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Selenium and its species in the aquatic moss *Fontinalis antipyretica*

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HIGHLIGHTS

- ► Se and its species were investigated in the aquatic moss *Fontinalis antipyretica*.
- ► Se is accumulated to 2250 ng g^{-1}, bioconcentration factor over 10^3.
- ► Enzymatic or water extraction gave 25% of soluble Se.
- ► Se(IV) and Se(VI) but no organic Se compounds were identified in extracts.
- ► *F. antipyretica* could be used for Se biomonitoring.

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ABSTRACT

The ability of the widely distributed aquatic moss *Fontinalis antipyretica* to take up Se from water was studied. Nine locations in the Notranjska region (Slovenia) with different land use in the catchment were sampled for water and moss in the year 2010 in spring, summer and autumn. The concentrations of Se in water at all locations did not exceed 0.2 ng mL^{-1}. *F. antipyretica* took up Se in the range between 345 and 2250 ng g^{-1}. All results for Se are expressed on dry matter basis. The Se content varied depending on the location and season. The highest concentration (2250 ± 170 ng g^{-1}) of Se was found in the Žerovniščica stream that flows through an agricultural area with dairy farming. The fraction of insoluble Se compounds in the residue after enzymatic hydrolysis using protease (XIV) was around 75%. Soluble Se compounds in the enzymatic extract of *F. antipyretica* were separated and measured using HPLC coupled to ICP-MS. Se(IV) and Se(VI) were found but no organic Se compounds were detected, even at the highest concentration.

1. Introduction

Large amounts of pollutants are released daily into the environment, including widespread discharges of soluble Se from agricultural and industrial sources (Fan et al., 2002). The addition of Se to feedstuffs and soil fertilisers is common practice (Keskinen et al., 2009). Part of the Se added to feed is incorporated by animals and part is lost or excreted and passed to the environment. In aquatic systems Se can occur in four oxidation states — elemental (Se^0), selenite (Se^{+4}), selenate (Se^{+6}) and selenide (Se^{-2}) (Canton and Van Derveer, 1997). The form of Se present in a given system depends on the redox potential and pH. The oxidised forms of Se, selenite and selenate, are the most soluble and predominate in natural water systems (Environmental Protection Agency, 1987). Because of their high solubility, these forms are more available for plants (Carvalho and Martin, 2001) and are potentially toxic to aquatic organisms. Once in the aquatic environment, Se can rapidly attain levels that are toxic to fish and wildlife because of its bioaccumulation in food chains and the resulting dietary exposure (Lemly, 2004). However toxicity does not affect all organisms, since some of them have tolerance to metals and metalloids, as a result of detoxification mechanisms. One way of detoxification is the synthesis of phytochelatins which can bind metals/metalloids (Bruns et al., 1997); the second mechanism is their accumulation outside the cytoplasm where metallic cations remain deposited on the surface and can be washed off (Fernández and Carballeira, 2000).

Mosses are widely distributed, long-lived, have a tremendous capacity to accumulate heavy metals and are relatively tolerant of metal pollution (Lopez and Carballeira, 1993). Therefore aquatic mosses are good indicators of the pollution of watercourses. Important characteristics of bioindicators are the following: they accumulate the pollutant without being killed, have a wide geographical distribution, are stationary, and are available all year round (Conti and Cecchetti, 2001). The true aquatic moss *Fontinalis antipyretica* possesses all these characteristics, and is widely used in metal toxicity studies (see for example: Bruns et al., 1997; Gapeeva et al., 2010; Vázquez et al., 2007; Vuori and Helisten, 2010; Díaz et al., 2012). Investigation of Se uptake from water in the natural environment by this aquatic moss was performed only in Russia (Gapeeva et al., 2010) and Spain (Vázquez et al., 2007). The aim of our study was to investigate the uptake of Se, the variability in Se uptake between sampling sites and seasons, and the Se compounds present (speciation) in the aquatic moss *F. antipyretica*. The purpose was to find if *F.*

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antipyretica can be used to indicate Se watercourse pollution. This moss species is common in the streams and rivers of Slovenia and elsewhere.

2. Materials and methods

2.1. Study areas and sample material

F. antipyretica Hedw. plants were taken from streams in the Notranjska region of Slovenia (Fig. 1). We chose eight sampling sites, where discharge of Se could be present due to dairy farming, stock raising and agricultural activities: one location each on the Ižica stream (in a small town in a Karst region) and the Lipsenjščica stream (surrounded by fields), two locations on the Pšata stream (sites P1 and P2 near a cattle farm), and four on the Žerovniščica stream (sites Ž1, Ž2, Ž3 and Ž4, in the village and nearby fields). The reference site was chosen upstream on the Lipsenjščica stream, located in an unpolluted, pristine area. The moss *F. antipyretica* was not always present at every sampling site. The moss samples were taken at each location from an area of about 1 m^2. Approximately 100 g fresh weight of moss was sampled from the base where it was attached to the substratum. Samples were not separated into green and brown parts.

We took moss samples for analysis of Se on the Pšata, Žerovniščica and Lipsenjščica streams once in 2009 and in April, July and October of 2010. The moss samples were rinsed immediately after sampling in the river to remove adhering material, placed in plastic bags and stored in a freezer until processed. Moss tips (less than 10 cm length) were then freeze-dried (ALPHA 1–4, Osterode am Herz, Germany) and milled in agate bowls (Fritsch, Pulverisette 7, Idar-Oberstein, Germany) (speed: 2600 rpm; time: 6 min). Samples of water were collected simultaneously with moss samples at each sampling site. The pH of water was between 7.3 and 8.5 and the temperature from 7.5 to 13.2 °C during the year.

2.2. Determination of Se in water

Water samples (in polyethylene bottles) were immediately filtered using a 0.45 μm Millex GV filter (Millipore Corporation), acidified to pH 2 and stored in a refrigerator at −20 °C until the total Se was measured by inductively coupled plasma-mass spectrometry (ICP-MS). The operational conditions were described elsewhere (Cuderman et al., 2008). The limit of detection for Se was 0.002 ng mL^{-1}. The accuracy of the method was checked with the certified reference material NIST Trace elements in water 1643d and good agreement was found between the obtained, 10.6 ± 0.1 ng mL^{-1}, and certified, 11.97 ± 0.14 ng mL^{-1}, values.

2.3. Determination of Se content in moss

To a 0.200 g of homogenised and lyophilised sample, a mixture of HNO_3 and H_2SO_4 was added and heated for 12 h at 80 °C in a closed tube. In later steps, H_2O_2, HF, and V_2O_5 in H_2SO_4 were added and heated again. Se(VI) was reduced to Se(IV) by the addition of concentrated HCl and heating at 90 °C. The method is described in detail by Smrkolj and Stibilj (2004). The solution was diluted before determining the Se content, which was carried out by hydride generation atomic fluorescence spectrometry (Smrkolj and Stibilj, 2004). Each sample was analysed at least in triplicate. The certified reference material spinach leaves, NIST 1570a was also analysed. Good agreement was found between the obtained, 108 ± 9 ng g^{-1} ($n = 10$), and certified, 117 ± 9 ng g^{-1}, values.

2.4. Extraction and speciation

For Se speciation, duplicate samples of about 0.5 g moss were extracted with 8 mL water with or without 60 mg of protease XIV by shaking for 24 h, followed by centrifugation at 11000 rpm at

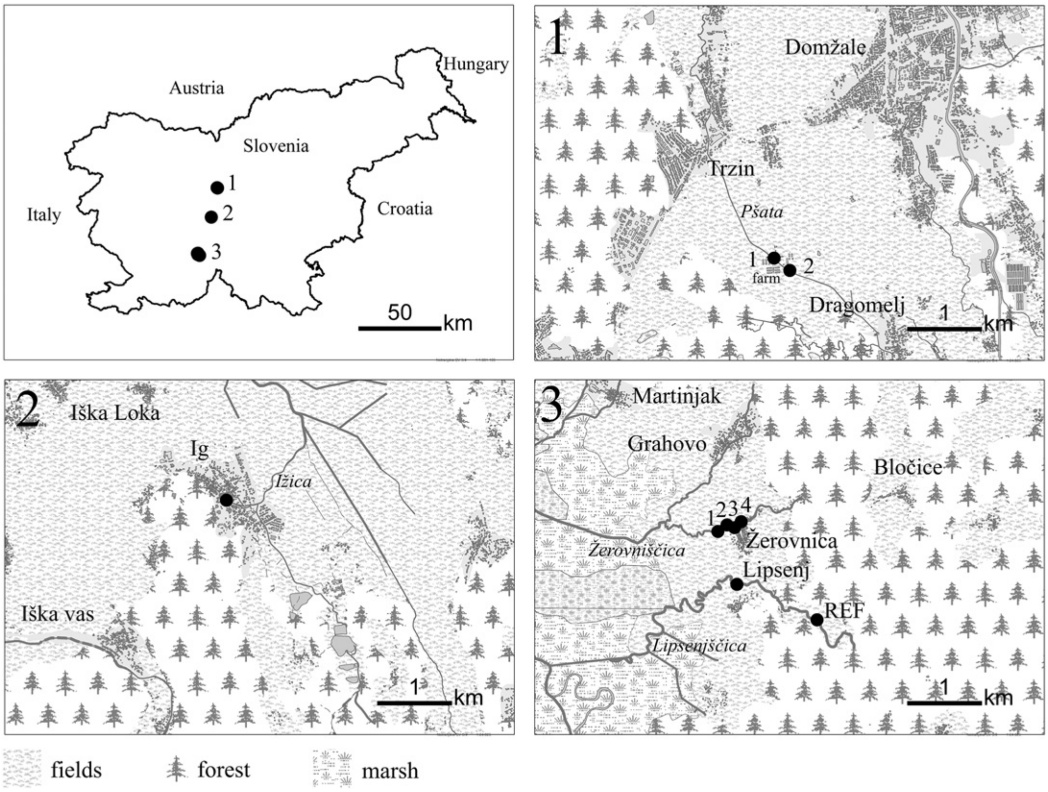

Fig. 1. Map of sampling areas. The names of the streams are written in italics. Sampling sites are marked with ●. Pšata stream had two sampling sites (P1 and P2) and Žerovniščica stream four sampling sites (Ž1, Ž2, Ž3, and Ž4).