



Testing different methods of estimating edaphic inputs in moss biomonitoring

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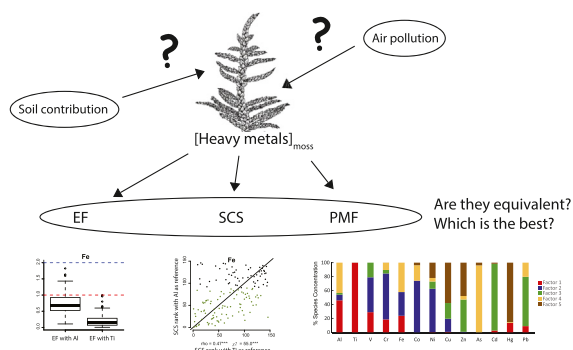
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HIGHLIGHTS

- Soil is known to contribute to the concentrations of elements in moss.
- Protocols for atmospheric biomonitoring with terrestrial mosses need to be improved.
- We compared EF, PMF and another technique for estimating soil contribution.
- Ti is not recommended as a reference element.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 13 January 2021

Received in revised form 4 March 2021

Accepted 4 March 2021

Available online 11 March 2021

Editor: Mae Sexauer Gustin

Keywords:

Atmospheric pollution

Bryophytes

Soil contribution

Heavy metals

Enrichment factor

Positive matrix factorization

ABSTRACT

Although soil is known to contribute to the concentrations of elements in moss, protocols for atmospheric biomonitoring with terrestrial moss do not include recommendations on how to address this factor. As a result, researchers indiscriminately use a wide range of detection/correction methods without considering whether the results are equivalent. In this study, three of these methods were compared: i) use of the enrichment factor (EF) index; ii) calculation of the ratios of different elements in soil and moss, and subtraction of the contribution of soil concentrations from the raw concentrations of elements in mosses (SCS); and iii) positive matrix factorization (PMF), a receptor modelling method for source apportioning based on multivariate analysis techniques. The aim of the comparison was to determine whether the methods produce equivalent results and, if not, which method is the most appropriate for use in moss biomonitoring surveys. The data used corresponded to 146 samples of *Pseudoscleropodium purum* collected from a regular sampling grid of 15 × 15 km in Galicia (NW Spain). Comparison of the methods revealed that, although they yield relatively similar results, the corresponding interpretations are not equivalent and none of the methods provides a reliable estimate of the soil contribution to the concentrations of elements in moss samples. Independently of the technique applied, use of Ti as a reference element is not recommended, because, at least in this study, it was present at unusually high levels in moss. Given the absence of a reliable correction method and the fact that most elements are present in fairly high amounts in the soil, we recommend using atmospheric biomonitoring with moss only for Cu, Zn and Cd, i.e. for those elements in moss for which the soil contributes very low amounts and corrections are not therefore necessary.

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1. Introduction

Terrestrial mosses have been used for many years as biomonitors of atmospheric pollution, but not all of the elements they contain are of atmospheric origin. As Aboal et al. (2010) pointed out (see reference for

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more details), in general, there are three different types of inputs: atmospheric (anthropogenic sources, including long-range atmospheric transport or local point sources of metals and metalloids and natural sources such as sea salts and biogenic emissions), plant (by leaching from plant material of vascular plants) and edaphic.

Some authors have identified this last input, the edaphic contamination, as one of the main problems in the moss biomonitoring technique (Wolterbeek and Bode, 1995; Steinnes, 1995). Moss chemistry sometimes appears to be largely dominated by inputs from local dust or airborne particulate matter, primarily reflecting local site geochemistry rather than major differences in atmospheric chemistry (Bargagli et al., 1995; Steinnes, 1995; Reimann et al., 2001, 2006). The edaphic inputs correspond to entrapped crustal materials, mainly windblown soil dust, or to soluble compounds that are transported from the soil into the moss tissues (i.e. by rain splash and capillary transport) during periods when there is close contact between soil and water (Steinnes, 1995; Berg and Steinnes, 1997). Field observations show the effect of rain splash, which causes soil particles to become layered on moss surfaces. Human activities such as open cast mining, transport and storage of ore and ore products may also influence the amount of edaphic particles deposited on moss tissues (Caritat et al., 2001). According to the results of factorial analysis (FA), these edaphic contributions involve numerous elements such as: Al, As, Ba, Ca, Ce, Co, Cr, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Hf, Ho, La, Li, Lu, Mn, Na, Nb, Nd, Ni, P, Pr, Rb, Sc, Si, Sm, Sr, Ta, Tb, Ti, Th, Tm, U, V, Yb, Y and Zr (Berg et al., 1995; Kuik and Wolterbeek, 1995; Steinnes, 1995; Berg and Steinnes, 1997; Reimann et al., 1999; Caritat et al., 2001). To this list we should probably add other elements such as B, Cs, K, Mg, Pb, Sn and Tl, whose concentration in the moss may have, at least partly, an edaphic origin. The existence of soil-derived particulate matter in moss has been demonstrated by direct observation of the particles in moss transplants (which are less likely to entrap soil particles than native mosses) by electron microscopy (i.e. scanning electron microscopy [SEM] and energy dispersive spectrometry [EDS]), and it was concluded that soil and rock dust were the main sources of particles adsorbed on moss tissues (Adamo et al., 2008).

The edaphic contribution to the total element load in mosses is an important problem when interpreting the results of the moss technique and has been recognized by various researchers. Soil-derived contaminants are difficult to remove (see Fernández et al., 2015 for cleaning methods), because particulate matter cannot be completely detached from moss surfaces (Spagnuolo et al., 2013). In addition, the level of contamination may be relatively high for many elements of interest, particularly in seasonally arid countries (Rühling, 1994). According to Bargagli (1995), comparison of data from regional surveys should consider only samples with comparable contents of Al, Ti or Fe (i.e. with a similar degree of soil-derived contamination).

Various methods have been used to determine the soil contribution to the concentration of elements in moss or, where this is not possible, to establish the source, origin (natural or anthropogenic) type of deposition (primary or secondary) of these elements: i) indices such as the enrichment factor (EF) index (Bargagli, 1995; Bargagli et al., 1995), the contamination factor (CF) index (e.g. Lazo et al., 2019), and the comparison index (Klos et al., 2010); ii) ratios between elements (e.g. Shoty et al., 2016) or between isotopes of the same element (e.g. Xiang et al., 2017); iii) edaphic correction of the raw data (Bargagli et al., 1995); iv) correlations between the concentration of the elements in the moss (e.g. Lazo et al., 2018) or between moss and soil (e.g. Yan et al., 2016); and v) multivariate methods, such as factorial analysis (FA) (e.g. Lazo et al., 2018), principal component analysis (e.g. Qarri et al., 2015), cluster analysis (e.g. Qarri et al., 2015) and positive matrix factorization (PMF) (e.g. Christensen et al., 2018). Techniques that enable interpretation of the soil contribution in individual samples are included in groups i, ii, iii and some group v methods. In addition, some methods in groups i and iii require analysis of soil samples, which is operationally more difficult and expensive.

Although dozens of studies have considered the edaphic contributions to the concentrations of elements in mosses, by the indiscriminate

or complementary use of the aforementioned methods, we have not found any studies that directly compare the methods. The lack of comparative studies probably explains why edaphic correction is not included in the moss biomonitoring protocols (see e.g. Fernández et al., 2015; Frontasyeva et al., 2014), as no correction method has been selected and/or standardized. Knowing whether soil samples should be obtained as part of moss biomonitoring surveys is a necessary requirement for establishing the protocols. As the existence of the soil contribution has been confirmed, a correction method must be included in the protocols to enable comparison of different studies or of data from biomonitoring networks. Three correction methods were compared: the enrichment factor (EF) method; subtraction of the contribution of soil from the raw concentrations of elements in mosses (SCS); and positive matrix factorization (PMF). Soil samples were analysed in the first two methods, but not in the third.

The objective of the study was to determine whether estimations of the soil contribution of elements in individual moss samples by the three different methods are equivalent and, if not, which of the methods is the most appropriate.

2. Material and methods

2.1. Sampling and processing

Samples of the moss *Pseudoscleropodium purum* (Hedw.) M. Fleisch were collected in March in 2014 from 146 sampling sites (SS) located in Galicia (NW Spain). The SS are located at the nodes of a regular 15×15 km grid that covers the entire area of the region (for more details, see Boquete et al., 2009). Whenever possible, the samples were collected at least 300 m from main roads, 100 m from small roads, 4 km from industries and 3 km from cities. The samples were collected in open areas, or where this was not possible, in forest clearings, as far as possible from trees, following the recommendations of Fernández et al. (2015). A minimum of 30 subsamples of similar weight were collected in each SS over an area of between 201 (circular area, $\varnothing > 16$ m) and 2500 m² (50×50 m) (Fernández et al., 2015). The subsamples were combined to form a single composite sample for each SS. Likewise, 10 subsamples of the first 5 cm of soil were collected in each SS and combined to make a composite sample.

Once in the laboratory, moss samples were cleaned to remove remains of adhered material, and the green parts were cut from the shoots. The material was dried in an oven at 45 °C and homogenized in a metal-free ultracentrifuge mill (Retsch ZM200, Retsch GmbH). Finally, the homogenized material was stored in glass vials until analysis. The soil samples were dried at room temperature and sieved with a 2 mm mesh sieve. They were then homogenized in a metal-free agate mill (Retsch Planetary Ball Mill PM 100) and stored in glass vials for analytical determination.

2.2. Chemical analysis

The moss and soil samples were digested in HNO₃ (Hiperpur) and H₂O₂, in a microwave oven (Ethos-1, Milestone). An undigested fraction of each moss sample was used to determine Hg, in a mercury analyzer (Milestone DMA80). The concentrations of Al, As, Cd, Co, Cr, Cu, Fe, Hg (soil only), Ni, Pb, Ti, V and Zn in the digested samples were determined by inductively coupled plasma mass spectrometry (Agilent 7700x). The determinations were carried out at the Research Support Services Unit and the Ecology Unit, University of Santiago de Compostela. The concentrations of almost all elements were above the limits of quantification (LOQ) of the analytical technique, except for Cu, in one sample and Cd and Co in 7 samples. In these cases, the data was replaced by half the corresponding LOQ.

To check the analytical quality, one sample of certified reference material (M2 and M3, *Pleurozium schreberi*, Steinnes et al., 1997) was analysed for every twenty samples. Analytical blanks were also analysed (one every ten samples) to test for possible contamination. The

analytical quality of the process was satisfactory; the overall percentage error was between 3% and 6% in most cases (except for Hg: 12%). The percentage recovery of the reference materials was between ca. 70–130% for Al, As, Cd, Cu, Fe, Ni, Hg, Pb, V and Zn. For Co and Cr, and ca. 69% and 40% respectively. Low recovery of Cr is normal, as it tends to be associated with silicates, which remain almost intact in the HNO₃ and H₂O₂ used in the digestion step. Ti is not certified in the reference material we have used because, as far as we know, there is no reference material with moss matrix that certifies the concentration of this element.

2.3. Statistical analysis

The methods compared were the calculation of enrichment factors, the method involving subtraction of the contribution of soil from the raw concentrations of elements in mosses (SCS) and positive matrix factorization (PMF).

2.3.1. Calculation of enrichment factors (EFs)

Bargagli (1995) and Bargagli et al. (1995) proposed this approach to deal with the problem of edaphic contamination. The raw concentrations of elements are normalized relative to the soil to estimate the edaphic contribution of metals present in mosses:

$$EF = \frac{[X_{\text{interest}}]_{\text{moss}}/[X_{\text{ref}}]_{\text{moss}}}{[X_{\text{interest}}]_{\text{soil}}/[X_{\text{ref}}]_{\text{soil}}} \quad (1)$$

where $[X_{\text{interest}}]$ is the concentration of the element of concern and $[X_{\text{ref}}]$ is the concentration of a selected reference element (usually Al and Ti, but also Fe, Li, Sc and rare earth elements). High EFs are expected for both macronutrients and typical anthropogenic elements, whereas EFs for lithophilic elements should approach a value of 1. This method assumes that if the reference element contents in the moss samples are derived completely from the soil, then an EF = 1 will correspond to 0% enrichment. EF values less than 1 are thus expected for elements that are only present due to edaphic inputs.

2.3.2. Subtraction of the contribution of soil from the raw concentrations of elements in mosses (SCS)

Edaphic correction was first described by Bargagli et al. (1995) and involves subtracting the substratum contribution to the moss concentration from the raw concentration of elements in mosses. In order to do this, Bargagli et al. (1995) relied on the use of a reference element characterized by having its main origin in the Earth's crust and limited metabolic significance in organisms. These researchers then used the following equations to estimate the substratum contribution to the concentration of an element in moss:

$$[X_{\text{ref}}]_{\text{background}} = [X_{\text{ref}}]_{\text{min}} \quad (2)$$

$$[X_{\text{ref}}]_{\text{edaphic}} = [X_{\text{ref}}]_{\text{moss}} - [X_{\text{ref}}]_{\text{background}} \quad (3)$$

$$[X_{\text{interest}}]_{\text{edaphic}} = \frac{[X_{\text{ref}}]_{\text{edaphic}}}{[X_{\text{ref}}]_{\text{soil}}} * [X_{\text{interest}}]_{\text{soil}} \quad (4)$$

where $[X_{\text{ref}}]_{\text{min}}$ is the lowest concentration of the reference element in the whole set of moss samples, $[X_{\text{ref}}]_{\text{background}}$ is the background concentration of the reference element (i.e. the non-particulate fraction), $[X_{\text{ref}}]_{\text{moss}}$ is the concentration of the reference element in the moss, $[X_{\text{ref}}]_{\text{edaphic}}$ is the concentration of the reference element of edaphic origin in the moss, $[X_{\text{ref}}]_{\text{soil}}$ is the concentration of the reference element in the soil, $[X_{\text{interest}}]_{\text{soil}}$ is the concentration of the element of interest in the soil, and $[X_{\text{interest}}]_{\text{edaphic}}$ is the estimated substratum contribution to the concentration of the element in moss.

After obtaining the estimated contribution of the substrate, this value is deducted from the initial concentration, ($[X_{\text{interest}}]_{\text{moss}} -$

$[X_{\text{interest}}]_{\text{edaphic}}$), to yield a new data set that does not include soil contamination.

The background concentration should be selected from an uncontaminated area that is not affected, or only slightly affected, by acid deposition. The excess reference element higher than this value will then correspond to the entrapped particles in each sample (i.e. the amount of soil-derived elements contaminating the moss sample). The concentrations of other metals in moss are estimated by subtracting the amount attributed to the soil from the total element concentration in mosses, relative to the reference element, collected at the same sampling location. Obviously, soil samples must be digested and analysed by the same procedures as the moss samples.

In this study, Al and Ti were used as reference elements, as they are the most commonly used in this type of study. All the Ti present in the moss was assumed to be of edaphic origin ($[X_{\text{ref}}]_{\text{background}} = 0$).

2.3.3. Positive matrix factorization (PMF)

This technique, as far as we know, has only been used in two bio-monitoring studies with terrestrial mosses (Christensen et al., 2018; Olise et al., 2019). However, the technique has been widely used to study air and water quality. Indeed, Hopke (2016) stated that PMF has become the most widely used receptor model, and a sharp rise in use occurred after release of the United States Environmental Protection Agency (EPA) version of the PMF (Norris et al., 2014).

The PMF is a weighted factorization problem with non-negativity constraints (Comero et al., 2009). It is a receptor model used to determine the sources of pollution, their profiles and their contributions to the samples proposed by Paatero and Tapper (1994). Receptor models are based on the mass balance principle, which is rooted in the law of conservation of mass. According to this, the concentration of an element in a sample can be described by the following equation:

$$x_{ij} = \sum_{k=1}^p g_{ik} f_{kj} \quad (5)$$

where x_{ij} is the concentration of the j^{th} element in the i^{th} sample, g_{ik} is the contribution of the k^{th} source to the i^{th} sample, and f_{kj} is the concentration of the j^{th} element in the emission of the k^{th} source. When this relationship is modelled, a new term e_{ij} (the residual for each sample/element) is added to the expression.

The PMF produces the profiles of the sources and their contributions to each sample by minimizing the Q function:

$$Q = \sum_{i=1}^n \sum_{j=1}^m \left[\frac{x_{ij} - \sum_{k=1}^p g_{ik} f_{kj}}{u_{ij}} \right]^2 \quad (6)$$

where u_{ij} is an estimate of the uncertainty for the j^{th} element in the i^{th} sample. The technique allows individually weighted data points.

Furthermore, as previously mentioned, this technique enables estimation of the real concentrations (Christensen et al., 2018), and therefore, in addition to detecting and interpreting the sources of contamination, it enables correction of the raw data to account for the edaphic contribution.

2.3.4. Implementation of statistical analysis

The SCS (Bargagli et al., 1995) and the EF (Bargagli, 1995; Bargagli et al., 1995) were developed following the steps described by their authors. The PMF, first proposed by Paatero and Tapper (1994), was developed following the indications of Norris et al. (2014).

For both the EFs and the estimates obtained by the SCS, the results obtained were compared for the two reference elements selected (Al and Ti). Moreover, as both techniques are based on the relationship between the elements of interest and reference elements of presumed edaphic origin, the ranges of the SS obtained with the FEs were

compared with the ranges of the SS according to the estimate obtained using the SCS method. As the PMF and the SCS provide estimates of soil input, these methods were also compared. In all comparisons, the Spearman correlation coefficient was calculated, and its significance determined.

In the comparison of the EF and the SCS, the SS classification/categorization was also considered. In the EF method, SS was considered enriched when the EF value was higher than the established enrichment threshold (in this case set at 1). In order to compare the two methods, an enrichment threshold was established so that if the SCS value was higher than the LOQ of the element of interest, the SS was considered enriched by that element. McNemar's test (Martínez-González et al., 2006) was used to detect any significant differences between the SS ranks obtained by the two techniques.

The SCS and the EF methods were implemented in the R statistical program (R Core Team, 2020), and the PMF method was implemented using the EPA's PMF 5.0 software (Norris et al., 2014). For calculation of the PMF, 16 SCS in which the concentrations of some elements were outliers were first eliminated from the original data set.

3. Results

The summary statistics of the concentrations in mosses and soil can be seen in Table A.1.

3.1. Enrichment factor

The rank of the SS changes according to the reference element considered (Fig. 1). The Spearman's correlation coefficients calculated for the different elements were significant ($p < 0.001$) but, according to the scale proposed by Mukaka (2012), they were low or moderate (0.42–0.69), except for Cu, which was high (0.74). The number of SCS classified as enriched was much higher when Al was used as a reference element than when Ti was used. The classification of SS depending on whether or not the EF is above the enrichment threshold (for a threshold of 1) differed significantly ($p < 0.01$) depending on the reference element considered. Both the reference element and the enrichment threshold can greatly affect the interpretation of a SS (Fig. A.1). In general, the EF values calculated for Al as the reference element were higher than when Ti was used as the reference element.

3.2. SCS

Similar to the EFs, the results of the SCS method also depend on the element used as the reference element (Fig. A.2). The correlations, most of which were statistically significant ($p < 0.01$; except for Ni with $p > 0.05$ and $\rho = 0.04$), were low to moderate (0.26–0.61). The SS classification differed significantly ($p < 0.001$) for all elements: the estimate made with Al as the reference element classified more SCS as enriched than the estimate made with Ti as the reference element.

3.3. Positive matrix factorization

The PMF yielded five factors. After rotation, these factors were illustrated as factor fingerprints (Fig. 2), which represented the composition of the emissions from the different sources (factors) detected by the PMF. Two clearly soil-based sources were identified: Factors 1 and 4. Factor 1 was dominated by Ti and also accounted for a large part of Al. Factor 4 was dominated by the As, which in this case was originated from the soil, as was also indicated by the EF and SCS methods. This

factor also seems to be the source of a large part of the Al and Fe, and it was thus categorized as an edaphic factor. Factors 3 and 5 were clearly of anthropogenic origin: the first was the main source of Cd and Pb and the second the main source of Hg. Factor 2, which accounted for a large part of the concentrations of V, Cr, Fe, Co, Ni and Cu, seems to be related to contamination derived from processes that mobilize soil or rocky material (e.g. mining) or other anthropogenic sources of pollution.

3.4. Comparing the different techniques

For most of the elements under study, the McNemar test did not detect any significant differences between the results of the EF and the SCS techniques when the same reference element was used. Both techniques yielded very similar results when Ti was used as the reference element (Fig. A.3), with high correlations in almost all cases and detection of few significant differences in concentrations of elements in the SS classification. When Al was used as the reference element (Fig. A.4), high correlations were observed for those elements in which there was a considerable soil input, but very low and sometimes not significant correlations for those elements of predominantly anthropogenic origin. Differences in SS classification were observed for those elements for which the soil contributed large amounts. On the other hand, when different reference elements were used to calculate the EFs and SCS (Figs. A.5 and A.6), the correlations for all of the elements of interest were moderate, low or even negligible, and in some cases were not significant ($p > 0.05$). In addition, the SS classification differed significantly for all elements.

Comparison of the soil inputs estimated by the SCS and the PMF methods showed that the SCS estimates were much higher than the PMF estimates (sum of Factors 1 and 4) (Fig. 3 and Fig. A.7); however, the estimates obtained when Al was used as the reference element were closer to the one-to-one line and lower than those obtained with Ti as the reference element. The Spearman's correlations for the comparison between both techniques were high or very high when Al was used as the reference element (0.73–0.98) and moderate or low when Ti was used (0.39–0.57) (Fig. 3).

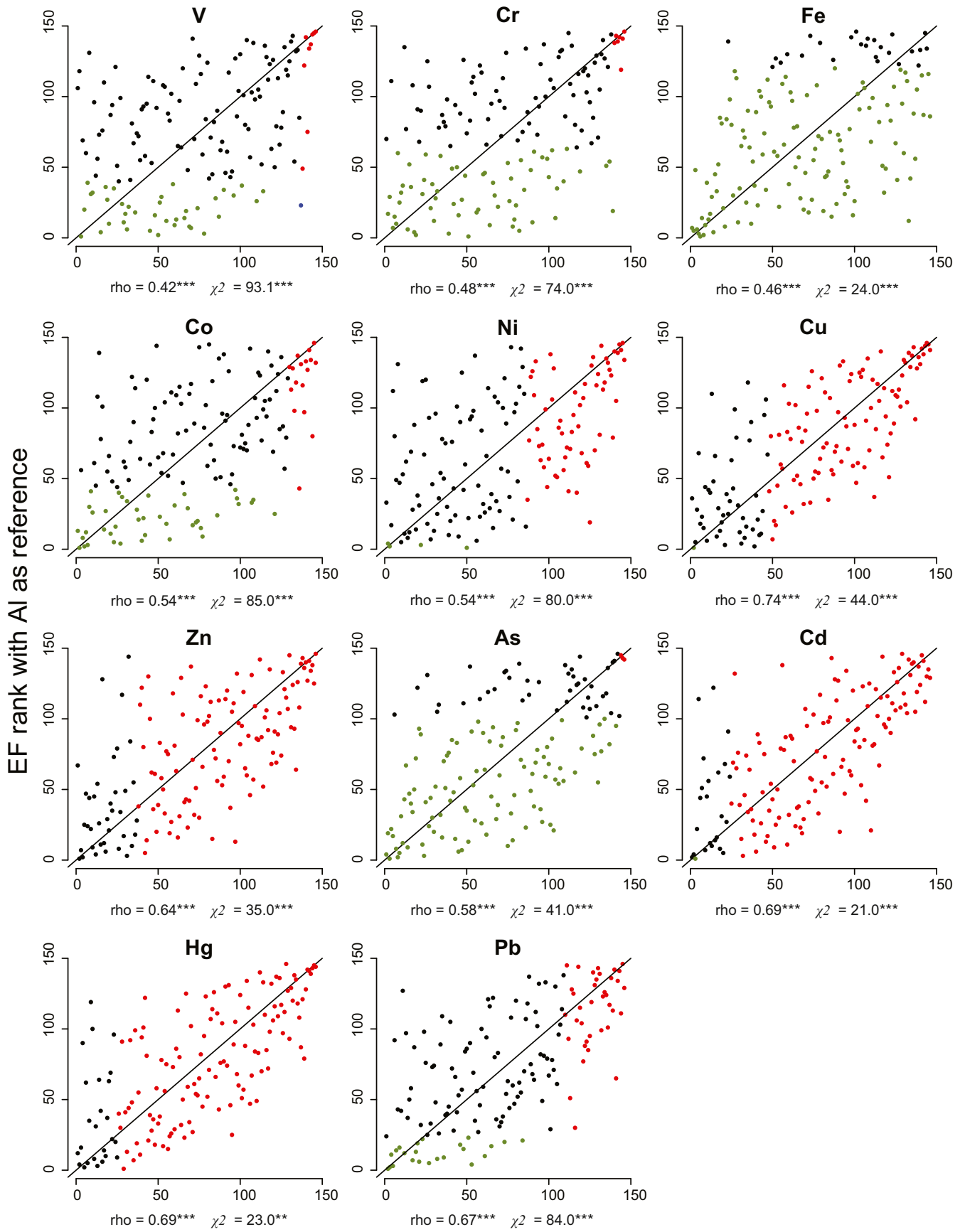
The three techniques enabled classification of most of the elements in three groups according to the edaphic contribution (Figs. A.1 and 4): i) Al, Fe and As, of largely edaphic origin; ii) Cu, Zn and Cd, of mainly anthropogenic origin; and iii) elements for which the soil contribution was intermediate (i.e. V, Cr, Co and Pb).

As mentioned in the previous comparisons, the estimates of soil input obtained with Ti as the reference element were extremely high, with percentages higher than 50,000% of the concentration observed in the sample (Fig. 4). These anomalies can be explained by the fact that the ratio between the concentration of Ti in moss and in the soil was one order of magnitude higher than most of the elements analysed in the study, especially those belonging to groups i and iii (Table 1).

4. Discussion

On the basis of our results, we conclude that the interpretations derived from the application of the three methods used, although similar and even complementary, are not equivalent. However, it was not possible to determine whether one of the methods is superior to the others and the choice will depend on the objective pursued by the researcher and on the data and resources available. For example, researchers might rule out the use of the EF and SCS methods (both of which require data on the concentration of the elements in the soil) on the basis of cost and opt for other less expensive methods. However, as the temporal

Fig. 1. Comparison of the ranks of the Enrichment Factors (EFs) obtained with Al and Ti as reference elements, for each element considered (ordered according to the atomic mass). Spearman's rho, and associated significance, and the McNemar test χ^2 statistic, and associated significance, carried out to determine if the classification of site as enriched depends on the technique used are shown for each element (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$). A color was assigned to each point (sampling site) to indicate whether the calculated EFs exceeded the enrichment threshold (set at 1). Green: both EFs are below the threshold; Red: both are above the threshold; Black: only the Al EF is above the threshold; Blue: only the Ti EF is above the threshold. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



EF rank with Ti as reference

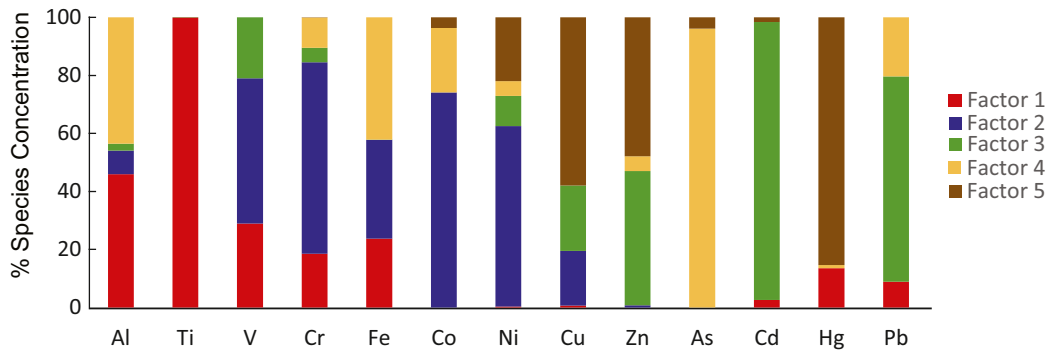


Fig. 2. Percentage of the concentration of each element that corresponds to each of the factors obtained with the Positive Matrix Factorization (PMF) method.

variation in soil concentrations is generally low, the results of a single soil sampling survey could be used to calculate EF and SCS over several years. Other factors to consider are whether qualitative (EF) or quantitative (SCS and PMF) analysis of the edaphic contributions of the elements in the moss is intended, or whether previous information is available about the possible sources of contamination.

The EF method has been the most widely used, being frequent even today (e.g. Ávila-Pérez et al., 2019; Svozilíková Krakovská et al., 2020). This is because the calculations involved are simple and interpretation is straightforward. The main difference between the EF method and other index-based methods is that soil sampling is conducted adjacent to the SSs where moss is sampled. Despite the higher cost involved,

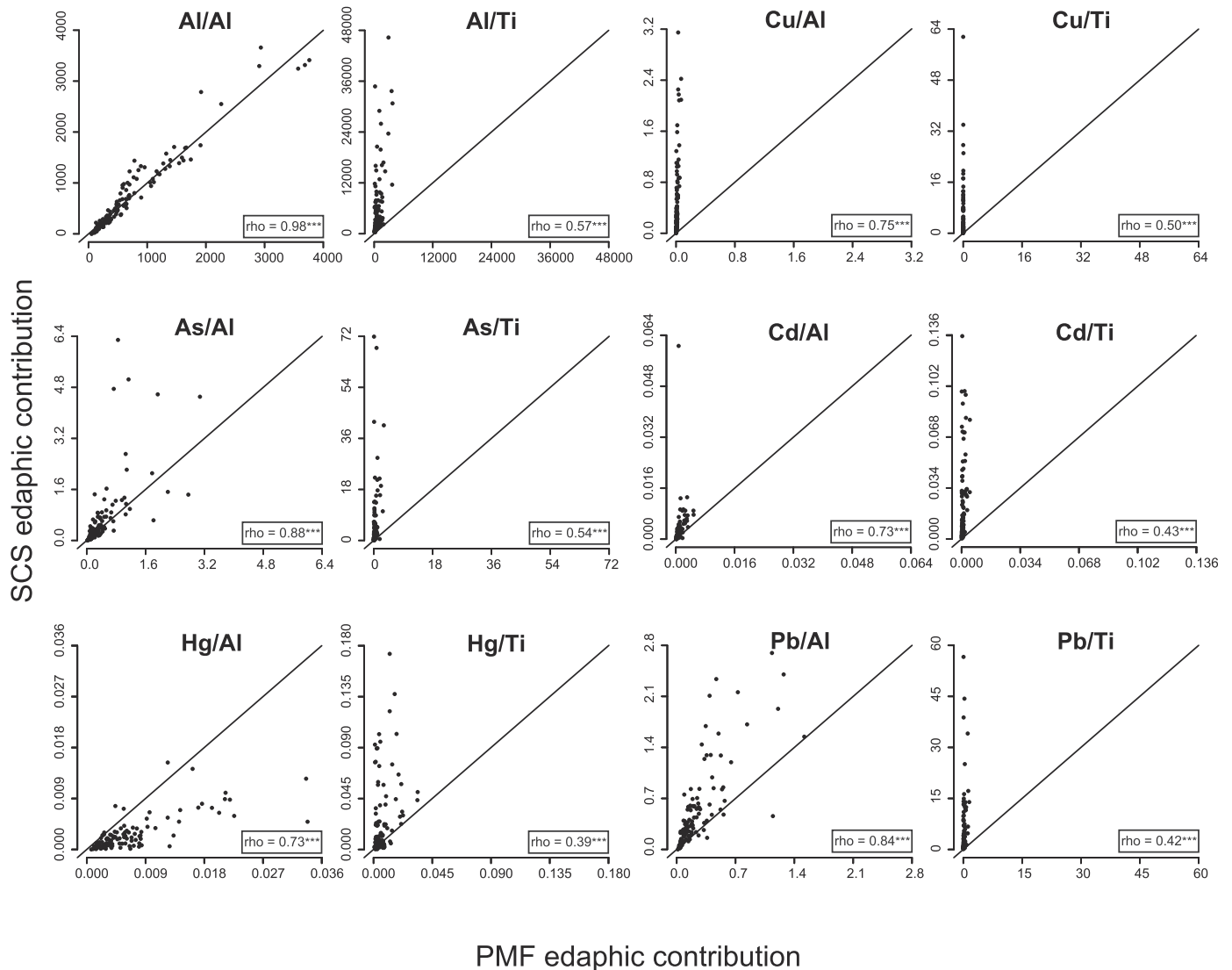


Fig. 3. Comparison of the soil contribution to element concentrations estimated using the Positive Matrix Factorization (PMF) method (A) and the SCS, with Al (B) and Ti (C) as reference elements (see text for further details). Spearman's rho value and the associated significance are shown for each element, (*: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$).

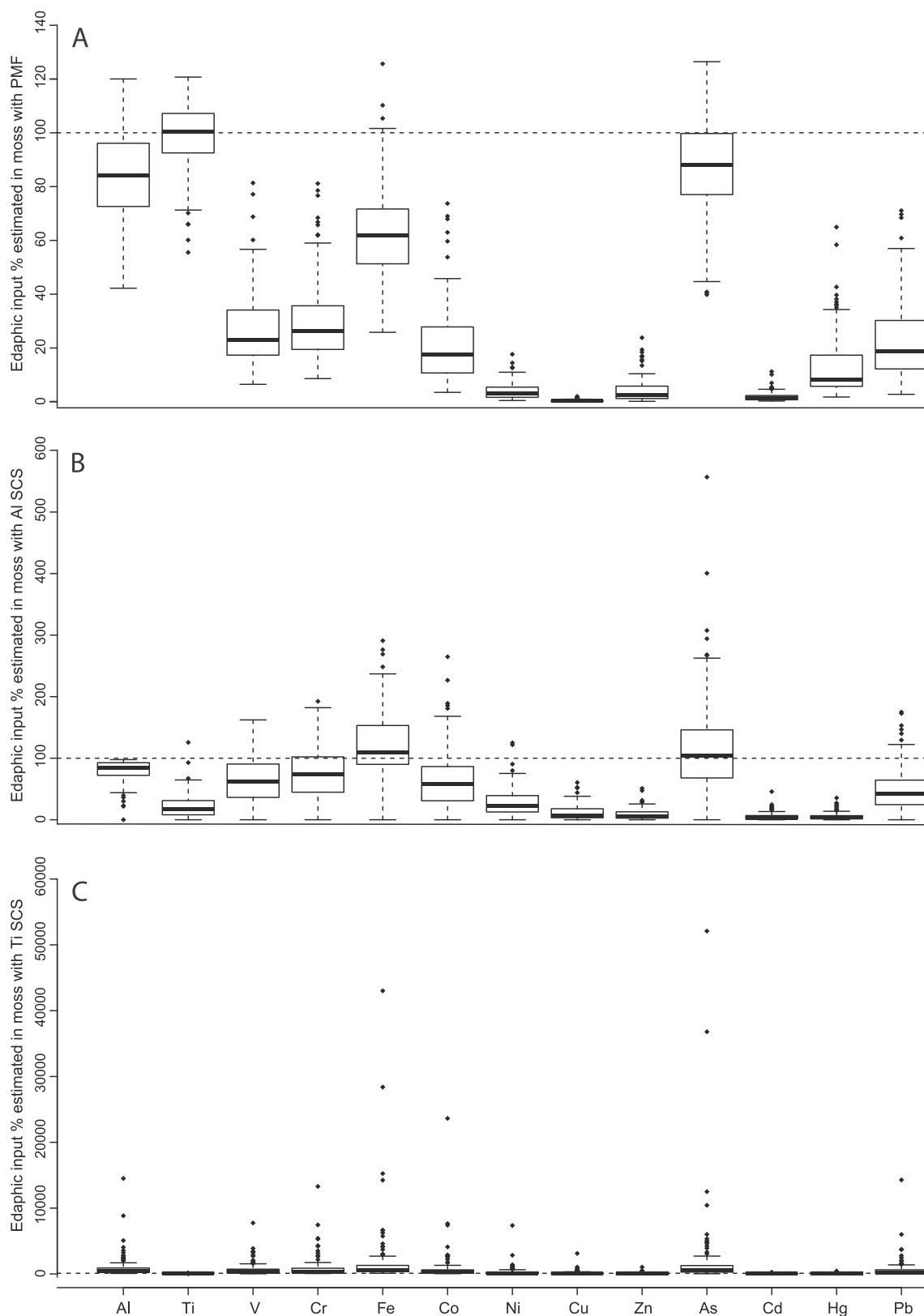


Fig. 4. Percentage of the total concentration of the elements in the moss corresponding to the soil contribution estimated by Positive Matrix Factorization (PMF) method, SCS, with Al as the reference element, and SCS, with Ti as the reference element (see text for further details).

this allows more accurate normalization than with other indices which use background concentrations as reference values (as in the CF), without an effective method of determining them having been developed. However, there is no consensus among researchers about the values

that should be used as enrichment thresholds, which vary between 1 (Jiang et al., 2018) and 10 (Dragović and Mihailović, 2009). Thus, the same SS could be considered enriched by an element or not enriched depending on the threshold selected (e.g. Co in Fig. A.1). This question

Table 1

Mean, median and range of ratios of the concentrations of different elements in moss and soil. The values were calculated from all the samples used in the study ($n = 146$).

| [element] _{moss} /[element] _{soil} | Mean | Median | Range |
|--|------|--------|------------|
| Al | 0.05 | 0.03 | 0.00–0.64 |
| Ti | 0.38 | 0.15 | 0.01–5.52 |
| V | 0.06 | 0.04 | 0.01–0.75 |
| Cr | 0.06 | 0.03 | 0.01–0.65 |
| Fe | 0.03 | 0.02 | 0.00–0.32 |
| Co | 0.06 | 0.04 | 0.01–0.41 |
| Ni | 0.14 | 0.10 | 0.02–0.73 |
| Cu | 0.43 | 0.29 | 0.04–1.97 |
| Zn | 0.52 | 0.40 | 0.08–2.65 |
| As | 0.03 | 0.02 | 0.00–0.28 |
| Cd | 1.06 | 0.79 | 0.06–6.97 |
| Hg | 1.06 | 0.66 | 0.13–12.97 |
| Pb | 0.07 | 0.06 | 0.00–0.35 |

remains unresolved, and careful interpretation of the results is required. Furthermore, the results obtained are not quantitative, and if the objective of a study is to compare sampling sites, this method does not allow comparison of levels of concentrations in different sites.

The concepts on which the calculation of EFs are based are very similar to those on which the SCS method is based. Both require use of a reference element, which as demonstrated in this study, can greatly modify the results obtained. As already mentioned, Ti and Al are the elements most commonly used as reference elements. In general, the calculations made relative to Ti produce higher estimates of the edaphic contribution to moss and, therefore, fewer SSs are classified as enriched. The overestimation is obvious for all of the elements in Fig. 4, especially for Fe and As (where it exceeds 40,000%), and is due to the fact that the Ti_{moss}/Ti_{soil} ratio is higher than the ratio of other elements of mainly edaphic origin, even up to one order of magnitude higher (e.g. Table 1 shows that the mean values of the moss/soil ratio is 0.38 for Ti and 0.04 for Al). These unusually high levels may be attributed to the physicochemical characteristics of Ti, specifically the ionic radius and covalence index (Nieboer and Richardson, 1980), which cause this element to have a higher affinity for binding sites on the moss surface than other elements of edaphic origin. Therefore, and according to our results, when Ti is present in moss at unusually high concentrations, its use as a reference element is not recommended, and other elements, such as Al, should be used. Thus, for ratio obtained for a reference element is lower (as with Al) than that obtained for Ti (due to unusually high levels of Ti in moss), it could be interpreted (wrongly) that the Ti in moss is not derived from the soil. However, even when Al was used as the reference element the SCS tended to overestimate the soil contribution (e.g. Fe and As in Fig. 4B). In addition, the lowest concentration of Al in the sample was used as the background concentration in the SCS method, even this procedure has not been validated.

In the comparison of the EF and SCS methods, both calculated with Al as the reference element (Fig. A.4), high correlations were obtained for V, Fe, Co, Cr, As and Pb. These elements coincide with groups “i” and “iii” identified in the results, i.e. those elements for which soil contributions are high or moderate. On the other hand, the correlations for elements belonging to group “ii” (Cu, Zn and Cd) and those that were not clearly classified in any of the three groups (Ti, Ni and Hg) were low or even non-significant. The high rank dispersion in the enriched SSs (Fig. A.4) is probably due to the differences between the EF and the SCS approaches. Thus, while the EF indicates the relationship between the concentrations of the elements, the SCS corrects the concentration by eliminating the estimated contribution from soil. When the soil contribution is high, the EFs are low (in this case less than or equal to 1) and the SCSs are also low (the remaining concentration, once the estimated soil input is eliminated, is low). However, when the soil contribution is low or negligible, the EFs are high, but the SCSs are not necessarily high. The SCSs may be low or high depending on

the initial concentration of the element in the moss, so that the rank of each SS varies depending on whether the EF or the SCS is applied. Although the correlations for the comparison of the two methods were low for elements with low soil input, both methods produced similar classifications (almost all SSs were enriched). The difference occurs for elements with large inputs from the soil, leading to significant differences in the classification of SSs, especially for those SSs which are close to the enrichment thresholds. This is because there are no standardized criteria for selecting the EF threshold and the SCS is not designed to have an enrichment threshold. In this study, the LOQ was established as the threshold for the SCS to enable comparison between the two techniques.

In addition, for application of the EF and SCS methods, the choice of the depth at which soil should be sampled is also a matter of discussion. Some authors (e.g. Kålås et al., 2000) consider that when the objective of a study is to evaluate the influence of local geochemistry on the concentrations of some metals, soil samples must be obtained at a relatively deep depth (e.g. 60 cm), corresponding to the C-horizon. At this depth, the influence of atmospheric deposition is negligible, whereas surface soils will be strongly affected by airborne metals. Nevertheless, in all of previous relevant studies, the upper 5 cm of soil was sampled (Bargagli, 1995; Bargagli et al., 1995; Fernández and Carballeira, 2001; Gerdol et al., 2002). At this depth, the samples could be influenced by airborne metals and the soil correction would include atmospheric deposition. In the present study, we wanted to take into account pollution of edaphic origin and secondary atmospheric pollution, caused by contamination of the moss by soil particles, which in turn would have been contaminated via atmospheric deposition of particles. We thus aimed to isolate the effect of primary air pollution on mosses by using the upper 5 cm of soil. In addition, it should be noted that the moss samples were digested with HNO_3 and H_2O_2 , which incompletely digest the soil particles in the moss, leaving the silicate matrix almost intact. As we did not want to release more elements than those released from the soil particles in the digested moss samples, the soil samples were also digested with HNO_3 and H_2O_2 (incomplete digestion).

Regarding the PMF method, this quantitative analysis has the advantage of being considerably cheaper than the others (no soil sampling is required) and it does not depend on the use of reference elements, thus avoiding the previously described problems. Furthermore, the PMF is very useful when the model assumptions are fulfilled, and its application has been reported in more than 1000 papers (see review article by Hopke, 2016). Nevertheless, the model assumptions are more stringent and restrictive, and like other statistical approaches, the method has some disadvantages: i) a greater number of SSs than used in a vast number of moss biomonitoring studies is required to produce robust results; ii) as deletion of SSs with outliers is a prerequisite for application of the method, the soil correction cannot be applied to the data from these SSs; iii) the PMF assumes that all SS are affected by common sources of pollution, which is probably not true in studies of extensive areas; iv) interpretation of the factors depends on the researcher; and v) like other receptor models, it is based on solving a mass balance equation (based on the principle of conservation of mass) by using multivariate analysis (Belis et al., 2014), and therefore it must be assumed that mosses retain all the of the elements that reach them, which is not the case (Aboal et al., 2010). The assumption of a common source would not be a problem in this study, as all SSs were in areas far from sources of pollution and included in a relatively small area (ca. 29,000 km²). The number of SSs is also not problematical, as the study included 146 sites, which is a large number for this type of study. However, the factors obtained in the PMF method are similar to those obtained by Varela et al. (2015) by means of Factorial Analysis. Thus, factor F_{b1} identified by Varela et al. (2015) was dominated by the same elements as Factor 2 in the present study (Co, Cr, Fe, Ni and V), factor F_{b2} was equivalent to Factors 3 and 5 (Cd, Pb, Zn, Hg and Cu) and factor F_{b3} was equivalent to Factors 1 and 4 (dominated by Al, As, Fe and, to a lesser extent, Co and Cr). This may indicate that the physicochemical characteristics

have a greater influence on the concentrations of the elements than their source of origin.

Comparison of the quantitative methods (PMF and SCS) of estimating the soil contribution, with Ti as the reference element (Figs. 3 and A.7), showed that SCS produced much higher estimates than PMF. This can be explained by the unusually high concentrations of Ti in moss. Although the estimates obtained when Al was used as the reference element are also higher than the PMF estimates (with the exceptions of Ti and Hg), they are much closer to the one-to-one line. For those elements for which almost all of the total amount in moss is derived from Factors 1 and 4 (identified as soil factors) or Factors 3 and 5 (anthropogenic factors), the SCS and PMF estimates (with Al as the reference element) are similar. The greatest discrepancies in the estimates produced by these two techniques involved the elements for which Factor 2 makes an important contribution. This factor may be related to activities that mobilize soil and rocky material leading to it being deposited on the soil surface. Given that in this study the upper 5 cm of the soil was sampled, the contribution by Factor 2 would be confused with the soil contribution in calculating the SCS (the same also applies to the EFs). This confusion could lead to overestimation of the soil input. Nonetheless, moss acts as a selective filter and does not accumulate all elements in the same way (Varela et al., 2015). In addition, moss has an extremely high level of temporal variability in the concentrations of different elements (Boquete et al., 2011). Quantitative interpretation of the element loads (i.e. concentrations) in moss is therefore not recommended, and a qualitative approach that indicates whether the moss is enriched/contaminated or not is more appropriate. Although use of the EF method with Al as a reference element might seem appropriate, this method provides an index and does not correct the concentrations. Moreover, this index does not take into account the overall data. Qualitative treatment of the data would ideally be done on the corrected concentrations, after elimination of the soil contribution.

On the basis of the study findings, we can conclude that none of the three methods used fully satisfies the requirements for applying the soil correction to the different concentrations of elements in the moss, for the following reasons: i) the results of the EF method depend on the reference element used. In addition, there is no standard enrichment threshold and the technique does not allow application of a soil correction to the data; ii) the SCS shares some of the problems of the EF method and tends to overestimate the soil contribution; and iii) the PMF has very restrictive assumptions, and interpretation of the results depends on the experience and prior knowledge of the researcher. Approximate estimates of the element content contributed by soil can be obtained using these techniques. However, as the methods have not been validated, we recommend applying all possible methods of estimating the soil inputs in moss samples in order to enable robust conclusions to be reached.

As there is no accurate and reliable method for estimating the proportion of elements in moss that are derived from soil, it is important to identify those elements for which the estimates should be made. In this study, most of the 13 elements considered were classified into one of three groups depending on the importance of the soil as a source of the element in the moss samples. The first group, in which the elements have a mainly edaphic origin, includes Al, As and Fe. Although As may be of soil origin, it is often attributed to agriculture activities and burning of fossil fuels (e.g. Khiem et al., 2020; Ștefănuț et al., 2019). In this study, As was assumed to be of edaphic origin due to the comparison of the PMF factors and the results obtained after the application of the EF and the SCS. The last two techniques indicated that the As in moss was of edaphic origin and, considering that Factor 4 of the PMF also included a high percentage of the variability in Al, the origin of the As is clearly edaphic (as also observed by e.g. Kuik and Wolterbeek, 1995). The second group, in which there is scarcely any contribution from the soil, comprises Cu, Zn and Cd. Enrichment of these elements was detected in all or almost all of the SSs, with very low contributions from Factors 1 and 4 (Fig. 2). The third group is

composed by V, Cr, Co and Pb, with intermediate contribution from the soil, enrichment in some SSs and part of the variability being explained by Factors 1 and 4 of the PMF. Within this group, Pb is unusual because it is generally associated with anthropogenic inputs (e.g. Lazo et al., 2018). However, Ti, Ni and Hg did not clearly belong to any of the groups. Titanium was present at unusually high levels in moss, but the entire concentration in moss is known to be derived from the soil (Bargagli, 1998). Nickel (Fig. A.4) enrichment was observed almost all of the SSs, although the percentage of the estimated soil contribution (SCS method) was not negligible (Fig. 4). A similar finding was found for Hg, as enrichment of this element was detected in almost all of the SSs, but the percentage of the estimated soil contribution (PMF method) was not negligible.

On the basis of the above, we recommend use of the moss biomonitoring technique for those metals for which edaphic corrections are not required, i.e. those elements in which the edaphic contribution is negligible (Cu, Zn and Cd). Interestingly, Cd is one of the few elements for which there is a correlation between bulk deposition and moss concentrations, possibly because of the absence of soil inputs (Aboal et al., 2010; Boquete et al., 2017). In fact, the most extensive studies of atmospheric biomonitoring with mosses focus on Cd and Pb (e.g. Harmens et al., 2012) and, although the Pb is one of the elements for which soil contributions are intermediate, Cd clearly falls in the group of elements with negligible contributions from the soil. Likely, there are more elements with a minimal edaphic contribution such as Ag, Bi, I, Pd, Pt, Rh, Sb and Se, which are elements with low concentrations in nature and they are released, in significant quantities, by anthropogenic sources.

5. Conclusions

Comparison of the EF, SCS, and PMF methods showed that the edaphic contribution to element concentrations in moss can be interpreted in different, not equivalent, ways. Although the interpretations may be relatively similar, none of the three methods are reliable, validated techniques for determining the edaphic input of elements in moss samples. Nonetheless, and regardless of the technique used, use of Ti as a reference element is not recommended because, at least in the present study, it is present at unusually high concentrations in moss.

Given the absence of reliable techniques for correcting the generally important edaphic contribution of elements in moss, we recommend only using moss for atmospheric biomonitoring of those elements with very low edaphic contribution in moss and for which corrections are not necessary (Cu, Cd and Zn in this study).

CRedit authorship contribution statement

Pablo Giráldez: Methodology, Software, Validation, Formal analysis, Writing – original draft, Visualization. **Zulema Varela:** Methodology, Investigation, Data curation, Writing – review & editing, Visualization, Supervision. **Jesús R. Aboal:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition. **J. Ángel Fernández:** Conceptualization, Methodology, Investigation, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors belong to the Galician Competitive Research Group GRC/GPC2016-002 and to the CRETUS Strategic Partnership (AGRUP2015/02) which are co-funded by FEDER (EU). Authors would like to thank RIAIDT-

USC for the use of analytical facilities. P. Giráldez is grateful to the Spanish Ministerio de Ciencia, Innovación y Universidades for a grant awarded within the Programa de Formación de Profesorado Universitario (FPU 2018 [grant number FPU18/04134]). Z. Varela was supported by a post-doctoral research grant awarded by the Autonomous Government of Galicia (Spain).

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2021.146332>.

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