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Best options for the exposure of traditional and innovative moss bags: A systematic evaluation in three European countries☆

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ABSTRACT

To develop an internationally standardized protocol for the moss bag technique application, the research team participating in the FP7 European project "MOSSclone" focused on the optimization of the moss bags exposure in terms of bag characteristics (shape of the bags, mesh size, weight/surface ratio), duration and height of exposure by comparing traditional moss bags to a new concept bag, "Mossphere". In particular, the effects of each variable on the metal uptake from the air were evaluated by a systematic experimental design carried out in urban, industrial, agricultural and background areas of three European countries with oceanic, Mediterranean and continental climate. The results evidenced that the shape, the mesh size of the bags and the exposure height (in the tested ranges), did not significantly influence the uptake capacity of the transplanted moss. The aspects more affecting the element uptake were represented by the density of the moss inside the bags and the relative ratio between its weight and the surface area of the bag. We found that, the lower the density, the higher the uptake recorded. Moreover, three weeks of exposure were not enough to have a consistent uptake signal in all the environments tested, thus we suggest an exposure period not shorter than 6 weeks, which is appropriate in most situations. The above results were confirmed in all the countries and scenarios tested. The adoption of a shared exposure protocol by the research community is strongly recommended since it is a key aspect to make biomonitoring surveys directly comparable, also in view of its recognition as a monitoring method by the EU legislation.

1. Introduction

Outdoor air pollutants are a complex mixture of primary and secondary compounds originating from a myriad of natural and anthropogenic sources. Even though evidence of specific components of this mixture to drive major risk for human health remained for long inconclusive, the particulate matter (PM) has recently been designed as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC). Fine airborne particles contain metals, polycyclic aromatic hydrocarbons (PAHs) and other toxic chemicals and can increase the natural-cause mortality even at concentrations well below the European annual mean limit value (Beelen et al., 2014). Even if some metals in (wet, dry and occult)

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atmospheric deposition are among potentially toxic pollutants, in Europe air quality target values have been established only for As, Cd, Ni and Pb and most automatic air quality monitoring stations measure the concentrations of particles based on size (aerodynamic diameter of PM_{10}: ≤10 μm or $PM_{2.5}$: ≤2.5 μm) and not their chemical composition. Moreover, when some data on metal deposition are available they have poor spatial coverage and local sources or variations in their fluxes remain hidden. Thus, to obtain quantitative information on the spatial pattern of deposition of metals (especially those not measured by monitoring devices) the monitoring with suitable organisms has become a very common approach (e.g., Harmens et al., 2015 and references therein). The moss bag technique is probably the most applied method for the active monitoring of airborne trace elements in urban and industrial environments. This technique was introduced by Goodman and Roberts (1971), later modified by Little and Martin (1974) and during the last decades there were several investigations pointing to the optimization and standardization of the method (Gailey and Lloyd, 1986a, b, c, d; Ares et al., 2012, 2014; Giordano et al., 2013). However, most studies considered only one of few methodological steps of the moss bag technique such as the duration or the height of bag exposure and were developed in areas with specific climatic and environmental conditions (e.g. Ares et al., 2012).

Taking advantage of the FP7 European project "MOSSclone" focused on the culture of a particularly performing moss clone, the production of a new concept bag ("Mossphere") for the moss exposure and the development of a standardized protocol for the moss bag technique, the research team involved in the project undertook a complex and systematic evaluation of the most important variables affecting the results of metal biomonitoring with moss bags. In particular, the effects of each variable (1. Shape of bags; 2. Net mesh size, 3. Ratio between moss weight and bag surface area; 4. Duration of the exposure, 5. Height of the exposure) on the metal uptake were evaluated separately. To develop an internationally standardized protocol for the moss bag technique it seemed necessary to test the variability in each of the methodological steps in a range of climatic conditions and land use classes. To this end an experimental design was, for the first time, applied in urban, industrial, agricultural and background areas of three European countries with oceanic, Mediterranean and continental climate.

2. Material and methods

2.1. Preparation of moss transplants

Moss transplants were prepared with *Pseudoscleropodium purum* (Hedw.) M. Fleisch., one of the most commonly used species in the moss bag technique (Ares et al., 2012). Samples were collected in a background area of SE Galicia (NW Spain; X: 596060, Y: 4709910 UTM 29N ETRS89) selected on the basis of previous results (Boquete et al., 2013).

All moss samples were carefully separated from litter and other exogenous materials and air-dried in laboratory. For the experiments, only 5 cm long green apices were selected. This material was firstly cleaned by placing in a plastic sieve (0.7 cm mesh size) and then subjected to one wash of 20 min with 10 mM EDTA (12.5 g d.w. moss/1 L EDTA with shaking) and three washes of 20 min each with distilled water (10 g d.w./1 L distilled water with shaking) and washed 3 times for 10 min in bidistilled water (10 g d.w./1 L bidistilled water with shaking) to remove adhering soil particles. The samples were then blotted on filter paper to remove excess moisture. Afterwards, moss apices were devitalized following three consecutive drying cycles of 8 h each at 50 °C, 80 °C and 100 °C (modified from: Adamo et al., 2007; Giordano et al., 2009). Finally, the bags were prepared (see below), vacuum packed and stored until use.

2.2. Experimental set-up

All the field experiments were carried out in NW Spain, SW Italy and E Austria (Fig. 1; Tables S1, S2, S3). The climate in Galicia (NW Spain) is influenced by the ocean and is temperate maritime; high rainfall (1000–2000 mm per year) and mild temperatures (annual average, 13 °C and spring average, 15 °C) characterize the investigated area (www.meteogalicia.es). The climate in Campania (SW Italy) is mild and influenced by the sea, (annual average temperature, 10.5 °C and spring average, 13.5 °C). The annual rainfall ranges between 900 and 1200 mm (http://www.sito.regione.campania.it/). Austria is not bordering the sea and presenting a temperate/continental climate in the investigated area (annual rainfall 550–900 mm; annual average temperature, 11.3 °C and spring average, 6.5 °C) (http://www.zamg.ac.at/).

In each country seven exposure sites (ESs) affected by different level and types of contamination were selected and classified accordingly as agricultural, background, industrial, and urban sites. The bags were hung vertically from sticks of an inert material fixed perpendicularly to a pole, or similar structures, at a height of 4 m above the ground, except in experiment 2.2.5. (see below). The moss bags were exposed for 3 weeks, except in experiment 2.2.4. (see below). Three replicates per ES for every single treatment were exposed. Ten moss bags, vacuum-packed in polyethylene bags and stored at 4 °C, were used to check contamination after exposure during transportation and laboratory handling.

2.2.1. Shape of the bags

Two couplets of moss-bags of different shape were compared at parity of mesh size (2 mm), quantity of devitalized moss filled in, and external surface of the device: rounded bag vs. Mossphere (S_{30}) – both made with a dry mass/surface ratio of 30 mg cm^{-2} – and flat bag vs. Mossphere (S_{15}) – both made with a dry mass/surface ratio of 15 mg cm^{-2} -, Fig. 2.

The Mossphere is a device designed by our team consisting of two coaxial empty spheres, each formed by two hemispheres, made of pierced high-density polyethylene (the internal sphere), and of a 2 mm mesh nylon net) (the external sphere). The internal sphere is 10 cm in diameter and has 3 mm long spikes homogeneously distributed on the convex side. The external sphere is 11 cm in diameter. The two spheres are closed with four plastic wires passing through four holes in the equatorial plastic border that delimits each hemisphere. The space between the two spheres (10 mm thick) is filled with moss, which is maintained in place by the spikes of the inner sphere.

The rounded bags were made as described by Ares et al. (2014). A square of plastic net of 22 × 22 cm was filled with the moss material, and secured with a nylon thread.

Rectangular flat bags (approximately 700 cm^2) were made with plastic net (2 mm mesh size). The moss was distributed homogeneously inside the bag, and to minimize overlapping and compression of the moss during the exposure (hanging vertically) (Temple et al., 1981), the bag was sewn in a zig-zag pattern with nylon thread to make 3 compartments.

Prior to use, the plastic net was washed in HNO_3 and then in distilled water to eliminate any trace contaminants. Transplants were exposed in triplicate for three weeks in all the ESs in March 2013; total number of samples = 189 (3 shapes × 3 countries × 7 ESs × 3 replicates).

2.2.2. Mesh size

Mosspheres with different mesh size (1 mm, 2 mm, and 4 mm)