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Cadmium-mediated oxidative stress and ultrastructural changes in root cells of poplar cultivars

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Abstract

To understand the phytoremediation capability of poplar 107 (*Populus*×*euramericana* cv. 'Neva') and poplar 118 (*Populus nigra*×*Populus ussuriensis*) stressed by cadmium (Cd), a hydroponic culture was performed. Cd accumulation and translocation were evaluated, activities of antioxidant enzymes and lipid peroxidation were assessed and ultrastructural alterations in root tip cells were investigated. The superoxide dismutase (SOD) activity in the leaves of the two cultivars exposed to 50 μM and 100 μM Cd was significantly higher ($P<0.05$) than that of the control and SOD activity in the roots was low. The levels of peroxisome (POD) and catalase (CAT) were significantly higher ($P<0.05$) than those of the control. The content of malondialdehyde (MDA) increased during the treatment. The morphological alterations in plasma membrane, dictyosomes and ER reflect the features of detoxification and tolerance under Cd stress. Reduction in number of ER, mitochondria and dictyosomes, swelling of rER and significant expansion of mitochondrial cristae were observed. The plasma membrane was damaged and cytoplasmic electron density increased, revealing a pool-like structure. Some electronic-dense granules occurred in the cytoplasm or cell wall. Plasmolysis occurred in some cells. The poplar cultivars used in the present study could be efficient phytoextraction plants as they have considerable ability to accumulate Cd (Cd concentration in shoot exceeding 0.01% (w/w)). The alterations of antioxidant enzymes and MDA content and ultrastructural changes in the poplar cultivars can serve as useful biomarkers in ecotoxicological tests with Cd.

Keywords: Cadmium (Cd); Oxidative stress; Phytoremediation; *Populus*; Ultrastructure

1. Introduction

Cadmium (Cd) is a particularly dangerous pollutant due to its high toxicity and solubility in water (Pinto et al., 2004). Cd is one of the most important factors limiting normal plant growth and can be readily absorbed by plants and accumulated in the human body through the food chain (Järup and Åkesson, 2009).

Owing to the rapid expansion of industrialization and the heavy use of chemical fertilizer, pesticides and herbicides in agriculture, Cd pollution is considered as one of the most serious environmental problems worldwide (Zacchini et al., 2009). Thus, there is an urgent and imperative need to develop efficient techniques for Cd removal from the environment. However, most conventional remediation approaches such as excavation and chemical leaching of metals are expensive and do not provide acceptable solutions to toxic metal pollution. The possibility of using specific plants which hyperaccumulate metals to selectively remove and recycle excessive soil metals was introduced by Chaney (1983). Phytoremediation, the use of plants to extract, sequester and/or detoxify hazardous heavy metal from medium (soil, water and air), is regarded as a feasible alternative with great potential for affordable remediation of polluted sites (Sun et al., 2009). This method has advantages such as lower costs, generation of a recyclable metal-rich plant residue, applicability to a range of toxic metals and radio nucleides, minimal environmental

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disturbance and elimination of secondary air or water-borne wastes (Raskin and Ensley, 2000).

In plants there are protective enzymatic and non-enzymatic mechanisms to scavenge reactive oxygen species (ROS) and alleviate their deleterious effects. Generation of ROS has been identified as an inevitable process of normal aerobic metabolism in plants and the four major types of ROS are singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) (Dinakar et al., 2010). To resist oxidative stress, plants can induce a series of detoxification reactions catalyzed by antioxidative enzymes, including superoxide dismutase (SOD), peroxisome (POD) and catalase (CAT) (Vitória et al., 2001; Zhang et al., 2005). Cadmium also induces oxidative stress of lipid peroxidation. Lipid peroxidation occurs in plants as a consequence of high ROS level when excessive ROS cannot be scavenged immediately and effectively, and finally resulting in the disruption of plant growth and development. Malondialdehyde (MDA) is one of the products resulting from lipid peroxidation damage and its concentration is related to the degree of membrane lipid peroxidation. Therefore, antioxidant enzyme activities and MDA contents serve as important physiological indicators to research the resistant abilities of plants under stress conditions.

The roots are the main route through which Cd enters plants. Although the effects of Cd on physiological and morphological features of poplars are known (Clemens, 2006; Conn and Gilliham, 2010), few investigations on toxic effects of Cd on ultrastructural alterations in constructing anti-Cd system in root tip cells under Cd stress have been reported. Electron microscopy (EM) techniques are very useful in localizing Cd in plant tissues, making it possible to identify the main accumulations of Cd in cells and cellular organelles and observe the range of potential mechanisms at different levels that might be involved in the detoxification and thus tolerance to heavy metal stress (Liu et al., 2009).

Poplars or cottonwood (*Populus*) are thought to be ideal plants with favorable characteristics such as fast growth, easy propagation and a deep rooting system and are currently used in phytoremediation programs (Malá et al., 2010). To understand the phytoremediation capability of the two selected *Populus* cultivars stressed by Cd, a hydroponic culture was performed in the present study. Cd accumulation and translocation were evaluated, tolerance was measured by growth and activities of antioxidant enzymes, lipid peroxidation was assessed and ultrastructural alterations in root tip cells were investigated.

2. Materials and methods

2.1. Culture condition and cadmium treatment

The two poplar cultivars, poplar 107 (*Populus* × *euramericana* cv. 'Neva') and poplar 118 (*Populus nigra* × *Populus ussuriensis*), were selected. Woody cuttings (15 cm in length and 1.5 cm in diameter) from year-old shoots were rooted in vermiculite for 1 month. Uniform roots and new shoots were selected and transferred to 1/2-strength Hoagland nutrient solution spiked with different concentrations of Cd (50 μM and 100 μM) and grown for 40 days. The Hoagland nutrient solution without Cd was used as the control treatment. Cadmium was provided as cadmium chloride ($CdCl_2$). The nutrient solution consisted of 0.75 mM K_2SO_4, 0.65 mM $MgSO_4$, 0.01 mM KCl, 0.25 mM KH_2PO_4, 2 mM $Ca(NO_3)_2$, 100 μM FeEDTA, 10 μM H_3BO_3, 1 μM $MnSO_4$, 0.1 μM $CuSO_4$, 0.05 μM $(NH_4)_6Mo_7O_4$ and 1 μM $ZnSO_4$ (Stephan and Prochazka, 1989), adjusted to pH 5.5. The experiments were conducted in a greenhouse under a 14 h photoperiod at 20/18 °C (day/night) and 65–75% humidity. The solutions were aerated by a pump connected to the containers with pump lines. The solutions were constantly aerated and replaced every 10 days. Root and shoot length was measured before the solutions were replaced as well as samples taken to determine antioxidant enzyme activities and MDA content. All treatments were done in three replicates.

2.2. Sampling procedure and Cd and determination

After 40 days, roots and shoots from each treatment were harvested from the cuttings. After removal of necrotic and putrid tissue, the roots were rinsed in tap water and deionized water to remove traces of nutrients and Cd ions from the root surfaces. The plants were divided into roots, new shoots (stems, young leaves, and mature leaves) and cutting stems (bark and wood). They were dried in a forced-air oven for 4 days at 45 °C, followed by 2 days at 80 °C and 12 h at 105 °C, and then ground with a cutting mill (IKA-Werke GMBH & CO. KG, Germany). All dried plant samples were prepared using wet-digestion method (Piper, 1942). The concentration of Cd was analyzed using inductively coupled plasma atomic emission spectrometry (ICP-AES) (Leeman Labs Inc., New Hampshire, USA).

2.3. Examination of antioxidant enzyme activities

Fresh roots (0.2 g FW) and leaves (0.05 g FW) in 10, 20, 30 and 40 d Cd treated plants were homogenized in 5 mL 0.05 M sodium phosphate buffer (pH 7.8) at the end of each time interval (10 d) of the Cd treatment. The homogenate was centrifuged at 10,000 × *g* for 20 min and the supernatant used for analyzing SOD, POD and CAT. The above steps were carried out at 4 °C. The SOD, POD and CAT activities were estimated according to the modified method of Zhang et al. (2005).

2.4. Examination of MDA content

The fresh samples from each treatment were homogenized in 5 mL 10% trichloroacetic acid (TCA) with a pestle and mortar at the end of each time interval (10 d). Homogenates were centrifuged at 4000 × *g* at 10 °C for 20 min. Aliquot of the supernatant, 2 mL 0.6% 2-thiobarbituric acid (TBA) in 10% TCA was added. The mixtures were heated in boiled water for 15 min and then quickly cooled in an ice bath. After centrifugation at 4000 × *g* 10 °C for 10 min, the absorbance of the supernatant was recorded at 532 nm and 450 nm. Lipid peroxidation was expressed as the MDA content in nmol/g FW (Zhang et al., 2005).

2.5. Transmission electron microscopy

According to the results from ICP-AES, seedlings of the two cultivars exposed to 50 μM and 100 μM Cd for 40 d were selected. The terminal portion (about 2 mm) of each root of the control and the treated groups were cut and fixed in a mixture of 2% formaldehyde and 2.5% glutaraldehyde in 0.2 M phosphate buffer (pH 7.2) for 2 h, and then thoroughly washed with the same buffer three times. This was followed by post-fixation with 2% osmium tetroxide in the same buffer for 2 h. The fixed samples were dehydrated in an acetone series (10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, and 100%), and embedded in Spurr's ERL resin. For ultrastructural observations, ultrathin sections of 75-nm thickness were cut on an ultramicrotome (Leica EM UC6, Germany) with a diamond knife, and were mounted on copper grids with 300 square mesh. The sections were stained with 2% uranyl acetate for 50 min and lead citrate for 15 min. Observation and photography were accomplished by transmission electron microscopy (JEM-1230, Joel Ltd, Tokyo, Japan).

Fig. 2. Large patches of dark color (visible necrosis) in the main vein and lateral veins of leaves in poplar 118 exposed to 100 μM Cd for 40 days. Arrows indicate visible necrosis.

2.6. Statistical analysis

Each treatment was replicated 3 times for statistical validity. SPSS computer software was used for statistical analyses (SPSS Japan Inc., Shibuya, Tokyo, Japan) and SigmaPlot 8.0 software was used for mapping. Any differences between treatments were determined using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test, and scored as significant if ($P<0.05$). The means and standard errors of the means mean±SE are reported.

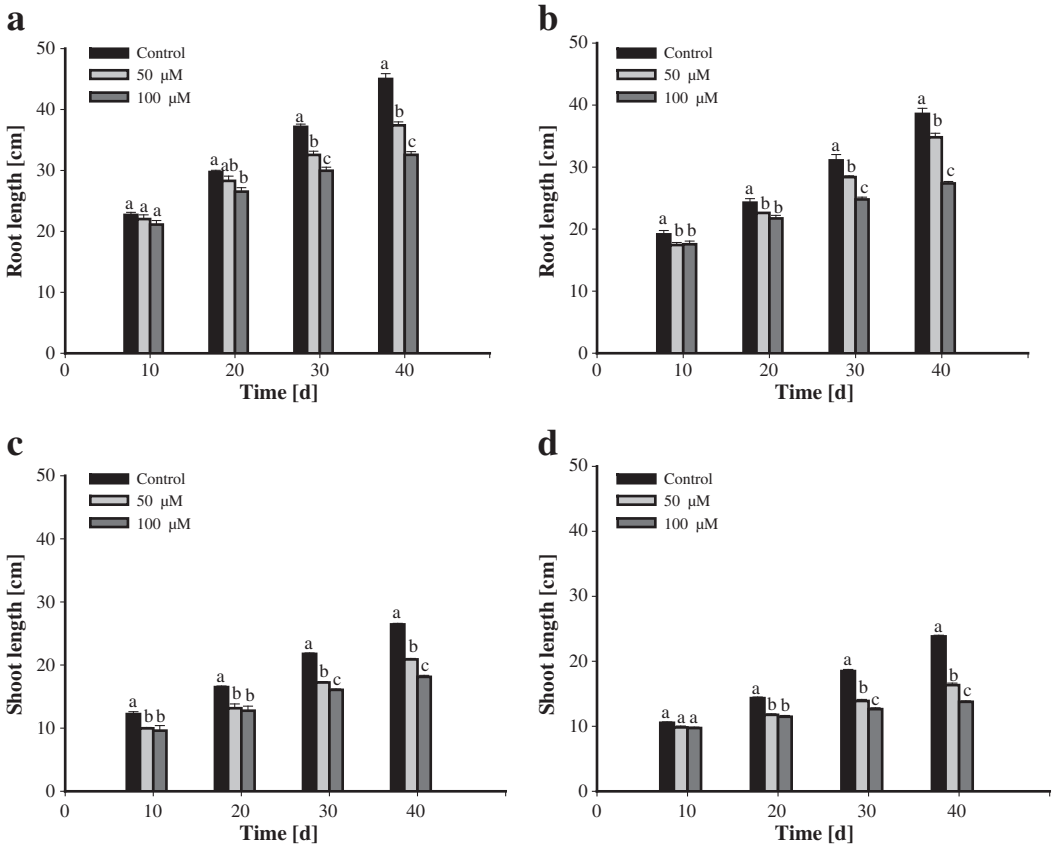

Fig. 1. Effects of different concentrations of Cd on root growth of poplar 107 (a) and poplar 118 (b) and shoot growth of poplar 107 (c) and poplar 118 (d). Vertical bars denote SE (n=3). Values with different letters differ significantly from each other by ANOVA and Tukey's test ($P<0.05$).

Table 1
Concentrations of Cd in different organs of poplar 107 and poplar 118 exposed to different Cd concentrations after 40 days of treatment *.

Elements	Species	Treatment	Organs (μg/g DW)					
			Young leaves	Mature leaves	Stems	Woods	Barks	Roots
Cd	Poplar 107	Control	1.88±0.02a	2.53±0.02a	1.07±0.01a	0.30±0.007a	1.99±0.03a	3.54±0.05a
		50 μM	361.92±0.24b	348.45±0.99b	297.84±1.91b	43.48±0.01b	644.47±0.75b	2730.54±19.05b
		100 μM	437.27±0.30c	387.99±0.25c	426.37±2.13c	52.70±0.34c	706.74±2.63c	2946.47±10.36c
	Poplar 118	Control	2.98±0.04a	3.58±0.006a	3.27±0.02a	0.03±0.003a	2.28±0.05a	4.26±0.04a
		50 μM	418.74±1.16b	233.70±0.77b	360.84±0.95b	11.61±0.15b	335.52±1.85b	2570.47±11.47b
		100 μM	694.84±1.85c	481.09±1.10c	584.51±1.22c	42.13±0.03c	662.68±2.04c	3046.81±13.50c

* Vertical bars denote SE (n=3). Values with different letters differ significantly from each other by ANOVA and Tukey's test ($P<0.05$).

3. Results

3.1. Effects of Cd on root and shoot growth

The effects of Cd on root growth varied with the concentration of Cd and treatment time (Fig. 1a,b). The root growth exposed to 50 μM and 100 μM Cd was inhibited significantly ($P<0.05$) during the whole treatment time versus the control, except for the group treated with 50 μM Cd for 20 days in poplar 107. The effects of Cd on shoot growth also varied with the Cd concentration and treatment time. Shoot growth in the two cultivars exposed to Cd concentrations was inhibited significantly ($P<0.05$), except for the group in poplar 118 treated with 50 μM Cd for 10 days (Fig. 1c,d). Morphology of the roots and leaves exposed to 50 μM Cd was similar to the control during the first 20 days. With prolonged duration of treatment, large patches of dark color (visible necrosis) in the main vein and lateral veins of leaves were observed in the two cultivars exposed to Cd (Fig. 2).

3.2. Cd accumulation

The Cd content of the roots, stems, and young and mature leaves and cutting stem bark and wood in the two poplar cultivars increased significantly ($P<0.05$) with increasing Cd concentration (Table 1). This investigation also assessed Cd content in stem cuttings and new shoots. Cd contents in bark were significantly higher when compared with wood. On average, the Cd contents of

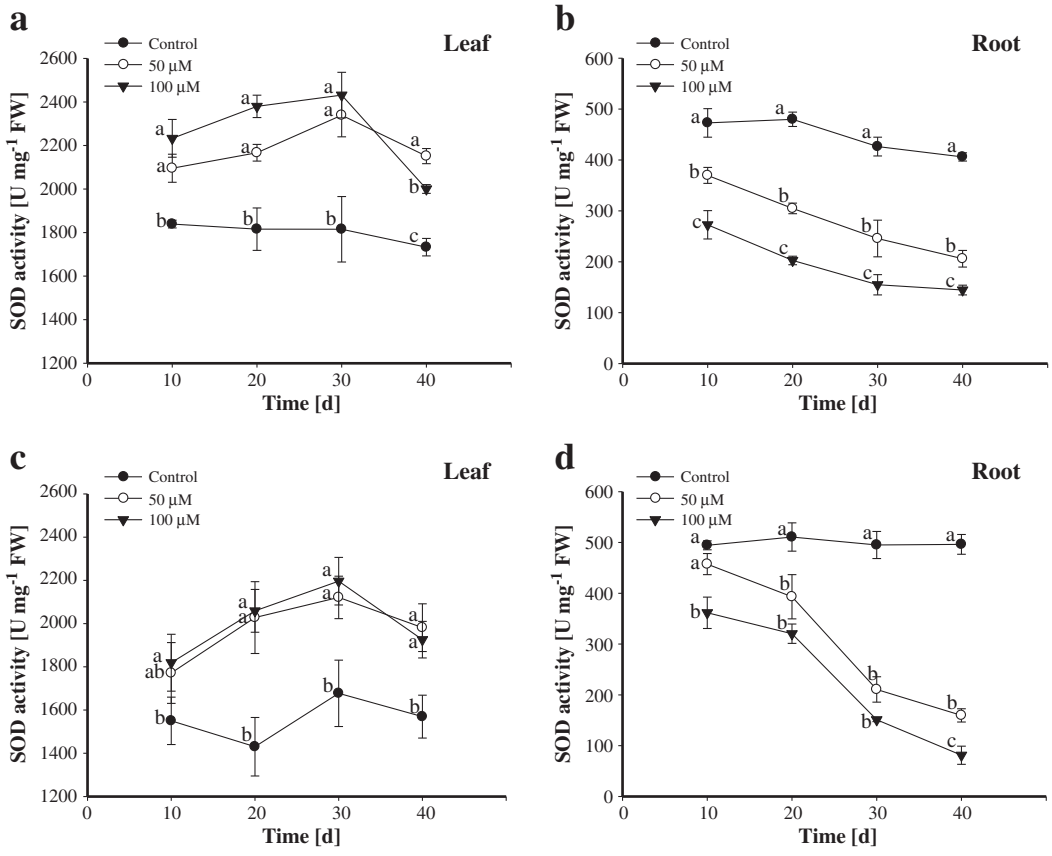

Fig. 3. Effects of different concentrations of Cd on the activities of SOD in poplar 107 (a, b) and poplar 118 (c, d) exposed to Cd stress in 40 days. a and c SOD in leaves, b and d SOD in roots. Vertical bars denote SE (n=3). Values with different letters differ significantly from each other by ANOVA and Tukey's test ($P<0.05$).

poplar 107 and poplar 118 in bark were 14 and 19 times the contents in wood, respectively. Cd contents in young leaves were significantly higher ($P<0.05$) than in mature leaves. About 60% of the Cd was accumulated in roots and the rest was transferred to the shoots.

3.3. Effects of Cd on activities of antioxidant enzymes

Effects of Cd on SOD activities of leaves and roots in the two cultivars varied with the different concentrations of Cd and the duration of treatment. SOD activities in leaves exposed to 50 μM and 100 μM Cd during the whole treatment were observed to be significantly higher ($P<0.05$) when compared with the control, except for poplar 118 exposed to 50 μM Cd for 10 days (Fig. 3a,c). The SOD activities in roots were lower than in leaves. The levels of SOD in roots exposed to 50 μM and 100 μM Cd were significantly lower ($P<0.05$) in comparison with the control, except for poplar 118 treated with 50 μM Cd for 10 days (Fig. 3b,d). The activity of SOD in roots treated with 100 μM Cd was the lowest and decreased progressively with prolonging duration of treatment. POD activity in leaves followed the same trend as SOD activity (Fig. 4a,c). POD activities in roots treated with 50 μM and 100 μM Cd were noted to be significantly higher ($P<0.05$) in comparison with the control, except for poplar 118 treated with 50 μM Cd for 10 days (Fig. 4b,d). The activity of POD in roots increased significantly with increasing Cd concentration during 20 to 30 day treatment. CAT activity in leaves was found to be significantly increased ($P<0.05$) for the duration of the treatment when compared with the control, except for poplar 118 exposed to 50 μM Cd for 10 days (Fig. 5a,c). There was a promoting effect ($P<0.05$) on the CAT activity in roots treated with 50 μM and 100 μM Cd during 20 to 40 day treatment in comparison with the control (Fig. 5b,d). CAT activities in leaves and roots declined at day 40, except for poplar 118 roots exposed to 50 μM Cd.

3.4. Effects of Cd on MDA contents

The MDA contents in roots and in leaves increased with prolonged treatment time (Fig. 6). The MDA content in leaves of the two cultivars exposed to 50 μM and 100 μM Cd was significantly higher ($P<0.05$) after 20 days when compared to the control (Fig. 6a,c). In roots, the same trend as in leaves was noted (Fig. 6b,d).

3.5. Effect of Cd on subcellular structures of root-tip meristems

A typical ultrastructure was exhibited in the control cells (Fig. 7a). The plasma membrane was unfolded with a uniform shape in all parts. Large amounts of endoplasmic reticulum (ER), dictyosomes, mitochondria and ribosomes were immersed in dense cytoplasm. The nucleus with well stained nucleoplasm and

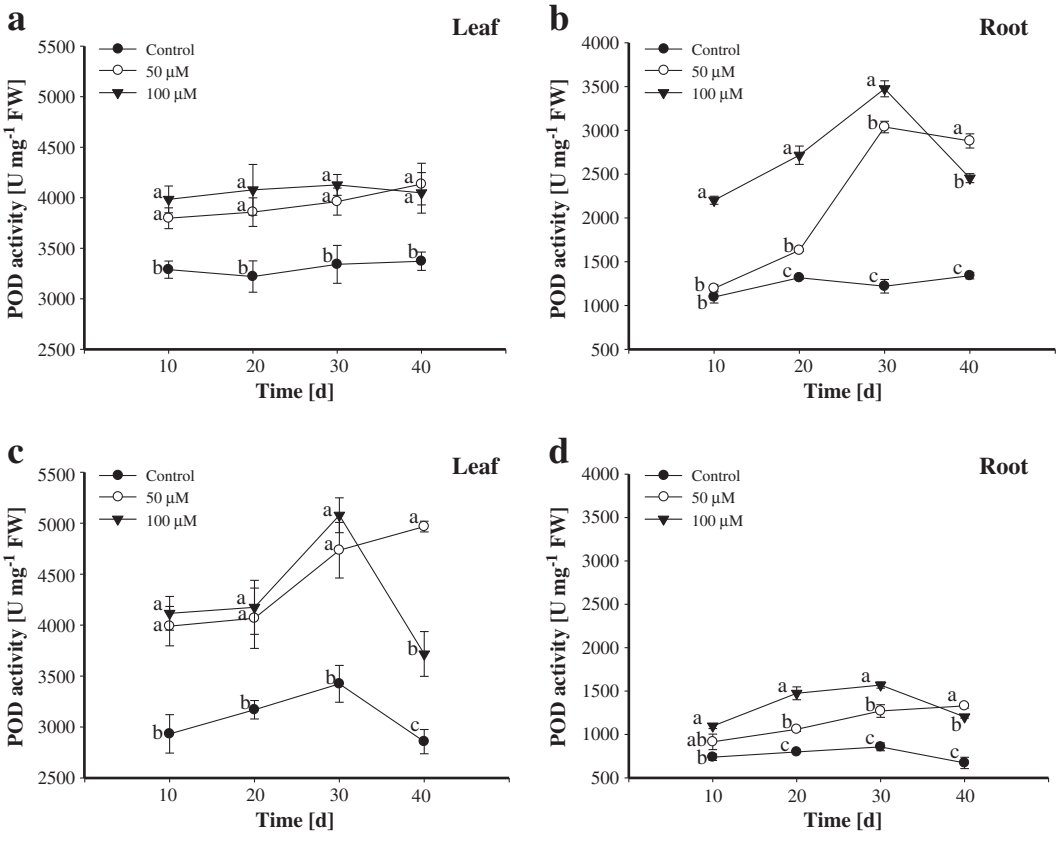

Fig. 4. Effects of different concentrations of Cd on the activities of POD in poplar 107 (a, b) and poplar 118 (c, d) exposed to Cd stress in 40 days. a and c POD in leaves, b and d POD in roots. Vertical bars denote SE (n=3). Values with different letters differ significantly from each other by ANOVA and Tukey's test ($P<0.05$).

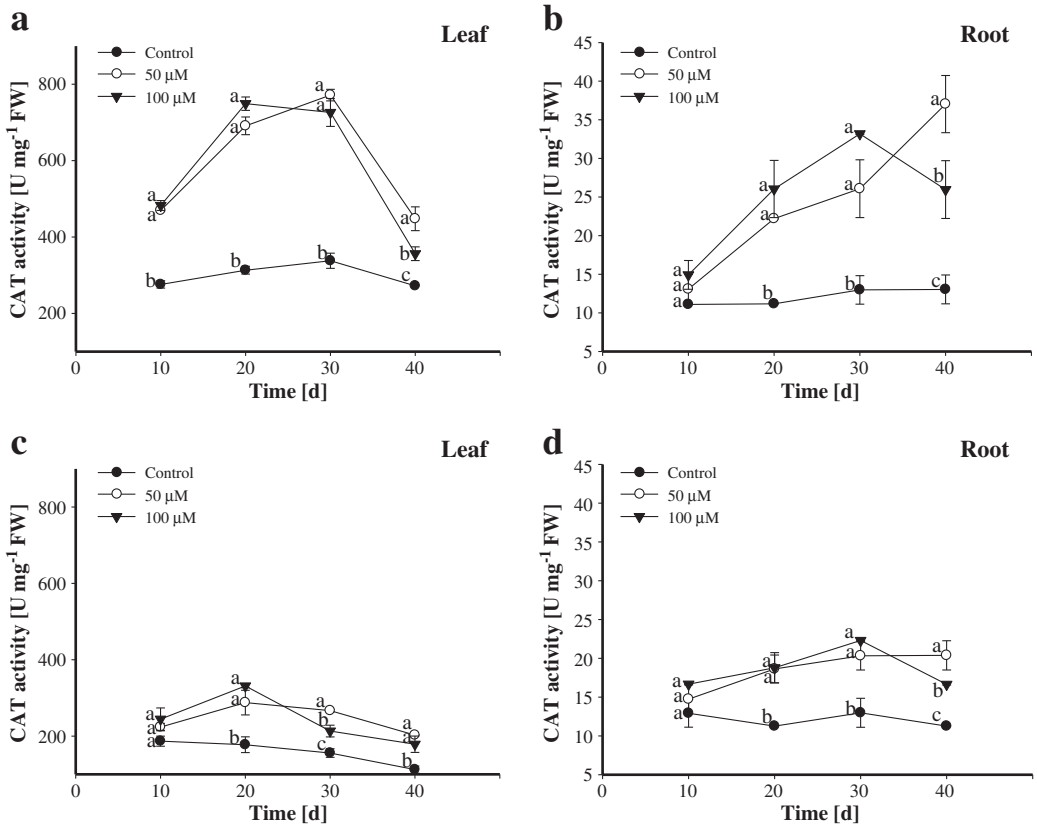

Fig. 5. Effects of different concentrations of Cd on the activities of CAT in poplar 107 (a, b) and poplar 118 (c, d) exposed to Cd stress in 40 days. a and c CAT in leaves, b and d CAT in roots. Vertical bars denote SE (n=3). Values with different letters differ significantly from each other by ANOVA and Tukey's test ($P<0.05$).

a distinct nucleolus was located in the center of cells, whereas vacuoles were distributed in root meristem cells. Root tip cells of the two cultivars exposed to Cd solutions showed several ultrastructural alterations in comparison with the control. Visible symptom of Cd toxicity was noted at 50 μM Cd after 40 day treatment. Some small vesicles containing electron-dense granules were distributed in the cytoplasm (Fig. 7b,c). They were formed by invaginations of the plasma membrane, or dictyosomes and ER secretion. Usually, several vesicles gradually fused together to produce a bigger cytoplasmic vacuole (Fig. 7d). Some vesicles were repeatedly wrapped and then formed "multivesicular bodies" (Fig. 7c) in the cytoplasm, which became clearly vacuolated. Electron-dense granules were always surrounded by vesicular systems. Some electronic-dense granules also appeared in the cytoplasm or cell wall. Severely toxic characteristics in ultrastructure of the root tip cells exposed to 100 μM Cd appeared. Plasmolysis occurred in some cells (Fig. 7e). Cell walls were dark and a large amount of electron-dense granules was precipitated in them (Fig. 7f). Content of cytoplasm in some cells became less and some cells were severely injured, eventually leading to death. The ultrastructural and morpholoIgical damage were noted after 40 d treatment, revealing reduction of ER, mitochondria and dictyosome number (Fig. 8a), swelling of rough endoplasmic reticulum (rER, Fig. 8b) and significant expansion of mitochondrial cristae (Fig. 8c). The plasma membrane was damaged and cytoplasmic electron density increased, revealing a pool-like structure (Fig. 8c,d).

4. Discussion

Adaptive responses in morphology and biomass production are the primary tolerance indicators which poplar can cope with the Cd contaminated environment. Stunting, chlorosis, necrosis and desiccation are typical toxic symptoms of Cd stress in foliage (Das et al., 1997). In the present investigation, the occurrence of toxic symptoms was positively correlated with the Cd concentration in leaves. The Cd level in young leaves was significantly higher than in old leaves, and all the symptoms were observed in young leaves, which was in agreement with the findings of Gu et al. (2007) and Pietrini et al. (2010) in willow and poplar.

Since Chaney (1983) suggested that some heavy-metal contaminated soils may be cleaned up by growing crop plants which accumulate the pollutants, the first feasibility experiments using 'hyperaccumulator' plants were conducted by McGrath et al. (1993), although Huiyi et al. (1991) had already shown that some forest species, including poplar, could be used to remove Cd from polluted soils. Data from the present investigation indicated that the two cultivars had the ability to accumulate Cd primarily in their roots (60%), with lower concentrations in the shoots. There are several definitions on hyperaccumulators (Baker and Brooks, 1989; Baker and Whiting, 2002). Most recognized standard criteria were based on metal concentrations in aboveground tissue of plant material sampled from its natural habitat (Pollard et al., 2002.). The currently accepted 0.01% (w/w) Cd concentration in the shoot defines hyperaccumulation (Baker

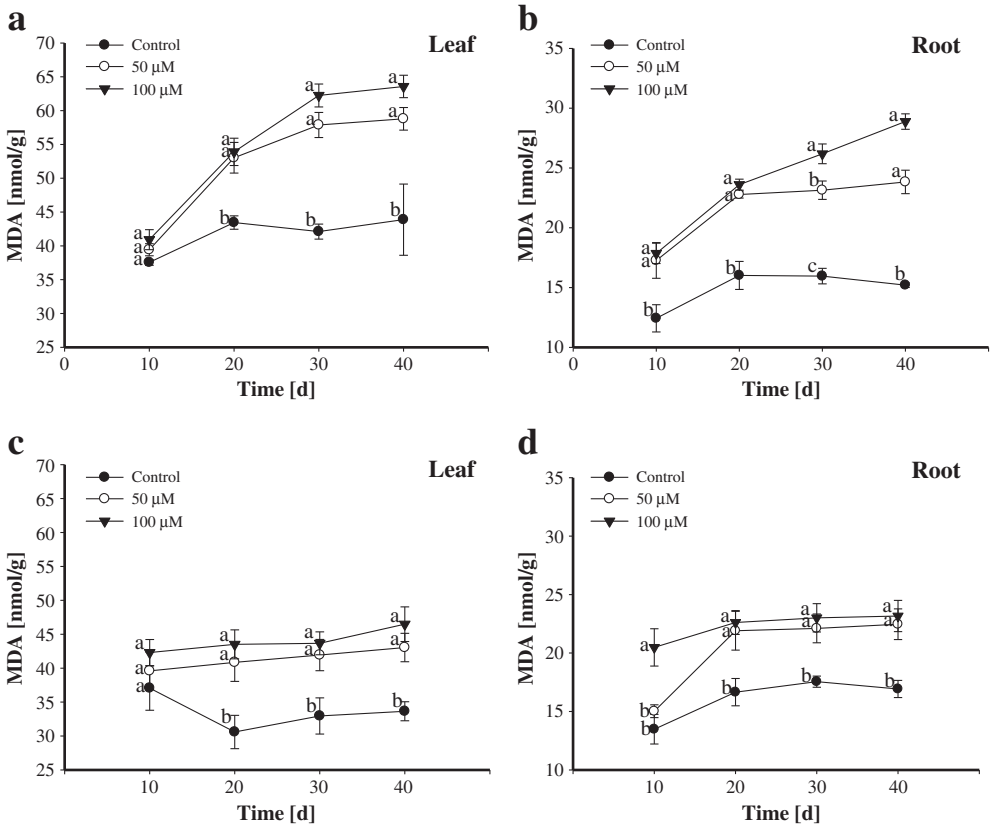

Fig. 6. Effects of different concentrations of Cd on the contents of MDA in poplar 107 (a, b) and poplar 118 (c, d) exposed to Cd stress over 40 days. a and c MDA in leaves, b and d MDA in roots. Vertical bars denote SE (n=3). Values with different letters differ significantly from each other by ANOVA and Tukey's test ($P<0.05$).

et al., 2000). Data from the present investigation implied that poplar trees could be efficient phytoextraction plants with considerable ability to accumulate Cd. This was in accordance with the findings of Jiao et al. (2012), Zacchini et al. (2009) and Wu et al. (2010).

The antioxidative system (consisting of enzymes and antioxidants) plays a protective role by stabilizing the amounts of ROS in plant cells (Yurekli and Porgali, 2006). Cd causes oxidative stress, due to an interaction with the antioxidative defense, disruption of the electron transport chain, or induction of lipid peroxidation (Nikolić et al., 2008).

SOD is the cell's first line of defense against ROS as the superoxide radical is a precursor to several other highly reactive species so that control over the steady state of superoxide concentration by SOD constitutes an important protective mechanism (Fridovich, 1997). The results from the investigation showed that the SOD activity in the leaves of two cultivars exposed to 50 μM and 100 μM Cd was significantly higher ($P<0.05$) than the control. High SOD activity has been associated with stress tolerance in plants, which may be attributed to the increased production of superoxide, resulting in the activation of existing enzyme pools or increased expression of genes encoding SOD (Mishra et al., 2006). However, the activity in roots was significantly lower ($P<0.05$) compared to the control in the present study. Vitória et al. (2001) indicated that reduction of SOD may be attributed to an inactivation of the enzyme by H_2O_2 produced in different compartments, where SOD catalyzes the disproportionation of superoxide radicals.

Peroxidase activity reflects the modified mechanical properties of the cell wall and cell membrane integrity of plant leaves under stress conditions (Ekmekçia et al., 2008). The activity of POD in the two cultivars increased in the present investigation. Increased POD activity may be due to Cd directly causing excessive production of H_2O_2 in cuttings and/or increased H_2O_2 was due to SOD. Thus, increased POD activity, in turn, scavenged excessive H_2O_2 and damage was limited.

Catalase is the most universal oxidoreductase, which scavenges H_2O_2 to O_2 and H_2O. Cd-induced inhibition of APX and CAT was also associated with H_2O_2 accumulation and growth retardation in poplar roots (Schutzendubel and Polle, 2002). Some reports indicated that CAT activity often decreased following exposure to elevated Cd concentrations (Shim et al., 2003). However, in the present investigation the CAT activities in leaves and roots declined only at day 40, except for poplar 118 roots exposed to 50 μM Cd. Vitória et al. (2001) reported that the activities of CAT, GR and specific isoenzymes of SOD increased in the leaves and roots of a resistant variety of radish, following exposure to increasing (between 0.25 and 1 mM) concentrations of Cd.

MDA formation is used as the general indicator of the extent of lipid peroxidation resulting from oxidative stress. In the present investigation the content of MDA increased compared

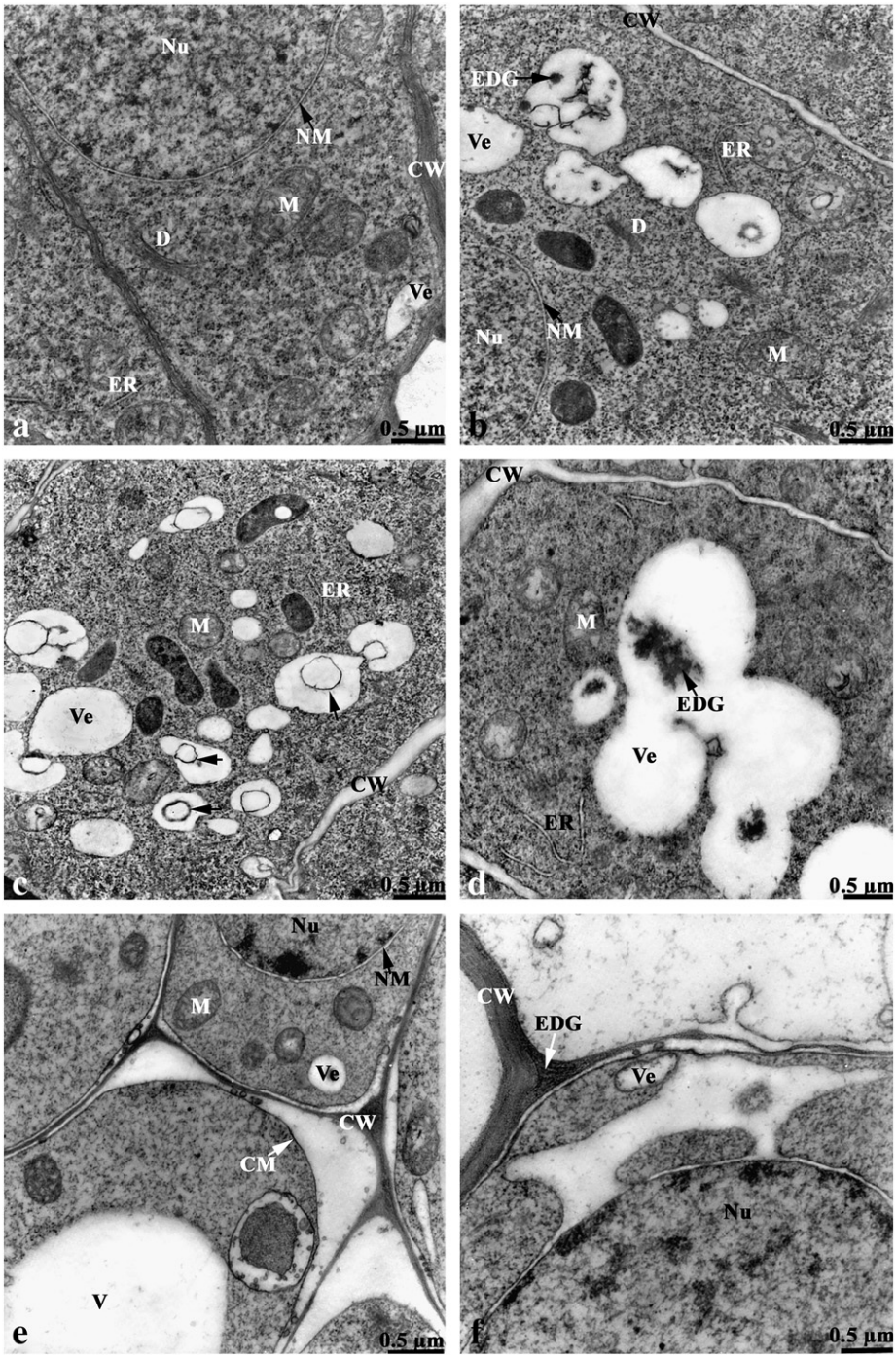

Fig. 7. TEM micrographs showing toxic effects of Cd on ultrastructure of the root meristematic cells of the two poplar cultivars after 40 d treatment. a: Control cells showing well-developed root tip cells. b–c. In poplar 107 exposed to 50 μM Cd, some small vesicles containing electron-dense granules were noted in cytoplasm. Vesicles repeatedly wrapped and formed "multivesicular bodies" in cytoplasm (arrows). d. Vesicles gradually fused together producing a big cytoplasmic vacuole (50 μM Cd, poplar 118). e. Plasmolysis occurred in some cells of poplar 107 exposed to 100 μM Cd. f. A large amount of electron-dense granules was precipitated in cell walls of poplar 107 treated with 100 μM Cd.CM = cytoplasm membrane, CW = cell wall, D = dictyosome, ER = endoplasmic reticulum, EDG = electron-dense granules, M = mitochondria, Nu = nucleus, NM = nuclear membrane, V = vacuole, Ve = vesicle.

to the control, indicating that Cd like other environmental stresses can generate the production of a powerful oxidation which in turn brings about lipid peroxidation (Hatata and Abdel-Aal, 2008).

The ultrastructural investigation in root cells of the two poplar cultivars after the treatment with Cd indicated that the structural alternation of root cells and metal accumulation in the cells are dependent on the concentration of the metal. At an ultrastructural level, 50 μM Cd did not cause significant cellular damage to root cells. At high Cd concentration (100 μM Cd), a series of cell structure injuries occurred. Dictyosomes were sensitive to Cd stress and the number of secreted vesicles increased. They

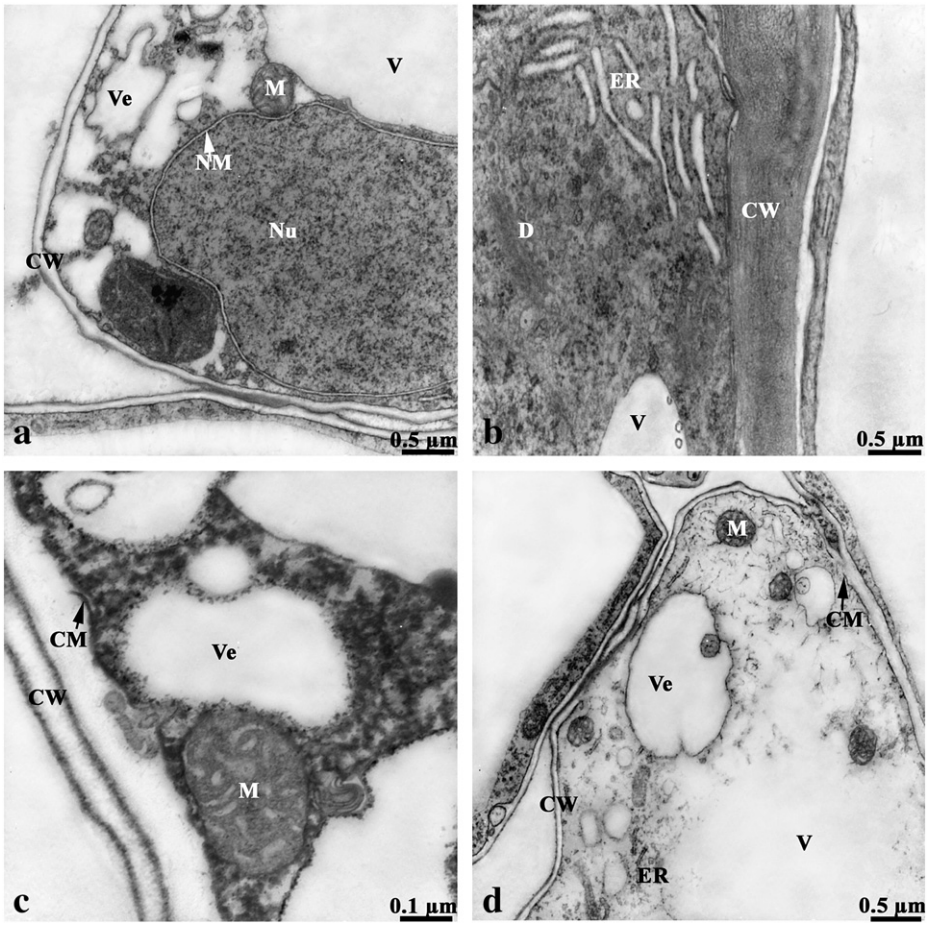

Fig. 8. TEM micrographs showing toxic effects of Cd on ultrastructure of the root meristematic cells of poplar 118 exposed to 100 μM Cd after 40 d treatment. a: Revealing reduction of ER, mitochondria and dictyosomes in number. b. Showing swelling of rER. c–d. Showing significant expansion of mitochondrial cristae. The plasma membrane was damaged (arrows) and cytoplasmic electron density increased, revealing pool-like structure.CM = cytoplasm membrane, CW = cell wall, D = dictyosome, ER = endoplasmic reticulum, M = mitochondria, Nu = nucleus, NM = nuclear membrane, V = vacuole, Ve = vesicle.

gradually disintegrated with increasing Cd concentration. At the same time, parallel arrays of rER with irregularly extended cisternae appeared in the cytoplasm. These vesicles from membranes of ER might carry some polysaccharides and proteins for Cd detoxification. Cd at high concentrations could cause a greater degree of cell vacuolization, increasing the compartmentation in meristems and cortical parenchyma cells. Sanità di Toppi and Gabbrielli (1999) indicated that a significant role in Cd detoxification and Cd tolerance was played by vacuolar compartmentalization preventing the free circulation of Cd ions in the cytosol forcing these ions into a limited area. The toxicity symptoms seen in the presence of excessive amounts of Cd may be due to destroying of the defense systems of cells. At a higher concentration of Cd (100 μM), the toxic symptoms of root cells in poplar are mainly the disintegration of cell organelles, disruption of membranes, withdrawal of plasma membrane from cell walls, and formation of multivesiculate bodies in cytoplasm (Figs. 7–8). Early researches reported that the occurrence of electron dense deposits in vacuoles and appearance of small vesicles in cytoplasm seemed to be common feature of metal-stressed plants (Jiang et al., 2009). The ultrastructural results in the present investigation showed some electron-dense granules in vacuoles and cytoplasm in the meristematic cells of two poplar cultivars after Cd treatment. These results supported the findings observed by X-ray microanalysis and by analytical EM (Liu et al., 2007; Rauser and Ackerley, 1987; Vázquez et al., 1992).

The increased amount of electron-dense granules in metal-exposed cells suggested that the formation of granules could be a detoxification pathway to prevent cell damage (Einicker-Lamas et al., 2002). Neumann et al. (1997) indicated that the cell wall played a role in metal tolerance when the cell wall volume was high compared to the cytosol and vacuole. However, the results from different authors are quite conflicting. Earlier studies reported that Cd mainly accumulated in cell walls when its content was high (Khan et al., 1984; Küpper et al., 2000), or it accumulated mainly in vacuoles and nuclei (Rauser and Ackerley, 1987). For example, Cd was localized mainly in the vacuoles and nucleoli of cortical cells of differentiating and mature root tissues in *Allium sativum* treated with 10 mM Cd for 9 d (Liu and Kottke, 2003) and in *Allium cepa* exposed to 1 mM and 10 mM Cd for 2 and 3 d (Liu and Kottke, 2004), while no Cd was detected in the cell walls.

The results in the present investigation revealed that Cd was detected in the cell wall. The conflicting results may be due to differences between the plant species and between their capacities to accumulate and sequester toxic metals, as well as

to differences in the used experimental methods and conditions (Turnau et al., 1996).

5. Conclusions

Heavy metal uptake by plant has its limitations as heavy metal toxicity can overpower the hyperaccumulator. It is very important to select better hyperaccumulators to improve phytoextraction efficiency in heavy metal contaminated soils. In view of the present findings, it is suggested that the examined poplar trees could be efficient phytoextraction plants with considerable ability to accumulate Cd. Poplar trees are fast-growing plants and exhibit high phytoextraction efficiency with higher biomass production and they could play an important role in treating Cd-contaminated soils.

In the two poplar cultivars investigated, cell walls immobilized some Cd ions. However, vacuoles were the main storage sites of Cd. The morphological alterations in plasma membrane, dictyosomes and ER reflected the features of detoxification and tolerance under Cd stress. Root meristematic cells of the examined poplar trees exposed to low Cd concentrations had a rapid and effective defense system, but at increased levels of Cd in the cytosol, cells were seriously injured.

The alterations of antioxidant enzymes and MDA content and ultrastructural changes in the poplar cultivars can serve as useful biomarkers in ecotoxicological tests with Cd. The data from these biomarkers can provide valuable information for monitoring and forecast early effects of exposure to Cd in real scenario conditions.

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