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In vitro ability of *Paulownia hybrid* as phytoremediator against some heavy metals pollution

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ABSTRACT

The in vitro study was carried out on Paulownia hybrid to examine the effect of different concentrations of heavy metals Pb, Cd and Ni in culture media on growth characters, photosynthesis pigments and accumulation of heavy metals occurred in shoots and roots, NPK absorption and total carbohydrates content. Culture medium supplemented with high concentration of Pb (100 ppm) led to the highest in vitro survival percentage (85%), rooting (100%) and longest both shootlets and roots, survival percentage of acclimatizaed shoots, longest adapted plants and highest number of leaves formed per adapted plant as well as the accumulation of Pb element which was in highest values in both shoots and roots, respectively. The highest number of shootlets formed per explant, numbers of leaves and roots per shootlet were in the highest values with high concentration of Ni (20ppm) treatment which also significantly increased chl.b, carotenoids, total chlorophyll contents as well as total carbohydrates content. Cd (5ppm) had pronounced effect on stem fresh, dry weights and leaves fresh weight of micropropagated plants. Addition of Pb at (25 ppm) to the culture media significantly increased chlorophyll a content to the highest value, highest content of N% and K% in both shoots and roots as well as P% shoots content. The highest accumulation of Cd element in both shoots and roots, highest contents of P% in roots were obtained as a result of cadmium supplement to culture medium at 20 ppm. High concentration of Ni (80 ppm) caused the highest accumulation of Ni element in both shoots and roots, respectively.

Key words: Paulownia, heavy metals, *in vitro*, phytoremediation

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INTRODUCTION

Paulownia is a deciduous, multipurpose tree (family *Paulowniaceae*, previously *Scrophulariaceae*) native to China. The rapid growth of the tree and its value in the timber market as well as new uses and related products developed the economic importance of *Paulownia*. The trees are used for reforestration, roadside and as ornamental tree. It grows well in a wide variety of soil types, notably poor ones [1]. A number of *Paulownia* species are valuable sources of secondary metabolites such as flavonoids with high antioxidant activities [2]. Mentioned the studies that have been conducted to evaluate its suitability for solid biofuel and cellulose pulp industry because of its high cellulose content (440 g. cellulose/Kg). A high demand for domestic and international markets for afforestation and bioenergy production has required the development of effective micropropagation approaches for use in reforestation programs.

Pollution of environment by toxic metals arises due to various industrial activities and has turned these metal ions into major health problem [3]. Heavy metals, such as cadmium, nickel, lead, copper, chromium and mercury are major environmental pollutants, and most of them are toxic even at low concentrations, play an important role in the environment pollution as a result of human activity [4].

Phytoremediation (the use of plants in metal extraction) is a promising alternative in the removal of heavy metals excess from the soil and water [5]. The plant metabolic activity affects the geological redistribution of heavy metals through pollution of air, water and soil. The exposure of plants to toxic levels of heavy metals triggers a wide range of physiological and metabolic alterations, since [6; 7]. That the effects of heavy metals on plants are complicated and related to many factors such as ion type and concentration, development stage of plant and nutrient conditions [8].

Paulownia species has been studied over the last two decades due to its ability to uptake land contaminants, namely heavy metals [9; 10].

In vitro culture technique is a key tool in phytoremediation research and can be used in studying of plant metal tolerance which then can be screened and tested for phytoremediation at polluted land [11].

Lead (Pb) is one of the ubiquitously distributed most abundant toxic elements in the soil. It causes adverse effect on morphology, growth and photosynthetic processes of plants. It inhibited seed germination of *Spartiana alterniflora* [12], *Pinus helipensis* [13]. Cadmium (Cd) has been to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plant [14]. Its inhibition effect of the nitrate reductase activity was found in *Silene cucbalus* [15]. Nickel (Ni) is a transition metal and found in natural soils at trace concentrations. It increases by human activities such as mining, emission of smelters, burning of coal and oil and pesticides [16].

The aim of this study was to investigate the *in vitro* propagation potential of *Paulownia* tree as a phytoremediation plant under the effect of various concentrations of some heavy metals (Pb, Cd and Ni) during developmental stages, given the current tree economic importance and potential future uses.

EXPERIMENTAL SECTION

This work was conducted at Tissue culture and Germplasm Conservation Research Laboratory, Horticulture Research Institute, Agriculture Research Center (ARC), and Department of Ornamental Plants and Woody Trees; National Research Center (NRC), Egypt during the years of 2014 and 2015 to evaluate some morphological and chemical changes of *in vitro Paulownia* plantlets treated with various concentration s of heavy metals (Pb at 25, 50 and 100 ppm, Cd at 5, 10 and 20 ppm and Ni at 20, 40 and 80 ppm) to understand the plant potential to grow and extract these metals as a phytoremediator plant.

Heavy metals tested
The heavy metal sources used in this study included lead sulphate (Aldrich), Cadmium sulphate {Laboratory Rasayan ($3Cd\ So_4.8H_2O$)} and Nickel oxide (Sigma- Aldrich). Stock solutions of each heavy metal salt were prepared at a concentration of 100 ppm.

Plant material and culture medium:
In vitro Stem node of Paulownia were used as explant source for micropropagation. MS- medium supplemented with 0.2 ppm of 6- benzylamino-purine (BAP) and 0.1ppm indole butyric acid (IBA), various concentrations of heavy metals were added (Pb at 25, 50 and 100 ppm, Cd at 5, 10 and 20 ppm and Ni at 20, 40 and 80 ppm) besides of control then enriched with sucrose 25g/L and solidified with 0.7% agar. The medium treatments were adjusted to pH 5.7 ± 0.1, then autoclaved at 121°C and 1.2kg/ cm2 for 15 min.). All the experiments had six gars replicates each included two explants.

Culture conditions:
Cultures were incubated in growth chamber at 24 ± 1°C under white cool florescent lamps with light intensity of 3k lux at 16 hr photoperiod. Twelve weeks after treatments, the following data were recorded:

Shooting behavior: Survival%, number of formed shootlets per explant, shootlet length (mm), Number of leaves per shootlet, fresh and dry weights of stems and leaves / shootlet (gm).

Rooting behavior: Percentage of roots formation (%) number of roots/shootlet root length (mm)**,** fresh and dry weights of roots/shootlet (gm).

Rooted plantlets were cultured into 0.2 liter capacity pots and filled with soil mixture sand: peat (1:1).

A**cclimatization behavior:** Survival of adapted plants%**,** number of formed leaves per plant and plant height (mm).

Extraction and chemical analysis

Photosynthetic pigments

Plant material after multiplication stage were collected and , photosynthesis pigments (chlorophyll a and b) as well as carotenoids were determined in shootlets tissues as mg/100g fresh weight by using spectrophotometer, according to the procedure achieved by [17].

Total carbohydrate percentage and mineral content

Total carbohydrate percentage in the dry samples after micropropagation were determined according to [18]. Nitrogen content was determined by modified micro-Kjeldahl method as described by Pregl [19]. Phosphorus content was estimated (after wet ashing) using ammonium molybdate method according to Snell and Snell [20]. Potassium was determined by using flame photometer according to Chapman and [21].

Determination of Pb, Cd and Ni (ppm)

Pb, Cd and Ni were determined using atomic absorption spectrophotometer according to [22].

Experimental design and data analysis

Complete randomized design was adopted with 3 replicates for each treatment. Data were statistically analyzed according to Duncan's multiple range test at 5% level of probability [23].

RESULTS AND DISCUSSION

Micro-propagation and acclimatization behavior

The effect of various concentrations of heavy metals (Pb, Cd and Ni) on *in vitro* propagation behavior of *Paulownia* plants is illustrated Table (1). It is observed that the high concentration of Pb (100ppm) in culture medium increased the survival percentage to 85%, rooting (100%) and produced the longest both shootlets (95.67mm) and roots (276.67mm) as well as the acclimatization survival percentage {with no significant difference between this treatment and those of Cd (5 and 10 ppm) or Ni (20 ppm)}, and longest adapted plants and highest number of leaves formed per adapted plant. While, Cd (20ppm) treatment led to the lowest *in vitro* survival percent (21.67%) and shortest shootlets (62.33mm) as compared to control treatment. However, the highest number of shootlets formed per explant, numbers of leaves and roots per shootlet were in the highest values (2.33, 18.67 and 6.33, respectively) were resulted due to the highest concentration of Ni (20ppm) treatment. Similar results were found by [24]. They mentioned that the most common effect of Cd toxicity in plants is the stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways. Also, [25] attributed the reduction in the growth of *Lemna polyrrhiza* to suppression of the elongation growth rate of cells, because of an irreversible inhibition exerted by Cd on the proton pump responsible for the process. Sharma and Dubey [26] observed stunted growth of plants and root hair development which are caused by the deposition of Pb. On the other hand, [27] pointed out that some of plants are sensitive and the others are more tolerant. Cd was found to inhibit lateral root formation while the main root became brown, rigid, and twisted [28; 29; 30].

Table (1): Micro-propagation and acclimatization behavior of Paulownia as effected by various concentrations of heavy metals

Charcter / Treatment	Survival%	Number of shootlets/ Explant	Shootlet length (mm)	Number of leaves /Shootlet	Rooting%	Number of roots/ plantlet	Length of roots (mm)	Survival of adapted plants	Height of adaptd Plant (mm)	Number of leaves / adapted plant
Control	33.33f	1.33 ab	67.46 cd	17.33 ab	66.67 ab	5.00 ab	175.33 bc	44.33 b	51.67 c	9.00 c
Pb (25ppm)	61.66 b	1.67 ab	84.67 abc	16.00 abc	50 b	5.67 ab	166.67 bc	41.67 b	72.67 c	12.67 ab
Pb (50ppm)	53.33 bc	1.33 ab	88.33 ab	16.00 abc	66.67 ab	3.67 ab	188.33 b	58.33 b	123.33 b	12.67 ab
Pb (100ppm)	85.00. a	1.00 b	95.67 a	14.67 bc	100 a	4.33 ab	276.67 a	80.56 a	185.00 a	14.00 a
Cd (5ppm)	45.53 bc	1.00 b	92.00 a	18.67 a	50 b	2.67 b	266.67 a	53.33 b	110.00 b	10. 00bc
Cd (10ppm)	31.67 de	1.33 ab	68.67 cd	16.00 abc	66.67 ab	3.00 ab	243.33 a	91.67 a	105.00 b	12.67 ab
Cd (20ppm)	21.67 e	1.67 ab	62.33 d	15.00 bc	66.67 ab	5.67 ab	172.5 bc	91.67 a	105.00 b	13.33 a
Ni (20ppm)	52.37 bc	2.33 a	85 abc	18.67 a	66.67 ab	6.33 a	193.33 b	93.33 a	106.67 b	11.33 abc
Ni (40ppm)	41.67cd	1.33 ab	71.67 bcd	16.00 abc	83.33 ab	5.33 ab	133.33 cd	86.67 a	123.33 b	12.00 abc
Ni (80ppm)	41.96cd	1.00 b	65.00 d	13.33 c	66.67 ab	4.00 ab	110.00 d	50.00 b	73.33 c	14.00 a

Stem, leaves and root weights of *in vitro* propagated plants

Results in Table 2 showed that Cd (5ppm) has pronounced effect on stem fresh, dry weights and leaves fresh weight of micro-propagated plants as compared to control. It can observe also that Pb, Cd and Ni at various concentrations has decreased root fresh, dry weights and leaves dry weights comparing with control which gave the highest values (4.88, 0.379 and 0.519g, respectively). Our study correlated to the study by [31] in which high concentration of Pb decreased the wheat shoot and root fresh weights. [32] revealed that Pb is a very toxic heavy metal and has inhibitory effect on biomass characteristics like fresh shoot and root weights, dry weights of wheat varieties due to changes in metabolism and physiology of plants. This inhibitory effect of heavy metals may be due to alteration in the activity of many key enzymes of various metabolic pathways [24]). [33] pointed out that plant material appropriate for phytoremediation should have ability to produce a large biomass in stress conditions.

Table 2: Fresh and dry weights (gm) of micro-propagated plants as effected by various concentration of heavy metals

Character / Treatment	Fresh weight (g)			Dry weights (g)		
	Stem F.W	**Leaves F.W.**	**Roots F.W.**	**Stem D.W.**	**Leaves D.W.**	**Roots D.W.**
Control	0.64 de	1.3 g	4.88 a	0.120 e	0.379 a	0.519 a
Pb (25ppm)	0.72 d	1.69 e	1.16 e	0.083 g	0.265 d	0.112 e
Pb (50ppm)	0.64 de	1.89 cd	0.52 h	0.108 f	0.279 d	0.095 fg
Pb (100ppm)	0.55 e	1.29 g	2.60 b	0.180 b	0.347 bc	0.246 b
Cd (5ppm)	1.61 a	2.63 a	2.54 b	0.212 a	0.344 c	0.220 c
Cd (10ppm)	1.47 b	2.04 bc	2.06 c	0.069 h	0.285 d	0.233 bc
Cd (20ppm)	0.59 de	1.53 f	0.87 g	0.153 d	0.235 e	0.090 g
Ni (20ppm)	1.15 b	1.93 cd	1.44 d	0.086 g	0.322 c	0.124 e
Ni (40ppm)	1.01 c	2.18 b	1.50 d	0.165 c	0.373 ab	0.173 e
Ni (80ppm)	0.65 de	1.85d	1.02 f	0.150 d	0.263 d	0.102 fg

Photosynthesis pigments

It is clearly observed (**Fig 1**) that adding Pb at (25 ppm) to the culture medium significantly increased chlorophyll a content to the highest value (544.07 mg/100g F.W.), while using of Ni at 80 ppm declined this value to the lowest value (282.86 mg/100g F.W.) and significantly reduced chl. a to about 13.05% of the control. Decreasing the concentration of Ni to 20 ppm in the culture medium significantly increased chl. b, carotenoids as well as total chlorophyll contents to the highest values (263.82, 122.97 and 750.58 mg/100 F.W., respectively), whereas using culture medium supplemented with Ni at 40 ppm significantly decreased these values to the lowest ones (128.43, 62.65 and 444.26 mg/100g F.W., respectively) as compared to control. Our results were in accordance to [34] who mentioned that low doses of Pb stimulated chlorophyll synthesis regardless an unbalanced uptake of essential elements [35]. Observed the depression effect of excessive nickel application on chlorophyll concentrations in some plant species, with the increase in nickel concentration the stimulatory effect decreases and toxicity increases. The inhibitory effect of Nickel in higher concentration were also observed in three poplar genotypes as reported by [36].

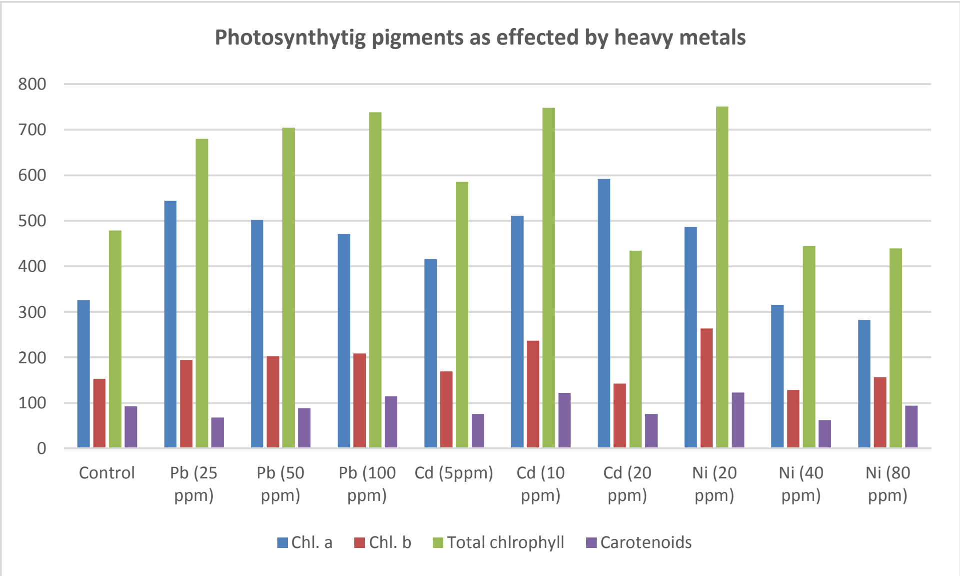

Fig (1): Effect of various concentrations of heavy metals on plant pigments content (mg/100g F.W.) of Paulownia shootlets

Fig (2): Development of *In vitro* plants of Paulownia as affected by Pb treatment at 100ppm

Fig (3): Development of acclimatized plants of Paulownia as affected by Pb treatment at 100ppm

Accumulation of heavy metals in shoots and roots after *in vitro* growth

The results in Table (3) showed that the accumulation of Pb element was in highest values (1.708 and 4.719 ppm, respectively) in both shoots and roots as a result of adding Pb at 100 ppm to culture medium. All concentrations of Pb applied in culture media (25, 50 and 100 ppm) decreased the accumulation of Ni significantly in both shoots and roots to lower values as control comparing with the other treatments . Data tabulated in Table (3) also revealed that the accumulation of Cd element in both shoots and roots were increased to highest values when Cd at 20 ppm was added to the culture media, lowered Ni content in both shoots and roots to the lowest ones (0.397 and 0.528 ppm, respectively) as compared to control. The same trend was observed when the high concentration of Ni (80 ppm) which caused the highest accumulation of Ni element (0.644 and 3.352 ppm) in both shoots and roots respectively and the lowest concentrations of Cd in both shoots and roots (0.113 and 0.123 ppm, respectively) as compared to control. It can also notice that, the metal content was increased in plant (shoots or roots) with increasing its concentration in the culture medium. Moreover, the metal accumulation in roots was always higher than in shoots. Our results go in line with those of [34] when they tested the effect of lead on microplants (shoots and roots) of *Daphne jasmine* and observed that lead accumulation would be higher in root system in the presence of growth toxic effect of lead. [37] indicated that lead uptake via root system is much more effective than via shoot base. The low solubility of most lead compounds in neutral medium and precipitation of lead by sulphate and phosphate at the root system may partly explain this [38]. For the phytoextraction process, substantial amounts of the heavy metals must be removed by the root from the medium, followed by their translocation to the harvestable plant parts, so that they can be completely removed from the contaminated site [39]. The bioaccumulation of single metal is known to be influenced by the presence of other metals, resulting in inhibited or enhanced bioaccumulation of one metal in the mixture [40]. Several studies reported that the presence of one metal influenced the uptake of another metal [41]. In another study by [42] on the bioconcentration of heavy metals in the plant structure, claimed that Cd and Ni are more toxic than Pb for plant.

Table (3): Heavy metals content of micropropagated plants as effected by various concentrations of them

Character / Treatment	Shootlets content (ppm)			Roots content (ppm)		
	Pb	**Cd**	**Ni**	**Pb**	**Cd**	**Ni**
Control	0.464 g	0.117 e	0.515 f	0.539 j	0.127 ef	0.672 d
Pb (25ppm)	0.832 c	0.111 f	0.510 f	2.167 c	0.129 e	0.589 g
Pb (50ppm)	0.902 b	0.114 ef	0.511 f	4.649 b	0.165 d	0.556 h
Pb (100ppm)	1.708 a	0.327 b	0.511 f	4.719 a	0.156 d	0.610 f
Cd (5ppm)	0.779 d	0.136 d	0.582 d	0.733 h	0.444 c	0.622 e
Cd (10ppm)	0.733 e	0.234 c	0.594 c	0.668 i	0.478 b	0.495 j
Cd (20ppm)	0.519 f	0.391 a	0.397 g	0.762 g	0.533 a	0.528 i
Ni (20ppm)	0.763 d	0.118 e	0.561 e	0.796 f	0.102 f	1.272 c
Ni (40ppm)	0.833 c	0.117 e	0.612 b	0.851 e	0.118 ef	1.657 b
Ni (80ppm)	0.824 c	0.113 ef	0.644 a	0.926 d	0.123 ef	3.352 a

NPK and total carbohydrates content (%)

The influence of heavy metals at various concentrations is shown in Table (4). The results revealed that the highest content of N% in both shoots and roots (0.61 and 0.38 %, respectively), P% shoots content (1.25%) as well as K% in both shoots and roots (1.49 and 1.29% ,respectively)were obtained by lead supplemented culture medium at low concentration (25 ppm), the same result was obtained in respect of K% element when the low concentration (5 ppm) of cadmium supplemented culture medium was used as compared to other treatments and control. The highest contents of P% in roots (0.291 and 0.293 %, respectively) were obtained as a result of cadmium supplemented culture medium at 10 and 20 ppm. The highest total carbohydrates (31.73%) in *in vitro* plants was obtained by adding Ni at low concentration (20 ppm). It is clear that uptake of N and total carbohydrates content were negatively influenced by increasing heavy metals concentration in culture medium. Similar results were obtained by [14] who observed that Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots [43]. [44] showed that, if soils' heavy metal contents were at acceptable level, there was no negative effect on yield and N contents of alfalfa plants. [45] indicated that increasing Cd, Pb concentrations (1 and 5 ppm) induced significant reduction in soluble sugars in *Albizia procera*. This result may be due to the cycles of carbohydrate catabolism and related enzymatic reactions [46].

Table (4): N, P, K and total carbohydrates (%) as effected by various concentrations of heavy metals in micropropagated plants

Treatment	N%		P%		K%		Total carbohydrates % Shootlets
	Shootles	Roots	Shootlets	Roots	Shootlets	Roots	
Control	0.53 b	0.189e	1.23 b	0.287 b	1.06 e	0.97 cd	8.33 h
Pb (25ppm)	0.61 a	0.38 a	1.25 a	0.242 f	1.49 a	1.29 a	15.43 e
Pb (50ppm)	0.53 b	0.25 c	0.241 g	0.229 g	1.31 c	0.99 cd	15.6 e
Pb (100ppm)	0.32 e	0.23 cd	0.209 j	0.217 i	1.11 d	0.94 d	10.5 g
Cd (5ppm)	0.46 c	0.34 b	0.360 c	0.275 c	1.36 b	1.29 a	30.53 b
Cd (10ppm)	0.32 e	0.34 b	0.334 d	0.291 a	1.31 c	1.11 b	16.49 c
Cd (20ppm)	0.25 f	0.21 de	0.268 f	0.293 a	1.28 c	1.02 c	12.33 f
Ni (20ppm)	0.40 d	0.25 c	0.228 i	0.222 h	0.94 f	1.14 b	31.73 a
Ni (40ppm)	0.38 d	0.25 c	0.237 h	0.255 d	1.09 de	1.11 b	15.95 d
Ni (80ppm)	0.27 ef	0.23 cd	0.293 e	0.248 e	1.36 b	0.85 e	15.95 d

CONCLUSION

Our results clearly indicate that *Paulownia* is a suitable species for Pb, Cd and Ni removal and be considered as promising plant phytoremediator for large scale of heavy metals.

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