



Phytotoxicity Of Cadmium: Recent Advances And Future Prospects

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ABSTRACT

Heavy metals are important environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional and environmental reasons. Heavy metal contamination of soil and water is one of the most serious environmental problem across the world due to their toxicity to human, animals, plants and microbes. Cadmium (Cd) is a trace element ubiquitous in the soil. However, anthropogenic activities such as the non-ferrous metal industry, mining, production, use and disposal of batteries, metal-contaminated wastes and sludge disposal, application of pesticides and phosphate fertilizers lead to dispersion of Cd. This non-essential element is taken up through the roots of many species and accumulated in all the plant parts including root, shoot, fruit and grain. Taken up in excess, Cd becomes poisonous and can cause serious health hazards to most living organisms. Cadmium accumulation through the tropic levels of the food chain constitutes a risk for humans. Cd directly or indirectly inhibits physiological processes such as respiration, photosynthesis, water relations and gas exchange. Cd may be preferentially accumulated in chloroplasts. Photosynthesis is inhibited at several levels. Cadmium disturbs cellular redox environment of the root causing oxidative stress. Various parts of root metabolism are affected as a consequence of Cd, including water and nutrient uptake and inhibition of several enzyme activities. Cd is also responsible to reduce nitrate reductase activity, carbonic anhydrase activity and proline content. 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 the plants. Additionally, the major forms of Cd in various staple plant foods need to be identified. This review emphasises cadmium toxicity on plants with regards to ecological, physiological and biochemical aspects.

Keywords: Oxidative stress, photosynthesis, respiration, cadmium toxicity, reductase, anhydrase, anthropogenic

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[1] and few are of biological importance. Heavy metal contamination of soil and water is one of the most serious environmental problem across the world due to

their toxicity to human, animals, plants and microbes[2]. Cadmium is released into the biosphere naturally by volcanoes, weathering of rocks[3] as well as anthropogenically through various urban/industrial wastes such as mining and metal refining[4], compost application[5] cadmium rich phosphate fertilizers[6] and waste water irrigation[7]. Like other toxic metals, Cd is also taken up by the plants and gets accumulated in various plant parts as free metal which may adversely affect the plant growth and metabolism[8]. In case, plant tolerate high level of the metal and get survived in the metal rich environment, it may enter in to the food chain and can cause serious health hazards to animals and human[9].

Heavy metals are important environmental pollutants and their toxicity is a problem of increasing significance for ecological, evolutionary, nutritional, and environmental reasons. Of all non-essential heavy metals, cadmium (Cd) is perhaps the metal that 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 emphasises Cd toxicity on plants with regards to ecological, physiological, and biochemical aspects. This non-essential element is taken up through the roots of many species and accumulated in all plant parts including root, shoot, fruit and grain[10]. Taken up in excess, Cd becomes poisonous and can cause serious health hazards to most living organisms[11]. Cadmium accumulation through the tropic levels of the food chain constitutes a risk for humans[12]. Cadmium decreases activities of various enzymes[13] and interfere with general and membrane physiology such as oxidative reactions and nitrogen metabolism[14].

Friedrich Stromeyer and Karl Hermann discovered cadmium (Cd) almost simultaneously in 1817 samples of zinc oxide obtained by roasting zinc carbonate from Salzgitter (Germany). Cd has no amphoteric properties and, although cadmate anions are found, it does not dissolve in bases[15]. Cd is a relatively rare element and is not found in a pure state in nature. In the air, Cd is rapidly oxidised into cadmium oxide. It easily reacts with carbon dioxide, water vapour, sulphur dioxide, sulphur trioxide, or hydrogen chloride and produces cadmium carbonate, hydroxide, sulphide, or chloride. Cd can undergo weak bonding to carbon and other more electronegative atoms.

Biological functions of Cd: The toxic effects of Cd on human health were first known in 1858, when Sovet reported that respiratory and gastrointestinal diseases occurred in people who worked with Cd-containing polishes and inhaled or swallowed these agents while working[16]. The first experiments on the effects of Cd in animals were conducted by [17] and [18]. They reported various clinical signs and morphological changes in organs of a variety of vertebrates including birds and dogs and mentioned that Cd intoxication can lead to kidney, bone, and pulmonary damage. Later, [19] reported that there was damage to the lungs, liver, and kidneys in cats and humans in Cd-exposed conditions. Humans normally absorb Cd into the body by ingestion or inhalation. Much of the Cd that enters the body by ingestion comes from terrestrial foods. It was estimated that 98% of the ingested Cd comes from terrestrial foods, while only 1% comes from aquatic foods such as fish and shellfish, and 1% arises from Cd in drinking water[20]. For acute exposure by ingestion, the principal effects are gastrointestinal disturbances such as nausea, vomiting, abdominal cramps, and diarrhoea. Acute poisoning by inhalation may lead to respiratory manifestations such as severe bronchial and pulmonary irritation, lung emphysema, and, in the most severe situations, even death may occur[21]. Excretion of Cd takes place via faeces and urine. However, uptake mechanisms responsible for the cellular accumulation of Cd remain to be identified. [22] found Cd and zinc containing protein in kidney tissues. This protein was named metallothionein (MT). MT functions in Cd detoxification primarily through the high affinity binding of the metal to MT, and thus sequestration of Cd away from critical macromolecules. Other proposed functions of MT, such as maintaining essential metal (zinc) homeostasis, scavenging reactive oxygen species, regulating gene expression, and tissue regeneration, could all contribute to MT protection against Cd[23].

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[24]. 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[25]. The latter property makes it susceptible to enter the atmosphere which is a major component of the global Cd cycle[26].

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[27]. 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[28]. Of the major heavy metals, Cd is a major industrial pollutant particularly in areas associated with smelting of zinc and heavy road traffic[29]. High concentrations of heavy metals in the soil are toxic to most plants[30]. 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[31] 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[32].

Heavy metal accumulation in plants has multiple direct and indirect effects on plant growth and alters many physiological functions[33] by forming complexes with O, N and S ligands[34]. They interfere with mineral uptake[35] protein metabolism[36] membrane functioning[37] water relations[38] and seed germination[39]. Cadmium inhibited net photosynthesis in green algae, corn, soybean, and pigeon pea[40], O₂ evolution in *Anacystis nedulens* and photo system II (PS II) in isolated chloroplasts of maize and spinach[40]. Moreover, they cause metabolic disturbance by altering essential biochemical reactions[41]. 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[42] and growth of leguminous plants[43] but also inhibited the growth of microorganisms present in the soil[44].

Cadmium homeostasis: The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms that includes uptake and accumulation of metals through binding to extracellular exudates and cell wall, complexation of ions inside the cell by various substances, for example, organic acids, amino acids, ferritins, phytochelatins, and metallothioneins; general biochemical stress defence responses such as the induction of antioxidative enzymes and activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures [45].

Cadmium Chelation: PCs are synthesized enzymatically by PC synthase in higher plants [46]. This enzyme removes a γ Glu-Cys residue from one molecule of glutathione (γ 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*[47]. Moreover, the rate of phytochelatins production elevated in rice and groundnut when exposed to cadmium[48]. PC synthase was purified to homogeneity in cell cultures of *Silene cucufalus*, *Beta vulgaris*, and *Equisetum giganteum*[49]. 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[50].

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[51]. More direct evidence of PCs in protecting plant enzymes was reported in suspensions cell cultures of *Rauvolfia serpentina* that were treated with Cd [52]. 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. 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[53]. Vacuolar compartmentalization prevents the free circulation of Cd ions in the cytosol and forces them into a limited area[28]. Several studies have shown that the vacuole is the site of accumulation of a number of heavy metals including Zn and Cd[54].

Cadmium Mobilization, Uptake and Transport: Mechanism of cadmium uptake, translocation 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 [55] 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[56]. 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[57]. 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[58], maize[59] and lettuce. An antagonism between zinc and cadmium and their active absorption was observed in lettuce roots[60]. 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 [28]. 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 Cd^{2+} in the cytosol and that in the root apoplasts. The large negative membrane potential alone provides more than enough energy to drive Cd^{2+} uptake even at low concentrations of Cd^{2+} . The kinetics of Cd^{2+} absorption by roots shows bio-phasic characteristics with saturable components at low Cd^{2+} activities in the absorption solution and a linear component at higher Cd activities[61]. Although the biphasic nature of Cd^{2+} 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 $CdCl^+$, $CdCl_2$ and $CdSO_4$ [62] or as organic complexes such as phytometallophore complexes. [63] 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 Zn^{2+} , 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[64]. Cd can easily penetrate the root system of xylem through the apoplastic and/or symplastic pathway[65] and reaches tissues of aerial parts of the plants[66]. 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[67]. Most Cd ions are retained in the roots and only small amounts are transported to shoots[68]. In general, the concentration of Cd in plants decreases in the order: root > leaves > fruits > seeds[69].

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[68]. Moreover, Cd was easily transported to the shoots and leaves of tomato plants but was not detected in fruits[70]. 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[71]. 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, [72] 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. [73] reported the occurrence of deoxymugineic and epihydroxymugineic acid phytometallophores in xylem sap collected from rice seedlings and [68] 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[73]. 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 nicotianamine 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)[74]. Zinc- binding substances similar to the phytochelatins have been reported in phloem sap of citrus[75].

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[76]. [77,78,79] 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[80]. 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 [81]. The bioavailability of some metals is limited because of low solubility in oxygenated water and strong binding to soil particles. Both the acidification of the rhizosphere and the exudation of carboxylates are considered potential targets for enhancing metal accumulation[82]. The degree to which higher plants are able to take up Cd depends on its concentration in the soil and its bioavailability, modulated by the presence of organic matter, pH, redox potential, temperature and concentrations of other elements. With the exception of Fe, which is solubilized by either reduction to Fe(II) or extrusion of Fe(III)-chelating phytosiderophores[83], little is known about active mobilization of trace elements by plant roots. In particular, the uptake of Cd ions seems to be in competition for the same transmembrane carrier with nutrients, such as K, Ca, Mg, Fe, Mn, Cu, Zn, Ni[84]. The cell membrane plays a role in metal homeostasis, preventing or reducing entry into the cell. However, examples of exclusion or reduced uptake

mechanisms in higher plants are limited. Cadmium is one of the most dangerous metals due to its high mobility and the small concentration at which its effects on plants begin to appear[85]. The apoplast continuum of the root epidermis and cortex is readily permeable for solutes. The cell walls of the endodermal cell layer act as a barrier for apoplastic diffusion into the vascular system. In general, solutes have to be taken up into the root symplasm before they can enter the xylem[86]. Subsequent to metal uptake into the root symplasm, three processes govern the movement of metals from the root into the xylem: sequestration of metals inside root cells, symplastic transport into the stele and release into the xylem[82]. The membrane potential, which is negative on the inside of the plasma membrane and might exceed -200mV in root epidermal cells, provides a strong driving force for the uptake of cations through secondary transporters[83].

Cadmium accumulation and detoxification: In general, plant accumulation of a given metal is a function of uptake capacity and intracellular binding sites. At every level, concentration and affinities of chelating molecules, as well as the presence and selectivity of transport activities, affect metal accumulation rates[82]. The strategies for avoiding heavy metal toxicity are diverse. A first barrier against Cd stress, operating mainly at the root level, can be the immobilization of Cd by means of the cell wall[87] and extracellular carbohydrates (mucilage, callose)[88]. In roots and leaves of bush bean, Cd ions seem to be mostly bound by pectic sites and hystidyl groups of the cell wall[89]. However, the importance of these mechanisms may vary in accordance with the concentration of Cd supplied, the species involved, the exposure time, etc. [28]. Preventing Cd ions from entering the cytosol through the action of the plasma membrane, that means exclusion of ions from plant cell walls, could theoretically represent the best defense mechanism. As a matter of fact, in early phases of radish seed germination Cd seems to enter the cells through Ca channels in the plasma membrane[90].

Toxicity Effects of Cd on Plants: Effect of Cd on growth and development Cd toxicity causes inhibition and abnormalities of general growth in many plant species. After long-term exposure to Cd, roots are mucilaginous, browning, and decomposing; reduction of shoots and root elongation, rolling of leaves, and chlorosis can occur. Cd was found to inhibit lateral root formation while the main root became brown, rigid, and twisted[91]. The main reason indicated is disordered division and abnormal enlargement of epiderma and cortical cell layers in the apical region. The changes in the leaf included alterations in chloroplast ultrastructure, low contents of chlorophylls, which caused chlorosis, and restricted activity of photosynthesis[92]. Effect of cadmium on the plant growth: Cadmium is not an essential nutrient and at high concentration inhibits plant growth[93]. It has also been reported that even at relatively low concentrations it alters plant metabolism[94]. The presence of cadmium in the soil decreases the growth of soybean[95] and chickpea plants[96]. High concentrations of Cd decreased cell growth as well as whole plant growth[81]. 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[33]. An increase in Cd concentration decreased the fresh mass in mungbean[97]. Moreover, a marked decrease in root and shoot mass of *Vigna ambacensis* was observed when treated with low concentration of Cd[98]. 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[99]. The presence of Cd decreased nodulation and nitrogenase activity in *Phaseolus vulgaris*[95], *Trifolium repens*[100]. Nitrogen assimilation in pea plants was severely affected on exposure to Cd[101]. A positive correlation was observed between leghemoglobin content and nitrogenase activity[102] and both these parameters exhibited a parallel decrease in the presence of Cd[103]. The oxidation stress generated by Cd²⁺ accelerated senescence of nodules in soybean plants[104].

Effect of cadmium on photosynthesis: Cadmium is an effective inhibitor of photosynthesis[105]. A linear relationship between photosynthesis and inhibition of transpiration was observed in clover, lucerne, and soybean that suggest Cd inhibited stomatal opening[106]. Cadmium damages the photosynthetic apparatus, in particular the light harvesting complex II [41] and photosystems I and II [107]. The inhibition

of root Fe(III) reductase induced by Cd leads to Fe(II) deficiency which seriously affects photosynthesis[108]. Cadmium also causes stomatal closure in higher plants[109] and an overall inhibition of photosynthesis[110]. In many species, such as oilseed rape (*Brassica napus*) [111], sunflower (*Helianthus annuus*) [112], *Thlaspi caerulescens*[113], maize, pea, barley[114]. the evidence showed that photosynthesis was inhibited after both long-term and short-term Cd exposure. A large number of studies have demonstrated that the primary sites of action of Cd are photosynthetic pigments, especially the biosynthesis of chlorophyll[115] and carotenoids[81]. According to [111], the observed chlorosis in oilseed rape was not due to a direct interaction of Cd with the chlorophyll biosynthesis pathway and most probably it was caused by decreasing of chloroplast density. The Cd-induced decrease in pigment content was more powerful at the leaf surface (stomatal guard cells) than it was in the mesophyll. In addition, the change of cell size, and the reducing of stomata density in the epidermis in Cd-treated leaves were observed. Thus, Cd might interfere directly with chloroplast replication and cell division in the leaf. This research also revealed that stomatal conductance was strongly reduced by Cd. Cd ions are known to affect the structure and function of chloroplasts in many plant species. The main target of the influence of Cd are 2 key enzymes of CO₂ fixation: ribulose-1,5-bisphosphate carboxylase (RuBPCase) and phosphoenolpyruvate carboxylase (PEPCase). It has been shown that Cd ions lower the activity of RuBPCase and damage its structure by substituting for Mg ions, which are important cofactors of carboxylation reactions and also Cd can shift RuBPCase activity towards oxygenation reactions[116].

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*[117]. Growth reduction associated with cadmium treatment was probably caused by inhibition of protein synthesis[118]. Moreover, the grains developed on the plants grown under Cd stress had lower protein content[119].

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[120]. The presence of Cd in the soil affected the assimilation of NO₃ in maize[121].

Effect of cadmium on carbonic anhydrase activity and proline content: Cadmium decreased the activity of carbonic anhydrase in plants[116]. Among the four tested heavy metals that induce proline accumulation, Cd was the strongest inducer[122] in rice[123]. In addition, proline could be involved in metal chelation in the cytoplasm[124]. Moreover, proline is a poor inducer of phytochelator synthesis[125]. An increase in constitutive proline levels have been observed in a copper-tolerant ecotype of *Armeria maritima* exposed to Cd[126]. The plants exposed to heavy metals seem to induce accumulation of free proline[127].

Effect of cadmium on antioxidant systems: Plants possess a number of antioxidant systems that protect them from oxidative damage[128]. Superoxidase (SOD) is the first enzyme in the detoxifying process that converts O²· radicals to H₂O₂ at a very rapid rate[129]. Cadmium was found to result in oxidative stress[130] by either inducing oxygen free radical production[131]. 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[132]

Effect of Cd on mineral nutrition: It has been reported that uptake, transport, and subsequent distribution of nutrient elements by the plants can be affected by the presence of Cd ions. 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[133]. In sugar beet, deficiency of Fe in roots induced by Cd was observed[134]. In pea plants, the uptake of P, K, S, Ca, Zn, Mn, and B was inhibited strongly after Cd exposure[135]. Treatment of barley plants with 1.0 μM Cd decreased the concentrations of P, K, Ca, Mg, Cu, Fe, Mn, Zn, Mo, and B in roots, whereas the concentrations of these elements in shoots were not decreased in comparison with the control[136]. A decrease in uptake of Ca and K by Cd has been found in a Cd-hyperaccumulator, *Atriplex*

halimus subsp. *schweinfurthii*[137]. Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting nitrate reductase activity in the shoots[138]. Appreciable inhibition of the nitrate reductase activity was also found in plants of *Silene cucubalus*[139]. Nitrogen fixation and primary ammonia assimilation decreased in nodules of soybean plants during Cd treatments[140].

Effect of Cd on ROS generation: Generally, heavy metals cause oxidative damage to plants, either directly or indirectly through reactive oxygen species (ROS) formation. Certain heavy metals such as copper and iron can be toxic through their participation in redox cycles like Fenton and/or Haber-Weiss reactions. In contrast, Cd is a non-redox metal unable to perform single electron transfer reactions, and does not produce ROS such as the superoxide anion ($O_2^{\bullet-}$), singlet oxygen (1O_2), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\bullet}), but generates oxidative stress by interfering with the antioxidant defence system[141]. Cd inhibits the photoactivation of photosystem 2 (PS2) by inhibiting electron transfer. Thus, Cd could lead to the generation of ROS indirectly by production of a disturbance in the chloroplasts. In addition, other reports suggested that Cd may stimulate the production of ROS in the mitochondrial electron transfer chain[142].

Effect of Cd on stress proteins: Extreme changes in environment could cause changes in gene expression, whereby leading to changes in the diversity of proteins in the cell. Therefore, changes in protein abundances under stressful conditions can be molecular markers for the manifestations of the responses to stress in organisms. In plants, the proteomics approach is developed as an important method for research on stress tolerance[143]. In recent years, much evident revealed that the response to stress in terms of proteomics occurred rather rapidly in plants after the exposure began. Heat-shock proteins (HSPs) are presently known as proteins that have functions to resist stress in eukaryotes. In Cd-treated maize plants a synthesis of 70 kDa phosphoprotein (HSP) was reported by [144]. In *Lycopersicon peruvianum* L., pre-treatment with a short heat stress before Cd exposure induced a protective effect by preventing membrane damage. HSP17 (molecular weight 17 kDa) and HSP70 proteins were also found in the cytosol of heat-shocked cells [145]. In Cd-treated pea plants, pathogen-related proteins PrP₄A and HSP71 were found, and they probably serve to protect cells against damages induced by Cd [146].

Defence mechanisms against Cd in plants: The mechanisms leading to heavy metal tolerance can be divided into avoidance strategies and tolerance strategies. Avoidance leads to limitation of Cd uptake. Plant tolerance mechanisms include accumulation and storing of Cd by binding it to amino acids, proteins, and peptides [147]. Other mechanisms that plants have developed to cope with damage caused by Cd are related to some stress signalling molecules, such as salicylic acid, jasmonic acid, nitric oxide, and ethylene. All these compounds were induced by Cd treatment, which suggests that they are involved in cell response to Cd toxicity [148]. Many plants survive, grow, and develop in Cd-polluted soils even in high concentrations of Cd. Investigations showed that some of these plants exhibit a hypertolerant capacity of their organelles and tissues. Strategies to cope with Cd toxicity involve the uptake and the distribution of Cd, defined as “hyperaccumulation”. On the other hand, some plants increased cleaning up of the ROS by antioxidants to protect cells and tissues from destruction. Thus, the mechanism of Cd tolerance in plants can include both antioxidant defence and/or hyperaccumulation defence [149]. Cd tolerance in plant by hyperaccumulation mechanism “Hyperaccumulator” is the term used for plants that actively take up exceedingly large amounts of one or more heavy metals from the soil. Moreover, the heavy metals are not retained in the roots but are translocated to the shoot and accumulated in aboveground organs, especially leaves, at concentrations 100-1000–folds higher than the accumulate in non-hyperaccumulating species. Hyperaccumulating plants show no symptoms of phytotoxicity. According to [149], about 450 angiosperm species have been defined as heavy metal (As, Cd, Co, Cu, Mn, Ni, Pb, Sb, Se, Tl, and Zn) hyperaccumulators until 2011, approximate 0.2% of all known species. However, new hyperaccumulating plants continue to be found [149]. In hyperaccumulating plants, the toxic effects of heavy metal at high accumulation are minimized, under the influence of detoxification mechanisms. Such mechanisms may be mainly based on chelation and sub-cellular compartmentalisation[150]. Root-to-shoot transportation of

heavy metals, including Cd, in hyperaccumulating plants is different to that in non-hyperaccumulating plants. This strategy retains in root cells most of the heavy metal ions taken up from the soil, detoxifies them by chelation in the cytoplasm or stores them in vacuoles, and rapidly translocates these elements to the shoot via the xylem. This involves specific features of root cell tonoplast, which enables heavy metals ion to readily efflux out of the vacuoles [149]. Many small organic molecules are present in hyperaccumulator roots that can operate as metalbinding ligands. However, the measure of contribution of different elements in hyperaccumulation strategies has not been defined yet. An important role in heavy metal hyperaccumulation seems to be played by free amino acids, such as histidine and nicotinamine, which form stable complexes with bivalent cations [151]. Enhanced metal xylem loading and translocation to the shoots is the next key physiological step in the metal hyperaccumulation trait that accounts for the increased metal flow towards the shoot. Storage and detoxification/ sequestration of heavy metals, including Cd, in the shoot are key strategies of hyperaccumulating plants. The heavy metal detoxification/sequestration occurs in locations such as the epidermis, trichomes, and even cuticle, where they do least damage to the photosynthetic machinery. In many cases, heavy metals are also excluded from both subsidiary and guard cells of the stomata. This may protect the functional stomatal cells from metal phytotoxic effects. The detoxifying/sequestering mechanisms in the aerial organs of hyperaccumulators consist mainly of heavy metal complexation with ligands and/or in their removal from metabolically active cytoplasm by moving them into inactive compartments, mainly vacuoles and cell walls[149]. A major plant strategy to detoxify nonessential metals is the synthesis of specific low-molecular-weight chelators to avoid binding to physiologically important proteins and to facilitate their transport into the vacuoles. The favored ligands of As(III) and Cd²⁺ are thiols, present in glutathione and phytochelatins (PC). The tripeptide glutathione (Glu- Cys-Gly), GSH, can bind to several metals and metalloids such as Cd, and is also involved in redox defence. However, increasing GSH (and PC) synthesis alone seems to be insufficient to achieve more than marginal enhancements of Cd and As tolerance or accumulation. The vascular Cd-GS2, which undertakes the transport of Cd, has been found in *Arabidopsis*. An ABC transporter that involves Cd-GS2 has been identified in *Arabidopsis*[152]. The small ligands, such as organic acids, have a major role as detoxifying factors. These ligands may be instrumental to prevent the persistence of heavy metals as free ions in the cytoplasm and even more in enabling their entrapment in vacuoles where the metal–organic acid chelates are primarily located. For example, in leaves of *Thlaspi goesingense*, citrate is the main ligand of Ni; in leaves of *Solanum nigrum*, citrate and acetate bind Cd; while most Zn in *Arabidopsis halleri* and Cd in *Thlaspi caerulescens* are complexed with malate[149].

Future prospects: There is a growing interest in problems concerning heavy metal contamination of cultivated lands and little is known regarding plant tolerance at the organism level. It is necessary to minimize the entry of Cd into the food chain because of the number of associated health risks. Many strategies have been devised to minimize Cd toxicity. Proper plant nutrition is a good strategy to alleviate the damaging effects of Cd on plants and to avoid its entry into the food chain. Use of plant nutrients to alleviate Cd toxicity in plants is a relatively inexpensive, time saving, and effective approach to avoid Cd contamination of food. Growers are already applying nutrients to obtain good crop yield, and so to alleviate Cd toxicity the proper management of these plant nutrients is needed, keeping in mind the interactions between Cd and plant nutrients. Crop rotation and the use of other organic and inorganic amendments are some other approaches being used to remediate Cd-contaminated soils, but these approaches are time consuming and require extra resources. Selection and breeding of crop plants/cultivars that accumulate low Cd in the grain and other edible plant parts is one of these approaches[153]. It seems an attractive approach to change the Cd profile of crop plants and the benefit continues generation after generation in plants through the seed. However, there are constraints to using this approach to produce low-Cd food, as it is very time consuming to develop and test a new cultivar. Phytoextraction is another approach to minimize Cd entry into the food chain; it involves the use of hyperaccumulator plants to remove Cd from soil. However, the problem is that hyper-accumulator plants are slow growing and produce very low biomass and a long time is required, perhaps several years, to remediate the contaminated site. Very little is known about the biochemistry of metal homeostasis factors. Physical

interaction of transporters, chelators, and chaperones is likely to play an important role. These results may provide a good background for strategies aimed at manipulating plants for decreased Cd content in order to develop crops capable of tolerating environmental changes with as little damage as possible. An improved knowledge in these crucial areas will help to further elucidate the molecular mechanisms that lie beyond plant metal tolerance and homeostasis.

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 thermo protection of heavy metals and heat shock protein induced by heavy metals needs critical investigation. Cadmium toxicity 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 non-essential ions and with the environmental factors. The phototoxic mechanisms involve different biochemical pathways in different plant species. Differential species tolerances to Cd toxicity almost certainly involves differences in the structure and functions of membranes. Other promising approaches in studying metal toxicity in tolerant and sensitive plant genotypes include determining the chemical compartmentalization of metals in various plant fractions, level and kinds of organic and amino acids which may act as metal chelators and detoxifiers, levels and forms of enzymes, and changes in route permeabilities to ions and molecules.

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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