

STUDIES ON CADMIUM TOXICITY IN PLANTS: A REVIEW

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Abstract

This review emphasises cadmium toxicity on plants with regards to ecological, physiological and biochemical aspects. Cadmium toxicity in plants and problems concerning tolerance and ecological performance are discussed briefly. Efforts have been made to compare the relative sensitivity of various plant groups including micro- and macro-flora. This review may help in interdisciplinary studies to assess the ecological significance of metal stress.

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INTRODUCTION

Heavy metals occur naturally in soils as rare elements. High concentrations are confined primarily to certain minerals usually present in forms which are not easily available but may be sensitive to higher concentrations. The release of heavy metals in biologically available forms, as a result of human activity, may damage or alter both natural and man made ecosystems (Tyler *et al.*, 1989). The chemical form (speciation) of heavy metals in soil solution is greatly dependent on the metal element concerned, pH and presence of other ions, etc. Toxic actions of heavy metal ions are essentially exerted on the enzymes. Inhibition of enzymes may be due to the making of catalytically active groups or protein denaturation. Prolonged exposure of soils to heavy metals may result in marked decreases in soil enzyme activity (Tyler *et al.*, 1989). Impeded litter decomposition and soil respiration are common features of heavy metal pollution of soils; several other studies also indicated toxic effects of heavy metals on soil mitochondria and extracellular soil enzymes (Tyler *et al.*, 1989). Cadmium is a non-essential heavy metal pollutant of the environment resulting from various agricultural, mining and industrial activities and also from the exhaust gases of automobiles (Foy *et al.*, 1978). It has been considered as an extremely significant pollutant due to its high toxicity and greater solubility in water which determines wide distributions in aquatic ecosystems (Lockwood, 1976). Aquatic organisms may acquire cadmium directly from contaminated water or through the food chain (Hart and Scaife, 1977). Water hyacinth is a good bioindicator of water pollution by heavy metals (Rosac

et al., 1981). The toxic effects of cadmium on biological systems were reported by various authors (Mukherjee *et al.*, 1984; Sharma *et al.*, 1985). Genotoxicity and ecotoxicity of cadmium in animals and other aspects were reported by various researchers (Webb, 1979; Nriagu, 1980; Degreave, 1981; Bhattacharya and Chaudhuri, 1995). Cadmium is naturally present in the environment; it is present in soil and sediments at concentrations which are generally more than 1 mg Kg^{-1} (Peterson and Alloway, 1979) and its total concentrations in unpolluted seawater, where it exists mainly as chloro-complexes is generally $< 1 \mu\text{g Kg}^{-1}$ (Mohlenberg and Jensen, 1980). In addition, indirect effects of the metal on the physical environment, such as variations in pH value and oxygen concentration in water resulting from toxic effects of the metal on photosynthesis were reported (Ravera, 1974, 1984). Recent progress in the study of heavy 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 review the salient features of cadmium metabolism and toxicity in micro- and macro-flora and their interactions with essential elements are highlighted, and their possible implications for plant systems elucidated.

CADMIUM TOXICITY

The symptoms of cadmium toxicity are easily identifiable. In plants, the most general symptoms are stunting and chlorosis. The chlorosis may appear to be Fe deficiency and the interaction of toxic metals and iron have been studied for many years. Chlorosis from excess cadmium appears to be due to a direct or an indirect interaction with foliar iron. Haghiri (1973) reported that high cadmium content in the growing medium suppresses the iron uptake by the plants. Root *et al.* (1975) felt that Cd-induced chlorosis in corn leaves could be due to changes in Fe:Zn ratios. In others, Cd toxicity appeared to induce phosphorous deficiency or reduce manganese transport problems (Godbold and Huttermann, 1985). Much of the physiological research on the mechanism of Cd toxicity has involved a single plant species or variety. In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants. Many factors affect the interactions between metals as reported earlier in biological systems (Sharma *et al.*, 1985).

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Toxicity of duckweed (*Lemna*) was independent of temperature within the range of 20–30°C (Nasu and Kugimoto, 1981). In many other species toxicity probably decreases with temperature. Increasing pH usually increases the mortality in a wide range of species.

Cytogenetic effects of cadmium

Cadmium is a non-essential heavy metal and a powerful enzyme inhibitor (Lockwood, 1976); it has been considered as an extremely significant pollutant due to its high toxicity and great solubility in water. Exposure of three species of green algae to CdCl₂ resulted in the formation of intramitochondrial granules containing cadmium (Silverberg, 1976). Swelling, vacuolization and degeneration of mitochondria were observed, implying significant cytotoxicity. Rosas *et al.* (1984) reported that cadmium inhibited cell proliferation and showed a low mitotic index of *Eichhornia crassipes* when it was exposed to the cadmium solution. Chromosomal aberrations have been found in onions (Avanzi, 1950), beans (Oehlkers, 1953), peas (Von Rosen, 1954) and barley (Degreave, 1971). The cytotoxic action of cadmium in plants was reported earlier by many workers (Levan, 1945; Avanzi, 1950; Von Rosen, 1954; Jacobs *et al.*, 1956; Hecht and Bock, 1971; Siegel, 1977). Rosas *et al.* (1984) reported that in plants exposed to cadmium for 24 h the cadmium penetrated into the cells inducing physiological and genetical damages, especially at concentrations of 1.5 to 10 mg litre⁻¹. They also mentioned that cadmium inhibited cell division and altered the chromosomes. The inhibition of cell proliferation, shown by the low mitotic index, was proportional to the concentration and time of exposure. The induction of pycnosis in root cells confirmed the cytotoxic action of cadmium. This effect has also been reported in the proplast of *Nicotiana tabacum* (Siegel, 1977).

DIFFERENTIAL CADMIUM TOLERANCE IN PLANTS

The phenomenon of heavy metal tolerance in plants has attracted the interest of plant ecologists and physiologists as well as evolutionary biologists (Baker, 1987). Development of metal tolerance is a major way to reduce the harmful effects of excessive exposure to heavy metal ions (Tyler *et al.*, 1989). Classical studies on vascular plant species do not unequivocally indicate that tolerant races should be competitively inferior in normal soils. The tolerance of vascular plants to heavy metals does not usually import a restricted uptake of these elements by the roots. This means that tolerant plants with a high capacity of immobilizing heavy metal ions in their root tissues might have to renew the most active parts of their below-ground biomass more often than non-tolerant plants in normal soils.

Plant species and varieties vary widely in tolerance to excess cadmium in the growth medium (Hertstein and Jager, 1986). In several species, these differences are

genetically controlled. Closely related genotypes are valuable tools for studying the physiological mechanisms of toxicity or tolerance. The interpopulation variation of plant species tolerant to Cd were reported by various authors (Bradshaw, 1984; Symeonidis *et al.*, 1985). Epstein (1969) pointed out that an element present in excess could interfere with metabolism through competition for uptake, inactivation of enzymes, displacement of essential elements from functional sites, or alteration of the structure of water. Many of these probably effect modification of membrane structure and function. The exact physiological mechanisms of cadmium toxicity or tolerance are still debated; these may vary with the plant species and varieties controlled by different genes through different biochemical pathways.

Obviously cadmium-tolerant plants must be able to prevent the absorption of excess cadmium or detoxify the Cd after it has been absorbed. Various physiological factors are associated with differential Cd tolerance, e.g. pH and other factors. In accordance with Antonovics *et al.* (1971) and Woolhouse (1983), it may be assumed that there exist different specific mechanisms of tolerance within each of the populations which are related to the contamination of their original habitat. Hertstein and Jager (1986) indicated that a principal mechanism for Cd tolerance in tolerant populations of *Agrostis tenuis* and *Arrhenatherum elatius* was associated after the protection of the shoots from excess cadmium. They also reported changes in the activities of enzymes like peroxidase and acid phosphatase to indicate metabolic responses of plants to stress conditions. Subsequently, Ernst (1980) reported that the acid phosphatase and peroxidase activity increased in cadmium-tolerant plants. Foy *et al.* (1978) and Jager *et al.* (1983) reported that Cd concentrations in plants were quite often different in roots and shoots. Metal-tolerant plants showing a diminished accumulation of the respective metal in shoots have been found to accumulate higher amounts in roots as compared to non-tolerant ones of the same species (Baker, 1984; Coughtrey and Martin, 1979; Woolhouse, 1983). Different species of aquatic macro- and micro-flora were reported to accumulate high concentrations of cadmium (Kumada *et al.*, 1973; Stokes *et al.*, 1973; Ravera, 1984).

EFFECT OF CADMIUM ON PLANT METABOLISM

In general, many of the plant species are resistant to or can be tolerant to certain amounts of heavy metals. This is probably achieved through trapping of these metals with metal-binding proteins. Overnell (1975) reported that (0.01–0.1 mg litre⁻¹) cadmium reduced the concentration of ATP and chlorophyll in many species, and decreased oxygen production. Li (1978) suggested that the algal membrane of *Thalassiosira* was the main site of cadmium action, although exposure of algae to heavy metals often resulted in the loss of cellular potassium. Unusual metal and cysteine-rich proteins, generally

named metallothioneins, have been recognised as some of the major metal-binding proteins in various kinds of plants and microorganisms (Fujita and Kawanishi, 1986). Nowadays it has been suggested that Cd-binding complexes similar to the metallothionein exists in several higher plants such as tomato (Bartolf *et al.*, 1980), bean (Weigel and Jager, 1980), tobacco (Wagner and Trotter, 1982), soyabean (Casterline and Barnett, 1982), rice (Kaneta *et al.*, 1983) and *Agrostis gigantea* (Rausser, 1984). Fujita and Kawanishi (1986) reported the purification and characterization of a Cd-binding complex from the root tissue of water hyacinth cultivated in a Cd^{+2} containing medium. They also indicated that water hyacinth root Cd-binding complex was identical to fission yeast Cd-BPI inorganic sulphur which was easily released as hydrogen sulphide by acidification.

PHYTOTOXICITY

To evaluate meaningful physiological and biochemical effects of toxic metals, one must examine conditions (different metals and their concentrations) which are relevant to phytotoxicity in nature (Cunnigham *et al.*, 1975). Many reports were not based on relevant metal concentrations related to the species. Before starting the phytotoxicity experiment one should be fully aware of the movement of metal in plant system and its absorption and translocation.

Availability of metal in soil depends on soil adsorption strength as well as plant effectors such as root exudates for metal chelation or reduction. Concepts of metal equilibrium in soil and metal movement from soil to root have been developed. Many reports on a model for the chemical activity of metal ions in soil are available (Hodgson, 1963, 1969; Lindsay, 1972, 1973, 1974; Santillan-Medrano and Jurinak, 1975; Street *et al.*, 1977). Studies on Cd have shown several implications for this chemical equilibrium model. Firstly, metals absorbed by organic matter, clays and hydrous oxide of Fe, Mn and Al cause the metal activity to be considerably lower than equilibrium values for known inorganic compounds. Soluble low-molecular weight chelates of the metals are formed in soil solution; this keeps the free metal activity low but increases the soluble metal concentration many-fold. Both adsorption and chelation are strongly affected by soil pH (Tiller *et al.*, 1972; Graham, 1973). In the pH range of productive agricultural soils, pH adjustment will affect sludge-borne Cd uptake by most field crops to only a minor extent. The highest grain-Cd concentrations occurred at a soil pH of about 6.0. Street *et al.* (1977) found that uptake of Cd by corn was less in one of the most acid soils that also had the highest organic matter content. They also reported that corn plants grown on soil amended with a Cd salt (CdSO_4) took up more of the metal than soil amended with sludge containing cadmium. Hinesly *et al.* (1984) reported decreased uptake of heavy metals by plants with the increase of soil pH.

Several other soil factors also play roles in metal binding, although these are less important than pH. The organic matter, clays and hydrous oxides absorb metals (Ellis and Knezek, 1972); soils with higher metal absorption capacity have lower potential for uptake of metals by plants (Miller *et al.*, 1976).

Metal phytotoxicity can result only if metals can move from the soil to plant roots. Several authors have summarized the movement process (Barber, 1974; Lindsay, 1974; Chaney *et al.*, 1975; Loneragan, 1975). Barber (1974) has described a model for this movement using heavy metals, while most other models have been applied only to macronutrients (Claassen and Barber, 1976). Metals in the soil solution (free ions, ion pairs of chelates) can move with the soil water (mass flow) as the plant absorbs water for transpiration. They also indicated that if the rate of absorption of metals by a plant exceeded the rate of arrival by convection, a depletion zone was created at the root and the concentration gradient promoted diffusion from the soil to the root. Besides the role of chelators, numerous other factors affected metal chelators: soil pH, metal concentration and strength of absorption, soil texture, soil bulk density, soil moisture content and concentrations of other nutrients and cations. Smiley (1974) reported that the change in soil pH near the root could strongly affect metal movement to the root. Very little study has been made on the movement of metals in the root zone.

PHYTOTOXICITY AND THEIR INTERACTIONS WITH NUTRIENTS

Ideally, each metal causing phytotoxicity would cause some characteristic symptom that would allow its diagnosis; further the symptoms would be apparent before substantial economic or ecological damages occurred (Hewitt, 1966). The most general symptoms are stunting and chlorosis. The chlorosis may appear to be due to Fe deficiency, and the interactions of toxic metals and Fe have been studied for many years. Chlorosis by the presence of excess of Cd appears to be due to a direct or an indirect interaction with foliar Fe content. Analysis of chlorotic young foliage showed low foliar Fe when soils or solutions contained high cadmium (Haghiri, 1973; Garty *et al.*, 1992). Chaney *et al.* (1975) reported that Cd caused chlorosis, which is observed only at low pH or neutral soils, and also low availability of iron (Brown and Jones, 1975). Symptom-bearing leaves usually have higher concentrations of iron than normal leaves (Root *et al.*, 1975; White, 1976). Root *et al.* (1975) reported that Cd-induced chlorosis might be due to changes in Fe:Zn ratios in corn leaves. Imai and Siegel (1973) reported Cd-induced curling of stems in the case of bean plants. Christensen (1984) reported that a decrease in Ca concentration from 10^{-3} to 10^{-2} M is found to reduce the Cd absorption in plants.

Interactions between Cd and other metals

Toxicity of cadmium cannot be considered without taking into account the translocation of several other essential elements. In the plant system, toxic effects of cadmium have been shown to be modified by essential elements like zinc, calcium, iron, copper and manganese. In addition, proteins and vitamins have also been reported to alter cadmium toxicity. Hewitt (1966) hypothesized that elements whose physical and chemical properties are similar will act antagonistically to each other biologically. Elements of similar type help to compete for the same transport and storage sites in the cell and displace each other from reactive enzymatic and receptor proteins. Selenium may act as a synergist to cadmium, whereas cadmium may inhibit copper toxicity. Moshe *et al.* (1972) observed no synergistic effect in *Chlorella* when Cd, Cu, Cr and Ni were added to culture media. Although low levels ($< 1.0 \text{ mg litre}^{-1}$) of lead increased the toxicity of cadmium ($0.1 \text{ mg litre}^{-1}$) in phytoplankton, antagonism occurs when the concentration of lead exceeds that of cadmium. Pretreatment of algae with metals (Ni and Hg) reduced cadmium toxicity; this may reflect competition among metals for cellular binding sites.

Interaction between cadmium and zinc

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 those two elements in the biological system is likely to be similar. The fact that cadmium is a toxic heavy metal and zinc an essential element makes this association interesting as it raises the possibility that the toxic effects of cadmium may be preventable or treatable by zinc. Hinesly *et al.* (1984) indicated that both cadmium and zinc uptake by plants were dependent on the pH of the growing media. Ravera (1984) showed that Cd had toxic effects in plants in the way of photosynthesis and also indicated various changes in biological activities. Subsequent studies have confirmed these findings and extended the interaction to other toxic effects of cadmium like inhibition of cell proliferation, and cytotoxic action (Rosas *et al.*, 1984) and growth suppression in plants (Chaney *et al.*, 1975). The biochemical mechanisms of Cd-Zn interaction are unknown, but various cellular and subcellular processes like the ratio of Cd to Zn in the tissues, induction of synthesis of different types of metallothionein, binding characteristics of metallothionein, alteration of absorption and tissue distribution of one metal by another, and competition at the level of zinc containing metalloenzymes are known to be involved in the interactions. In recent years it has been suggested that Cd-binding complex similar to the metallothionein exists in several higher plants such as tomato (Bartolf *et al.*, 1980), kidney bean (Weigel and Jager, 1980) and cabbage (Wagner, 1984). Subsequently, Fujita and Kawanishi (1986) reported the presence of another type of Cd-binding complex named Cd-BPI in root tissue of water hyacinth cultivated in a Cd^{2+} -containing medium. It is

liable to be degraded by both acid and mercaptoethanol. Dabin *et al.* (1978) concluded that zinc and cadmium are bound to different ligands in rice roots.

CADMIUM UPTAKE AND TRANSPORTATION

Cadmium is one of the most dangerous heavy metals due to its high mobility and the small concentration at which its effects on plants begin to show (Barcelo and Poschenrieder, 1992). Jarvis *et al.* (1976) found that the roots of lettuce released much more of their absorbed Cd for translocation to the shoots than other crops (ryegrass and orchardgrass). The greater translocation is due to active transport or lack of metal absorption to fixed or soluble chelators in the root or perhaps due to exchange with the Ca, Mn and Zn moving through the roots (John, 1976). Moral *et al.* (1994) reported that Cd was easily transported to aerial parts of tomato and was not detected in fruits. Hinesly *et al.* (1984) reported that pH of the soils had great influential on cadmium transportation in corn (*Zea mays* L.). John *et al.* (1972) found that soil pH interacted significantly with sludge-borne soil Cd contents to affect corn leaf Cd concentrations. The highest grain-Cd concentrations occurred at a soil pH at about 6.0. Uptake of cadmium by corn was less from the most acid soil that also had the highest organic matter content (Street *et al.*, 1977). Garcia-Miragaya and Page (1976) found that the ratios of complexed to uncomplexed Cd were independent of Cd concentration and slightly affected by pH over a range of 6.0–8.5. Many factors in soil have been shown to influence the uptake of heavy metals by plants. Cadmium uptake increased with decreasing soil pH (Lagerwerff, 1971; Miller *et al.*, 1976) and decreased with increasing soil cation exchange capacity (Haghiri, 1974).

Cadmium appears to be absorbed passively (Cutler and Rains, 1974) and translocated freely (Jarvis *et al.*, 1976). Chelators in nutrient solutions can help in cadmium uptake (Francis and Rush, 1974). Pronounced interactions between Zn and Cd occurred in cadmium uptake and translocation (Chaney *et al.*, 1976; Chaney and Harnick, 1978). Apparently, part of Cd toxicity was a result of Cd interference in a Zn-dependent process (Falchuk *et al.*, 1975).

The differences in the ability of plants to accumulate heavy metals have been related to differences in their root morphology (Hemphill, 1972; Schierup and Larsen, 1981). The latter suggested that a plant with numerous thin roots would accumulate more metals than one with few thick roots. Wahbeh (1984) compared the absorption and accumulation of Cd, Mn, Zn, Mg and Fe in three species of sea grasses. The two species *Halophila ovalis* and *Halophila uninervis* had significantly higher photosynthetic and respiratory rates than *Halophila stipulacea* (Wahbeh, 1983). Chukwuma (1993) compared the accumulation of cadmium, lead and zinc in cultivated and wild plant species in the derelict lead-zinc mine. There was an overall reduction in the potential toxicity of Cd by Zn through simple

mass action effect specifically for cultivated plants, while other additional tolerant or adaptive mechanisms might be operative in the wild plants. McKenna *et al.* (1993) reported the interactions between Zn and Cd in nutrient solution and their effects on the accumulation of both metals in plant roots and leaves. They reported higher Cd concentration in the older than in younger leaves of lettuce and spinach. The potential accumulation of Cd in old leaves could not be solely due to the transpiration rate. Metal-binding peptides were present in older leaves in higher amounts than in younger leaves in tobacco and cadmium was transported into the vacuoles as a means of detoxification (Vogeli-Lange and Wagner, 1990). Cadmium concentrations were reported to be higher in roots than in shoots (Cataldo *et al.*, 1981; Rauser, 1986). As per the recommendation of the National Research Council of the United States (1980) the Cd content of forage crops should not be more than $0.5 \mu\text{g g}^{-1}$ in order to limit the concentration of Cd in the liver and kidney of animals feeding on these crops to protect humans against cadmium toxicity through concentration of these organs.

PHYSIOLOGY AND BIOCHEMISTRY OF CD PHYTOTOXICITY

Studies on the physiology of metal toxicity are difficult. The activity of a given metal in the cytoplasm is affected by chelating carboxylic and amino acids, and the pH. Foy *et al.* (1978) reported that the metal phytotoxicity depended on soil pH, organic matter and phosphate status of the soil. The physiology of metal toxicity begins with the increased metal supplied to the root which proceeds to a failure of a clearly defined 'essential-to-life' plant process. Cadmium availability is strongly influenced by soil pH, especially presence of chelators, presence of Cl^- etc. (Hahne and Kroontje, 1973; Takijima *et al.*, 1973; Garcia-Miragaya and Page, 1976; Chaney and Giordano, 1977; Street *et al.*, 1977). Although many physiological studies on plants have been reported (Bazzaz *et al.*, 1974a,b; Koeppe *et al.*, 1975), Cd appears to be absorbed passively (Cutler and Rains, 1974) and translocated freely (Jarvis *et al.*, 1976). Simon (1977) reported that some of the ecotypes were Cd-tolerant because of natural adaptation. Pronounced interactions occur between Zn and Cd, and Ca and Cd uptake and translocation in plants have been reported (Largerwerff and Biersdorf, 1972; John *et al.*, 1976; Chaney and Hundeman, 1977). Apparently, part of Cd toxicity is a result of Cd interference in a zinc-dependent process (Falchuk *et al.*, 1975; Rorison, 1975). Certainly, a study at chronic toxic level is more meaningful than a work only at acute toxic levels (Collins *et al.*, 1976; Cunningham *et al.*, 1977).

CONCLUSION

Cadmium toxicities in plants are often clearly identifiable entities; instead, they may be the results of complex interactions of the major toxic ions with other essential

or nonessential ions and with other environmental factors. The phytotoxic mechanisms involve different biochemical pathways in different plant species. Differential species tolerances to Cd toxicity almost certainly involves differences in the structure and function of membranes. Other promising approaches in studying metal toxicity in tolerant and sensitive plant genotypes include determining the chemical compartmentation of metals in various plant fractions, levels and kinds of organic and amino acids which may act as metal chelators and detoxifiers, levels and forms of enzymes, and changes in root permeabilities to ions and molecules.

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