

1-1-2013

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Recommended Citation

TRAN, TUAN ANH and POPOVA, LOSANKA PETROVA (2013) "Functions and toxicity of cadmium in plants: recent advances and future prospects," *Turkish Journal of Botany*. Vol. 37: No. 1, Article 1. <https://doi.org/10.3906/bot-1112-16>

Available at: <https://journals.tubitak.gov.tr/botany/vol37/iss1/1>

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Functions and toxicity of cadmium in plants: recent advances and future prospects

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Received: 15.12.2011 • Accepted: 23.07.2012 • Published Online: 26.12.2012 • Printed: 22.01.2013

Abstract: 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. It summarises the toxic symptoms of Cd in plants (i.e. growth and plant development, alterations in photosynthesis, stomatal regulation, enzymatic activities, water relation, mineral uptake, protein metabolism, membrane functioning, etc.). The main barriers against Cd entrance to the cell, as well as some aspects related to phytochelatin-base sequestration and compartmentalisation processes, are also reviewed. Cd-induced oxidative stress is also considered one of the most widely studied topic in this review. This review may help in interdisciplinary studies to assess the ecological significance of Cd stress.

Key words: Growth, cadmium hyperaccumulation, mineral nutrition, oxidative stress, photosynthesis, stress proteins

1. Brief historical notes and discovery of cadmium

Friedrich Stromeyer and Karl Hermann discovered cadmium (Cd) almost simultaneously in 1817 in 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 (Borsari, 2011).

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.

2. Some characteristics of Cd distribution in soil

Cd in soils is derived from both natural and anthropogenic sources. Natural sources include underlying bedrock or transported parent material such as glacial till and alluvium. The major natural sources for mobilisations of Cd from the earth's crust are volcanoes and weathering of rocks. Within the biosphere the Cd is translocated by different processes. Naturally a very large amount of Cd is released into the environment, about 25,000 t a year. About half of this Cd is released into rivers through weathering of

rocks and some Cd is released into the air through forest fires and volcanoes. The rest of the Cd is released through human activities.

The main anthropogenic input of Cd to soils occurs by industrial waste from processes such as electroplating, manufacturing of plastics, mining, paint pigments, alloy preparation, and batteries that contain Cd, composts, or fertilisers. Even domestic sewage sludge, which originated from the strictest control sources, contains Cd and adds it to pollution. From the sewage systems, Cd enters rivers and streams and therefore contaminates other places or accumulates in the sludge. The addition of Cd in metal-rich sewage sludge may also result in contamination of groundwater (Moradi et al., 2005).

The average natural abundance of Cd in the earth's crust has most often been reported from 0.1 to 0.5 ppm, but much higher and much lower values have also been cited depending on a large number of factors. Igneous and metamorphic rocks tend to show lower values, from 0.02 to 0.2 ppm, whereas sedimentary rocks have much higher values, from 0.1 to 25 ppm. Fossil fuels contain 0.5 to 1.5 ppm Cd, but phosphate fertilisers contain from 10 to 200 ppm Cd (Cook & Morrow, 1995). According to Wagner (1993), non-polluted soil solutions contain Cd concentrations ranging from 0.04 to 0.32 mM. Soil

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solutions that have a Cd concentration varying from 0.32 to about 1 mM can be regarded as polluted to a moderate level. Because Cd is a naturally occurring component of all soils, all food stuffs will contain some Cd and therefore all humans are exposed to natural levels of Cd. It has been reported that leafy vegetables and potato tubers naturally accumulate higher levels of Cd than do fruits and cereals. Moreover, tillage and crop rotation practices similarly have a greater impact upon the Cd content of food than does the concentration of Cd in soils (Mench et al., 1998).

3. Soil factors and Cd uptake in plants

Among various soil parameters known to affect the availability of Cd, soil pH was considered the most important. Many investigations showed that there was a linear trend between soil pH and Cd uptake: the decreasing of soil pH leads to increasing concentration of Cd in plants, provided that other soil properties remain unchanged (Kirkham, 2006). Soil pH affects the availability of Cd present in soil solution but increasing of soil pH does not always reduce Cd uptake by plants (Eriksson, 1989; Singh et al., 1995). Soil pH is also one of the important factors regulating Cd extractability in soils. The increase in soil pH increases the adsorption of Cd by soils and thus reduces its extractability (Christensen, 1984). Under field conditions, the uptake of Cd by plants may be affected by many variable soil and climatic parameters. Adams et al. (2004) investigated the relationship between soil properties and the concentration of Cd in wheat and barley grain by analysing 162 wheat and 215 barley grain samples collected from paired soil and crop surveys in Britain, and wheat and barley samples from 2 long-term sewage sludge experiments. The results showed that soil total Cd and pH were the significant factors influencing the Cd concentration in grain. Li et al. (2005) conducted field experiments with rice plants grown on the acidic red soil in China, and showed that, at soil pH of 4.95, Cd content in grain was 0.36 mg kg^{-1} , while, at pH of 6.54, Cd content in grain was 0.43 mg kg^{-1} . According to Chaudri et al. (2007), among the main factors responsible for Cd accumulation in wheat grain are Cd, pH, and organic carbon in soil. In barley grains, Singh and Myhr (1998) did not observe any significant correlation between the extractable Cd in soil, soil pH, and Cd accumulation. It is not easy to extrapolate results from greenhouse studies to field conditions. In greenhouse experiments application of NPK-fertilisers containing Cd increase its concentration in crops (He & Singh, 1994), but no such increase in Cd concentration in crops grown under field conditions was found as a result of long-term application of phosphate fertilisers. Many greenhouse and pot experiments also have yielded results that showed that the uptake of Cd in plants was affected by soil pH. The extent of Cd accumulation also depends on plant genotype (Li et al., 2005).

Some researchers consider that Cd uptake is by active transport, but most evidence points to the hypothesis of passive uptake. The active transport across the cell membranes depends on metabolic energy (ATP) to transport ions via carriers, which are molecules that serve as binding sites (Mengel & Kirkby, 1982; Marschner, 1995). Each carrier has affinity to a certain ion and regulates the content within the plant. Passive uptake, on the other hand, is independent of ATP (Larcher, 1995; Marschner, 1995). It is hypothesised that the transport of Cd within the plant occurs in the xylem as it follows water transport upwards in the xylem (Greger & Landberg, 1995). The effects of transpiration on Cd uptake have both been confirmed (Hardiman & Jacoby, 1984; Salt et al., 1995) and not confirmed (Perfus-Barbeoch et al., 2002).

The uptake of ions takes place in competition with that of elements such as Zn (Zhao et al., 2002), P (Dheri et al., 2007), Cl^- (Li et al., 1994; Oporto et al., 2009), Ca (Choi & Harada, 2005), and Cu (Kudo et al., 2011). Soil, environmental, and management factors impact on the amount of Cd accumulated in plants (Hart et al., 1998). Much of the Cd taken up by plants is retained in the roots, but a portion is translocated to the aerial portions of the plant and into the seed. The amount of Cd accumulated and translocated in plants varies with species and with cultivars within species.

Shen et al. (2006) reported that mycorrhizal inoculation increased plant growth with enhancement of P nutrition, and thus may increase plant tolerance to Zn and Cd by a dilution effect.

4. 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 (Sovet, 1858). The first experiments on the effects of Cd in animals were conducted by Alsberg and Schwartz (1919) and Schwartz and Alsberg (1923). 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, Prodan (1932) 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 (van Assche, 1998). 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 (Lauwerys, 1986). Excretion of Cd takes place via faeces and urine. However, uptake mechanisms responsible for the cellular accumulation of Cd remain to be identified.

Margoshes and Vallee (1957) 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 (Nordberg, 2009).

In plants, toxic effects of Cd were studied in the 1950s. Most of these effects will be discussed in the next subchapters.

5. Toxic effects of Cd in plants

5.1. 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 (Krantev et al., 2008; Yadav, 2010; Rascio & Navari-Izzo, 2011). 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 (He et al., 2008; Rascio et al., 2008; Lee et al., 2010; Liu et al., 2010; Miyadate et al., 2011). Rascio et al. (2008) reported that treatment of rice seedlings with Cd led to inhibition of root growth and alterations in their morphogenesis. In pea plants, the Cd stress also caused disorders in root elongation and the mitotic process and caused chromosomal aberrations of root tips. The observation showed that in these abnormalities as lagands, bridges, stickiness, precocious separation, and fragments were most common (Siddiqui et al., 2009). At high Cd concentration (250 μ M), the disorder of mitosis of roots in pea happens rapidly, even after 24 h of treatment. An unusual number of nucleus populations in the differentiated roots were found (Fusconi et al., 2006, 2007). In *Allium cepa*, the inhibition of mitotic index, induction of chromosome aberration, mitotic aberrations,

and micronucleus formation were observed after 24 h of treatment with Cd. In addition, damage to the DNA in root-cap cells has been found (Seth et al., 2008).

5.2. Effects of Cd on photosynthesis

In many species, such as oilseed rape (*Brassica napus*) (Baryla et al., 2001), sunflower (*Helianthus annuus*) (Di Cagno et al., 2001), *Thlaspi caerulescens* (Küpper et al., 2007), maize, pea, barley (Popova et al., 2008), mungbean (*Vigna radiate*) (Wahid et al., 2008), and wheat (Moussa & El-Gamal, 2010), 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 (Baszynski et al., 1980) and carotenoids (Prasad, 1995). According to Baryla et al. (2001), 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 such as *Triticum aestivum* (Atal et al., 1991), *Beta vulgaris* (Greger & Ögren, 1991), *Vigna radiata* (Keshan & Mukherji, 1992), *Spinacea oleracea* (Sersen & Kralova, 2001), and *Phaseolus vulgaris* (Padmaja et al., 1990). 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 (Siedlecka et al., 1998). Stiborova (1988) and Malik et al. (1992) demonstrated that Cd caused an irreversible dissociation of the large and small subunits of RuBPCase, thus leading to total inhibition of the enzyme. In addition to the negative effects of Cd on the photosynthetic carboxylation reactions PSII electron transport and especially oxygen-evolving complex were found to be very sensitive to the effect of Cd (Clijsters & Assche, 1985). As regards the site and mechanism of inhibition of Cd, it is generally accepted that the water-oxidising complex (OEC) of PS2 is affected by Cd by replacing the Ca²⁺ in Ca/Mn clusters constituting the oxygen-evolving centres (Sigfridsson et al., 2004) or by some modifications in the Qb-binding site (Geiken et al.,

1998). Cd also produces alterations in the functionality of membranes by inducing changes in their lipid and fatty acid composition (Ouariti et al., 1997; Popova et al., 2009).

5.3. 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 (Das et al., 1997). In sugar beet, deficiency of Fe in roots induced by Cd was observed (Chang et al., 2003). In pea plants, the uptake of P, K, S, Ca, Zn, Mn, and B was inhibited strongly after Cd exposure (Metwally et al., 2005). 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 (Guo et al., 2007). A decrease in uptake of Ca and K by Cd has been found in a Cd-hyperaccumulator, *Atriplex halimus* subsp. *schweinfurthii* (Nedjimi & Daoud, 2009).

Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting nitrate reductase activity in the shoots (Hernandez et al., 1996). Appreciable inhibition of the nitrate reductase activity was also found in plants of *Silene cucubalus* (Mathys, 1975). Nitrogen fixation and primary ammonia assimilation decreased in nodules of soybean plants during Cd treatments (Karina et al., 2003).

The observation of Cd-treated soybean seedlings showed that there was an increase in laccase activity (laccases are responsible for lignin biosynthesis), during the early stage of Cd treatment, whereby Cd induced the lignin synthesis in early stage of root growth and as a result might cause inhibition of root elongation (Yang et al., 2007).

How Cd inhibits the uptake of other elements is not yet completely clear. In maize, Cd treatment induced an inhibition of H⁺ATPase in root cells. Many studies revealed that H⁺ATPase is an integral protein associated with the plasma membrane and is located preferentially at the epidermal and cortical cell layers of roots. H⁺ATPase functions as an ion transporter across the plasmalemma and this is dependent on the electrochemical gradient generated by the plasma membrane H⁺ATPase. Thus, Cd, which causes a decrease in activity of H⁺ATPase, might inhibit absorption of some essential elements (Astolfi et al., 2005). In addition, data on poplar (*Populus jaquemontiana* var. *glauca*) showed that Cd can inhibit mineral nutrition by competition between this metal and other metal ions (Solti et al., 2011). The authors have suggested 2 mechanisms. In the first type (type 1), the mechanism was like the influence of Cd on Fe. It is known that Cd might inhibit the chelating process of Fe and the loading of Fe

into the xylem. Thus, the metals that are transported in the xylem, like as occurred with Fe, were influenced by Cd as type 1. In the second type (type 2), the mechanism was like the influence of Cd on Ca in competition for Ca-transporters. The alkaline earth metals (except Mg) belong to type 2.

It should be mentioned that several plant nutrients have many direct as well as indirect effects on Cd availability and toxicity. Direct effects include decreased Cd solubility in soil by favouring precipitation and adsorption (Matusik et al., 2008), competition between Cd and plant nutrients for the same membrane transporters (Zhao et al., 2005), and Cd sequestration in the vegetative parts to avoid its accumulation in the grain/edible parts (Hall, 2002). Indirect effects include dilution of Cd concentration by increasing plant biomass and alleviation of physiological stress.

5.4. 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 ($\text{O}_2^{\cdot-}$), singlet oxygen ($^1\text{O}_2$), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH^{\cdot}), but generates oxidative stress by interfering with the antioxidant defence system (Benavides et al., 2005; Cho & Seo, 2005; Gratao et al., 2005).

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 (Heyno et al., 2008).

Treatment of pea and rice plants with Cd stimulates the plasma-membrane-bound NADPH oxidase in peroxisomes and thus generates ROS. The activation of ROS generation is fast. For example, in Scots pine (*Pinus sylvestris*) seedlings, treatment with 50 mM Cd led to an increase in ROS in 6 h. In *Medicago sativa* exposure to Cd for 6–24 h caused a rapid accumulation of peroxides and depletion of glutathione (GSH) and homoglutathione (hGSH), and led to redox imbalance. The Cd-induced cell death in bright yellow-2 (BY-2) tobacco cells was preceded by NADPH-oxidase-dependent accumulation of H_2O_2 followed by cellular O_2 and fatty acid hydroperoxide accumulation (Gill & Tuteja, 2010).

The manifestations of ROS damages in plants involve lipid peroxidation, protein peroxidation, and DNA damage. Cd produced an enhancement of lipid

peroxidation in *Phaseolus vulgaris* (Chaoui et al., 1997), *Helianthus annuus* (Gallego et al., 1996), and *Pisum sativum* (Lozano-Rodriguez et al., 1997). DNA damage caused by Cd involved destruction of nucleic acids, cell membrane, lipids, and proteins; reduction of protein synthesis; and damage of photosynthetic proteins, which affects growth and development of the whole organism. DNA damage has also been defined via determination of frequency of abnormalities such as fragments, precocious separation, laggards, single and double bridges, and stickiness (Gill & Tuteja, 2010; Kranner & Colville, 2011).

A variety of proteins function as scavengers of superoxide and hydrogen peroxide. These include, among others, superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), peroxidases (POD), and glutathione reductase (GR), and non-enzymatic scavengers, including, but not limited to, glutathione (GSH), ascorbic acid (ASA), carotenoids, and tocopherols.

SOD, GR, APX, POD, and CAT showed variations in their activities that depend on the Cd concentration and plant species used. Increased activity of SOD has been detected in many Cd-treated plants, such as pea (Sandalio et al., 2001), wheat (Milone et al., 2003), and bean (Cardinaels et al., 1984). Decline in the enzymatic activity of CAT and SOD has been associated with Cd toxicity in *Phaseolus vulgaris* (Chaoui et al., 1997), *Phaseolus aureus* (Shaw, 1995), *H. annuus* (Gallego et al., 1996), and *Pisum sativum* (Sandalio et al., 2001). Variable activity of CAT has been observed under Cd stress. Yilmaz and Parlak (2011) reported that the observed high tolerance of *Groenlandia densa* to Cd stress was partially due to high activity of CAT. Its activity increased in rice, mustard, wheat, chickpea, and black bean (*Vigna unguiculata* subsp. *cylindrica*) roots and declined in soybean, *Phragmites australis*, *Capsicum annuum*, and *Arabidopsis* under Cd stress (Gill & Tuteja, 2010). APX and GPX are scavengers of H₂O₂ in ROS detoxification. An increase in leaf APX activity under Cd stress has been reported in *Ceratophyllum demersum*, mustard, wheat, and black bean. An increase in GPX activity in Cd-exposed plants was reported in wheat, *Arabidopsis*, and *Ceratophyllum demersum*. It was found that an initial increase in GPX activity in spruce needles subjected to Cd stress and subsequent Cd treatments caused a decline in the activity (Gill & Tuteja, 2010). A decrease in POD activity caused by Cd was reported in mustard (*Brassica juncea*) (Markovska et al., 2009). An increase in GR activity was found in cotton, *Arabidopsis*, blackgram, wheat, and mustard upon Cd treatment (Markovska et al., 2009; Gill & Tuteja, 2010). The activities of MDHAR and DHAR were found to increase in mustard plants exposed to 10 µM Cd (Markovska et al., 2009). An increase of

glutathione-S-transferases (GST) activity was found in rice shoots, while in roots the activity of the enzyme was inhibited by Cd treatments. Compared with shoots, rice roots had higher GST activity, indicating that the ability of Cd detoxification was much higher in roots than in shoots (Zhang & Ge, 2008). Dixit et al. (2011) reported the cloning of a GST gene from *Trichoderma virens*, a biocontrol fungus, and introducing it into tobacco plants by *Agrobacterium*-mediated gene transfer. Their results showed that the transgenic plants expressing the TvGST gene, under exposure to different Cd concentrations, were more tolerant in comparison with wild-type plants. The levels of GST showed enhanced values in transgenic plants expressing TvGST compared to control plants, when exposed to Cd, although Cd accumulation in the plant biomass in transgenic plants was similar or lower than that in wild-type plants. Cd stress increases the activity of POD in radish (*Raphanus sativus* L.) (El-Beltagi et al., 2010) and causes no significant change in the leaves of pea plants.

An increase in ASH content during Cd exposure was found in barley. In contrast, a decrease in ASH in the roots and nodules of soybean under Cd stress was also observed. Cd also decreased the ASH content in cucumber chloroplasts and in the leaves of *Arabidopsis* and pea, whereas it remained unaffected in *Populus canescens* roots (Gill & Tuteja, 2010).

An increase in GSH levels, which resulted in enhanced antioxidant activity against Cd toxicity, has been found in the leaves and chloroplasts of *Phragmites australis* Trin. (Cav.) ex Steudel. Increased concentration of GSH has been observed with increasing Cd concentration in pea, *Sedum alfredii*, and black bean. A decrease in GSH, which could weaken the antioxidative response and defensive strength against Cd stress in the more sensitive genotypes, was also found in pea (Metwally et al., 2005).

Accumulation of large amounts of osmolytes (proline) is an adaptive response in plants exposed to a stressful environment. Proline accumulation appeared to be a suitable indicator of heavy metal stress. The role of proline as an antioxidant was reported in tobacco (*Nicotiana tabacum* L.) cells exposed to Cd stress. Islam et al. (2009) reported that tobacco cells exposed to Cd treatment accumulated high levels of proline and by this way they can alleviate the inhibitory effect of Cd on cell growth.

5.5. 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 (Nanjo et al., 2011). 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 Reddy and Prasad (1993). 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 (Neumann et al., 1994). 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 (Rodríguez-Serrano et al., 2009).

In wheat seedlings treated with 50 μM CdCl_2 for 48 h, a 51-kDa soluble protein was found. This protein was designated as a Cd stress-associated protein. It was generated mainly in the root tissue of treated and control seedlings and located below the plasma membrane and outer periphery of the tonoplast (Mittra et al., 2008). In poplar (*Populus tremula* L.) exposed to Cd for a short-term (14 days) or a longer term (56 days) treatment, it was found that stress-related proteins, like HSPs, proteinases, and pathogenesis-related proteins, increased in abundance in leaves. The abundance of many typical stress-related proteins like HSPs or glutathione-S-transferases was increased, whereas most of the proteins from the primary metabolism (glycolysis, tricarboxylic acid cycle, nitrogen metabolism, and sulphur metabolism) were severely decreased in abundance (Kieffer et al., 2009). Lee et al. (2010) reported that Cd affected the synthesis of 36 proteins in rice. In roots, the synthesis of 16 proteins was increased, while the synthesis of 1 protein was reduced. In leaves, the synthesis of 16 proteins was up-regulated, while the synthesis of 3 proteins was down-regulated. Treatment of tomato plants with a low Cd concentration (10 μM) induced changes in 36 polypeptides, while higher Cd concentration (100 μM) induced changes in 41 polypeptides (Rodríguez-Celma et al., 2010). In 3-week-old *Arabidopsis thaliana* seedlings exposed to 10 μM Cd, it was found that among 730 determined proteins 21 were up-regulated in response to Cd. These proteins can be classed into 5 groups in accordance with their functions: 8 proteins involved in group (1) that involve ROS detoxification, 6 proteins belong to group (2) that involve carbon metabolism and photosynthesis, 4 proteins belong to group (3) that involve protein metabolism, and 5 proteins are classed in group (4) and group (5) that involve gene expression and with various or unknown function (Semane et al., 2010). Studying barley tolerance to boron, Atik et al. (2011) determined 7 proteins that were up-regulated in response to boron treatment. Some of the

proteins were related to photosynthesis and others were located in the vacuole.

6. 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 (Pál et al., 2006). 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 (Rodríguez-Serrano et al., 2006; Popova et al., 2012).

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 (Rascio & Navari-Izzo, 2011).

6.1. 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 Rascio and Navari-Izzo (2011), 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 (Rascio & Navari-Izzo, 2011; Altinözülü et al., 2012). In hyperaccumulating plants, the toxic effects of heavy metal at high accumulation are minimised, under the influence of detoxification mechanisms. Such mechanisms may be mainly based on chelation and sub-cellular compartmentalisation (Yadav, 2010).

The uptake of Cd in plants from the soil seems to occur mainly via Ca^{2+} , Fe^{2+} , Mn^{2+} , and Zn^{2+} transporters (Rascio & Navari-Izzo, 2011). In non-hyperaccumulating plants, Cd uptake is nonspecific. For example, in maize, the strong

adsorption of Cd on root apoplast might act as a main driving force to uptake this metal from the soil (Redjala et al., 2009); however, the result was contrary to this in rice (Lu et al., 2009).

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 (Rascio & Navari-Izzo, 2011). Many small organic molecules are present in hyperaccumulator roots that can operate as metal-binding 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 (Hassan & Aarts, 2011).

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 (Rascio & Navari-Izzo, 2011).

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 favoured 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 vacuolar 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* (Verbruggen et al., 2009). 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 (Rascio & Navari-Izzo, 2011).

7. Factors alleviating Cd toxicity in plants

Survival under stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals, and induce biochemical changes that adjust the metabolism accordingly. Therefore, the search for signal molecules that mediate stress tolerance is an important step in our better understanding of how plants acclimate to the adverse environment.

In general, Cd has been shown to interfere with the uptake, transport, and use of several elements (Ca, Mg, P, and K). The application of 10 mM Mg in the nutrient solution of Japanese mustard spinach (*Brassica rapa* L. var. *pervirdis*) can alleviate Cd toxicity (Kashem & Kawai, 2007). The results showed that additional Mg in the nutrient solution enhanced the growth of plants suffering from Cd toxicity, resulting in a reduced Cd concentration in the plant. The authors suggested that additional Mg counteracted and detoxified physiological Cd toxicity in plants, especially in shoots. In a transcriptomic study of Mg starvation in *Arabidopsis*, Hermans et al. (2011) showed that a Mg pretreatment of 7 days alleviated the bleaching of young leaves caused by Cd. No or little difference in Cd tissue concentration between the +Mg and -Mg plants was observed. The authors suggested that lower Cd toxicity was probably not attributable to modified root to shoot translocation. A protective effect of Mg pretreatment was also observed on Fe starvation. However, Fe foliar spray partially alleviated Cd-induced chloroses, while it almost completely restored chlorophyll content in Fe-deficient leaves. The author's conclusion was that the protective effect of Mg against Cd toxicity could be attributable partly to the maintenance of Fe status but also to the increase in antioxidative capacity, detoxification, and/or protection of the photosynthetic apparatus. A hydroponic experiment with 2 rice cultivars differing in Cd tolerance was conducted by Hassan et al. (2005) to investigate the alleviating effect of Zn on growth inhibition and oxidative stress caused by Cd. The data showed that the addition of Zn to the medium solution alleviated Cd toxicity, which was reflected in a significant increase in

plant height, biomass, chlorophyll concentration, and photosynthetic rate, and a marked decrease in MDA and activity of anti-oxidative enzymes. It was also noted that Zn increased shoot Cd concentration at higher Cd supply, probably due to the enhancement of Cd translocation from roots to shoots. Similar results have been shown for maize plants (Adiloglu et al., 2005). Köleli et al. (2004) reported that in wheat plants grown on Zn-deficient soil Cd toxicity in the shoot was alleviated by Zn treatment, but this was not accompanied by a corresponding decrease in shoot concentrations of Cd. The results are compatible with the hypothesis that Zn protects plants from Cd toxicity by improving plant defence against Cd-induced oxidative stress and by competing with Cd for binding to critical cell constituents such as enzymes and membrane protein and lipids. Shi et al. (2010) reported that silicon (Si) supply significantly alleviated the toxicity of Cd in peanut seedlings; this was correlated with a reduction in shoot Cd accumulation, an alteration of Cd subcellular distribution in leaves, and a stimulation of antioxidative enzymes. The mechanisms of Si amelioration of Cd stress were cultivar and tissue dependent. Pedrero et al. (2007) reported the protective effect of selenium in alleviation of Cd toxicity in broccoli (*Brassica oleracea*).

Plant resistance can be induced by adopting various strategies. One of these, exogenous use of various growth regulators and other chemicals, has been proven worthwhile in producing resistance to many stresses in a number of plants. Salicylic acid (SA) response against heavy metal stress is a new study subject in the field of crop physiology. Results indicated that seed imbibition with SA affected physiological processes related to growth and development and photosynthesis in maize plants. The beneficial effect of SA during the earlier growth period may help plants to avoid cumulative damage upon exposure to Cd. Alternatively, SA could be involved in the expression of specific proteins or defence-related enzymes. 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 (Krantev et al., 2008; Popova et al., 2008, 2009, 2012).

Several hypothetical explanations may account for the positive effect of SA on Cd-induced stress in plants. SA prevented cumulative damage development in response to Cd. The suggestion was supported by the data of the lowered root level of Cd in SA- pretreated maize plants (Krantev et al. 2008). Similar data have been reported by Szalai et al. (2005) in maize and by Popova et al. (2009) in pea plants. Obviously, the lowered root level of Cd in SA-pretreated plants reduced the harmful effect of Cd and exerted a beneficial effect on growth and photosynthesis. SA alleviated the oxidative damage caused by Cd. The

values of MDA, electrolyte leakage, and proline content of SA- pretreated plants were lower compared with those of Cd-exposed plants (Krantev et al., 2008). Pretreatment with SA exerted a protective effect on the membrane stability judging by the increased total lipids level and by changes in their fatty acid composition (Popova et al., 2008, 2012). Taken together these data support the conclusion that SA may indirectly attenuate Cd toxicity through the development of a general antistress response in plants, which probably includes the regulation of the antioxidant system and lipid metabolism, leading to maintenance of membrane integrity.

A protective effect of abscisic acid (ABA) against Cd toxicity has been suggested by Hsu and Kao (2003). The authors showed that exogenous application of ABA reduced the transpiration rate, decreased Cd content, and enhanced Cd tolerance of rice seedlings. There are data that another phytohormone, gibberellin, is also involved in plant adaptation to Cd stress. Ghorbanli et al. (2000) showed that the addition of 10 mg m⁻³ gibberellin reduced the negative effects of Cd²⁺ in shoot and root growth of soybean plants. The addition of gibberellin caused a partial elimination of the Cd effects on the roots and shoots and increased leaf area and length of stem.

NO is a free radical that can react with O₂⁻ and, thus, regulate its accumulation in the tissue. NO is also a signal molecule involved in triggering the defence response of cells against different stress conditions. A protective role of NO has also been observed in sunflower (Laspina et al., 2005), soybean (Kopyra et al., 2006), pea (Tran et al., 2011), and wheat (Singh et al., 2008) under Cd toxicity.

8. 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 minimise the entry of Cd into the food chain because of the number of associated health risks. Many strategies have been devised to minimise Cd toxicity. Proper plant nutrition is a good strategies 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 (Chaney et al., 1999; Liu et al., 2007). 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 minimise Cd entry into the food chain; it involves the use of hyper-accumulator 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.

Acknowledgement

This work was in part supported by the National Science Foundation at the Ministry of Education and Science, Bulgaria (grant NTS-09-01676/ 2010).

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