



Review Paper

Cadmium: Toxicity and tolerance in plants

S. Aiman Hasan¹, Q. Fariduddin¹, B. Ali¹, S. Hayat*¹ and A. Ahmad²

¹Plant Physiology Section, Department of Botany, Aligarh Muslim University, Aligarh - 202 002, India

²Department of Applied Sciences, Higher College of Technology, Al-Khuwair-133, Sultanate of Oman

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Abstract: Of all the non-essential heavy metals, cadmium (Cd) is perhaps the metal which has attracted the most attention in soil science and plant nutrition due to its potential toxicity to humans, and also its relative mobility in the soil-plant system. This review summarizes the toxic symptoms of Cd in plants (i.e. growth retardation, alterations of photosynthesis, stomatal movement, enzymatic activities, water relations, interferences with mineral uptake, protein metabolism, membrane functioning, etc.) but also includes the mechanisms of cadmium uptake, translocation and deposition. Moreover, it also throws light on chelation, including identification of Cd ligands present in cytosol and vascular tissue. Cadmium-induced oxidative stress is also considered as one of the most widely studied topics in this review.

Key words: Cadmium, Carbonic anhydrase, Chelation, Chlorophyll, Net photosynthetic rate
PDF of full length paper is available with author (*hayat_68@yahoo.co.in)

Introduction

Heavy metals are defined as metals having a density higher than 5 g cm³. Of the total 90 naturally occurring elements, 53 are considered heavy metals (Weast, 1984) and few are of biological importance. Based on their solubility under physiological conditions, 17 heavy metals may be available to living cells and have significance for the plant and animal communities within various ecosystems (Weast, 1984). Among the heavy metals Zn, Ni, Cu, V, Co, W and Cr are non toxic heavy elements at low concentration. As, Hg, Ag, Sb, Cd, Pb and Al have no known function as nutrients and seems to be more or less toxic to plants and microorganisms (Goldbol and Huttermann, 1985; Niess, 1999; Sogut *et al.*, 2005; Beak *et al.*, 2006).

Heavy metals are significant environmental pollutants (Angelone and Bini, 1992; Berry, 1986; Kevresan *et al.*, 1998) and their availability in soils depends on natural process, especially lithogenic and pedogenic, but also on anthropogenic factors such as mining, combustion of fossil fuels, urban waste disposal, soil runoff, metal working industries, boating activities, phosphate fertilizer application, sewage treatment plant effluents, and municipal solid waste disposal sites Table 1.

Increase in levels of heavy metals in soils could also be attributed to factors such as soil properties or different agricultural practices, for example application of sludge to agricultural land (Foy *et al.*, 1978). Household waste, municipal and industrial waste are also sources of heavy metals to soil Table 2 (Alloway, 1995). Soil contaminated with the heavy metals above the permissible limit lead to declines in agricultural yields (Nellessen and Fletcher, 1993; Salt and Rauser, 1995; Akinola and Ekiyoyo, 2006). The accumulation of heavy metals in the environment is now becoming a major cause of environmental pollution.

Toxicity of heavy metals: Of the known metals Cd²⁺, Ni²⁺, Zn²⁺, Cu²⁺ are toxic to plants at elevated levels, whereas Pb²⁺ has been generally observed to cause phytotoxicity (Foy *et al.*, 1978). The concentration of Cd in non-polluted soil solution ranges from 0.04 mM to 0.32 mM, and its concentration in the range of 0.32 to about 1 mM may be categorized as polluted (Sanita di Toppi and Gobbielli, 1990). Of the major heavy metals, Cd is a major industrial pollutant particularly in areas associated with smelting of zinc and heavy road traffic (Somasekaraiah *et al.*, 1992; Das *et al.*, 1997). High concentrations of heavy metals in the soil are toxic to most plants (Baker, 1986; Ernst, 1980; Macnair, 1993; Wool House, 1983). There are two types of causal relationships existing between the high concentration of heavy metals in the soil and the expression of toxicity symptoms. On the one hand, heavy metals compete with essential mineral nutrients for uptake thereby disturbing the mineral nutrition of plants (Clarkson and Luttge, 1989) and on the other hand, after uptake by the plant, it accumulates in plant tissue and cell compartments and hampers the general metabolism of the plant (Turner, 1997; Thurman and Collins, 1983; Taylor, 1988).

Heavy metal accumulation in plants has multiple direct and indirect effects on plant growth and alters many physiological functions (Wool House, 1983) by forming complexes with O, N and S ligands (Van Assche and Clijsters, 1990). They interfere with mineral uptake (Yang *et al.*, 1998; Zhang *et al.*, 2002; Kim *et al.*, 2003; Shukla *et al.*, 2003; Drazic *et al.*, 2004; Adhikari *et al.*, 2006) protein metabolism (Tamas *et al.*, 1997) membrane functioning (Quariti *et al.*, 1997; Azevedo *et al.*, 2005) water relations (Kastori *et al.*, 1992) and seed germination (Iqbal and Siddiqui, 1992; Al-Hellal, 1995). Cadmium inhibited net photosynthesis in green algae, corn, soybean, and pigeon pea (Bazzas *et al.*, 1974; Sheoran *et al.*, 1990; Krupa *et al.*, 1993), O₂ evolution in *Anacystis nedulens* and photosystem II (PS II) in isolated chloroplasts of maize and spinach (Bazzaz *et al.*,

1974). Moreover, they cause metabolic disturbance by altering essential biochemical reactions (Krupa, 1988; Hermens *et al.*, 2004; Epstein and Bloom, 2005). Contrary to this potato plants grown in soil with high concentrations of heavy metals led to an increase in nitrate reductase activity.

Accumulation of heavy metals not only decreased nodulation (Casella *et al.*, 1988; Lorenzo *et al.*, 1994; Gogoreena *et al.*, 1995, 1997; Farnandez *et al.*, 1996; Comba *et al.*, 1998) and growth of leguminous plants (Hasan *et al.*, 2007a) but also inhibited the growth of microorganisms present in the soil (Coppola *et al.*, 1988; Lorenz *et al.*, 1994).

Basic chemical properties of cadmium: Cadmium (Cd) is the element of group IIB in the periodic table and its atomic number is 48. It shows chemical similarity with the other elements of group IIB especially with zinc (Zn) and mercury (Hg). Cadmium is commonly associated with Zn and Hg in natural geologic settings. Cd (II) is relatively more stable in a positive valence of two and occurs in most of natural aquatic system in this state (Baes and Mesoner, 1976). The ability of Cd to form complexes with ammonia, amines, halide ions and cyanide indicate similarities with most of the transition metals series ions.

Cadmium is a white lustrous and tarnishable relatively volatile element with melting and boiling points of 321 and 767 °C, respectively, and a heat of vaporization of 26.8 K cal mol⁻¹ (Cotton and Wilkinson, 1966). The latter property makes it susceptible to enter the atmosphere which is a major component of the global Cd cycle (Laws, 1993).

Chelation of cadmium: In order to cope with highly toxic metals or to maintain the level of essential metals within the physiological range, plants have evolved a variety of complex mechanisms for metals tolerance. Among these, Berry (1986) suggested that accumulation and detoxification as the main strategies that serve to control the uptake and accumulation of heavy metals. Different plant species, commercial varieties, cultivars and ecotypes vary widely in their tolerance to excess concentrations of heavy metals (Al-Hellal, 1995).

One recurrent general mechanism for heavy metal detoxification in plants and other organisms is chelation by a ligand and in some cases, the subsequent compartmentalization of the ligand-metal complex. Formation of these complexes is termed chelation and the complexes are known as chelates. Two major groups of complexes have been isolated from different higher plants/cultures *in vitro* –

- (i) 8-14 kDa complexes, similar to those of metal 40-thioneine (MTs).
- (ii) 1.5-4 kDa complexes, the PCs = (y Glu-Cys)_n Gly (Steffens, 1990).

These complexes contain a number of amino acids with glutamic acid, cysteine and glycine as the major constituents.

PCs are synthesized enzymatically by PC synthase in higher plants (Gekeler *et al.*, 1989; Kneer and Zenk, 1992). This enzyme removes a y Glu-Cys residue from one molecule of glutathione (y Glu-Cys-Gly) and couples to another glutathione. Co-production of PCs and MTs upon exposure to trace metals was reported in yeast

Candida glabrata (Mehra *et al.*, 1988). Moreover, the rate of phytochelatin production elevated in rice and groundnut when exposed to cadmium (Shanthala *et al.*, 2006)

PC synthase was purified to homogeneity in cell cultures of *Silene cucufalus*, *Beta vulgaris*, and *Equisetum giganteum* (Robinson *et al.*, 1993). PC synthase catalyzes the formation of metal-chelating peptides (=PCs) from glutathione in the presence of heavy metal ions. Incubation of PC under specific conditions in the absence of heavy metal ions did not lead to the formation of PC peptides. However, addition of Cd to the incubation mixture instantaneously reactivated this enzyme (Loffler *et al.*, 1989).

It has been shown that heavy metals cause cell death in plants by inactivating enzymes, through metal sensitive groups, rendering them catalytically inactive (Van Assche and Clifsters, 1990). Therefore, plants as an adaptive strategy might have developed certain physiological and biochemical mechanisms to tolerate metal toxicity. PCs could reduce cytoplasmic toxicity by complexing intracellular metals; the PC metal complexes would be expected to be less toxic to cellular metabolism than free metal ions. The best indirect evidence for such an assumption comes from tomato cells selected for Cd tolerance; these cells accumulated PCs to considerably higher levels than did Cd-sensitive cells (Steffens, 1990). More direct evidence of PCs in protecting plant enzymes was reported in suspensions cell cultures of *Rauvolfia serpentina* that were treated with Cd (Meuwly and Rauser, 1992).

In addition to PCs, other intracellular ligands may play a role in complexing Cd (glutathione and various polycarboxylic acids). Barley and maize seedlings exhibited retardation in shoot and root growth after exposure to Cd. The total protein and glutathione content of barley and maize roots declined with an increase in heavy metal concentration however, this decrease was more in the roots than in the shoots (Shanthala *et al.*, 2006). Thus, glutathione is somehow involved in the biosynthesis of PCs (Robinson *et al.*, 1993) which in turn confers tolerance to the cells (Jackson *et al.*, 1987). Sulphate salts have also been reported to afford protection to Cd toxicity by enhancing sulphate uptake, leading to increased synthesis of glutathione, a precursor of PCs. Complexation by organic acids, e.g., citrate and malate, with Cd in vacuoles was reported in tobacco suspension cultures (Kortz *et al.*, 1989).

Vacuolar compartmentalization prevents the free circulation of Cd ions in the cytosol and forces them into a limited area (Sanita di Toppi and Gabrielli, 1999). Several studies have shown that the vacuole is the site of accumulation of a number of heavy metals including Zn and Cd (Ernst *et al.*, 1992). Extracellular chelation by organic acids such as citrate and malate is important in mechanisms of aluminum tolerance. For example, malate efflux from root apices is stimulated by exposure to aluminum and is correlated with aluminum tolerance in wheat (Delhaize and Ryan, 1995). Some aluminum – resistant mutants of *Arabidopsis* also have increased organic acid efflux from roots (Larsen *et al.*, 1998).

Table - 1: Summary of the major anthropogenic inputs of cadmium to soils (Berry, 1986)

Source of cadmium	Concentration in soil (mg kg ⁻¹)	Input to soil (g ha ⁻¹ year ⁻¹)
Wet/Dry deposition, general	-	<1.10 – 9.0
Wet/Dry deposition, smelters	-	25.0 – 1000
Street dust	1.5 – 12.0	-
Rubber tyre wear	20 - 90	-
Incenerator fly-ash	2.6 - 68	-
Direct application		
Phosphate fertilizers	0.2 – 3451.0 – 641.0 (kg ⁻¹ P)	0.3 – 8.9
Byproduct gypsum	<6.0	-
Sewage sludge	<1.0 – 3410	Up to 150
Compost	0.26 – 11.7	-

Table - 2: Range of concentration (mg kg⁻¹ dry matter) for selected trace metals in sewage sludge (Alloway, 1995)

Metals	Minimum	Maximum
Arsenic (As)	1.0	960.0
Cadmium (Cd)	<1.0	34.0
Chromium (Cr)	8.0	40600.0
Copper (Cu)	50.0	8000.0
Mercury (Hg)	0.01	55.0
Molybdenum (Mo)	1.0	40.0
Nickel (Ni)	6.0	5300.0
Lead (Pb)	29.0	3600.0
Zinc (Zn)	91.0	4900.0

Mechanism of cadmium uptake, translation and deposition:

Plant response to increased levels of Cd in soil differs in terms of the ability of various plants species to take up and transport increased levels of Cd. Cd can be easily transported within plants (Epstein and Bloom, 2005) in the form of metallo-organic complexes, but mechanisms of uptake, translocation and deposition are quite complex; the bio-availability of Cd in soil depends upon its concentration, pH, temperature, redox potential and concentration of other elements. The acidification of the rhizosphere and exudation of carboxylase are considered to be potential targets for enhancing metal accumulation (Clemens *et al.*, 2002). The mechanism for the uptake of Cd by the plant root generally involves competition for absorption sites between the heavy metals and several mineral nutrients sharing similar chemical properties (Jarvis *et al.*, 1976). The reduction of K, calcium (Ca) and magnesium (Mg) in tissue due to high concentrations of Cd has been reported in cucumber and tomato plants (Burzynski, 1988), maize (Walker *et al.*, 1977) and lettuce. An antagonism between zinc and cadmium and their active absorption was observed in lettuce roots (Costa and Morel, 1994). Moreover, other mineral nutrients such as nitrate, not sharing similar chemical characteristics with Cd, are also affected by its presence. In most environmental conditions, Cd enters first into the

roots and damages the root system first (Sanita di Toppi and Gabbrielli, 1998).

The mechanisms that control the uptake of Cd by plant roots and accumulation in edible parts of the plant are not well understood. Cadmium absorption across the plasma membrane of root cells is controlled by the electrochemical potential difference between the activity of Cd²⁺ in the cytosol and that in the root apoplasts. The large negative membrane potential alone provides more than enough energy to drive Cd²⁺ uptake even at low concentrations of Cd²⁺. The kinetics of Cd²⁺ absorption by roots shows bio-phasic characteristics with saturable components at low Cd²⁺ activities in the absorption solution and a linear component at higher Cd activities (Costa and Morel, 1993). Although the biphasic nature of Cd²⁺ transport is open to speculation, it should be related to two separate types of membrane transport systems *eg.* (i) Movement via a saturable cation transported in the plasma membrane and (ii) diffusive movement channels with linear concentration kinetics. Absorption of Cd could also occur as inorganic complexes of Cd such as CdCl⁺, CdCl₂ and CdSO₄ (McLaughlin *et al.*, 1996) or as organic complexes such as phytometallophore complexes. Von Wirén *et al.* (1996) speculated that Zn(II) phytometallophore complexes were readily absorbed by maize roots but the binding sites present in the plasma membrane of the root are not highly specific for Fe(III) phytometallophores, allowing the transport of other metals like Cd. However, there is no direct evidence in support of Cd binding with phytometallophores during its transport in root cells.

Moreover, other metals, especially Zn²⁺, interact with Cd and reduce uptake during Zn-deficient conditions. Zinc's role in maintaining the integrity of the root cell plasma membrane is evident from the fact that cereal roots grown under Zn-deficient conditions are implicated in reduced Cd uptake following Zn application (Welch, 1995). Cd can easily penetrate the root system of xylem through the apoplastic and/or symplastic pathway (Salt Rauser, 1995) and reaches tissues of aerial parts of the plants (Yang *et al.*, 1998). Despite the difference in mobility of metal ions in the plants the metal content is generally greater in the root than in the above-ground tissues (Ramas *et al.*, 2002). Most Cd ions are retained in the roots and only small amounts are transported to shoots (Cataldo *et al.*, 1983). In general, the concentration of Cd in plants decreases in the order: root > leaves > fruits > seeds (Blum, 1997; Sharma *et al.*, 2006).

The extent of Cd transport into edible organs differs widely among crops. In soybean more than 98% of the accumulated Cd was retained by roots and only 2% was transported to shoots (Calatdo *et al.*, 1983). Moreover, Cd was easily transported to the shoots and leaves of tomato plants but was not detected in fruits (Morel *et al.*, 1994). After uptake by the roots Cd is transferred to the shoots, through the cells of vascular bundles. Movement of the trace metal is also regulated by vascular tissues (Kuppelwieser and Feller, 1991). There are numerous cell membrane barriers that Cd must cross to enter edible plant organs and this is especially true for seeds and grains.

Xylem transport: The chemical composition of xylem sap is very different from phloem sap, xylem sap having a pH ranging from 5.0 – 6.0, a more oxidizing redox potential and a much lower concentration of organic compounds, such as sugars, peptides and proteins. As described earlier Cd not only prefers to form bonds with sulphhydryl ligand groups, but also binds to N and O ligand groups. Thus, cysteine and other sulphhydryl- containing compounds (phytochelatins, glutathione etc.) and various organic acids (citrate) and other amino acids in xylem sap could be important in transporting Cd from roots to shoots. Although there are no definitive studies on the forms of Cd in xylem sap, Senden *et al.* (1994) reported that treating xylem sap with citric acid increased Cd transport through xylem vessels of excised tomato stem/leaf systems. Furthermore, when citric acid was supplied to the roots of tomato plants, Cd(II) uptake increased two- fold and Cd transport from roots to shoots increased 6- to 8- fold. Thus, citrate can stimulate Cd uptake in the root and transports it in xylem sap. Mori *et al.* (1991) reported the occurrence of deoxymugineic and epihydroxymugineic acid phytometallophores in xylem sap collected from rice seedlings and Cataldo *et al.* (1983) reported finding Cd(II) associated primarily with components of the amino acid/peptide fraction of xylem sap collected from xylem exudates of soybean, while polyvalent ions such as Fe(III) were found associated with organic acid complexes.

Phloem transport: The abundance of organic ligands (organic acids, amino acids, sugars, peptides and proteins) and the alkaline pH (pH 7.0-8.0) of phloem sap ensures that virtually all Cd²⁺ carried in the phloem is present in complexed conditions that favour the stability of sulphhydryl-containing ligands, which are likely to be carriers of Cd. However, there is no direct evidence for any specific Cd complexes in phloem sap, but they could include phytometallophores, such as nicotinamine, metallothioneins, the phytochelatins as well as glutathione, cysteine and other sulphhydryl-containing molecules. Deoxymugineic acid a phytometallophone was identified in phloem sap of rice plants (Mori *et al.*, 1991). It is presumed that phytochelatins and phytometallophores play a role in Cd movement in phloem sap and in loading Cd into seeds and grains. Moreover, nicotinamine could also function as an iron transporter in the phloem of all higher plants as nicotinamine was essential for iron mobilization in plants from phloem sources (like mature leaves) to phloem sinks (reproductive organs, newly forming roots leaves and growing points) (Scholz, 1989). Zinc- binding substances similar to the phytochelatins have been reported in phloem sap of citrus (Taylor *et al.*, 1988).

Deposition: The compounds that bind Cd in mature seeds during their development are not known. Cadmium may bind to phytate (myo-inositol hexaphosphate) in globoid crystals within the protein bodies of developing seeds. Other metals (Fe, Zn, Mn, Mg and Ca) have been reported to be associated with phytate within globoid crystals of these organelles (Welch, 1986). Van Steveninck *et al.* (1990, 1992 and 1994) reported that phytate globular deposits containing Zn was formed in small vacuoles of root cells within the elongation zone of roots of soybean, maize and wheat. However,

Cd was not bound to phytic acid in these small root-cell vacuoles. Alternatively, Cd could be found to 2nd class metallothioneins in developing seeds and grains because genes for the expression of these sulphhydryl-rich proteins (known to bind Zn) have been reported in seeds of some plant species like wheat and maize (White and Rivin, 1995; Hsieh *et al.*, 1996).

Further research conducted to determine the major form of Cd in edible portions of important crops showed that in oat (*Avena sativa* L.) roots, Cd transport from cytosol to the vacuole across the tonoplast is demonstrated through Cd²⁺/H⁺ antiport activity. After the uptake of the heavy metal by the plants it is deposited/accumulated in plant tissue and cell compartments (Prasad, 1995).

Effect of cadmium on the plant growth: Cadmium is not an essential nutrient and at high concentration inhibits plant growth (Anita *et al.*, 1990; Aery and Rana, 2003). It has also been reported that even at relatively low concentrations it alters plant metabolism (Van Assche and Clijsters, 1990). The presence of cadmium in the soil decreases the growth of soybean (Dewdy and Ham, 1997, Cataldo *et al.*, 1983) and chickpea plants (Hasan *et al.*, 2007b). High concentrations of Cd decreased cell growth as well as whole plant growth (Prasad, 1995).

Effect of cadmium on fresh and dry mass: The interaction of *Rhizobium* in the nodules of chickpea was found to be very sensitive to heavy metals resulting in a decrease in dry mass of chickpea and greengram (Woolhouse, 1983; Rana and Ahmad, 2002). An increase in Cd concentration decreased the fresh mass in mungbean (Shen *et al.*, 1990). Moreover, a marked decrease in root and shoot mass of *Vigna ambacensis* was observed when treated with low concentration of Cd (Al-Yemens, 2001).

Effect of cadmium on nodulation: The presence of heavy metals in the soil decreased the yield of symbiotic nitrogen- fixing organisms and the number of nodules per plant (Vigue *et al.*, 1981). The presence of Cd decreased nodulation and nitrogenase activity in *Phaseolus vulgaris* (Dewdy and Ham, 1997; Vigue *et al.*, 1981), *Trifolium repens* (McGrath *et al.*, 1988), soybean (Vespa *et al.*, 1978), *Alnus rubra* (Wickliff *et al.*, 1980) and in *Pisum sativum* (Dhingra and Priefer, 2006). Nitrogen assimilation in pea plants was severely affected on exposure to Cd (Hernandez *et al.*, 1995; Dhingra and Priefer, 2006). A positive correlation was observed between leghemoglobin content and nitrogenase activity (Darkaro, 1995; Comba *et al.*, 1998) and both these parameters exhibited a parallel decrease in the presence of Cd (Farnandez *et al.*, 1996). The oxidation stress generated by Cd²⁺ accelerated senescence of nodules in soybean plants (Balestrasse *et al.*, 2001).

Effect of cadmium on photosynthesis: Cadmium is an effective inhibitor of photosynthesis (Greger *et al.*, 1994; Chugh and Sawhney, 1999; Vassilev *et al.*, 2005). A linear relationship between photosynthesis and inhibition of transpiration was observed in clover, lucerne, and soybean that suggest Cd inhibited stomatal opening (Barcelo *et al.*, 1986). Cadmium damages the photosynthetic

apparatus, in particular the light harvesting complex II (Krupa, 1988) and photosystems I and II (Siedlecka and Baszynsky, 1993; Siedlecka and Krupa, 1996). The inhibition of root Fe(III) reductase induced by Cd leads to Fe(II) deficiency which seriously affects photosynthesis (Alkantara *et al.*, 1994). Cadmium also causes stomatal closure in higher plants (Poschenreder *et al.*, 1989) and an overall inhibition of photosynthesis (Sheoran *et al.*, 1990; Krupa *et al.*, 1993; Chaug and Sawhny, 1999).

Effect of cadmium on chlorophyll and protein content: The presence of Cd decreased the content of chlorophyll and carotenoids, and increased non-photochemical quenching in *Brassica napus* (Larsen *et al.*, 1998). Similarly, the synthesis and level of chlorophyll decreased in other plant species under the influence of the cadmium (Czuba and Ormond, 1973; Griffiths *et al.*, 1995; Stiborova *et al.*, 1986; Bishnoi *et al.*, 1993; Ferretti *et al.*, 1993; Imai *et al.*, 1996; Phetsombat *et al.*, 2006; Pandey *et al.*, 2007).

Growth reduction associated with cadmium treatment was probably caused by inhibition of protein synthesis (Foy *et al.*, 1978). Phytotoxicity of the metal in other crop plants has been observed in the form of a loss in protein levels (Dubey and Dwivedi, 1987; Tamas *et al.*, 1997). Moreover, the grains developed on the plants grown under Cd stress had lower protein content (Salgare and Achareke, 1992).

Effect of cadmium on nitrate reductase activity: Nitrate reductase (NR), the primary enzyme in the nitrate assimilation pathway, is the limiting factor in plant growth and development (Solomonson and Barber, 1990) and its level is influenced by a variety of environmental factors (Andrew, 1986; Murphy *et al.*, 1997a). The presence of Cd in the soil affected the assimilation of NO₃ in maize (Nassabaum *et al.*, 1988; Hernandez *et al.*, 1996), pea (Burzynski, 1988), *Silene vulgaris* (Mathys, 1975), bean and tomato (Quariti *et al.*, 1997) and in *Cicer arietinum* (Ali *et al.*, 2007; Hasan *et al.*, 2007a).

Effect of cadmium on carbonic anhydrase activity and proline content: Cadmium decreased the activity of carbonic anhydrase in plants (Siedlecka and Krupa, 1996). Among the four tested heavy metals that induce proline accumulation, Cd was the strongest inducer (Saradhi and Saradhi, 1981) in rice (Roy *et al.*, 1992), *Brassica napus* callus (Chandner and Thorpe, 1987), *Armeria maritima* (Farago, 1981), and sunflower (Kastori *et al.*, 1992) and *Brassica juncea* (Singh and Tiwari, 2003). In addition, proline could be involved in metal chelation in the cytoplasm (Farago and Mullen, 1979). Moreover, proline is a poor inducer of phytochelator synthesis (Grill *et al.*, 1987). An increase in constitutive proline levels have been observed in a copper-tolerant ecotype of *Armeria maritima* exposed to Cd (Farago, 1981). The plants exposed to heavy metals seem to induce accumulation of free proline (Bassi and Sharma, 1993; Costa and Morel, 1994).

Effect of cadmium on antioxidant systems: Plants possess a number of antioxidant systems that protect them from oxidative

damage (Smeets *et al.*, 2005; Pal *et al.*, 2006). Superoxidase (SOD) is the first enzyme in the detoxifying process that converts O₂ radicals to H₂O₂ at a very rapid rate (Polle and Rennenberg, 1994). Cadmium was found to result in oxidative stress (Hendy *et al.*, 1992; Somashekaraiah *et al.*, 1992) by either inducing oxygen free radical production (Balaknina *et al.*, 2005; Demirevska-Kepava *et al.*, 2006) or by decreasing concentrations of enzymatic and non-enzymatic antioxidants (Somashekaraiah *et al.*, 1992; Stohs and Bagchi, 1995; Shaw, 1995; Gallego *et al.*, 1996; Sandalio *et al.*, 2001; Balestrasse *et al.*, 2001; Fornazier *et al.*, 2002; Cho and Seo, 2004; Mohan and Hosetti, 2006). These defense systems are composed of metabolites such as ascorbate, glutathione, tocopherol, *etc.*, and enzymatic scavengers of activated oxygen such as peroxidases, catalases and superoxide dismutases (Noctor and Fayer, 1998; Asada, 1999; Sandalio *et al.*, 2001; Khan *et al.*, 2002; Borctal *et al.*, 2003; Panda and Khan, 2003; Chaoui *et al.*, 2004; Demiral and Turkan, 2005; Mandhanian *et al.*, 2006).

Peroxidase induction is a general response of higher plants after uptake of toxic quantities of metals (Van Assche and Clijsters, 1990). Cd ions can inhibit or sometimes stimulate the activity of several antioxidative enzymes. In *Halianthus annuus* leaves, Cd enhanced lipid peroxidation, increased lipoxygenase activity and decreased the activity of superoxidase (Sandalio *et al.*, 2001; Khan *et al.*, 2002; Panda and Khan, 2003), glutathione reductase, catalase, ascorbate peroxidase, glutathione reductase and dehydroascorbate reductase (Gallego *et al.*, 1996). Cadmium induced the activity of peroxidase (POX) in soybean (Fuhrer, 1982), bean leaves (Lee *et al.*, 1996) and in roots and leaves of *Oryza sativa* (Reddy and Prasad, 1993), *Brassica juncea* (Singh and Tiwari, 2003; Hayat *et al.*, 2007), *Cicer arietinum* (Hasan *et al.*, 2007b), *Bacopa monniera* (Mishra *et al.*, 2006) and in the leaves of *Calamus tenuis* (Khan and Patra, 2007). In *Phaseolus aureus*, Cd ions decreased catalase activity and increased the activities of guaiacol peroxidase and ascorbate peroxidase (Shaw, 1995; John *et al.*, 2007). Membrane damage due to lipid peroxidation caused by metals is mediated by activated oxygen radicals (hydrogen peroxidase, hydroxyl and superoxide radicals) and could be quenched by the induction of specific enzymes like peroxidase, superoxide dismutase and catalase (De Vos and Schat, 1981). *Phaseolus vulgaris* roots exposed to 5 mM Cd had higher activities of guaiacol and ascorbate peroxidase and elevated levels of lipid peroxidation (Chaomi *et al.*, 1997). Cadmium treatment also increased lipid peroxidation (Lazono-Rodriguez *et al.*, 1997; Sandalio *et al.*, 2001; Astolfi *et al.*, 2004; Chaoui *et al.*, 2004; Srivastava *et al.*, 2004) whereas no effect on lipid peroxidation was noticed in the roots of *Daucus carota* plants exposed to Cd (Sanita di Toppi *et al.*, 1998).

In addition to these antioxidant molecules, thiols also possess strong antioxidative properties and are able to counteract oxidative stress imposed by Cd (Pichorner *et al.*, 1993; Shanthala *et al.*, 2006). Germinating pigeon pea seedlings exposed to Cd²⁺ had

altered enzyme activity and mobilizations of food reserves (Bishnoi et al., 1993).

Conclusions: Although our knowledge of Cd toxicity in higher plants as well as in the soil-plant system has increased considerably in the recent years, there are still many gaps in our knowledge about the basic mechanisms that control Cd movement and its accumulation in plants. Certainly more research is needed regarding the mechanism of Cd uptake by the root, translocation, and its deposition within plants. Additionally, the major forms of Cd in various staple plant foods (e.g. rice, wheat, corn, bean, and potato) need to be identified. We must elaborate the knowledge about the biochemistry of metal homeostasis factors, physical interaction of transporters, chelators and chaperones. A genetic approach as opposed to physiological/ biochemical investigations may assist in understanding the mechanism of metal tolerance. Some studies have been conducted on the mechanism of Cd tolerance by selecting Cd-sensitive and Cd-tolerant strains. Genetic improvement of Cd-hypersensitive genotypes of agricultural, horticultural and silvicultural plants may emerge as a challenging subject. Transgenic production of Cd-excluders might emerge as a priority area. *In vitro* (cell culture) investigations are relevant not only to understand metal tolerance but also enzymological aspects and metal ion homeostasis. The cellular and molecular basis of thermoprotection of heavy metals and heat shock protein induced by heavy metals needs critical investigation. An improved knowledge in these crucial areas will help to elucidate the molecular mechanisms that lie beyond plant metal tolerance and homeostasis.

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