CADMIUM AND LEAD EFFECTS ON *EX VITRO* GROWTH AND ANTIOXIDATIVE RESPONSE OF TWO *PAULOWNIA* CLONES

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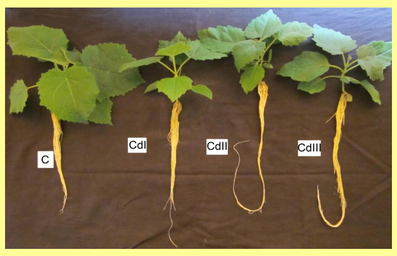
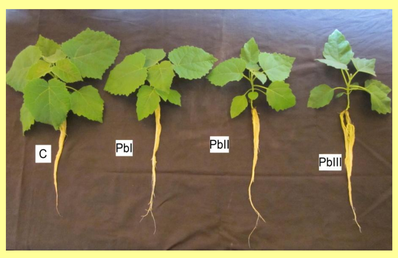
ABSTRACT

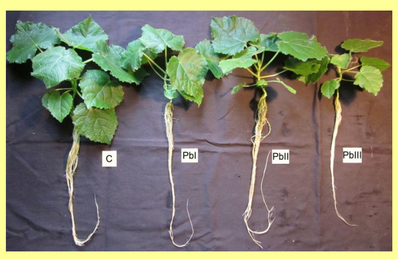
The tolerance of *Paulownia tomentosa x fortunei* clone TF 01 and *Paulownia elongata x fortunei* clone EF 02, grown hydroponically at different levels of heavy metals (0.5, 2.5, 5.0 mg/l Cd and 5, 10, 20 mg/l Pb was compared. These treatments caused significant reduction in total leaf area of both clones. The leaf number, root and stem lenght changed in different manner. The elevated levels of lipid peroxidation at higher Cd and Pb concentrations indicated oxidative stress in leaf tissues. The activity of phenylalanine ammonia-lyase (PAL), which participate in the control of phenolic metabolism, showed pronounced stimulation in the leaves of *Paulownia tomentosa x fortunei* after all treatments. The content of some biologically active compounds, such as phenols and flavonoids increased in the leaves of *Paulownia elongata x fortunei*.

Paulownia tomentosa x fortunei





Paulownia elongata x fortunei



MATERIAL AND METHODS

Plant material. Seeds and *in vivo* explants from *P. tomentosa x fortunei and P. elongata* x *P. fortunei* were used for developing of *in vitro* multiplication protocol. Shoots were cultured on Murashige and Skoog's (1962) nutrient medium supplemented with 3 % (w/v) sucrose, 0,8 % agar, vitamins, 4,439 µM BAP and 0,537 µM IAA. After multiplication the shoots were transferred to rooting medium based on half strength basal salts MS medium, 2 % sucrose, 6 % agar and vitamins suplemented with 4,92 µM IBA and 1,075 µM IAA. The pH of all media was adjusted to 5,7 before autoclaving. All cultures were incubated under controlled conditions – 16 h photoperiod, light intensity of 35 µmol/m²/s and 24/18ºC day/night temperature. After three weeks of rooting the shoots were washed from the medium with tap water and roots were rinsed with 1,5 ml/l Proplant solution.

Hydroponic experiment. *Ex vitro* seedlings were transplanted to *Hellriegel* (1898) nutrient solution with an addition of A-Z microelements in growth chamber. The heavy metal treatment was applied on the 48-th day after transplanting and one of the following heavy metals was added: 0.5, 2.5 and 5.0 mg/l of Cd supplied as Cd(NO3)2.4H2O and 5.0, 10.0 and 20.0 mg/l of Pb supplied as Pb(NO3)2. Plants grown in nutrient solution without metals served as controls. Plants were harvested after 10 days of treatment. Fresh mass of each of the plant sample was measured gravimetrically. Dry mass of shoots and roots was determined after oven-drying (60°C) for two days until constant weight. Leaf area was calculated using SigmaScan Pro 5 software. The bioaccumulation coefficient (BC) was calculated as the heavy metal content in plant divided by heavy metal concentration in the solution (*Nanda-Kumar et al., 1995*).

Enzyme and metabolite assayes. PAL activity was measured according to the procedure of *Yuan et al.* (2002). Total protein content was measured using the method of *Lowry et al.* (1951). The level of lipid peroxidation was determind as described previously *Heath et al.* (1968). Total phenol content was determind by the method of *Pfeffer et al.* (1998) and total flavonoid content was measured by the method of *Zhishen* (1999).

Hydroponic culture of *ex vitro Paulownia* plants

Treatments	Root lenght [sm]	Stem lenght [sm]	Leaf number	Leaf area [sm²]	BC
P. tomentosa x fortunei					
Control	28.25±1.06b	8.57±0.46b	12±0.85b	429±8b	
0.5 mg/l Cd	24.92±1.11a	6.87±0.47a	10±0.3a	203±19a	58.4
2.5 mg/l Cd	32.11±1.50a	8.02±0.19a	10±0.45a	195±11a	222.3
5.0 mg/l Cd	28.75±1.78b	8.01±0.32a	11±0.25a	207±19a	95.9
5.0 mg/l Pb	32.37±1.06b	8.12±0.32a	13±0.60a	241±9.5a	318.8
10.0 mg/l Pb	25.87±0.93a	7.32±0.27a	10±0.30a	257±14.5a	263.9
20.0 mg/l Pb	26.07±0.73a	7.62±0.28a	9±0.45a	242±11a	398.9
P. elongata x fortunei					
Control	37.51±1.06b	10.51±0.26b	10±0.40b	502±19.5d	
0.5 mg/l Cd	38.11±0.93b	9.41±0.91a	10±0.65b	462±32.5cd	111.4
2.5 mg/l Cd	37.00±0.81b	9.61±0.41a	8±0.40a	376±11.5bc	60.2
5.0 mg/l Cd	27.11±0.84a	8.62±0.25a	8±0.30a	271±23.5ab	113.3
5.0 mg/l Pb	30.41±0.89a	8.62±0.22a	10±0.65b	357±41ab	110.7
10.0 mg/l Pb	30.07±0.61a	7.92±0.28a	9±0.2b	303±9.5ab	160.4
20.0 mg/l Pb	17.72±1.06a	7.45±0.45a	8±0.85a	143±28.5a	211.6

Table 1. Mean values ± SD (n = 5-6) of root and stem lenght, leaf number and total leaf area of *Paulownia tomentosa x fortunei* and *Paulownia elongata x fortunei*, grown in hydroponic in response to heavy metal stress. The bioaccumulation coefficient (BC) was obtained as the heavy metal content in plant divided by heavy metal concentration in the solution. Values with the same letter are not significantly different when are separated by Fisher's LSD test (P < 0.05).

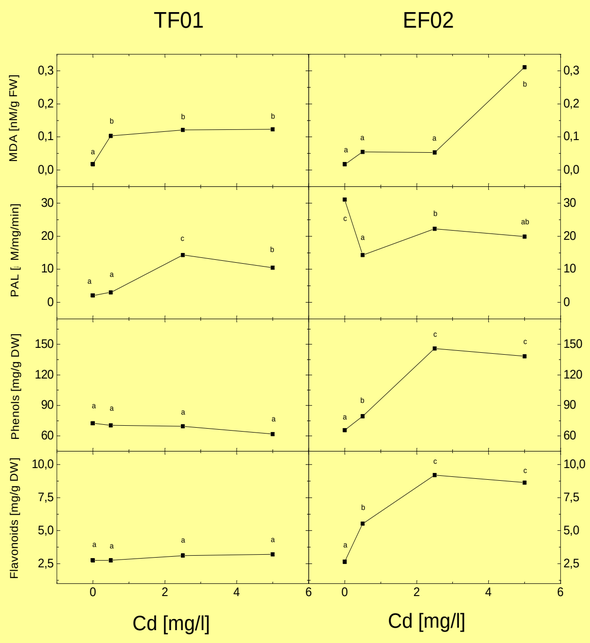


Fig. 1: Changes in MDA level, PAL activity, phenol and flavonoid content in leaves of *P. tomentosa x fortunei* and *P. elongata x fortunei*, grown at different levels of Cd exposition. Values with the same letter are not significantly different when means are separated by Fiaher's LSD test (P<0.05).

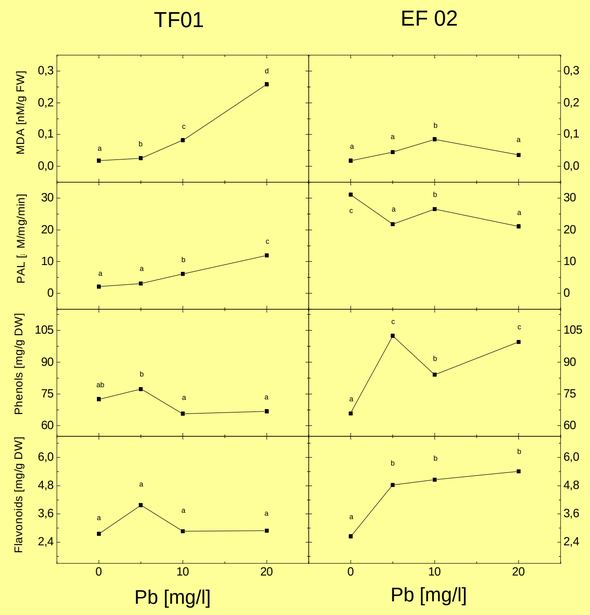


Fig. 2. Changes in MDA level, PAL activity, phenol and flavonoid content in leaves of *P. tomentosa x fortunei* and *P. elongata x fortunei*, grown hydroponically at different levels of Pb exposition. Values with the same letter are not significantly different when means are separeted by Fisher's LSD test (P<0.05).

RESULTS AND DISCUSSION

Effect of heavy metal stress on plants growth. Heavy metals are known to cause curtailing of all growth parameters and productivity of plants (*Wu et al., 2004*). In our study, with increasing heavy metal levels, the root and stem lenght, leaf number and total leaf area of both clones were reduced (table 1). We established slight increase only of root elongation of *P. tomentosa x fortunei* at 2.5 mg/l, 5.0 mg/l Cd and 5.0 mg/l Pb. With increasing Cd and Pb contents total leaf area of *P. tomentosa x fortunei* was reduced by about 53 %, and total leaf area of *P. elongata x fortunei* decreased by 8%, 25% and 46%, respectively with increasing concentration of Cd and by 29%, 40% and 72%, respectively with increasing concentration of Pb. *P. tomentosa x fortunei* accumulated approximately 2-fold most Pb in the roots than *P. elongata x fortunei* (data not shown). The bioacumulation coefficient increased in the order of Pb>Cd (Table 1).

Effect of heavy metal stress on plants malondialdehyde. The increased accumulation of lipid peroxides is indicative of encanced production of toxic oxygen (*Markovska et al., 2009*). In our study, MDA concentration is raised significant in leaves of *P. elongata x fortunei* with increasing Cd levels (fig. 1), and in leaves of *P. tomentosa x fortunei* with increasing Pb levels (fig. 2).

Effect of heavy metal stress on plants secondary metabolism. In our study, PAL activity in the leaves of control *P. elongata x fortunei* is higher in comparison to that in the leaves of control *P. tomentosa x fortunei*. PAL activity increased in leaves of *P. tomentosa x fortunei*, but decreased in leaves of *P. elongata x fortunei* after all treatments. There is some evidence for induction of phenolic metabolism in plants as a response to multiple stress (*Michalak, 2006*). Flavonoids and phenolic substances have been shown to act as antioxidants and antimicrobials in a wide range of plants (*Pietta, 2000*). They play pivotal roles in absorbing free radicals, quenching singlet oxygen, and decomposing peroxides. Flavonoids are also able to function as chelators for metals (*Brown et al., 1998*). In our study phenols and flavonoids contents increased singificant in the leaves of *P. elongata x fortunei* with increasing Cd and Pb levels (fig. 1 and fig. 2).

CONCLUSION

P. tomentosa x fortunei is more tolerant to heavy metal stress than P. elongata x fortunei. It is characterized with a lower reduction of root, stem length and leaf number and enhanced PAL activity after treatments.

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