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The contents of risk elements, arsenic speciation, and possible interactions of elements and betalains in beetroot (*Beta vulgaris*, L.) growing in contaminated soil

Research Article

Jiřina Száková[1,*], Jaroslav Havlík[2], Barbora Valterová[1], Pavel Tlustoš[1], Walter Goessler[3]

[1]*Department of Agroenvironmental Chemistry and Plant Nutrition, Czech University of Agriculture, CZ-165 21 Prague 6, Czech Republic*

[2]*Department of Microbiology, Nutrition and Dietetics, Czech University of Agriculture, CZ-165 21 Prague 6, Czech Republic*

[3]*Institute of Chemistry, Analytical Chemistry, Karl-Franzens-University Graz, A-8010 Graz, Austria*

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Abstract: The effect of enhanced soil risk element contents on the uptake of As, Cd, Pb, and Zn was determined in two pot experiments. Simultaneously, transformation of arsenic and its compounds in beetroot (*Beta vulgaris* L.) plants was investigated. The mobile fractions of elements were determined in 0.05 mol L^{-1} $(NH_4)_2SO_4$ extracts and did not exceed 2% of total soil arsenic, 9% of total cadmium, 3% of total lead, and 8% of total zinc, respectively. Although the soils were extremely contaminated the mobile portions of the elements represented only a small fragment of the total element content. Arsenic contents in beet plants reached up to 25 mg As kg^{-1} in roots and 48 mg As kg^{-1} in leaves in the soil characterized by the highest mobile arsenic portion. Arsenic portions extractable with water and phosphate buffer from the beetroot samples did not show significant differences between the extraction agents but the extractability was affected by the arsenic concentration. Arsenic was almost quantitatively extractable from the samples with the lowest total arsenic concentration, whereas in the samples with the highest total arsenic concentration less than 25% was extractable. Arsenate was the dominant arsenic compound in the extracts (70% in phosphate buffer, 50% in water extracts). A small portion of dimethylarsinic acid, not exceeding 0.5%, was detected only in the sample growing in the soil with the highest arsenic concentration. The role of betalains (betanin, isobetanin, vulgaxanthin I and vulgaxanthin II) in transformation/detoxification of arsenic in plants was not confirmed in this experiment because the plants were able to grow in the contaminated soil without any symptoms of arsenic toxicity.

Keywords: *Beetroot (Beta vulgaris L.) • Arsenic • Speciation • Cadmium • Lead • Zinc • Betalains*

1. Introduction

Among common plant pigments such as carotenoids and chlorophylls, anthocyanins and betalains are frequently discussed as a consequence of exogenic stress or senescence and of an ecological adaptation to a changing environment [1]. Anthocyanins are a subgroup of flavonoids, which are phenolic compounds that appeared sequentially during plant evolution. Anthocyanins occur in the most recently evolved plants, predominantly in angiosperms but also in gymnosperm and in a number of fern species [2]. Betalains are nitrogen-containing pigments replacing the anthocyanins in plants of the Caryophyllales families. They are immonium conjugates of betalamic acid with *cyclo*-dopa and amino acids or amines, respectively, comprising red-violet phenolic betacyanins and yellow non-phenolic betaxanthins [3,4].

Among various functions of anthocyanins and betalains in plants [1], complementary roles of the pigments were reported. In this context, the relationships between flavonoids and especially anthocyanins, and

* *E-mail: szakova@af.czu.cz*

essential macro- and micronutrients were frequently investigated. The ability of magnesium treatment to increase the stability of anthocyanins through formation of complexes at elevated temperature (29°C/21°C day/night) in *Aster spp*. flowers was described by Shaked-Sachray *et al*. [5]. The metal-anthocyanin (or polyphenol) complexes or positive correlations between anthocyanin and metal contents that were observed in *Brassica spp*. (molybdenum, tungsten), *Vitis vinifera* (iron, copper), and *Betula pendula* (zinc) were described by Hale *et al*., Esparza *et al*., and Weber and Konieczyński, respectively [6-9]. The function of anthocyanins in plants against oxidative stress induced by risk elements was investigated as well. Increasing anthocyanin contents were determined in *Azolla imbricata* cultivated in Cd-treated growth medium [10]. Similarly, M-Kalantari and Oloumi [11] observed a significant increase of anthocyanin content in *Brassica napus* plants treated by $CdCl_2$ in a pot experiment, and Al-Aboudi *et al*. [12] determined complexes between the Pb and Cd ions, and flavanoid glycosides in *Phragmites australis* plants. Although the functions of betalains are analogous to anthocyanins, possible interactions of betalains with essential or risk elements have not been systematically investigated.

While inorganic arsenic compounds are the most important ones in soil [13-15], both inorganic and organic (methylated) arsenic compounds were isolated from different parts of higher plants [16]. Transformation and translocation of arsenic compounds among plant tissues is strongly dependent on plant species and/or soil characteristics [17]. The occurrence of individual arsenic compounds in higher plants and their distribution into different plant tissues are strongly dependent on plant species [18,19]. As(III), As(V), DMA, MA, trimethylarsine oxide, the tetramethylarsonium ion, and one arsenoribose were identified in green plant species grown at arsenic contaminated sites; the compounds and their concentrations differ with plant species [19,20]. The presence of arsenic phytochelatin complexes in *Holcus lanatus, Pteris cretica* [21], *Cicer arietinum* [22], and *Helianthus annuus* [23] was discussed as mechanism of plant tolerance to enhanced arsenic content. In the context of biochemical stress responses caused by enhanced arsenic content in plants, decreases in chlorophyll and carotenoid concentrations in *Trifolium pratense* were observed (Mascher *et al*., 2002) [24], whereas only sporadic information was published concerning possible arsenic-flavonoid interactions. Uniquely, Aziz [25] described increasing red pigmentation of *Azolla filiculoides* with increasing arsenic content in growing medium where the intensity of red colour of the plants correlated significantly with arsenic concentration in water.

In our experiment, the effect of arsenic level in soil on its uptake by beetroot (*Beta vulgaris* L.) plants was investigated in two pot experiments. The main objectives of the experiments were: i) to describe translocation and transformation of individual arsenic compounds within red beet plants with regard to the arsenic level in soil, and ii) to identify individual red pigments in beetroot and to assess possible relations with the arsenic contents in these plants. Because the multi-element long-term contaminated soils were used in our experiment, the Cd, Pb, and Zn levels in beetroot were determined and the red pigments-element relationships evaluated, as well.

2. Experimental Procedures

2.1 Pot experiments

2.1.1 Experimental soils

Příbram arable was taken from the field, polluted by lead from the mining and smelting industry. The vicinity of the smelter is among the most polluted areas in the Czech Republic. The main source of Pb contamination was the atmospheric deposition of trace elements by galenite mining followed by ore smelting and lead processing. Mining and metallurgical activities in this area led to enhancement of As, Cd and Zn contents in soil, due to the high content of trace elements in parent rock [26]. The area of Mokrsko (located 35 km south of Prague) has a high geogenic As content due to gold arsenopyrite occurrence [27]. Kutná Hora (70 km east of Prague) soils are contaminated by arsenic, cadmium, and zinc mainly due to tailings of silver mining in the Middle Ages and furthermore the concentrations of Cd and Zn were always substantially elevated [28]. Fluvisols slightly contaminated by river floods from Píšťany (80 km north-west from Prague) were added to the set of experimental soils. Total element contents and main soil characteristics are summarized in Table 1, available contents of macro- and micronutrients according to the Mehlich III extraction procedure [29] are given in Table 2.

In the first pot experiment the soils from Příbram, Mokrsko, and three soils from the location Kutná Hora were applied. For the second pot experiment two soils from the location Kutná Hora, the soil from Mokrsko, and the slightly contaminated soil from Píšťany were chosen. Before planting, soil samples were collected from individual pots, air dried at 20°C, ground in a mortar, passed through a 2-mm plastic sieve and analyzed for total and plant-available arsenic contents. Plants were cultivated in 6 litre plastic pots with 5 kg of air-dry soil with four replicates for each soil type. NPK (0.5 g N, 0.16 g P, 0.4 g K per pot) was added before sowing and beetroot (*B. vulgaris* cv. Bona) was cultivated for

Soil		As mg kg^{-1}	Cd mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}	pH	TOC %	CEC mmol kg^{-1}
Příbram	Loam	83±2	8.5±0.4	805±11.5	492±3	4.52±0.02	1.91±0.01	130±15
Mokrsko	Loamy-clay sand	839±112	0.03±0.01	11±0.5	238±2	5.36±0.04	0.78±0.02	116±8
Kutná Hora arable	Loam	1312±103	19.5±0.36	79±0.1	1931±56	7.41±0.03	0.95±0.00	380±15
Kutná Hora meadow	Sandy loam	1573±46	4.9±0.03	30±0.5	499±61	6.97±0.03	4.02±0.25	148±12
Kutná Hora waterside	Sandy loam	81±5	0.70±0.07	33±5.8	123±23	7.29±0.02	3.49±0.21	295±21
Píšťany	Loamy-clay sand	21±1	0.83±0.06	46±8.9	100±4	6.87±0.02	1.50±0.03	127±5

Table 1. Total element contents and basic characteristics of experimental soils; data are presented as mean±standard deviation, n=3.
TOC…total organic carbon
CEC…cation exchange capacity

Soil	P mg kg^{-1}	K mg kg^{-1}	Ca mg kg^{-1}	Mg mg kg^{-1}	Fe mg kg^{-1}	Mn mg kg^{-1}	B mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}
Příbram	283±5	160±2	951±15	48.9±2.3	210±2	117±3	5.67±1.63	17.0±0.3	47.6±0.8
Mokrsko	33.8±1.1	90.7±16.9	1715±24	196±12	230±1	57.1±0.0	3.29±1.06	3.74±0.53	7.06±0.67
Kutná Hora arable	56.2±1.1	610±11	7210±224	543±8	164±9	32.7±1.0	7.88±0.20	25.3±0.4	408±1
Kutná Hora meadow	1.10±0.12	103±7	14060±659	201±6	191±4	30.8±2.2	4.17±1.08	36.7±4	227±1
Kutná Hora waterside	1.50±0.09	217±15	10628±303	642±3	254±15	52.4±0.7	5.95±0.07	17.6±1.5	68.5±0.8
Píšťany	123±2	173±9	3091±1	220±5	372±3	97.4±3.3	1.99±0.38	11.3±0.3	31.7±0.4

Table 2. Available contents of macro-and micronutrients in soils determined by Mehlich III extraction procedure [29]; data are presented as mean±standard deviation, n=3.

6 months. Pots were placed in an outdoor weather-controlled vegetation hall. Soil moisture was kept at 60% of water holding capacity (WHC) by watering daily with deionised water. Leachate was collected and re-circulated to the system. The aboveground biomass and beetroots were harvested in the end of vegetation period, gently washed by deionised water, checked for fresh biomass, freeze-dried (Lyovac GT-2, Germany), ground and analyzed.

2.2 Analytical methods

2.2.1 Sample decomposition and extraction

Total element concentrations in soil were determined in digests obtained by two-step decomposition as follows: 0.5 g of the sample was decomposed by dry ashing in a mixture of oxidizing gases (O_2+O_3+NO_x) in an Apion Dry Mode Mineralizer (Tessek, CZ) at 400°C for 10 h; the ash was then dissolved in a mixture of HNO_3 + HF, evaporated to dryness at 160°C and dissolved in diluted Aqua Regia [30]. Certified reference material RM 7001 Light Sandy Soil (Analytika, CZ) containing 12.3±1.1 mg As kg^{-1} was used for quality assurance of the analytical data of total arsenic determination, and 13.2±1.0 mg As kg^{-1} was determined in this sample. For determination of mobile element portions, aliquots of the soil samples were extracted with 0.05 mol L^{-1} aqueous $(NH_4)_2SO_4$ solution at a solid/liquid ratio of 1/25 (1 g + 25 ml) for 4 h [31]. Each extraction was done three times. All the chemicals used were of electronic grade purity and were purchased from Analytika and Lach-Ner Ltd., Czech Republic. For the centrifugation of the extracts, the Hettich Universal 30 RF (Germany) device was used. Total organic carbon (TOC) was determined spectrophotometrically after the oxidation of organic matter by $K_2Cr_2O_7$ [32]. Values of pH were measured in a 1/20 (1 g + 20 ml) 0.01 mol L^{-1} $CaCl_2$ soluble extract at 20±1°C. Cation exchange capacity (CEC) was calculated as the sum of Ca, Mg, K, Na and Al extractable in 0.1 M $BaCl_2$ (1/20 (1 g + 20 ml)). Available contents of nutrients were determined by the Mehlich III soil extraction procedure [29] using flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-300, Australia) (for Ca, K and Mg) and ICP-OES (for P, Fe, B, Cu, Mn, and Zn). Before extraction and total element content determination, the soil samples were air-dried at 20°C, ground in a mortar, and passed through a 2-mm plastic sieve.

Plant samples were decomposed using the dry ashing procedure as follows: an aliquot (~1 g) of the

dried and powdered biomass was weighed to 1 mg into 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 a Dry Mode Mineralizer Apion (Tessek, Czech Republic). The ash was dissolved in 20 mL of 1.5% HNO_3 (electronic grade purity, Analytika Ltd., Czech Republic) and kept in glass tubes until the analysis [33]. Aliquots of the certified reference material RM NCS DC 73350 poplar leaves (purchased from Analytika, CZ) were mineralized under the same conditions for quality assurance of the total arsenic contents in experimental plants. In this material, containing 0.37±0.06 mg As kg^{-1}, 0.32±0.07 mg Cd kg^{-1}, 1.5±0.3 mg Pb kg^{-1}, and 37±3 mg Zn kg^{-1} was determined 0.38±0.04 mg As kg^{-1}, 0.32±0.07 mg Cd kg^{-1}, 1.5±0.3 mg Pb kg^{-1}, and 36±1 mg Zn kg^{-1}.

For determination of arsenic compounds in redbeet biomass aliquots (~500 mg) of the dried and powdered aboveground biomass or roots were weighed to 0.1 mg were placed into 10-mL screw-capped polyethylene tubes and 10 mL of one of the extraction agents was added. In the case of roots pure water was applied as extraction agent whereas for aboveground biomass a 20 mmol L^{-1} ammonium phosphate buffer was used [34]. The closed tubes were fastened to the arms of a cross-shaped rotor and turned top over bottom at 45 rpm for 14 h. For determination of arsenic compounds in soil samples, aliquots of the dried soil samples were extracted with 0.05 mol L^{-1} aqueous $(NH_4)_2SO_4$ solution in ratio 1/25 (1 g + 25 ml) for 4 h [31]. The mixtures were then centrifuged for 10 min at 3000 rpm, and filtered through a 0.22 µm cellulose-nitrate ester filter (Millex-GS, Milipore, Bedford, MA, USA). Aliquots of this solution (20 µl) were chromatographed.

For determination of individual red pigments in beetroot samples, 0.3 g of each freeze-dried sample were finely ground using a Waring blender. 30 mg of each sample were extracted in 10 ml of ultrapurified water for 30 min by vigorous shaking on an orbital shaker, followed by an extraction for 10 min in an ultrasonic bath. Afterwards, crude extracts were centrifuged in an Eppendorf minispin plus at 14.500 rpm for 10 min and filtered through 0.2 µm filter for HPLC analysis.

Individual compounds used as chromatography standards were isolated from crude extract by semi-preparative HPLC, as stated below. Crude extract, which was prepared in the same way as for analysis, was first pretreated by solid phase extraction on a C18 cartridge (Separon SGX, 50 µm particle size, Tessek, CZ), elution with 100% MeOH to remove interferences.

The total arsenic concentrations in the plant biomass decomposed by dry ashing procedure and in soil digests, soil and plant extracts, and individual pigment fractions were determined by hydride generation atomic absorption spectrometry (Varian AA280Z, Varian, Australia), equipped with continuous hydride generator VGA-77 [35] where a mixture of potassium iodide and ascorbic acid was used for pre-reduction of the sample and the extract was acidified by HCl before measurement. High arsenic concentrations as well as cadmium, lead, and zinc were determined by ICP-OES with axial plasma configuration (Varian VistaPro, Varian, Australia). For determination of elements in individual pigment fractions ICP-MS (PQ ExCell, VG Elemental, Winsford, UK) was applied.

2.2.2 Chromatographic systems

A Hewlett Packard 1100 solvent delivery unit (Germany) together with a Hamilton PRP-X100 (USA) anion-exchange column (250 mm x 4.1 mm i.d., spherical 10-µm particles of a styrene-divinylbenzene copolymer with trimethylammonium exchange sites) was used for the separation of As(III), dimethylarsinic acid (DMA), methylarsonic acid (MA), and As(V). An aqueous 0.02 mol L^{-1} $NH_4H_2PO_4$ solution pH 6.0 at a flow rate of 1.5 mL min^{-1} served as mobile phase. The column effluent was introduced into the plasma of the ICPMS (Agilent 7500) for arsenic selective-detection [36].

Individual beet pigments were determined using a RP-HPLC method with UV detection [3]. The separation of four pigments was achieved with an analytical HPLC system Dionex Summit (Dionex corp, US), consisting of P680 quaternary gradient pump, diode array detector UVD340U, column thermostat, interfaced with the Waters 717 autosampler (Waters Inc., US), using a Gemini C18 column (250 × 4.6 mm I.D., 5 µm particle size). Column temperature was 30°C. Mobile phase: A: KH_2PO_4 at pH 2.75, B: 50% ACN (Lach-Ner., CZ), gradient elution profile A/B 0 min: 100/0, 5 min 100/0; 30 min 70/30. Peak integration was performed at 477 nm and all compounds were quantified based on calibration curves obtained from individual previously separated standards of betanin and betanidin and vulgaxanthin II and vulgaxanthin I. All samples were prepared and measured in triplicate.

Semi-preparative column Phenomenex Luna C18 (250 × 10 mm I.D., 15 µm particle size, Phenomenex, US) was used in the HPLC system mentioned above to obtain pure chromatographic standards. Mobile phase consisted of A: 0.05% formic acid and B: 100% MeOH gradient elution profile A/B 0 min: 100/0, 5 min 100/0; 30 min 60/40, flow 3 ml/min. Individual peak fractions were repeatedly collected in dark, ice-chilled tubes,

evaporated by freeze drying and briefly kept in darkness under nitrogen for immediate analysis on an analytical HPLC.

3. Results and Discussion

3.1 Contents of investigated elements and arsenic compounds in red beet samples

Table 1 documents the high level of soil arsenic contamination in the Kutná Hora location rising up to 1573±46 mg As kg^{-1}. Previous studies have also confirmed [37] high arsenic contents in upper layers of arable soils in this location. According to the public notice [38] the „pseudo-total" element concentrations *(i.e. Aqua Regia* soluble) in loamy soils cannot exceed a maximum of 30 mg kg^{-1} As, 1.0 mg kg^{-1} Cd, 140 mg kg^{-1} Pb and 200 mg kg^{-1} Zn. In this case, total arsenic concentration reached the legislation threshold limit even in the control in all the investigated soils except the soil Píšťany [38]. Moreover, enhanced contents of Cd and Zn were determined in the soils from the locations Příbram, Kutná Hora arable and Kutná Hora meadow, and also Pb in the soil Příbram. The behavior of the risk elements in soils is affected by a whole complex of soil physicochemical parameters such as pH, content of soil organic matter, clay minerals, source of contamination, *etc*. [39]. In our case, the 0.05 mol L^{-1} $(NH_4)_2SO_4$ extractable arsenic portion in the soils Kutná Hora characterized by high total organic matter content and/or high sorption capacity did not exceed 0.3% of total arsenic content in soil. On the contrary, the mobile content of arsenic in the sandy soil Píšťany reached 2% of total arsenic content due to the low level of CEC. For cadmium, the mobile contents varied between 0.5% (Kutná Hora) and 9% (Mokrsko) of the total contents. In the case of Pb the mobile contents varied from 0.1% (Příbram) to 3% (Píšťany) and Zn varied from 0.2% (Píšťany) to 8% (Mokrsko). Although the soils were extremely contaminated the mobile portions of the elements represented only a small fragment of the total element content.

Total arsenic contents in beetroot digests varied between 0.1 and 25 mg kg^{-1} and in leaves between 0.2 and 48 mg kg^{-1} (Table 3) in correlation to the mobile soil arsenic concentration (r=0.87 for beetroot and r=0.85 for leaves). Comparing different vegetable species, Warren *et al*. [40] determined the following order of arsenic uptake: potatoes < spinach < cauliflower < lettuce < red beet < radish. In contrast to pepper plants where arsenic is accumulated in the roots blocking translocation within the plant [41], within the red beet plants the translocation

	As mg kg^{-1}	Cd mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}
Beetroot				
Pot experiment 1				
Příbram arable	0.48 ± 0.09	4.96 ± 0.43	9.29 ±1.79	374 ±32
Mokrsko arable	24.8 ± 1.9	0.38 ± 0.04	1.52 ±0.35	71.5 ±5.7
Kutná Hora arable	5.36 ± 0.55	0.94 ± 0.23	1.32 ±0.28	102 ±21
Kutná Hora meadow	8.72 ± 1.19	0.48 ± 0.08	1.57 ±0.73	62.0 ±7.1
Kutná Hora waterside	0.16 ± 0.0101	0.50 ± 0.25	1.41 ±0.38	31.3 ±4.2
Pot experiment 2				
Mokrsko arable	17.3 ± 0.2	0.46 ± 0.01	0.10 ± 0.01	57.2 ± 2.1
Kutná Hora arable	6.7 ± 0.8	1.89 ± 0.09	0.20 ± 0.01	105 ± 1
Kutná Hora meadow	8.2 ± 0.4	1.16 ± 0.02	0.10 ± 0.02	39.6 ± 5.0
Píšťany arable	0.6 ± 0.1	0.23 ± 0.00	0.25 ± 0.07	33.0 ± 0.9
Leaves				
Pot experiment 1				
Příbram arable	2.86 ± 0.80	23.6 ±4.3	10.1 ±2.1	1622 ±270
Mokrsko arable	48.0 ± 1.4	1.88 ±0.305	2.69 ±0.892	186 ±27
Kutná Hora arable	9.10 ± 2.81	9.92 ±1.30	3.63 ±0.709	439 ±29
Kutná Hora meadow	13.4 ± 2.01	4.88 ±0.315	4.04 ±0.931	168 ±9
Kutná Hora waterside	0.24 ± 0.02	1.33 ±0.246	4.72 ±1.87	75.5 ±17.5
Pot experiment 2				
Mokrsko arable	18.1 ± 0.9	1.23 ± 0.09	0.28 ± 0.04	171 ± 10
Kutná Hora arable	20.1 ± 1.5	4.69 ± 0.22	0.44 ± 0.03	213 ± 19
Kutná Hora meadow	10.2 ± 0.6	3.80 ± 0.13	0.43 ± 0.03	52.9 ± 3.8
Píšťany arable	3.2 ± 0.6	0.57 ± 0.24	0.37 ± 0.03	35.0 ± 1.9

Table 3. Total element contents in red beet samples; data are presented as mean±standard deviation, n=3.

of arsenic was not affected. In the case of cadmium and lead, higher element contents were observed in leaves according to Sekara *et al*. [42]. They reported that the red beet was characterized by the highest cadmium and lead concentration ratio (shoots/roots) among the vegetable species examined (field pumpkin, chicory, common bean, white cabbage and parsnip). McGrath *et al*. [43] observed the linear relationships between total soil and crop Cd and Zn contents with no evidence of a plateau across the range of soil metal concentrations achieved in long-term field experiment at sewage sludge amended soils. The slopes of the soil-plant relationships depended on the type of crop or crop part examined, but were generally in the order red beet > sugar beet > carrot > barley. Our results, however, showed limited uptake of these elements at the highly contaminated soil Kutná Hora where low mobility of these elements occurred. Therefore, red beet belongs among plants with relatively high ability to take up soil trace elements. In our case no visible symptoms of risk element phytotoxicity and no decrease of plant yield were observed regardless of risk element contents in soil where the highest beetroot yield was observed in Kutná Hora soil (57±9 g per pot) whereas the slightly contaminated soil Píšťany was the lowest (36±6 g per pot).

Various extraction procedures were described for determination of arsenic compounds in plant material ranging from water extraction at ambient temperature to pressurized liquid extraction or microwave–assisted extraction at elevated temperatures using different extractants. In our experiments, the influence of the extractants for releasing the arsenic compounds from *B. vulgaris* growing in arsenic contaminated soils was investigated within the first vegetation period (Experiment 1). Arsenic portions extractable by water and phosphate buffer did not show significant differences between the extraction agents but the extractability was affected by the arsenic concentration in beetroot (Figure 1). Arsenic was almost quantitatively extracted from the samples with the lowest total arsenic concentration, whereas in the samples with the highest total arsenic concentration less than 25% was extractable. In the case of aboveground plant biomass, poor extractability of arsenic compounds not exceeding 50% of total arsenic content was observed similarly as in our previous experiments [34] and reported by other authors [44]. Similarly, Ruiz-Chancho *et al.* [45] reported extraction efficiencies ranging from 3.0% to 41.4%, with good agreement between samples from the same plant species where different extraction procedures with different ratios of water and methanol were compared. Schmidt *et al.* [46] reported the highest extraction yield of 90% for ground leaf material of *Tropaeolum*

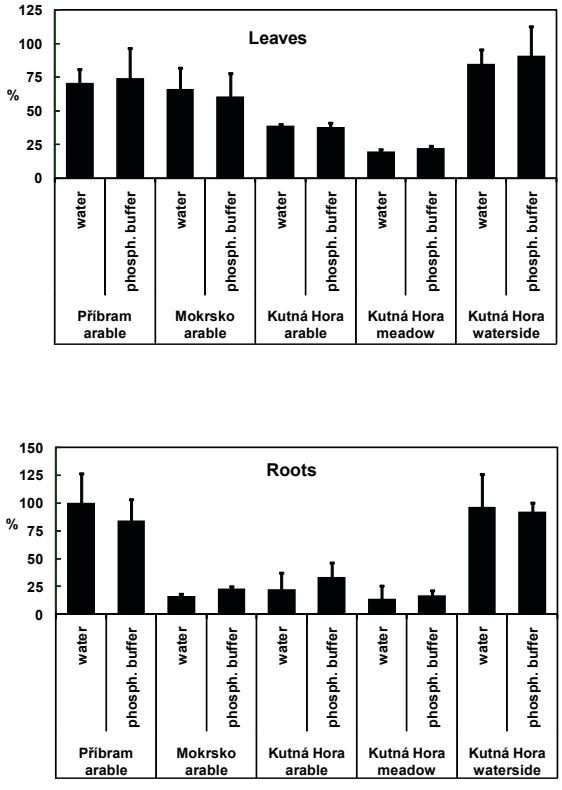

Figure 1. Average recoveries of arsenic in beet plants with individual extraction agents – pot experiment 1 (data are presented as bar mean and standard deviation n=5).

majus with 0.1 mol L^{-1} phosphate buffer, pH 7.7, in a two-step extraction with a total extraction time of 24 h. They also compared different sample pretreatment approaches where the extraction of higher amounts of arsenic (50–70% of total arsenic) from non-ground leaves with deionized water in comparison with the buffer (20–40% of total arsenic) was observed because of the osmotic effects in this case. Variable extractability of arsenic, as affected by the type of arsenic species accumulated in the tissue as well as on the type of the tissue (leaf, leaf stalk, stem), was found as well. For the extraction of 5 mm long segments cut from individual leaves without previous homogenization of the plant parts yields between 75 and 93% depending on arsenic species prevailing in the cells, obtained using 1 or 10 mmol L^{-1} phosphate buffer [47]. Sequential extraction procedure where the plants are extracted first with 1:1 water-methanol followed by 0.1 mol L^{-1} hydrochloric acid can lead to almost quantitative release of arsenic from terrestrial plant samples [48]. However, the wide range of arsenic extractability within the set of plant samples confirmed different tightness of arsenic bounds in plant cells. Different components of immobilization/

detoxification mechanisms such as phytochelatin complexes [21,23] can be expected in different plant species and individual plant tissues. However, potential presence of arsenic complexes with various plant pigments can also be taken into account.

Arsenate was the dominant arsenic compound in the extracts (Figure 2) within Experiment 1, especially in the leaves where abundance of arsenate reached up to almost 90%. A small portion of dimethylarsinic acid, not exceeding 0.5%, was detected in leaves growing in the soils from Mokrsko and Kutná Hora. In roots only arsenate and arsenite were detected and the percentage of arsenate did not exceed 75% of extractable arsenic. In our previous experiments arsenic uptake and speciation in radish (*Raphanus sativus*) plants were investigated [49]. As(III) was the dominant compound (63%) followed by As(V) and DMA (20 and 17%, respectively) in radish roots while in leaves most of the arsenic present was arsenate (42%), arsenite (40%), and DMA was also detected (18%) indicating the role of biomethylation processes within the plant. In stems+leaves and roots of pepper plants (*Capsicum annum)* arsenate and arsenite were the dominant arsenic compounds, and low portions of MA and DMA were detected, as well [34]. Therefore, the abundance and distribution of arsenic compounds within red beet plants did not differ significantly from the behavior of arsenic compounds in other plant species and the results were expectable. Comparable results have been presented by other authors within different plant species [16,19,20,44].

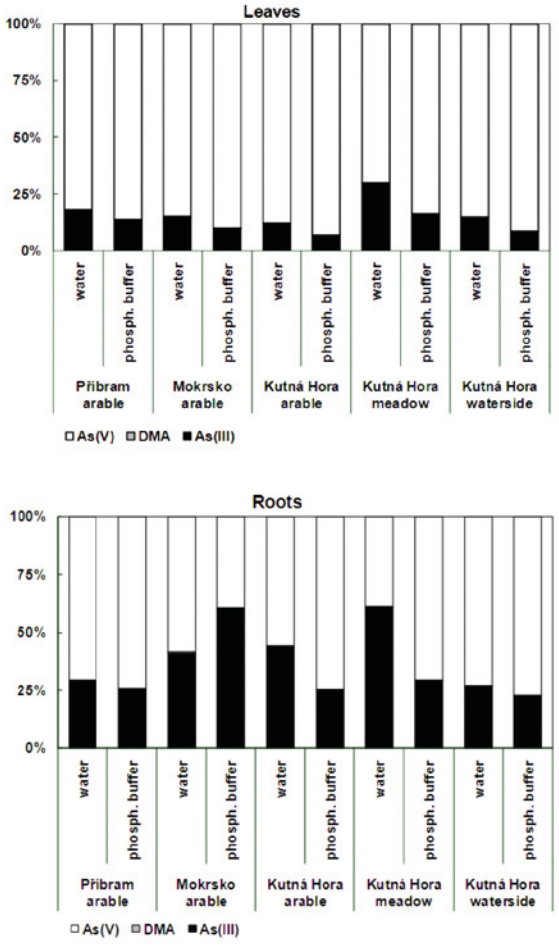

Figure 2. Distribution of arsenic compounds within beet plants – pot experiment 1 (n=5).

3.2 Contents of betalains in beetroot samples

For an evaluation of potential interactions of arsenic with red pigments in red beet plants the experiment was repeated in the second vegetation period where two contaminated soils from the Kutná Hora location and one from Mokrsko were applied and uncontaminated soil from Píšťany was added to the set of the experimental soils (Experiment 2, see Table 3). Although total arsenic contents in leaves and beetroot of the plants differed from Experiment 1 due to different weather conditions within the second vegetation period, the abundance of individual arsenic compounds within the red beet plants

	As(III)		DMA		As(V)		sum
	mg/kg	% of sum	mg/kg	% of sum	mg/kg	% of sum	mg/kg
Leaves							
Mokrsko arable	0.597 ±0.144	25.2	0.023 ±0.004	0.992	1.718 ±0.088	73.2	2.352
Kutná Hora arable	0.313 ±0.057	7.28	0.004 ±0.001	0.096	4.088 ±1.273	92.6	4.405
Píšťany arable	0.038 ±0.037	2.53	0.004 ±0.000	0.369	1.272 ±0.769	97.1	1.314
Beetroot							
Mokrsko arable	2.494 ±0.092	69.4	0.024 ±0.003	0.681	1.082 ±0.161	29.9	3.600
Kutná Hora arable	0.211 ±0.063	27.3	0.005 ±0.001	0.732	0.545 ±0.030	71.9	0.761
Kutná Hora meadow	0.741 ±0.008	48.2	0.017 ±0.004	1.139	0.778 ±0.005	50.6	1.536
Píšťany arable	0.025 ±0.011	14.2	0.005 ±0.001	2.924	0.147 ±0.022	82.8	0.177

Table 4. Arsenic compounds in water extracts of red beet samples – pot experiment 2; data are presented as mean±standard deviation, n=3.

Sample	vulgaxanthine II	vulgaxanthine I	betanin	isobetanin
Mokrsko arable	9.41±0.94	5.12±0.66	10.22±0.92	5.27±0.53
Kutná Hora arable	6.84±0.52	0.68±0.81	15.02±1.97	8.70±1.52
Kutná Hora meadow	8.42±0.37	0.30±0.03	20.18±1.25	8.78±0.70
Píšťany arable	2.49±0.32	0.58±0.06	20.67±2.29	10.82±0.97

Table 5. Red pigment contents in raw extract of redbeet – pot experiment 2 (mg/g sample d.w.); data are presented as mean±standard deviation, n=3.

determined by water extracts of the plant biomass was comparable to the first experiment (Table 4). The extractability of arsenic varied in leaves between 12 and 40%, in roots between 10 and 30% where the highest value corresponded in both cases to the uncontaminated soil Píšťany. In this point the results are comparable to Experiment 1 (Figure 1). However, for other plant species such as pepper plants higher water extractable portions (up to 100%) were determined for arsenic in roots [34]. As stated by Raab *et al.* [21], the different arsenic compounds can be present in the plant cell as free molecules, adsorbed to various cell compartments such as the cell wall, mitochondria, vacuoles or chemically bound as a part of arsenic-phytochelatin complexes. In our case, free molecules of arsenic compounds are not the dominant part of arsenic in red beet plants. However, the HPLC-ICPMS system is not able to distinguish other types of arsenic bounds.

Betalains are, like flavonoids, complex water soluble molecules but, unlike flavonoids, they are all indoles, contain nitrogen and are derived from the amino acid tyrosine. Like anthocyanins, the pigments are stored in the vacuole, mainly as glycosides. Unlike most anthocyanins, betalains have the ability to react with amino acids. We still do not know how their synthesis is controlled and how they are transported to and into the vacuoles [50]. The role of betalains in possible interactions with chemical elements was not yet investigated, unlike anthocyanin-metal complexes identified for molybdenum, tungsten, cadmium and also arsenic in various plant species [7,10,25]. Because of high content of betalains in the beetroot the role of these pigments was evaluated in our experiment. In the raw extract of the beetroot samples vulgaxanthin I, vulgaxanthin II, betanin, and isobetanin were identified (Table 5) where different amounts of individual pigments were observed for the sample from the uncontaminated Píšťany soil compared to the beetroot samples from the risk element contaminated soils. Although high variability of betalain contents were observed among individual red beet varieties and different parts of the roots, betanin seems to be predominant in the common samples [3] consistent with our observations.

The determination of elements in the beetroot samples (Table 3) suggested lower concentrations of As but higher concentrations of Pb in the samples dominated by betanin (R=0.85 and 0.76, P<0.05) and isobetanin (R=0.99 and 0.42), respectively. By contrast, samples that were rich in vulgaxanthin showed a positive correlation with As content (R=0.89). For other elements no relationship was found.

Although the measurement of arsenic compounds in leaves and beetroot of red beet plants showed no differences from other terrestrial plants, some aspects indicated more complex behavior of arsenic within the red beet plants. As this correlates seem too preliminary due to the sample amounts, a more detailed investigation of the beetroot extracts will be necessary to elucidate detailed composition of the extracts followed by investigation of possible arsenic bounds to these structures. We can conclude that our results confirmed the ability of *B. vulgaris* to accumulate risk elements in their tissues without apparent symptoms of element phytotoxicity at the available element levels contained in our experimental soils. The relationships of betalains with element contents in plants were not unambiguously confirmed at these contamination levels. If we are to suppose possible plant detoxification mechanisms involving the interactions of risk elements with plant pigments, further investigations should focus on plant growth in higher mobile element concentrations in soil.

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