The response of chamomile (*Matricaria chamomilla* L.) plants to soil zinc supply

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ABSTRACT

A pot experiment was conducted to investigate the influence of varying supplies of Zn (50–150–300 mg/kg soil – Orthic Luvisol) on the uptake of Zn by plants, selected productive parameters and production of secondary metabolites in *Matricaria chamomilla* L., diploid cv. Novbona. Chamomile takes up Zn easily and accumulates it in all its organs. The maximum supply of Zn resulted in an 18-fold increase in chamomile shoots where it reached the level of 271.0 mg/kg dry matter. Such a treatment resulted in a 5-fold increase of Zn in chamomile anthodia (*Matricariae flos* drug) reaching a level of 159.8 mg/kg dry matter. During cultivation, experimental plants showed no signs indicating an excess of Zn. Of the selected productive parameters, the increasing concentration of Zn in the soil affected significantly only the plant height. A weak, positive, insignificant effect of Zn was observed in the production of the plant biomass. When supplying Zn at a rate of 50 mg/kg soil the biomass of the shoots dry matter increased by 17% and anthodia by 8%, respectively ($P > 0.05$). However, an additional increase in Zn supply reduced production of anthodia, while the dose of 300 mg Zn/kg soil resulted in a significant, 17% decrease of anthodia yield in comparison with the maximum production achieved with the treatment by 50 mg Zn/kg soil. The application of Zn into the soil affected only slightly the content of essential oil and proportion of chamazulene, (*E*)-β-farnesene, and ene-yne-dicycloethers. An increased supply of Zn did not affect the concentration of flavone apigenin and coumarin herniarin in chamomile anthodia. Zn fertilization decreased the accumulation of Cd in chamomile plants; supply of 50 mg Zn/kg soil and caused an decrease in Cd concentration by 10% in shoots ($P > 0.05$) and by 37% (from 0.280 to 0.176 mg Cd/kg dry matter) in anthodia ($P < 0.01$), respectively. An additional increase in soil Zn decreased significantly with an accumulation of Cd by 18% (at a dose of 300 mg Zn/kg soil) only in chamomile shoots.

Keywords: accumulation; Cd; growth; interaction; *Matricariae flos*; secondary metabolites; Zn

Zinc is one of the essential micronutrients required for the growth of plants. It is an integral component or activator of a number of enzymes that represent almost all enzymatic groups in plants. Zinc is highly mobile in the soil and is easily taken up by plants and thus can accumulate in the biomass up to extremely high phytotoxic concentrations (Vysloužilová et al. 2003a, b). The accumulation rate depends on soil conditions, plant species, genotype, climate and agronomic practice (Tiller 1989, Tlustoš et al. 2001). High concentrations of Zn in the soil, a frequent consequence of anthropogenic activities (Alloway 1995), affect adversely the life processes of plants (e.g. Vangronsveld and Clijsters 1994) and can result in decreased production of biomass (Vysloužilová et al. 2003a, b) and deteriorated quality of production.

Chamomile [*Matricaria chamomilla* L., syn. *Chamomilla recutita* (L.) Rauschert] (Compositae) is one of the most important and most frequently cultivated medicinal herbs, particularly in Europe. Chamomile anthodia (*Matricariae flos* drug) contain a range of pharmacologically effective secondary metabolites with anti-inflammatory and spasmolytic action. The unique medicinal effect of chamomile results from combined action of all inherent substances: sesquiterpenes [(-)-α-bisabolol, matricin or chamazulene], flavonoids (apigenin glucosides), polyacetylenes [(*Z*)-ene-yne-dicycloether], coumarins (herniarin and umbelliferone), mucilages, etc. (Schilcher 1987).

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Despite all these positive properties, chamomile can also accumulate cadmium (Marquard and Schneider 1999, Grejtovský and Pirč 2000) and zinc (Plescher et al. 1995). Up until now we have little information on the response of chamomile plants to increasing concentrations of heavy metals in the soil and the potential influence of metals on production of specific secondary metabolites (De Pasquale et al. 1988, Grejtovský et al. 2001).

The aim of our study was to investigate the effect of increasing concentrations of soil Zn on the following: (i) growth and selected productive parameters of chamomile; (ii) the content of therapeutically relevant secondary metabolites in the anthodia of this important medicinal plant; (iii) effectiveness of Zn fertilization in relation to decreasing levels of Cd in *Matricariae flos* drug.

MATERIAL AND METHODS

Plant culture. Chamomile (*Matricaria chamomilla* L.) plants, diploid cv. Novbona, were cultivated in plastic pots under natural climatic conditions in the Botanical garden of P.J. Šafárik University in Košice. The pots were filled up with soil (Orthic Luvisol – Table 1) enriched with increasing doses of Zn added in the form of $ZnSO_4.7\ H_2O$ (0–50–150–300 mg Zn/kg dry soil). For each concentration the necessary amount of Zn-sulphate was dissolved in water and added to the soil at constant mixing to ensure a uniform distribution of Zn. Each pot containing 10 kg dry soil was prepared separately and each treatment was repeated 5 times. After 2-week equilibration of soil at 60% of MWHC, 50-day old chamomile plants, grown previously under laboratory conditions, were planted to the pots, five plants in each. During the cultivation, the plants were fertilized with nutrients using the following quantities (mg per kg of soil): N – 39.7 (as NH_4NO_3), P – 7.2 (as KH_2PO_4), and K – 51.8 (as KH_2PO_4 + KCl). The soil water content was checked regularly and maintained at 60% MWHC using distilled water. The temperature during vegetation ranged from 23 to 28°C during the day and between 14 and 18°C at night. Chamomile anthodia were collected at the stage when 1/2–2/3 of tubular flowers were in full flower. Cultivation was terminated on day 80 after potting the plants when the age of plants reached 130 days. The drug (*Matricariae flos*) yield was determined by weighing the anthodia air-dried at room temperature. The production of shoot dry matter was determined after drying at 80°C for 72 h.

Chemical analyses. Soil characteristics (Table 1) were determined using standard procedures. The pH was determined in 1M KCl suspension (soil:solution ratio = 1:2.5) with SenTix combined electrode (WTW). Organic C was determined spectrophotometrically after the oxidation of organic matter by $K_2Cr_2O_7$ (Walinga et al. 1992). The available nutrients were extracted by Mehlich 2 procedure (Fiala 1999); K was determined by flame photometer (Flapho 4), Ca and Mg by complexometric titration method and P colorimetrically. Cation

Table 1. Some chemical and physical characteristics of the soil used

	Character	Content
	pH/KCl	6.4
	C_{ox} (%)	1.00
	CEC [cmol (+)/kg]	10.89
Available nutrients by Mehlich II method	P (mg/kg)	65
	K (mg/kg)	157
	Ca (mg/kg)	3 742
	Mg (mg/kg)	393
Particle size distribution	coarse sand (%)	4.7
	fine sand (%)	52.4
	coarse silt (%)	21.1
	fine silt (%)	10.4
	clay (%)	12.3
Heavy metals – in *aqua regia* extract	Cd (mg/kg)	0.16
	Zn (mg/kg)	43.2

exchange capacity (CEC), soil texture, and total contents of Cd and Zn (in *aqua regia* extract) were determined by the Soil Science and Conservation Research Institute, Bratislava, Slovak Republic.

Determination of Cd and Zn concentrations in plants. Powdered plant material (shoots and anthodia) was decomposed by dry ashing procedure (Mader et al. 1998). The determination of Zn and Cd concentration was performed by differential pulse anodic stripping voltametry method using a polarographic analyser EP 100 (HSC Service, Bratislava) with HMDE electrode. The accuracy of plant analyses was checked by reference material RM 12-02-03 Lucerne with a certified content of Cd = 0.136 ± 0.003 mg/kg and Zn = 33.2 ± 0.5 mg/kg for which we obtained Cd = 0.142 ± 0.026 mg/kg and Zn = 33.9 ± 0.8 mg/kg.

Essential oil. Powdered anthodia (2 g) were extracted by steam distillation for two hours and the essential oil was determined gravimetrically. The principal components of essential oil: (–)-α-bisabolol, chamazulene, (*E*)-β-farnesene and ene-yne-dicycloethers were determined by high performance liquid chromatography (HPLC). The chromatographic conditions were described by Repčák et al. (1999). The content of the substances mentioned was described as percentage of dry matter of the drug.

Apigenin and herniarin content in chamomile anthodia was determined as aglycones by HPLC after extraction with methanol and acid hydrolysis with 6% HCl (Repčák et al. 1998). Standards for apigenin (Sigma, Taufkirchen, Germany) and herniarin (Extrasynthese, Genay, France) were used to obtain quantitative results for the components investigated.

Statistical evaluation. Analysis of variance (ANOVA) was performed on all experimental data and the least significant differences (*LSD*) were determined to compare the treatments.

RESULTS AND DISCUSSION

Accumulation of Zn

The results obtained by chemical analysis of specimens of experimental plants are presented in Table 2. The addition of Zn into the soil caused a significant increase in its concentration in the shoots from 14.9 mg/kg dry matter (control plants) to 271.0 mg/kg dry matter at the highest Zn addition. In comparison with the control the Zn content in the shoots increased 18-fold. Similar significant effect of Zn addition was reflected in its concentration in chamomile anthodia. While the control anthodia contained 32.7 mg Zn/kg, the highest addition of this metal to the soil (300 mg/kg) caused that the respective anthodia accumulated 159.8 mg Zn/kg dry matter. This is almost a 5-fold increase in comparison with the control. Moreover, this concentration of Zn in chamomile anthodia exceeds considerably the highest acceptable level (80 mg/kg) of this metal in consumables set by the "Food Code of the Slovak Republic" (1998). As the "Food Code" does not specify the tea species, we assume that the concentration determined may not pose an immediate risk to the users.

According to our earlier observations the commercial chamomile drug, which is sold in pharmacy contains approximately 20–50 mg Zn/kg dry matter (unpublished results). Comparable values (from 30 to 50 mg/kg dry matter) were reported by Chizzola (1989) in the samples of chamomile anthodia obtained from growers in Austria.

Among the usually studied potentially toxic elements, Zn belongs to the most mobile trace elements with high bioavailability and high transfer to the plants. This corresponds fully to our results of determination of Zn in the chamomile plants. The maximum concentration of Zn in chamomile shoot dry matter (271.0 mg/kg) most likely did not

Table 2. Accumulation of Zn in *Matricaria chamomilla* L. as influenced by soil-applied Zn

Zn treatment (mg/kg soil)	Zn concentration (mg/kg dry matter)	
	shoots	anthodia
Control	14.67 ± 5.97	32.68 ± 3.09
50	54.36 ± 12.89	61.10 ± 5.11
150	155.40 ± 15.47	100.26 ± 12.02
300	271.00 ± 67.02	159.80 ± 29.74
$LSD_{0.05}$	47.09	21.88

Data represent means ± *SD*, *n* = 5

Table 3. The influence of Zn supply on some production parameters of *Matricaria chamomilla* L.

Zn treatment (mg/kg soil)	High of plant (cm)	Tillers per plant	Dry matter yield (g per pot)	
			shoots	anthodia
Control	54.8 ± 5.4	8.3 ± 0.9	24.8 ± 3.0	12.5 ± 1.4
50	61.5 ± 4.1	7.8 ± 1.1	29.1 ± 5.1	13.5 ± 2.0
150	57.1 ± 2.2	7.9 ± 1.4	27.8 ± 3.7	11.6 ± 2.2
300	59.1 ± 5.0	7.7 ± 0.7	28.8 ± 5.1	11.2 ± 1.8
$LSD_{0.05}$	6.5	1.6	6.5	2.2

Data represent means ± *SD*, *n* = 5

reach the phytotoxic level as the plants did not show signs of chlorosis nor any visual symptoms indicating excess of Zn. However, Kabata-Pendias and Pendias (1992) assumed potential phytotoxic effects already at Zn concentrations exceeding 100 mg/kg dry plant matter.

Plant growth

The influence of Zn on some growth and productive chamomile characteristics is illustrated in Table 3. The data indicated that the level of Zn supply had a slightly positive effect on the plant height with significantly highest growth ($P < 0.05$) observed in plants treated with 50 mg Zn/kg soil. A similar effect of Zn supply on this parameter was reported also by Misra (1992) who also observed a positive effect of this element on production of tillers and branches of *Mentha arvensis* L. plants.

Moreover, the addition of Zn to the soil affected positively the yield of shoot dry matter which, compared to the control, was in all treatment groups higher by 12–17% (Table 3). Unfortunately, the increase of biomass was insignificant ($P > 0.05$). Chamomile is a flower drug and therefore flowers are the principal product of this important medicinal plant. The control chamomile plants produced 12.5 g anthodia dry matter per pot. The addition of Zn to the soil (50 mg/kg soil) increased yield of anthodia dry matter by 8% ($P > 0.05$). Application of additional Zn (150 mg/kg soil) caused a slight decrease in the yield (–7.2% in comparison with the control) and the adverse effect was still more intensive at the highest dose of 300 mg Zn/kg soil. Although the plants produced 11.2 g anthodia dry matter, this represents a significant 17% decrease ($P < 0.05$) in the principal product compared to the maximum yield of 13.5 g obtained at the rate of 50 mg Zn/kg soil.

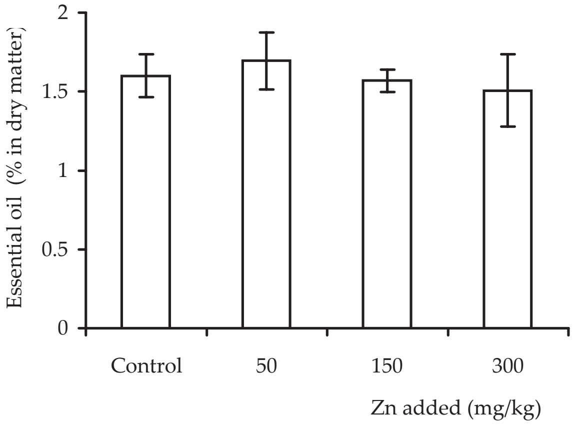

Figure 1. The content of essential oil in anthodia of *Matricaria chamomilla* L. affected by application of Zn into the soil; error bars represent standard deviation ($n = 5$)

The knowledge about the influence of Zn and other trace metals on productive parameters of chamomile is scanty. While Dovjak (1988) failed to observe any effect of B and Mo fertilization on production of chamomile drug in a field experiment, lately Masarovičová et al. (2003) observed drastic depression of root and shoot biomass in chamomile cultivated in a solution culture containing 240 μM Zn.

The content of secondary metabolites in chamomile anthodia

All metabolites observed in chamomile anthodia (essential oil – Figure 1 and its principal components chamazulene, (*E*)-β-farnesene and ene-yne-dicycloethers – Figure 2) showed a common tendency: evidence of culmination of the positive effect of Zn supply at a rate of 50 mg/kg and subsequent decrease with increasing supply, however, only insignificantly ($P > 0.05$). This weak response of chamomile to the level of Zn supply indicates

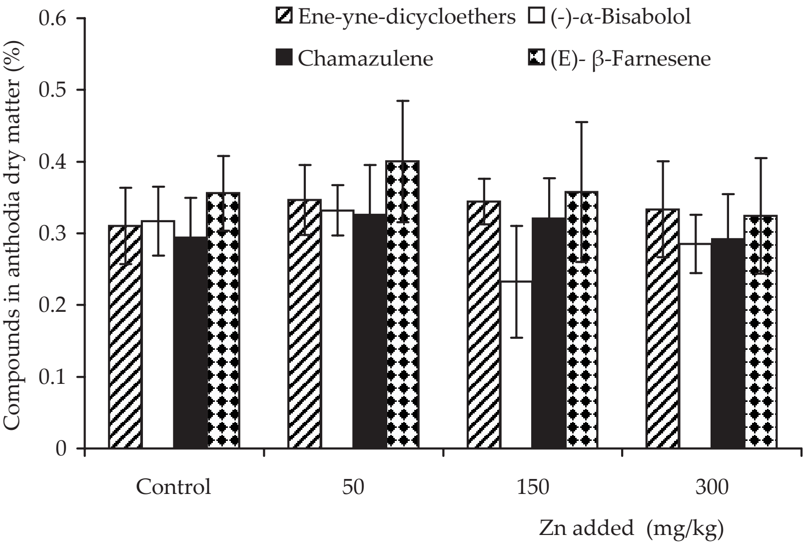

Figure 2. The effect of increasing Zn supply on the level of main essential oil components in chamomile anthodia dry matter; error bars represent standard deviation (n = 5)

its modest nutritional requirements on this trace element (the soil used was itself deficient in Zn) but, on the other hand, there is inherited fixation with regard to effective ingredients. Moreover, Zn is a metal with low toxicity (McBride 1994) and despite the fact that the dose of 300 mg Zn/kg soil resulted in accumulation of 271 mg Zn/kg shoot dry matter this concentration failed to disrupt plant metabolism or result in decline of its metabolites.

The data on the influence of trace elements or heavy metals on the active substances in chamomile plants are limited. While Dovjak (1988) did not observe any effect of Mo on the content of oil and chamazulene, application of toxic Cd to the soil in the study performed by Grejtovský et al. (2001) caused a significant decrease in the content of essential oil and sesquiterpenes chamazulene and (–)-α-bisabolol and an increase in (*E*)-β-farnesene and polyacetylenes ene-yne-dicycloethers. Similar to that of De Pasquale et al. (1988) observed a reduction of chamazulene in Cd-treated chamomile anthodia but, contrary to our observations, recorded a marked increase of the level of bisabolol.

A positive correlation between Zn supply and the content of essential oil and menthol in *Mentha arvensis* L. was reported by Misra (1992). Ren et al. (1993) observed increased synthesis of ginsenosides in *Panax quinquefolium* L. plants. On the other hand, field experiments with *Mentha piperita* L. and *Mentha arvensis* L. grown on strongly contaminated soil in the vicinity of a smelting plant for non-ferrous metals, showed a decreased yield of fresh herbage but the content of essential oil was not affected by contamination of soil with heavy metals (Zheljazkov and Nielsen 1996).

As far as the other two therapeutically important constituents of the chamomile drug, flavone apigenin and coumarin herniarin were concerned, no effect of soil Zn supply was observed (Table 4). The present observations agree with those obtained in our previous study which failed to show any changes in the content of these substances even after application of a considerable quantity of Cd into the soil (Grejtovský et al. 2001).

Effectiveness of Zn supply on the Cd uptake

While Zn is an essential nutrient for living organisms, Cd is non-essential and potentially toxic for higher plants, animals and humans. Plants take up Cd easily and accumulate it in relevant edible parts and because of that Cd transfer into the environment and the food chain is a subject of constant concern. From this point of view proper attention should be paid to chamomile plants, which accumulate Cd (Marquard and Schneider 1999, Grejtovský and Pirč 2000). The accumulative capacity of this medicinal plant was proven also in our experiment in which the total soil Cd content of 0.16 mg/kg (in *aqua regia* extract) resulted in 0.272 mg Cd/kg in control shoots and 0.280 mg Cd/kg in anthodia dry matter collected from these plants (Figure 3). Although this concentration does not exceed the maximum acceptable

Table 4. Effect of increasing Zn supply on the content of apigenin and heriarin in *Matricaria chamomilla* L. anthodia

Zn treatment (mg/kg soil)	Apigenin	Herniarin
	(mg/g dry matter)	
Control	5.05 ± 0.54	4.24 ± 0.71
50	5.41 ± 0.82	3.67 ± 0.62
150	5.22 ± 0.47	4.00 ± 0.48
300	5.07 ± 1.07	3.84 ± 0.47
$LSD_{0.05}$	1.02	0.77

Data represent means ± *SD*, *n* = 5

concentration of Cd in tea species (1 mg/kg dry matter) specified by the "Food Code of the Slovak Republic" (1998) it can result in problems on the market with herbal drugs (Kabelitz 1998).

The addition of Zn to the soil at a rate of 50 mg/kg decreased concentration of Cd in the shoots by 10% and in anthodia even by 37% ($P < 0.01$). An additional increase in soil Zn concentration caused a decrease in the accumulation of Cd in chamomile shoots by 18% ($P < 0.05$) at a rate of 300 mg Zn/kg soil.

Our results agree in general with the data obtained by other of authors who state that the Zn supply has an antagonistic effect on the accumulation of Cd by crop plants (Abdel-Sabour et al. 1988, Oliver et al. 1994, Choudhary et al. 1995). However, no interactions between Zn and Cd were observed in older studies (e.g. White and Chaney 1980), as was also indicated by Nan et al. (2002). The status of Zn nutrition of plants may play an important role in Cd accumulation by crop plants. However, it appears that the suppressive effect of Zn supply on Cd bioavailability is the most effective at low levels of Zn in the soil or in the Zn-deficient conditions (Abdel-Sabour et al. 1988, Oliver et al. 1994, Choudhary et al. 1995) as already low doses of Zn suppress considerably the Cd uptake. The use of higher doses of Zn may raise problems because it can inhibit soil micro-organisms and depress crop yield or cause the accumulation of Zn to high levels in relevant plant parts as observed in our experiment in chamomile anthodia, as a herbal drug intended for therapeutical purposes.

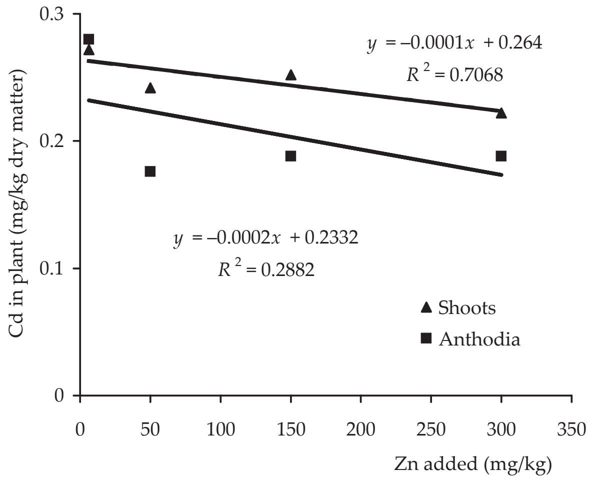

Figure 3. The influence of increasing Zn doses applied to the soil on accumulation of Cd in *Matricaria chamomilla* L. plants (*n* = 5)

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REFERENCES

Abdel-Sabour M.F., Mortvedt J.J., Kelsoe J.J. (1988): Cd-Zn interactions in plants and extractable Cd and Zn fractions in soil. Soil Sci., *145*: 424–431.

Alloway J.B. (ed.) (1995): Heavy Metals in Soils. 2nd ed. Blackie Academic and Professional, London, Glasgow.

Chizzola R. (1989): Metalic trace elements in herbs and spices grown in Austria. Acta Hort., *249*: 89–96.

Choudhary M., Bailey L.D., Grant C.A., Leisle D. (1995): Effect of Zn on the concentration of Cd and Zn in plant tissue of two durum wheat lines. Can. J. Plant Sci., *75*: 445–448.

De Pasquale R., Ragusa S., Iauk L., Barbera R., Galati E.M. (1988): Effect of cadmium on germination, growth and active principle contents of *Matricaria recutita* L. Pharm. Res. Commun., *20* (Suppl. V): 151–154.

Dovjak V. (1988): Fertilization effect by NPK-nutrients, molybden and boron on the dry matter formation dynamic, nutrient consumption, drug yield and essential oil formation in camomile (*Matricaria chamomilla* L.) plant. Poľnohospodárstvo, *34*: 906–924. (In Slovak)

Fiala K. (1999): Záväzné metódy rozborov pôd. Čiastkový monitorovací systém – Pôda. (Standard Methods of Soil Analysis.) VÚPOP, Bratislava.

Food Code of the Slovak Republik. (Potravinový kódex SR.) (1998): Part II. Epos, Bratislava. (In Slovak)

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., Eliašová A., Markušová K. (2001): Effect of cadmium on active principle contents of *Matricaria recutita* L. Herba Pol., *47*: 203–208.

Kabata-Pendias A., Pendias H. (1992): Trace Elements in Soils and Plants. 2nd ed. CRC Press, Boca Raton.

Kabelitz L. (1998): Heavy metal in herbal drugs. Pharm. Ind., *60*: 444–451. (In German)

Mader P., Száková J., Miholová D. (1998): Classical dry ashing of biological and agricultural materials. Part II. Losses of analytes due to their retention in an insoluble residue. Analusis, *26*: 121–129.

Marquard R., Schneider M. (1999): Problem of cadmium in cultivation of medicinal plants. In: Marquard R., Schubert E. (eds.): Fachtagung Arznei- und Gewürzpflanzen. Justus-Liebig-Universität Giessen, Fachverlag Köhler, Giessen: 5–15. (In German)

Masarovičová E., Králová K., Streško V. (2003): Effect of metal ions on some medicinal plants. Chem. Inz. Ekol., *10*: 275–279.

McBride M.B. (1994): Environmental Chemistry of Soils. Oxford University Press, Inc., New York.

Misra A. (1992): Effect of zinc stress in Japanese mint as related to growth, photosynthesis, chlorophyll content and secondary plant products – the monoterpenes. Photosynthetica, *26*: 225–234.

Nan Z., Li J., Zhang J., Cheng G. (2002): Cadmium and zinc interactions and their transfer in soil-crop system under actual field conditions. Sci. Total Environ., *285*: 187–195.

Oliver D.P., Schultz J.E., Tiller K.G., Wilhelm N.S., Merry R.H., Cozens G.D. (1994): The effect of zinc fertilization on cadmium concentration in wheat grain. J. Environ. Qual., *23*: 705–711.

Plescher A., Pohl H., Vetter A., Förtsch U. (1995): Transfer of heavy metals from soil into medicinal and spice herbs. Herba Germ., *3*: 116–125. (In German)

Ren F.C., Liu T.C., Liu H.Q., Hu B.Y. (1993): Influence of zinc on the growth, distribution of elements, and metabolism of one-year old American ginseng plants. J. Plant Nutr., *16*: 393–405.

Repčák M., Eliašová A., Ruščančinová A. (1998): Production of herniarin by diploid and tetraploid *Chamomilla recutita*. Pharmazie, *53*: 278–279.

Repčák M., Imrich J., Garčar J. (1999): Quantitative evaluation of the main sesquiterpenes and polyacetylenes of *Chamomilla recutita* essential oil by high-peformance liquid chromatography. Phytochem. Anal., *10*: 335–338.

Schilcher H. (1987): Die Kamille. Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Tiller K.G. (1989): Heavy metals in soils and their environmental significance. Adv. Soil Sci., *9*: 113–142.

Tlustoš P., Balík J., Dvořák J., Száková J., Pavlíková D. (2001): Zinc and lead uptake by three crops planted on different soils treated by sewage sludge. Rostl. Výr., *47*: 129–134.

Vangronsveld J., Clijsters H. (1994): Toxic effects of metals. In: Farago M. (ed.): Plants and the chemical elements. VCH Verlagsgesellschaft mbH, Weinheim: 150–177.

Vysloužilová M., Tlustoš P., Száková J. (2003a): Cadmium and zinc phytoextraction potential of seven clones of *Salix* spp. planted on heavy metal contaminated soils. Plant Soil Environ., *49*: 542–547.

Vysloužilová M., Tlustoš P., Száková J., Pavlíková D. (2003b): As, Cd, Pb and Zn uptake by *Salix* spp. clones grown in soils enriched by high loads of these elements. Plant Soil Environ., *49*: 191–196.

Walinga I., Kithome M., Novozamsky I., Houba V.J.G., van der Lee J.J. (1992): Spectrophotometric determination of organic carbon in soil. Commun. Soil Sci. Plant Anal., *23*: 1935–1944.

White M.C., Chaney R.L. (1980): Zinc, Cd and Mn uptake by soybean from two Zn- and Cd-amended coastal plain soils. Soil Sci. Soc. Am. J., *44*: 308–313.

Zheljazkov V.D., Nielsen N.E. (1996): Effect of heavy metals on pepermint and cornmint. Plant Soil, *178*: 59–66.

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