



Mobility of arsenic and its compounds in soil and soil solution: The effect of soil pretreatment and extraction methods

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ABSTRACT

The effect of soil extraction procedures and/or sample pretreatment (drying, freezing of the soil sample) on the extractability of arsenic and its compounds was tested. In the first part, five extraction procedures were compared with following order of extractable arsenic portions: 2 M HNO₃ ≫ 0.43 M CH₃COOH ≥ 0.05 M EDTA ≥ Mehlich III (0.2 M CH₃COOH + 0.25 M NH₄NO₃ + 0.013 M HNO₃ + 0.015 M NH₄F + 0.001 M EDTA) extraction ≫ water). Additionally, two methods of soil solution sampling were compared, centrifugation of saturated soil and the use of suction cups. The results showed that different sample pretreatments including soil solution sampling could lead to different absolute values of mobile arsenic content in soils. However, the interpretation of the data can lead to similar conclusions as apparent from the comparison of the soil solution sampling methods ($r=0.79$). For determination of arsenic compounds mild extraction procedures (0.05 M (NH₄)₂SO₄, 0.01 M CaCl₂, and water) and soil solution sampling using suction cups were compared. Regarding the real soil conditions the extraction of fresh samples and/or *in situ* collection of soil solution are preferred among the sample pretreatments and/or soil extraction procedures. However, chemical stabilization of the solutions should be allowed and included in the analytical procedures for determination of individual arsenic compounds.

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1. Introduction

The mobile portions of arsenic in soils are relatively low compared to most mobile elements such as cadmium and zinc. Baroni et al. [1] determined the water extractable arsenic to be between 0.010 and 0.040 mg kg⁻¹ although the soils contained 5.3–1226 mg kg⁻¹ total arsenic. In highly contaminated grassland soil (2035 mg kg⁻¹) up to 8.48 mg kg⁻¹ was extractable with water. According to the literature, arsenic in soil is present mostly as arsenate and small percentages of methylated arsenic compounds in soils were also reported [2,3]. The key components responsible for the mobility of arsenic in soil include the crystalline layer silicate minerals and hydroxides of iron (Fe), aluminum (Al) and manganese (Mn). Significant amounts are also adsorbed by secondary aluminosilicates, imogolite, and allophane and ferrihydrite [4,5]. Moreover, competitive relationships were observed between As and P [6,7] and organic anions derived from dissolved organic carbon [8,9] for the same sorption sites. Root–soil interactions can strongly affect the soil solution chemistry in the rhizosphere. The concentrations of organic acids (citrate, oxalate, acetate) are signif-

icantly larger in the rhizosphere soil solution than in the bulk soil [10]. In this context, increasing mobility and plant-availability of arsenic was observed in soils treated with oxalate [11].

Various soil extraction procedures were developed and tested for the determination of plant-available, mobile, and potentially mobilizable pools of trace elements in soils. However, a general consensus by the authors on which extractant is the most suitable in this case was not established. Moreover, the effectiveness of individual extractants to predict the plant-available elements depends on soil physicochemical parameters and contamination levels [12–15]. For identification and quantification of elemental fractions associated with individual soil components and for better understanding of the behavior of these elements in soil, the methods of sequential extraction of soils were developed. Complete summary of existing sequential extraction procedures including detailed comparison of these methods and/or extracting agents applied within individual extraction schemes was recently done by Filgueiras et al. [16] and Bacon and Davidson [17]. Exceptional position of arsenic among the other potentially toxic elements in soil chemistry led to individual approach in development of sequential extraction schemes. The sequential release of loosely and strongly adsorbed arsenic, arsenic coprecipitated with metal oxides or amorphous monosulfides, As coprecipitated with crystalline iron oxyhydroxides, As oxides, As coprecipitated with pyrite,

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Table 1Basic physical-chemical properties and total arsenic content in the experimental soils according to the soil locations; data are presented as mean \pm standard deviation.

	CEC ^b (mmol kg ⁻¹)	TOC ^c (%)	pH	P ^a (mg kg ⁻¹)	K ^a (mg kg ⁻¹)	Ca ^a (mg kg ⁻¹)	Fe (total) (mg kg ⁻¹)	Mn (total) (mg kg ⁻¹)	As (total) (mg kg ⁻¹)
Experiment 1									
Píšťany	201 \pm 4	2.6 \pm 0.1	6.8 \pm 0.5	151 \pm 1	148 \pm 5	4565 \pm 131	16400 \pm 790	975 \pm 13	37.2 \pm 2.2
Mikulov	99.1 \pm 8.2	4.2 \pm 0.1	4.2 \pm 0.2	251 \pm 37	72.3 \pm 0.3	1194 \pm 124	18330 \pm 1662	1185 \pm 161	392 \pm 30
Pramenáč	157 \pm 1	3.9 \pm 0.2	3.5 \pm 0.1	65.1 \pm 3.2	109 \pm 9	253 \pm 122	26697 \pm 3465	1500 \pm 98	254 \pm 1
Příbram meadow	166 \pm 20	3.6 \pm 0.4	5.2 \pm 0.1	37.5 \pm 1.3	91.9 \pm 4.5	2272 \pm 33	13123 \pm 415	1042 \pm 43	117 \pm 1
Kbely	299 \pm 4	1.9 \pm 0.1	7.2 \pm 0.4	45.4 \pm 0.0	175 \pm 8	7840 \pm 131	15101 \pm 174	440 \pm 23	43.2 \pm 2.7
Příbram arable	151 \pm 2	2.1 \pm 0.1	6.0 \pm 0.2	62.6 \pm 1.0	97.6 \pm 1.3	2139 \pm 0	14751 \pm 1895	991 \pm 146	71 \pm 4.1
Litavka	134 \pm 3	1.9 \pm 0.2	4.6 \pm 0.1	8.22 \pm 0.96	90.7 \pm 1.2	724 \pm 76	21617 \pm 2280	3831 \pm 546	437 \pm 67
Kutná Hora	295 \pm 13	2.9 \pm 0.1	7.1 \pm 0.2	9.32 \pm 0.82	263 \pm 1	6877 \pm 41	16676 \pm 177	434 \pm 26	115 \pm 3
Experiment 2									
Kutná Hora arable	380 \pm 25	4.0 \pm 0.3	7.4 \pm 0.3	56.2 \pm 1.1	610 \pm 11	7210 \pm 224	12949 \pm 723	259 \pm 14	473 \pm 10
Kutná Hora meadow	148 \pm 18	1.0 \pm 0.0	7.0 \pm 0.1	1.1 \pm 0.1	103 \pm 7	14060 \pm 659	18137 \pm 1274	476 \pm 18	1428 \pm 17
Příbram	123 \pm 12	1.9 \pm 0.0	4.5 \pm 0.1	283 \pm 5	160 \pm 2	951 \pm 15	12533 \pm 1000	802 \pm 38	36 \pm 1

^a Available contents of nutrients determined by Mehlich III extraction procedure (Mehlich [31]).^b CEC: Cation exchange capacity.^c TOC: Total content of carbon.

and As sulfides was described by Keon et al. [18]. The sequential extraction procedures regarding specific behavior of arsenic in soil were described also by Hall et al. [19] and Wenzel et al. [20].

The sample pretreatment and/or storage before extraction can affect the composition of soil solution which is related to element mobility in soils [21,22]. Various methods were developed for collection of soil solutions [23] such as centrifugation, suction cups or lysimeters where *in situ* sampling is considered to better represent solution extracted by plants and the physical structure of the sample remains intact. However, problems can occur with the stability of element species in the solution [24]. The influence of pH [25], soil microbial populations [26,27], and/or root exudates [28] on the stability of arsenic species in soil solution, wastewater or cultivation medium was observed.

Evidently, the different soil extraction procedures will result in different portions of released arsenic. This situation is given by an extraction agent applied and/or experimental conditions within the individual extraction procedure. However, the combined effect of soil extraction procedures and sample pretreatments remains questionable, especially in the case of mild extraction procedures and soil solution sampling methods. Finally, what can be said about the behavior of individual arsenic species in such solutions?

In our first experiment the extractability of As with five different extracting agents as well as the effect of sample pretreatment was evaluated on eight soil samples differing in their physicochemical parameters. In the second part of the experiment we looked at the effect of extraction methods and/or sample pretreatments on the distribution of arsenic compounds in soil arsenic portion extractable by mild extraction procedures. Three soils differing in their arsenic content were tested in this case. Among the large number of soil extractants available, pure water and 0.01 M of aqueous CaCl₂ were chosen as universal agents used for a wide scale of elements and 0.05 M aqueous (NH₄)₂SO₄ as a representant of the extracting agents developed especially for the assessment of arsenic mobility. The extractability of arsenic compounds was tested in the end of pot experiment where *Mentha aquatica* L. was planted to simulate the real soil conditions. The main goal of the study was to evaluate and compare some of the most widely used methods of soil extraction and soil solution collection. Moreover, the effect of sample preparation methods will be discussed especially if the determination of arsenic compounds is necessary for an evaluation of the potential plant-availability of soil arsenic. Therefore, the limits of the interpretation of the analytical data obtained by different procedures will be highlighted in this case.

2. Materials and methods

2.1. Experiment 1

2.1.1. Soil sample pretreatment

Eight soil samples differing in physical-chemical properties and total arsenic contents (Table 1) were tested within this experiment. Experimental soils: Příbram soils (loamy Cambisols) were taken from the field and meadow polluted by lead from mining and smelting industry. Fluvisol from the alluvium of the Litavka river, Czech Republic was heavily polluted by wastes from smelter setting pits. The area of Mokrsko (sandy Cambisols) has a high geogenic As content due to gold arsenopyrite occurrence. Geogenic source of contamination was also in the case of the Chernozems Mokrsko and Fluvisols Pramenáč. Kutná Hora soils (Luvisols) are contaminated by arsenic, cadmium, and zinc mainly due to tailings of silver mining in the middle ages. Slightly contaminated Fluvisols from Píšťany and Chernozems from Kbely were added to the set of experimental soils. At the individual locations cca 10 kg of soil from 0 to 25 cm depth after removing plant residues were sampled.

The pH was determined using deionized water or 0.2 M KCl (w/v = 1:2.5). Cation exchange capacity (CEC) was calculated as the sum of Ca, Mg, K, Na and Al extractable in 0.1 M BaCl₂ (w/v = 1:20 for 2 h) [29]. Total organic carbon (TOC) was determined spectrophotometrically after the oxidation of organic matter by K₂Cr₂O₇ [30]. Available contents of nutrients were determined by the Mehlich III soil extraction procedure [31] using flame atomic absorption spectroscopy (FAAS, VARIAN SpectrAA-300, Australia) (for Ca, K and Mg) and ICP-OES (for P). 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. This pretreatment represents the most common procedure used within soil analyses in the Czech republic. Alternatively, aliquots of the samples were extracted as moist samples (saturated to 100% of its maximal water holding capacity) immediately after the soil sample collection where a dry mass of the soils was determined separately. Finally, aliquots of the samples were frozen at -18 °C for 14 days and than air-dried, ground, and sieved. In this case, the effect of sample pretreatment was tested for 0.05 M EDTA, and water extracts.

2.1.2. Extraction procedures

The soil samples were extracted by the following extraction procedures:

1. extraction with 2 M solution of HNO_3 at a solid/liquid ratio 1/10 (3 g + 30 ml) at 20 °C for 6 h [32],
2. extraction with 0.43 M solution of CH_3COOH at a solid/liquid ratio 1/40 (1 g + 40 ml) for 5 h [33],
3. extraction with 0.05 M NH_4^- -EDTA solution at pH 7 at a solid/liquid ratio of 1/10 (3 g + 30 ml) for 1 h [34],
4. Mehlich III extraction procedure (0.2 M CH_3COOH + 0.25 M NH_4NO_3 + 0.013 mol l⁻¹ HNO_3 + 0.015 M NH_4F + 0.001 M EDTA at a solid/liquid ratio of 1/10 (3 g + 30 ml) for 10 min) [31],
5. extraction with deionized water at a solid/liquid ratio of 1/10 (3 g + 30 ml) overnight [34].

Each extraction was provided in three replications using a reciprocal shaker, 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 reaction mixture was centrifuged at 3000 rpm (i.e. 460 g) for 10 min and supernatants were kept at 6 °C before measurement. Blank extracts representing 5% of the total number of extracts were prepared using the same batch of reagents and the same apparatus analyzed at the same time and in the same way as soil extracts. Except for EDTA extraction procedure the level of pH of individual extractants was not adjusted.

The total concentration of arsenic in the soils was determined in the digests obtained by the following decomposition procedure: Aliquots (0.5 g) of air-dried soil samples were decomposed in a digestion vessel with a mixture of 8 ml concentrated nitric acid, 5 ml of hydrochloric acid, and 2 ml of concentrated hydrofluoric acid. The mixture was heated in an Ethos 1 (MLS GmbH, Germany) microwave assisted wet digestion system for 33 min at 210 °C. After cooling, the digest was quantitatively transferred into a 50 ml Teflon® vessel and evaporated to dryness at 160 °C. The digest was then dissolved in a 3 ml nitric and hydrochloric acid mixture (1 + 3), transferred into a 25 ml glass tube, filled up by deionized water, and kept at laboratory temperature until measurement. A certified reference material RM 7003 Silty Clay Loam containing 16.7 ± 3.1 mg As kg⁻¹ was applied for the quality assurance of analytical data and 18.1 ± 0.5 mg As kg⁻¹ was obtained. In the case of 2 mol l⁻¹ solution of HNO_3 this reference material contains 1.3 ± 0.19 mg As kg⁻¹ and 1.2 ± 0.1 was obtained.

2.1.3. Soil solution

Elemental concentrations in soil solution represent the plant-available portion of these elements. However, there are different approaches to soil solution collection [23]. We tested (i) centrifugation of fully saturated soil at 10,000 rpm for 10 min and (ii) the application of suction cups, where specialized nylon suction cups (DI Gottfried Wieshammer, Wien, Austria) were fixed into pots containing cca 350 g of the soil at the beginning of the experiment to get a soil solution. The pots in three replications with installed suction cups were watered with deionized water to full water capacity one day before suction and left for 24 h to equilibrate. The design of suction cup application and soil solution sampling was described in details by Jaklová Dyrtrtová et al. [35]. An aliquot, 10 ml of soil solution, was sampled from each pot and immediately analyzed for As concentrations. The collection of the soil solution was repeated twice (14 and 28 days after beginning of the experiment).

2.1.4. Analytical methods

The contents of As in soil digests and extracts were determined by optical emission spectroscopy with inductively coupled plasma (ICP-OES), and with axial plasma configuration, Varian, VistaPro, equipped with autosampler SPS-5 (Australia). Calibration solutions were prepared in corresponding extraction agents with concentrations of 100–1000 µg l⁻¹ As. The operating measurement

wavelength for ICP-OES was 188.9 nm. Measurement conditions were for all lines: power 1.2 kW, plasma flow 15.0 l min⁻¹, auxiliary flow 0.75 l min⁻¹, nebulizer flow 0.9 l min⁻¹. For the determination of low arsenic concentrations in soil solutions, water and 0.01 M CaCl_2 extracts hydride generation atomic absorption spectrometry (VARIAN AA280Z, Varian, Australia), equipped with continuous hydride generator VGA-77 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 [36].

2.2. Experiment 2

2.2.1. Pot experiment

Three soil samples differing in physical–chemical properties and total arsenic contents (Table 1) were selected for this experiment. *M. aquatica* L. was cultivated in 6 l plastic pots with 5 kg of air-dried soil. Four replicates were made for each treatment. The plants were watered with deionized water and the soil moisture was kept at 60% of its maximal water holding capacity (MWHC). 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 and analyzed for total arsenic content. In the middle of the vegetation period soil solution samples were collected using suction cups (see above). At the end of the vegetation period soil samples were taken from the bulk, then aliquots of the samples were air-dried at 20 °C, ground in a mortar and passed through a 2-mm plastic sieve, and aliquots were extracted as moist samples immediately after soil sample collection. These samples were used for determination of extractable concentrations of arsenic compounds. Because of the low uptake arsenic uptake by *M. aquatica* biomass [37] no significant changes in arsenic contents in experimental pots were expected.

2.2.2. Extraction procedures

For determination of arsenic compounds, aliquots of the dried and fresh soil samples were extracted with (i) 0.01 M aqueous CaCl_2 solution at a solid/liquid ratio of 1/10 (3 g + 30 ml) for 6 h [38], (ii) deionized water at a solid/liquid ratio of 1/10 (3 g + 30 ml) overnight [34], (iii) 0.05 M aqueous $(\text{NH}_4)_2\text{SO}_4$ solution at a solid/liquid ratio of 1/25 (1 g + 25 ml) for 4 h [20]. Each extraction was provided in three replications, 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 as mentioned above. For determination of arsenic compounds, the extracts were filtered through 0.22 µm cellulose-nitrate ester filters and aliquots of these solutions (40 µl) were chromatographed. Before measurement the solutions were stored in the dark.

2.2.3. 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 ($\text{O}_2 + \text{O}_3 + \text{NO}_x$) in an Apion Dry Mode Mineralizer (Tessek, CZ) at 400 °C for 10 h; the ash was then decomposed in a mixture of $\text{HNO}_3 + \text{HF}$, evaporated to dryness at 160 °C and dissolved in diluted *Aqua Regia* [39]. 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 12.5 ± 1.4 mg As kg⁻¹ was determined in this sample. The total arsenic concentrations in the soil digests and extracts were determined by hydride generation atomic absorption spectrometry (VARIAN SpectraAA-300, Varian, Australia), equipped with a continuous hydride generator VGA-76 where a mixture of potassium iodide and ascorbic acid was used for pre-reduction of the sample, then the extract was acidified by HCl before measurement and in

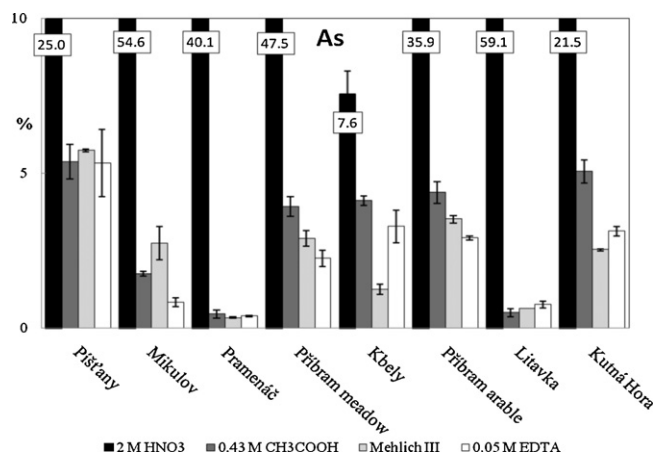


Fig. 1. Comparison of the set of strong extraction procedures (% of total element content in soil).

the case of high arsenic contents by ICP-OES with axial plasma configuration (Varian VistaPro, Varian, Australia) as mentioned above.

A Hewlett Packard 1100 solvent delivery unit (Germany) together with a Hamilton PRP-X100 (USA) anion-exchange column (250 mm × 4.1 mm i.d., spherical 10- μ m 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 NH₄H₂PO₄ 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 [40]. Behavior of individual extractants during chromatographic analysis as well as recovery of individual arsenic compounds was published elsewhere [41].

3. Results and discussion

3.1. Comparison of soil extraction procedures

As evident from Table 1, all in Experiment 1 are arsenic contaminated soils with different arsenic concentrations ranging from 37 to 437 mg As kg⁻¹. The highest total arsenic content was determined in the soil Litavka representing serious anthropogenic contamination. Fig. 1 summarizes the arsenic portions extractable by strong extraction procedures. HNO₃ (2 M) was able to extract arsenic portions ranging between 7.5 and 59% where higher extractability was observed for highly contaminated soils suggesting an anthropogenic origin of the soil pollution. 2 M solution of HNO₃ is able to dissolve the element portion comparable to the sum of labile soil element portion representing the element fractions associated with individual soil components [32] and arsenic is released only when iron oxides are dissolved by strong chemical extractant [15]. Soil amorphous aluminum and iron oxyhydroxides have the most significant effect on its ability to retain arsenic [42]. Low pH of this extractant can also be responsible for low extractability of arsenic compared to other elements such as cadmium and zinc [43].

The remaining extraction procedures (0.43 M solution of CH₃COOH, Mehlich III extraction procedure, and 0.05 M EDTA) showed comparable portions of extractable arsenic regardless of the experimental soil. In the case of the 0.05 M EDTA extraction procedure, a method widely used as an assessment of the wide scale of elements in soil, it seems to be questionable in the case of arsenic. Arsenic is present in soil mostly in anions, however the extractant based on chelating agent and developed for the release of cationic forms of elements is not useful in this case. The extractability of arsenic did not exceed 6% and was affected predominantly by

different physicochemical parameters of the soils. Evidently, the sandy soil Příšt'any (total As content 37.2 mg kg⁻¹) demonstrated the highest extractability in this case (between 5.3 and 5.7% of total arsenic content in the soil) and a higher release of arsenic from the silt and sand fractions was confirmed [44]. Contrary to that, the lowest extractability of arsenic was observed for the soils with the highest total As content (Pramenáč and Litavka with 254 and 437 mg kg⁻¹ of As, respectively) without an unambiguous effect of some of the soil characteristics. Generally, the arsenic mobility within the set of experimental soil was not related significantly to some of the individual soil characteristics but was affected by a whole complex of soil parameters [45]. The potential effect of soil iron oxyhydroxides can be discussed in this case because total iron content in the soils Pramenáč and Litavka was 2.7 and 2.2%, respectively, whereas in all the remaining soils the total iron content did not exceed 1.9% (Table 1). The effect of Fe-bearing additives in various rates on potential immobilization of arsenic in soil was frequently investigated and confirmed [46,47] indicating that a relatively small addition of Fe-oxides can significantly affect the As mobility in soil.

3.2. The effect of soil sample pretreatment

In the case of arsenic portions extracted with 0.05 M EDTA the effect of sample pretreatment was tested (Fig. 2) and no significant differences were obtained between air-dried, frozen and subsequently air-dried samples, and fresh samples. Evidently, the relatively strong extractant is able to overlap the potential effect of slightly changing physicochemical parameters of the soils due to different pretreatments of the soil samples. Moreover, this extractant is not optimal for the evaluation of the mobile portions of arsenic in soil as discussed above.

Single soil extraction procedures were recently evaluated by Menzies et al. [12]. They confirmed the suitability of neutral salt extractants (0.01 M CaCl₂, 0.1 M NaNO₃) for the assessment of the available pool of elements in soil. According to previous experiments most of the mild extracting procedures including pure water were developed predominantly for a wide range of elements, especially heavy metals [34]. The water extractable arsenic portions varied from 0.4 to 1.8% of total arsenic (Fig. 3). Similarly as in the case 0.43 M solution of CH₃COOH, Mehlich III extraction procedure, and 0.05 M EDTA extraction procedure, the sandy soil Příšt'any demonstrated the highest extractability of arsenic whereas the lowest extractability of arsenic was observed for the soils Litavka and Pramenáč. However, the effect of sample pretreatment was evident in this case (Fig. 3). In most cases the fresh samples tended to

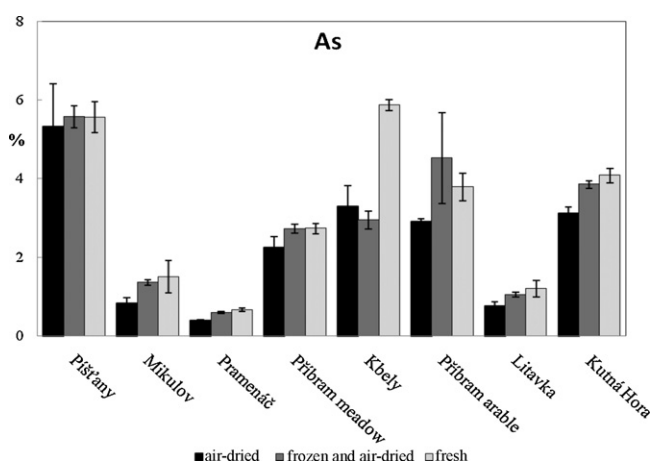


Fig. 2. The effect of sample pretreatment on the 0.05 M EDTA extractable portion of arsenic in soils (% of total element content in soil).

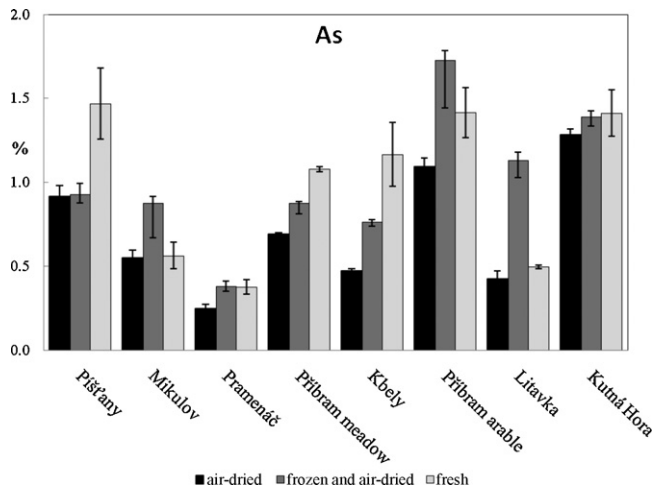


Fig. 3. The effect of sample pretreatment on the water extractable portion of arsenic in soils (% of total element content in soil).

have better extractability of arsenic compared to the air-dried ones. The effect of air-drying or sample storage on the mobility of various elements was already described. For rare earth elements (La, Ce, Pr, Nd) the air-drying process increased the element contents in water soluble, exchangeable, carbonate bound, and Fe–Mn oxide bound fractions whereas the fraction bound on organic matter decreased [48]. Air-drying leads to a decrease in Cu, Ni, and Zn concentrations in soil solution as well as a decrease in soluble organic carbon [21]. Compared to these elements the behavior of arsenic seems to be substantially affected by soil redox potential [49] where the presence of more mobile As(III) is expected in the 100% saturated soil sample. Reynolds et al. [50] observed low sorption of arsenic on soil solids under anaerobic conditions whereas during aeration As was retained on freshly precipitated Fe (hydro)oxides. The effect of sample pretreatment on arsenic extractability was observed also in plant samples [51]. In this case potential changes in the abundance of arsenic compounds in the extracts in the freeze-dried samples compared to fresh ones are discussed. For assessment of the differences between frozen and subsequently dried soil and other tested variants (air-dried and fresh samples) the possible effect of soil microflora and/or presence of individual arsenic compounds should be taken into account. Freezing of the sample can result in a possible release of arsenic from soil microbial populations although subsequent immobilization of released arsenic on soil particles can occur, as well. The determination of arsenic in soil solution samples (Fig. 4)

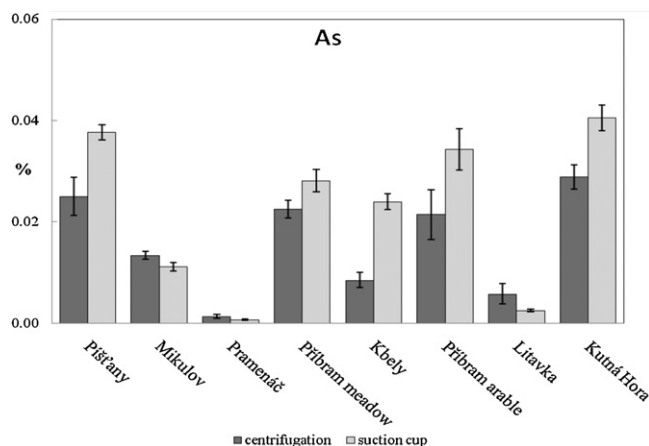


Fig. 4. Comparison of the methods of soil solution sampling (% of total element content in soil).

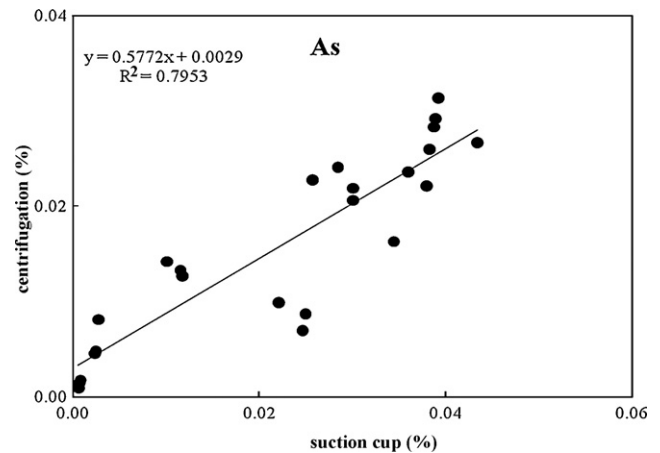


Fig. 5. Linear correlation between element portions (% of total element content in soil) obtained by individual methods of soil solution sampling.

showed similar trends in arsenic mobility as in the case of water extractable arsenic. However, the mobile arsenic portions released by the water extraction of the sample are significantly higher compared to the soil solution sampling methods most probably due to intensive shaking of the extracted sample. In contrast to findings summarized by Nolan et al. [23] suction cups showed higher As concentrations compared to the centrifugation of saturated soil.

Wang et al. [48] and Gray and McLaren [21] recommended applying the extraction and/or soil solution sampling of field-moist soil samples reflecting more closely *in situ* conditions for better correlations with plant-available element portions. As documented by our results, different sample pretreatment and/or different mild soil extraction procedures can lead to different absolute values of mobile arsenic content in soils. However, the interpretation of the data can lead to similar conclusions comparing the individual soils as apparent from the comparison of the soil solution sampling methods where fairly good correlation was observed (Fig. 5).

3.3. The effect of soil pretreatment and/or extraction method on mobile arsenic compounds

For an evaluation of possible plant-availability of arsenic, the presence of individual arsenic compounds within a mobile pool of arsenic should be determined. In our previous work [40] three mild extraction agents for the determination of plant-available portions of elements in soil were evaluated for arsenic speciation in soil samples. These samples were amended by different arsenic compounds (As(III), As(V), DMA, MA). Deionized water, 0.01 M CaCl₂, and 0.05 M (NH₄)₂SO₄ were used for the extraction of the plant-available portion of the arsenic compounds in air-dried soil samples. Air-drying of the samples was chosen to allow possible comparison of soils under uniform conditions where uniform sample pretreatment is applied [52]. Arsenate was the dominant compound followed by small portions of arsenite, methylarsonic acid, and dimethylarsinic acid depending on the individual soil treatments. In all the experiments where methylarsonic acid was added to the soil, methylarsonous acid was detected in the extracts suggesting that the soil bacteria are capable of reducing methylarsonic acid. No significant differences were observed in the interpretation of analytical data obtained by using different extracting procedures. In this experiment, soils contaminated by former mining and smelting activities differing in both total arsenic content and main physicochemical parameters (Table 1) were investigated. According to individual extracting agents used, the arsenic portions extractable by individual extracting agents represented 0.063–0.187% for 0.01 M CaCl₂, 0.273–1.11% for 0.05 M

Table 2

The contents of arsenic compounds extractable with individual extractants—Experiment 2, samples collected from the pots before sowing; data are presented as mean \pm standard deviation.

Soil location	Extractant	As(III) (mg kg ⁻¹)	DMA (mg kg ⁻¹)	As(V) (mg kg ⁻¹)	Sum (mg kg ⁻¹)	% of total
Kutná Hora arable	Water	0.005 \pm 0.001	0.004 \pm 0.000	1.03 \pm 0.033	1.04 \pm 0.032	0.220
Kutná Hora meadow	Water	0.039 \pm 0.003	0.025 \pm 0.002	0.723 \pm 0.032	0.787 \pm 0.036	0.055
Příbram	Water	0.001 \pm 0.000	<0.003	0.043 \pm 0.002	0.044 \pm 0.002	0.123
Kutná Hora arable	(NH ₄) ₂ SO ₄	0.021 \pm 0.005	0.038 \pm 0.002	4.20 \pm 0.073	4.26 \pm 0.073	0.900
Kutná Hora meadow	(NH ₄) ₂ SO ₄	0.105 \pm 0.006	0.064 \pm 0.005	3.74 \pm 0.102	3.90 \pm 0.094	0.273
Příbram	(NH ₄) ₂ SO ₄	0.039 \pm 0.008	<0.003	0.360 \pm 0.009	0.400 \pm 0.017	1.11
Kutná Hora arable	CaCl ₂	0.006 \pm 0.002	0.003 \pm 0.001	0.291 \pm 0.025	0.299 \pm 0.024	0.063
Kutná Hora meadow	CaCl ₂	0.047 \pm 0.008	0.020 \pm 0.001	0.369 \pm 0.019	0.436 \pm 0.009	0.031
Příbram	CaCl ₂	0.017 \pm 0.002	<0.003	0.050 \pm 0.004	0.067 \pm 0.004	0.187

Table 3

The contents of arsenic compounds extractable with 0.05 mol l⁻¹ (NH₄)₂SO₄ as affected by sample pretreatment—Experiment 2, samples collected from the pots at the end of the experiment; data are presented as mean \pm standard deviation.

Soil location	Pretreatment	As(III) (mg kg ⁻¹)	DMA (mg kg ⁻¹)	MA (mg kg ⁻¹)	As(V) (mg kg ⁻¹)	Sum (mg kg ⁻¹)	% of total
Kutná Hora arable	Fresh	0.117 \pm 0.006	<0.003	<0.001	2.84 \pm 0.65	3.55 \pm 0.83	0.751
Kutná Hora meadow	Fresh	0.159 \pm 0.004	0.034 \pm 0.007	<0.001	1.16 \pm 0.26	1.62 \pm 0.29	0.113
Příbram	Fresh	0.023 \pm 0.003	<0.003	<0.001	0.290 \pm 0.059	0.376 \pm 0.070	1.04
Kutná Hora arable	Air-dried	0.207 \pm 0.010	0.042 \pm 0.005	<0.001	4.02 \pm 1.28	5.00 \pm 1.66	1.06
Kutná Hora meadow	Air-dried	0.082 \pm 0.012	0.049 \pm 0.001	0.017 \pm 0.002	4.05 \pm 0.55	5.03 \pm 0.66	0.352
Příbram	Air-dried	0.047 \pm 0.006	<0.003	<0.001	0.287 \pm 0.056	0.401 \pm 0.064	1.12

(NH₄)₂SO₄ and 0.055–0.220% for deionized water, respectively (Table 2). Therefore, the effectivity of the extraction procedure decreased in order 0.05 M (NH₄)₂SO₄ > water > 0.01 M CaCl₂ regardless of the analyzed soil. The abundance of arsenic compounds in the extracts was expected for oxidizing soil conditions [49] where As(V) was the dominant arsenic compound with a small percentage of As(III). Small amounts of DMA were already present in the extracts of highly contaminated soil whereas in the less contaminated soil Příbram, this compound was not detected.

A slightly different behavior of arsenic compounds was observed in extracts of soil samples collected from pot experiment at the end of the vegetation period of *M. aquatica* (Table 3). The total extractable arsenic contents in the extracts slightly increased in the air-dried samples at the end of vegetation. Primarily As(III) and DMA portions increased and small amounts of MA occurred in the most contaminated soil Kutná Hora meadow. The role of root exudates and predominant soil microbial activity can be discussed in this case [28]. Moreover, the effect of sample pretreatment was evident in this case where slightly lower extractability of arsenic from fresh soil samples (60% of its MWHC) was reported even when

compared to the samples extracted before the start the experiment. Among the individual arsenic compounds the As(III) portion increased in both the Kutná Hora contaminated soils. Compared to Experiment 1 where the fresh samples extracted were fully saturated, the redox conditions in this case were different resulting in different behavior of mobile As. The effect of soil microbial activity can be speculated as indicated by our previous results [37]. Macur et al. [27] stated that both arsenic oxidizing and reducing bacteria coexist in soil environments, and the relative abundance and metabolic activity of specific microbial populations plays an important role in the speciation of As in soil solution. The concentrations of arsenic compounds in soil solutions (sampled using suction cups) from the pots in the middle of the vegetation period seemed to support this theory. The As(V) concentrations in soil solutions sampled from individual pots varied between 205 and 398 $\mu\text{g l}^{-1}$ in the soil Kutná Hora arable, between 530 and 805 $\mu\text{g l}^{-1}$ in the soil Kutná Hora meadow, and between 15 and 40 $\mu\text{g l}^{-1}$ in the soil Příbram, respectively. With respect to As(III) concentrations the values varied between 0.5 and 5.6 $\mu\text{g l}^{-1}$ in the soil Kutná Hora arable, between 6.0 and 14.6 $\mu\text{g l}^{-1}$ in the soil Kutná Hora meadow, and between 0.6 and 3.8 $\mu\text{g l}^{-1}$ in the soil Příbram, respectively. DMA was detected only in the soil Kutná Hora arable and varied between 1.0 and 3.2 $\mu\text{g l}^{-1}$. In principle the abundance of individual arsenic compounds reflected these in soil extracts (Tables 2 and 3) but the data were quite varied and the assessment of the data is limited. Stability of arsenic compounds as well as macronutrient cations, anions and soluble organic carbon concentrations in soil solution should be discussed in this context [21,53]. Fig. 6 documents conversion of analytical standard containing 10 $\mu\text{g l}^{-1}$ of As(III), DMA, MA, and As(V) at room temperature over a 13 h period. Ambiguous results from a number of researchers concerning instability of individual arsenic compounds were reviewed by Francesconi and Kuehnelt [24]. The effect of sample matrix, sample preparation, storage, microbial activity, etc. can be taken into account. McCleskey et al. [54] summarized possible methods of the preservation of water samples for arsenic III/V determinations. They summarized that the samples should be filtered, preserved with HCl, H₂SO₄, or EDTA to stabilize Fe, and stored in the dark. Other possibilities to stabilize As(III), and As(V) in the soil solutions can be phosphoric acid used alone and with the addition of reducing

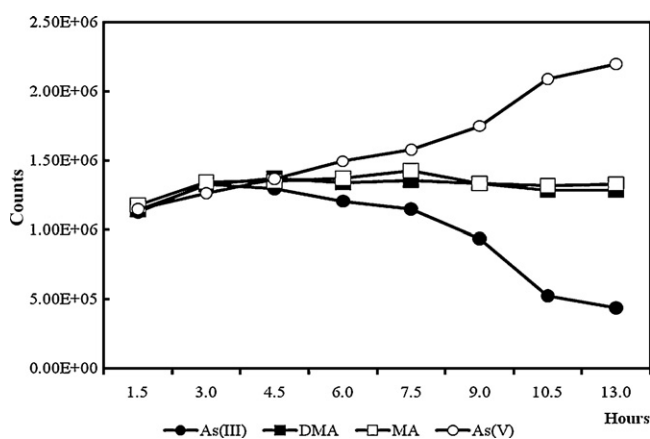


Fig. 6. The stability of the intensity of the ICPMS signal of the aqueous solutions (10 $\mu\text{g l}^{-1}$ of each compound) of individual arsenic compounds (solutions kept at laboratory room temperature).

agents (ascorbic acid and hydroxylammonium hydrochloride) [53]. In our case, the solutions were filtered (0.45 µm cellulose-nitrate ester filters) and stored in the dark but chemical stabilization was not applied because of possible interferences during HPLC-ICPMS determination of arsenic compounds. These measures will be tested in further research to stabilize and improve the quality of the analytical data including the optimization of analytical procedures.

We can conclude that the application of different sample pretreatments and/or different soil extraction procedures can lead, in the case of the determination of individual arsenic compounds, to serious variation of the results especially in the case of inorganic arsenic species. Therefore, more attention must be given to the soil extract/solution preparation and its adequate preservation before measurement. For more detailed assessment of bioavailability of arsenic compounds in soil and especially soil solution the investigation of arsenic behavior in actual conditions (i.e. preferably in fresh soils and/or in soil solutions) will be necessary. The findings can be summarized as follows: (i) Within the investigated set of soil samples characterized by wide range of soil properties no unambiguous effect of these properties on arsenic mobility was observed. Arsenic contamination level as well as source of contamination (anthropogenic versus geogenic) is also not the parameter determining the arsenic mobility in individual soils. (ii) Although individual sample pretreatments showed different absolute results the data correlated fairly good ($r=0.79$). However, the extraction of fresh samples can be recommended to catch up the real soil conditions. (iii) Determination of arsenic compounds in mild soil extracts and soil solution require chemical stabilization to preserve especially the ratio of inorganic arsenic compounds.

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