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Towards the methodological optimization of the moss bag technique in terms of contaminants concentrations and replicability values

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HIGHLIGHTS

- Moss bag optimization is done on the basis of metal concentration and replicability.
- The species *Sphagnum denticulatum* is more suitable for most of the metals.
- The use of 5.68 mg of moss tissue for each cm^{-2} of bag surface shows the best results.
- The duration of 8 weeks of exposure was the best option for most of the metals.
- Taking into account practical considerations, an exposure height of 4 m is recommended.

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ABSTRACT

The moss bag technique is a simple and economical environmental monitoring tool used to monitor air quality. However, routine use of the method is not possible because the protocols involved have not yet been standardized. Some of the most variable methodological aspects include (i) selection of moss species, (ii) ratio of moss weight to surface area of the bag, (iii) duration of exposure, and (iv) height of exposure. In the present study, the best option for each of these aspects was selected on the basis of the mean concentrations and data replicability of Cd, Cu, Hg, Pb and Zn measured during at least two exposure periods in environments affected by different degrees of contamination. The optimal choices for the studied aspects were the following: (i) *Sphagnum denticulatum*, (ii) 5.68 mg of moss tissue for each cm^{-2} of bag surface, (iii) 8 weeks of exposure, and (iv) 4 m height of exposure. Duration of exposure and height of exposure accounted for most of the variability in the data. The aim of this methodological study was to provide data to help establish a standardized protocol that will enable use of the moss bag technique by public authorities.

1. Introduction

The moss bag technique is the most commonly reported method of active biomonitoring with terrestrial mosses. The technique is a simple and cost-effective way of evaluating air quality, among other advantages mentioned by numerous authors (see e.g. Sun et al., 2009; Rivera et al., 2011; Saitanis et al., 2013). However, use of the moss bag technique remains restricted to the scientific field because of the lack of internationally standardized protocols (see e.g. Little and Martin, 1974; Gailey and Lloyd, 1986a,b,c; Giordano et al., 2009), while, in the case of native moss a norm for biomonitoring air quality exists (CEN/TC 264/WG 31). This lack of standardization hampers the comparison of the results and conclusions of different studies, which in turn precludes use of the technique by official institutions in the implementation of environmental monitoring programs.

Several well-defined steps must be taken into account while applying the moss bag technique for biomonitoring purposes. These steps mainly involve the selection and preparation of moss, preparation of bags, exposure conditions and post-exposure treatments (Ares et al., 2012). Only a very few of the methodological aspects of the technique have been standardized or investigated in detail (e.g. devitalizing treatment during moss preparation; Giordano et al., 2009). The high degree of variability in each of the methodological steps greatly influences the results obtained. The following are some of the most variable steps (for review see Ares et al., 2012): i) selection of species; ii) ratio between the weight of moss and surface area of the bag; iii) duration of exposure and iv) height of exposure.

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In previous studies, selection of the different options involved in the application of the moss bag technique has been carried out on the basis of practical considerations or, in some cases, by taking into account the maximum accumulation of contaminants in the moss tissues. In this way: i) it was demonstrated that *Sphagnum* spp. is able to accumulate higher quantities of contaminants than other commonly used biomonitoring species due to their morphological and physiological characteristics (Temple et al., 1981; Bargagli, 1998; González and Pokrovsky 2014); ii) an optimal quantity of moss per surface area is considered that where a thin layer of moss is placed within the bag, with all the shoots with the same exposed surface (Gailey and Lloyd, 1986a; Zechmeister et al., 2006), however Temple et al. (1981) consider than 30 mg cm^{-2} provide the optimal accumulation rates; iii) longer duration of exposure than 1 or 2 weeks are recommended as the contaminant uptake increase with the duration of exposure (Gailey and Lloyd, 1986b) iv) and regarding the height of exposure, 4 m are recommended because of some practical considerations and because Vuković et al. (2013) pointed out that samples exposed at this height showed higher element accumulation than 8 or 16 m. However, the best option must be selected on the basis of other key requirements (Ratcliffe, 1975; Ares et al., 2012): (i) transplants should be easy to prepare and handle; (ii) they should enable replicable results to be obtained; and (iii) they should be efficient at capturing contaminants, indicating occurrence in the atmosphere within a reasonable time. The present study focuses on optimization of the different methodological steps on the basis of contaminant concentrations and replicability of the results.

The within-site replicability of samples has not been studied in depth in relation to any of the above-mentioned methodological aspects, except the duration of exposure (Gailey and Lloyd, 1986b). As these studies were carried out with non-devitalized moss and as growth of moss during the exposure period affects the metal uptake measured in different portions of the shoot (Fernández et al., 2010), it is advisable to test different times of exposure with devitalized moss (Giordano et al., 2009). However, little attention has been paid to the influence of the type of site on the sample replicability. Indeed, different microenvironments or sites affected by different degrees of contamination (i.e. urban, industrial, rural) may represent important sources of variability (Tretiach et al., 2011).

Investigation of data replicability also enables evaluation of the total variability associated with different methodological steps, and it is possible to rank these aspects in terms of variability. Thus, efforts should be focused on standardizing the most variable aspects of the moss bag technique.

In the present study, we determined in moss bags the concentrations of Cd, Cu, Hg, Pb and Zn as the concentrations of these elements in the atmosphere and moss are generally well correlated owing to the characteristics of the ionic radius and covalent bond index (Aboal et al., 2010). Moreover, monitoring of Cd, Hg and Pb is contemplated in current legislation (EU Directive 1999/30/EC and 2004/107/EC).

Table 1
Summary of the study area characteristics showing the sampling sites (SS) codes, geographical coordinates (UTM 29T ED50), altitude (h), description of the area and sampling sites where each experiment was carried out. (SP: selection of species; QS: quantity of moss per unit of bag surface area; DE: duration of exposure and HE: height of exposure.)

SS Codes	Coordinates		*h* (m)	Area description	Methodological aspect			
	X	*Y*			SP	QS	DE	HE
SS1	567178	4817834	26	Steel-works factory	x	x	x	
SS2	567147	4818261	5	Steel-works factory	x	x	x	x
SS3	567938	4818376	7	Steel-works factory	x			
SS4	567447	4818989	9	Steel-works factory	x			
SS5	583842	4837331	288	Coal-fire power plant		x	x	
SS6	486249	4754973	21	Fe–Mn smelter		x	x	
SS7	622773	4839371	13	Aluminia-aluminium smelter		x	x	x
SS8	536390	4747182	232	Periurban		x	x	x
SS9	539494	4747484	303	Highway roadside				x
SS10	566344	4826193	30	Rural area	x			
SS11	616468	4684220	974	Rural area		x	x	
SS12	586485	4710232	694	Rural area		x	x	
SS13	549827	4733561	306	Rural area				x

Table 2
Main climatological parameters recorded by the nearest automated monitoring stations present in the surrounding areas of the sampling sites (SS) during the different periods of exposure. Distance between the nearest automated monitoring station and SS is also indicated. Data provide by the regional meteorological agency (www.meteogalicia.es) (AT: average temperature in °C; WD: wind direction in °; P: Precipitation in L/m^2; n.a. = data not available).

	Distance (m)		2010							2011
			March	April	May	September	October	November	December	January
SS1-4, 10	4463–6728	AT	n.a.	12.28	13.31	16.86	13.79	9.71	8.42	11.6
		WD	225	90	90	90	45	225	90	90
		P	71.7	48.6	85.2	36.2	185.9	214.9	127.3	134
SS5	7744	AT	9.74	12.39	13.25	17.01	14.27	10.39	9.32	9.44
		WD	n.a.	45	45	45	45	225	180	45
		P	77.3	43.9	32.5	18.8	96.4	144.6	110.6	90
SS6	7341	AT	n.a.	15.39	15.1	18.1	15.07	11.27	10.06	10.13
		WD	n.a.	90	45	45	180	45	180	45
		P	n.a.	n.a.	62.5	22.2	186	201.3	122.8	126
SS7	7852	AT	6.15	9.11	9.98	15.17	12.16	7.93	6.69	6.83
		WD	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		P	65.1	31.9	71.9	26.3	122.7	171.1	246.72	151
SS8,9,13	336–4463	AT	9.36	13.07	14.21	17.6	13.69	9.43	7.83	8.8
		WD	45	45	45	45	45	45	45	45
		P	134.7	70	84	31.4	212.8	180.1	228.4	227.4
SS11	200	AT	5.23	10.05	10.29	15.44	10.19	5.33	4.01	4.13
		WD	45	45	45	45	45	225	45	45
		P	142.6	37.7	81.2	28	237.3	223.57	122.5	180.8
SS12	19459	AT	4.56	9.46	9.94	15.17	9.86	5.01	3.29	3.74
		WD	225	90	0	90	180	270	90	180
		P	165.3	67.2	101.5	18.7	418.2	220.3	210.23	315.8