

Effect of Metal Toxicity on Plant Growth and Metabolism: I. Zinc

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Abstract Zinc toxicity and problems with regard to tolerance and ecological significance are briefly discussed. Differential tolerance of plant genotypes exposed to zinc toxicity is a promising approach to enrich our understanding of zinc tolerance in plants. Knowledge concerning the physiology and biochemistry with regard to phytotoxicity, uptake and transport of zinc and tolerance and its characterization are also discussed. The cytotoxic effects of zinc on plants are elucidated. The major change was seen in the nucleus of the root tip cells due to zinc toxicity. The chromatin material was highly condensed and some of the cortical cells showed disruption and dilation of nuclear membrane in presence of 7.5 mM zinc. The cytoplasm became structureless, disintegration of cell organelles and the development of vacuoles were also observed. The number of nucleoli also increased in response to zinc resulting in the synthesis of new protein involved in heavy metal tolerance. This review may help in interdisciplinary studies to assess the ecological significance of metal stress.

Keywords Accumulation • Phytotoxicity • Tolerance • Transport • Uptake • Zinc

Résumé Effet de la toxicité des métaux sur la croissance et le métabolisme des plantes: I. Zinc. La toxicité du zinc et les problèmes de tolérance ou de conséquence écologique liés sont rarement discutés. L'approche en terme de tolérance différentielle des

génotypes de plantes exposées à la toxicité du zinc est prometteuse pour l'enrichissement de notre compréhension de la tolérance des plantes au zinc. Les connaissances de la physiologie et la biochimie face à la phytotoxicité, à l'absorption et au transport du zinc, ainsi que la tolérance et sa caractérisation sont aussi discutées dans ce papier. Les effets cytotoxiques du zinc sur les plantes sont maintenant élucidés. La modification majeure concerne la noyau des cellules de l'extrémité des racines. La chromatine est fortement condensée et certaines des cellules corticales montrent la rupture et la dilatation de leur membrane nucléaire en présence de 7.5 mM de zinc. De plus, le cytoplasme perd sa structure, la désintégration d'organites et le développement de vacuoles sont aussi observés. Enfin, le nombre de nucléoles augmente en réponse au zinc. Ils résultent de la synthèse d'une nouvelle protéine impliquée dans la tolérance aux métaux lourds. Cette synthèse bibliographique pourra aider les études interdisciplinaires à évaluer les conséquences écologiques des stress dus aux métaux.

Mots clés Accumulation • Phytotoxicité • Tolérance • Transport • Absorption • Zinc

1 Introduction

The occurrence of heavy metals in soils may be beneficial or toxic to the environment. Excess of metals may produce some common effects of individual metals on different plants (i.e. both macro- and microflora). The biota may require some of these elements in trace quantities but higher concentrations there may be toxicity problems. Metal toxicity in plants have been

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reported by various authors (Bollard and Butler, 1966; Brown et al., 1972; Brown and Jones, 1975; Foy et al., 1978; Gerloff, 1963). Generally, zinc is an essential element which belongs to Group-II of the periodic table. It acts as a plant nutrient (Shier, 1994; Welch, 1995) but at higher concentrations, it is toxic. Since it is assimilated early by plants, it can be highly phytotoxic. Growth inhibition is a general phenomenon associated with zinc toxicity (Collins, 1981). Zinc is also a constituent of metalloenzyme or a cofactor for several enzymes such as anhydases, dehydrogenases, oxidases and peroxidases (Hewitt, 1983) and plays an important role in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants (Shier, 1994). It also plays an important role in the synthesis of nucleic acid and proteins and helps in the utilization of phosphorous and nitrogen during seed formation. Hyper-accumulation of zinc has been observed in many plant species (Baker and Walker, 1990; Verkleij and Schat, 1990), accumulations of 1% of the dry weight being common among plants growing on zinc minewastes (Rascio, 1977). The toxic effects of zinc on plants have been reported by many researchers (Beckett and Davis, 1977, 1978; Davis and Parker, 1993; Taylor et al., 1991; Webber, 1981; Wheeler and Power, 1995; White et al., 1974). Genotoxicity of zinc in micro- and macroflora were also elucidated by various researchers (Mukherjee and Sharma, 1985; Subhadra and Panda, 1994). In addition, the toxic effects of the metals on photosynthesis in plants were elaborated (Clijsters and VanAssche, 1985; Garty et al., 1992; Stiborova et al., 1986; Van Assche and Clijsters, 1986; Van Assche et al., 1979, 1980). Recent progress in the study of toxic metals and their interactions with essential elements has greatly increased our understanding of the mechanism of toxicity at the biochemical level (Abdulla et al., 1985). In this communication, the salient features of zinc toxicity, the effects on plant metabolism and its interaction with other essential elements are discussed; their possible implications in the plant ecosystem are highlighted.

2 Zinc Toxicity

Zinc toxicity depends on pH, which controls the concentration of zinc in solution. High concentrations of zinc can cause toxicity in plants (Daviscarter and

Shuman, 1993). The general symptoms are stunting of shoot, curling and rolling of young leaves, death of leaf tips and chlorosis. Ye et al. (1997) reported that the seedlings of *Typha latifolia* were chlorotic in the presence of $\sim 80 \mu\text{M}$ zinc. Foliar chlorosis appeared on *Thlaspi ochroleucum* seedlings treated with 1 mM Zn after 4 days of culture as reported by Shen et al. (1997).

2.1 Effect on Germination

Zinc is an essential nutrient for plant growth, although elevated concentrations resulted in growth inhibition and toxicity symptoms. It does not affect seed germination but helps in plumule and radicle development. Baker (1978) reported that the seeds of *Silene maritima* were germinated better and rapidly on calcium nitrate solutions containing different concentrations of zinc.

2.2 Effect on Root

Zinc though an essential element for plant growth, showed toxicity symptoms at higher concentrations inhibit root growth (Baker, 1978; Bradshaw and McNeilly, 1981). Zinc toxicity was marked in root system particularly in root blunt, thickening and caused restraint on both cell division and cell elongation (Barcelo and Poschenrieder, 1990; Wainwright and Woolhouse, 1976). Doyar and Van Hai Tang (1980) reported that the nitrogen and phosphorous increased with the increase in zinc content in the roots. The concentration of zinc in the roots decreased with plant age as reported by Pearson and Rengel (1995). Sresty and Madhava Rao (1999) based on transmission electron microscopy concluded that radicle elongation was more adversely affected than the plumule extension. The root elongation of *Cajanus cajan* cv. ICPL 87 was completely inhibited after 24 h treatment with 7.5 mM zinc. They also reported that root cortical cells were extensively damaged and major changes took place in the nucleus of the root tip cells treated with 7.5 mM zinc.

2.3 Effect on Reproductive Growth

Metal sensitive plants have great difficulties in reaching the reproductive phase when exposed to metals.

The seeds of most plant species growing on heavy metal enriched soils have very low metal concentrations (Ernst, 1974; Lepp and Dickison, 1994). Zinc-resistant genotypes of *Silene vulgaris* were stimulated by increased levels of zinc, not only in vegetative growth but also in seed production (Ernst, 1998). Surpassing a critical zinc concentration resulted in reduced growth the no flower production.

2.4 Effect on Plant Physiology and Morphology

The physiology of metal toxicity in plants was mainly concerned with metal movement from soil to root and metal absorption and translocation. Plant availability of a metal in the soil depends on soil adsorption strength as well as plant effectors such as root exudates for metal chelation or reduction. Lindsay (1972) and others developed models for the chemical activity of metal ions in soil. The chemical activity was usually dependent on soil pH, CO₂ concentration and redox potential of the soil (Hodgson 1963, 1969; Lindsay, 1972, 1974). The concept of metal equilibrium in soil, and of metal movement from soil to root were well documented (Foy et al., 1978). Studies on the physiological and biochemical metal toxicity were almost unknown. The activity of a metal in the cytoplasm was affected by chelating carboxylic and aminoacids and the pH. Zinc inhibited Fe translocation in some cases (Ambler et al., 1970) and the young chlorotic leaves showing Zn²⁺ toxicity generally contained more than 100 ppm Fe; Fe deficiency generally occurred at concentrations lower than 40 ppm Fe in the young leaves. The metal toxicity resulted with the increased metal supplied to the root which affect the disintegration of cell organelles, disruption of membranes and condensation of chromatin material and increase in number of nucleoli were major events during zinc toxicity (Sresty and Madhava Rao, 1999). Ernst (1998) elaborated details on mechanism of heavy metal toxicity in plants at the cellular and organism level. He also reported that the plasma membrane was the cell compartment which regulated metal entry into the cell, in addition its proteins, especially the SH groups might be affected in their activity causing damage to membrane stability due to zinc toxicity. As soon as heavy metals passed through the plasma membrane, they could immediately

interact with all metabolic processes in the cytosol. Godbold and Huttermann (1985) reported that increasing zinc levels in culture solution decreased the shoot to root ratios and translocation of Zn, Fe, Mg, K, P and Ca and caused accumulation of these nutrients in the root. Pearson and Rengel (1995) indicated that higher concentration of zinc affected the leaf and the root morphology. They suggested that the zinc supply from the roots into the leaves of different ages might be determined by the relative transpiration rate of the leaves. Further, Malea et al. (1995) tested the effect of zinc on leaf cell mortality of *Halophila stipulacea*. The mortality of the cells increased along with the increase of zinc concentrations (10⁻⁴ to 10⁻⁷ M) and the increase of incubation time (2nd–12th days). Necrosis was evident in the epidermal cells at all concentrations on the 12th day of culture. Necrosis was also detectable in the mesophyll cells at the highest concentration (10⁻⁴ M) after the eighth day. Zinc toxicity was primarily associated with alteration of root physiology, thereby inhibiting the root elongation (Woolhouse, 1983).

3 Differential Zinc Tolerance in Plants

3.1 Differential Tolerance In vitro and In vivo

Zinc toxicity and differential zinc tolerance in various plant groups were reported (Ambler et al., 1970; Brown and Jones, 1975; Earley, 1943; Polson and Adams, 1970; Symeonidis et al., 1985; White et al., 1974). Earley (1943) reported that different soybean cultivars responded differentially to toxic Zn in sand culture. White (1976) studied the differential varietal tolerance in soybean and found that they were associated with differences in susceptibility to zinc-induced Fe deficiency, Zn uptake and translocation, and susceptibility to toxicity unrelated to Fe deficiency. Gregory and Bradshaw (1965) developed Zn-tolerant efficiency in bentgrasses. Further, Ernst (1977) and Mathys (1973) accepted the compartmentalization hypothesis and suggested that malate might chelate zinc in the vacuoles of tolerant plants (Mathys, 1977a). The fact that high malate, Zn-tolerant ecotypes were not tolerant to Cu and was difficult to understand since malate would be expected to bind Cu much

more strongly than it does in Zn. Mathys (1977b) and Ernst (1977) conducted detailed studies on metal tolerant enzymes in tolerant and non-tolerant ecotypes. However, in in-vitro studies, the activities of several enzymes remained at normal levels in tolerant ecotypes as metal supply increased, but activities fell markedly in nontolerant ecotypes. They also confirmed that tolerant plants were able to maintain the normal metabolic processes when subjected to metal stress which severely interfered with the metabolism of nontolerant plants. Hertstein and Jager (1986) studied the tolerances of different populations of three grass species (*Agrostis tenuis*, *Festuca rubra* and *Anthoxanthum odoratum*) to zinc and other metals. They observed that all populations originating from metal contaminated habitats possessed multiple-metal-tolerance. Genecological and physiological aspects of tolerance were studied extensively with regard to zinc and other metals tolerance (Antonovics et al., 1971; Woolhouse, 1983). Baker (1978) selected zinc tolerant *Silene maritima* populations in water-culture experiments. Subsequently, Amado-Filho et al. (1997) screened six seaweed species for zinc tolerance for a period of 21 days using rapid hydroponic methods. They reported that all species died at 76 μM of zinc, two species (*Ulva lactuca* and *Enteromorpha flexuosa*) died at 152 μM and one, *Hypnea musciformis* died at 0.152×10^{-2} mM. Ye et al. (1997) tested four populations of *Typha latifolia* collected from metal-contaminated and uncontaminated sites grown in nutrient solution containing 1.0–5.0 $\mu\text{g}/\text{ml}$ Zn under controlled environmental conditions. They also reported that seedlings from metal contaminated populations showed three times more tolerance to zinc as compared to the uncontaminated population. Shen et al. (1997) compared the growth of hyperaccumulator species *Thlaspi caerulescens* and non-hyperaccumulator species *Thlaspi ochroleucum* in nutrient culture experiment with zinc. They reported that *Thlaspi caerulescens* was able to tolerate 0.5 mM Zn in solution without growth reduction, and up to 1 mM Zn without showing visible toxic symptoms but with a 25% decrease in dry matter yield. Sresty and Madhava Rao (1999) and Madhava Rao and Sresty (2000) tested two genotypes of pigeonpea (*Cajanus cajan*) in nutrient solution containing zinc (2.5, 5.0 and 7.5 mM) to assess Zn tolerance in term of root and shoot tolerance index, metal uptake and dry mass population. Rout et al. (1999) selected Zn tolerant cal-

lus lines of *Brassica* species using in vitro techniques. They assessed various growth parameters such as fresh and dry weight of callus, growth tolerance index and metal uptake to determine the tolerant line. The tolerant calli had the maximum peroxidase and catalase activity and higher rate of metal uptake as compared to non-tolerant calli. Subsequently, Samantaray et al. (2000) developed zinc tolerant calli and regenerated of plantlets from tolerant calli of *Setaria italica* using in vitro technique. The tolerant plants showed luxuriant growth in zinc rich minespoil. Rengel (2000) observed that the ecotypes of *Holcus lanatus* tolerant to zinc toxicity also tolerated Zn-deficiency better than the Zn-sensitive ecotypes because of their greater ability to taking up zinc from Zn-deficient soil.

4 Effect of Zinc on Nuclear Activity

Growth of plants was mainly dependent on cell division. Fujii (1954) found that zinc played an important role in mitosis. Gebhart (1984) observed higher frequency of chromosome aberrations in presence of heavy metals. Heavy metals, which constituted one of the important groups of environmental pollutants, were mostly genotoxic (Sharma and Talukdar, 1987). Further study indicated that the heavy metals like nickel, cadmium and zinc had genotoxic activity through oxidative pathways involving free radicals (Michaelis et al., 1986; Ochi et al., 1983). Sresty and Madhava Rao (1999) observed a major change in the nucleus of the root tip cells in response to zinc. The chromatin material was highly condensed and some of the cortical cells showed disruption and dilation of nuclear membrane in presence of 7.5 mM zinc. The cytoplasm became structureless; disintegration of cell organelles and the development of vacuoles were also observed. They also noted that the number of nucleoli increased in response to zinc resulting in the synthesis of new proteins involved in heavy metal tolerance. Further, the information of SH-rich phytochelatins appears to play a role in heavy metal detoxification in different organisms (Grill et al., 1987; Rauser, 1995; Robinson et al., 1988; Tomsett and Thurman, 1998). Ernst (1998) compared the nuclear activity and cell division in Zn-sensitive and Zn-resistant ecotypes of the perennial grass *Festuca rubra* in presence of different level of zinc. He observed that the nuclear volume decreased

by 30% and doubled the length of the cell cycle when Zn-sensitive ecotypes were exposed to 3 μM Zn for 4 days (Powell et al., 1986); the nuclear volume increased by 50% in Zn-resistant ecotypes exposed to same Zn level.

5 Effect of Zinc on Metabolism

In general, a number of plant species were resistant to certain amounts of metals. This was probably achieved through trapping of these metals within the metal-binding proteins. Zinc was known to be a constituent of many enzymes which stimulated various metabolic activities such as nucleic acid metabolism, protein synthesis, photosynthesis, respiration and carbohydrate metabolism (Shkolnik, 1984). Zinc was easily assimilated by plants, and could also be strongly phytotoxic; growth inhibition was a general phenomenon to zinc toxicity (Collins, 1981). Many authors observed inhibition of photosynthesis by heavy metal ions but the mechanism of action was not known in details (Stiborova et al., 1986; Tripathy and Mohanty, 1980). Van Assche (1973) reported that the high concentration of zinc inhibited metabolic activity. In some cases, the concentration ranged from 0.4–1.0 mM inhibited photosystem (Baker et al., 1982; Hampp et al., 1976; Tripathy and Mohanty, 1980; Van Assche and Clijsters, 1986). Lorimer (1981) and Lorimer and Miziorko (1981) reported that bivalent cations (Zn^{2+}) played a major role in the activation of the key enzyme of the Calvin cycle and in the equilibrium between CO_2 and O_2 binding by protein and inhibition of photosynthesis reactions localized in the thylakoid membranes of chloroplasts (Van Assche and Clijsters, 1984). Interference of zinc in photochemical reactions of chloroplast was intensively studied (Van Assche and Clijsters, 1986). Garty et al. (1992) studied the effects of low pH and Zn on chlorophyll degradation in lichen (*Ramalina duriaei* Bagl.). They found that Zn decreased photosynthesis in lichens having chlorophycean photobionts under pH 2.0. Verkleij and Schat (1990) and Meharg (1993) reported that the plasma membrane was the compartment of the cell which might at least partially regulate the entry of a heavy metal ion into the cell. This direct exposure might have consequences for the adaptation and selection of plant species and genotypes. Marschner

(1986) indicated that cation and anion uptake by the cell could be actively regulated by electrogenic proton pumps ($\text{H}^+ - \text{ATPase}$), transmembrane redox pumps (NAD (P) oxidase), and ion channels (Marschner, 1995). Davis et al. (1995) indicated that the plants exposed to higher concentrations of zinc disturbed the mitochondrial structure and reduces the energy.

6 Zinc Uptake and Transport

Zinc an essential element for the normal growth and metabolism of plants played very important role in enzyme activation and was also involved in the biosynthesis of some enzymes and growth hormones (Devlin, 1967; Nanson and McElroy, 1963). The transport and distribution of zinc and other metals in plants were reported by various researchers (Longnecker and Robson, 1993; Marschner, 1986; Wahbeh, 1984). The accumulation of zinc in the roots in the tolerance races were reported (Peterson, 1969; Turner and Marshall, 1972a, b). Baker (1978) reported that the populations of *Silene maritima* accumulated zinc to a high degree in the roots relative to the shoots. Turner and Marshall (1972a, b) detected a correlation between the uptake of ^{65}Zn by cell wall and mitochondrial fractions from the roots of a range of *Agrostis tenuis* populations and the indices of zinc tolerance. Wahbeh (1984) reported that the distribution and abundance of zinc in various vegetative tissues of the sea grasses *Halophila ovalis*, *Halophila stipulacea* and *Halodule uninervis*. He also indicated that both the root and the leaf absorbed metals, and that translocation was low (Lyngby et al., 1982). Availability of heavy metals in soil and their uptake by plants not only was dependant on the total metal content in the soil but also upon other factors i.e. soil organic matter and cation exchange capacity (Bjerre and Schierup, 1985; MacLean, 1976; Miner et al., 1997; Strickland et al., 1979). Hinesly et al. (1984) found that the uptake of heavy metals (Zn and Cd) by *Zea mays* generally decreased as the soil pH increased. They also assumed that the increased solubility of Zn in the alkaline pH range was due to the dispersion of organic matter containing complexed zinc form. Adriano (1970) demonstrated that the distribution of metals in the fractions depended on the soil properties, and, for most mineral soils, the largest amounts of zinc were present in the residual fraction.

Whitehead (1987) found that the accumulations of zinc (Zn^{2+}) varied in different plant species. The ratio between the accumulation of root and the shoot was always greater than 1.0 in ryegrass and clover. McKenna et al. (1993) found a complex interaction between Zn^{2+} and Cd^{2+} on the accumulation in the root and leaf of lettuce and spinach; the interactions were dependent on the relative concentration of those metals in the growth medium. Zinc accumulation was higher in the younger than the older leaves. Shetty et al. (1994) reported that the application of mycorrhizal fungi helped in higher accumulation of zinc in the roots than in the shoots. The influence of arbuscular mycorrhizae (AM) on plant growth and zinc uptake by *Lygeum spartum* and *Anthyllis cytisoides* was studied in soils with different levels of heavy metals (Diaz et al., 1996). Rout et al. (1999) and Samantaray et al. (2000) conducted in vitro studies on zinc accumulation in tolerant and non-tolerant calli of *Setaria italica* and *Echinochloa colona* respectively and observed that the uptake and accumulation was more in tolerant calli than the non-tolerant ones. Bert et al. (2000) investigated zinc tolerance in five populations of *Arbidopsis halleri* raised from seed collected from contaminated and *Arbidopsis thaliana* from non-contaminated sites. They observed that the populations of *Arbidopsis thaliana* from non-contaminated area accumulated zinc in shoots and roots more quickly than the populations from the contaminated sites. Frey et al. (2000) measured zinc concentration in shoot which was higher and reached a maximum value of 83 mM kg^{-1} dry mass whereas total concentration of zinc in roots were lower upto 13 mM kg^{-1} . They observed that the distribution of zinc in *Thlaspi caerulescens*; Zn got mainly accumulated in the vacuoles of epidermal leaf cells and was totally absent from the vacuoles of the cells from the stomatal complex, thereby protecting the guard and subsidiary cells from high zinc concentrations. They concluded that zinc also got accumulated in high concentrations in both the cell walls of epidermal cells and in the mesophyll cells, indicating that apoplastic compartmentation was another important mechanism involved in zinc tolerance in the leaves of *T. caerulescens*. Chardonens et al. (1975) demonstrated that the tonoplast vesicles derived from

Zn-tolerant ecotype of *Silene vulgaris* accumulated more Zn than the Zn-sensitive ecotype. They also characterized the tonoplast-transport system that caused this difference in the uptake and demonstrated its genetic correlation. The most prominent differences being its insensitivity to protonophores and ortho-vanadate and its stimulation by Mg-CTP. They concluded that in both Zn-tolerant and Zn-sensitive ecotypes, Zn was actively transported across the tonoplast (temperature coefficient >1.6), most likely as a free ion, since citrate did not accumulate in vesicles. Lasat et al. (2000) reported the molecular physiology and zinc transport in the Zn-hyperaccumulator *Thlaspi caerulescens* and a non-accumulator. They reported that Zn transport was stimulated at a number of sites in *T. caerulescens* contributing to the hyperaccumulation trait. The transport processes that were stimulated included Zn influx into both root and leaf cells, and Zn loading into the xylem. They also hypothesized that the stimulation of Zn influx was 4–5 fold into the root because of an increased abundance of Zn transporters in *Thlaspi caerulescens* root cells. Zinc was sequestered in the vacuoles of non accumulator *T. arvense* root cells which retarded Zn translocation to the shoot. Subsequently, they characterized the Zn transport genes in *T. caerulescens* and reported that ZNT1 (Zn transporter) gene was highly expressed in roots and shoots of *T. caerulescens*. But in *T. arvense*, ZNT1 was expressed at far lower levels and this expression was stimulated by imposition of Zn deficiency. Further, Whiting et al. (2000) indicated the positive responses to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. Hacısalihoglu et al. (2001) reported the high and low-affinity zinc transport systems and their possible role in zinc efficiency in wheat (*Triticum aestivum*). The low-affinity system showed apparent K_m values similar to those previously reported for wheat (2–5 mM). High-affinity Zn transport system with apparent K_m values were found in the range of 0.6–2 nM. Because it functioned in the low range of the available Zn level found in most soils, this novel high affinity uptake system was likely to be the predominant Zn^{2+} uptake system. Zn^{2+} uptake was similar for cv. Dagdas and cv. BDME-10 over both the high- and low-affinity Zn^{2+} activity ranges.

6.1 Mechanisms Involved in Zn Tolerance

Mechanism of metal tolerance by plants has been studied by various authors (Baker, 1987; Cunningham et al., 1975; Foy et al., 1978). At first, exclusion of metals from roots seemed a likely mechanism because root cell walls could bind metals; the extent of binding by the cell wall was related to the degree of tolerance to a specific metal (Turner, 1970; Turner and Marshall, 1972a, b). Jones et al. (1971) found that Zn complex was formed in roots of Zn-tolerant bentgrass after digesting the roots with cellulase. Although binding of metals to root cell walls might contribute to metal tolerance, it was not adequate enough to enable the plants to prevent metal transport to their leaves (Wainwright and Woolhouse, 1977). Woolhouse (1983) felt that there existed different specific mechanisms of tolerance within each of the populations which were related to the contamination of their original habitat. The mechanism of tolerance depended possibly on three major factors i.e. an exclusion of heavy metals from uptake, i.e. an “avoidance” (Levitt, 1980) or translocation to the shoots (Baker, 1978), a protection of sensitive structures in the cytoplasm either by immobilization of metals in the cell wall (Turner and Marshall, 1972a, b) and metal complexation with soluble compounds, e.g. organic acids and aminoacids (Hertstein and Jager, 1986) or by binding to specific proteins (Rauser, 1984). Cumming and Taylor (1990) described the signal transduction and acclimation mechanisms on metal tolerance in plants. They also indicated that the mechanism of metal tolerance were due to exclusion and external metal detoxification mechanism, internal metal detoxification mechanisms, and multiple mechanisms and co-tolerances. The production of metal-chelating ligands high in thiol groups might also render co-tolerance to Zn^{2+} . Further, Neumann et al. (1997) found by electron microscopy that most of the heavy metals were tightly bound to the cell wall in tolerant plant populations as compared to non-tolerant ones. Frey et al. (2000) elucidated tolerance mechanism at the cellular and subcellular level for the detoxification of the accumulated zinc within the leaf of hyperaccumulator *Thlaspi caerulescens*. They also observed that there was apoplastic compartmentation in the leaves of *T. caerulescens*. Hall (2002) reported the range of potential cellular mechanisms that may be involved in the detoxification of heavy metals and thus

tolerance to metal stress. These includes the binding to cell wall and extracellular exudates, reduced uptake or efflux pumping of metals at the plasma membrane, chelation of metals in the cytosol by peptides such as phytochelatins, repair of stress-damaged proteins and the compartmentation of metals in the vacuole by tonoplast located transporters.

7 Phytotoxicity

To evaluate meaningful physiological and biochemical effects of toxicity, one must know the metals which are phytotoxic in nature and interactions with other metals (Cunningham et al., 1975). Before starting a phytotoxicity experiment one should be fully aware of the movement of the metal including its absorption and translocation in the plant system. Availability of metal in the soil depends on soil adsorption strength as well as plant effectors such as root exudates for metal chelation or reduction. Metal phytotoxicity can result only if metals can move from the soil to root systems (Foy et al., 1978). Phytotoxicity levels of zinc in different crop plants were reported by many workers (Chardonnens et al., 1999; Staker and Cummings, 1941). The most significant phytotoxicity symptoms were stunting of growth, chlorosis and reduction in biomass yield. The phytotoxicity caused by a wide variety of metals has been well documented; however, models designed to quantify the relationship between exposure to metal ions and progressive yield losses are lacking (Taylor et al., 1991).

7.1 Phytotoxicity and Its Interaction with Other Nutrients

Ambler et al. (1970) indicated that zinc induced inhibition of Fe translocation from root to tops which causes chlorosis in plants. Zinc causes phytotoxicity like chlorosis at low pH level as reported by Chaney et al. (Chavan and Banerjee, 1980). Chavan and Banerjee (Chaudhry et al., 1977) reported that Zn toxicity appear to be due to Fe deficiency. Cayton et al. (1985) reported that the absorption and translocation of plant nutrients like Fe, Mg, K, P and Ca depended on Zn concentration in soil. They reported that zinc

was an antagonist to Cu at the primary absorption site, in contrast with its action on Fe, P, Mg, K and Ca. Zinc interfered at the loading site of the roots and decreased the rate of translocation or absorption of essential nutrients to plants or caused mineral imbalances (Brown, 1979; Chaney, 1975; Chaudhry et al., 1977; Warnock, 1970; Watanabe et al., 1965). White et al. (1976) observed that increased levels of Zn in soil greatly increased translocation of Mn to tops which indicate the appearance of chlorosis. They hypothesized that the Zn and Mn interfere with Fe utilization in the leaves for chlorophyll synthesis. Accumulation of zinc in the roots or shoots was generally accompanied by accumulation of calcium (Baker, 1978). He also compared the interaction of zinc and calcium with regard to uptake by zinc-tolerant and non-tolerant population of *Silene maritima* by conducting solution culture experiment. The result indicated that the total zinc uptake was not affected by calcium level in the tolerant population but was decreased significantly in the non-tolerant population. The stimulation by calcium of zinc uptake in the tolerant plants may reflect an involvement of calcium in the zinc tolerance mechanism sited in the roots. Davis and Parker (1993) reported that zinc toxicity were highly correlated with Ca: Zn ratio and reduced stem biomass. Shetty et al. (1994) pointed out that growth inhibition was due to interference of zinc with phosphorous uptake by plants. They also reported that the application of vesicular-arbuscular mycorrhizae (VAM) fungi at zinc contaminated sites increased plant biomass even at elevated levels of zinc in the soil. Synergism was observed between boron and zinc when both were in excess together as excess accelerated the effect of high zinc by lowering the biomass, economic yield and carbonic anhydrase activity in mustard (Sinha et al., 2000). Gianquinto et al. (2000) concluded that the Zn concentration in leaf of *Phaseolus vulgaris* cv. Borlotto nano was reduced by the addition of phosphorous to plants grown at low Zn supply. Further, Rengel and Romheld (2000) reported that the zinc deficiency depressed the rate of Fe transport to shoots at early stages of *Triticum aestivum*. Kaya and Higgs (2001) tested the effectiveness of phosphorus and iron supplemented in nutrient solution on growth of tomato plant at high zinc (77 μM) concentration. They also reported that the application of supplementary P and Fe in the nutrient solution resulted in increase of both dry weight and chlorophyll concentration in *Lycopersicon esculentum*

cv. Blizzard, Liberto, Calypso and also decreased zinc concentration in the leaves and roots of plants grown at high Zn treatment.

7.2 Phytotoxicity and Its Interaction with Other Heavy Metals

Zinc and cadmium have many physical and chemical similarities as they both belong to Group II of the Periodic Table. They are usually found together in the ores and compete with each other for various ligands. Thus interaction between Zn and Cd in the biological system is likely to be similar. The fact that cadmium is a toxic heavy metal and zinc is an essential element which makes this association interesting as it raises the possibility that the toxic effects of cadmium may be preventable or treatable by zinc (Chowdhury and Chandra, 1987). Hinsely et al. (1984) suggested that both cadmium and zinc uptake by plants were dependent on the pH of the growing media. The basic mechanisms of Cd-Zn in the tissues induced the synthesis of different types of metallothionein, binding characteristics of metallothionein, alteration in absorption and tissue distribution of one metal by another, and competition at the level of zinc containing metallozymes known to be involved in the interactions. McKenna et al. (1993) reported the interactions between zinc and cadmium and the concentration and tissue distribution in spinach and lettuce. They observed that the cadmium concentration in young leaves of lettuce and spinach decreased exponentially in the solution containing Zn at low (0.0316 μM) but not at high (0.316 μM) concentration of Cd. The Zn:Cd concentration ratios in young leaves of lettuce and spinach grown at 0.316 μM Cd was greater as the concentration of Zn increased. Cayton et al. (1985) indicated that Zn enhanced translocation of Mn and Cu but decreased Cu absorption by the root.

8 Conclusion

This review mostly concerns with the role of zinc toxicity in micro- and macroflora. Zinc acts as a plant nutrient (Shen et al., 1997; Watanabe et al., 1965);

but at higher concentrations it is toxic. Zinc toxicity in plants is clearly visible with the inhibition of growth and decrease in biomass production; severe toxicity can also be fatal. Zn-toxicity might be the result of complex interactions of the major toxic ions i.e. Cd^{+2} , Cu^{+2} , Pb^{+2} with Ca, Mg, Fe and P and other environmental factors. Zinc toxicity was almost certainly involved with metabolism through competition for uptake, inactivation of enzymes, displacement of essential elements from functional sites. Generally, Zn toxicity caused chlorosis and inhibited Fe translocation in some cases (Ambler et al., 1970). The physiology and biochemistry of zinc toxicity have been less studied in intact plants.

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