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Measurement uncertainty from physical sample preparation of moss samples: Estimation of mechanical cleaning vs. rinsing

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ABSTRACT

Next to sampling, the physical sample preparation step is a second large source of uncertainty. To assess the level of uncertainty from sampling, sample preparation and analysis of moss material, 27 combined and duplicate samples of moss species *Pleurozium schreberi* (Brid.) Mitt were collected and prepared for analysis using two different treatment methods After sampling had been done, samples were dried at an ambient temperature and then each primary and duplicate sample was divided into two sub-samples for preparation. The first sub-sample was manually cleaned whereas the second one was triple rinsed with deionized water and left to dry. Subsequently, the samples were milled and digested in a close microwave system with 8 mL of HNO_3 (1:1) and 1 mL of 30% H_2O_2. In all samples Cu, Fe and Zn were determined using GFAAS and FAAS techniques. Each sample was analyzed twice. Sampling, sample preparation and analytical uncertainty were calculated using ANOVA, RANOVA, modified RANOVA and range statistics methods. Sampling and sample preparation uncertainty varied from 3.8 to 19.8% and from 3.6 to 11.2%, respectively. For all the elements examined analytical uncertainty was below 1.1%. The comparison of element concentrations in manually cleaned and rinsed samples showed that rinsed samples were enriched in Cu and Zn, as opposed to manually cleaned that in turn displayed raised levels of Fe. However, except for Zn, these differences were not statistically significant.

1. Introduction

Bryophytes encompass a large group of organisms such as: mosses, hornworts and liverworts that have no vascular system, true roots and shoots. They are anchored to substrate by rhizoids that are not able to absorb water and nutrients from the substrate (Szczepaniak and Biziuk, 2003; Ötvös et al., 2004; Gerdol and Bragazza, 2006; González and Pokrovsky, 2014). Bryophytes take up essential nutrients from dust and rainfall. They are sensitive to the levels of contaminants in the atmosphere, so can be considered as good indicators of habitat quality. Of this group of organisms, mosses have extensively been used as air quality indicators since the 1960s (Onianwa, 2001; Fernández and Carballeira, 2002; Gałuszka, 2006; Harmens et al., 2010; Boquete et al., 2011; Spagnuolo et al., 2013). Their leaves have only one layer of cells, so their surface:volume ratio is high, and metals can easily exchange sites in their cell walls. Moreover, they can trap airborne particulates, which is very useful in studying atmospheric transport and deposition (Aboal et al., 2008, 2011; Spagnuolo et al., 2013). The absorption of elements and particulates by mosses is limited to rainfall, snowmelt and dry deposition and makes them the most effective collectors reflecting both the local pollution and the long-term conversion of atmospheric pollutants (Prevedouros et al., 2004; Harmens et al., 2004, 2008, 2010; Coşkun et al., 2009; Poikolainen et al., 2009; Castorina and Masi, 2015). Their abundance and presence in various ecosystems make them almost ideal bioindicators. Unfortunately, concentration of elements in moss tissues depends on several factors such as: environmental conditions, sampling procedure, sample preparation and analysis. All of them must be taken under consideration because they can be a source of partial uncertainty (Dołęgowska and Migaszewski, 2015).

Uncertainty characterizes the dispersion of results that can be attributed to the measurand (Lyn et al., 2003). The procedure for individual component estimation of measurement uncertainty, such as sampling and analytical uncertainty has successfully been applied by several authors (Thompson, 1998; Ramsey, 1998; Lyn et al., 2007; Rostron and Ramsey, 2012). However most of these studies have been centered on foodstuff and soil samples (Lyn et al., 2003; Kurfürst et al., 2003). Aside from sampling and analysis, physical sample preparation process is another source of uncertainty. Like sampling, physical sample preparation is out of our control and the error covering this step may reach even 300% (Markert, 1995). The methodology used for estimation of uncertainty derived from sample preparation of foodstuffs was proposed by Lyn et al.

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(2003). According to this model, sample preparation uncertainty, as an additional component of measurement uncertainty, can be calculated as follows:

$$s_{meas} = \sqrt{s_{samp}^2 + s_{prep}^2 + s_{anal}^2}$$

In the relevant literature we can find solid information how this step may affect the permeability of moss membrane and element concentrations (Aboal et al., 2008, 2011; Fernández et al., 2010; Pérez-Llamazares et al., 2011; Vázquez et al., 2015). There are no data on the level of uncertainty which is related with the physical sample preparation stage, and how this step affects the measurement uncertainty.

The plant samples have not been cleaned prior to chemical analysis for a long time. Nowadays, the decision on washing or not washing of plant samples is taken based on the study objectives. The samples are washed if the level of atmospheric contamination and potential phytotoxicity of contaminants on organism are determined whereas analysis of unwashed samples allows us to assess the deposition of contaminants (Markert et al., 1999). According to Fernández et al. (2010), washing allows us to avoid dependence on some environmental factors (mainly soil-related effects) by standardization of samples. However, the efficiency of this step and its impact on moss tissues is difficult to assess. The study conducted by Vázquez et al. (2015) showed that long washing procedure might change bioconcentrated intra- and extracellular fractions. What is more, because mosses are deprived of protective structures, the washing step may lead to losses in some elements (Dunn et al., 1992). The chemical analysis of washed and unwashed moss samples gives ambiguous results affecting data interpretation (Aboal et al., 2011). Türkan et al. (1995) noted lower concentrations of several elements (Cd, Cr, Fe, Pb, Zn) in moss samples which were washed with tap and then with distilled water. Significant differences in element concentrations between unwashed and above 30-s washed *Pseudoscleropodium purum* moss samples were also recorded by Fernández et al. (2010). The comparison of chemical composition of particles deposited onto moss tissues collected from unpolluted and polluted areas has revealed that washing of samples for less than 30 s does not remove all the particles, whereas longer washing leads to changes in the equilibrium of extracellularly bound cations (Aboal et al., 2011). Additionally, the efficiency of washing effect is also dependent on the type of sample storage (freezing, drying, and acclimatization). Washing of dried samples is more effective than washing of frozen samples, because the last ones reveal greater adhesive properties in relation to deposited particles.

Apart from washing, cleaning involves also manual techniques like: shaking, blowing or wiping. These techniques also allow removal of loosely attached material without any interference in equilibrium of moss membrane. However, these techniques have occasionally been applied. As mentioned before, most studies have focused on differences of element concentrations in washed and unwashed plant samples stored at different conditions (frozen, dried, acclimatized) (Aboal et al., 2008; Fernández et al., 2010). Considering this, the principal objectives of this study were to: (1) estimate the uncertainty of sampling, sample preparation and analysis of *Pleurozium schreberi* (Brid.) Mitt. moss samples and (2) compare the chemical composition of moss samples using two different treatment methods.

2. Sampling

In all, 54 composite samples of moss species *Pleurozium schreberi* (Brid.) Mitt were collected in September of 2014 within three forested areas. Sampling site 1 (SS1-Wierna Rzeka) is situated 37 km west of Kielce (N 50°50′814″, E 20°18′745″). Due to its location (far from urban and industrial centers) this area was chosen as an unpolluted and reference site. Sampling site 2 (SS2-Posłowice Range) (N 50°54′946″, E 20°38′849″) and 3 (SS3-Piaski) (N 50°50′768″, E 20°34′380″) are located in the southwestern and northwestern parts of the city. SS2 lies in the neighborhood of housing developments and the main road. Sampling site 3 is situated close to the penitentiary and the local road. This area is characterized by the lowest moss coverage and the highest environmental degradation.

Each sample consisted of 8–10 sub-samples taken from an open space area of 10 m^2. Only apical green parts of moss samples were collected. Duplicate samples were collected at a distance of 1–2 m. All samples were similar in weight (20 g) and taken homogenously throughout each primary and duplicate sampling site. Samples were *in situ* cleaned from foreign organic matter, placed in disposable polyethylene bag and transported to the laboratory.

3. Sample preparation and chemical analysis

At the laboratory, each sample (20 g) was removed from its bag and dried at an ambient temperature. Each primary sample (S1) was divided into two equal sub-samples for preparation (10 g) and described as P1 (manually cleaned sample) and P2 (rinsed sample). The same procedure was undertaken for duplicate samples (S2). All P1 sub-samples were manually cleaned by triple shaking (shoots were hold by the distal part and shook three times, the whole process took 30 s), whereas P2 sub-samples were triple rinsed with deionized water (to avoid changes in membrane permeability the whole process also took 30 s) and left to dry at an ambient temperature. To avoid uncertainty from sampling homogenization, all sub-samples were prepared on the same day by the same person using the same equipment. Subsequently, all P1 and P2 sub-samples were milled using IKA WERKE mill to a pass <0.5 mm and digested with 8 mL of 1:1 HNO_3 and 1 mL of 30% H_2O_2 (Suprapur®) in a close microwave system Multiwave 3000 (Anton Paar GmbH). After digestion, each sample was diluted to 25 mL with deionized water and analyzed for Cu, Fe and Zn using the GFAAS (for Cu) and FAAS (for Fe and Zn) techniques (Thermo Scientific model iCE 3500Z spectrometer). For all samples duplicate chemical analysis was performed. As a standard reference material, Tomato leaves SRM-1573a was applied. The recalibration process was done after a series of 10 samples analyzed. The recovery was in the range of 97–99%, whereas LOQ and LOD were as follows: Cu – 0.058 μg L^{-1}, 0.174 μg L^{-1}; Fe – 0.049 mg L^{-1}, 0.147 mg L^{-1}; Zn – 0.004 mg L^{-1}, 0.012 mg L^{-1}. All the analytical details are summarized in Table S1 of ESM.

4. Results

4.1. Differences in element concentrations in moss samples prepared with two different treatment methods

Differences between Cu, Fe and Zn concentrations in moss samples (both manually cleaned and triple rinsed with deionized water) are depicted in Fig. 1. There is no explicit trend in element contents. Higher concentrations of Fe correspond mainly to P1 samples, whereas P2 samples are enriched in Cu and Zn (Fig. 1). Summary statistics and statistical parameters calculated for elements examined were presented in Table 1. The differences expressed as: $\left|\left(treatment\ 1 - treatment\ 2/treatment\ 1\right)\right| \cdot 100\%$ were as follows: 14.7 (SS1), 10.6 (SS2) and 5.8% (SS3) for Cu; 8.0 (SS1), 9.4 (SS2), 6.8% (SS3) for Fe; and 6.7 (SS1), 8.7 (SS2) 2.7% (SS3) for Zn. It is interesting to note that except for Zn from SS1 and SS2, these differences were not statistically significant ($p < 0.05$). Testing was done using the Mann–Whitney *U* and Student *t*-tests. The highest contents of