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THE EFFECT OF SOIL CONTAMINATION LEVEL AND PLANT ORIGIN ON CONTENTS OF ARSENIC, CADMIUM, ZINC, AND ARSENIC COMPOUNDS IN *MENTHA AQUATICA* L.

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Abstract: Water mint (*Mentha aquatica* L.) belongs to the arsenic tolerant plant species suitable for cultivation in Central European climate conditions. Therefore, its possible application for remediation of contaminated soil was investigated in pot and field experiments. Two *M. aquatica* plants of different origin, i) commercially market-available mint plants, and ii) plants habituated at the arsenic contaminated former mining area in southern Tuscany (Italy) were tested for their arsenic uptake, transformation, and speciation. The total arsenic concentrations in the experimental soils varied from 21 to 1573 mg As kg^{-1}, the mobile fractions did not exceed 2% of total soil arsenic. The mint plants originating from the contaminated area were able to remove ~400 µg of arsenic per pot, whereas the commercial plant removed a significantly lower amount (~300 µg of arsenic per pot). Only arsenite and arsenate, but no organoarsenic compounds were identified in both stems and leaves. Arsenate was the predominant arsenic compound and reached up to 80% regardless of the origin of the mint plants. Although *M. aquatica* seems to be able to grow in contaminated soils without symptoms of phytotoxicity, its efficiency to remove arsenic from the soil is limited as can be demonstrated by total elimination of As from individual pots not exceeding 0.1%. Moreover, the application of plants originating from the contaminated site did not result in sufficient increase of potential phytoextraction efficiency of *M. aquatica.* Although not suitable for phytoextraction the *M. aquatica* plants can be used as vegetation cover of the contaminated soil at the former mining areas.

INTRODUCTION

Surrounding the city Kutná Hora, Central Bohemia, the area of medieval silver mining, is characterized by high level of soil contamination with risk elements (especially As, Cd, and Zn). The available analytical data [1] confirmed high arsenic contents of As in soils ranging between 50 and 330 $mg\cdot kg^{-1}$. Therefore, possible remediation of these soils must be taken into account and phytoremediation suggests itself a relatively low cost and environmental friendly method leading to decrease of soil contamination level.

However, the spectrum of plant species suitable for phytoextraction of arsenic is relatively limited. Ma *et al.* [2] described the fern species *Pteris vittata* L. as capable of accumulating up to 23000 mg kg^{-1} of As in aboveground biomass, most of which was present as inorganic As (47–80%). Thereafter, more fern species were described as As hyperaccumulators such as *Pityrogramma calomelanos* [3], and *Pteris cretica*, *Pteris longifolia* and *Pteris umbrosa* [4]. The results suggested that *P. calomelanos* is able to release 2% of the total As from soil. However, these plants are not able to survive in Central European climatic conditions and their applicability is negligible.

Mentha aquatica L. (*Lamiaceae*) was described as the plant characterized by a high content of essential oil, phenolics, and flavanoids resulting in anti-inflammatory, antioxidant, and antibacterial action of this traditional medicinal plant [5–7]. Moreover, *Mentha spp*. is frequently discussed among the other aquatic macrophytes as the potential bioindicator of risk element pollution of water [8] and soil [9].

Zurayk *et al.* [10, 11] compared twelve Mediterranean plant species characterized as hydrophytes and found that only *M. aquatica* was able to accumulate sufficient amounts of Cd, Cr, and Ni and it was considered as a bioindicator for these elements. Growth rates remained high during the experimental period, indicating that the plants were little affected by the presence of metal at the experimental concentration. In the case of lead, low root-leaf transport was observed resulting in low lead accumulation in aboveground biomass [12]. The plants are usually able to change their tolerance to risk elements according to the environmental conditions. The populations of plants growing in contaminated soil are able to adapt their tolerance to actual metal contents in soil [13]. *M. aquatica* plants sampled at an arsenic contaminated site (total content of arsenic in soil was 266 mg·kg-1) demonstrated arsenic content in aboveground biomass up to 216 mg·kg-1 and in roots up to 540 mg·kg-1 [14] without apparent symptoms of arsenic phytotoxicity. Therefore, *M. aquatica* seems to be a promising plant to be tested for potential phytoremediation of the arsenic contaminated soils.

In our previous experiments [15] the mint plants tested in rhizobox experiments did not reach the arsenic contents presented by Baroni *et al.* [14], although both total and mobile arsenic contents in soil were comparable to the levels documented in the mentioned paper. Moreover, the dominant portion of arsenic retained in roots limits the potential use of this plant for phytoremediation technologies. To verify the findings ascertained in model conditions, field experiments under real conditions of the contaminated site are necessary.

The main objectives of our pot and field experiments were i) to compare the growth potential and arsenic uptake by commercially available plants of *M. aquatica* and arsenic-tolerant plants originating from the arsenic contaminated site; ii) to compare the arsenic compound transformations within aboveground biomass of both experimental plants; and iii) to assess potential phytoremediation ability of both plants in field conditions.

MATERIAL AND METHODS

Pot experiment

The area of Mokrsko (located 35 km south of Prague) has a high geogenic As content due to gold arsenopyrite occurrence. Kutná Hora (70 km east of Prague) soils are contaminated with 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 higher [1]. The sampling areas were selected at the suburb of the Malín municipality close to a medieval silver mine (so-called gallery of 14 assistants) close to a small streamlet named

Beránka. The location was described and mapped elsewhere [16]. Slightly contaminated Fluvisols from Píšťany (80 km north-west from Prague) was added to the set of experimental soils. Total element contents and main soil characteristics are summarized in Table 1, the available contents of macro- and micronutrients according to Mehlich III extraction procedure [17] are given in Table 2.

Table 1. Total element contents and basic characteristics of experimental soils; data are presented as mean ± standard deviation, n = 3

Soil	As mg kg^{-1}	Cd mg kg^{-1}	Pb mg kg^{-1}	Zn mg kg^{-1}	pH	TOC %	CEC mmol kg^{-1}
Kutná Hora meadow	1573 ± 46	4.9 ± 0.03	30.0 ± 0.5	499 ± 61	6.97 ± 0.03	4.02 ± 0.25	148 ± 12
Kutná Hora arable	1312 ± 103	19.5 ± 0.4	79.0 ± 0.1	1931 ± 56	7.41 ± 0.03	0.95 ± 0.00	380 ± 15
Mokrsko	839 ± 112	0.03 ± 0.01	11.0 ± 0.5	238 ± 2	5.36 ± 0.04	0.78 ± 0.02	116 ± 8
Píšťany	21 ± 1	0.83 ± 0.06	46 ± 9	100 ± 4	6.87 ± 0.02	1.50 ± 0.03	127 ± 5

TOC – total organic carbon
CEC – cation-exchange capacity

Table 2. Available contents of macro-and micronutrients in soils determined by Mehlich III extraction procedure [17]; data are presented as mean±standard deviation, n = 3

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}
Kutná Hora meadow	1.10 ± 0.12	103 ± 7	14060 ± 659	201 ± 6	191 ± 4	30.8 ± 2.2	4.17 ± 1.08
Kutná Hora arable	56.2 ± 1.1	610 ± 11	7210 ± 224	543 ± 8	164 ± 9	32.7 ± 1.0	7.88 ± 0.20
Mokrsko	33.8 ± 1.1	90.7 ± 16.9	1715 ± 24	196 ± 12	230 ± 1	57.1 ± 0.0	3.29 ± 1.06
Píšťany	123 ± 2	173 ± 9	3091 ± 1	220 ± 5	372 ± 3	97.4 ± 3.3	1.99 ± 0.38

Before planting, soil samples were collected from individual pots, air dried at 20°C, ground in a mortar and passed through a 2-mm plastic sieve [18] and analyzed for its total and mobile element content. Plants were cultivated in 6-litre plastic pots with 5 kg of air-dry soil and four replicates were used for each soil. NPK (0.5 g N, 0.16 g P, 0.4 g K per pot as inorganic salts solutions) was added before sowing and *M. aquatica* was cultivated for two vegetation periods. The *M. aquatica* plants were of different origin, i) commercially market-available mint plants and ii) plants habituated at the arsenic contaminated former mining area in southern Tuscany (Italy) purchased by the University of Siena, Dipartimento Sci. Ambientali, Sez. Geochim. Ambientale, Siena, Italy. The plants were prepared by vegetative propagation from one plant cultivated in uncontaminated soil. Soil moisture was regularly controlled and kept at 60% of the maximum water holding capacity (MWHC). The aboveground biomass was harvested at the end of the vegetation period, gently washed by deionized water, checked for fresh biomass, freeze-dried (Lyovac GT-2, Germany), ground and analyzed.

Field experiment

The same treatments as in the pot experiment were performed in a precise field experiment (plot size: 4 m^2) at the locations of Kutná Hora arable and Kutná Hora meadow for three vegetation periods. Twenty five plants of the commercial mint in 5 rows and 12 plants of the mint originating from the contaminated site in 3 rows were planted in each plot. The plants were planted under natural conditions, only weed plants were regularly removed. No fertilizers and/or pesticides were applied for the entire duration of the experiment. The aboveground biomass was harvested at the end of each vegetation period, gently washed by deionised water, checked for fresh biomass, freeze-dried (Lyovac GT-2, Germany), ground and analyzed.

Analytical methods

Total element concentrations in soil were determined in digests obtained by two-step decomposition as follows: 0.5 g of 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 [19]. Certified reference material RM 7001 Light Sandy Soil (Analytika, CZ) containing 12.3 ± 1.1 mg As kg^{-1}, 0.32 ± 0.05 mg Cd kg^{-1}, 43.8 ± 3.7 mg Pb kg^{-1}, and 120 ± 7 mg Zn kg^{-1} was used for quality assurance of the analytical data of total element determination, and 12.8 ± 1.5 mg As kg^{-1}, 0.30 ± 0.01 mg Cd kg^{-1}, 39.9 ± 1.3 mg Pb kg^{-1}, 113±6 mg Zn 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 hours [20]. 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. The pH was determined using deionised water or 0.2 M KCl (w/v = 1 + 2.5). Cation-exchange capacity (CEC) was calculated as the sum of Ca, Mg, K, and Al extractable in 0.1 M $BaCl_2$ (w/v = 1+20 for 2 hours) [21]. Total organic carbon (TOC) was determined spectrophotometrically after the oxidation of organic matter by $K_2Cr_2O_7$ [22]. The available contents of nutrients were determined by the Mehlich III soil extraction procedure [17] using flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-300, Australia) (for Ca, K and Mg) and ICP-OES with axial plasma configuration (Varian VistaPro, Varian, Australia, for P, Fe, B, Mn), see Table 2.

Plant samples were decomposed using the dry ashing procedure as follows: An aliquot (~1 g) of the dried and powdered aboveground 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 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 [23]. 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. This material was certified to contain the following: 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 0.36 ± 0.02 mg As kg^{-1}, 0.34 ± 0.05 mg Cd kg^{-1}, 1.6 ± 0.2 mg Pb kg^{-1}, and 35 ± 3 mg Zn kg^{-1}.

For determination of arsenic compounds in *M. aquatica* biomass aliquots (~500 mg) of the dried and powdered aboveground biomass or roots were weighed to 0.1 mg into 10-mL screw-capped polyethylene tubes and 10 mL of 20 mmol L^{-1} ammonium phosphate buffer was added [24]. The closed tubes were fastened to the arms of a cross-shaped rotor and turned top over bottom at 45 rpm for 14 hours. 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.

The total arsenic concentrations in the plant biomass decomposed by dry ashing procedure and in soil digests, soil, and plant extracts were determined by hydride generation atomic absorption spectrometry (Varian AA280Z, Varian, Australia), equipped with a continuous hydride generator VGA-77 [25] 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, and zinc were determined by ICP-OES with axial plasma configuration (Varian VistaPro, Varian, Australia).

An Agilent 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 7500ce) for arsenic selective-detection [26].

RESULTS AND DISCUSSION

Pot experiment

Table 1 documents a high level of soil arsenic contamination in the Kutná Hora location reaching 1573 ± 46 mg As kg^{-1} as also reported by other authors [27]. According to the public notice released by Czech Ministry of the Environment [18] the element concentrations in 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. The total arsenic and zinc concentrations reached the legislation threshold limit in all the investigated soils with the exception of the soil Píšťany. Elevated concentrations of cadmium were determined in both soils from the Kutná Hora location. However, mobile portions of arsenic in the soils are relatively low. The water extractable arsenic portions in the soils of Kutná Hora characterized by high total organic matter content and/or high sorption capacity do 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, most probably due to a low level of CEC. No effect of changing pH levels was observed although a decreasing As mobility with increasing sorption capacity of the soil occurred in our previous experiment [28]. The arsenic mobility within the set of experimental soil is usually not related significantly to some of the individual soil characteristics but is affected by a whole complex of soil parameters [19, 29]. Therefore, there is a big problem in finding the soil parameter unambiguously responsible for changing As mobility in individual soils. For cadmium, the mobile contents varied between 0.5% (Kutná Hora) and 9% (Mokrsko) of the total contents. In the case of Zn, the mobile contents varied from 0.2% (Píšťany) to 8% (Mokrsko).

Comparing the yield of aboveground plant biomass (Table 3) a higher mass of leaves vs. stems was reported where no effect of soil contamination level was reported.

Table 3. Total yield of aboveground plant biomass per pot (g); data are presented as mean ± standard deviation, n = 4; The averages marked by the same letter did not significantly differ at α = 0.05 within individual columns

1st vegetation period				
Soil	commercial plants		plants from contaminated site	
	leaves	stems	leaves	stems
Kutná Hora meadow	8.2 ± 1.9 ^{b}	6.5 ± 2.7 ^{ab}	13.6 ± 1.2 ^{c}	8.4 ± 1.9 ^{bcd}
Kutná Hora arable	12.6 ± 1.0 ^{c}	10.4 ± 1.0 ^{cd}	16.5 ± 1.9 ^{d}	11.1 ± 1.8 ^{d}
Mokrsko	5. 8 ± 1.5 ^{ab}	7.4 ± 1.5 ^{b}	4. 5 ± 1.7 ^{a}	3.88 ± 0.30 ^{a}
Píšťany	11.9 ± 1.3 ^{c}	8.1 ± 1. 7 ^{bc}	14.00 ± 2.4 ^{cd}	8.6 ± 1.9 ^{bcd}
2nd vegetation period				
Soil	commercial plants		plants from contaminated site	
	leaves	stems	leaves	stems
Kutná Hora meadow	3.85 ± 0.50 ^{a}	0.95 ± 0.11 ^{a}	10.3 ± 2.2 ^{cd}	4.1 ± 1.2 ^{bc}
Kutná Hora arable	7. 8 ± 2.5 ^{bc}	6.7 ± 2.6 ^{cd}	11. 8 ± 0.2 ^{d}	8.8 ± 1.5 ^{d}
Mokrsko	3.7 ± 1.2 ^{a}	3. 5 ± 1.1 ^{ab}	5.2 ± 1.9 ^{ab}	4.6 ± 2.0 ^{bc}
Píšťany	6.7 ± 2.8 ^{abc}	3.38 ± 0.95 ^{ab}	19.5 ± 3.5 ^{e}	8.1 ± 1.7 ^{d}

In most cases, a higher yield of aboveground biomass was produced by the *M. aquatica* plants originating from the contaminated site. The highest total arsenic contents (Table 4) were determined in the soils with lower sorption capacity (Kutná Hora meadow, Mokrsko) compared to Kutná Hora arable with a CEC level of 380 ± 15 mmol.kg^{-1} confirming that the increasing element content in plants is not affected solely by the increasing total element content in soil [30].

Table 4. Total arsenic contents in aboveground plant biomass – pot experiment (mg.kg^{-1}); data are presented as mean ± standard deviation, n = 4. The averages marked by the same letter did not significantly differ at α = 0.05 within individual columns

1st vegetation period				
Soil	commercial plants		plants from contaminated site	
	leaves	stems	leaves	stems
Kutná Hora meadow	13.7 ± 5.0 ^{d}	3.65 ± 1.42 ^{b}	10.2 ± 4.2 ^{cd}	2.44 ± 0.96 ^{ab}
Kutná Hora arable	5.03 ± 0.38 ^{ab}	1.60 ± 0.53 ^{ab}	8.13 ± 2.43 ^{bc}	1.49 ± 0.46 ^{a}
Mokrsko	10.0 ± 0.9 ^{cd}	1.94 ± 0.31 ^{ab}	12.4 ± 4.5 ^{cd}	7.30 ± 3.01 ^{c}
Píšťany	1.28 ± 0.51 ^{a}	1.12 ± 0.43 ^{a}	1.71 ± 0.83 ^{a}	0.75 ± 0.59 ^{a}
2nd vegetation period				
Soil	commercial plants		plants from contaminated site	
	leaves	stems	leaves	stems
Kutná Hora meadow	42.2 ± 6.8 ^{d}	13.8 ± 5.6 ^{a}	20.24 ± 5.58 ^{c}	8.85 ± 1.33 ^{a}
Kutná Hora arable	7.20 ± 1.03 ^{ab}	2.98 ± 0.93 ^{a}	4.60 ± 0.98 ^{a}	1.77 ± 0.12 ^{a}
Mokrsko	37.7 ± 12.3 ^{c}	41.4± 11.4 ^{b}	17.1 ± 7.7 ^{bc}	3.15 ± 2.35 ^{a}
Píšťany	4.46 ± 2.21 ^{a}	3.50 ± 2.313 ^{a}	4.39 ± 1.29 ^{a}	2.27 ± 0.35 ^{a}

The effect of P on the increasing mobility of arsenate due to a competitive sorption of arsenate and phosphate in soils was highlighted by many authors [31, 32]. In our case, however, the plants were fertilized and the potential effect of different P levels in soils was suppressed. Apparently**,** leaves are able to accumulate arsenic more intensively than stems, and better rooting of plants in the second vegetation period resulted in better arsenic uptake, especially in the case of commercial plants. Due to a higher yield of plant biomass originating from the contaminated site, the total arsenic uptake per pot tended to higher values in these samples (Figure 1).

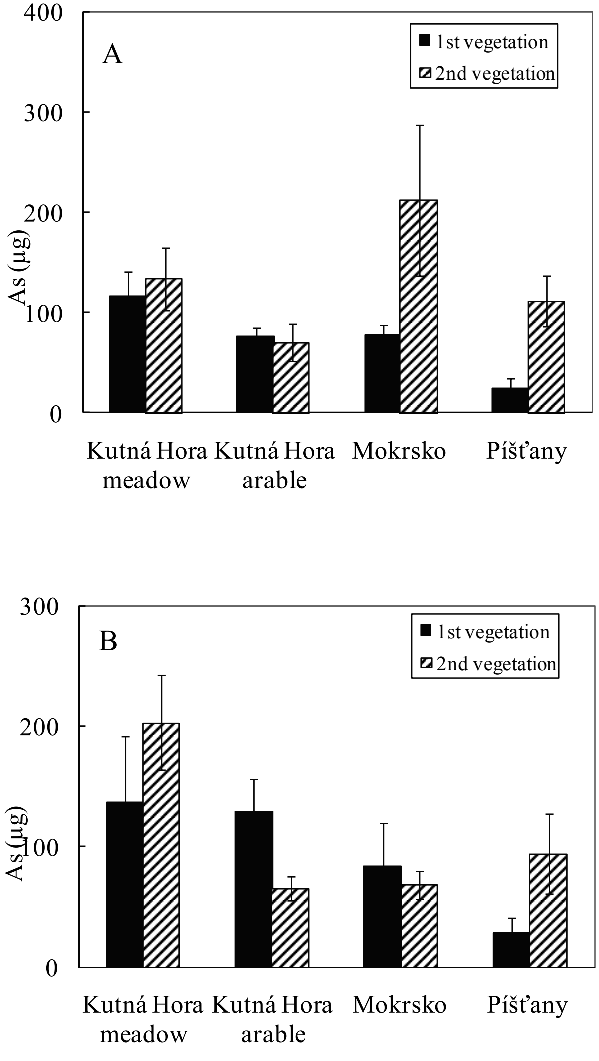

Figure 1. Total uptake of arsenic by aboveground biomass of M. aquatica – pot experiment; A) commercial plants, B) plants from the contaminated site

Chaney [33] argued that plant arsenic levels between 3 and 10 mg kg^{-1} can be considered as phytotoxic. Our experimental plants demonstrated no visible symptoms of phytotoxicity although the arsenic contents in mint leaves exceeded these levels even 4-fold in some cases. According to Directive No. 2002/32/ES [34] the maximum values of elements in raw feedstuffs (we calculated 12% of humidity) are 2 mg kg^{-1} of arsenic. Therefore, the potential transfer of the elements originating from the *M. aquatica* plants growing in the contaminated soil to the food chain by means of herbivorous animals should be taken into account. Zheljazkov *et al.* [35] found no detectable amount of Cd, Cu, or Pb in the essential oils of peppermint, basil, and dill and supported the use of aromatic plants as alternative crops for Cd, Pb, and Cu enriched soils. The potential effect of previous adaptation of a particular water mint sample collected directly at the extremely contaminated site was not observed. The highest As concentration in plants was described in submerged roots and stems, an order of magnitude higher than the aerial stems and leaves due to physicochemical adsorption, most probably due to co-precipitation with Fe at the submerged parts of stems [36, 37]. In our case, the plants were planted in the soils where the humidity was kept at 60% of MWHC. This was not representative of typical wetlands with submerged parts of the plants and presence of „iron plaque“ at plant stems cannot be expected.

The results of determination of As compounds in mint plants showed dominating pools of arsenate in both stems and leaves of *M. aquatica* plants (Table 5) followed by arsenite and small percentages of DMA (not exceeding 1.5%) and occasionally MA.

In our previous rhizobox experiment only arsenite and arsenate (no organoAs) compounds were identified in both aboveground biomass and roots [15]. In this pot experiment the presence of organoarsenicals can be explained by a longer duration of the experiment resulting in a higher uptake of arsenic compared to the rhizobox experiment. A small percentage of organic compounds of As were determined, for example, in stems and leaves of pepper plants [24] and described by other authors in many different plant species [38, 39]. No significant differences occurred between commercial plants and plants originating from the contaminated site.

For potential plant use in phytoextraction of risk element tolerance of plants to the high soil contamination level, high yields of aboveground biomass and predominant accumulation of the elements in aboveground plant biomass are necessary [40]. In our case, during two vegetation periods, the *M. aquatica* plants were able to remove only 393 μg As per pot in the case of the plants originating from the contaminated site and 299 μg As per pot for the commercial plants. As apparent from Figure 1, the plants tended in most cases to a higher total uptake of arsenic in the second vegetation period suggesting gentle adaptation of the plants to the cultivation conditions. The effective removal of the risk element from the soil is usually evaluated using transfer factor values given as a ratio of element concentration in the aboveground biomass of plant and in the soil. In this context, Iskandar and Kirkham [41] found the levels of the transfer factors of risk elements decreasing in order Cd > Zn > Mn > Cu > Co > Pb > Cr > As . In our experiment the transfer factors varied between 0.006 and 0.379 for the commercial plants and between 0.006 and 0.317 for the plants originating from the contaminated site confirming a limited ability of mint plants to accumulate arsenic in the aboveground biomass. Moreover, our previous rhizobox experiment [24] showed that a significant portion of the element (60% at the highly contaminated soil) was retained in roots. Therefore, the potential effectivity

Table 5. Arsenic compounds in aboveground plant biomass – pot experiment, average of both vegetation periods; data are presented as mean±standard deviation, n = 3

Stems	As(III)		DMA		MA		As(V)		sum
commercial plants	$mg.kg^{-1}$	% of sum	$mg.kg^{-1}$	% of sum	$mg.kg^{-1}$	% of sum	$mg.kg^{-1}$	% of sum	$mg.kg^{-1}$
Kutná Hora meadow	1.69 ± 0.01	33.0	0.034 ± 0.00	0.7	0.016 ± 0.01	0.31	3.39 ± 0.00	66.1	5.14 ± 0.01
Kutná Hora arable	0.229 ± 0.03	13.9	0.024 ± 0.00	1.4	0.008 ± 0.00	0.46	1.40 ± 0.11	84.2	1.66 ± 0.10
Mokrsko	6.1 ± 1.08	8.2	0.215 ± 0.03	0.3	< 0.001	-	67.0 ± 7.70	91.5	73.4 ± 8.72
Píšťany	0.347 ± 0.21	24.8	0.009 ± 0.00	0.7	< 0.001	-	0.95 ± 0.09	74.7	1.31 ± 0.29
plants from contaminated site									
Kutná Hora meadow	1.70 ± 0.30	29.3	0.049 ± 0.00	0.8	0.005 ± 0.00	0.09	4.13 ± 0.79	69.8	5.88 ± 0.64
Kutná Hora arable	0.200 ± 0.00	17.5	0.012 ± 0.00	1.1	0.001 ± 0.00	0.06	0.93 ± 0.09	81.4	1.15 ± 0.09
Mokrsko	1.85 ± 0.77	15.1	0.060 ± 0.02	0.5	0.004 ± 0.00	0.03	10.7 ± 2.48	84.4	12.6 ± 2.23
Píšťany	0.126 ± 0.01	14.8	0.007 ± 0.00	0.8	< 0.001	-	0.73 ± 0.15	84.4	0.868 ± 0.15
Leaves	As(III)		DMA		MA		As(V)		sum
commercial plants	mg/kg	% of sum	mg/kg	% of sum	ug/kg	% of sum	Mg/kg	% of sum	mg/kg
Kutná Hora meadow	8.74 ± 1.01	46.1	0.170 ± 0.06	0.90	0.057 ± 0.05	0.30	9.98 ± 0.62	52.7	18.9 ± 0.89
Kutná Hora arable	0.946 ± 0.08	28.9	0.027 ± 0.00	0.83	< 0.001	-	2.30 ± 0.19	70.2	3.28 ± 1.42
Mokrsko	8.03 ± 1.23	29.8	0.307 ± 0.02	1.14	0.018 ± 0.01	0.06	18.6 ± 1.6	69.0	27.0 ± 1.53
Píšťany	0.175 ± 0.02	17.7	0.006 ± 0.00	0.58	< 0.001	-	0.81 ± 0.06	81.7	0.989 ± 0.07
plants from contaminated site									
Kutná Hora meadow	5.20 ± 0.03	47.1	0.085 ± 0.02	0.77	0.008 ± 0.002	0.1	5.76 ± 0.61	52.0	11.1 ± 0.54
Kutná Hora arable	0.972 ± 0.04	42.0	0.028 ± 0.00	1.21	< 0.001	-	1.31 ± 0.08	56.8	2.3 ± 0.04
Mokrsko	1.51 ± 0.28	21.7	0.104 ± 0.03	1.46	< 0.001	-	5.53 ± 1.78	76.8	7.1 ± 2.06
Píšťany	0.606 ± 0.16	23.2	0.014 ± 0.00	0.53	< 0.001	-	1.97 ± 0.11	76.2	2.6 ± 0.23

of the *M. aquatica* plants for arsenic phytoextraction is negligible but the plants showed high tolerance to arsenic contamination of soil regardless of plant origin and soil arsenic level. Similarly, *Agrostis tenuis* plants growing without phytotoxicity symptoms in the soil containing up to 26500 mg kg^{-1} As were able to accumulate 3470 mg kg^{-1} As [42] confirming a high tolerance of these plants to elevated arsenic contents in soil without their hyperaccumulation parameters.

Field experiment

For verification of the phytoremediation potential of plants, a controlled field experiment is necessary compared with the pot experiment. Plant growth in the Kutná Hora arable soil occurred without any problems and/or any phytotoxicity symptoms. The yields of aboveground biomass of commercial plants varied between 8.1 and 13.5 g of dry matter per plant while plants originating from the contaminated site yielded between 7.0 and 16.0 g of dry matter per plant. In contrast to the pot experiment, plant growth at the Kutná Hora meadow site deteriorated and the yields varied between 2.5 and 7.6 g of dry matter per plant of commercial plants, and between 0.6 and 7.6 g of dry matter per plant of plants originating from the contaminated site. The lower yield can be affected by different growing conditions at individual sites where the Kutná Hora meadow experimental plot was located (on a shady and sloping field).

The arsenic contents in commercial *M. aquatica* plants varied between 1.81 and 8.07 mg As kg^{-1} of dry matter from the Kutná Hora arable soil and between 3.41 and 9.45 mg As kg^{-1} of dry matter from the Kutná Hora meadow soil. In the case of plants originating from the contaminated site the arsenic contents varied between 3.02 and 100 mg kg^{-1} of dry matter from the Kutná Hora meadow soil and between 3.24 and 7.08 mg kg^{-1} of dry matter from the Kutná Hora arable soil (Table 6).

Table 6. Total arsenic, cadmium, and zinc contents in aboveground plant biomass – field experiment (mg.kg^{-1}); data are presented as mean ± standard deviation, n = 3.

location	season	plant	As	Cd	Zn
			mg.kg^{-1}	mg.kg^{-1}	mg.kg^{-1}
Kutná Hora meadow	1st	commercial plants	9.45 ± 3.53	0.29 ± 0.08	60.0 ± 2.9
		plants from contaminated site	100 ± 4	1.66 ± 0.09	178 ± 2
	2nd	commercial plants	7.38 ± 2.92	0.16 ± 0.03	80.7 ± 16.7
		plants from contaminated site	15.0 ± 0.5	0.31 ± 0.08	181 ± 11
	3rd	commercial plants	3.41 ± 0.66	0.13 ± 0.04	117 ± 17
		plants from contaminated site	3.02 ± 1.80	0.16 ± 0.02	190 ± 18
Kutná Hora arable	1st	commercial plants	3.78 ± 1.19	0.15 ± 0.02	30.1 ± 5.4
		plants from contaminated site	3.24 ± 0.95	0.09 ± 0.03	24.6 ± 1.5
	2nd	commercial plants	1.81 ± 0.05	0.07 ± 0.02	71.3 ± 3.6
		plants from contaminated site	5.25 ± 0.21	0.43 ± 0.09	73.1 ± 4.8
	3rd	commercial plants	8.07 ± 0.36	0.05 ± 0.01	87.2 ± 8.1
		plants from contaminated site	7.08 ± 0.24	0.06 ± 0.01	82.3 ± 4.9

In the Kutná Hora arable soil, the arsenic concentrations increased gradually during the three vegetation periods, the highest As concentrations in the Kutná Hora meadow soil were found in the first vegetation period, while the next seasons showed significantly lower concentrations ($\alpha = 0.05$). However, a higher element concentration was associated with a lower biomass yield resulting in a comparable total removal of arsenic within individual growing seasons. Due to the elevated concentrations of Cd and Zn in the experimental soils, the contents of these elements in plant biomass was determined to assess the potential source of additional stress. In contrast to arsenic, cadmium belongs to the elements that is easily translocated to the aboveground biomass of plants [43]. In our case, however, the cadmium contents in mint aboveground biomass did not exceed 2 $mg\cdot kg^{-1}$ confirming low mobility and plant-availability of cadmium with an increasing pH level of the soils [44]. Zinc concentrations in plant leaves can be classified as follows: zinc content below 10 $mg\cdot kg^{-1}$ of dry matter results in symptoms of its deficit in plant tissues; zinc content between 10 and 25 $mg\cdot kg^{-1}$ of dry matter is still insufficient for plants; zinc content between 26 and 150 $mg\cdot kg^{-1}$ represents a normal level of the element; the content between 151 and 400 $mg\cdot kg^{-1}$ of dry matter indicates an enhanced level, and more than 400 $mg\cdot kg^{-1}$ of dry matter represents a toxic level of zinc [45]. In our case the zinc concentrations in plants varied between 25 and 190 $mg\cdot kg^{-1}$ allowing us to suggest that these values did not reach the potentially toxic levels of zinc in the aboveground biomass.

The highest total removal of arsenic from 1 m^2 of the plot after three vegetation periods represented 21.5 mg. This level was reached for the plants originating from the contaminated site at Kutná Hora arable soil. Transfer factors of arsenic were lower compared to the pot experiments and did not exceed 0.1, confirming that the application of *M. aquatica* plants for phytoextraction use is unreliable. Both pot and field experiments can be compared as follows: *M. aquatica* plants demonstrated good tolerance to elevated contents of arsenic in soil regardless of plant origin and cultivation conditions. The pot experiment, under more stable and controlled cultivation conditions, expectedly showed less variation of the results than was observed in the field. Both experiments, however, clearly showed that *M. aquatica* is an unsuitable plant for effective phytoextraction of arsenic in contaminated soil.

Baroni *et al.* [14] determined arsenic contents in 64 plant species in the area of the former mine in south Tuscany (Italy), and the highest arsenic contents were determined in the leaves and roots of *M. aquatica* (as mentioned above) and in roots of *Phragmites australis.* The mint plants did not reach the arsenic contents presented by Baroni *et al.* [14] in both pot and field experiments, although both total and mobile arsenic contents in soil were comparable to the levels documented in the mentioned paper. Therefore, the growth of *M. aquatica* at the extremely contaminated site did not lead to the change in arsenic uptake by these plants compared to the plants cultivated in uncontaminated soil. Due to the lack of relevant information, there was no possibility to compare physicochemical parameters of soils at our locations with the location described by Baroni *et al.* [14], although a possible presence of “iron plaque” at the stems can be taken into account.

Although not suitable for phytoextraction, the *M. aquatica* plants demonstrated high tolerance and good growth ability in the highly contaminated soil. The plants are able to create thick and permanent vegetation cover capable of surviving more subsequent vegetation periods, and may be applied towards the stabilization of the extremely contaminated sites. Moreover, the vegetation cover is stable for several vegetation periods without intensive fertilizers and/or pesticide application. However, potential environmental risks arise from elevated plant biomass arsenic content which exceeds the maximum permissible limits for feedstuffs. If applied towards the stabilization of contaminated land, protection against herbivorous animals as well as suitable disposal of the contaminated biomass should be achieved.

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