Environmental Pollution 213 (2016) 841–849

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Evaluation of the use of moss transplants (*Pseudoscleropodium purum*) for biomonitoring different forms of air pollutant nitrogen compounds☆

Z. Varela [a, *], R. García-Seoane [a], M. Arróniz-Crespo [b], A. Carballeira [a], J.A. Fernández [a], J.R. Aboal [a]

[a] Área de Ecología, Facultad de Biología, Campus Vida, Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Spain
[b] School of Environment, Natural Resources and Geography, Deniol Road, Bangor, Gwynedd, LL572UN, United Kingdom

ARTICLE INFO

Article history:
Received 24 January 2016
Received in revised form
19 March 2016
Accepted 21 March 2016
Available online 31 March 2016

Keywords:
Biomonitoring
N pollution
$\delta^{15}N$
Nitrate reductase
Phosphomonoesterase
Pseudoscleropodium purum

ABSTRACT

We investigated whether three different types of moss transplants (devitalized moss bags with and without cover and auto-irrigated moss transplants) are suitable for use as biomonitors of the deposition of oxidised and/or reduced forms of N. For this purpose, we determined whether the concentration of atmospheric NO_2 was related to the % N, $\delta^{15}N$ and the activity of the enzyme biomarkers phosphomonoesterase (PME) and nitrate reductase (NR) in the tissues of moss transplants. We exposed the transplants in 5 different environments of Galicia (NW Spain) and Cataluña (NE Spain): industrial environments, urban and periurban environments, the surroundings of a cattle farm and in a monitoring site included in the sampling network of the European Monitoring Programme. The results showed that the moss in the auto-irrigated transplants was able of incorporating the N in its tissues because it was metabolically active, whereas in devitalized moss bags transplants, moss simply intercepts physically the N compounds that reached it in particulate or gaseous form. In addition, this devitalization could limit the capacity of moss to capture gaseous compounds (*i.e.* reduced N) and to reduce the oxidised compounds that reach the specimens. These findings indicate that devitalized moss transplants cannot be used to monitor either oxidised or reduced N compounds, whereas transplants of metabolically active moss can be used for this purpose. Finally, the NR and PME biomarkers should be used with caution because of the high variability in their activities and the limits of quantification should be evaluated in each case.

1. Introduction

Globalization and the growing demand for resources have favoured the increase in atmospheric emissions of nitrogenous compounds (*i.e.* NO_x and NH_y) that has occurred in the last century. Such is the magnitude of the emissions of anthropogenic sources that the deposition of N from these sources has reached similar levels to those due to natural processes such as animal excretions, nitrification/denitrification, forest fires and volcanic eruptions (Vitousek et al., 1997; Galloway et al., 2003). The increased concentrations of these compounds in the atmosphere, soils and aquatic systems have serious effects on human and ecosystem health (Townsend et al., 2003; Galloway et al., 2004). In response to this problem, the European Parliament has established maximum limits for total emissions of nitrogen oxides and ammonia, via Directive 2001/81/EC and the Gothenburg Protocol of the Convention of Long-range Transboundary Air Pollution (LRTAP). Although reductions in NO_x and NH_3 emissions of respectively 44% and 25% were detected between 1990 and 2011, many European countries continue to exceed the permitted levels of emission of these compounds (European Environment Agency, 2015). There is therefore a need for public authorities to control the atmospheric emissions of nitrogenous pollutants.

As demonstrated on numerous occasions, terrestrial mosses can be used to biomonitor atmospheric pollutants (Triulzi et al., 1996; Orlinski, 2002; Real et al., 2003; Varela et al., 2013). Indeed, numerous studies have used mosses to determine, more or less

☆ This paper has been recommended for acceptance by Charles Wong.

* Corresponding author.
E-mail address: zulema.varela@usc.es (Z. Varela).

http://dx.doi.org/10.1016/j.envpol.2016.03.056
0269-7491/

successfully, the atmospheric deposition of N by analyzing the concentrations of this element in the moss tissues (*e.g.* Solga et al., 2005; Solga and Frahm, 2006, Solga et al., 2006a, b; Liu et al., 2008a, b; Harmens et al., 2011; Varela et al., 2013; Meyer et al., 2015). These studies have used passive biomonitoring techniques, i.e. sampling and analysis of the N in samples of moss growing naturally in the study area (i.e. native moss). However, this type of biomonitoring may be limited by various factors: i) the scarcity or absence of moss in some environments (i.e. urban and industrial areas); ii) the high degree of variability in the adsorption of pollutants by moss growing at the same sampling site (Fernández et al., 2002; Aboal et al., 2006); and iii) possible phenotypic and/or genotypic adaptations of mosses growing in polluted environments, which may modify the tissue concentrations of the pollutants (Fernández and Carballeria, 2000; Tabors et al., 2004; Boquete et al., 2013).

Given these limitations, some authors have suggested the use of different types of moss transplants in relation to the study objectives (Ares et al., 2012). Thus, if the aim is to determine the concentrations of pollutants in a particular area, the use of devitalized moss transplants (moss bags) is recommended. If, however, the aim is to determine physiological parameters, irrigated moss transplants are more appropriate (the moss remains metabolically active in them). The use of transplants has some important benefits: studies can be carried out in urban and industrial areas, the site and exposure period can be controlled and the conditions being monitored can be standardized. However, to date no studies have been carried out to determine whether moss transplants (of whatever type) are good biomonitors of atmospheric N or whether the different forms in which the N occurs in the atmosphere (i.e. NO_x and NH_y) influence the uptake by moss transplants. Some studies have demonstrated that, as a macronutrient, N affects the physiology of the native moss, even to the extent of regulating their concentrations in the tissues (*e.g.* Koranda et al., 2007; Arróniz-Crespo et al., 2008), and that N may have different effects on moss metabolism depending on the form in which it occurs in the environment (Soares and Pearson, 1997). Indeed, some studies have demonstrated that bryophytes preferably take up reduced forms of N, as the uptake is metabolically less costly than that of oxidised forms due to the high cation exchange capacity of these organisms (*e.g.* Solga and Frahm, 2006; Stevens et al., 2011a; Varela et al., 2013). In addition, the $^{15}N/^{14}N$ ratios (i.e. the ratios of the stable isotopes: $\delta^{15}N$) vary in different types of N compounds (Freyer, 1978; Garten, 1992; Heaton et al., 1997). Determination of $\delta^{15}N$ values enables differentiation of the various anthropogenic sources of N emissions, as the oxidised forms of N are basically emitted to the atmosphere due to transport and industrial activities, whereas the reduced forms (such as NH_y) are derived from agricultural and livestock-related activities. It is therefore essential to determine whether moss transplants are suitable for biomonitoring pollution involving different N compounds.

The possibility of being able to exert greater control over the exposure of moss transplants to particular pollutants makes this type of biomonitoring ideal for determining the effects of these pollutants on physiological parameters used as biomarkers (*i.e.* enzymes). The bryophyte's own enzymes have been shown to be useful indicators of the impacts of N on the organisms (Arróniz-Crespo et al., 2008).

For all of the above reasons, the aim of the present study was to determine whether different types of moss transplants are suitable for use as biomonitors of the deposition of oxidised and/or reduced forms of N. For this purpose, we investigated whether the concentration of atmospheric NO_2 (measured by physicochemical techniques) was related to the % N, $\delta^{15}N$ and the activity of the enzyme biomarkers phosphomonoesterase (PME) and nitrate reductase (NR), in three different types of moss transplants.

2. Material and methods

2.1. Sampling

A total of 15 sampling sites (SS), situated in Galicia (NW Spain) and Cataluña (NE Spain), were used in the study (Fig. 1): 6 were located in industrial environments (SS06-10 and SS15), 7 in urban and periurban environments (SS001-05 and SS13-14), 1 on a cattle farm (SS12) and 1 in a monitoring site included in the sampling network of the European Monitoring and Evaluation Programme (EMEP/VAG/CAMP; http://www.emep.int/; SS11). The following industrial sites were selected because they emit high levels of NO_x to the atmosphere: a non-ferrous smelter (SS06-09), which emits 0.155 Mg of NO_x/NO_2 year^1; a power station of output 563 MW (SS15), which emits 1.62 Mg of NO_x/NO_2 year^{-1}; and power station of output 1468 MW (SS10), which emits 8.87 Mg of NO_x/NO_2 year^{-1} (E-PRTR, 2011; www.prtr-es.es). Both power stations mainly burn coal as a fuel.

The experimental exposures were carried out between February and March 2013 at SS01-05 and between May and June 2013 at the other SS. In each SS, three replicates of each type of transplant (see section 2.2.) and passive/diffusive air samplers (see section 2.3.) were exposed for 21 days at a height of 4 m.

2.2. Preparation of moss bags

Moss bags were prepared using *Pseudoscleropodium purum* (Hedw.) M. Fleisch. which has been widely used in biomonitoring of various types of contaminants (*e.g.* Kunert et al., 1999; Fernández and Carballeira, 2000; Orlinski, 2002; Real et al., 2003; Tsikritzis et al., 2003; Gerdol and Bragazza, 2006; Harmens et al., 2011). The moss samples were collected from an unpolluted rural area on the basis of previous results (Boquete et al., 2013). Once in the laboratory, the samples were cleaned to remove other plant remains, and the apical portions (5 cm) were cut from the shoots. These portions were washed 3 times with bidistilled water (10 L H_2O for each 100 g dry weight (d.w.), with shaking (20 min, 100 rpm), and then once with 10 mM EDTA (1 L of solution per 200 g d.w.) with shaking (20 min, 100 rpm), as suggested by Ares et al. (2012). The samples were then blotted on filter paper to remove excess moisture.

Prior to exposure of the transplants, 3 samples of each type of moss bag were stored for subsequent determination of the initial concentrations of the pollutants (Couto et al., 2004). Another three bags were reserved for use as control samples during analysis of the transplants after the experimental exposures.

After exposure, the moss in the different types of bag was dried at 45 °C and homogenized in an ultracentrifugal mill (Retsh ZM-200) for analysis to determine the % N and $\delta^{15}N$ (see section 2.5.). One sample of each of the 3 auto-irrigated transplants was dried at ambient temperature for analysis of the enzyme activity in relation to N metabolism (see section 2.4.).

2.2.1. Auto-irrigated moss transplants (AT)

Aliquots of moss (wet weight, 10 g) were placed on capillary mats (Ø = 15 cm) at a density of approximately 105 mg w.w. cm^{-2}. Each capillary mat was placed on top of a plastic container; the short ends of the mat were inserted through slits positioned close to the edge of the lid of the container. The container was filled with distilled water, so that the moss was maintained at high relative humidity, to prevent water stress. Each container with the capillary mat and moss sample was covered with a plastic mesh (mesh size 0.8 cm), to ensure that the moss remained in contact with the water and also to prevent it being blown away by the wind (Fernández et al., 2010).