Bull. Environ. Contam. Toxicol. (2003) 70:576–585

DOI: 10.1007/s00128-003-0024-2

Bulletin of Environmental Contamination and Toxicology

Response of Fast Growing Woody Plants from Family Salicaceae to Cadmium Treatment

L. Lunáčková,[1] E. Masarovičová,[1] K. Král'ová,[2] V. Streško[3]

[1] Department of Plant Physiology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská Dolina B-2, Sk-84215 Bratislava, Slovak Republic
[2] Institute of Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská Dolina B-2, Sk-84215 Bratislava, Slovak Republic
[3] Institute of Geology, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská Dolina B-2, Sk-84215 Bratislava, Slovak Republic

Received: 20 February 2002/Accepted: 20 October 2002

Cadmium as a trace element and toxic metal is one of the major environmental pollutants. Detrimental effects on human health are also well documented (McLaughlin and Sing 1999). The toxic effect of cadmium on plants acts as a stress factor and causes many responses at different levels. In most environmental conditions Cd enters first the roots and consequently they are likely to experience Cd damage first. There occurs inhibition of root growth, damage of root hairs and root tips (Hagemeyer et al. 1986). Cd is believed to penetrate the root through the cortical tissue. As soon as Cd enters the roots, it can reach the xylem through an apoplastic and/or a symplastic pathway (Salt et al. 1995), complexed by several ligands, such as organic acids and/or phytochelatins (Cataldo et al. 1988). Normally Cd ions are mainly retained in the roots, and only small amounts are transported to the shoots (Cataldo et al. 1983). In shoots there occur leaf roll, chlorosis, leaf growth inhibition, disturbances in stomata movements as well as acceleration of senescence (Barceló et al. 1986). Cadmium interacts with the water balance and mineral nutrition balance as the uptake of Cd ions seems to be in competition for the same transmembrane carrier with nutrients such as K, Ca, Mg, Fe (Clarson and Lüttge 1989). Natural mineral deposits containing particularly large quantities of heavy metals often support very characteristic plant assemblages and species that thrive in these metal-enriched environments. Metal levels in the tissues of indicator species generally reflect metal levels in the soil. Hyperaccumulators can concentrate metals in their above-ground tissues to levels far exceeding those present in the soil or in the non-accumulating species without significant inhibition or damage of metabolic processes. Hyperaccumulation is very important assumption for cleaning up of contaminated substrates by plants – phytoremediation (Raskin and Ensley 2000). However, many of described hyperaccumulators lack characteristics important for effective phytoextraction: perennial habit, high biomass production, extensive root mass and high transpiration rate. The woody plants have above mentioned attributes and therefore the woody plants are target for application in remediation, e.g. poplar trees have already been used for cleaning up of Se (Pilon-Smits et al. 1998). Effective phytoremediation properties combined with high biomass productivity, fast metal transport to the both roots and shoots as well as metal specificity were also confirmed for some *Salix* sp. (Greger 1999).

Correspondence to: E. Masarovičová

The aim of this paper was the screening of some fast growing woody plants (six clones of *Salix* sp. and *Populus* sp.) so as to find potentially suitable clones able to tolerate and accumulate high Cd concentration in their organs without significant inhibition or damage of metabolic processes. We studied the Cd effect on physiological and production characteristics and primary metabolite content in response to the uptake and accumulation of Cd.

MATERIALS AND METHODS

Six species of fast growing trees or shrubs (*Salix viminalis*, clone Olča, *Salix alba* 21, *Salix purpurea, Salix cinerea, Populus x euroamericana* Robusta - later only *P.* Robusta and *Populus x euroamericana* Gigant - later only *P.* Gigant) providing from Research Station Gabčíkovo, Institute of Forest Research, Zvolen were used for experiment. Stem cuttings cca 20 cm long from last year shoots were cut before the beginning of growing season (March). The cuttings were grown hydroponically in growth cabinet under the following conditions: air temperature 25 °C, relative air humidity 70%, 12 hours photoperiod with irradiance 100 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR). The cuttings were rooted and grown in control 100 μ*M* $Ca(NO_3)_2$ and in Cd concentration 10 μ*M* $Cd(NO_3)_2$ combined with 100 μ*M* $Ca(NO_3)_2$ treatment (Greger and Landberg 1999). The used Cd concentration was chosen as limited concentration that is considered as pollution to a high level (Sanita di Toppi and Gabrielli 1999) but makes studied *Salix* sp. and *Populus* sp. possible to grow. The solutions were changed every 3 days to prevent depletion of metals, nutrients and oxygen. Twenty one-day-old plants were washed in distilled water and used for experimental evaluation.

Shoot and root CO_2 exchange (photosynthesis and respiration) was determined gasometrically using a closed measurement system. A shoot of an intact plant was placed in the thermostabilised chamber and CO_2 exchange rate was measured at the air temperature in the chamber: 25 °C and irradiance: 350 μmol m^{-2} s^{-1} PAR (saturating irradiance). Finally, respiration rates (R_D) of the leaves and roots were measured in the dark. Leaf area was measured by areameter (Planix 7, SR). The values of specific leaf mass (SLM) were calculated from the leaf area and leaf dry mass. The measurement and equipment used have been described in detail by Masarovičová (1997).

Chlorophylls and carotenoids were extracted with the 80 % acetone p.a. and determined spectrophotometrically (Jenway 6405 UV/Visible, Great Britain): chlorophyll *a* at 663.2 nm, chlorophyll *b* at 646.8 nm, carotenoids at 470 nm. Chlorophyll content was calculated according to Lichtenthaler (1987). Stomata density, length and width were determined by microrelief method and evaluated with projective microscope – lanameter (MP3, PZO, Poland).

Leaf starch content was determined in fresh plant material (1 g), extracted with 80 % ethyl alcohol (4 mL) and centrifuged at 2000 *g* for 10 min. The precipitate was solubilized in 52 % $HClO_4$ (4 mL), left to stand on ice for 15 min and centrifuged at 3000 *g* for 15 min. Starch content in supernatant was determined spectrophotometrically (Jenway 6405 UV/Visible, Great Britain) with anthrone solution (0.1 g anthrone in 50 mL 95 % H_2SO_4) at 630 nm (Davidek 1981). Soluble saccharide content in the leaves was determined in fresh plant material (1

g), extracted with 75 % ethyl alcohol (1 mL), toluene (1 drop) and left to stand for 3 hr and centrifuged at 12000 *g* for 30 min. For reducing sugar evaluation Somogy solution (containing Na_2CO_3, $NaHCO_3$, $C_4H_4O_6KNa$ x $4H_2O$, Na_2SO_4, $CuSO_4$ x $5H_2O$ and Na_2SO_4) was added to the supernatant. After 15 min in water bath (100 °C) Nelson solution (containing $(NH_4)_2MoO_4$, H_2SO_4 and Na_2AsO_4 x $7H_2O$) was added and samples were left to stand for 30 min at 25 °C. Reducing sugar content was determined in diluted samples spectrophotometrically (Jenway 6405 UV/Visible, Great Britain) at 710 nm. Non reducing sugars were hydrolyzed by oxalic acid and NaOH and consequently the same method was used for determination of total sugar content. Non reducing sugar content was calculated from total sugar and reducing sugar content (Oser 1971).

Dried powdered samples were digested with HNO_3, HF and H_3BO_3 and FAAS (Perkin Elmer 1100, USA) was used for determination of Cd content.

In all experiments 5 replicates were done and for evaluation of the data significance Excel 2000 was used.

RESULTS AND DISCUSSION

For screening of chosen fast growing woody plants the following characteristics were estimated: maximal net photosynthetic rate (P_{Nmax}), mitochondrial respiration rate in the dark (R_D) for leaves and roots, reducing sugar, non reducing sugar, starch and assimilation pigment content, leaf area, specific leaf mass and stomata parameters as well as Cd uptake and accumulation.

Photosynthesis is considered to be sensitive to Cd administration. Several authors (e.g. Krupa 1988, Siedlecka and Baszynski 1993) confirmed toxic effect of Cd on photosynthetic apparatus and inhibition of its activity. In our experiment, significant decrease of P_{Nmax} was established in *S. viminalis, S. purpurea* and *P.* Gigant. However, photosynthetic rate was not influenced in *S. alba, S. cinerea* and *P.* Robusta (Table 1). Not affected P_N was also found by Haag-Kerwer et al. (1999) in *Brassica juncea* L. at 25 μM $Cd(NO_3)_2$ (similar to Cd concentration used in our experiments).

Kessler and Brand (1995) reported inhibition of mitochondrial oxidative phosphorylation by Cd ions. According to these authors cadmium inhibited this biochemical process probably by increasing the passive permeability to H^+ of the mitochondria inner membrane. In our experiment, leaf R_D significantly decreased only in *P.* Gigant, but increased in *S. cinerea.* Values of root R_D significantly increased in *S. viminalis, S. purpurea, S. cinerea* and *P.* Robusta (Table 1). Higher R_D values could be explained by the fact that toxic effect of heavy metal induced energy requires for increased metal uptake into the roots and for repairing mechanisms as a consequence of metabolism damages. There is also need of energy for increased exclusion of toxic substances or chelating processes (Procházka et al. 1998). Cadmium concentration used in our experiment did not significantly change root R_D of *S. alba*, which showed higher tolerance of this species. Root R_D of *P.* Gigant was not determined because of strong inhibition of root growth in this species.

Table 1. Values of maximal photosynthetic rate (P_{Nmax}) and respiration rate (R_D) in relation to Cd treatment.

	Control P_{Nmax} ± SE	Cd treatment P_{Nmax} ± SE	Control Leaf R_D ± SE	Cd treatment Leaf R_D ± SE	Control Root R_D ± SE	Cd treatment Root R_D ± SE
	(μmol CO_2 m^{-2} s^{-1})			(μmol CO_2 kg^{-1} d.w. s^{-1})		
S. viminalis	9.34 ± 1.43	3.11 ± 0.09*	1.32 ± 0.07	1.73 ± 0.25	89.81 ± 11.02	196.07 ± 29.76*
S. alba	5.07 ± 0.45	3.89 ± 0.18	1.52 ± 0.18	1.41 ± 0.11	106.72 ± 14.77	170.74 ± 28.81
S. purpurea	7.63 ± 0.14	2.16 ± 0.11**	1.64 ± 0.27	1.32 ± 0.11	145.16 ± 26.65	331.35 ± 11.47**
S. cinerea	4.27 ± 0.07	4.59 ± 0.32	1.14 ± 0.02	1.77 ± 0.182*	112.15 ± 9.11	427.48 ± 23.13**
P. Robusta	5.98 ± 0.68	3.73 ± 0.5	1.09 ± 0.07	1.18 ± 0.11	220.84 ± 15.4	1760.89 ± 309.38*
P. Gigant	5.77 ± 0.75	3.20 ± 0.36*	1.41 ± 0.05	0.93 ± 0.09*	522.70 ± 92.47	—

d.w. – dry weight; SE – standard deviation; * significant difference at P<0.05; ** significant difference at P<0.01

Table 2. Values of primary metabolite contents in relation to Cd treatment.

	Control Reducing sugars ± SE	Cd treatment Reducing sugars ± SE	Control Non reducing sugars ± SE	Cd treatment Non reducing sugars ± SE	Control Starch ± SE	Cd treatment Starch ± SE
			(mg g^{-1}d.w.)			
S. viminalis	7.57 ± 0.43	37.81 ± 2.17**	12.99 ± 0.5	9.97 ± 0.91	2.03 ± 0.1	3.11 ± 0.30*
S. alba	9.16 ± 0.31	21.51 ± 1.85*	13.34 ± 0.97	11.74 ± 1.12	2.81 ± 0.16	2.20 ± 0.18
S. purpurea	14.19 ± 0.96	39.33 ± 0.24**	13.96 ± 1.38	9.52 ± 1.99	3.83 ± 0.24	5.33 ± 0.47*
S. cinerea	8.41 ± 1.41	15.33 ± 1.88*	11.97 ± 0.36	8.65 ± 0.71*	5.40 ± 0.02	5.17 ± 0.45
P. Robusta	8.46 ± 0.49	21.01 ± 1.06**	14.35 ± 1.37	8.01 ± 0.95*	5.96 ± 0.26	7.00 ± 0.46
P. Gigant	14.60 ± 1.76	30.84 ± 1.43**	12.92 ± 0.84	11.55 ± 0.48	5.88 ± 0.25	5.82 ± 0.49

d.w. – dry weight; SE – standard error; * significant difference at P<0.05; ** significant difference at P<0.01

Disturbances in saccharide metabolism due to Cd-dependent inhibition of many enzymes were observed by several authors (e.g. Greger and Bertell 1992, Vassilev et al. 1997, Verma and Dubey 2001). In our experiments, the analyses of primary metabolites in the leaves showed unambiguous increase of reducing sugar content in all Cd treated plants. Non reducing sugar content was not influenced, except for *S. cinerea* and *P.* Robusta. In these two species significant decrease of non reducing sugars was found. Starch content in the leaves was not affected in most studied species. Significantly increased level of starch was established only in *S. viminalis* and *S. purpurea* (Table 2).

From above mentioned results follows that in studied species the strongest inhibition of P_{Nmax} by Cd (*S. viminalis* and *S. purpurea*) correlated with the greatest increase of the saccharide content in the leaves. This result could be explained by the fact that nutrient deficiencies and heavy metal toxicities are known to induce both the starch accumulation (Vazquez 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. This presumption was confirmed also by significantly increased specific leaf mass in studied species of our experiment (Table 3). In our previous paper (Šottníková et al. 2003) also strong inhibition of root growth was found in *S. viminalis* and *S. purpurea*. The further reasons of sugar accumulation in the leaves might be Cd-induced water stress where accumulating sugars could provide an adaptive mechanism in maintaining a favorable osmotic potential and stress-induced sufficient carbohydrate storage reserves (Shah and Dubey 1997, Dubey and Singh 1999).

In more tolerant species (*S. cinerea, S. alba, P.* Robusta), P_{Nmax} was not negatively influenced and starch content was not changed. However, reducing sugar level also increased in these species and therefore higher SLM values were estimated, too. In *S. cinerea*, the SLM was not increased probably due to higher leaf R_D what might cause increased oxidation of primary metabolites. Decrease of non reducing sugars in *S. cinerea* and *P.* Robusta may indicate disturbances in enzyme activity (Lohaus et al. 1995). In these more tolerant species, lower inhibition of root growth was found (Šottníková et al. 2003). In above mentioned relationship it should be stressed that the wooden parts of trees are source of water and organic as well as inorganic compounds.

Cadmium had negative effect on leaf expansion, which also confirmed Haag-Kerwer et al. (1999). Reduced leaf area was observed in *S. viminalis*, *S. alba*, *S. cinerea* and *P.* Robusta (Table 3). Assimilation pigment content is considered to decrease as a result of Cd effect (Larrson et al. 1998). However, no significant decrease of assimilation pigment content was established in our experiment. Total chlorophylls $\underline{a+b}$ and carotenoid content were not negatively affected by $10\mu M$ $Cd(NO_3)_2$ concentration (Table 4). Similar results found Kummerová and Brandejsová (1994) in *Zea mays* leaves grown at $10\mu M$ $Cd(NO_3)_2$. The above mentioned different Cd effects on chlorophyll content is in agreement with the general finding that chlorophylls are not specific biomarkers (Pessarakli 1997).

Table 3. Values of specific leaf mass (SLM) and leaf area in relation to Cd treatment.

	Control SLM ± SE (mg d.w. m^{-2})	Cd treatment	Control Leaf area ± SE (m^2)	Cd treatment
S. viminalis	20.6 ± 0.3	26.8 ± 0.6**	0.075 ± 0.008	0.039 ± 0.002**
S. alba	22.3 ± 0.4	27.3 ± 0.7**	0.051 ± 0.005	0.033 ± 0.002*
S. purpurea	21.2 ± 0.9	25.6 ± 0.1*	0.062 ± 0.009	0.044 ± 0.006
S. cinerea	22.7 ± 0.7	28.1 ± 1.8	0.065 ± 0.004	0.034 ± 0.004**
P. Robusta	23.4 ± 1.0	32.5 ± 3.7*	0.116 ± 0.013	0.081 ± 0.005*
P. Gigant	23.6 ± 0.7	31.9 ± 1.0**	0.088 ± 0.011	0.102 ± 0.011

d.w. – dry weight; SE – standard error; * significant difference at $P<0.05$;
** significant difference at $P<0.01$

Table 4. Chlorophyll (Chl) and carotenoid content in relation to Cd treatment.

	Control Chl *a+b* ± SE	Cd treatment Chl *a+b* ± SE (mg g^{-1}d.w.)	Control Carot. ± SE	Cd treatment Carot. ± SE
S. viminalis	11.25 ± 0.52	10.34 ± 0.20	1.16 ± 0.05	1.17 ± 0.03
S. alba	11.75 ± 0.74	10.33 ± 0.36	1.56 ± 0.04	1.41 ± 0.07
S. purpurea	14.94 ± 0.31	12.80 ± 0.92	2.34 ± 0.16	1.83 ±0.13
S. cinerea	10.24 ± 0.50	11.79 ± 0.48	1.59 ± 0.05	1.60 ± 0.06
P. Robusta	14.81 ± 0.48	14.68 ± 0.28	1.88 ± 0.11	1.94 ± 0.10
P. Gigant	13.74 ± 0.78	13.60 ± 0.16	1.86 ± 0.12	1.82 ± 0.06

d.w. – dry weight; SE – standard error

According to Watson and Dallwitz (1992) stomata of family Salicaceae are present either on both surfaces (frequently) or mainly confined to one surface. In our experiment, we observed stomata on both surfaces in *S. alba*, *P.* Robusta and *P.* Gigant. Other *Salix* species had stomata confined to abaxial surface. According to several literature data (e.g. Rawson et al. 1980, Barceló et al. 1986) cadmium negatively influenced water balance and transpiration, which was connected with disturbances in stomata parameters and their function. Cadmium and water stress increased stomata density but reduced stomata sizes, which is known as xeromorphic character of leaves. We observed the effect of Cd mainly on stomata density, which increased in *S. alba*, *S. purpurea* and *P.* Robusta. The most sensitive species was *S. purpurea*, where decrease of stomata length and width was found as well. No significant effect of Cd was observed in *S. cinerea* and *P.* Gigant (Table 5, 6).

The analyses of Cd content in the roots, cuttings and shoots showed that Cd was accumulated mainly in the roots. This fact was confirmed by several authors (e.g. Cataldo et al. 1983; Fargašová 1998). However, relatively higher accumulation in the leaves was estimated in *S. purpurea*, *S. alba* and *P.* Robusta in comparison to *S. cinerea*, *S. viminalis* and *P.* Gigant (Table 7).

Table 5. Values of stomata density in relation to Cd treatment.

	Control	Cd treatment	Control	Cd treatment
	stomata density (No. mm^{-2}) ± SE			
	adaxial	adaxial	abaxial	abaxial
S. viminalis	no stomata	no stomata	?	?
S. alba	77.24 ± 3.69	117.89 ± 6.56 **	106.23 ± 5.11	120.87 ± 4.76*
S. purpurea	no stomata	no stomata	329 ± 7.53	410.3 ± 12.12**
S. cinerea	no stomata	no stomata	91.6 ± 27	109.49 ± 48.85
P. Robusta	91.06 ± 5.12	90.24 ± 4.77	139.84 ± 5.28	173.44 ± 7.81**
P. Gigant	113.82 ± 10.36	121.95 ± 8.05	188.47 ± 8.21	171.47 ± 0.11

SE – standard error; * significant difference at P<0.05; ** significant difference at P<0.01

Table 6. Values of stomata parameters (length and width) in relation to Cd treatment.

	Control stomata length ± SE (µm)	Cd treatment	Control	Cd treatment	Control stomata width ± SE (µm)	Cd treatment	Control	Cd treatment
	adaxial	adaxial	abaxial	abaxial	adaxial	adaxial	abaxial	abaxial
S. viminalis	no stomata	no stomata	?	?	no stomata	no stomata	?	?
S. alba	27.25 ± 0.69	27.5 ± 1.05	26 ± 0.84	26.83 ± 0.62	20 ± 0.53	18.75 ± 0.42	20 ± 0.69	18.17 ± 0.62
S. purpurea	no stomata	no stomata	21.5 ± 0.72	18.2 ± 0.8**	no stomata	no stomata	13.17 ± 0.38	11.67 ± 0.5*
S. cinerea	no stomata	no stomata	8.67 ± 2.07	9.67 ± 2.27	no stomata	no stomata	5.33 ± 1.26	6.17 ± 1.46
P. Robusta	23.25 ± 0.75	22.5 ± 0.83	27 ± 1.12	26.17 ± 0.54	18 ± 0.73	19 ± 0.41	17.67 ± 0.96	19.5 ± 0.61
P. Gigant	20.75 ± 1.06	21.75 ± 0.92	22 ± 0.78	24.5 ± 0.89	15.75 ± 0.38	16.5 ± 0.67	16.17 ± 0.59	15.83 ± 0.58

SE – standard error; * significant difference at P<0.05; ** significant difference at P<0.01

Table 7. Cadmium accumulation in roots, cuttings and shoots.

	Roots	Cuttings	Shoots
		Cd ($\mu g.g^{-1}$ d.w.)	
S. viminalis	4658.2	42.8	3.1
S. alba	4135.7	106.7	24.5
S. purpurea	2561.0	80.7	62.1
S. cinerea	2307.0	120.0	9.4
P. Robusta	5014.3	116.7	29.2
P. Gigant	—	86.4	2.0

d.w. – dry weight

It could be concluded that from all studied species grown at 10 μM $Cd(NO_3)_2$ *S. viminalis* and *P.* Gigant seemed to be the most sensitive ones with the lowest accumulation of Cd into their shoots. The highest Cd accumulation in the shoots was found in *S. purpurea* but sensitivity of this species was also quite high. *S. cinerea, S. alba* and *P.* Robusta were considered as more Cd tolerant species. Relatively higher accumulation in the leaves together with better root growth was established in *S. alba*. According to Baker (1995) Cd hyperaccumulators are defined as plants that accumulate more than 0.01 % (100 $\mu g\ g^{-1}$) of Cd in dry weight of their shoots. In regard to this definition studied fast growing woody plants do not belong to this group of plants. However, they are able to accumulate quite high levels of toxic metals because of their high biomass production, high transpiration rate and extensive root system. This makes the tolerant species potentially utilizable for phytoextraction of toxic metals from contaminated substrates.

Acknowledgments. The research was supported by Grant of Comenius University in Bratislava No. 61/2002, Grant Agency VEGA No. 1/7258/20 and EU Project COST Action 837. We thank to Ing. L. Varga, Forest Research Institute, Zvolen, Research Station Gabčíkovo, Slovakia for supplying the willow and poplar cuttings.

REFERENCES

Baker AJM (1995) Metal hyperaccumulation by plants: our present knowledge of the ecophysiological phenomenon. In: Randall D, Raskin I, Baker A, Blevins D, Smith R (eds) Current Topics in Plant Biochemistry, Physiology and Molecular Biology, University of Missouri, Columbia, Missouri, p 7-8

Barceló J, Poschenrieder C, Andreu I, Gunsé B (1986) Cadmium-induced decrease of water stress resistance in bush bean plants (*Phaseolus vulgaris* L. cv. Contender). I. Effects of Cd on water potential, relative water content and cell wall elasticity. J Plant Physiol 125:17-25

Cataldo DA, Garland TR, Wildung RE (1983) Cadmium uptake kinetics in intact soybean plants. Plant Physiol 73:844-848

Cataldo DA, McFadden KM, Garland TR, Wildung RE (1988) Organic constituents and complexation of nickel, iron, cadmium and plutonium in soybean xylem exudates. Plant Physiol 86:734-739

Clarkson DT, Lűttge U (1989) Mineral nutrition. Divalent cations, transport and compartmentalization. Progr Bot 51:93-112

Davidek J (1981) Laboratory manual of food analyses. SNTL, Prague
Dubey RS, Singh AK (1999) Salinity induces accumulation of soluble sugars and alters the activity of sugar metabolising enzymes in rice plants. Biol Plant 42:233-239.
Fargašová A (1998) Root growth inhibition, photosynthetic pigments production, and metal accumulation in *Sinapis alba* as the parameters for trace metals effect determination. Bull Environ Contam Toxicol 61:762-769
Greger M (1999) *Salix* as phytoextractor. Abstracts of COST 837 Working Group 4, 28th and 29th January 1999, Warsaw, p. 12
Greger M, Bertell G 1992. Effects of Ca^{2+} and Cd^{2+} on the carbohydrate metabolism in sugar beet (*Beta vulgaris*). J Exp Bot 43:167-173
Greger M, Landberg T (1999) Use of willow in phytoextraction. Int J Phytorem 2:1-10
Haag-Kerwer A, Schafer HJ, Heiss S, Walter C, Rausch T (1999) Cadmium exposure in Brassica juncea causes a decline in transpiration rate and leaf expansion without effect on photosynthesis. J Exp Bot 50:1827-1835
Hagemeyer J, Kahle H, Breckle SW, Waisel Y (1986) Cd in *Fagus sylvatica* L. trees and seedlings: leaching, uptake and interconnection with transpiration. Wat Air Soil Pollut 29:347-359
Kessler A, Brand MD (1995) The mechanism of the stimulation of state 4 respiration by cadmium in potato tuber (*Solanum tuberosum*) mitochondria. Plant Physiol Biochem 33:519-528
Krupa Z (1988) Cadmium-induced changes in the composition and structure of the light-harvesting chlorophyll a/b protein complex II in radish cotyledons. Physiol Plant 73:518-524
Kummerová M, Brandejsová R (1994) Project TOCOEN. The fate of selected pollutants in the environment. Part XIX. The phytotoxicity of organic and inorganic pollutants-cadmium. The effect of cadmium on the growth of germinating maize plants. Toxicol Environ Chem 42:115-122
Larrson EH, Bornman JF, Asp H (1998) Influence of UV-B radiation and Cd^{2+}on chlorophyll fluorescence, growth and nutrient content in *Brassica napus*. J Exp Bot 49:1031-1039
Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol 148:350-382
Lohaus G, Winter H, Reins B, Heldt HW (1995) Further studies on phloem loading process in leaves of barley and spinach. The comparison of metabolite concentration in the apoplastic compartment with those in the cytosolic compartment and in sieve tubes. Bot Acta 108:270-275.
Masarovičová E (1997) Measurements of plant photosynthetic activity. In Pessarakli M (ed) Handbook of photosynthesis, Marcel Dekker Inc., New York, Basel, Hong Kong, p 769-801
McLaughlin MJ, Singh BR (1999) Cadmium in Soils and Plants. Kluwer Academic Publishers, Dortrecht, Boston, London
Pessarakli M (1997) Handbook of photosynthesis. Marcel Dekker Inc., New York, Basel, Hong Kong

Pilon-Smits EAH,de Souza MP, Lytle CM, Shang C, Lugo T, Terry N (1998) Selenium volatilization and assimilation by hybrid poplar (*Populus tremula* x *alba*). J Exp Bot 49:1889-1892

Procházka S, Macháčková I, Krekule J, Šebánek J, et al. (1998) Plant Physiology. Academia Prague

Raskin I, Ensley BD (2000) Phytoremediation of toxic metals. John Wiley and sons, Inc., New York

Rauser WE, Samarakoon AB (1980) Vein loading in seedlings of *Phaseolus vulgaris* exposed to excess cobalt, nickel and zinc. Plant Physiol 65:578-583

Salt DE, Blaylock M, Nanda Kumar PBA, Dushenkov V, Ensley B D, Chet I, Raskin I (1995) Phytoremediation: A novel strategy for the removal of toxic metals from environment using plants. Biotechnol 13:468-474

Sanita di Toppi L, Gabrielli R 1999. Response to cadmium in higher plants. Environ Exp Bot 41:105-130

Siedlecka A, Baszynski T 1993. Inhibition of electron fow around photosystem I in chloroplasts of Cd-treated maize plants is due to Cd-induced iron deficiency. Physiol Plant 87:199-202

Shah K, Dubey RS (1997) Effect of cadmium on proline accumulation and ribonuclease activity in rice seedlings, role of proline as a possible enzyme protectant. Biol Plant 40:121-130.

Šottníková A, Lunáčková L, Masarovičová E, Lux A, Streško V (2003) Changes in the rooting and growth of willows and poplars induced by cadmium. Biol Plant in press

Vassilev A, Yordanov I, Tsonev T (1997) Effects of Cd^{2+} on the physiological state and photosynthetic activity of young barley plants. Photosynthetica 34:293-302

Vazquez MD, Poschenrieder Ch, Barcelo J (1987) Chromium VI induced structural and ultrastructural changes in bush bean plants. Ann Bot 59:427-438

Verma S, Dubey RS (2001) Effect of cadmium on soluble sugars and enzymes of their metabolism in rice. Biol Plant 44:117-123.

Watson L, Dallwitz MJ (1992) The families of angiosperms: automated descriptions with interactive identification and information retrieval. Aust Syst Bot 4:681-695