TOXICITY OF HEAVY METALS (Zn, Cu, Cd, Pb) TO VASCULAR PLANTS

A Literature Review

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Abstract. The literature on heavy metal toxicity to vascular plants is reviewed. Special attention is given to forest plant species, especially trees, and effects at low metal concentrations, including growth, physiological, biochemical and cytological responses. Interactions between the metals in toxicity are considered and the role of mycorrhizal infection as well. Of the metals reviewed, Zn is the least toxic. Generally plant growth is affected at 1000 μg Zn L^{-1} or more in a nutrient solution, though 100 to 200 μg L^{-1} may give cytological disorders. At concentrations of 100 to 200 μg L^{-1}, Cu and Cd disturb metabolic processes and growth, whereas the phytotoxicity of Pb generally is lower. Although a great variation between plant species, critical leaf tissue concentrations affecting growth in most species being 200 to 300 μg Zn g^{-1} dry weight, 15 to 20 μg Cu g^{-1} and 8–12 μg Cd g^{-1}. With our present knowledge it is difficult to propose a limit for toxic concentrations of Zn, Cu, Cd and Pb in soils. Besides time of exposure, the degree of toxicity is influenced by biological availability of the metals and interactions with other metals in the soil, nutritional status, age and mycorrhizal infection of the plant.

1. Introduction

Zinc and Cu are essential elements to higher plants and are involved in several metabolic processes, whereas Pb and Cd are not known to have any function in plants.

A deficiency of Zn and Cu causes changes in fundamental processes of plant metabolism, leading to growth retardations. These are well known from agricultural plant species. Less is known about Cu and Zn deficiencies in wild, naturally growing plant species. As a consequence of air pollution and the increasing acidity of, e.g., forest soils in northern Europe, leaching of minerals like Zn is increased. In the future a deficit of Zn in certain soils cannot be ruled out, leading to a lack of this metal in forest vegetation.

Initially, the increasing acidity of soils has proved to liberate the bound pool of metals, which may lead to increased availability and uptake of metal ions in plants. Related to forest die-back, trees grown in acid soil or showing symptoms of damages contain increased levels of heavy metals in both roots and aerial parts (Mayer and Heinrichs, 1981; Von Bredow *et al.*, 1986). Although several plant species, including grasses, herbs and trees, are able to evolve tolerance, sensitive species or genotypes, however, are supposed to be affected by heavy metals at relatively low concentrations in the soil solution.

The aim of this work is to compile and evaluate minimal toxicity of Zn, Cu,

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Cd, and Pb to vascular plants from literature studies. Special attention is given to forest species and especially trees.

2. Zinc

Generally, Zn is supposed to be the least toxic of the heavy metals. Like Cu, Zn is an essential metal to higher plants and is involved in several important metabolic processes. It activates enzymes or is incorporated into metalloenzymes in the electron transport system. Further, Zn plays an important role in the protein synthesis, as in the carbohydrate, nucleic acid and lipid metabolism. As Zn forms stable complexes with DNA and RNA it might also affect DNA and RNA stability (Collins, 1981).

A deficiency of Zn in plants may cause significant changes in the plant metabolism, resulting in growth retardation, stunted growth and chlorosis. Zinc deficiency among crop plants is very common and widespread all over the world. Less is known about wild-growing plant species although it might be a future problem in some forests with increasing acidity of the soils. In south Swedish soils the exchangeable pool of Zn ions has decreased significantly during the period 1949–1985 (Falkengren-Grerup *et al.*, 1987).

On the contrary, soils may locally contain large amounts of Zn, either naturally, overlaying Zn-rich minerals, or made by man. The increasing acidity of soils may also liberate the bound pool of Zn, initially leading to increased concentrations of free Zn ions in the soil solution. Several plant species, both grasses and herbs, and also tree species as Betula pubescens and B. pendula are capable of evolving tolerance to Zn, but not necessarily to other heavy metals. Sensitive species or genotypes may, however, show toxicity symptoms already at relatively low Zn levels.

2.1. GROWTH RESPONSES AND SEED GERMINATION

Generally, growth symptoms of Zn toxicity in plants are similar to those of Zn deficiency. An excess supply of Zn affects both shoot and root growth and the shoots become stunted and chlorotic. Further, the epidermis of roots and the cells in the epidermis may become lignified (Päivöke, 1983a).

Under laboratory conditions with nutrient solutions usually at least 1000 μg Zn L^{-1} is needed to affect growth. This is several times the concentrations generally found in soil waters from lysimeters under tree stands in south Sweden (Bergkvist, 1987). In a study with Picea abies seedlings grown for 5 weeks, shoot and root dry weights decreased at c. 1100 μg Zn L^{-1} (Godbold *et al.*, 1985) as did root elongation of Deschampsia caespitosa (Godbold *et al.*, 1983). Root elongation of Picea abies seemed to be less sensitive at this Zn concentration but was greatly retarded at 2000 μg L^{-1}. This concentration decreases shoot and root growth of rye grass (Lolium perenne). When adding competing Ca ions the toxicity of Zn was reduced, an effect observed in other studies too (Wainwright and Woolhouse, 1977; Wong and Bradshaw, 1982; Brown and Wilkins, 1985). Similarly, additions of complexing agents as humic acid and EDTA may reduce toxicity compared to if only ionic Zn is present in the growth medium (Marquenie-Van der Werf and Out, 1981).

As soil conditions and other environmental factors play a decisive role in metal uptake and toxicity, many scientists prefer tissue concentration as a more valid measurement tool, when predicting toxicity in plants than soil or solution concentrations. In a comprehensive literature survey, MacNicol and Beckett (1985) have extracted critical Zn concentrations in tissues of a great number of agricultural crops. These cricital values are defined as the lowest tissue concentration, at which a metal has toxic effects. In tops and leaves of different species, critical Zn concentrations varied from 60 to 900 $\mu g\ g^{-1}$ dry weight but most species are sensitive in the range 200 to 300 $\mu g\ g^{-1}$. Documented critical Zn tissue concentrations for tree species are few but will probably be in the same range. Thus 226 μg Zn g^{-1} dry weight was found to be critical in the needles of sitka-spruce (Picea sitchensis), whereas 250 $\mu g\ g^{-1}$ was needed for minimum toxicity in Quercus rubra (Jordan, 1975; Burton *et al.*, 1983). These levels are several times found in leaves of naturally growing trees in unpolluted areas.

Compared to data concerning effects of heavy metals on seedling growth, very few studies have been devoted to reproduction and seed germination. Usually very high Zn concentrations have been used in the growth medium and the results are somewhat conflicting. The seed germination itself seems to be rather insensitive to Zn also at different pH. At Zn levels lower than $10^4\ \mu g\ L^{-1}$ in the germination medium, seed germination from both tree and grass species has proved to be unaffected (Jordan, 1975; Scherbatskoy *et al.*, 1987). An objection to these studies is, however, that further development and survival of the seedlings were not followed up. To Zn non-tolerant seeds of Betula pendula and B. verrucosa, $10^4\ \mu g$ Zn L^{-1} was enough to significantly reduce, not only germination, but also the number of survivors and the elongation of hypocotyls and radicles (Brown and Wilkins, 1986). As lower Zn concentrations were not used in the study, the lowest limit to interfere with seed germination and survival of the seedlings could not be stated.

2.2. PHYSIOLOGICAL RESPONSES

Like other heavy metals, an excess of Zn may, in different ways, affect physiological processes. Whether the effect is a direct or an indirect one is often unclear and more work is needed in this important field.

Usually the water relations in the plant are altered by heavy metals, leading to water stress and wilting. The influence of Zn on the water content in plants is obscure, however. After 5 weeks exposure to c. 2000 μg Zn L^{-1} or more, the water content in the needles of Picea abies seedlings was only slightly, not significantly reduced (Schlegel *et al.*, 1987). On the contrary, both water potential and water content in bean (Phaseolus vulgaris) was increased when treated with Zn (Rauser and Dumbroff, 1981). Similar results were obtained in experiments with pea (Pisum sativum) seedlings, in which water uptake increased, when exposed up to 6500 μg Zn L^{-1}. At higher Zn concentrations, however, uptake was reduced resulting in a water deficit (Pävöke, 1983b).

Other well documented symptoms of Zn toxicity are decreased leaf chlorophyll content and rate of photosynthesis (Van Assche *et al.*, 1979; Porter and Sheridan, 1981). Usually very high Zn levels are used in the growth medium which are far from natural background levels.

At some stages in the biosynthesis of chlorophyll, Zn is supposed to compete with Fe, leading to chlorosis. In ten-weeks-old seedlings of Picea abies, the chlorophyll decrease in primary needles and hypocotyls was dependent on Zn levels in the range 2000 to 6500 μg L^{-1} and the decrease was significant in the hypocotyls at 3900 μg Zn L^{-1} in the growth medium. The chlorophyll loss was accompanied by a reduced net photosynthetic rate, measured as CO_2-uptake. In the spruce seedlings, uptake of CO_2 was significantly affected at c. 2000 μg Zn L^{-1}. Neither transpiration nor dark respiration was influenced by Zn (Schlegel *et al.*, 1987). When compared to maize seedlings, the chlorophyll synthesis was stimulated at these Zn levels, indicating that the chlorophyll content in leaves would not be the site for inhibition of photosynthesis by Zn in C_4-plants (Stiborova *et al.*, 1986c).

Among other processes of importance in plant metabolism is a functioning transport system of the photosynthetic assimilates to sinks in the plant and uptake of nutrients. Zinc has been reported to cause callose deposits on sieve tube plates in the phloem (Peterson and Rauser, 1979). Although not supposed to be responsible, translocation of carbohydrates will be markedly restricted by higher Zn levels, leading to accumulation of sugars and starch in the leaves and a reduced transport to growing parts (Rauser and Samarakoon, 1980).

More primary mechanisms of Zn toxicity may be an increased permeability of root membranes, which will cause nutrients to leak out from the roots. Thus 650 μg Zn L^{-1} proved to be enough to enhance leachage of K from maize roots (Collins, 1981). As only a low deficit of K may influence sugar transport and accumulation of carbohydrates (Bergmann, 1983; Huber, 1984) this might be an indirect effect of Zn in the carbohydrate transport. Potassium release from the root tips of Agrostis capillaris, however, was not affected at Zn concentrations in the range 65 to 6500 μg Zn L^{-1} nor was K-uptake and translocation in barley (Hordeum vulgare) at $\leq$ 6500 μg L^{-1} (Wainwright and Woolhouse, 1977; Oberländer and Roth, 1978).

The chlorosis in Zn-poisoned plants is often attributed to Fe-deficiency. Interferences with Fe-metabolism, where Zn is supposed to inhibit the reducing capacity of the roots, are shown to arise early at Zn treatments. Thus translocation in decapitated soy bean (Glycine max) shoots was decreased at 65 μg Zn L^{-1} in the growth medium (Ambler *et al.*, 1970). However, as roots will be an effective barrier to further Zn transport up to the shoots, this low Zn level may probably not influence Fe uptake and translocation in whole plants.

2.3. BIOCHEMICAL RESPONSES

At toxic concentrations of Zn in the plant tissues, the metal has proved, from a number of studies, to seriously affect activity of several enzymes and other fundamental metabolic processes.

Established reduced photosynthetic rate by an excess of Zn has brought about more detailed studies of the enzymes involved in the photosynthesis. In a comparative study it was found that phosphoenolpyrovatecarboxylate (PEPC), a key enzyme of photosynthesis in C_4-plants, was more sensitive to Zn than the corresponding enzyme, ribulose-1,5-bisphosphate carboxylase (RuBPC) in C_3-plants (Stiborova *et al.*, 1986a). At 650 μg L^{-1}, the activity of PEPC was significantly decreased, whereas no obvious effect on biomass and root/shoot ratio was observed up to 65 000 μg Zn L^{-1} (Stiborova *et al.*, 1986c).

Van Assche and Clijsters (1986) observed that toxic concentrations of Zn also negatively affect photosynthetic electron transport and photophosphorylation in bean (Phaseolus vulgaris) seedlings. Essential to the electron transport are enzymes of the Krebs cycle. The activity of some of the enzymes involved, as malatedehydrogenase (MDH) and isocitratedehydrogenase (ICDH) change by an excess supply of Zn. Dependent on Zn level and plant part, both stimulated and inhibited enzyme activity was obtained in Phaseolus vulgaris, when treated with 160 and 1600 μg Zn L^{-1} (Weigel and Jäger, 1980). Similar results though at higher Zn levels were noted by Mathys (1975), when studying non-Zn resistant populations of Silene cucubalus.

Other enzymes, which may be affected by heavy metals are hydrolytic enzymes like phosphatase and ribonuclease (Agarwala *et al.*, 1977; Malhotra and Khan, 1980). In cotyledons of pea (Pisum sativum) acid phosphatase activity was significantly reduced at 650 μg Zn L^{-1} and the effect was markedly more pronounced in nutrient deficient medium (Päivöke, 1983a). Although not convincingly shown at lower Zn levels, at least 10 times more Zn in the growth medium seems to be needed, to interfere in carbohydrate metabolism. As shown by Samarakoon and Rauser (1979), translocation of photoassimilates from the leaves to sinks in the plants, as roots and expanding buds and leaves, may be seriously restricted. The reduced transport usually leads to accumulation of sugars and starch in the leaves (Agarwala *et al.*, 1977; Rauser 1978). The same Zn concentration also caused an increase of soluble phenolic compounds in the plant tissues and a shift from polar to more non-polar components (Rauser, 1978).

Nitrogen metabolism is disturbed in various ways by an excess of Zn. The protein content in bean (Phaseolus vulgaris) roots significantly decreased at 1600 μg Zn L^{-1} and at higher additions N-fixation in Medicago sativa was reduced (Porter and Sheridan, 1981). Further, nitrate reductase activity is suppressed. From studies with Picea rubens, it was concluded, however, that the roots have the ability to adjust to toxic metal levels in the root environment. Therefore, Zn toxicity was not supposed to be an important factor in nitrogen transformations in red spruce (Yandow and Klein, 1986).

2.4. CYTOLOGICAL RESPONSES

Root growth and elongation are shown to be affected by Zn at relatively low levels (1000 μg L^{-1}) in the growth medium. In root segments of non-Zn-tolerant Agrostis capillaris, also the cell elongation was reduced at 6500 μg L^{-1} but the membrane permeability was unaffected (Wainwright and Woolhouse, 1977). However, although fundamental to root growth and probably a primary site for Zn toxicity, more detailed studies at root cellular level are very few, especially at low Zn concentration.

It is well known from Zn treatment studies, that abbreviations and abnormalities increase at root tip mitosis in, e.g. Allium cepa. Cell extension is also affected, whereas the number of cells undergoing mitosis are relatively less influenced (Nag *et al.*, 1980; Singh and Sharma, 1980).

Somewhat contrary to these results are two recently published works, where cell function has been studied in Zn-tolerant and non-tolerant Festuca rubra. The results clearly show disturbances from Zn at significantly lower concentrations than earlier reported. One explanation for these results may be that the growth solutions were renewed more often than in other toxicity studies. At 100 μg Zn L^{-1}, the mean area of the mitotic cells was reduced as was the nuclear and nucleolar volume. Cellular RNA and the protein content was also lowered (Powell *et al.*, 1986b). Further root extension and the number of cells undergoing mitosis were seriously affected (Powell *et al.*, 1986a). Compared to control, the mitotic index was halved at 200 μg Zn L^{-1}. At 500 μg L^{-1} less than 10% of the cells were undergoing mitosis and at 1000 μg L^{-1} no cells in visible stages of mitosis could be observed. The cell doubling time and cell cycle were also markedly increased. Although more studies are needed with other species too, these results from Festuca indicate that low Zn concentrations may induce changes in the root cell function, leading to growth retardation.

3. Copper

Like Zn, Cu is an essential micronutrient for normal plant metabolism. Copper acts as a component of several enzymes, involved in carbohydrate, N and cell wall metabolism. Further, Cu is important to seed production, disease resistance and the water relations in the plant (Bussler, 1981).

Although Cu deficiency may not be unusual in for example agricultural plants and fruit trees, the amounts necessary for normal growth and development are generally supplied by available amounts in the soil.

At higher soil levels, Cu could be extremely toxic, although several plant species are able to evolve tolerant genotypes. However, like other heavy metals, the degree of toxicity is dependent on, e.g., the nutritional status of the plant. Phosphorus deficiency in the plant may enhance the toxicity of Cu (Wallace, 1984).

3.1. GROWTH RESPONSES AND SEED GERMINATION

Generally visible symptoms of heavy metal toxicity, like that of Cu, are small chlorotic leaves and early leaf fall. Further, the growth is stunted and initiation of roots and development of root laterals are poor. The reducted root development may result in a lowered water and nutrient uptake, leading to disturbances in the metabolism and growth retardations.

However, despite the well known toxicity of Cu to plants very few studies concern tree species. In a study by Heale and Ormrod (1982) four woody plant species were grown in culture solutions supplied with two concentrations of Cu (4000, 20 000 μg L^{-1}). Visible symptoms of toxicity in leaves and roots were similar to those mentioned above. At 4000 μg Cu L^{-1}, 2-yr-old seedlings of Pinus resinosa were wiltering. The Cu concentration in the needles was 16 μg g^{-1} dry weight and in the roots more than 3000 μg g^{-1} dw. At these tissue levels, the roots had a red-brown color and the lateral roots were strongly dwarfed. Compared to control, the weight of Cu treated seedlings was reduced by 30%. The dry weights of other species studied were markedly affected too, but the uptake of Cu differed significantly between species. At 4000 μg Cu L^{-1}, roots of Lonicera tatarica contained only 279 μg Cu g^{-1} dry weight but like Pinus, they were seriously damaged. The weight of the seedlings was almost 75% lower than that of the controls. In a later study by Burton *et al.* (1986) similar concentrations of Cu proved to affect seedlings of sitka-spruce (Picea sitchensis). Both shoot and root growth was significantly decreased by 5000 μg Cu L^{-1} in the growth medium.

Some more works are reported from studies with grass and herbaceous species. Comparatively low levels of Cu in the growth medium are shown to give rise to growth retardations. When treated with 64 μg Cu L^{-1}, growth of root segments of a non-tolerant genotype of Agrostis capillaris was reduced by 50 to 60%, compared to control. In the presence of Ca in the cultures, the growth decrease was only 30 to 40% (Wainwright and Woolhouse, 1977). The same results have been obtained by Symeonidis *et al.* (1985). Similarly, Chrysanthemum morifolium proved to be affected. The decrease in dry weight of leaves and stem was only 13%, but reached 45% in the roots. The Cu concentrations in the plant organs were 10, 7.1 and 57 μg g^{-1} dry weight, respectively. Still lower Cu levels have been shown to affect shoot and root lengths of rye grass (Lolium perenne) seedlings. Grown for 14 days in culture solutions containing only 20 μg Cu L^{-1}, root growth decreased by 50%. Also shoot growth was retarded at this comparatively low Cu level (Wong and Bradshaw, 1982).

This great difference in response between and within species grown at fairly similar growth conditions, normally complicates attempts to set a concentration limit in a culture solution, at which Cu may be toxic to plants. In soil still more factors must be considered, like availability and uptake conditions.

Therefore, leaf tissue concentrations of a metal would be a better measurement tool. Critical leaf tissue concentrations of Cu are limited to a comparatively narrow interval, in which the metal is shown to be toxic. Generally, most species are affected between 15 and 25 μg Cu g^{-1} dry weight, but corresponding concentrations of Cu in culture solutions are shown to vary considerably (Beckett and Davis, 1977; Davis and Beckett, 1978; MacNicol and Beckett, 1985). Some species may respond below 10 μg Cu g^{-1} of leaf tissue, whereas others like sitka-spruce (Picea sitchensis) is comparatively insensitive, enduring 88 μg Cu g^{-1} d w of the needles (Burton *et al.*, 1983).

Like the other heavy metals, seed and germination is fairly insensitive to Cu (Patterson and Olson, 1983; Scherbatskoy *et al.*, 1987). Although there are great differences between species, the elongation and growth of the radicles may be easily affected (Patterson and Olson, 1983). In Betula papyrifera and Picea mariana, these functions were retarded by 25% at c. 30 μg Cu L^{-1} in water extracts of filter papers, used as germination medium. In mineral soil, the same reductions were obtained for Betula at a water extractable concentration of 60 μg Cu L^{-1}. At relatively low Cu concentrations, the formation of root hairs was also affected and at higher Cu levels, the roots of all the tree species in the study were shorter than the control. The study also showed that seeds of deciduous trees were more sensitive to Cu than those of the conifers, as were tree species with smaller seeds. Further, germinating seeds, treated with Cu, were more susceptible to fungus infection.

3.2. PHYSIOLOGICAL RESPONSES

Physiological studies in intact vascular plants treated with Cu are almost lacking. Until now, known published work has usually been focused on *in vitro* studies with isolated chloroplasts and unicellar green algae.

From several of these studies, Cu-ions are shown to inhibit photosynthesis and respiration. The photosynthesis is considerably more sensitive, however, and photosystem I more than photosystem II. The chlorophyll content is also decreased in the leaf cells. On the contrary, in intact seedlings of Zea mays exposed to 64 to 64 000 μg Cu L^{-1} in the culture solutions, the chlorophyll content was significantly increased. Accompanied by a reduced root/shoot ratio, the activity of the enzyme phosphoenolpyrovate carboxylase (PEPC), a key enzyme in photosynthesis in C_4-plants, was significantly affected at 64 μg Cu L^{-1}, already. Similarly, ribulose-1,5-bisphosphate carboxylase (RuBPC) involved in the photosynthesis of C_3-plants, was inhibited in young barley seedlings (Stiborova *et al.*, 1986a, c).

In the presence of Cu-ions, the permeability of cells is shown to increase, leading to leakage of, among others, K-ions. The leakage in detached root tips of Agrostis capillaris was 9 to 28 times that of the control, when plants were exposed to 640 to 64 000 μg Cu L^{-1}, whereas in an intact root system, the increase was 4 to 13 times the control (Wainwright and Woolhouse, 1977). In other studies concentrations of K too, in both shoots and roots, were usually markedly lowered by Cu in the growth medium (200 to 6400 μg L^{-1}). Similarly, the uptake and metabolism of Fe were affected (Lingle *et al.*, 1963; Oberländer and Roth, 1978; Sharma *et al.*, 1978; Bujtas and Cseh, 1981). On the contrary, the K content in seedlings of sitka-spruce (Picea sitchensis) was unaffected, when plants were grown in soil supplied with 0.5 to 32 μg Cu g^{-1} dry weight of soil. The same was true for Ca (Veltrup, 1981; Burton *et al.*, 1984).

3.3. BIOCHEMICAL RESPONSES

From relatively few published studies, generally *in vitro*, an excess of Cu is known to disturb both the carbohydrate and N metabolism but also the activity of certain enzymes. As earlier mentioned, photosynthesis may be indirectly influenced, as the key enzymes involved, PEPC and RuBPC are inhibited by Cu (Stiborova *et al.*, 1976a, c).

At several times the higher Cu levels (1000 to 10 000 μg L^{-1} the growth medium, the N metabolism in soybean (Glycine max) was disturbed. Both N-fixation (measured as acetylene reduction) and the contents of leghemoglobin, active in the N-fixation, were decreased by Cu, but compared to Cd, the effects were far less pronounced. Inversely related to N-fixation rate, was an accumulation of NH_4. According to the authors this increase would be due to an altered translocation of amino acids to or from nodules, increased breakdown in the cells and decreased assimilation (Vesper and Weidensaul, 1978).

The observed altered activity in the nodules may, however, be an indirect effect of Cu. When reclaiming, for example, heavy metal polluted soils, N-fixing plant species are usually used. In order to compare the sensitivity between species to Cu, Alnus crispa, a N-fixing non-leguminous shrub, was grown in soil with different levels of Cu. At the lower Cu levels (<40 μg^{-1} g^{-1} soil) the activity of the N-fixing nodules was only slightly affected but was seriously inhibited above this level. However, the seedling growth and nodule weight were decreased by only 20 μg Cu g^{-1}, indicating that N-fixation was affected less than growth (Fessenden and Sutherland, 1979). Thus, the obtained activity of nodules may be an indirect effect of Cu, due to decreased vitality and growth of the plant.

3.4. CYTOLOGICAL RESPONSES

Copper plays an important role in cell function and is essential in structural stability of chromosomes and energy transfer. An excess of the metal may however like other metals, affect viscosity of the plasma resulting in cell abnormalities and functional disorders. Despite the fact that Cu has proved to be one of the most active metals causing chromosome damages (Singh and Sharma, 1980; Fiskesjö, 1988) published studies on cellular effects are very few.

Comparatively low levels are shown to disturb cell function and structure in excised root segments as well as intact roots. In non-tolerant clones of Agrostis capillaris both root growth and cell elongation were seriously retarded when treated with 64 μg Cu L^{-1} growth medium. Similar results have been obtained with onion (Allium cepa) roots. Copper is also shown to increase the permeability of the cell membranes, leading to leakage of nutrients as K, and also sugars and amino acids (Wainwright and Woohouse, 1977, Nag *et al.*, 1980, Fiskesjö, 1988).

Like Zn, an excess of Cu increases abbreviations and abnormalities at root tip mitosis. The number of cells undergoing mitosis are decreased and the mitotic activity is disturbed. In e.g. Cu treated (64 μg L^{-1}) onion roots, the mitotic index was only 26 compared to a control index of 41 (Fiskesjö, 1988). In the presence of Cu, cells also showed several abnormalities as aggregation of chromosomes during metaphases, stickiness and production of binucleate cells, disturbances which are increased with increasing levels of the metal (Nag *et al.*, 1980; Fiskesjö, 1988).

4. Cadmium

The toxicity of Cd to plants is well documented. Chemically Cd is similar to Zn and available Cd in the growth medium is easily taken up by the plants. Like Pb, Cd is not known to be an essential element to plants. Although a limited transport of Cd to shoots and binding to cell walls occur in the roots, Cd is relatively more toxic to plants than Pb. The strong affinity of Cd ions for sulhydryl groups of several compounds and phosphate groups involved in plant metabolism might explain the great toxicity.

4.1. GROWTH RESPONSES AND SEED GERMINATION

Plants treated with higher concentrations of Cd usually become stunted in growth. The leaves are smaller, curled and chlorotic and leaf margins and veins show a red-brown coloration.

A number of growth and biomass studies on exposure of plants to elevated Cd levels show similar results. When growing Alnus rubra seedlings for 11 weeks in a nutrient solution, containing 3 to 122 μg Cd L^{-1}, the growth of both leaves, stem and roots was stimulated slightly at the lower Cd concentrations. A certain biomass decrease was noted at c. 60 μg Cd L^{-1}, but leaf biomass was not significantly reduced below 122 μg L^{-1}. The corresponding concentration in leaf tissue was 9.4 μg g^{-1} dry weight and 212 μg g^{-1} d w in the roots (Wickliff *et al.*, 1980). In another experiment with the same species grown in a N-free nutrient solution, root and stem dry weights were significantly decreased at a Cd concentration of 62 μg L^{-1}. Effects on the roots were already obtained at 31 μg L^{-1}. At this level, the biomass decrease was 44% and root tissue concentration 60.9 μg g^{-1} dw. On the contrary, the number of N-fixating nodules per plant was increased by 25 to 55% at all the Cd concentrations used (Wickliff and Evans, 1980). A species in the same family, Alnus glutinosa, proved to be far less sensitive. Treatments with up to 8 000 μg Cd L^{-1} did not affect shoot or nodule dry weights. Increased root biomass was significantly positively correlated with increasing metal levels, a response not earlier reported in Cd studies with woody species (Smith and Brennan, 1984).

Leaves of 2-yr-old Quercus palustris seedlings were chlorotic, when grown for seven weeks in a nutrient solution supplied with 100 μg Cd L^{-1}. The volume of the roots was also significantly affected (Russo and Brennan, 1979). In a repeated experiment, in which growth and development of the seedlings were followed for

2 yr, a biomass increase was obtained during the first year of Cd treatment. In the second year, Cd caused no effect on tree growth, but the percentage of foliar toxicity symptoms was increased (Kazimir and Brennan, 1986).

When comparing the sensitivity of tree species to Cd, a species of Picea appeared to be most sensitive. Exposed to Cd and grown under the same conditions, seedlings of Acer rubrum showed damaged leaves as chlorosis and reduced leaf area at 23 μg Cd g^{-1} dry weight of leaf tissue. The development of needles of the Pinus species involved, was not inhibited until exposed to 61 μg Cd g^{-1} dry needle weight, whereas developing needles of Picea abies had chlorotic needle tips at 7.5 μg g^{-1} d w. Although not statistically proved, Godbold *et al.* (1985) noted a biomass decrease of both shoots and roots in Picea abies exposed to 112 μg Cd L^{-1} for 5 weeks. Comparatively low Cd levels (250 μg L^{-1}) significantly decreased biomass of shoots and roots of Picea sitchensis (Burton *et al.*, 1986). As lower levels of Cd were not used in the two studies, the lower limit which may affect growth can not be stated.

The sensitivity of grasses and herbaceous species to Cd, of which especially agricultural plants have been studied, are similar to that of tree species. As with trees, both the interspecies and the intraspecies variation may be great, however. A tissue concentration of 2 μg Cd g^{-1} dry weight reduced stem biomass of Chrysanthemum morifolium and at 2.8 μg g^{-1} stem and leaf biomass of soybean (Glycine max) was significantly reduced. In contrast, growth of radish (Raphanus sativa) was still stimulated at 144 μg Cd g^{-1} shoot dry weight (Turner, 1973; Patel *et al.*, 1976).

According to Beckett and Davis (1977) the critical tissue concentration of a heavy metal, at which the metal causes a biomass decrease, is fairly independent of growth conditions. Although the variation between species is great, the lower limit to affect growth ranges from 3 to 10 μg Cd g^{-1} dry weight of leaf or shoot tissue. In the same range, 4 to 8 μg g^{-1} d w also appeared to be critical to naturally growing meadow-sweet (Filipendula ulmaria). When applying Cd-salt solutions to the meadow soil, the biomass production was fairly unaffected, but the length of aboveground shoots was significantly reduced. The development of rhizomes was seriously inhibited and the yearly production of roots in the uppermost part of the soil horizon was halved compared to the control. This growth decrease was partly compensated by a more intense root growth deeper in the soil (Balsberg, 1982 a, b).

The number of fertile shoots per unit area of Filipendula was not affected but seed weight was significantly lower (58%) than the control. Seed quality and seed germination was not studied in this field experiment. Other studies show, however, that germination of seeds of, e.g., Lolium perenne is unaffected by high Cd levels (20 000 μg L^{-1}) and lower levels may rather stimulate germination as shown by seeds from tree species (Wong and Bradshaw, 1982; Scherbatskoy *et al.*, 1987).

4.2. PHYSIOLOGICAL RESPONSES

Disorders in the physiological processes can be demonstrated at slightly lower or approximately the same Cd concentrations in the growth medium and/or the plant organs as growth disorders.

Photosynthesis and transpiration are inhibited by Cd (Huang *et al.*, 1974; Carlson and Bazzaz, 1977). Measured as CO_2-uptake, the photosynthetic rate in spruce (Picea abies) was significantly decreased by 112 μg Cd L^{-1} in the growth medium. The transpiration rate was lowered at 560 μg L^{-1}, whereas the respiration rate remained unchanged at both these Cd levels (Schlegel *et al.*, 1987). On the other hand Cd did significantly increase the respiration rate in soybean (Glycine max) et c. 100 μg L^{-1} (Lee *et al.*, 1976b).

According to Hampp *et al.* (1976) the CO_2-fixation in the chloroplasts is inhibited by Cd. This inhibition may be related to disturbances in the non-cyclic electron transport. The effects of Cd were more pronounced than those of Zn. As both metal form stable complexes with, e.g., phosphates, the formation of ATP is supposed to be affected. The increased respiration rate in Cd-treated plants also suggests an increased demand for ATP production through the oxidative photophosphorylation (Lee *et al.*, 1976b).

Reduction in transpiration rate by Cd is also supposed to be due to a lower water content in the plant and stomatal closure. As the metal has proved to cause changes in xylem tissues and blockages in the xylem elements, water transport in the plant may be indirectly affected (Lamoureaux and Chance, 1977). Root dry weight of spruce (Picea abies) was more decreased by Cd than shoot dry weight, leading to a decreased root/shoot ratio (Godbold *et al.*, 1985). As later proposed by Schlegel *et al.* (1987) this could result in a reduced water uptake followed by a lowered water content and transpiration rate of the shoots.

Accompanied by reduced photosynthetic rate, the chlorophyll content of primary needles and cotyledons of Picea abies was significantly decreased by 112 μg Cd L^{-1} (Schlegel *et al.*, 1987). Iron is of fundamental importance in plant metabolic processes, especially in the chlorophyll synthesis, but also in activating enzymes, involved in the photosynthesis and respiration. A number of studies with agricultural species show that the content of Fe in shoots and leaves usually is decreased at Cd treatments of c. 100 μg L^{-1} or more in the growth medium.

Magnesium is an irreplaceable component in the chlorophyll molecule. Further, enzymes involved in the carbohydrate metabolism, are activated by Mg. Cd-Mg interferences are, however, more difficult to evaluate as both increases and decreases of Mg content in Cd-treated plants are reported. In sitka-spruce (Picea sitchensis) needles, the concentration of Mg was unaffected by 250 μg Cd L^{-1} in the growth medium (Burton *et al.*, 1986). Similarly Cd has no effect on Ca and Zn concentrations, whereas that of Mn was significantly decreased. This interaction of Cd with Mn is known from a number of other studies, as well. In Cd-treated plants Ca is usually reported to decrease, whereas the content of K has appeared to increase, often markedly (Patel *et al.*, 1976; Cunningham, 1977; Wallace *et al.*, 1977; Grünhage and Jäger, 1981, Smith and Brennan, 1984).

4.3. BIOCHEMICAL RESPONSES

Disorders in biochemical processes, as in the carbohydrate and nitrogen metabolism could seriously affect growth and vitality of plants.

Cadmium might replace other metals like Zn in metal-activated enzymes. Both stimulations and inhibitions of enzyme activity in Cd treated plant are reported. The metal is also known to form stable complexes with certain proteins and has a high affinity for low-molecular metalloproteins in the cytoplasma (Jastrow and Koeppe, 1980). Compared to man and animals the knowledge about biochemical effects caused by Cd in plants is weak.

A great interest has been devoted to effects of Cd, in particular, on N-fixating plants. Higher concentrations of Cd have proved to affect the development of N-fixating nodules, but also the N-fixation itself and the activity of nitrogenase. Further, leghemoglobin involved in the N-fixation is increased by Cd. The increased concentrations of NH_4 obtained in Cd treated plants are also supposed to inhibit the activity of nitrogenase (Huang *et al.*, 1974, Vesper and Weidensaul, 1978).

On the contrary, on exposure of soybean (Glycine max) to lower Cd concentrations (up to 100 $\mu g\ L^{-1}$) the N-fixation, measured as acetylene reduction, was stimulated. The amino acid content was increased, too, especially of those containing sulphur (Roucoux and Dabin, 1978). In Alnus rubra seedlings grown in culture solutions with 6 to 62 μg Cd L^{-1}, the activity of nitrogenase decreased with increasing concentration of Cd. Strong negative correlations between the enzymatic activity and Cd concentration in the nodules indicate that Cd may selectively affect nitrogenase. The increased number of nodules in the Cd treated seedlings was supposed to compensate for the lowered enzyme activity (Wickliff and Evans, 1980).

Another enzyme involved in the N metabolism is nitrate reductase. In primary root tips of spruce (Picea abies) seedlings, the activity of the enzyme decreased on exposure of the seedlings to a culture solution containing 100 μg Cd L^{-1} and with a pH of 3.0. This metal concentration exceeds by a factor of 20 to 30 the highest ones found in lysimeter soil solutions under spruce stands in southern Sweden (Bergkvist, 1987). Thus, as concluded by the authors, pH and Cd in soil solutions from unpolluted areas might hardly affect the formation and activity of nitrogenase (Yandow and Klein, 1986).

The activity of carbonic anhydrase, catalyzing the decomposition of carbonic acid to CO_2 and H_2O, was decreased in the Cd concentration range 50 to 151 $\mu g\ L^{-1}$ culture solution. As this enzyme is dependent on the presence of Zn, Cd is supposed to interfere with Zn. In the range 100 to 151 μg Cd L^{-1}, the activity of hydrolytic enzymes, as ribonuclease, deoxyribonuclease and acid phosphatase, was stimulated. The activity of peroxidase was increased, too, indicating that Cd might induce a too early senescence in plants (Lee *et al.*, 1976b).

4.4. CYTOLOGICAL RESPONSES

Published studies on the action of Cd on cell function and structure in higher plants are relatively few. According to Singh and Sharma (1980) Cd is one of the most active metals on chromosome substances and in poisoning of the cell cytoplasma. Thus, cytological abnormalities have been observed in Cd treated species like Allium cepa and Crepis capillaris.

From studies with woody species cellular changes may arise at relatively low Cd levels. Seedlings of Acer saccharinum grown in sand with additions of Cd-salts, showed reductions of H_2O conducting xylem tissue and diameter of tracheids and vessel elements. Further, the cell wall metabolism and the cell elongation were inhibited and the cellular volyme and size were reduced by Cd (Lamoureaux and Chaney, 1977).

From a detailed study by Wickliff and Evans (1980) of Alnus rubra, Cd proved to seriously affect root and nodule ultra structure at comparatively low levels in the growth medium. At 12 μg Cd L^{-1} no effects was observed in nodule ultra structure, but at 31 μg L^{-1}, numerous septa were seen in the vesicles and the cytoplasm of the vesicles. Host cells appeared to collapse. In a quarter of the root cells, nucleoli and starch grains were observed in the seedlings treated with as low as 12 μg Cd L^{-1}. When increasing the exposure to 62 μg L^{-1}, these abbreviations could be seen in nearly every xylem parenchymatic cells of the roots together with a densely stained endoplasmatic reticulum and nucleus. The appearance of nucleoli and starch grains in the Cd treated plants was supposed to be due to a lowered cell division and cellular growth.

5. Lead

Like Cd, Pb is considered to be a nonessential metal to plants, although at lower Pb concentrations, a stimulation effect has been observed in many studies, especially older ones. Later this effect was supposed to be an indirect one, through a probable release of nutrients by the Pb-addition to a sub-optimal growth medium. Compared to Cd the phytotoxicity of Pb to plants is relatively low, due to a very limited availability and uptake of Pb from soil and soil solutions. However, plant roots are usually able to take up and accumulate large quantities of Pb^{2+} in soil and culture solutions but translocation to aerial shoots is generally limited, due to binding at root surfaces and cell walls. Found deposits of Pb, especially as pyrophosphate, in the cell walls of the roots but also similar deposits in stems and leaves and the occurrence of Pb granules may further explain the low toxicity (Peterson, 1978; Kabata-Pendias and Pendias, 1985).

Lead toxicity studies, especially at higher Pb levels, are numerous. Due to, e.g., species and growth conditions, the toxic effects of Pb may vary considerably and are quite often conflicting. Thus, the effect of Pb varies inversely with phosphate concentration of the nutrient solution and with the phosphate status of the plant. Other factors of great importance are plant age and time of treatment (Koeppe, 1981). Reports of toxicity in plants, growing in natural ecosystems are few, as are laboratory studies with lower Pb levels in the growth medium.

5.1. GROWTH RESPONSES AND SEED GERMINATION

Visible symptoms of toxicity, though unspecific to Pb, are smaller leaves and a stunted growth. Leaves may become chlorotic and reddish with necrosis and the roots turn black.

In most studies, generally only very high levels of Pb in the growth medium has proved to affect growth in plants. The results may be misleading, however, as precipitation of Pb in the solutions is not unusual at higher Pb concentrations. Thus, presence of anions as phosphate and sulfate may reduce Pb uptake. Outstanding are the works by Godbold and his colleagues. The initial liberation of heavy metal ions into the soil solution by increasing soil acidification warranted them to more detailed studies about heavy metals, as Pb, one of several possible contributing factors in forest decline.

In nutrient solutions containing 104 μg Pb L^{-1}, as the chloride, the root elongation of Picea abies seedlings decreased by more than 30% compared to the control. At four times higher Pb levels, the root elongation was half the control. Noteworthy too, is that growth retardations were greater in an artificial soil solution than in full strength nutrient solution (Godbold and Hütterman, 1985). As proposed by the authors, further studies are needed to ascertain the influence of Pb at a lower pH and nutrient regime. The data may indicate, however, that comparatively low levels of Pb in a soil solution affect growth. These results are supported by Stiborova *et al.* (1986b, c) studying Hordeum vulgare and Zea mays. At treatment wth 207 μg Pb L^{-1}, biomass was decreased and root/shoot ratio was significantly lower than the control. About 10 times more Pb in solution was needed to influence root length of Agrostis capillaris (Symeonidis *et al.*, 1985). However, the concentration of free Pb^{2+} will rarely exceed 5 μg L^{-1} even in greatly acid forest soils, due to complexation with organic matter.

Comparisons of the toxicity effects from culture solutions with those obtained at Pb treatments of soils are usually not possible. Lead supply to soil, as used in many Pb toxicity studies, is also of limited interest when predicting the influence of Pb on plants growing in natural ecosystems. Soil type and other growth conditions must be considered. So, root length of Picea sitchensis seedlings was significantly decreased by 5 μg Pb g^{-1} dry weight of a peaty gley soil with additions of quartz sand, whereas 10 to 20 times more Pb was needed to cause a growth decrease of roots and shoots of Plantanus occidentalis (Carlson and Bazzaz, 1977). Similar results were obtained by Dutta (1980), exposing Sorghum vulgare to Pb in a sandy soil.

The great influence of environmental factors on availability, uptake and toxicity of Pb to plants brought MacNicol and Beckett (1985) to omit Pb in their review on critical tissue concentrations of heavy metals. Although, Pb has proved to be toxic in culture solutions, the risk of Pb poisoning of plants in soil was supposed to be very small. Grown in culture solutions with additions of Pb-salts, 19 μg Pb g^{-1} dry weight in the needles of Picea sitchensis caused a biomass decrease

(Burton *et al.*, 1983), whereas under similar growth conditions 35 μg g^{-1} dw was needed to alter growth in Hordeum vulgare (Davis *et al.*, 1978). Critical concentrations in leaf tissues of plants grown in culture solutions might, however, in most cases never be comparable to those obtained when analyzing leaves from naturally growing plants. The contribution of Pb from direct aerial pollution of leaf surfaces compared to that taken up by the roots is usually great. Although entering of smaller Pb particulates through stomata and cuticular cracks into the leaves cannot be excluded, most foliar applied Pb has proved to be effectively immobilized at the leaf surface (Zimdahl and Arvik, 1973).

Like other heavy metals, Pb is a little toxic to seed germination. Concentrations as high as 20 000 μg Pb L^{-1} in the medium did not affect seed germination of four tree species, nor was pH found to interfere (Scherbatskoy *et al.*, 1987). The same results were obtained with germinating Lolium perenne seeds (Wong and Bradshaw, 1982). As the further development of radicles and hypocotyls was not followed, the results may be doubtful, when predicting seed germination and establishment of plants from seeds under natural conditions. Only in very polluted areas with high soil solution concentrations of Pb might the influence of this metal on seed germination be of interest.

5.2. PHYSIOLOGICAL RESPONSES

Lead is well known from numerous studies, to interfere with and inhibit various physiological processes. Exposed plants show decreased photosynthetic and transpiration rates with increasing supply of the metal. The responses are suggested to be, indirectly or directly, related to changes in resistance of the stomata to CO_2 and diffusion of water (Bazzaz *et al.*, 1974). Lead ions are also shown to inhibit chlorophyll biosynthesis (Hampp and Lendzian, 1974) leading to lowered chlorophyll contents. Thus, the inhibited photosynthesis could partly be related to reduced chlorophyll contents of the leaves.

As roots are effective barriers against further transport of Pb to the shoots, usually very high Pb concentrations in the growth medium are needed to affect photosynthesis in intact plants. Although the root elongation of Picea abies seedlings was greatly inhibited by c. 400 μg Pb L^{-1}, Godbold and Hütterman (1987) did not obtain any effect on the net photosynthesis, measured as CO_2-uptake. Nor were transpiration rate or chlorophyll content affected. On the contrary, the decrease of both biomass and growth of Platanus occidentalis seedlings, grown in a soil culture, supplied with 50 μg Pb g^{-1}, was accompanied by reduced photosynthesis and transpiration (Carlson and Bazzaz, 1977). Other studies show, however, that low concentrations of Pb may rather result in stimulation effects. Exposed to 100 μg Pb L^{-1}, the net photosynthesis in Medicago sativa increased and a decreased rate was only observed at 10^5 μg Pb L^{-1} (Porter and Sheridan, 1981). Low Pb levels (200 μg L^{-1}) resulted also in significantly increased chlorophyll contents in Zea mays leaves, an effect which remained up to 5×10^5 μg L^{-1}, whereas the chlorophyll a/b ratio was decreased in the same concentration range (Stiborova *et al.*, 1986c).

Other physiological processes, known as sensitive to heavy metals are H_2O and nutrient uptake. Although comparatively few studies have been published, fairly high levels of Pb seem to be needed to affect these processes (Oberländer and Roth, 1978; Päivöke, 1983c). Most likely by blocking the entry or binding of the ions to the ion-carrier, concentrations of Fe, Mn and Zn were lowered in both shoots and roots of young pea (Pisum sativum) seedlings by treatment with c. 2000 μg Pb L^{-1} (Kannan and Keppel, 1976). Similar effects were obtained by Walker *et al.* (1977) but the degree of heavy metal influence upon the nutritional status was dependent on the stage of development of the plant.

5.3. BIOCHEMICAL RESPONSES

Several biochemical processes are affected by an excess of Pb. As the metal reacts with important functional groups, the activity of several enzymes is influenced, some of which are of fundamental importance in the photosynthesis and N metabolism. The activity of hydrolytic enzymes and peroxidase is shown to be altered, too, indicating an enhanced senescence in plants treated with Pb. Increased soluble protein and free amino acid contents have been observed at Pb treatments, suggesting a shift to an increased degradation of proteins (Lee *et al.*, 1976a).

Probably, due to the limited transport of Pb from roots to shoots, the biochemical, like the physiological responses, is often more pronounced in the roots. Accompanied by a significant decrease of the root/shoot ratio in Zea mays, exposed to ≥ 200 μg Pb L^{-1}, the protein content decreased in the roots but was unchanged in the shoots (Stiborova *et al.*, 1986c)). The activity of phosphoenolpyrovatecarboxylase (PEPC) in the leaves, however, proved to be very sensitive to Pb and was significantly reduced at this concentration in the growth medium. As PEPC is a key enzyme and its activity is limiting to photosynthesis in C_4-plants, the photosynthetic rate in maize leaves would be affected at relatively low Pb treatments too. Quite in contrast to these results are those of Dutta (1980). Grown in soil, supplied with 50 to 400 μg Pb L^{-1}, the chlorophyll content in Sorghum vulgare decreased significantly, whereas the activity of PEPC was significantly stimulated. Due to species or experimental growth conditions, further studies are needed to ascertain these differences in results.

Associated with the initial steps in the chlorophyll synthesis is a metal-sensitive enzyme, d-aminolevulinic acid dehydratase (ALAD). Like the chlorophyll contents, the activity of this enzyme decreased in the leaves of both Pennisetum typhoideum and Phaseolus vulgaris, when exposed to higher levels of Pb, while the contents of d-aminolevulinic acid (ALA) was unchanged (Prasad and Prasad, 1987a, b). The decreased activity was supposed to be due to an interaction of Pb with -SH-groups at the active sites of the enzyme. Although not measured in these studies, it can be concluded that lower levels of Pb in the growth medium might hardly affect the activity of ALAD and the chlorophyll synthesis.

The same is true of the enzymes involved in the N metabolism. At least 2000 μg Pb L^{-1} in the growth medium is needed to significantly decrese nitrogenase activity in roots of Pisum sativum (Päivöke, 1983c). In Medicago sativa the activity was still more insensitive (Porter and Sheridan, 1981). Nitrate reductase in Picea rubens is also initially affected by Pb, but as concluded by Yandow and Klein (1986), Pb, like Zn, is unlikely to be an important negative factor in nitrogen transformations in red spruce, as the roots are capable of adjusting to toxic metals in the growth medium.

5.4. CYTOLOGICAL RESPONSES

Various forms of Pb may cause cell disturbances and chromosomal leisons in plant tissue. Included in these are effects by the highly toxic organic Pb compounds, as tri- and tetraethyllead, discussed as a possible factor in forest decline (Backhaus and Backhaus, 1986; Faulstich *et al.*, 1987).

Reports on cellular effects, at exposure of plants to solutions of lead salts or organic compounds are numerous. With increasing concentration of Pb and time of exposure, tetramethyl-Pb inhibits the cell division. Of damaged cell organels, the mitrochondria proved to be the most sensitive (Sekerka and Bobak, 1974). At low levels of organic Pb (21 μg L^{-1}) spindle disturbances occurred in onion (Allium cepa) root tip cells. Further, the number of cell divisions was seriously affected. As much as 20000 μg Pb L^{-1} as $Pb(NO_3)_2$ did not interfere with cell division, although this concentration caused significant spindle disturbances (Ahlberg *et al.*, 1972). These results show that alkyle Pb compounds are more toxic to cell functions than inorganic ones. In a similar experiment with Allium sativum root tip cells only using inorganic Pb, chromosomal abnormalities increased with dose and duration of treatment. By c. 6000 μg Pb L^{-1} cell division was unaffected, whereas the number of chromosomal leisons were significantly higher than in the control. Minimum threshold concentration to affect the root cells was 600 μg L^{-1}. Genetical effects, such as those observed, are supposed to be due to Pb interaction with, e.g., DNA and RNA (Dhir *et al.*, 1986).

6. Role of Interactions Between Zn, Cu, Cd and Pb in Toxicity

In soil and soil solutions, plants are exposed to many elements simultaneously and to other factors, which could interact in different ways. Thus, the integrated effects may be quite different from those obtained, when any of the elements or factors is acting alone.

A basic condition for normal development of plants is a chemical balance of elements in the soil and plants. The conditions may, however, vary due to species, genotypes, age of the plant and a lot of other factors.

Interactions between elements do occur naturally in soils and plants. Usually, the effects are antagonistic, that is when the combined effects of two or more elements are less than the sum effects of the elements alone. An excess of macronutrients like Ca, P, Mg may interfere with trace nutrients. On the other hand one or more trace elements may affect uptake and metabolism of macro and other trace elements in the plant. Especially, this could be true in soils with chemical imbalance due to contamination by for example heavy metals. Such effects in plants have been numerously reported both from studies with heavy metal contaminated soils and in laboratory studies, as discussed earlier under metal toxicity.

Often, effects of heavy metals are the sum of or more than the sum of the effects of each metal alone. This type of interactions are named additive and synergistic, respectively. Thus 'safe' levels of single metals as those mostly obtained in laboratory studies, may be misleading, when applied to soil solution because of probable interactions with other heavy metals.

Although interactions between heavy metals are commonly observed, they are often complex and contradictory. At Cd treatments of several vegetable species, uptake and concentrations of Zn increased in the plant shoots (Turner, 1973). On the other hand, as Cd concentration increased in corn (Zea mays), the Zn concentration decreased (Root *et al.*, 1975). However, effects are shown to be dependent on metal level and pH in the growth medium. Thus, at low Zn levels and low pH, the Zn concentration in bean (Phaseolus vulgaris) leaves were unaffected by Cd, whereas higher levels of both Zn and Cd significantly decreased the Zn concentration in the leaves. Interactions between Cd and Cu in barley (Hordeum vulgare) were somewhat similar (Wallace *et al.*, 1977).

In another study, the amount of Zn in barley shoots was only slightly affected by Cu. Copper was influenced in the same way by Zn at or below critical levels in the culture solution (Beckett and Davis, 1978).

Positive interactions are often reported between Pb and Cd. In corn (Zea mays) Pb increased Cd uptake, whereas, conversely, Cd reduced Pb uptake (Miller *et al.*, 1977). Further, combinations of Pb and Cd proved to synergistically inhibit root growth (Hassett *et al.*, 1976). Similar effects have been reported in American sycamore (Platanus occidentalis) (Carlson and Bazzaz, 1977). Both root, stem and leaf growth were decreased synergistically by Pb-Cd treatment. However, photosynthesis and transpiration were not affected in the same manner. The additions of Cd to Pb-treated plants did not further reduce the photosymthetic and transpiration rates.

Thus, interaction effects may be inconsistent, depending on the parameter measured in the plant, but also on plant organ, plant species and soil as shown by Miles and Parker (1979). Although some evidence of synergism and antagonism was found, this could not be conclusively proved.

7. Role of Mycorrhizal Infection in Toxicity

The role of mycorrhizal infection in nutrient uptake in plants is well established. Present at trace concentrations, the uptake of essential metals, like Cu and Zn, is enhanced in mycorrhizal infected plants (Lambert *et al.*, 1970; Gildon and Tinker, 1983).

However, in soils contaminated by heavy metals, naturally or man-made, such increases would be of great disadvantage to the host plant. Therefore, the role of infection by mycorrhiza and heavy metal resistance is of greatest interest. Calluna vulgaris is a successful colonizer of heavy metal contaminated soils in northern Europe. From plants growing in such soils and in a natural heathland soil, Bradley *et al.* (1981) collected seeds. The seeds were germinated and grown in sand cultures, supplied with dilute nutrient solution containing Cu and Zn. The obtained results clearly show, that mycorrhizal infected (M) plants are more resistant to heavy metal toxicity than non-mycorrhizal (NM) plants. One explanation could be the lowered concentration of the metals in shoots of the M plants. On the contrary, roots of the M plants contained significantly higher heavy metal levels than NM plants, indicating a retention of the metals by the fungus. When repeating the experiments involving two other ericaceous species, Vaccinium macrocarpon and Rhododendron ponticum, similar results were obtained (Bradley *et al.*, 1982).

In two grass species, Festuca rubra and Calamagrostis epigeios, root growth in M plants was not or only slightly affected by elevated Zn levels, whereas shoot growth, like the NM plants, was decreased by Zn. Neither did the mycorrhizal infection hamper uptake and translocation of Zn to the shoots, which may explain the influence of Zn in the shoots (Dueck *et al.*, 1986).

A direct adverse effect of mycorrhizal infection was observed in a study with a perennial grass species (Ehrharta calycina). Exposed to a range of acidic and heavy metal (Cu, Ni, Pb, Zn, Fe and Co) additions to sand cultures, metal concentrations in shoots and roots were greater in M plants than in NM plants. Increasing the external acidity and heavy metal level resulted in an increased uptake of the metals in the M plants. Acidity, heavy metals or mycorrhizal infection alone was not responsible for the plant growth retardations. However, the combinations of heavy metals and acidity did reduce growth more in M plants than in NM plants. As proposed this reduced growth may be due to the enhancement of heavy metal concentrations in the plant (Kilham and Firestone, 1983).

In spite of the fairly widespread opinion that mycorrhizal infection in plants improves resistance to heavy metals, similar conflicting results have been obtained from studies with tree species. In the light that pine seedlings with roots inoculated with fungus survived better on strip-mine soils than non-inoculated seedlings, Miller (1982) studied the location and concentrations of heavy metals inside and on the surfaces of the roots more in detail. The different concentrations of some elements between M and NM roots, indicate that mycorrhiza can affect metal uptake.

Other species known to colonize heavy metal contaminated sites are the two birch species, Betula pendula and B. pubescens. With and without mycorrhizal infection, and at various levels of Zn, plant seedlings with known tolerance were compared. The growth of both NM and M birch seedlings was reduced by Zn. Coupled to lower concentrations of Zn in M seedlings, the growth retardations were less in these than in the NM seedlings (Brown and Wilkins, 1985). In contrast, paper birch (Betula papyrifera) seedlings, inoculated with four fungus species, reacted with lower growth of both shoots and roots than not inoculated seedlings when exposed to Cu. As supposed by the authors, these differences in growth were not due to differences in uptake of Cu, rather an increased need for photosynthates by the fungus from the host (Jones *et al.*, 1986).

These contradictory results make generalizations and conclusions about the influence of mycorrhizal infection and heavy metal resistance very difficult. According to plant and fungus species, host plant tolerance and external metal levels, the interaction between host plant and mycorrhiza may be very complex and further research is needed. Of special interest would be the role of mycorrhizal infection at lower metal levels, which in culture solutions have proved to affect the metabolism and growth in higher plants.

8. Summary and Conclusions

Numerous investigations have been made on the toxic effects of Cd, but also Zn, Cu and Pb on higher plants. However, the metal concentrations used are mostly so high that they are not realistic for a natural forest land or its vegetation. Usually they are at least 10 to 100 times higher than the normal contents in the soil solution (lysimeter water) from forest soils in southern Sweden. Most of the investigations are also made in traditional liquid cultures where the stated concentration only concern the situation at the start of the experiment or immediately after each replacement of the solution. Further, consideration is not taken to the metal ion acitivity in the soil or nutrient solution not to chelated elements or interactions with other ions. The experimental periods are also usually very short ranging from a few hours to a few weeks. This complicates the possibilities of assessing whether a longer period of exposure (months or years) to considerably lower metal concentrations in the plant medium causes growth disorders.

ZINC

Zinc is, like Cu, an essential metal to higher plants and is involved in several metabolic processes. The minimum concentrations of Zn are given in Table I. In excess these processes may be affected but of the metals reviewed, Zn is the least toxic (Figure 1).

- To affect growth at least 1000 μg Zn L^{-1} in a nutrient solution is needed. This is several times the concentration generally found in soil waters from lysimeters under tree stands in south Sweden.
- Critical leaf tissue concentrations of Zn is also several times those found in leaves of naturally growing trees in unpolluted areas. Most crop species are sensitive in the range 200 to 300 μg g^{-1} dry weight (Figure 2), so are the few tree species studied.
- Seed germination itself is rather unsusceptible to Zn like also to the other metals in this review. An objection to the germination studies is, however, that further development and survival of the seedlings are generally not followed up.
- Exposure to at least 2000 μg Zn L^{-1} is needed to affect physiological processes

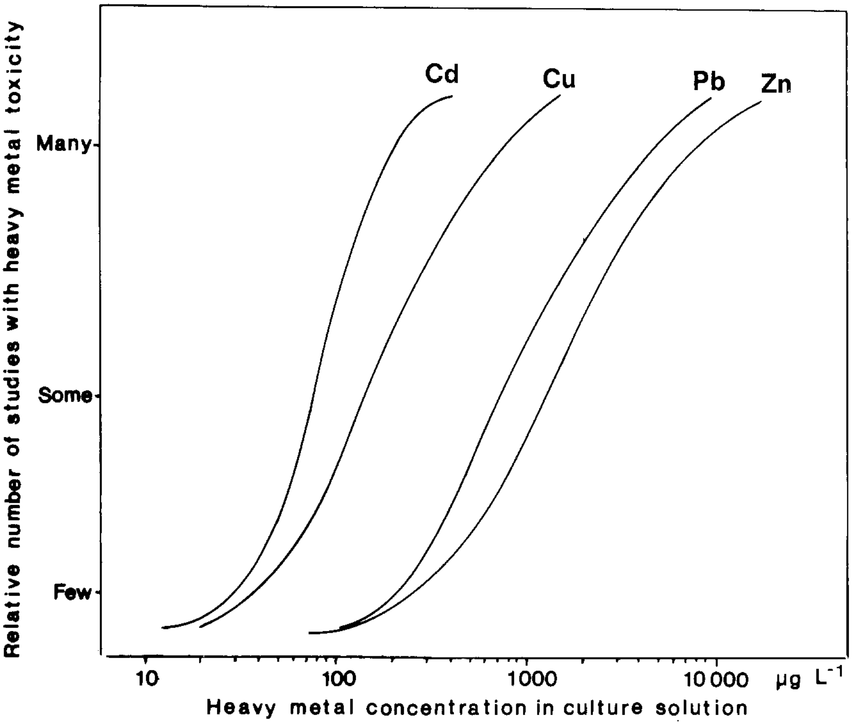

Fig. 1. Relative number of studies with reported symptoms of heavy metal toxicity in vascular plants, exposed to different concentrations of heavy metals in culture solutions.

like photosynthesis, chlorophyll content and water relations. Enzyme activity may be more susceptible. As low as 650 µg Zn L^{-1} is shown to decrease activity of e.g. enzymes involved in photosynthesis and hydrolytic enzymes.

- Still lower Zn concentrations (100 to 200 µg L^{-1}) disturb cell function as the mitotic activity in root tips. Although more studies are needed in this field, one explanation to these results may be that the growth medium was renewed more often than in other toxicity studies.

COPPER

Growth disorders resulting from higher copper additions are fairly well documented in crop plants but there are only a few investigations dealing with lower additions, <100 µg L^{-1} in the growth medium (nutrient solution) (Figure 1). The effects of copper on physiological processes and cell function in particular, but also biochemical processes, have been fairly little investigated. This complicates the possibilities to arrive at conclusions. The Cu minimum concentrations are given in Table II. However, the results from the few investigations available indicate that:

- Germination of seeds of different trees species is influenced slightly or not at all despite high contents of Cu in the plant medium. The elongation and growth of the primary root (radicle) are however, more easily affected and can be inhibited

TABLE I

Minimum Zn concentrations toxic to vascular plants. Effects followed by *, statistical significant at $p \leq 0.05$ (NS = nutrient solution)

Minimum toxic conc. ppm (μM)	Growth conditions	Plant species studied	Effects observed	References
0.06 (1.0)	$ZnSO_4$, NS, 3 weeks	Chrysanthemum morifolium	P-conc.(leaves,stem) increase* Ca-conc.(leaves) increase Cu-conc.(stem) increase Mg-conc.(roots) decrease	Patel *et al.*, 1976
0.65 (10)			Mg-conc.(stem) decrease* Cu-conc.(stem) increase*	
0.06 (1.0)	$ZnSO_4$-sol., pH 6.5	Zea mays	Chlorophyll content changes*	Stiborova *et al.*, 1986b
0.65 (10)			Phosphoenolpyrovate carboxylase activity decrease*	
0.06 (1.0)	$ZnSO_4$-sol., 7 days	Hordeum vulgare	Root/shoot ratio increase* Root protein decrease* Chlorophyll content changes*	Stiborova *et al.*, 1986c
0.10 (1.5)	$ZnNO_3$-sol., change every 2 d., 4 days	Festuca rubra (non-tolerant)	Root elongation decrease Cytological changes	Powell *et al.*, 1986a, b
0.16 (2.5)	$ZnSO_4$, NS	Phaseolus vulgaris	Shoot and root fresh wt. decrease Enzymes in the citrate cycle, changed activity	Weigel and Jäger, 1980
1.6 (25)			Root protein conc. decrease	
0.50 (7.7)	$ZnCl_2$, NS pH 3,4,5 change every 2 d., up to 40 days	Picea rubens	Nitrate reductase activity decrease at pH 3 and pH 5, stimulation at pH 4	Yandow and Klein, 1986
0.65 (10)	ZnAc, NS pH 6.2–6.3	Pisum sativum	Nitrogenase activity decrease* Acid phosphatase activity decrease*	Päivöke, 1983a, b
1.1 (20)	$ZnSO_4$, NS, 5 weeks	Picea abies	Root dry wt. decrease Shoot dry wt. decrease	Godbold *et al.*, 1985
2.0 (30)			Chlorophyll decrease	
1.6 (25)	$ZnSO_4$, NS, 10 hr	Deschampsia caespitosa (non-tolerant)	Root elongation rate decrease	Godbold *et al.*, 1983
1.8 (28)	$ZnSO_4$, $Ca(NO_3)_2$-sol., pH 7, change every 3 d., 14 days	Lolium perenne	Shoot and root growth decrease	Wong and Bradshaw, 1982
2.0 (30)	$ZnSO_4$, NS, pH 4.3, 7 days	Picea abies	Root elongation decrease	Godbold and Hütterman, 1985
2.0 (30)	$ZnSO_4$, NS, 5 weeks	Picea abies	Photosynthesis decrease* Needle water content decrease*	Schlegel *et al.*, 1987
4.0			Chlorophyll decrease*	

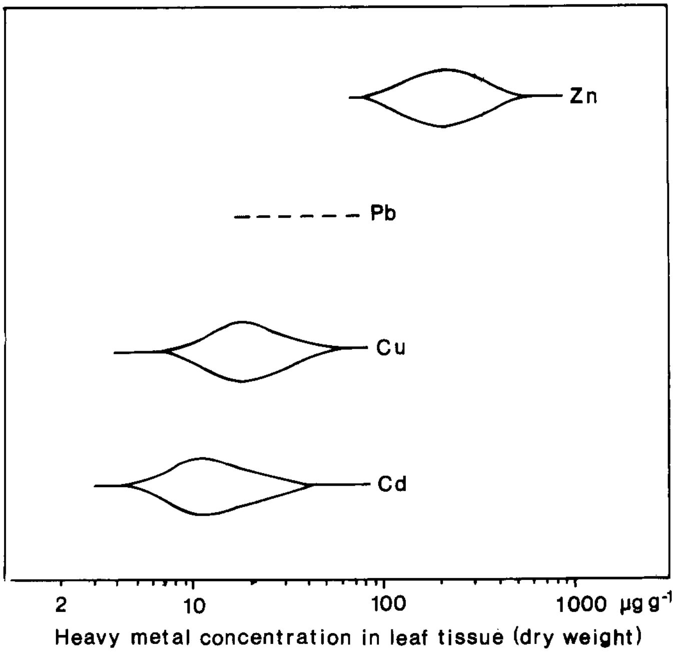

Fig. 2. Critical leaf tissue concentrations of heavy metals shown to give symptoms of toxicity. With the exception for Pb, the critical concentration of which is rarely reported, the width of the figures reflects the concentration range in which most vascular plant species are susceptible to the metal.

at relatively low Cu concentrations, c. 60 µg L^{-1} water extract of a mineral soil.

- Growth disorders have been noted in crop plants when the growth medium has contained c. 60 µg Cu L^{-1} or lower but most investigations report that this does only occur at considerably higher concentrations. Large variations occur between different species but in most crop plants the critical concentration limit of Cu in leaf tissue is 15 to 20 µg g^{-1} dry weight (Figure 2), thus at the upper limit for normal concentrations. That a toxic effect has been obtained at these tissue concentrations probably depends on Cu being bound to the cell walls of the roots and that transports to the shoots are restricted. Therefore, very high concentrations of Cu are required in the plant medium before the root cells become saturated and lose their ability to regulate further transport of Cu to the shoots. The contents in the roots are then usually so high that the roots are damaged, which indirectly influences also the leaves.
- Excessive concentrations of Cu may disturb the N metabolism of N-fixing plants as well as the activity of several enzymes. However, too few investigations have concerned this aspect to make possible any determination of toxic concentration limits in the plant medium or plant tissues.
- Copper in excess reduces the Fe contents of various plant organs, particularly the uptake of K, whereas the calcium uptake appears to be less susceptible.

TABLE II

Minimum Cu concentrations toxic to vascular plants. Effects followed by *, statistical significant at $p \leq 0.05$ (NS = nutrient solution)

Minimum toxic conc. ppm (μM)	Growth conditions	Plant species studied	Effects observed	References
0.02 (0.50)	$CuSO_4$, $Ca(NO_3)_2$-sol., pH 7, change every 3 d., 14 days	Lolium perenne	Shoot and root growth decrease	Wong and Bradshaw, 1982
Waterextract:	Cu-salt in solution	Betula papyrifera	(F)Radicle elongation decrease	Patterson and Olson, 1983
≥0.03(F)(0.50)	Filterpaper (F)		(M)Radicle elongation decrease	
≥0.06(M)(1.0)	Organic soil(O) Mineral soil (M)	Picea glauca Picea mariana	(M)Radicle elongation decrease (F)Radicle elongation decrease	
>0.23(O)(3.6)			(O)Radicle elongation decrease	
		Pinus banksiana	(F)Radicle elongation decrease (M)Radicle elongation decrease	
		Pinus resinosa	(F)Radicle elongation decrease (at >0.03 ppm) (O)Radicle elongation decrease	
		Pinus strobus	(M)Radicle elongation decrease	
0.06 (1.0)	$CuSO_4$, NS, 3 weeks	Chrysanthemum morifolium	Yield(roots) decrease* Ca- and Mg-conc.(roots) decrease* P- and Fe-conc.(roots) increase* Zn-conc.(roots) increase	Patel *et al.*, 1976
0.64 (10)			Yield(leaves,stem) decrease* Mg-conc.(stem) decrease* Ca-conc.(leaves) increase*	
0.06 (1.0)	Metalsalt-sol.	Agrostis capillaris (non-tolerant)	Root growth decrease (root elongation)	Wainwright and Woolhouse, 1977
0.06 (1.0)	$CuSO_4$, NS, 14 days	Agrostis capillaris (non-tolerant)	Root length decrease	Symeonidis *et al.*, 1985
0.06 (1.0)	$CuSO_4$-sol. pH 6.5	Zea mays	Root/shoot ratio decrease Chlorophyll content changes* Phosphoenolpyrovatecarboxylase activity decrease*	Stiborova *et al.*, 1986b
0.64 (10)			Root protein decrease*, shoot protein increase*	
0.06 (1.0)	$CuSO_4$-sol. 7 days	Hordeum vulgare	Root/shoot ratio decrease* protein content decrease*	Stiborova *et al.*, 1986c
0.64 (10)			Ribulose-1.5-bisphosphate carboxylase activity decrease*	
0.06 (1.0)	$CuSO_4$-sol. 2,4 days	Allium cepa	Root length decrease Mitotic index decrease	Fiskesjö, 1988
0.22 (3.5)	$CuSO_4$, NS	Phaseolus vulgaris	Isocitratedehydrogenase activity(shoot) decrease, (roots)increase Protein content(shoot) decrease	Weigel and Jäger, 1980
0.25 (0.4)	Metalsalt-sol.	Festuca rubra (non-tolerant)	Root length decrease	Karataglis, 1981
20	$CuSO_4$, sand pH 5.87, 13–14 weeks	Alnus crispa	Biomass (dry wt.) decrease Nodule biomass decrease Nodule activity slight decrease	Fessenden and Sutherland, 1979

TABLE III

Minimum Cd concentrations toxic to vascular plants. Effects followed by *, statistical significant at $p \leq 0.05$ (NS = nutrient solution)

Minimum toxic conc. ppm (μM)	Growth conditions	Plant species studied	Effects observed	References
0.01 (0.11)	$CdCl_2$, sand+NS, 11 weeks	Alnus rubra	Cytological changes	Wickliff and Evans, 1980
0.03 (0.25)				Wickliff *et al.*, 1980
0.06 (0.50)			Leaf, stem and root dry wt. decrease Nodule number decrease Nitrogenase activity decrease	
0.05 (0.45)	$CdSO_4$, NS, 10 days	Glycine max	Carbonic anhydrase activity decrease*	Lee *et al.*, 1976b
0.10 (0.90)			Other enzyme activity increased * at 0.10 ppm	
0.15 (1.35)			Respiration increase* Growth decrease, chlorosis, curled leaves, leaf fall	
0.05 (0.45)	NS, pH 5.2, change every 4 d., 14,21 days	Glycine max	Yield decrease*	Cunningham, 1977
0.10 (0.90)			Zn-conc. (leaves,stem) increase K-conc. increase Fe-conc. (leaves,stem) decrease, (roots) increase Mg-conc. (leaves,roots) decrease	
0.10 (0.90)	$CdCl_2$, NS, 3 days	Zea mays	Lateral root initiation decrease*	Malone *et al.*, 1978
0.10 (0.90)	$CdSO_4$, NS, 53 days	Chrysanthemum morifolium	Chlorotic leaves, black roots Turgor pressure and transpiration rate decrease Stomatal resistance increase	Kirkham, 1978
0.10 (0.90)	$CdCl_2$, NS, application 5 times a week, 16 weeks	Quercus palustris	Root volyme decrease*	Russo and Brennan, 1979
0.10 (0.90)	$CdCl_2$, NS, pH 3,4,5 change every 2 d., up to 42 days	Picea rubens	Nitrate reductase activity decrease at pH 3 and pH 5, stimulation at pH 5	Yandow and Klein, 1986
0.11 (1.0)	$CdSO_4$, NS 3 weeks	Chrysanthemum morifolium	Yield decrease* Ca-conc.(leaves) increase Ca-conc.(roots) decrease* Mg- and Mn-conc.(roots) decrease* P, Fe-, Cu-conc.(roots) increase	Patel *et al.*, 1976
0.11 (1.0)	$CdSO_4$, NS	Glycine max	Yield(leaf,stem) decrease* Mn-conc. decrease* Fe-conc.(leaf,stem) decrease* Ca-conc.(leaf,stem) decrease	Wallace *et al.*, 1977 Wallace, 1979

Table III (continued)

Minimum toxic conc. ppm (μM)	Growth conditions	Plant species studied	Effects observed	References
0.11 (1.0)	$CdSO_4$-sol., pH 6.5	Zea mays	Root/shoot ratio decrease* Shoot protein increase* Chlorophyll content changes*	Stiborova *et al.*, 1986b
0.11 (1.0)	$CdSO_4$-sol., 7 days	Hordeum vulgare	Root/shoot ratio increase* Root protein content decrease* Chlorophyll content changes*	Stiborova *et al.*, 1986c
0.11 (1.0)	$CdSO_4$, NS, 5 weeks	Picea abies	Root dry weight decrease Shoot dry weight decrease Chlorophyll content decrease	Godbold *et al.*, 1985
0.11 (1.0)	$CdSO_4$, NS, 5 weeks	Picea abies	Needle water content decrease* Chlorophyll content decrease*, Photosynthesis decrease	Schlegel *et al.*, 1987
0.56 (5.0)			Transpiration decrease*	
0.25 (2.2)	$CdSO_4$, NS, pH 5.5, 18 days	Phaseolus vulgaris	Shoot and root weight decrease Enzymes in the citrate cycle, generally increased activity Root protein content decrease	Weigel and Jäger, 1980
0.25 (2.2) 0.50 (4.5)	$CdCl_2$, NS, 6 weeks	Picea sitchensis	Yield decrease*	Burton *et al.*, 1986
0.56 (5.0)	$CdSO_4$, NS, pH 4.3, 7 days	Picea abies	Root elongation decrease	Godbold and Hütterman, 1980
5	$CdCl_2$, sand+ NS	Acer sacharinum	Leaf, stem and root dry wt. decrease* Height decrease* Chlorophyll content changes*	Lamoureaux and Chaney, 1977
5	CdO, sand mixture, 55 days	Pisum sativum	Yield decrease K-, P-, Zn-conc.(roots) increase* Na- and Ca-conc.(roots) decrease* Fructose, glucose conc. decrease Sucrose conc. increase Peroxidase activity increase	Grünhage and Jäger, 1981, 1982 Grünhage *et al.* 1981

CADMIUM

The inhibiting effect of Cd on growth, uptake of nutrients and physiological and biochemical processes is well documented at higher Cd concentrations in the plant medium. Studies of effects with lower concentrations, < 100 μg L^{-1} nutrient solution (Figure 1) or < 10 μg Cd g^{-1} soil, are considerably less frequent. The experiments are usually made on various crop plants, though a number of studies have also included tree species. The Cd minimum concentrations are given in Table III. The results from the various investigations with both crop plants and trees indicate that:

- Lower Cd concentrations, < 2 μg g^{-1} dry weight in leaf tissues or < 50 μg L^{-1}

in the growth medium may stimulate growth. This is assumed to depend on e.g., the fact that Cd at these concentrations causes increased turgor and transpiration.

- Growth is inhibited at higher Cd concentrations. Both cell elongation and plant cell size are influenced. In addition, the photosynthesis and transpiration rates as well as the turgor decrease, whereas dark respiration increases. A premature senescence occurs also supported by the increasing activities of acid phosphatase and peroxidase. In most plant species investigated these toxic effects have been obtained when shoots or leaf tissue contain between 3 and 10 μg Cd g^{-1} dry weight, in a nutrient solution corresponding to c. 100 μg Cd L^{-1}. Many plant species have a critical limit in the leaf tissue at c. 10 μg Cd g^{-1} dry weight (Figure 2).
- In N-fixing plant species Cd decreases the formation of N-fixing nodules on the roots. The fixation of N is inhibited as well as the activity of nitrogenase. These effects were obtained when the growth medium contained c. 60 μg Cd L^{-1}.
- Cadmium increases the contents of Fe in the roots but decreases it in the leaves. The Mn content decreases in both leaves and roots, whereas the content of Ca and K usually increase.

LEAD

Compared to Cd, the phytotoxicity of Pb to plants is relatively low, due to a very limited availability and uptake from soil and soil solution. The Pb minimum concentrations are given in Table IV. Further binding of Pb to root surfaces and the cell walls, limits the translocation to aerial shoots.

- Nutrient solutions containing 100 to 200 μg Pb L^{-1} is shown to affect root growth and elongation. These growth retardations are greater in an artificial soil solution than in full strength solution. Free Pb-ions will, however, rarely exceed 5 μg L^{-1} in forest soils, due to complexation with organic matter.
- Critical leaf tissue concentrations data are few (Figure 2). The risk of Pb poisoning of plants in soil is supposed to be very small due to the great influence of environmental factors on avilability and uptake.
- Like other heavy metals, the toxicity to seed germination is very small.
- Both physiological and biochemical processes are affected by an excess of Pb. As the metal reacts with important functional groups, the activity of several enzymes is influenced, some important in the photosynthesis and N metabolism. Published studies with lower Pb levels are few, however (Figure 1), but 200 μg L^{-1} is shown to affect an enzyme in photosynthesis as well as root protein content.
- Cytological disturbances in root cells are noted at 600 μg Pb L^{-1} or more. The genetical effects observed are supposed to be due to Pb interaction with DNA and RNA.

TABLE IV

Minimum Pb concentrations toxic to vascular plants. Effects followed by *, statistical significant at $p \leq 0.05$ (NS = nutrient solution)

Minimum toxic conc. ppm (μM)	Growth conditions	Plant species studied	Effects observed	References
0.10 (0.50)	$PbbCl_2$, NS, pH 4.5,7 days	Picea abies	Root elongation decrease	Godbold and Hütterman, 1985
0.21 (1.0)	$Pb(NO_3)_2$-sol., pH 6.5	Zea mays	Root/shoot ratio decrease* Root protein decrease* Chlorophyll content changes* Phosphoenolpyrovatecarboxylase activity decrease*	Stiborova *et al.*, 1986b
0.21 (1.0)	$Pb(NO_3)_2$-sol., pH 6.5	Hordeum vulgare	Root/shoot ratio increase* Root protein decrease* Chlorophyll content changes*	Stiborova *et al.*, 1986c
0.50 (2.4)	$PbCl_2$, NS, pH 3,4,5 change every 2 d., up to 42 days	Picea rubens	Nitrate reductase activity decrease at pH 3 and pH 5, stimulation at pH 4	Yandow and Klein, 1986
0.62 (3.0)	$Pb(NO_3)_2$-sol., 2–72 days	Allium cepa	Chromosomal abnormalities	Dhir *et al.*, 1986
50	$PbCl_2$, silty clay loam from A-horizon, 90 days	Plantanus occidentalus	Foliage biomass decrease New stem growth decrease Photosynthesis and transpiration decrease	Carlson and Bazzaz, 1977
50	$PbCl_2$, sandy soil, pH 8.5, 7 weeks	Sorghum vulgare	Fresh and dry weight decrease Shoot and root length decrease Chlorophyll content decrease Soluble protein (leaves) increase	Dutta, 1980
125	$PbCl_2$, loamy sand, pH 6.0, 24, 31 days	Zea mays	Cu- and Zn-conc. decrease	Walker *et al.*, 1977

CONCLUSIONS

With our present knowledge it is difficult to propose a limit for toxic concentrations of Zn, Cu, Cd and Pb. There is no completely unambiguous method of determining the biologically available fraction of the metals in the soil. Neither is it possible to use the contents in the roots as a measure since most of the metals are bound in the cell walls or outside the roots and are not biologically active or can be transported up to the above-ground parts. Some studies have given the metal concentrations in the leaf tissues as toxic limit values. As regards Zn and Cd, this measure is probable the most reliable since the metals are easily mobile in the plant. Initially, Cu and Pb appear to be accumulated in the roots and the contents in the leaves remain at a normal level until the roots are saturated. In situations where the metals in the growth medium are increased, the roots are probably damaged first, which indirectly causes toxic effects in the leaves.

- In combination with other heavy metals and/or gases the toxic effects may be synergistic and the critical limit reduced. External conditions such as drought, parasite attacks, etc., work in the same direction.
- Factors as age and nutritional status of the plant is important when assessing its tolerance. A tree seedling or a nutrient deficient plant will probably be damaged considerably earlier and at lower metal concentrations than an older tree or a plant with a balanced nutritional status.
- Most tree species have mycorrhiza which at least at lower metal concentrations can protect the root from taking up heavy metals. The susceptibility of the mycorrhiza to heavy metals is poorly known but the absence of mycorrhiza may increase the metal uptake and thus the risk for root injury.
- There are, thus, several factors capable of counteracting or enhancing a toxic effect. The metal contents in the plant medium or leaf tissue at which a toxic effect has been demonstrated in laboratory experiments with pure nutrient solutions, is at least a factor of ten or more the normal contents in natural forest land or vegetation. However, it must be strongly emphasized that the average degree of exposure during the experimental period in most trials with nutrient solutions has not been as high as the one stated. Under unfavorable external plant conditions and a longer period of exposure we cannot exclude the possibility of a toxic effect arising on the vegetation. Particularly tree seedlings and the fine root system should be susceptible.

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