CHAPTER 5

Trace Elements in Plants

I. INTRODUCTION

The trace element metabolism of plants has been extensively studied and the basic information on many topics is available in monographs on plant physiology or plant nutrition. The metabolic fate and role of each trace element in plants can be characterized in relation to some basic processes such as:

1. Uptake (absorption), and transport within a plant
2. Enzymatic processes
3. Concentrations and forms of occurrence
4. Deficiency and toxicity
5. Ion competition and interaction

These topics are relatively well-understood for certain micronutrients, but further investigations are needed for many other trace elements. The reaction of plants to chemical stresses that are caused by both deficiencies and excesses of trace elements cannot be defined exactly because plants have developed during their evolution and course of life (ontogeny and phylogeny) several biochemical mechanisms that have resulted in adaptation to and tolerance of new or chemically imbalanced environments. Therefore, plant responses to trace elements in the soil and ambient air should always be investigated for the particular soil–plant system.

Plants reveal various tendencies in the uptake of trace elements. Three general uptake characteristics can be distinguished: accumulation, indication, and exclusion. To a large extent, this depends on the specific ability of plants and huge differences in metal uptake between plant species. Also, between genotypes of a species, great variabilities have been demonstrated in many studies. The most common plants tested recently for phytoremediation have been listed by Felix et al.[1298]: *Alyssum murale, Thlaspi caerulescens, Nicotiana tabacum, Zea mays, Brassica juncea*, and *Salix viminalis*. The ability of several agricultural crop plant species, such as mustard, radish, turnip, rape, and amaranth, to accumulate higher amounts of some metals (Cd, Cr, Cu, Ni, and Zn) has been investigated as well.[1492]

Plants able to take up metals above established background concentrations and more than other species from the same soils are called *hyperaccumulators.* These are mainly populations of species found in soils rich in metals either due to geochemical parameters or due to pollution. Hyperaccumulators usually have a low biomass because they use more energy in the mechanisms necessary to adapt to the high metal concentrations in the tissues. There are a number of studies on hyperaccumulators because of their specific physiologic processes and practical aspects. Plants that highly accumulate metals are promising in phytoremediation programs (see Chapter 2, Section IV.B). This has been a highlighted topic at several recent conferences, and has been widely reviewed in recent publications and books.[1244,1459]

A number of various plants are known as medicinal herbs and have been used for a very long time (possibly since Neanderthal man) to cure illness. Although a curative agent is mainly associated with organic compounds such as glycosides or alkaloids, trace elements can have additional impact. Most medicinal plants belong to a kind of weed that can accumulate a greater amount of trace elements than other plants. A good example is dandelion, often used in herb medicine and also as a nutritional plant. However, when dandelion grows in a polluted environment, it is known to take up, from both aerial and soil sources, many more trace metals than other plants.[1356] Thus, trace element content in those plants should be of special concern.

Hyperaccumulators are plants and/or genotypes that accumulate metals above certain concentrations in leaves. As Greger[1316] presented, based on a literature review, hyperaccumulators should contain trace metals in leaves above the following levels (in ppm):

$>$100 — Cd
$>$1000 — Co, Cu, Ni, Pb
$>$10,000 — Mn, Zn

In polluted regions, however, some plants (not hyperaccumulators) may concentrate metals at those levels. Therefore, increased levels of a given metal in a hyperaccumulating plant must be related to their contents in other plants grown in the same environment.[1356]

The chemical composition of plants reflects, in general, the elemental composition of the growth media. The extent to which this relation exists, however, is highly variable and is governed by many different factors. The common concentrations of trace elements in plants growing on various, but nonpolluted, soils show quite a large variation for each element.

A large variety of possible ligands for metals exist in plants, especially in xylem and phloem. Thus, metal ions form complexes with small and macromolecular substances, mainly organic. Inorganic ligands, however, are also very important complexants for metals. Depending on the size and physical and chemical characteristics, metals can form with ligands in plants either easily transported or strongly bound forms, and complexes. Both are of great metabolic importance because they control the transport of nutrients within the plant organs, and also protect the plant against an excess, especially of trace metals.[1456,1459]

It is observed with some regularity that sea plants contain more Al, As, Br, Cl, I, Sr, V, and Fe (on a dry matter basis) than terrestrial plants.[1333a] This is thought to be a general rule in chemical element distribution among sea and terrestrial plants that has also been emphasized by Dobrovolsky,[1280] who calculated the variable abundance of trace elements that are involved in biogeochemical cycling in different climatic zones of the globe. There is an increase of over tenfold when comparing element cycling in a tundra zone with that in a tropical forest.

II. ABSORPTION

The main sources of trace elements in plants are their growth media, for example, nutrient solutions or soils. One of the most important factors that determines the biological availability of a trace element is its binding to soil constituents. In general, plants readily take up the species of trace elements that are dissolved in the soil solutions in either ionic or chelated and complexed forms. Much has been written on the absorption of trace elements from solutions by Moore,[548] Loneragan,[489] Mengel and Kirkby,[531] Wild and Jones,[1184] and others, and this absorption can be summarized as follows:

1. It usually operates at very low concentrations in solutions.
2. It largely depends on the concentrations in the solution, especially at low ranges.
3. The rate depends strongly on the occurrence of H^+ and other ions.
4. The intensity varies with plant species and stage of development.

5. The processes are sensitive to some properties of the soil environment such as temperature, aeration, and redox potential.
6. It may be selective for a particular ion.
7. The accumulation of some ions can take place against a concentration gradient.
8. Mycorrhizae play an important role in cycling between external media and roots.

Generalizations of plant processes operative in the absorption of trace elements rest on the evidence for one or a few of the elements, and most often can represent some approximation to processes acting in a natural plant-soil system. Absorption by roots is the main pathway of trace elements to plants; however, the ability of other tissues to readily absorb some nutrients, including trace elements, has also been observed.

In general, the uptake of trace elements by plants is affected, in addition to plant-specific ability, by soils factors, of which the most significant are pH, Eh, water regime, clay content, organic matter content, cation exchange capacity, nutrient balance, and concentration of other trace elements. Also, climatic conditions are shown to influence the rate of trace metal uptake, which may be partly an indirect impact due to the water flow phenomenon. Generally, a higher ambient temperature influences a greater uptake of trace elements by plants.

Plant ability to take up chemical elements from growth media is evaluated by a ratio of element concentration in plants to element concentration in soils and is called: Biological Absorption Coefficient (BAC), Index of Bioaccumulation (IBA), or Transfer Factors (TF). Some elements are more susceptible to phytoavailability than others (see Figure 23). There is, however, great variability among plants, which is indicated by the data of Bini et al.[1226] for four tree species (*Populus nigra* L., *Platanus orientalis* L., *Laurus nobilis* L., *Pinus picea* L). Ranges of BAC values of these trees varied from 209 to 2000 for Cu, and from 10 to 100 for Ni.

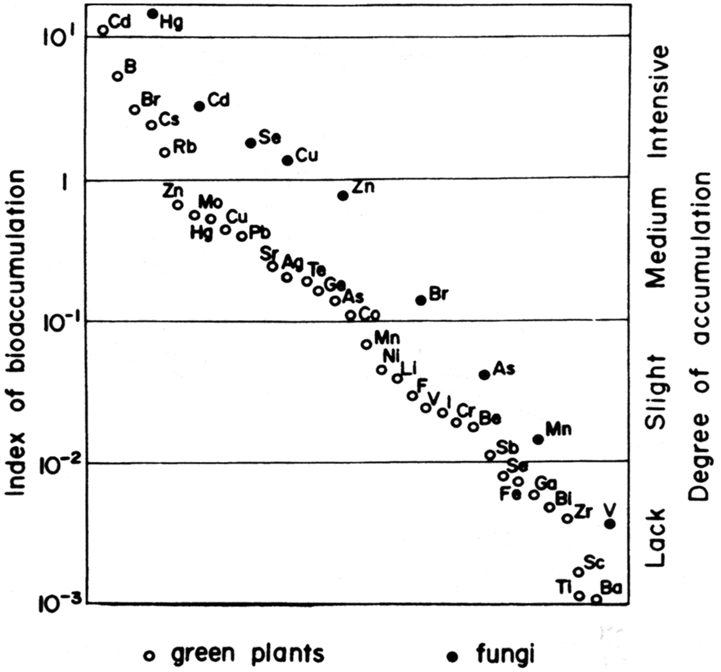

Figure 23 Bioaccumulation of trace elements by plants from soils. Index of bioaccumulation (BAC, IBA) was calculated as the ratio of trace elements in plants to their concentration in soils. The calculation is based on data for different plants and soils. Values for fungi are based on data from Byrne and Ravnik.[117]

A. Root Uptake

The absorption of trace elements by roots can be both passive (nonmetabolic) and active (metabolic), but there are some disagreements reported in the literature concerning which method is involved in certain elements. Despite controversies, in each case the rate of trace element uptake will positively correlate with its available pool at the root surface.

Passive uptake is the diffusion of ions from the external solution into the root endodermis. Active uptake requires metabolic energy and takes place against a chemical gradient. Several data support the suggestion that, at the concentration generally present in soil solutions, the absorption of trace elements by plant roots is controlled by metabolic processes within roots.[489,548,788] The ion activity in the solution is believed to be one of the significant factors that influence plant uptake of ions. Presumably, this is an important factor when the uptake is active, but may not be important when the uptake is passive.

Mechanisms of uptake differ, depending on the given element. Pb and Ni are preferably absorbed passively, while Cu, Mo, and Zn are preferably absorbed actively. When biological and structural properties of root cells are altered, however, all elements are taken up passively. This is the case when concentrations of elements pass over a threshold value for a physiological barrier.

Much evidence indicates that roots exhibit great activity in the mobilization of trace elements that are bound by various soil constituents. Roots and associated microorganisms are known to produce various organic compounds which are very effective in releasing the trace elements from firmly fixed species in soil. The trace elements most readily available to plants are, in general, those that are adsorbed on clay minerals (especially, montmorillonite and illite), while those fixed by oxides and bound onto microorganisms are much less readily available. The depletion of trace elements in solution from the root-soil interface reflects a higher rate of their uptake by roots than mass-flow and diffusion mechanisms in certain soils.[531,638] The mechanisms of uptake of trace elements by roots involve several processes:

1. Cation exchange by roots
2. Transport inside cells by chelating agents or other carriers
3. Rhizosphere effects

Ions and other materials released from roots to rooting media control nutrient uptake by roots. Root exudates of plants are composed mainly of amino acids (e.g., aspartic, glutamic, prolinic) and vary with plant species (and varieties), microorganism association, and conditions of plant growth. Cation oxidation states around roots are believed to be of great importance in these processes. Changes in the pH of the root ambient solution may play an especially significant role in the rate of availability of certain trace elements.[241] Root exudates (e.g., phytosiderophore 2′-deoxymugineic acid) of metal-deficient cereal plants (e.g., Zn, Fe) are especially effective in mobilizing these metals from various precipitations within the root media.[1004a] The exudates released from roots of graminaceous species are of great importance since they are also active in mobilizing other trace metals from soils.

Specific physical and chemical properties of rhizospheres controlled by root exudates and microflora govern processes of the absorption of chemical elements. Apparently, the significant role played by low-molecular-weight organic acids (e.g., oxalic, acetic, fumaric, citric, tartaric) is dissolution and/or complexation processes that mobilize slightly soluble forms of trace elements.[1262,1380]

Roots can also act as a "barrier" in the uptake or transport of trace elements. Otte et al.[1107] described interesting properties of roots of the plants growing in marsh (e.g., *Spartica anglica*). The Fe-plaque formed on the roots consists mainly of Fe (hydro) oxides with a large capacity to adsorb metals. Above certain threshold concentrations, the Fe-plaque becomes saturated with the trace metal, and the root is confronted with higher metal contents exceeding that of the surrounding medium.

Chaney et al.[128] believed that the reduction step is obligatory in root uptake of Fe. The reduction of other metals such as Mn, Cu, Sn, or Hg in the uptake step apparently has not been clearly observed. Rice roots, on the other hand, exhibit a peculiar mechanism to absorb Si and Se in the form of oxides.[395]

The ability of different plants to absorb trace elements varies greatly; when compared on a large scale, however, the index of their accumulating ability illustrates some general trends. Some elements such as Cd, B, Br, Cs, and Rb are extremely easily taken up, while Ba, Ti, Zr, Sc, Bi, Ga and, to an extent, Fe and Se, are but slightly available to plants (Figure 23). This trend, however, will differ a great deal for particular soil-plant systems. Marked differences in the metal uptake between both plant species and varieties open up new aspects for plant breeding programs for the biodepletion in metal transport to the food chain.

Fungi are nongreen plants with quite a diverse mechanism of nutrient uptake, and they have a specific affinity for some trace elements. They may accumulate Hg and also other elements such as Cd, Se, Cu, and Zn to high levels (Figure 23). Concentrations of several trace elements in plants from a pine forest in Japan indicate that there is a preferable uptake by mushrooms of such elements as Cd, Zn, Cu, Cs, and Rb.[1564,1565] Concentrations of these elements were one order of magnitude higher than those in plants growing in the same forest. Concentrations of Ca and Sr in mushrooms, on the other hand, were lower than those in plants.

B. Foliar Uptake

The bioavailability of trace elements from aerial sources through the leaves may have a significant impact on plant contamination and it is also of practical importance in foliar applications of fertilizers, especially of elements such as Fe, Mn, Zn, and Cu. Foliar absorption of radionuclides released into the atmosphere from nuclear weapons testing and nuclear power installations is of especially great concern.

Foliar uptake is believed to consist of two phases—nonmetabolic cuticular penetration, which is generally considered to be the major route of entry, and metabolic mechanisms, which account for element accumulation against a concentration gradient. The second process is responsible for transporting ions across the plasma membrane and into the cell protoplast. Wyttenbach et al.[1553] studied the uptake of As, Br, and I by Norway spruce in order to distinguish between the uptake from soils and from gaseous or soluble compounds in the air. They found a strong positive correlation between the endogenous needle concentrations and the surface loadings, and also insignificant correlations with total soil concentrations of these elements.

Trace elements taken up by leaves can be translocated to other plant tissues, including roots where the excesses of some metals seem to be stored. The rate of trace element movement among tissues varies greatly, depending on the plant organ, its age, and the element involved. Results illustrated in Figure 24 show that Cd, Zn, and Pb absorbed by the tops of brome grass were not likely to move readily to the roots, whereas Cu was very mobile.

A fraction of the trace elements absorbed by leaves may be leached from plant foliage by rainwater. Differences in leaching of trace elements can be related to their function or metabolic association. For example, the easy removal of Pb by washing suggests that the metal was largely a superficial deposit on the leaf surface. In contrast, the small fraction of Cu, Zn, and Cd that can be washed off indicates a greater leaf penetration of these metals than was noted for Pb by Little and Martin[482] and Kabata-Pendias.[374] Moreover, significant absorption of foliar-applied Zn, Fe, Cd, and Hg were reported by Roberts.[657] Foliar leaching by acid rain may involve cation exchange processes, in which the H^+ ion of rainwater replaces microcations held on binding sites in leaf cuticle.[885]

The absorption of trace metals, directly from wet (and dry) deposition by above-ground parts of plants, has been reported often. Morphology of the surface of leaves is an important factor governing foliar uptake of trace elements. Some plants (e.g., lichens, mosses, mushrooms, dandelion, etc.) are especially susceptible to absorb elements and some compounds from aerial sources.

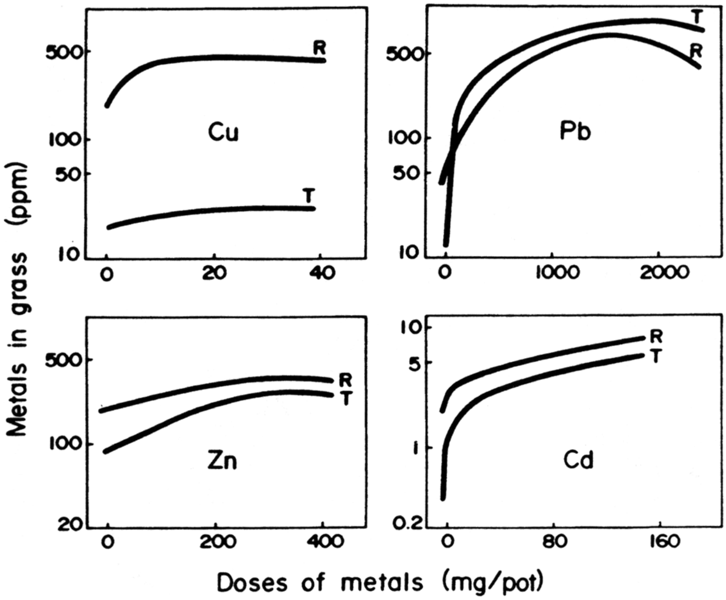

Figure 24 Distribution of heavy metals from aerial sources between the tops (T) and roots (R) of brome grass.[376]

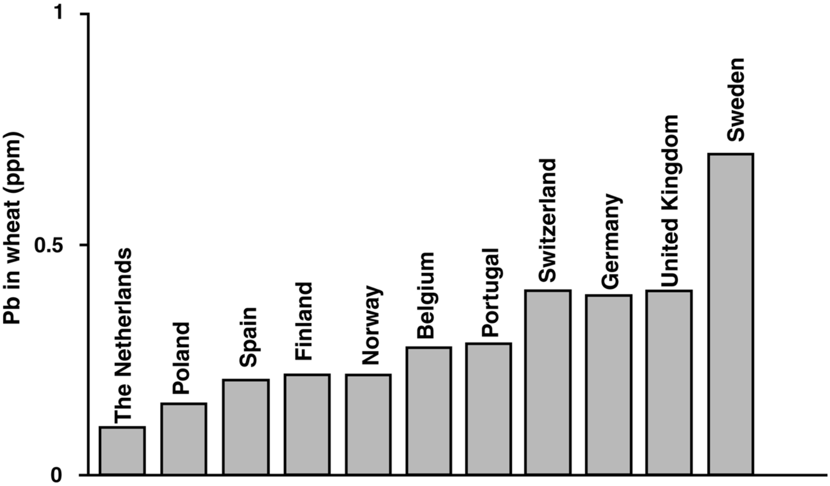

Figure 25 Geometric mean contents of Pb in winter wheat (*Triticale vulgare* L.) collected in 1985 from various countries in Europe. (FAO Cooperative Project Network on Trace Elements—Report, 1991, by M. Verloo *vide* Kabata-Pendias et al.[1358])

Such plants are very suitable for the phytoindication of atmospheric pollution. Also, cereal tops show a relative sensitivity to aerial pollution, revealing the variation in trace metal contents (especially Pb and Ni) mainly due to the absorption from aerial particles (Figures 25 and 26). Dalenberg and van Driel[1270] studied the uptake and translocation of ^{210}Pb from atmospheric deposition. Their results have indicated that 73 to 95% of the total Pb content in plants are taken up by leaves and transported to other plant organs. This was observed not only for leafy plants, such as spinach, but also for cereals.

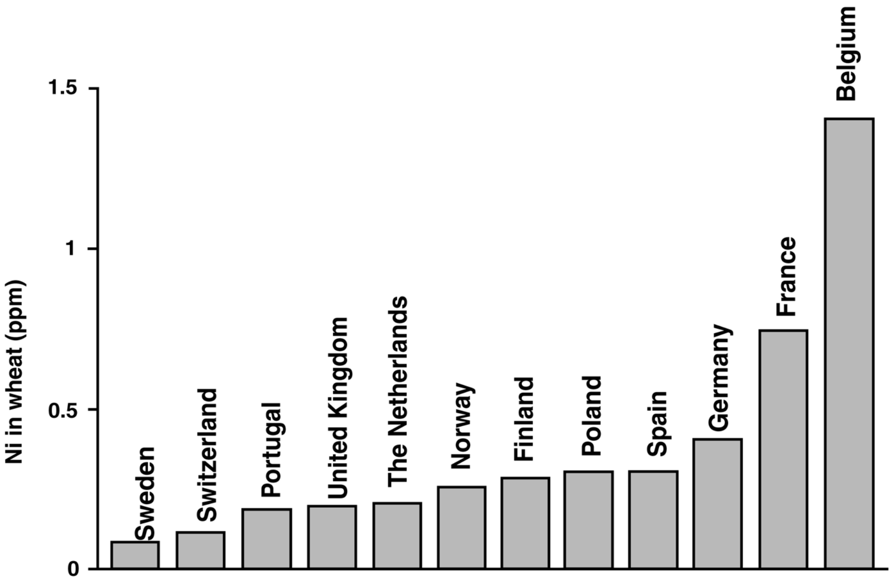

Figure 26 Geometric mean contents of Ni in winter wheat (*Triticale vulgare* L.) collected in 1985 from various countries in Europe. (FAO Cooperative Project Network on Trace Elements—Report, 1991, by M. Verloo *vide* Kabata-Pendias et al.[1358])

III. TRANSLOCATION

The transport of ions within plant tissues and organs involves many processes:

1. Movement in xylem
2. Movement in phloem
3. Storage, accumulation, and immobilization

The chelating ligands are most important in the control of cation translocation in plants. However, numerous other factors such as pH, the oxidation-reduction state, competing cations, hydrolysis, polymerization, and the formation of insoluble salts (e.g., phosphate, oxalate, etc.) govern metal mobility within plant tissues.

Tiffin[788] gave a detailed review of the mechanisms involved in the translocation of micronutrients in plants. It can be summarized that long-distance transport of trace elements in higher plants depends on the vascular tissues (xylem and phloem) and is partly related to the transpiration intensity. Chemical forms of trace metals in phloem exudates differ for each element. Van Goor and Wiersma,[822] for example, reported that Zn was almost all bound to organic compounds, while Mn was only partly complexed.

The distribution and accumulation patterns of trace elements vary considerably for each element, kind of plant, and growth season. As reported by Scheffer et al.,[688a] in the phases of intensive growth of summer barley the amount of Fe and Mn is relatively low, whereas the amount of Cu and Zn is very high. While the first two metals are accumulated mainly in old leaves and leaf sheaths, Cu and Zn seem to be distributed more uniformly through the plant. Differentiation in trace element distribution between various parts of pine trees is also clearly shown by data presented in Table 33. A relatively common phenomenon, however, is the accumulation and immobilization of trace metals in roots, especially when their metal supply is sufficient. The mechanism of exclusion seems also to control the transport of cations from roots to aerial parts.[417]

Table 33 Variation in Trace Element Contents in Pine Trees (ppm DW)[186]

Plant Part	Al	B	Co	Cr	Cu	Fe	Mn	Ni	Pb	Ti	V
Needles											
1 year old	400	18.0	0.9	4.8	4.2	150	430	6.0	0.2	15	0.6
Older	200	24.0	0.8	4.0	2.5	370	740	2.1	0.5	30	1.2
Branches	400	6.0	0.6	1.6	3.0	650	430	1.1	0.6	25	1.8
Knots	120	4.5	0.2	0.8	1.2	78	185	0.3	0.1	6	0.8
Bark	230	4.5	0.4	1.0	2.0	100	123	0.4	0.3	15	2.8
Wood	7	0.9	0.1	0.3	0.6	5	61	0.3	0.1	1	0.2
Roots											
1-mm diameter	1430	6.5	0.1	0.9	3.5	7171	134	1.1	0.3	46	0.6
5-mm diameter	82	3.2	0.7	0.6	1.2	46	50	0.4	0.1	6	0.5

Note: Samples from a pine forest on old alluvial sands in Ukraine.

Brundin et al.[953] have shown that roots of plants growing along stream channels (mainly *Carex* species) as well as aquatic mosses are barrier-free with respect to trace metal uptake. However, physiological barriers are evident with respect to the transport of some metals (e.g., Cu, Zn, and Co) from roots to aerial parts. Most *Carex* species are barrier-free with respect to the translocation of Pb, and partly of Ni, Cr, and Mo.

Horiguchi and Nishihara[1028] studied the association of trace metals with the major constituents of potato tubers and peanut seeds. They found that only a small proportion of metals was distributed in the soluble components of a low molecular weight of peanut seeds, while potato tubers contained higher proportions of the metals in the low-molecular-weight fraction. It is presumed that starch as well as lipid fractions have only a weak affinity for trace metals, and that proteins are the major ligands that form complexes with metals in plants.

The transport of trace elements among plant organs also depends on the electrochemical variables of elements. In general, easily transported from roots to above-earth parts are Ag, B, Li, Mo, and Se; moderately mobile are Mn, Ni, Cd, and Zn; and strongly bound in root cells are Co, Cu, Cr, Pb, Hg, and Fe.

IV. AVAILABILITY

The linear responses of trace element absorption by several plant species in increasing their tissue concentrations from nutrient and soil solutions are illustrated in Figure 27. These responses support the statement that the more reliable methods in diagnosing the available trace element status of soils are those based on element concentrations in the soil solutions rather than methods based on the pool of soluble and/or extractable trace elements. The phytoavailability of trace metals correlates best with the concentration of cationic species in the liquid phase. Table 34 summarizes these relations.

A number of extraction methods have been suggested in recent years for the evaluation of trace element concentrations in soils. In general, they can be classified into separate groups as follows: acids (HCl, NHO_3, aqua regia), chelating agents (EDTA, DTPA [+TEA]), buffered salt solutions (AAAc buffer), and unbuffered salt solutions ($CaCl_2$, $MgCl_2$, $NaNO_3$, NH_4NO_3). Acid extractants, depending on the strength and soil mineralogical composition, can extract nearly total amounts of trace metals. Chelating agents and buffered salt solutions are believed to extract potentially mobile portions of metals. Neutral salt solutions have been introduced as simulating the natural soil solutions and therefore are useful to evaluate the ecological relevance of metals.

Much work has been done on universal extractants for soil tests to assess micronutrient availability. Barber,[930] Cox and Kamprath,[151] and Walsh and Beaton[847] prepared comprehensive reviews

Table 34 Relative Phytoavailability of Different Species of Metals from Soils

Metal Species	Phytoavailability
Simple or complex cations in solution phase	Easy
Exchangeable cations in organic and inorganic complexes	Medium
Chelated cations	Slight
Metal compounds precipitated on soil particles	Available after dissolution
Metals bound or fixed inside organic substances	Available after decomposition
Metals bound or fixed inside mineral particles (primary or secondary soil minerals)	Available after weathering and/or decompositon

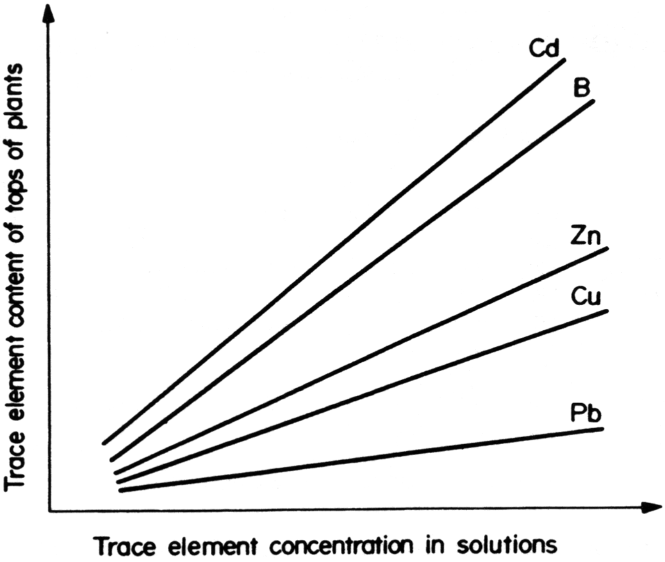

FIGURE 27 Trace element uptake by plants as a function of their concentrations in nutrient solutions.

of chemical tests for the determination of water-soluble, acid-extractable, exchangeable, and complexed or chelated trace elements in soils.

A wide overview of trace element soil tests was recently presented in *Method of Soil Analysis*, edited by Bingham and Bartels.[1225b] In this volume, Amacher discussed extractants used to estimate plant-available Cd, Ni, and Pb in soils which are the same as for other trace metals. Good correlation was obtained for extracted and available metals added at elevated levels to the soils; whereas the correlation was less suitable for the prediction of plant-available quantities of metals under field conditions. Humez et al.[1339] presented different approaches to assessment of mobility of elements in sediments, including a method of kinetics extraction. Neutral salt solutions (e.g., $CaCl_2$, $MgCl_2$, $NaNO_3$) are also proposed as a simulated solution phase of soil for the estimation of bioavailable trace element contents. Barber[930] concluded that 0.01 M $CaCl_2$ at any soil/solution dilution was particularly applicable to trace metal analyses where chelation effects are important. The interpretation of soil testing results is by no means an easy problem and may give reliable information for only a particular soil-plant system. These methods, nevertheless, are widely used, with various results, in agricultural practices. Minnich et al.[1094] stated that extraction procedures are easier and probably provide a better predictive ability for plant Cu uptake than Cu^{2+} activity measurements. The DTPA-extractable Cu and Zn correlated well with metal contents of plant tops.[1047,1094] Minnich et al.[1094] emphasized that continual monitoring of soil solution ion activity or a buffering index is needed to improve predictions of plant ion uptake. The uptake of elements by plant roots is restricted to the liquid phase, therefore the content of metals in the soil solution is of primary importance.[1046] However, more information is needed about multiple ion activity ratios and about antagonistic or synergistic effects of accompanying cations and anions in the soil solution, in relation to uptake and physiological effects of trace metals in plants. Also, there is insufficient information on the availability of organic metal complexes in the solution phase.

Complexing compounds produced on a base of acetic acid (e.g., EDTA, DTPA, EDDHA) and their mixtures with other compounds are more widely used for identifying deficiency problems, mainly with Mn, Cu, Zn, and Fe.[1225b,1401] All soil tests were calibrated to determine deficient levels of micronutrients. Thus, most of these methods should be calibrated to determine phytotoxic levels of soil trace elements. Recently renewed is the earlier concept (Barber[930]) of using extractants that simulate natural soil solution.[151,320] These are solutions of neutral salts, mainly $CaCl_2$, in various concentrations (most frequently 0.01 M). The suitability of this method has been discussed in some publications.[1302,1335] The final conclusion is that $CaCl_2$ extractant can be considered universal because it has more or less the same ionic strength (0.03 M) as the average salt concentration in many soil solutions. It cannot however, serve as a soil test to estimate both deficient and phytotoxic levels of trace metals. To do this, calibration to biological indices is necessary. Possible formation of complex ions with Cl^- in certain soil conditions should be considered. Such complex ions (e.g., $CdCl^+$, $CdCl_3$) can increase the solubility of some trace metals, as observed in the case of Cd.[1330]

The mixed extractant AAAc-EDTA was applied in the large world project on available trace element contents of soils in 30 countries, as carried out by Sillanpää and Jansson.[1441] The relation between Cd, Pb, Co, and Se in crop plants and the pool of soluble forms of elements in soils, despite differences in soil variables and climatic conditions, was acceptably close.

Recently, Coca Cola has been proposed as a relatively simple extractant and is easily applied in soil testing procedures for mobile trace elements.[1482] The active compound in Coca Cola is phosphoric acid, which dissolves 27% of Fe, 38% of Cu, 86% of Zn, and 165% of Mn compared to those extracted by DTPA solution. Due to the relatively stable composition of Coca Cola, such an extractant might be suitable for specific experiments and projects.

The specific properties of plants are very significant in determining the bioavailability of trace elements and are quite variable with changing soil and plant conditions. The availability of different plant species to absorb certain trace elements from the same soil environment is illustrated in Table 35. In fact, different genotypes of the same species uptake different amounts of the trace elements. The wide variation (within the range of a factor of 100) in trace element contents of *Artemisia* species was observed in one meadow ecosystem.[1097] To provide an effective evaluation of the pool of bioavailable trace elements, therefore, techniques based on both soil tests and plant analyses should be used together.

Conditions of plant growth media also have a significant impact on the absorption of trace elements by roots. Findings of Miyasaka and Grunes[1421] indicated that increasing the soil temperature from 8°C to 16°C influenced, by twofold higher, the contents of Cu, Zn, and Mn in leaves of winter wheat. Thus, during a cold season, plants may have low levels of some trace elements that may limit or affect their growth, and also cause a deficiency in grazing animals.

The sampling procedures for each field, each crop, and a specific plant part in the same stage of growth must be standardized for obtaining compatible results that could be classified as deficient, sufficient, or excessive or toxic for plants. Existing soil and plant tests, however, do not satisfactorily predict trace element deficiencies in crop plants that would respond to application of micronutrients.

Ranges of trace element concentrations and their classification for the mature leaf tissue presented in Table 36 are overall approximations and can differ widely for particular soil-plant systems. It is necessary to emphasize that ranges of concentrations of trace elements required by plants are often very close to the content that exerts a harmful influence on plant metabolism. It is not easy, therefore, to make a clear division between sufficient and excessive quantities of trace elements in plants.

Table 35 Variation in Trace Element Content among Various Plants from One Site in the Same Forest Ecosystem[a] (ppm DW)[1045]

Plants	B	Cd	Cu	Fe	Mn	Pb	Zn	Cr	Ni
Grass *Agrostis alba*	3.4	0.6	4.2	80	740	1.2	59	1.0	1.8
Clover *Trifolium pratense*	9.0	0.7	6.0	115	136	2.8	99	1.0	2.2
Plantain *Plantago major*	7.0	1.9	9.8	135	100	2.4	97	1.4	3.0
Mosses									
Polytrichum juniperinum	3.4	0.8	9.2	800	176	22.4	69	2.0	2.0
Entodon schreberi	3.2	0.7	10.3	425	180	13.0	77	2.8	1.6
Lichens									
Parmelia physodes	2.4	0.4	5.0	1100	62	17.0	78	3.2	4.8
Lobaria pulmonaria	2.4	0.5	7.5	1450	66	28.0	74	3.2	2.4
Edible fungi									
Cantharellus cibarius	4.0	1.0	24.5	49	19	1.2	150	0.4	2.2
Leccinum scabra	0.8	2.7	18.0	44	6	<0.1	125	0.4	1.8
Inedible fungi									
Tylopilus felleus	3.5	1.6	35.0	50	14	0.4	180	0.4	1.2
Russula veternosa	6.4	1.0	32.0	28	18	1.0	175	0.4	1.0

[a] Pine and birch forest on light sandy soil near Warsaw, Poland.

Table 36 Approximate Concentrations of Trace Elements in Mature Leaf Tissue Generalized for Various Species (ppm DW)[66,171,279,322,369,381,395,531,916]

Element	Deficient (if less than the stated amounts of essential elements)	Sufficient or Normal	Excessive or Toxic	Tolerable in Agronomic Crops[1081,1357]
Ag	—	0.5	5–10	—
As	—	1–1.7	5–20	0.2[a]
B	5–30	10–100	50–200	100
Ba	—	—	500	—
Be	—	<1–7	10–50	—
Cd	—	0.05–0.2	5–30	0.05–0.5
Co	—	0.02–1	15–50	5
Cr	—	0.1–0.5	5–30	2
Cu	2–5	5–30	20–100	5–20
F	—	5–30	50–500	—
Hg	—	—	1–3	0.2[a]
Li	—	3	5–50	—
Mn	10–30	30–300	400–1000	300
Mo	0.1–0.3	0.2–5	10–50	—
Ni	—	0.1–5	10–100	1–10
Pb	—	5–10	30–300	0.5–10
Se	—	0.01–2	5–30	—
Sn	—	—	60	—
Sb	—	7–50	150	—
Ti	—	—	50–200	—
Tl	—	—	20	—
V	—	0.2–1.5	5–10	—
Zn	10–20	27–150	100–400	50–100
Zr	—	—	15	—

Note: Values are not given for very sensitive or highly tolerant plant species.

[a] FW basis.

Houba and Uittenbogaard[1334] published results of determining 27 trace elements in 140 plant kinds from 66 countries during the period from 1981 to 1993. Each plant kind was represented by a number of samples (a few tens) from various locations, and therefore it is difficult to interpret the variability in trace element contents among plants. Some general trends, however, indicate that Pb is likely to be concentrated in cabbage and tomato leaves, Mo in sugar beet leaves and Chinese cabbage, Se in mushrooms, and Li in potato leaves. Onion bulbs contain elevated amounts of Cr, As, and Ni; lettuce leaves—Cr, Pb, and Cd; and spinach leaves—Li, Ni, and Pb.

V. ESSENTIALITY, DEFICIENCY, AND EXCESS

Knowledge of the importance of certain trace elements for healthy growth and development of plants dates from the last century. At present, 17 trace elements (Al, B, Br, Cl, Co, Cu, F, Fe, I, Mn, Mo, Ni, Rb, Si, Ti, V, and Zn) are known to be essential for all plants, several are proved necessary for a few species only, and others are known to have stimulating effects on plant growth, but their functions are not yet recognized (Table 37). A feature of the physiology of these elements is that although many are essential for growth, they can also have toxic effects on cells at higher concentrations. Hypothetical schemes of the reactions of plants to increasing concentrations of the essential and nonessential trace elements are presented in Figures 28 and 29.

The trace elements essential for plants are those which cannot be substituted by others in their specific biochemical roles and that have a direct influence on the organism so that it can neither grow nor complete some metabolic cycle. The elements needing more evidence to establish their essentiality usually are those thought to be required in very low concentrations (at μg kg^{-1} or ng kg^{-1} ranges) or that seem to be essential for only some groups or a few species of plants.

Bowen[94] classified the functions and forms of the elements in organisms, based on the current state of knowledge, by dividing the trace elements that occur in plants into the following groups:

1. Those incorporated into structural materials—Si, Fe, and rarely Ba and Sr
2. Those bound into miscellaneous small molecules, including antibiotics, and porphyrins—As, B, Br, Cu, Co, F, Fe, Hg, I, Se, Si, and V
3. Those combined with large molecules, mainly proteins, including enzymes with catalytic properties—Co, Cr (?), Cu, Fe, Mn, Mo, Se, Ni (?), and Zn
4. Those fixed by large molecules having storage, transport, or unknown functions—Cd, Co, Cu, Fe, Hg, I, Mn, Ni, Se, and Zn
5. Those related to organelles or their parts (e.g., mitochondria, chloroplasts, some enzyme systems)—Cu, Fe, Mn, Mo, and Zn

In summary, based on extensive literature, trace elements are involved in key metabolic events such as respiration, photosynthesis, and fixation and assimilation of some major nutrients (e.g., N, S). Trace metals of the transition metal group are known to activate enzymes or to be incorporated into metalloenzymes as electron transfer systems (Cu, Fe, Mn, and Zn) and also to catalyze valence changes in the substrate (Cu, Co, Fe, and Mo). Some particular roles of several trace elements (Al, Cu, Co, Mo, Mn, and Zn) which seem to be involved in protection mechanisms of frost-hardy and drought-resistant plant varieties are also reported.[511,718]

The requirements of plants and even of individual species for a given micronutrient have been well-demonstrated by Hewitt[317] and Chapman.[131] If the supply of an essential trace element is inadequate, the growth of the plant is abnormal or stunted and its further development, especially its metabolic cycles, are disordered. Although deficiency symptoms cannot be generalized, they may be quite characteristic for the particular element. Bergmann and Čumakov[66] presented comprehensive illustrations of deficiency (and some toxicity) symptoms in cultivars. The descriptions of deficiency symptoms summed up in Table 38 indicate that chlorosis is the most frequent symptom. Visible symptoms are important in diagnosis of deficiencies; however, disturbance of metabolic processes and consequent losses in production of biomass may occur before the deficiency symptoms are recognized. In order to develop a better diagnostic method, biochemical indicators based on enzymatic assays were proposed by Ruszkowska et al.,[672] Rajaratinam et al.,[639] and Gartrell et al.[258] as a sensitive test for a hidden deficiency of a given micronutrient. The activity of some enzymes

Table 37 Forms and Principal Functions of Trace Elements That Are Essential for Plants[94,142,349,531,614,859]

Element	Constituent of	Involved in
Al[a]	—	Controlling colloidal properties in the cell, possible activation of some dehydrogenases and oxidases
As[a]	Phospholipid (in algae)	Metabolism of carbohydrates in algae and fungi
B	Phosphogluconates	Metabolism and transport of carbohydrates, flavonoid synthesis, nucleic acid synthesis, phosphate utilization, and polyphenol production
Br[a]	Bromophenols (in algae)	—
Co	Cobamide coenzyme	Symbiotic N_2 fixation, possibly also in non-nodulating plants, and valence changes stimulation synthesis of chlorophyll and proteins (?)
Cu	Various oxidases, plastocyanins, and ceniloplasmin	Oxidation, photosynthesis, protein and carbohydrate metabolism, possibly involved in symbiotic N_2 fixation, and valence changes, cell wall metabolism
F[a]	Fluoracetate (in a few species)	Citrate conversions
Fe	Hemo-proteins and nonheme iron proteins, dehydrogenases, and ferrodoxins	Photosynthesis, N_2 fixation, and valence changes
I[a]	Tyrosine and its derivatives (in angiosperms and algae)	—
Li[a]	—	Metabolism in halophytes
Mn	Many enzyme systems	Photoproduction of oxygen in chloroplasts and, indirectly, in No_3^- reduction
Mo	Nitrate reductase, nitrogenase, oxidases, and molybdoferredoxin	N_2 fixation, No_3^- reduction, and valence changes
Ni[a]	Enzyme urease (in *Canavalia* seeds)	Possibly in action of hydrogenase and translocation of N
Rb[a]	—	Function similar to that of K in some plants
Se[a]	Glycene reductase (in *Clostridium* cells) combined with cysteine and methionine	Can replace S in some plants
Si	Structural components	—
Sr[a]	—	Function similar to that of Ca in some plants
Ti[a]	—	Possibly photosynthesis and N_2 fixation
V[a]	Porphyrins, hemoproteins	Lipid metabolism, photosynthesis (in green algae), and, possibly, in N_2 fixation
Zn	Anhydrases, dehydrogenases, proteinases, and peptidases	Carbohydrate, nucleic acid, and lipid metabolism

[a] Elements known to be essential for some groups or species and whose general essentiality needs confirmation.

is correlated mainly with Cu, Fe, and Mo levels in plant tissues. The practical use of the enzymatic assays is, however, greatly limited because of a high rate of variation and of technical difficulties in the determination of the enzymatic activity.

The most widely used diagnostic tests are soil and plant analyses. More specific diagnosis of critical levels of some trace metals in plant tissues should also be related to the ratio of antagonistic elements, as described by Nambiar and Motiramani[560] for Fe:Zn ratios in maize. Concentrations of immobile trace elements in old leaves or in whole plants, unlike those of mobile micronutrients,

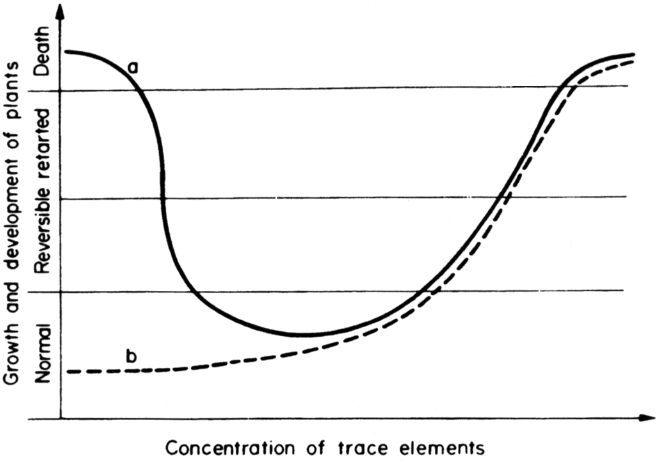

Figure 28 Schematic diagram of plant response to stress from deficiency and toxicity of trace elements: (a) essential trace elements; (b) non-essential trace elements.

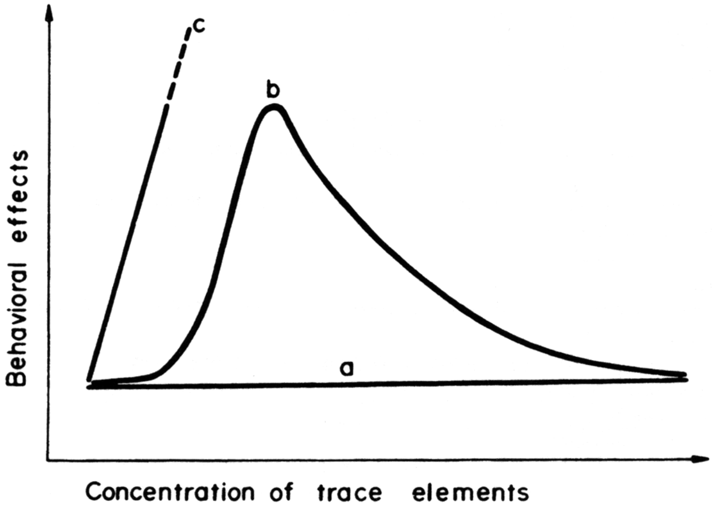

Figure 29 Schematic diagram of behavioral plasticity of plants under chemical stress: (a) no behavioral change of entirely tolerant species; (b) development of behavioral tolerance; (c) reaction of nontolerant species leading to damage of organisms followed by no recovery.

may be misleading in assessing the nutrient status of plants. Nevertheless, plant tissue analysis has been used successfully for assessing deficiencies when based on the normal tissue contents for plant genotypes or species and organs and the stage of plant development. A comprehensive literature has been published in various countries on the diagnosis of trace element deficiencies and their correction through the application of particular micronutrients. Mengel and Kirkby[531] presented the most current information on micronutrients and indicated the need for their application to some cultivars. It should be emphasized, however, that the application of a given micronutrient is effective only if the soil content or the availability of the element is low. High-yield crop plants can suffer from acute or light deficiencies of some micronutrients. Finck[995] gave an example of the extent of the deficiencies (both medium and light) in high-yield grain crops: (1) Mn supply to oats—on brown earths 40% and on calcareous marsh soils about 50% of fields, (2) Cu supply to oats—about 20% of fields on both soil types, and (3) Zn supply to wheat—about 15% of fields on both soil types.

Table 38 Symptoms of Micronutrient Deficiency in Some Common Cultivars[66,114,531,718]

Element	Symptoms	Sensitive crop
B	Chlorosis and browning of young leaves; killed growing points; distorted blossom development; lesions in pith and roots, and multiplication of cell division	Legumes, *Brassica* (cabbage and relatives), beets, celery, grapes, and fruit trees (apples and pears)
Cu	Wilting, melanism, white twisted tips, reduction in panicle formation, and disturbance of lignification and of development and fertility of pollen	Cereals (oats), sunflower, spinach, and lucerne (alfalfa)
Fe	Interveinal chlorosis of young organs	Fruit trees (citrus), grapes, and several calcifuge species
Mn	Chlorotic spots and necrosis of young leaves and reduced turgor	Cereals (oats), legumes, and fruit trees (apples, cherries, and citrus)
Mo	Chlorosis of leaf margins, "whiptail" of leaves and distorted curding of cauliflower, "fired" margin and deformation of leaves due to NO_3 excess, and destruction of embryonic tissues	*Brassica* (cabbage and relatives) and legumes
Zn	Interveinal chlorosis (mainly of monocots), stunted growth, "little leaf" rosette of trees, and violet-red points on leaves	Cereals (corn), legumes, grasses, hops, flax, grapes, and fruit trees (citrus)

Both deficiencies and toxicities of trace elements for plants most commonly result from complex factors that vary with the specific environment. However, many observations and experiments conducted on various soil types in different countries have clearly demonstrated that soil genesis and soil properties are the main factors controlling micronutrient deficiencies. The data summarized in Table 39 present the general relationship between the occurrence of micronutrient deficiencies in plants and soil properties. The most frequently occurring deficiencies are related to extremely acid soils (light sandy) or to alkaline soils (calcareous) with improper water regimes and with excesses of phosphate, N, and Ca, as well as Fe and Mn oxides.

VI. TOXICITY AND TOLERANCE

Metabolic disorders of plants are affected not only by micronutrient deficiencies, but also by their excesses. In general, plants are much more resistant to an increased concentration than to an insufficient content of a given element. Although many observations have been published on the harmful effects of trace element excesses, the nature of these processes is still poorly understood. Basic reactions, as reviewed by Peterson,[609] Foy et al.,[241] Bowen,[94] and Prasad and Strzalka,[1461] related to toxic effects of element excesses are the following:

1. Changes in permeability of the cell membrane—Ag, Au, Br, Cd, Cu, F, Hg, I, Pb, UO_2
2. Reactions of thiol groups with cations—Ag, Hg, Pb
3. Competition for sites with essential metabolites—As, Sb, Se, Te, W, F
4. Affinity for reacting with phosphate groups and active groups of ADP or ATP—A1, Be, Sc, Y, Zr, lanthanides and, possibly, all other heavy metals
5. Replacement of essential ions (mainly major cations)—Cs, Li, Rb, Se, Sr
6. Occupation of sites for essential groups such as phosphate and nitrate—arsenate, fluorate, borate, bromate, selenate, tellurate, and tungstate
7. The damage to photosynthesis apparatus involved in several metabolic alterations is the most significant effect of the excess of trace metals

Table 39 Soil Factors Contributing to Micronutrient Deficiency

			Contributing soil factors					
Element	Soil Units[a]	pH Range	Organic Matter	Water Regime	Other Factors	Critical Deficiency Limit in Soil[b] (ppm)	Plants Responding	Some Countries of Occurrence
B	Podzols, rendzinas, gleysols, nitosols, and ferralsols	Acid and neutral	Very low or very high	Flooded soils	Light texture, free $CaCO_3$	0.1–0.3 (HW)	Beets, legumes, crucifers, and grapes	Australia, Egypt, France, Poland, Taiwan, U.S., Russia, China, and India
Co	Podzols, histosols, rendzinas, and solonetz	Neutral, alkaline, or strongly acid	High	High moisture	Free $CaCO_3$, high Fe and Mn	0.02—0.3 (AA)[c]	Legumes	Australia, France, Germany, Poland, Sweden, U.S., and Russia
Cu	Histosols, podzols, rendzinas, solonchaks, and solonetz	—	Low or high	HIgh moisture	Light leached soil, high N, P, and Zn, free $CaCO_3$	1–2 (NA, 0.5 *N*) 0.8—3 (NA, 1 *N*) 0.8—1 (Ac-ED) 0.2 (AC)	Cereals, legumes, and citrus	Australia, Egypt, U.S., Russia, and European countries
Fe	Rendzinas, ferralsols, solonetz, arenosols, and chernozems	Alkaline	High with free $CaCO_3$ or low in acid soil	Poor drainage, moisture extremes	Free $CaCo_3$, high P, Mn, and HCO_3^-	0.2—1.5 (DT) 2.5—4.5 (DT) <30—35 (Ac-ED)	Citrus, grapes, pineapples, and tomatoes	Throughout the world, and especially in arid and semiarid regions
Mn	Podzols, rendzinas, and histosols	Strongly acid or alkaline	High, e.g., alkaline peats	Moisture extremes	Free $CaCO_3$ and high Fe	1—5 (DT) 20—100 (HR) 1—2 (AC) 14–70 (PA)	Cereals, legumes, beets, and citrus	Australia, France, U.S., and Russia

Mo	Podzols and ferralsols decalcified	Strongly acid to acid	—	Good drainage	High Fe and Al oxides, and high SO_4 -S	0.01—0.6 (AO)[d] <0.1 (HW)	Crucifers, cucurbits, and legumes	Australia, India, U.S., and Russia
Se	Podzols, histosols, and ferralsols	Acid	High	Waterlogging	High Fe oxides, and SO_4 -S	<0.04 (T)[c]	—	Australia, U.S., and some European countries
Zn	Podzols, rendzinas, and solonetz	Strongly acid or alkaline	Low	—	Free $CaCO_3$, high N and P	1–8 (HA) 1.5–3 (ED) 0.4–1.5 (DT) 0.3–2 (AC)	Cereals, legumes, and citrus	Australia, France, India, U.S., and Russia

Note: Explanation of symbols used for extraction methods determining contents of soluble elements: AA, 2.5% acetic acid; AC, ammonium acetate; AO, ammonium oxalate; DT, DTPA; ED, EDTA; Ac-ED, ammonium acetate-EDTA; HA, 0.1 *N* hydrochloric acid; HR, hydroquinone reducible method; HW, hot water; NA, 0.5 or 1 *N* nitric acid; PA, 0.1 *N* phosphate acid; and T, total.

[a] Generalized soil types assigned to soil units as given in Table 8.

[b] Critical deficiency limits very highly among plant species and soil kinds.

[c] Animal response.

[d] Highly pH dependent.

Recent findings support the idea that metals induce biochemical changes in plants similar to responses elicited by pathogen attack.[1378]

An assessment of toxic concentrations and effects of trace elements on plants is very complex because it depends on so many factors that it cannot be measured on a linear scale. Some of the most important factors are the proportions of related ions that are present in solution and their compounds. For example, the toxicity of arsenate and selenate is markedly reduced in the presence of excess phosphate or sulfate, and metallo-organic compounds may be either much more toxic than cations or much less so. It should also be noted that certain compounds (e.g., oxygenated anions of metals) may be more toxic than their simple cations. Van Assche and Glijsters[1175] reported that enzyme measurements in plants (2-week-old test bean) can be a criterion for the phytotoxic effect of soil pollution with trace metals. The metal toxicity is reflected by the increase in capacity of various enzymes (e.g., peroxidase, dehydrogenase). The specific plant (iso)enzyme reactions on different trace metals were observed.

Several orders of trace element toxicity to plants are presented in the literature and they vary with each experiment and each plant; however, they correlate fairly well with the following factors:

1. Electronegativity of divalent metals
2. Solubility products of sulfides
3. Stability of chelates
4. Bioavailability

Despite the reported diversity in toxicity levels, it can be stated that the most toxic metals for both higher plants and certain microorganisms are Hg, Cu, Ni, Pb, Co, Cd, and possibly also Ag, Be, and Sn.

Although plants adapt rather readily to chemical stress, they also may be very sensitive to an excess of a particular trace element. Toxic concentrations of these elements in plant tissues are very difficult to establish. The values presented in Table 36 give very broad approximations of possibly harmful amounts of trace elements in plants. Visible symptoms of toxicity vary for each species and even for individual plants, but most common and nonspecific symptoms of phytotoxicity are chlorotic or brown points of leaves and leaf margins, and brown, stunted, coralloid roots (Table 40).

A common feature of plants is their ability to prolong survival under conditions of trace element excesses in their environments, mainly in soils. Lower plants especially, such as microorganisms, mosses, liverworts, and lichens, reveal an extremely high level of adaptation to toxic concentrations of certain trace elements. Zajic,[898] Weinberg,[856] and Iverson and Brinckman[344] presented comprehensive reviews on microorganisms involved in the cycling of trace metals and of their resistance to high metal concentrations. Tyler et al.[1171] reported that the depressions by 20 to 40% of most biological activities in forest litter/soil, compared to controls, were measured when concentrations of trace metals (Cu, Zn, and Pb) amounted to 2 to 10 times those of regional baseline samples. McGrath et al.[1089] found that the process of N-fixation is sensitive to increased concentrations of metals in soils. The inhibition by 50% of N-fixation by white clover rhizobium bacteria was at concentrations (ppm DW) of Zn 334, Cu 99, Ni 27, and Cd 10. These authors stated that mechanisms of the inhibition are still unknown and need to be investigated further, because the toxic effects can occur at metal concentrations close to or below the current guidelines for soil protection (see Chapters 2. IV. A and 4. IV. A).

Although the higher plants are believed to be less tolerant of increased concentrations of trace elements, they are also widely known to accumulate these elements and to survive on soils contaminated by large quantities of various trace elements. Antonovics et al.,[33] Peterson,[609] Bradshaw,[101] Woolhouse and Walker,[886] and Tyler et al.[1171] attempted to summarize and define what is implied by the term “tolerance” of plants. This term refers to both the population occurring in an area highly contaminated by trace elements, and to individual plants or species which are able to withstand greater levels of toxicity than are others. Extreme tolerance of many species of bryophytes, lichens, and fungi is widely reported.[1037,1171]

Table 40 General Effects of Trace Element Toxicity on Common Cultivars[66,241,381,395,531,731,916,1009]

Element	Symptoms	Sensitive Crop
Al	Overall stunting, dark green leaves, purpling of stems, death of leaf tips, and coralloid and damaged root system	Cereals
As	Red-brown necrotic spots on old leaves, yellowing or browning of roots, depressed tillering, wilting of new leaves	Legumes, onion, spinach, cucumbers, bromgrass, apricots, peaches
B	Margin or leaf tip chlorosis, browning of leaf points, decaying growing points, and wilting and dying-off of older leaves In severely affected pine trees, necrosis occurs on needles near the ends of shoots and in upper half on the tree	Cereals, potatoes, tomatoes, cucumbers, sunflowers, and mustard, apple, apricots, citrus, walnut
Be	Inhibition of seed germination and reduced growth, degradation of protein enzymes	—
Cd	Brown margin of leaves, chlorosis, reddish veins and petioles, curled leaves, and brown stunted roots Severe reduction in growth of roots, tops, and number of tillers (in rice). Reduced conductivity of stem, caused by deterioration of xylem tissues. Reduction of chlorophyll and carotenoids	Legumes (bean, soybean), spinach, radish, carrots, and oats
Co	Interveinal chlorosis in new leaves followed by induced Fe chlorosis and white leaf margins and tips, and damaged root tips	—
Cr	Chlorosis of new leaves, necrotic spots and purpling tissues, injured root growth	—
Cu	Dark green leaves followed by induced Fe chlorosis, thick, short, or barbed-wire roots, depressed tillering. Changes in lipid content and losses of polypeptides involved in photochemical activities	Cereals and legumes, spinach, citrus seedlings, and gladiolus
F	Margin and leaf tip necrosis, and chlorotic and red-brown points of leaves	Gladiolus, grapes, fruit trees, and pine trees
Fe	Dark green foliage, stunted growth of tops and roots, dark brown to purple leaves of some plants (e.g., “bronzing” disease of rice)	Rice and tobacco
Hg	Severe stunting of seedlings and roots, leaf chlorosis and browning of leaf points	Sugar beets, maize, and roses
Li	Chlorotic and necrotic spots on leaves and injured root growth	Citrus
Mn	Chlorosis and necrotic lesions on old leaves, blackish-brown or red necrotic spots, accumulation of MnO_2 particles in epidermal cells, drying tips of leaves, and stunted roots and plant growth	Cereals, legumes, potatoes, and cabbage
Mo	Yellowing or browning of leaves, depressed root growth, depressed tillering	Cereals
Ni	Interveinal chlorosis (caused by Fe-induced deficiency) in new leaves, gray-green leaves, and brown and stunted roots and plant growth	Cereals
Pb	Dark green leaves, wilting of older leaves, stunted foliage, and brown short roots	—
Rb	Dark green leaves, stunted foliage, and increasing amount of shoots	—
Se	Interveinal chlorosis or black spots at Se content at about 4 ppm, and complete bleaching or yellowing of younger leaves at higher Se content, pinkish spots on roots	—
Ti	Chlorosis and necrosis of leaves, stunted growth	Beans
Tl	Impairment of chlorophyll synthesis, mild chlorosis and slight cupping of leaves, reduced germination of seeds and growth of plants	Tobacco and cereals
Zn	Chlorotic and necrotic leaf tips, interveinal chlorosis in new leaves, retarded growth of entire plant, and injured roots resemble barbed wire	Cereals and spinach

The heavy-metal resistance in plants is of special concern. Practical problems and implications concerning metal-tolerant organisms can be related to:

1. Microbial origin of metal ore deposits
2. Metal cycling in the environment
3. Geobotanical prospecting (i.e., the use of tolerant and sensitive plants to locate natural deposits of metal ores)
4. Microbiological extraction of metals from low-grade ores
5. Establishment of vegetation on toxic waste materials
6. Microbiological treatment of waste waters
7. Development of resistance in microorganisms to metal-containing fungicides and other pesticides

The evolution of metal tolerance is believed to be quite rapid in both microorganisms and sometimes in vascular plants. As stated by Tyler et al.,[1171] tolerant genotypes of some vascular plants may develop within a few years. Developed metal-tolerance of plants may be both phenotypically and genotypically acquired. Evolutionary changes caused by heavy metals have now been recorded in a large number of species occurring on metalliferous soils that differ from populations of the same species growing on ordinary soils. Species of higher plants that show a tolerance to trace elements belong most commonly to the following families: Caryophyllaceae, Cruciferae, Cyperaceae, Gramineae, Leguminosae, and Chenopodiaceae.

The ranges of highest concentrations of trace elements found in various plant species are presented in Table 41. Various fungi are also well-known to be able to accumulate a high proportion of easily soluble and/or easily volatile elements such as Hg, Se, Cd, Cu, and Zn. The "upper critical level" of an element is the lowest tissue concentration at which it has toxic effects. Macnicol and Beckett[1081] made an extensive survey of literature to establish critical levels of 30 elements, most predominant of which are Al, As, Cd, Cu, Li, Mn, Ni, Se, and Zn. The values established by these authors for the "upper critical levels" are quite similar to those presented by others as "excessive or toxic levels" (Tables 36 and 42). They stated that the critical levels for a given element are

Table 41 The Greatest Accumulation of Some Metals (% AW) Reported in Various Plant Species[609,613]

Element	Plant
	>10%
Ni	*Alyssum bertolonii*
Zn	*Thlaspi calaminare, caerulescens*
	1 to 3%
Cr	*Pimelea suteri and Leptospermum scoparium*
Co	*Crotalaria cobaltica*
Ni	*Alyssum bertolonii*
Se	*Astragalus recemosus*
Sr	*Arabis stricta*
U	*Uncinia leptostachya* and *Coprosma arborea*
Zn	*Viola calaminaria*
Mn	*Macadamia neurophylla*
	0.1 to 1%
Cu	*Becium homblei, Aeollantus biformifolius*
Hg	*Betula papyrifera*
W	*Pinus sibiricus*
Zn	*Equisetum arvense*
Ni	*Berkheya coddi*
Pb	*Minuratia verna*

Table 42 Critical Concentrations of Trace Metals in Plant Tissues (ppm DW)

	Levels of Growth Depression	
Metal	**In Sensitive Plant Species[1052]**	**For 10% Yield Loss[1081]**
As	—	1–20
Cd	5–10	10–20
Co	10–20	20–40
Cr	1–2	1–10
Cu	15–20	10–30
Hg	0.5–1	1–8
Ni	20–30	10–30
Zn	150–200	100–500

variable, which reflects both effects of interactions with other elements and developing plant resistance to a high tissue concentration of certain elements.

Microorganisms can very rapidly develop mechanisms of tolerance to excesses of trace metals. Fungi are usually more resistant than bacteria. Lichens reveal a relatively high tolerance to trace metals due to the presence of fungi hyphae. Fungi of mycorrhiza developed at the surface of roots of several plant species, mainly trees, can protect against the transfer of trace metals into root cells from surroundings. These phenomena are observed especially on trees in soil polluted with Cu and Zn.[1525] Turnau et al.[1524] observed in vacuoles of fungi hyphae (*Paxillus involutus*) granules with high contents of P, S, Ca, Al, and Cd. The Cd accumulation inside fungal vacuoles suggests the possibility of immobilization of metals by the symbiotic fungus. Hamon et al.[1321a] reported that Cd and Zn in the rhizosphere of radish existed mainly as immobile forms complexed by organic compounds, while dissolved Cd and Zn were largely uncomplexed in unplanted soil. The authors concluded that plants mainly absorb the free metal ions from soil solution.

Toxicity of trace metals to microorganisms and microbial processes in agricultural soils has been extensively reviewed by Giller et al.[1310] The authors emphasized that the results of numerous laboratory ecotoxicological studies are the most difficult to meaningfully extrapolate to evaluate data on toxic effects that are likely to occur in the field.

A concept of "metal (Mn) equivalent" (related to "Zn equivalent" of trace metals to crop plants) in the toxicity to soil respiration has been proposed by Saviozzi et al.[1480] and is calculated as the sum of the amounts of the available metals, and related to the least toxic one: $\text{Mn equivalent} = \text{Mn} + 1.95\ \text{Pb} + 2.1\ \text{Ni} + 2.5\ \text{Zn} + 6.7\ \text{Cd} + 6.7\ \text{Cu}$. Pb, Ni, and Zn show about twice and Cd and Cu about seven times the toxic effects of Mn.

Mechanisms of trace element resistance in plants have been the subject of several detailed studies which indicated that both highly specific and multiple metal tolerance may appear, as reported by Antonovics et al.,[33] Bradshaw,[101] Simon,[728] Foy et al.,[241] and Cox and Hutchinson.[149] These authors summed up possible mechanisms involved in metal tolerance. They distinguished external factors, such as low solubility and mobility of cations surrounding plant roots, as well as effects of metal ion antagonisms. The real tolerance, however, is related to internal factors. This is not a mechanism of tolerance in a simple sense, but consists of several metabolic processes:

1. Selective uptake of ions
2. Decreased permeability of cell walls or other differences in the structure and function of membranes
3. Immobilization of ions in various organs (synthesis of immobilizing compounds including the formation of minerals, and/or fixation by charged ligands)
4. Alteration in metabolic patterns—increased enzyme system that is inhibited, or increased antagonistic metabolite, or reduced metabolic pathway by passing an inhibited site, or decreased requirement for products of inhibited synthesis
5. Adaptation to toxic metal replacement of a physiological metal in an enzyme

6. Release of ions from plants by leaching from foliage, guttation, leaf shedding, and excretion from roots
7. Release of volatile organic metal compounds (e.g., Hg, Pb, and Sn), mainly as methylated metals that are very toxic species to most organisms
8. Excretion from leaf tips in the form of salts

In addition to the mechanisms mentioned, phenomena of avoidance of polluted substrates are reported. This is observed especially in the behavior of microorganisms which are capable of developing on better (less contaminated) substrate. Some vascular plants, while growing on topsoil heavily contaminated by trace metals, can develop roots in deeper soil layers.[1171]

Selective uptake of ions is related to a capability of plants for active selective sorption and discrimination of available ions or compounds in the soil. Selective transport of ions to the tops taken up by roots is also observed. This selectivity depends most probably on immobilization mechanisms. Mechanisms of selective sorption and transport of trace ions can be broken down under a critical concentration of trace elements, and a passive flux of ions within a plant organism takes place (see Chapter 5, Sections II and III).

Decreased permeability of cell walls is closely associated with the immobilization. These mechanisms of the plant tolerance to metals (Al, Cd, Zn, Mn, and Pb) are broadly reviewed by Foy[998] and Tyler et al.[1171] Binding to the cell wall, including wall impermeability, is observed in various vascular plants, bryophytes, lichens, fungi, and microoganisms. Several compounds such as thioneins, sulfides, pectic substances, and organic acids (e.g., uronic, mannuronic, norstictic—in lichens only) are involved in metal complexation. Thus, synthesis of immobilizing compounds and/or fixation by charged ligands are responsible for the removal of trace ions from plant metabolism by deposition (storage) in fixed and insoluble forms in various organs and organelles. Roots are the most common storage for the excess of trace metals, but also foliage and seeds are known to accumulate deposited forms of different trace elements. Tyler et al.[1171] stated that the immobilization in cell wall ligands is most probably a key reaction in reducing the toxicity of trace metal excess.

Kvalevsky[1373,1374] reported that in old above-earth parts of plants, several mineral forms of trace elements can be distinguished; for example, FeAsS (arsenopyrite), ZnS (sphalerite), PbS (galena), MoS (molybdenite), HgS (cinnabar), and also some other trace element minerals, in the forms of carbonates, sulfates, fluorides, wolframites, etc. Other metals like Ag and Au were observed in the form of thin microscopic flakes ($< 2\ \mu$m). He reported that more than 30 elements can be temporarily excluded from physiological processes and stored in plant tissues as inactive minerals.

The biochemical resistance is not yet well-recognized, and may involve several other mechanisms of the immobilization. The alteration in metabolic patterns is related to an increase of the enzyme system that is inhibited, or to an increase of antagonistic metabolite. This also may be associated with a decreased requirement for products of the inhibited syntheses or with a reduction of metabolic pathways by passing an inhibited site of a part of the metabolic system. The nature of an adaptation to toxic metal contents induced into an enzymatic system needs a better understanding. The great flexibility of enzymatic systems and variability among plant species are involved in the evolution of metal tolerance of which several points are still not clear. Considerable evidence was given by Antonovics et al.[33] and Cox and Hutchinson[149] that tolerant plants may also be stimulated in their growth by higher amounts of metal, which reveals a physiological need for an excess of a particular metal by a single plant genotype or species.

Tyler et al.[1171] discussed whether plants that become tolerant to trace metals may be equally productive as nontolerant species or populations. These authors concluded that: “The action of the tolerance mechanism often imparts a demand for metabolic energy, which would make tolerant forms less competitive under normal conditions than their nontolerant counterparts.” Therefore, the growth of plants under the stress of elevated concentrations of trace metals is highly limited. In most cases, the morphology of those plants is altered, and salt excretion can be observed at the tips of leaves in some plants. In oats grown in soil heavily polluted with trace metals (mainly Zn and Pb), the composition of salt extraction was (in % of oven-dried weight): Zn 0.76, Pb 0.15, Mn 0.068, and Cu 0.039.[1399]

The tolerance of some plants to elevated concentrations of trace metals in growth media and in tissues creates a health risk to humans and animals. This is a special problem in cases of trace elements that are easily tolerated by plants and highly toxic to humans, as for example Cd. Therefore, health-related limits for certain elements in food plants are carefully controlled by national and international legislation. The contemporary limits can be changed in the future as more pertinent data are collected. Tolerant plants, due to their ability to grow in contaminated substrates, and due to the accumulation of extremely high amounts of trace metals, may create a great health risk by forming a polluted link in the food chain.

The excess of trace elements—both essential and nonessential to plants —has a deteriorating impact on metabolic processes. In some cases, toxic effects are specific for a given element (see Table 40). Plants develop different mechanisms to protect against their excess. These mechanisms are, in general, related to the root (mainly root tips, meristems) exudates containing polygalacturonic acid that fix metals outside or within root cells, and to the production of phytochelates (various derivatives of glutathione) fixing metals and displacing either in vacuoles or on cell membranes.[1459,1542,1551]

VII. INTERACTION

A chemical balance in living organisms is a basic condition for their proper growth and development. Interactions of chemical elements are also of similar importance to deficiency and toxicity in the physiology of plants. Interactions between chemical elements may be both antagonistic and synergistic, and their imbalanced reactions may cause a real chemical stress in plants.

Antagonism occurs when the combined physiological effect of two or more elements is less than the sum of their independent effects, and synergism occurs when the combined effects of these elements is greater. These interactions may also refer to the ability of one element to inhibit or stimulate the absorption of other elements in plants (Figure 30). All these reactions are quite variable and may occur inside the cells, within the membrane surfaces, and also surrounding plant roots. Interaction processes are controlled by several factors and these mechanisms are still poorly understood, although some data are available.[241,581,840]

Interactions between major and trace elements summarized in Table 43 clearly show that Ca, P, and Mg are the main antagonistic elements against the absorption and metabolism of several trace elements. Some synergistic effects, however, have also been observed for antagonistic pairs of elements, depending on the specific reaction of the plant genotype or species.

Antagonistic effects occur most often in two ways—the macronutrient may inhibit trace element absorption and, in turn, the trace element may inhibit absorption of a macronutrient. These reactions have been observed especially for phosphate, but have also been reported for other macronutrients whose uptake and metabolic activity may be inhibited by several trace elements.[395,463]

Most important for practical application are the antagonistic effects of Ca and P on heavy metals such as Be, Cd, Pb, and Ni that often constitute a health hazard. Both major nutrients, Ca and P, are known to play a significant role in the integrity of cell membranes, and therefore any imbalance in these nutrients may affect interaction processes either in the nutrient solution external to or at the surface of root-cell membranes. It is noteworthy that although the antagonistic effects of P and Ca on many trace cations and anions are frequently reviewed in the literature, the antagonistic impact of Mg on trace metals is only occasionally reported. The addition of Mn and Mg to a solution reduced the mortality of nematodes (*Steinernema carpocapsae*) due to the excess of several trace metals.[1346]

Deficiency of one trace element essential to a plant can facilitate uptake of other micronutrients. The compensatory absorption of micronutrients under deficiency stress is reported for several

Table 43 Interactions Between Major Elements and Trace Elements in Plants[251,381,531,554,663]

Major Element	Antagonistic Elements	Synergistic Elements
Ca	Al, B, Ba, Be, Cd, Co, Cr, Cs, Cu, F, Fe, Li, Mn, Ni, Pb, Sr, and Zn	Cu, Mn, and Zn
Mg	Al, Be, Ba, Cr, Mn, F. Zn, Ni[a], Co[a], Cu[a] and Fe[a]	Al and Zn
P	Al, As, B, Be, Cd, Cr, Cu, F, Fe, Hg, Mo, Mn, Ni, Pb, Rb, Se, Si, Sr, and Zn	Al, B, Cu, F, Fe, Mo, Mn, and Zn
K	Al, B, Hg, Cd, Cr, F, Mo, Mn, and Rb	—
S	As, Ba, Fe, Mo, Pb, Se, and Zn	F[b] and Fe
N	B, F, Cu, and Mn	B, Cu, Fe, and Mo
Cl	Br and I	—
Na	Mn	—
Si	B, Mn	—

[a] Reported for microorganisms.

[b] Mutual pollution causes significant injury.

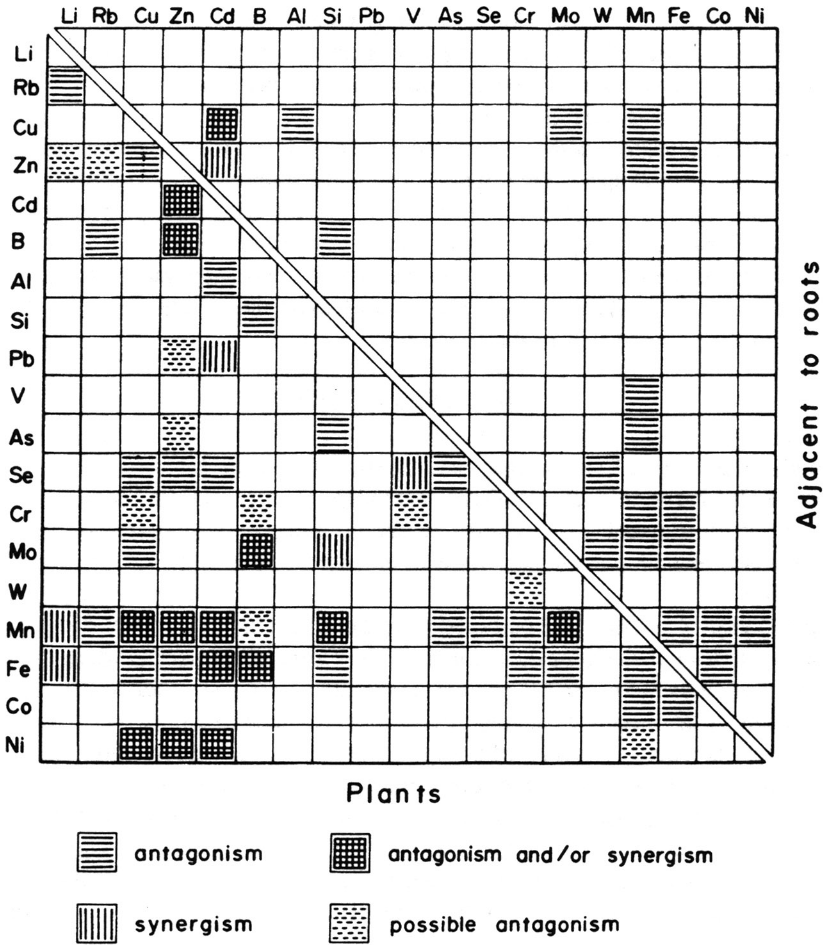

Figure 30 Interactions of trace elements within plant organisms and adjacent to plant roots.

elements and most commonly for Mn and Cu, and for Fe and Cu, Zn, and Mn.[1466] Antagonistic, additive or synergistic, reponse of plants to binary mixtures of elements seems to be a concentration-dependent effect. It initially was observed by Chaney and Hornick[129] for the Cd/Zn relationship. Recent findings presented by Sharma et al.[1485] indicated that when only one element of binary mixtures, Cu/Cd, Cu/Zn, and Cd/Zn, exceeds some critical level of toxicity, the joint effect is either synergistic or additive. This clearly indicates that interaction effects are less metal-specific at high concentrations than at low metal concentrations.

Interrelationships between elements in different soils and/or plant species are of multivariant character. These relationships may be attributed to the ionic competition at the sorption sites of soil particles and also to properties of the surface root cell membrane and root exudates.

Interactions observed within plants between trace elements have also indicated that these processes are quite complex, being at times both antagonistic and synergistic in nature, and occasionally are involved in the metabolism of more than two elements (Figure 30). The greatest number of antagonistic reactions have been observed for Fe, Mn, Cu, and Zn, which are, obviously, the key elements in plant physiology (Table 37). These trace metals are linked to processes of absorption by plants and to the enzymatic pathway. The other trace elements often involved in antagonistic processes with these four trace metals are Cr, Mo, and Se.

Synergistic interactions between trace elements are not commonly observed. Those reported for Cd and other trace metals such as Pb, Fe, and Ni may be artifacts resulting from the destruction of physiological barriers under the stress of excessive concentrations of heavy metals. Moreover, several reactions that occur in the external root media and affect root uptake should not be directly related to metabolic interactions, but the two reactions are not easily separated. Some interactions among trace metals are still unpredictable; for example, the addition of Ni- (organic and inorganic forms) enhanced Cu, Zn, and Mn uptake by winter wheat, whereas the addition of Pb increased Cu and Zn.[1442]

VIII. BIOINDICATION

Indicator species are organisms that serve as a measure of the environmental conditions that exist in a given locale. These may be various animal and plant organisms and their organs, or products (e.g., honey). Earlier indicator plants were used in geobotanical prospecting to give evidence of a certain geological phenomenon. At present, it is a widely used method in environmental studies. Also monitoring, which is systematically checking the chemical state of the environment, is often based on bioindicators/biomonitors. Several characteristics of an ideal bioindicator have been given by various authors, and are summarized by Wittig.[1546] In general, an acceptable bioindicator requires the following:

- Suitable accumulation rate of some or selected elements
- Toxitolerance with no sensitivity to the accumulating element/substance
- Present in large amounts in the ecosystem under investigation
- Wide distribution in various environments, and wide geographic range
- Easy to identify and sample
- No seasonal differences in availability and applicability
- Existence of a correlation between accumulation and input to the ecosystem

There are several other conditions required for suitable bioindication and biomonitoring methods. These conditions are related to standardized sampling and analytical methods, comparing results with other monitoring methods, and possible retrospective and long-time investigation periods.

Dandelion is reported quite frequently as a useful environmental indicator, since this plant fulfills most of the listed demands.[1278,1356,1470] An evaluation of metal pollution in selected regions of Poland using dandelion is presented on Table 44. Dandelion leaves grown in the industrial region (Region SW) contain more trace metals than dandelion leaves from other areas. The RDB value identifies a very high relative deviation from background values of all metals in plants from industrial regions. The order of relative accumulation of metals is the following: Ni > Cd > Zn >

Table 44 Metals in Leaves of Dandelion Grown in Different Regions of Poland (ppm)[1356]

Metal[a]	Country N = 780	All Regions N = 240	Region SW N = 60	Region SE N = 70	Region NW N = 60	Region NE N = 50
Cd	0.5	0.6	1.2	0.6	0.5	0.4
	−20	0	50	0	−20	−50
Cu	9.4	9.4	13.4	10	8.4	7
	−27	−22	10	−20	−42	−71
Cr	0.8	0.8	0.7	1.3	0.7	0.4
	25	25	14	53	14	−50
Mn	60	65	74	103	69	42
	0	7	18	41	13	−18
Ni	3.4	1.3	4.2	6.4	3.1	1.9
	50	−53	52	68	35	−5
Pb	1.1	1.2	3	1	1	0.5
	−81	−66	33	−100	−100	−300
Zn	45	50	72	67	35	40
	0	10	37	32	−28	−12

[a] Upper values for metals are Geometric Means, and lover are RDB (Relative Deviation to Background) values, RDB = {[AM − RF]/RF} × 100. RF, reference contents (mg/kg DW): Cd 0.6, Cr 0.6, Cu 12, Mn 60, Ni 2, Pb 2, Zn 45.

Pb > Mn > Cu. Also, needles of pine trees (e.g., Scots pine, Norway spruce) and chicory roots and shoots are often used for evaluation and monitoring of environmental pollution.[1279,1496,1553] A good indication of industrial pollution in eastern Siberia was obtained by Arzhanova and Elpatevsky[1206] analyzing oak leaves which contain, after washing, up to (in ppm): Cd 7.7; Cu 21; Zn 264; and Pb 1155. Roots of aquatic plants as well as aquatic mosses have also been reported as very suitable phytoindicators in certain environments.[1483]

Soil microorganisms are considered good candidates for bioindication of soil metal pollution. However, Parkhurst et al.[1449] have described some problems with using a population of microorganisms or microbial activity as soil biomonitors. These include a large natural variation in population size and activity of microorganisms in soils and their sensitivity to all chemical and physical soil factors. Some examples, however, indicate that the bioassay could be used to assess the bioavailability and harmful effects of trace metals.[1462] Screening for the presence of rhizobia can be a rapid method for the soil bioassay of toxic effects of trace metals and allows the determination of the bioavailable pool of metals in soils.[1310,1332]

The inhibitory effect on soil bioactivity expressed as the soil respiration, differs considerably among trace metals but is always a function of metal contents. The general order of toxicity of metals to microorganisms can be presented as follows: Ag > Cu > Cd > Ni > Zn > Pb > Mn. Two metals, Cu and Zn applied as sulfates to the soil at concentrations up to 2000 ppm, affect soil microbiota: fungi (glucophilic, cellulose-decomposing, thermophilic, and thermotolerant), bacteria, and actinomycetes, generally causing reduction in their populations and soil enzyme activities.[1325] Soil enzyme activity has been used as a sensitive indicator of the effect of trace metals on soils. Most soil enzymes are inhibited by metals (e.g., the contents of Cu, Cr, and As at the levels (in ppm) 900, 842, and 1124, respectively) decrease enzyme activity in the following order (relative to control values): phosphatase 70%; urease, 31%; sulphatase, 25%; and dehydrogenase, 17%.[1461] Alkaline and acid phosphatase is reported to be most sensitive to an excess of Cd, Co, and Zn.[1443]