PLANT ROOTS

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edited by
Yoav Waisel
Amram Eshel
Tel Aviv University
Tel Aviv, Israel
Uzi Kafkafi
The Hebrew University of Jerusalem
Rehovot, Israel

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Trace Element Stress in Roots

Jürgen Hagemeyer and Siegmar-W. Breckle
versity of Bielefeld, Bielefeld, Germany

I. INTRODUCTION

Heavy metals comprise a small part of the earth's crust. Nevertheless, these elements play an important role in plant ecology and affect growth and performance of plant roots even in small quantities as trace elements. Trace elements can be divided into three groups. Some of them are rare and others are more abundant. The presence of both is not essential to plants. The third, smaller group includes those elements that are essential for some or for all organisms. The dose response curves of the effects of essential trace elements on organisms consist of three parts: at very low concentration organisms suffer from deficiency exhibiting characteristic symptoms; (2) at a range of medium concentrations organisms grow normally; and (3) at concentrations above a critical level the elements are toxic (Berry and Wallace, 1981). High concentrations of trace elements in the rhizosphere of higher plants primarily damage the roots. Consequently, they also affect other plant parts. The threshold levels of deficiency and of toxicity differ widely for the various elements (Baker and Walker, 1989). In the nonessential elements, only normal and toxic concentration ranges (2) and (3) are found.

In most ecosystems potentially toxic trace elements occur naturally in an active form only in very small quantities. Human mining activities have resulted in an ever-increasing contamination of the biosphere with potentially toxic trace metals (Nriagu, 1996). An outstanding example is lead. Once this element was rather rare. It is now one of the most widely distributed trace metals and it is more evenly distributed in the terrestrial ecosystems than before (Neite et al., 1992; Nriagu, 1992; Singh et al., 1997). The worldwide use of leaded gasoline, which began in 1923, has resulted in contamination of all ecosystems.

II. ESSENTIAL, BENEFICIAL, AND TOXIC TRACE METALS

Several trace metals are essential for higher plants. This means that (1) plants cannot complete their life-cycle without the respective elements; (2) they cannot be replaced by other elements; and (3) they have a specific function in plant metabolism (Marschner, 1986). For such elements the concentration range between deficiency and toxicity varies widely. Currently six trace elements are considered essential for higher plants: boron, copper, iron, manganese, molybdenum, and zinc. Two additional candidates are chlorine and nickel (Marschner, 1986). Nickel seems to be an essential micronutrient, although the failure to complete the life-cycle without nickel has only been confirmed in few plant species (Gerendas et al., 1999). Some elements, like cobalt, iodine, sodium, silicon, and vanadium, are considered beneficial but not essential for plant growth (Marschner, 1986). All beneficial and essential elements cause toxicity symptoms when concentrations are high. Nevertheless, they are not regarded as toxic elements.

Only those exhibiting no essentiality are regarded as toxic elements.

Because of the variability in the responses of various plants to some elements, essentiality is sometimes difficult to ascertain. An insufficient supply of a plant with an essential element causes specific deficiency symptoms. Nonessential elements never cause deficiency symptoms, even at extremely low concentrations. However, in a number of studies, unexplained growth enhancements were observed in plants subjected to mild stress from toxic trace elements. For example, root growth stimulation of *Betula pendula* seedlings was induced by low concentrations of cadmium (Gussarsson, 1994). Enhanced root elongation, root biomass gain, and root hair formation were found in plants of a tolerant population of *Silene vulgaris* treated with lead chloride (Wierzbicka and Panufnik, 1998). Jiang and Liu (1999) reported stimulated root growth of *Brassica juncea* plants treated with low concentrations of lead nitrate.

III. EFFECTS OF TRACE ELEMENTS ON EXTENSION GROWTH AND DIFFERENTIATION OF ROOTS

Trace element effects on plants also depend on whether the element is an essential nutrient or not. Inhibition of root extension growth can result from interference with cell division or with cell elongation. Trace elements were shown to affect both processes. Slowing down of the mitotic rate of root cells of *Allium cepa*, *Zea mays*, and *Lupinus luteus* was caused by high lead concentrations (Hammett, 1929; Przymusinski and Wozny, 1985). It was hypothesized that such inhibition is related to a lead-induced reduction of cell cycle proteins, like cyclin (Deckert and Gwozdz, 1999). Apparently lead has several effects. However, its main influence on root growth was on root cell elongation (Garland and Wilkins, 1981; Sieghardt, 1981). The elasticity of cell walls is so much reduced by lead or by cadmium that under mechanical stress they may break (Lane and Martin, 1982; Barcelo et al., 1986).

A physiological explanation of root growth inhibition under lead stress suggests an increased level of free radicals and of reactive oxygen species, which exceeds the capacity of the antioxidant enzymes (Rucinska et al., 1999). This may result in reduced root growth. Lead nitrate (10^{-2} M) had no effect on the emergence of the seminal roots of maize (*Zea mays*) seedlings but did affect root elongation after emergence. At 10^{-3} M lead nitrate, the growth of primary and of other seminal roots was slowed down as a result of partial inhibition of cell division and cell elongation (Obroucheva et al., 1998). Lead stress also had marked effects on root branching pattern and on root system morphology.

Lead toxicity to *Fagus sylvatica* seedlings started at a concentration of 48 μmol lead/kg soil. At lower lead concentrations, root growth was slightly enhanced (Breckle et al., 1988). Similar results were observed in a culture experiment with young *Picea abies* trees (Hagemeyer et al., 1994). Root growth increased at low soil concentrations of cadmium or zinc. However, at higher concentrations of both metals root growth of *Picea* and *Fagus* saplings was strongly inhibited (Hagemeyer et al., 1994). A slight growth enhancement by low metal concentrations depends on the specific metal and is known in general already since Sachs (1874).

Lead caused reductions in root elongation of *Allium cepa* already at concentrations of 0.1 μM lead nitrate (Liu et al., 1994a). The lead treatment caused irregularities in mitoses. Nickel sulfate treatments up to 10 μM resulted in stimulated root growth, but higher concentrations inhibited it. At high nickel concentrations irregularly shaped nucleoli were observed in root cells (Liu et al., 1994b).

Structural and ultrastructural effects of copper stress on *Zea mays* roots were reported by Ouzounidou et al. (1995). Seedlings grown at higher copper concentrations (80 μm) exhibit damaged epidermal cells of their roots. In other root tissues the effects of such a copper level were varied. Cortical or stelar cells with disintegrated cytoplasm were observed next to cells with well-preserved structure. The root ultrastructure was less affected by copper than their morphology and physiology. The occurrence of healthy cells in copper-stressed roots indicated a varied response of the cells to such harmful conditions.

Inhibition of root elongation of *Picea abies* seedlings grown in nutrient solutions containing mercury, cadmium, or zinc was reported by Godbold and Hüttermann (1985). The order of toxicity was Hg > Cd > Zn. Toxicity symptoms of mercury in spruce seedlings, like decreased transpiration rates and lowered chlorophyll contents in needles, were attributed primarily to root damage (Godbold and Hüttermann, 1988).

Ultrastructural alterations of root cells of *Cajanus cajan* treated with zinc sulfate or with nickel sulfate and inhibition of radicle elongation in seedlings were described by Sresty and Madhava Rao (1999). Toxic effects of Zn and Ni were correlated with their concentrations. Extensive damage to root cells grown under metal stress was shown by EM. The nuclei of root tip cells showed condensed chromatin strands. Some cortical cells showed disruption and dilation of their nuclear membranes. Other toxicity symptoms were expressed by structureless cytoplasm, by disintegration of organelles, and by the development of vacuoles. Some cortical cells showed two nucleoli. The authors suggested that this might be a result of the stimulation of the nucleolus to increase the production of ribosomes and mRNA, which enhance the synthesis of new proteins involved in the trace metal tolerance.

Effects of nickel sulfate ($\geq 10\,\mu$M) on root growth of *Pisum sativum* plants resulted in reductions of root ension growth and a reduction of potassium concentrations (Gabbrielli et al., 1999). The water content of roots was negatively correlated with the tissue nickel concentration. Roots under nickel stress had increased phenol contents and higher extracellular peroxidase activities. Such effects were indicative to a rapid senescence. A selective cell death of damaged tissues may be part of a defense strategy.

The chemistry as well as the physiological effects of chromium are rather complicated (Barcelo and Poschenrieder, 1997; Mishra et al., 1997). Trivalent and hexavalent chromium had somewhat different effects on root growth of onion plants (Liu et al., 1992). At concentrations of 0.2–20 μM hexavalent dichromates reduced root growth more than Cr^{3+}, mostly by inhibition of cell division. Chromium interfered with mitoses and caused chromosome aberrations.

The effects of trace elements were also studied with l-grown plants. Soil treatments are usually better comparable to natural or field conditions than nutrient solution experiments. However, such conditions are more difficult to control and direct observations of roots are restricted.

A standardized "artificial soil" consisting of an ion exchange resin embedded in an inert sand matrix was proposed for studies of trace element effects on root growth (Köhl, 1997). The metal ions are buffered by the ion exchanger and the sand provides the mechanical impedance like in natural soils. Implementation of this experimental technique should advance our understanding of the root–soil–trace element relationships.

Under natural conditions plant roots are rarely exposed to stress from a single toxic element in the soil. Various ions usually affect plant growth simultaneously (Hagemeyer, 1999). The interactions of the effects of different metals can be described as *independent*, *additive*, *synergistic*, or *antagonistic* (Berry and Wallace, 1981; Wallace, 1982). Such interactions should also be considered in experimental studies. In combination treatments, cadmium and lead showed additive or even synergistic effects on root growth of *Fagus sylvatica* seedlings (Kahle, 1988; Breckle et al., 1988; Kahle and Breckle, 1989). For instance, in a treatment with 2.4 mmol Pb (kg soil)$^{-1}$ the roots had only 35% of the dry matter of the control. At 45 μmol Cd (kg soil)$^{-1}$, the root dry matter was 71% of the control. However, the two metals applied at the same time reduced root growth to 29% of the control. The combination of the two metals (269 μmol Pb (kg soil)$^{-1}$ + 178 μmol Cd (kg soil)$^{-1}$, ammonium acetate-extractable fraction) reduced the root mass of saplings significantly more than application of each of the ions separately. Another example for additive effects was found with saplings of *Picea abies* (Hagemeyer et al., 1994). Combined treatments with Cd + Zn reduced root growth much more than either of the separate treatments.

The effects of combinations of copper, cadmium, and zinc on root growth of *Silene vulgaris* exposed to single metals or binary combinations in hydroculture were nonadditive (Cu + Zn, Cu + Cd) or antagonistic (Zn + Cd), when applied concentrations were rather low. The nature of the combination effects depended on the metal concentrations (Sharma et al., 1999). When one of the metals of the combinations was applied in concentrations above a critical toxicity level, synergism was the predominant interaction.

Synergistic effects in seedlings of *Sinapis alba*, i.e., greater growth inhibition, were found in combinations of vanadium with nickel, molybdenum, or copper (Fargasova, 1999). Vanadium and manganese had mutually antagonistic effects. Manganese, molybdenum, and copper were antagonists of nickel. In some combinations no interactions of the metals were found. Many such interactions between trace elements are encountered in studies using soil or other complex substrates. Although they pose additional complicating factors, they should not be neglected in realistic studies.

Reactions of plant roots to trace elements can be very sensitive. However, the outlined results demonstrate the large variability in effective critical concentrations, which cause toxicity symptoms. This can be observed even within the same species. Obviously the effects of a certain element depend largely on substrate conditions, particularly the presence of interacting ions. It therefore seems questionable to establish critical upper levels of toxic elements, which can be tolerated by plants.

IV. EFFECTS OF TRACE ELEMENTS ON PLANTS OF VARIOUS LIFE FORMS AND SYSTEMATIC CATEGORIES

Genotypic differences in resistance to trace elements were described for various wild plants (Ernst, 1982; Baker and Proctor, 1990; Macnair, 1993). It has been well known for a long time that the flora of mining areas is resistant to toxic levels of such mostly metallic elements. Those plants were named metallophytes (Duvigneaud and Denaeyer–De Smet, 1963). In some species or ecotypes resistance is restricted to one particular element; in others, cotolerance to two or several trace elements occurs (Cox and Hutchinson, 1979).

Very few species of trees are found among metallophytes (Ernst, 1985). During their long life span, trees accumulate large amounts of toxic elements when growing on contaminated soils. They generally lack the morphological or physiological adaptations that regulate internal concentrations of toxic trace elements, which are found in various herbaceous plants. Some exceptions can be found among mangroves and other halophytic trees (Hagemeyer, 1990, 1997; Breckle, 2000). Therefore, most trees can survive only on less contaminated substrates, where trace element concentrations in their tissues do not exceed critical levels (Ernst, 1985). There are, however, a very few specialized tree species which thrive on metal-enriched soils. An outstanding example is *Sebertia acuminata* (Sapotaceae), a Ni hyperaccumulator from New Caledonia. It has a remarkable capacity for nickel accumulation (Jaffre et al., 1976; Sagner et al., 1998).

The survival strategy of most trees on metal-rich sites seems to rely on the phenotypic plasticity, which enables tree root systems to avoid soil regions of high contamination (Dickinson et al., 1991; Turner and Dickinson, 1993). Plants vary in their response to trace elements not only among various life forms but also among various taxa. The subclass Caryophyllidae is a systematic group with many resistant members. A variety of trace element–resistant genera belong to the Caryophyllaceae and Plumbaginaceae. Some Brassicaceae are also typical metallophytes. Root growth can be used as an indicator of trace metal resistance. In this way the lead responses of 23 different plant taxa were compared by Wierzbicka (1999). Under the applied experimental conditions four groups of plants were distinguished: (1) species with the highest tolerance growing on mine waste heaps, like *Silene vulgaris* or *Leontodon hispidus*; (2) species with high constitutional tolerance, like *Biscutella laevigata* or *Zea mays*; (3) species with intermediate constitutional tolerance, like *Allium cepa* grown from seeds, *Triticum vulgare*, *Pisum sativum*, or *Secale cereale*; and (4) species with low constitutional tolerance, like *Brassica napus* or *Phaseolus vulgaris*.

Taxonomic relations are, however, not always reliable indications for the trace element resistance of a species. The necessary mechanisms have apparently evolved several times independently. The physiotype concept (Albert and Kinzel, 1973; Kinzel, 1982; Choo and Albert, 1999), proposed for the classification of halophytes, has not yet been applied to metallophytes, with the exception of cadmium (Kuboi et al., 1986). This concept of a chemical characterization of various groups of plants by distinct ion patterns deserves further consideration in the case of trace element–resistant plants.

V. PLASTICITY OF ROOT DEVELOPMENT IN RESPONSE TO ENVIRONMENTAL CONDITIONS

Root development within a species is a result of a broad genetic disposition enabling the species to cope with a wide range of soil factors (Carlson and Bazzaz, 1977; Taylor and Allison, 1981; Stienen, 1985; Fitter, 1991; Chapter 2 by Fitter in this volume). In different horizons of the soil profile concentrations of nutrient and trace elements can show spatial as well as temporal variations. Such edaphic variations stimulated the evolution of the remarkable plasticity of root systems.

The effects of different soil layers on the development of *Fagus sylvatica* roots were investigated using growth chambers. In homogeneous substrate, root development of beech plants was faster than that of roots growing through three horizontal layers of soils, which differed in metal concentrations (Fig. 1) (Breckle and Kahle, 1992; Breckle, 1996). Stressed roots ($240\,\mu$mol Pb (kg soil)$^{-1}$), after reaching a horizon of low heavy metal stress (48 or $14\,\mu$mol Pb (kg soil)$^{-1}$), grew slightly faster than unstressed roots from the control growth chambers. This indicates an enhanced recovery growth. Characteristics of a distinct soil layer affect root growth and architecture. Also, the heterogeneity and patchiness of the soil profile have an effect.

Different parts of the root system of a plant, i.e., tap roots, basal roots, or lateral roots, can react differently to metal toxicity. This is another aspect of the plasticity of root development under stress. In an experiment with aluminum the lower layer in containers with stratified soil had toxic concentrations, whereas the upper

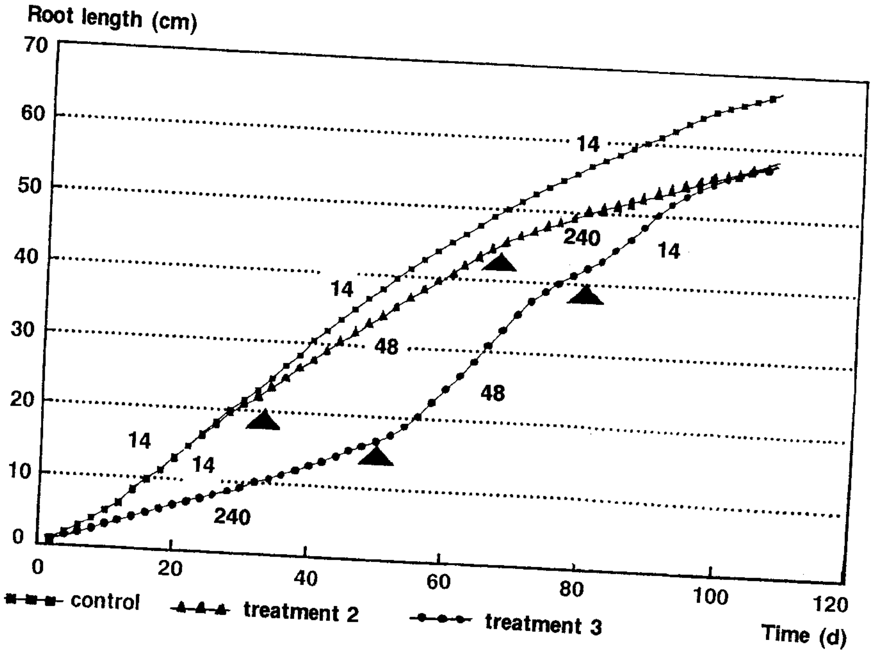

Figure 1 Effects of lead-contaminated soil layers on elongation of primary roots of *Fagus sylvativa* seedlings grown for 110 days in root growth chambers with soils differing in lead contamination. The numbers at each curve indicate the contamination of the layer with lead (μmol/kg, extractable fraction). Triangles indicate the time, resp. root-length, when the root reaches the next layer. 14-14-14 treatment: control. The 14-48-240 treatment 2 indicates increasing lead availability; the 240-48-14 treatment 3 ndicates decreasing lead availability. (From Breckle and Kahle, 1992.)

part of the soil was nontoxic (Bushamuka and Zobel, 1998). Recordings of root elongation of maize and soybean cultivars showed that in some aluminum-tolerant cultivars only part of the root system was able to grow in the aluminum toxic layer. Some cultivars increased lateral root production in the nontoxic topsoil layer, thus avoiding toxicity. It was concluded that individual parts of the root system could respond independently to aluminum stress in the soil. Therefore, measurements of total root production may produce misleading results in assays of metal tolerance of plant species.

The root systems of metal hyperaccumulating plants (see Section IX) also show adaptive responses to uneven distributions of metals in the soil. Zinc-hyperaccumulating *Thlaspi caerulescens* plants were grown in transparent containers with different types of soils differing in zinc (Schwartz et al., 1999) or cadmium concentrations (Whiting et al., 2000). The distribution of the metal in the containers was either homogeneous, ered, or patchy. Root distribution in the soil profile ended on the type of soil and the contaminating tal. While in a zinc-contaminated soil roots were clustered in the 5–10 cm of the profile, in a lead-contaminated soil more vertical roots explored the 10–15 cm layer. Nevertheless, in both treatments the mean lengths of the root systems were similar. When spots of zinc-contaminated soil were included into uncontaminated soil profiles, roots colonized the zinc-enriched zones strongly and almost exclusively. Unlike the roots of nonhyperaccumulating plants, the roots of *Thlaspi caerulescens* explored mainly zinc-enriched soil areas. The plants consistently allocated ~ 70% of their total root biomass and length in the Zn-enriched soil (Whiting et al., 2000). Zinc availability in the soil was the main factor influencing the root architecture. This is of particular importance when plants are used for phytoremediation of contaminated soils (Saxena et al., 1999).

The effects of low concentrations of trace elements on root growth depend also on the growth conditions. When comparing the effect of lead on *Phaseolus vulgaris* in aeroponic and hydroponic cultures it was found that seedlings grown in aeroponic chambers exhibited a significant decrease of growth with only 24 μM lead in nutrient solution. The roots were the organ with the highest percentage of growth inhibition with values reaching only 55% of the controls (Engenhart, 1984). On the other hand, in hydroponics all organs had increased their biomass in the 24-μM lead treatment, whereas with 48-μM lead root growth was slightly inhibited. Aeroponic plants had a stronger and larger root system with well-developed root hairs. In contrast, hydroponic plants had stronger and larger shoots and fewer roots with no root hairs. This can partly explain why aeroponic plants, which develop a larger root surface, were much more sensitive to lead (Christlieb and Weber, 1980; Engenhart, 1984).

VI. DEVELOPMENT OF ROOT HAIRS

The development and turnover of root hairs play a major role in the establishment of an efficient water- and mineral-absorbing root system (Gilroy and Jones, 2000; Chapter 5 by Ridge and Katzumi in this volume). The root hair density of *Raphanus sativus*, when grown in hydroponic culture, decreased with increasing lead concentrations (Lane and Martin, 1980). The lower density coincided with their earlier collapse under relatively low concentrations of copper, nickel, or cobalt (Blaschke, 1977; Patterson and Olson, 1983). Thus, absorption capacity for the toxic element and effective absorption time of the root hairs were distinctly reduced. This was shown for various crop plants as well as for young *Betula* trees. At the same time, the lower root hair density will exert a negative effect on the absorption capacity of water and of nutrients (Engenhart, 1984).

Plants of a lead-tolerant population of *Silene vulgaris* from southern Poland and of a nontolerant population were treated with 2.5 mg Pb L^{-1}. After 10 days of treatment, roots of tolerant plants were covered with root hairs on 100% of their surface, while nontolerant plants had hairs only on 60–68% of the root surface (Wierzbicka and Panufnik, 1998). At higher lead concentrations of 5 mg L^{-1}, the root hair length of the tolerant plants was somewhat smaller, but not as much as that of the nontolerant plants.

VII. DEVELOPMENT OF LATERAL ROOTS

It is generally accepted that the development of laterals determines the architecture of growing root systems. Adaptational changes of root architecture may be important for plants to survive on sites with a strong metal stress, where soil is a heterogeneous mosaic of patches with different chemical and physical properties. In seedlings of *Picea abies* grown for 4 weeks in solutions containing 0.5 μM Pb, growth of primary, secondary, and tertiary roots was reduced (Godbold and Kettner, 1991). The initiation of lateral roots was more sensitive to lead than the growth of already established older roots.

The development of laterals of second and third order in *Fagus sylvatica* roots was slightly stimulated by increased lead concentrations (Table 1). Such an increase can be explained by the decrease of the growth of the primary roots. The number of laterals of second and third order was higher. Therefore, the total length of all the roots of a system remained constant, although the primary root was shorter (Table 1). Dense branching of the root system is a typical response to damage of root tips. The architecture of root systems of beech trees was altered by lead from a loose to a more compact, branched structure. This was also demonstrated by experiments with cadmium and with combined applications of cadmium and lead (Bertels et al., 1989). In contrast, the density of lateral roots of maize decreased in a nutrient solution with 24 μM Pb, but increased again to the original level in

Table 1 Total Number of Lateral Roots of Various Orders per Plant, Percentage of Short Roots (< 2 mm long) Among All Laterals, and Length of the Unbranched Main Root of *Fagus sylvatica* Seedlings Grown for 40 Days in Root Chambers in Soil with Various Ammonium Acetate–Extractable Lead Concentrations

	Pb (μmol/kg)				
	14.4	48	115	211	1360
Number of laterals					
first order	110	87	70	47	4.1
second order	127	195	189	180	1.2
third order	1.5	1.0	9.6	14.0	0.3
Total number of lateral roots	238	283	268	241	5.6
Percentage of short roots	36.6	39.4	43.5	46.7	53.8
Length of main root from tip to first laterals (mm)	35.4	37.6	33.3	24.3	6.0

Source: Breckle (1996).

a higher lead treatment (Malone et al., 1978). This was explained by the need for a minimum stimulus to activate dictyosomes to export lead ions actively out of the cells.

Stimulated branching of roots under trace metal stress as a result of damage to the root tips by cadmium, lead, or zinc with effects on the formation of lateral roots in the herb *Ocimum sanctum* and in a metal-tolerant cultivar of the grass *Festuca rubra* was also reported by Cadiz and Davies (1997). In both species the metals at concentrations up to 10 μM stimulated the formation of lateral root primordia. Zinc had the strongest inducing effect. The authors also observed a reduction in the size of the apical root meristem under metal stress. They suggested that this effect is similar to a chemical decapitation, which releases the dominance effect of the root apex and thus increases the number of lateral root primordia.

An enhanced development of laterals was observed in various crop plants grown with increased but nontoxic copper levels (Blaschke, 1977). Under such conditions, the root systems showed a denser and more compact structure and the rates of water uptake had decreased. This was shown to occur in *Fagus* under lead stress (see above) and in *Trifolium* under manganese, copper, and zinc stress (Vogel, 1973). Indications for the same phenomenon were given by Rastin et al. (1985) showing an enhanced dieback of lateral roots in spruce under increased levels of trace metals in forest soils. To some extent such a dieback can be balanced by the growth of new laterals. Under strong lead stress ($> 211\,\mu$mol (kg soil)$^{-1}$) an enhanced development of second-order laterals was observed (Breckle et al., 1988). Such flushes might cause periodic cycles of growth, development, and death as part of a survival strategy (Altgayer, 1979). Control plants and stressed plants apparently had different growth cycles.

The density of laterals is not only determined by the number of developing primordia, but also by the elongation of the main roots. The architecture of the root systems of *Zea mays* was changed by solution of 1 mM lead nitrate. The growth and number of laterals was not altered, but their distribution along the roots was denser and the branching zone of the main root was shorter. This was caused by a reduced length of the mature cells in the primary roots. Thus, the lead-induced inhibition of primary root growth resulted in a more dense arrangement of laterals (Obroucheva et al., 1998), which is almost a general rule.

Results obtained so far show that the root architecture of plants under trace element stress is altered to a more dense and compact structure. The metal-induced stimulation of the development of laterals often leads to a more densely branched root system.

VIII. UPTAKE AND ACCUMULATION OF TRACE ELEMENTS

Root uptake of lead, cadmium, or other trace elements depends on pH, the mobility of the trace elements, and the developmental stage of the plants. The binding capacity of the soil and, thus, the extent of the plant-available fraction of an element is a soil characteristic, but the latter depends also on the uptake abilities of specific roots.

An element of comparatively low mobility in soils and in plants is lead. Lead is passively absorbed into the root tip of seedlings of *Zea mays*, mainly by thin epidermal cell walls in the meristemic region (Tung and Temple, 1996). Only limited quantities of lead entered into protoplasts. As cells matured, accumulation of lead in cell walls increased. Both short- and long-distance transport of lead were apoplastic. When the conducting vascular tissue in the root center had differentiated, lead entered into the conducting systems. It was also absorbed from the water-absorbing zone of the roots, but these quantities remained in the root cortex. The Casparian strip was an effective barrier for lead, but transport through passage cells was possible. Such results underline the comparatively low mobility of lead in plant tissues. The same was observed in roots of *Brassica juncea* where considerable amounts of lead from the treatment solutions were accumulated in the roots, while only small quantities were transported into hypocotyls and shoots (Liu et al., 2000).

The localization of trace metals in cells and tissues of plant roots can be determined with x-ray microanalysis and similar techniques. The distribution of metals in roots of water hyacinth (*Eichhornia crassipes*) showed distinct patterns (Vesk et al., 1999). Iron was found accumulated at the root surface, e.g., in root plaques known from wetland plants (see Section XV; Ye et al., 1997). Concentrations of iron decreased centripetally. They were higher in cell walls than within cells. In contrast, the trace metals copper, zinc, and lead were not found on the root surface. Their levels increased centripetally and were higher inside the cells than in the walls. Highest levels were found inside the cells of the stele. Although some distribution patterns of elements could be described, the authors caution against general interpretations, since the individual variability of the sampled plants was large.

The complicated solution chemistry and speciation of chromium also affects root absorption. Uptake and translocation of chromium by 11 species of common vegetable crop plants supplied with either Cr^{3+} or CrO_4^{2-} were studied (Zayed et al., 1998). A speciation analysis indicated that in the roots of all tested plants CrO_4^{2-} was converted to Cr^{3+}. The translocation of chromium from roots to shoots was limited. Accumulation in roots was about 2 orders of magnitude higher than in shoots, regardless of the chromium species in the nutrient solution. Highest concentrations were found in crop species of the Brassicaceae family, e.g., cauliflower, kale, or cabbage.

Silicon is a major inorganic constituent of many higher plants (grasses, conifers, *Equisetum*, etc.), but it is rarely considered in biological studies. It might turn out to react as a trace element in some plant groups. In nutrient solutions silicon absorption of the roots of *Triticum aestivum* was rapid (Rafi and Epstein, 1999). Nearly mature plants that were preloaded with silicon showed the same absorption rates as plants previously grown in solutions without silicon addition. The authors give two reasons for this observation: (1) About 90% of the absorbed silicon was transported to the shoots and the roots maintained a low silicon status which promoted further uptake. (2) The absorbed silicon is largely immobilized in insoluble form. Thus, there was no negative feedback from shoots to roots and the silicon uptake continued unabated.

There is growing concern about the radioactive contamination of the environment, particularly after accidents in nuclear power stations and industries. Investigations of the mobility of radionuclides, e.g., isotopes of cesium or strontium, in ecosystems also have to consider root uptake and mobility of these isotopes in plants with respect to their alkaline earth or alkali metal chemistry (Carini and Lombi, 1997; Jones et al., 1998; Entry et al., 1999; Guivarch et al., 1999; Zhu et al., 1999). A comparative study of the root uptake of cesium-134 of 30 different plant taxa was presented by Broadley and Willey (1997). They found lowest accumulations in slow-growing Gramineae and highest accumulations in fast-growing Chenopodiaceae. This was observed after a short-term exposure of the plants. If radiocesium uptake of Chenopodiaceae is also high during long-term exposures, implications for food contamination and the potential of such plants for phytoremediation of contaminated soils should be considered.

The uptake of trace elements into roots depends on the ionic milieu of the rhizosphere. With hydroponically grown *Sinapis alba* plants interactions in the uptake of various trace metals were found (Fargasova and Beinrohr, 1998). Root accumulation of vanadium was inhibited by nickel, manganese, and copper, and the accumulations of nickel or manganese were both inhibited by copper. However, none of the tested metals inhibited copper accumulation.

Many toxic effects of trace elements result from their replacement of calcium at vital sites of cell membranes in root cells. Absorption of Cd^{2+} by roots of *Tamarix aphylla* was markedly inhibited by increasing concentrations of Ca^{2+} in the solution (Hagemeyer and Waisel, 1989). Magnesium ions were less effective in reducing Cd^{2+} uptake. Monovalent ions (Na^+, K^+, Li^+) also reduced Cd^{2+} uptake, but to a lesser extent than divalent ions (Hagemeyer, 1990). An optimal supply of calcium or magnesium can considerably alleviate the toxicity of some trace elements (Wilkins, 1957; Wallace et al., 1980; Hagemeyer and Waisel, 1989; Hagemeyer, 1990; Skorzynska-Polit et al., 1998; Saleh et al., 1999).

Not only the calcium status of a plant but also the phosphorus supply determines uptake of trace elements in a specific way (Wallace et al., 1978). Furthermore, the form of nitrogen supply can affect the toxicity of trace metals (Zornoza et al., 1999). The nickel tolerance of *Helianthus annuus* was lowest when grown with nitrate alone. Simultaneous supply with nitrate and ammonium reduced nickel toxicity.

IX. RESPONSE TO LONG-TERM EXPOSURE OF PLANTS TO TRACE ELEMENTS FROM GEOGENIC SOURCES

A constant, long-term exposure of *Artemisia vulgaris* plants to slightly increased concentrations of lead along roadsides has given rise to the selection of lead-resistant ecotypes (Helming and Runge, 1979). Similar ecotypic variation was also found in *Salix* and *Betula* in biotopes affected by ore mining (Denny and Wilkins, 1987a–c).

Ore deposits and similar remnants from mining operations have a rather high total content of various trace elements. However, the plant-available fraction is usually much lower (Wenzel and Jockwer, 1999). This is the reason that sometimes a great variety of plant species can grow on ore deposits rich in lead, zinc, or other trace elements. For example, in the area of Stolberg, an old mining site south of Aachen, Germany, birch trees are growing well on mounds of ore smelter ash with total lead concentrations reaching 10–20 g (kg substrate)$^{-1}$.

High zinc concentrations are often found on ore outcrops in mining areas. There are impressive genotypic differences in Zn resistance among certain species of the natural vegetation (Ernst, 1982; Macnair, 1993). The mechanisms of zinc resistance are in principle similar to those of copper resistance. Compartmentation plays a major role at the cellular, tissue, or organ level. At the cellular level, zinc is accumulated in the vacuoles (Harmens et al., 1993b).

Serpentine is a common name for a number of rock types that contain ferromagnesian minerals. Owing to high concentrations of magnesium and iron, such rocks are called ultramafic (Proctor, 1999). After weathering, ultramafic rocks produce soils with naturally elevated levels of trace elements. They are found on all continents (Roberts and Proctor, 1992). Serpentine soils have increased concentrations of nickel, chromium, or cobalt as well as unfavorably low calcium/magnesium ratios (Menezes de Sequeira and Pinto da Silva, 1992; Rodenkirchen and Roberts, 1993; Shallari et al., 1998; Proctor, 1999; Ater et al., 2000). This poses severe problems to plant growth. A specialized flora with many endemic species has developed on ultramafic sites (Arianoutsou et al., 1993). Some species have a remarkable potential for trace element accumulation; nickel concentrations in their leaves can reach the 1% range. Plants are called hyperaccumulators if the concentration of a trace metal regularly exceeds 0.1% under natural conditions (Baker and Brooks, 1989; Greger, 1999). Many hyperaccumulating species were found in the genus *Alyssum* (Morrison et al., 1980). Some accumulating species of this genus showed root growth in solutions with nickel concentrations up to 1 mM, whereas root growth of a nonaccumulating species was inhibited even by traces of nickel. Two serpentine species with differing resistance strategies were studied by Gabbrielli et al. (1990). *Silene italica* limits its nickel uptake. Root growth was inhibited by a suppression of mitotic activity in root tips at 7.5 μM nickel in the culture solution. The same concentration did not affect root growth in *Alyssum bertolonii*, which is a nickel accumulator. A calcium supply of 25 mM reversed the effects of nickel on root growth in *Silene*, but in *Alyssum* the addition of calcium reduced root growth. This finding demonstrates that *Alyssum bertolonii* is also adapted to low calcium concentrations in the substrate, which is typical for serpentine soils. Concentrations of nickel in roots and shoots of this species reached 0.3% and 0.6% of the dry weight, respectively (Pandolfini and Pancaro, 1992). It is amazing that a plant can tolerate such high concentrations of a toxic element in its live organs.

In several hyperaccumulating *Alyssum* spp. it was shown that exposure to nickel caused an increase of free histidine in the xylem sap (Krämer et al., 1996). The authors suggested that nickel tolerance is based on an enhanced production of histidine, which serves as a chelator of nickel in the xylem sap. The transport of the toxic ions from the roots into the shoot is thus promoted.

Hyperaccumulators of other elements, like cobalt, copper, or chromium, were also found (Baker and Brooks, 1989). For instance, *Brassica pekinensis* was identified as a hyperaccumulator of lead (Xiong, 1998). Such plants have a potential use in phytoremediation of metal-contaminated soils (Saxena et al., 1999). Furthermore, recent research demonstrated a defensive function of accumulated metal ions against herbivores and pathogens (Boyd and Martens, 1998; Sagner et al., 1998; Boyd and Moar, 1999).

X. EFFECTS OF RARE EARTH ELEMENTS ON ROOT GROWTH

The rare earth elements (REE), or lanthanides, are often left out of the biological studies. This group includes the 14 elements with atomic numbers from 58 (cerium) to 71 (lutetium) as well as the elements lanthanum, scandium, and yttrium (Greenwood and Earnshaw, 1986). In spite of their name, most of these elements are not really rare in nature. For instance, in the earth crust cerium is five times more abundant than lead. The total concentration of the REE in the earth crust is ~ 0.01% (Holleman and Wiberg, 1995). In general, the REE with even atomic numbers are more abundant than those with odd atomic numbers, a fact known as "Harkins rule" (Holleman and Wiberg, 1995). The physical and chemical properties of the different REE are similar. The ions of REE are mostly trivalent. There are > 100 minerals known to contain REE, and the different elements often occur in groups in the minerals (Greenwood and Earnshaw, 1986).

In comparatively few studies root uptake and accumulation of REE in higher plants were investigated. One reason may be insufficient detection limits of older analytical methods, since the REE occur in plants in rather low concentrations (Markert, 1987; Breckle, 1997; Fu et al., 1998). Several studies were made in China, owing to widespread use of REE in agriculture and industry in that country (Yang et al., 1999).

The uptake of scandium (Sc^{3+}) into root tips of three different cultivars of *Sorghum bicolor* was determined by short-term (10-min) uptake studies (Wilkinson and Duncan, 1992). In root tips of the acid soil stress sensitive cultivar accumulation of nonextractable (water, EDTA), scandium ions increased with decreasing pH of the growth medium. However, nonextractable scandium did not increase in root tips of the tested cultivars that were tolerant to acid soil stress. The study showed a strong effect of pH on scandium accumulation.

The REE levels in *Citrus* tree samples in Florida were correlated with soil concentrations (Wutscher and Perkins, 1993). The highest concentrations were found in feeder roots, ranging from 4.6 to 585 $\mu g\ g^{-1}$.

Accumulation of REE by sugarcane (*Saccharum officinarum*) was possible via the leaves when sprayed on leaf surfaces or via the roots after soil application (Chua et al., 1998). Root uptake was determined in plants grown on soil sprayed with a microelement fertilizer containing nitrates of various REE, including cerium and lanthanum. Root uptake showed a linear correlation with REE concentrations of the soil. The absorbed quantities of REE were mobile in the plants and were accumulated in all parts. The authors concluded that high concentrations of REE in the soil could result in harmful effects for humans consuming sugarcane products.

The chemical speciation in the soil, chelation, binding forms, and bioavailability of REE were addressed in several studies. Sun et al. (1997) studied the effects of EDTA on the uptake of lanthanum, gadolinium, and yttrium from nutrient solutions by 2-week-old wheat (*Triticum* sp.) seedlings. Addition of EDTA to the nutrient solution reduced the accumulation of the elements in the roots, but increased their accumulation in stems and leaves. Roots showed a comparatively higher accumulation capacity than the aboveground parts. The REE concentrations in the roots were linearly correlated with concentrations in the nutrient solutions in the tested range of 0–4 ppm. This was found with both the ionic and the EDTA-complexed forms of the elements.

A similar result was described for wheat (*Triticum* sp.) plants grown in soil (Yang et al., 1999). Bioaccumulation of all the elements (lanthanum, cerium, samarium, gadolinium, and yttrium) in roots was much higher than in stems and leaves. Uptake was increased by EDTA addition to the soil, which resulted from desorption of REE ions from soil compounds.

A sequential extraction procedure was used to determine the distribution of REE in different chemical fractions of Mollisols in China. Samples of *Zea mays* and *Oryza sativa* plants growing on the soils were analyzed (Li et al., 1998). Concentrations in plant parts followed the order root > leaf > stem > grain. Total soil concentrations or the sum of all extracts of the sequential extraction procedure were not useful indicators of REE plant uptake. The acetate-extractable fraction of REE in the soil might play a role in controlling plant uptake. However, the authors concluded that a more accurate speciation analysis method is necessary to establish a relationship between REE speciation in soil and the bioavailability.

Comparatively little is known about the effects of the REE on growth and development of roots of higher plants. An extensive study with roots of maize (*Zea mays*) and mungbean (*Vigna radiata*) seedlings revealed that the relative root elongation of both species was inversely correlated with lanthanum or cerium concentrations in the solution (Diatloff and Smith, 1995a). Mungbean was more sensitive than maize. Cerium was more toxic than lanthanum to mungbean. The concentration causing 50% reduction of mungbean root elongation was 0.9 μM cerium and 3.1 μM lanthanum. To maize roots lanthanum was more toxic. Concentrations causing 50% reduction of maize root elongation were 12.2 μM cerium and 4.8 μM lanthanum at pH 5.5.

Lanthanum at concentration $< 1\ \mu$M enhanced elongation of maize and mungbean roots but not root dry matter accumulation. Transport of La to the shoot was blocked and it accumulated in the roots. Similar results were obtained for cerium (Diatloff and Smith, 1995b, c).

The effects of REE were examined by REE spraying at the end of the tillering phase of sugarcane, *Saccharum officinarum* (Pan et al., 1993). It increased the activity of Na-K-ATPase in root cells. The vigor of root systems, the amount of rhizosphere bacteria and enzyme activities in the soil were increased. As a result, growth rate and yield of the plants were increased.

The cytological effects of praseodymium oxide and neodymium oxide (1–5 ppb) on root tip cells of *Vicia faba* caused chromosomal aberrations and mitotic anomalies in various proportions (Singh et al., 1997). Depending on the concentration and duration of the treatment, abnormal meta- and anaphase cells were recorded. These effects were similar to aberrations induced by radiation and radiomimetic chemicals.

Europium ions (Eu^{3+}) influenced the contents and composition of anthraquinones in root cultures of the

Chinese medicinal plant *Cassia obtusifolia* (Guo et al., 1998). Thus, the production of secondary metabolites in plants can be affected by REE.

Roots of water hyacinth (*Eichhornia crassipes*) remove large quantities of europium(III) from water. This species is used for decontamination of polluted water. In the roots, intracellular europium ions are probably complexed by organic acids (Kelley et al., 2000).

The varied effects of the different REE on root growth of plants require further attention. Research should particularly focus on synergistic or antagonistic effects of combinations of several REE on plant growth, since the naturally occurring REE minerals usually contain combinations of various elements.

XI. PHYSIOLOGICAL BASIS OF TRACE ELEMENT TOLERANCE

The evolution of trace element resistance is still a matter of discussion. The basic question is whether the trace element resistance in higher plants is controlled by one or a few genes or by the combined action of many genes (polygenic control). Macnair (1983) presented evidence that the copper tolerance of *Mimulus guttatus* is controlled by a single major gene. Accordingly, he argued that there must be a single physiological or biochemical process which generates the tolerance required for the colonization of toxic soil. Other physiological differences between tolerant and nontolerant plants may occur which are manifestations of subsequent genetic changes to improve the degree of adaptation. A similar result was found for the copper tolerance of *Silene vulgaris* (Schat and Ten Bookum, 1992). With results from crossing experiments of *Silene vulgaris* plants from populations differing in copper tolerance it was suggested that copper tolerance of this species is under the control of two major genes (Schat et al., 1993), and the tolerance level seems to be controlled by two additional genes. The authors concluded that all these genes are involved in the control of an exclusion mechanism operating at the plasma membrane.

In a review of the responses of higher plants to cadmium it was concluded that cadmium detoxification is probably a complex phenomenon under polygenic control (Sanita di Toppi and Gabbrielli, 1999). The results of earlier studies using grasses and herbs were taken as evidence for polygenic control of trace metal tolerance (Wilkins, 1960; Bröker, 1963; Urquhart, 1971; Gartside and McNeilly, 1974).

Besides investigation of the genetic basis of trace element tolerance, it is also necessary to further elucidate the physiology of mechanisms that enable metallophytes to survive on contaminated substrates. Furthermore, it will be important to clarify the regulatory mechanisms involved in uptake and turnover of essential trace elements that are required in very low concentrations. Toxic effects of high internal concentrations can be alleviated by a number of physiological mechanisms. These depend on rates and mechanisms of uptake, on translocation along root tissues, and on the properties of the trace element.

The biological effects of trace elements on water relations, especially the primary toxicity mechanisms of the different metal ions, may be as different as their chemical properties—e.g., valency, ion radius, redox potential, and stability of organic complexes (Barcelo and Poschenrieder, 1990; Poschenrieder and Barcelo, 1999). The metal ions can induce a sequence of biochemical and physiological alterations (Foy et al., 1978; Lepp, 1981) by damaging membranes and altering enzyme activities (Kennedy and Gonsalves, 1987). A multitude of secondary effects have been observed, such as disturbances of the hormone balance, deficiency of essential nutrients, inhibition of photosynthesis, or changes in carbon allocation.

One mechanism of trace element resistance is to avoid toxic accumulations of such elements in sensitive plant parts, like meristems. In some plant species, this is achieved by reduced root uptake. Also, the translocation from root cortex to xylem vessels can be slowed down. Toxic elements are therefore sequestered outside the root symplasm or in specialized tissues outside the endodermis. In roots of *Betula*, Zn accumulated in cells of the endodermis when the roots were subjected to concentrations below the lethal threshold (Denny and Wilkins, 1987a,b). The distribution of cadmium in roots of *Phaseolus vulgaris* plants was investigated after cultivation in cadmium containing nutrient solutions (Vazquez et al., 1992). The accumulation of cadmium decreased from outer to inner parts of the root cortex. Only small amounts were detected in the endodermis. As the endodermis constitutes a barrier to ion transport, root cortex cells usually contain higher element concentrations than cells in the central vascular cylinder.

A study of the ultrastructural localization of lead in roots of *Allium cepa* suggested protective mechanisms against lead in root tip cells (Wierzbicka, 1998). Plants were treated with lead added to nutrient solutions as chloride or nitrate. Lead accumulated in the apoplast of root tips. Based on ultrastructural observations, the author hypothesized that there are two protective mechanisms against lead in the root tips of onion: (1) The amount of polysaccharides in the cell walls and the thickness of the walls increase, which allows a larger retention of lead outside the cytoplasm. (2) Lead is exported from the cells through plasmatubules to the root tip apoplast. In this way lead levels in the symplast are kept low.

Another strategy of metal resistance found in certain cases is the accumulation of toxic ions in cell vacuoles. A zinc-malate shuttle mechanism was proposed for the transport of zinc ions through the cytoplasm into the vacuole (Mathys, 1977; Ernst et al., 1992). This hypothesis needs further investigation. Using isolated oat (*Avena sativa*) root tonoplast vesicles, it was found that ions of zinc, manganese, cadmium, or calcium could be transported by metal/H$^+$ antiport mechanism into the plant vacuole (Gonzalez et al., 1999). This antiport mechanism seems to be metal specific, since in the same system of oat root tonoplast vesicles no Ni/H antiport was found (Gries and Wagner, 1998). In this study the vacuoles were not a major compartment for nickel accumulation. Also, no heavy metal accumulation was found in leaf cell vacuoles of *Silene vulgaris* plants from a heavy metal–polluted mine dump (Bringezu et al., 1999).

Plants can avoid detrimental effects on sensitive physiological processes in cells by immobilization and sequestration of toxic trace elements. Specialized molecules with large numbers of negatively charged groups capture trace element cations in the cytoplasm. Metallothioneinlike proteins were found in roots of plants, which are similar to metal-binding metallothioneins from animals and fungi (Tomsett and Thurman, 1988; Robinson et al., 1993; Prasad, 1999). In general, such molecules have large numbers of sulfur-containing amino acids, like cystein, which bind cations to –SH groups. A copper-binding thionein was found in roots of a copper-resistant strain of *Agrostis gigantea* (Rauser and Curvetto, 1980). In roots of maize a Cd binding protein appeared after subjecting the plants to cadmium stress. This protein contained 40% cystein (Rauser and Glover, 1984).

Additionally, smaller metal-binding polypeptides were discovered in plants and named phytochelatins (Grill et al., 1985). In most cases they consist of only three different amino acids: glutamic acid, cystein, and glycine. The polypeptide chain is a repetitive sequence of poly(gamma-glutamylcysteinyl)glycine. Chain length and proportions of the constituents vary in different plants (Robinson and Jackson, 1986; Narender Reddy and Prasad, 1990; Rauser, 1990; Prasad, 1999). Such compounds were found in various members of the Fabales (e.g., *Phaseolus vulgaris*, *Glycine max*) and assumed to bind and immobilize toxic trace metals (Grill et al., 1986).

Similar cadmium-binding polypeptides were observed in root tissue of six different plant species including sunflower, soybean, and potato grown under cadmium stress (Fujita and Kawanishi, 1987). The authors concluded that low-molecular-weight cadmium-binding complexes play a role in the trace element resistance of plant roots, the organ directly in contact with the noxious metals. Also, roots of pepper plants (*Capsicum annuum*) exposed to cadmium stress responded with increased phytochelatin concentrations (Jemal et al., 1998). Higher levels of phytochelatins were found in roots of cadmium stressed *Silene vulgaris* plants than in control plants. More than 60% of total cadmium in the roots was bound to polypeptides. It was suggested that the cadmium sequestration by polypeptides plays a role in cadmium resistance of the plants (Verkleij et al., 1990). However, this assumption was challenged by the findings of other authors. When subjected to copper stress, both sensitive and resistant genotypes of *S. vulgaris* produced phytochelatins in the root tip. Thus, the differential copper resistance of this species did not depend on differential phytochelatin production (Schat and Kalff, 1992).

Also, the zinc resistance of *S. vulgaris* was not due to increased phytochelatin production (Harmens et al., 1993a). Under cadmium stress, sensitive strains of *S. vulgaris* produced more phytochelatins in root tips than resistant genotypes. Thus, phytochelatins may serve to chelate and detoxify cadmium. However, the cadmium resistance of some varieties did not depend on an increased production of such polypeptides (De Knecht et al., 1994). Synthesis and accumulation of phytochelatins in *Phaseolus coccineus* plants depended on the growth stage in which cadmium was added to the nutrient solution (Tukendorf et al., 1997). Only when cadmium was applied in an early growth stage was a high accumulation of phytochelatins observed. The contributions of trace element–binding polypeptides or proteins to metal resistance appear to be not fully understood. This problem should be tackled in future research.

XII. TOXICITY TESTS USING ROOT GROWTH PARAMETERS

The growth of roots of higher plants is a sensitive indicator of trace element toxicity (Hagemeyer, 1999). Effects of trace elements on different growth parameters of roots, like elongation or branching, are detectable at low substrate concentrations. Based on this observation, a number of short-term test systems for biotoxicity of metals were proposed (Köhl and Lösch, 1999). Wilkins (1957) described a method to determine the lead tolerance of different genotypes of *Festuca ovina* originating from soils with different lead concentrations. Tillers of the test plants were grown in hydroculture with added lead. Root elongation was measured at daily intervals. Differences in the growth rates indicated varied degrees of lead tolerance of the plants.

More recently, a rapid screening method for chemicals involved in environmental hazards was developed (Liu et al., 1995). A test using roots of onion (*Allium cepa*) was proposed. It is based on a previously described "*Allium* test" (Fiskesjö, 1985). Onion bulbs are placed in the test liquids which are daily renewed. The bulbs are allowed to sprout and to produce roots for 24–96 h in beakers, protected from light. Then root samples are cut and fixed in Carnoy's reagent. For the examination of chromosome and nucleus morphology the fixed roots are squashed in carbol-fuchsin solution. A silver-staining procedure is used to examine changes in the nucleoli.

In order to examine the described *Allium* test technique, Liu et al. (1995) used salt solutions of 11 different trace elements in tap water at pH 6.5. The applied concentrations ranged from 10^{-7} to 10^{-1} M. The effects of the tested metals on cell division and nucleoli in root tip cells depended on the concentration and the duration of the treatment. The metals caused irregularities of chromosomes, nuclei and nucleoli, like c-mitosis, chromosome bridges, chromosome stickiness, or irregularly shaped nuclei and nucleoli to varying degrees. Based on the concentrations that caused serious toxic effects the metals could be divided into three groups: the first group of highest toxicity included mercury and cadmium (10^{-7}–10^{-5} M); the second group of medium toxicity included zinc, lead, copper, nickel, cobalt, aluminum, and chromium (10^{-4}–10^{-3} M); and the third group of low toxicity included manganese and magnesium (10^{-2} M). This test appears to be a simple and fast, but sensitive, screening technique.

A biomonitoring method for the cadmium contamination of soil and water using growth parameters of mungbean (*Phaseolus aureus*) roots was devised by Geuns et al. (1997). The toxicity threshold value for root elongation was an internal cadmium concentration of 25 μg/g root dry weight. Above this concentration sterol synthesis in roots was reduced. Cadmium increased the stigmasterol-sitosterol ratio and induced a redistribution of sugars in roots. At internal cadmium levels of 100 μg/g dry weight an increase of polyunsaturated fatty acids in roots was found. The polyamine synthesis was strongly affected. At a cadmium concentration of the medium of 100 μM the synthesis of putrescine was sixfold increased. When mungbean seedlings are used as biomonitors of cadmium toxicity, the most sensitive parameters are the root growth reduction and the putrescine accumulation in the roots that occurs before any growth reduction is detectable. In situations where funds are limited and an extensive laboratory infrastructure is not available, the uncomplicated root tests can offer reasonable alternatives to microorganism-based toxicity tests.

XIII. MYCORRHIZA AND TRACE ELEMENT RELATIONS OF PLANTS

The symbioses of plant roots and fungi, called mycorrhizae, are a widespread phenomenon (Varma and Hock, 1999; see also the Chapters 50 by Kottke and 49 by Sieber in this volume). Effects of mycorrhizae on trace element relations of plants have been repeatedly investigated (Leyval et al., 1997). Two aspects were intensively studied: effects of mycorrhizae on trace element resistance of higher plants, and enhancement of nutrient absorption by mycorrhizae.

Ectomycorrhizae with *Paxillus involutus* could ameliorate Zn toxicity in some varieties of birch (Brown and Wilkins, 1985; Denny and Wilkins, 1987c,d). The mycorrhizal association of *Pinus sylvestris* with *Paxillus involutus* reduced the toxic effects of cadmium and zinc on root elongation (Hartley-Whitaker et al., 2000). The infection of the roots with the fungus decreased the transport of cadmium or zinc to the trees shoots. Grown on contaminated substrates, the mycorrhizal hyphae contained vacuoles with accumulated cadmium (Turnau et al., 1993). This is considered a detoxification mechanism that reduces the trace element burden. The localization of trace metals in roots of *Picea abies* seedlings colonized with the fungus *Hebeloma crustuliniforme* was studied with x-ray microanalysis (Brunner and Frey, 2000). Cadmium was found mainly in the Hartig net. Nickel was detected in the Hartig net and in cell walls of the cortex. Zinc occurred in the Hartig net, cortical cell walls, and the fungal mantle.

In laboratory experiments ectomycorrhizae of various fungi with roots of *Pinus sylvestris* seedlings were investigated (Colpaert and Van Assche, 1993). Uptake of cadmium was highest in the nonmycorrhizal control; thus, a protective effect of the symbiosis against cadmium toxicity was concluded. In contrast, the mycorrhizal association of *Corylus avellana* roots with *Tuber albidum* did not prevent chromium accumulation in the roots (Strati et al., 1999). *Agrostis capillaris* showed a negative correlation between copper concentrations of the soil and the degree of mycorrhizal infection of the roots (Griffioen et al., 1994). However, in an area contaminated with cadmium and zinc the mycorrhizal fungi had evolved resistance to these metals and can thus play a role in the trace element resistance of this grass. The symbiotic ectomycorrhiza does not always reduce metal toxicity in forest trees. The amelioration depends on the species and strain of the ectomycorrhizae as well as on the metal (Godbold et al., 1998).

Mycorrhizae can also stimulate the absorption of trace elements by plant roots (Ernst, 1985). The uptake of zinc by endomycorrhizal roots of *Araucaria cunninghamii* and by ectomycorrhizal roots of *Pinus radiata* was enhanced as compared to uninfected controls (Bowen et al., 1974). In this same way the symbiosis can enhance root acquisition of mineral nutrients in soils of poor nutrient availability (Dehn and Schüepp, 1989; Faber et al., 1990; Clark and Zeto, 1996; Caris et al., 1998; Clark et al., 1999). However, transport of cadmium and zinc to the shoots of lettuce was lower in mycorrhizal plants. The metal retention in roots was attributed to complexation by cystein-containing ligands of fungal proteins (Dehn and Schüepp, 1989). Such a mechanism can also support the trace element resistance of plants.

The enhancement of plant metal uptake by mycorrhizae could improve the efficiency of the phytoremediation of contaminated soils. In the three grass species *Paspalum notatum*, *Sorghum halpense*, and *Panicum virginatum*, plants inoculated with the fungus *Glomus* sp. had higher radioisotope concentrations (^{137}Cs and ^{90}Sr) in the plant tissue (Entry et al., 1999). Plant bioconcentration ratios were higher in mycorrhizal plants than in uninoculated plants. The authors concluded that sites contaminated with radionuclides can be effectively cleaned with mycorrhizal plants.

The outlined results show that there is no simple and straightforward interpretation of the effects of mycorrhizae on trace element relations of plants. Apparently, these effects depend on both partners of the symbiosis as well as on environmental conditions. The assumption that a plant gains additional resistance to toxic trace elements from the association with a symbiotic partner needs detailed investigation in each particular case.

XIV. EFFECTS OF PLANTS ON SOILS

It is well known that trace elements in the soil affect the development of plant roots. On the other hand, the roots of plants can affect the concentrations of plant-available trace metal fractions in the soil. Plant roots can mobilize or immobilize soil minerals (McCully, 1999; Chapter 36 by Neumann and Römheld in this volume). Consequently, root growth of all plants growing in such a soil is then influenced.

For elements like iron and manganese the mobility and the availability for root absorption is controlled by the oxidation status of the soil. Plant roots that penetrate the soil layers can alter the oxygen partial pressure in the rhizosphere. Thus, the roots can affect the accumulation and the mobility of elements in their vicinity. This was shown for *Spartina townsendii* and *Atriplex portulacoides* effect on a wetland soil from a salt marsh in Ireland (Doyle and Otte, 1997). The salt marsh soils are effective sinks for trace elements in ecosystems. The soil pool of trace elements that is influenced by the studied plants can have considerable effects on the biogeochemistry, accumulation, and plant availability of the trace elements (Doyle and Otte, 1997).

Another aspect of plant effects on soils concerns the interaction with rhizosphere bacteria (Wenzel et al., 1999). The role of such microorganisms in facilitating selenium and mercury accumulation in roots of the wetland plants *Scirpus robustus* and *Polypogon monspeliensis* was studied by De Souza et al. (1999). In a laboratory experiment the plants were treated with the antibiotic ampicilline to inhibit growth of rhizobacteria. The root uptake of selenium and mercury was significantly lower than in plants without ampicilline treatment. When axenic *Scirpus* plants were inoculated with bacteria isolated from the rhizosphere of field-grown plants the accumulation of selenium and mercury was significantly higher than in axenic controls. The authors concluded that rhizosphere bacteria can promote the accumulation of selenium and mercury in roots and shoots of wetland plants. The nature of the stimulating effect is as yet unknown. Several possible effects of the rhizobacteria were discussed: (1) stimulation of production of compounds like siderophores which facilitate metal absorption by roots; (2) increase of root surface area by stimulation of root hair growth; (3) transformation of the trace elements into more readily absorbable forms; (4) increase of selenium uptake by stimulation of the sulfate transport protein which also transports selenate; and (5) reduction of the rhizosphere pH, which enhances mercury absorption into roots. The stimulating effect of rhizobacteria on trace element absorption is important when plants are used for phytoremediation of contaminated soils (Wenzel et al., 1999; Saxena et al., 1999).

Plant roots can also reduce the mobility of trace elements in the soil. In pots with *Agrostis* plants copper activities in the soil solution were 2 orders of magnitude lower than in bare pots without vegetation. It was concluded that the plant growth affects soil pH, the dissolved organic carbon concentration, and the calcium concentration of the soil solution to such an extent that the total dissolved copper concentration and the free metal activity in the soil were reduced. In this way potentially toxic metals can be immobilized in the soil by the action of plant roots (Römkens et al., 1999).

The multiple interactions among plant roots, soil microorganisms, and the soil matrix are complex. Nevertheless, such relations should be studied in detail under realistic field conditions. This will improve our understanding of trace element effects on plant growth.

XV. TRACE ELEMENT DEFICIENCIES

The key symptom of iron deficiency in leaves is chlorosis, caused by an inhibition of chloroplast development. In many plant species iron deficiency is also associated with inhibition of root elongation, increase in the diameter of apical root zones and an abundant root hair formation (Römheld and Marschner, 1981). It can also be connected with the development of a typical rhizodermal cell wall labyrinth as in other transfer cells. This seems to be part of a regulatory mechanism which enhances iron uptake (Kramer et al., 1980). It was found only in those plant species that can acidify the rhizosphere (Römheld and Kramer, 1983). Iron deficiency stress can stimulate the activity of the enzyme Fe(III)-reductase, which is associated with plasma membranes in root cells. The enzym catalyzes the reduction of Fe(III) to Fe(II), which is more readily absorbed by roots. This was shown for *Pisum sativum* plants (Grusak et al., 1993). The plasma membrane bound reductase system can also increase the uptake of other trace elements, like copper and manganese. It has been suggested that it plays a general role in cation absorption of roots (Norvell et al., 1993; Welch et al., 1993).

Under conditions of iron deficiency graminoid species can release substances from their roots, which mobilize Fe(III) by chelation (Marschner et al., 1986, 1987; Römheld and Marschner, 1990; Chapters 35 by Jungk and 36 by Neumann and Römheld in this volume). The chemical nature of these substances, sometimes called low-molecular-weight organic acids, or phytosiderophores, varies with species. Compounds like mugineic acid or avenic acid were found in root exudates. Studies with iron-deficient barley (*Hordeum vulgare*) roots suggest that phytosiderophores of the mugineic acid family are secreted from roots as monovalent anions through anion channels (Sakaguchi et al., 1999). The Fe(III) chelates are transported into root cells (Takagi et al., 1984; Marschner, 1986). The release of phytosiderophores is stimulated by iron deficiency. They can also mobilize other trace elements, like copper, zinc, and manganese (Römheld and Marschner, 1990).

In iron-deficient wheat plants the released phytosiderophores mobilized also zinc in the rhizosphere and in the root apoplast (Zhang et al., 1991). The same was found for cadmium (Cieslinski, et al. 1998). In different cultivars of wheat (*Triticum turgidum*), cadmium accumulation was proportional to the levels of low-molecular-weight organic acids in the rhizosphere. Apparently these substances have a role in the solubilization of cadmium in the soil solution. The phytosiderophore release of the efficient cultivar of wheat (with respect to zinc absorption efficiency) under conditions of zinc deficiency was higher than the inefficient variety (Cakmak et al., 1994). The principal compound was 2′-deoxyimugineic acid, which was also released under iron deficiency. The authors concluded that an enhanced release of phytosiderophores under zinc deficiency stress may be the reason for an efficient zinc uptake in certain gramineous species.

While most authors report stimulation of metal uptake by secreted low-molecular-weight organic substances, Saber et al. (1999) reached a different conclusion. In hydroculture experiments with sunflower (*Helianthus annuus*), they found that aluminum and zinc ions stimulated the release of malic and citric acid from roots. Experimental additions of these organic acids into the growth medium to some extent alleviated inhibitory effects of the toxic ions on plant growth. The authors concluded that the tolerance mechanism of sunflower plants against toxic ions includes metal exclusion through secretion of organic acids into the rhizosphere, which reduces the uptake of the toxic ions. An additional mechanism is internal tolerance by chelation of toxic ions in the cytoplasm.

Most metal ions are much more readily absorbed in a reduced form (Mn^{2+}, Fe^{2+}, etc.) than in oxidized form. Apparently, most plants are able to reduce metal ions in the vicinity of their roots. This is facilitated by changes of pH of the rhizosphere, but also by exudation of reducing substances. Many questions remain open: How can plants achieve a balanced ion uptake to serve their particular needs with the help of rather unspecific chelators? In which way do the exuded chelating substances interact with ion-complexing compounds of the soil? Are there other plant groups with particular substances such as the iron-siderophores of grasses? For example, mugineic acid and avenic acid are typical phytosiderophores of all grasses, but not of members of other graminoid families, as Commelinaceae, Cyperaceae, and Juncaceae (Mino et al., 1983; Takagi et al., 1984; Marschner, 1986).

The special iron mobilization of grasses is important under a normal or low supply of iron (see Chapter 36 by Neumann and Römheld in this volume). What happens under conditions of high iron concentrations, such as at low pH and under low O_2? Whereas iron deficiency occurs more frequently, iron toxicity is rare. In habitats with periodically submerged soils, which are poor in O_2, iron and other metallic ions are present in a reduced state. Fe(II) is more soluble than Fe(III) and can thus cause toxicity symptoms. Such toxicity can appear in paddy fields. The roots of such plants are covered with thick crusts of brownish Fe(III) compounds (see also Chapter 56 by Beyrouty in this volume). Iron deposits on roots were shown to ameliorate toxic effects of excess copper (Greipsson, 1994). On the surfaces of roots and rhizomes of *Spartina maritima* plants of intertidal areas in Portugal metal-enriched rhizoconcretions were found (Vale et al., 1990). They contained up to 11% iron, large amounts of manganese, and variable amounts of other trace elements. The ecological implications of trace element accumulations in the rhizospheres of some plant species deserve further investigation.

Data on the effects of either deficiency or toxicity of Mn on roots are scarce. In Mn-deficient plants the formation of lateral roots ceased completely (Abbott, 1967). There was an increase in the number of small nonvacuolated cells in these roots. This would indicate that cell elongation is more sensitive to manganese deficiency than cell division. The formation of manganese oxide plaques was described for rice (*Oryza sativa*) plants in hydroculture (Crowder and Coltman, 1993). Special adaptations to high levels of manganese supply are not known.

XVI. OPEN QUESTIONS

An interesting but as yet unsolved question is how and why very low concentrations of toxic trace elements can stimulate plant growth to some extent. The described alleviation of trace element toxicity by calcium and other major nutrients also deserves a special attention.

Some plant groups have evolved their own peculiar spectrum of ecological and physiological adaptations. For instance, grasses have developed effective regulatory mechanisms at the root level. In grass shoots, the element content, even on ore soils, tends to be much lower than in broadleaf herbs. The search for comparable, special adaptations, on the physiological or biochemical level, in other plant families should be intensified.

In view of the widespread occurrence of mycorrhizae, the effects of such symbiosis on trace element relations of higher plants should be considered in all studies. For reasons of practicality, these phenomena are often neglected in laboratory experiments. Therefore, it seems necessary to conduct more experiments under realistic field conditions, which include such important factors.

Owing to rapid developments in molecular biological studies, the investigation of the genetic control of trace metal tolerance of higher plants will certainly be pushed forward in the future. The major questions are still whether the tolerance mechanisms are under mono-, oligo-, or polygenic control and how these mechanisms have evolved.

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