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Possibility for using of two *Paulownia* lines as a tool for remediation of heavy metal contaminated soil

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Abstract

One-year-old two *Paulownia* lines (*P.tomentosa x fortunei* – TF 01 and *P. elongata x fortunei* – EF 02) were grown, as pot experiment, in soil collected from the field of waste depository of Kremikovtzi ferrous metallurgical industry near Sofia. The soil was heavily polluted with Cd. Metals content (Ca, Mg, K, Na, Cd, Cu, Pb, Zn and Fe) in soil and its distribution in roots, stems and leaves of both lines was studied. The results showed that Ca and K accumulated more in stem, Mg, Na, Fe and Cd in root, while Pb, Cu and Zn in the leaves of both lines. The bioaccumulation factor (BF) and translocation factor (TF) were evaluated in order to determine the potential of plants in removing metals from contaminated 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. 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. The success of phytoremediation depends on plant growth and restricted distribution of heavy metals in shoots. Our results showed that stem length and total leaf area of *Paulownia elongata x fortunei* were higher than *Paulownia tomentosa x fortunei* but BF for Cu and Zn and TF for Pb was less. BF for Cd was 1.7 times higher and TF for Zn was 1.03 times higher in Paulownia elongata x fortunei. Selected two lines (*P. tomentosa x fortunei* – TF 01 and *P. elongata x fortunei* – EF 02) were accumulators of Cu, Zn and Cd. *Paulownia tomentosa* x *fortunei* accumulated more Pb and Zn in aboveground parts, while *Paulownia elongata* x *fortunei* – accumulated Zn only. These lines proved to be a promising species for phytoremediation of heavy metal polluted soils due to high biomass productivity.

Key words

Phytoextraction, Heavy metals, *Paulownia* lines, Stem length, Leaf area

Introduction

Heavy metal contamination of soil is one of the most important environmental problems throughout the world (Doumett *et al.,* 2008; Wuana *et al.,* 2010; Ashraf *et al.,* 2010; Ozturk *et al.,* 2008, 2010, 2012). Heavy metals are non-biodegradable and can accumulate in biological systems– humans, animals, microorganisms and plants, causing toxicity (D`amore *et al*., 2005). The metal-contaminated soil are often hard to remediate and the conventional engineering methods used are expensive and non-effective (Salt *et al.,* 1995; Aksoy and Ozturk, 1996, 1997; Aksoy *et al*., 2000; Zhou and Song, 2004; Yilmaz *et al.,* 2006; Gucel *et al.,* 2009a, b; Uysal *et al*., 2012). 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 demonstrates that the cost of phytoextraction of heavy metals by plants is only a fraction 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 tolerance for multiple heavy metals and easy to harvest (Clements *et al*., 2002; Hsiao *et al*., 2007). Unfortunately, no plant has been found to fulfill all these standards.

Some plant species endemic to metalliferous soils 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 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 rooted superficially. As an alternative to the use of herbaceous hyper accumulators, some woody species are metal tolerant and have a fast growth rate, deep root system and show ability to accumulate number of metals. Only few studies are available in this connection, most concerning is the use of poplar and willow for remediation of Cd polluted soils (Robinson *et al*., 2000; Klang-Westin and Eriksson, 2003; Dickinson and Pulford, 2005; Celik *et al*., 2010). *Paulownia tomentosa* (Thunb.) Steud and other tree species belonging to genus *Paulownia* (*Paulownia fortunei* Hems., *Paulownia elongata* S.Y. Hu) are also used for phytoremediation (Doumett *et al*., 2008, 2011; Stankovic *et al.,* 2009; Wang *et al*., 2009, 2010) due to their ability to accumulate heavy metals along with fast growth rate. A 5-7 year-old tree can grow up to 15-20 m height and produce annual biomass up to 150 t ha^{-1} (Caparròs *et al*., 2008). Massive production of *P. tomentosa* biomass within a short time leads to a significant removal of contaminants from polluted soil, despite low rate of metal absorption (Doumett *et al*., 2008).

In Bulgaria, *Paulownia* spp. are selected by the BIOTREE Company, according to the technology registered by Biotree Ltd. This company is largest producer and supplier of genetically superior *Paulownia* tissue-cultures – *in vitro* seedlings, which are preferred by the farmers due to fast development and uniform and regular growth. Two lines (*P. tomentosa x fortunei* – TF 01 and *P. elongata x fortunei* – EF 02) have been selected and patented with the purpose of obtaining two types of plants viz., short and branchy individuals and taller tree individuals, less branchy for the purpose of wood material formation. As there is no information regarding heavy metal tolerance of these lines and possibility to use as phytoremediators of contaminated soils. Thus present study was undertaken to enlighten this aspect.

Materials and Methods

Plant material : *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* lines were chosen as plant materials for study. One-year-old plantlets derived from *in vitro* micropropagation seedlings were initially cultivated in plastic pots (d = 10 cm) filled with 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 with natural sunlight from 20th April to 20th July, 2012. The glasshouse temperature was maintained between 15ºC to 35ºC with 40% to 65% humidity . The plants were harvested (six-eight replicates for each test) at the end of July and their parts were separated. In order to remove the substrate from radical system, roots were washed carefully.

The dry mass of plant parts (leaf, stem and root) were gravimetrically determined after drying at 60ºC, until a constant weight was obtained. Heavy metal content in each plant part was analyzed after sample homogenization in a blender. Leaf area was calculated using Sigma Scan Pro 5 Software.

Before planting and after harvesting of plant, total Cd, Cu, Pb, Zn, Fe, Ca, Mg, Na and K contents were determined in the samples, obtained by collecting three soil aliquots, from each pot which were combined and dried at 105°C until a constant weight was obtained. 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 end of the study. Portion of (25 g) soil aliquots were transferred into 1000 ml polyethylene bottles and 500 ml redistilled water was added. The bottles were mixed in a mixer at room temperature for 48 hr. After mixing, each sample was centrifuged for 5 min at 10,000 g and the supernatant was filtered by 0.2 µm pore size filters.

Soil sampling and characterization : The polluted soil was collected from a field near the territory of waste depository of Kremikovtzi Ferrous Metallurgical Industry. Soil samples were collected from the surface and at depth of 20 and 40 cm. The soil was air dried and sieved through nylon mesh and then mixed with sand in 3:1 ratio. Soil aliquots with 2.5 kg dry weight were used to fill 30 experimental pots. The pot experiments were carried out to investigate the difference in growth and heavy metal accumulation between one-year-old *Paulownia tomentosa* x *fortune* and *Paulownia elongata* x *fortunei* plants grown on soils heavily polluted with Cd.

Metal analysis : Heavy metal content (Ca, Mg, K, Na, Cd, Cu, Pb, Zn and Fe) in soil, roots, stems and leaves of both plants were analyzed. The bioaccumulation factors (BF) and translocation factors (TF) were calculated in order to determine the effectiveness of plants in removing heavy metals from soil. Total metal content in soil and plant organ samples was estimated by Atomic Absorption Spectrophotometric (AAS) analysis, after acidic digestion with Suprapur grade Fluka reagents. The values for U_{plant} was obtained when Me_{plant} was divided to biovailable amount of metals in the soil ($Me_{soil\ t=0}$) before using in pot experiments: $U_{plant} = 100 \times (Me_{plant}) / (Me_{soil\ t=0})$.

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 and Discussion

The physico-chemical properties of the soil is as follow: 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 heavy metal content in these samples were: Cd–4.8; Cu–69.5; Pb–115.5; Zn–199.5; Fe–48730; Ca–2015; Mg–3645; Na–325; K-5020 (mg kg^{-1} d.wt.). According to the Bulgarian permissible limit, at pH (H_2O) - 8.00, heavy metal concentration as follows: Cd 3.0, Cu < 300, Pb < 120 and Zn < 400 mg kg^{-1} d.wt., revealing that soil was heavily polluted with Cd. Cd^{2+} ion exceeded 1.6 times the permissible limit.

Treated plants did not show any morphological variations, like discoloration, pigmentation, yellowing or stunting. The length of stem of *P. elongata* x *fortunei* was longer, but leaf numbers were lesser than that of *P. tomentosa* x *fortunei*. Nevertheless, total leaf area of *P. elongata* x *fortunei was,* approximately 2-fold greater as compared to *P. tomentosa* x *fortunei*. The fresh and dry matter of both clones differed insignificantly (Table 1).

In order to evaluate the extent of competition of soil constituents, metal bioavailable fraction was calculated. The percentage (n = 6-8) of bioavailable Ca, Mg, K, Na, Fe, Pb, Cu, Zn and Cd ions in metal polluted soil with respect to their total concentrations is presented in Table 2.

Table 1 : Mean values (n=6-8 ± SD) of stem length, leaf number, total leaf area and ratios of fresh and 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^{-1})	5.25±0.42	4.91±0.47

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

Heavy metal concentration in plant roots was found in the following order: Cd < Pb < Cu < Zn < Fe with significant difference between each other (Fig.1). The results showed that accumulation of Ca and K was more in stems, Mg, Na, Fe and Cd in roots, while Pb, Cu and Zn in leaves of both plants. Data obtained for shoots (stem and leaves) indicated that metal accumulation of Pb, Cu, Zn and Cd was higher than roots of both plants.

It was interesting to note that the metal removal efficiency, metal accumulation in whole plants (Me_{plant}) and plant uptake percentage (U_{plant}) for all metals of, both clones, differed significantly (Table 3).

The results showed that Ca^{2+}, Mg^{2+} and K^+ ions were present in *Paulownia tomentosa* x *fortunei*, but accumulation of Pb, Cu and Cd was higher in *Paulownia elongata* x *fortunei*. The plant uptake percentage for Mg, K, Zn and Ca was higher in *Paulownia tomentosa* x *fortunei,* while for Pb, Cu and Cd it was found to be more in *Paulownia elongata* x *fortunei* plants.

Paulownia tomentosa x *fortunei* plants accumulated high quantity of Fe and Zn in comparison to *Paulownia elongata* x *fortunei* plants. Fe and Zn, uptake by plants, was noted to be higher than Pb, Cu and Cd ions, respectively.

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		Final concentration			
	(mg kg^{-1} d.wt.)	(%)	*P.t.* (mg kg^{-1} d.wt.)	(%)	*P.el.* (mg kg^{-1} d.wt.)	(%)
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
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

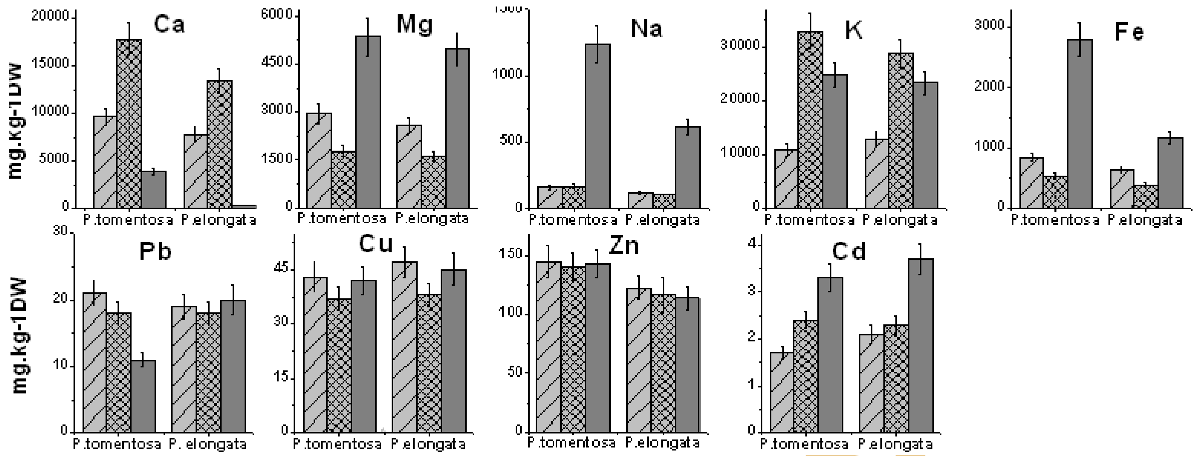

Fig. 1 : Mean values (n=6-8 ± SD) of metal accumulation in roots, stems and leaves of *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* plants, grown on polluted soil

The term bioaccumulation factor (BF), defined as the ratio of heavy metal concentrations in plant dry mass to those in soil was used to determine the efficiency of plants to remove metals from soil. BF was > 1 for Cu, Zn and Cd in both plants. *Paulownia elongata* x *fortunei* accumulated high amount of Cd than *Paulownia tomentosa* x *fortunei*. Translocation factor (TF) or shoot/root ratio, stating total element concentration in the shoot tissue to total element concentration in the root tissue, changed in the present study for Pb and Zn in *Paulownia tomentosa* x *fortunei* and for Zn in *Paulownia elongata* x *fortunei* (Table 4).

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

Heavy metal concentration in plant tissue changes depending on their concentration in the environment (Xiong, 1998). Markert (1994) proposed the normal level of Cd, Pb and Zn in plant to be 0.05, 1.0 and 50.0 mg kg^{-1} d.wt., respectively. The results showed that Fe accumulation, by roots of both plants grown on polluted soil, was highest. Pb, Cu, Zn and Cd also accumulated in leaves of both plants (Fig. 1). To consider a plant as hyperaccumulator, the minimum threshold tissue concentration for Cd is 0.01% and for Zn is 1% d.wt. of the plant (Gardea – Torresdey *et al.*, 2005). In the present study, the total Zn concentration was 4.3% for *Paulownia tomentosa* x *fortunei* and 3.6% for *Paulownia elongata* x *fortunei, respectively*.

Bioaccumulation factor (BF) and Translocation factor (TF) reveals the phytoremediation potential of plants. On the basis of BF values plants are classified as excluders, accumulators or hyperaccumulators, if their BF value is < 1, >1 and > 10, respectively (McGrath and Zhao, 2003). BF value obtained for both lines grown on polluted soil were higher than 1.00 for Cu, Zn and Cd (Table 4). Therefore, *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* lines

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})

	Paulownia tomentosa* x *fortunei		***Paulownia elongata* x *fortunei***	
	(Me_{plant})	**(U_{plant})**	**(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

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

Heavy metals	**BF*(mg kg^{-1} plant dw/ mg kg^{-1} soil)**		**TF**(mg shoot / mg root)**	
	P. t	**P. el.**	**P. t.**	**P. el.**
Fe	0.23	0.23	0.24	0.24
Pb	0.96	0.93	1.82	0.95
Cu	2.52	2.09	0.93	0.96
Zn	3.84	3.37	1.01	1.05
Cd	1.57	2.68	0.61	0.59

appeared to be accumulators of Cu, Zn and Cd. McGrath and Zhao (2003) reported that plants having TF values > 1.00 are considered to be hyperaccumulators. The results showed that TF in *Paulownia tomentosa* x *fortunei* was more than 1.00 only for Pb and Zn, while for *Paulownia elongata* x *fortunei* plants TF level was more for Zn (Table 4). Pb, Cu, Zn and Cd accumulation in aboveground parts and Fe level in roots showed that *Paulownia tomentosa* x *fortunei* and *Paulownia elongata* x *fortunei* lines are ideal for phytoremediation. The mechanisms involved in heavy metal uptake are phytostabilization and phytoextraction. Before planting Na, Pb, Cd and Ca were presented in the extractable fraction of soil. After harvesting, solubility of Ca, Fe, Cu and Zn in soil increased (Table 2). Comprising plant metal accumulation and extractable metal concentration in soil showed that heavy metal uptake and translocation was 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} value calculated for both plants indicated low metal uptake efficiency and translocation as compared to the amount mobilized in soil. Mg, K, Zn and Ca ion uptake predominated in both plants, while Cu, Pb, Fe and Cd accumulation was found less. It is known that only a part from the total amount of heavy metal in soil is accessible for living organisms.

This part is represented as free ions, soluble forms and ions adsorbed to the inorganic soil components (Greger, 2004). Some metals, as Zn and Cd, were observed predominantly in soluble form, while others, like Pb, were much more approachable due to 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 soluble metals in soil. Some root-release organic components like phenols, organic acids, alcohols and proteins that might serve as carbon and nitrogen source for the growth and development of microorganisms, participating in the degradation process (Giller *et al.,* 1998). The plants release about 10-20 % from their photosynthetic products as root secretion, which stimulate microbial activity in the rhizospheric zone (Schmidt, 2003). Among various soil parameters known to affect the availability of metals, soil pH is considered as most important. Many investigations have shown that there is a linear trend between soil pH and Cd uptake: decreasing soil pH leads to increased concentration of Cd in plants, provided other soil properties remain unchanged (Kirkham, 2006). Soil pH affects the availability of Cd present in soil solution, but increased of soil pH does not always reduce Cd uptake by plants (Eriksson, 1989; Singh *et al.,* 1995). Under field conditions, Cd uptake by plants may be affected by many variable soil and climatic parameters.

It was interesting to observe that BF for *Paulownia elongata* x *fortunei* was 1.7 times higher than that for *Paulownia tomentosa* x *fortunei*, but TF was approximately similar (Table 4). However, the interactive effect of heavy metals is very complicated in plants, due to their dependence on many factors, including combinations of elements, plant species and plants part. Probably, the competitive effect of Ca and Mg in formation of heavy metal chelating complexes with organic components secreted by plants is also involved. It has been reported that uptake, transport and subsequent distribution of nutrient elements by plants can be affected in the presence of Cd ions (Eriksson, 1989). 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). Solti *et al. (*2011) reported that in *Populus jaquemontiana* var. *glauca*, Cd ions inhibited mineral nutrition due to competition between Cd^{2+} and other metal ions. The mechanism involve the influence of Cd on Fe as Cd might inhibit the chelating process of Fe and loading of Fe into xylem. That is why metals that are transported into xylem are influenced by Cd. Secondly, the influence of Cd^{2+} on Ca^{2+} is in competition for Ca-transporters. Except for Mg, all other alkaline earth metals behave in a similar manner.

Heavy metals have different mobility and are transported from roots to shoots in different ways. Cd and Zn are more mobile than Cu and Pb (Greger, 2004). Zn is translocated extensively as it is essential to 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 same membrane transporters (Zhao *et al.,* 2005) and Cd sequestration in the vegetative parts, to avoid its accumulation in grain/edible parts (Hall, 2002). Indirect effect 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 was less than one.

Results of the present study revealed that the selected two lines (*P. tomentosa x fortunei* – TF 01 and *P. elongata x fortunei* – EF 02) were accumulators of Cu, Zn and Cd. *Paulownia tomentosa* x *fortunei* accumulated more Pb and Zn in aboveground parts, while *Paulownia elongata* x *fortunei* accumulated only Zn. These lines are promising species for phytoremediation of heavy metal polluted soils due to high biomass production, rather than its metal accumulation potential. 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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