Environmental Pollution 220 (2017) 828–836

Contents lists available at ScienceDirect

Environmental Pollution

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

Assessing temporal trends of trace metal concentrations in mosses over France between 1996 and 2011: A flexible and robust method to account for heterogeneous sampling strategies☆

Emeline Lequy [a, *], Nicolas Dubos [b], Isabelle Witté [a], Aude Pascaud [c, d], Stéphane Sauvage [c, d], Sébastien Leblond [a]

[a] Natural Heritage Department, National Museum of Natural History, 12 rue Buffon, F-75005, Paris, France
[b] Centre d'Ecologie et des Sciences de la Conservation (CESCO UMR 7204) & Mécanismes adaptatifs et évolution (MECADEV UMR 7179), Sorbonne Universités, MNHN, CNRS, UPMC, CP51, 55 rue Buffon, 75005 Paris, France
[c] Mines Douai, Département Sciences de l'Atmosphère et Génie de l'Environment, SAGE, F-59508, Douai, France
[d] Université de Lille, F-59650, Villeneuve-d'Ascq, France

A R T I C L E   I N F O

Article history:
Received 16 August 2016
Received in revised form
17 October 2016
Accepted 22 October 2016
Available online 9 November 2016

Keywords:
Moss survey
Bio-monitoring
Generalized additive mixed modeling
Heavy metals

A B S T R A C T

Air quality biomonitoring has been successfully assessed using mosses for decades in Europe, particularly regarding heavy metals (HM). Assessing robust temporal variations of HM concentrations in mosses requires to better understand to what extent they are affected by the sampling protocol and the moss species. This study used the concentrations of 14 elements measured during four surveys over 15 years in France. Analyses of variance (ANOVA) and a modeling approach were used to decipher temporal variations for each element and adjust them with parameters known to affect concentrations. ANOVA followed by post hoc analyses did not allow to estimate clear trends. A generalized additive mixed modeling approach including the sampling period, the collector and the moss species, plus quadratic effects, was used to analyze temporal variations on repeated sampling sites. This approach highlighted the importance of accounting for non-linear temporal variations in HM, and adjusting for confounding factors such as moss species, species-specific differences between sampling periods, collector and methodological differences in sampling campaigns. For instance, lead concentrations in mosses decreased between 1996 and 2011 following quadratic functions, with faster declines for the most contaminated sites in 1996. On the other hand, other HM showed double trends with U-shaped or hill-shaped curves. The effect of the moss was complex to handle and our results advocate for using one moss species by repeated site to better analyze temporal variations.

1. Introduction

Air quality has been a major issue tackled by the European authorities for decades, and recently by the Directive 2008/50/EC (EU, 2008), or the Clean Air Policy Package adopted in 2013. Air pollutants are suspected to induce respiratory pathologies especially in the case of particulate matter (Brook et al., 2010; III and Dockery, 2006; WHO, 2014), and they also threaten natural ecosystems and agricultural systems by accumulation of toxic components, notably heavy metals (HM) (Kidd et al., 2015; Nagajyoti et al., 2010; Sandilyan and Kathiresan, 2014). Biomonitoring studies have been used all over Europe to monitor air quality by measuring HM concentrations in several organisms. The ICP-Vegetation (International Coordinated Program on Effects of Air Pollution on Natural Vegetation and Crops) was created in the late 1980s under the Convention on Long-Range Transboundary Air Pollution. The ICP-Vegetation uses mosses to bio-monitor air quality in HM, Indeed, mosses have numerous physiological and morphological properties making them efficient bio-monitors of air pollution (Bates, 1992; Harmens et al., 2012; Markert et al., 2003; Ruhling and Tyler, 1968; Schröder et al., 2010). They lack proper root and vascular systems, and they have no developed cuticle on their leaves. This makes mosses very prone to take up elements from wet and dry deposition, and in addition mosses have a well-developed cation

☆ This paper has been recommended for acceptance by Klaus Kummerer.

* Corresponding author.
E-mail address: e.lequy@gmail.com (E. Lequy).

http://dx.doi.org/10.1016/j.envpol.2016.10.065
0269-7491/

exchange properties (Puustjärv, 1955). Concentrations in mosses are higher than in atmospheric deposition and usually above detection levels of analyzers. Besides, their ubiquity and easy-sampling make it possible to collect mosses on very large territories.

Moss bio-monitoring of air quality has already been successfully conducted (Ares et al., 2011; Balabanova et al., 2010; Bekteshi et al., 2015; Berg and Steinnes, 1997; Genoni et al., 2000; Herpin et al., 2001; Varela et al., 2014). For example, a global decrease of HM concentrations in mosses was shown over Europe, suggesting air quality improves (Harmens et al., 2015). Furthermore, relationships between modelled atmospheric deposition and concentrations in mosses were found for lead and cadmium, (Harmens et al., 2012; Schröder et al., 2014, 2010).

However, unlike physical devices used to directly measure atmospheric deposition, mosses are living organisms affected by their environment. Several aspects of the sampling protocol used by the ICP-Vegetation were proven to affect element concentrations in mosses, such as the sampling period, the moss species, or the intra-site variability (Fernández et al., 2015; Fernández and Carballeira, 2002; Lequy et al., 2016). In this respect, Dołęgowska and Migaszewski (2015) and Lequy et al. (2016) stressed the need to account for protocol and environmental parameters to better interpret results of moss bio-monitoring. Yet previous studies describing temporal trends over successive campaigns (Harmens et al., 2015) did not include features of the sampling protocol, despite their contribution to the variability of the concentrations in mosses.

The objectives of this article are to analyze trends of concentrations in mosses in the French contribution to ICP-Vegetation by (i) using the method usually applied by the ICP-Vegetation members on the data to assess trends and (ii) propose a model that analyze temporal trends of concentrations in mosses and that takes into account the possible effects of the protocol, here the collector, the sampling period and the moss species.

To do so, we will (i) use ANOVA and post-hoc Tukey's Honest Significant Differences to globally assess the evolution of concentrations in mosses, and (ii) use mixed modeling on repeated measurements of concentrations during the four French surveys as response variable, with explanatory variables based on features of the protocol.

2. Materials and methods

2.1. The BRAMM dataset

2.1.1. The BRAMM program

The BRAMM program (Bio-monitoring of HM atmospheric deposition using mosses) is the French contribution to the ICP-Vegetation. It consists in four surveys conducted in 1996, 2000, 2006 and 2011, described in Lequy et al. (2016). They include between 449 and 559 sites. The ICP-Vegetation guidelines recommend to sample 5 moss species as gametophytes: *Hylocomium splendens* (Hedw.) Schimp., *Hypnum cupressiforme* Hedw., *Pseudoscleropodium purum* (Hedw.) M.Fleisch. in Engl. et al., *Pleurozium schreberi* (Brid.) Mitt., and *Thuidium tamariscinum* (Hedw.) Schimp., respectively referred to as Hs, Hc, Pp, Ps, and Tt. Details about the sampling protocols and analytical procedures are described in (Harmens, 2010).

Different other data were recorded, notably the site identification, the year of the survey, the date of sampling, the moss species, and the name of the collector of each sampling site for each survey.

This study focuses on the following HM and other elements: Al, As, Ca, Cd, Cr, Cu, Fe, Hg, Na, Ni, Pb, Sb, V and Zn. These elements were measured in the 4 surveys, except Hg which has been measured since 2000, and As and Sb which were not available in the 2000 survey.

In the BRAMM dataset, 280 sites are considered a repetition of measurements over the four surveys (Fig. 1). Such sites were less than 500 m apart between surveys, in similar biotopes (here, the type of forest). Indeed, the BRAMM program only collect mosses under forests, which corresponds to the optimal biotope of the 5 moss species recommended by the ICP-Vegetation guidelines. These 280 sites constitute the "repeated sites" dataset.

2.1.2. Quality of the chemical analyzes

The accuracy of the concentration was assessed for the four campaigns using a reference material, following the standardized procedure described in the AFNOR ISO 5472-25 (Lequy et al., 2016). The reference material is based on a large sample of the moss *Pleurozium schreberi*, named M2, collected in 1993 in a forested background site in Finland as described by Steinnes et al. (1997). For each element, the reference values were computed from the analysis of M2 by 16 European laboratories in 2011. Then the bias between the chemical analyses form a laboratory is determined relatively to the reference value. When the bias is significant, concentrations of the element i were corrected proportionally to the reference concentrations as follows.

Corrected concentration_survey_i = measured concentration_survey_i * M2_reference_2011/M2measured by the French laboratory_survey_i.

A value by default were attributed to values under the detection or the quantification limits. This value by default was calculated as the minimum of the distribution of the considered BRAMM survey divided by three. Elements As and Sb had no available measurement in 2000.

2.2. Analytical approach and statistics

2.2.1. ANOVA and Tukey's Honest significance difference

To highlight an effect of the time on the concentrations in mosses, ANOVA was performed on the four surveys, including all the sampling sites. Differences between the surveys and the regions were assessed using the post hoc test Tukey's Honest Significant Differences (HSD). As all the studied elements were log-normally distributed, they were log-transformed to validate the normality hypothesis needed to use ANOVA and Tukey's HSD. This analysis

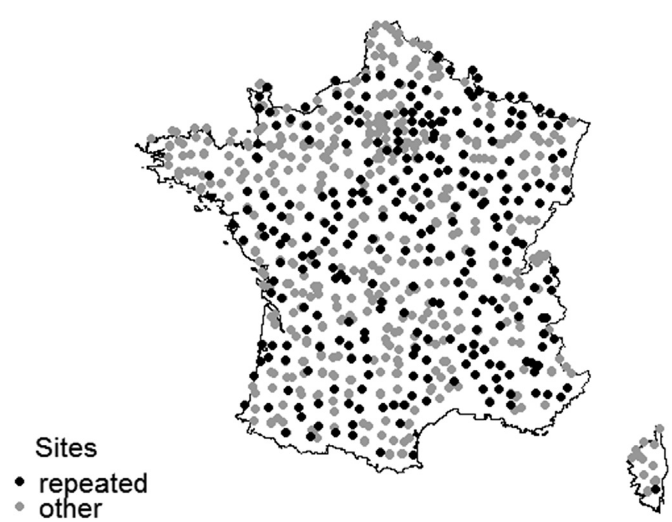

Fig. 1. Distribution of the repeated sites of the BRAMM datasets relatively to all the sampling sites pooled from the four surveys.