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Comparison of cadmium effect on willow and poplar in response to different cultivation conditions

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Abstract

Salix alba L. and *Populus* × *euroamericana* cv. Robusta cuttings were grown in 10 µM $Cd(NO_3)_2$ (direct treatment) or in Knop solution and afterwards in $Cd(NO_3)_2$ (indirect treatment). Cd impact on rooting of directly treated plants and its impact on normally formed roots and shoots of indirectly treated plants were studied. The cumulative length, number and biomass of willow roots, pigment and starch contents, leaf net photosynthetic rate and dry mass/leaf area ratio of willow leaves were positively influenced by indirect treatment. However, indirectly treated poplars were more sensitive to Cd than directly treated ones. Indirect treatment lowered root Cd uptake in willow, Cd accumulation in cuttings of both species and Cd accumulation in poplar shoots. Cd-caused structural changes were similar in both species and in both treatments. Root apices, rhizodermis and cortex were the most seriously damaged root parts. In directly treated willow, the structure of central cylinder (0.5 - 1 cm from apex) remained unchanged in contrast to indirectly treated plants. Formation of cambium close to the apex indicated shortening of root elongation zone of indirectly treated plants. Directly Cd-treated poplar roots exhibited unusual defence activity of root apical meristem and accumulation of darkly stained material around central cylinder.

Additional key words: Cd uptake and accumulation, growth, lateral root primordium, root apex, saccharide content.

Introduction

Large areas of land are contaminated, many with heavy metals, so novel technological approaches are required to remove excess toxic metals. New bioremediation strategies concerning cleaning up of contaminated substrates using phytoextraction led to enhance interest of physiological study of potentially (hyper)accumulating plants. One of approaches, which are being developed to extract toxic metals from soil, is the use of fast growing trees. Perspective clones able to accumulate metals (Zn and Cd) and many kinds of toxic organic compounds were found in the *Salix* (Greger and Landberg 1999) and the *Populus* species (Predieri and Gatti 2000). *Salix phylicofolia* belongs to shoot accumulators of Zn and Cd (Stolz and Greger 2000). Woody plants have characteristics important for effective phytoextraction: perennial habit, high biomass production, large amount of deeply penetrating roots and high transpiration rate (Stomp 1993).

Structural and ultrastructural alterations are either cause or consequence of metabolic and physiological dysfunction (*e.g.*, Verma and Dubey 2001, Fornazier *et al.* 2002) in metal stressed plants, mainly after relative large exposure time to toxic metal (Prasad and Hagemeyer 1999). Root tip damage is, together with decrease of root elongation rate, collapsing of root hairs or decrease of their number, decrease of root biomass, increase or decrease of lateral root formation, one of the main morphological and structural effects caused by Cd (Hagemeyer and Breckle 1996). Surface damage (Vogelei and Rothe 1988) and plasmolysis of epidermal cells (Wagatsuma *et al.* 1987) have been found after severe Al-stress. Increased vacuolation, the occurrence of autophagic vacuoles, accumulation of electron-dense

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Abbreviations: Car - carotenoid; Chl - chlorophyll; PAR - photosynthetic active radiation; P_N - net photosynthetic rate; RAM - root apical meristem; R_D - dark respiration rate; SLM - dry mass per leaf area (specific leaf mass).

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deposits in vacuoles, altered Golgi apparatus activity and decrease of meristem length seem to be common features of metal stressed root tips (Prasad and Hagemeyer 1999).

Inhibition of root extension growth can be the result of interference with cell division or with cell elongation. Trace elements have been shown to affect both processes. However, the result of stress depends not only on its cause, but also on its intensity (Hagemeyer and Breckle 1996). The dry mass production of roots was increased by low soil concentration of Cd or Zn. But, at higher concentration of both metals, root growth was strongly inhibited (Hagemeyer *et al.* 1994). Röder (1987) observed increase of root length by 45 µmol(Cd) kg^{-1}(soil) and consequent reduction of their dry mass. The extension growth of primary roots of beech seedlings was reduced by 15 % after exposure to 62 µmol(Cd) kg^{-1}(soil), a treatment with 187 µmol(Cd) kg^{-1}(soil) reduced growth by 85 % (Bertels 1989). Experimental data indicate that a limitation of water availability and a lower regulation capacity of stomata as well as an apparent decrease in hydraulic conductance in growing stem and roots seem to be the major constraints of Cd stress (Hagemeyer and Waisel 1987, 1989).

Different conditions of root formation may cause alterations in their structure and growth characteristics, which may influence the uptake and translocation of toxic ions. These changes can result in modification of physiological processes and growth parameters of the whole plant. The aim of our study was to compare some physiological and anatomical characteristics of the plants of two fast growing trees - willow and poplar - rooted and grown directly in Cd solution with plants rooted in optimal nutrient conditions (Knop solution) and afterwards transferred to Cd.

Materials and methods

Two species of fast growing trees, *Salix alba* L. cv. 21 and *Populus* × *euroamericana* cv. Robusta, providing from the Institute of Forest Research, Research Station Gabčíkovo, were used for experiments. Stem cuttings, 18 cm long, from last year shoots cut in March before beginning of growing season, were grown hydroponically in growth cabinet under: air temperature of 25 °C, relative air humidity 70 %, 12-h-photoperiod with irradiance 100 µmol m^{-2} s^{-1} (PAR). First part of cuttings (direct treatment) was directly rooted and grown in solution of 100 µM $Ca(NO_3)_2$ (control) or in the same solution with addition of 10 µM $Cd(NO_3)_2$ for twenty one days (Greger and Landberg 1999). Second part of cuttings (indirect treatment) was firstly rooted in Knop nutrient solution for 10 d. Then the plants were transferred into 100 µM $Ca(NO_3)_2$ and after 3 d half of them was placed into 10 µM $Cd(NO_3)_2$ for 8 d. The solutions were changed every 3 d to prevent depletion of metals, nutrients and oxygen.

Shoots of intact plants were placed in the thermostabilised chamber and CO_2 exchange rate was measured by infrared gas analyser (IRGA - *Infralyt*, Dessau, Germany) at the air temperature in the chamber of 25 °C and irradiance of 350 µmol m^{-2} s^{-1} PAR (saturating irradiance as determined in previous experiments). Finally, root and leaf dark respiration (R_D) were measured. Specific leaf mass (SLM) was calculated as the ratio of leaf dry mass per leaf area. The measurement and equipment used have been described in detail by Masarovičová (1997).

Chlorophylls and carotenoids were extracted with the 80 % acetone and their contents were determined spectrophotometrically (*Jenway 6405 UV/Visible*, London, Great Britain): chlorophyll *a* at 663.2 nm, chlorophyll *b* at 646.8 nm, carotenoids at 470 nm. Chlorophyll and carotenoid contents were calculated according to Lichtenthaler (1987). Stomata density and stomata length were detected using microrelief method and evaluated with microscope-lanameter (*MP3*, *PZO*, Cracow, Poland).

Leaf starch content was determined in fresh material, extracted with 80 % ethyl alcohol and centrifuged at 2 000 *g* for 10 min. The precipitate was solubilised in 52 % $HClO_4$, left to stand on ice for 15 min and centrifuged at 3 000 *g* for 15 min. Starch content in supernatant was determined spectrophotometrically (*Jenway 6405 UV/Visible*) with anthron solution (0.1 g anthron in 50 cm^3 95 % H_2SO_4) at 630 nm (Davídek 1981). Soluble saccharides were extracted with 75 % ethyl alcohol, toluene and left to stand for 3 h and then centrifuged at 12 000 *g* for 30 min. For reducing sugar evaluation Somogy solution was added to the supernatant. After 15 min in water bath Nelson solution was added and samples were left to stand for 30 min. Reducing sugar content was determined in diluted samples spectrophotometrically (*Jenway 6405 UV/Visible*) at 710 nm. Non-reducing sugars were hydrolysed with $C_2H_2O_4.2\ H_2O$, neutralised with NaOH and, consequently, the same method was used for determination of total sugars. Non-reducing sugar content was calculated from total sugar and reducing sugar content afterwards (Oser 1971).

Production parameters included cumulative length, number and dry mass of roots and shoots (newly growing parts without the original cuttings).

Root apices and root segments (1 - 1.5 mm long) from region 0.5 - 1 cm from the root tip were fixed in 5 % glutaraldehyde in 0.1 M phosphate buffer, postfixed in 0.5 % OsO_4 and dehydrated in ethyl alcohol through 10 % step graded series and finally in pure propylene oxide. Samples were embedded in Spurr resin (*Sigma*, Taufkirchen, Germany). Semithin sections (1 µm) were cut with ultramicrotome *LKB Nova* (Bromma, Sweden) with glass knives. Sections were stained with toluidine blue and basic fuchsine (Lux 1981) and observed using a *Zeiss Axiolab* microscope (Jena, Germany). Photographs were taken with 100 ASA black and white negative films.

Dried powdered samples were digested with HNO_3, HF and H_3BO_3 and flame atomic absorption spectrometer (*Perkin Elmer 1100*, USA) was used to determine Cd content. To evaluate the significance of obtained data the Statgraphic software (Student's *t*-test) was used.

Results and discussion

The physiological and production parameters of the control plants were compared with plants grown at Cd treatment, and the plants rooted directly in $Cd(NO_3)_2$ (direct treatment) were compared with plants firstly rooted in Knop nutrient solution and transferred to $Cd(NO_3)_2$ afterwards (indirect treatment).

It was found that Chl *a*+*b* and Car contents in leaves were not influenced by Cd treatment. However, nutrient supply positively influenced pigment contents in willow (Table 1). P_N of willow was not negatively influenced by Cd, but significant decrease of P_N in relation to Cd was found in poplar grown directly in $Cd(NO_3)_2$. This finding could be related to decrease in chlorophyll content in poplar leaves. According to several authors (*e.g.*, Krupa 1988, Siedlecka and Baszynski 1993, Bőddi *et al.* 1995, Siedlecka and Krupa 1996, Larrson *et al.* 1998) pigment content and photosynthetic rate are negatively affected by Cd. Pre-growing of the plants in Knop nutrient solution positively influenced P_N only in willow.

Leaf R_D of indirectly Cd treated willow decreased in comparison to both the control and directly Cd treated plants. Leaf R_D of poplar was not influenced by Cd and by nutrient supply. Root R_D of poplar was higher in Cd treated plants than in control plants in both variants. Roots of willow were influenced neither by Cd nor by nutrient supply.

Cd increased reducing sugar content and decreased non-reducing sugar content in leaves of studied plants. Starch content was not influenced by Cd in both variants (Table 1). Greger and Bertell (1992), Vassilev *et al.* (1997) and Stoyanova and Tschakalova (1997) found that Cd could increase content of soluble saccharides or starch. Similar results were obtained in *Salix viminalis* L., *S. alba* L. cv. 21, *S. purpurea* L. and *S. cinerea* L. and *Populus* × *euroamericana* cv. Gigant and cv. Robusta (Lunáčková *et al.* 2003). Conditions of indirect Cd cultivation enhanced reducing sugars in plants of both willow and poplar. Non-reducing sugar content was variable. Starch content significantly increased in indirectly treated willow. This fact could be also related to enhanced P_N and lowered leaf R_D. Increase of starch content under indirect treatment conditions was also found in control plants of this species (Table 1). Cadmium enhanced SLM values in both species and caused xeromorphic character of leaves – increased stomata density but reduced stomata sizes, what was also confirmed by Rawson and Craven (1975). This finding might be caused by disturbances in water balance

Table 1. Physiological, production and biochemical characteristics of willow and poplar plants in response to two different Cd-treatments (direct and indirect). Means ± SD. Comparisons were done between control (C) and direct Cd treatment (Cd) at $P = 0.05$ (*) and $P = 0.01$ (**), and between plants firstly rooted in Knop solution (K) and direct Cd treatment at $P = 0.05$ (♠) and $P = 0.01$ (♠♠).

Parameters	Treatment	Direct		Indirect	
		willow	poplar	willow	poplar
Chl *a*+*b* [mg g^{-1}(d.m.)]	C	11.75 ± 0.74	14.81 ± 0.48	15.79 ± 0.19♠♠	13.21 ± 0.69♠♠
	Cd	10.33 ± 0.36	14.68 ± 0.28	19.85 ± 1.36♠♠	13.20 ± 0.40♠♠
Car [mg g^{-1}(d.m.)]	C	1.56 ± 0.04	1.88 ± 0.11	2.32 ± 0.26	2.17 ± 0.002
	Cd	1.41 ± 0.07	1.94 ± 0.10	2.82 ± 0.23♠♠	2.35 ± 0.07♠
P_N [µmol(CO_2) $m^{-2}s^{-1}$]	C	5.07 ± 0.45	5.96 ± 0.68	5.99 ± 0.27	6.98 ± 0.68
	Cd	3.89 ± 0.18	3.73 ± 0.50	5.36 ± 0.39♠♠	2.57 ± 0.52**
Reducing sugars [mg g^{-1}(d.m.)]	C	9.16 ± 0.31	8.46 ± 0.49	12.48 ± 2.12	16.34 ± 2.23*
	Cd	21.51 ± 1.85*	21.01 ± 1.06**	32.48 ± 1.74**♠	27.52 ± 1.77*♠
Non-reducing sugars [mg g^{-1}(d.m.)]	C	13.34 ± 0.97	14.35 ± 1.37	9.91 ± 1.38♠	5.99 ± 1.21♠
	Cd	11.74 ± 1.12	8.01 ± 0.95*	1.96 ± 0.75**♠♠	12.65 ± 1.35*♠
Starch [mg g^{-1}(d.m.)]	C	2.81 ± 0.16	5.96 ± 0.26	5.23 ± 0.60♠	6.17 ± 0.41
	Cd	2.20 ± 0.18	7.00 ± 0.46	5.66 ± 0.36♠♠	6.05 ± 0.48
SLM [g(d.m.) m^{-2}]	C	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00♠♠	0.02 ± 0.00
	Cd	0.03 ± 0.00**	0.03 ± 0.02*	0.02 ± 0.001*♠♠	0.03 ± 0.00*

(Poschenrieder and Barceló 1999) and by enhanced accumulation of primary metabolites in the leaves. Different cultivation conditions did not significantly influenced SLM in poplar; however, positive effect of indirect cultivation was observed in willow (Table 1). These results could be explained by the fact, that nutrient deficiencies and heavy metal toxicities are known to induce both the starch accumulation (Vasquez *et al.* 1987) and probably also the reducing sugar accumulation within the leaves. This may be due to both an inhibitory effect on vein loading (Rauser and Samarakoon 1980) and a decrease of the sink force because of reduced root growth. Correlation between strong inhibition of root growth in *S. viminalis* and *S. purpurea* (Šottníková *et al.* 2003) and strong inhibition of P_N together with great increase of the saccharide content in leaves in these species caused by Cd was found (Lunáčková *et al.* 2003). Knop nutrient solution decreased stomata density and increased stomata length in poplar. These findings might be connected with potential effect of nutrients on water balance.

Cadmium negatively influenced cumulative length and, in some cases, both number and dry mass of roots. The negative effect of Cd on root growth was observed by several authors (*e.g.* Hagemeyer *et al.* 1986, Fargašová 2001). Shoots were not so strongly negatively influenced by Cd as roots, however, reduction of leaf area, shoot length and, in some cases, decrease of number and dry mass of shoots were also found.

From the beginning of direct cultivation there were obvious differences between the control and Cd-treated roots. When observed macroscopically roots treated with Cd had typical signs of Cd impact (see also Hagemeyer and Breckle 1996), they were shorter, thicker than control roots and their apices changed their colour from white to brownish. In indirectly treated plants the Cd influence after one-week treatment was less evident macroscopically due to a good state of root system before exposure to Cd. However, the root apices became brownish and softer at the end of the experiment.

The structure of root was similarly influenced by Cd treatment in both willow (Fig. 1) and poplar (Fig. 2). The root apex of willow in control conditions (in both direct and indirect cultivation) was formed by a large number of highly cytoplasmic meristematic cells. The root apical meristem (RAM) can be classified as the open (Fahn 1990), or intermediate open type (Groot and Rost 2001) with no clear boundary between the root proper and root cap (Fig. 1*A*). The root cap was approximately 220 µm long. Transversal section at the distance of 0.5 - 1 cm from the apex (Fig. 1*B*) showed characteristic structure of willow root with small epidermal cells, developing aerenchyma in relatively narrow cortex and central cylinder with four strands of xylem and phloem. The centre of the root was occupied by parenchymatous pith. Cd treatment influenced the root apex in both treatment variants and changed the root structure at the distance of 0.5 - 1 cm from the root tip (Fig. 1*C*-*F*). In indirectly treated plants, the cells of the root apex were highly vacuolated and pycnotic degeneration of nuclei was observed (Fig. 1*C*). Similar effect of Cd was also found in root apices of directly treated willows (Fig. 1*E*). Ontogenetic development of individual tissues was more advanced in Cd treated roots in both variants at the distance of 0.5 - 1 cm from the root tip (Fig. 1*D*,*F*) than it was in control (Fig. 1*B*). This was caused by reduced growth rate and tissue maturation closer to the apex, the typical signs of stress effect on root (Hagemeyer and Breckle 1996). Cd effect was evident mostly in peripheral root parts at this distance; epidermal and cortical cells were plasmolysed and irregularly shaped in comparison with rounded, isodiametric cells in the control. However, the central cylinder was not evidently affected in directly Cd-treated roots. On the other hand, changes in cell shape in central cylinder of the indirect Cd variant occurred. Primordia of lateral roots were developed in both Cd treatments, but cambium was formed at this distance only under indirect cultivation. Nor root primordia, neither cambium was observed at this distance in the control roots. Cells of lateral root primordia in indirect Cd variant were edged and vacuolated in comparison with young root primordia of directly Cd-treated plants containing isodiametric, non-vacuolated meristematic cells. Lateral root primordia did not emerge at the root surface after Cd treatment (in both variants), they remained hidden in the cortex. After transferring the cuttings to control conditions some lateral root primordia continued to grow and emerged on the root surface (not documented here). These new laterals substituted damaged adventitious root apices.

The histological observation of poplar roots showed similar effects of Cd as in the case of willow. Control roots of poplar had open or intermediate open type of RAM organization (Fig. 2*A*), similar to willow. The root cap of this species was approximately 340 µm long. Transversal section at the distance of 0.5 - 1 cm from the root tip showed similar pattern of organization as in willows (Fig. 2*B*). Epidermal cells were small; the aerenchyma was formed in the cortex by lysigenous cavities. Central cylinder contained four strands of xylem and phloem tissues and centrally located parenchymatous pith.

Cd caused pycnotic degeneration of nuclei, vacuolation and plasmolysis in cells of apical meristem in both indirect and direct treatment variant (Fig. 2*C*). In indirectly Cd-treated plants the organisation of RAM was not changed due to the complete stop of both cell division and growth. On the other hand, the whole organisation of the root apex was changed in plants directly cultivated in Cd. More extensive meristematic zone between the root proper and the root cap indicated the abnormal activity of RAM or reactivation of the quiescent centre induced by damage of meristematic cells (Fig. 2*E*). This behaviour of RAM demonstrates defence activity induced by

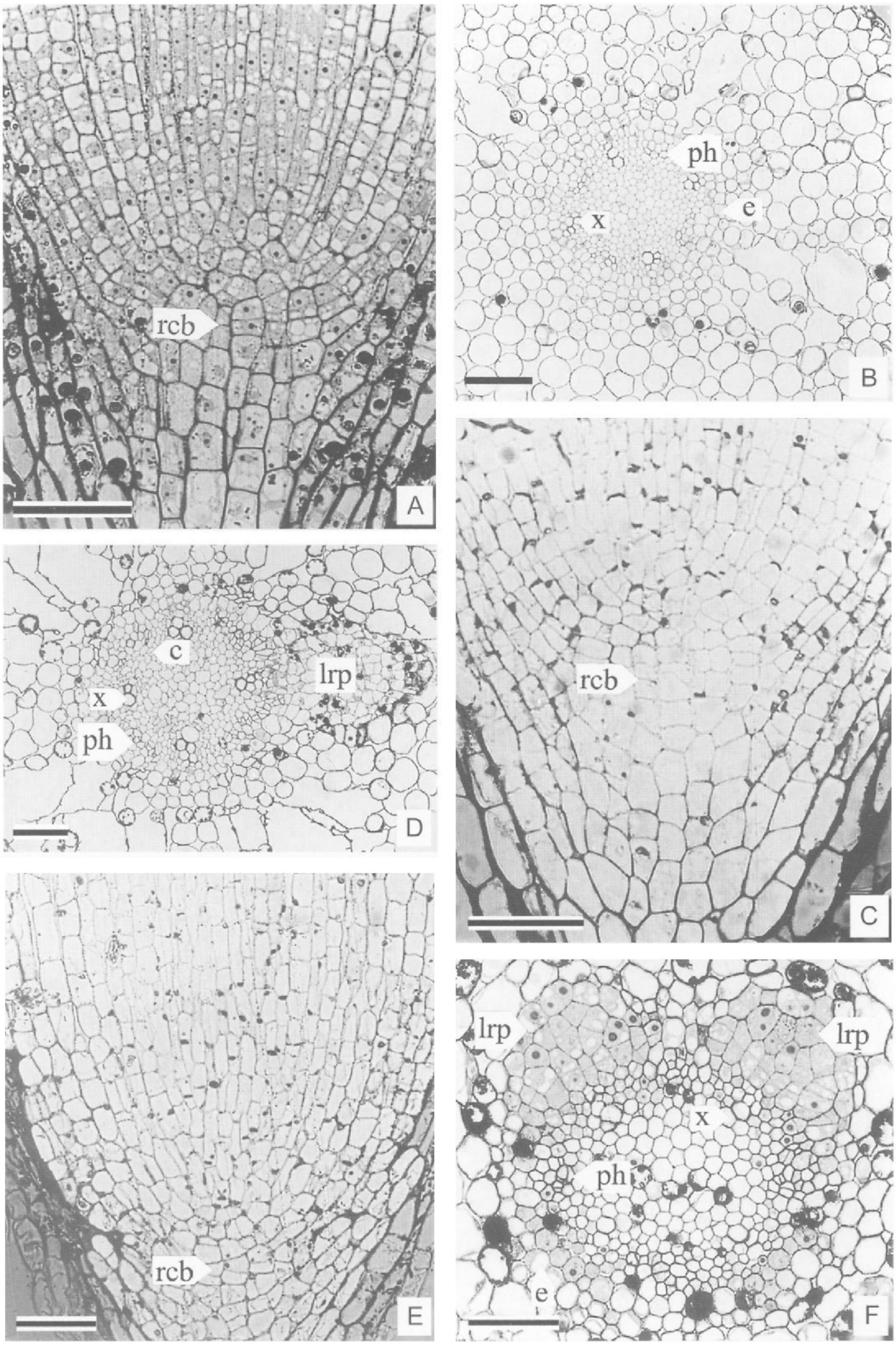

Fig. 1. Semithin sections of willow root: *A* - longitudinal section of apical meristem - indirect treatment control; *B* - cross section of root at 0.5 - 1 cm from root apex - indirect treatment control; *C* - longitudinal section of apical meristem - indirect Cd-treatment; *D* - cross section of root at 0.5 - 1 cm from root apex - indirect Cd-treatment; *E* - longitudinal section of apical meristem - direct Cd-treatment; *F* - cross section of root at 0.5 - 1 cm from root apex - direct Cd-treatment; (c - cambium, e - endodermis, lrp - lateral root primordium, ph - phloem, rcb - root cap boundary, x - xylem, *bars* = 50 μm).

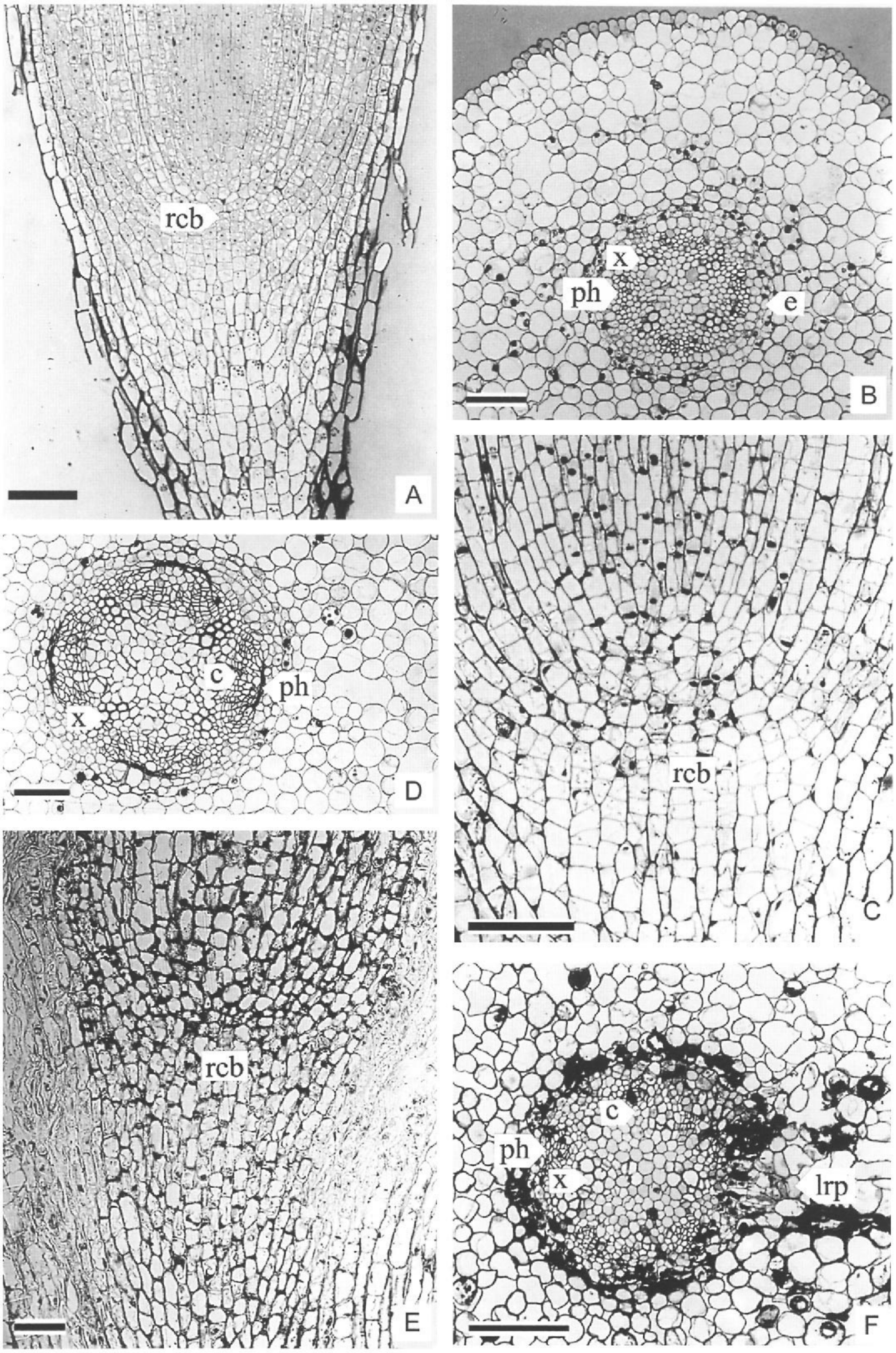

Fig. 2. Semithin sections of poplar root: *A* - longitudinal section of apical meristem - indirect treatment control; *B* - cross section of root at 0.5 - 1 cm from root apex - indirect treatment control; *C* - longitudinal section of apical meristem - indirect Cd-treatment; *D* - cross section of root at 0.5 - 1 cm from root apex - indirect Cd-treatment; *E* - longitudinal section of apical meristem - direct Cd-treatment; *F* - cross section of root at 0.5 - 1 cm from root apex - direct Cd-treatment; (c - cambium, e - endodermis, lrp - lateral root primordium, ph - phloem, rcb - root cap boundary, x - xylem, *bars* = 50 μm).

Cd-stress. The RAM resembled the open type of organization in this case. Its cells were highly vacuolated and plasmolysed. The root cap was bigger than in control and it was approximately 1 mm long. The effect of Cd resulted also in earlier maturation of tissues closer to the root apex (Fig. 2*D,F*). Cambium was formed and lateral root primordia were also developed already at the distance of 0.5 - 1 cm from the apex in both Cd variants. In directly Cd-treated poplars accumulation of darkly stained material in inner cortical layers resulted in formation of thick compact barrier surrounding the root central cylinder (Fig. 2*F*). It can be supposed that such isolation of vascular tissues has a protective character.

Table 2. Production parameters of of willow and poplar plants in response to two different Cd-treatments (direct and indirect). Means ± SD. Comparisons were done between control (C) and direct Cd treatment (Cd) at $P = 0.05$ (*) and $P = 0.01$ (**), and between plants firstly rooted in Knop solution (K) and direct Cd treatment at $P = 0.05$ (♠) and $P = 0.01$ (♠♠).

Parameters	Treatment	Direct willow	poplar	Indirect willow	poplar
Root length [m]	K	-	-	0.983 ± 0.151	0.384 ± 0.088
	C	1.774 ± 0.08	0.429 ± 0.05	1.442 ± 0.174	0.556 ± 0.085
	Cd	0.596 ± 0.07*	0.035 ± 0.01**	1.193 ± 0.148	0.249 ± 0.075*
Root number	K	-	-	17.25 ± 3.15	5.75 ± 1.65
	C	45.5 ± 3	6.7 ± 0.3	25.9 ± 3.2	5.57 ± 0.9
	Cd	40.6 ± 4	2.7 ± 1*	28.1 ± 2.3	4.30 ± 1
Root dry mass [g seedling^{-1}]	C	0.022 ± 0.002	0.011 ± 0.0007	0.026 ± 0.004	0.016 ± 0.002♠
	Cd	0.008 ± 0.001**	0.005 ± 0.002*	0.020 ± 0.002♠♠	0.008 ± 0.002**
Shoot length [m]	K	-	-	0.112 ± 0.01	0.056 ± 0.003
	C	0.206 ± 0.009	0.077 ± 0.01	0.181 ± 0.02	0.118 ± 0.003
	Cd	0.140 ± 0.010*	0.075 ± 0.01	0.139 ± 0.0159	0.083 ± 0.006**
Shoot number	K	-	-	2.5 ± 2.88	1.0 ± 0.0
	C	3.8 ± 0.60	1.7 ± 0.3	2.0 ± 0.00	1.5 ± 0.68
	Cd	2.2 ± 0.20*	1.3 ± 0.3	1.75 ± 0.80	1.25 ± 0.3
Shoot dry mass [g seedling^{-1}]	C	0.137 ± 0.011	0.195 ± 0.015	0.193 ± 0.022♠	0.403 ± 0.025♠♠
	Cd	0.168 ± 0.011	0.262 ± 0.074	0.158 ± 0.01	0.237 ± 0.03**

Cumulative length of roots was higher in indirectly treated plants in both species as explained before. However, the growth of both roots and shoots of poplar was more reduced by indirect Cd-treatment than by direct one. This indicated higher sensitivity of pre-growing poplars in comparison to plants rooted directly in Cd. Similar results were found in willow shoots. In cases, where dying back and consequent loss of roots or shoots occurred, the obtained values achieved more than 100 %. On the other hand, better root growth of indirectly Cd-treated willow was observed. These results were also confirmed by dry mass evaluation. No significant differences were found between Cd treated plants of both variants in root dry mass of poplar and shoot dry mass of both species, except for enhanced willow root dry mass in indirect Cd treatment in comparison to direct one. However, significant differences between the two types of cultivation were found in control plants (Table 2).

The highest Cd accumulation was determined in roots, then in the cuttings and the lowest accumulation was found in the newly grown shoots. Pre-growing in Knop nutrient solution did not affect root uptake of Cd in poplar; however, Cd uptake in willow was reduced by approximately 30 %. Accumulation of Cd in indirectly treated cuttings was lower about app. 50 % in comparison to direcly Cd-treated cuttings in both species. This fact was presumably connected with different time of exposition to Cd in each cultivation variant. Cd translocation and accumulation into the shoots was not affected by different cultivation conditions in willow, however, it lowered about app. 50 % in indirectly treated poplar plants in comparison to directly treated ones (Table 3).

Table 3. Cadmium accumulation [μg g^{-1}(d.m.)] in the plant organs in response to direct and indirect Cd-treatment.

Parameters	Direct willow	poplar	Indirect willow	poplar
Roots	4135.7	5014.3	2910.0	4869.0
Cuttings	106.7	116.7	62.6	45.4
Shoots	24.5	29.2	28.7	15.6

It could be concluded, that rooting in Knop nutrient solution when compared with direct cultivation in Cd had positive impact on some production parameters of *S. alba* roots (root cumulative length, number and biomass production) and some physiological characteristics of

S. alba leaves (assimilation pigment and starch contents, P_N, SLM). Roots and shoots of *P.* Robusta rooted in Knop nutrient solution were more sensitive to toxic effect of Cd than plants cultivated directly in Cd treatment. In general, cadmium negatively influenced root apices of both species in both experimental variants. On the other hand, mainly central cylinders of more distant root parts were not seriously influenced by Cd treatment. Cambial activity started and lateral root primordia were formed close to the root apex. Structural changes induced by Cd indicated better adaptation of roots of directly Cd-treated plants of both species than of roots of indirectly Cd-treated plants.

On the basis of all obtained results, as well as, above-mentioned physiological and production characteristics of fast growing woody plants, *S. alba* could be potentially used for phytoextraction of toxic metals from contaminated substrates.

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