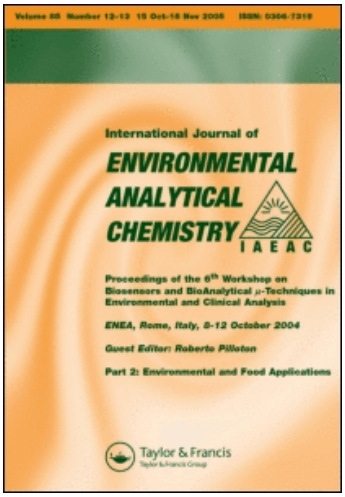

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Factors affecting arsenic speciation in environmental samples: sample drying and storage

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Factors affecting arsenic speciation in environmental samples: sample drying and storage

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Arsenic is a ubiquitous element. Its toxicity, mobility, and bioaccumulation depend usually on its chemical form, and therefore, arsenic speciation is indispensable for the assessment of environmental risk and human hazard. Little is known about the effect of sample preparation procedures, such as drying and storage, on the resulting arsenic speciation. In this study, we investigated the influence of different drying methods and storage conditions on the arsenic speciation in mineral soils, organic soils, and plants. Drying soils and plants using different methods may change the concentrations of the total methanol–water (20%, v/v) extractable arsenic, the proportion of organic arsenic and the ratio of arsenite-to-arsenate. Loss of methanol–water extractable arsenic compounds (up to 63%) was observed particularly in the samples rich in water. Following drying, the speciation of organic arsenic changed less than that of inorganic arsenic. Drying showed little influence on the total arsenic determination. None of the storage methods tested could preserve the arsenic speciation in organic soils and plants, although arsenic speciation after one-month storage varied less in freeze-dried samples than wet samples. Storage of the samples at low temperatures (2 or −20°C) had the largest impact on the samples rich in organic matters, leading to less arsenic being extractable by methanol–water. Both drying and storage of the soil and plant samples changed apparently the arsenic speciation. Therefore, we recommend conducting the arsenic speciation possibly with fresh and wet samples, so that the results of arsenic speciation may be more approaching the original states.

Keywords: Arsenic; Sample drying; Storage; Speciation

1. Introduction

Arsenic is an ubiquitous trace metalloid found in virtually all environmental media. Arsenic is distributed widely throughout the earth's crust and is introduced into water through the dissolution of minerals and ores. Arsenic concentrations in groundwater in some areas are elevated as a result of erosion from local rocks. Anthropogenic sources of arsenic include mining and smelting processes, combustion of fossil fuels and commercial usage of e.g. alloying agents, wood preservatives, insecticides, herbicides, and fungicides [1].

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Arsenic creates adverse effects on the environment and human health due to its toxicity and bioaccumulation. While the toxicity of arsenic is dependent on the chemical form or species, the determination of total arsenic in an environmental sample is not sufficient to access its environmental risk. Thus, speciation analysis is necessary in order to determine the form of arsenic in the sample. In the past, most studies were focused on arsenic speciation in aquatic and terrestrial environmental samples [2] and much efforts were also made to improve the arsenic extraction from environmental samples [3, 4]. However, the influence of the sample preparation process, e.g. drying and storage of the solid samples on the speciation was usually ignored. Freeze-drying seemed the most common and accepted method [5, 6]. The storage and handling of freeze-dried samples is easier as compared to fresh samples and is most probably the reason that extraction of freeze-dried samples was the absolutely dominant method in the studies of speciation. However, Emons *et al*. [7] suggested that freeze-drying of solid samples should be avoided because of possible species transformation and loss of volatile species. Hjorth [8] demonstrated that freeze-drying does not preserve the speciation pattern of major element, trace metal in anoxic lake sediments. Huang and Ilgen [9] observed large losses of inorganic and organic arsenic during freeze-drying of water samples, suggesting the potential loss of arsenic compounds from solid samples. In the past, information about the influence of storing solid samples on arsenic speciation was little available. Therefore, the objectives of this study were to investigate (i) the influence of different drying methods on the arsenic speciation and total arsenic concentrations and (ii) the variation of arsenic speciation in the samples, which were long-term stored at different temperatures.

2. Experimental

2.1 *Instrumentation*

A high performance liquid chromatograph (HPLC) instrument (BIOTEK Instruments, USA), consisting of a gradient pump (System 525), capillary PEEK tubing (0.25 mm i.d.), a 200 μL injection loop (Stainless Steel), and an HPLC autosampler 465 (Kontron Instruments, Germany) was connected to an anion-exchange column (IonPak AG7 and AS7, both Dionex) and coupled to an ICP-MS (Agilent 7500c, Japan), equipped with a concentric nebulizer (Glass Expansion, Australia) and a Scott-type glass spray chamber.

The separation was performed at a flow rate of $1\,mL\,min^{-1}$, using a nitric acid gradient between pH 3.4 and 1.8. Dipotassium salt of benzene-1,2-disulfonic acid (0.05 mM) was added to the eluent as an ion-pairing reagent [10]. At outlet of the separation column, an internal standard ($10\,\mu g\,Ge\,L^{-1}$ in 0.01 M nitric acid) was added by means of a Y-connector.

2.2 *Reagents and standards*

Arsenate (As(V)), arsenite (As (III)) and dimethylarsinic acid (DMA) were purchased from Merck. Arsenbetaine (AsB) was obtained from Fluka and monomethylarsonic acid (MMA), arsencholine (AsC), trimethylarsine oxide (TMAO) and tetramethylarsonium ion (TETRA) as iodide from Argus Chemicals, Italy. De-ionized water used throughout the work was purified in a Milli-Q system (Millipore, Milford, MA).

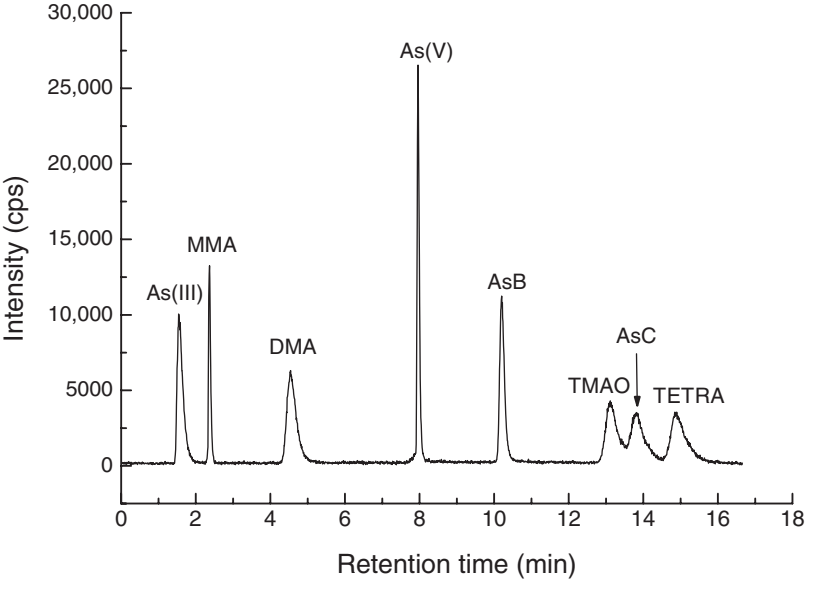

Figure 1. HPLC-Chromatograms of 5 μg As L^{-1} arsenite (As(III)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenate (As(V)), trimethylarsine oxide (TMAO) arsencholine (AsC) and tetramethylarsonium ion (TETRA) standards.

Individual stock solutions (50 mg As L^{-1}) of As(III), As(V), MMA, DMA, AsB, AsC, TMAO and TETRA were prepared in Milli-Q water and stored at 4°C in the dark. A multi-compound working solution with a total concentration of 40 μg As L^{-1} for external calibration was prepared before each use by dilution of the stock solutions with Milli-Q water (figure 1).

2.3 *Sampling and sample preparation*

We chose samples from mineral soils, forest floors, wetland soils, needles and mosses to examine the influence of the drying process and storage on arsenic speciation. They represented the factors: plants (needles and mosses) and soils (mineral soils, forest floors, wetland soils), samples rich (forest floors, wetland soils, plants) or poor (mineral soils) in water, samples rich (forest floors, wetland soils, plants) or poor (mineral soils) in carbon, samples easily or hardly (needles) torn into fine pieces and samples with low (mineral soils and needles) or high (forest floors, wetland soils, mosses) proportion of organic arsenic.

All soils and plants were taken in April 2004 from Lehstenbach catchment in NE-Bavaria, Germany. Mineral soils and forest floors were sampled from Bw-C (brown-colored mineral soil and starting rock enriched) and Oa (forest floor with well-decomposed organic substances) horizons in an upland soil profile at the stand Coulissenhieb. Wetland soils (0–20 cm deep) were taken from a fen (Histosol). Green needles of Norway spruce (*Picea abies* [L.] Karst.) were collected. *Sphagnum* moss was collected in a wetland area. Samples of mineral soil and organic soil (forest floors and wetland soils) were homogenized and sieved to 2 and 5 mm, respectively. Plant samples were shortly rinsed with distilled water.

Part of each fresh sample was analysed without drying. Soils (2.0–10 g) and plants (1.5–3.0 g) were first transferred to 100 mL polyethylene bottles. After adding 10 mL methanol–water (MeOH–H_2O) solution (20%, v/v), the samples were homogenized and torn into fine pieces using an Ultra Turrax (IKA Words, USA). After ultrasonic treatment for 10 min, the samples were centrifuged (8800 g) for 10 min and the supernatant was analysed using HPLC-ICP-MS. The sampling, preparation and analysis of wet samples were all finished within 48 h to prevent transformations of arsenic compounds.

In order to investigate the influence of different drying methods on the arsenic speciation in soils and plants, we dried the samples with freeze drying, nitrogen drying (at 25°C), and air drying at 25 and 60°C. For freeze drying, the soil samples were frozen in an acetone bath cooled with dry ice. The plant samples were directly frozen at −20°C in the deep freezer. All frozen samples were then transferred to a freeze dryer (L05, Wkf, Germany) for drying. Nitrogen-drying was conducted using a Turbo Vap II (Zymark, USA). In principle, N_2-purging at 1 bar evaporates water at 25°C controlled by a water bath. Air drying was made in an oven at 25 and 60°C, respectively. The drying procedure for N_2-drying and air-drying was stopped when the sample weight reached a constant value. After drying, the soil and plant samples were ground.

For analysis of MeOH–H_2O extractable arsenic in dried samples, soil (1.0 g) and plant samples (0.15–0.3 g) were transferred to 15 mL polyethylene tubes. After adding 4 mL MeOH–H_2O solution (20%, v/v), the samples were treated with ultrasound for 10 min and centrifuged (8800 g) for 10 min. The supernatant was analysed with HPLC-ICP-MS.

Quality control of arsenic speciation was conducted using reference material DORM-2 (National Research Council, Canada). Concentrations of AsB (16.4 ± 0.5 μg As g^{-1}) and TETRA (0.23 ± 0.04 μg As g^{-1}) extracted with MeOH–H_2O solution (20%, v/v) and 10 min ultrasound corresponded to 100 and 92% recovery of the certificated values, respectively.

2.4 *Total arsenic analysis*

Soil (0.5 g) and plant (0.2 g) samples were first digested with 3 mL nitric acid (65%) by High Pressure Accelerated Solvent (HPA-S, Anton Paar, Austria) [11]. In the 3-step program, a first heating to 80°C, is followed by heating to 170°C and finally to 270°C, lasting for 90 min. The supernatant was then filtered with membrane filter, diluted to 25 mL with Milli-Q water for further analysis with ICP-MS (Agilent 7500c, Japan). Recovery of total arsenic from the certificated tomato leaf 1573a (NIST) was 95%.

3. Results and discussion

3.1 *Arsenic speciation in soil and plant samples dried with different methods*

In the needles and mosses, As(III) was the predominant form of arsenic. Beside As(III) and As(V), organic arsenic (DMA, AsB, TMAO, and two unidentified organic arsenic) were detected (table 1), which account up to 3.3 and 38% of total MeOH–H_2O extractable arsenic. In needles, the concentrations of total extractable arsenic were

Table 1. Concentrations (ng As g^{-1} dry weight) of methanol–water extractable arsenic compounds in soils and plants dried with different methods.

	As(III)	As(V)	MMA	DMA	Unknown 1	AsB	Unknown 2	TMAO	Total As	Org As (%)	As extracted (%)
Needle											
Wet sample	27.6 ± 1.2	7.08 ± 0.58	<DL	1.95 ± 0.28	<DL	0.61 ± 0.19	0.67 ± 0.12	<DL	37.9 ± 2.1	1.62	27.1
N_2-dry at 25°C	34.3 ± 4.4	11.9 ± 0.5	<DL	3.31 ± 0.28	<DL	2.42 ± 0.63	1.87 ± 0.76	<DL	53.8 ± 4.3	3.04	38.4
Freeze-dry	26.2 ± 0.2	10.6 ± 0.9	<DL	3.70 ± 0.08	<DL	0.37 ± 0.52	1.59 ± 0.11	<DL	42.5 ± 1.0	3.30	30.4
Air-dry at 25°C	36.3 ± 3.6	13.3 ± 1.1	<DL	3.21 ± 0.46	<DL	1.15 ± 0.21	1.88 ± 0.39	<DL	55.8 ± 5.8	3.03	39.9
Air-dry at 60°C	69.9 ± 3.1	9.78 ± 0.33	<DL	4.05 ± 0.05	<DL	1.38 ± 0.92	3.03 ± 0.76	<DL	88.2 ± 4.4	3.14	63.0
Moss											
Wet sample	132 ± 25	37.6 ± 5.0	<DL	7.40 ± 0.18	11.2 ± 0.9	3.89 ± 0.53	7.75 ± 1.60	3.32 ± 1.09	204 ± 28	16.5	70.4
N_2-dry at 25°C	54.1 ± 4.6	21.9 ± 0.3	<DL	8.18 ± 1.07	22.1 ± 1.5	3.97 ± 0.87	8.19 ± 1.73	5.31 ± 1.43	123 ± 12	38.6	42.4
Freeze-dry	50.5 ± 12.3	22.7 ± 4.2	<DL	4.87 ± 0.98	18.6 ± 3.8	3.19 ± 0.18	6.59 ± 0.57	0.56 ± 0.80	107 ± 22	31.6	36.9
Air-dry at 25°C	50.1 ± 2.3	25.4 ± 1.3	<DL	8.93 ± 0.07	12.6 ± 2.0	3.44 ± 1.04	5.26 ± 0.84	4.02 ± 1.05	110 ± 8	31.2	37.9
Air-dry at 60°C	36.4 ± 2.9	54.7 ± 7.8	<DL	6.68 ± 1.16	19.7 ± 4.3	1.76 ± 2.50	5.97 ± 1.26	5.88 ± 1.60	131 ± 17	30.5	45.2
Forest floor											
Wet sample	15.0 ± 1.9	152 ± 6	<DL	6.78 ± 0.27	<DL	8.42 ± 0.58	<DL	0.69 ± 0.45	183 ± 8	8.67	1.11
N_2-dry at 25°C	5.53 ± 0.33	48.0 ± 1.1	<DL	4.28 ± 0.10	<DL	8.21 ± 0.46	<DL	1.22 ± 0.09	67.2 ± 1.0	20.4	0.41
Freeze-dry	13.3 ± 3.6	59.9 ± 0.4	<DL	4.11 ± 0.39	<DL	9.57 ± 1.83	<DL	0.88 ± 0.11	87.8 ± 5.5	16.6	0.53
Air-dry at 25°C	11.0 ± 0.1	53.5 ± 0.8	<DL	4.55 ± 0.09	<DL	9.30 ± 0.26	<DL	1.40 ± 0.05	79.7 ± 1.3	19.1	0.48
Air-dry at 60°C	39.8 ± 4.2	71.0 ± 2.3	<DL	5.17 ± 0.22	<DL	11.3 ± 1.1	<DL	2.86 ± 0.20	130 ± 8	14.8	0.79
Wetland soil											
Wet sample	2.43 ± 0.10	23.9 ± 0.9	1.36 ± 0.08	0.54 ± 0.03	0.88 ± 0.05	2.34 ± 0.10	<DL	<DL	31.4 ± 1.0	16.3	0.09
N_2-dry at 25°C	2.46 ± 0.2	10.5 ± 0.1	0.84 ± 0.01	0.19 ± 0.03	0.80 ± 0.03	3.95 ± 0.15	<DL	0.65 ± 0.14	19.4 ± 0.2	33.2	0.05
Freeze-dry	10.4 ± 1.7	11.1 ± 0.4	0.86 ± 0.18	0.16 ± 0.03	0.42 ± 0.08	4.25 ± 0.98	<DL	0.32 ± 0.32	27.4 ± 2.9	21.9	0.07
Air-dry at 25°C	5.29 ± 0.20	12.5 ± 0.5	0.75 ± 0.09	0.16 ± 0.06	0.47 ± 0.07	3.39 ± 0.02	<DL	0.41 ± 0.52	23.0 ± 1.3	22.6	0.06
Air-dry at 60°C	20.3 ± 1.6	12.6 ± 0.5	0.83 ± 0.09	0.34 ± 0.01	0.51 ± 0.05	3.80 ± 0.35	<DL	1.30 ± 0.01	39.6 ± 2.6	17.1	0.11
Mineral soil											
Wet sample	3.48 ± 1.01	1.53 ± 0.13	<DL	<DL	<DL	<DL	<DL	<DL	5.10 ± 1.04	–	0.09
N_2-dry at 25°C	3.29 ± 0.32	2.46 ± 0.77	<DL	<DL	<DL	<DL	<DL	<DL	6.02 ± 0.26	–	0.10
Freeze-dry	2.92 ± 0.19	2.34 ± 0.10	<DL	<DL	<DL	<DL	<DL	<DL	5.50 ± 0.32	–	0.09
Air-dry at 25°C	3.32 ± 0.01	2.20 ± 0.10	<DL	<DL	<DL	<DL	<DL	<DL	5.85 ± 0.09	–	0.10
Air-dry at 60°C	3.24 ± 0.02	2.10 ± 0.23	<DL	<DL	<DL	<DL	<DL	<DL	5.77 ± 0.19	–	0.10

Mean values and standard derivations of three extraction replicates are shown. DL: detection limit = 0.1 ng As g^{-1}.

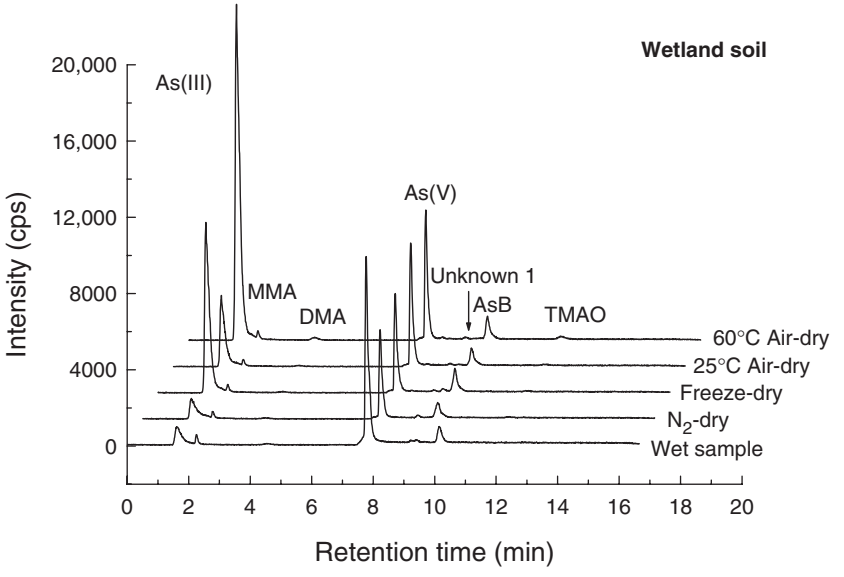

Figure 2. HPLC-Chromatograms of methanol–water extractable arsenic compounds (arsenite (As(III)), monomethylarsonic acid (MMA), dimethylarsinic acid (DMA), arsenate (As(V)) and trimethylarsine oxide (TMAO)) in wetland soils dried with different methods and wet samples.

in the order: 60°C air-dried > 25°C air-dried and N_2-dried > freeze-dried and wet samples. We observed higher concentrations of extractable organic arsenic in most dried needles and mosses compared to the wet samples.

The wetland soils and forest floors consist of dead plant tissues decomposed to different extents and with carbon contents >40% [12]. Organic arsenic (MMA, DMA, AsB, and TMAO) accounted up to 20 and 33% of the total extractable arsenic in forest floors and wetland soils (figure 2), respectively. For both organic soils, the proportion of organic arsenic was in the order: N_2-dried > 25°C air-dried ≥ freeze-dried > 60°C air-dried > wet samples. The concentrations of MMA, DMA, and 'unknown 1' decreased after drying, whereas the concentrations of AsB and TMAO increased. The total MeOH–H_2O extractable arsenic was in the order: wet or 60°C air-dried > freeze-dried > 25°C air-dried > N_2-dried organic soils.

Arsenic speciation in mineral soils showed only inorganic arsenic. The concentrations of total MeOH–H_2O extractable arsenic showed a small variation among the different drying methods (5.10–6.02 ng As g^{-1}).

We have demonstrated that drying using different methods result in different arsenic speciation, including the As(III)/As(V) ratio, total extractable arsenic, and percentages of organic arsenic. The mineral soils showed less variation of arsenic speciation than the organic samples (plants and organic soils). The lower concentrations of total extractable arsenic in wet needles as related to the dried needles may reflect the difficulty to tear the wet needles into fine pieces using an Ultra Turrax. The dried mosses and organic soils had much lower concentrations of extractable total arsenic than the wet samples. We suspect loss of MeOH–H_2O extractable arsenic during the drying [9], which usually took several days because mosses, forest floors, and wetland soils contained large amounts of water (ca. 90, 50, and 75%, respectively). On the other hand, drying may result in a change of the sample composition or redistribution

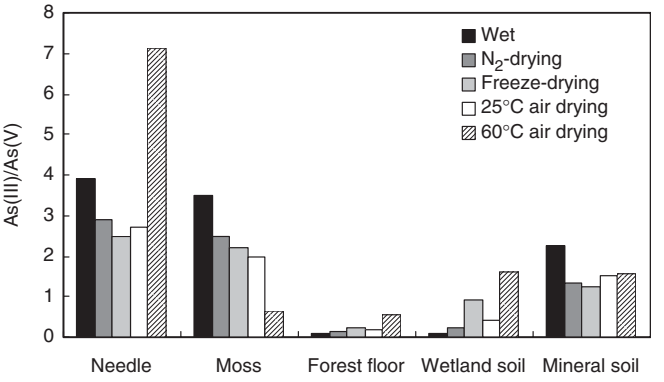

Figure 3. Ratio of methanol–water extractable arsenite (As(III)) to arsenate (As(V)) in soil and plant samples dried with different methods or wet samples.

of arsenic compounds into other phases, which immobilize parts of the extractable arsenic, especially inorganic arsenic, from the samples. Krachler and Emons [13] suggested that extraction yields for freeze-dried samples might be lower because the organic structure of the samples is altered upon freeze-drying and may lead to the formation of agglomerates that hamper the extraction of the analyte. Rapin *et al.* [14] suggested that some minerals become more crystalline during the freeze-drying. The concentrations of total extractable arsenic in organic samples dried at 60°C were much higher than those dried under other conditions, suggesting a collapse of organic matters in the samples at high temperatures. Subsequently, more arsenic may be released.

The ratio of extractable As(III)/As(V) sank in needles, mosses, and mineral soils after drying (figure 3), and decreased dramatically in mosses air dried at 60°C. However, the extractable As(III)/As(V) ratio increased largely in needles air-dried at 60°C. In organic soils, the ratio of extractable As(III)/As(V) increased after drying. The ratio increased especially in the cases of 60°C air-dried and freeze-dried wetland soils (figures 2 and 3) and 60°C air-dried forest floor materials (figure 3). Arsenite may oxidize to As(V) in contact with the atmospheric oxygen during drying. This took place more likely in the case of mineral soils. In contrast, reduction of As(V) to As(III) may be accompanied with the oxidation of the organic matters in samples rich in organic matters, for example forest floors and wetland soils. Arsenite is usually more soluble and mobile than As(V), thereby, change of the valency of inorganic arsenic may alert the mobility of inorganic arsenic in soils. Subsequently such conversion may lead to different arsenic release from samples into extracts. Conversion between As(III) and As(V) together with the modification of arsenic binding to solid phases after drying may result in the shift of As(III)/As(V) ratios. However, we were not able to differentiate their relative relevance.

The variation in speciation of organic arsenic was less as compared to that of the inorganic arsenic, probably because the higher stability and mobility of organic arsenic over inorganic arsenic [15]. However, a slight increase or decrease of extractable organic arsenic concentrations still could be observed in some cases. Such variation seemed to be caused by organic arsenic degradation or mobilization after drying. We observed the strongest decrease of extractable TMAO and DMA in freeze-dried samples (table 1), indicating freeze-drying may result in demethylation of organic arsenic. Similar dealkylation of organotin compounds was reported by Shawky *et al.* [16]. The increase of organic arsenic in the dried samples may result from mobilization

Table 2. Concentrations of total arsenic in wet and dried soil and plant samples.

	Total As ($\mu g\,As\,g^{-1}$ dry weight)
Needle	
Wet sample	–
N_2-dry at 25°C	–
Freeze-dry	0.14
Air-dry at 25°C	0.18
Air-dry at 60°C	0.11
Moss	
Wet sample	–
N_2-dry at 25°C	–
Freeze-dry	0.29
Air-dry at 25°C	0.32
Air-dry at 60°C	0.34
Forest floor	
Wet sample	15.9
N_2-dry at 25°C	16.3
Freeze-dry	16.5
Air-dry at 25°C	15.4
Air-dry at 60°C	17.5
Wetland soil	
Wet sample	3.57
N_2-dry at 25°C	2.92
Freeze-dry	3.69
Air-dry at 25°C	2.90
Air-dry at 60°C	3.44
Mineral soil	
Wet sample	6.53
N_2-dry at 25°C	5.89
Freeze-dry	5.99
Air-dry at 25°C	6.29
Air-dry at 60°C	6.00

–: not determined. All standard derivations were $< 3\%$.

of organic arsenic after drying or methylation during drying. However, arsenic methylation under similar conditions has not yet been reported.

Arsenic speciation in solid samples using MeOH–H_2O extraction cannot represent 100% of the total arsenic in the samples [17]. Therefore, there is large uncertainty of the result of arsenic speciation, if the sample is prepared differently. The extraction recovery of arsenic in soil are especially low (<1%, table 1), because arsenic in soils is specifically-sorbed (≈9.5%) or partitions mostly in the mineral phases (≈90%) [18]. However, MeOH–H_2O extractable arsenic in soils represented the mobile fraction [19], which is relevant to ecotoxicology. Our results indicated that arsenic speciation in soils and plants is strongly affected by the drying methods. Consequently, the different results of arsenic speciation may lead to different risk assessment for the environment and human health.

3.2 *Total arsenic in soil and plant samples dried with different methods*

The different drying methods exhibited little influence on determining total arsenic concentrations in samples with different materials by digesting with strong acid (table 2). However, determination of total arsenic in plants seemed to be more difficult

than soils because smaller amounts of plant samples could be used for digestion and the total arsenic concentrations were generally lower in plants than in soils. Additionally, analysis of wet plant samples may be disturbed by their high water contents (e.g. 91% for moss).

3.3 *Arsenic speciation in soil and plant samples stored at different temperatures*

We choose wet and freeze-dried samples for the storage test, because wet samples may give a better representation of arsenic speciation under natural condition (see aforesaid) and freeze-drying is the mostly used method for arsenic speciation. We tested the storage of these samples at 2 and −20°C for one month, since these temperatures are usually available in most laboratories.

The concentrations of total MeOH–H_2O extractable arsenic and inorganic arsenic in wet and freeze-dried mosses, needles, forest floors, and wetland soils decreased after storage, except for wet needles (table 3). The total extractable arsenic was in the order: 25 > 2 > −20°C stored fresh needles. The speciation of organic arsenic in most samples differed from their original states after one month storage. In wet needles, 'unknown 2' was no more detected in the samples stored at 25 and 2°C and only small amounts were found in the samples stored at −20°C. Dimethylarsinic acid in wet needles decreased after storage in the order 25 > 2 and −20°C storage. The concentrations of DMA and 'unknown 2' in freeze-dried plants decreased slightly after storage at 2 and −20°C, whereas loss of 'unknown 1' and TMAO was observed in mosses. The AsB concentrations decreased after storage in all cases. In wet forest floors, TMAO disappeared after storage. In wet wetland soils, MMA and DMA were no more detected in the sample stored at 25°C. Storage of wet forest floors under N_2 atmosphere did not hamper the change of the samples. However, loss of MeOH–H_2O extractable arsenic was much smaller as compared to those samples stored under ambient atmosphere. In general, storing wet samples resulted in a higher loss of MeOH–H_2O extractable arsenic than when storing them as freeze-dried samples.

In freeze-dried mineral soils, the concentrations of total MeOH–H_2O extractable arsenic varied little from the freshly prepared samples after one month storage (table 4). Nevertheless, higher amounts of As(III) and As(V) could be extracted from wet mineral soils than from fresh ones stored for one month at 2°C.

In summary, none of the storage methods tested could preserve the arsenic speciation in organic soils and plants, although arsenic speciation after one month storage varied less in freeze-dried samples than the wet samples. Treatment of samples at very low temperatures (−20 and −78°C) seems to reduce strongly the microbial activity, which may lead to further transformation of arsenic compounds. The freeze-dried mineral soils was the only one which may be stored long-term at low temperatures without large changes in arsenic speciation, probably because of the low contents of organic matters.

The MeOH–H_2O extractable arsenic increased in wet needles and mineral soils after a month storage in the order 25°C storage > 2 > −20°C. The microbial activity may help to release arsenic compounds by decomposing samples or changing the binding of arsenic compounds to solid phases. Such effect was especially obvious in the case of wet needles, which was hardly torn into fine pieces using an Ultra Turrax. Conversely, the concentrations of MeOH–H_2O extractable arsenic in mosses and organic soils reduced after storage. The effect was more apparent when the samples

Table 3. Concentrations (ng As g^{-1} dry weight) of methanol–water extractable arsenic compounds in soils and plants stored under different conditions.

		As(III)	As(V)	MMA	DMA	Unknown 1	AsB	Unknown 2	TMAO	Total As	Org As (%)
Needle											
Wet sample	25°C	51.1 ± 22.8	10.2 ± 0.6	<DL	1.96 ± 1.34	<DL	<DL	<DL	<DL	88.8	2.20
	2°C	42.5 ± 0.5	10.4 ± 1.2	<DL	2.39 ± 0.36	<DL	<DL	<DL	<DL	55.2	4.32
	−20°C	24.1 ± 0.1	10.63 ± 0.2	<DL	2.10 ± 0.29	<DL	<DL	0.91 ± 0.24	<DL	37.8	7.96
Freeze dried sample	2°C	19.5 ± 0.3	14.4 ± 0.1	<DL	2.25 ± 0.26	<DL	<DL	0.66 ± 0.16	<DL	36.8	7.92
	−20°C	16.9 ± 1.4	13.7 ± 0.9	<DL	1.88 ± 0.07	0.23 ± 0.03	<DL	1.08 ± 0.05	<DL	33.7	9.45
Moss											
Wet sample	25°C	114 ± 5	21.5 ± 3.0	<DL	3.24 ± 0.06	13.2 ± 2.8	1.08 ± 0.47	8.15 ± 0.60	1.41 ± 1.22	163	15.8
	2°C	83.2 ± 1.1	24.9 ± 3.0	<DL	14.7 ± 0.1	14.0 ± 2.3	2.15 ± 0.23	10.3 ± 1.4	<DL	149	27.6
	−20°C	83.0 ± 20.7	47.7 ± 7.2	<DL	11.4 ± 0.6	15.5 ± 2.2	3.48 ± 0.30	7.99 ± 5.31	0.67 ± 0.94	162	22.6
Freeze dried sample	2°C	35.8 ± 1.9	24.9 ± 0.5	<DL	4.78 ± 0.41	9.91 ± 1.97	2.37 ± 1.20	6.11 ± 1.46	<DL	83.9	27.6
	−20°C	32.1 ± 2.1	23.5 ± 0.5	<DL	4.62 ± 0.26	14.3 ± 0.7	<DL	6.81 ± 0.48	<DL	81.5	31.6
Forest floor											
Wet sample	25°C	9.32 ± 0.82	22.7 ± 1.7	<DL	1.93 ± 0.04	<DL	3.00 ± 0.04	<DL	<DL	36.9	8.30
	2°C	5.91 ± 0.12	25.5 ± 2.0	<DL	2.10 ± 0.10	<DL	2.51 ± 0.56	<DL	<DL	36.0	13.4
	−20°C	4.11 ± 0.02	31.0 ± 1.9	<DL	2.14 ± 0.02	<DL	3.07 ± 0.48	<DL	<DL	40.5	12.8
	2°C (N_2)	6.15 ± 0.41	54.6 ± 13.4	<DL	2.99 ± 0.02	<DL	4.30 ± 0.19	<DL	<DL	68.1	10.7
	−20°C (N_2)	7.10 ± 0.07	50.2 ± 0.3	<DL	3.11 ± 0.07	<DL	5.11 ± 0.02	<DL	<DL	65.5	12.5
Freeze dried sample	2°C	9.35 ± 0.96	29.3 ± 2.8	<DL	2.21 ± 0.06	<DL	5.05 ± 0.55	<DL	0.96 ± 0.05	46.9	17.5
	−20°C	10.1 ± 0.2	27.5 ± 2.3	<DL	2.30 ± 0.09	<DL	5.47 ± 0.07	<DL	1.21 ± 0.23	46.6	19.3
Wetland soil											
Wet sample	25°C	5.25 ± 0.11	4.44 ± 0.16	<DL	<DL	0.39 ± 0.04	1.47 ± 0.01	<DL	<DL	12.0	18.8
	2°C	2.85 ± 0.02	6.47 ± 0.20	0.83 ± 0.04	0.22 ± 0.21	0.30 ± 0.03	1.44 ± 0.06	<DL	<DL	12.1	23.1
	−20°C	2.19 ± 0.15	8.34 ± 0.19	0.88 ± 0.05	0.52 ± 0.05	0.76 ± 0.24	1.84 ± 0.02	<DL	<DL	14.7	28.5
Freeze dried sample	2°C	6.14 ± 1.21	7.09 ± 0.31	0.85 ± 0.22	0.13 ± 0.01	0.30 ± 0.10	2.08 ± 0.12	<DL	<DL	16.6	20.2
	−20°C	6.03 ± 0.07	6.14 ± 0.36	1.78 ± 1.24	0.09 ± 0.01	0.26 ± 0.05	2.59 ± 0.01	<DL	<DL	17.0	27.9

Mean values and standard derivations of three extraction replicates are shown.

Table 4. Concentrations (ng As g^{-1} dry weight) of methanol–water extractable arsenic compounds in untreated and freeze-dried mineral soils stored at different temperatures.

	As(III)	As(V)	Total As
Wet sample			
2°C	6.67 ± 0.27	1.81 ± 0.08	8.47
−20°C	4.31 ± 0.20	2.04 ± 0.14	6.34
Freeze-dried			
2°C	3.26 ± 0.06	2.52 ± 0.01	5.77
−20°C	2.71 ± 0.12	2.51 ± 0.04	5.11

Mean values and standard derivation of three extraction replicates are shown.

were stored at lower temperatures. Again, we suspect that storage at low temperatures seems to lead to the formation of agglomerates that hamper the extraction of the arsenic compounds. This effect may refer to organic matter, since these samples are all rich in organic substances. The most remarkable case should be wet wetland soils stored at −20°C. We observed large amounts of water released from wetland soils after one-month storage at −20°C. The water contents in the wetland soils was up to 90%. The freezing process seems to change the distribution of water in wetland soils.

Storage of wet and freeze-dried samples result in loss of organic arsenic, especially in wet organic soils and plants stored at 25°C. Besides the change of sample materials during storage at low temperatures, this may be due to decomposition caused by micro-organisms. In contrast, the DMA concentrations in the wet mosses increased after storage at 2 and −20°C. The excessive amount of DMA may originate from AsB or TMAO, since DMA may be one of the intermediates of e.g. AsC and AsB decomposition [20].

4. Conclusions

Drying and long-term storage of soil and plant samples may change the arsenic speciation in the samples. Such variation depends on sample preparation and the properties of samples, such as carbon and water contents. We recommend to conduct arsenic speciation in fresh and wet samples, so that the results may be more approaching their original states.

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