

RESPONSES OF HYBRID POPLAR TO CADMIUM STRESS: PHOTOSYNTHETIC CHARACTERISTICS, CADMIUM AND PROLINE ACCUMULATION, AND ANTIOXIDANT ENZYME ACTIVITY

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The effect of two cadmium concentrations (10^{-5} M and 10^{-4} M) on growth, Cd accumulation and within-plant distribution, proline (Pro) content, and the activity of superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST) and glutathione peroxidase (GSH-Px) was examined in leaves and roots of poplar plants. Symptoms of Cd toxicity were evident in Cd-treated plants: stunted growth (plant height and biomass), decreased root length, and chlorosis of young leaves. Stem and leaf growth was more affected than root growth. The decreased photosynthetic activity of treated plants may have been due to lowered chlorophyll synthesis. The activity of SOD, CAT, GST and GSH-Px was altered, as was Pro accumulation. The increment of pollutant concentration in the nutrient medium was associated with higher Cd accumulation in plants. Cd in roots was ~40 times higher than in leaves and stems of plants exposed to 10^{-5} M Cd. Although Cd accumulation in roots was high, the potential use of this hybrid poplar for remediation of Cd-contaminated sites seems low. This is because, apart from the described growth disturbances, translocation of Cd from roots to aboveground parts is low.

Key words: Antioxidant enzymes, biomass production, cadmium, chlorophyll, poplar, proline.

INTRODUCTION

Beside the ability to take up essential nutrients, plants are able to absorb and accumulate other metals, even those with unknown metabolic function. The presence of heavy metals (HM) in excess amounts is a global problem, threatening the health of vegetation, wildlife and humans (Heckathorn et al., 2004). Cadmium (Cd) is a heavy metal (specific density exceeding 5 g/cm^3 , and relative atomic mass above 40; Seregin and Ivanov, 2001) constituting a nonliving component of the biosphere, and occurs naturally in soils and plants (Page et al., 2006). It is a nonessential element ranked as one of the most hazardous HM (Kim et al., 2003). The main sources of Cd in the environment are industrial processes and phosphate fertilizers (Wagner, 1993). Elevated concentrations of both essential and nonessential HM can lead to symptoms of toxicity and growth inhibition in most plants (Hall, 2002). The most common symptoms of Cd phytotoxicity are leaf chlorosis,

growth inhibition, disturbance of respiration and nitrogen metabolism, and reduction of photosynthesis and of water and mineral element uptake (Sanità di Toppi and Gabbrielli, 1999).

The principal mechanisms of plant response to Cd stress include phytochelatin-based sequestration and compartmentalization, and additional defense mechanisms based on cell wall immobilization, plasma membrane exclusion, and induction of stress proteins (Dražić et al., 2006). HM stress stimulates free proline accumulation in some plant species (Schat et al., 1997; Pandey and Sharma, 2002; Zengin and Munzuroglu, 2005). Free Pro increases plant tolerance to stress by osmoregulation, stabilizing protein synthesis, and protecting enzymes against denaturation (Kuznetsov and Shevyakova, 1997).

The effects of Cd on plant growth (Chen and Kao, 1995; Ouzounidou et al., 1997; Ali et al., 2001),

Abbreviations: CAT – catalase; Cd – cadmium; GSH-Px – glutathione peroxidase; GST – glutathione-S-transferase; HM – heavy metals; Pro – proline; ROS – reactive oxygen species; SOD – superoxide dismutase.

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TABLE 1. Comparison of growth characteristics of poplar plants grown in the presence of 0, 10^{-5} and 10^{-4} M Cd. Values are means of five replicates. Different letters in each column indicate significant differences between treatments at $p < 0.05$ using Duncan's multiple range test. R/S – root/shoot ratio

Treatment Cd [M]	Mass			R/S	Plant height [cm]	Leaf area [cm ² plant ⁻¹]
	roots [g]	stem [g]	leaves [g plant ⁻¹]			
0 (control)	6.60 a	9.95 a	20.00 a	0.30	64.60 a	1259 a
10^{-5}	3.69 b	2.75 b	7.54 b	0.36	36.60 b	527 b
10^{-4}	3.68 b	2.30 b	5.83 b	0.45	24.80 c	407 b

structure (Barceló et al., 1988), photosynthetic characteristics (Ali et al., 2001; Zengin and Munzuroglu, 2005), mineral nutrition (Zornoza et al., 2002; Kim et al., 2003), enzyme activity (Schützendübel et al., 2001; Scabba et al., 2006) and water relations (Poschenrieder et al., 1989) are widely studied and well documented in various species.

One of the responses of plants to various stress factors, including HM, is overproduction of free radicals and reactive oxygen species (ROS) which may result in oxidative stress (Yurekli and Porgali, 2006). Along with hydrophilic (GSH, ascorbate) and lipophilic (α -tocopherol, β -carotene) antioxidants, plants also possess antioxidant enzymes that protect them from oxidative damage (Gallego et al., 2002). Superoxide dismutase (SOD) converts the superoxide anion to H_2O_2 and O_2 , catalase (CAT) converts H_2O_2 to H_2O and O_2 , and glutathione peroxidase (GSH-Px) catalyzes reduction of organic peroxides and H_2O_2 to H_2O using reduced glutathione (GSH). Unlike copper or iron which directly produce free radicals (Van Assche and Clijsters, 1990), Cd is not a redox-active metal, and it increases levels of ROS probably via indirect mechanisms (Michalak, 2006). Oxidative stress in Cd-treated plants is an early symptom of toxicity (Sandalio et al., 2001), and Cd-induced modifications of antioxidative enzyme activity are well known (Schützendübel et al., 2001). The activity of antioxidative enzymes under cadmium stress is concentration-dependent and can be either inhibited or stimulated (Milone et al., 2003). Cd decreased the activity of CAT and increased the total activity of SOD in *Pisum sativum* (Dalurzo et al., 1997). The activity of SOD, CAT, ascorbate peroxidase and GR was diminished in sunflower leaves due to Cd exposure (Gallego et al., 1996). The activity of CAT and GR was increased in roots and leaves of radish after 24 h exposure to Cd (Vitória et al., 2001). In duckweed plants exposed to cadmium the activity of guaiacol peroxidase (GPX) increased, but there was no effect on ascorbate peroxidase (APX) and SOD (Paczkowska et al., 2007). Glutathione transferase plays an important role in the removal of products of lipid and protein peroxidation because this enzyme catalyzes the conjugation of endogenous or exogenous electrophilic substrates to reduced glutathione (Gajewska et al., 2006).

Hybrid poplars were originally bred and grown as a cash crop for pulpwood and as a renewable energy source. Because of their rapid growth and high transpiration rate, they may be useful for phytoremediation, that is, decontamination of sites. According to Chappell (1997), the advantages of the genus *Populus* in phytoremediation are the great number of species that enter no food chains, fast growth (3–5 m/year), and a high transpiration rate. Detoxification of soil and water contaminated with heavy metals has been demonstrated for various poplar species. The aim of this paper was to analyze the impact of two Cd concentrations (10^{-5} M, 10^{-4} M) upon the activity of CAT, GSH-Px, SOD and GST, as well as proline content in roots and leaves of poplar plants. Also studied were growth parameters, photosynthetic characteristics, metal accumulation and within-plant Cd distribution.

MATERIALS AND METHODS

PLANT CULTIVATION

Hybrid poplar (*Populus nigra* \times *maximowiczii* \times *P. nigra* var. *Italica*) clone 9111/93 was grown using the method of water culture. Woody cuttings (taken before the beginning of vegetation and kept in a cool place) were obtained from the Institute of Lowland Forestry and Environment (Novi Sad, Serbia) collection. The experiment was carried out in semi-controlled greenhouse conditions. Cuttings were placed in 40 l plastic pots (15 cuttings per pot) and grown in deionized water for six weeks (completion of root development). After that period, homogeneously rooted cuttings were kept in Hoagland's nutrient solution at full strength (Hoagland and Arnon, 1950). The nutrient solution was continuously aerated and renewed every two weeks. Cadmium was applied as $CdCl_2$ dissolved in deionized water and added to nutrient solution. Three treatments were applied: 0 M Cd (control) and nutrient solutions containing 10^{-5} and 10^{-4} M Cd. All treatments were made in triplicate with one-stemmed plants. Growth, Cd accumulation and biochemical characteristics were analyzed after six weeks of Cd exposure.

Analyses were performed on leaves of the same physiological status. The seventh leaf from the top was sampled from all plants of the same treatment, measured (leaf area and mass), and mixed to obtain a representative sample used in all biochemical analyses. Only healthy, undamaged leaves were used. After plant harvest, all leaves were collected, dried, milled and used for chemical analyses.

PLANT GROWTH ANALYSIS

After sampling, plant organs were separated and measured. Total root, stem, and leaf biomass was measured in treated and control plants, and presented as fresh mass (g). Number of leaves per plant was counted. Weight of leaves per plant (g plant^{-1}) were estimated and leaf area (cm^2) was measured with a photoelectric leaf area meter (LI-COR 3000, Lambda Instruments, Lincoln, Neb., U.S.A.).

ENZYME ASSAYS AND PROLINE DETERMINATION

Root and leaf tissues were ground and homogenized with a mortar and pestle in liquid nitrogen and extracted in 5 volumes of 50 mM phosphate buffer, pH 7.0, containing 1 mM EDTA and 1% polyvinylpyrrolidone (PVP). The extract was centrifuged at 4°C at 15,000 rpm for 15 min, and the supernatants were used for biochemical analyses.

Total protein concentration in the supernatant was determined by the method of Bradford (1976) with bovine serum albumin as the standard. Catalase (CAT) activity was determined by the rate of disappearance of hydrogen peroxide measured spectrophotometrically according to the method of Claiborne (1984). Glutathione-S-transferase (GST) activity was measured by monitoring the formation of the product of the reaction between reduced glutathione and 1-chloro-2,4-dinitrobenzene (CDNB) at 340 nm as described by Habig et al. (1974). The activity of superoxide dismutase (SOD) was measured by inhibition of superoxide radical production in the xanthine-xanthine oxidase reaction, according to the method described by McCord and Fridovich (1968). The activity of glutathione peroxidase (GSH-Px) was assayed as described by Paglia and Valentine (1967). The activity of GSH-Px was defined as 1U = 1 nmol NADPH oxidized per min. Proline was estimated spectrophotometrically as ninhydrin complex extracted in toluene (Bates, 1973).

DETERMINATION OF CHLOROPLAST PIGMENT CONCENTRATION AND PHOTOSYNTHETIC ACTIVITY

The concentration of chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids was measured spectrophotometrically (DU-65 BECKMAN spectrophotometer) in absolute acetone extracts of 0.2 g leaf

TABLE 2. Cd accumulation in roots, stem and leaves of poplar plants grown in the presence of 0, 10^{-5} and 10^{-4} M Cd. Values are means of three replicates. Different letters in each column indicate significant differences between treatments at $p < 0.05$ using Duncan's multiple range test. R/S – root/shoot ratio

Treatment Cd [M]	Cd concentration ($\mu\text{g g}^{-1}$ d.m.)			R/S
	leaves	stem	roots	
0 (control)	1.6 c	0.15 c	4.23 c	2.42
10^{-5}	85.8 b	109.5 b	3956.1 b	21.46
10^{-4}	214.0 a	371.3 a	6778.3 a	11.58

(Wettstein, 1957) and expressed as mg g^{-1} dry weight. The rate of net photosynthesis was determined polarographically, using a Hansatech DW1 electrode. Leaf segments were cut from the middle of the leaf lamina, avoiding the main veins, and suspended in buffer, pH 7.6–7.8, containing 10 mM NaHCO_3 (Walker, 1987).

Cd CONCENTRATION

Plant material was dried at 100°C, milled, dry-ashed at 450°C and treated with HCl. Cd concentration in plant organs was determined using a standard atomic absorption spectrophotometer (VARIAN SPECTRAA). The amount of Cd was calculated on the basis of dry weight and expressed as $\mu\text{g g}^{-1}$.

STATISTICAL ANALYSES

The data were calculated with Microsoft Office Excel, and processed statistically by ANOVA using STATISTICA for Windows. Means of the studied parameters were compared with the least significant difference (LSD) values by Duncan's test and ranked. $P < 0.05$ was taken as the significance level.

RESULTS

PLANT GROWTH AND Cd ACCUMULATION

Visual symptoms of Cd toxicity such as stunted growth (plant height and biomass), decreased root length and chlorosis of younger leaves were evident in Cd-treated plants. Stem and leaf growth was more affected than root growth (Tab. 1). At 10^{-5} and 10^{-4} M Cd, leaf mass per plant decreased by 62.30 and 70.85%, respectively, stem mass by 72.36 and 76.88%, and root mass by 44.09 and 44.24%. In the presence of 10^{-5} and 10^{-4} M Cd, leaf area decreased from 1259 to 527 and 407 cm^2 , respectively. Plant height also significantly decreased.

As shown in Table 2, an increase of the Cd concentration in the nutrient medium enhanced Cd

TABLE 3. Effect of cadmium supply on chlorophyll content and rate of photosynthesis ($\mu\text{mol O}_2 \text{ g}^{-1} \text{ h}^{-1}$) of poplar plants grown in the presence of 0, 10^{-5} and 10^{-4} M Cd. Values are means of three replicates. Different letters in each column indicate significant differences between treatments at $p < 0.05$ using Duncan's multiple range test

Treatment Cd [M]	Chloroplast pigment concentration (mg g^{-1} dry weight)				Chl a/b ratio	Rate of photosynthesis
	Chl a	Chl b	Chl a+b	Carotenoids		
0 (control)	8.85 c	3.27 d	12.12 c	2.18 c	2.71	918.1 a
10^{-5}	6.56 d	2.04 e	8.59 d	1.39 d	3.21	547.2 c
10^{-4}	1.74 e	4.88 ab	6.61 e	2.39 c	0.35	735.3 b

accumulation in the plants. Control plants contained $1.60 \mu\text{g g}^{-1}$ Cd in leaves, $0.15 \mu\text{g g}^{-1}$ Cd in stem, and $4.23 \mu\text{g g}^{-1}$ Cd in roots. In the treatments the Cd content of the different plant organs ranked as follows: leaves < stems < roots. Plants grown in the presence of 10^{-4} M Cd accumulated $214.0 \mu\text{g g}^{-1}$ Cd in leaves, $371.3 \mu\text{g g}^{-1}$ Cd in stem and $6778.3 \mu\text{g g}^{-1}$ Cd in roots.

PHOTOSYNTHETIC CHARACTERISTICS

Total chlorophyll content was significantly altered in leaves of treated plants (Tab. 3). Content of Chl a, Chl b and carotenoids increased dramatically in plants exposed to 10^{-5} M Cd. In plants exposed to 10^{-4} M Cd, Chl b increased by 49% and carotenoids by 10%. Treatment of poplar plants with 10^{-5} M Cd slightly increased the Chl a/b ratio versus the control; the 10^{-4} M Cd treatment led to a huge decrease (87%). Photosynthetic oxygen evolution was also altered (Tab. 3). Control plants and Cd-treated plants were distinguished on the basis of their photosynthetic activity expressed per unit of leaf mass. The rate of oxygen evolution decreased by 40.4 and 19.91% in plants treated with 10^{-5} and 10^{-4} M Cd, respectively.

ANTIOXIDANT ENZYME ACTIVITY AND PROLINE ACCUMULATION

The activity of enzymes involved in antioxidative protection increased in plant leaves (Fig. 1a): CAT and SOD activity was significantly higher than the control in plants exposed to 10^{-5} M Cd; in 10^{-4} M Cd-treated plants the increase was not significant. CAT and GST activity increased in roots of treated plants (Fig. 1b): there was a large increase of GST activity at both of the Cd doses applied, and of CAT only at 10^{-4} M Cd. SOD activity was inhibited at both Cd concentrations, while GSH-Px activity was not significantly affected.

Proline content changed in both leaves and roots of treated plants (Fig. 2). In plants grown at 10^{-4} M Cd, proline accumulation in leaves was significantly reduced (from 85.60 to $67.00 \mu\text{g g}^{-1}$ d.w.), while in roots it was almost doubled. In contrast, Pro accu-

mulation in leaves and roots of plants treated with 10^{-5} M Cd did not change significantly versus the control.

DISCUSSION

Poplar plants exposed to different levels of Cd in the growing medium exhibited morphological, physiological and biochemical changes. There were sharp decreases in biomass production, leaf number, weight per plant, leaf area per plant, stem diameter and height. Although Cd accumulation was higher in roots than in other plant organs, root growth apparently was less affected than leaf and stem growth. Root fresh mass decreased by $\sim 44\%$, but shoot (stem + leaves) mass by more than 60% (Tab. 1). The lower susceptibility of roots might be explained by accumulation of the metal in inactive form or else by mobility of Cd within the plant, facilitating its translocation to aerial parts (Barceló et al., 1988). In contrast to our results, Titov et al. (1996) reported higher sensitivity of roots in HM-treated wheat and barley seedlings. In maize cultivars, increased Cd in nutrient solution significantly reduced the dry weight of both leaves and roots (Ekmekçi et al., 2008). Apart from the impact of Cd in the growth medium and concomitant accumulation in roots, it seems that root growth in poplars also depends on the genotype. Pilipović et al. (2005) grew poplar plants hydroponically in the presence of 0, 10^{-5} and 10^{-7} M Cd. The growth responses of roots and shoots exhibited differences between four poplar clones, and in some cases biomass production was stimulated. Lower biomass production (especially in leaves) and shorter roots are symptoms of Cd toxicity described previously in tomato plants (Chaffei et al., 2004). Cd-induced inhibition of fresh and dry mass, plant height, root length, leaf area and other biometric parameters has been reported in almost all investigations (Vassilev and Yordanov, 1997).

The distribution of biomass and Cd varied between plant organs, and tended to increase in treated plants (Tab. 1). The root/shoot ratio of biomass increased with the Cd concentration in the nutrient solution. The root/shoot ratio of Cd con-

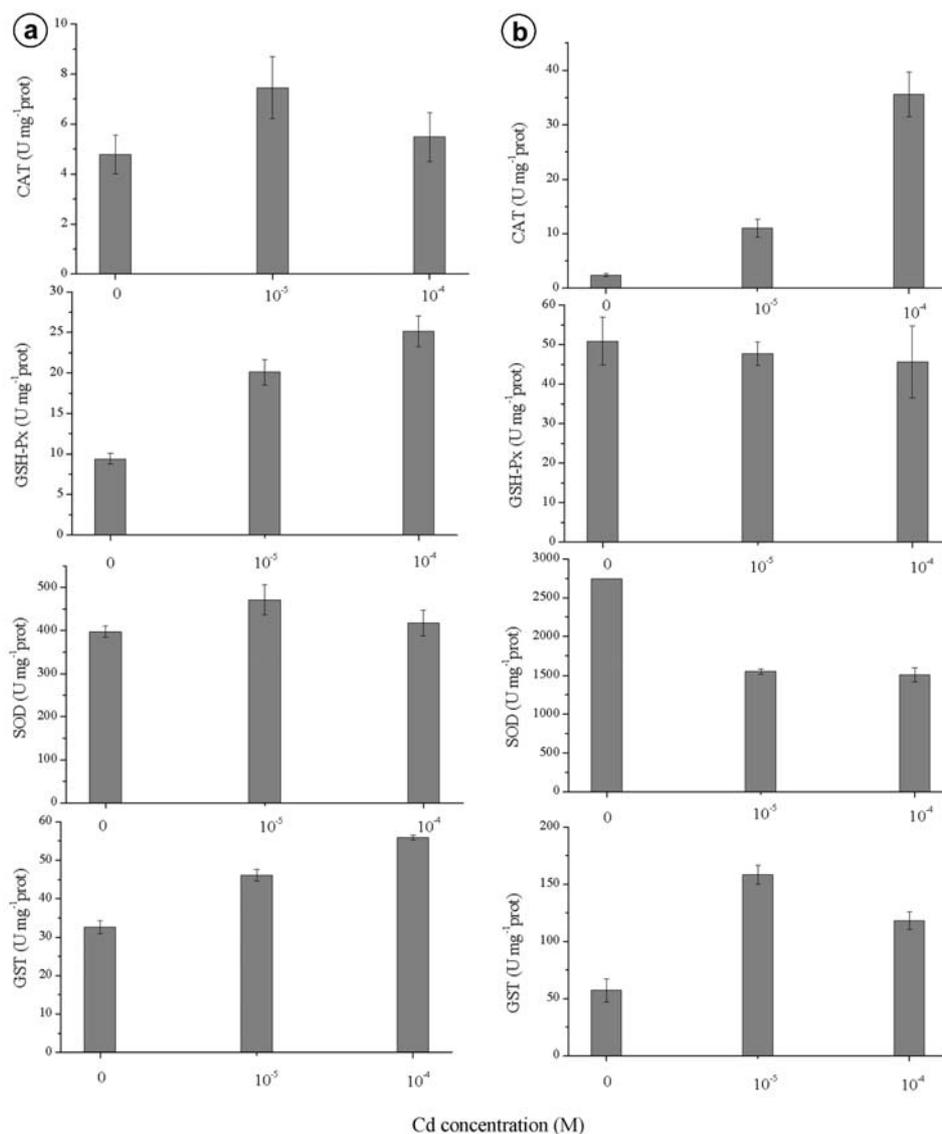


Fig. 1. Catalase (CAT), glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and glutathione-S-transferase (GST) activity in leaves (a) and roots (b) of poplar plants treated with 0, 10⁻⁵ and 10⁻⁴ M Cd. Values are means of four replicates \pm SD.

centrations was higher than that of biomass, and was higher at 10⁻⁵ than at 10⁻⁴ M Cd. Heavy metal and biomass allocation patterns differing from our results have been reported in other woody species. For example, in *Salix viminalis* the root/shoot biomass ratio was slightly higher than the root/shoot Cd ratio, indicating that the relative allocation of Cd to shoot was higher than that of biomass (Nylund, 2003).

The increment of pollutant concentration in the nutrient medium was associated with the increment of Cd accumulation in the plants (Tab. 2). Root amounts of Cd were \sim 40 times higher than in leaves

and stems of plants grown at 10⁻⁵ M Cd. The distribution of Cd differed slightly between the treatments. In plants grown at the lower Cd concentration, 95.3% of total accumulated Cd was in roots, 2.6% in stems and 2.1% in leaves; plants grown at the higher Cd concentration showed increased translocation from roots to shoots: 2.9% of total Cd was accumulated in leaves, 5.0% in stems and 92.1% in roots. In both treatments the Cd distribution within poplar plants decreased in the following order: root > stem > leaf. Higher accumulation of Cd in roots has been found in *Nerium oleander* (Kadukova et al., 2006), *Triticum aestivum* and

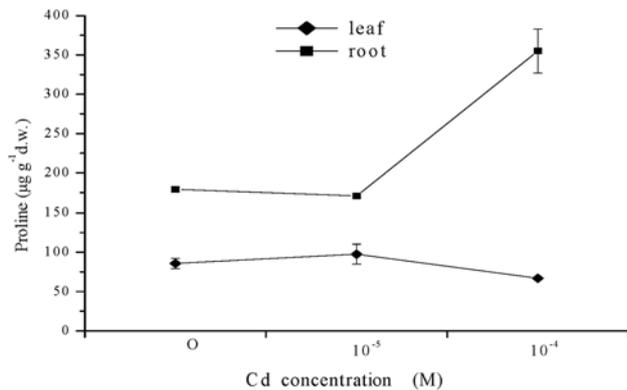


Fig 2. Proline accumulation in leaves and roots of poplar plants treated with 0, 10^{-5} and 10^{-4} M Cd. Values are means of four replicates \pm SD.

T. durum (Kovačević et al., 1999; Ozturk et al., 2003), *Pinus sylvestris* (Kim et al., 2003), *Cannabis sativa* (Linger et al., 2005), cabbage (Pandey and Sharma, 2002), maize (Ekmekçi et al., 2008), willow and poplar (Lunáčeková et al., 2003/4). Except in the control plants, Cd levels in all organs exceeded levels considered toxic, that is, 5–30 ppm (Orcutt and Nilsen, 2000).

Our results also show that the Cd treatments markedly altered the concentrations of photosynthetic pigments in the poplar plants (Tab. 3). The amounts of chlorophyll *a* and consequently total chlorophylls were lower than in control plants. Cadmium-induced reduction of chlorophyll amount has been reported in Indian mustard and mung bean (Šimonová et al., 2007), cape gooseberry, pepino, pepper, tobacco and tomato (Thiebeauld et al., 2005), *Phaseolus vulgaris* (Zengin and Munzuroglu, 2005) and *Alternanthera philoxeroides* (Deng et al., 2007). In contrast, Lunáčeková et al. (2003) found no significant decrease of chloroplast pigment content in fast-growing woody plants exposed to Cd. Chlorosis, rolling and stunting are the main and most visible symptoms of Cd toxicity in plants (Benavides et al., 2005). In our experiment only the young leaves were visibly whitened in Cd-treated poplar plants. This may have been due to disturbed chlorophyll synthesis rather than enhanced degradation. The mechanism by which Cd induces leaf chlorosis is unclear (Barylá et al., 2001). According to van Assche and Clijsters (1990), cadmium alters chlorophyll biosynthesis by inhibiting protochlorophyllide reductase, likely through interaction of Cd with SH groups. It could also affect the integration of chlorophyll molecules into pigment-protein complexes of photosystems (Horvath et al., 1996). Substitution of Mg ions by Cd in chlorophyll molecules could be another possible mechanism resulting in destruction of chlorophyll

(Küpper et al., 1998). Barylá et al. (2001) found that chlorosis in Cd-treated oilseed rape plants was not due to a direct effect of the metal on the biosynthesis of photosynthetic pigments and the photosynthetic apparatus; their results showed that Cd alters cell division and chloroplast replication, resulting in a drastic decrease in the number of chloroplasts and consequently in the amount of chlorophyll (per unit leaf area).

The amount of chlorophyll *b* tended to increase in poplar plants exposed to 10^{-4} M Cd. These results are in agreement with findings from Zhou and Qiu (2005), who demonstrated that Cd treatments resulted in an increase of chlorophyll content of *Sedum alfredii* Hance plants, due to a Cd-induced increase of Fe uptake. The rate of formation of δ -aminolevulinic acid, a precursor of chlorophyll and heme synthesis, is controlled by Fe (Pushnik and Miller, 1989). In our study the concentration of Fe in young leaves used for pigment determination was not considerably different in poplar plants treated with 10^{-5} and 10^{-4} M Cd (data not shown). The specific effects of Cd on chlorophyll *a* and *b* synthesis in poplar plants require further study.

Photosynthetic evolution of oxygen was lower in Cd-treated plants (Tab. 3); this seems in agreement with earlier reports (Chug and Sawhney, 1999). Impairment of photosynthesis is considered one of the most important factors limiting plant growth under Cd stress (Vassilev and Yordanov, 1997). The recorded decrease of photosynthetic activity in poplar plants is partly attributable to lower chlorophyll content (Tab. 3). Reduced chlorophyll content is suggested to be a primary cause of diminished photosynthetic activity, since it has been found to precede decreased CO_2 fixation (Baszynski et al., 1980). In addition to disturbed chlorophyll synthesis as a cause, Ernst (1980) noted that inhibition of photosynthesis could occur due to Cd-induced changes in CO_2 fixation, stomatal conductance, electron transport and Calvin cycle enzyme activity. The variety of suggestions in the literature concerning the effects of Cd on the photosynthetic apparatus is related to the variety of plant species, Cd treatments and experimental conditions (Barylá et al., 2001). The exact site of the action of Cd on photosynthesis remains to be established in poplar.

On average proline accumulated more in roots ($235.09 \mu\text{g g}^{-1} \text{d.w.}$) than in leaves ($83.09 \mu\text{g g}^{-1} \text{d.w.}$) (Fig. 2). Pro content was not markedly altered in plants exposed to 10^{-5} M Cd in the nutrient solution; an increase to 10^{-4} M Cd lowered Pro accumulation in leaves by 21.7%, and increased it in roots by 97.8%. Stimulation of Pro accumulation after exposure to excess HM has been reported in other plant species, and seems to correspond with Cd-induced changes in the water regime. Water deficit changes

the regulation of pyrroline-5-carboxylate (an enzyme involved in Pro synthesis), resulting in high Pro accumulation (Delauney and Verma, 1993; Ali et al., 2001). Pandey and Sharma (2002) demonstrated that Co, Ni and Cd induced an increase of Pro concentration in cabbage leaves, and suggested an association with the changed water status of the treated plants. Schat and coworkers (1997) attributed Pro accumulation in leaves of Cd-treated *Silene vulgaris* to HM-induced water deficit. Pro is also implicated in HM detoxification processes. Free Pro chelates Cd ions, resulting in the formation of a non-toxic Cd-proline complex (Sharma et al., 1998). Pro is also involved in antioxidant defense. Matysik et al. (2002) reviewed the role of this amino acid in protecting plants against damage by reactive oxygen species.

The antioxidative system (consisting of enzymes and antioxidants) plays a protective role by stabilizing the amounts of ROS in plant cells (Sies, 1993; Yurekli and Porgali, 2006). Cd causes oxidative stress, probably through an interaction with the antioxidative defense, disruption of the electron transport chain, or induction of lipid peroxidation (Michalak, 2006). Stimulation of antioxidant enzyme activity at low Cd levels could play a significant role in protecting cells against Cd-induced oxidative stress (Scebba et al., 2006). In our experiments the antioxidant defense was altered in both roots and leaves at the two Cd levels tested. The effects on leaf CAT and SOD were similar (Fig. 1): at the lower concentration their activity increased, and at the higher concentration it decreased. This means that 10^{-4} M Cd in the nutrient medium, that is, $214 \mu\text{g g}^{-1}$ Cd in leaf tissue, is a critical level of this HM for the treated plants, inducing a significant decrease in the activity of these enzymes. Such critical values may vary between species. Scebba et al. (2006) reported a critical external Cd concentration of $4.4 \mu\text{M}$ for SOD in *Mischanthus sinensis* plants. In other plant species the activity of CAT was either increased (Brej, 1998; Vitória et al., 2001) or decreased (Shaw, 1995; Gallego et al., 1996; Chaoui et al., 1997). Versus the control, leaf GST activity increased by 41% at the lower and 72% at the higher Cd levels. Root GST was more stimulated at the lower than at the higher Cd concentration. Thus, 10^{-4} M Cd in the nutrient medium, that is, $6778 \mu\text{g g}^{-1}$ Cd in roots, is a critical level of it for this enzyme as well, inducing a significant decrease of GST activity. Generally, leaf antioxidative enzymes were more sensitive to Cd; lower tissue Cd concentrations markedly affected their activity. In treated plants the activity of SOD was decreased in roots and increased in leaves. Cadmium had no effect on SOD activity in duckweed plants (Paczkowska et al., 2007); increased activity of SOD was reported in *Pisum sativum* (Dalurzo et al., 1997); in sunflower

(Gallego et al., 1996) and pea (Sandalio et al., 2001), SOD activity decreased following exposure to Cd.

In this work the values of all the studied parameters changed in relation to the Cd concentration. A significant decrease in both plant height and biomass production was accompanied by reduction of stem diameter, leaf area and weight of leaves per plant. For the studied plants, $214 \mu\text{g g}^{-1}$ Cd in leaf tissue apparently represents a critical level of this HM, inducing a significant decrease in CAT and SOD activity as well as Pro accumulation. SOD activity decreased in roots and increased in the leaves of treated plants. Cd-induced toxic effects (stunted growth, leaf chlorosis, oxidative stress) were observed at both Cd doses, indicating that this clone was vulnerable to these pollutant levels. High amounts of Cd accumulated in roots, but in view of its low translocation from roots to aboveground parts, along with the disturbances in plant growth, this hybrid poplar shows little potential for use in remediation of sites contaminated with Cd.

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