# Arsenic accumulation in various plant types



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# Abstract

Arsenic (As) is ubiquitous in nature appearing in various chemical forms, mainly due to differences in soil redox potential, pH and biological activity. Inorganic As species are considered more toxic than organic ones. As-speciation analysis of specific food crops is an important issue, since food and drinking water mainly contain inorganic As species and, thereby, constitute a threat to human health.

The aim of the present study was to achieve a more complete view of the habitat influence on the accumulation and speciation of As in plants in a temperate region. Also, the aim was to identify the risks of consuming vegetables cultivated in As contaminated soil. The hypothesis was that both the plant habitat and the soil concentration of As may influence the level of As accumulated, and reveal which As species that predominate in plants.

Ninety two terrestrial, emergent and submerged plants naturally occurring in As contaminated soil/sediment at six different localities in Sweden were collected and screened for As accumulation. Similarly, 22 terrestrial and emergent plants were collected at four different localities in Slovakia. Plants were analysed for total As concentrations and the As species arsenite, arsenate, methylarsonic acid (MMA) and dimethylarsinic acid (DMA). A greenhouse cultivation with three different species of vegetables was performed to determine the risks of dietary As intake from vegetables cultivated in soil with elevated levels of As.

In general, with increasing $[As]_{soil}$, there was a concomitant increase in $[As]_{plant}$. The relatively higher levels of internal arsenite, compared with other As species, might promote a higher root to shoot translocation of the total As in plants. Terrestrial plants showed a higher shoot to root As ratio than emergent plants. Submerged plants had a high accumulation of As

and a high $[As]_{shoot}:[As]_{root}$ ratio. This is possibly due to foliar uptake of As and a lower redox potential in the submerged habitat than in the emergent and terrestrial habitats.

The inorganic As species arsenate and arsenite predominated ($\geq$75 %) in all six plant species analysed. Moreover, MMA was found in up to 25 % of the total As species in five out of six analysed plant species. Other As species, for example DMA, was not detected in any of the plants tested.

Vegetables cultivated in As containing soil had elevated concentrations of As in the biomass. Furthermore, the data showed that consumption of one normal sized carrot would result in 5-10 times higher As intake than the daily limit level of As in drinking water (10 µg As $L^{-1}$) in the European Union. The risk of As entering the food chain is accentuated because the more toxic inorganic As species are predominating in plants.

I conclusion, plants in temperate regions accumulate increasing levels of As from the surrounding soil/sediment with increasing soil-As concentration especially under submerged conditions. However, individual plant species can have properties which result in higher or lower As accumulation compared to this general trend. Cultivation of crops in As containing soil/sediment might exert elevated risks of intake of toxic inorganic As.

# Contents

# 1. Background

## 1.1 Arsenic in nature

Arsenic (As) originating from both natural and anthropogenic sources is present at trace levels everywhere in nature (Lombi *et al.* 2002). The major reservoirs for As in the environment are rocks, soils and oceans. More than 99 % of the As in the world is found in rocks, mainly in silicate minerals, where As substitutes Al, Fe and Si (Bhumbla & Keefer, 1994), and sulfur commonly associates to As (Duker *et al.* 2005). In soils, Al, Ca, Fe, Mg and Ni form solids with As (Bhumbla & Keefer, 1994). There are several As species found in soil, water and plants (Fig. 1).
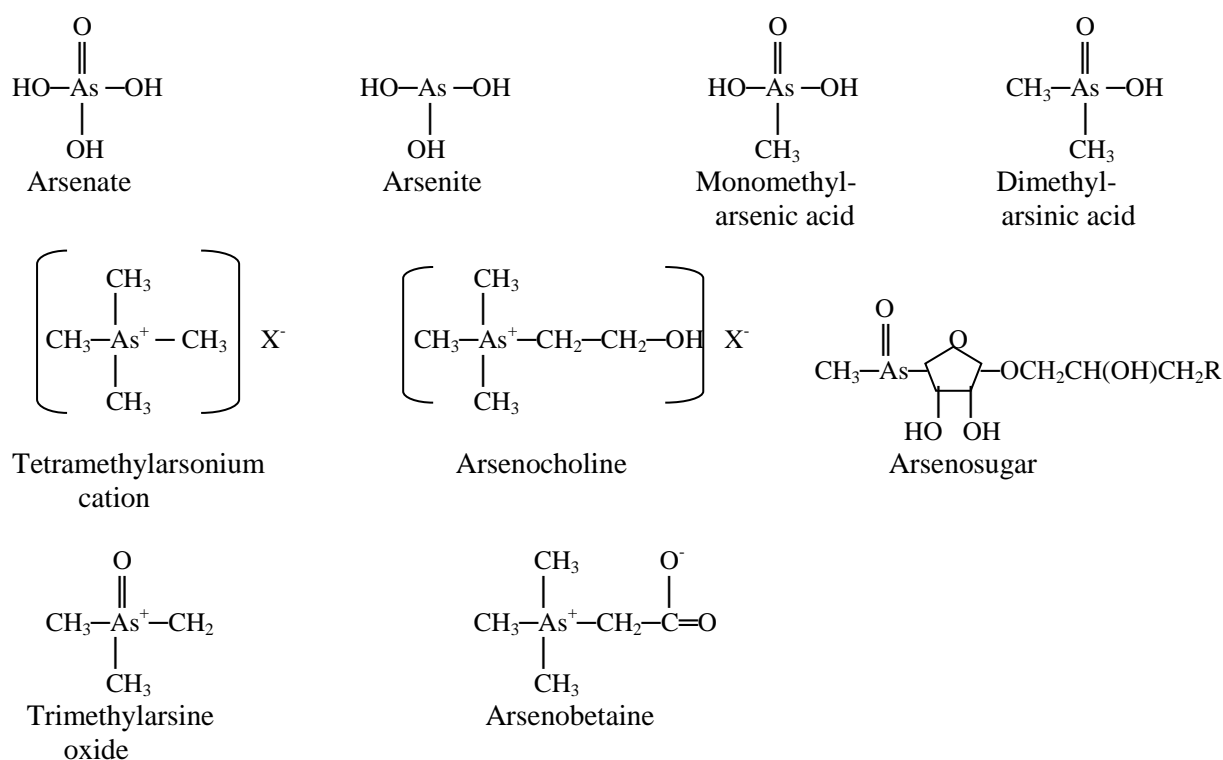


Figure 1. Arsenic species found in soil, water and plants (modified from Meharg & Hartley-Whitaker, 2002).

Arsenate is the predominant species of As under oxic conditions, while arsenite species dominates under anoxic conditions (Sadiq, 1997). Both arsenate and arsenite consist of several As oxyanionic species with the common factor that the As atoms are in oxidation

states III or V (Sadiq, 1997). Several factors influence the solubility and speciation of As, the major ones are pH and redox potential. With increasing pH the solubility of arsenate increases, while the solubility of arsenite decreases, and the opposite occurs when pH is decreasing (Raven *et al*. 1998). The dominating forms of As in soil solutions are arsenate and arsenite. Small amounts of methylated As species like methylarsonic acid (MMA) and dimethylarsinic acid (DMA) are usually also present in the soil (Bowell, 1994). The methylated As species are produced through biological activity, primarily by bacteria but also by fungi (Wood, 1974). In water there is a gradient from the surface water where almost all As is in the form of arsenate to the sediment-pore water, where almost all As is in the form of arsenite (Zheng *et al.* 2003).

## 1.2 Arsenic release by anthropogenic activities

The limit for As in agricultural soil, or soil used for domestic living, is set to 10 mg kg$^{-1}$ (DW) by the Swedish environmental protection agency (Naturvårdsverket, 2009a). It is estimated that there are approximately 80000 locations in Sweden polluted by anthropogenic activities, and that elevated levels of As are found in 25 % of these sites (Naturvårdsverket, 2009b). In Swedish industry, the main As compound was used as an impregnating agent in the wood industry. From the 1$^{st}$ of January 2004, new stricter rules were applied in Sweden towards the use of As as an impregnating agent, that lead to a drastically decreased use of As (Fig. 2) (KemI, 2005).
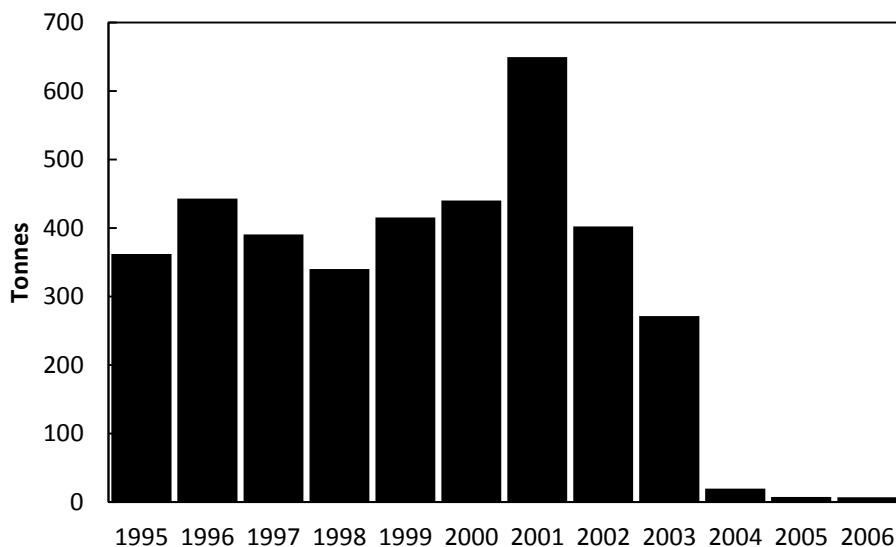
Figure 2. The use of arsenic and arsenic compounds in all products in Sweden between the years 1995 to 2006 (Produkt- & bekämpningmedelsregistret, 2009)

## 1.3 Routes for arsenic intake

Drinking water contaminated with As is a major problem. The most common route for As intake for humans is through drinking water. All continents have problems with As contaminated groundwater, but the problems are especially potent in South East Asia like Bangladesh and West Bengal where millions of people are at risk of exposure of As-contaminated drinking water (Nordstrom, 2002). In Sweden, the Swedish Geological Survey has identified several areas with elevated levels of As in the groundwater, especially in Västerbotten but also in the eastern parts of middle Sweden (SGU, 2005). In Sweden as well as in other countries of the European Union the limit for drinking water is set to 10 µg l$^{-1}$ based on the risk for developing cancer during a lifetime exposure to As (Livsmedelsverket, 2001; Commission Directive 2003/40/EC).

Besides drinking water, food may also make a significant contribution to the dietary intake of As. For example, As intake from rice may contribute to the total As intake in equal amounts as As-contaminated drinking water, particularly if cooked in As polluted water (Meharg,

2005). Rice may take up As from the surrounding soil and the concentration of As in rice grains can reach elevated levels (Juhasz *et al.* 2006; Williams *et al.* 2007). The concentration of As in rice is usually below 0.5 mg kg$^{-1}$ (DW), but since it is common to eat approximately 200 g (DW) of rice per day in Asian diets (Zhu *et al*. 2008), the total amount of ingested As can reach levels 5-10 times higher than the daily limit set for drinking water. Processed rice products like puffed rice and rice crackers may also contain elevated levels of As (Sun *et al*. 2009). A significant part of the total concentration of As in rice consists of arsenite and arsenate and these inorganic forms of As have a bioassesability of approximately 90 % (Juhasz *et al.* 2006). Other crops have been tested for accumulation properties of As; for example beetroot, lettuce, potato and radish (Smith *et al*. 2009; Warren *et al*. 2003). The amount of As in the edible parts of these vegetables can reach relatively high levels of As, for example more than 30 mg As kg$^{-1}$ (DW) in radish (Smith *et al*. 2009), which could pose a problem for heavy consumers of particular vegetables cultivated in As contaminated soils. This problem is, however, a more limited one, since these vegetables are not staple food like rice.

## 1.4 Arsenic exposure to humans

There is no threshold value when As becomes toxic to humans and exposure may give rise to cancer in liver, lung, skin, bladder and kidney even at low concentrations (Smith *et al.*1992). Ingested inorganic As species are absorbed in the intestine and methylated in the liver mainly to DMA, and the methylated species are finally being excreted via the urine (Suzuki *et al.* 2001). In a study of six human volunteers, the process of excretion took place in three phases; 66 % of the ingested As had a half-time of approximately 2 days, 30 % had a half-time of approximately 9 days and 4 % had a half-time of approximately 1 month (Pomroy *et al*. 1980).

Inorganic species of As are generally considered to be more toxic to living organisms than organic As (Meharg & Hartley-Whitaker, 2002), and of the inorganic As species, arsenite is considered to be more toxic than arsenate (Bhumbla & Keefer, 1994)**.** An exception is the methylated intermediate methylarsonous acid ($CH_3As(OH)_2$) in the metabolic detoxification pathways in the human liver, which has a trivalent oxidation state of the As atom. This arsenic species is more cyto- and genotoxic and exerts more inhibition of enzymes than inorganic arsenicals (Thomas *et al.* 2001). Trivalent methylated arsenicals are believed to induce their genotoxic effect by DNA damage due to the production of reactive oxygen species like hydrogen peroxide, hydroxyl radicals and superoxide anions (Nesnow *et al.* 2002). Organic As compounds found in marine organisms, mainly arsenobetaine, are generally considered to have low or no toxicity (Kaize *et al.* 1985). Hence, when determining the risk of As intake, the total intake of toxic As species is more important than the total amount of As intake as such.

## 1.5 Arsenic uptake by plants

In general, plants take up As through the roots, but submerged plants may also take up As by the leaves from the water column (Wolterbeek & van der Meer, 2002) (Fig. 3). An understanding of how plants behave in As contaminated environments could reduce As related problems and prevent As intake for humans and livestock. General differences in the accumulation and translocation of As in plant tissue depend for example on plant species and habitat. Crop and vegetable production can benefit from knowledge of habitats and external conditions which might promote a higher accumulation of As in edible parts of the plants.
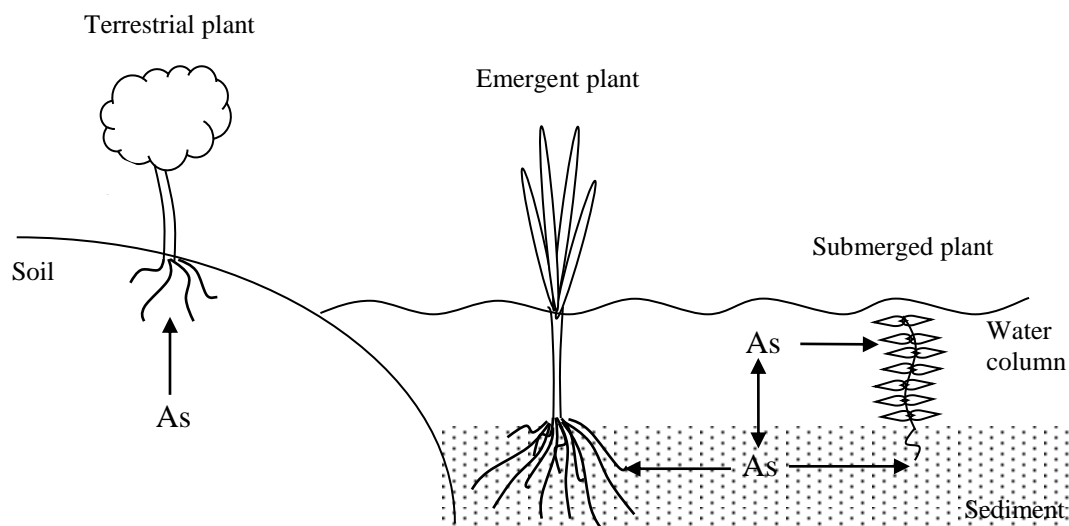
Fig. 3. Uptake routes of As in terrestrial, emergent and submerged plants.

### 1.5.1 Distibution of As in plants

Generally, arsenic is primarily found in the roots rather than in the shoots of plants. For example, a more than 15 times higher As concentration in *Spartina pectinata* (Rofkar & Dwyer, 2011), and a 28-75 times higher As concentration in rice were found in the roots than in the shoots (Azizur Rahman *et al.* 2007). However, some species, for example radish have a higher concentration of As in the shoots compared with the roots (Smith *et al.* 2008).

### 1.5.2 Apoplastic uptake of As

The first compartment of the plant body subjected to a substance via the roots is the apoplast. Localization of As in the apoplast may make a significant contribution to the total amount of As in a plant. Roots of rice grown under reducing conditions were separated into an apoplastic fraction containing 173 mg As kg$^{-1}$ (60 % of total As), a non-apoplastic fraction containing 112 mg As kg$^{-1}$ (39 % of total As) and the shoot tissue containing 2.3 mg As kg$^{-1}$ (1 % of total As) (Bravin et al. 2008). In addition, in the hyperaccumulating fern *Pteris vittata* approximately 1/6 of total As was found in the apoplast (Chen et al. 2005).

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### 1.5.3 Cellular uptake of As

From the apoplast As can enter into the plant cytosol. Plant uptake of arsenate into the cell cytoplasm has been suggested to occur by high-affinity phosphate transporters, while arsenite has been suggested to be taken up by aquaglyceroporins (Meharg & Macnair 1992; Meharg & Jardine, 2003). Also organic As like MMA and DMA can be taken up by plants by aquaglyceroporins (Azizur Rahman *et al*. 2011). Figure 4 describes a general overview of the cellular uptake, detoxification and transport of As into the root cells of plants (Tripathi *et al.* 2007). The main processes include the reduction of arsenate to arsenite by gluthatione and a complexation of arsenite to phytochelatins. The complex is then translocated to the shoot or stored in the vacuoles of the root cells. Members of the Nodulin26-like Intrinsic Proteins (NIP) subfamily of aquaporins in the plasma membrane, with important roles in the uptake of metalloids like boron and silicon, also facilitate the disposal of arsenite from the cell by efflux (Bienert *et al.* 2008).
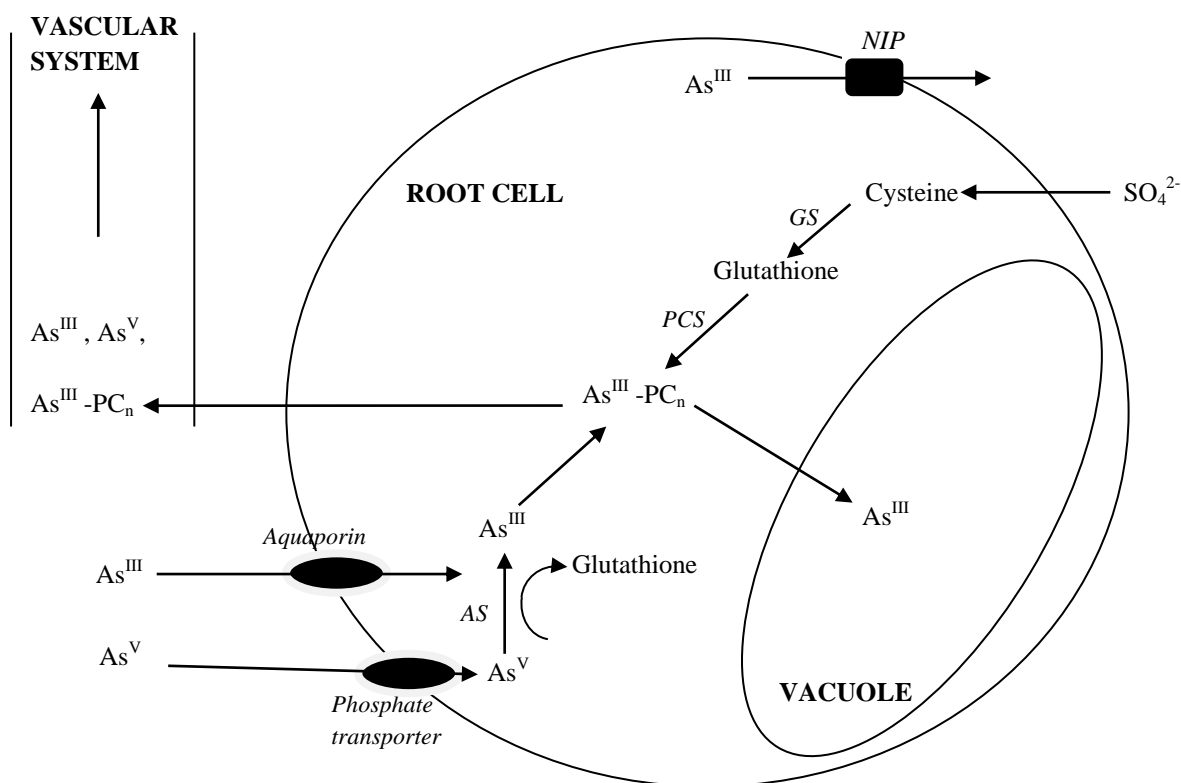
Fig 4. Uptake, detoxification and transport of arsenic in the root cell and vascular system of plants. Arsenite=As$^{III}$, arsenate=As$^{V}$. Cysteine is synthesised from sulfate and is transformed into glutathione by glutathione synthetase (GS). Phytochelatin synthase (PCS) produces phytochelatins from glutathione. Arsenite binds to phytochelatins (As$^{III}$ -PC$_n$) and is transported into vacuoles or to the shoot like arsenite or as a phytochelatin-arsenite complex. Efflux of arsenite from the cell can also be performed by NIP aquaporins. Arsenate reductase (AS) reduces arsenate to arsenite by using glutathione as a reductant (Modified from Tripathi *et al.* 2007).

## 1.5.4 Toxicity of As to plants

Production of reactive oxygen species (ROS) like hydrogen peroxide and superoxide by plants exposed to environmental stresses causes damage to DNA, proteins and lipids (Singh *et al.* 2006). Plants exposed to either arsenate or arsenite produce ROS (Srivastava *et al.* 2007). Arsenate and arsenite are also toxic to plants by disturbing central cellular functions. Arsenate can replace phosphate in aerobic phosphorylation, disturbing the cellular energy flow (Hughes, 2002), and arsenite can disturb protein functioning by binding to sulphydryl groups in the proteins (Ullrich-Eberius *et al.* 1989; Ozturk *et al.* 2010).

### 1.5.5 Hyperaccumulation of As

Hyperaccumulation of As by a plant means that the concentration of As in the plant biomass exceeds 1 g kg$^{-1}$ (DW) (Branquinho *et al.* 2007). The first hyperaccumulator to be discovered was the fern *Pteris vittata* with a frond concentration of more than 20 g kg$^{-1}$ (DW) of As (Ma *et al.* 2001). In *Pteris vittata*, arsenate predominates in the roots and arsenite in the fronds (Ma *et al*. 2001). In the fronds, the arsenite is stored in the vacuoles (Lombi *et al.* 2002). The conversion of arsenate to arsenite may be performed by a cylindrical sheath of thiolates surrounding the veins in *Pteris vittata* (Pickering *et al*. 2006). So far, 12 species of ferns from the family Pteridaceae show hyperaccumulating abilities of As (Zhao *et al*. 2009).

## 2. Objectives

This work has two aims.

1. To clarify the habitat´s importance for As accumulation in plants. Studies of As accumulation in plants are usually performed on a single or few plants species. In the present study, As accumulation in plants was investigated by analysis on a large dataset of submerged, emergent and terrestrial plants from different regions.

2. To investigate the As accumulation and speciation in edible vegetables cultivated in soil with elevated levels of As.

Plant types were given special attention, since the speciation of As in the environment depends on physicochemical factors like redox potential and pH, parameters which can vary significantly, for example, between submerged and terrestrial habitats. The hypothesis was that the plant habitats could affect the accumulation and speciation of As in plants.

The understanding of the general patterns of accumulation and speciation of As in plants could help to elucidate the implications for dietary uptake of As from crops and vegetables cultivated in As containing soil. Arsenic accumulation in crops may depend on the pH, $[As]_{soil}$ and the plant species and if the edible part is the root or the shoot. The hypothesis was that plants cultivated in As-containing soil will accumulate As into the edible plant parts.

## 3. Comments on material and methods

### 3.1 Plant species used

Sweden has a relatively cold climate and a short vegetation season. Naturally occurring plant species, which have adapted to local conditions are, therefore, the best suitable candidates for understanding As uptake in plants in Sweden. Self-sown plants in areas where both natural and anthropogenic processes have led to elevated levels of As in the soil/sediment were collected for analysis of total concentration of As, accumulation factor $[As]_{plant}:[As]_{soil}$, shoot to root ratio $[As]_{shoot}:[As]_{root}$ and speciation of As. The plants were typed as terrestrial, emergent or submerged plants. Figure 5 shows the sampling locations in Sweden. Also, results from plants collected in As containing mine sites in Slovakia were analysed and compared with the plants collected in the Swedish mine sites.
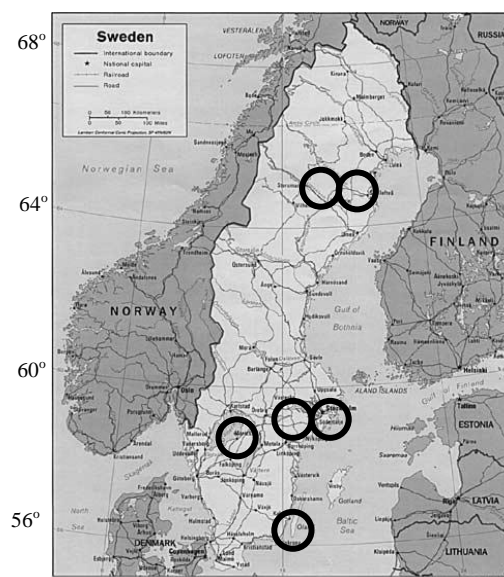


Fig. 5. Sampling locations in Sweden.

Individual plant species were only collected at one location since many factors might influence the uptake of As of this particular species, for example longitude-latitude and degree and composition of pollution in the soil. The concentration of As in the soil has a major influence on the concentration of As in the plant tissue, and since plants were collected only at one location, it is important to consider the soil-As concentration when drawing conclusions about individual plant species.

## 3.2 Arsenic-species analysis

Arsenic species were extracted from the plant material using a MeOH:$H_2$O(1:1)-solution followed by a 0.1 M HCl-solution. The extraction efficiency ranged from approximately 20-110 % depending on plant species and plant part. The low extraction efficiencies in some cases have not been elucidated in detail, but they are likely to depend on problems with getting all the As in the plant material in solution. In some cases small fragments of plant material, especially from woody plants, were seen after the extraction, and it is likely that these fragments contained some As. Analysis of the As species arsenate, arsenite, MMA and DMA was performed using a HPLC-AAS (high-pressure liquid chromatography-atomic absorption spectroscopy) technique. The arsenic species were separated from each other using HPLC and the amounts of each species were determined by AAS. A successful separation of the As species in the HPLC was highly dependent on which column, eluent and flow rate that was used. First, Ionpac AG9-HC, Dionex anionic column with NaCO$_3$ eluent was tested. By this experimental setup, however, it was not possible to separate arsenite from DMA. Changing the pH of the eluent did not have any effect on the overlap of the peaks of arsenite and DMA. Gradients of various eluents (methanol+acetonitril, methanol+$H_2$O, methanol+NaCO$_3$) were then tried without success of separating arsenite and DMA. Methanol is not useful as eluent in As-species separation, since adsorption abilities of some of the As

species are negatively affected. Then no concentrated peaks are produced in the detector. A new column (Hamilton PRP X-100 anion exchange column, 250mm x 4.6 mm) was then tried with 30 mM phosphate buffer (pH 6) as eluent. Still some overlap of arsenite and DMA peaks were detected, but after adjusting the eluent concentration to 20 mM phosphate buffer (pH 5.8) the peaks were separated. For optimal separation of peaks a flow rate of 1 ml min$^{-1}$ was employed.

The As peaks separated by HPLC was at first detected, manually by extracting fractions of the outlet eluent from the column. Fractions were then analysed for As concentration in AAS. However, this method was found to be tedious and in-accurate. Instead, the outlet from the column was connected to the pump facilities in the hydrid generator (VGA-77) and pumped directly into AAS. This provided a smooth, continuous signal with little background disturbance (fig. 6). To eliminate interaction effects of the matrix, chemical standards were added to each sample in the analysis. The detection limit for arsenite was 1.5 µg L$^{-1}$, for DMA 7 µg L$^{-1}$, for MMA 3 µg L$^{-1}$ and for arsenate 9 µg L$^{-1}$.
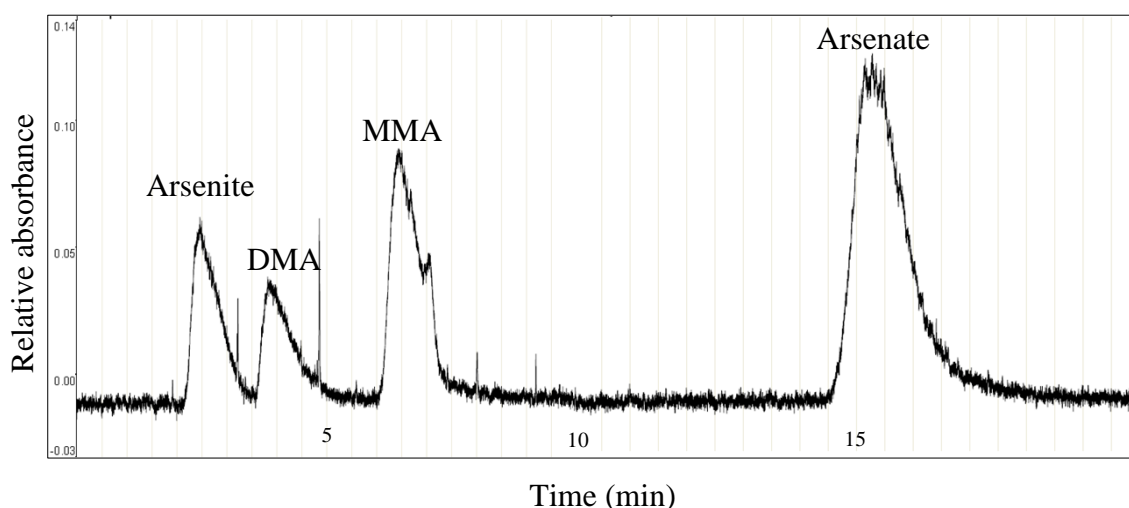


*Fig. 6. Arsenic standard peaks separated with a Hamilton PRP X-100 (250mm x 4.6 mm) anion exchange column and detected with atomic absorption spectrophotometer vapour generation technique.*

# 4. Results and discussion

## 4.1 Soil and plant concentrations of As in terrestrial and emergent plants

The plants in this study accumulated As to various degrees. The concentration ranges of As in plants and soil from Sweden and Slovakia are listed in table 1.

*Table 1. The span of As (mg As kg-1 DW) in the shoots and roots of submerged, emergent and terrestrial plants and in soil from Sweden and Slovakia. Mean±SE. No submerged plants were collected in Slovakia.*

|  | Slovakia | | Sweden | |
|---|---|---|---|---|
| Soil | 45-100000 | | 2-2400 | |
|  | Shoots | Roots | Shoots | Roots |
| Submerged | - | - | 4 – 33 | 65 – 273 |
| Emergent | 1.6 - 642 | 8 - 1623 | 0.2 - 22 | 5 - 514 |
| Terrestrial | 2 – 49 | 3.7 - 377 | nd - 24 | nd - 92 |

The $[As]_{soil}$ has a direct influence on plants. For example, a reduction in crop yield could be seen in soil with an As concentration ranging in between 25-85 mg kg$^{-1}$ (DW) (SGU, 2005). The $[As]_{shoot}$ in emergent plants from mine sites in both Sweden and Slovakia and terrestrial plants from Slovakia were correlated with the $[As]_{soil}$ ($p<0.05$) (Fig.7). Previous results in 14 analysed plant species showed the same pattern (Martinez-Sánchez *et al*. 2011). Terrestrial plants from Sweden show an indication of correlation between the $[As]_{soil}$ and the $[As]_{shoot}$ ($p>0.05$) (fig. 7).
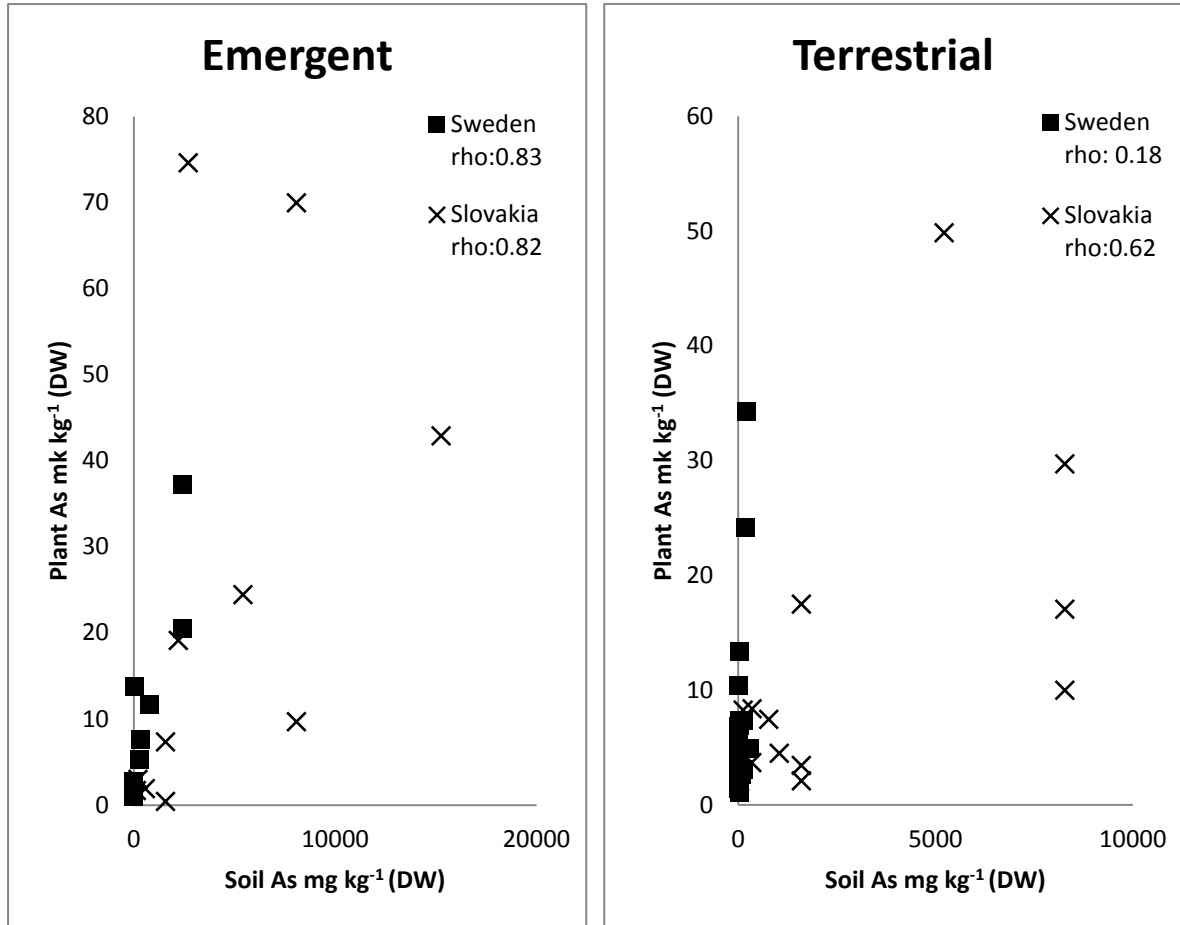
Figure 7. [As]$_{shoot}$ versus [As]$_{soil}$ in emergent and terrestrial plants from mine sites. The results show a positive correlation between [As]$_{shoot}$ and [As]$_{soil}$ for emergent plants from both Sweden and Slovakia and for terrestrial plants from Slovakia ($p<0.05$). Terrestrial plant from Sweden show an indication of a positive correlation ($p>0.05$).

## 4.2 Arsenic speciation

Inorganic species of As are generally considered to be the most toxic to humans. Inorganic forms of As were predominating in plants collected at Boliden and Sjösa (Paper 1). The same As species predominated both in the roots and the shoots (Paper I). Similar processes may occur during transport of As throughout the plant body, for example after binding of As to phytochelatins (Tripathi *et al*. 2007), and could result in a similar As speciation pattern between roots and shoots. Inorganic arsenate usually predominates in plants (Jedynak *et al*.

2009; Smith *et* al. 2008; Zheng *et al.* 2003), and also in a number of different crops and vegetables (Signes-Pastor *et al.* 2008; Smith *et al.* 2009). The predomination of arsenate in plants fails to correspond to the general agreement that arsenite is the main storage form of As in plants as presented in figure 4. A lack of enzymatic activity for the reduction of arsenate to arsenite in some plants has been proposed as an explanation for these results (Mattusch et al. 2000). The bioaccessibility of inorganic As in rice is high (Juhasz *et al.* 2006). Therefore, the risks of intake of inorganic As from crops like rice is imminent.

Methylarsonic acid (MMA) was found in the roots and shoots in five out of six investigated plant species, indicating that organic As also was a common feature in the plants (Paper I).

## 4.3 Distribution of As in plants

The $[As]_{shoot}:[As]_{root}$ ratio was low for most plants analysed (Paper 1). This has previously been shown also in *Spartina pectinata, Oryza sativa* and *Silene vulgaris* (Rofkar & Dwyer, 2011; Azizur Rahman *et al.* 2007; Sneller *et al.* 1999). Terrestrial plants showed a higher $[As]_{shoot}:[As]_{root}$ ratio than emergent plants (Paper 1). Terrestrial plants from both Sweden and Slovakia had a higher $[As]_{shoot}:[As]_{root}$ ratio than emergent plants ($p<0.05$) (Table 2). Differences between the different habitats, for example a lower soil redox potential in the emergent habitats, leading to the formation of iron plaque on the roots of emergent plants, could explain the lower $[As]_{shoot}:[As]_{root}$ ratio in these plants. It was proposed that As is restricted from the vascular system through adsorbance and precipitation on the iron plaque (Bravin et al. 2008).

*Table 2. [As]$_{shoot}$:[As]$_{root}$ ratio of emergent and terrestrial plants from mine and non-mine sites in Slovakia and Sweden. Mean ± SE. Mine sites: Sweden n=35, Slovakia n=24, non-mine sites: Sweden n=42.*

| | Mine sites | | Non-mine sites | |
|---|---|---|---|---|
| | Emergent | Terrestrial | Emergent | Terrestrial |
| Slovakia [As$_{shoot}$]: [As$_{root}$] | 0.13±0.04 | 0.45±0.10 | | |
| Sweden [As$_{shoot}$]: [As$_{root}$] | 0.15±0.05 | 1.22±0.42 | 0.10±0.06 | 0.39±0.15 |

A few plant species had a [As]$_{shoot}$:[As]$_{root}$ ratio above one, for example *Arabis arenosa, Picea abies, Empetrum nigrum* and *Taraxacum sp.* (Paper 1). Arsenic speciation might explain these results. For example, *Empetrum nigrum,* had a relatively high amount of arsenite and MMA, compared with arsenate (Paper 1). Plant species with predominantly arsenate all had a low [As]$_{shoot}$:[As]$_{root}$ ratio. With arsenite as the predominate As species, a higher translocation of As from the roots to the shoots may occur due to chelating of arsenite by phytochelatins (Smith *et al.* 2008). Addition of dimercaptosuccinate, an arsenic chelator with similar chemical properties as phytochelatins, to hydroponic cultures of Indian mustard, led to a 5-fold increase of As translocation to the shoot compared to control plants (Pickering *et al.* 2000).

## 4.4 Accumulation of arsenic in roots and shoots

Arsenic accumulation differs between plant species and individuals. For example, different cultivars of rice differ in the accumulation of As in the grains, as well as in the relative amounts of organic and inorganic As in the grain (Norton *et al.* 2009). Both emergent and terrestrial plants from Swedish mine sites had a higher accumulation factor (AF) than plants from Slovakian mine sites ($p < 0.05$) (Fig. 8). Plants from Swedish mine sites also had a higher AF than plants from other locations in Sweden ($p < 0.05$).
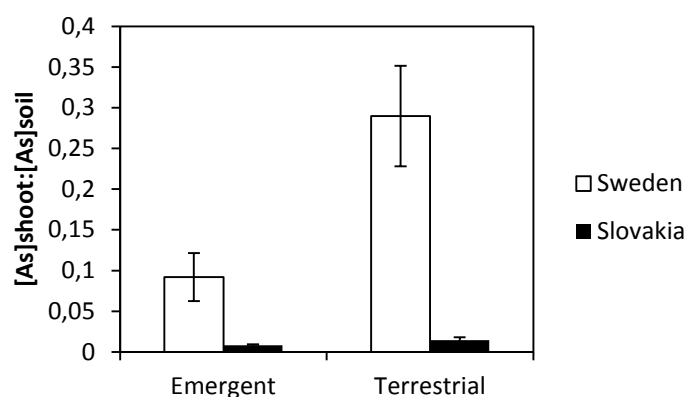
*Fig. 8. Accumulation factor [As]<sub>shoot</sub>:[As]<sub>soil</sub> for the shoots of emergent and terrestrial plants collected in mine sites in Sweden and Slovakia*

The plants from the Swedish mine sites are all located in the same area in Sweden (the Skellefteå field). Differences in the plant cultivars from the Skellefteå field could explain the higher AF. For example, *Taraxacum sp.* collected in the Skellefteå field had a 25-35 times higher $[As]_{shoot}$:$[As]_{root}$ ratio than *Taraxacum sp.* collected in Slovakia (Table 3). *Taraxacum officinale* was earlier shown to accumulate high levels of Pb and Cd (Pichtel *et al.* 2000).

*Table 3. Arsenic concentration (mg As kg$^{-1}$ DW), accumulation factors and [As]$_{shoot}$:[As]$_{root}$ ratio in shoots, roots and soil of Taraxacum sp.. Mean± SE. Plants collected in 3 replicates. Soil collected in pooled samples.*

| Species | | Taraxacum sp.1 Slovakia | Taraxacum sp.2 Slovakia | Taraxacum sp. Sweden |
|---|---|---|---|---|
| Soil | | 348.89 | 5228.83 | 218.23 |
| As concentration | Shoots | 3.69 ±2.11 | 49.82 ±19.82 | 34.30 ±11.56 |
| | Roots | 13.76 ±4.10 | 377.59 ±155.37 | 3.92 ±0.42 |
| Accumulation factor | Shoots | 0.01 ±0.01 | 0.01 ±0.00 | 0.16 ±0.05 |
| | Roots | 0.04 ±0.01 | 0.07 ±0.03 | 0.02 ±0.00 |
| [As]<sub>shoot</sub>:[As]<sub>root</sub> | | 0.25 ±0.08 | 0.34 ±0.29 | 8.71 ±3.08 |

### 4.4.1 Accumulation of As in submerged plants

Submerged plants had a higher AF in both roots and shoots than emergent and terrestrial plants (Paper 1). One reason for the high As accumulation in submerged plants could be that,

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in addition to As uptake via roots, uptake of As also occurs via submerged leaves from the water column (Fig. 3). The high AF could also be explained by higher levels of arsenite in the submerged habitats compared with emergent and terrestrial habitats. Plants can accumulate higher amounts of As when exposed to arsenite compared with arsenate at similar concentrations (Abedin *et al.* 2002; Srivastava *et al.* 2007). The redox potential has a strong influence on As speciation where the presence of oxygen (pE > 10), arsenate is the dominating species, while under poor oxygen conditions (pE < 6), like in submerged soils, arsenite is the dominating species of As (Sadiq, 1997).

A general overview of the accumulation and distribution of As in submerged, emergent and terrestrial plants is shown in figure 9.
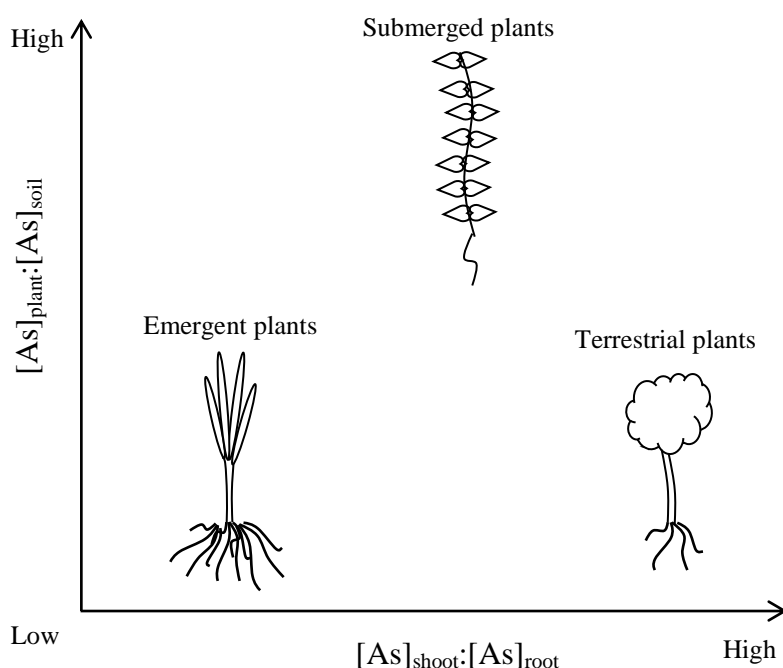


Fig. 9. Relative distribution ($[As]_{shoot}:[As]_{root}$) and accumulation ($[As]_{plant}:[As]_{soil}$) of As in submerged, emergent and terrestrial plants.

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## 4.5 Accumulation and speciation in crops and vegetables

Arsenic-speciation analysis of lettuce cultivated for 24 hours in 10 µM arsenite or arsenate-solutions for the study of As uptake, showed that the inorganic As species arsenate and arsenite predominated in the edible plant parts (Table 4). No organic As was detected (Table 4).

Table 4. Arsenic species found in edible parts of lettuce cultivated in 10 µM arsenate or 10 µM arsenite for 4 days in hydroponic conditions. No organic As was detected. Mean ±SE. (mg kg-1 DW)

*Table 4. Arsenic species found in edible parts of lettuce cultivated in 10 µM arsenate or 10 µM arsenite for 4 days in hydroponic conditions. No organic As was detected. Mean ±SE. (mg kg$^{-1}$ DW).*

| Treatment (10 µM) | Shoots | | Roots | |
|---|---|---|---|---|
| | Arsenite | Arsenate | Arsenite | Arsenate |
| Arsenate | 7.69 ±0.25 | 2.45 ±0.55 | 358.8 ±116.7 | 38.78 ±14.40 |
| Arsenite | 6.57 ±0.53 | 0.61 ±0.31 | 427.5 ±17.70 | 36.37 ±2.17 |

The amount of arsenite was significantly higher than arsenate in both shoots and roots regardless of treatment ($p < 0.05$) (Table 4). These results in lettuce could be explained by a specific As species conversion and the binding of As in the cell walls or to phytochelatins and stored in the vacuole according to the scheme presented in figure 4.

The limit for daily intake of As from drinking water is 10 µg L$^{-1}$ (Commission Directive 2003/40/EC). This limit is based on calculations of inorganic As. Analysis of the As species in lettuce showed that inorganic As was predominating (Table 4). These results highlight the risks that the more toxic inorganic As species can enter into the food chain. Lettuce cultivated in As containing agricultural alum-shale soil in the greenhouse contained 1.47±0.26 mg/kg (DW) (Greger, 2006). Consumption of approximately 50 g (FW) of this lettuce equals to the exposure of 10 µg of As.

Edible parts of carrot, lettuce and spinach contained 27, 20 and 7 mg As kg$^{-1}$ (DW), respectively, after cultivation in As contaminated soil from an abandoned glass works (Fig 10). The AF for the carrot, lettuce and spinach was 0.05, 0.04 and 0.01, respectively. Spinach, which had the lowest As concentration, was harvested after three weeks to prevent blooming, while the carrots and lettuce were harvested after five weeks when the edible parts were fully developed.
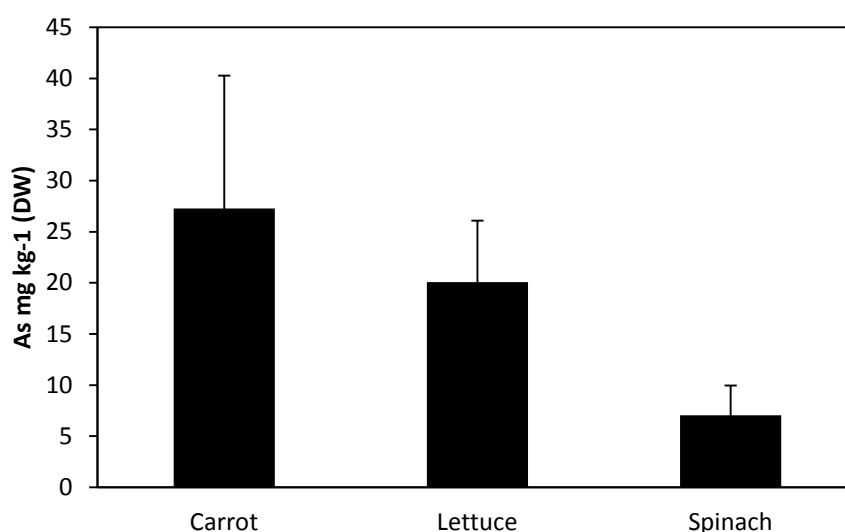


Fig. 10. Total As concentration (mg kg$^{-1}$ DW) in edible parts of plants cultivated in greenhouse grown in soil (514 mg As kg$^{-1}$ DW).

A normal sized carrot of 75 g (fresh weight) cultivated in the greenhouse soil used in this study contained approximately 100 μg of As (Fig. 10), which is 10 times higher than the limit daily intake of As from drinking water. Assuming no other sources of As, a normal sized fresh weight carrot of 75 g should not contain more than approximately 2.5 mg As kg$^{-1}$ (DW) to keep the daily intake below 10 μg of As.

Alfalfa (*Medicago sativa*) and oats (*Avena sativa*) grown in As containing agricultural alum-shale soil in Sweden contained 0.37±0.14 and 0.29±0.05 mg As kg$^{-1}$ (DW), respectively

(Paper 1). These plant species could be a potential source of As intake for both humans and livestock. For example, approximately 35 g (DW) of the oats mentioned above, which corresponds to about 1 dl of porridge oats, equals to the exposure of 10 µg of As.

## 5. Conclusions

With increasing $[As]_{soil}$, the $[As]_{plant}$ increases, except for submerged plants, where accumulation of As may be high even at low $[As]_{sediment}$, probably due to foliar uptake of As.

Inorganic species of As predominate in all plants analysed. The intake of As via food could pose a threat to humans or animals via food chains, for example via rice (South East Asia) and oats (Sweden).

Plants from Swedish mine sites had a higher As accumulation factor then plants from Slovakian mine sites, possibly due to differences between plant cultivars.

Plant species with an unusual high $[As]_{shoot}:[As]_{root}$ ratio like *Arabis arenosa, Taraxacum sp.*, and *Empetrum nigrum* may be used to explore the underlying mechanisms for As transport from roots to shoots.

Selection and breeding processes to achieve cultivars of different crops and vegetables with low As accumulation, low levels of inorganic As and low translocation of As from roots to shoots is of great importance in order to minimize problems with As.

## 6. Future perspectives

The possible influence of external oxygen levels on accumulation and speciation of As in plants needs to be investigated further in experiments performed under controlled oxygen concentrations. Also, the extraction abilities of As by submerged plant species have to be elucidated more thoroughly. The As-extraction abilities of submerged plants could prove to be interesting for phytoremediation of water. Moreover, the predomination of inorganic As species in plants used for human consumption also needs further attention. The risks of As intake through vegetables and crops needs additional investigation.

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