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BRIEF COMMUNICATION

Zinc and cadmium effects on growth and ion distribution in *Populus tremula* × *Populus alba*

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Abstract

The effects of cadmium and zinc on growth and mineral distribution in *Populus tremula* × *P. alba* genotype 717-1B4 were investigated. Exposure to 360 mg(Cd) kg^{-1}(soil) resulted in accumulation of Cd in all organs and inhibition of primary and secondary growth as well as of the net photosynthetic rate. No growth inhibition occurred under zinc exposure. Cd was mainly stored in the woody parts of stem, whereas zinc was preferentially localized in the leaves. Cd treatment also altered distribution of Zn^{2+}, Ca^{2+}, Mg^{2+}, K^{+}, and Fe^{2+} in different organs.

Additional key words: poplar, mineral distribution, net photosynthetic rate, stomatal conductance.

Plants responses to heavy metals are drawing growing attention, as metal pollution is still increasing worldwide and threatens the stability of our ecosystems (Menon *et al.* 2007). Metals exist naturally in the environment and take part in the normal metabolism of plants. Yet beyond a certain threshold, that is variable according to the metals and plant species, phytotoxicity can occur (Shamsi *et al.* 2008, Markovska *et al.* 2009). Cadmium (Cd) is a toxic heavy metal albeit plants often easily absorb it. This bioavailability is most likely due to its similarities with zinc (Zn), an essential element for life. Though essential for plant metabolism, zinc can also be present at an excessive level in the environment, adversely affecting ecosystems. Besides, a contamination of the environment by cadmium often occurs as a result of zinc pollution, since Cd is a by-product of the Zn industry.

The first strategy for a plant to resist to a high amount of toxic metal ions in its environment is to hamper their entrance, or to develop active effluxes from roots. However airborne particles deposition on leaves accounts for a substantial contribution to metal pollution of plants that cannot be prevented by root efflux (Yaaqub *et al.* 1991). Plants unable to impede entry of toxic ions must then possess inner mechanisms of detoxification and mostly compartmentalisation. This is sometimes insufficient, at least partly due to lack of specificity of ions transporters. Essential ions are conveyed *in planta* by specific and multiple transporters, *e.g.* P-type ATPases, cation diffusion facilitator, ZIP, *etc.* (Colangelo and Guerinot 2006). Meanwhile the translocation of toxic elements like cadmium is not fully explained yet.

Metals are reported to be unevenly distributed among species and among plant organs and tissues. The roots are most often directly in contact with the pollutants, especially in the case of edaphic pollutions. This is why roots usually exhibit the greatest metal content in the plant (*e.g.* Wojcik and Tukiendorf 2005, Deram *et al.* 2006, Unterbrunner *et al.* 2007). Yet, metal ions can also concentrate in aboveground organs, especially in hyperaccumulator plants like *Thlaspi caerulescens*

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Abbreviations: g_s - stomatal conductance; P_N - net photosynthetic rate.
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(Roosens *et al.* 2003). The metal distribution inside the plant represents *per se* an indicator of the tolerance and/or accumulation mechanisms. Besides supplying an important biomass, tree species would provide a practical material: the wood, from which little dispersal is likely to occur. So, the storage of heavy metals in woody parts appears to be prominent for the validation of a proper woody phytoremediator.

This study describes primary and secondary growth and gas exchange of young pot-grown poplars submitted to Cd or Zn, and the consecutive stress they endured. The paper provides the profiles of the content of major elements, namely Ca, Mg, K and Fe, as for the two heavy metals Cd an Zn, in the poplar organs, including different tissues of the cutting.

Woody stem cuttings obtained from 1-year-old cut-back stems of *Populus tremula* L. × *P. alba* L. [*Populus* × *canescens* (Aiton) Smith] genotype INRA 717-1B4 were planted into sand. After rooting (1 month), plants were placed during 6 weeks in 1 dm^3 pots composed of sand and peat moss (25:75, v/v, pH 6.9) to allow stem and leaf growth. Then the plants were transferred into 10 dm^3 pots containing uncontaminated or contaminated soil. Concomitantly plants were pruned in order to make sure that the new leafy stems were entirely formed while plants were exposed to metal. The heavy metal constraints consisted in the addition of 1 mmol of Cd or Zn at the beginning of the treatment (day 0). The actual concentration of metal in soils and in soil solutions was measured (Table 1). The temperature was 21 ± 2 °C, relative humidity 70 ± 5 % and an irradiance 1000 µmol m^{-2} s^{-1} provided during 16-h photoperiod. At the end of the treatment (day 61), plants were divided into roots, cutting (initial stem utilized to root the plant), stem (newly formed stem during the treatment) and leaves. The cuttings were lyophilised and separated into 3 parts: xylem, cambial zone and bark.

During the treatment, the total leaf area and the diameter of the cuttings (radial growth) were measured as described by Morabito *et al.* (2006). The net photo-synthetic rate (P_N) and the leaf stomatal conductance to water vapour (g_s) were measured on the last fully expanded leaf. Measurements were conducted with a portable photosynthetic system (*Ciras-2, PP Systems*, Hitchin, Herts, UK). Zn, Cd, K, Ca, Mg and Fe were quantified as described by Marchand *et al*. (2006). Dry samples (100 mg) were grinded into a fine powder and resuspended in 10 cm^3 of an acid mixture containing $HClO_4$ (60 %) + HNO_3 (85 %) + H_2O (1:1:1, v/v). Subsequently 1 cm^3 H_2O_2 (30 %) was added in each sample. Digestion was completed in a *MDS 2000* (*CEM Corp*.®, Mathews, NC, USA) microwave, 15 min at 190°C and 1.3 MPa. The final volume was brought to 100 cm^3 with double distilled water. Heavy metals and ions contents were determined on a *Jobin-Yvon® HR-ICP-AES* (Edison, NJ, USA) from three individual plants per treatment. The heavy metal content in soil as well as the concentration in the soil solution were also measured.

Measurements were carried out on 3 to 5 biological replicates. A Student's *t*-test was performed to determine significance of differences.

Normal Cd soil content is about 1 mg kg^{-1} (Peer *et al.* 2005) but less than 2 % of this metal is usually in bioavailable form (Ma and Rao 1997). The Cd treatment applied in the present study, 360 mg(Cd) kg^{-1}(soil DM), with a soil solution close to 20 µM Cd, represented a severe pollution. The resulting soil zinc content, 265 mg(Zn)kg^{-1}(soil DM) or 140 µM Zn in soil solution was at least 2-fold higher than in nature. However, this concentration was lower than the phytotoxicity thresholds (*e.g*. Paschke *et al.* 2006). *In vitro*-cultured *Populus alba* showed toxicity when exposed to 0.5 to 3 mM Zn (Castiglione *et al.* 2007).

Under Zinc treatment, Zn content in the soil was approximately 9-fold higher than in control pots and corresponded to 265 mg kg^{-1}(DM). Under cadmium treatment the zinc content did not significantly differ from control soil and corresponded to 37.1 mg kg^{-1}(DM), whereas Cd content in the soil was 360 mg kg^{-1}(DM). Cd was detected neither in control nor in Zn-treated soils. Under control conditions, soil solution contained 8.3 µM Zn^{2+}. When Zn treatment was applied, this concentration reached 140.4 µM Zn^{2+}. The Cd treatment resulted in a soil solution containing 20.8 µM Cd^{2+} and 7.3 µM Zn^{2+}.

Table 1. Soil constraint characterization and physiological parameters of *Populus tremula* × *P. alba* genotype 717-1B4 exposed 61 d to 360 mg(Cd) kg^{-1}(soil), to 265(Zn) mg kg^{-1}(soil) or to control condition. Means ± SE (n = 3 to 5; significant differences in comparison with control at * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$ according to Student *t*-test; nd - not detectable).

Parameter	Control	Zinc	Cadmium
Zn in soil [mg kg^{-1}]	29.7±0.5	265.0±17.3***	37.1±7.7
Zn^{2+} in solution [µM]	8.3±2.3	140.4±10.6***	7.3±1.1
Cd in soil [mg kg^{-1}]	nd	nd	360.0±15***
Cd^{2+} in solution [µM]	nd	nd	20.8±0.5***
K in soil [g kg^{-1}]	3.1±0.1	2.9±0.2	2.5±0.1
Mg in soil [g kg^{-1}]	2.2±0.1	2.2±0.1	2.1±0.1
Ca in soil [g kg^{-1}]	2.9±0.1	3.6±0.6	3.5±0.7
Total leaf area [dm^2]	21.8±3.1	25.0±3.6	7.8±1.3**
Diameter increase [mm]	2.7±0.4	2.3±0.2	0.1±0.2
Root dry mass [g]	3.8±1.8	5.7±2.0	0.5±0.1*
Cutting dry mass [g]	12.6±0.4	13.1±2.1	7.8±0.3***
Stem dry mass [g]	7.9±2.9	9.1±1.8	1.3±0.1*
Leaves dry mass [g]	19.0±6.4	22.3±4.3	2.8±0.2*
g_s [mmol(H_2O) m^{-2} s^{-1}]	156.0±20	141.0±16	68.0±20*
P_N [µmol(CO_2) m^{-2} s^{-1}]	22.7±0.9	19.8±2.0	1.7±0.2***

After stem pruning on day 0, which corresponds to the beginning of the metal treatments, bud break occurred on day 21. The primary growth, estimated by the total leaf area, was not altered under Zn treatment whereas a 64 % decrease occurred in Cd-treated plants on day 61

(Table 1). Zn treatment had no effect on the dry mass of the different plant organs, however, DM of all organs exhibited a significant reduction caused by Cd exposure (Table 1).

Zn treatment had no significant effect on P_N and g_s. Cd treatment caused an important inhibition of P_N and g_s (93 and 56 %, respectively; Table 1).

Under control conditions, the stem presented the highest zinc content (Table 2). After Zn exposure, all organs presented a significant increase in Zn content. When exploring the different tissues of the cuttings, Zn treatment did not change Zn content in xylem nor in the cambial zone, while the Zn contant was almost doubled in bark compared to control plants. Under Cd exposure, Zn content in leaves was 2.3 fold higher than in control (Table 3).

Table 2. Contents of Cd, Zn, Fe [mg kg^{-1}], Ca, Mg and K [g kg^{-1}] in dry mass of poplar 717-1B4 exposed to 360 mg(Cd) kg^{-1}(soil), to 265(Zn) mg kg^{-1}(soil) or to control condition (C) for 61 d. Means ± SE (n = 3; significant differences in comparison with control at ** - P < 0.01, *** - P < 0.001 according to Student t-test; nd - not detectable).

Ions		Roots	Cutting	Stem	Leaves
Cd	C	nd	nd	nd	nd
	Zn	nd	nd	nd	nd
	Cd	255.1±68***	85.6±18***	92.7±4***	84.0±7.5***
Zn	C	46.6±7.5	33.3±5.8	60.1±7.3	35.3±5.4
	Zn	194.4±36**	83.8±8.7**	157.3±25**	325.6±16***
	Cd	40.1±3.5	36.3±7.8	46.1±4.5	79.6±3.6**
Fe	C	478.0±156	103.0±7.0	49.0±7.0	102.0±14
	Zn	352.0±34	109.0±9.0	42.0±11	111.0±4.0
	Cd	608.0±100	114.0±9.0	49.0±13	110.0±31
Ca	C	8.3±0.7	8.4±0.4	8.0±0.4	7.5±0.6
	Zn	8.8±0.4	9.7±0.7	8.2±0.4	9.0±0.3
	Cd	9.9±0.6	9.3±1.5	12.0±0.1**	11.2±0.7**
Mg	C	1.6±0.1	0.7±0.1	1.7±0.01	2.1±0.1
	Zn	1.5±0.1	0.8±0.0	1.8±0.1	2.5±0.1
	Cd	1.2±0.0**	0.5±0.0	1.8±0.2	4.1±0.2***
K	C	23.1±2.6	6.5±1.1	21.6±0.7	18.6±1.5
	Zn	19.7±2.2	7.2±0.1	21.3±0.2	18.4±0.6
	Cd	10.5±0.7**	3.3±0.12*	20.4±1.2	43.2±5.2**

Under control and Zn constraints, no Cd was detected in plants (Table 2). Under Cd treatment, the highest Cd content occurred in the roots. In the cutting, Cd accumulated more in the bark and in the cambial zone than in the xylem (Table 3).

Iron content remained unchanged under both treatments (Table 2). Under Zn exposure, no change occurred in potassium, calcium or magnesium content. Under Cd exposure, roots exhibited a reduction in K^+ and Mg^{2+} content. K^+ content also decreased in the cuttings whereas it increased in leaves. Calcium content increased in the stem and leaves. A modified status of metal homeostasis in response to Cd was reported in hyperaccumulator species (Küpper *et al.* 2001) and a Cd-induced increase of K^+ content in leaves was observed in also soybean (Drazic *et al.* 2004). These changes may aim to alleviate Cd toxicity. Indeed, Zn^{2+}, Mg^{2+}, and Ca^{2+} were reported to reduce Cd phytotoxicity by partly impeding Cd uptake from soil (Zoghlami *et al.* 2006) and protective effect against Cd toxicity *in planta* have been ascribed to Zn^{2+} (Aravind and Prasad 2005).

Table 3. Zinc and cadmium content [mg kg^{-1}] in the xylem, cambial zone and bark of poplar 717-1B4 stems exposed 61 d to 360 mg(Cd) kg^{-1}(soil), to 265(Zn) mg kg^{-1}(soil) or to control conditions. Means ± SE (n = 3; significant differences in comparison with control at ** - P < 0.01, *** - P < 0.001 according to Student t-test; nd - not detectable).

Metal	Treatment	Xylem	Cambial zone	Bark
Zn	control	32.8±7.1	169.7±28.6	104.9±7.90
	Zn	28.7±1.8	184.9±32.5	199.6±18.4**
	Cd	27.2±4.1	168.4±16.7	89.3±1.90
Cd	control	nd	nd	nd
	Zn	nd	nd	nd
	Cd	16.5±6.2***	122.9±28.8***	158.1±40.9***

Although cadmium is absorbed near to hyper-accumulation level, its handling by the plant is problematical. Indeed, an unefficient Cd compart-mentalisation can lead to impaired functioning of meristems, necrotic spots on leaves, and reduced growth under severe Cd exposure. In roots, Cd toxicity can induce cytoskeleton perturbation (Xu *et al.* 2009). Survival of 717-1B4 poplar genotype under such drastic condition is not granted. On the other hand, the results confirm the ability of this poplar genotype to pursue growth and development in Zn-polluted soils. The plant appeared to be able to uptake and to compartmentalise efficiently Zn in its leaves and bark under a moderate Zn contamination. Poplar biomass production and metal bioaccumulation factors in tissues plead for its use in Zn phytoextraction.

Beyond this study, it would be worth exploring the physiological response of such trees at the tissue level where specific protein profiles could be associated to ion transport and storage.

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