Photosynthesis and growth response of Paulownia tomentosa x fortunei hybrid plants to different levels of heavy metals Cd, Pb and Zn

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Photosynthesis and growth response of *Paulownia tomentosa* x *fortunei* hybrid plants to different levels of heavy metals Cd, Pb and Zn

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ABSTRACT

Paulownia tomentosa x *fortunei* hybrid exposed to different concentrations of Cd, Pb and Zn were analyzed with reference to study the metal`s effects on the photosynthetic and antioxidants parameters. The maximum accumulation of heavy metals is occurred in the roots. These treatments caused significant reduction in total plant dry biomass, stomatal conductance, transpiration rate and water use efficiency, but increased net photosynthetic rate. An enhanced level of lipid peroxidation and increased concentration of H_2O_2 at higher concentrations of Pb and Zn indicated that they caused oxidative stress in the leaf tissues. Ascorbate peroxidase showed maximum stimulation at 0.5 mg L^{-1} Cd, 20 mg L^{-1} Pb, and 30 mg L^{-1} Zn, while catalase prominently increased. The great increase was observed in the levels of glutathione and

flavonoids. Increased activities of antioxidant enzymes suggest that they have some additive function in the mechanism of metal tolerance in *Paulownia tomentosa* x *fortunei* hybrid.

Key words: Cadmium, gas exchange, growth, lead, lipid peroxidation, *Paulownia tomentosa* x *fortunei* hybrid, protective enzymes, zinc,

Abbreviations:

Running title: *Paulownia* hybrid response to Cd, Pb and Zn

INTRODUCTION

Increasing emissions of heavy metals are dangerous because they may get into the food chain with risk for human health (Galloway et al., 1982; Angelone and Bini, 1992). Cadmium (Cd), lead (Pb), copper (Cu) and zinc (Zn) are some of the most widespread heavy metal contaminants of soils and act toxic in animals and plants at elevated concentrations (Kabata-Pendias and Pendias, 1991). Three different molecular mechanisms of metal toxicity are induced as heavy metals bind to the oxygen, nitrogen and sulphur atoms in the plant tissues: 1/ production of reactive oxygen species by autooxidation and Fenton reaction; 2/ blocking of essential functional groups in biomolecules; 3/ displacement of essential metal ions from biomolecules (Schutzendubel and Polle, 2002).

The heavy metal phytoremediation (phytoextraction) refers to the use of plants that can remove contaminants from soil and accumulate them in harvestable parts. Plant biomass production and the metal concentration in the biomass are important factors for the practical efficiency of phytoextraction (McGrath and Zhao, 2003). One strategy is to use high biomass crop plants like *Brassica juncea*, *Helianthus annuus*, *Zea mays*, *Nicotiana tabacum* (Meers et al., 2005; Tassi et al., 2007) or hyperaccumulator plants (Baker et al., 2000; Robinson et al.,

2000).Widely used for phytoremediation of contaminated soils herbaceous hyperaccumulators are slow-growing plants and low-biomass producers which accumulate only one specific element and possess low depth roots. Deeper pollution and contamination caused by a number of metals require using as an alternative fast-growing woody species with deep root system and the ability to grow on nutrient-poor soil. Some of them (poplar, willow, black locust, ash, or alder) are successfully used for remediation of substrates contaminated by inorganic and organic pollutants. Poplar and willow are mainly used for the remediation of Cd polluted soils (Robinson et al., 2000; Lei et al., 2007), but their heavy metal tolerance is limited (Dietz and Schnoor, 2001). *Paulownia* is a promising woody species for the remediation of Pb, Zn, Cu and Cd polluted soils owing to its very high biomass productivity, rather than its metal accumulation potential (Wang et al., 2010; Doumett et al., 2011).

Paulownia is native from the China. *Paulownia tomentosa* has been introduced into USA and Europe as an ornamental plant and is still widely used for this purpose. Trees introduced in Bulgaria reach 12 m average height and 13.4 cm average diameter during 7 years (Kalmukov, 1995). Over the last two decades *Paulownia* species has been extensively studied due to its ability to uptake nitrates and land contaminants, namely heavy metals (Wang et al., 2010; Doumett et al., 2011). This high-yielding tree can be used for the production of energy, paper pulp and wooden building materials. The genetically tissue–cultured *Paulownia* seedlings produced by The World Paulownia Institute (WPI) allow production of biofuels after introducing of cultivars without detrimental impacts on food supply or the environment. Research on *in vitro* propagation of *P. elongata* and *P. fortunei* has been reported by Bergmann et al. (1997). Application of this technology for micropropagation of tree species offers a rapid means of producing clonal planting stock for afforestation, woody biomass production and it is effective way to maintain the genetic gain (Park and Bonga, 1992). The use of nodal segments for *in vitro*

propagation promoted a higher rate of multiplication of *Paulownia tomentosa* (Ozaslan et al., 2005).

Plants used in the current paper are propagated and rooted according technology registered of Biotree Ltd., Bulgaria. This laboratory is largest producer and supplier of genetically superior *Paulownia* tissue-cultures – *in vitro* seedlings. *Paulownia tomentosa* x *fortunei* hybrid plants is preferred by the farmers due to fast development a uniform regular growth. There is no information about it tolerance to heavy metals and possibilities to use as phytoremediator of contaminated soils. The results derived from tissue cultures can be used to predict the responses of plants to environmental contaminants and to improve the design and thus reduce the cost of subsequent conventional whole plant experiments (Doran, 2009).

In this research, the effects of Cd, Pb and Zn on growth, photosynthetic gas exchange, and protective enzyme activities of *Paulownia tomentosa* x *fortunei* hybrid plants, grown in hydroponic after transplant the explants were studied to provide fundamental base for vegetation restoration in contaminated soils.

MATERIALS AND METHODS

Plant material and source of explants

Seeds and *in vivo* explants from the species *P. tomentosa* and their hybrids with *P. fortunei* are used for developing of *in vitro* multiplication protocol. The explants were washed with Tween and subsequently sterilized with 0.1% mercury chloride ($HgCl_2$) solution for 5 minutes and then rinsed tree times in sterile distilled water for 5, 10 and 15 minutes. For induction of shoots, explants are cultured on Murashige and Skoog (1962) (MS) nutrient medium included 2.5% (w/v) sucrose, 0.8% (w/v) agar and vitamins. For a multiplication of shoots MS medium was used supplemented with 4.439 µM 6-benzylaminopurine (BAP) and 0.537 µM indolil acetic acid

(IAA). The medium included 3.0% sucrose (w/v), 0.8% agar and vitamins. After multiplication the shoots were transferred to rooting medium based on half strength basal salts MS medium, 2 % sucrose, 6 % agar and vitamins supplemented with 4.92 µM indole-3-butyric acid (IBA) and 1.075 µM IAA. The pH of all media was adjusted to 5.7 using 0.1 N HCl and 0.1 N NaOH before autoclaving. All cultures were incubated under controlled conditions – 16 h photoperiod, light intensity of 35 µmol m^{-2} s^{-1} and 24/18±1ºC day/night temperature. After three weeks of rooting the shoots were washed from the medium with tap water and the roots were rinsed with 1.5 ml L^{-1} Proplant solution.

Hydroponic experiment

The uniform explants were selected and transplanted to polyethylene vessels containing 1.2 l of 1/4 Hellriegel solution (1898) with an addition of A-Z microelements after Hoagland (pH 5.9) except ZnSO4 in growth chamber with a 16-h photoperiod (PAR 100 µmol m^{-2} s^{-1} on the upper leaf surface, 25/23±1 ºC day/night temperature, relative humidity 60/70%). Each vessel contained two explants. After 21 days of cultivation the plants were transferred to 1/2 Hellriegel solution with microelements (pH 5.9) except ZnSO4. The heavy metal treatment was applied on the 48th day after transplanting of explants at the following concentrations: Cd [0.5; 2.5 and 5.0 mg L^{-1} Cd supplied as $Cd(NO_3)_2.4H_2O$]; Pb [5.0; 10.0 and 20.0 mg L^{-1} Pb supplied as $Pb(NO_3)_2$] and Zn [10.0; 20.0 and 30.0 mg L^{-1} Zn supplied as $Zn(NO_3)_2.6H_2O$]. Plants grown in nutrient solution without metals served as controls. The solutions were aerated every day and were changed every 3 d. Plants were harvested after 10 d of heavy metals treatment. Dry mass of shoots and roots were determined after oven-drying (60^{o}C). Leaf area was calculated using software SigmaScan Pro 5. Leaf area ratio (LAR) was described as the leaf area (cm^2) divided by the total plant dry biomass (Hunt, 1982).

Analysis of heavy metals

Total metal content in each organ was analyzed after sample homogenization in a Blender. Dry vegetal tissues (0.5 g) were treated with 2 ml of 30% hydrogen peroxide and 3 ml 65% nitric acid and 9 ml of 37% hydrochloric acid. The samples were heated on a heating block at 200°C to evaporate to dryness. The residue was taken up in 25 ml of 1N HCl. Metal concentration were determined on the inductively – coupled Plasma Mass Spectrometer (CCD Simultaneus ICP OES, Varian, Australia).

Gas exchange measurement

Net photosynthetic rate (P_n, µmol CO_2 m^{-2} s^{-1}), transpiration rate (T_r, mmol H_2O m^{-2} s^{-1}), stomatal conductance (G_s, mmol m^{-2} s^{-1}) were measured simultaneously on 10th day after heavy metal treatments using a portable gas analyzer Li-6400, (Li-Cor Inc., Lincoln, NE, USA), with a red-blue LED light source. Measurements were made in the late morning hours under standardized conditions (air temperature of 25°C, humidity of 50% inside the gas exchange cuvette, 1000 µmol m^{-2} s^{-1} PAR and 350 µmol ambient concentration of CO_2. The third basal leaf of plants at each heavy metal treatment were measured and averaged. The following indices were calculated: water use efficiency (WUE, µmol CO_2 mmol H_2O^{-1} = P_n/T_r (Nijs et al., 1997).

Quantitative pigments determination

Leaves of three replicates of 58-day-old plants from each treatment were used for the determination of photosynthetic pigments (chlorophyll a, chlorophyll b, chlorophyll a+b, and carotenoids) (Lichtenthaler, 1987).

MDA and H_2O_2 assays

0.3 g FW of the third basal leaf were homogenized in a mortar at 4°C with 3 ml 0.1% trichloroacetic acid and centrifuged for 20 min at 15 000 rpm. Malondialdehyde (MDA) estimation was measured by Dhindsa et al. (1981) and Heath and Packer (1968). For the Hydrogen peroxide (H_2O_2) was assay by Jessup et al. (1994).

Nonenzymatic antioxidant metabolites assays

For the low molecular antioxidant metabolites extraction was made by method of Doulis et al. (1997). The concentrations of reduced (GSH) and oxidized (GSSG) glutathione were determined with an enzyme recycling assay (Griffith 1980). Reduced form of ascorbic acid (ASC) was estimated enzymatically as described by Foyer et al. (1983).

Content of total phenolic compounds were determined spectrofotometrically using Folin-Ciocalteu reagent and calculated as chlorogenic acid equivalents (Pfeffer et al. 1998). Total flavonoids content was measured using a colorimetric assay in accordance with the method of Zhishen (1999). The total flavonoids of the samples were expressed in mg of (+) catechin equivalent per g DW of the sample.

Determination of enzymatic antioxidants

In order to prepare crude extracts for determination of enzymes glutathione reductase (GR), guaiacol peroxidase (GPO) and catalase (CAT) the plant material were grinded with 4 ml of the extraction buffer (100 mM potassium phosphate buffer, pH 7.8; 5 mM EDTA; 2% PVP) that was added to 0.3 g of tissue powder. The extraction buffer for the determination of ascorbate peroxidase (APX) contained: 50 mM potassium phosphate buffer, pH 7.0; 1 mM ascorbic acid; 1 mM EDTA; 0.2% PVP and was added to 0.15 g of tissue powder. The suspensions were centrifuged (16 000g, 15 min, 4 ^{o}C). All enzymes were assayed spectrophotometrically. GPO (EC 1.11.1.7) was assayed according to Polle et al. (1994). CAT (EC 1.11.1.6) was assayed according to Aebi (1984). GR (EC 1.6.4.2) was assayed according to Sherwin and Farrant (1998). APX (EC 1.11.1.11) was assayed according to Nakano and Asada (1981).

The protein content was determined after Lowry et al. (1951).

Statistical evaluation

Data are expressed as means ±SD, where n=3-6. Comparison of means was performed by Fisher's LSD test ($P \leq 0.05$) after performing ANOVA analysis (Statgraphics Plus, v. 2.1).

RESULTS

Biomass production

Visual assessment of plants treated with heavy metals did not show any phytotoxic symptoms and treated plants had the same appearance as control plants. Mean values of total dry biomass determined in *Paulownia tomentosa* x *fortunei* hybrid 58 days after transplanting of explants to the Hellriegel nutrient solution and 10 days after treatment with heavy metals are reported in Table 1. Total dry biomass showed strong reduction after treatment with heavy metals. The highest dry biomass reduction was observed in the variants when plants are treated with 2.5 and 5.0 mg L^{-1} Cd. Leaf area values in response to the type and concentration of heavy metals followed the same trend as total dry biomass. Leaf area ratios showed the capability of a plant in forming of photosynthetic surface and increased slightly after treatment with the highest concentrations of Cd and Zn. Treatment with 10 mg L^{-1} Pb lead to the highest values of LAR. Root/shoot dry biomass ratio increased after all treatments in comparison with control.

Heavy metal accumulation

The concentrations of heavy metals in different parts of the plants are shown in Table 2. The highest accumulation of Cd, Pb and Zn was found in roots. In general, the metal content in plants increased with the increase of metal concentrations in solution, and the metal accumulation in roots is always significantly higher than that in stems and leaves.

Gas exchange

The addition of heavy metals in the growth medium enhanced net P_n of *Paulownia tomentosa* x *fortunei* hybrid plants compared to the control, whereas G_s and T_r decreased sharply with an arising of the metal concentrations (Figure 1). All heavy metal treatments lead to significant increase in WUE compared to the control (Figure 1). WUE reached maximal value at the lowest Cd and Pb and the middle Zn concentrations.

Pigment composition

The chlorophyll a+b and carotenoid contents in the leaves of *Paulownia tomentosa* x *fortunei* hybrid plants in response to the addition of heavy metals are shown in Table 3. The values of chlorophyll a+b remained higher than the control, but the difference was significant only for the variant with 10 mg L^{-1} Zn. An increase in the carotenoid content was found only after applying of 20 mg L^{-1} Pb compared to the variant with 10 mg L^{-1}. Chlorophyll a/b ratios in the leaves of *Paulownia* hybrid in response to the addition of heavy metals were near to the control, but chlorophyll/carotenoid ratios showed significant differences. The highest chlorophyll/carotenoid ratios were observed in *Paulownia tomentosa* x *fortunei* hybrid leaves when 10 mg L^{-1} Pb was added to the nutrient medium.

Nonenzymatic antioxidants

The heavy metal treatment caused oxidative stress, as indicated by the significant increase of MDA contents in the *Paulownia tomentosa* x *fortunei* hybrid leaves especially at higher concentrations of Cd and Pb and the highest concentration of Zn (Table 4). Content of H_2O_2 was significantly higher than the control at 20 mg L^{-1} Pb, 20 and 30 mg L^{-1} Zn (Table 4). The level of GSH + GSSG sharply increased in the variants with 2.5 mg L^{-1} Cd , 20 mg L^{-1} Pb and 10 mg L^{-1} Zn (Table 4). The ASC content changed in a different manner too, but the values were lower or closely to the control (Table 4). Total phenols decreased slightly in parallel with the increasing concentrations of Cd and Pb. The highest values were observed at 10 mg L^{-1} Pb and 30 mg L^{-1} Zn

(Table 4). Similarly content of total flavonoids increased at the variants with 10 and 30 mg L^{-1} Zn in the nutrient solution. The changes of total flavonoid content are similar to that of total phenolic content after treatment with different concentrations of Pb and Zn.

Enzymatic antioxidants

The level of the antioxidant enzymes, such as SOD, POD and CAT in the *Paulownia tomentosa* x *fortunei* hybrid leaves may determine the sensitivity of plants to lipid peroxidation. The increasing of Cd, Pb and Zn levels in nutrient medium activated CAT, with the exception of variants 10 and 20 mg L^{-1} Zn (Table 5). The GPO activity decreased in a different manner. The highest decrease in GPO activity was observed in 5 mg L^{-1} Cd and 5 and 10 mg L^{-1} Pb and no significant effect in variants with Zn. There is a correlation between Pb content in the nutrient medium and GPO activity which indicate that the ability of this enzyme to eliminate ROS is limited. GR was affected by applying of Cd, Pb and Zn in the nutrient medium. It was enhanced most significantly in the variants with 10 mg L^{-1} Pb and 20 mg L^{-1} Zn (Table 5). APX activity increased with addition of Cd, Pb and Zn in the nutrient medium. There is a correlation between Pb and Zn concentration in the nutrient medium and APX activity while in the variants with Cd addition in the nutrient medium the correlation is inversely proportional (Table 5). Increased activities of CAT, GR and APX provide a better defense mechanism against heavy metal-induced oxidative damage in *Paulownia tomentosa* x *fortunei hybrid* plants.

DISCUSSION

The effects of metals (especially Cd, Pb and Cu) on the structure and function of trees are still intensively studied. The roots of four willow and two poplar species responded to Cd treatment more sensitively than the shoots. Cd ions accumulated in roots suppressed their growth in all

tested species (Šottnikova et al., 2003). The main morphological and structural effects of Cd on roots was summarized by Barcelo and Poschenrieder (1999): decrease of root elongation and biomass, root tip damage, collapsing of root hairs or decrease of their number, decrease of lateral root formation. Our results showed that root elongation and biomass of *Paulownia tomentosa* x *fortunei* hybrid plants were slightly suppressed, namely with the increase in Cd and Pb concentrations in solution (data are not represented), but strong reduction of total plant dry biomass after all treatments is established (Table 1). Willow and poplar species growing directly in Cd $(NO_3)_2$ showed better adaptation of roots than these growing in Knop nutrient solution and transferred to Cd $(NO_3)_2$. The positive effect of indirect treatment on pigment and starch contents, net photosynthetic rate and specific leaf mass are reported (Lunačkova et al., 2003). These results showed that Cd impact is depended on the cultivation conditions. We established slight increase of stem elongation and dry biomass of *Paulownia tomentosa* x *fortunei* hybrid plants with increase in heavy metal concentration of solution, but the values were remained lower than control. The number of leaves and their dry biomass were lowered after treatments with increasing concentrations of Cd and Pb (data are not presented). Leaf areas decreased about twice after all treatments (Table 1). Leaf area ratios were also calculated (Table 1) for the evaluating the capability of plant to invest biomass in the photosynthetic surface. Interestingly, the treatment with the highest concentration of Cd and with middle concentration of Pb showed one of the highest values in spite of the lowest total dry weight. The need of an improved leaf surface per dry weight unit was probably related to a increased photosynthetic efficiency. Root/shoot dry biomass ratio confirmed the above-mentioned results (Table 1): control plants showed the lowest values. Plants treated with increasing concentrations of heavy metals had the highest investment in root biomass compared to the aerial section.

The metal concentrations of untreated plant were 1, 0.05 and 50 mg kg^{-1} DW for Pb, Cd and Zn, respectively (Markert, 1994). Our data obtained for metal accumulation in *Paulownia tomentosa* x *fortunei* hybrid shoots indicated lower content than those measured in the roots (Table 2). The results showed a more pronounced root accumulation of the toxic element Pb and the essential metal Zn compared to Cd. The accumulation of Pb, Cd and Zn in whole plants during the hydroponic experiments of the present study increased in the order of Cd<Zn<Pb. Several studies reported different results on accumulation and translocation patterns even in the same plant species. The accumulation pattern in roots of *Brassica juncea* plants grown under hydroponics increased in the order of Cd<Pb<Cu<Zn (Dushenkov et al., 1995). Shoots of *B. juncea* grown in a fertilized sand:perlite mixture showed an accumulation pattern Cu<Cd<Pb<Zn (Nanda-Kumar et al., 1995). This discrepancy arises due to variation in heavy metal concentration, form of metal present, and plant species (Kim et al., 2003). The metals have a different mobility and they are transported from roots to shoots to different manner. Cd and Zn are more mobile than Cu and Pb (Greger, 2004). Zn is translocated extensively as it is essential to the plant metalloenzymes (Delhaize et al., 1985; Van Assche and Clijsters, 1990) and photosynthesis (Hsu and Lee, 1988), while Pb and Cd are toxic to plants.

Gas exchange measurements are often useful to detect the more sensitive site of action of heavy metals. In *Zea mays* L. simultaneous inhibition of photosynthesis and transpiration by Cd (Carlson et al., 1975) suggested that the primary effect should be on stomatal function and as a result decreased the water use efficiency. Our results showed that stomatal conductance and T_r decreased sharply with increasing of metal concentrations in solution, but P_n was enhanced. Hermle et al. (2007) found that seedlings of *Salix viminalis* cope with heavy metal stress by increased photosynthetic rate and without growth reduction.

Higher WUE in the variants with slight and moderate metal concentrations can be interpreted as an attempt of plants to improve their water regime. May be reduced water uptake by damaged roots reflected in higher WUE. Improved WUE on the basis of reduced water use is generally achieved by plant when there is need to balance water use against a limited water reserve. High WUE is largely a function of reduced water use rather then a net improvement in plant production or biochemistry of assimilation. WUE commonly achieved via moderated growth, reduced leaf area, and short growth duration. Barcelò and Poschenrieder (1990) reported that the disturbed water relations to plants comprised one of the main reasons for the heavy metal phytotoxicity. Heavy metal contamination limits root growth (limiting water uptake) and increases the degree of root suberization and lignification (increased resistance to water flow) as well as leaf senescence and abscission.

Transpiration is a key process for the improvement of water-soluble components or contaminants uptake and flux to the upper parts of plants. Nikolič et al. (2008) observed symptoms of Cd toxicity in Cd-treated hybrid poplar plants: stunted growth, decreased root length and chlorosis of voting leaves. Stem and leaf growth is more affected than root growth and the decreased photosynthetic activity of treated plants may have been due to lowered chlorophyll synthesis. It is known that Cd (Baszynski et al., 1980) and Pb (Humpp and Lendzian, 1974) decreased total chlorophyll content and chlorophyll a/b ratio in higher plants. Generally, carotenoids were less affected by heavy metals; this resulted in a lower chlorophyll/carotenoid ratio for higher plants (Baszynski et al., 1980). Our results showed slightly increase in the chlorophyll a+b content after treatment with Cd and Pb. The highest values were reached after treatment with Zn. The chlorophyll a/b ratios were closed to control. The carotenoid content slightly decreased with the increase in concentration of heavy metals in solution, this resulted in greater changes in chlorophyll/carotenoid ratio (Table 3). No changes of the pigment

concentration or ratio were observed in *Zea mays* L. leaves after treatment with Zn (Cottenie et al., 1976).

Cd, Pb and Zn are not redox active, but induce oxidative stress in growing plant parts due to the enhanced production of ROS resulting in unbalanced cellular redox status. Such production depends on the intensity of stress, repeated stress periods, species and age of plants (Asada, 1994; Verma and Dubey, 2003). The activities of antioxidative enzymes SODs, CAT, APX and the levels of low molecular antioxidants, particularly GSH increased concomitantly with increasing Pb concentration and the duration of treatment (Malecka et al., 2009). Our results showed that GSH + GSSG increased in a different manner with increasing the oxidative stress induced by heavy metals (Table 4). The values were higher than of control. The level of ASC was changed too, but the values after treatment with increasing concentrations of Cd were lower than the control (Table 4). Total phenolic and flavonoid contents changed in the same manner after treatment with Pb and Zn and flavonoid content rised slightly after treatment with increasing concentrations of Cd (Table 4). Besides being radical scavengers, flavonoids are also able to function as chelators for metals (Brown et al., 1998). In callus cultures of the legume plant *Ononis arvensis*, flavonoid level increased after both Cu and Cd treatments (Tůmova and Ruskova, 1998).

Pb and Cd can indirectly initiate the production of different ROS and increases the activity of enzymes of antioxidant defense system in several plant species (Shah et al., 2001; Metwally et al., 2003). The changes in activities of antioxidant enzymes depended on the duration, the type of metal and the strength of stress treatment. Young seedlings of *Paulownia fortunei* have effective detoxific mechanisms to Cd and Pb and react with increasing antioxidant enzyme activities; phytochelatin production and proline content, but had un-effective detoxic mechanisms to Zn and Cu stress. That is why the low concentrations of Cu, presented in mine tailings may be the major

constraint for the survival of these seedlings (Wang et al., 2011). Our results showed that CAT, GR and APX activities increased in a different manner with the increase concentration of heavy metals in solution (Table 5). These enzymes provide a better defense mechanism against heavy metal-induced oxidative damage in *Paulownia tomentosa* x *fortunei* hybrid plants.

CONCLUSIONS

It was concluded that 58-day-old *Paulownia tomentosa x fortunei* hybrid plants grown under hydroponic-culture conditions possessed a high potential for heavy metal accumulation in the roots which increased in the order of Cd<Zn< Pb. Strong biomass and leaf area reduction, but increased root/shoot dry biomass was observed after treatment with increasing of heavy metal concentrations in the nutrient medium. LARs increased slightly after treatment with the highest concentrations of Cd and Zn and with middle concentration of Pb. P_n was enhanced, but G_s, T_r and WUE decreased sharply with the increase of metal concentrations in solution. The increased levels of hydrogen peroxide and malondialdehyde showed that the uptake of heavy metals from the solution caused oxidative stress in the leaf tissues. The greatest increase was observed in the levels of reduced and oxidized glutathione forms and flavonoids. High H_2O_2 level could be neutralized mainly through the increased activities of catalase, glutathione reductase and ascorbate peroxidase. High activities of two enzymes from the ascorbate-glutathione cycle allowed to suppose that H_2O_2 neutralization is rather enzymatic than non-enzymatic process.

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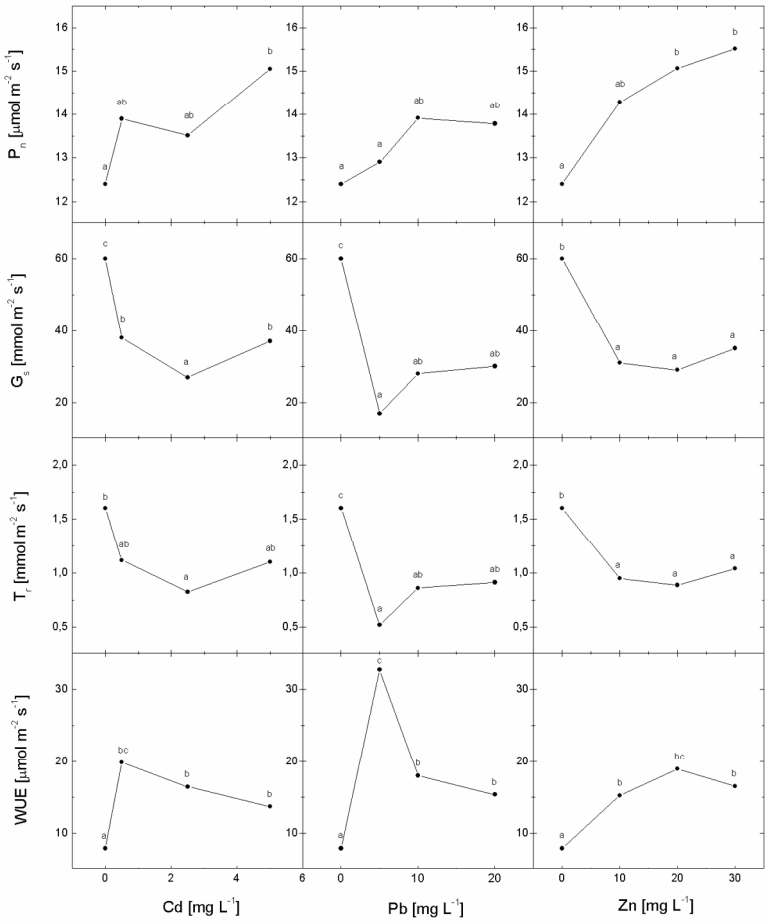

Gas-exchange parameters in leaves of Paulownia tomentosa x fortunei hybrid plants grown grown as hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn. Data are presented as means of five replications ± standard error. Different letters indicate significant differences assessed by Fisher LSD test (P≤0.05) after performing one-way ANOVA analysis
287x374mm (150 x 150 DPI)

Table1. Total dry biomass, root/shoot biomass ratio, leaf area and leaf area ratio (LAR) of the *Paulownia tomentosa* x *fortunei* hybrid grown as hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn

Treatments	Total DW	Root/shoot DW	Leaf area	LAR
	g		cm^2	$cm^2\ g^{-1}$
Control	0.908 ± 0.050^b	0.232	429 ± 16^b	497 ± 25^a
0.5 mg L^{-1} Cd	0.455 ± 0.045^a	0.326	218 ± 24^a	474 ± 57^a
2.5 mg L^{-1} Cd	0.419 ± 0.032^a	0.328	195 ± 40^a	473 ± 42^a
5.0 mg L^{-1} Cd	0.427 ± 0.060^a	0.349	207 ± 38^a	510 ± 35^a
5.0 mg L^{-1} Pb	0.483 ± 0.066^a	0.328	241 ± 39^a	523 ± 27^a
10.0 mg L^{-1} Pb	0.521 ± 0.070^a	0.327	257 ± 39^a	531 ± 29^a
20.0 mg L^{-1} Pb	0.497 ± 0.053^a	0.359	241 ± 33^a	504 ± 33^a
10.0 mg L^{-1} Zn	0.445 ± 0.063^a	0.349	198 ± 27^a	472 ± 22^a
20.0 mg L^{-1} Zn	0.597 ± 0.075^a	0.315	277 ± 38^a	487 ± 32^a
30.0 mg L^{-1} Zn	0.553 ± 0.055^a	0.329	268 ± 27^a	498 ± 35^a

Data are presented as means of five replications ± standard error. Different letters indicate significant differences assessed by Fisher LSD test ($P\leq0.05$) after performing one-way ANOVA analysis

Table 2. Heavy metal accumulation of different organs of *Paulownia tomentosa* x *fortunei* hybrid grown as hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn

Metal concentration	Metal content			
	Leaf	Stem	Shoot	Root
mg L^{-1}	mg kg DW^{-1}	mg kg DW^{-1}	mg kg DW^{-1}	mg kg DW^{-1}
Cd				
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
0.5	1.8±0.2^{a}	1.1±0.1^{a}	1.6±0.2^{a}	26.3±2.4^{a}
2.5	3.9±0.4^{b}	10.1±1.4^{b}	5.3±0.4^{b}	541.7±46.8^{b}
5.0	11.9±1.3^{c}	9.5±0.9^{c}	11.4±1.3^{c}	458.2±32.7^{b}
Pb				
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5.0	5.6±0.6^{a}	7.6±0.8^{a}	6.2±0.4^{a}	1580.8±91.5^{a}
10.0	19.6±2.8^{b}	11.6±1.9^{b}	17.8±2.7^{b}	2608.0±141.7^{b}
20.0	24.0±2.5^{c}	14.4±3.8^{c}	21.8±3.5^{c}	7941.2±263.8^{c}
Zn				
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
10.0	177.6±12.8^{a}	141.6±10.5^{a}	167.9±12.8^{a}	488.0±34.2^{a}
20.0	274.8±23.5^{bc}	201.2±25.9^{b}	262.6±24.5^{b}	843.2±67.3^{b}
30.0	240.0±23.4^{b}	221.6±22.8^{bc}	758.8±65.9^{c}	1566.8±103.5^{c}

Data are presented as means of five replications ± standard error. Different letters indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing one-way ANOVA analysis

Table 3. Chlorophyll a+b content, chlorophyll a/b ratio, carotenoid content and chlorophyll/carotenoid ratio of *Paulownia tomentosa* x *fortunei* hybrid grown as hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn

Treatments	Chl a+b	Chl a/b	Carotenoids	Chl a+b/carotenoids
	mg g FW $^{-1}$		mg g FW $^{-1}$	
Control	4.36±0.22^{a}	1.56	0.26±0.05^{bcd}	16.91
0.5 mg L^{-1} Cd	5.28±0.39^{bc}	1.59	0.30±0.03^{cd}	17.76
2.5 mg L^{-1} Cd	4.40±0.18^{a}	1.52	0.32±0.03^{d}	13.67
5.0 mg L^{-1} Cd	4.96±0.16^{abc}	1.59	0.19±0.02^{ab}	26.56
5.0 mg L^{-1} Pb	5.45±0.28^{bc}	1.63	0.22±0.04^{abc}	25.02
10.0 mg L^{-1} Pb	5.66±0.19^{c}	1.50	0.17±0.05^{a}	35.40
20.0 mg L^{-1} Pb	4.89±0.38^{ab}	1.57	0.31±0.01^{d}	15.93
10.0 mg L^{-1} Zn	5.57±0.34^{bc}	1.51	0.27±0.04^{cd}	20.90
20.0 mg L^{-1} Zn	5.37±0.22^{bc}	1.58	0.31±0.04^{d}	17.85
30.0 mg L^{-1} Zn	5.33±0.35^{bc}	1.53	0.26±0.03^{bcd}	23.71

Data are presented as means of five replications ± standard error. Different letters indicate significant differences assessed by Fisher LSD test (P≤0.05) after performing one-way ANOVA analysis

Table 4. MDA, H_2O_2, GSH+GSSG, ASC, total phenols and flavonoids contents in leaves of *Paulownia tomentosa* x *fortunei* hybrid grown as hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn

Treatments	MDA	H_2O_2	GSH+GSSG	ASC	Phenols	Flavonoids
	nM g^{-1} FW	nM g^{-1} FW	nM g^{-1} FW	nM g^{-1} FW	mg g^{-1} DW	mg g^{-1} DW
Control	0.017±0.001^{a}	141±14^{b}	4.67±0.32^{a}	3.71±0.29^{cd}	72.5±2.9^{abc}	2.76±0.17^{a}
0.5 mg L^{-1} Cd	0.103±0.009^{b}	62±7^{a}	4.67±0.36^{a}	1.31±0.26^{a}	70.6±4.3^{abc}	2.76±0.16^{a}
2.5 mg L^{-1} Cd	0.121±0.009^{c}	78±14^{a}	19.84±1.02^{d}	1.96±0.18^{ab}	69.6±4.8^{abc}	3.12±0.22^{ab}
5.0 mg L^{-1} Cd	0.123±0.005^{c}	86±8^{a}	9.14±0.39^{bc}	0.87±0.13^{a}	61.9±4.9^{a}	3.22±0.10^{ab}
5.0 mg L^{-1} Pb	0.026±0.007^{a}	83±7^{a}	7.39±0.42^{abc}	1.74±0.45^{ab}	77.3±5.3^{bcd}	3.97±0.21^{cd}
10.0 mg L^{-1} Pb	0.082±0.006^{b}	89±5^{a}	10.51±0.54^{c}	3.71±0.46^{cd}	65.7±4.7^{ab}	2.87±0.15^{ab}
20.0 mg L^{-1} Pb	0.258±0.009^{d}	238±13^{c}	22.55±0.43^{d}	3.92±0.46^{d}	66.7±5.8^{abc}	2.89±0.15^{ab}
10.0 mg L^{-1} Zn	0.041±0.009^{a}	82±35^{a}	27.84±0.99^{e}	1.53±0.37^{ab}	80.2±4.4^{cd}	4.19±0.19^{ab}
20.0 mg L^{-1} Zn	0.043±0.005^{a}	214±31^{c}	8.96±0.59^{bc}	2.28±0.32^{ab}	67.7±4.6^{abc}	2.76±0.20^{d}
30.0 mg L^{-1} Zn	0.072±0.006^{b}	301±38^{a}	6.22±0.33^{ab}	3.26±0.23^{bc}	87.1±5.8^{d}	3.79±0.21^{bc}

Data are presented as means of five replications ± standard error. Different letters indicate significant differences assessed by Fisher LSD test ($P \leq 0.05$) after performing one-way ANOVA analysis

Table 5. CAT, GPO, GR and APX activities determinate in leaves of *Paulownia tomentosa* x *fortunei* hybrid grown as hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn

Treatments	CAT	GPO	GR	APX
	μM mg pr.$^{-1}$ min^{-1}	μM mg pr.$^{-1}$ min^{-1}	μM mg pr.$^{-1}$ min^{-1}	μM mg pr.$^{-1}$ min^{-1}
Control	4.1±0.2^{a}	8.9±0.4^{bc}	45.8±2.8^{bc}	49.8±2.3^{a}
0.5 mg L^{-1} Cd	5.3±0.4^{a}	7.1±0.3^{b}	44.7±2.1^{ab}	200.9±15.1^{c}
2.5 mg L^{-1} Cd	12.4±0.4^{c}	16.8±0.3^{d}	55.8±2.2^{d}	178.5±9.6^{c}
5.0 mg L^{-1} Cd	12.6±0.5^{c}	4.2±0.1^{a}	56.5±2.8^{d}	67.5±6.7^{ab}
5.0 mg L^{-1} Pb	13.6±0.4^{c}	4.1±0.3^{a}	36.4±2.8^{a}	72.3±4.4^{ab}
10.0 mg L^{-1} Pb	13.5±0.8^{c}	6.5±0.4^{ab}	61.1±3.5^{d}	96.6±6.8^{b}
20.0 mg L^{-1} Pb	9.4±0.7^{b}	8.1±0.6^{b}	38.7±2.7^{ab}	105.7±4.5^{b}
10.0 mg L^{-1} Zn	3.4±0.5^{a}	8.9±0.4^{b}	54.6±2.7^{cd}	30.2±2.1^{a}
20.0 mg L^{-1} Zn	4.3±0.5^{a}	8.8±0.6^{b}	93.5±3.8^{c}	93.3±8.3^{b}
30.0 mg L^{-1} Zn	12.4±0.3^{c}	11.6±0.4^{c}	40.3±2.2^{ab}	95.7±6.8^{b}

Data are presented as means of five replications ± standard error. Different letters indicate significant differences assessed by Fisher LSD test (P≤0.05) after performing one-way ANOVA analysis