Lead uptake by *Matricaria chamomilla* L.

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ABSTRACT

Investigations were carried out under laboratory conditions in a nutrient solution according to Knop to observe the influence of increasing concentrations of Pb (5, 25, 50, 75 μmol/l) on its uptake and accumulation in chamomile (*Matricaria chamomilla* L.), diploid cv. Novbona. The essential part of Pb taken up by chamomile plants accumulated in roots; only minor portion of the metal was translocated to the above-ground part of the plant. Addition of Pb to the growth medium reduced significantly the root biomass (–46.3% at the highest supply of Pb); reduction in the above-ground dry matter (–18.3%) was insignificant. Pb treatment also reduced chlorophyll content in leaves ($P < 0.01$). The highest level of Pb resulted in a decrease of Chl *a* by 52% and of Chl *b* by 48%. Lead in the nutrient medium induced accumulation of free proline (Pro) in leaf rosette tissues ($P < 0.01$). Distribution of Pb in chamomile plants (cv. Novbona) and accumulation of Pb with focus on accumulation in inflorescences (drug *Flos chamomillae*) was investigated in a pot experiment with soil (Orthic Luvisol) supplemented with 50 mg Pb/kg dry soil. At this treatment, the content of Pb in chamomile inflorescences was 3-fold higher in comparison with the control ($P < 0.05$) but the level of accumulated Pb (2.08 mg Pb/kg dm flowers) was far below the limit (10 mg/kg) set by the WHO as the highest acceptable level of Pb in the chamomile drug.

Keywords: chamomile; chlorophyll; growth; *Flos chamomillae*; Pb accumulation; proline

Lead (Pb) is probably the most frequently occurring heavy metal in contaminated environment. Sources of Pb contamination include various human activities. Despite the effort of many advanced countries to reduce emissions of Pb by using lead-free automobile fuel, the worldwide emissions of Pb continue to increase (Adriano 2001).

Pb is the least mobile trace element which is strongly bound in the soil, particularly through complexation with organic matter, chemisorption on oxides, hydroxides and clay silicates and precipitation as sulphides, carbonates or phosphates (McBride 1994). Lead is not essential for plant metabolism although its occurrence in plants is common (Kabata-Pendias and Pendias 1992). Pb is taken up by plants only in very small quantities, its largest proportion being accumulated in roots and only small portion transported into shoots (Jones et al. 1973, Xiong 1998, Wierzbicka 1999, Fargašová 2004). Heavy metals of anthropogenic origin accumulate in the upper layer of soil, i.e. the root zone. They are not fixated strongly to the soil (Filipinski and Grupe 1990) so higher proportion of their mobile fraction (Šichorová et al. 2004) can penetrate to plants via roots (Borůvka et al. 1997, Tlustoš et al. 2001). The intensity of Pb uptake by plant biomass is affected significantly by plant species, soil pH, organic matter content and type and content of clay minerals (Tlustoš et al. 2001). Plants can tolerate even higher concentrations of Pb in the substrate without visual symptoms of damage (Xiong 1997). However, excessive accumulation of this toxic metal in plant tissues results in numerous disturbances of physiological processes (e.g. Vangronsveld and Clijsters 1994), causing eventually inhibition of growth, decreased yield and decreased quality of cultivated crops.

Accumulation of heavy metals in the useful parts of plants grown on highly contaminated soil is particularly important with medicinal plants because extreme demands are placed not only on the content of effective ingredients in these plants but also on their harmlessness, including the content of heavy metals, namely Cd and Pb

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(WHO 1999). We focused on chamomile plants (*Matricaria chamomilla* L.) as one of the most important and most frequently cultivated medicinal herb. Chamomile flowers (*Flos chamomillae* drug) are widely used in pharmacy and cosmetics for their unique composition and content of active ingredients with antispasmodic and antiinflamatory activity as well as for their action as a mild antiseptic and adstringent (Schilcher 1987). The aim of the present study was to investigate (i) the effect of increasing concentrations of Pb on growth and accumulation of Pb in the biomass of plants cultivated in a nutrient solution, (ii) the effect of the Pb concentrations used on selected biochemical parameters (content of chlorophyll and free proline) in chamomile leaves and, (iii) in a pot experiment (soil culture), to observe the distribution of Pb in the plant at a non-phytotoxic concentration in the substrate with special stress on Pb content in chamomile inflorescences.

MATERIAL AND METHODS

Solution culture. The experiment was carried out on chamomile (*Matricaria chamomilla* L.), diploid cv. Novbona. Fifteen days after germination in siliceous sand approximately uniformly developed seedlings were transferred to a common polyethylene vessel which served for the first pre-cultivation in a 5-fold diluted nutrient solution according to Knop, containing the following trace elements (μmol/l): B – 4.6, Cu – 0.03, Mn – 0.9, Zn – 0.08 and FeNaEDTA – 5 μmol. After 14 days of pre-cultivation (at continuous aeration), during which the nutrient solution was replaced 3 times, every time using solution with pH adjusted to 5.4–5.6 with KOH, the plants were transferred to polyethylene vessels (three plants per pot) filled with 660 ml of nutrient solution according to Knop, diluted 1:4, and pH of the solution was adjusted to 5.6 with KOH. After seven days of the second pre-cultivation, the nutrient solution was replaced with a fresh one of the same concentration but without FeNaEDTA (to avoid the formation of nontoxic metal-EDTA complexes); Fe was added as $FeCl_3$ (5% solution). Increasing quantities of Pb in the form of $PbCl_2$ were added to the nutrient solution to obtain respective increasing concentrations of Pb (5, 25, 50, 75 μmol/l). The experimental plants were cultivated for 25 days at continuous aeration under following conditions: photon flux density of 120 μmol/m^2/s at the leaf level, photoperiod 16 h, day/night temperature 24–25/18–20°C and relative humidity 65–70%. The nutrient solution was replaced every 3 days.

Prior to harvest, the roots were desorbed by placing the plants in an ice-cold 5 mmol/l $Ca(NO_3)_2$ solution for 1 h. After separating the root system from the leaf rosette, it was washed under running distilled water and finally rinsed with redistilled water. After taking the samples of fresh leaves (to determine the content of chlorophyll and free proline), both parts of the plants were dried immediately at 80°C.

Soil culture. The diploid cv. Novbona of *Matricaria chamomilla* L. was grown in plastic pots under natural climatic conditions in the Botanical Garden of P.J. Šafárik University in Košice. The pots were filled with 10 kg of soil (Orthic Luvisol) with the following agrochemical and physical parameters: pH/KCl – 6.7, Cox – 1.0%, CEC – 16.0 cmol(+)/kg; available nutrients (Mehlich II) (mg/kg): P – 65, K – 157, Ca – 3740, Mg – 392; particle size composition (%): sand – 11.6, silt – 65.7, clay – 22.7. The experiment consisted of the control – soil with the natural content of Pb 17.2 mg/kg (in Aqua regia extract), or 0.016 mg Pb/kg soil in mobile form (in 1 mol/l NH_4NO_3 extract), and the Pb treatment that consisted of soil supplemented with Pb at a rate of 50 mg Pb/kg dry soil. Each pot with treated soil was prepared individually by constant mixing of soil while adding solution of $PbCl_2$ to ensure uniform distribution of Pb. All the treatments were repeated 4 times. After 2-week equilibration of soil at 60% 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 moisture of soil was adjusted daily by adding distilled water to maintain 60% MWHC. Temperature during vegetation ranged between 23 and 28°C during the day and between 14 and 18°C at night. Chamomile inflorescences were collected at the stage when about 2/3 tubular flowers were in full flower. Cultivation was terminated on day 75 after potting the plants, i.e. when the age of plants was 125 days. The plants were cut and their roots were separated by washing with tap water and rinsing with redistilled water. The production of biomass (shoots and roots) was determined after 72 h drying at 80°C. The yield of drug (*Flos chamomillae*) was determined by weighing the inflorescences dried in the air at room temperature.

Determination of Pb in plants. Powdered plant material was decomposed by dry ashing procedure (Mader et al. 1998). Pb concentration was determined 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 a reference material RM 12-02-03 Lucerne.

Chlorophyll determination. Leaf samples (~0.5 g) were selected randomly from the plants and were homogenized in a porcelain mortar in 10 ml 80% (v/v) acetone with a small quantity of $CaCO_3$. The extract was centrifuged for 5 min at 2000 g. A portion of extract (1 ml) was made up to 5 ml and its absorbance was recorded at 663 and 646 nm spectrophotometrically. Chlorophyll (Chl) *a* and *b* were calculated according to Lichtenthaler (1987).

Determination of proline content. Fresh leaf tissues (~0.250 g) were homogenised in 10 ml 3% (w/v) sulfosalicylic acid and centrifuged at 2000 g for 10 min. Proline was measured exactly according to Bates et al. (1973). Concentration of free proline was calculated from a standard curve for L-proline (Merck, Darmstadt, FRG) and expressed as μmol/g leaf dm.

Statistical evaluation. Experimental data obtained by solution culture were evaluated by one-way analysis of variance (ANOVA). Kruskal-Wallis test was performed to detect differences between treatments for each measured parameter ($P < 0.05$). When a difference was detected, multiple comparisons were performed to separate the means. The pot experiment with soil supplemented with Pb was evaluated by Student *t*-test.

RESULTS AND DISCUSSION

Uptake and accumulation of Pb (solution culture)

The concentration of Pb in roots and tops (leaf rosette) of experimental chamomile plants (*Matricaria chamomilla* L.) was affected significantly by the amount of Pb added to the nutrient solution ($P < 0.01$, Table 1). Considerably high concentration of Pb (15856 mg/kg dm, $P < 0.001$, when the nutrient solution contained 75 μmol/l Pb) accumulated in chamomile roots and this concentration increased strongly and almost proportionally with increasing level of Pb in the nutrient medium. This observation is in full agreement with the data reported by a number of authors who proved by numerous studies that Pb accumulated preferentially in roots capable of retaining more than 90% Pb taken up by the plant (e.g. Jones et al. 1973, Xiong 1998, Wierzbicka 1999, Geebelen et al. 2002, Fargašová 2004 and others).

Usually some portion of Pb taken up by the roots is translocated via xylem into the above-ground plant organs (e.g. Jones et al. 1973, Fargašová 2004). The content of Pb in the leaf rosette of chamomile plants (Table 1) increased significantly with increasing concentration of Pb in the nutrient solution ($P < 0.01$), however, accumulation of Pb in this part of the plant was generally very low, reaching only 2.72 mg Pb/kg dm at the highest concentration of Pb in the nutrient solution (75 μmol). The reason for such low accumulation of this metal can probably be seen in elimination of EDTA from the nutrient solution during exposure of plants to Pb, when Fe contained in the nutrient medium during pre-cultivation in the form of FeNaEDTA was replaced by addition of $FeCl_3$ solution. Sequestration of Pb by synthetic chelates (e.g. EDTA) increases bioavailability, uptake and translocation of this metal in plants that are then capable to accumulate in their tops increased amounts of Pb in the form of Pb-EDTA (e.g. Vassil et al. 1998). Geebelen et al. (2002) observed the accumulation of 928 mg Pb/kg dm in the leaves of beans growing in solution containing 200 μmol Pb-EDTA without significant morphological changes in leaves while other authors (Kabata-Pendias and Pendias 1992) considered 30 to 300 mg Pb/kg dm as a critical concentration. Evidently, in addition to the concentration of the metal in the growth

Table 1. The effect of increasing concentrations of Pb in nutrient solution on accumulation of Pb in roots and shoots of *Matricaria chamomilla* L.

Pb in solution (μmol/l)	Pb concentration (mg/kg dm)	
	roots	shoots*
Control	8.9 ± 1.5^{a}	0.28 ± 0.19^{a}
5	1453 ± 151^{c}	0.71 ± 0.31$^{a, b}$
25	6280 ± 1 149$^{c, d}$	1.08 ± 0.25^{b}
50	10850 ± 2 330^{d}	2.16 ± 0.48^{c}
75	15856 ± 2 933^{e}	2.72 ± 0.79^{c}

*leaf rosette

Values are means ± SD (standard deviation), $n = 5$; mean values with different letters in the same column are significant ($P < 0.05$) according to Kruskal-Wallis multiple range test

Table 2. Production of biomass by *Matricaria chamomilla* L. growing in a nutrient solution with increasing concentrations of Pb and dry matter content in leaf rosette tissues

Pb treatment (μmol/l)	Biomass production (mg dm per plant)		Shoot/root ratio	Dry matter content in leaves (%)
	roots	shoots		
Control	137.1 ± 13.4[a]	475.6 ± 92.7[a]	3.44[a]	9.87 ± 1.24[a]
5	104.4 ± 19.7[b]	397.6 ± 42.7[a]	3.85[a, b]	11.30 ± 3.38[a]
25	91.7 ± 26.3[b]	414.5 ± 87.7[a]	4.56[b, c]	10.87 ± 2.50[a]
50	85.1 ± 36.0[b, c]	398.8 ± 160.9[a]	4.69[c]	9.72 ± 0.98[a]
75	73.6 ± 14.3[c]	387.5 ± 77.6[a]	5.19[c]	10.47 ± 1.40[a]

Values are means ± SD, $n = 5$; values followed by the same letter in the same column are not significantly different ($P < 0.05$) as measured by the Kruskal-Wallis test

medium and the exposure time, also biological specificity of the respective plant species should be considered.

Growth response

Addition of Pb to the growth medium resulted in a gradual reduction of root mass (Table 2). When 75 μmol/l Pb was added, the biomass of roots decreased significantly ($P < 0.01$) by 46.3%. Roots of plants treated with Pb also showed slight but evident morphological changes, such as shorter lateral roots and reduction of root hair. The majority of authors involved in similar research observed an inhibition of root growth in plants grown in substrates contaminated with Pb (e.g. Stiborová et al. 1986, 1987, Punz and Sieghardt 1993, Geebelen et al. 2002, Fargašová 2004). Reduction of root growth may be induced by disturbances to metabolic processes in the roots, which can occur already at low Pb levels in a growth substrate. Geebelen et al. (2002) observed a significant induction of syringaldazine peroxidase (EC 1.11.1.7) in the roots of beans already at 10 μmol Pb-EDTA in the nutrient solution. Increased activity of peroxidases indicates an initiation of oxidative stress (Vangronsveld and Clijsters 1994). In addition, Stiborová et al. (1986, 1987) presumed that reduction of root growth might be associated with metal-induced inhibition of protein synthesis in roots.

Supply of Pb had no significant effect on the weight of dry biomass of chamomile leaf rosette (Table 2). In comparison with the control, the highest concentration of Pb in the nutrient solution (75 μmol/l) caused a decrease in the aboveground part dm by 18.5% but the difference was insignificant ($P > 0.05$). Similarly, some decrease

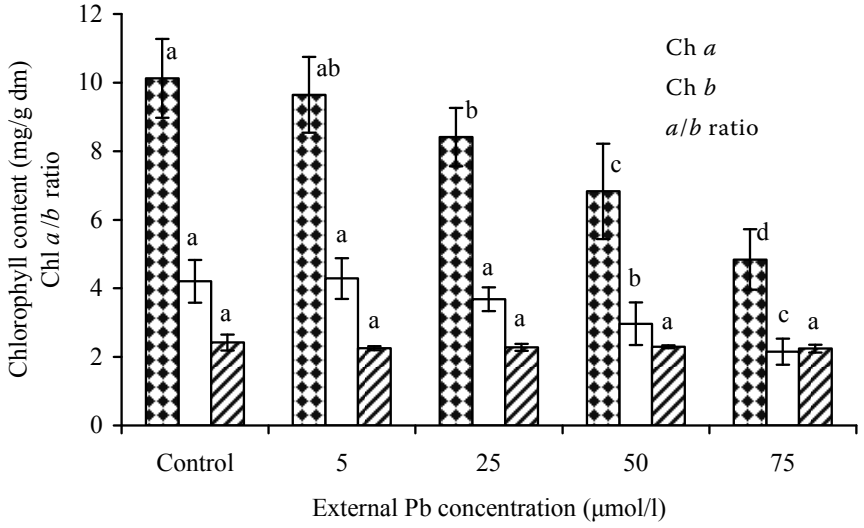

Figure 1. Changes in chlorophyll (Chl *a*, Chl *b*) content and chlorophyll *a*:*b* ratio in leaves of *Matricaria chamomilla* L. after 25-day exposure to increasing concentrations of Pb. Values are means ± SD ($n = 5$), indicated by bars

in production, observed with other treatments, indicated relationship with external concentration of Pb. The shoot/root ratio increased significantly ($P < 0.01$) with increasing concentration of metal in the nutrient solution, which indicated a higher sensitivity of chamomile roots compared to the shoots. However, the extent of retardation of roots or above-ground parts depends on the concentration of metal, plant species and other factors that affect the plant growth. In addition to marked negative effects of Pb on plant growth, also a significant stimulation of plant biomass production was reported by some authors (e.g. Khan and Khan 1983, Xiong 1998).

Pb influence on chlorophyll content

The effect of Pb on chlorophyll content in leaf rosette tissues is presented in Figure 1. In comparison with the control, the content of chlorophylls (Chl *a* and Chl *b*) was reduced significantly ($P < 0.01$) as a result of increasing concentrations of Pb. The inhibition of Chl *a*, at the highest treatment with Pb was somewhat higher as it decreased by 52% in comparison with the control while the decrease in Chl *b* was only 48%. Due to a more intensive decrease in Chl *a*, the ratio of both components (Chl *a*:Chl *b*) declined from 2.42 (control plants) to 2.24 (treatment with 75 µmol/l Pb), however, the difference was statistically insignificant ($P > 0.05$). Similar observations were reported by Fargašová (2004) who compared toxicity of heavy metals (Cd, Cu, Pb, Se, Zn) to seedlings of *Sinapsis alba* L. A significant reduction in the content of chlorophyll in the leaves of beans after exposure to Pb-EDTA, although with stronger decrease in Chl *b*, was observed by Geebelen et al. (2002) as well as by Stiborová et al. (1986) in barley treated with $Pb(NO_3)_2$; contrary to that, maize leaves showed a significant increase in chlorophyll (Stiborová et al. 1987).

Despite the fact that chlorophyll content and the ratio of its components (*a*:*b*) can be modified by internal factors and environmental conditions, chlorophyll is one of the most frequently investigated (although non-specific) characteristics used for identification of physiological disorders induced by the impact of pollutants (Masarovičová et al. 1999).

In our experiment the content of Chl *a* and Chl *b* decreased proportionally with increasing concentration of Pb in the growth medium while the ratio of these components changed only insignificantly (*a*:*b*); hence, we assume that the decrease in chlorophyll could be caused by inhibition of its synthesis due to interaction of Pb with – SH groups of δ-aminolevulinic acid dehydratase (ALA-D), an enzyme catalysing the conversion of δ-ALA into porphobilinogen in the synthesis of chlorophyll (Prassad and Prassad 1987).

Content of free proline

The level of accumulation of free proline (Pro) in the leaf rosette tissues in relation to external concentration of Pb is presented in Figure 2. While control plants contained 2.39 µmol Pro/g dm after 25-day cultivation period, plants exposed to Pb showed an increase in proline content proportional to increasing concentration of Pb in the nutrient solution. At the highest supply of Pb (75 µmol/l), the Pro content in leaf rosette tissues reached 5.24 µmol/g dm which is a significant 2.2-fold increase in comparison with the control ($P < 0.01$). Generally, the accumulation of free Pro is a function of the length of exposure to stress and depends also on the type of stress. Therefore, it seemed rather difficult to compare the magnitude of accumulation of free Pro in our chamomile plants with the values reported by other authors, particularly with regard to diversity of experimental conditions, plant species, type of heavy metals and their concentrations (e.g. Alia and Pardha Saradhi 1991, Bassi and Sharma 1993, Schat et al. 1997).

Exposure to heavy metals disturbs water balance in plants and results in water deficit in plants (Barceló and Poschenrieder 1990). Schat et al.

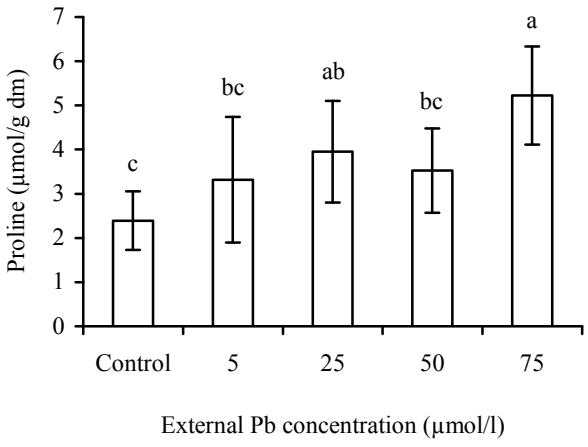

Figure 2. Free proline in leaves *Matricaria chamomilla* L. after 25-day exposure to increasing concentration of Pb in the nutrient solution. Each value is the mean (± SD) of 5 plants

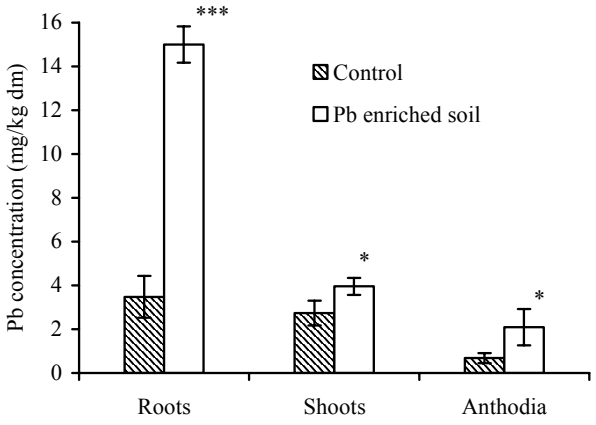

Figure 3. Distribution and accumulation of Pb in chamomile plants (*Matricaria chamomilla* L.); pot experiment with soil supplemented with 50 mg Pb/kg dry soil.

Control – soil with natural content of Pb (17.2 mg/kg in Aqua regia extract). Error bars represent SD, $n = 4$

*, ***significant at the $P \leq 0.05$, or $P \leq 0.001$ probability levels, respectively

The highest acceptable concentration of Pb in drug *Flos chamomillae,* determined by the World Health Organization, is 10 mg/kg dm (WHO 1999)

(1997) proved that metal-induced proline accumulation depends on the development of a metal-induced water deficit in the leaves rather than by a toxic heavy metal accumulation per se. Although the production of the above-ground biomass of experimental plants was reduced, the reduction was insignificant ($P > 0.05$, Table 2). Variations in dry matter content in chamomile leaves failed to show its relationship to Pb concentration in the nutrient medium, which indicated that water supply to leaf rosette tissues was not affected (Table 2). The proportionally increasing values of free Pro allowed us to assume that during the exposure to Pb the chamomile plants maintained their active physiological status which we consider essential for the „via root" intake of Pb.

Distribution and accumulation of Pb in chamomile plants (soil culture)

This part of our study focused exclusively on distribution and accumulation of Pb in individual chamomile plant parts with special stress on accumulation of Pb in inflorescences (anthodia; drug *Flos chamomillae*). Determination of Pb level in individual parts of the plant showed considerably decreasing accumulation of Pb in the sequence root > shoot > inflorescence and reflected logically the small mobility of this metal in the plant (Figure 3). The level of accumulation of Pb in inflorescences (anthodia) of control plants (0.68 mg/kg dm) was comparable with values reported by Dragland (1996) and Chizzola et al. (2003), and was far below the values of 90% percentile determined by Kabelitz (1998) by evaluation of Pb content in 321 samples of chamomile drug in Germany.

The soil used in our experiment contained 17.2 mg Pb/kg (in Aqua regia extract), which is lower than the median value of Pb in arable soils in the Slovak Republic (Wilcke et al. 2005). Adding Pb to this soil at a rate of 50 mg/kg resulted in an increased concentration of this metal in all analysed chamomile parts (Figure 3). The content of Pb in the utility part (inflorescence) of chamomile plants grown on contaminated soil was increased 3-fold in comparison with the control. However, from the hygienic and toxicological point of view, the level of accumulated Pb (2.08 mg/kg dm) was far below the highest acceptable value – 10 mg/kg dm – prescribed for *Flos chamomillae* by the World Health Organization (WHO 1999). The same limit

Table 3. The effect of Pb addition to soil on production of biomass of chamomile (*Matricaria chamomilla* L.) – pot experiment

Treatment	Dry matter yield (g per pot)		
	roots	shoots	anthodia
Control	4.5 ± 0.9	30.3 ± 4.8	14.6 ± 1.2
Pb (50 mg/kg soil)	4.7 ± 0.4	31.2 ± 3.4	16.5 ± 0.8
t-test	NS	NS	*

Control – soil with natural content of Pb (17.2 mg/kg in Aqua regia extract)

Values are means ± SD of four replicates

NS – not significant, *significant at the $P \leq 0.05$ probability level; for both treatments, control and Pb, mean values were evaluated by Student's *t*-test

is set in the Food Code of the Slovak Republic (Anonymous 1998).

From our point of view, the increased accumulation of Pb in inflorescences of chamomile plants grown on soil supplied with Pb can be closely related to the low content of soil organic matter, lower proportion of clay fraction in the soil used and related weaker binding of Pb to soil particles. On the other hand, anthropogenic contamination of soil increases proportion of mobile forms of heavy metals in the surface horizon (Šichorová et al. 2004) and, because of that, the subsequent translocation of Pb taken up by plants depends to a considerable degree on its physiological status. The concentration of Pb used in our study (50 mg/kg soil) had no toxic effect on chamomile plants and failed to affect production of plant biomass although the slightly increased yield of inflorescences by contaminated plants was significant (Table 3). Similar positive effect of Pb on plant growth, particularly of its low concentrations, was reported by other authors (e.g. Khan and Khan 1983, Xiong 1998).

Based on the results presented in this study as well as on information published by the above mentioned authors we can conclude that under common cultivation conditions, at common (normal) content of Pb in the soil, the concentration of this toxic metal in *Flos chamomillae* drug can hardly reach the limit value of 10 mg/kg dm (WHO 1999). Increased levels of Pb in the chamomile drug indicate either wrong technology of cultivation (e.g. irrigation with water contaminated with heavy metals) (Abou-Arab and Abou Donia 2000), improper processing of the drug, or unsuitable location affected by high loads of atmospheric pollutants (Baranowska et al. 2002).

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