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Determination of arsenic species in water, soils and plants

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Abstract Ion chromatographic separation coupled with ICP-MS was used to determine arsenic species in plant and soil extracts. A scheme for growth, harvesting, sample pre-treatment and analysis was developed for the arsenic species to enable determination. Preliminary results obtained with ten herb plants grown on arsenic-contaminated soil compared to non-contaminated soil show a heterogeneous pattern of accumulation rate, metabolization and detoxification mechanisms in monocots and dicots. Arsenite appears to be the major component in plants with good growth. Organic arsenic species were even detected at very low concentrations (< 150 μg kg^{-1} (dry mass)).

1 Introduction

Arsenic is a major contaminant in groundwater and soils in mining areas. Fine-grained waste material from tin-ore mining near Altenberg (Saxony) deposited in tailings emits seepage contaminated by arsenite. Plants began to grow on the tailings surface about five years ago. The tailings contain circa 0.02–0.03% total arsenic in the solid phase and the ferric oxyhydroxide precipitate, forming sediments with up to 8% of arsenic in the downstream river [1]. Plants provides a suitable way of accumulating arsenic from the soil in order to decontaminate it [2–6]. However, although knowledge of the total arsenic can be used to calculate the overall efficiency of arsenic input, it does not indicate anything concerning its transfer mechanism from the soil to the leaves or its behavior in the plants. Possible pathways for the metabolization of arsenic in soil and aquatic organisms by methylation were intensively discussed [7–9].

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Previously developed analytical techniques combining a separation step with an element-specific detector to determine arsenic species [10–15] might enable the arsenic pathways through the plants to be monitored, so that the capabilities and efficiency of soil remediation by growing plants can be estimated.

This paper describes an analytical procedure for determining arsenic species and present selected results of the study of plants grown on mixtures of standard soil containing arsenic-contaminated tailings material.

2 Experimental

2.1 IC-ICP-MS procedure. The analytical equipment and the procedure used for these investigations were described previously [11]. Figure 1 shows a typical plot for the application of this ion chromatographic separation coupled with an ICP-MS as a detector (IC-ICP-MS) for a standard solution of eight arsenic species with gradient elution by dilute nitric acid. Calibration was performed with standard solutions containing all arsenic components of interest in the concentration range of 1 to 100 μg As L^{-1} each. The linearity of the calibration plots was better than 0.998. The LODs calculated with the software SQS (Perkin Elmer) were as follows: arsenite (As(III)) 0.64 μg As L^{-1}, methylarsonic acid (MA) 1.67 μg As L^{-1}, dimethylarsinic acid (DMA) 0.78 μg As L^{-1}, arsenate (As(V)) 2.19 μg As L^{-1}, arsenobetaine (AsB) 0.76 μg As L^{-1} and arsenocholine (AsC) 1.69 μg As L^{-1}.

2.2 Plant materials and growth. Herb plants occurring under the geographical and meteorological conditions of Altenberg, Saxony, were selected for the greenhouse experiments. A series of 10 plant species cultivated on a reference soil (RS; University of Leipzig, Botanical Garden) and a 1 : 1 (by volume) mixture of this reference soil and tailings material (MST) contained monocots and dicots of the herbs listed in Table 1. This table also contains a qualitative estimation of the plants' growth on MST compared to growth on RS. The pre-germinated seedlings were homogeneously distributed on the soil-filled petri dishes (i.d. 20 cm, height 5 cm) and watered with bidistilled water twice weekly. After 12 weeks' growth, the relevant amounts of plant material were harvested and treated as described below.

2.3 Sample pre-treatment and extraction procedures. A microwave digestion procedure using HNO_3 and H_2O_2 was used to determine the total arsenic in plant and soil materials. Regarding the determination of arsenic species, a moderate extraction method consisting of shaking the grinded plant tissues with bidistilled water (2 h, over-

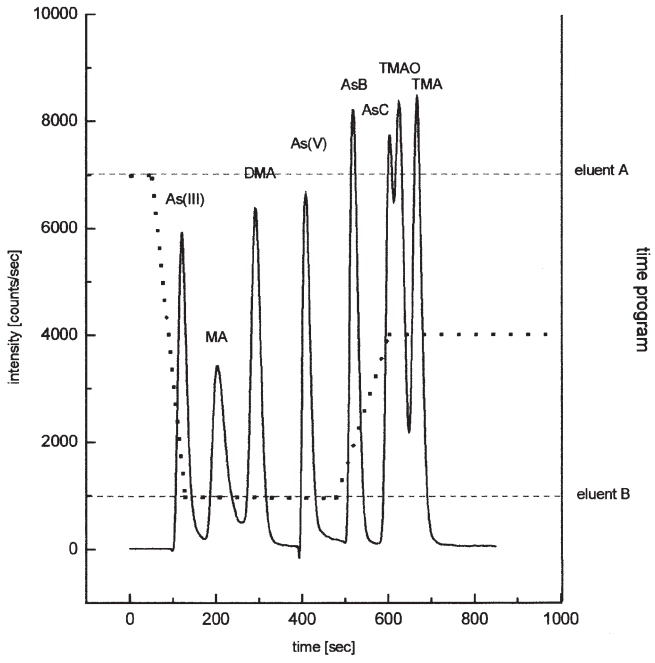

Fig. 1 Chromatogram (IC-ICP-MS) of a standard solution of eight arsenic species. Conditions: Eluent A: 0.4 mM HNO_3, 0.05 mM benzene-1,2-disulfonic acid, Eluent B: 50 mM HNO_3, 0.05 mM benzene-1,2-disulfonic acid, 200 µL injected, ICP-MS detection: m/z 75, cross-flow nebulizer, concentration of each arsenic species 20 µg L^{-1}, TMAO (trimethylarsine oxide), TMA (tetramethylarsonium ion)

Table 1 Plants under greenhouse investigations

Number[a]	Plant species	Identification of growth on MST[b]
D,1	*Artemisia vulgaris*	– –
D,2	*Polygonum persicaria*	–
M,3	*Phleum pratense*	–
D,4	*Silene vulgaris*	+ +
M,5	*Agrostis capillaris*	–
M,6	*Carex leporina*	+
D,7	*Trifolium repens*	– –
D,8	*Rumex acetosella*	– –
M,9	*Calamagrostis epigejos*	+ +
D,10	*Plantago major*	– –

[a] M...monocots, D...dicots,
[b] – –... died, –... poor growth, +... moderate growth, ++... normal growth

head shaking) was preferred to prevent species transformation during this process. The same extracts were also used for the ICP-MS determination of total amounts of water-extractable arsenic. The extracts were filtered (0.45 µm) and/or centrifuged (10 min, 1600 g), and aliquots of the supernatant were immediately subjected to chromatographic analysis.

3 Results and discussion

The uptake of arsenic by plants grown on MST was studied and compared with those grown on RS. In addition, the arsenic in both soils (RS and MST) available to the plants was determined in water extracts from the soil samples. The mean concentration of arsenic in reference soil was 0.20 ± 0.03 mg kg^{-1} (dry mass). The concentrations in the plants grown on this soil varied between 0.1 and 1.9 mg kg^{-1} (dry mass). In contrast to this reference soil, the MST contained a higher water-extractable arsenic level of 10.0 ± 0.5 mg kg^{-1} (dry mass), which is reflected in a substantially greater arsenic concentration of 10 to 95 mg kg^{-1} (dry mass) in the plants. However, the different concentrations of arsenic in plants grown on the contaminated soil do not correspond with their botanical properties (size, number of leaves, color) at the time of harvesting. From the results in Fig. 2 illustrating the uptake efficiency of arsenic depending on the soil (A – RS, B – MST) and on the type of plant (M – monocots, D – dicots), it can be seen that the amounts of arsenic accumulated during the growth are not uniform and do not correlate with the plant type (M or D). Moreover, the order of accumulation efficiency concluded for plants on RS does not hold for MST, as can be seen from the small number of plant species which survived under these conditions, as well as the differences in the arsenic concentration levels in the same plant species grown on different soil conditions. Therefore, it can be assumed that the efficiency of arsenic up-

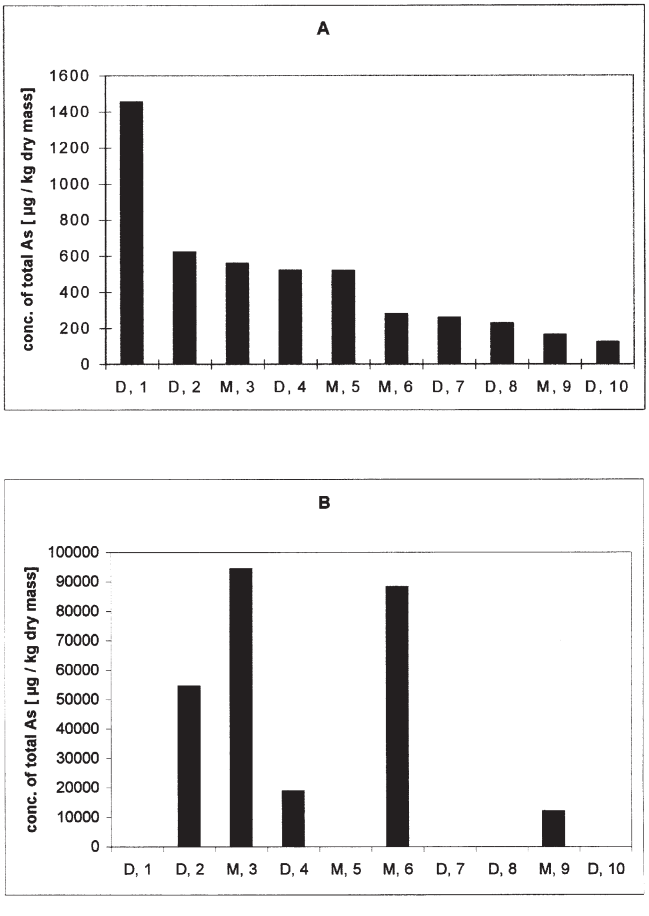

Fig. 2 A, B Uptake efficiencies of water-extractable arsenic by mono- and dicots grown on RS **(A)** and MST **(B)**

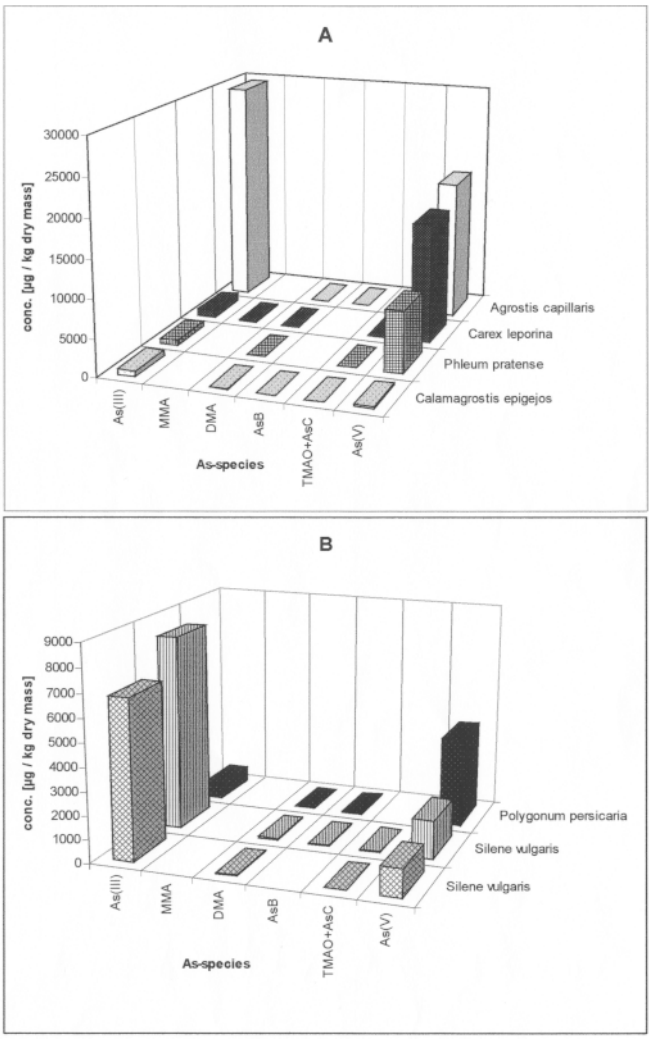

Fig. 3 A, B Arsenic species concentrations determined in overground vegetative organs of mono- **(A)** and dicots **(B)** grown on MST

take depends on both the form of arsenic in the soil and on the ability to activate it for uptake. In the case of RS, arsenic mainly exists as soluble arsenate or adsorbed on humic substances. The binding of arsenic on the MST matrix also includes adsorbates on ferric oxyhydroxides and in crystalline forms such as arsenopyrite.

The broad variety of accumulation efficiencies and compatibility of arsenic among the plants investigated could be explained by differences in the detoxification mechanism. More detailed information can be obtained by determining arsenic species in the various parts of the plants such as the leaves and stalks, and in the corresponding soil.

Although arsenate is the most common form of arsenic occurring in the soil extract (MST), the main arsenic compound in leaves of *Silene vulgaris* is arsenite. Furthermore, other arsenic species were also detected in *Silene vulgaris*, indicating the low ability of plants to detoxify the most toxic inorganic arsenic by methylation. A more efficient way for the plants to protect themselves from interruption of the oxidative phosphorylation [16] by arsenate would seem to be reduction to arsenite and storage in this valance state in the leaves, which closely tallies with the observations of Van den Broeck et al. [17]. After the plant's death, arsenite can be rapidly oxidized again to arsenate by the loss of enzymatic activity. Therefore, it is likely that the arsenite/arsenate ratio is also shifted to arsenate in poorly grown plants. Figure 3 shows the concentration of arsenic species in monocots and dicots. In both cases the major components are arsenate and arsenite. Organic arsenic species were also found, albeit in much smaller concentrations than the inorganic ones. The plants *Calamagrostis epigejos* (monocot) and *Silene vulgaris* (dicot), which are normally grown on MST, demonstrate the probable typical behavior of the accumulation of arsenite in overground vegetative organs of plants. *Carex leporina*, *Phleum pratense* (monocots) and *Polygonum persicaria* (dicot) show a bad plant development stage which can also be identified from the higher arsenate concentration. Despite poor growth, equally high concentrations of arsenite and arsenate were observed in *Agrostis capillaris* (monocot).

Additionally, naturally occurring monocots grown on ferric oxyhydrate precipitate (FeP) formed in the seepage of the dam foot of these tailings, which contains about 8% w/w total arsenic, were also investigated with respect to their arsenic species distribution. In contrast to the high uptake rate of plants grown on MST, the *Holcus lanatus*, *Juncus effusus* and an unidentified grass only accumulated rather moderate total arsenic concentrations in their stalks and leaves. In these plants, too, the predominant arsenic species found in the stalks, leaves and blooms was arsenite (Fig. 4), even though the roots are mainly surrounded by arsenate adsorbed on ferric oxyhydrates. In comparison to the concentration of inorganic arsenic in these plants, relatively high concentrations of organic arsenic species were found. These plants seem to be very well adapted to these growth conditions.

Conclusion

Ion chromatography coupled with ICP-MS is a powerful tool to investigate the distributions of arsenic species in plants and corresponding soil extracts. Using a simple species-preserving extraction method involving water, the proposed gradient separation of eight arsenic species is robust and affords long-term stability for the analysis of aqueous extracts of plant material. The results presented show that in the case of well-grown plants, arsenic exists mainly in the three-valence state. The main arsenic component in plants with poor growth or which have died was found to be arsenate. Organic arsenic compounds were also found in both soils and plants. The plants themselves and the available form of arsenic in the soil (or pore water) is likely to be responsible for their different accumulation rate and compatibility. Further investigations will focus on micro-sample handling to directly analyze arsenic species in certain plant tissues.

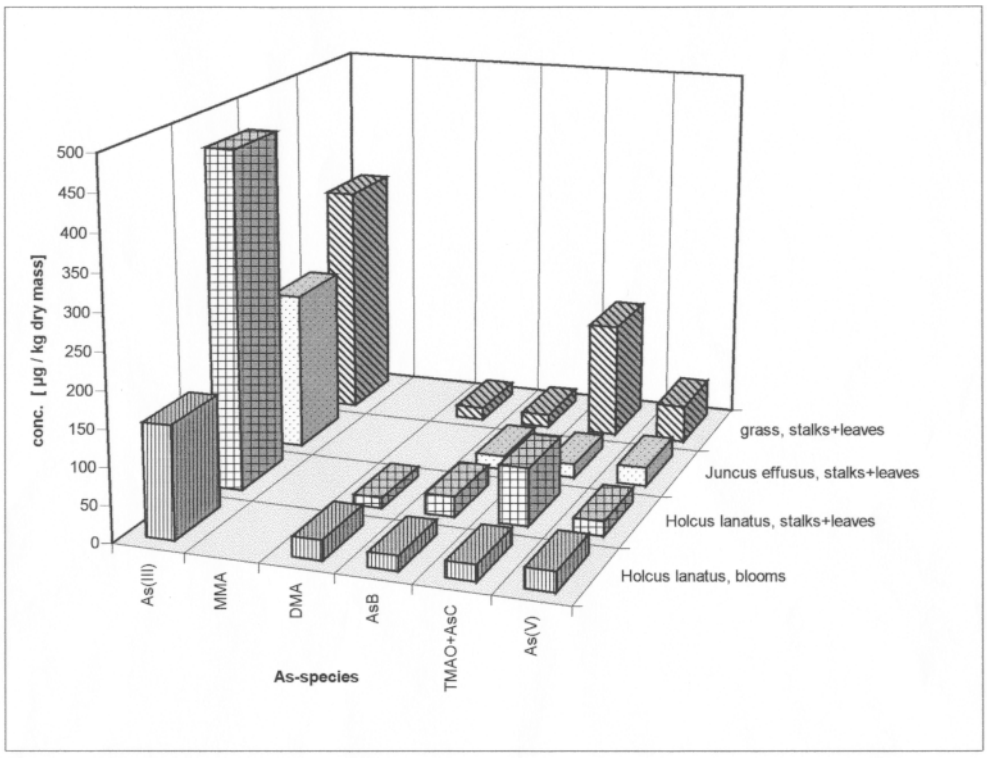

Fig. 4 Arsenic species concentrations in plants grown on ferric oxyhydroxide- precipitates

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