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Trace element concentrations in the moss *Hypnum cupressiforme* growing in a presumably unpolluted area

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HIGHLIGHTS

- We determined the levels of pollutants in H. *cupressiforme* at a regional level.
- FA was used to explore their spatiotemporal variability.
- The origin of the pollutants was not the factor underlying this variability.
- Interpreting this variability as the pollutants' origin is an oversimplification.
- This leads to erroneous interpretation of the results of biomonitoring studies.

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ABSTRACT

In this study we determined the concentrations of As, Cd, Hg, Ni and Pb in samples of the moss *Hypnum cupressiforme* collected during 5 different sampling surveys (2006–2014) in a presumably unpolluted area in northern Spain (25 sampling sites). We then applied factor analysis (FA) to the data to explore the factors underlying the spatial and temporal variability in the concentrations. The percentage of variance explained by the FA ranged between 34 and 98%, and was usually higher than 70%. The FA yielded 5 factors that explained the variance in the concentrations of Cd, As, Hg and Pb in all sampling surveys and also a single factor that explained the variance in Hg and Pb concentrations in 2006. Although the lack of obvious sources of pollution in the study region (at least for the elements considered) suggests that most elements (except perhaps Ni) probably originated from long-range atmospheric transport, this would not explain the results of the FA. We suggest that rather than being due to the origin of the pollutants (as frequently assumed), the spatio-temporal variability in the concentrations of these elements is probably determined by a series of other factors: the physicochemical characteristics of the pollutants and of the moss binding surfaces, physiological processes (e.g. moss growth), and the characteristics of the sampling sites (e.g. vegetation cover, elevation, slope, aspect). We therefore conclude that the assumption that variations in element concentrations in moss tissues are due to the origin of the pollutants is an oversimplification that leads to erroneous interpretation of the results of biomonitoring studies with terrestrial mosses.

1. Introduction

The passive moss biomonitoring technique has been used on numerous occasions to evaluate atmospheric inputs of metals and metalloids at regional, national and transnational scales in Europe and, less commonly, outside of Europe (e.g. Viet et al., 2010; Coskun et al., 2011; Thöni et al., 2011; Špirić et al., 2013, etc.). Such studies and surveys usually involve detecting temporal and spatial trends in airborne pollutants and using this information to identify possible sources of the pollutants in the areas concerned. The potential sources of emission within study regions have been ranked amongst the most important factors determining the

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bioaccumulation of metals in mosses (Schröder et al., 2008; Kleppin et al., 2008; Holy et al., 2009).

The variations in the concentrations of atmospheric pollutants determined in biomonitoring studies with terrestrial mosses should therefore mainly be shaped by the emission sources present in the survey area. However, the bioaccumulation of elements in moss tissues not only depends on the atmospheric concentrations of contaminants, but also on other factors that affect the amount of pollutants that reach the mosses (e.g. elevation, topography of the sampling site and vegetation cover: Fernández et al., 2015) and the uptake of pollutants by these organisms (e.g. physicochemical characteristics of the pollutants, physiological processes in mosses: Aboal et al., 2010). In a recent study carried out by our research group, factor analysis (FA) was used to identify patterns of covariance in the accumulation of elements in moss tissues in unpolluted and polluted sites (Varela et al., 2015). The findings demonstrated that the variations in the concentrations of elements in moss samples are more closely related to the physicochemical characteristics of the pollutants and of the moss tissues than to the atmospheric inputs, and it was concluded that the origin of the pollutants was not the underlying factor explaining the relationships between the heavy metals studied (op. cit.). Factor analysis has also been used in studies carried out in the surroundings of other industrial areas (e.g. Kuik and Wolterbeek, 1995; Bačeva et al., 2012). Although the authors of these studies attributed the variations in concentrations of elements to the different sources of contaminants in the areas under study, the results could also be explained by the physicochemical characteristics of the elements and their interactions with the functional groups of the moss and/or particles.

Factor Analysis (FA) and Principal Component Analysis (PCA) have also been applied to regional-scale data on pollutant accumulation in moss samples (e.g. Ermakova et al., 2004a, b; Barandovski et al., 2012; Špirić et al., 2013). In these studies, the large areas considered encompassed several industries supposedly acting as sources of pollutants to the atmosphere. Thus, the authors associated some of the factors identified by FA with the presence of e.g. smelters, refining plants and power plants. However, apart from the previously mentioned study (Varela et al., 2015), we do not know of any studies in which FA has been applied to regional-scale data obtained in areas free of pollution sources.

The aim of the present study was to evaluate whether the spatial and temporal variability in the concentrations of five heavy metals and metalloids in mosses collected in a presumably unpolluted area were due to the common origin of the pollutants studied or to other factors. We applied factor analysis to the data and interpreted the findings (i.e. the variability in the concentrations) in relation to the origin of the pollutants and other possible explanatory factors. The five elements studied were selected because they are explicitly included in the European legislation on ambient air quality.

2. Material and methods

2.1. Sampling and processing

Samples of the terrestrial moss *Hypnum cupressiforme* Hedw. were collected at 25 sampling sites (SS) located at the nodes of a 25 × 25 km grid (i.e. approximately 2 SS/1000 km^2), which mainly lies in the region of La Rioja (N Spain), during 5 different sampling surveys (2006, 2008, 2010, 2012, 2014). The sampling zone is mapped in Fig. 1 and the main geographic and ecological characteristics of the sampling sites are shown in Table 1. The sampling zone has a clear agricultural vocation in the lowlands, with cattle and forestry activities in higher altitudes. Significant industrial activities are restricted to the neighbouring areas of the most important cities: Vitoria (244,000 inhabitants), Pamplona (196,000) and Miranda de Ebro (36,000) in the northern third of the sampling zone, and Logroño (151,000) in the centre. Other populated places in the zone that sustain industrial activities are Calahorra (24,000), Arnedo (15,000), Estella (14,000) and Alfaro (9500), all of them located in the eastern third, and Haro (11,000) in the centre-west. The main highway crossing the sampling zone runs parallel to the river Ebro. The moss samples were preferentially collected in open areas and otherwise in forest clearings as far as possible from trees. At each SS, around 30 subsamples of similar weight and uniformly distributed over an area of approximately 30 × 30 m were collected and combined into a composite sample (Fernández et al., 2015). For each survey, sampling was carried out twice a year (April and October) and the composite samples from each SS were further combined into single (yearly) samples to improve their temporal representativeness (Couto et al., 2003).

In the laboratory, the moss samples were cleaned by removing plant remains and other foreign material. The green parts of the moss shoots were separated and retained for analysis. These samples were then homogenised in an ultracentrifugal metal-free mill (Retsch ZM 200) and dried at 45 °C.

2.2. Chemical analysis

In the samples collected during the 2006, 2008 and 2010 surveys (and also in the aliquots from 2012 to 2014 surveys in which Cd and Pb were determined), trace elements were extracted by acid treatment (2% HNO_3 v/v) and ultrasound (SONICS Vibra cell VCX 130). The concentrations of As, Cd, Ni and Pb were then determined by graphite furnace atomic absorption spectrometry (Perkin Elmer AAnalyst 600). The samples collected in 2012 and 2014 were digested in 1 mL H_2O_2 (30%) plus 5 ml aqua regia (1HNO_3:3HCl) in a microwave oven (CEM Mars 5) before being filtered. The concentrations of As and Ni were then determined by inductively coupled plasma mass spectrometry (ICP-MS - Varian 820-MS). Mercury was always determined directly in powdered material in an elemental analyzer (Milestone DMA80).

For analytical quality control, duplicate samples were analyzed once every nine samples. Two certified reference materials (M2 and M3, *Pleurozium schreberi*; Steinnes et al., 1997) were also analyzed once every nine samples. Analytical blanks were analyzed at the same frequency to control for possible contamination.

The analytical quality of the process was generally satisfactory. The overall percentage error (Čeburnis and Steinnes, 2000) ranged between 1 and 15% (except for Pb in 2006) and was usually below 10%. The percentage recovery usually ranged between 80 and 110%. Despite the use of various analytical techniques, the results from the different surveys were comparable.

2.3. Statistical analysis

Principal component factorial analysis (PC-FA) was applied to the data (according to Mardia et al., 1979) to assess the relationships between the concentrations of elements in moss samples collected during each of the sampling surveys. In the final step, varimax rotation was applied to simplify the loading structure. The analyses were implemented using R statistical software (R Development Core Team, 2008).

3. Results

The concentrations of As, Cd, Hg, Ni and Pb determined in the moss samples from each of the sampling surveys are shown in Fig. 2. The concentrations of the same elements (except for Cd) measured in samples of *H. cupressiforme* collected from unpolluted