PHOTOSYNTHESIS and GROWTH RESPONSE of TWO PAULOWNIA LINES to HEAVY METALS Cd, Pb and Zn

K. Miladinova**, Y.Markovska*** N. Tzvetkova*, K. Ivanova***, M. Geneva****, T. Georgieva**,

* Biotree, 8 Iliensko shoes str., 1220 Sofia, Bulgaria;
** Faculty of Biology, University of Sofia, 8 Dragan Tsankov Blvd., Sofia 1164, Bulgaria Bulgaria;
*** University of Forestry, Faculty of Forestry, 10 Kliment Ochridskii str., 1756 Sofia, Bulgaria;
**** Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. Georgi Bonchev str., bl. 21, Sofia 1113, Bulgaria

K. Miladinova: kameca@abv.bg
Y. Markovska: pmmaster2001@yahoo.com
N. Tzvetkova: nikolina_tzvetkova@mail.bg
K. Ivanova: ivanova.katya@abv.bg
M. Geneva: boykova2@yahoo.com
T. Georgieva: t.georgieva@biotree.bg

ABSTRACT

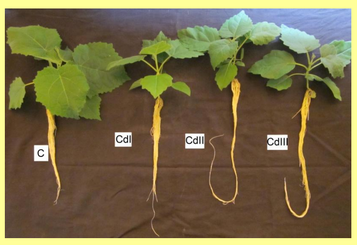
Paulownia tomentosa x fortunei and Paulownia elongata x fortunei lines exposed to different concentrations of Cd, Pb and Zn in hydroponic experiment were analyzed with reference to study the metal`s effects on the growth and photosynthetic parameters. Maximal accumulation of heavy metals was occurred in the roots. These treatments caused significant reduction in total dry biomass, leaf area, stomatal conductance and transpiration rate, but increased leaf area ratio (LAR), Chl a+b, net photosynthetic rate and water use efficiency. The accumulation of heavy metals in both lines increased in order Pb < Zn < Cd, while bioaccumulation coefficients (BC) rose in order Pb < Cd < Zn. The BC changed in different manner with increasing concentration of heavy metals in nutrient solution. The bioaccumulation coefficients of Pb and Cd were greater than 100. These results confirm both lines as accumulators which grow rapidly and have a potential for phytoremediation of metal contaminated soils showing phytostabilization mechanism.

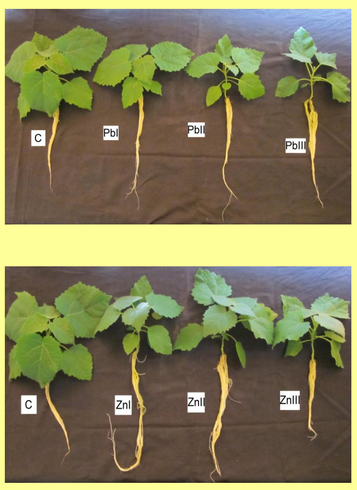
MATERIAL AND METHODS

Plant material: Seeds and in vivo explants from the species P. tomentosa, P. elongata and their hybrids with P. fortunei were used for developing of in vitro multiplication protocol. Design, growth conditions and Cd, Pb, Zn treatment: The experiments were set as ten treatments including control, each treatment with 3 replications. The heavy metal treatment was applied on the 48th day after transplanting when the plants had adapted to the conditions of 1/2 diluted Hellriegel nutrient solution and one of the following heavy metals: Cd [0.5; 2.5 and 5.0 mg l-1 of Cd supplied as Cd(NO3)2.4H2O: MERCK]; Pb [5.0; 10.0 and 20.0 mg l-1 of Pb supplied as Pb(NO3)2: MERCK] and Zn [10.0; 20.0 and 30.0 mg l-1 of Zn supplied as Zn(NO3)2.6H2O: MERCK] was added. Plants grown in nutrient solution without metals served as controls. At the end of the experiment the plant samples were collected, washed with tap water and rinsed with distilled water before being separated into leaf, petiole, stem and root and fresh mass of each plant sample were measured gravimetrically. Dry mass of shoots and roots were determined after oven-drying (60oC) for 2 d until constant weight was obtained. Leaf area was calculated using software SigmaScan Pro 5. Leaf area ratio (LAR) was described as the leaf area (cm2) divided by the total plant dry weight (Hunt 1982).

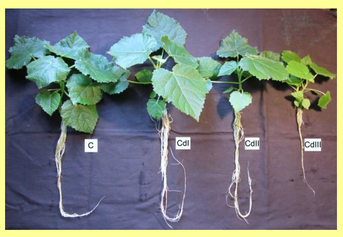
Analysis of heavy metals: Total metal content in each organ was analyzed after sample homogenization in a Blender. Dry vegetal tissues (0.5 g) were treated with 2 ml of 30 % hydrogen peroxide and 3 ml 65 % nitric acid and 9 ml of 37 % hydrochloric acid (Doumett et al. 2008). The samples were heated on a heating block at 200oC 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). The metal content in shoots was calculated on the basis of the relative dry mass (DM) of each aerial part of plants ([HM]leaves x DMleaves) + ([HM]stems x DMstems)/(DMleaves + DMstems) (Tassi et al. 2007). Since stems mainly act as a transport organ of heavy metal from the roots to the upper parts, above results in terms of leaves accumulation will be stressed, as such results are more suitable for comparison with the various treatments. 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).
Photosynthetic pigment content: Ch a, Ch b, and carotenoid content from the upper most fully-expanded leaves at 58 days after transplanting the explants to the Hellriegel solution were determined according to the method of Lichtenthaler (1987).
Gas-exchange measurement: Net photosynthetic rate (PN, μmol m-2 s-1), transpiration rate (E, mmol m-2 s-1), and stomatal conductance (gs, 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.

Paulownia tomentosa x fortunei





Paulownia elongata x fortunei



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 (Schűtzendubel and Polle 2002). The physiological effects of heavy metals on plants included: 1/ perturbation of stomatal functions to changes in water relations and rate of gas exchange (Papadakis et al. 2004); 2/ reduction of photosynthetic pigments and activity (Alaoui-Sossé et al. 2004; Lou et al. 2004); 3/ disruption of the integrity of cellular membranes (Hall 2002).
Phytoextraction is an environmentally friendly in situ technique for remediation of heavy metal contaminated soils which involves the removal of toxins by the roots of the plants with subsequent transport to aerial plant organs. 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). Populus nigra has shown to be useful as a biomonitor in heavy metal contaminated regions (Djingova et al. 1995) and now Populus has been internationally accepted as a model system for physiological and molecular tree studies (Tuscan et al. 2006). 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 (Doumett et al. 2008; 2011; Wang et al. 2010).

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). The correlation between Zn accumulation in the leaves and photosynthetic rate during adaptation at low CO2 conditions is established (Lazova et al. 2003). Over the last two decades Paulownia species has been extensively studied due to its ability to uptake nitrates and land contaminants, namely heavy metals (Doumett et al. 2008; 2011; Wang et al. 2010). This high-yielding tree can be used for the production of energy, paper pulp and wooden building materials. 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. The company's laboratory is largest producer and supplier of genetically superior Paulownia tissue-cultures – in vitro seedlings. The farmers prefer Paulownia tomentosa x fortunei line TF 01 due to fast development and uniform regular growth. Paulownia elongata x fortunei line EF 02 is less branchy and is used for the purpose of wood material production. There is no information about its tolerance to heavy metal stress and possibilities of management of soil pollution with these lines.

In this research, the effects of Cd, Pb and Zn on growth and photosynthetic gas exchange of Paulownia tomentosa x fortunei line TF 01 and Paulownia elongata x fortunei line EF 02, grown in hydroponic after transplant the explants are compared so as to provide fundamental base for vegetation restoration in contaminated soils.

RESULTS AND DISCUSSION

Treatments [mg.l-1]	Total Dry Mass [g]	Root/ shoot DM	Total Leaf Area [cm2]	LAR [cm2.g-1]
Control	0.908 0.050b	0.232	429 16b	497 25a
0.5 Cd	0.455 0.045a	0.326	218 24a	474 57ab
2.5 Cd	0.419 0.032a	0.328	195 40a	473 42a
5.0 Cd	0.427 0.060a	0.349	207 38a	510 35b
5.0 Pb	0.483 0.066a	0.328	241 39a	523 27a
10.0 Pb	0.521 0.070a	0.327	257 39a	531 29a
20.0 Pb	0.497 0.053a	0.359	241 33a	504 33a
10.0 Zn	0.445 0.063a	0.349	198 27a	472 22a
20.0 Zn	0.597 0.075a	0.315	277 38a	487 32a
30.0 Zn	0.553 0.055a	0.329	268 27a	498 35a

Table 1. Total dry biomass, root/ shoot biomass ratio, total leaf area and leaf area ratio (LAR) of the Paulownia tomentosa x fortunei line TF 01 grown in hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn

Treatments [mg. l-1]	Total Dry Mass [g]	Root/ Shoot DM	Total Leaf Area [cm2]	LAR [cm2.g-1]
Control	1.233 0.092bc	0.196	502 26d	415 54ab
0.5 Cd	1.298 0.041c	0.214	462 26cd	484 27abc
2.5 Cd	0.929 0.043abc	0.258	376 36bcd	437 53abc
5.0 Cd	0.486 0.065ab	0.226	271 48abc	560 73c
5.0 Pb	0.785 0.030abc	0.234	357 42abcd	480 88abc
10.0 Pb	0.581 0.037abc	0.240	303 19abcd	433 35abc
20.0 Pb	0.287 0.016a	0.199	143 17a	530 72bc
10.0 Zn	1.228 0.189c	0.217	534 21bcd	415 32a
20.0 Zn	0.511 0.020ab	0.242	225 29ab	451 62abc
30.0 Zn	0.409 0.021abc	0.227	171 25abcd	419 29abc

Table 2. Total dry biomass, root/ shoot biomass ratio, total leaf area and leaf area ratio (LAR) of the Paulownia elongata x fortunei line EF 02 grown in hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn

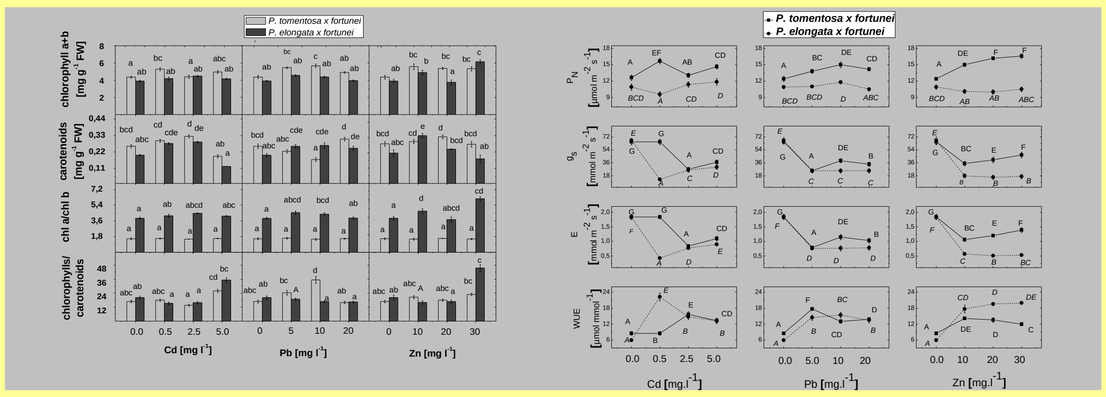


Fig. 1. Chlorophyll a+b content, chlorophyll a/b ratio, carotenoid content and chlorophyll/ carotenoid ratio in the leaves of Paulownia tomentosa x fortunei line TF 01 and Paulownia elongata x fortunei line EF 02 grown in hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn. Data are presented as means of five replications standard error. Different letters indicate significant differences assessed by Fisher´ s LSD test (P ≤ 0.05) after performing of one-way ANOVA analysis

Fig. 2. Gas-exchange parameters in the leaves of Paulownia tomentosa x fortunei line TF 01 and Paulownia elongata x fortunei line EF 02 grown in hydroponic and exposed 10 days to different concentrations of Cd, Pb and Zn. Fs, line effect; Fm, Cd, Pb or Zn effect; Fs x Fm, line x metal interaction effect. Data are presented as means of twenty replications ± standard error. The different letters above the bars are significantly different from each other at P ≤ 0.05.

	Metal Concentration [mg. l-1]	Metal Content [mg. kg DM-1] Leaf	Stem	Shoots	Roots	BC
Cd	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	-
	0.5	1.8±0.2ab	1.1±0.1ab	2.9±0.2a	26.3±2.4a	58.4
	2.5	3.9±0.4ab	10.1±1.4ab	14.0±0.4a	541.7±56.8b	222.3
	5.0	11.9±1.3abc	9.5±0.9ab	21.4±1.3a	458.2±32.7b	95.9
Pb	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	-
	5.0	5.6±0.6abc	7.6±0.8ab	13.2±0.4a	1580.8±231.5c	318.8
	10.0	19.6±2.8bc	11.6±1.9ab	31.2±2.7a	2608.0±341.7d	263.9
	20.0	24.0±2.5c	14.4±3.8b	38.4±3.5a	7941.2±563.8e	398.9
Zn	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	-
	10.0	177.6±12.8d	141.6±10.5c	319.2±12.8b	488.0±34.2b	80.7
	20.0	274.8±23.5f	201.2±15.9d	476.0±24.5c	843.2±67.3b	65.9
	30.0	240.0±23.4e	221.6±12.8e	461.6±65.9c	1566.8±123.5c	67.6

Table 3. Metal contents in Paulownia tomentosa x fortunei line TF 01 exposed 10 days to different concentrations of Cd, Pb and Zn and bioaccumulation coefficients

	Metal Concentration [mg. l-1]	Metal Content [mg kg DW-1] Leaf	Stem	Shoots	Roots	BC
Cd	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	-
	0.5	0.3±0.02a	0.5±0.03a	0.8±0.02a	55.0±3.4a	111.6
	2.5	0.9±0.04a	3.1±0.04a	4.0±0.24ab	146.5±26.8b	60.2
	5.0	2.6±0.03a	12.0±0.91a	14.6±1.30b	551.8±52.7e	113.3
Pb	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	-
	5.0	1.2±0.61a	2.4±0.03a	3.6±1.40ab	540.0±23.1e	108.7
	10.0	2.4±0.08a	9.6±0.09a	12.0±0.70ab	1592.0±34.2g	160.4
	20.0	6.4±0.05a	4.8±0.04a	11.2±0.05ab	4220.8±56.3h	211.6
Zn	0	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	-
	10.0	92.1±2.8b	112.4±10.5b	204.5±12.8c	234.5±31.2c	43.9
	20.0	173.4±21.5c	155.6±15.9c	329.0±14.5d	457.8±47.3d	39.3
	30.0	177.4±23.4c	243.6±12.8d	421.0±15.9e	622.8±56.5f	34.8

Table 4. Metal contents in Paulownia elongata x fortunei line EF 02 exposed 10 days to different concentrations of Cd, Pb and Zn and bioaccumulation coefficients

Conclusion