Bull. Environ. Contam. Toxicol. (2006) 77:763–771

DOI: 10.1007/s00128-006-1129-1

Bulletin of Environmental Contamination and Toxicology

Response of Chamomile Plants (*Matricaria recutita* L.) to Cadmium Treatment

A. Pavlovič,[1] E. Masarovičová,[1] K. Král'ová,[2] J. Kubová[3]

[1] Department of Plant Physiology, Faculty of Natural Sciences, Comenius University, Mlynská Dolina B-2, SK-84215 Bratislava, Slovakia
[2] Institute of Chemistry, Faculty of Natural Sciences, Comenius University, Mlynská Dolina CH-1, SK-84215 Bratislava, Slovakia
[3] Institute of Geology, Faculty of Natural Sciences, Comenius University, Mlynská Dolina G, SK-842 15, Bratislava, Slovakia

Received: 3 April 2006/Accepted: 10 October 2006

Cadmium is a metal that has no biological function and is highly toxic to plants and animals. All vascular plants take up Cd through roots to varying degrees from the soil. The aim of our previous study (Lunáčková et al. 2003) was to screen some fast growing woody plants from the family Salicaceae to find potentially suitable clones able to tolerate and accumulate high Cd concentration in their organs without significant inhibition of growth or physiological function. Now, we give our attention to medicinal plants such as *Hypericum perforatum* (Masarovičová et al. 2004), *Salvia officinalis* and *Matricaria recutita* (Pavlovič et al. 2005). Some of them have the ability to accumulate high levels of toxic metals in the shoot and have been classified as Cd accumulators (Kráľová and Masarovičová 2003) according to Baker (1995). Although hyperaccumulation of Cd by plants for the purpose of phytoremediation be a very useful tool for remediating contaminated sites (Raskin and Ensley 2000), there is also another side of the coin. The above-mentioned medicinal species are widely used in pharmaceutical, food and cosmetics industries, therefore higher content of cadmium in their shoot is undesirable. But a novel approach, termed biofortification, use plants with enhanced capacity to accumulate minerals (e.g. selenium) to improve human health through balanced mineral nutrition. Marquard and Schneider (1998) were the first to confirm that chamomile plants had the potential to accumulate high levels of cadmium from the soil. However, there is little information about effects of Cd on growth and production characteristics (De Pasquale et al. 1988, Grejtovský and Pirč 2000) and no information about physiological processes such as photosynthesis, respiration or pigment content, except for our previous study with *Hypericum perforatum* (Kráľová and Masarovičová 2003, Masarovičová et al. 2004), in plant species producing specific secondary metabolites. We also assumed (Kráľová and Masarovičová 2003, Kráľová et al. 2004) possible sequestration of Cd by some of specific secondary metabolites (such as hypericin, apigenin, quercetine). Palivan et al. (2001) found the formation of copper complexes with hypericin in *Hypericum perforatum* that has been classified as Cd accumulator species previously (Kráľová and Masarovičová 2003). Grejtovský and Pirč (2000) found no necroses, only small inhibition of growth in Cd-treated chamomile plants, despite relatively high values of accumulated Cd. This enhanced Cd tolerance of chamomile (similarly to *H. perforatum*) could also contribute the formation of Cd complexes

Correspondence to: E. Masarovičová

with its specific secondary metabolites such as -(-) a-bisabolol oxide B, and some flavonoids e.g. quercetin, apigenin that can also play a role in the detoxification mechanism (Kráľová et al. 2004). The aim of our present study was to investigate the effect of Cd on physiological and production characteristics in two tetraploid cultivars *Matricaria recutita* in response to the uptake and accumulation of Cd under different cultivation conditions.

MATERIALS AND METHODS

Two tetraploid cultivars of *Matricaria recutita* L., cv. Goral and cv. Lutea were used for our experiments. Seeds were obtained from Institute of Agroecology in Michalovce (Slovakia) and were germinated and grown in soil in growth chamber under standard conditions: 25 °C, 80% relative humidity and 100 μmol m^{-2} s^{-1} PAR irradiance with day/night photoperiod of 16 h light/8 h dark. Fourteen days after germination, 20 seedlings of each variant were gently washed to remove the soil adhering to the roots. Than the roots were put on the strips of filter paper. The primary root length was measured. The filter papers were coiled, put into flask and 3 cm submerged in Hoagland solution with the following Cd concentrations: 3, 6, 12, 24, 60 μM $Cd(NO_3)_2 \times 4H_2O$ p.a. (w/v) (Lachema, Czech Republic). The plants growing in Hoagland solution without Cd served as control. The plants were cultivated at 25 °C, 80% relative humidity and 200 μmol m^{-2} s^{-1} PAR for 7 days. Primary root length, root increment (root length after treatment – root length before treatment) was calculated [(root increment of Cd-treated plants / root increment of control plants).100] in %, root and shoot dry mass (n = 20), as well as Cd content in plant organs were determined after 7 days of treatment. In another experiment the older plants were grown in the greenhouse conditions in the soil for 7 weeks after germination. Then, the plants were gently washed in water to remove the soil adhering to the roots and transferred to hydroponic Hoagland solutions (control) and Hoagland solution containing the following Cd concentration: 3, 6, 12, 24, 60, 120, 240 μM $Cd(NO_3)_2 \times 4H_2O$ (n = 12) and were grown in a growth chamber under conditions described above. Root and shoot dry weight and Cd content in plants organs were determined after 7 days of exposure.

Plants used for photosynthetic and respiration measurements (n = 4) as well as for analysis of assimilation pigment concentration were grown under greenhouse conditions for 9 weeks after germination. Then their roots were washed, transferred to hydroponic Hoagland solutions (control) and Hoagland solution with 12 μM $Cd(NO_3)_2 \times 4H_2O$ and placed in the growth chamber under above-mentioned condition for 10 days. The solutions were continuously aerated and changed every 3 days to avoid depletion of Cd and oxygen. After 10 days, shoots of intact plants were placed in the thermostabilised chamber and CO_2 exchange rates were determined by infrared gas analyser (IRGA) using a closed measurement system at the air temperature of 25 °C, irradiance of 300 μmol m^{-2} s^{-1} PAR and 350 vpm CO_2. Finally root and shoot dark respiration rates (R_D) were determined. Four plants were used for each variant. The measurement and equipment used have been described in detail by Masarovičová and Kráľová (2005). The remaining part of the plants was used for determination of

assimilation pigment concentration. Concentration of assimilation pigment was determined after Cd treatment (12 and 120 μM Cd) in the leaves, which had grown before treatment (old leaves) and young leaves growing during experiment. The plants growing in Hoagland solution without Cd served as control. Chlorophylls and carotenoids were extracted with 80% chilled acetone p.a. (v/v) (Fluka, Czech Republic) and determined spectrophotometrically (Jenway 6400 UV/Visible, London, Great Britain) for chlorophyll *a* at 663,2 nm, chlorophyll *b* at 646,8 nm and carotenoids at 470 nm against 80% acetone blank. Detection limit was 0,1 nm. Three plants were used for each variant and pigments contents were calculated according to Lichtenthaler (1987).

All plants were processed for Cd analysis. Plants were harvested and thoroughly washed under running water to remove the test solution from the exterior of the roots and total root and shoot accumulation of Cd was determined. Plant samples were dried at 70°C and cut to small pieces (<1 mm). Digestions of plants were carried out in the stainless steel coated PTFE pressure vessels ZA-1 (Czech Republic). 0.1-0.5 g of plant sample was weighted to the vessel and 5.0 ml of conc. HNO_3 p.a. (Lachema, Czech Republic) was added. Vessels were closed and heated in the oven at 160°C for 6 hours. After digestion the solution was diluted to 50 ml with redistilled water and stored in a 100 ml polyethylene (PE) bottle. Cadmium contents were determined by flame atomic absorption spectrometry method (AAS Perkin Elmer Model 1100, at 228.8 nm with deuterium background correction). The accuracy of analytical results for Cd in plant samples was checked by the analysis of the certified standard reference materials DC 73350 Poplar Leaves and DC 73349 Bush Branches and Leaves (both NCS, China). The precision of Cd determination expressed by relative standard deviation (corresponding to four independent replicates) varied in the range from 1 to 5 %. Recovery of Cd found in various types of plant samples varied from 98.3 to 100.6 %. The detection limit of cadmium was 2 $\mu g\ L^{-1}$.

The Cd concentration in solution - Cd content in plants organs and Cd content in plants organs - growth parameter (root length, shoot and root weight) relationships were processed by linear regression analyses (statistiXL ver.1.6 for Excel). In same cases, Cd concentration (μM) in solution or Cd accumulation in tissue ($\mu g\ g^{-1}$) was log transformed (marked*, in table 1) to equalize the variance of the data. Where it was possible, we used the ANCOVA (statistiXL ver.1.6 for Excel) to test the homogeneity of slopes and y-intercepts of the relationship between the Cd concentrations in solution - Cd content in plants organs and Cd content in plants organs - growth parameter (root length, shoot and root weight) for cv. Goral vs. cv. Lutea. Data from photosynthetic and respiration measurements were processed by t-test. One-way ANOVA (Statgraphics) was used for data from concentration of assimilation pigment.

RESULTS AND DISCUSSION

The concentration gradient that was used in our experiments reflects Cd content in the soil from non-contaminated to highly contaminated sites (Linkeš et al. 1997).

For estimation of Cd toxicity to roots, we used primary root length and increment in root length, which are considered to be a reliable parameter for heavy metal tolerance (Murphy and Taiz 1995). At the beginning of Cd-treatment, non-significant variance in root length was observed (cv. Lutea, P = 0.07, cv. Goral, P = 0.7, ANOVA). Significant inhibition of root growth was observed in both chamomile cultivars after Cd-treatment (Table 1). We did not find any differences in Cd accumulation in roots (slope and y-intercepts P>0.05) between cultivars, but cv. Lutea accumulated slightly higher amount of Cd in the shoots (y-intercepts P = 0.025). No differences in shoot weight between cultivars were recorded after Cd-treatment (slope P>0.05) (Table 1). Limited elongation of roots after Cd application could result from inhibited mitosis, decreased synthesis of the components of cell walls, damaged Golgi apparatus or changes in the metabolism of polysaccharides in the root cap (Punz and Sieghardt 1993). We also observed fragility, browning and twisting of roots. In shoots leaf roll, chlorosis and leaf growth inhibition occurred. During the root test chamomile plants cv. Goral formed the anthodia in all concentrations except control, despite the fact that the plants were only 3 weeks old. According to our observation, the plants started blossoming when they are 8 – 12 weeks old, however Cd treatment resulted in reduced size of flowers. From 4 to 5 weeks earlier blossoming under Cd administration was also recorded for Cd hyperaccumulator *Arabidopsis halleri* (Küpper et al. 2000). Lower Cd content in plants cultivated in paper rolls than plants cultivated in hydroponic solution is due to bind between ions and cellulose in the paper rolls (Tamás et al. 2006). These conditions are similar to soil, where metals are bound to the soil particles. Thus insoluble metals are not available for plant uptake (Raskin and Ensley 2000). The plants growing in paper rolls exceeded the limit of Cd hyperaccumulator (100 μg g^{-1}) sensu Baker (1995) at 60 μM Cd in solution. Grejtovský and Pirč (2000) also found over 100 μg g^{-1}. d.w. in shoots of plants growing in contaminated substrates. In another experiment, seven weeks old plants growing 7 days in Cd-hydroponic solution exhibited no significant changes in root biomass (cv. Lutea, P = 0.92, cv. Goral, P = 0.83) and shoot biomass (cv. Lutea, P = 0.81, cv. Goral, P = 0.45), which has already been observed in our earlier paper for *Hypericum perforatum* and *Matricaria recutita* cv. Novbona (Kráľová and Masarovičová 2003). Experimental plants growing in hydroponic solution exceeded the threshold for Cd hyperaccumulator at 6 μM Cd and accumulated up to 5-times more Cd in the shoots than the plants growing in paper rolls (root cv. Goral: slope = 21.97, y-intercept = 389.4, R^2 = 0.95, root cv. Lutea: slope = 27.43, y-intercept = 315.1, R^2 = 0.98, shoot cv. Goral: slope = 4.22, y-intercept = 145.8, R^2 = 0.91, shoot cv. Lutea slope = 4.16, y-intercept = 170.1, R^2 = 0.83, non-significant differences in slopes and y-intercepts between cultivars using ANCOVA, data not shown). Comparable Cd accumulation in the shoots was recorded also for accumulator *Brassica juncea* (Zhu et al. 1999) and the medicinal species *Hypericum perforatum* (Masarovičová et al. 2004). Conversely, woody plants from the family Salicaceae (Lunáčková et al. 2003) as well as the medicinal species *Salvia officinalis*, that are not Cd accumulator species, accumulated considerably less Cd in the shoot under the same cultivation conditions (no common slope exists for *Salvia* and *Matricaria*, P = 0.001, Pavlovič et al. 2005).

Table. 1 The summary of responses of *M. recutita* exposed to various concentration of Cd, mean ± SE, n = 20. Concentration of Cd in solution in relation to Cd accumulation (* Cd concentration in solution was log transformed) and Cd accumulation in tissues in relation to growth parameter (root length, shoot and root weight, * Cd accumulation in tissues was log transformed).

Species and cultivar	Cd in solution (μM)	Root Cd accumulation (μg g^{-1} d.w.)*	Shoot Cd accumulation (μg g^{-1} d.w.)*	Root weight (mg d.w.)	Shoot weight (mg d.w.)*	Primary root length (cm)	Root increment (%)
Matricaria recutita cv. Goral	control	12.2 ± 1.0	6.0 ± 0.9	3.92 ± 0.29	14.21± 1.55	8.73 ± 0.44	100
	3	103.8 ± 5.1	32.1 ± 1.5	3.01 ± 0.28	10.62 ± 1.15	7.83 ± 0.36	78
	6	236.0 ± 8.8	41.8 ± 1.6	3.16 ± 0.41	9.52 ± 1.21	8.01 ± 0.55	91
	12	323.0 ± 10.5	50.2 ± 2.0	2.87 ± 0.53	9.76 ± 1.12	7.60 ± 0.55	71
	24	412.5 ± 16.5	66.2 ± 3.3	2.08 ± 0.13	8.89 ± 1.05	7.14 ± 0.54	65
	60	695.0 ± 23.0	117.3 ± 5.2	1.53 ± 0.12	5.99 ± 0.59	5.60 ± 0.21	39
Regression equation		$y = 370.8x - 42.4$ $R^2 = 0.95$ $P < 0.001$	$y = 56.4x + 0.6$ $R^2 = 0.91$ $P = 0.002$	$y = -0.003x + 3.7$ $R^2 = 0.85$ $P = 0.009$	$y = -5.94x + 19.11$ $R^2 = 0.91$ $P = 0.003$	$y = -0.004x + 8.7$ $R^2 = 0.93$ $P = 0.002$	
		*	*	*	*	*	
Matricaria recutita cv. Lutea	control	10.6 ± 1.1	5.5 ± 0.5	6.29 ± 0.76	13.29 ± 1.33	11.93 ± 0.35	100
	3	139.3 ± 6.9	51.8 ± 2.4	4.81 ± 0.62	10.37± 1.31	10.62 ± 0.42	77
	6	226.0 ± 9.0	68.0 ± 2.0	3.73 ± 0.57	8.55 ± 1.09	9.37 ± 0.36	72
	12	313.0 ± 12.5	75.4 ± 2.2	2.51 ± 0.17	8.36 ± 0.99	7.80 ± 0.41	42
	24	381.2 ± 17.1	91.2 ± 5.1	2.92 ± 0.53	8.42 ± 1.23	7.40 ± 0.31	42
	60	702.0 ± 28.1	140.5 ± 6.6	3.62 ± 0.78	9.24 ± 0.80	7.37 ± 0.30	38
Regression equation		$y = 359.6x - 33.9$ $R^2 = 0.95$ $P < 0.001$	$y = 68.2x + 9.6$ $R^2 = 0.95$ $P < 0.001$	$y = -1.89x + 8.22$ $R^2 = 0.77$ $P = 0.022$	$y = -3.52x + 15.73$ $R^2 = 0.84$ $P = 0.01$	$y = -2.69x + 15.12$ $R^2 = 0.83$ $P = 0.012$	
ANCOVA		slope P = 0.867 y-inter P = 0.962	slope P = 0.962 y-inter P = 0.025		slope P = 0.084 y-inter P = 0.351		

Table 2 Values of net photosynthetic rate (P_N) and dark respiration rate (R_D), dry weight, root length and Cd accumulation in the plants used for photosynthetic measurement.

Parameter	Variant	cv. Goral	cv. Lutea
P_N (nmol CO_2 g^{-1} d.w.s^{-1})	control	148.48 ± 4.77	177.39 ± 8.63 ♠
n= 4	Cd 12 μM	110.12 ± 8.27*	124.85 ± 10.91**
R_D shoot (nmol CO_2 g^{-1} d.w.s^{-1})	control	34.22 ± 1.45	38.62 ± 1.59
n = 4	Cd 12 μM	36.75 ± 3.03	40.61 ± 3.81
R_D root (nmol CO_2 g^{-1} d.w.s^{-1})	control	132.36 ± 21.43	44.19 ± 5.12 ♠
n = 4	Cd 12 μM	181.87 ± 22.42	55.83 ± 9.60 ♠♠
Shoot biomass (mg)	control	116.92 ± 11.61	136.92 ± 8.36
n = 15	Cd 12 μM	85.00 ± 11.81	101.18 ± 15.88
Root biomass (mg)	control	25.86 ± 3.08	24.56 ± 2.77
n= 15	Cd 12 μM	15.44 ± 2.41*	12.33 ± 1.83**
Root length (cm)	control	14.14 ± 1.07	13.97 ± 0.81
n = 15	Cd 12 μM	10.50 ± 0.84*	9.65 ± 1.11**
Shoot Cd content (μg g^{-1} d.w.)	control	15.9 ± 3.7	4.6 ± 0.9
n = 4	Cd 12 μM	360.5 ± 23.6**	248.3 ± 19.6** ♠
Root Cd content (μg g^{-1} d.w.)	control	19.9 ± 1.5	14.8 ± 1.0
n = 4	Cd 12 μM	1081.0 ± 54.3**	895.0 ± 55.0**

Mean ± SE, comparisons were done between control variant and Cd treatment (Cd 12 μM) at P = 0.05 (*) and P = 0.01 (**) and between cultivars at P= 0.05 (♠) and P = 0.01 (♠♠). Student's t-test was used.

Table 3. Chlorophyll *a* (Chl *a*), *b* (Chl *b*) and carotenoid content in relation to Cd treatment.

Chl *a*	Control	12 μM-o	12 μM-y	120 μM-y	ANOVA
cv. Goral	8.45 ± 0.43 ^{a}	6.58 ± 0.73 ^{b}	4.56 ± 0.26 ^{c}	6.30 ± 0.28 ^{b}	P = 0.003
cv. Lutea	4.84 ± 0.29 ^{a}	4.07 ± 0.17 ^{b}	1.88 ± 0.17 ^{c}	3.04 ± 0.13 ^{d}	P < 0.001

Chl *b*	Control	12 μM-o	12 μM-y	120 μM-y	ANOVA
cv. Goral	3.79 ± 0.18^a	3.04 ± 0.19^b	1.82 ± 0.14^c	2.73 ± 0.16^b	P < 0.001
cv. Lutea	2.15 ± 0.14^a	1.96 ± 0.09^a	0.85 ± 0.10^b	1.55 ± 0.02^c	P < 0.001

carotenoid	Control	12 μM-o	12 μM-y	120 μM-y	ANOVA
cv. Goral	3.36 ± 0.15^a	2.70 ± 0.20^b	1.92 ± 0.16^c	3.22 ± 0.16^{ab}	P = 0.001
cv. Lutea	2.08 ± 0.16^a	1.89 ± 0.09^{ab}	1.00 ± 0.08^c	1.55 ± 0.02^b	P < 0.001

Mean ± SE, ANOVA, identical superscripts denote no significant difference in a row. o - old leaves growing before treatment, y - young leaves growing during treatment.

Twelve μM Cd concentration in hydroponic solution represents strong contaminated soil (Linkeš et al. 1997), however the Cd effect on plant was stronger in comparison to the soil, because Cd are not bound to the soil particles and so all ions are available for plant uptake. Cv. Lutea seemed to be more sensitive to Cd treatment. The measurements confirmed higher inhibition of

photosynthesis in cv. Lutea (70% of control P_N, P = 0.01), although they accumulated less Cd than cv. Goral (75% of control P_N, P = 0.05). Similar decrease of shoot dry weight (72% of control) in both cultivars was also detected (Table 2). Decrease of net photosynthetic rate could be due to structural and functional disorders in many different levels. Inhibition of P_N in Cd treated plants has been well described in Vassilev and Yordanov (1997). P_N was not effected in *Hypericum perforatum* after application of Cd (Kráľová and Masarovičová 2003), but it was inhibited to a greater extent in some species and cultivars of Salicaceae, despite lower Cd concentration in the leaves (from 2,0 to 62,1 μg g^{-1} d.w., Lunáčková et al. 2003). Shoot and root respiration rates were not changed significantly in both chamomile cultivars (Table 2). Data about effect of Cd upon dark respiration are often controversial. Lee et al. (1976) established that Cd increased dark respiration rate and activity of the following enzymes: isocitrate dehydrogenase, glutamate dehydrogenase, malate dehydrogenase. On the contrary, Cd inhibited enzymes activity directly - by interaction with SH-groups, or indirectly - by disturbing cation balance at the subcellular level (Vassilev and Yordanov 1997). Lunáčková et al. (2003) confirmed higher root respiration rate in Cd variant of some species of Salicaceae as well as Masarovičová et al. (2005) in *Hypericum perforatum*. Significant decrease of chlorophyll *a* and *b* content was confirmed in young chamomile leaves grown during Cd treatment. Lower decrease was observed in older leaves which had grown before Cd application. Therefore inhibition of chlorophyll biosynthesis was more affected than its degradation. This finding is consistent with Stobart et al. (1985) that reduction of chlorophyll in Cd treated plants is connected with inhibition of its biosynthesis. On the other hand, Somaskekaraiah et al. (1992) established that chlorophyll concentration could be lowered by the activation of its enzyme degradation, e.g. increased lipoxygenase activity. Significantly higher pigment content was detected in young leaves of chamomile growing at 120 μM Cd than at 12 μM Cd (Table 3), despite higher Cd concentration in the shoot at 120 μM (1957 μg Cd g^{-1} d.w. in shoot at 120 μM Cd, 521 μg Cd g^{-1} d.w. in shoot at 12 μM Cd). Except well-known defense mechanisms, another explanation of this phenomenon might be increased production of specific secondary metabolites that could play a supplementary role in detoxification mechanism induced by Cd. Cadmium application induced higher production of some of them in chamomile plants such as: α-bisabolol (De Pasquale et al. 1988) polyacetylenes ene-yne-dicycloethers and sesquiterpene (E)-β-farnesene (Grejtovský et al. 2001), herniarine, umbelliferone (Eliašová et al. 2004).

We confirmed that chamomile belongs to the group of Cd accumulator species. If we take into account high content of Cd in chamomile shoot (over 300 μg g^{-1} at 12 μM Cd in solution), only small extent of damages occurred in Cd treated plants. Therefore this plant species exhibited high tolerance to Cd treatment.

Acknowledgments. This work was financially supported by the Grant Agency VEGA No. 1/3489/06 and No. 1/1312/04 and EU COST Action 859. We thanks to RNDr. Jana Kohanová and Jana Kovaríková for technical assistance and Assoc. Prof. Ivan Šalamon for supplying the chamomile seeds.

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

De Pasquale R, Raqusa S, Iauk L, Barbera R, Galati EM (1988) Effect of cadmium on germination, growth and active principle contents of *Matricaria recutita* L. Pharmacol Res Commun 20:151-154

Eliašová A, Repčák M, Pastírová A (2004) Quantitative changes of secondary metabolites of *Matricaria chamomilla* by abiotic stress. Z Naturforsch 59: 543-548

Grejtovský A, Pirč R (2000) Effect of high cadmium concentrations in soil on growth, uptake of nutrients and some heavy metals of *Chamomilla recutita* (L.) Rauschert. J Appl Bot 74:169-174

Grejtovský A, Repčák M, Eliášová A, Markušová K (2001) Effect of cadmium on active principle contents of *Matricaria recutita* L. Herba Polonica 48:203 -208

Kráľová K, Masarovičová E (2003) *Hypericum perforatum* L. and *Chamomilla recutita* (L.) Rausch. - accumulator of some toxic metals. Pharmazie 58: 359-360

Kráľová K, Masarovičová E, Pavlovič A (2004) Are secondary metabolites of medicinal plants responsible for tolerance to toxic metals? In: Habán M. (ed.) Book of abstracts, 3rd Conference on Medicinal and Aromatic Plants of Southeast European Countries, Nitra, Slovakia, p 98

Küpper H, Lombi E, Zhao F, McGrath SP (2000) Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. Planta 212:75-84

Lee K, Gunnigham B, Paulsen G, Liang G, Moore R (1976) Effects of cadmium on respiration rate and activities of several enzymes in soybean seedlings. Physiol Plant 36:4-6

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Methods Enzymol 148:350-382

Linkeš V, Kobza J, Švec M, Ilka P, Pavlenda P, Barančíková G, Matúšková L (1997) Monitoring pôd Slovenskej republiky, Súčastný stav monitorovaných pôd 1992 - 1996 [Soil monitoring in Slovakia, Actual state of soil 1992 - 1996], VUPU, Bratislava

Lunáčková L, Masarovičová E, Kráľová K, Streško V (2003) Response of fast growing woody plants from family Salicaceae to cadmium treatment. Bull Environ Contam Toxicol 70:576-585

Masarovičová E, Kráľová K (2005) Approaches to measuring plant photosynthetic activity. In: Pessarakli M (ed) Handbook of Photosynthesis, LLC CRC Press, Taylor and Francis Group, Boca Raton, USA, p 617-656

Masarovičová E, Kráľová K, Kummerová M, Kmentová E (2004) The effect of cadmium on root growth and respiration rate of two medicinal plant species. Biologia Bratislava 59/Suppl. 13:211-214

Marqaurd R, Schneider M (1998) Zur cadmiumproblematik im Arzneipflanzenbau. In: Marqaurd R, Schubert E (eds) Fachtagung Arznei- und Gewűrzpflanzen, 1.-2. Okt., Giessen, p. 9-15 (in German)

Murphy A, Taiz L (1995) A new vertical mesh transfer technique for metal tolerance studies in *Arabidopsis* ecotypic variation and copper-sensitive mutants. Plant Physiol 108:29-38

Palivan CG, Geschidt G, Weiner L (2001) The formation of copper complexes with hypericin in solutions: An APR study. J Inorg Biochem 86:369

Pavlovič A, Masarovičová E, Kráľová K, Kubová J (2005) Photosynthetic activity of some medicinal plants under cadmium stress. In: Book of abstracts Photosynthesis and stress, 15.-16. Sept. 2005, Brno, Czech Republic, p 96

Punz WF, Sieghardt H. (1993) The response of roots of herbaceous plant species to heavy metals. Environ Exp Bot 33:85-98

Raskin I, Ensley BD (2000) Phytoremediation of toxic metals. Using plants to clean up the environment. John Wiley & Sons, Inc

Somashekaraiah B, Padmaja K, Prasad A (1992) Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): Involvement of lipid peroxides in chlorophyll degradation. Physiol Plant 85:85-89

Stobart AK, Griffiths WT, Ameen-Bukhari I, Sherwood RP (1985) The effect of Cd biosynthesis of chlorophyll in leaves of barley. Physiol Plant 63:293-298

Tamás L, Budíková S, Šimonovičová M, Huttová J, Široká B, Mistrík I (2006) Rapid and simple method for Al-toxicity analysis in emerging barley roots during germination. Biol Plant 50:87-93

Vassilev A, Yordanov I (1997) Reductive analysis of factors limiting growth of cadmium-treated plants: a review. Bulgarian J Plant Physiol 23:114-133

Zhu YL, Pilon-Smits EAH, Tarun AS, Weber SU, Jouanin L, Terry N (1999) Cadmium tolerance and accumulation in indian mustard is enhanced by overexpressing γ-glutamylcysteine synthetase. Plant Physiol 121:1169-1117