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Original Articles

Improving active biomonitoring in aquatic environments: The optimal number and position of moss bags

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ABSTRACT

The present study was carried out to determine the optimal number of moss bags of *Fontinalis antipyretica* required for biomonitoring stream water pollutants. With this aim, we examined the variability in the concentrations of Al, Cd, Co, Cu, Fe, Ni, Pb and Zn in 50 moss bags exposed in 4 different stream sampling sites (SS). In general, there were no significant differences in the element concentrations between groups of moss bags (n = 5), either along the 50 m length of each stream, or between the different sides of the streams. Considering errors of 10, 15 and 20%, the maximum number of moss bags required to estimate the mean tissue concentrations of the elements at the SS was respectively 26, 11 and 6. For most of the pairs of SS and elements studied, 5 or fewer moss bags were sufficient to differentiate between the mean concentrations. These findings allow us to conclude that a greater number of moss bags (at least 6) than those generally used until present should be utilized for biomonitoring water pollution with aquatic bryophytes.

1. Introduction

Water is one of the most essential natural resources: it is necessary for life and livelihoods. Notwithstanding, barely 20% of the world's population is free from threat to water security and less than half of habitats associated with continental waters are slightly threatened (Vörösmarty et al., 2010). Managing water is essential for sustainable development worldwide, and the needs of people and ecosystems must also be balanced (UNESCO, 2009; UNDP, 2006). The EU Water Framework Directive (Directive 2000/60/CE), which coordinates the objectives of European water policy regarding the protection of waters, recommends using biota as a matrix for pollutant monitoring to enable evaluation of the impact of the bioavailable pollutants in aquatic environments.

Biomonitoring techniques have been used successfully for several decades to detect water pollution. In particular, the use of transplanted aquatic bryophytes (i.e. active biomonitoring, also called the “moss bag technique”) enables simple, reliable and economical assessment of water quality. The advantages of the technique have been reported by numerous authors (Cesa et al., 2013; Fernández et al., 2006; Samecka-Cymerman et al., 2005; Yurukova and Gecheva, 2003; López et al., 1994; Kelly et al., 1987). However, for the moss bag technique to be considered suitable for biomonitoring, the moss must be able to capture elements from the environment and the results obtained must represent the local variability in the concentrations of different elements at the sampling site during exposure of the moss bags. Furthermore, the validity of the technique also depends on its capacity to demonstrate that two sampling sites are significantly different, in terms of the concentrations of contaminants in the exposed moss. All of these factors are directly related to the number of moss bags exposed at a sampling site.

Few studies have determined the optimal number that should be used in biomonitoring studies, despite the importance of this aspect of the technique (Debén et al., 2017). Thus, the number of moss bags used is generally very variable (Bruns et al., 1995; Yurukova and Gecheva, 2003). The variability in the concentrations of different elements in moss bags exposed at sampling sites appears to range between less than 10% and more than 100% (Rasmussen and Andersen, 1999; Mersch and Reichard, 1998; Samecka-Cymerman et al., 2005; Rabnecz et al., 2008), although this information is not generally reported (and cannot usually determined from the data provided).

With the aim of proposing a harmonized protocol, Debén et al. (2017) recommended employing at least 3 bags per site based on the option most frequently used to date (Diviš et al., 2012; Cesa et al., 2009; Vázquez et al., 2000), while highlighting the need for a specific study on the topic. The aims of the present study were therefore as follows: (i) to determine the optimal position of the moss bags in the river based on the local variability of element concentrations; (ii) to calculate the number of moss bags required per site based on the error level of the

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estimated mean metal concentrations; (iii) to calculate the number of moss bags needed to differentiate sampling sites in terms of the concentrations of elements in moss tissues; and (iv) to establish the optimal number of moss bags that should be used in biomonitoring studies.

2. Material and methods

2.1. Preparation of the transplants and exposure

Samples of the aquatic moss *Fontinalis antipyretica* Hedw. were collected from an unpolluted stream in Galicia (NW Spain). The plants were rinsed first at the site with stream water and washed once again in the laboratory (5 L water per 150 g f.w. of moss, for 1 min, with shaking). Basal parts, as well as material in poor condition, most epiphytes, plant remains and particles attached to the surface of the moss were discarded. The remaining material was devitalized by oven-drying with temperature ramp (50 °C for 5 h, 80 °C for 5 h and 100 °C for 10 h).

Flat bags (10 × 20 cm) were made with fibreglass mesh (aperture 4 mm^2) free of trace contaminants due to a previous wash in HNO_3. The ratio between the moss weight and the surface area of the bag ranged between 3 and 6 mg cm^{-2}. In total, 215 moss bags were prepared and 50 of these were placed in each of the 4 sampling sites (SS). For each SS, 3 control moss bags were treated in the same way as the transplants but were not exposed in the streams. Finally, another 3 moss bags were vacuum-packed and stored for subsequent determination of the initial concentrations of elements.

The exposure sites were 4 stretches (length 50 m) of streams (width between 4 and 2 m) located in NW Spain and SW Poland (Fig. 1). Sampling site 01 and SS04 were slightly affected by agricultural and urban contamination. Sampling site 02 and SS03 were located downstream of respectively a disused copper and a uranium mine. In each stream, ten plastic lines were attached to river rocks at 5 m intervals and five moss bags were tied with plastic ties to each line (n = 50 bags). The exact locations of each line on each side of the streams are shown in Fig. 1D.

After being exposed *in situ* for 7 days, the moss bags were removed from the SS and the moss samples were dried at 40 °C for 48 h. The moss was then homogenized in an ultracentrifuge mill (Restch ZM200, heavy metal-free) and stored in glass vials until chemical analysis.

2.2. Analytical procedures

The moss samples (ca. 0.2 g) were digested with 8 mL of HNO_3 (Hiperpur) and 2 mL of H_2O_2 (30%) in a microwave oven (Ethos-1, Millestone) in Teflon vessels at high pressure, by increasing the temperature to 190 °C over 25 min and maintaining this temperature for 15 min. The cold extracts were then made up to a final volume of 25 mL with MilliQ water. The concentrations of Al, Cd, Co, Cu, Fe, Ni, Pb and Zn in the extracts were then determined spectrometrically in an ICP-MS (Agilent 7700x).

As control of the analytical quality, certified reference material M3 (*Pleurozium schreberi*, Steinnes et al., 1997), analytical blanks and duplicate samples were analyzed once every ten samples. The percentage of recovery from the reference material ranged between 80% and 106% for Al, Cd, Fe and Ni, and between 71% and 75% for Co, Cu, Pb and Zn. The percentage difference between duplicates was < 11% for all elements except Co (13%) and Pb (15%).

2.3. Statistical analysis

Lilliefors modifications of the Kolmogorov-Smirnov test was used to check the normality of the raw data. When necessary, Box-Cox transformations were used to normalize data. Enrichment factors (EFs) were calculated as the ratio between the concentration at the end of the

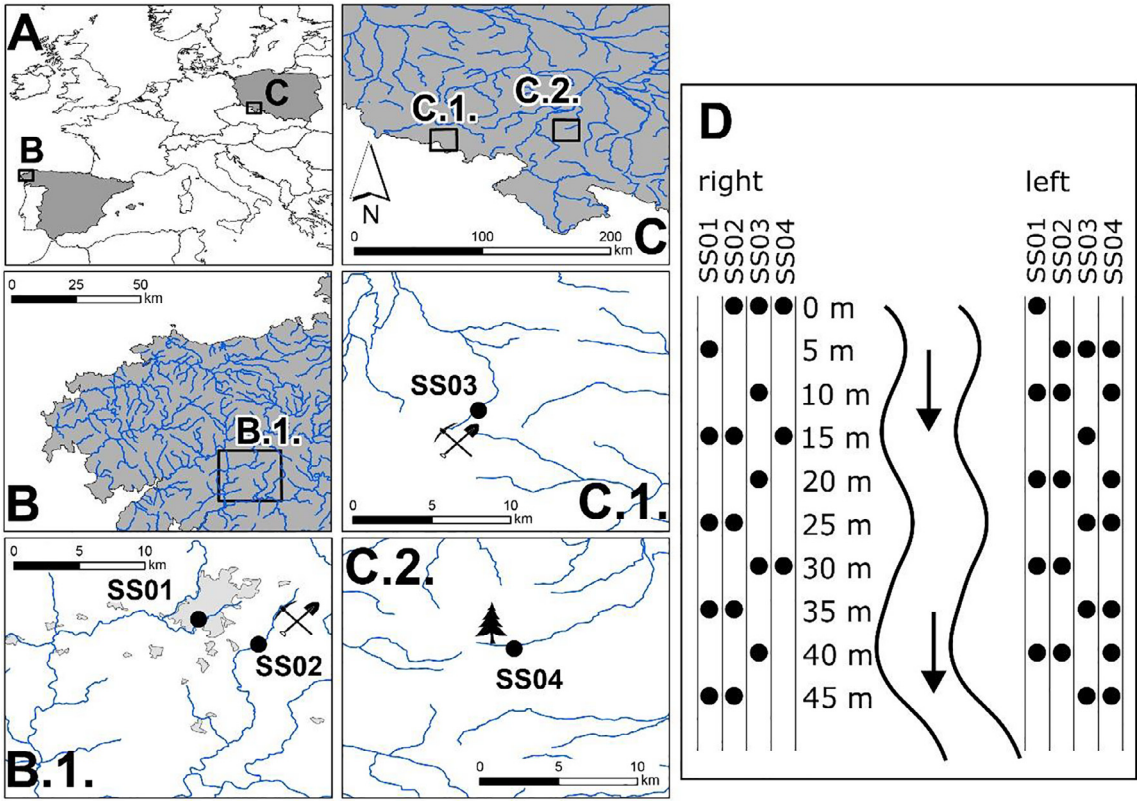

Fig. 1. Map showing the location of the sampling sites. (A) Location of the study areas in Europe. (B and C) Details of the study areas in NW Spain and SW Poland respectively. The exact locations of the sampling sites are shown in B.1., C.1. and C.2. (D) Diagram of the exact location of the groups of moss bags (n = 5; represented by black circles) on the different halves of each stream sampling site; the arrows represent the direction of water flow.