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Arsenic accumulation and speciation in plants from different habitats

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Abstract

Understanding As accumulation in plants is necessary in order to alleviate problems with As in the environment and to improve sustainable As phytotechnologies. To find suitable candidates for phytoremediation purposes and to investigate specific accumulation patterns due to growth habitat and plant groups, As accumulation in 124 plant species collected from different habitats and speciation in 6 of these plant species, was determined. The data show that submerged plants have a higher accumulation than emergent and terrestrial plants. The As concentration in terrestrial and emergent plants were correlated with the $[As]_{soil}$, while the accumulation factor correlated negatively with $[As]_{soil.}$ Gymnosperms had a high $[As]_{shoot}$:$[As]_{root}$ ratio. The inorganic As species, arsenate and arsenite were found in plants from all habitats and methylarsonic acid (MMA) in all but one plant species. Arsenate predominated in submerged plants. The results suggest that the habitat and the $[As]_{soil}$ have a strong influence on the As accumulation in plants and that submerged plants and/or gymnosperms might be suitable for phytoremediation of As.

1. Introduction

Arsenic pollution is a serious problem around the world. Accumulation of As by plants in areas with elevated levels of As could pose a risk of As transfer to human beings and grazing animals. Also, millions of people are at risk of exposure to As-enriched drinking water, especially in areas in SE Asia like Bangladesh and West Bengal, but all continents have areas with elevated levels of As in the ground water (Nordstrom, 2002).

Accumulation of As in plants varies between plant species and habitats. The uptake of As from soil by terrestrial plants is usually low. Soils normally have a concentration below 10 mg As kg^{-1} (Fitz and Wenzel, 2002) and $[As]_{plants}$ usually ranges between 0.009-1.7 mg As kg^{-1} (Pais and Jones, 2000). However, where $[As]_{soil}$ is elevated, for example in mining areas, $[As]_{plant}$ is higher compared with plants in reference areas (Rodushkin, 1999). Most terrestrial plants have a low $[As]_{shoot}$:$[As]_{root}$ ratio (Sneller et al., 1999). Terrestrial ferns belonging to the family Pteridaceae show a particularly high uptake of As and a high $[As]_{shoot}$:$[As]_{root}$ ratio. So far 12 As-hyperaccumulating species of ferns have been discovered (Zhao et al., 2009). Trees and shrubs show low As accumulation (Craw et al., 2007). However, among gymnosperms, *Pseudotsuga menziesie* shows a relatively high As accumulation in the stem and needles (Haug et al. 2004). The As concentration is low in crops like vegetables, spices and cereals (Signes-Pastor et al., 2008). However, rice grains may contain elevated levels of As which could

lead to a high As intake in human beings from high rice consumption (Williams et al., 2007).

Phytoextraction is a method to remove a contaminant from the soil/sediment into the harvestable part of a plant and phytostabilization involves long term stabilisation of a contaminant using plants (Ward and Singh, 2004). Trees and shrubs are of interest for phytoextraction due to the relatively high biomass production and easily harvestable plant parts. Emergent plant species like *Eriophorum angustifolium* may contain higher levels of As in the roots than that recorded in the surrounding soil (Stoltz and Greger, 2002). Submerged plants may have high As accumulation, for example *Callitriche stagnalis* and *Myriophyllum propinquum* in New Zealand with concentrations exceeding 1000 mg kg^{-1} (DW) (Robinson et al. 2006). Uptake of As in submerged plants can occur either in roots or in shoots or in both (Wolterbeek and van der Meer, 2002).

For a range of organisms the inorganic As species arsenate and arsenite are generally considered to be more toxic than organic species (Meharg and Hartley-Whitaker, 2002). The solubility and speciation of As are influenced by several factors of which the most important ones are the redox potential and pH. Arsenate predominates at a high redox potential, while arsenite predominates at a low redox potential (Sadiq, 1997). Due to pH induced changes in the solubility of ferrihydrite and oxides/hydroxides of Fe, Al and Mn, the solubility of arsenate increases with increasing pH, while the solubility of arsenite increases with decreasing pH (Raven et al. 1998; Sadiq, 1997).

The root to shoot translocation of As is carried out in the vascular system either as arsenite, arsenate, or as arsenite-phytochelatin complexes (Tripathi et al., 2007). For most plant species, the As species that dominate in the roots usually also dominate in the shoots (Zheng et al., 2003). Localization of As in the plant tissue differs between individual plants depending on the external conditions and the plant species. Tripathi et al. (2007) have produced an overview of the basic steps of cellular As uptake and detoxification in plant-root cells. The basic steps include the reduction of arsenate to arsenite by gluthatione, complexation of arsenite with phytochelatins and sequestration of the complex into the vacuols of the root cells (Tripathi et al. 2007). The binding of As to the thiol-containing peptides could function as a detoxification mechanism in the plant cell (Sneller et al., 1999). The level of cationic As is generally so low that the addition to the total amount of As inside the plant is negligible (Zheng and Hintelmann, 2009).

The aim of the present study was to investigate the influence of plant habitats on the accumulation and speciation of As in individual plants and groups like terrestrial, emergent and submerged plants. In addition, the plant accumulation and speciation of As was needed to identify plant species, which could serve as suitable candidates for As phytoextraction and/or phytostabilization in temperate regions. Ferns and trees/shrubs, in particular, were investigated with regard to As accumulation. The hypothesis was that the external conditions, for example the redox potential, in the various habitats and the individual plant species could influence As accumulation and speciation in plants. To

the authors' knowledge, this is the first time that habitats have been investigated on a large scale to understand the patterns of As accumulation in plants.

2. Materials and methods

2.1. Field material

Submerged, emergent and terrestrial plants were collected as 3 replicates at 6 different localities in Sweden and 4 different localities in Slovakia with $As_{[soil]}$ levels >2 mg kg^{-1} (DW). In total 124 plant species were collected. The Swedish localities were two Zn mine sites (Boliden [N 64º 52.18', E 020º 21.15'] and Kristineberg [N 65º 04.54', E 018º 35.53']), two abandoned wood impregnation sites (Katrineholm [N 59º 00.02', E 016º 09.43'] and Sjösa [N 58º 46.88', E 017º 05.78']) and two sites with alum shale soil (Kinne-Kleva [N 58º 34.33' , E 013º 26.19'] and Öland [N 56º 25.37', E 016º 25.86']). The Slovakian localities were 4 Sb mine sites [Dubrava [N 48°58.19´, E 19°30.24´], Medzibrod [N 48°49.33´, E 19°20.62´], Pezinok [N 48°19.08´,E 17°14.15´] and Poporc [N 48°43.25´, E 20°58.54´]). Sampling was carried out in July and August 2007 and May 2009 for plants analysed for total As concentration, and in July and August 2008 for As speciation. The soil was collected in connection with plant roots. Plants were thoroughly washed in de-ionized H_2O for approximately 5 mins, until all visible soil was removed, and divided into above soil/sediment and below soil/sediment parts. Crop plants were separated into edible and non-edible plant parts and only the edible plant parts were analysed.

2.2. Total arsenic analysis

Plants and soil destined for analysis of total As concentration were dried at 80°C for 48h. A complementary drying at 105°C for 24h was also performed to get the correct dry-weights. The dried plant material was wet digested in HNO_3:$HClO_4$ (7:3, V/V). Soil was sieved to <2mm and wet digested in 7 M HNO_3.

A hydride generation technique (VGA-77) using atomic absorption spectrophotometry (Varian SpectAA 55B) was performed to determine the total concentration of As in the wet digested plant and soil materials. The detection limit for analysis was 7 µg As L^{-1}. Three replicates of plant material were analysed and the soil was analysed as one pooled sample from 3 replicates. *Phalaris* (NJV 94-4, Analytical Standards, Sweden) were used as reference material. For hydride generation Na borohydride (3 %; Merck), NaOH (2.5%; EKA Chemicals) and HCl (6M; VWR International) were used. Standards were added to the samples to eliminate interaction effects of the matrix.

2.3 Arsenic species analysis

Six plant species were selected for As species analysis, two submerged, two emergent and two terrestrial plant species. Plants were prepared by a modified extraction protocol originally developed by Mir et al. (2007). The basic idea, as presented by those authors, is a sequential extraction of As, first with MeOH to maximize extraction of organic As species, followed by HCl to maximize the extraction

of inorganic As species. The collected plant material was thoroughly washed in de-ionized H_2O for approximately 5 min and dried at room temperature. Of the dried plant material, 0.5 g was placed in 10 mL MeOH:H_2O(1:1)-solution in a 15 mL Falcon tube and ultra-mixed (Polytron, model no. PT2000, Kinematica AG) at maximum speed for approximately 10 s in the first extraction round, followed by heavy shaking for 5 min. The tube was then put in a sonicator for 20 min followed by centrifugation at 1700 x *g* for 10 min. The supernatant was transferred into a 50 mL Falcon tube. Again 10 mL of MeOH:H_2O(1:1) was added to the 15 mL Falcon tube, the pellet was re-suspended in the liquid and the extraction procedure was repeated. In total, this extraction procedure was performed 3 times rendering 30 mL MeOH:H_2O(1:1)-solution. The same procedure was then repeated 3 additional times with the same pellet with 0.1 M HCl rendering 30 mL of HCl-solution. Extracted MeOH:H_2O(1:1)-solution was reduced to a dry pellet at 60°C and re-suspended in de-ionized H_2O to 3 mL, while the HCl fraction was left unaltered.

A high-pressure liquid chromatograph (HPLC) coupled to an atomic absorption spectrophotometer (AAS) was used to determine the amount of As species in the samples. Before injection of a 100 µL sample into the HPLC, the samples were filtered through a 0.22 µm filter. The As species were separated using a HPLC with a Hamilton PRP X-100 (250mm x 4.6 mm) anion exchange column. Ammonium-phosphate buffer (pH 5.8) with a flow rate of 1 mL min^{-1} was used as the eluent in the HPLC. For peak detection, an atomic absorption spectrophotometer (Varian SpectAA 55B) hydride generation technique (VGA-77) was used. Quantifications of As in the peaks were performed by external calibration using standard solutions for arsenite, arsenate, methylarsonic acid (MMA) and dimethylarsinic acid (DMA). Chemicals used were sodium-meta-arsenite (Merck) for arsenite, sodium arsenate dibasic heptahydrate (Sigma-Aldrich) for arsenate, sodium methylarsonate (Sigma-Aldrich) for MMA and cacodylic acid (Sigma Aldrich) for DMA. Varian software (SpectrAA Worksheet Oriented AA Software, Version 5.1) was used to detect peaks (Fig. 1).

2.4 Calculations and statistical analysis

Calculations of accumulation factor (AF) and the shoot to root-ratio (S/R) were performed using the following formulae:

$$AF_{root} = [As]_{root} / [As]_{soil} \quad (1)$$

$$AF_{shoot} = [As]_{shoot} / [As]_{soil} \quad (2)$$

$$S/R = [As]_{shoot} / [As]_{root} \quad (3)$$

Statistical analysis was performed with statistical software R. Comparisons between mean values of AF, S/R and As species were made using the Wilcoxon signed rank test for non-normally distributed data and ANOVA for normally distributed data. For significance, p-values < 0.05 were used. The correlation between the $[As]_{plant}$ and the $[As]_{soil}$ was calculated using a Spearman's rank correlation test.

Calculation of the As-species peak area (A) was performed in Microsoft Office Excel 2007 using the formula presented by Burriel-Marti et al. (1968):

$A = (1/0.9387) * t_{50\%} * h_0$

where $t_{50\%}$ is the width of the peak in seconds at 50 % of the height and h_0 is the height of the peak.

3. Results

The As concentration was much higher in the Slovakian sites (45-100000 mg kg^{-1} DW) compared with the Swedish sites (2-2400 mg kg^{-1} DW). This magnitude in difference between countries i.e. up to 400 times, does not match the difference in magnitude found in plants of up to 25 times (Table 1). This is also clearly shown in the comparison with *Taraxacum* sp. from the two countries where the highest accumulation factor is found in the Swedish case where the lowest As concentration was found (Table 2). The accumulation factor decreases with increasing soil As concentration for both roots and shoot and for all plant types ($p<0.05$ for terrestrial and emergent plant root and shoot; not shown),

The concentrations in submerged, emergent and terrestrial plants ranged from 4 - 33, 0.2 - 642 and 0 - 49 mg kg^{-1} (DW) in shoots and 65 - 273, 5 - 1623 and 0 - 377 mg kg^{-1} (DW) in roots, respectively (Table 3). The $[As]_{soil}$ was correlated with the $[As]_{plant}$, both in the shoots and in the roots of emergent and terrestrial plants ($p<0.05$) (Fig. 2). However, no such correlation was shown for submerged plants.

Submerged plants had a higher $[As]_{root}$ and $[As]_{shoot}$ ratio than the $[As]_{sediment,}$ i.e. an accumulation factor (AF) above one for both roots and shoots (Fig. 3). The AF for submerged plants was higher than the AF for both emergent and terrestrial plants ($p<0.05$) (Fig. 3). None of the emergent and terrestrial plants had an AF above one in the shoots (Table 3). All submerged plants analysed had a root AF above one (Table 3). Two of the emergent species (*R. tomentosum, V. beccabunga*) and 5 of the terrestrial species (*A. vulneraria, L. pilosa, M. pratense, V. myrtillus, P. sylvestris*) also had a root AF above one. Moreover, the gymnosperms *Picea abies* and *Pinus sylvestris* showed a relatively high AF in the shoots, 0.34 and 0.63, respectively.

The inorganic forms of As, arsenite and arsenate, predominated in all plant tissues analysed (Table 4; Fig. 4). Organic As in the form of MMA was found in all analysed plant species except for *Sparganium sp*. The predominant As species in submerged plants was arsenate ($p<0.05$) while arsenite seems to the predominant As species in the shoots of terrestrial plants (not statistically significant) (Fig. 4). A somewhat higher MMA content in terrestrial plants than in submerged and emergent plants was observed (Fig 4). In the emergent plants there was a tendency for arsenate to be the predominant As species.

The As in plants was mainly distributed in the roots (Fig. 5). Terrestrial plants had a higher $[As]_{shoot}$:$[As]_{root}$ ratio than emergent plants ($p<0.05$) and showed a tendency to higher $[As]_{shoot}$:$[As]_{root}$ ratios than submerged plants (Fig. 5). High $[As]_{shoot}$ to $[As]_{root}$ ratios of As were found in a few terrestrial plant species (*Arabis arenosa, Empetrum nigrum, Picea abies, Pinus sylvestris, Taraxacum sp.*) (Table 3). Also, in 75 % of the analysed trees, the $[As]_{shoot}$: $[As]_{root}$ ratio was above one (*Picea abies, Pinus sylvestris, Sorbus aucuparia*) (Table 3). Of the individual As species, arsenite had a relatively high $[As]_{shoot}$:$[As]_{root}$ ratio in both emergent and terrestrial plants (Table 5). The

$[As]_{shoot}$:$[As]_{root}$ for the separate As species was similar between the individual terrestrial and emergent plant species, i.e. arsenite had the highest $[As]_{shoot}$:$[As]_{root}$. For submerged plants, arsenite had the highest $[As]_{shoot}$:$[As]_{root}$ in *Sparganium natans*, but for *Sparganium sp.* arsenate had the highest $[As]_{shoot}$:$[As]_{root}$ (Table 5). In *Empetrum nigrum*, MMA also had a relatively high $[As]_{shoot}$:$[As]_{root}$ ratio (Table 5).

Crops, trees and shrubs all had a relatively low As concentrations in shoots, < 6 mg As kg^{-1} (DW) (Table 3). However, among crops, oats (*Avena sativa*) and alfalfa (*Medicago sativa*) had a concentration of 0.29 and 0.37 mg As kg^{-1} (DW), respectively, in the edible parts. Ferns had a low accumulation of As in the shoots and roots (<0.2 and <2 mg As kg^{-1} (DW) respectively) (Table 3).

4. Discussion

The accumulation of As in plants is strongly influenced by the plant´s habitat, especially in submerged habitats where plants have a higher accumulation factor (AF) than emergent and terrestrial plants (Fig. 3). The influence of the habitat on the As accumulation in plants was stronger than any plant relationship, for example the submerged *Ranunulus flammula* had a higher AF compared to the emergent *Aconitum napellus* and *Caltha palustris*, all members of the family Ranunculaceae (Table 3). No differences in As accumulation were detected within the submerged, emergent and terrestrial habitats between genus's, families, monocotyledons-eudicotyledons or phanerogams-cryptogams.

The accumulation of As by emergent and terrestrial plants was mainly determined by the $[As]_{soil}$ as shown by the correlation between $[As]_{plant}$ and the $[As]_{soil}$ (Fig 2). However, the accumulation factor decreased with increasing As concentration in soil (Table 2; not shown). Accumulation of As also depends on the plant species, for example the higher As accumulation by *Equisetum fluviatile* than by *Juncus filiformis* living in similar conditions (Table 3). The difference in As accumulation could depend on a different regulation of the high-affinity phosphate transporters or the aquaglyceroporins, which are involved in the cellular uptake of arsenate and arsenite (Meharg and Macnair, 1992; Meharg and Jardine, 2003), or different regulation of As in the cell walls.

Arsenic accumulation by the submerged leaves, in addition to the As uptake via roots, could result in the higher AF of submerged plants. Also physicochemical adsorption of Fe oxides which co-precipitate with As on submerged plant surfaces could result in a higher AF in submerged plants (Robinson et al., 2006). These authors found a correlation between As and Fe contents in aquatic macrophytes. Alternatively, differences in the As speciation in the submerged habitat could result in the higher AF of submerged plants. Arsenate is the predominating species in conditions with high redox potential, while arsenite is the predominating species in conditions with low redox potential, like wetland sediments (Sadiq, 1997). Previous experiments have shown that plants accumulate higher amounts of As when exposed to arsenite compared with arsenate in submersed plants (Srivastava et al. 2007).

External As species could influence the As species within the plants. The presumption for the analysis of As species in plants was that arsenate would be the dominating species in the terrestrial habitats and arsenite the dominating As species in the submerged habitats. However, the As speciation analysis showed a trend of arsenate as the predominating As species in submerged plants and arsenite in terrestrial plants (Fig. 4), i.e. an opposite trend to the expected results. These results indicate differences between submerged and terrestrial plants regarding the internal modifications of As, possibly due to different binding of As to phytochelatins (Tripathi et al., 2007) or modifications in the cell walls.

The relative abundance of the different As species was similar between roots and shoots of the plants analysed (Fig. 4). These results indicate that similar plant processes occur throughout the plant body in response to As, or that no internal changes of the As species occur within the plants. Methylarsonic acid (MMA) was found in 5 out of 6 plant species analysed (Table 4). Dimethylarsinic acid (DMA) was not detected in any samples (Table 4). Plants can methylate inorganic As (Raab et al. 2007), but it is not clear whether the MMA in this study originates from the internal metabolism of the plants or from external sources, for example microbial activity.

The higher $[As]_{shoot}$:$[As]_{root}$ ratio of terrestrial plants (Fig. 5) is mainly due to the high $[As]_{shoot}$:$[As]_{root}$ ratios of *Arabis arenosa*, *Empetrum nigrum*, *Picea abies*, *Pinus sylvestris* and *Taraxacum sp.* (Table 3). Interestingly, all these plant species were collected at the same location, in an area called the Skellefteå field in Sweden. Elevated levels of As (>15 mg kg^{-1}) are naturally occurring in this area. Plants from this area may show local adaptations to persistently high levels of internal As in the shoots. A higher amount of arsenite could also promote a higher $[As]_{shoot}$:$[As]_{root}$ ratio in plants. The relative amount of arsenite and MMA compared with arsenate is high in *Empetrum nigrum* (Fig 4). The $[As]_{shoot}$:$[As]_{root}$ ratio of the individual As species indicates that a higher portion of arsenite and MMA is distributed in the shoots in *Empetrum nigrum* compared with the other plant species analysed, but the results are not statistically significant (Table 5). Submerged and emergent plant species analysed all had low $[As]_{shoot}$:$[As]_{root}$ ratios (Table 5). A higher amount of internal arsenite could promote a higher $[As]_{shoot}$:$[As]_{root}$ ratio in *Empetrum nigrum*, possibly due to the chelation of arsenite by phytochelatins (Tripathi et al. 2007). Addition of dimercaptosuccinate, which has similar binding abilities to arsenite as phytochelatins, to hydroponic cultures containing Indian mustard (*Brassica juncea*), increased the $[As]_{shoot}$:$[As]_{root}$ ratio 5 times (Pickering et al. 2000). Further studies investigating plant species with a high $[As]_{shoot}$:$[As]_{root}$ ratios could prove interesting, for example *Arabis arenosa*, a close relative to the well-studied *Arabidopsis thaliana*.

Trees, shrubs and crops had low $[As]_{shoot}$ and $[As]_{root}$ ratios (Table 3). However, the crops alfalfa (*Medicago sativa*) and oats (*Avena sativa*) had a concentration of 0.37 and 0.29 mg As kg DW^{-1} in the edible parts, respectively. A daily consumption of 34 g (DW) of oats (Table 3) is equal to 10 µg of As. This quantity of oats is approximately the amount needed for one portion of oatmeal or muesli. Consumption of oats from this study could, therefore, result in ingestion of elevated levels of As.

Ferns did not show any great accumulating properties of As (Table 3). In a screening study of 45 fern species regarding As accumulation, only members of the genus *Pteris* were shown to accumulate high concentrations of As indicating that high As accumulation is not a general fern characteristic (Meharg, 2003).

The observed characteristics of submerged plants, for example high AF, suggest that these plants would be suitable for phytoremediation of As in temperate regions. Phytofiltration using submerged plants to clean water may benefit by putting areas of interest under water, or to lead groundwater from contaminated areas into controlled submerged systems. Arsenite is the predominant As-species in low redox conditions provided by flooded situations. The reductive dissolution of As-Fe oxides and the lower adsorption of arsenite compared with arsenate in such situations (Zhao et al. 2010), would lead to a higher bioavailability of As, reducing the time needed for phytoremediation. Three out of 4 trees had $[As]_{shoot}$:$[As]_{root}$ ratios above one (*Picea abies*, *Pinus sylvestris, Sorbus aucuparia*) (Table 3). In addition, the gymnosperms *Picea abies* and *Pinus sylvestris* show a relatively high AF in the shoots in combination with a high biomass, which could make them suitable for phytoextraction purposes. Gymnosperms like *Pseudotsuga menziesie* have previously been shown to contain relatively high levels of As in stems and needles (Haug et al. 2004). Plants used for phytofiltration or phytoextraction can, after harvest, for example be used for bioenergy providing additional benefits after phytoremediation. Individual plant species like *R. tomentosum* and *V. beccabunga* in the present study show properties that might make them suitable for phytostabilizaton purposes. The properties include root accumulation of As, i.e. a root AF above one and a low $As]_{shoot}$:$[As]_{root}$ ratio.

In conclusion, the key "take home" message provided by this study is that the habitat of a plant and the $[As]_{soil/sediment}$ is more important in As accumulation in plants than individual plant characteristics and systematic relationships. Taking into account the plant habitats is a prerequisite for the development of suitable conditions, which might facilitate successful phytoremediation of As. Based on the results of this study, the practical recommendation to achieve effective phytoremediation of As, is to use submerged plants for phytofiltration.

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Figure captions

Figure 1. Arsenic speciation chart showing As standard peaks separated with a Hamilton PRP X-100 (250mm x 4.6 mm) anion exchange column and detected by the hydride generation technique, using atomic absorption spectrophometry.

Figure 2. Arsenic concentrations in submerged, emergent and terrestrial plants in relation to As concentration in soil or sediment. There is a positive correlation between $[As]_{plant}$ and $[As]_{soil}$ for emergent and terrestrial plants ($p<0.05$) but no correlation for submerged plants ($p>0.05$).

Figure 3. Shoot to soil and root to soil ratios, $[As]_{shoot}$:$[As]_{soil}$, $[As]_{root}$:$[As]_{soil}$ in submerged, emergent and terrestrial plants with n=4, 10 and 67 respectively. Mean ±SE. Significant difference between different letters a-c in roots and shoots separately (Wilcoxon signed rank test: $p<0.05$).

Figure 4. Relative amounts of As species in shoots and roots of terrestrial, emergent and submerged plants. n=2 for terrestrial, emergent and submerged plants, respectively. MA: methylarsonic acid, DMA: dimethylarsinic acid. Mean ±SE. Significant difference between different letters a-e (ANOVA: $p<0.05$).

Figure 5. Shoot to root concentration ratio, $[As]_{shoot}$:$[As]_{root}$, in submerged, emergent and terrestrial plants with n=4, 10 and 67, respectively. Mean ±SE. Significant difference between different letters a-b (Wilcoxon signed rank test: $p<0.05$).

Table 1. Ranges of As in the roots and shoots of submerged, emergent and terrestrial plants and in soil from Sweden and Slovakia. Mean±SE. No submerged plants were collected in Slovakia.

	Sweden (mg As kg^{-1} DW)			Slovakia (mg As kg^{-1} DW)	
Soil	2-2400			45-100000	
	Submerged	Emergent	Terrestrial	Emergent	Terrestrial
Roots	65 – 273	5 - 514	nd - 92	8 - 1620	3.7 - 377
Shoots	4 – 33	0.2 - 22	nd - 24	1.6 - 642	2 – 49

Table 2. Arsenic concentration in shoots, roots and soil of field samples and accumulation factors in shoots and root. Arsenic concentration in shoots relative to roots ± SE. Three replicates of plant samples were collected. Soil/sediment samples were collected in pooled samples.

Species	As concentration(mg kg^{-1} DW)			Accumulation factor		$[As]_{shoot}$:$[As]_{root}$
	Shoots	Roots	Soil	Shoots	Roots	
Taraxacum sp. Sweden	34.3 ±11.6	3.92 ±0.42	218	0.16 ±0.05	0.02 ±<0.01	8.71 ±3.08
Taraxacum sp.1 Slovakia	3.69 ±2.11	13.8 ±4.1	349	0.01 ±0.01	0.04 ±0.01	0.25 ±0.08
Taraxacum sp.2 Slovakia	49.8 ±19.8	378 ±155	5230	0.01 ±<0.01	0.07 ±0.03	0.34 ±0.29

Table 3. Arsenic concentration in shoots, roots and surrounding soil of field samples, accumulation factor of As in shoots and roots and As concentration in shoots in relation to roots. Mean ± SE. nd=not detected. Plant samples were collected as 3 replicates. Results presented without SE are collected without replicates. Soil/sediment samples were collected as 3 replicates and analysed as one pooled sample.

Habitats	Plant type	*Species*	As concentration mg kg DW^{-1}			Accumulation factor		$[As_{shoot}]$: $[As_{root}]$
			Shoots	Roots	Soil/ Sediment	Shoots	Roots	
Submerged		*Callitriche sp.*	32.9 ±11.5	88.0	6.68	4.93 ±1.73	13.2	0.34
Plants		*Ranunculus flammula*	3.63 ±1.58	97.1 ±62.6	6.68	0.54 ±0.24	14.6 ±9.4	0.03 ±0.01
		Sparganium natans	9.09 ±2.28	70.01 ±7.43	28.5	0.32 ±0.08	2.46 ±0.26	0.14 ±0.04
		Sparganium sp.	8.16 ±0.93	273 ±71	7.21	0.66 ±0.21	16.9 ±5.1	0.08 ±0.04
Emergent		*Aconitum napellus*	1.87 ±0.20	67.8 ±15.8	580	0.12 ±0.03	<0.01 ±<0.01	0.03 ±<0.01
Plants		*Allium ursinum*	74.5 ±16.2	953 ±140	2720	0.03 ±0.01	0.35 ±0.05	0.08 ±0.02
		Alnus glutinosa	0.22 ±0.03	—	60.0	nd	—	—
		Caltha palustris	2.94 ±1.01	125 ±93	226	0.01 ±<0.01	0.55 ±0.41	0.05 ±0.03
		Cardamine flexuosa	7.29 ±1.49	225 ±75	1580	0.01 ±0.01	0.14 ±0.05	0.04 ±0.02
		Cirsium palustre	642 ±325	927 ±157	100200	0.01 ±<0.01	0.01 ±<0.00	0.62 ±0.26
		Equisetum fluviatile	22.1 ±3.6	514 ±76	792	0.02 ±<0.01	0.49 ±0.10	0.04 ±0.01
		Equisetum palustre	20.5 ±5.0	88.8 ±30.6	2423	0.01 ±<0.01	0.04 ±0.01	0.33 ±0.18
		Eriophorum angustifolium	4.47 ±0.72	82.3 ±21.5	320	0.01 ±<0.01	0.26 ±0.07	0.09 ±0.03
		Juncus articulatus	19.1 ±0.7	143 ±24	2228	0.01 ±<0.01	0.06 ±0.01	0.14 ±0.03
		Juncus effusus	42.9 ±18.6	1232 ±437	15300	<0.01 ±<0.01	0.08 ±0.03	0.04 ±0.01
		Juncus filiformis	11.6 ±7.7	55.5 ±26.5	791	0.01 ±0.01	0.07 ±0.03	0.20 ±0.05
		Menyanthes trifoliata	0.95 ±0.55	8.39 ±1.89	19.3	0.05 ±0.03	0.44 ±0.10	0.09 ±0.06
		Petasites albus	1.65 ±0.55	7.90 ±1.67	143	0.01 ±0.01	0.05 ±0.01	0.22 ±0.06
		Rhododendron tomentosum	13.8 ±2.3	148 ±82	35	0.40 ±0.07	4.26 ±2.36	0.17 ±0.09
		Scirpus sylvatica	24.4 ±11.6	1623 ±963	5427	<0.01 ±<0.01	0.30 ±0.18	0.03 ±0.01
		Stachys sylvatica	69.9 ±29.7	808 ±106	8086	0.01 ±<0.01	0.10 ±0.01	0.08 ±0.03
		Vaccinium oxycoccus	2.75 ±0.92	5.11 ±4.66	12.8	0.22 ±0.07	0.40 ±0.36	0.07 ±0.33
		Vaccinium uliginosum	7.58 ±1.43	102 ±61	359	0.02 ±0.00	0.28 ±0.17	0.22 ±0.14
		Veronica beccabunga	1.58 ±0.33	30.2 ±15.9	11.9	0.13 ±0.03	2.54 ±1.34	0.10 ±0.06

Table 3. Continued

Habitats	Plant type	Species	As concentration mg kg DW^{-1}			Accumulation factor		$[As_{shoot}]$: $[As_{root}]$
			Shoots	Roots	Soil/ Sediment	Shoots	Roots	
Terrestrial		*Achillea millefolium*	2.46 ±1.18	3.30 ±1.07	11.6	0.21 ±0.10	0.15 ±0.14	0.48 ±0.51
Plants		*Aegopodium podagraria*	0.14 ±0.03	0.53 ±0.32	2.68	0.05 ±0.01	0.20 ±0.12	0.47 ±0.22
		Agrostis capillaris	7.38 ±0.84	54.9 ±12.7	143	0.05 ±0.01	0.38 ±0.09	0.15 ±0.04
		Anthyllis vulneraria	3.24 ±0.38	12.7 ±5.5	8.6	0.38 ±0.04	1.47 ±0.63	0.40 ±0.19
		Arabis arenosa	24.1 ±2.7	17.2 ±7.2	181	0.13 ±0.01	0.10 ±0.04	2.79 ±1.71
		Armoracia rusticana	nd	0.08 ±0.02	5.51	nd	0.01 ±0.00	0.00 ±0.00
		Arrhenatherum elatius	0.21 ±0.06	0.55 ±0.15	7.19	0.03 ±0.01	0.08 ±0.02	0.55 ±0.33
		Bunias orientalis	0.27 ±0.21	0.28 ±0.34	7.93	0.03 ±0.03	0.03 ±0.04	nd
		Calamagrostis arundinacea	0.09 ±0.03	0.56 ±0.24	2.68	0.03 ±0.01	0.21 ±0.09	0.20 ±0.07
		Calluna vulgaris	7.36 ±1.26	7.25 ±5.21	48.93	0.15 ±0.03	0.15 ±0.11	0.49 ±1.19
		Capsella bursa-pastoris	38.1 ±8.1	4.50 ±1.72	1052	<0.01 ±0.01	0.04 ±0.01	0.11 ±0.02
		Chelidonium majus	nd	0.14 ±0.12	5.01	nd	0.03 ±0.02	nd
		Cirsium arvense	nd	nd	5.22	nd	nd	nd
		Daucus carota ssp. carota	0.12 ±0.07	0.18 ±0.13	28.2	nd	0.01 ±0.01	0.13 ±0.07
		Deschampsia cespitosa	2.91 ±0.59	4.19 ±1.36	—	—	—	0.96 ±0.42
		Deschampsia flexuosa	5.95 ±3.86	11.0 ±3.2	35.1	0.17 ±0.11	0.31 ±0.09	0.62 ±0.41
		Empetrum nigrum	5.41 ±1.13	2.89 ±0.56	36.2	0.63 ±0.32	0.29 ±0.16	4.21 ±2.60
		Epilobium angustifolium	1.07 ±0.64	4.24 ±0.10	47.6	0.02 ±0.01	0.09 ±<0.00	0.25 ±0.15
		Equisetum hyemale	0.11 ±0.09	20.9 ±12.3	161	nd	0.13 ±0.08	nd
		Equisetum sylvaticum	1.49 ±1.61	7.85 ±0.17	9.10	0.16 ±0.18	0.86 ±0.02	0.18 ±0.20
		Fragaria vesca	nd	nd	2.68	nd	nd	nd
		Galium odoratum	8.24 ±1.14	19.6 ±8.55	136	0.06 ±0.01	0.14 ±0.06	0.60 ±0.26
		Hieracium umbellatum	0.15 ±0.04	0.47 ±0.13	8.25	0.02 ±<0.01	0.06 ±0.02	0.39 ±0.18
		Hypochaeris radicata	nd	nd	4.32	nd	nd	nd
		Impatiens glandulifera	0.15 ±0.04	nd	3.71	0.04 ±0.01	nd	nd
		Leontodon autumnalis	1.15 ±0.64	1.24 ±1.54	6.91	0.17 ±0.09	0.18 ±0.22	0.55 ±0.92

Table 3. Continued

Habitats	Plant type	Species	As concentration mg kg DW^{-1}			Accumulation factor		$[As_{shoot}]$: $[As_{root}]$
			Shoots	Roots	Soil/ Sediment	Shoots	Roots	
		Linnaea borealis	13.4 ±3.92	24.61 ±2.28	25.6	0.52 ±0.15	0.96 ±0.09	0.55 ±0.16
		Lupinus polyphyllos	nd	nd	2.75	nd	nd	nd
		Luzula pilosa	4.61 ±0.96	17.0 ±8.5	16.2	0.28 ±0.06	1.04 ±0.53	0.42 ±0.17
		Luzula sylvatica	2.03 ±0.68	3.68 ±2.20	45.0	0.05 ±0.02	0.08 ±0.05	0.76 ±0.20
		Matricaria perforata	0.26 ±0.05	1.07 ±0.08	5.01	0.05 ±0.01	0.21 ±0.02	0.24 ±0.02
		Melampyrum pratense	4.62 ±1.47	92.3 ±76.1	24.1	0.19 ±0.06	3.84 ±3.16	0.26 ±0.16
		Melampyrum sylvaticum	4.31 ±1.05	16.5 ±19.4	26.4	0.16 ±0.04	0.62 ±0.73	0.11 ±0.15
		Melilotus altissimus	0.30 ±0.15	0.85 ±0.15	116	nd	0.01 ±<0.00	0.38 ±0.16
		Oxalis acetosella	8.37 ±3.45	7.92 ±1.35	361	0.02 ±0.01	0.02 ±0.01	0.96 ±0.29
		Rubus caesius	0.56 ±0.13	0.37 ±0.01	17.2	0.03 ±0.01	0.02 ±0.00	1.52 ±0.34
		Rubus chamaemorus	1.54 ±0.58	16.8 ±7.75	38.0	0.04 ±0.02	0.44 ±0.20	0.34 ±0.28
		Rubus idaeus	0.28 ±0.07	0.74 ±0.23	21.8	0.01 ±<0.01	0.03 ±0.01	0.39 ±0.02
		Sagina procumbens	1.90 ±1.21	43.0 ±32.1	138	0.01 ±0.01	0.31 ±0.23	0.34 ±0.24
		Solanum dulcamara	0.27 ±0.13	0.65 ±0.07	19.9	0.01 ±0.01	0.03 ±<0.00	0.45 ±0.25
		Tanacetum vulgare	nd	nd	8.25	nd	nd	nd
		Taraxacum sp.	34.3 ±11.6	3.92 ±0.42	218	0.16 ±0.05	0.02 ±<0.01	8.71 ±3.08
		Trifolium pratense	1.14 ±0.14	4.59 ±2.41	19.1	0.14 ±0.08	0.19 ±0.09	0.24 ±0.18
		Trifolium repens	4.68 ±0.78	8.24 ±1.05	52.7	0.09 ±0.01	0.16 ±0.02	0.61 ±0.18
		Tussilago farfara	nd	0.23 ±0.14	120	nd	nd	nd
		Urtica dioica	0.51 ±0.05	0.40 ±0.05	5.43	0.09 ±0.01	0.07 ±0.01	1.27 ±0.06
		Vaccinium myrtillus	6.84 ±2.07	15.6 ±2.1	11.5	0.59 ±0.18	1.35 ±0.18	0.50 ±0.12
		Vaccinium vitis-idaea	4.93 ±1.50	8.10 ±4.77	297	0.02 ±0.01	0.03 ±0.02	0.52 ±1.29
	Crops	*Allium cepa*	—	nd	30.8	—	nd	—
		Avena sativa	0.29 ±0.05	—	170	nd	—	—
		Hordeum vulgare	nd	—	nd	—	—	—
		Medicago sativa	0.37 ±0.14	0.95 ±0.16	142	nd	0.01 ±<0.01	0.35 ±0.12

Table 3. Continued

Habitats	Plant type	Species	As concentration mg kg DW^{-1}			Accumulation factor		$[As_{shoot}]$: $[As_{root}]$
			Shoots	Roots	Soil/ Sediment	Shoots	Roots	
		Pisum sativum	nd	—	—	—	—	—
		Triticum aestivum	nd	—	—	—	—	—
	Ferns	*Athyrium felix-femina*	nd	0.02 ±0.06	3.47	nd	0.01 ±0.02	nd
		Dryopteris filix-mas	nd	0.46 ±0.51	2.63	nd	0.17 ±0.19	nd
		Pteridium aquilinum	0.17 ±0.16	1.73 ±0.87	21.8	0.01 ±0.01	0.08 ±0.04	0.07 ±0.05
	Trees and Shrubs	*Betula pendula*	nd	—	8.25	nd	—	—
		Betula pubescens	1.89 ±0.24	9.50 ±5.52	79.8	0.02 ±<0.01	0.12 ±0.07	0.54 ±0.38
		Carpinus betulus	2.08 ±0.18	—	±1612	<0.01 ±0.01	—	—
		Fraxinus excelsior	0.02 ±0.03	—	10.1	nd	—	—
		Ligustrum vulgare	0.10 ±0.06	—	28.2	nd	—	—
		Malus domestica	0.08 ±0.14	—	24.3	nd	—	—
		Picea abies	5.18 ±1.34	3.17 ±1.12	15.4	0.34 ±0.09	0.21 ±0.07	2.14 ±0.76
		Pinus sylvestris	5.51 ±0.79	9.21 ±4.58	8.71	0.63 ±0.09	1.06 ±0.53	1.04 ±0.57
		Populus x canescens	9.99 ±1.13	—	8287	<0.01 ±<0.01	—	—
		Prunus avium	nd	—	4.57	nd	—	—
		Prunus padus	0.01 ±0.06	—	23.8	nd	—	—
		Rosa rugosa	nd	—	8.58	nd	—	—
		Rosa villosa	nd	—	30.9	nd	—	—
		Salix alba	29.0 ±3.76	—	8287	<0.01 ±<0.01	—	—
		Salix caprea	0.20 ±0.08	—	21.8	0.01 ±<0.01	—	—
		Sorbus aucuparia	3.50 ±0.87	2.81 ±0.02	33.5	0.10 ±0.03	0.08 ±<0.01	1.25 ±0.32
		Ulmus glabra	0.18 ±0.15	—	5.01	0.04 ±0.03	—	—

Table 4. Concentrations of total As and As species in shoots and roots, total As concentration in soil, accumulation factor and shoot to root ratio. MMA: methylarsonic acid, DMA: dimethylarsinic acid. Extraction efficiency equals the ratio between total As content in plant part and the sum of As species in the plant part. Plant samples were analysed as three replicates. Mean ±SE.

Habitat	Species	Plant part	Total As (mg As kg^{-1})	Arsenite (mg As kg^{-1})	Arsenate (mg As kg^{-1})	MMA (mg As kg^{-1})	DMA (mg As kg^{-1})	Extraction efficiency (%)
Submerged	*Sparganium natans*	shoot	10.8 ±4.6	0.63 ±0.26	2.07 ±0.72	0.07 ±0.07	nd	32.8
		root	59.8 ±2.1	13.0 ±6.7	43.6 ±9.1	14.2 ±5.8	nd	111
	Sparganium sp.	shoot	5.24 ±0.94	0.00 ±<0.01	3.12 ±0.29	nd	nd	61.4
		root	303 ±39	5.21 ±5.81	94.0 ±41.7	nd	nd	36.6
Emergent	*Equisetum fluviatile*	shoot	12.5 ±2.8	2.19 ±1.05	5.92 ±2.41	0.25 ±0.24	nd	64.5
		root	531 ±255	12.6 ±2.8	68.3 ±47.6	2.41 ±2.41	nd	22.1
	Eriophorum angustifolium	shoot	4.46 ±0.70	0.45 ±0.18	1.30 ±1.19	0.19 ±0.19	nd	45.5
		root	59.9 ±30.4	2.39 ±0.84	29.2 ±19.7	0.90 ±0.90	nd	47.5
Terrestrial	*Empetrum nigrum*	shoot	3.93 ±0.21	0.41 ±0.16	0.36 ±0.36	0.62 ±0.38	nd	36.5
		root	2.97 ±0.74	0.78 ±0.11	0.93 ±0.56	0.40 ±0.37	nd	78.5
	Pinus sylvestris	shoot	2.38 ±0.82	0.99 ±0.14	0.25 ±0.25	0.03 ±0.03	nd	70.4
		root	34.4 ±20.7	4.56 ±1.53	4.18 ±0.71	0.21 ±0.21	nd	40.4

Table 5. Shoot to root ratio, $[As]_{Shoot}$:$[As]_{root}$, of the separate As species. MMA: methylarsonic acid, DMA: dimethylarsinic acid. Due to differences in the extraction efficiencies between shoots and roots, the total $[As]_{shoot}$:$[As]_{root}$ may differ from the $[As]_{shoot}$:$[As]_{root}$ for the individual As species. Mean ± SE. nd=not detected.

Habitat	Species	$[As]_{Shoot}$: $[As]_{root}$	Arsenite	Arsenate	MMA	DMA
Emergent	*Equisetum fluviatile*	0.03 ±0.01	0.24 ±0.14	0.18 ±0.13	<0.01 ±<0.01	nd
	Eriophorum angustifolium	0.12 ±0.04	0.36 ±0.24	0.02 ±0.02	0.07 ±0.07	nd
Terrestrial	*Empetrum nigrum*	1.52 ±0.39	0.54 ±0.19	0.19 ±0.19	0.39 ±0.39	nd
	Pinus sylvestris	0.10 ±0.03	0.31 ±0.14	0.08 ±0.08	0.05 ±0.05	nd
Submerged	*Sparganium natans*	0.19 ±0.09	0.08 ±0.04	0.05 ±0.01	0.02 ±0.01	nd
	Sparganium sp.	0.02 ±<0.01	<0.01 ±<0.01	0.05 ±0.02	nd	nd

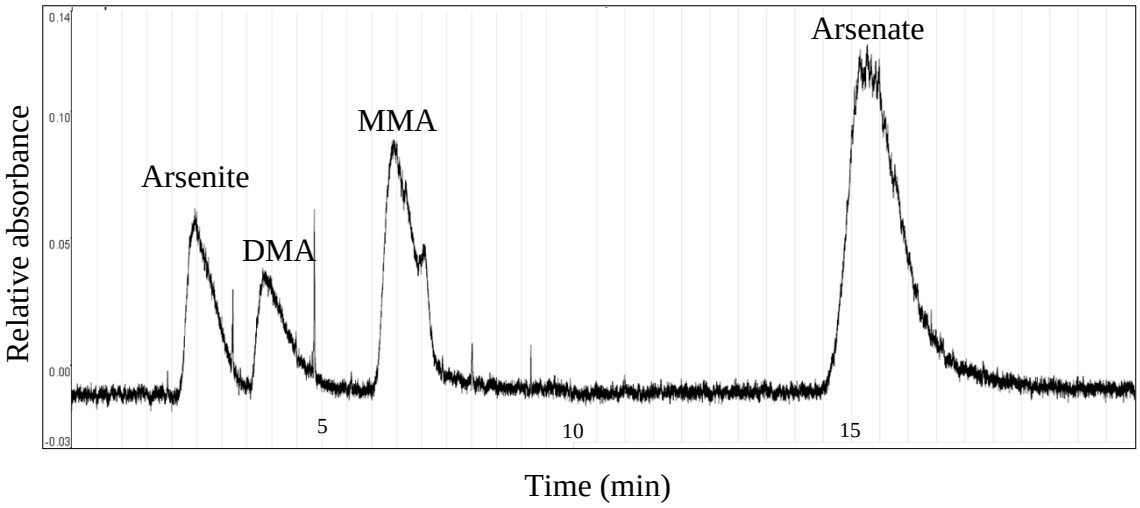
Arsenite
DMA
MMA
Arsenate
Relative absorbance
Time (min)
0.14
0.10
0.05
0.00
-0.03
5
10
15

Fig. 1.

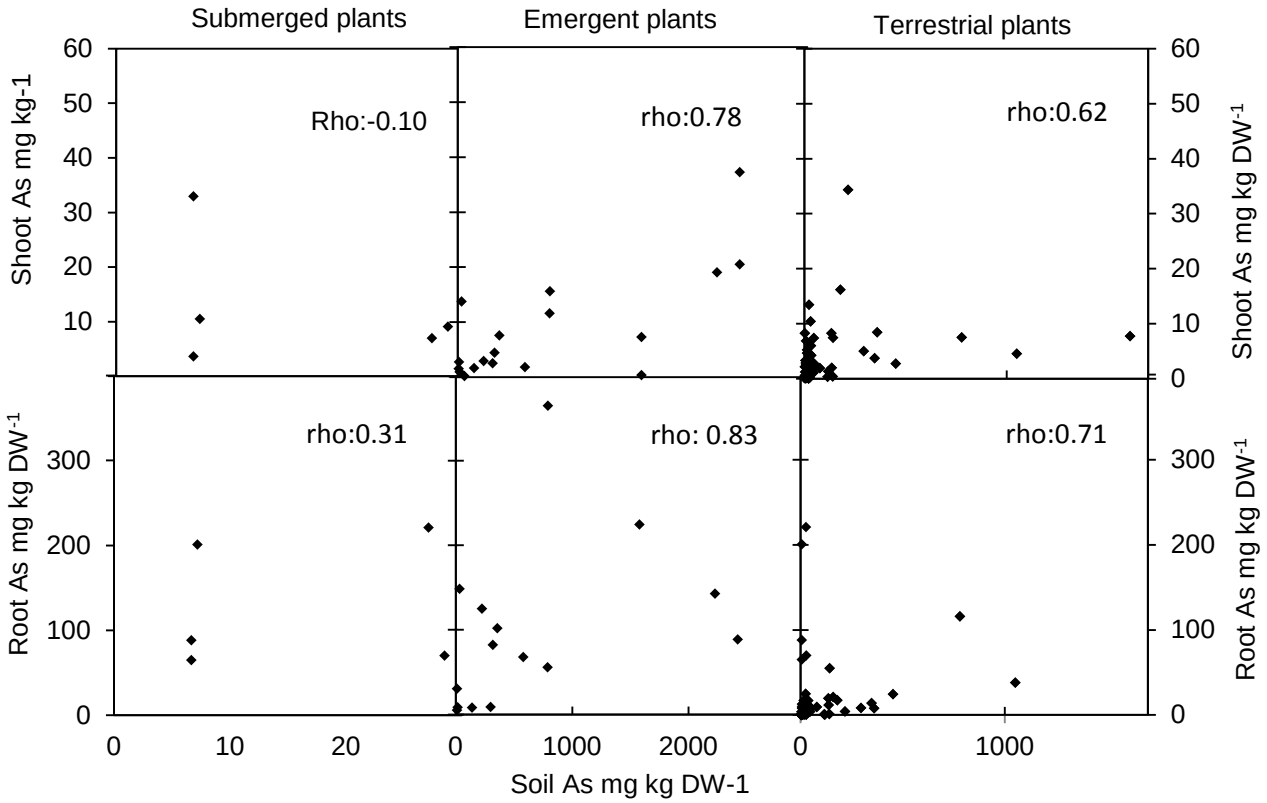
Submerged plants
Emergent plants
Terrestrial plants
Shoot As mg kg-1
Root As mg kg DW-1
Shoot As mg kg DW-1
Root As mg kg DW-1
Rho:-0.10
rho:0.78
rho:0.62
rho:0.31
rho: 0.83
rho:0.71
Soil As mg kg DW-1

Fig. 2.

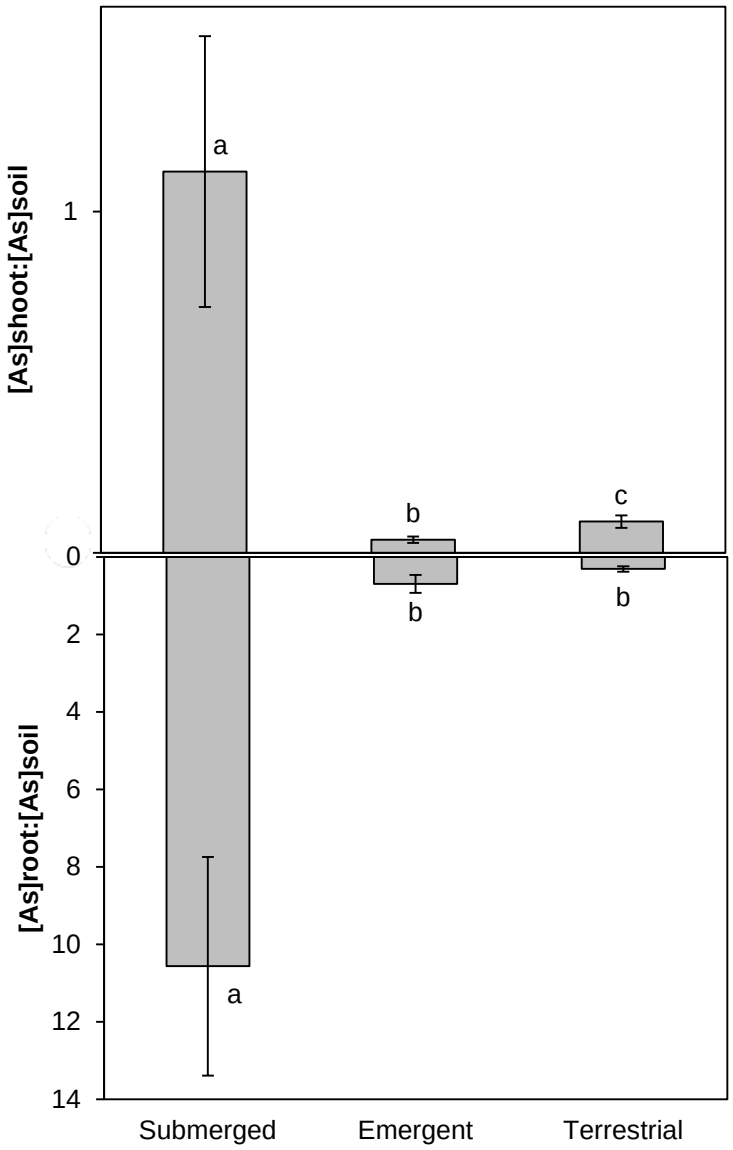
[As]shoot:[As]soil
[As]root:[As]soil
1
0
2
4
6
8
10
12
14
a
b
c
b
a
Submerged
Emergent
Terrestrial

Fig. 3.

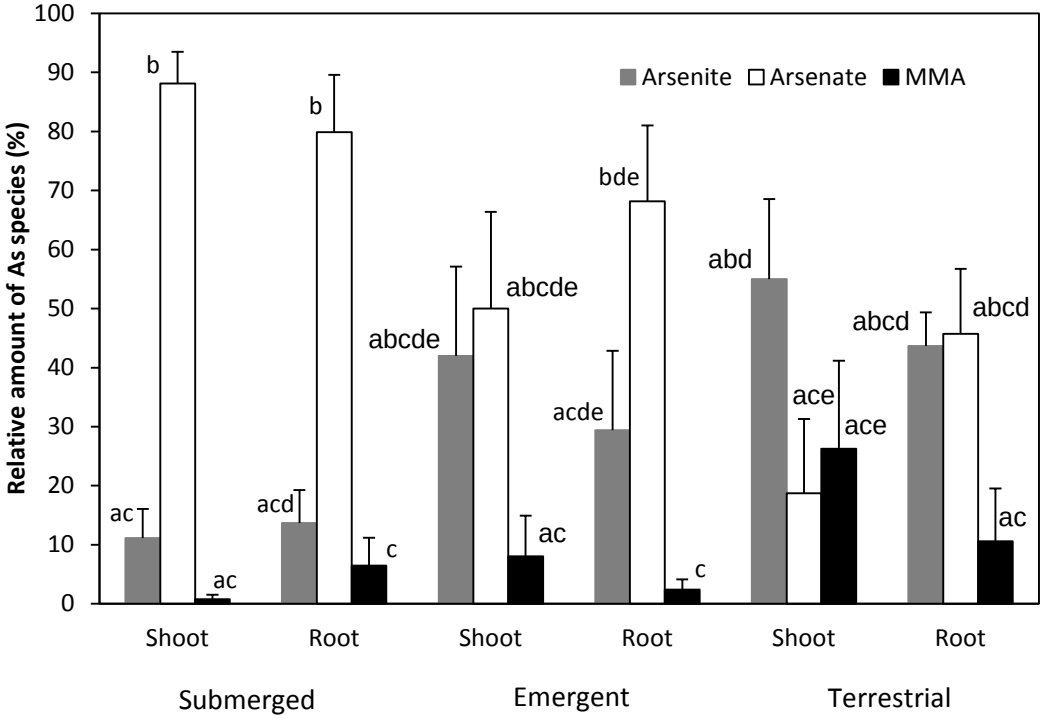
Relative amount of As species (%)
100
90
80
70
60
50
40
30
20
10
0
Arsenite
Arsenate
MMA
b
b
bde
abd
abcd
abcde
abcde
acde
ace
ace
abcd
ac
acd
c
ac
c
ac
Shoot
Root
Shoot
Root
Shoot
Root
Submerged
Emergent
Terrestrial

Fig. 4.

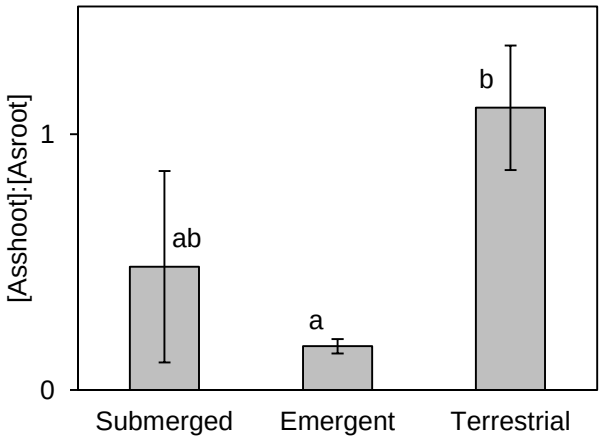

Fig. 5.