EDTA MEDIATED PHYTOEXTRAXTION OF Fe, Zn, Cu, Pb and Cd BY TWO PAULOWNIA HYBRIDS PLANTS

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ABSTRACT

Phytoextraction is an environmentally friendly in situ technique for remediation of contaminated soils which involves the removal of toxins, especially heavy metals and metalloids, by the roots of the plants with subsequent transport to aerial plant organs. The aim of the present investigation was to study the effect of ethylenediaminetetraacetic acid (EDTA) on accumulation potential of two Paulownia hybrids (P. tomentosa x fortunei – TF 01 and P. elongata x fortunei – EF 02) to Fe, Zn, Cu, Pb and Cd. Paulownia is an important woody species using in reforestation programs because it is high-biomass producer and promising heavy metal accumulator. One year-aged plants were grown as a pot experiment on the soil collected from the vicinities (1 km) of a Kremikovtzi ferrous metallurgical combine near to Sofia. The influence of the addition of EDTA, at 1 and 5 mM concentration, on metal accumulation by the plants and metal mobilization in soil was evaluated. A significant enhancement of metal uptake in response to 5 mM EDTA application was mainly obtained in roots of P. tomentosa x fortunei hydrid for Fe (i.e. 5.150 mg kg-1). Metal accumulation produced by 1 mM EDTA in the shoots of P. tomentosa x fortunei hybrid was higher than in the shoots of P. elongata x fortunei hybrid.

We examined the physiological indicators in order to develop practicable strategy for selecting heavy metal tolerant hydrids of Paulownia, which are produced by BioTree Ltd., Bulgaria. During the treatment the root and stem dry mass of P. elongata x fortunei – EF 02 hybrid was reduced more in comparison with these of P. tomentosa x fortunei – TF 01 hybrid. Control of EF 02 hybrid was characterized by higher leaf dry mass and area than that of TF 01 hybrid. The leaf dry mass and area of EF 02 decreased gradually with increasing of EDTA concentration. The leaf dry mass and area of TF 01 rose at 5 mM EDTA. Total antioxidant capacity (free radicals scavenging activity) changed in the same manner in both plants. Despite the accumulated levels of Fe, Cu, Cd, Pb and Zn in the shoots, P. tomentosa x fortunei – TF 01 hybrid was more tolerant to heavy metal stress than P. elongata x fortunei – EF 02 and could be successfully used for phytoremediation of polluted soil.

MATERIAL AND METHODS

The Paulownia tomentosa x fortunei and Paulownia elongata x fortunei hydrids 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 hybrids. 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 15oC to 35oC, relative humidity ranged from 40% to 65%. The plants were harvested in the end of July.

Experiment set-up and pot monitoring

The influence of the addition of complexing agent was evaluated using EDTA (ethylenediaminetetraacetic disodium salt dehydrate, purity ≥ 97%, Fluka), applied 60 days after planting (20th June, 2012) at 1, 5 and 10 mM kg-1 soil DW, at pH 8.00 (the natural pH of the soil). The comlexing agent was applied in a single dose via manual aspersion of 150 ml of their aqueous solutions with concentrations of 0.04, 0.2 and 0.4M. Untreated pots were used as a control. Thirty days after EDTA application plants (five replicates for each test) were harvested and their organs 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 60oC 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 in the soil were determined by atomic absorption spectrophotometer (AAS) on samples obtained by collecting three soil aliquots from each pot which were combined and heated at 105oC 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 EDTA (pH adjusted to 8.00) were added at the same concentrations as those applied to the pots. 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. A similar procedure was carried out for the determination of the bioavailable metal fraction after plant removal, by adding 500 ml aliquots of redistilled water to the soil of each pot.

Metal analyses

Total metal content in soil and plant organ samples was determined by 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 200oC and the residual were dissolved in 50 ml 1N HCl.

Detrmination of antioxidant activity

Total antioxidant capacity (free radicals scavenging activity) was measured from the bleaching of the purple-colored methanol solution of free stable radical (diphenylpycril-hydrazyl, DPPH·) inhibition after Tepe et al. (2006). DPPH· radical is a stable radical with a maximum absorption at 517 nm that can readily undergo reduction by an antioxidant. The inhibition of free radical DPPH· in percent (I%) was calculated in the following way:

I% = (Ablank-Asampe/Ablank) × 100,

where Ablank is the absorbance of the control reaction (containing all reagents except the test compound), Asample is the absorbance of the test compound, i.e. paulownia leaf extracts.

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.

Introduction

The chemically improved phytoextraction known as induced phytoextraction is based of highly yielding plants that extract high amount metals when their mobility in the soil has been increased by chemical treatments. Several chelating agents such as organic acids (oxalic, citric, malic, succinic, tartaric, glutamic), EDTA (ethylendiamino-tetraacetic acid), NTA (nitrylacetic acid), ets, are investigated for their ability to mobilize metals and increase the metal accumulation by different plant species such as corn, pea, soybean, including fast growth woody species poplar, willow, black locust, ash, alder and paulownia. The ideal plants for phytoremediation should possess multiple traits, especially they should have a fast growth rate, large biomass, deep roots, and should tolerate and accumulate a range of heavy metals in their aerial or harvestable parts.

In our contry Paulownia spp. are selected by BIO TREE company, Bulgaria according to technology registered of 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. There is scarce information about its tolerance to heavy metals and possibilities to use Paulownia spp. as phytoremediator of contaminated soils.

In this work the tolerance to high concentrations of heavy metals of one-year-old Paulownia tomentosa x fortunei and Paulownia elongata x fortunei plants in glass house studies is compared in order to evaluate their phytoremediation potential and improve him using chelating agents (namely EDTA) from the view of practical application. The aim of this research is to evaluate the influence of addition of different concentrations (1, 5 and 10 mM) of EDTA on the distribution of Cd, Cu, Zn, Pb, Zn and Fe between the water soluble and insoluble fractions in soil and the organs of two hybrids. The allocation of biomass in different organs and the antioxidative response of investigated plants to action of heavy metals are studied too.

RESULTS AND DISCUSSION

P.elongata	*Control*	*EDTA 1mM*	*EDTA 5mM*
roots (g DW)	3.51 0.15b	3.18 0.23 b	1.55 0.54 a
stems (g DW)	2.084 0.73 b	1.179 0.32 ab	0.949 0.29 a
leaves (g DW)	4.31 1.26 b	3.08 0.15 b	1.39 0.12 a
leaf area (cm2)	889.7 88.5 c	465 48.4 b	178 40.8 a
DPPH (%)	43 3.2 a	95 8.5 c	75 6.9 b

Table 2. Mean values SD of root, stem, leaf dry weights, total leaf area and antioxidative capacity, measured at the end of the experiment on Paulownia elongata x fortunei grown in a metal polluted soil in response to the addition of EDTA as a complexing agent

P. tomentosa	Control	EDTA 1mM	EDTA 5mM
roots (g DW)	3.243 0.68 b	1.368 0.11 a	1.775 0.96 a
stems (g DW)	1.466 0.31 b	0.89 0.08 a	1.074 0.09 ab
leaves (g DW)	3.353 0.71 b	2.416 0.32 a	2.289 0.03 a
leaf area (cm^2)	482 30 b	622 59 c	360 55 a
DPPH (%)	73 6.5 a	97 8.4 b	96 8.5 b

Table 1. Mean values SD of root, stem, leaf dry weights, total leaf area and antioxidative capacity, measured at the end of the experiment on Paulownia tomentosa x fortunei grown in a metal polluted soil in response to the addition of EDTA as a complexing agent

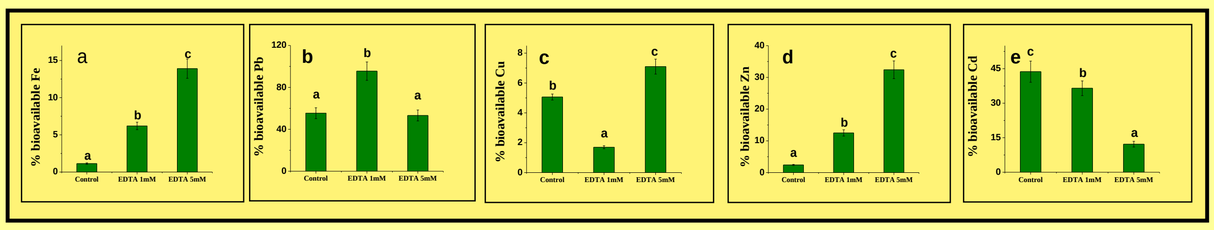


Fig.1.

Mean percentages (n=5) of bioavailable Fe, Pb, Cu, Zn, Cd in metal with respect to their total concentrations, after extraction with 1 and 5 mM EDTA

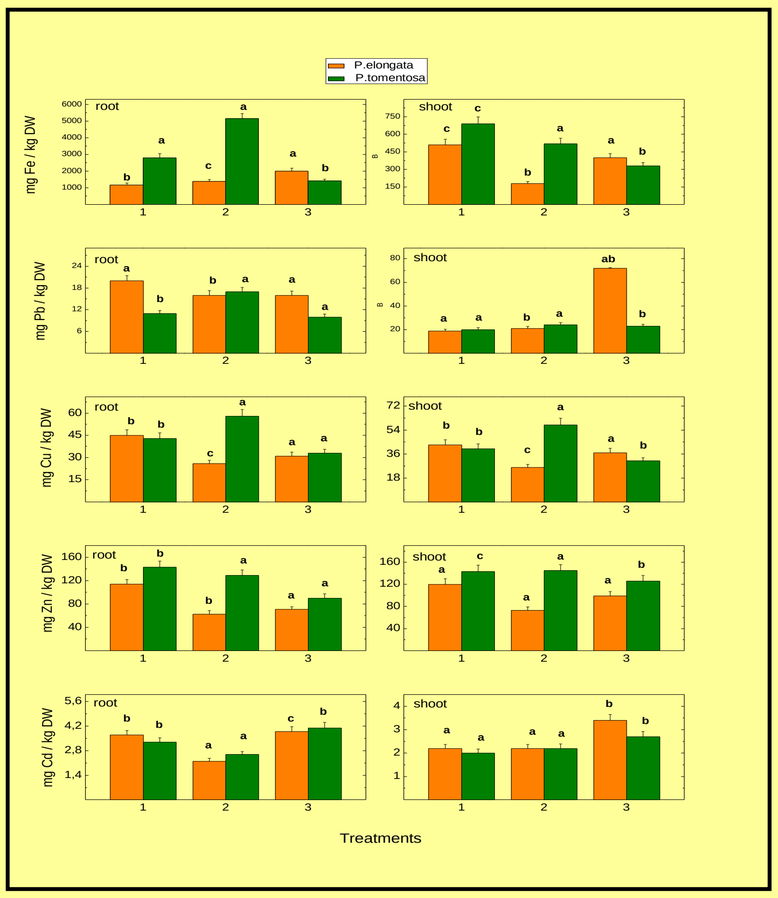
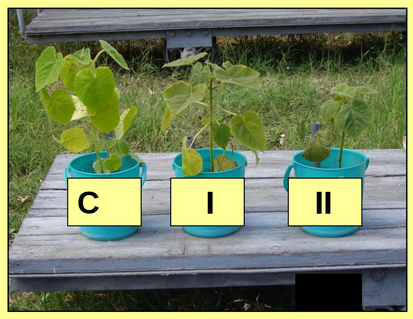
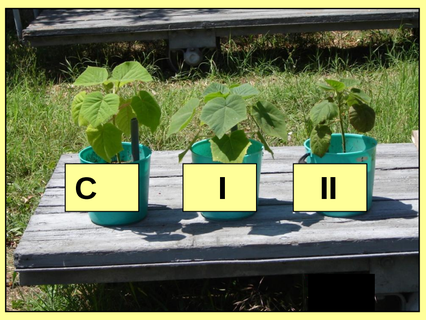


Fig. 2. Mean values (n=5) of metal accumulation in roots and shoots of Paulownia tomentosa x fortunei and Paulownia elongata x fortunei in polluted soil (1 – Control) in response to the addition of complexing agent at (2 - 1 mM EDTA) and (3 – 5 mM EDTA).



P. elongata x fortunei
Treatment with EDTA:
Control, 1mM EDTA(I) and 5mM EDTA(II)



P. tomentosa x fortunei
Treatment with EDTA:
Control, 1mM EDTA(I) and 5mM EDTA(II)

Conclusion

The results of this study indicate that both Paulownia hybrids are a promising species for phytoremediation of heavy metal polluted soil owing its high biomass productivity, rather than its metal accumulation potential. Three-months-old plants of Paulownia elongata x fortunei possess higher total leaf area compared to that of Paulownia tomentosa x fortunei. In agreement with the current status of research on induced phytoremediation, we conclude that the application of 1 mM EDTA improves metal accumulation without increasing environmental impact, but reduces investment in root and stem dry biomass of plants. The total leaf area and antioxidant activity of Paulownia tomentosa x fortunei are enhanced after this treatment.