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The risk element uptake by chamomile (*Matricaria recutita* (L.) Rauschert) growing in four different soils

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Abstract: German chamomile (*Matricaria recutita* (L.) Rauschert) belongs to the plants with a high tolerance to toxic elements. The ability of chamomile to accumulate risk elements was tested in a pot experiment in which four soils contaminated by different levels of arsenic (As), cadmium (Cd), lead (Pb), and zinc (Zn), differing in their physicochemical parameters, were used. The element mobility in the soils was affected predominantly by the cation exchange capacity (CEC) of the soils. Whereas As, Pb, and Zn were retained in roots, Cd showed good ability to translocate to the shoots, including anthodia, even in extremely Cd-contaminated soil without symptoms of phytotoxicity. The bioaccumulation factor for Cd calculated as the ratio of element content in the plant and the soil was the highest among the investigated elements. Between 5.5 and 35% of the total Cd was released to infusion, and its extractability decreased with increasing Cd content in anthodia. The essential oil composition suggested an alteration of the abundance of the individual compounds. However, no detectable contents of risk elements were found in the oil. Chamomile can be recommended as a suitable alternative crop for risk element-contaminated soils tested within this experiment, but only for production of essential oil.

Introduction

Chamomile is one of the oldest known medicinal herbs (Shrivastava et al. 2010). The German chamomile (*Matricariae flos* drug) is one of the most famous medicinal plants and has been known for more than 2,500 years. The plant contains 0.24–1.9 % volatile oil and is composed of a variety of separate oils. Approximately 120 secondary metabolites have been identified in chamomile, including 28 terpenoids and 36 flavonoids. The principal components of the essential oil extracted from German chamomile flowers are the terpenoids α-bisabolol and their oxide azulenes, including chamazulene and acetylene derivatives (McKay and Blumberg 2006; Srivastava et al. 2010). The unique pharmacological effect of German chamomile results from the combined action of all inherent active compounds: sesquiterpenes (/-/-α – bisabolol and its oxides, chamazulene), flavonoids (apigenin glucosides, luteolin, quercetin), polyacetylenes ((Z)-en-in-dicycloether), coumarins (herniarin and umbelliferone), mucins, phytosterols, choline, phenol carboxylic acids, and mineral substances. The most important ones are the components of the essential oil (sesquiterpenes) and the flavonoid fraction. The major phenolic compounds in the chamomile infusions identified by Raal et al. (2012) were chlorogenic acids, ferulic acid glycosides, dicaffeoyl quinic acids, and apigenin glycosides. However, high variability of the amounts of active compounds was observed according to the sampling location. The influence of soil and climatic conditions, particularly the altitude and fertility of the environment, must be taken into account (Formisano et al. 2015). Animal model studies indicate potent anti-inflammatory action, some antimutagenic and cholesterol-lowering activities, as well as antispasmodic and anxiolytic effects (McKay and Blumberg 2006). Although numerous preparations of chamomile have been developed, the most popular use seems to be in the form of herbal tea (Srivastava et al. 2010).

Chamomile appears to be tolerant to Cd, because its overall metabolism is only slightly altered by high concentrations of this metal. In this context, Pavlovič et al. (2006) reported over 300 mg·kg^{-1} of Cd in chamomile shoots growing in soilless culture containing 12 µM Cd with only small damages in the treated plants. However, because it has been confirmed that chamomile has the ability to accumulate Cd (Chizzola et al. 2003, Masarovičová et al. 2010), the soils used for the production of chamomile drugs must be checked. On the contrary, the uptake of other important risk elements (e.g. Pb) is lower compared to other medicinal plant species (Grejtovský et al. 2008, Kaličanin and Velimirović 2013). However, adverse effects of risk elements (Cu, Mn, Ni, Pb, and Zn) on the number of inflorescences, leaf length and width, and seed production were reported by Prokop'ev et al. (2014). Scientific results indicate

that risk elements stimulate an increase in ROS (reactive oxygen species) formation, being affected especially by the given metal and exposure concentration (Petö et al. 2011).

The plant-availability of elements in soil is affected by the physicochemical parameters of the soils, climatic conditions, plant genotype, and plant management (Kabata-Pendias and Pendias 2001, Tokalıoğlu et al. 2003). Thus, the element content in plants is not directly related to the total element content in the soil (Schwartz et al. 2001). Among the physicochemical and biological parameters affecting the plant-availability of risk elements, soil pH, redox potential, cation exchange capacity (CEC), content of carbonates, hydroxides and oxides of Fe and Mn, clay minerals, organic matter, plant species, vegetation cover and the activity of soil organisms and microorganisms should be mentioned (Alloway 1990, Ross 1994, Cheng and Mulla 1999, Adriano 2001, Kabata-Pendias and Pendias 2001). The effects of nutrient content on the growth and yield of German chamomile have already been reported (Mosleh et al. 2013). The connections between the nutrient supplement of the chamomile plants and the content of essential oil was published as well. Nasiri et al. (2010) proved the beneficial effect of foliar application of Fe and Zn on both plant yield and essential oil content.

In our experiment, the ability of German chamomile to accumulate potential risk elements was tested in a pot experiment in which four soils contaminated by different levels of As, Cd, Pb, and Zn and characterized by different physicochemical parameters were used. Moreover, the potential interactions with essential elements such as Cu, Fe, and Mn were assessed, as well as the extractability of both risk and essential elements into the infusion. Simultaneously, the composition of essential oil and reachability of the risk elements into the oil were assessed. The main objectives of the experiment were i) to verify the tolerance of chamomile plants to increased risk element contents in soils as affected by various soil properties and risk element contamination levels and ii) to estimate the potential risk of enhanced element contents for chamomile production as a medicinal plant as well as the possibility of chamomile cultivation in arable soil contaminated by risk elements.

Material and methods

Experimental design

The plants were cultivated in pots with four soils differing in their main physicochemical characteristics and pseudototal (i.e. *Aqua Regia* soluble) risk element contents (Table 1). For the experiment the soils affected either by industrial activity (non-ferrous metals mining and smelting, chemical industry) – Chernozem 2 (50.69°N, 13.72°E), Fluvisol (50.52°N, 14.07°E), Cambisol (49.69°N, 14.01°E), or by land application of sewage sludge in the case of Chernozem 1 (50.12°N, 14.54°E) were used. All the soils were already investigated and the more detailed description of the soils and/or locations was published elsewhere (Száková et al. 1999, 2000). The soils were collected in each sampling point from a depth of 0–25 cm, air dried, sieved through a 5-mm plastic sieve, and homogenized. Laboratory soil samples for the determination of total and mobile concentrations of elements were air dried at 20°C, ground in a mortar, and passed through a 2-mm plastic sieve. The *M. recutita* (diploid variety Bohemia widely planted in the Czech Republic) plants were cultivated in 6-liter plastic pots with 5 kg of air-dry soil, and six replicates were used for each treatment. The Czech diploid variety Bohemia was licensed in 1952 and is classified as the bisabololoxid genotype. The chamomile of the variety Bohemia has the certification trademark no. CZ/00411/PDO – "Chamomilla Bohemica." Bohemia typically contains 1.2% of essential oil. Mineral fertilizer NPK (0.5 g N, 0.16 g P, 0.4 g K per pot as inorganic salt solutions, representing ca. 300 kg N, 96 kg P, and 240 kg K per ha, respectively) was added before sowing. The experiment started at April 2008, and 5 plants were sown per pot. Soil moisture was regularly controlled and kept at 60% of the maximum water holding capacity (MWHC). Pots were placed in an outdoor weather-controlled vegetation hall protecting the pots against rainfall. Weed plants were regularly manually removed; other cultivation conditions such as light and temperature were not managed. The experiment was terminated at July 2008. The harvested biomass was divided into anthodias, shoots, and roots. Plant material was carefully washed in deionized water, dried at 60°C in a drying oven, homogenized, and analyzed.

Analytical methods

Determination of soil physicochemical parameters

The pH value was determined in 0.01 M $CaCl_2$ extract 1/10 (w/v = 5 g + 50 ml, Novozamsky et al. 1993). Cation-exchange capacity (CEC) was calculated as the sum of Ca, Mg, K, Na, Fe, Mn, and Al extractable in 0.1 M $BaCl_2$ (w/v = 1 g + 20 ml for 2 hours) (ISO 1994). Total organic carbon (TOC) was determined spectrophotometrically after the oxidation of organic matter by $K_2Cr_2O_7$ (Sims and Haby 1971). For the determination of potentially available portions of main nutrients in soils, the Mehlich III extraction procedure (0.2 M CH_3COOH + 0.25 M NH_4NO_3 + 0.013 M HNO_3 + 0.015 M NH_4F + 0.001 M

Table 1. The main characteristics of the experimental soils

Soil type	Texture	Cox	pH	Ca#	Mg#	K#	P#	CEC¶	As$	Cd$	Cr$	Cu$	Ni$	Pb$	Zn$
		%		mg/kg	mg/kg	mg/kg	mg/kg	mmol/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
Chernozem 1	loam	3.45^a	7.3^a	5828^a	146^a	199^a	82^a	69.2^a	20.7^a	14.6^c	82.1^b	68.2^b	34.8^c	34.0^a	133^a
Chernozem 2	loam	3.95^a	6.8^a	2668^b	156^a	192^a	128^b	224^c	362^c	1.0^a	19.5^a	24.4^a	13.0^a	99.6^b	112^a
Fluvisol	sandy loam	5.93^b	4.4^b	1026^c	35^b	120^a	248^c	176^{bc}	27.6^a	1.6^a	100^b	46.0^b	21.3^b	48.6^a	207^b
Cambisol	loam	3.88^a	6.3^a	2420^b	108^a	363^b	81^a	165^b	124^b	4.8^b	27.7^a	23.6^a	17.8^b	1276^c	190^b

plant-available element contents determined by Mehlich III extraction procedure (Mehlich 1984); ¶ cation exchange capacity; $ the pseudototal (*Aqua regia* soluble) concentrations of the investigated elements in soils; the averages marked by the same letter superscripts did not significantly differ at p<0.05 within individual columns; n=6

ethylenediamine acetic acid (EDTA) at a solid/liquid ratio of 1/10 [3 g + 30 ml] for 10 minutes) was applied (Mehlich 1984).

Determination of essential and risk elements in soils and plants

Plant samples were decomposed using the dry ashing procedure as follows: An aliquot (~1 g) of the dried and powdered plant material was weighed into a borosilicate glass test tube and decomposed in a mixture of oxidizing gases (O_2+O_3+NO_x) in an Apion Dry Mode Mineralizer (Tessek, Czech Republic) at 400°C for 10 hours. The ash was than dissolved in 20 ml of 1.5% HNO_3 (Miholová et al. 1993). A certified reference material CRM CTA-OTL-1 Tobacco leaves was applied for the quality assurance of analytical data. For the determination of the pseudototal element contents on soil, aliquots (~0.5 g) of air-dried soil samples were decomposed in a digestion vessel with 10 ml of *Aqua Regia* (i.e. nitric and hydrochloric acid mixture in a ratio of 1+3). The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave-assisted wet digestion system for 33 minutes at 210°C. After cooling, the digest was quantitatively transferred into a 25 ml glass tube, topped up by deionized water, and kept at laboratory temperature until measurement. A certified reference material RM 7001 Light Sandy Soil was applied for the quality assurance of analytical data.

For the determination of mobile and element proportions, the soil samples were extracted with 0.11 mol l^{-1} solution of CH_3COOH at a solid/liquid ratio of 1/20 (1 g + 20 ml) for 16 hours (Quevauviller et al. 1993).

Chamomile infusions were prepared as follows: 1±0.001 g of dried anthodias was weighed out and put into standardized glass beakers. Then, 50 ml of boiled distilled water was poured into the glass beakers, after which they were covered by watch glasses. After 15 min, the extracted solution was filtered through filter paper (blue label) into test tubes and immediately measured (Street et al. 2006). All samples were analyzed in triplicates, and blanks represented 10% of the total number of samples.

The determination of As, Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn in soil extracts, soil and plant digests, and plant infusions was carried out by inductively coupled plasma optical emission spectrometry with axial plasma configuration (ICP-OES, Varian VistaPro, Australia) equipped with an autosampler SPS-5 at spectral lines λ = 193.7 nm for As, λ = 226.5 nm for Cd, λ = 205.6 nm for Cr, λ = 327.4 nm for Cu, λ = 238.3 nm for Fe, λ = 257.6 nm for Mn, λ = 231.6 nm for Ni, λ = 220.0 nm for Pb, and λ = 206.2 nm for Zn. Calibration solutions were prepared in corresponding extraction agents as follows: 5–50 µg/l for Cd; 50–500 µg/l for As, Cr, and Ni; 1–10 µg/l for Mn, Pb, and Zn; 0.1–1 µg/l for Cu; and 5–50 µg/l for Fe.

Determination of the essential oil

The chamomile anthodias (20 g of dry matter) were placed in the distillation flasks and immersed in 0.4 L of water. The reactor flask was than heated at 100°C for 4 hours. The essential oil obtained by hydrodistillation was separated out from the distilled water by 0.001 µm of natrium sulphate (Na_2SO_4). The samples were centrifuged for 5 minutes sequentially. Possible residues of hexane were insufflated by nitrogen. Essential oil composition was determined using Agilent Technologies 6890 N, Series II gas chromatography, fitted with a flame ionization detector (FID) and capillary column HP – 101 (25.0 × 0.2 mm id × 0.20 µm nominal) split/splitless system. The operation conditions were as follows: the GC injector temperature was 280°C and the detector temperature 300°C, nitrogen was used as carrier gas. The sample size 1.0 µl and automatic type of injection were used. Major active components of volatile oil were determined using standard compounds chamazulene, -(-)-α bisabolol (Roth, Analytika Praha Ltd.), mode: constant flow. The oven temperature was programmed from 120°C, rising at 2.0°C/min to 150°C. The second slower method was used to obtain better separation of some peaks. In this case, the oven was programmed from 150°C, rising at 1°C/min to 155°C and continuing at 50°C/min until 260°C with 3-minute hold.

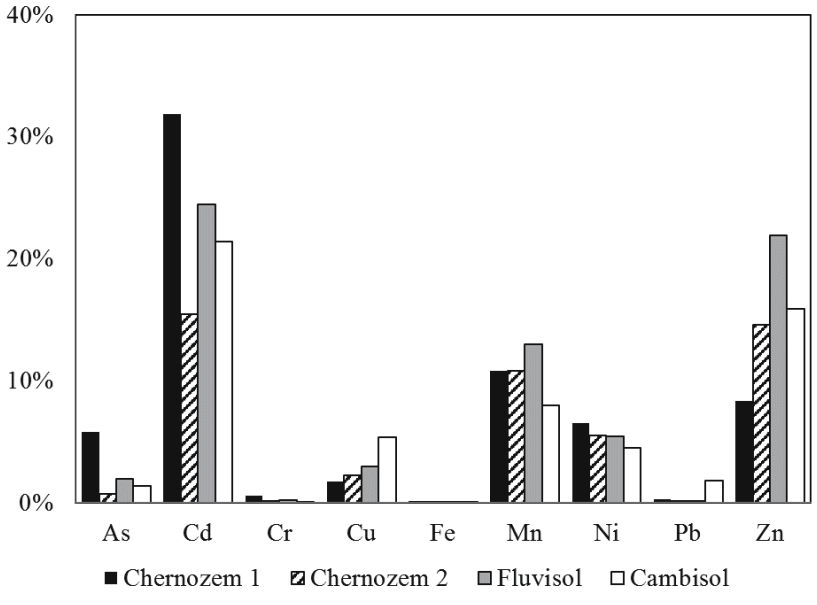

Fig. 1. The proportions of soil elements extractable with 0.11 mol/L acetic acid solution, respectively, as related to the pseudototal content of the elements in the soils

The component identification was also based on comparison of their Kováts indices (KI) (obtained using a series of n-alkanes [C8–C25]), retention times (RT), and mass spectra with those obtained from authentic samples (Sigma, US) and/or the NIST/NBS, Wiley libraries, and the literature (Adams 2001). To assess the effect of the elevated risk element contents in soil, the results were compared with a "control" sample of var. Bohemia anthodias obtained from the location with bio management. The "control" sample was prepared in the same way and within the same set as the experimental samples.

Statistics

The analytical data were processed using Statistica 10 Cz statistical software (StatSoft, USA). One-and two way analyses of variance at the significance level $\alpha = 0.05$ as well as correlation analysis, where Pearson correlation coefficients were applied, were used for the assessment of relationships between variables (Meloun and Militký 2004). The so-called bioaccumulation factor (BAF) quantifying the element transfer from soil to plants was used as the ratio of the weighed mean of the element content in whole aboveground biomass of plants to the pseudototal element content in soil.

Results and discussion

Table 1 documents the variability of both soil physicochemical parameters, including nutrient contents and risk element contents, where the differences for all the parameters were significant at ($p < 0.05$). According to the public notice characterising the conditions for the protection of the agricultural soil quality in the Czech Republic (Anonymous 2016), the maximum values of As, Cd, Cr, Cu, Pb, and Zn exceeded the preventive values of these elements in soil (20 mg/kg for As, 0.5 mg/kg for Cd, 90 mg/kg for Cr, 60 mg/kg for Cu, 50 mg/kg for Ni, 60 mg/kg for Pb, and 120 mg/kg for Zn). For As, Cd, and Pb, the maximum levels exceeded the indicative values, where the soil element contents represent a potential risk for crop contamination (*i.e.*, 40 mg/kg for As, 2 mg/kg for Cd, and 300 mg/kg for Pb). Both Fluvisol and Cambisol are multi-element contaminated soils, where Fluvisol represented slightly and Cambisol extremely contaminated soil. The maximum permissible contents of other elements determined in chamomile plants (i.e. Fe and Mn) are not defined by the Czech public notice (Anonymous 2016). The mobile proportions of the investigated elements are presented in Figure 1. Diluted acetic acid can be used for the estimation of the plant-available pool of elements (Sastre et al. 2004). The results showed low potential mobility of Cr, Fe, and As, whereas higher mobility of As in the case of Chernozem 2. The higher As mobility is due to an anthropogenic source of As contamination at this location, as already proved in other experiments (Száková et al. 2009). On the contrary, the acetic acid extractable pool was the lowest in the case of Chernozem 2. For Cd, Cr, Cu, and Ni, the highest potentially mobilizable pool was documented for Chernozem 1. It could be related to the differences in the CEC values (Table 1), where Chernozem 2 is characterized by the highest CEC level, and Chernozem 1 by the lowest value. As expected, the highest acetic acid extractable element portions were observed for Cd, with the highest level for the highly Cd-contaminated Chernozem 1. The high mobility of Cd in all experimental soils suggested the potential plant availability of this element. The percentages of mobile element contents were also observed for Mn and Zn, whereas the acetic acid extractable Fe, Cr, and Pb were negligible, except for the Pb content in the extremely contaminated Cambisol.

The average yields of individual parts of the chamomile plants are summarized in Table 2, showing no significant differences ($p < 0.05$) among the experimental variants. The results indicated higher yields of the aboveground biomass in Cambisol and the lowest yields in Chernozem 2. For roots, the highest yield of dry biomass was reported in Fluvisol. Thus, the chamomile yields were unaffected by the risk element contents in the soils, and/or by the soil physicochemical parameters. The results confirmed high tolerance of German chamomile to relatively high contents of risk elements in the soil. However, the risk element contents in plants reflected the different total element contents in the individual soils (Table 3). The World Health Organization (WHO 2005) recommends maximum values of 10 mg/kg Pb and 0.3 mg/kg Cd in herbal medicinal products. In our case, the Cd contents in the aboveground biomass of chamomile exceeded this recommendation in all samples. Voyslavov et al. (2013) surveyed Cd and Pb contents in chamomile plant samples from 26 locations in Bulgaria affected by several anthropogenic sources. The Cd contents varied between 0.12 and 3.1 mg/kg in flowers, 0.16 and 3.4 mg/kg in stems, and 0.28 and 3.9 mg/kg in roots. For Pb, the element contents were in the range of 0.3–2.3 mg/kg, 0.3–2.4 mg/kg, and 1.0–6.5 mg/kg in flowers, stems, and roots, respectively. In our case, the Cd contents were similar to these from contaminated areas, whereas enhanced Pb content in shoots was observed only in the extremely contaminated Cambisol but did not reach the level recommended by the WHO.

The uptake variability of the risk elements was predominantly affected by the content of the individual elements in soil, confirming the classification of chamomile plants as the species indicating the elevated element contents

Table 2. The average yields of chamomile plants dry matter per pot

	Anthodia g	Shoots g	Roots g
Chernozem 1	5.5±0.5^a	8.7±1.1^a	5.2±1.1^a
Chernozem 2	4.6±0.4^a	7.9±0.6^a	5.1±0.6^a
Fluvisol	5.7±0.4^a	8.0±1.2^a	5.9±1.0^a
Cambisol	6.0±0.6^a	9.0±0.5^a	5.6±0.9^a

the averages marked by the same letter superscripts did not significantly differ at $p<0.05$ within individual columns; n=6

in soil regardless of the essentiality of these elements. Mihaljev et al. (2014) surveyed the trace element contents in 14 species of wildlife medicinal plants, including chamomile. The contents of microelements in the examined samples varied in a wide range: Mn 23.8–6454 mg/kg, Fe 61.8–673 mg/kg, Cu 6.68–24.5 mg/kg, Zn 16.1–114 mg/kg, Ni 0.738–6.03 mg/kg. Tokalioğlu (2012) reported a decreasing sequence of the mean metal levels in medicinal herbs as follows: Fe > Mn > Zn > Cu > Ni > Cr > Pb. Although affected by the different contents of individual elements in soil, our results allowed us to follow a similar order for the aboveground biomass of chamomile. Table 4 presents the Pearson's correlation coefficients characterizing the response of the element contents in chamomile plants on the mobile (i.e. acetic acid extractable) pool of the individual elements in soil. For the essential elements, the correlations were weak, suggesting the element uptake according to the requirements of the plant rather than the element contents in the soils. For As and Cd, the element contents in all parts of the plant increased with the increasing mobile pool of these elements in the soil. Fairly good correlations between Cd in individual parts of chamomile plants and mobile content of this element in soil was already reported by Voyslavov et al. (2013). Weak correlations were observed for Cr and Pb contents in anthodia, suggesting limited translocation of these elements to the aboveground biomass, as showed also by Růžičková et al. (2015) in the case of Pb in *Ocimum basilicum* plants.

The element distribution within individual parts of plants is presented in detail in Figure 2, where total amounts of elements in the individual parts of plants calculated per one pot are presented. We can summarize that whereas As, Cr, and Pb were retained in roots, Cd showed good ability to translocate to the aboveground biomass, including flowers, even in extremely Cd-contaminated soil without symptoms of phytotoxicity. Except for Fe, the essential elements are relatively easily

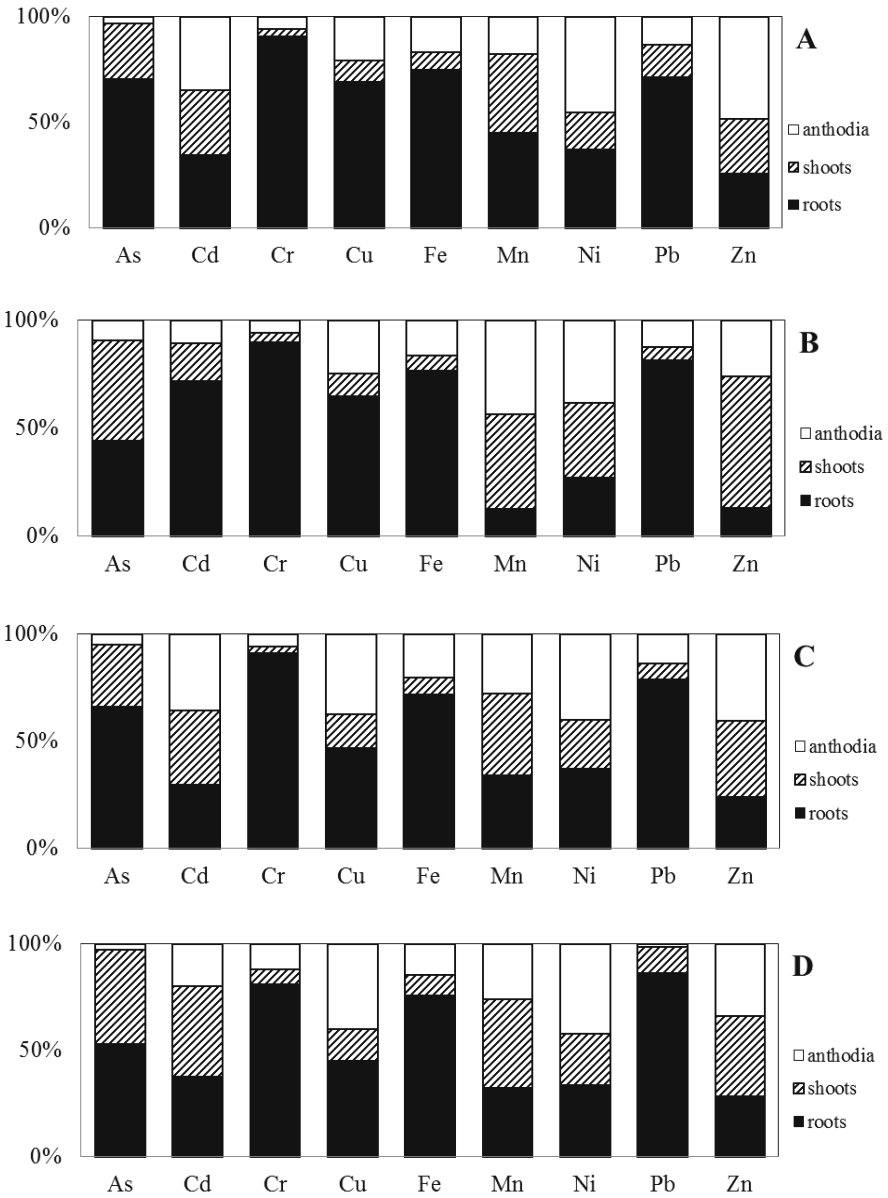

Fig. 2. The element distribution within the whole chamomile plants (100 %) according to the individual soils, calculated as the total amount of the elements in the individual plant parts related to the total amount of the elements in the whole plant (calculated as averages per one pot); A – Chernozem 1, B – Chernozem 2, C – Fluvisol, D – Cambisol

Table 3. The contents of investigated elements in chamomile plant dry matter

	As mg/kg	Cd mg/kg	Cr mg/kg	Cu mg/kg	Fe mg/kg	Mn mg/kg	Ni mg/kg	Pb mg/kg	Zn mg/kg
					Chernozem 1				
anthodia	0.15±0.05^{aA}	18.7±1.4^{aC}	0.35±0.07^{aA}	31.2±1.2^{aA}	106±7^{bA}	19.2±2.5^{aA}	6.48±0.5^{bB}	0.46±0.04^{aA}	65.91±3.3^{bA}
shoots	1.35±0.2^{bA}	16.7±2.2^{aC}	0.23±0.1^{aA}	15.6±5.7^{aA}	50.5±5.6^{aA}	41.2±9.7^{bA}	2.46±0.5^{aA}	0.53±0.4^{aA}	35.28±4.9^{aA}
roots	3.57±0.7^{cA}	18.6±5.2^{aC}	5.78±1.5^{bB}	105±13^{bB}	472±132^{cA}	50.2±19.6^{bA}	5.36±2.67^{bB}	2.46±1^{bA}	34.9±15.6^{aA}
					Chernozem 2				
anthodia	1.20±0.20^{aB}	2.14±0.5^{aB}	0.32±0.05^{aA}	26.1±1.4^{bA}	97.9±5.8^{bA}	610±64^{bC}	8.31±0.4^{aB}	0.44±0.1^{aA}	142±9^{aB}
shoots	6.10±1.00^{bC}	3.65±0.3^{aB}	0.24±0.05^{aA}	11.0±3.0^{aA}	40.2±12^{aA}	613±71^{bC}	7.60±1.0^{aB}	0.22±0.05^{aA}	332±51^{bB}
roots	15.4±3.0^{cB}	2.81±1.0^{aB}	1.95±0.5^{bA}	52.2±17.0^{cA}	426±77^{cA}	347±95^{aB}	7.72±2.0^{aB}	2.74±1^{bA}	183±30^{aB}
					Fluvisol				
anthodia	0.19±0.1^{aA}	1.35±0.1^{aA}	0.32±0.04^{aA}	29.6±2.1^{bA}	121±13^{bA}	58.7±11.0^{aB}	2.95±0.34^{bA}	0.42±0.1^{aA}	62.9±6.1^{bA}
shoots	1.19±0.3^{bA}	1.31±0.2^{aA}	0.18±0.02^{aA}	12.7±6.0^{aA}	48.3±8.7^{aA}	81.0±17.6^{bB}	1.69±0.3^{aA}	0.24±0.1^{aA}	55.6±7.9^{bA}
roots	2.74±0.4^{cA}	1.14±0.2^{aA}	5.04±1.7^{bB}	37.3±5.4^{cA}	436±103^{cA}	72.1±23.8^{bA}	2.74±0.7^{bA}	2.47±0.9^{bA}	37.9±6^{aA}
					Cambisol				
anthodia	0.22±0.1^{aA}	2.63±1.2^{aB}	0.28±0.03^{aA}	28.3±4.9^{bA}	91.9±11.2^{aA}	51.9±23.6^{aB}	2.99±0.5^{bA}	0.37±0.1^{aA}	56.3±17.1^{aA}
shoots	3.76±1.00^{bB}	5.69±0.6^{bB}	0.16±0.03^{aA}	10.7±3.3^{aA}	61.3±22.8^{aA}	82.2±15.2^{bB}	1.68±0.3^{aA}	3.06±0.8^{bB}	62.7±10.7^{aA}
roots	4.54±0.70^{cA}	4.78±1.4^{bB}	1.94±0.5^{bA}	32.3±12.0^{bA}	476±96^{bA}	65.0±19.7^{aA}	2.40±0.4^{bA}	22.1±4.3^{cB}	47.6±7.6^{aA}

means with standard deviations followed by the same letters (lower case superscripts – among plant parts, upper case superscripts – among soils) did not significantly differ at $p < 0.05$; n=6

Table 4. The Pearson's correlation coefficients of investigated element contents in chamomile plants and 0.11 mol/L acetic acid extractable (i.e. plant-available) proportion of these elements in soil

	As	Cd	Cr	Cu	Fe	Mn	Ni	Pb	Zn
anthodia	0.901*	0.986*	0.382	0.441	0.558	0.205	0.008	-0.176	-0.308
shoots	0.778*	0.959*	0.207	0.292	-0.045	0.216	-0.447	0.950*	-0.245
roots	0.951*	0.920*	0.784*	0.101	-0.179	0.167	-0.040	0.967*	-0.284

the values marked by * are significant at $p < 0.05$; n=24

Table 4. The concentrations of the investigated elements in chamomile infusions; n=6

	Chernozem 1	Chernozem 2	Fluvisol	Cambisol
As (mg/L)	0.001±0.001	0.014±0.002	0.002±0.000	0.002±0.000
Cd (mg/L)	0.021±0.003	0.002±0.000	0.009±0.001	0.018±0.002
Cr (mg/L)	0.003±0.006	0.005±0.002	0.003±0.002	0.004±0.001
Cu (mg/L)	0.079±0.013	0.038±0.013	0.343±0.028	0.343±0.076
Fe (mg/L)	0.014±0.001	0.014±0.03	0.088±0.007	0.103±0.026
Mn (mg/L)	0.031±0.003	0.482±0.017	0.055±0.002	0.077±0.005
Ni (mg/L)	0.018±0.001	0.018±0.006	0.014±0.007	0.016±0.007
Pb (mg/L)	0.004±0.000	0.007±0.000	0.004±0.002	0.004±0.000
Zn (mg/L)	0.183±0.041	0.229±0.013	0.151±0.000	0.179±0.004

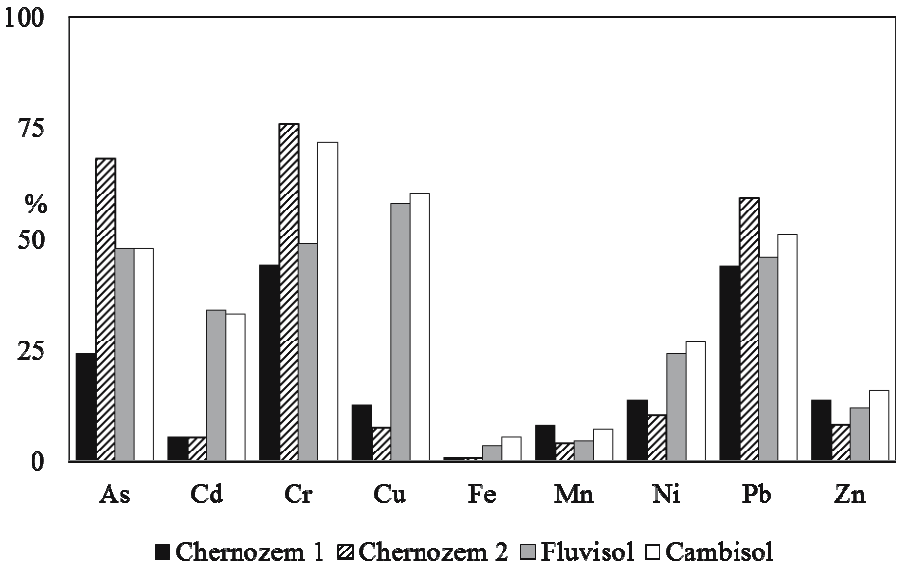

Fig. 3. The relative element proportions leached from the anthodias with hot water during preparation of chamomile infusion as related to the total element content in these anthodias

translocated to the aboveground biomass. Regardless of the soil, the abundance of the elements in the individual parts of plants can be summarized as follows: roots Cr > Pb > Fe > As > Cu > Cd > Ni > Zn > Mn; shoots Zn > Mn > Cd > As > Ni > Cu > Fe > Pb≈ Cr; anthodia Ni > Mn > Cd > Zn, Cu > Fe > Pb > Cr > As. The ratio of Cd translocation up to the chamomile flowers was comparable to the behavior of micronutrient elements such as Mn, Zn, and Cu. Except for Chernozem 2, the Cd distributions in our samples were similar to those for Mn and Zn, but Cu was more retained in roots. Pb remained in roots in the extremely contaminated Cambisol to a higher extent compared to the other soils. For Cd, no clear trend in Cd distribution in relation to soil Cd level was observed.

For most of the elements, the highest risk element contents are retained in roots, and their translocation to the aboveground biomass is limited. The lowest risk element contents are found in the generative organs of the plants (Sauerbeck and Lubben 1996). Borůvka et al. (1997) investigated the Cd, Pb, and Zn uptake by plants growing in anthropogenically contaminated soil where the element translocation rate to the aboveground biomass decreased in the order of Zn > Cd > Pb. Their results showed that whereas Zn content in the aboveground biomass represented 40% of its content in roots, for Cd and Pb these ratios represented only 13% and 7.5%, respectively. In our experiment, a different pattern of Cd compared to other risk elements, especially Pb and Cr, resulting in predominant Cd accumulation in the aboveground biomass was observed. These results confirmed the excellent ability of chamomile to accumulate Cd as already reported by many other authors (Chizzola et al. 2003, Masarovičová et al. 2010).

The BAF values can be affected by various factors such as plant species, soil contamination level and element mobility in the soils (Iyengar et al. 1981; King 1988). The role of potential antagonisms of elements such as Zn *vs.* Cd could be taken into account (Alloway 1990). In our case, the highest average BAFs were observed for Cd (BAF=1.4), followed by Zn (BAF=0.8) and Cu (BAF=0.6). For other elements, the BAFs were less than 0.5, and for As, Cr, Fe, and Pb they were even less than 0.05. Whereas plant accumulation of essential elements such as Cu and Zn is beneficial for plants, a high BAF_{Cd} level

requires further attention regarding the potential medicinal use of chamomile plants. In the opposite, Zheljazkov (2008b) observed low BAFs (<1) for other medicinal plants such as *Bidens tripartita*, *Leonurus cardiaca*, *Marrubium vulgare*, *Melissa officinalis*, and *Origanum heracleoticum*, confirming a low risk-element accumulation potential of common medicinal plants.

As mentioned above, the most popular use of chamomile is infusion of the anthodia (Srivastava et al. 2010). The concentrations of investigated elements in the infusions are summarized in Table 5, and the percentages of extracted elements from the total dry mass of anthodia used for the infusion preparation are presented in Figure 3. Pytlakowska et al. (2012) classified the extractability of the elements to the infusion into three specific groups: highly extractable (>55%), including K; moderately extractable (20–55%), including Mg, Na, P, B, Zn, and Cu; and poorly extractable (<20%), including Al, Fe, Mn, Ba, Ca, and Sr. Among the risk elements, the highest concentrations in the infusions were observed for Cd, where the concentrations reflected the differences in the total Cd contents in anthodia in the case of Chernozem 1 and Cambisol. For less contaminated samples, higher extractable concentrations of Cd were found for anthodia from Fluvisol, where the total Cd contents were the lowest. The concentrations of As, Cr, and Pb were low, except for the As level in the infusion from anthodia growing in As-contaminated Chernozem 2, where the total As contents were enhanced as well (Table 3). The concentrations of essential elements were higher *due to* higher total contents of these elements in anthodia.

Therefore, the soil Cd level and mobility needs to be carefully checked for cultivation of German chamomile for pharmaceutical production of high-quality chamomile drugs.

Between 5.5 and 35% of the total Cd was released to infusion, and its extractability decreased with increasing Cd content in flowers. The results are comparable to those published by Chizzola et al. (2008), where extractable Cd contents varied in the range of 15–21%. In the opposite, negligible contents of elements (Cd, Pb, Cu, Zn, and Mn) were observed by Zheljazkov et al. (2008) in infusions of other medicinal plants (*B. tripartita*, *L. cardiaca*, *M. vulgare*, *M. officinalis*, *O. heracleoticum)*. Within the investigations of Carrara Vulcano et al. (2008), the average levels of Pb and Cd in samples of chamomile infusion did not exceed the value 0.2 mg/L, and negligible Cd concentrations were also reported by Başgel and Erdemoğlu (2006). In our case, the As, Cr, and Pb contents in the chamomile infusions can be neglected, but the Cd levels should be investigated in more detail, especially in the Cd-contaminated soils.

The composition of chamomile essential oil is presented in Table 5. Orav et al. (2010) monitored the composition of essential oil within Europe and stated that the predominant compound of the essential oil in the Czech Republic is α-bisabolol (taking between 23.9 and 44.2% of the essential oil). However, as mentioned above, var. Bohemia belongs to bisabololoxid genotypes. Thus, bisabolol oxide A and bisabolol oxide B were the predominant compounds in the essential oil (Table 5). The results showed significant ($p < 0.05$) differences for chamazulene, spiroethers, and spathulenol, where chamazulene increased in contaminated soils compared to the "control", and an opposite pattern was reported for spiroethers and spathulenol. The bisabolol and β-farnesene contents tended to decrease in the contaminated soils, but the differences were not significant at $p < 0.05$. Among the samples originating from the contaminated soils, no significant differences were observed. Zheljazkov et al. (2006) tested the effects of soil enriched with risk elements (Cd, Pb, Cu) on the composition of essential oils of *Anethum graveolens*, *Ocimum basilicum*, and *Mentha piperita*. Similarly, Stancheva (2010) investigated the content and composition of essential oils of *Salvia officinalis* grown in contaminated soil. As a result, the essential oil content was slightly lowered and the oil composition altered (not significantly). The soil amendment with Ni and Cd resulted in an increase of these elements in chamomile plants but not in the essential oils (Chand et al. 2012). In our case, the essential oil composition was altered as well, and the risk element contents were under the detection limits of the analytical technique. The perceptible aspects of stress in plants, in terms of the elicitation and priming of defensive strategies, is the question of what precise factor or condition is actually sensed by the plant to induce a specific defensive response (Berglund et al. 2015).

According to the findings of Zheljazkov et al. (2006, 2008a), our results support the use of aromatic plants as alternative crops for risk element-contaminated soils. However, these conclusions are valid only for the soils tested within this study. Changing mobility of elements as related to the CEC indicates potential differences in the risk element mobility and plant uptake at the locations with different soil physicochemical parameters. The high ability of chamomile plants to accumulate Cd in the aboveground biomass brings some limitations: i) the choice of the contaminated areas for

Table 5. The relative abundance of the individual essential oil components (% of the total essential oil volume)

	Chernozem 1	Chernozem 2	Fluvisol	Cambisol	control
beta-Farnesene	3.90	4.09	4.17	2.97	5.14
Spathulenol	2.73	2.82	2.85	2.84	6.36
Bisabolol oxide B	32.32	39.52	32.35	36.79	28.67
Bisabolon	7.70	7.44	11.33	7.42	7.54
Bisabolol	2.04	4.05	1.05	0.85	5.91
Chamazulene	22.29	20.75	21.75	16.73	8.95
Bisabolol oxide A	21.94	17.15	20.33	25.21	23.26
Spiroethers	7.08	4.18	6.16	7.19	14.18

potential chamomile cultivation should not include soils extremely contaminated with Cd. Moreover, the effects of soil and cultivation conditions on Cd extractability into infusion should be elucidated in further research; ii) if cultivated in the Cd-contaminated soil, the plants can be used only for essential oil production, where the risk of elevated Cd content is negligible. In this context, however, the disposal of the Cd contaminated aboveground biomass will require special attention and could be hazardous during its handling and processing.

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