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The effect of soil risk element contamination level on the element contents in *Ocimum basilicum* L.

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Abstract: Red basil (*Ocimum basilicum* L.) cv. Red Rubin was cultivated in model pot experiment in the soil amended by arsenic, cadmium and lead solutions in stepwise concentrations representing the soil concentration levels of former mining area in the vicinity of Příbram, Czech Republic. The element levels added to the soil reached up to 40 mg Cd, 100 mg As, and 2000 mg Pb per kg of soil. Moreover, essential macro-and microelements as well as cyanidine contents were investigated to assess their potential interactions with the risk elements. The extractable element portions in soils determined at the end of vegetation period differed according to the individual elements. Whereas the plant-available (extractable with 0.11M CH_3COOH) content of Cd represented 70–100% of the added Cd, the mobile portion of Pb did not exceed 1%. The risk element content in plants reflected the increasing element contents in soil. The dominant element portions remained in plant roots indicating the limited translocation ability of risk elements to the aboveground biomass of this plant species. Although the risk element contents in amended plants significantly increased, no visible symptoms of phytotoxicity occurred. However, the effect of enhanced risk element contents on the essential element uptake was assessed. Considering inter-element relationships, elevated sulphur levels were seen in amended plants, indicating its possible role of phytochelatin synthesis in the plants. Moreover, the molybdenum contents in plant biomass dropped down with increasing risk element uptake by plants confirming As-Mo and Cd-Mo antagonism. The increasing content of cyanidine in the plant biomass confirmed possible role of anthocyanins in detoxification mechanism of risk element contaminated plants and suggested the importance of anthocyanin pigments for risk element tolerance of plants growing in contaminated areas.

Introduction

Plants have various protective mechanisms and strategies against enhanced contents of risk elements in soils. Natural sensitivity or tolerance of plants to accumulate metals is substantially affected by plant species and genotypes. Plant species can be divided into three groups: i) excludors, i.e. plants with limited uptake and accumulation of potentially toxic elements (mainly monocotyledon grasses, such as sudangrass, fescue belong into this group); ii) indicators, i.e. majority of agricultural crops whose element contents more or less linearly responds to increasing available content of trace elements in soil (wheat, oats, maize); iii) accumulators, i.e. plants accumulating higher contents of elements in their tissues in response to their accumulation in the soil (Soudek et al. 2008, Bhargava et al. 2012). Sainger et al. investigated the element uptake by plants growing in highly contaminated soils and reported a high adaptatability of some plant species growing in such environment. For example, Bosiacki et al. (2013) observed increasing yield of *Ricinus communis* aboveground biomass with increasing Cd content in cultivation substrate. Clearly, plants growing at the highly contaminated sites developed a range of mechanisms that may be involved in the detoxification of risk elements such as reduced uptake of these elements, their binding to the cell wall, immobilization, exclusion of the plasma membrane, reduction of heavy metal transport, compartmentalisation and metal chelation by tonoplast located transporters, expression of stress proteins, role of organic acids and aminoacids, etc. (Clemens 2006, Hall 2002, Solanki and Dhankar 2011).

Anthocyanins are responsible for the red, purple, and blue colors of fruits, grains, and flowers of various plants. These phenolic compounds occur as aglycons called anthocyanidins but more prevalent is their glycosidic anthocyanin form. More than 23 anthocyanidins and 500 of their corresponding glycosides have been isolated, however, only 6 are the most common in vascular plants, i.e., pelargonidin, peonidin, cyanidin, malvidin, petunidin, and delphinidin (Castañeda-Ovando et al. 2009, Iwashina 200, Welch et al. 2008). Plant anthocyanins play an important role in human health and nutrition. In this context, their potential antioxidant, anti-inflammatory, and anticancer properties are discussed (Welch et al. 2008, Phippen and Simon 1998). Moreover, anthocyanins from *Aronia melanocarpa* were used to reduce the harmful effects of cadmium toxicity in rats. Administering anthocyanins with cadmium chloride resulted in a statistically significant decrease of aspartate

aminotransferase (AST) and alanine aminotransferase (ALT) activity, concentration of bilirubin and urea in blood serum and decreased cadmium cumulation in liver and kidneys in relation to animals receiving cadmiumchloride only (Kowalczyk et al. 2003). The metal-anthocyanin (or polyphenol) complexes or positive correlations between anthocyanin and metal contents were observed in *Brassica* spp. (molybdenum, tungsten), *Vitis vinifera* (iron, copper), and *Betula pendula* (zinc) and were described by Hale et al. (Hale et al. 2001, Hale et al. 2002), Esparza et al. (Esparza et al. 2004), and Weber and Konieczyński (Weber and Konieczyński 2003), respectively. Not only are the complexes more stable uder acidic pH conditions but self-complexation or complexation with other phenolics and metals leads to the formation of metallocyanins. These complexes are more stable and show spectral shifts and express more intense colors under unfavourable pH (Castañeda-Ovando et al. 2009). Cyanidin readily complexes with Pb, Hg, Cd, As and Ni ions which leads to shifts in absorbtion spectra of cyanidin for which reason, cyanidine flavylium ion was recently suggested for spectrophotometric determination of these metal ions (Okoye et al. 2013).

The function of anthocyanins in response to oxidative stress of plants induced by risk elements was also investigated. Increasing anthocyanin contents were determined in *Azolla imbricata* cultivated in Cd-treated growth medium (Dai et al. 2006) and red cabbage (*Brassica oleracea* var. Rubrum) cultivated in copper-enriched medium (Posmyk et al. 2009). Although the functions of betalains are analogous to anthocyanins, the potential role of betalains (betanin, isobetanin, vulgaxanthin I and vulgaxanthin II) in transformation/detoxification of arsenic in beetroot (*Beta vulgaris*) was not confirmed (Száková et al. 2010).

The genus *Ocimum* contains around 30 species native to the tropics and subtropics, with some species naturalized and/or cultivated in temperate areas and includes species used as condiments, sources of essential oils or medicine (Vieira and Simon 2006). Moreover, in particular red varieties of basil are characterized by high contents of anthocyanins. In red basil varieties, 11 cyanidin-based and 3 peonidin-based pigments were isolated. Compared to other anthocyanin rich plant species such as *Vitis vinifera, Brassica oleracea* var. *Rubrum*, *Rubus idaeus, Salvia officinalis etc.* the red basil varieties are characterized as an abundant source of acylated and glycosylated anthocyanins and could provide a unique source of stable red pigments to the food industry (Phippen and Simon 1998, Simon et al. 1999).

In our investigation, *O. basilicum* cv. Red Rubin was cultivated in model pot experiment in the soil with stepwise increasing content of As, Cd, and Pb to assess i) the uptake of both risk elements and micronutrients by plants as affected by increasing risk element content in soil, and ii) the response of the plants on increasing risk element content in plant biomass *via* inter-element interactions and/or plant anthocyanin contents.

Experimental procedures

Pot experiment

Red basil (*Ocimum basilicum* L.) cv. Red Rubin plants were cultivated in 6-litre plastic pots with 5 kg of air-dry soil and three replicates were used for each treatment. NPK (0.5 g N, 0.16 g P, 0.4 g K per pot as inorganic salts solutions) was added before sowing. Soil moisture was regularly controlled and kept at 60% of the maximum water holding capacity (MWHC). The experimental soil was uncontaminated Chernozem with cation exchange capacity (CEC) 255 mmol kg^{-1}, pH level 7.2, and C-org. 2.3 %, 27±2mg kg^{-1} As, 0.686±0.072 mg kg^{-1} Cd, 47±2 mg kg^{-1} Pb. According to Public notice (Public notice 1994) giving the maximum levels of risk elements in agricultural soils in the Czech Republic, the "pseudo-total" (*Aqua Regia* soluble) element concentrations in loamy soils cannot exceed a maximum of 30 mg kg^{-1} As,1.0 mg kg^{-1} Cd, and 140 mg kg^{-1} Pb confirming the acceptable risk element levels in the experimental soil. Before sowing, water solutions of $CdCl_2$, Na_2HAsO_4, and $Pb(CH_3COO)_2$ were added in the following concentrations according to the individual treatments: treatment I: 0 mg · kg^{-1} As, 0 mg · kg^{-1} Cd, 0 mg · kg^{-1} Pb; treatment II: 25 mg · kg^{-1}As, 10 mg · kg^{-1} Cd, 500 mg · kg^{-1} Pb; treatment III: 50 mg · kg^{-1}As, 20 mg · kg^{-1} Cd, 1000 mg · kg^{-1} Pb; treatment IV: 75 mg · kg^{-1}As, 30 mg · kg^{-1} Cd, 1500 mg · kg^{-1} Pb; treatment V: 100 mg · kg^{-1}As, 40 mg · kg^{-1} Cd, 2000 mg · kg^{-1} Pb.

The aboveground biomass and roots were harvested immediately before start of flowering where maximum level of anthocyanins is expected (Phippen and Simon 1998), gently washed in deionised water, checked for fresh biomass, freeze-dried (Lyovac GT-2, Germany), ground and analyzed.

Before the analysis, root samples were grouped according to treatments because of low amount of the root biomass for the chemical analyses whereas the aboveground biomass was analyzed after individual pots. The soil samples were collected after harvest from individual pots, air dried at 20°C, ground in a mortar and passed through a 2-mm plastic sieve (Public notice 1994) and analyzed for its mobile element contents.

Analytical methods

For determination of mobile portions of elements in soils, two soil extraction procedures were applied as follows:

1. extraction with 2 mol · L^{-1} solution of HNO_3 at a ratio of 1 : 10 (w/v) at 20°C for 6 hours (Public notice 1994),
2. extraction with 0.11 mol · L^{-1} solution of CH_3COOH at a ratio of 1 : 20 (w/v) for 16 hours (Quevauviller et al. 1993).

Each extraction was done in three replicates, all the chemicals used were of analytical grade purity, and were purchased from Analytika and Lach-Ner Ltd., CzechRepublic. For the centrifugation of the extracts, the Hettich Universal 30 RF (Germany) device was used. The reaction mixture was centrifuged at 3000 rpm for 10 minutes at the end of each extraction procedure, and supernatants were kept at 6°C prior to measurement.

Plant samples were decomposed using the dry ashing procedure as follows: an aliquot (1 g) of the dried and powdered aboveground biomass and roots was weighed in a borosilicate glass test-tube and decomposed in a mixture of oxidizing gases ($O_2 + O_3 + NO_x$) at 400°C for 10 hours in Dry Mode Mineralizer Apion (Tessek, Czech Republic). The ash was dissolved in 20 mL of 1.5% HNO_3 (Analytika, Prague, Czech Republic) and kept in glass tubes until the analysis (Miholová et al. 1993). A certified reference material CRM CTA-OTL-1 Tobacco leaves was applied for quality assurance of the results. According

to the guidance, this material contains 0.539±0.060 mg · kg^{-1} As, 1.12±0.12 mg · kg^{-1} Cd, and 4.91±0.80 mg · kg^{-1} Pb while our system determined 0.590±0.020 mg · kg^{-1} As, 1.13±0.04 mg · kg^{-1} Cd, and 4.65±0.15 mg · kg^{-1} Pb. Inductively coupled plasma-atomic emission spectrometry (ICP-OES, Varian, VistaPro, Australia) was used for determination of elements in soil extracts and plant digests.

For determination of individual hydrolyzed red pigments in the aboveground biomass 0.3 g of each freeze-dried sample was finely ground using the Waring blender. Thirty mg of each sample were extracted with 10 ml of 4 mol · L^{-1} HCl in 50% methanol for 50 min at 80°C. Afterwards, aliquots of extracts were centrifuged in Eppendorf minispin plus at 14 500 rpm for 10 min and filtered through 0.45 µm Minisart RC filter. Finally, the reaction mixture was evaporated to dryness *in vacuo* in a rotary evaporator and the residue was dissolved in 1 ml of mixture consisting of 50% methanol and 0.1% aquaeous trifluoracetic acid, and kept under nitrogen until analysis. The separation of cyanidin as the only anthocyanine present under these analytical conditions was achieved with an analytical HPLC system Dionex Summit (Dionex corp, US), consisting of P680 quaternary gradient pump, diode array detector UVD340U and column thermostat, interfaced with the Waters 717 autosampler (Waters Inc., US), For separation, a Phenomenex Gemini C18 column (250 × 4.6 mm I.D., 5 µm particle size) was used, mobile phase: A: 10% formic acid, B: 100% acetonitrile (Lach-Ner., CZ). The cyanidin was well separated from the matrix using a gradient elution from 15 to 25% B in 20 min. Peak integration and quantitation was performed at 285 and 539 nm (Nyman and Kumpulainen 2001). The peaks were quantified using external standard method. All samples were prepared and measured in triplicate.

Statistics

The analytical data were processed using Microsoft Office Excel 2007 and Statistica 10 CZ statistical softwares. One--way Analysis of Variance (ANOVA) at a significance level of α=0.05 followed by Tukey-Kramer test were applied for the data characterized by the normal data distribution. Correlation analysis was used for assessment of the relationships among the variables where Pearson's correlation coefficients were applied (Meloun and Militký 2004).

Results and discussion

The extractable portions of risk elements in soil determined after harvest clearly show their variance in the soil mobility (Table 1). The potentially mobilisable element content ranged between 69–98% of the total content for Cd, it ranged between 41–60% for Pb, and between 16–52% for As confirming high mobility of Cd in soil (Száková et al. 2000, Tlustoš et al. 1994, Šmejkalová et al. 2003). Moreover, the extractable element contents tended to decrease with increasing element amendment documenting high sorption ability of the experimental soil especially in the case of As and Pb. Diluted acetic acid is characterized as the extractant releasing exchangeable element fractions specifically sorbed on soil clay minerals. Therefore, this extractant is recommended as a suitable test to predict the changes in element mobility in soil (Sastre et al. 2004). In our case, the 0.11mol · L^{-1} CH_3COOH extractable element contents reached up to 77% for Cd, 41% for As and less than 1% for Pb. As seen from Table 1, although the element concentrations added to the soil differed among the elements, the 0.11 mol · L^{-1} CH_3COOH extractable contents of As, Cd, and Pb were comparable.

The element contents in plant roots and aboveground biomass are summarized in Tables 2 and 3. The As, Cd, and Pb contents in plants reflected increasing contents of these elements in soils whereas essential micronutrients remained significantly unchanged. Similarly, the biomass yield varying between 45 and 62 g of fresh matter did not indicate any significant differences among the treatments. With an exception of molybdenum in aboveground biomass, which lowered with the increasing risk element content in plants. These relationships were confirmed by using correlation analysis (Tables 4 and 5), as well. Similarly, Zhang et al. (Zhang et al. 2000) observed decreasing molybdenum content in wheat plants growing in soilless culture with elevated cadmium concentration. The adverse Cd-Mo relationships were also reported in animals. Smith and White (Smith and White 1997) observed that molybdenum addition to the sheep diet resulted in lower cadmium accumulation in the tissues of the animals. Antagonistic relationships between As and P, and Cd and Zn, frequently discussed in scientific literature (Soudek et al. 2008, Soudek et al. 2006) were not confirmed in our case.

The results showed predominant accumulation of the risk elements in roots with limited translocation to the aboveground biomass (Tables 2 and 3).The element contents in the aboveground biomass were 6-fold lower than in the roots in the case of As, 9-fold lower for Pb and even 13-fold lower for Cd at the highest soil risk element level. Similarly, Zheljazkov et al. (Zheljazkov et al. 2008) studied the cadmium effect on *O. basilicum* plants and reported higher Cd accumulation in plant roots. They also observed competitive relationships between

Table 1. The element contents in soils extractable with the individual extracting agents (mg·kg^{-1})

Treatment	2 mol·L^{-1} HNO_3			0.11 mol·L^{-1} CH_3COOH		
	As	Cd	Pb	As	Cd	Pb
I.	1.72±0.12	0.35±0.05	16.1±1.9	1.51±0.42	0.32±0.00	8.20±0.49
II.	11.1±0.3	9.34±0.98	251±30	10.3±0.7	3.64±0.00	5.80±1.12
III.	21.6±1.1	19.6±1.7	472±31	14.5±0.4	8.74±0.50	9.28±0.29
IV.	32.3±1.8	21.6±0.9	620±32	18.9±0.5	9.03±0.14	15.3±1.4
V.	51.9±0.1	27.5±1.4	859±20	25.7±0.1	11.5±0.5	21.4±5.0

Table 2. Total element contents in roots of the experimental plants (mg·kg^{-1}); data are presented as mean ± standard deviation, n=2; The means marked by the same letter did not significantly differ at α = 0.05 within individual columns (one-way ANOVA followed by Tukey-Kramer test)

Treatment	As	Cd	Pb	Cu	Fe
I.	4.21±0.24^{a}	1.56±0.54^{a}	17.8±4.14^{a}	41.7±6.3^{a}	3447±846^{a}
II.	49.2±2.2^{b}	18.5±0.88^{b}	47.8±3.96^{d}	37.6±2.1^{a}	2877±201^{a}
III.	69.9±2.4^{b,c}	27.5±1.59^{b,c}	96.6±6.78^{b,c}	53.9±4.1^{a}	3892±280^{a}
IV.	85.4±4.6^{c}	40.2±1.66^{c,d}	128±12.21^{a,b}	40.8±6.4^{a}	2958±862^{a}
V.	85.1±10.4^{c}	42.5±6.70^{d}	179±26.30^{b}	41.0±4.4^{a}	2128±158^{a}
Treatment	Mn	Mo	Zn	P	S
I.	117.5±34.1^{a}	0.676±0.02^{a}	58.7±17.6^{a}	984±161^{a}	1522±13^{a}
II.	104.7±8.63^{a}	0.677±0.00^{a}	36.8±2.4^{a}	947±8^{a}	1521±40^{a}
III.	90.2±5.54^{a}	0.606±0.09^{a}	45.4±2.5^{a}	1003±25^{a}	1681±40^{a}
IV.	108.3±20.59^{a}	0.61±0.02^{a}	41.8±3.9^{a}	1092±37^{a}	1765±129^{a}
V.	81.8±12.98^{a}	0.607±0.01^{a}	40.9±4.7^{a}	1109±47^{a}	1687±29^{a}

Table 3. Total element contents in aboveground biomass of the experimental plants (mg·kg^{-1}); data are presented as mean ± standard deviation, n=6; The means marked by the same letter did not significantly differ at α = 0.05 within individual columns (one-way ANOVA followed by Tukey-Kramer test)

Treatment	As	Cd	Pb	Cu	Fe
I.	1.43±0.15^{a}	0.10±0.01^{a}	1.3±0.9^{a}	16.8±2.1^{a}	621±112^{a}
II.	10±0.89^{b}	1.6±0.28^{b}	5.0±0.7^{b}	18.4±3.1^{a}	632±83^{a}
III.	12.9±2.35^{c}	2.3±0.38^{c}	12.9±5.5^{c}	18.4±2.1^{a}	801±303^{a}
IV.	13±0.78^{c}	2.7±0.28^{c}	14±1.8^{c}	17.5±1.2^{a}	685±113^{a}
V.	13.2±1.69^{c}	3.7±0.64^{d}	19±1.1^{d}	18.1±2.6^{a}	719±90^{a}
Treatment	Mn	Mo	Zn	P	S
I.	101±3.75^{a}	3.02±0.35^{d}	18±1.19^{a}	1922±288^{a}	1513±78^{a}
II.	103.4±10.03^{a}	2.33±0.17^{c,d}	22.2±2.8^{b}	2423±296^{b}	1572±64^{a}
III.	94.7±6.24^{a}	2.18±0.51^{b,c}	20.4±2.3^{a,b}	2251±331^{a,b}	1704±89^{a,b}
IV.	92.9±10.87^{a}	1.63±0.52^{a,b}	20.4±2.2^{a,b}	2304±190^{a,b}	1850±101^{b}
V.	84.8±5.21^{a}	1.47±0.41^{a}	21.4±3.0^{a,b}	2484±345^{b}	1905±225^{b}

Cd and Pb, and Cd and Cu in the aboveground biomass not confirmed in our study (most probably due to relatively low content of Cu in plants and/or limited translocation of Pb to the shoots). Among the elements, Cd demonstrated the highest plant uptake ability according to its high mobility in the soil. Vysloužilová et.al (Vysloužilová et al. 2003) investigated the accumulation of As, Cd, and Pb in willow (*Salix* spp.) leaves and twigs in the same soil at comparable element levels as in our experiment. They observed high cadmium accumulation predominantly in leaves. However, willows belong to the plant species accumulating this element especially in leaves (Pulford and Watson 2002). Thus, the differences among individual plant species should be taken into account in this case (Ducsay 2011).

Although the plants accumulated enhanced contents of risk elements, no potential visible symptoms of phytotoxicity occurred even at the highest soil risk element level. The element uptake by *O. basilicum* plants can be assessed in the context of potential production of essential oils and/or plant pigments. Stancheva et al. (Stancheva et al. 2010) cultivated *Salvia officinalis* plants in the risk element-polluted soil which successfully accumulated cadmium and lead (0.200 ± 0.010 mg·kg^{-1} Cd, and 8.4 ± 0.4 mg·kg^{-1} Pb in shoots, 15.6 ± 0.8 mg·kg^{-1} Cd,

Table 4. Pearson's correlation coefficients among individual elements – roots (n=10, bold coefficients are significant at α=0,05)

	As	Cu	Fe	Mn	Mo	Zn	P	S	Cd	Pb
As	1	0.177	0.310	0.433	-0.631	-0.558	0.560	**0.800**	**0.981**	**0.897**
Cu	0.177	1	**0.768**	0.206	-0.450	0.416	0.261	0.405	0.101	0.137
Fe	-0.310	**0.768**	1	0.165	-0.016	0.622	-0.041	0.040	-0.402	-0.441
Mn	-0.433	-0.206	0.165	1	0.429	0.037	-0.487	-0.082	-0.422	-0.477
Mo	-0.631	-0.450	-0.016	0.429	1	0.070	-0.562	**-0.654**	**-0.638**	**-0.642**
Zn	-0.558	0.416	0.622	0.037	0.070	1	0.286	0.181	0.494	-0.356
P	0.560	0.261	-0.041	0.487	0.562	0.286	1	0.631	**0.653**	**0.717**
S	**0.800**	0.405	0.040	0.082	**-0.654**	-0.181	-0.631	1	**0.812**	**0.775**
Cd	**0.981**	0.101	-0.402	0.422	**-0.638**	-0.494	**-0.653**	**0.812**	1	**0.954**
Pb	**0.897**	0.137	-0.441	0.477	**-0.642**	-0.356	**-0.717**	**0.775**	**0.954**	1

Table 5. Pearson's correlation coefficients among individual elements – aboveground biomass (n=30, bold coefficients are significant at α=0,05)

	As	Cd	Cu	Fe	Mn	Mo	Pb	Zn	P	S
As	1	**0.818**	0.047	0.269	**-0.388**	**-0.710**	**0.805**	0.242	0.355	**0.579**
Cd	**0.818**	1	0.230	0.246	**-0.583**	**-0.826**	**0.859**	**0.418**	**0.549**	**0.832**
Cu	0.047	0.230	1	0.265	0.262	-0.192	0.120	**0.774**	**0.543**	0.332
Fe	0.269	0.246	0.265	1	-0.018	**-0.468**	**0.527**	-0.161	-0.218	0.178
Mn	**-0.388**	**-0.583**	0.262	-0.018	1	**0.551**	**-0.553**	0.161	-0.015	**-0.438**
Mo	**-0.710**	**-0.826**	-0.192	**-0.468**	**0.551**	1	**-0.854**	-0.164	-0.198	**-0.703**
Pb	**0.805**	**0.859**	0.120	**0.527**	**-0.553**	**-0.854**	1	0.077	0.196	**0.703**
Zn	0.242	**0.418**	**0.774**	-0.161	0.161	-0.164	0.077	1	**0.900**	**0.452**
P	0.355	**0.549**	**0.543**	-0.218	-0.015	-0.198	0.196	**0.900**	1	**0.530**
S	**0.579**	**0.832**	0.332	0.178	**-0.438**	**-0.703**	**0.703**	**0.452**	**0.530**	1

and 69.6 ± 3.5 mg · kg^{-1} Pb in roots). Evidently, *S. officinalis* plants accumulated lower element contents compared to our experiment but resulted in plant biomass growth suppression. However, essential oil yield and quality was not affected. Chand et al. (Chand et al. 2012) cultivated chamomile (*Matricaria chamomilla*) in the risk element-contaminated soil and although a substantial amount of risk elements were being translocated to flowers, the essential oil extracted from the flowers did not contain measurable contents of these elements. Similar conclusions were drawn by Zheljazkov and Nielsen (Zheljazkov et al. 1996) and Zheljazkov et al. (Zheljazkov et al. 2008) for *Coriandrum sativum*, *Anethum graveolens*, *M. chamomilla, Mentha* x *piperita*, *O. basilicum, Hyssopus officinalis, Melissa officinalis*, and *S. officinalis*. Therefore, potential cultivation of essential oil-producing crops should be a reasonable way for agricultural use of risk element contaminated soils.

As mentioned above, anthocyanins play an important role in plant protection against oxidative stress due to enhanced risk element concentration (Dai et al. 2006, Posmyk et al. 2009). Phippen and Simon (Phippen and Simon 1998) documented presence of cyanidin and peonidin-based pigments in *O. basilicum* plants as confirmed in our experiment. Because cyanidin was the predominant anthocyanidine in our samples this pigment was quantified and applied for further evaluation. The contents of cyanidin in *O. basilicum* aboveground biomass were as follows: 7.5 ± 2.3 mg · g^{-1} for treatment I, 9.3 ± 2.6 mg · g^{-1} for treatment II, 7.9 ± 2.8 mg · g^{-1} for treatment III, 9.4 ± 1.4 mg · g^{-1} for treatment IV, and 10.8 ± 1.6 mg · g^{-1} for treatment V, respectively. Therefore, the cyanidin contents tended to increase in risk element treated variants suggesting possible cyanidin-element interrelationships. Similarly, Posmyk et al. (Posmyk et al. 2009) reported increasing anthocyanin content in red cabbage (*Brassica oleracea* var. Rubrum) with increasing copper concentrations in the cultivation media. Hale et al. (Hale et al. 2001) speculated possible formation of molybdenum-anthocyanin complexes in plant vacuoles. Also Phippen and Simon (Phippen and Simon 1998) mentioned possibility of anthocyanin bindings with iron, zinc, copper, etc. Takeda et al. (Takeda et al. 1994) described blue pigment complex, protodelphin, in *Salvia patens* flowers as metallo-

-anthocyanin complex with magnesium. Moreover, similar blue complexes were formed by the use of manganese, cobalt, nickel, zinc and cadmium instead of magnesium.

Conclusions

The results confirmed that the risk element content in plants reflecs the increasing element contents in soil. The dominant element portions remained in plant roots indicating the limited translocation ability of risk elements to the aboveground biomass of this plant species. Although the risk element contents in amended plants significantly increased, no visible symptoms of phytotoxicity occurred. Moreover, Valterová et al. (Valterová et al. 2012) studied possible relationships among risk element (arsenic, cadmium, zinc) contents and anthocyanins in aboveground biomass of *Noccaea caerulescens*. Except for As, the results indicated the interactions in this context. In our experiment, the protective effect of anthocyanins against the risk element stress can be speculated, as well. The possible element-anthocyanin complexes in the plant biomass remains for further research as well as the possible interactions of risk elements with selected essential elements as indicated for instance in the case of As-Mo and Cd-Mo antagonism.

Therefore, *O. basilicum* seems to be a suitable crop for cultivation in risk element contaminated soil due to its ability to grow in these soils without yield suppression, the plants can be used for essential oil production and/or for ornamental purpose. These conclusions are similar to Zheljazkov and Nielsen (Zheljazkov and Nielsen 1996) recommending *Mentha x piperita* as a very profitable crop which be used as substitute for the other highly contaminated crops.

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