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Responses of two poplar species (*Populus alba* and *Populus x canadensis*) to high copper concentrations

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Abstract

With the aim to examine their potentials as renewable resources to decontaminate polluted soils, growth, photosynthesis and nitrogen balance were analyzed in two poplar species (*Populus x canadensis*, *Adda* clone and *Populus alba*, *Villafranca* clone) to investigate the tolerance to high copper (Cu) concentrations. The two clones showed different responses to Cu in terms of tolerance and metal allocation: *P. x canadensis* accumulated Cu in roots, displaying features sought in plants suitable for phytostabilization, while *P. alba* accumulated the metal in leaves, like an indicator species.

Keywords: Poplar; Heavy metals; Gas exchange; Phytotoxicity

1. Introduction

Pollution of soil and water with heavy metals has serious consequences for environment and human health. In particular, human health is affected through direct exposure via skin and inhalation, and through oral exposure, with potential for systemic accumulation by consumption of contaminated food and groundwater (Hrudey et al., 1995).

Plants show different degrees of tolerance to heavy metals, being able to exclude or accumulate and store them in particular organs, tissues, modified cells (trichomes) and subcellular compartments (vacuoles). Several species are known to accumulate large amounts of heavy metal and are therefore called hyperaccumulators. This ability can be used in phytoextraction technologies to bring the level of soil pollution below the limits established by environmental rules (Salt et al., 1998). Responses to high levels of heavy metals have been extensively studied in hyperaccumulators, in model plants, and to a lesser extent also in trees (Lasat, 2002; Maegher, 2000; Pulford and Watson, 2003).

In trees, levels of tolerance have been proved for many contaminants, so that together with morphological and physiological characteristics (fast growth, deep root system, high hydraulic control capacity and long life) and commercial uses (paper and wood industry), trees appear suitable for phytoremediation purposes (Fischerová et al., 2006; Rokwood et al., 2004).

However, tolerance characteristics and metal uptake and compartmentalization are highly variable among tree species and hybrids (Kopponen et al., 2001; Laureysens et al., 2005; Sebastiani et al., 2004). Responses of trees to high heavy metal concentrations have been studied on polluted fields like mines and disposal sites (Álvarez et al., 2003; Djingova et al., 1999; Madejón et al., 2004), as well as in controlled environments particularly suitable for preliminary screenings (Di Baccio et al., 2003; Punshon et al., 1995).

In this context, the aim of this work was to determine the ability of poplar trees to tolerate high copper (Cu) concentrations and to accumulate Cu ions in aerial plant organs or conversely to store them in the root system. Since metal tolerance and translocation capacity are traits highly dependent on species and clones, we performed our investigation on two different poplar

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species: the white poplar *Populus alba*, *Villafranca* clone and the hybrid *Populus x canadensis*, *Adda* clone. The main physiological responses, with particular emphasis to those mostly disturbed by heavy metals (growth, photosynthesis and nitrogen balance), were discussed.

2. Materials and methods

2.1. Plant material and treatments

Woody cuttings of *P. x canadensis*, *Adda* clone and *P. alba*, *Villafranca* clone were rooted and grown in pots containing a universal loam:perlite mixture (1:1, v:v) and irrigated daily with deionized water for about 30 days. Plants were successively transferred to a growth chamber (temperature 23 °C; relative humidity 55–75% day–night; photoperiod 16–8 h light–dark; maximum light intensity 400–500 $\mu mol\, m^{-2}\, s^{-1}$), grown in pots filled with clay suitable for hydroponic culture (diameter 13–15 mm; pH 7.0; AgriLeca, Milano, Italia) and supplied with Hoagland's solution, pH 6.7 (Arnon and Hoagland, 1940). After 15 days of acclimation to the hydroponic system, 24 homogeneous plants per genotype were selected and randomly assigned to three groups of treatment with Hoagland's solution containing different concentrations of $CuSO_4{\cdot}5H_2O$: 0.4 (control), 25 and 75 μM and monitored for 30 (T_1) and 80 (T_2) days. To avoid root necrosis the solution was completely replaced twice per week and aerated by diffused air aeration systems. Four plants randomly selected at the beginning of the experiment were used to determine the initial biomass (T_0) after oven drying at 70 °C until constant weight.

2.2. Growth analysis

At times T_1 and T_2 of sampling, four plants from each treatment group were randomly selected and divided into leaves, stem and roots, separately weighted and oven-dried at 70 °C until constant weight was reached. To remove Cu ions adsorbed at the root surface, root apparatus was carefully rinsed with deionised water soon after harvesting. Stem length (SL) and stem basal diameter (SD) were measured weekly on each plant. Leaf area (LA) was determined by the regression equations $y = 0.7121x$ ($R^2 = 0.99$) for *P. x canadensis* and $y = 0.6327x$ ($R^2 = 0.94$) for *P. alba*. Both equations were forced through the origin; (y) represents leaf area and (x) the product of the two main orthogonal diameters of leaves. Each equation was previously determined by measuring the surface area of 215 (*P. x canadensis*) and 184 (*P. alba*) randomly selected leaves and analyzed using the software Scion Image 4.0.2 (Scion Corporation, MD, USA). Specific leaf area (SLA) was calculated as the ratio of leaf area to corresponding leaf dry mass. Relative growth rate (RGR) was calculated as $RGR = [(\log_e MT_x - \log_e MT_0)/(T_x - T_0)]$ where MT_x is the total dry mass at sampling time T_x (T_1 and T_2) and MT_0 is the initial (T_0) total plant dry mass average (Hunt, 1978). Metal resistance was calculated as tolerance index (TI; %) accordingly to Landberg and Greger (2002) on the basis of total dry weight of shoot and root.

2.3. Copper and nitrogen determinations

Samples of leaves, stem and roots were oven-dried at 70 °C and ground to powder in a laboratory mill. The plant material (0.2 g) was mineralized in HNO_3–$HClO_4$ (5:1, v:v), clarified with ultra pure water and used for measurements of total Cu in an atomic absorption spectrophotometer (Analyst 200, Perkin-Elmer, Norwalk, CT, USA).

Organic nitrogen content of leaves was determined with the Kijeldhal method using a 2100 Kjeltec System Distillation Unit (Foss Tecator AB, Höganäs, Sweden).

2.4. Chlorophyll analysis

Chlorophyll *a* and *b* (Chl_a and Chl_b) contents were determined by reading the absorbance at 664 and 647 nm of extracts obtained from two disks of 10 mm in diameter from the fourth to fifth leaf from the apex. The extraction was performed with *N*,*N*-dimethylformamide (1:20, w:v) for 48 h at 4 °C in the dark and chlorophyll content determined according to Moran (1982). Spectrophotometer measurements were monitored on a Lambda 25 Spectrophotometer (Perkin-Elmer, Norwalk, CT, USA).

2.5. Gas exchange and chlorophyll fluorescence measurements

Gas exchange was measured with a portable photosynthesis system (LI-6400, Li-Cor, Lincoln, NE, USA) equipped with CO_2 control module and light source consisting of blue-red light-emitting diodes (LI-6400-02B). Net photosynthesis (A), stomatal conductance to H_2O (g_s) and intercellular CO_2 concentration (C_i) were measured on the fourth and fifth fully expanded leaves from the apex (Leaf Plastochron Index 4-5) (Dickmann, 1971). All gas exchange measurements were conducted at time T_2 on four replicate plants for each clone-treatment combination. Leaf chamber temperature and humidity were adjusted to maintain a leaf-to-air vapour pressure difference of about 1.2 kPa, with irradiance maintained at a previously determined saturating value of 500 $\mu mol\, m^{-2}\, s^{-1}$. Photosynthetic CO_2 response curves (A/C_i) were determined by measuring the response of A to varying C_i. External CO_2 partial pressures (C_a) were supplied in steps; the CO_2 assimilation rate was first measured by setting the reference CO_2 concentration near ambient (40 Pa) and then at 30, 20, 10, 5, 40, 40, 60 and 80 Pa. Measurements were recorded automatically at each C_a set point when A had equilibrated, which was typically less than 3 min. The A/C_i curves were analyzed with Photosynthesis Assistant software (Version 1.1, Dundee Scientific, Dundee, Scotland). Photosynthesis Assistant analyses A/C_i curves were based on the model of Farquhar et al. (1980), as subsequently modified by von Caemmerer and Farquhar (1981), Sharkey (1985), and Harley and Sharkey (1991). A/C_i curves were analyzed to estimate two biochemical parameters potentially limiting to photosynthesis: V_{cmax} (maximum rate of carboxylation) and J_{max} (light-saturated rate of electron transport). We analyzed the A/C_i curves using the Michaelis–Menten constants of Rubisco described in Harley et al. (1992a,b) and used by Wullschleger (1993). The speci-

ficity factor for Rubisco (Jordan and Ogren, 1984) was also analyzed. The relative stomatal limitation to photosynthesis (l_g), an estimate of the proportion of the reduction in photosynthesis attributable to CO_2 diffusion between the atmosphere and the site of carboxylation, was calculated from A/C_i curves by the method of Farquhar and Sharkey (1982) as $l_g = (1 - A/A_o) \times 100$ where A is the rate of net photosynthesis at the growth C_a and A_o is the photosynthetic rate when C_i equals the growth C_a. Under these conditions, A_o is the rate of photosynthesis that would occur if there were no diffusive limitations to CO_2 transfer from the bulk atmosphere to the site of carboxylation. For this calculation, mesophyll conductance was considered to be infinitely large.

Contemporary to gas exchange determinations, chlorophyll fluorescence emissions in 30-min dark-adapted leaves were measured with a portable pulse amplitude modulation fluorometer (PAM-2000, Walz, Effeltrich, Germany) to estimate the effect of treatments on photosystem II (PSII) efficiency. Background fluorescence signal (F_o), the maximum fluorescence (F_m), and potential quantum yield of PSII photochemistry [$F_v/F_m = (F_m - F_o)/F_m$] were determined as well.

2.6. Statistical analysis

The experiment had a completely randomized design with four replicates per clone for each time point. Data were subjected to analysis of variance (ANOVA) to examine the effect of time, treatment and clone. Statistical analysis was conducted using the software CoStat 6.2 (CoHort Software, CA, USA). Separation of means was performed using LSD test at $P = 0.05$ significance level.

3. Results

3.1. Growth traits

Increase in the biomass of all plant organs (leaves, stems and roots) from time T_1 to time T_2 was observed in both *P. x canadensis*, *Adda* clone and *P. alba*, *Villafranca* clone under all Cu concentrations tested (Table 1). At time T_2 and 75 μM Cu, *P. x canadensis* showed a 22% reduction of total biomass compared to control plants, while no statistically significant differences were observed at 25 μM Cu. In *P. alba* at 25 and 75 μM Cu, growth was, respectively, 57 and 73% less than in control plants.

A similar trend was observed in leaf growth (leaf area, LA; specific leaf area, SLA), stem growth (stem length, SL; stem diameter, SD), and total growth (relative growth rate, RGR), as shown in Table 2. Therefore, *P. x canadensis*, *Adda* clone appeared to be less responsive than *P. alba*, *Villfranca* clone to Cu treatments.

Table 1
Leaves, stems, roots and total biomass of *Populus x canadensis*, *Adda* clone and *Populus alba*, *Villafranca* clone grown with 0.4 (control), 25 and 75 μM Cu for 30 (T_1) or 80 (T_2) days

Time	Organ	Species	Cu treatment		
			0.4 μM	25 μM	75 μM
T_1	Leaves	*P. x canadensis*	5.630 ± 0.564	6.341 ± 0.779	3.854 ± 0.790
		P. alba	3.336 ± 0.547	2.973 ± 0.377	2.104 ± 0.584
	Stem	*P. x canadensis*	2.046 ± 0.258	2.661 ± 0.389	1.464 ± 0.364
		P. alba	1.214 ± 0.248	1.182 ± 0.155	0.835 ± 0.230
	Roots	*P. x canadensis*	0.804 ± 0.105	0.627 ± 0.104	0.363 ± 0.104
		P. alba	0.398 ± 0.099	0.383 ± 0.044	0.409 ± 0.092
	Total	*P. x canadensis*	8.484 ± 0.902	9.629 ± 1.251	5.680 ± 1.247
		P. alba	4.948 ± 0.872	4.537 ± 0.552	3.348 ± 0.903
T_2	Leaves	*P. x canadensis*	22.130 ± 2.169	22.369 ± 3.345	18.336 ± 3.345
		P. alba	27.701 ± 3.486	12.127 ± 2.983	7.291 ± 1.831
	Stem	*P. x canadensis*	15.530 ± 0.453	11.918 ± 1.654	9.257 ± 1.655
		P. alba	16.542 ± 2.317	5.913 ± 1.178	4.190 ± 0.999
	Roots	*P. x canadensis*	3.381 ± 0.348	3.884 ± 0.885	4.103 ± 0.759
		P. alba	3.593 ± 0.685	2.387 ± 0.519	1.111 ± 0.198
	Total	*P. x canadensis*	41.040 ± 1.933	38.170 ± 5.812	31.696 ± 5.789
		P. alba	47.835 ± 6.336	20.427 ± 4.595	12.592 ± 2.995

Organ	Main effects			Interactions			
	Clone	Metal	Time	C × M	C × T	M × T	C × M × T
ANOVA *P* values							
Leaves	.0034	.0003	.0000	.0123	ns	.0035	.0148
Stem	.0013	.0000	.0000	.0421	ns	.0000	ns
Roots	.0028	ns	.0000	ns	.0209	ns	.0206
Total	.0002	.0020	.0000	ns	.0000	ns	ns

Values are the mean ± standard error ($n = 4$). Units are g of dry mass. ANOVA: *P* values for main effects and interactions clone × metal (C × M), clone × time (C × T), metal × time (M × T), clone × metal × time (C × M × T) are shown.

Table 2
Stem length (SL), stem diameter (SD), leaf area (LA), specific leaf area (SLA), relative growth rate (RGR) of *P. x canadensis*, *Adda* clone and *P. alba*, *Villafranca* clone grown with 0.4 (control), 25 and 75 μM Cu for 30 (T_1) or 80 (T_2) days

Time	Parameter	Species	Cu treatment		
			0.4 μM	25 μM	75 μM
T_1	SL	*P. x canadensis*	0.531 ± 0.030	0.596 ± 0.022	0.425 ± 0.044
		P. alba	0.404 ± 0.035	0.394 ± 0.022	0.285 ± 0.041
	SD	*P. x canadensis*	6.975 ± 0.184	7.250 ± 0.433	6.325 ± 0.506
		P. alba	5.000 ± 0.000	5.500 ± 0.000	4.500 ± 0.354
	LA	*P. x canadensis*	0.157 ± 0.012	0.174 ± 0.022	0.098 ± 0.017
		P. alba	0.105 ± 0.014	0.092 ± 0.012	0.051 ± 0.015
	SLA	*P. x canadensis*	0.028 ± 0.001	0.027 ± 0.001	0.026 ± 0.001
		P. alba	0.032 ± 0.002	0.031 ± 0.001	0.024 ± 0.000
	RGR	*P. x canadensis*	0.089 ± 0.004	0.085 ± 0.005	0.066 ± 0.007
		P. alba	0.094 ± 0.006	0.078 ± 0.004	0.066 ± 0.009
T_2	SL	*P. x canadensis*	1.123 ± 0.039	0.958 ± 0.052	0.819 ± 0.065
		P. alba	1.027 ± 0.005	0.789 ± 0.085	0.755 ± 0.088
	SD	*P. x canadensis*	11.945 ± 0.419	9.860 ± 0.326	9.710 ± 0.785
		P. alba	12.183 ± 0.848	7.673 ± 0.393	7.140 ± 0.902
	LA	*P. x canadensis*	0.433 ± 0.050	0.380 ± 0.046	0.291 ± 0.049
		P. alba	0.579 ± 0.043	0.239 ± 0.052	0.101 ± 0.044
	SLA	*P. x canadensis*	0.020 ± 0.002	0.017 ± 0.001	0.016 ± 0.001
		P. alba	0.021 ± 0.002	0.020 ± 0.001	0.015 ± 0.005
	RGR	*P. x canadensis*	0.050 ± 0.001	0.049 ± 0.002	0.046 ± 0.003
		P. alba	0.059 ± 0.002	0.047 ± 0.003	0.041 ± 0.003

Parameter	Main effects			Interactions			
	Clone	Metal	Time	C × M	C × T	M × T	C × M × T
ANOVA *P* values							
SL	.0001	.0000	.0000	ns	ns	.0068	ns
SD	.0000	.0000	.0000	ns	ns	.0001	ns
LA	.0049	.0000	.0000	.0027	ns	.0000	.0044
SLA	.1618	.0016	.0000	ns	ns	ns	ns
RGR	ns	.0007	.0000	ns	ns	ns	ns

Values are mean ± standard error ($n = 4$). Units are m (SL), mm (SD), m^2 (LA), $m^2\ g^{-1}$ (SLA) and $days^{-1}$ (RGR). ANOVA: *P* values for main effects and interactions clone × metal (C × M), clone × time (C × T), metal × time (M × T), clone × metal × time (C × M × T) are shown.

3.2. Nitrogen and chlorophyll concentrations

Nitrogen content in leaves decreased through the treatments in both species. At time T_2, it decreased more in *P. x canadensis* than in *P. alba*, which showed a slight decrease only in the plants treated with 75 μM Cu (Table 3).

The two poplar species revealed significant differences in chlorophyll content in the response to Cu treatment (Table 4; see *P* values for metal and interaction). *P. x canadensis* showed a slight increase in Chl_a and Chl_b contents in treated plants compared to controls. In *P. alba* Chl_a and Chl_b contents slightly decreased in plants treated with 75 μM Cu. $Chl_{a/b}$ ratio did not change through the treatments in both species, indicating that the synthesis of photosystem cores and of pigments of light harvesting complexes was not affected.

3.3. Photosynthetic capacity

Photosynthetic rates maintained stable across Cu treatments in *P. x canadensis* (within 17–14% positive variation compared with control plants), while showed a decrease (about 28%) with increasing Cu concentration in *P. alba* (Fig. 1). Accordingly,

Table 3
N concentration (N%) in leaves of *P. x canadensis*, *Adda* clone and *P. alba*, *Villafranca* clone grown with 0.4 (control), 25 and 75 μM Cu for 30 (T_1) or 80 (T_2) days

Time	Species	Cu treatment		
		0.4 μM	25 μM	75 μM
T_1	*P. x canadensis*	3.61 ± 0.10	3.51 ± 0.11	3.11 ± 0.12
	P. alba	3.35 ± 0.20	3.33 ± 0.09	2.83 ± 0.09
T_2	*P. x canadensis*	3.10 ± 0.11	2.44 ± 0.12	2.11 ± 0.11
	P. alba	2.71 ± 0.08	2.72 ± 0.03	2.61 ± 0.11

Main effects			Interactions			
Clone	Metal	Time	C × M	C × T	M × T	C × M × T
ANOVA *P* values						
ns	.0000	.0001	.0118	.0034	ns	.0351

Values are mean ± standard error ($n = 4$). Units are expressed as %, w:w (N). ANOVA: *P* values for main effects and interactions clone × metal (C × M), clone × time (C × T), metal × time (M × T), clone × metal × time (C × M × T) are shown.

Table 4
Chlorophyll *a* (Chl_a), *b* (Chl_b), total (Chl_{tot}) and chlorophyll *a*/*b* ratio ($Chl_{a/b}$) in leaves of *P. x canadensis*, *Adda* clone and *P. alba*, *Villafranca* clone grown with 0.4 (control), 25 and 75 μM Cu for 80 (T_2) days

Time	Chl	Species	Cu treatment		
			0.4 μM	25 μM	75 μM
T_2	Chl_a	*P. x canadensis*	8.436 ± 0.672	13.056 ± 0.592	10.248 ± 0.729
		P. alba	10.200 ± 0.475	10.323 ± 0.676	9.101 ± 0.490
	Chl_b	*P. x canadensis*	2.371 ± 0.158	3.544 ± 0.165	3.003 ± 0.216
		P. alba	2.883 ± 0.131	3.001 ± 0.196	2.658 ± 0.166
	$Chl_{a/b}$	*P. x canadensis*	3.530 ± 0.061	3.686 ± 0.041	3.443 ± 0.124
		P. alba	3.540 ± 0.037	3.440 ± 0.018	3.438 ± 0.031
	Chl_{tot}	*P. x canadensis*	10.807 ± 0.829	16.600 ± 0.754	13.251 ± 0.922
		P. alba	13.083 ± 0.604	13.324 ± 0.872	11.759 ± 0.656

Chl	Main effects		Interaction
	Clone	Metal	C × M
ANOVA *P* values			
Chl_a	ns	.0006	.0023
Chl_b	ns	.0019	.0092
$Chl_{a/b}$	ns	ns	.0808
Chl_{tot}	ns	.0007	.0030

Values are mean ± standard error ($n = 4$). Units are expressed as μg mm^{-2}. ANOVA: *P* values for main effects and interaction clone × metal (C × M) are shown.

stomatal conductance decreased in *P. x canadensis* (44 and 25% negative variation in 25 and 75 μM treated plants, respectively), while remained fairly steady in *P. alba* (within 2–15% negative variation). Susceptibility for stomata closure with increasing Cu treatment was confirmed in *P. x canadensis* by the decrease of intercellular CO_2 concentration, which was not the case in *P. alba*. However, instantaneous gas exchange parameters did not change significantly with treatment or genotype. Maximum rate of carboxylation (V_{cmax}) and light-saturated rate of electron transport (J_{max}) showed an increase in 25 μM Cu treated plants of *P. x canadensis*; whereas, in *P. alba* values of both parameters progressively decreased from control to 75 μM Cu (Table 5). The J_{max}/V_{cmax} ratio remained steady across treatments in *P. x canadensis* ranging about 2.5, while it decreased in *P. alba* with increasing Cu because of the relatively stronger decrease in J_{max} compared with V_{cmax}. Relative stomatal limitation to photosyn-

Table 5
Maximum rate of carboxylation (V_{cmax}), light-saturated rate of electron transport (J_{max}), relative stomatal gas-phase limitation (l_g) estimated from A/C_i curves and chlorophyll fluorescence in leaves of *P. x canadensis*, *Adda* clone and *P. alba*, *Villafranca* clone grown with 0.4 (control), 25 and 75 μM Cu for 80 (T_2) days

Time	Parameter	Species	Cu treatment		
			0.4 μM	25 μM	75 μM
T_2	V_{cmax}	*P. x canadensis*	27.67 ± 2.01	42.73 ± 2.66	35.37 ± 5.57
		P. alba	45.33 ± 4.97	41.77 ± 4.97	36.63 ± 3.01
	J_{max}	*P. x canadensis*	69.90 ± 2.29	106.63 ± 7.34	89.23 ± 11.87
		P. alba	153.00 ± 14.08	111.93 ± 14.01	75.60 ± 26.47
	J_{max}/V_{cmax}	*P. x canadensis*	2.54 ± 0.11	2.52 ± 0.28	2.55 ± 0.16
		P. alba	3.38 ± 0.25	2.65 ± 0.30	2.07 ± 0.42
	l_g	*P. x canadensis*	10.08 ± 1.60	22.52 ± 2.79	32.17 ± 9.74
		P. alba	26.58 ± 8.38	22.26 ± 3.48	15.22 ± 7.60
	F_v/F_m	*P. x canadensis*	0.75 ± 0.08	0.82 ± 0.00	0.82 ± 0.01
		P. alba	0.79 ± 0.01	0.80 ± 0.00	0.80 ± 0.01

Parameter	Main effects		Interaction
	Clone	Metal	C × M
ANOVA *P* values			
V_{cmax}	.0364	ns	.0220
J_{max}	.0242	ns	.0035
J_{max}/V_{cmax}	ns	.0411	.0398
l_g	ns	ns	.1772
F_v/F_m	ns	ns	ns

Values are mean ± standard error ($n = 4$). Units are expressed as μmol of CO_2 m^{-2} s^{-1} (V_{cmax}), μmol of electrons m^{-2} s^{-1} (J_{max}), % (l_g), relative units (F_v/F_m). ANOVA: *P* values for main effects and interactions clone × metal (C × M) are shown.

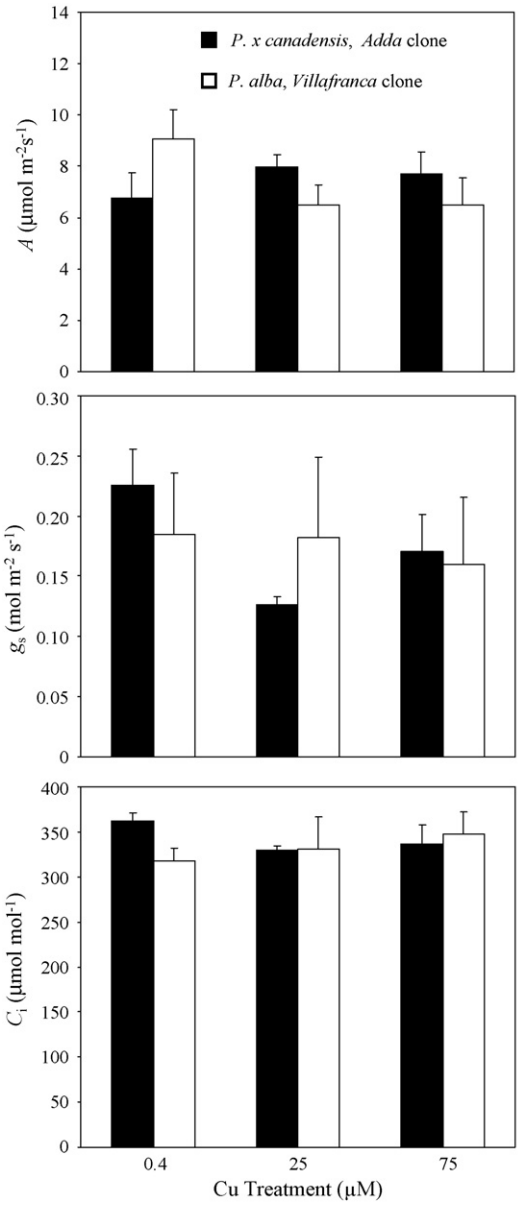


Fig. 1. Photosynthetic rate (A), stomatal conductance (g_s) and intercellular CO_2 concentration (C_i) in leaves of *Populus x canadensis*, *Adda* clone (black bars) and *Populus alba*, *Villafranca* clone (white bars) grown with 0.4 (control), 25 and 75 μM Cu for 80 (T_2) days. Units are μmol m^{-2} s^{-1} (A), mol m^{-2} s^{-1} (g_s), μmol mol^{-1} (C_i). Values are mean ± standard error ($n = 4$).

thesis appeared to increase with increasing Cu treatment in *P. x canadensis*, while decreased in *P. alba*, confirming the genotypic difference of the two species in terms of gas exchange and photosynthetic capacity. Cu did not induce a significant variation in F_v/F_m values averaging 0.8 across treatments and clones (Table 5).

3.4. Copper content

Cu concentration of *P. x canadensis* and *P. alba* sampled at T_1 had similar values, averaging 8.8 and 5.9 mg kg^{-1}, respectively, in leaves and in stem of 25 and 75 μM Cu treated plants. Cu root concentration increased in both species (Fig. 2). However, after 80 days the two species showed significant differences in term of metal allocation in leaves: in *P. alba*, Cu concentration kept increasing, while in *P. x canadensis* it averaged between 3.0 and 4.0 mg kg^{-1} through all the treatments. In both species, Cu concentration in the roots increased with the treatment.

At time T_1, Cu content was higher in *P. x canadensis* than in *P. alba* in all plant organs (Fig. 3), and at time T_2 in stem and roots of 25 and 75 μM Cu treated plants. Leaf Cu content at time T_2 was higher in *P. alba* than in *P. x canadensis* because of the higher roots to shoot translocation rate in this genotype.

4. Discussion

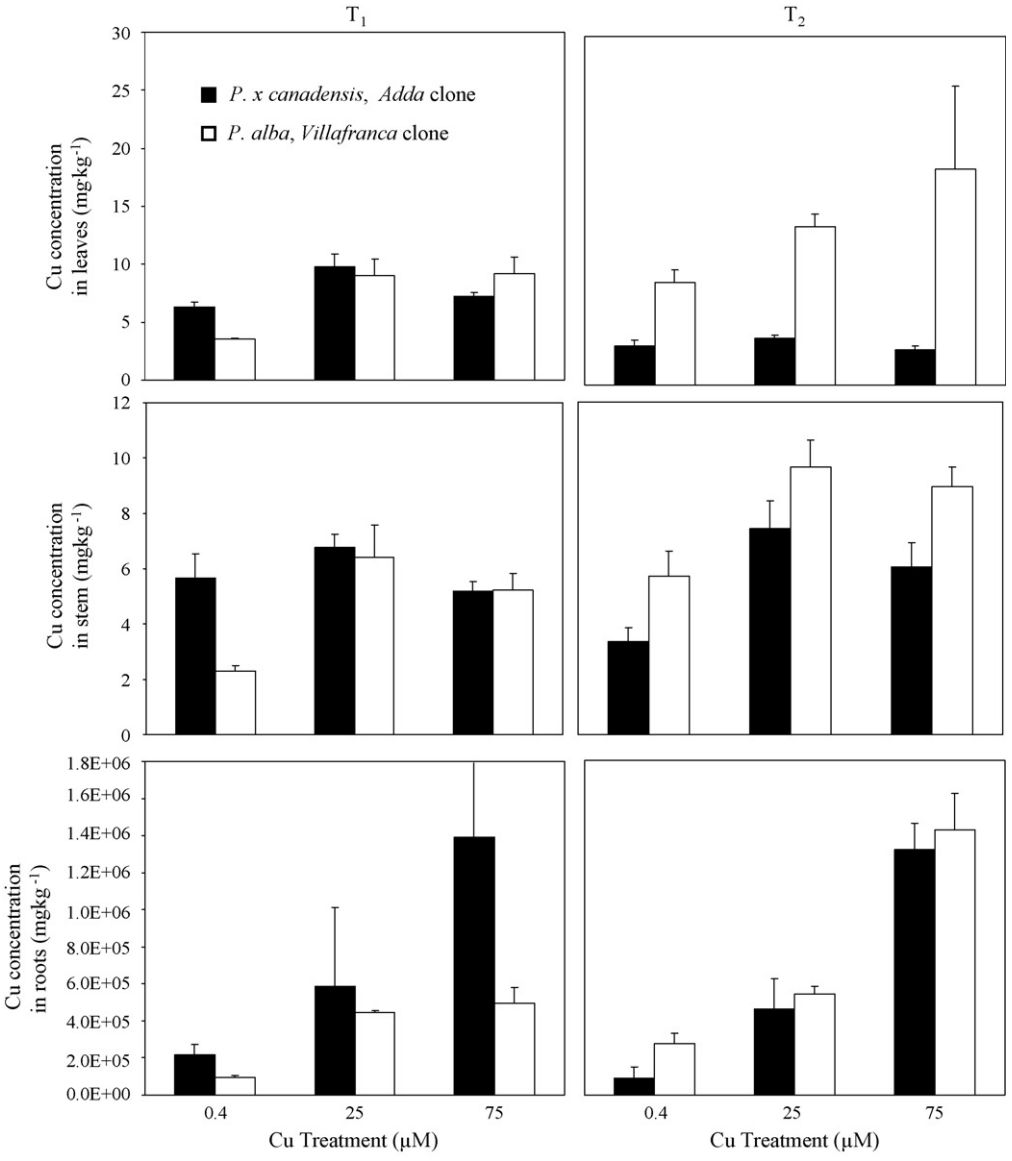
A way to evaluate the tolerance to heavy metals in plants is to measure the survival rate in heavily toxic substrates or the reduction in growth rate (Hunt, 1978) and the impairment of main physiological functions such as assimilation rate and nitrogen balance (Tognetti et al., 2004). Using this approach, in this study we observed that two different poplar species *P. x canadensis*, *Adda* clone and *P. alba*, *Villafranca* clone differ in their response to Cu treatment in term of growth and metal allocation.

P. x canadensis proved to be more tolerant to high Cu: plant growth was significantly reduced only at concentration of 75 μM Cu, while no differences were observed between control and 25 μM Cu treated plants. On the other hand, *P. alba* showed a remarkable reduction of growth throughout all levels of treatment.

It has been long known that in plants the application of toxic concentrations of metal can induce growth reduction because of the interference with nutrient uptake, and photosynthetic activity. Cu affects nitrogen uptake as it impairs plasma membrane functionality (Burzyński and Buczek, 1994), and it also affects nitrogen assimilation by blocking the cytosolic enzyme nitrate reductase (Kłobus et al., 2002). In accordance with these observations we found that nitrogen concentration decreased in plants supplied with Cu (Table 3), thus implying a possible interference between the metal and the nitrogen uptake.

To evaluate whether the two poplar species were similarly affected by Cu treatment in term of photosynthetic efficiency, we measured the chlorophyll content and the main photosynthetic parameters. It is known that interveinal chlorosis of leaves is one of the firstly visible symptoms of Cu toxicity (Taylor and Foy, 1985), and that it is due to decreased rate of chlorophyll biosynthesis and chlorophyll content, caused by damages to thylacoid membranes (Quartacci et al., 2000). In tolerant plants, however, it has been reported that chlorophyll content increases or does not significantly change in response to treatment with metals (Baszyński et al., 1982; Stiborova et al., 1986). When we measured the chlorophyll content after 80 days of Cu treatment, we noticed an increase in Chl_a and Chl_b content in *P. x canadensis* grown with 25 and 75 μM Cu, and a decrease in *P. alba* grown with 75 μM Cu (Table 4). That points out a higher Cu tolerance in *P. x canadensis* than in *P. alba*. Cu in leaves causes toxicity mainly because it negatively interferes with the electron transport chain and the oxygen evolving complex of the PSII

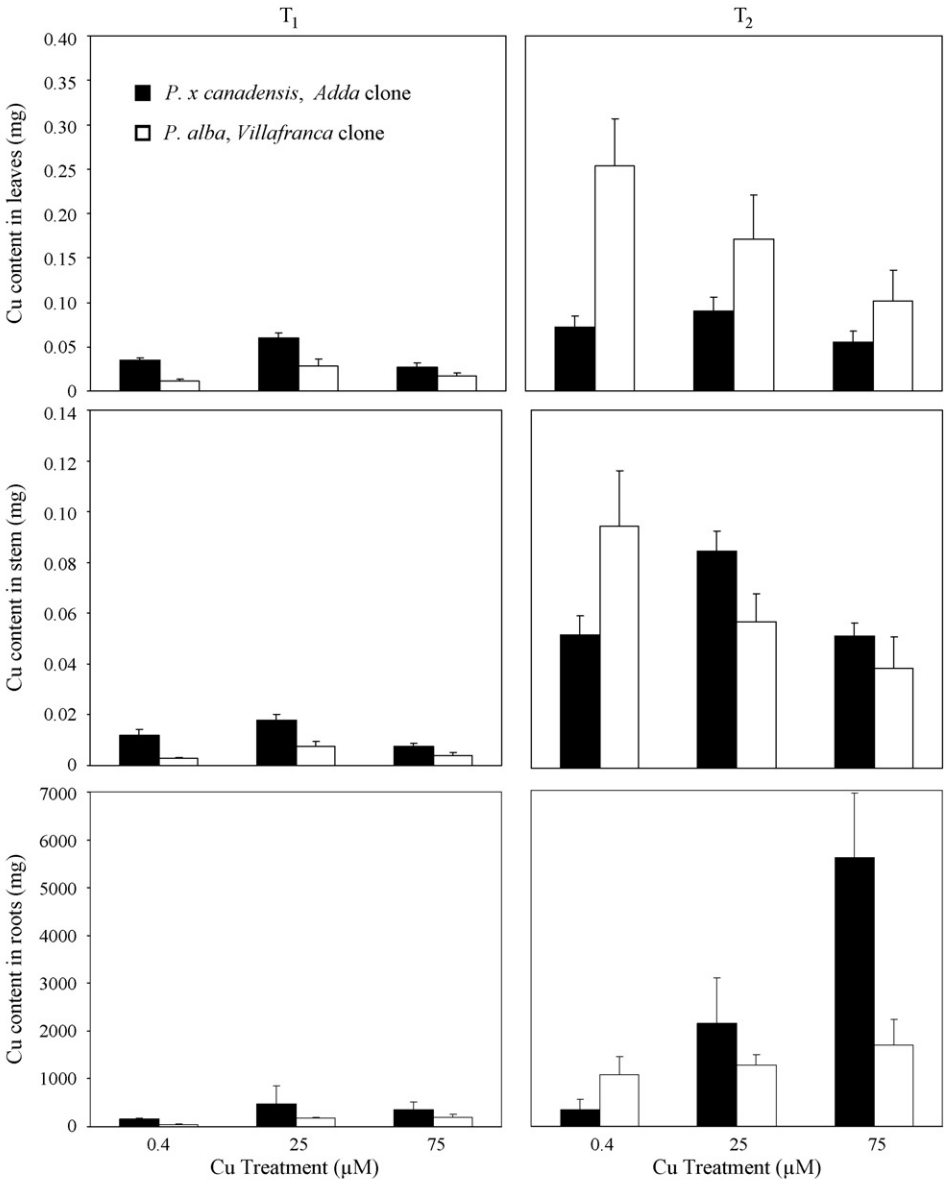
(Romanowska, 2002). Moreover, it induces a decrease of initial (F_o) and maximal (F_m) fluorescence (Boucher and Carpentier, 1999), and it inhibits variable fluorescence (F_v) (Ouzounidou et al., 1993). No significant differences were reported for F_v and F_m in the two poplar species (Table 5); however, light-saturated rate of electron transport (J_{max}) and maximum rate of carboxylation (V_{cmax}) revealed that the two clones had different responses to Cu (Table 5; interaction C × M; $P = .0398$). In *P. x canadensis*, J_{max} and V_{cmax} slightly increased along the treatment, while the opposite was reported for *P. alba*. Accordingly, the photosynthetic rate increased in *P. x canadensis* while it decreased in *P. alba* (Fig. 1), possibly explaining the reduction of growth reported in *P. alba*. Altogether these results showed that the photosynthetic apparatus of *P. alba* is more sensitive to high Cu than that of *P. x canadensis*, in which the stomata conductance only seemed to be slightly affected by the treatment with the metal (Fig. 1). An explanation of these different behaviours was found by measuring Cu concentration in different plant organs, which revealed that the two species differed in term of metal allocation. Cu concentration in roots increased with increasing Cu treatment



ANOVA *P-value*	Main effects			Interactions			
	Clone	Metal	Time	C*M	C*T	M*T	C*M*T
Leaves	.0069	.0421	.0000	ns	.0001	ns	ns
Stem	ns	.0000	.0012	ns	.0002	ns	ns
Roots	ns	.0000	ns	ns	ns	ns	ns

Fig. 2. Cu concentration in leaves, stem and roots of *P. x canadensis*, *Adda* clone (black bars) and *P. alba*, *Villafranca* clone (white bars) grown with 0.4 (control), 25 and 75 µM Cu for 30 (T_1) or 80 (T_2) days. Units are mg kg^{-1}. Values are mean ± standard error ($n = 4$). ANOVA: P values for main effects and interactions clone × metal (C × M), clone × time (C × T), metal × time (M × T), clone × metal × time (C × M × T) are shown.

level in both species, while in leaf it increased only in *P. alba* (Fig. 2; time T_2). Plants that preferentially accumulate heavy metals in roots generally show a wide range of metal tolerance, as the photosynthetic apparatus is protected from toxicity (Pulford et al., 2002). Accordingly photosynthesis was highly impaired in *P. alba*, while *P. x canadensis* retained good photosynthesis capacity. On the other hand, heavy metals accumulated in roots often imply root toxicity, resulting in a reduction of root growth. Root biomass reduction and changes in root morphology have been described as a feature of Cu toxicity (Arduini et al., 1995; Patterson and Olson, 1983), while the redistribution of roots to less contaminated zones of soil has been reported as an example of facultative tolerance in trees (Pulford and Watson, 2003). In our study we observed both responses: reduction of root biomass and root thickening was observed at increasing levels of Cu in *P. alba*, while root growth seemed marginally affected in *P. x canadensis*. At the same time, at high Cu concentration, the root system in *P. x canadensis* developed preferentially on the pot



ANOVA *P-value*	Main effects			Interactions			
	Clone	Metal	Time	C*M	C*T	M*T	C*M*T
Leaves	.0001	.0095	ns	.0346	.0000	ns	ns
Stem	ns	.0244	.0000	.0265	ns	ns	.0210
Roots	.0156	.0008	.0000	.0121	ns	.0024	.0120

Fig. 3. Total Cu content in leaves, stem and roots of *P. x canadensis*, *Adda* clone (black bars) and *P. alba*, *Villafranca* clone (white bars) grown with 0.4 (control), 25 and 75 µM Cu for 30 (T_1) or 80 (T_2) days. Units are mg. Values are the mean ± standard error ($n = 4$). ANOVA: *P* values for main effects and interactions clone × metal (C × M), clone × time (C × T), metal × time (M × T), clone × metal × time (C × M × T) are shown in the table.

surface instead than deeply, which may be interpreted as a plant attempt to avoid metal toxicity.

The tolerance of *P. x canadensis* towards high Cu concentrations (TI_{25} = 93.0 and TI_{75} = 77.2) was remarkable, as well as its ability to accumulate the metal in the root system without suffering from toxicity. The wide and well-expanded root apparatus of this clone could be advantageously exploited in phytostabilization programs to avoid the spreading of pollutants through the environment. Because the accumulation of Cu in stems of plants treated with 25 μM Cu was quite high as well (0.08 mg on average; Fig. 3), the utilization of *P. x canadensis* in phytoextraction processes could also be feasible. Furthermore, as Cu concentration and total Cu content in leaves did not increase with increasing metal concentration in the treatment (Figs. 2 and 3; leaves at time T_2), the return of the metal to soil by annual leaf shedding could be avoided.

Tolerance to Cu was lower (TI_{25} = 42.7 and TI_{75} = 26.3) in *P. alba*, as expected in species that accumulate heavy metals in shoots more than in roots, while the root to shoot translocation rate was high, as desired for phytoextraction technologies. When heavy metals are accumulated in leaves, the risk of spreading and maintaining pollutants into the environment by leaf fall becomes an important consideration. Mertens et al. (2004) noticed that for this reason white poplar is not suitable for Cd and Zn phytoextraction. However, plants with a specific leaf accumulation profile are often used as biomonitors, because they give information on the quality of the environment (Markert et al., 2003). Biomonitoring using trees has been extensively exploited to evaluate the level of environmental pollution from soil and atmosphere, as well as the pollutant availability to living organisms. Among trees, poplars have been successfully employed in USA and in EU (Djingova et al., 1999; Wagner, 1993). Madejón et al. (2004) used *P. alba* leaves to monitor trace elements in soil contaminated by mine spill-out and they found that Cu concentration in leaves correlates with metal availability in surface and deep soil layers. We report here that *P. alba*, *Villafranca* clone and the hybrid *P. x canadensis*, *Adda* clone responded differently to high Cu treatments in terms of tolerance and metal allocation. *P. x canadensis*, *Adda* clone, accumulating Cu mainly in roots, present a trait sought in plants suitable for phytostabilization, while *P. alba*, *Villafranca* clone, with a high root to shoot translocation rate, could be exploited as an indicator of Cu polluted soils.

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