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Growth performance, photosynthetic status and bioaccumulation of heavy metals by *Paulownia tomentosa* (Thunb.) Steud growing on contaminated soils

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Abstract

This work focuses on the study of the potential of a woody specie *Paulownia tomentosa* (Thunb.) Steud in the phytoremediation of soils polluted by heavy metals. Total metal concentrations in soil samples as well as their bioaccumulation in plant tissues were performed by Atomic Absorption Spectrometry. Bioaccumulation factors (BF) and translocation factors (TF) were calculated in order to determine the effectiveness of plants in removing heavy metals from soil. Results showed that heavy metals significantly affected the root biomass production compared to the leaf biomass and caused slight reductions in all growth parameters. However, the presence of high amounts of ETM in polluted substratum restricted the synthesis of chlorophyll pigments and lead to the deterioration of photosynthetic parameters. Zn, Pb and Cd were found in plant shoots and roots at different levels, between 5.083 and 205.33 mg kg^{-1} DMW for Zn, 23.22 and 50.13 mg kg^{-1} DM for Pb and between 0 and 3.88 mg kg^{-1} DMW for Cd. Translocation and bioaccumulation factors indicated that *Paulownia tomentosa* could be used in the phytoextraction of Zn and Pb.

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Introduction

Heavy metals, such as zinc (Zn), lead (Pb), and cadmium (Cd) are among the most common pollutants found in both industrial and urban effluents and hence, pose a great potential threat to the environment and human health.In fact, Zn is an essential element or micronutrient for plants but can be highly toxic and impairs their growth when present at excessive concentration (Ebbes and Kochian, 1997; Hanschand Mendel, 2009). In contrast, Pb and Cd are considered to have extremely toxic effect even at low concentration (Willams *et al.,* 2000).

Heavy metal stress can exert a negative effect on physiological functions within plants by inducing an oxidative stress, alter the membrane permeability and impair the mineral nutrition (Reddy *et al.,* 2005). These effects lead to impairment of photosynthesis by changing chloroplast ultrastructure and disassembly of the thylakoids and hence, the inhibition of photosynthetic pigments biosynthesis (Azzarello *et al.*, 2012; Vassilev *et al.*, 2011). It results a visual toxicity symptoms in higher plants like growth inhibition, chlorosis, browning of roots, senescence and death of plants (Ebbs and Kochian, 1997).

Accordingly, several processes such as physical, thermal and chemical treatments have developed in order to remediate the soil contaminated with heavy metals. However, these technologies are costly and damage the soil structure and fertility (Simonnot and Cruz, 2008).

Thus, phytoremediation has been proposed as an environment friendly and cost-effective alternative for removing metals and remediating contaminated soils through plants. It includes two main techniques namely the phytoextraction which is based on the use of hyperaccumulating plants able to concentrate pollutants in their aerial parts destined for harvest and phytostabilization which uses plants that can reduce the mobility and bioavailability of metals in their rhizosphere (Gisbert *et al.,* 2003).

Suitable plants for phytoremediation must produce high biomass, have a rapid growth and have the potential to tolerate and accumulate high amounts of heavy metals into their aboveground biomass (Chaney *et al.*, 1997). Thus, woody species are considered to be a good candidate for this purpose, since the most hyperaccumulator are herbaceous species, which are slow-growing, produce low biomass and have shallow root systems (Pulford and Watson, 2003).

Paulownia tomentosa (Thunb.) Steud is a tree species belonging to the genus *Paulownia* which included the woody species. These lasts are known and proved for their effectiveness in phytoremediation purposes due to their rapid growth, a massive production of biomass, a deep root system and an elevated tolerance to high levels of heavy metals in soil (Wang *et al.*, 2010; Doumett *et al.*, 2010).

Therefore, the present study was designed to assess growth performance, photosynthetic status and bioaccumulation of Zn, Pb and Cd of *Paulownia Tomentosa* growing on heavy metal contaminated Tunisian soils.

Materials and methods

Plant material and growth conditions

Paulownia tomentosa plantlets derived from micropropagation were cultivated in plastic pots filled with Zn, Pb and Cd naturally rich substrata provided from two Tunisian mines, namely Jebel Ressas (JR) (36°61' N;10°32' E; 97m above sea level) and Ghezala (GH) (37°5’ N; 9°32’ E; 30 m above sea level), whereas the control one (TU) was collected from the experimental plot of the National Agronomic Institute of Tunisia (INAT) (36°51' N; 10°11' E; 10 m above sea level).The pot-cultures were placed under glass house supplied with natural sunlight (photoperiod varying from 13 to 16 h) from May, 1^{st} to 30^{th} June, 2012. The glass house temperatures varied from 17°C to 29°C, whereas the relative humidity ranged between 43% and 67%. Pots were irrigated daily with distilled water to maintain vigorous plant growth.

The experiment was conducted at the Horticulture

Science Laboratory of INAT and was arranged in a completely randomized design with 6 replicates for each substratum.

Soil analysis

The soils samples were collected with a hand auger at the 0-20 cm depth soils. These samples were placed into plastic bags, transported to the laboratory, and air-dried prior to analysis.

The soil texture was determined according to the sedimentation method. The soil pH was measured in 1:2.5 soil: water extracts using a pH-meter (pH Meter pH538). The electrical conductivity (EC) was determined from saturated soil-paste on extract (soil: water) by a conductivity meter (Conductivity Meter LF538). Calcium carbonates $CaCO_3$ (%) were determined by hydrochloric acid (HCl) using a calcimeter of Bernard (NF ISO 10693). The determination of the organic matter (% OM) was carried out by the Walkley and Black (1934) method modified by Naânaâ and Susini (1988). Finally, available P_2O_5 was measured by the Olsen method (NF ISO 11263).

Heavy metal analysis

One gram of each air-dried plant part samples was burned in a muffle furnace at 450 °C for 4 hours. The ash was completely digested with concentrated HCL and then made up to the volume (50 ml) with distilled water according to the Alloway (1995) procedure. Similarly, air-dried and sieved soil samples (1 g) were digested with concentrated HCl acid and placed on a hot plate for 3 hours. On cooling, the digest was allowed to cool and filtered through a Whatman filter paper. The filtrate was collected into a 50 ml volumetric flask and diluted to the mark with distilled water. The Zn, Pb and Cd concentrations in the diluted digests were measured by an atomic absorption spectrophotometer (AA-6300, Shimadzu Corporation, Kyoto, Japan).

Bioaccumulation and translocation factors

In order to evaluate the phytoremediation potential of *P.tomentosa*, the bioaccumulation factor (BCF) as well as the translocation factor (TF) were calculated as following:

BCF= C_{roots} (mgKg^{-1}DMW)/ C_{sub} (mgKg^{-1}DMW)

TF= C_{shoot} (mg Kg^{-1}DMW)/ C_{roots} (mg Kg^{-1}DMW)

Where DMW: dry matter weight, C_{roots} , C_{shoot} and C_{sub} are metals concentration in the plant roots, shoots (mg/kg DMW) and soil (mg/kg DMW), respectively. Plants was categorized as phytoextractor when TF >1 (Fitz and Wenzel, 2002) and as phytostabilizer when BCF > 1 and TF < 1, respectively (Mendez and Maier, 2008).

Growth parameters

For each treatment, measurements of plant height, fresh and dry matter weights were evaluated by destructive harvests. In fact, the plants samples were harvested after 60 days of pot culture and then divided into roots, leaves and stems. These plants parts were gently washed with distilled water and immediately weighed (fresh matter weight). After that, they were wrapped in paper bags, labeled and then oven-dried at 65°C for 48h to constant weight and reweighed (dry matter weight).Their dry matter contents were computed using the following equation:

$$\%DM = \frac{DMW}{FMW} \times 100$$

Where DM: dry matter (%), FMW: fresh matter weight (g) and DMW: dry matter weight (g).

Leaf area of *P. tomentosa* plants growing on the three substrata was measured using a planimeter (Li-Cor area meter, model 3100, Li-Cor USA). The leaf area ratio (LAR) was determined by dividing the leaf area (cm^2) by the dry matter weight (g) of the aerial plant part (Hunt, 2003).

Chlorophyll fluorescence measurements

Chlorophyll fluorescence was measured on healthy leaves, using a portable system: Fluorescence Induction Monitor (The IMF 1500, Analytical Development Company Limited, Adc).The analysis of measurements is related to the relative initial fluorescence values (F0) and the maximum quantum yield of the photochemistry of the PSII (Fv/Fm)

(Baker and Rosenqvist, 2004).

Photosynthetic pigment analysis

The photosynthetic pigments, namely chlorophyll a, chlorophyll b and total chlorophyll as well as carotenoids were extracted according to Torrecillas *et al.* (1984) method. Five milliliters of 80% acetone were added to fresh leaf samples (approximately 100 mg). The total extraction took place in darkness at 4°C for 72 h. The optical density was measured by a UV-Visible spectrophotometer (Labomed, Inc., USA) at 460 nm, 645 nm and 665 nm. The photosynthetic pigment contents were estimated according to Mc Kinney (1941) and Arnon (1949) equations.

Statistical analysis

All experiments were carried out in triplicate.The two-way analysis of variance (ANOVA) for all measured parameters was performed by the statistical package SAS 8.00 version (SAS, 1999). Significant different means were compared by using the Least Significance Difference (L.S.D.) test at $p<0.05$.

Results and discussion

Substrata characteristics-Zn, Pb and Cd bioavailability in substrata

Selected physico-chemical characteristics of the three substrata: Tunis (TU), Jebel Ressas (JR) and Ghezala (GH) were determined and their values are summarized in Table 1.

Table 1. Physico-chemical characteristic of the three experimental substrata (TU: Tunis; JR: Jebel Ressas, GH: Ghezala, EC: electrical conductivity, OM: organic matter).

	Substratum		
	TU (Control)	JR	GH
pH (H_2O)	8.1	8.0	8.1
EC (mmhos/cm)	2.7	3.9	1.2
$CaCO_3$ (%)	34	31	34
OM (%)	1.4	0.8	0.5
AvailableP_2O_5 (ppm)	14	2	6
Clay (%)	12	8	15
Silt (%)	50	18	35
Sand (%)	36	71	48
Texture	Sandy loam	Sandy	Sandy loam

Soil samples analyses showed that all substrata have high pH (8.00) and hence are alkaline (Table 1).Thus, the soil alkalinity were probably caused by elevated carbonates levels, which ranging from 31 to 34%. Soil pH is considered to be one of the most important parameters that affect directly the availability of trace metals (Sauve *et al.*, 1997).

Table 2. Heavy metal contents (mg kg^{-1} DMW) of Zn, Pb and Cd in the three experimental substrata (TU: Tunis; JR: Jebel Ressas, GH: Ghezala).

Substrata	Content (mg kg^{-1} DMW)		
	Zn	Pb	Cd
TU (Control)	2.45	27.25	0.15
JR	3460.00	2763.00	17.90
GH	286.00	314.50	4.75
Phytotoxicity levels	150-200	10-20	5-10
Hyperaccumulation thresholds	>10,000	>1,000	>100

As shown in Table 2, total heavy metal contents in the soil obtained from the three experimental sites TU, JR and GH were in the range of 2.45 to 3460 mg Kg^{-1} DMW for Zn, 27.25 to 2763 mg Kg^{-1} DMW for Pb and 0.15 to 17.9 mg Kg^{-1} DMW for Cd, respectively.

Zn, Pb and Cd uptake by roots, stems and leaves of Paulownia tomentosa (Thunb.) Steud

As shown in Table 3, the bioaccumulation of heavy metals Zn, Pb and Cd in all plant parts increased significantly with the increasing of substrata metal contents. Metal concentrations in plants growing in uncontaminated soil (TU) varied between 5.08-9.23, 23.22-35.22 and 0.00-0.07 mg Kg^{-1} DMW for Zn, Pb and Cd, respectively. However, in plants growing in contaminated soils JR and GH, a significant accumulation of these three metals was observed in comparison to the control, reaching 205.33, 50.13 and 3.88 mg Kg^{-1} DMW for Zn, Pb and Cd, respectively.

Table 4. Zn, Pb and Cd bioaccumulation factors in *Paulownia tomentosa* (Thunb.) Steud. plant parts growing on the three substrata (TU: Tunis, JR: Jebel Ressas and GH: Ghezala).

Substratum	BCF		
	Zn	Pb	Cd
TU (control)	3.77 ± 0.030^{a}	1.29 ± 0.010^{a}	0.00
JR	0.05 ± 0.000^{c}	0.02 ± 0.000^{c}	0.15 ± 0.003^{b}
GH	0.27 ± 0.010^{b}	0.12 ± 0.000^{b}	0.86 ± 0.006^{a}

Mean values of the same column followed by the same letter were not significantly different according to the LSD test at 5%.

Furthermore, heavy metals uptake by the plant parts (roots, stems and leaves) showed remarkably different trends (Table 3). In fact, Zn was mainly partitioned in leaves and roots. However, Pb was partitioned between all plant organs and particularly stems and roots. Indeed, the content of this heavy metal in stems of plants growing in JR and GH substrata was 46.23 and 50.13 mg kg^{-1} DMW, respectively. Cd is not easily transferred to above-ground plant biomass and the highest accumulations were found in roots of plants growing on the substrata JR (2.65 mg kg^{-1} DMW) and GH (3.88 mg kg^{-1} DMW). However, none of *P.Tomentosa* samples reached the hyperaccumulation thresholds for the three metals Zn, Pb and Cd. Nevertheless, *P.Tomentosa* test samples were able to accumulate up to twice the toxic limits of Zn in the leaves, while it accumulated Pb in stems tissues nearly four times the thresholds. In the case of Cd, the accumulation in plant tissues was than the toxic limits (Table 2).

Table 5. Zn, Pb and Cd translocation factors (TF) in *Paulownia tomentosa* (Thunb.) Steud aerial plant parts growing on the three substrata (TU: Tunis, JR: Jebel Ressas and GH: Ghezala).

Substratum	TF		
	Zn	Pb	Cd
TU (control)	1.45 ± 0.010^{a}	1.52 ± 0.020^{c}	0.00
JR	1.36 ± 0.010^{b}	1.76 ± 0.030^{b}	0.28 ± 0.030^{a}
GH	0.58 ± 0.020^{c}	2.22 ± 0.020^{a}	0.01 ± 0.007^{b}

Mean values of the same column followed by the same letter were not significantly different according to the LSD test at 5%.

In our research, the high pH values limited the uptake of Zn, Pb and Cd by the roots of *Paulownia tomentosa* (Jung and thornton, 1996).

In fact, the accumulation of trace metals by *Paulownia* tissues decreased in the order of Zn > Pb > Cd. Our findings agree with literature data, which confirm the phytoremediator potential of tree species belonging to the genus *Paulownia* (Wang *et al.*, 2010;

Doumett *et al.*, 2010). Nevertheless, *Paulownia tomentosa* is able to grow under contaminated sites through its adaptive mechanisms that give him the opportunity to concentrate within its tissues several metals, such us Zn, Pb and Cd.

Bioaccumulation and translocation factors of Zn, Pb and Cd in Paulownia tomentosa (Thunb.)Steud.plant parts.

The bioaccumulation and translocation factors are calculated in order to assess the suitability of this plant for phytoextraction and phytostabilization. As can be seen in Table 4, the bioaccumulation factor (BCF) values were very low and varied from 0.05 to 0.27 for Zn, 0.02 to 0.12 for Pb and 0.15 to 0.86 for Cd both in the two mines soils JR and GH , respectively. However, the highest BCF values for Zn and Pb were measured in the uncontaminated substratum (the control) and ranged from 3.77 for Zn and 1.29 for Pb. The BCF mean values in *P. tomentosa* roots was ranged as follow: Cd (0.86) > Zn (0.27) >Pb (0.12). These results indicated that *P.tomentosa* plant limited the mobility of trace metals in the rhizosphere.

Table 6. Zn, Pb and Cd translocation factors (TF) in *Paulownia tomentosa* (Thunb.) Steud aerial plant parts growing on the three substrata (TU: Tunis, JR: Jebel Ressas and GH: Ghezala).

Substratum	Plant parts	TF		
		Zn	Pb	Cd
TU(Control)	Leaves	0.90 ± 0.01[b]	0.66 ± 0.01[e]	0.00 ± 0.00[b]
	Stems	0.55 ± 0.00[c]	0.86 ± 0.01[c]	0.00 ± 0.00[b]
JR	Leaves	1.23 ± 0.01[a]	0.69 ± 0.01[d]	0.28 ± 0.00[a]
	Stems	0.13 ± 0.01[f]	1.07 ± 0.02[b]	0.00 ± 0.00[b]
GH	Leaves	0.37 ± 0.01[d]	0.87 ± 0.00[c]	0.01 ± 0.01[b]
	Stems	0.21 ± 0.01[e]	1.35± 0.02[a]	0.00 ± 0.00[b]

Mean values of the same column followed by the same letter were not significantly different according to the LSD test at 5%.

On the other hand, the translocation factor (TF) values for Zn determined in *P. tomentosa* plants growing on substrata JR and TU were > 1 and less than one on GH substratum. However, TF values were > 1 in all substrata and varied from 1.52 to 2.22 for Pb but they were very low for Cd (Table 5).

Based on our experimental data, *P. tomentosa* is most efficient in Pb and Zn translocation from roots to shoots (Ma *et al.,* 2001). These results are in agreement with those reported by Tzvetkova *et al.* (2013), who demonstrated that TF are higher than one in *Paulownia tomentosa x fortune* for Zn and Pb and *Paulownia elongate x fortunei* but only for Zn. However, the low TF values for Cd indicates that plant developed specific internal mechanisms that restricted the uptake of this toxic metal into roots by avoiding its translocation in aboveground parts, which constitute the metabolic activities sites (Djebali *et al.*, 2002). Furthermore, two basic strategies can explain this Cd tolerance phenomenon: either by its compartmentalization in root vacuoles and producing chelators for metal binding or by its exclusion (Gosh and Spingh, 2005).

Overall, these results indicated that *P. tomentosa* is suitable for phytoextraction of Zn and Pb from roots to aerial parts, but is inefficient for phytostabilisation of Zn, Pb and Cd (Yoon *et al.,*. 2006; D'Souza *et al.*,2013).

Zn, Pb and Cd effects on plant growth

Mean values of growth parameters which are the fresh, the dry matter weights (g) and the dry matter content (%) of leaves, stems and roots as well as the stem length (cm) and the leaf area were determined in *Paulownia tomentosa* 60 days after planting in the three substrata(Tables 6and 7).

Table 7. Effect of the three substrata (TU: Tunis, JR: Jebel Ressas and GH: Ghezala) on the leaf area (cm²) and the leaf area ratio (LAR cm²/g) of *Paulownia tomentosa* (Thunb.) Steud.

Substratum	Leaf area (cm²)	LAR (cm²/g)
TU (control)	579.50 ± 32.20[b]	98.94 ± 4.26[c]
JR	522.39 ± 8.80[c]	112.56 ± 1.27[b]
GH	632.20 ± 3.12[a]	131.99 ± 2.89[a]

Mean values of the same column followed by the same letter were not significantly different according to the LSD. test at 5%.

As can be seen in Table 6, the two contaminated substrata (JR and GH) affected all growth parameters by reducing the fresh and dry matter weight in all plant parts and this effect is more pronounced in roots and stems than in leaves.The most marked fresh matter weight reduction was observed in plants growing in the substrata JR (9.28g, 11.17g and 11.32g in leaves, stems and roots, respectively).These reductions were of 41.16% and 25.98%, both in the roots and stems, respectively, in comparison to the control (TU). However, a slight reduction (9%) was observed in the leaves.

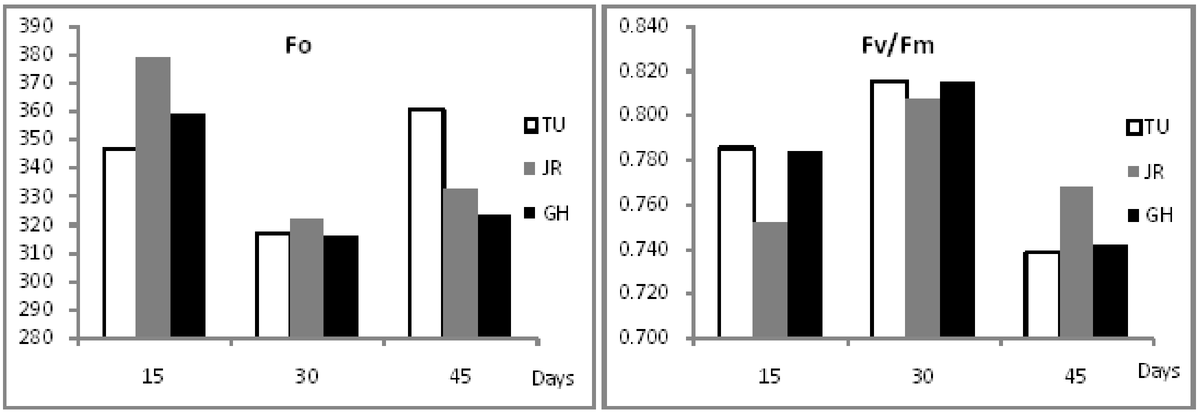

Fig. 1. Effect of the three substrata (TU: Tunis, JR: Jebel Ressas and GH: Ghezala) on the chlorophyll fluorescence parameters in *Paulownia tomentosa* (Thunb.) Steud.

On the other hand, the root dry matter content is much lower than that of the aerial parts (leaves and stems). In fact, the inhibition of root growth may be explained by the accumulation of high levels of trace metals Zn, Pb and Cd in roots (Sottnikova *et al*., 2003).

Also, the stem length of plants growing on substrata JR and GH decreased significantly, compared to the control. The elongations were of 19.92, 17.5 and 17.58 cm in plants growing in TU, JR and GH, respectively (Table 6).

Our findings indicated that heavy metal stress limited the growth of *Paulownia tomentosa* and its biomass production, which was in agreement with those of Azzarello *et al.* (2012). According to these authors, a decrease in plants growth at high levels of Zn is explained by damage of chloroplasts and hence, leading to inhibition of photosynthesis. Furthermore, Wang *et al.* (2010) reported that Zn, Pb and Cd stress severely affected plant height of *Paulownia fortunei* at different degree. In fact, Pb and Cd had little negative effect on elongation. However, Zn treatments lead to a substantial decrease on this parameter. Besides, Tzvetkova *et al.* (2013) compared the effect of high Cd levels on growth parameters of two *Paulownia* lines. Results showed that shoot length of *Paulownia elongate x fortunei* is higher than *Paulownia tomentosa x fortunei*, with lengths falling to 23 cm and 19.3 cm, respectively.

As reported in Table 7, the leaf area decreased slightly in plants growing in JR substrata of the order of 9.85% in comparison to the control TU. However, the highest leaf area was observed in substrata GH by an increasing of about 9.09% as compared to the control (Table 7). Furthermore, the Leaf Area Ratio (LAR) increased with the highest of Zn, Pb and Cd levels in substrata JR and GH, despite of the lowest total dry matter weight (Table 7). This result indicated the ability of *Paulownia* to invest its biomass in the photosynthetic surface (Miladinova *et al.,* 2014).

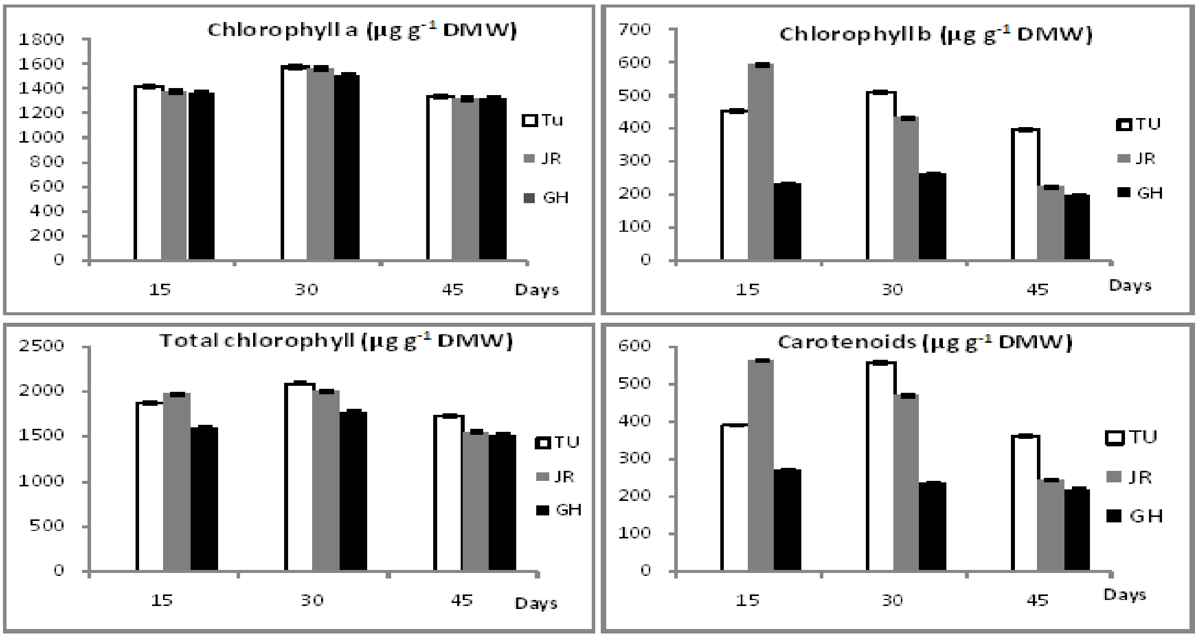

Fig. 2. Chlorophyll pigments and carotenoid contents (µg g^{-1} DMW) in *Paulownia tomentosa* (Thunb.) Steud plants growing on the three substrata (TU: Tunis, JR: Jebel Ressas and GH: Ghezala).

Zn, Pb and Cd effect on photosynthetic status of Paulownia tomentosa (Thunb.) Steud

Chlorophyll fluorescence

Chlorophyll fluorescence parameters are good biomarkers of plant tolerance to several biotic and abiotic stresses. Results showed that initial fluorescence (Fo) and the maximum quantum yield of PSII (Fv/Fm) were affected by several factors such as heavy metal concentrations, the time of exposure to stress and the elevated temperature in the glasshouse (Fig. 1.).

In this study, the maximum damage of the leaf photosynthetic was observed at the 2nd and the 6th weeks, since a reduction in the ratio Fv/Fm (0.74-0.78) and an increase in Fo values were recorded. However at the 4th week, an increase in the ratio Fv/Fm (0.81 to 0.82) indicating that all plantlet samples were healthy and not suffering from any stress condition (Demming and Bjorkman, 1987).

Higher values of Fo could be induced by inactivation of some PSII reaction center, which resulted in a decrease in the electron capture by the chlorophylls antennas and have led to a decline in the energy transfer (Yamane *et al.*,. 1997).

Moreover, Zn, Pb and Cd affect photosynthesis by inhibiting the oxygen evolution and the electron transfer reaction in PSII, which are the component of photosynthetic apparatus and are the most sensitive target for metallic stress (Juneau *et al.*, 2001). In fact heavy metals induce several alterations of photosystem, which resulting in a reduction of energy transfer of ATP and NADPH production. However, PSII is the most sensitive target to metals by substitution of the toxic ones to essential cofactors of enzymes involved in the water photolysis and lead to photo inhibition (Faller *et al.,* 2005). Furthermore, the decrease in the Fv/Fm ratio is related to the denaturation of chlorophyll proteins of PSII and structural changes in the thylakoid by increasing degradation of thylakoid proteins (Sigfridsson, 2004; Pastikka *et al.*, 2001).

Chlorophyll pigments and carotenoid biosynthesis

As can be seen in fig. 2. , accumulation of Zn, Pband Cd at high level in the substratum induced decreases in chlorophyll pigments and carotenoid content in leaves. However, the biosynthesis of chlorophyll a, chlorophyll b, total chlorophyll as well as carotenoids was altered. This fact was in agreement with previous research in some other plant species such as *Brassica juncea*, *Phaseolus vulgaris*, *Pisumsativum* (Zengin and Munzuroglu, 2005; Hattab *et al.*, 2009; Sinha and Shrivastave, 2012).

Indeed, chlorophyllb biosynthesis was declined substantially in response to heavy metal concentration in substrata. A linear decrease was shown in contaminated substrata JR and GH, which varied between 15.29% and 50.08%, when compared to the control at the 4th and the 6th week after planting (Fig. 2.).

Chlorophyll a concentration is higher than that of chlorophyll b. In fact, Chlorophyll a biosynthesis was more pronounced at the 4th week of culture and increased by 11.01%, 14.02% and 10.25% for plants growing in TU, JR and GH substrata, respectively when compared to values recorded at the 2nd week. However a slight decrease was observed at the 6th week by 15.15%, 15.77% and 12.65% for plants growing in TU, JR and GH substrata, respectively when compared to levels recorded at the 4th week (Fig. 2.).

Total chlorophyll content in *Paulownia* leaves increased significantly at the 4th week, when compared to levels recorded at the 2nd week of culture by 11.5%, 16.92% and 10.81% for plants growing in TU, JR and GH substratum, respectively. However these levels decreased significantly at the 6th week by 16.93%, 22.82% and 14.43% for plants growing in TU, JR and GH substrata, respectively when compared to values recorded at the 4th week after planting (Fig. 2.).

According to Mysliwa-Kurdziel and Strzalka (2002), heavy metal stress disturbs physiological metabolisms and affected primary photosynthetic parameters in higher plants. Indeed,Cd is reported to have negative effect on chlorophyll content by reduction of the energy consumption by Calvin's cycle reactions (Stobart *et al,*. 1985) and a decrease in the net CO_2 assimilation (Mysliwa-Kurdziel and Strzalka, 2002). Furthermore, Azzarello *et al.* (2012) reported that high Zn levels (above 1000 µM) affected chloroplasts, which are sensitive to oxidative stress. Consequently, changes in cellular organization were detected, which affected negatively the photosynthesis and lead to depressed growth. Besides, Lei *et al.* (2012) noted that metal stress (Zn and Cd) imposed during the culture period of *Seagrass thalassia hemprichii* decreased biosynthesis of chlorophyll pigments. This is a consequence of the disorganization and the damage of chloroplasts, the disassembly of the thylakoids and a consequent decrease in nutrient absorption by plants.

The highest carotenoid content (564.14 µg g^{-1} DMW) was measured in the control plants and these contents were found to be affected by the highest levels of heavy metals and the duration of exposure to the stress**.** Indeed, at the 6th week after planting, carotenoid content decreased by 35.08%, 47.96% and 7.94% in TU, JR and GH substrata, respectively when compared to the levels measured at the 4th week (Fig. .2).

In fact, these substances are non-enzymatic antioxidants, which play an important role in the protection of chlorophyll pigments (Sinha *et al.*, 2010). Heavy metal stress decreased the carotenoid biosynthesis and lead to a reduction in the protection of photosystems II (PSII) against photo-oxidation.

Conclusions

Overall results showed that the different parameters of growth (fresh and dry matter weight, leaf area, plant height) of *Paulownia tomentosa* plants growing on heavy metal contaminated Tunisian soils were declined. Additionally, a negative effect in photosynthetic status was also observed in these plants, which is the consequence of the alteration in the photosystem II by the decrease in the ratio Fv/Fm and an increase in F0 values. Also a reduction of chlorophyll pigments and carotenoid biosynthesis were recorded. However, the uptake of both Zn and Pb was higher than that of Cd, without visible symptoms of toxicity. Furthermore, the comparison between the translocation and bioaccumulation factor values indicated the phytoremediation potential of this specie which could be used for the phytoextraction of Zn and Pb.

Henceforth, *Paulownia tomentosa* is promising for phytoremediation of soils, due to its high biomass productivity and its deep root system, giving it a major interest in phytoremediation.

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