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Photosynthetic Performance of *Paulownia tomentosa* (Thunb) Steud. Exposed to Heavy Metals Zinc and Cadmium

Nada Ben Bahri[1]*, Yousr Zaouchi[1], Bochra Laribi[1,2], Salah Rezgui[1], Taoufik Bettaieb[1]

[1]National Agronomic Institute of Tunisia. 43, Av. Charles Nicolle-1082, Tunis, Tunisia.
[2]Higher Agronomic Institute of Chott-Mariem, BP 47, 4042- ChottMeriem- Sousse, Tunisia.

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***Corresponding Author**
Nada Ben Bahri
E-mail: *nbenbahri@ yahoo.fr*
Phone: *(+216) 71 28 71 10*
Fax: *(+216) 71 79 93 91*

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The present study was carried out to assess the alteration in photosynthetic performance of *Paulownia tomentosa* seedlings produced *in vitro* and cultivated under glass house when exposed to trace metals: zinc (Zn) and cadmium (Cd). In this respect, Zn and Cd were added to the substrates of culture at various concentrations: Zn (250, 500, 750 and 1000 µM) and Cd (25, 50 and 75 µM). A non-supplemented substrate with metal salts was served as a control. The photosynthetic activity was evaluated through measurements of chlorophyll fluorescence and the photosynthetic pigments, namely chlorophyll a, chlorophyll b and total chlorophyll as well as the carotenoids. Main results showed that the initial fluorescence (F0) values were higher in plants grown on Zn added substrate compared to those grown in the presence of Cd. However, the Fv/Fm ratio which indicates the efficiency of photosystem II, ranged from 0.78 to 0.82 for all treatments. Additionally, the presence of 75µM Cd in the substrate stimulates the biosynthesis of chlorophyll pigments by increasing their proportions about approximately 196.77%, in comparison to the control. On the contrary, Zn significantly reduced the contents of these pigments by 9.45% compared to the control.

Besides, when the Cd concentrations were 25, 50 and 75µM and Zn concentration was 250µM, the carotenoid contents increased up to 115.51%, 253.07%, 239.19% and 87.56% respectively, in comparison to the control, was noted. Overall, results of this study proved the ability of *Paulownia tomentosa* to maintain its photosystem activity even on Zn and Cd contaminated sites, despite the restrictive effect of Zn on the biosynthesis of photosynthetic pigments when its concentration exceeds 500 µM.

INTRODUCTION

During the last two centuries, anthropogenic activities have contributed to the enrichment of soil by trace metals. The main sources of contamination are emitted by industrial activities such as metallurgy, fossil fuels, means of transport and agricultural activities.

Trace metals can be classified according to their essential characteristics for living organisms; they must not exceed a certain level and must be maintained in the animal feed or soil, to allow development and normal reproduction of organisms. However, excessive levels of these essential elements cause toxicity in plants. Hence, the non-essential elements such as Mercury (Hg), Cadmium (Cd) or Lead (Pb), in addition to their toxicity to living beings, can induce deficiencies of essential elements by competition on the active sites of important molecules in the organism physiology (Walker et al., 1996).

Indeed, Zn toxicity towards plants is found in the soils contents of 150 mg/Kg (Adriano, 2001) and results in an iron chlorosis likely to reach tissue necrosis following reductions in chlorophyll synthesis and degradation of chloroplasts (Prasad and Prasad, 1987).

Regarding Cd, it is considered as a very toxic element and very soluble in the liquid phase of the soil (Pinto et al., 2004). However, the plants roots are affected at the level of 1 mg/Kg Cd in the soil and symptoms of toxicity appear from 5mg/Kg (Adriano, 2001). Toxicity by this element is mainly intracellular, following its physical and chemical properties similar to those of calcium, which allow it to cross biological barriers and accumulate in tissues (Hall, 2002). Consequently, the general symptoms of Cd phytotoxicity are stunting, iron chlorosis, anatomical, morphological, and physiological as well as biochemical alterations in different plant organs such as leaves, stems and roots. This toxicity generates mainly in plants, oxidative stress due to increased levels of reactive oxygen species (ROS) within cells (Fornazier et al., 2002).

Phytoremediation is considered as an alternative for removing unwanted metals and remediating soils contaminated by toxic elements through plants. It includes several techniques namely the phytoextraction which is based on the use of hyper accumulating plants able to concentrate pollutants in their aerial parts (stems, leaves, etc.) destined for harvest (Gisbert et al., 2003).

In this context, Stankovic et al. (2009) analyzed the content of heavy metals in the leaves of Paulownia trees growing in the urban environmental conditions. They concluded that *Paulownia elongata* S.Y.Hu is a tolerant species, which endures urban environmental conditions well and also that it can be recommended for growing in tree alleys and wind protection zones along urban and regional traffic lines.

Recently, *Paulownia tomentosa* has been introduced in the phytoremediation studies owing to its quick growth, strong biomass production and its high tolerance to stress conditions. In fact, this species proved a real tolerance to high concentrations of metals during hydroponics studies and on ground (Doumett et al., 2010). Indeed, Azzarello et al. (2012) studied of the effects of increasing Zn concentrations on the cellular ultrastructure and the photosynthetic parameters of *Paulownia tomentosa* grown in hydroponic conditions, with particular attention given to the main strategies for Zn detoxification used by this promising phytoremediating tree species.

Therefore, this study aims to assess the alteration in photosynthetic performance of *Paulownia tomentosa* seedlings in the presence of trace metals zinc (Zn) and cadmium (Cd) in the soil, through measurements of chlorophyll fluorescence and photosynthetic pigments.

MATERIAL AND METHODS

Plant material and growth conditions

After acclimatization, seedlings of *Paulownia tomentosa* obtained from micropropagation, were transplanted into 1 liter volume pots and placed under the glass green house of the Horticultural Sciences Laboratory at the National Agronomic Institute of Tunisia (36°51' N; 10°11' E; 10 m above sea level), from May, 1st to 30th June, 2012 (2 months of culture). The temperature (°C) and relative humidity (%) during the cultivation period of *Paulownia tomentosa* seedlings are presented in Table 1.

Table (1): Temperature (°C) and relative humidity (%) during the cultivation period of *Paulownia tomentosa* seedlings

		Month	
		May	**June**
Temperature (°C)	Day	26,15 ± 2,98	29,15 ± 2,95
	Night	17,17 ± 1,76	20,07 ± 1,07
Relative humidity (%)	Day	48,87 ± 7,77	43,61 ± 9,41
	Night	67,03 ± 6,22	61,22 ± 8,10

Experimental design, Zn and Cd treatments

The experimental design was conducted as a randomized block with four replications. Each treatment (Zn and Cd) was applied to each experimental unit. The culture substrate was mainly consisted of ¾ brown peats and ¼ sand. It was then supplemented with the metal salt Zinc Sulfate 7-

hydrate ($ZnSO_4.7H_2O$) at various concentrations (250, 500, 750 and 1000 µM) and $CdCl_2$ (25, 50 and 75 µM). A non-supplemented substrate with metal salts was served as a control. The following parameters were then determined.

Chlorophyll fluorescence measurements

Chlorophyll fluorescence measurements were assessed on healthy leaves using a portable fluorometer: Fluorescence Induction Monitor (FIM 1500, Analytical Development Company Limited, ADC). The relative fluorescence values of initial (F0), maximal (Fm) and variable (Fv) fluorescence were determined. The PSII efficiency was expressed as the Fv/Fm ratio (Baker and Rosenqvist, 2004). A single pulse of actinic light with duration of one second was applied. All measurements were taken using 20 min dark-adapted intact leaves.

Extraction and determination of chlorophyll and carotenoid pigments

Extraction of chlorophyll and carotenoid pigments consisted in placing approximately 100 mg of fresh and healthy leaves in 5 ml of acetone at 80%. After 72 hours in the dark and at a temperature of 4 °C, the optical density (OD) was measured at 460 nm, 645 nm and 665 nm by a UV-Visible spectrophotometer (Labomed, Inc., USA). The contents of the photosynthetic pigments, namely chlorophyll a, chlorophyll b and total chlorophyll as well as the carotenoids were calculated according to the following equations (Arnon, 1949):

- Chlorophyll a: 12, 7* DO (663 nm) – 2, 69*DO (645 nm)
- Chlorophyll b: 22, 9* DO (645 nm) – 4, 68*DO (663 nm)
- Total chlorophyll: 20, 2* DO (645 nm) + 8, 02*DO (663 nm)
- Carotenoids: 5*DO (460) [(Chlorophyll a *3,19) + (Chlorophyll b*30,3)]/200

Statistical analyses

Data analysis for all measured parameters was performed using PROC ANOVA of SAS 8.00 version (SAS Institute, 1999). Mean comparison was carried out using the $LSD_{0.05}$ statistical test.

RESULT

Chlorophyll fluorescence

Chlorophyll fluorescence measurements are shown in Table 2. As shown in this table, a slight variation was noted regarding the chlorophyll fluorescence values which is depending on the type of trace metals Zn or Cd. Indeed, the seedlings subjected to different Zn solution showed slightly higher F0 values than those irrigated with Cd (Table 2).

The Fv/Fm values measured for all treatments are not much variable and included between 0.78 and 0.82 (Table 2).

Table (2): Effects of different concentrations of Zn and Cd heavy metals on the chlorophyll fluorescence parameters in *Paulownia tomentosa* seedlings.

Treatments		F0	Fv/Fm
0 (control)		360.67^{abc}	0.79^{c}
	250	367.00^{ab}	0.80^{bc}
	500	371.59^{a}	0.79^{c}
	750	367.00^{ab}	0.78^{d}
Zn (µM)	1000	360.17^{abc}	0.78^{d}
	25	351.92^{c}	0.80^{bc}
	50	329.50^{d}	0.82^{a}
Cd(µM)	75	353.08^{bc}	0.81^{b}
$LSD_{0.05}$		14.921	0.0075
CV		5.12	1.16

Values in the same columns with same superscripts are not significantly differentat $P≤0.05$.

Determination of chlorophyll and carotenoid pigments

The total chlorophyll (a + b) and the carotenoid contents in the leaves of *Paulownia tomentosa* seedlings in response to the addition of heavy metals Zn and Cd are shown in Figure 1. As shown in this figure, the values of chlorophyll (a, b and total) are higher in plants grown on Cd added substrates (25, 50 and 75µM) as those sprayed with solutions of Zn (250, 500, 750 and 100µM). However, the differences between the plants exposed to Zn and the control are not significant (Figure 1).

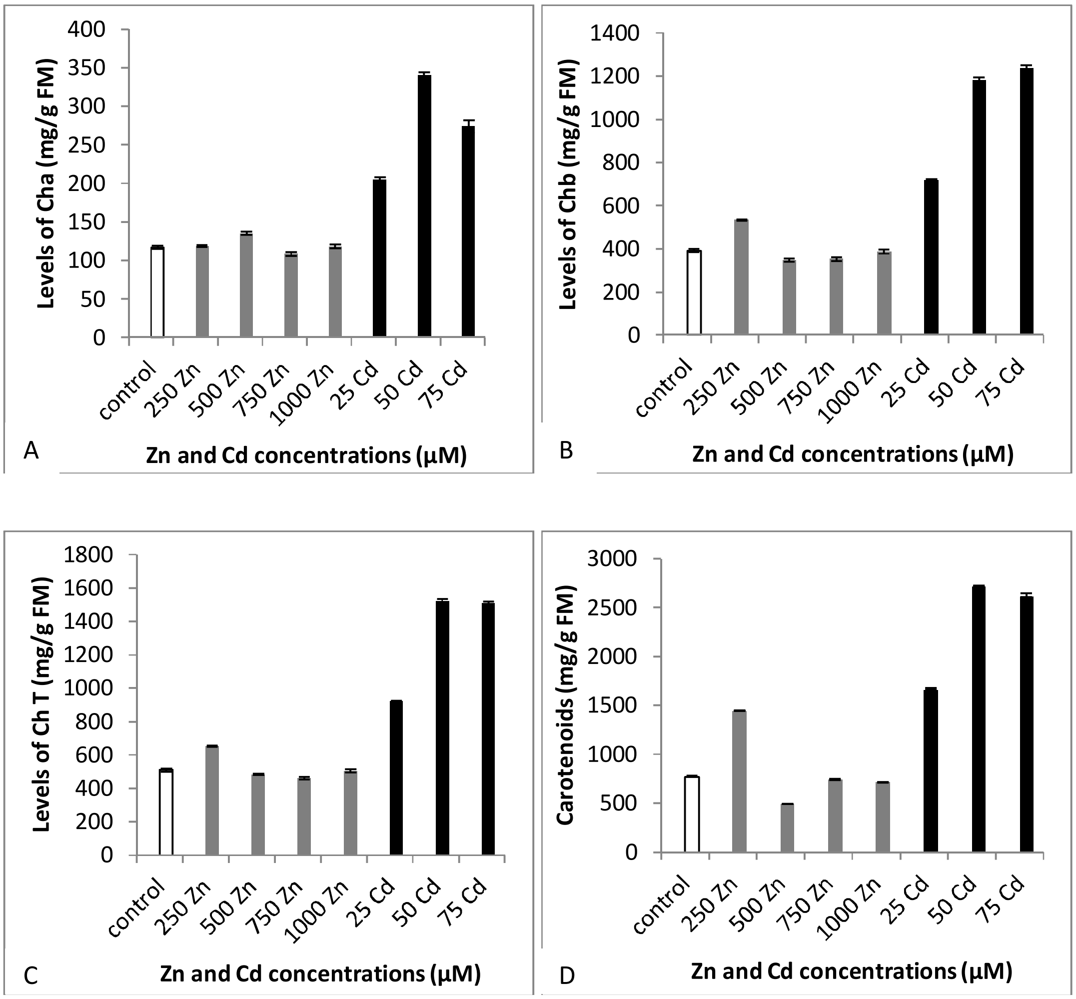

Figure 1: Levels of chlorophyll pigments and carotenoids (A: Chlorophyll a content; B: Chlorophyll b content; C: Total chlorophyll content; D: Carotenoids content in *Paulownia tomentosa* seedlings according to the Zn and Cd at different concentrations.

The presence of Zn in the substrate culture significantly reduced the levels of Chlorophyll a, Chlorophyll b and Chlorophyll (a + b), especially at 750µM.However, plants treated with Cd, show little change in chlorophyll content and carotenoids (Figure 2).

Figure 2: *Paulownia tomentosa* seedlings cultured in the presence of different concentrations of Zn and Cd. A: Seedlings grown in the presence of Zn and Cd. B: *Paulownia* seedlings cultured in the presence of different Zn concentrations and C: *Paulownia* seedling grown in the presence of different Cd concentrations.

The carotenoid synthesis is induced by Cd (25, 50 and 75µM) and by Zn at low doses (250µM), causing a significant increase of approximately 115.51%, 253.066%, 239.19% and 8 7 . 5 6 %, respectively relative to the control. Neverthless, high concentrations of Zn (500, 750 and 1000µM) decrease the synthesis of these pigments (Figure 2).

DISCUSSION

Chlorophyll fluorescence parameters are often useful to detect the presence of biotic and abiotic stress in plants. Exposing plants to different levels of Zn and Cd resulted in changes in these parameters such us initial fluorescence F0 and the Fv/Fm ratio.

In fact, the higher values of F0 (initial fluorescence) in seedlings subjected to heat stress have resulted in a decrease in the electron capture by the chlorophylls antennas and have led to a decline in the energy transfer to the reaction centers (Bettaieb et al., 2008), since F0 is sensitive to all the factors that may affect the structure of antennas and thus altering the distance between the chlorophyll molecules which ensure the excitation transfer. This causes a structural alteration in the reaction centers of the primary photosystem II (PSII) (Bjorkmann and Demming, 1987).

Fv/Fm values measured for all treatments are included between 0.78 and 0.82. According to Maxwell and Johnson (2000), the ratio Fv/Fm reflects the general physiological state and especially the degree of alteration of the structure of the photosynthetic apparatus.

These values are usually obtained under optimal growth conditions and in the absence of any environmental stress (Bounaqba, 1998). As reported by Perreault (2008), heavy metals induce several alterations of photosystem, which resulting in a reduction of energy transfer and 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 (Faller et al., 2005).

Henceforth, it appears that *Paulownia tomentosa* can tolerate the presence of Cd and Zn in the substrate culture.

In the present investigation, the photosynthetic pigments content in *Paulownia tomentosa* declined in leaves of plants exposed to Zn. Instead, plants sprayed with Cd solutions show little change in chlorophyll contents.

Our results agrees with those reported by Wang et al., (2010) who showed that exposure of *Paulownia fortunei* seedlings to several trace metals, such as Zn and Cd reduced significantly the chlorophyll pigments and carotenoids contents.

In a recent study, Azzarello et al. (2012) reported that electron and confocal microscopy analysis showed differences in the cellular ultrastructure between control and treated (above 2000 µM) plants, which exhibited an accumulation of electron-dense materials. The major toxic effects of high Zn concentrations were related to damages to the cell functionality, i.e., the chloroplast ultrastructure, which negatively affected the photosynthetic performance, thus leading to a significant growth inhibition. *Paulownia tomentosa* plants are able to limit Zn induced damages by activating effective mechanisms of Zn sequestration and accumulation of excess Zn in dedicated structures, such as petiole cell walls and root hairs, or by excluding part of the Zn in exudates located on the petiole surface.

However, our findings are in contrast to those reported by Sbartai et al., (2012) who showed that exposure of tomato plants to Cd induced a decrease in the content of Chlorophyll a and b, while the low concentration Zn stimulated their biosynthesis. Otherwise, Lei et al., (2012) noted a decline in chlorophyll content after exposure of *Seagrass Thalassia hemprichii* species to higher Zn and Cd concentrations which could be due to the disruption and damage of chloroplasts and the reduced number of thylakoids as well as the inhibition of nutrient absorption. Indeed, it has been reported that the decrease in chlorophyll content is one of the primary events that occur in plants under metal stress and is due to the inhibition of enzymes involved in chlorophyll biosynthesis (Mysliwa-Kurdziel and Strzalka, 2002).

The carotenoid contents decreased with the increase in concentration of Zn in solution, and increased with all treatments of Cd and Zn at 250µM.

In fact, these molecules play an important role in protecting chlorophyll pigments (Sinha et al., 2010). They are essential constituents of the chlorophyll-protein complexes which provide on the one hand the transfer of light energy to the chlorophyll and which on the other hand, recover the energy of the chlorophyll particularly in case of excessive light (Paulsen, 1997).

Our results confirm the work of Singh et al., (1996), who demonstrated that the trace metal elements usually affect the synthesis of chlorophyll much more than carotenoids. Besides, the levels of carotenoids in the species *Seagrass Thalassia hemprichii*, at low Cd concentrations, increased significantly, but decreased at high concentrations.

The reduction of the carotenoids synthesis in the presence of high Zn concentrations leads to a decrease in the protection of photosystems II in the leaves against photo-oxidation, thereby causing oxidative stress and even leaf senescence (Yang et al.,2002). Furthermore, Vassilev et al.,(2011) attributed the reduction of chlorophylls and carotenoids in the leaves of *Phaseolus vulgaris* L. to a disorder of the integral of net photosynthesis due to a limitation in the number of stomata and the reducing flow of CO_2 in leaves. It has been suggested that the decrease in chlorophyll pigments and carotenoids proportions could be attributed to many causes, such as the disruption of their biosynthesis or increased degradation, and a sharp reduction in thylakoids.

CONCLUSION

Overall, results proved the tolerance of *Paulownia tomentosa* to heavy metal stress, particularly Cd and Zn. Indeed, this species maintained its photosystem activity even on Zn and Cd contaminated sites, despite the restrictive effect of Zn on the biosynthesis of photosynthetic pigments when its concentration exceeds 500 µM. Furthers investigations are needed in order to examine the ability of *Paulownia tomentosa* to extract and accumulate heavy metals in its tissues and hence, confirm its use in phytoremediation programs.

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