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SCIENCE DIRECT
Talanta 69 (2006) 251–258
Talanta
www.elsevier.com/locate/talanta

# Extraction of arsenic compounds from lichens

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Received 19 April 2005; received in revised form 2 September 2005; accepted 4 October 2005
Available online 15 November 2005

**Abstract**

Different extraction procedures were applied to improve the extraction efficiency of arsenic compounds from lichens. Two lichen species were chosen from an arsenic-contaminated environment: epiphytic *Hypogymnia physodes* (L.) Nyl. and terricolous *Cladonia rei* Schaer. Samples were extracted with water at temperatures of 20, 60 and 90 °C, using mixtures of methanol/water (9:1, 1:1 and 1:9), Tris buffer and acetone and the extracts speciated. Water and Tris buffer showed the best extraction efficiency of all extractants used; however, the extraction efficiency was still less than 23%. Since a major fraction of arsenic appeared to be associated with trapped soil particles, a sequential extraction procedure originally designed for soils (extraction steps: (1) 0.05 mol $l^{-1}$ $(NH_4)_2SO_4$; (2) 0.05 mol $l^{-1}$ $(NH)_4H_2PO_4$; (3) 0.2 mol $l^{-1}$ $NH_4$-oxalate buffer, pH 3.25; (4) mixture of 0.2 mol $l^{-1}$ $NH_4$-oxalate buffer and 0.1 mol $l^{-1}$ ascorbic acid, pH 3.25; (5) 0.5 mol $l^{-1}$ KOH) was applied and found to remove 45% of the total arsenic from *H. physodes* and 83% from *C. rei*. The lipid-soluble fraction of arsenic was estimated by $k_0$-INAA analysis of diethylether extracts and was found to be negligible. An HPLC-UV-HGAFS system was used to determine the arsenic compounds extracted. In both lichen species, arsenous acid, arsenic acid, monomethylarsonic acid, dimethylarsinic acid, arsenobetaine, trimethylarsine oxide and glycerol-ribose were detected. In addition, phosphate-ribose was found in *H. physodes*.


*Keywords:* Lichens; Arsenic compounds; Extraction efficiency; Sequential extraction; Bioindication

## 1. Introduction

According to the International Association for Lichenology, a lichen is an association between a fungus and a photosynthetic symbiont, which results in a stable thallus of specific structure. Lichens are able to acquire substances from the environment through the whole surface of the thallus in two ways: (a) by entrapment of particles from the air or substrate and (b) by extra- and/or intracellular uptake of ions from solution [1–6]. As at least epiphytic lichens are supposed to reflect atmospheric concentrations of pollutants [6–8], they are often used as bioindicators of air pollution. Usually total concentrations of pollutants are monitored in lichens, and studies dealing with this problem are numerous (e.g. [8–14]). However, in this way no information on the chemical form of elements present in the environment is obtained. It is also not known how the compounds from the environment are reflected in lichens, if they are taken up selectively by the lichens, and if they are in any way metabolized in this association. Therefore, the study of speciation of chemical compounds is necessary, not only in lichens, but also in the bioavailable portions of the environment from where lichens are able to acquire those compounds (e.g. soil and air). To improve our knowledge of lichen behaviour in relation to their environment, efficient isolation/extraction of compounds of the elements of interest from samples of lichens is needed.

Investigations dealing with arsenic in lichens are few in number and mostly do not concentrate predominantly on lichens. Due to the chemical complexity of lichens, the extraction efficiencies of arsenic are low and therefore limiting in the study of arsenic speciation, defined as the determination of arsenic compounds (for arsenic compounds, see Table 1). In most cases, methanol/water (1:1 and 9:1) has been used as extractant with lichens [15–17]. Extraction efficiencies obtained in those cases ranged from 1.1 to 42% and were lichen species dependent. The highest extraction efficiency was achieved for an undetermined species from the genus *Cladonia* (42%) [16]. Kuehnelt et al. [17] intercompared the extraction of two lichen samples of different species (*Alectoria ochroleuca* and *Usnea articulata*) with methanol/water (9:1) and pure water at 25 °C

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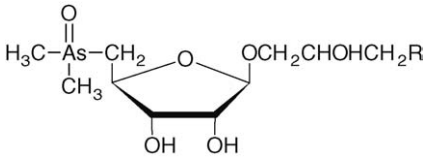
doi:10.1016/j.talanta.2005.10.011

Table 1
Arsenic compounds investigated in this study

| | Abbreviation | Formula |
|---|---|---|
| Anionic arsenic compounds | | |
| Arsenous acid | As(III) | $H_3AsO_3$ |
| Arsenic acid | As(V) | $H_3AsO_4$ |
| Monomethylarsonic acid | MMAA | $CH_3AsO(OH)_2$ |
| Dimethylarsinic acid | DMAA | $(CH_3)_2AsO(OH)$ |
| Cationic arsenic compounds | | |
| Arsenobetaine | AsB | $(CH_3)_3As^+CH_2COOH$ |
| Arsenocholine | AsC | $(CH_3)_3As^+CH_2CH_2OH$ |
| Tetramethylarsonium ion | TETRA | $(CH_3)_4As^+$ |
| Trimethylarsine oxide | TMAO | $(CH_3)_3As^+OH$ |
| Arsenosugars | | |
| Glycerol-ribose (R = OH) | | |
| Phosphate-ribose (R = $OPO_3HCH_2CHOHCH_2OH$) | | |
| Sulfate-ribose (R = $SO_3H$) | | |
| Sulfonate-ribose (R = $OSO_3H$) | |  |

and found higher extraction yields for extraction with pure water (7 and 25%, respectively). The better extractability with water was explained by the statement that glycerol-ribose is better removed by water than by a 9:1 methanol/water mixture. Sequential extraction with Milli-Q water–$CaCl_2$–$H_3PO_4$ was applied by Farinha et al. [18] to transplants of *Parmelia sulcata*, where extraction efficiencies of 0.7–9.3% were achieved.

Regarding speciation of arsenic in lichens, the literature was reviewed by Dembitsky and Rezanka [19] and by Dembitsky and Levitsky [20]. In lichens, both inorganic and organic arsenic compounds were recorded. Besides the dominant inorganic arsenic, Koch et al. [15] reported phosphate-ribose and trace amounts of DMAA and glycerol-ribose in lichen samples from the genera *Bryoria*, *Alectoria* and *Cladonia*. Arsenite and arsenate were the major arsenic compounds in lichens (mostly species from the genus *Cladonia*) from Yellowknife, Canada, and represented as much as 62–93% of total extracted arsenic. In this study, arsenobetaine was reported for the first time for lichens and was present in all analysed samples [16]. Besides that, glycerol-ribose was detected in two lichen samples and the presence of an unknown compound was recorded. Kuehnelt et al. [17] reported arsenobetaine as the major compound in the lichen *A. ochroleuca* and as one of the major compounds in the lichen *U. articulata*. Glycerol-ribose was the major compound in *Usnea*. Farinha et al. [18] found only anionic arsenic compounds (inorganic arsenic, DMAA and MMAA) in samples of the transplanted lichen *P. sulcata*. Variations in arsenic compounds present in lichens studied so far were explained by two possibilities: (a) differences in lichen species and therefore the metabolism of arsenic and (b) differences in their environments [16].

Our present study was aimed to improve the extraction efficiency of arsenic in lichens. Comparison was made between two species of lichens, one corticolous (*Hypogymnia physodes* (L.) Nyl.) and one terricolous (*Cladonia rei* Schaer.). A soil sample from the area where the lichens were obtained was also examined, to see if arsenic content of lichens could be directly ascribed to soil. In extracts, arsenic speciation was investigated to see the influence of extractant on speciation and to get information on the arsenic compounds present.

## 2. Experimental

Samples of the two lichen species *H. physodes* and *C. rei* collected in April 2003, and one representative soil sample collected in September 2004, were taken. Samples were collected in Žerjav, Slovenia, close to the former lead–zinc mine and smelter, where preliminary $k_0$-INAA analyses have shown an increased content of arsenic in samples from the environment.

### 2.1. Sample preparation

Lichen samples were moistened and substrate was removed by nylon tweezers. Subsequently, they were freeze-dried and made brittle by immersion in liquid nitrogen and then crushed and ground in a mortar. For *Cladonia*, only podetia (=vertical parts of thalli) were used to make a powder in order to avoid contamination from soil. The soil sample was freeze-dried and then sieved at 0.5 mm mesh and divided into fractions >0.5 mm (soil 1) and <0.5 mm (soil 2). Fractions were homogenized in liquid nitrogen.

### 2.2. Determination of total As

For determination of total As in samples, the $k_0$-INAA method was employed. About 100 mg of homogenized soil and about 180 mg of powdered lichen were pressed into tablets, sealed into polyethylene ampules and irradiated in the TRIGA Mark II Reactor, Ljubljana, at a neutron fluence rate of