POSSIBILITY FOR USING OF TWO PAULOWNIA LINES AS A TOOL FOR REMEDIATION OF HEAVY METAL CONTAMINATED SOIL

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ABSTRACT

One year-aged two *Paulownia* lines (*P. tomentosa x fortunei* – TF 01 and *P. elongata x fortunei* – EF 02) were grown as a pot experiment on the soil collected from the field near waste depository of Kremikovtzi ferrous metallurgical combine by Sofia. The soil was heavily polluted with Cd. The content of metals (Ca, Mg, K, Na, Cd, Cu, Pb, Zn and Fe) in the soil and its distribution in the roots, stems and leaves of both plants were traced out. The results showed that Ca and K were accumulated more in the stems, Mg, Na, Fe and Cd in the roots, while Pb, Cu and Zn in the leaves of both plants. We examined bioaccumulation factor (BF) and translation factor (TF) in order to determine the effectiveness of plants in removing metals from soil. The BF for Fe, Pb, Cu and Zn in TF 01 line exceeded that of EF 02 line - 5.6; 1.03; 1.20; 1.14 times, respectively. The TF was higher in TF 01 line for Fe, Pb and Cd (6.0; 1.92 and 1.03, respectively), but not for Cu and Zn.

P. tomentosa x fortunei line responded to action of heavy metals with reference to increase in fresh mass/dry mass ratio, but decrease stem length, leaf number and total leaf area in comparison with *P. elongata x fortunei* line. That is why it successfully using for phytoremediation of polluted soil is disputable.

Key words: Phytoextraction, heavy metals, *Paulownia* lines, stem lenght, leaf area

INTRODUCTION

Heavy metal contamination of soils is one of the most important environmental problems throughtout world (Doumett et al., 2008; Wuana et al., 2010). Heavy metals are non-biodegradable and can accumulate in biological systems – humans, animals, microorganisms and plants causing toxicity (Nouri et al., 1980; D`amore et al., 2005). However, metal-contaminated soils are often hard to remediate and the used conventional engineering methods are expensive and non-effective (Salt et al., 1995; Zhou and Song, 2004). In contrast, the use of plants to remove heavy metal contaminants from soils, known as “phytoremediation“, offers economic and environmental advantages and is a promising technology (Salt et al., 1995). Phytoremediation is an alternative which demonstrated that the cost of phytoextraction of heavy metals by plants is only a fraction of these of conventional engineering technologies (Anderson et al., 1999; McGrath et al., 2002). The ideal plants for phytoremediation should possess multiple traits like fast growth rate, large biomass production, deep root system, great potential for accumulation and toleration for multiple heavy metals and could be easy to harvest (Clements et al., 2002; Hsiao et al., 2007). Unfortunately, no plant that has been described can fulfill all these standards.

Some plant species endemic to metalliferous soil accumulate large amounts of heavy metals in their shoots and show great potential for cleaning up of metal contaminated soils (Baker and Brooks, 1989; Xiong, 1997). They are identified as hyperaccumulators, but most of them are not suitable for phytoremediation application in the field due to their small biomass production and slow growth (Shen et al., 2002). In addition, they generally accumulate only one specific element and are low-depth rooted. As an alternative to the use of herbaceous hyperaccumulators, selected woody species are metal tolerant and have a fast growth rate, a deep root system and ability to accumulate number of metals. Only a few studies are present in literature, most of them concerning the use of poplar and willow for the remediation of Cd polluted soils (Robinson et al., 2000; Klang-Westin and Eriksson, 2003; Dickinson and Pulford, 2005). *Paulownia tomentosa* (Thunb.) Steud and other tree species belonging to the genus *Paulownia* (*Paulownia fortunei* Hems., *Paulownia elongata* S.Y. Hu) are used for phytoremediation (Doumett et al., 2008, 2011; Stankovic et al., 2009; Wang et al., 2009, 2011) due to their tolerance to heavy metals in combination with fast growth rate. A 5-7 year-old tree can grow up to 15-20 m of height and produced annual biomass to a 150 t ha^{-1} (Caparròs et al., 2008). The massive production of biomass of *P. tomentosa* within a short time lead to a significant removal of contaminants from polluted soil possible despite the low rate of metal absorption (Doumett et al., 2008).

In our country *Paulownia spp.* are selected by BIOTREE company, Bulgaria according to technology registered by Biotree Ltd. This company is largest producer and supplier of genetically superior *Paulownia* tissue-cultures – *in vitro* seedlings, which are preferred from the farmers due to its fast development and an uniform and regular growth. Two lines (*P. tomentosa x fortunei* – TF 01 and *P. elongata x fortunei* – EF 02) are selected and patented with the purpose of obtaining two types of plants: *(i)* lower and branchy individuals and *(ii)* trees higher individuals, less branchy for the purpose of wood material formation. There is no information about heavy metal tolerance of these lines and possibilities to use as phytoremediators of contaminated soils.

In the present study, a pot experiments were carried out to investigate the differences in growth and heavy metals accumulation between one-year-old *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* plants grown on heavily polluted with Pb and Cd soil. The distribution of metals (Ca, Mg, K, Na, Cd, Cu, Pb, Zn and Fe) between the soil and the roots, stems and leaves of both plants are traced out. The bioaccumulation factors (BF) and translation factors (TF) are calculated in order to determine the effectiveness of plants in removing heavy metals from soil.

MATERIALS AND METHODS

Soil sampling and characterization

The polluted soil was taken from the surface and at depths of 20 and 40 cm of a field near waste depository of Kremikovtzi ferrous metallurgical combine and have the following agrochemical characteristics: pH(H_2O) - 8.00, 9 mg kg^{-1} soil total mobile nitrogen ($N\text{-}NO_3^-$ + $N\text{-}NH_4^+$), 26.0 mg kg^{-1} soil mobile phosphorus (P_2O_5). The following content of studied heavy metals (mg kg^{-1}DW) was measured: Cd –4.8; Cu – 69.5; Pb -115.5; Zn –199.5 ; Fe – 48730; Ca – 2015; Mg – 3645; Na – 325; K - 5020. Because the Bulgarian maximal limit concentrations (MLC) at pH (H_2O) - 8.00 are Cd - 3.0, Cu < 300, Pb < 120 and Zn < 400 mg kg^{-1}DW, the soil are heavily polluted with Cd. The content of Cd exceeded MLC 1.6 times, respectively. The soil was air dried and sieved through nylon mesh and than mixed with sand in the ratio 3:1. Soil aliquots with 2.5 kg dry weight (DW) were used to fill 30 experimental pots.

Plant material

The *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* lines was chosen as a plant models for the experiment described. One-year-old plantlets derived from *in vitro* micropropagation seedlings were initially cultivated in plastic pots (d = 10 cm) filled with a peat-perlite mixture (2:1, v:v), placed in greenhouse and irrigated daily prior to being transplanted into experimental pots. Each pot was planted with one plantlet of two lines. All pots were adjusted daily by weight to 60% water holding capacity with tap water to maintain vigorous plant growth.The experiment was conducted in a glasshouse supplied with natural sunlight from 20th April to 20th July, 2012. The glasshouse temperatures were from 15^{o}C to 35^{o}C, relative humidity ranged from 40% to 65%. The plants were harvested (six-eight replicates for each test) in the end of July and their organs were separated. In order to remove the substrate from the radical system, roots were carefully washed.

The dry mass of plant organ samples (leaf, stem and root) was gravimetrically determined after heating at 60^{o}C until a constant weight was obtained. Heavy metal content in each organ was analyzed after sample homogenization in a blender. Leaf area was calculated using SigmaScan Pro 5 software.

Before planting and after removal of plants, total Cd, Cu, Pb, Zn, Fe, Ca, Mg, Na and K contents were determined on samples obtained by collecting three soil aliquots from each pot which were combined and heated at 105^{o}C until a constant weight. The soil bioavailable metal fraction (free metal ions, soluble metal complexes and metals adsorbed to inorganic soil constituents at ion exchange sites) was also determined by extraction tests at the beginning and at the end of the study. Portions (25g) soil aliquots were transferred into 1000 ml polyethylene bottles and 500 ml redistilled water were added. The bottles were mixed in a mixer at laboratory temperature for 48 h. After mixing, each sample was centrifuged for 5 min at 10 000 g and the supernatant was filtered on 0.2 μm pore size filters.

Metal analyses

Total metal content in soil and plant organ samples was determined by Atomic Absorption Spectrophotometric (AAS) analysis, after acidic digestion with Suprapur grade Fluka reagents. The plant and soil samples were digested with a solution of 1:3 HNO_3 : HCl (v/v), heated to a 200^{o}C and the residual were dissolved in 50 ml 1N HCl.

Statistical analysis

The mean values ±SD and exact number of experiments are given in the figures and tables. The significance of differences between control and each treatment was analyzed by Fisher LSD test ($P \leq 0.05$) after performing ANOVA multifactor analysis.

RESULTS

Biomass production

Visual assessment of plants did not report any phytotoxic symptoms (e.g. discoloration, pigmentation, yellowing or stunting). Stem length of *P. elongata* x *fortunei* is higher, but leaf number is smaller than that of *P. tomentosa* x *fortunei*. Nevertheless, total leaf area of *P. elongata* x *fortunei* is approximately 2-fold higher compared to *P. tomentosa* x *fortunei*. The fresh matter/ dry matter of both clones are differed insignificantly (Table 1).

Table 1. Mean values (n=6-8 ± SD) of stem lenght, leaf number, total leaf area and ratios fresh matter/ dry matter, measured at the end of the experiment in *Paulownia tomentosa* x *fortunei* and *P. elongata* x *fortunei*, grown on metal polluted soil

Parameter	*P. tomentosa* x *fortunei*	*P. elongata* x *fortunei*
Stem length (cm)	19.9±3.8	23.0±8.3
Leaf number	9.5±1.0	8.4±1.5
Total leaf area (cm^2)	482.00±30.00	889.70±88.50
FM/DM (g/g)	5.25±0.42	4.91±0.47

Evalution of metal bioavailability is soil before planting and after removal of plants

In order to evaluate the extent of competition of soil constituent, metals biovailable fraction are calculated. The mean percentages (n = 6-8) of bioavailable Ca, Mg, K, Na, Fe, Pb, Cu, Zn and Cd in metal polluted soil with respect to their total concentrations, after extraction with redistilled water at the beginning and the end of the experiment are reported in Table 2.

Table 2. Total and bioavailable Ca, Mg, K, Na, Fe, Pb, Cu, Zn and Cd in the soil before planting and after removal of *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* at the end of the experiment

Metal	Initial concentration		End concentration			
	(mg kg^{-1}DW)	(%)	*P.t.* (mg kg^{-1}DW)	(%)	*P.el.* (mg kg^{-1}DW)	(%)
Ca	2015±342	26.6	805±67	76.7	1130±156	35.9
Mg	3645±389	2.3	2290±312	4.7	2715±365	2.5
K	5020±499	12.2	2655±285	7.5	3565±378	4.3
Na	325±46	96.5	140±29	29.6	160±27	40.0
Fe	48730±456	1.2	14975±385	30.7	30070±673	61.7
Pb	115.5±34.7	55.4	32.0±2.4	27.7	41.5±3.6	35.9
Cu	69.5±5.4	5.1	33.0±2.9	47.5	42.0±3.7	60.4
Zn	199.5±23.6	2.4	74.5±7.6	37.3	99.0±8.7	49.6
Cd	4.8±0.3	43.7	0.7±0.02	14.6	2.2±0.4	45.8

The results showed that before planting highest concentrations of Na, Pb, Cd and Ca in bioavailable fraction is observed. The presence of Fe in soluble form is negligible despite of its highest soil concentration. The total soil concentrations of all metals decreased at the end of the experiment. The soil concentrations of metals after removal of *Paulownia tomentosa* x *fortunei* plants are smaller than that of *Paulownia elongata* x *fortunei* as compared to initial concentrations. The percentages of biovailable metals are differed at the end of the experiment. Solubilization of Ca, Fe, Cu and Zn increased, but that of Na and Pb decreased. The concentration of Cd is smaller in the extractable fraction after removal of *Paulownia tomentosa* x *fortunei*, but rose after harvesting of *Paulownia elongata* x *fortunei*.

Plant uptake and translocation

Heavy metal concentrations in plant roots are in the order of Cd < Pb < Cu < Zn < Fe with significant differences one to the other (Figure 1). The results showed that Ca and K are accumulated more in the stems, Mg, Na, Fe and Cd in the roots, while Pb, Cu and Zn in the leaves of both plants. Data obtained for shoots (stem and leaves) indicated that metal accumulation of Pb, Cu, Zn and Cd are higher than those measured in roots of both plants.

It is interesting to compare the metal removal efficiency of both clones. Table 3 shows the metal accumulation in the whole plants (Me_{plant}) and plant uptake percentages (U_{plant}) for all metals. The values for U_{plant} is obtained as Me_{plant} is divided to biovailable amount of metals in the soil ($Me_{soil\ t=0}$) before it using in pot experiments: $U_{plant} = 100 \times (Me_{plant}) / (Me_{soil\ t=0})$.

Table 3. Mean values (n = 6-8 ± SD) of metal accumulation in the whole plants of *Paulownia tomentosa* x *fortunei* and *Paulownia. elongata* x *fortunei* (Me_{plant} in g kg^{-1} DW) and plant uptake percentages (U_{plant})

Metal	*Paulownia tomentosa* x *fortunei* (Me_{plant})	(U_{plant})	*Paulownia elongata* x *fortunei* (Me_{plant})	(U_{plant})
Ca	31.4±2.5	5.84±0.71	21.5±1.7	4.01±0.5
Mg	10.4±1.3	12.07±1.12	9.2±0.8	11.02±1.2
K	68.5±5.8	11.16±1.65	64.8±6.9	10.54±1.8
Na	1.6±0.5	0.50±0.02	0.8±0.09	0.27±0.02
Fe	4.2±0.3	0.75±0.08	2.2±0.32	0.39±0.04
Pb	0.050±0.003	0.78±0.08	0.058±0.006	0.91±0.08
Cu	0.123±0.015	3.49±0.29	0.131±0.017	3.72±0.43
Zn	0.429±0.039	8.94±0.96	0.354±0.038	7.37±0.69
Cd	0.007±0.0002	0.15±0.03	0.008±0.0004	0.17±0.03

The results showed that alkaline earth metals (Ca, Mg) and K prevail in *Paulownia tomentosa* x *fortunei*, but accumulation of heavy metals (Pb, Cu and Cd) are higher in *Paulownia elongata* x *fortunei*. The plant uptake percentage for Mg, K, Zn and Ca is higher in *Paulownia tomentosa* x *fortunei*, while Pb, Cu and Cd is more in *Paulownia elongata* x *fortunei* plants.

If is judged by remain heavy metal concentrations in soils after plant harvesting, *Paulownia tomentosa* x *fortunei* plants accumulated higher quantity of Fe and Zn in comparison with *Paulownia elongata* x *fortunei* plants. Research demonstrated that Fe and Zn absorption by plants is higher than Pb, Cu and Cd.

The term bioaccumulation factor (BF), defined as the ratio of heavy metal concentrations in plant dry mass to those in soils is used to determine the effectiveness of plants in removing metals from soil. BF is higher > 1 for Cu, Zn and Cd in both plants. *Paulownia elongata* x *fortunei* accumulated more Cd than *Paulownia tomentosa* x *fortunei*. Translation factor (TF) or shoot/root ratio stating total element concentration in the shoot tissue to total element concentration in the root tissue is changed in our experiment for Pb and Zn in *Paulownia tomentosa* x *fortunei* and for Zn in *Paulownia elongata* x *fortunei* (Table 4).

Table 4. Effectiveness in removing heavy metals from the soils and metal translocation from the root to the shoot of *Paulownia tomentosa* x *fortunei* and *P. elongata* x *fortunei* plants, grown on polluted soil.

Heavy metals	BF* $\frac{\text{mg kg}^{-1}\text{ plant DW}}{\text{mg kg}^{-1}\text{ soil}}$	TF** $\frac{\text{mg shoot}}{\text{mg root}}$
	Paulownia tomentosa x *fortunei*	
Fe	0.23	0.24
Pb	0.96	1.82
Cu	2.52	0.93
Zn	3.84	1.01
Cd	1.57	0.61
	Paulownia elongata x *fortunei*	
Fe	0.04	0.04

Pb	0.93	0.95
Cu	2.09	0.96
Zn	3.37	1.05
Cd	2.68	0.59

The phytoremediation success depends on plant growth and restricted distribution of heavy metals in the shoots. Our results showed that stem length and total leaf area of *Paulownia elongata* x *fortunei* are higher than *Paulownia tomentosa* x *fortunei*, but BF for Cu and Zn and TF for Pb are smaller. BF for Cd is highest with 1.7 times, and TF for Zn – with 1.03 times.

DISCUSSION

Heavy metal concentration in plant tissues changed on dependence of their content in the environment (Xiong, 1998). As Markert (1994) proposed the normal compositions of Cd, Pb and Zn in plant are 0.05, 1.0 and 50.0 mg/kg dry weight, respectively. Our results showed that quantities of Fe accumulated in the roots of both plants grown on polluted soil are highest. Pb, Cu, Zn and Cd prevailed in the leaves of both plants (Figure 1). To consider a plant as a hyperaccumulator, the minimum threshold tissue concentration is 0.01% for Cd and 1% for Zn per dry weight of plant (Gardea – Torresdey et al., 2005). In our experiment the total Zn concentration is 4.3% for *Paulownia tomentosa* x *fortunei* and 3.6% for *Paulownia elongata* x *fortunei*.

Recently plant ability for phytoremediation is improved by bioaccumulation factor (BF) and translocation factor (TF). According to Anderson (2007) the plants are accepted as exluders, accumulators or hyperaccumulators, if its the BF values are as follows < 1, >1 and > 10. The obtained BF values for both plant grown on polluted soil are higher than 1.00 for Cu, Zn and Cd (Table 4). Therefore *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* lines appeared to be accumulators for Cu, Zn and Cd. According to Anderson (2007) TF values for accumulators are > 1.00. Our results showed that TF in *Paulownia tomentosa* x *fortunei* exceeded 1.00 only for Pb and Zn, while for *Paulownia elongata* x *fortunei* plants TF more than one is estimated for Zn (Table 4). The levels of Pb, Cu, Zn and Cd accumulation in aboveground parts and Fe in the roots allowed supposing that *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* are plants that could be used for phytoremediation. The mechanisms that are involved in heavy metal uptake are phytostabilization and phytoextraction. Before planting Na, Pb, Cd and Ca prevail in the extractable fraction of soil. After harvesting solubility of Ca, Fe, Cu and Zn in soil increased (Table 2). The comparison between plant metal accumulation and extractable metal concentrations in soil showed that the heavy metal uptake and translocation is not dependent on the extent of bioavailable fraction and the predominant mechanism for metal accumulation is not concentration gradient between soil and plant tissues. U_{plant} values calculated for both plants indicated very low efficiency in metal uptake and translocation compared to the amounts mobilized in soil. Mg, K, Zn and Ca uptake predominate in both plants, while Cu, Pb, Fe and Cd accumulation is smaller. It is known that only a part from the total amount heavy metals in the soil is accessible for the living organisms. This part is represented as free ions, soluble forms and ions adsorbed to the inorganic soil components. Some metals, as Zn and Cd, are observed predominantly in soluble form, while others, as Pb, are much more approachable because of their presence as weakly soluble forms (Poschenreiter et al., 2001). In order to reach the required phytoextraction level it is necessary to increase the concentration of the soluble metals inside the soil. Some root-released organic components – phenols, organic acids, alcohols, and proteins, might serve as carbon and nitrogen sources for the growth and development of the microorganisms participating in the degradation process (Giller et al., 1998). The plants release about 10-20 % from their photosynthetic products as root secretion, which stimulates the microbial activity in the rhizospheric zone (Schmidt, 2003). Among various soil parameters known to affect the availability of metals, soil pH is considered the most important. Many investigations showed that there is a linear trend between soil pH and Cd uptake: the decreasing of soil pH leads to increasing concentration of Cd in plants, provided that other soil properties remain unchanged (Kirkham, 2006). Soil pH affects the availability of Cd present in soil solution, but increasing of soil pH does not always reduce Cd uptake by plants (Eriksson, 1989; Singh et al., 1995). Under field conditions, the uptake of Cd by plants may be affected by many variable soil and climatic parameters. It is interesting that BF for *Paulownia elongata* x *fortunei* is 1.7 times higher than that for *Paulownia tomentosa* x *fortunei*, but TF are approximately similar (Table 4). However, the interactive effects of heavy metals in plants are complicated, not only additive, antagonistic or synergistic, but also related to many factors including concentration combinations of elements, plant species and various parts of plants. Probably the competitive effect of Ca and Mg in the formation of heavy metal chelating complexes with organic components secreted by plants is showed too. It has been reported that uptake, transport, and subsequent distribution of nutrient elements by the plants can be affected by the presence of Cd ions. In general, Cd has been shown to interfere with the uptake, transport, and use of several elements (Ca, Mg, P, and K) and water by plants (Das et al., 1997). Data on poplar (*Populus jaquemontiana* var. *glauca*) showed that Cd can inhibit mineral nutrition by competition between this metal and other metal ions (Solti et al., 2011). The investigators suggested two mechanisms. In the first type, the mechanisms is like the influence of Cd on Fe. It is known that Cd might inhibit the chelating process of Fe and the loading of Fe into the xylem. That is why, the metals that are transported in the xylem are influenced by Cd. In the second type, the mechanism is like the influence of Cd on Ca in competition for Ca-transporters. Except Mg, alkaline earth metals act in the same manner.

The heavy metals have a different mobility and they are transported from roots to shoots to different way. 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. Several plant nutrients have many direct or indirect effects on Cd availability and toxicity. Direct effects include decreased Cd solubility in soil by precipitation and adsorption on inorganic components (Matusik et al., 2008), competition between Cd and plant nutrients for the same membrane transporters(Zhao et al., 2005) and Cd sequestration in the vegetative parts to avoid its accumulation in the grain/edible parts (Hall, 2002). Indirect effects include dilution of Cd concentration by increasing plant biomass and alleviation of physiological stress. Our results showed that *Paulownia elongata* x *fortunei* possessed suitable growth parameters, but TF for all heavy metals except Zn are less than one.

CONCLUSION

Selected two lines (*P. tomentosa x fortunei* – TF 01 and *P. elongata x fortunei* – EF 02) are accumulators for Cu, Zn and Cd. *Paulownia tomentosa* x *fortunei* accumulated more Pb and Zn in aboveground parts, while *Paulownia elongata* x *fortunei* – only Zn. These lines are a promising species for phytoremediation of heavy metal polluted soils due to very high biomass productivity, rather than its metal accumulation potential. The comparison between plant metal accumulation and bioavailable metal concentration in soil showed that the mechanism for metal accumulation is not concentration gradient between soil and plant tissues.

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CAPTION TO ILLUSTRATIONS

Fig. 1. Mean values (n=6-8 ± SD) of metal accumulation in roots, stems and leaves of *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* plants, grown on polluted soil.