of a large plant biomass before the chelate is applied. In contrast, such metals as Cu, Zn, and Cd are usually more bioavailable in soil and can cause severe phytotoxicity at levels that require remediation, particularly to dicotyledonous species such as *Brassica juncea* [92]. It was shown that *B. juncea* seedlings were able to accumulate 875 mg kg^{-1} Cd with chelating agent vs. 164 mg kg^{-1} without a chelator [93]. This amount of Cd may be very toxic to the plant and result in the inhibition of plant growth, thus limiting the chance of successful chemically assisted phytoextraction on mixed pollution sites. The other issue of concern is the possible leaching of metal–chelate complexes to groundwater, which may represent an environmental hazard [94]. For example, it was reported that application of more than 1 g kg^{-1} EDTA becomes inefficient because Pb concentration in crops is not enhanced and the leaching rate increases [95].

The chelate-induced metal hyperaccumulation may be fatal to the plant. An accumulation of elevated Pb levels is highly toxic and can cause plant death. Because of the toxic effects, it is recommended that chelates should be applied only after a maximum amount of plant biomass has been produced. Prompt harvesting (within 1 week of treatment) is required. Huang et al. [88] suggested the following field application protocol when growing a high biomass crop that is sensitive to chelates. After the crop has become well established and reached a sufficient biomass, a selected chelate may be applied to the root zone to facilitate rapid Pb accumulation. The plants should be harvested shortly after chelate addition to reduce environmental risk.

28.5 ORGANIC MATERIALS

An application of organic materials such as animal manure, poultry litter, and pig slurries can stimulate plant yield and increase aggregate stability, infiltration rate, retention of water and decrease soil bulk density. In many cases, the plant yield may be progressively increased as the rate of the organic materials increases [96]. The enhanced soil fertility and improved soil physical conditions may also be due to the macronutrients contained in the organic materials [68].

However, nutrients are not the only components of organic materials that can cause environmental concern. Heavy metal accumulation in soils fertilized with animal wastes is also possible. The trace metals contained in the organic materials may affect the level of metal concentrations in the soil solution. For example, long-term application of Cu and Zn rich poultry litter was found to increase concentrations of levels of these metals in soil up to toxic [23]. Concentrations of Cu and Zn in animal wastes can vary due to the type of animal and feeding practices [50]. For example, the amounts of Cu and Zn applied to the soil with poultry litter exceeded the annual nutrient requirements of plants, with estimates of 6.6 times more Zn applied than plants require [97]. Thus, the organic fertilizers used for remediation of contaminated soils must be rather clean to exclude the possibility of adding new metals to the soil. At the same time, they must be able to stimulate transfer of certain elements, including toxic metals, to more available to plants forms with a consequent accumulation of the metals in plants.

Application of organic materials is known to affect soil pH; therefore, these materials may influence soil metal mobility through their influence on solubility or dissociation kinetics of metals and changes in the solid/liquid phase equilibrium [98]. Nitrogen, the most important component of organic fertilizers, can highly correlate with P, Cu, and Zn concentrations in above-ground plant parts, suggesting that improvements in N fertility can increase P, Cu, and Zn concentrations in plants [50]. It was shown that Cu is able to form $Cu–NH_4$ complexes that are very mobile in soil

TABLE 28.4
Concentrations of Metals[a] in Initial Non-fertilized (Control) and Fertilized Soils

	Cd	Cu	Pb	Zn
Site 1 — control	0.44 ± 0.06	39.3 ± 0.5	53.1 ± 2.9	128 ± 10
Site 1 + urea	0.43 ± 0.03	42.1 ± 3.7	49.3 ± 5.2	135 ± 9
Site 1 + manure	0.45 ± 0.02	37.6 ± 4	59.5 ± 25	129 ± 11
Site 1 + ispolin	0.45 ± 0.02	38.3 ± 3.3	74.1 ± 44.5	121 ± 10
Site 2 — control	2.4 ± 0.5	159 ± 19	208 ± 31	514 ± 74
Site 2 + urea	2.2 ± 0.1	134 ± 19	195 ± 24	470 ± 50
Site 2 + manure	2.5 ± 1.1	148 ± 12	179 ± 40	499 ± 30
Site 2 + ispolin	2.7 ± 0.2	159 ± 11	181 ± 31	486 ± 43

[a] mg kg^{-1}.

solutions [99]. Thus, an excess of N application may also result in an excess of trace metal contents in plants grown in the fertilized soils. It should also be noted that no direct correlation between N uptake and plant yield has been shown. As reported by Trapeznikov et al. [100], an increase of 24 to 101% (depending on plant species) of N uptake was accompanied by an increase of above-ground biomass of 18 to 52%. However, it is clear that the increased plant biomass resulting from the treatment may be attributed first of all to enhanced soil fertility and not only to N uptake.

An ability of cereal crops to take up large amounts of nutrients (N, P, and K) has been shown in numerous studies [101]. Cereal crops also demonstrated a high tolerance to several metals, so they might use the metal surpluses that may originate from soil contamination. It may be assumed that certain species of cereals, especially in combination with appropriate soil amendments, may be successfully used to improve the processes of metal phytoextraction and phytoremediation of contaminated soils.

28.6 EXPERIMENTAL STUDIES ON THE EFFECTS OF DIFFERENT FERTILIZERS ON METAL REMOVAL FROM CONTAMINATED SOILS USING WHEAT

To optimize and manipulate the process of metal phytoextraction successfully, the author studied the effects of three fertilizers (urea, horse manure, and ispolin — fertilizer on the basis of mixture of organic fertilizers, humic acids, and industrial population of worms) on yields and physiological characteristics of wheat (*Triticum aestivum*) and removal of metals from two Podzol soils with loam (site 1) and sandy loam (site 2) textures. Soil in site 2 was contaminated with several metals (Cd, Cu, Pb, and Zn), and soil in site 1 was relatively clean. Wheat seedlings were grown in the two soils for 36 days. Urea, manure, and ispolin were incorporated into the soils at rates of 10 mg kg^{-1}, 100 mg kg^{-1}, and 100 mg kg^{-1}, respectively.

Metal concentrations in the initial soils before and after application of different fertilizers are presented in Table 28.4. No statistically significant differences were present between concentrations of Cd, Cu, Pb, and Zn in the fertilized and nonfertilized soils. Therefore, soil contamination with the metals by applied fertilizers was negligible.

The amounts of metals taken by plants during the vegetation test are shown in Figure 28.7. Although treatment of soil with all the fertilizers did not automatically result in an increase of metal concentrations in plants, it is seen that application of ispolin led to an increase of Cu and application of urea led to increased uptake of Cd, Cu, Pb, and Zn in the wheat grown in the loam (clean) soil. Amendment of sandy loam (contaminated) soil with manure and ispolin resulted in

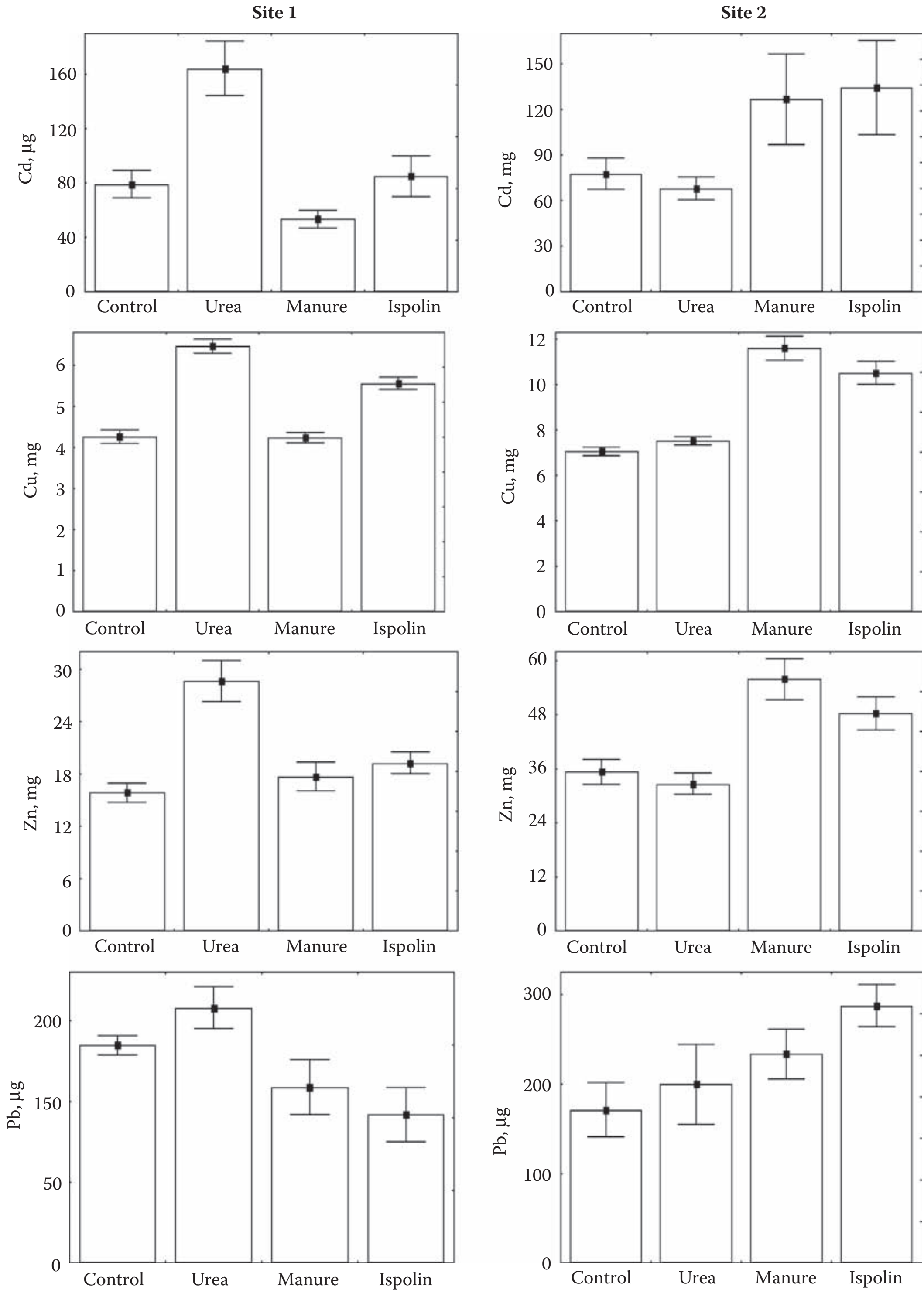

FIGURE 28.7 Variations in amounts of metals taken by wheat grown in clean (site 1) and contaminated (site 2) soils after application of different fertilizers. Control wheat was grown in non-fertilized soil.

TABLE 28.5
Effects of Wheat Growth and Application of Different Fertilizers on Concentrations of Metals[a] in Contaminated Soil

	Without fertilizers		Wheat + fertilizers					
	Control		Urea		Manure		Ispolin	
Element	**1a**[b]	**2a**[c]	**1b**[d]	**2b**[e]	**1b**[d]	**2b**[e]	**1b**[d]	**2b**[e]
Cd	2.4 ± 0.5	1.7 ± 0.4[f]	2.2 ± 0.1	1.3 ± 0.4[g]	2.5 ± 1.1	1.6 ± 0.6	2.7 ± 0.2	1.4 ± 0.4[g]
Cu	159 ± 19	164 ± 39	134 ± 19	151 ± 19	148 ± 12	169 ± 15	159 ± 11	143 ± 14[f]
Pb	208 ± 31	170 ± 42	195 ± 24	155 ± 47	179 ± 40	169 ± 33	181 ± 31	143 ± 21[f]
Zn	514 ± 74	507 ± 178	470 ± 50	555 ± 188	499 ± 30	426 ± 79	486 ± 43	404 ± 72[f]

[a] mg kg^{-1}.

[b] Initial nonfertilized soil.

[c] Soil after the end of the experiment (only wheat).

[d] Initial fertilized soil.

[e] Soil after the end of the experiment (wheat + fertilizer).

[f] Differences between metal concentrations in the initial and experimental soils are significant at $P < 0.05$.

[g] Differences between metal concentrations in the initial and experimental soils are significant at $P < 0.01$.

an increased metal uptake by the plants grown in the soil. The differences between control and the treatments were significant at $P < 0.05$.

It is known that fertilizers may affect soil pH and therefore mobility of metals in soil [70,71]. The pH of the loam soil amended with urea was the lowest compared to the pH values of the control (without application of fertilizers) and loam soil amended with other fertilizers. In this case, it may be assumed that the decrease in the soil pH was mainly responsible for the variations in metal uptake. On the other hand, pH of the sandy loam soil decreased significantly ($P < 0.05$) only after application of ispolin. Because pH of the soil fertilized with manure did not change compared to that of the control, in this case soil pH cannot be used for explanation of the observed variations in the metal uptake.

The highly variable process of element uptake depends on many factors and pH is not the only parameter determining mobility of trace elements in soil. In particular, these two soils differed significantly in soil texture and content of total carbon (8.4% in loam soil and 3.6% in sandy loam soil). Soil texture and soil organic matter content, like pH, can have a significant influence on metal uptake. Finally, as a function of many factors, metal mobilization, in certain environmental conditions, may be efficient for one or another metal, but may also result in immobilization of others.

The experiments showed that growth of wheat in the nonfertilized, contaminated soil led to a decrease (1.4 times) of Cd content in the soil (Table 28.5). Amendment of the soil with urea enhanced the effect: the decrease of soil Cd was more significant. The best effect was demonstrated after application of ispolin: over a short period (36 days) concentrations of Cd, Cu, Pb, and Zn in the contaminated soil decreased 1.2 to 1.4 times compared with those in the initial soil (the differences were statistically significant). It should be noted that calculations made by McGrath et al. [102] showed that it would take 9 years to reduce Zn concentration in soil from 440 mg kg^{-1} to 300 mg kg^{-1} using plant hyperaccumulators. Application of wheat together with ispolin for remediation of metal-contaminated soil allowed them to affect simultaneously the uptake of several metals and, more importantly, to reduce time required for the soil cleanup. In particular, if the decrease of Zn content in this soil continued with approximately the same rate, the level of 300 mg kg^{-1} may be reached within several months.

To estimate the effect of the soil type and amendment of soils with ispolin on element behavior in plants, a principal component analysis of the plant samples was performed (Figure 28.8). Roots and leaves of the plants grown in the two soils were separated from each other. The PC1 was

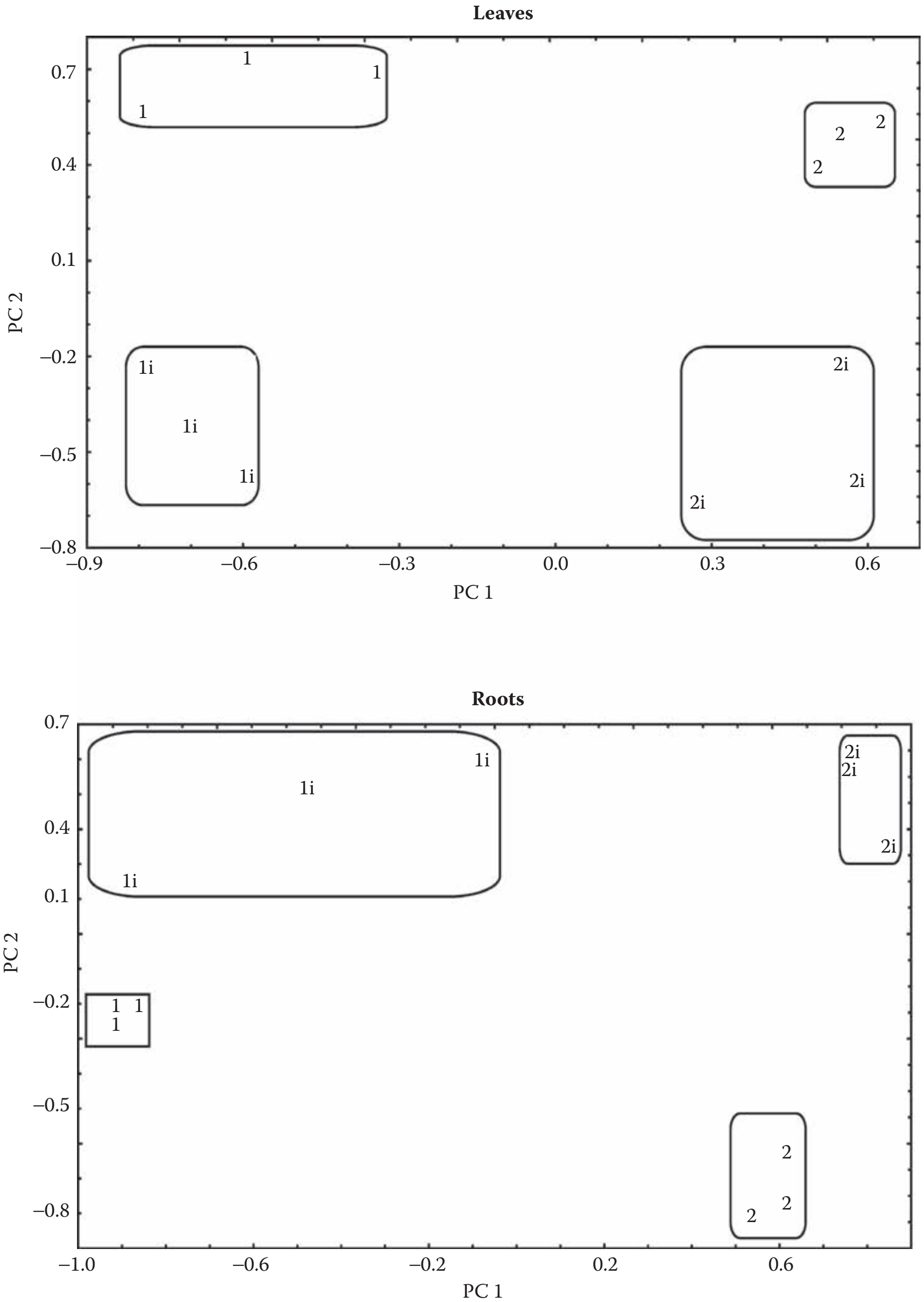

FIGURE 28.8 Score plot of the PC1 and PC2 for leaves and roots of the wheat seedlings grown in clean (1) and contaminated (2) soils. 1 and 2: plants were grown in nonfertilized soils; 1i and 2i: plants were grown in soil fertilized with ispolin.

responsible for the separation. In roots, Na, Cu, S, Pb, Zn, Sb, and P were highly correlated with the first PC. In leaves S, Sb, Mo, Na, Cu, Ca, As, and K provided the main contribution to the PC1. As one might expect, with small exceptions (P in roots), concentrations of these elements were different in plants grown in the two soils. Although the list of the elements responsible for the separation of the plant samples into different groups was similar for roots and for leaves, certain differences between different plant parts were observed. This might reflect different functions of roots and leaves in a plant and, as a result, different behavior of certain elements in the two plant parts.

The second PC was responsible for separation of the plants grown in the control and fertilized soils. Highly correlated with the PC2 elements were K, Rb, Mo, P, Pb, B, and Sb in roots and Rb, K, Mo, As, Mg, Sr, and Cd in leaves. In both cases, K, Rb, and Mo provided the most significant contribution to separation of the plant samples. In ispolin, potassium and molybdenum were presented in a form that may be easily taken by a plant. An application of ispolin resulted in an increase of the level of potassium in soil. This may be explained by rather high (5 to 8%) concentration of potassium in ispolin. Rb is a chemical analogue of K and similarity of physicochemical parameters of K^+ and Rb^+ might result in a similar behavior of the two ions in the plant. In particular, it is known that, under K^+ deficiency, plants can take up higher amounts of Rb [103]. Other elements were rather different for different plant parts. As it was in the previous case, concentrations of many of these elements in plants grown in fertilized and nonfertilized soils differed significantly, though an addition of ispolin to both soils did not result in an increase of concentrations of these elements in the soils.

28.6.1 EFFECTS OF MINERAL ELEMENTS ON PLANT BIOMASS AND METAL UPTAKE

Deficiency and excess of mineral elements in soil can significantly affect plant yield. It is known that nonsufficient nutrient availability can reduce growth of leaf biomass [104]. In addition, metal mobility in soil solution and, as a result, the rate and extent of metal phytoextraction will also depend on the presence and amounts of various inorganic chemicals in the rhizosphere. For example, phosphorus is a major nutrient, and plants respond favorably to the application of P fertilizer by increasing biomass production. The application of P fertilizer, however, can inhibit the uptake of some major metals due to metal precipitation as pyromorphite and chloropyromorphite [105].

Potassium has been found to enhance yielding capacity, resistance to stresses and diseases and crop quality, though it was also reported that potassium fertilizer depending on the K/Mg ratio in the soil can increase or decrease the plant yield. (When this ratio is near 1, the yield increases, but when it is over 3, the yield can decrease [106].) High bioavailable potassium content in soil may also result in Mg and Ca deficiency in plants. This may be illustrated by data presented in Table 28.6 and Figure 28.9.

An application of ispolin, fertilizer, which has a very high content of available potassium, resulted in a significant increase of K concentration in soil. Total amount of K and content of exchangeable K were higher in the soils amended with this fertilizer. As a result, concentration of K in the plants grown in the fertilized soils was higher than in the control plants. Although Ca and Mg concentrations in both soils remained unchanged after application of ispolin, concentrations of these two nutrients in plants decreased. The most significant decrease was observed in leaves. When the K/Mg ratio in soil was the highest, a statistically significant decrease of Mg concentration in roots was also observed. Figure 28.9 shows variations in plant biomass resulting from application of ispolin. The variations were soil dependent.

In loam soil, an application of ispolin stimulated plant yield, but fertilization of sandy loam soil did not affect the growth of plant biomass (only in this case the K/Mg ratio in the soil was the highest: 6.2). Other characteristics of the physiological state of the plants — for example, chlorophyll index — demonstrated significant effects from ispolin application in the plants grown in loam soil and an absence of such an effect in the plants grown in sandy loam soil (Figure 28.9).

Zhao et al. [24] studied the effects of soil amendments with KNO_3, KCl, and K_2SO_4 on plant yield and metal uptake. They found that shoot and root dry weight of wheat was reduced significantly by the addition of K fertilizer in KCl and K_2SO_4 forms, but that only minimal changes occurred when soil was treated by KNO_3. This experiment showed that anions such as Cl^- and

TABLE 28.6
Total Amounts and Concentrations of Exchangeable Mg, K, and Ca[a] in Fertilized and Nonfertilized Soils and in Plants Grown in the Soils

	Loam soil		Sandy loam soil	
Element	**Control**	**+ Ispolin**	**Control**	**+ Ispolin**
Mg total	5465 ± 140	5460 ± 358	4077 ± 793	4057 ± 575
Mg fraction	315 ± 10	333 ± 11	54 ± 4	72 ± 8[b]
Mg roots	2437 ± 138	2873 ± 698	2237 ± 107	1903 ± 134[b]
Mg leaves	3747 ± 165	3113 ± 230[b]	3560 ± 79	2443 ± 253[b]
K total	3883 ± 156	4170 ± 150[b]	1400 ± 125	1720 ± 149[b]
K fraction	333 ± 10	702 ± 51[b]	80 ± 3	447 ± 83[b]
K roots	17,700 ± 1300	34,700 ± 6447[b]	11,300 ± 600	29,100 ± 500[b]
K leaves	80,400 ± 5500	98,500 ± 3051[b]	59,600 ± 1900	81,200 ± 7200[b]
Ca total	35,200 ± 1600	33,500 ± 3400	9265 ± 1525	9170 ± 1378
Ca fraction	5110 ± 192	5123 ± 125	962 ± 69	992 ± 65
Ca roots	5660 ± 175	6633 ± 1731	4403 ± 286	3897 ± 231
Ca leaves	6633 ± 439	4593 ± 153[b]	4063 ± 301	2370 ± 160[b]
K/Mg fraction	1.1	2.1	1.5	6.2

[a] mg kg^{-1}.

[b] Differences between control and ispolin-fertilized samples are significant at $P < 0.05$.

SO_4^{2-} increased Cd uptake by plants; this may be explained as a result of Cl^- and SO_4^{2-} complexing with Cd^{2+} and thereby increasing the bioavailability of Cd^{2+} in soil. Similar results were reported by Norvell et al. [30], who showed that accumulation of Cd in wheat may be enhanced by presence of Cl^- ions in soil. Although the mechanism of the ion interactions was not clear, the authors suggested that it is likely to involve increased solubility or availability of soil Cd resulting from the formation of chlorocomplexes in soil solution.

Potassium also has an effect on plant uptake of Cd. In particular, Cd concentrations in shoots increased two times with increasing K addition [24]. Lorenz et al. [107] reported that an excess addition of such cations as K^+ and NH_4^+ to soil caused a substantial increase of major and heavy metal ions in soil solution as well as their uptake by plants. This may be explained by the fact that NH_4^+ ions have an acidifying effect in soil, due to the nitrification processes or the release of H^+ ions to soil solution during plant uptake of NH_4^+ ions.

An application of amendments such as lime, apatite, and zeolite will increase Ca content in soil and also increase soil pH [49]. Because the increase of pH may lead to decrease of metal mobility in soil, these Ca fertilizers may be used for metal stabilization in contaminated soils. It is also known that many enzymatic and physiological processes in plant cells are under the regulatory control of Ca^{2+} [108]. In particular, externally supplied Ca^{2+} can affect the toxicity of some elements, perhaps by competing with metal uptake [104].

Kim et al. [36] studied the effects of the presence of K^+, Ca^{2+}, or Mg^{2+} in soil on transport and toxicity of Pb^{2+} and Cd^{2+} in rice. In contrast to the data cited previously, in this experiment K^+ had little effect on uptake or toxicity of Pb^{2+} and Cd^{2+}. Ca^{2+} and Mg^{2+} blocked Cd^{2+} transport into rice roots and Cd^{2+} toxicity for the root growth, which suggested that detoxification effect of these ions is directly related to their blocking the entry of the metal. Similarly, Ca^{2+} blocked Pb^{2+} transport into the roots and Pb^{2+} toxicity for the root growth. The authors suggested that the protective effect of Ca^{2+} on Pb^{2+} toxicity may be related to inhibition of the metal accumulation in the root tip, a potential target site of Pb^{2+} toxicity. Mg^{2+} did not ameliorate the Pb^{2+} toxicity for the root growth as much as Ca^{2+} did, though it decreased Pb^{2+} uptake by roots in the same manner as Ca^{2+}. These results suggest that the protective effect of Ca^{2+} on Pb^{2+} toxicity may involve multiple mechanisms,

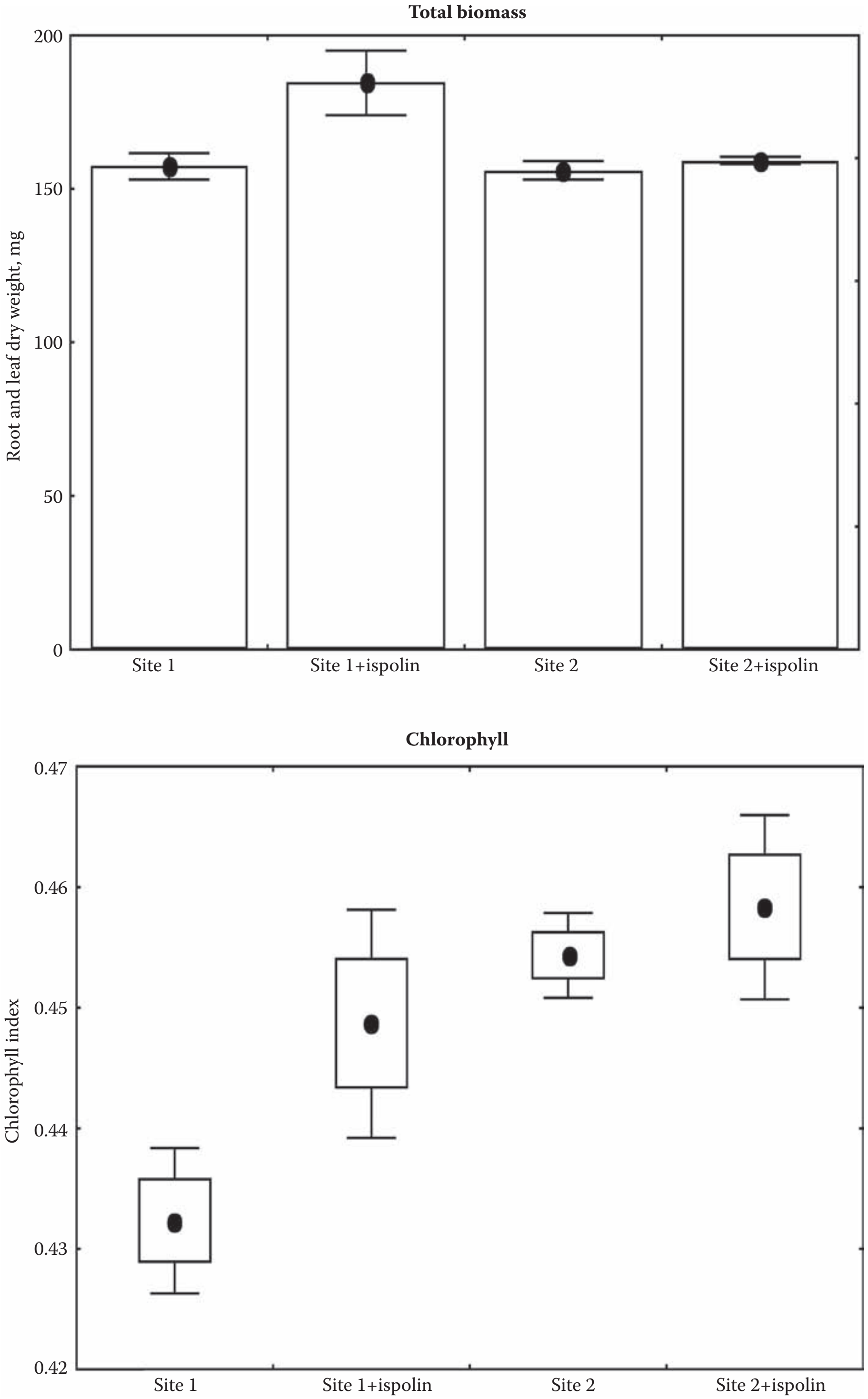

FIGURE 28.9 Variations in root and leaf biomass and chlorophyll index in wheat seedlings grown in fertilized and nonfertilized loam soil (site 1) and sandy loam soil (site 2).

including competition at the entry level, and that Pb^{2+} and Cd^{2+} may compete with divalent cations during transport of the metals into roots of rice plants.

Thus, soil amendments capable of enhancing the transfer of metals from soil solid phase to soil solution and increasing the metal uptake by plants can help to enhance metal phytoextraction. Application of fertilizers is very sensitive to soil parameters and plants growing in the soils; thus, the success of soil fertilization for enhancement of plant production and metal phytoextraction will depend on the selection of appropriate combination of the plant species and soil amendment.

28.7 SOIL BIOTA AS A PROMISING MEANS TO AFFECT METAL PHYTOEXTRACTION

Another possible way to stimulate yield of plants and increase plant metal uptake is an appropriate manipulation of plant—microorganism interactions. From 20 to 50% of plant biomass is below the ground [109]; from 30 to 60% of assimilatory products of photosynthesis are in the root zone, where they are used for root growth, root respiration, ion uptake, and release of organic exudates into the rhizosphere [53]. Unfortunately, due to the high complexity of the soil ecosystem, many aspects of the interactions between plants and soil microorganisms are still poorly understood. Numerous studies describing uptake of metals by plants often ignore the impact of soil biota on the processes of plant growth and metal uptake.

The structure of the soil microorganism community depends significantly on the physicochemical parameters of soil. For example, it was reported that the highest biomass of microorganisms in Podzol soil is presented by mycelium of fungi; the contribution of bacteria is less than 2.3% of the total amount of all microorganisms and the amount of actinomycetes may be very small [110]. Chernozem is enriched with bacteria (up to 90% of the total amount of soil microorganisms), the part of actinomycetes is 2 to 5%, and contribution of fungi may be less that 1%. The microbial colonization is also specific to certain plant species.

Elevated levels of trace metals can affect qualitative and quantitative structure of microbial communities. Many reports have demonstrated that heavy metals adversely influenced microorganism growth, morphology, and biochemical activities resulting in the decrease of microbial biomass and diversity [111,112]. In particular, it was shown that such trace metals as Cu, Ni, Pb, and Zn inhibited the extent of mycorrhizal colonization of cereal plants [113]. Cd, Cu, and Ni also significantly inhibited the *Azotobacter* population [21].

Moreover, it seems that any, even insignificant, disturbance in metal concentrations in the growth medium can negatively affect the soil microbial population. Pot experiments performed by the author with wheat seedlings grown in soil to which a small amount of nontoxic Cr^{3+} was added demonstrated considerable variations in the diversity of the rhizosphere microorganisms [114]. In particular, a large part of ordinary microbial strains died within the first hours after Cr^{3+} was added to soil; the number of the microorganisms that were previously presented in negligible amounts increased significantly. It is interesting that total amounts of the rhizosphere microorganisms remained at approximately the same level as in the control. It would be reasonable to suggest that, as a result of the variations in the microbial community, certain changes in chemical situation in the rhizosphere may be expected.

Microbial processes play an important role in defining the speciation and mobility of toxic metals and radionuclides in soil [115]. It has been reported that soil microorganisms, primarily bacteria and fungi, can significantly affect metal uptake by plants [116,117]. Rhizosphere biota can interact symbiotically with roots to enhance the potential for metal uptake and directly influence metal solubility by altering their chemical properties. In particular, plants and associated microorganisms can change soil pH, thus affecting the availability of main nutrients and trace metals [118].

Superior uptake properties to facilitate the movement of mineral elements into plants were demonstrated for different microbial strains. Preliminary evidence indicates that soil microbes are also important in the metal translocation from roots to upper plant parts [40,119]. Therefore, it is quite possible to manipulate the soil environment in such a way that metal uptake would be increased and, as a result, the process of metal removal from contaminated soils would be enhanced. In addition, it is clear that an improvement of nutrient availability as a result of the physiological activity of the rhizosphere microorganisms will increase plant growth, with a consequent removal of more metals from soil.

28.7.1 ARBUSCULAR MYCORRHIZAL FUNGI

Despite the important role that rhizosphere microorganisms play in plant interactions with the soil environment in general, and toxic metals in particular, relatively few studies have focused on the effects of these microorganisms on metal remediation efforts [120]. Among the rhizosphere organisms used in the phytoremediation studies, the arbuscular mycorrhizal fungi (AMF) have received special attention.

Data on metal uptake by mycorrhizal plants and the role of AMF in the metal uptake by host plants are rather contradictory. It is assumed that, in general, plant–AMF symbiosis may play an important role by increasing metal stress tolerance [121]. It was reported that fungi may constitute a biological barrier against transfer of toxic metals to the shoot [122]. In some cases, AMF can reduce excess plant uptake of elements like Zn, Cd, and Mn. The mechanisms by which AMF provide protection against metal toxicity to themselves and to plants are still relatively unclear. A number of mechanisms have been postulated; however, in some cases, data have been ambiguous and circumstantial [123]. In other cases, AMF have been shown to enhance or have no effect on the uptake of Zn, Pb, Ni, and Cu [113].

Inconsistent results on the effects of AMF on metal uptake may be due to a range of factors that are not controlled or accounted for, such as an inherent metal uptake capacity of plants, corresponding fungal properties, and soil adsorption/desorption characteristics [121].

28.7.2 BACTERIA

Bacteria have the ability to produce metabolites that, in suitable plant hosts, show activity similar to auxins, giberellins, cytokinins, and ethylene [124]. The role of bacteria in the rhizosphere chemistry is of particular interest. The specific exudates that root-colonizing bacteria excrete into surrounding soil catalyze the oxidation-reduction reactions that can alter mobility of metal ions [125]. Therefore, bacteria potentially may be used to facilitate movement of metals that would otherwise be unavailable to plants. It was shown that, in some cases, bacteria are perhaps more proficient than root exudates at solubilizing and absorbing certain metals [126]. For example, Zhou et al. [127] used bacterial extracellular polymers (BEP) to affect fate of uptake and translocation of inorganic pollutants in terrestrial ecosystems. An addition of BEP to metal-contaminated soil resulted in 1.6- to 12.8-fold higher soil-soluble Cu concentration over the control. Consequently, the Cu uptake by the ryegrass grown in the soil was increased by 31%.

However, the question remains whether the rhizosphere population can be altered in such a way that metal uptake by host plants would be increased. Preliminary studies with different bacterial systems showed that it is very difficult to overcome the competitiveness of the indigenous population and that inoculation is rarely successful [128]. Meanwhile, it may be assumed that the problem lies in the way by which interactions between bacteria and plant will be affected. Why it is desirable to change species populations in the rhizosphere and the consequences of such variations must be considered.

It seems that modification of the soil microbial biomass by conventional agricultural practices would be easier than attempts to modify it by means of direct addition of microorganisms to soil. In particular, in many experimental studies, the new population of microorganisms was just added to soil. If the experimental design is changed slightly — for example, if seeds are treated before sowing with certain strains of microorganisms — the proposed results may show considerable promise as a means to enhance the metal uptake. Seed germination is an important stage of plant growth. The conditions during the germination may have a crucial significance for further life of the plant.

28.8 EFFECTS OF *CELLULOMONAS* AND *MYCOBACTERIUM* STRAINS ON METAL PHYTOEXTRACTION BY CEREAL CROPS

In the author's experiments, seeds of wheat, oats, and barley were germinated on a moist filter paper to which culture of phosphate-mobilizing bacteria *Cellulomonas* sp. 32 SPBTI or *Mycobacterium* sp. 12 was added. It was shown that the bacteria are able to affect the uptake of different macro- and trace elements [119]. As an example, Figure 28.10 illustrates transfer of potassium from soil solution to leaves of oats treated and not treated with *Cellulomonas*. Potassium content in leaves of the plants increased and concentration of exchangeable K^+ in the soil solution decreased as a result of the bacterial treatment. Thus, *Cellulomonas* was favorable for supply of the plants with potassium. Similar variations in K content were observed after treatment of wheat seeds with *Mycobacterium.* However, treatment of barley seeds did not significantly change concentrations of K in soil or in different plant parts.

The ability of microorganisms to accumulate large amounts of potassium is well known [129]. Growth rate of bacteria also depends upon K^+ [130]. As a consequence of the metabolic activity of plants and microorganisms, concentration of K^+ in soil solution near the root surface can decrease, thereby inducing desorption of K^+ held on the external surface of soil particles. Therefore, the increase of K content in the plants and decrease of K concentration in soil are quite appreciable.

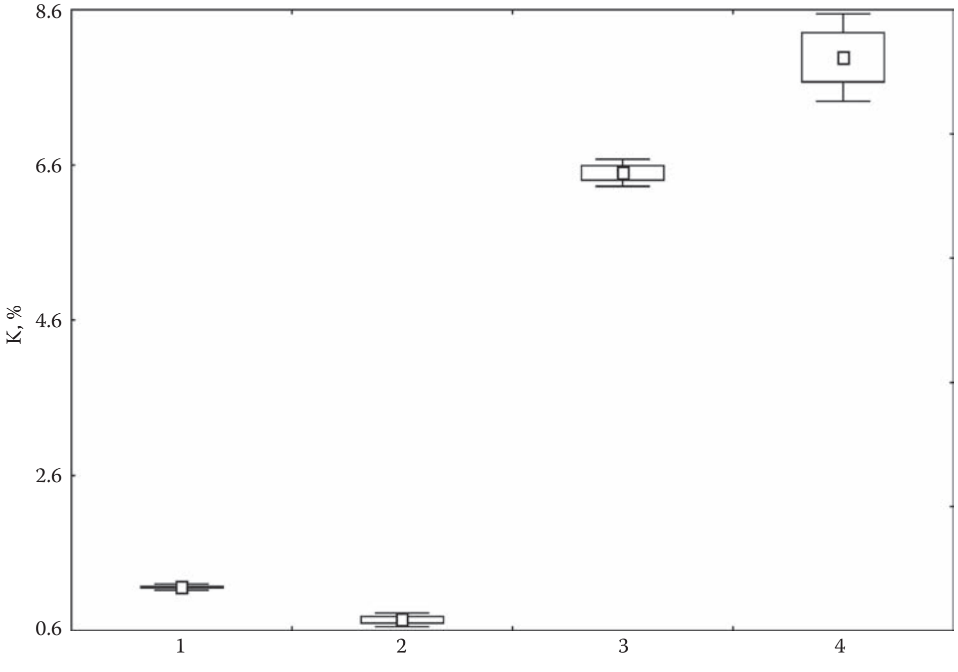

FIGURE 28.10 Concentration of exchangeable K^+ in soil solutions after cultivation of soil with nontreated (1) and treated with *Cellulomonas* (2) oats and K content in leaves of nontreated (3) and treated with *Cellulomonas* (4) plants.

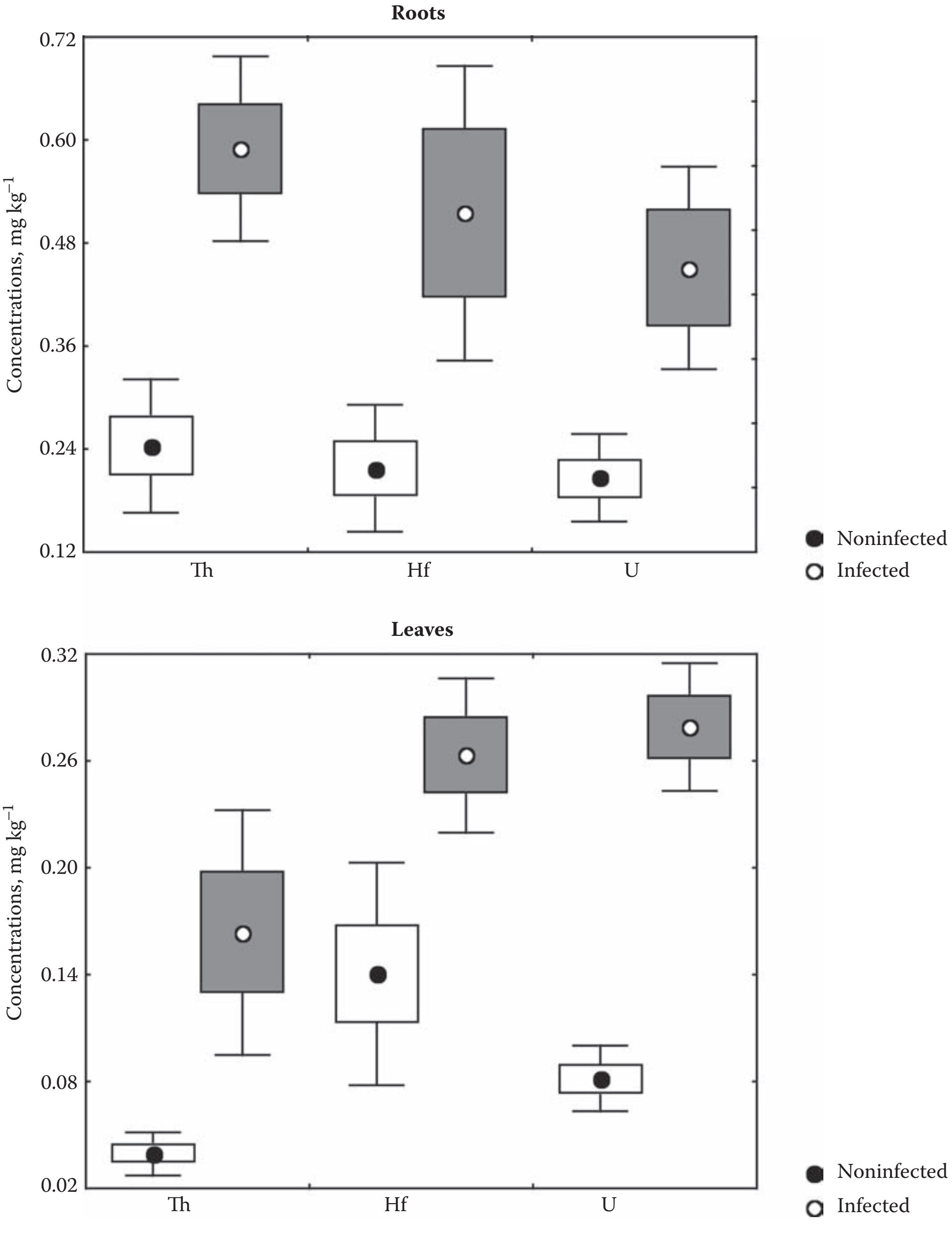

FIGURE 28.11 Concentrations of Hf, Th, and U in roots and leaves of the wheat seedlings infected and noninfected with *Cellulomonas*.

It was also found that presowing treatment of wheat seeds by bacteria may result in significant variations in uptake of different elements and their translocation from roots to leaves. Concentrations of many elements in roots and leaves of the plants treated by bacteria were much higher than those in the control plants. In particular, concentrations of Cr, Rb, Ag, Sb, Cs, Hf, Th, and U increased in roots and in leaves of the wheat seedlings treated with *Cellulomonas* compared with those in the control. Figure 28.11 illustrates such variations in the example of three elements: Hf, Th, and U. On the other hand, concentrations of many rare earth elements (La, Ce, Sm, Yb, and Lu) and

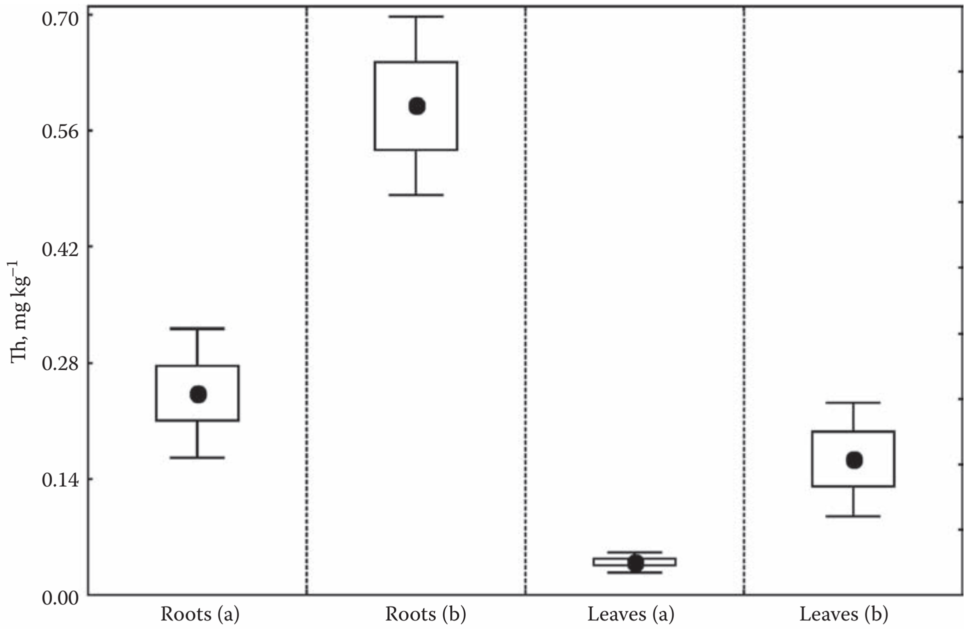

FIGURE 28.12 Thorium in roots and leaves of wheat seedlings noninfected (a) and infected (b) with *Mycobacterium*.

Fe increased only in roots, and contents of K, Sc, Zn, and Eu were higher only in leaves, of the plants treated by the bacteria.

Similar variations in element uptake were observed after treatment of seeds with *Mycobacterium*. Figure 28.12 shows concentrations of Th in roots and leaves of wheat (*Triticum astevum*) infected and non-infected with *Mycobacterium*. The differences in Th uptake by treated and nontreated with *Mycobacterium* plants are statistically significant. Figure 28.13 illustrates the dynamics of Th in soil artificially contaminated with this metal after growth in the soil wheat seedlings inoculated with *Mycobacterium*.

It is known that, because of their high complexing ability, all radionuclides can be bound tightly to solid surfaces [131]. Therefore, one might expect that an addition of Th to the experimental soil may result in a quite rapid absorption of Th by soil organic matter and Th adsorption on the surface of soil particles. The observed variations in Th content in the soil might be caused solely by physiological activity of the experimental plants and microorganisms. Thus, it may be assumed that the changes in species diversity in the rhizosphere and, as a result, variations in the organic exudates into the soil can stimulate transfer of certain elements to more bioavailable forms. As a consequence, roots will be able to take up more metals and the rate of metal translocation to leaves may also be increased.

28.9 CONCLUSIONS

Cereal crops in combination with certain soil/plant treatments were shown as perspective "tools" to improve metal phytoextraction and removal of metals from contaminated soils. In conclusion, it may be said that a greenhouse experiment is the key stage in identifying the optimal procedures required to increase metal bioavailability and to stimulate metal phytoextraction, though only during a field trial, when the results of the previous model experiments are tested in real environmental conditions, can the feasibility of the manipulation measures finally be estimated. At this stage,

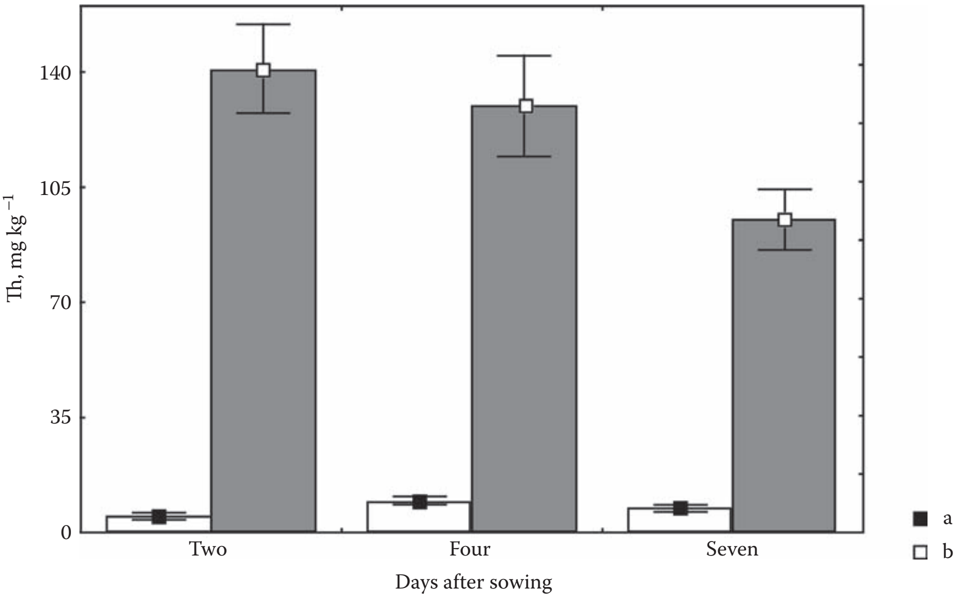

FIGURE 28.13 Dynamics of Th concentration in clean (a) and artificially contaminated with Th (b) soils after growing in the soils of wheat seedlings infected with *Mycobacterium*.

necessary modifications and corrections may be made, taking into account the local situation. The plant species and soil treatments found to be promising under batch experiment and/or greenhouse conditions may be less effective in the field.

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