Background element content of the lichen Pseudevernia furfuracea: A supra-national state of art implemented by novel field data from Italy

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Background element content of a highly performing lichen biomonitor: a methodological review integrated by field data

Authors:

Elva Cecconi[1], Guido Incerti[2], Fiore Capozzi[3], Paola Adamo[4], Roberto Bargagli[5], Renato Benesperi[6], Fabio Candotto Carniel[1], Fabiana Cristofolini[7], Sergio Enrico Favero-Longo[8], Simonetta Giordano[3], Domenico Puntillo[9], Sonia Ravera[10], Valeria Spagnuolo[3], Mauro Tretiach[1]*

[1]Department of Life Sciences, University of Trieste, Italy
[2]Department of Agri-Food, Animal and Environmental Sciences (DI4A), University of Udine, Italy
[3]Department of Biology, University of Naples Federico II, Italy
[4]Department of Agricultural Sciences, University of Naples Federico II, Italy
[5]Department of Physical, Earth and Environmental Sciences, University of Siena, Italy
[6]Department of Biology, University of Florence, Italy
[7]Research and Innovation Centre, Fondazione Edmund Mach (FEM), San Michele all'Adige, Italy
[8]Department of Life Sciences and System Biology, University of Torino, Italy
[9]Natural History Museum and Botanical Garden, University of Calabria, Italy
[10]via del Labaro 54, Rome, Italy

**Corresponding author contact details: Mauro Tretiach*
Address: Via L. Giorgieri 10, I-34127 Trieste, Italy
E-mail: tretiach@units.it

Abstract

In biomonitoring, the knowledge of background element content (BEC) values is an essential pre-requisite for the correct assessment of pollution levels. Here, we assessed the BEC values of a highly performing biomonitor, the epiphytic lichen *Pseudevernia furfuracea*, by means of a careful review of literature data, integrated by an extensive field survey. Methodologically homogeneous element content datasets, reflecting different exposure conditions across European and extra-European countries, were compiled and comparatively analysed. Element content in samples collected in remote areas was compared to that of potentially enriched samples, testing differences between medians for 25 elements. This analysis confirmed that the former samples were substantially unaffected by anthropogenic contributions, and their metrics were therefore proposed as a first overview on supranational background levels. We also showed that bioaccumulation studies suffer a huge methodological variability. Limited to original field data, we investigated the background variability of 43 elements in 62 remote Italian sites, characterized in GIS environment for anthropization, land use, climate and lithology at different scale resolution. The predictivity of selected environmental descriptors on BEC was tested using Principal Component Regression (PCR) modelling. Land use, climate and lithology resulted highly predictive of the elemental composition. In the case of lithogenic elements, regression models consistently predicted the lichen content throughout the country at randomly selected locations. Further predictors should be considered only for As, Co, and V. Through a multivariate approach we also identified three geographically homogeneous macroregions at national level for which specific BECs were provided, for use as reference in biomonitoring applications.

Keywords: air pollution; baseline; bioaccumulation; particulate matter; *Pseudevernia furfuracea*

1. Introduction

Lichens are widely used as passive (native) or active (transplanted) bioaccumulators (Herzig et al., 1989; Garty, 2001) to monitor deposition of airborne persistent pollutants (Brunialti and Frati, 2014), because their pollutant content is significantly related to the bulk atmospheric depositions (e.g. Herzig et al., 1989; van Dobben et al., 2001). In both cases, a key issue is the interpretation of data in terms of deviation from unaltered, "natural" references. Several approaches have been suggested to quantitatively assess such deviation: (i) interpretative scales, i.e. ranks of increasing alteration matched to corresponding element concentration ranges, based on meta-analysis of available data of different species (Nimis and Bargagli, 1999) or single species (Nimis et al., 2001; Tretiach and Baruffo, 2001a); (ii) "Exposed-to-Control ratio" (EC ratio), limited to transplant applications, as the element concentration ratio in exposed to unexposed samples (Frati et al., 2005); (iii) comparison with "background values", i.e. baseline element concentration values measured in samples collected in remote areas, far distant from known emission sources (Bargagli, 1998). In this framework, the knowledge of background content of persistent chemicals is of primary importance for the evaluation of pollution phenomena, in several ecological compartments (Reimann and Garrett, 2005).

While chemical backgrounds are frequently reported for soils and sediments (e.g. Chen et al., 1999; Rodríguez et al., 2006), biological matrices have been less investigated, with exceptions regarding mostly mosses and vascular plants (Markert and De Li, 1991; Chiarenzelli et al., 2001). In the case of lichens, background element content (BEC) values were reported for pools of epiphytic foliose or fruticose species (Bargagli, 1998) and for a single species (*Hypogymnia physodes*: Bennet, 2000) based on literature reviews. BEC values derived from *ad hoc* designed field campaigns were reported for pooled epiphytic species collected in different mountain systems of the world (Bergamaschi et al., 2004), for the epilithic *Umbilicaria decussata* in Antarctica (Bargagli et al., 1999), and for two species of *Nephroma* and *Usnea* from Patagonia (Monaci et al., 2012). Several criticalities affect current available lichen BEC values:

(a) data pooled for different taxa may be problematic (Djingova et al., 2004), since the species may accumulate differently (Nimis et al., 2001; Tretiach and Baruffo, 2001b; Minganti et al., 2003); (b) the analysed species are not used in standard biomonitoring surveys; (c) previous reviews did not consider methodological differences in sample pre-processing (e.g. washing, drying) and analytical procedures (e.g. acid digestion procedure) among data sources (Adamo et al., 2008; Baffi et al., 2002); (d) even when based on purposed field survey, the fairly low number of sites in single remote areas does not ensure the representativeness of the overall element background variation in the target macroregion. Furthermore (e), it is known that element composition in remote areas predominantly reflects local environmental conditions such as lithology, climate and their possible interactions (Incerti et al., 2017), suggesting that reliable BEC values should be proposed for ecologically homogenous contexts.

In order to overcome such issues, here we assessed the BEC values of a single highly performing biomonitor, the epiphytic lichen *Pseudevernia furfuracea* (L.) Zopf, selected because it is one of the most commonly used lichen in active and passive biomonitoring surveys throughout European and extra-European countries (for details, see section S1.1 in Supplementary Material). We surveyed the literature to compile and comparatively analyse methodologically homogeneous datasets encompassing a supra-continental spatial scale. Moreover, we integrated the literature data with an extensive field survey and a climatic, lithological, and land use characterization of the collection sites, thus providing BEC data representative of different environmental contexts.

Specific aims were threefold: to (i) provide a preliminary broad reference on BEC in the target species for biomonitoring application in Europe; (ii) explore BEC pattern at national level, in relation to anthropization, land use, climate and lithological variables, assessed by a GIS-based environmental characterization of the sampling sites; (iii) test the predictivity of target environmental descriptors on BEC in *P. furfuracea*, using multiple regressive modelling.

2. Materials and methods

2.1 Literature survey data

Literature was searched on Scopus, Google Scholar and lichen-specific search engine ("Recent Literature on Lichens"; Culberson et al., 2015) for eligible active and passive biomonitoring studies reporting element content data concerning *Pseudevernia furfuracea*. Details on search methods and parameters, data gathering and ancillary information considered for further analyses are reported in section S1.2 in Supplementary Material.

2.2 Field data and environmental characterization of field sites

For this study, thalli of *P. furfuracea* without distinction of the two varieties (Incerti et al., 2017) were collected at 62 sites located in the main Italian mountain ranges (Supplementary Table S1). Selection of sites, sample pre-processing, chemical analyses, and quality assessment procedures follow Incerti et al. (2017). The collection sites were characterized in terms of anthropization (population density, built-up area cover), land use (occurrence of artificial surfaces, agricultural areas, and forest and semi-natural areas) climate (precipitation and temperature), and lithology (occurrence of igneous, metamorphic, sedimentary carbonate and sedimentary clastic rocks), using thematic maps in a GIS environment, as reported in section S1.3 in Supplementary Material.

2.3 Element content datasets

Three different datasets, based on lichen exposure conditions and duration, were considered. Our field data were merged with pre-exposure control data of methodologically consistent active biomonitoring studies (section S1.4 in Supplementary Material), constituting a dataset named *'background'* (henceforth, BG), under the assumption that in both cases lichen thalli were purposely collected in remote, proximate-natural conditions far from known anthropogenic emission sources. Differently, data of transplanted samples from active biomonitoring studies were included into a dataset named *'transplant'* (T). Finally, data from passive biomonitoring studies referring to native thalli collected in differently polluted areas were included into a dataset named *'passive'* (P).

2.4 Data analysis

Descriptive statistics of element content were separately calculated for each element in datasets BG, T and P. BG medians were tested for significant differences compared to T and P medians using Mann-Whitney's U test, considering either all data pooled within each dataset or separately for different land use types.

Limited to field data, the matrix of collection sites × element content, with data standardized for each element, was submitted to Principal Component Analysis (PCA) and Cluster Analysis (CA) using Euclidean distance as distance measure and Ward's method as grouping algorithm. For the resulting clusters of sites, descriptive statistics of element content were calculated for each element. Significant environmental differences among clusters of sites were tested using Kruskal-Wallis ANOVA and non-parametric Dunn's post hoc test, for 13 environmental variables preliminarily selected as potential predictors of lichen element content (section S1.3 in Supplementary Material).

A Principal Component Regression (PCR) model was fitted for each element to assess the relationships between environmental variables and lichen BEC, while avoiding possible collinearity among the predictors. First, a matrix of collection sites × environmental variables was submitted to PCA. Then, a multiple linear regression model was fitted for each element, in which the Principal Components (PCs) were considered as independent predictors (Jolliffe, 2002). PCR models were fitted on data from 40 randomly selected sites (i.e. fitting dataset), and tested on the remaining 22 sites (i.e. validation dataset).

All data analyses and graphics were performed with the software package Statistica v. 10 (StatSoft Inc., Tulsa, OK). Statistical significance was tested at $\alpha = 0.05$ in all cases.

3. Results

3.1 Literature survey on *P. furfuracea* element content

The literature search produced 62 studies of active (70%) and passive (29%) biomonitoring carried out in 14 European and 2 non-European countries (Fig. 1; Supplementary Table S2). Expectedly, studies were widely variable in terms of targeted elements, type of biomonitoring application, and lichen exposure conditions as related to specific objectives (Supplementary Fig. S1). Less obvious was the remarkable variability of methods detected for pre-treatment of lichen material, acid digestion protocols, analytical techniques and quality assurance/control, as well as a lack of technical detail in several reports (Table 1).

3.2 Element content in background and exposure conditions

The three datasets BG, P and T contained different sets of elements, also showing very different record counts. Overall, dataset BG included 2950 data for 43 elements, dataset P included 513 data for 44 elements, and dataset T included 3760 data for 43 elements (Supplementary Table S3). Expectedly, BG samples showed significantly lower median element content than P and T samples for 10 out of 12, and 21 out of 24 tested elements, respectively (Table 2). When these elements were ranked according to the ratio of median values of T to BG datasets, Na showed the highest value (14.4), followed by Pb (6.1), and by terrigenous elements such as Ti and Al (4.8 and 3.4, respectively). All the other elements ranged between 1.5 and 3, with the exception of Hg (1.1), although with significant BG vs T difference. Non-significant differences between BG and P or T samples were limited to Mn, K and Se, whereas, in the case of S, median content of BG samples was lower than in T samples, but higher than in P ones.

When stratified by land use, results of the comparative analysis among the three datasets confirmed the general pattern, with T and P samples exposed to rural, urban or industrial conditions showing consistently higher element content compared to BG conditions, although with some exceptions (Fig. 2). In particular, samples of dataset P showed not significantly different content of Cu and Zn at industrialized sites, and of Fe at urbanized sites, compared to BG samples, as also observed for Hg in T samples at urban and industrial sites (Fig. 2).

3.3 Context-dependency of background element content at national level

The cluster analysis of element content data from the field sampling produced three main site clusters (Fig. 3A-B; Supplementary Fig. S2), well separated for geographical location, climatic conditions and lithological substrates. Cluster I included 22 sites generally characterized by metamorphic substrates in western Alps (except for sites 35 in eastern Alps, and 23 with sedimentary substrate); cluster II included 20 sites characterized by sedimentary carbonate substrates in eastern Alps and northern Apennines (with the exceptions of sites 4 in western Alps, and 27 with metamorphic substrate); cluster III included 20 sites characterized by different lithological substrates in the Apennines (with the exception of sites 7, 10 and 22, located in western Alps).

A clear pattern of lichen element composition along environmental gradients emerged from the PCA (Fig. 3C). In particular, elements of group 1 (Ag, Au, Cs, Rb, Bi, Sb, Sn, Cu, Mo, Zn) were consistently placed at high scores on the second PC axis, inversely related to temperature, and positively and negatively associated to the occurrence of metamorphic and carbonate substrates at the sampling sites, respectively. Differently, elements of groups 3 (Al, Ce, La, Y, Fe, Li, Ti, Th, Nb, U, Ca, Sr, Na, Hf, Zr) and 4 (As, Ge, Sc, V, Cd, Pb, S, Se, Hg) were positively associated to the first PC axis, consistent to prevalence of agricultural areas and low forest cover, high temperatures, and low precipitations. Elements of group 2 (Ba, Mn, Pd, Co, Cr, Ni, Mg, K, P) were not clearly associated to the first two PC axes, but inversely related to the abundance of carbonate substrates at the sampling sites, which mainly contributed to the third PC axis (8% of the total variance). In addition, K and P were negatively correlated with the fourth PC axis (6% of the total variance), hence positively to agricultural land cover and temperatures, and negatively to precipitations (data not shown). Interestingly, such environmental patterns of lichen element composition were generally corresponding to geographical gradients, as elements of group 1 showed also positive correlation with latitude and negative with longitude, whereas elements of groups 3 and 4 showed the opposite geographical pattern. Such geographical correspondence is better noticeable in the plot of collection sites in the ordination space (Fig. 3B).

Consistent with the PCA results, clusters of sites at different geographic location (Supplementary Fig. S2) showed significant differences of standardized content of the three groups of elements (Fig. 4). In detail, sites of cluster I showed the highest mean content of elements of group 1, and intermediate values for the other element groups. Sites of cluster II showed the lowest content of all the element groups, while sites of cluster III showed the highest content of all the groups of elements, with the exception of group 1. Such pattern was consistent with environmental differences between clusters (Supplementary Table S4).

When removing from the clusters the few sites inconsistent for lithological substrate and/or location (i.e. sites 35, 23 from cluster I; sites 4, 27 from cluster II; sites 7, 10, 22 from cluster III), three sets of lichen samples were obtained, fully consistent for experienced environmental conditions, collected respectively from: (1) western Alps, over metamorphic siliceous substrates, (2) eastern Alps and northern Apennines, over sedimentary rocks, and (3) central and southern Apennines, on different substrate types. Descriptive statistics for these sets of samples are proposed as BEC estimates at sub-national scale (Table 3). Notably, these showed significant between-group differences for 40 elements out of 43 (Table 3), with the exception of Ba, K and Pd. For comparison, comprehensive estimates at national scale are also provided (Supplementary Table S5).

3.4 Relationships between environmental predictors and background element content

All environmental variables were significantly associated with lichen element content, being predictive for at least three chemical elements (Fig. 5). A consistent pattern of correlation was found between single environmental variables and elements in the same groups. Indeed, population density was consistently positively associated to lichen element content, particularly with element groups 3 and 4 (Fig. 5). Land use in the surroundings of the sampling sites differently affected the lichen content of different groups of elements, with values increasing with increasing agricultural land cover, and decreasing with increasing forest cover, for all elements of group 3, and most elements of groups 4 and 2, whereas elements of groups 1 were inversely or not affected (Fig. 5).

Lichen content of elements of groups 2, 3, and 4 was also significantly affected by climatic variables, with a pattern of positive and negative correlation for temperatures and precipitations, respectively. These were consistent for all elements of groups 3 and 4, except for As, with the formers more frequently associated to mean annual temperature, the latter to minimum temperature of the coldest month, and both groups to total precipitations of the wettest month (Fig. 5). A very similar pattern to that of group 4 was found for most elements of group 2 (Ba, Co, Cr, Mg, K, P and, limited to temperatures, Ni), while lichen content of those of group 1 was generally not (Ag, Au, Cs, Bi, Sb, Sn, Cu) or negatively (Rb, Mo, Zn) correlated to mean and maximum temperatures, and positively associated to precipitations (limited to Bi, Sb, Sn, Mo, Zn).

Lichen content of elements of group 1 were positively and negatively associated to metamorphic and carbonate rocks, respectively. A similar pattern of correlation was found for some elements of group 2 (Mn, Ni and, limited to carbonate substrate, Co and Cr), while others were positively associated to metamorphic rocks (Mg) or negatively to igneous rocks (Co, Cr, Ni, Mg). Differently, lichen content of elements of groups 3 and 4 was barely affected by lithology, with few significant correlation scores of opposite signs scattered within the groups for metamorphic (positive for Pb and negative for Sr and Na), carbonate (positive for Na and negative for As and V) and clastic (positive for Hf and Zr and negative for Ti) rocks. Finally, igneous rocks were positively correlated with typical terrigenous elements (Al, Fe, Ti) and V.

Considering multivariate environmental relationships as assessed by PCR modelling, statistically significant outcomes were found for 31 out of the 43 tested elements (Fig. 5, detailed results in Supplementary Table S6). Exceptions, unsatisfactorily related to environmental predictors, were Au and Rb (group 1), Ba, Mn, Pd, Cr and Mg (group 2) and As, Sc, Pb, S, and Hg (group 4). Interestingly, statistically significant PCR models were found for all elements of group 3, mostly including elements of lithogenic origin and rare earth ones. In 10 cases (i.e. Al, Ca, Hf, La, Li, Na, Nb, Th, Ti and Zr) the PCR models were also significantly predictive of the validation datasets (Supplementary Fig. S3-S6). Differently, PCR models for elements of other groups, although significantly predictive of the fitting datasets, did not provide satisfactory performance when applied to the validation datasets (Supplementary Fig. S3-S6) in terms of predicted vs observed comparisons.

4. Discussion

4.1 Estimate of background element content at large scale: methodological limitations

The papers selected for BEC assessment at supranational level obviously differed for specific scope and objectives, with unequal representation of countries, periods and environmental conditions. Surprisingly, they also revealed a huge methodological diversity, with application of widely different procedures for sample pre-processing, digestion, analytical technique, and QC assessment. Moreover, important methodological information was often missing, with 52% and 31% of the studies even failing to report QC methods and digestion protocols, respectively. This could heavily affect the comparability of analytical results (Baffi et al., 2002; Bettinelli et al., 2002). In this framework, our BG dataset provides for the first time a broad overview of the BEC ranges for most elements and for a single highly performing lichen biomonitor at supranational scale. Moreover, it illustrates a novel, reliable methodological frame, which can be recommended for the analysis of future biomonitoring data.

4.2 Comparison of background and enriched element content at large scale

Expectedly, our comparative analysis of element content data indirectly confirmed that BG samples were substantially unaffected by anthropogenic contributions, as they showed significantly lower levels for most elements in comparison to P and T samples. The only exceptions were K, Mn, S, Se and, partially, Hg. Our observations for K can be easily explained, as the content of K fluctuates following the physiological state of the thalli, so that it often decrease in transplanted lichens due to membrane leakage (e.g. Garty et al., 1998; Häffner et al., 2001). Mn content can also decrease in transplanted lichens, contextually with vitality loss (Bari et al., 2001), as a result of washing effects by rainfalls (Gallo et al., 2017). Concerning S and Se, their distribution can be greatly heterogeneous, being influenced by different natural and anthropical sources, transport phenomena, and by the physiological state of the thalli (Låg and Steinnes, 1974; Vingiani et al., 2004; Brenot et al., 2007; Wen and Carignan, 2007). Finally, high Hg levels are usually related to wet depositions and altitude (Bargagli, 2016; Zechmeister, 1995). Our observation of higher Hg in lichen samples from rural areas can be ascribed to local geochemical anomalies (e.g. samples transplanted nearby volcanic areas; Carasci and Cataldo, 2016) or to the emission of single industrial plants (e.g. samples transplanted around a waste incinerator; Tretiach et al., 2011).

4.3 Relationships between environmental descriptors and background element content

At confirmation that *P. furfuracea* is an excellent biomonitor, we found a pattern of association between the (generally low) BECs and the environmental conditions at the remote sampling sites. In particular, land use, climate and lithology were satisfactorily predictive, confirming the findings of Incerti et al. (2017). The effects of these factors on lichen bioaccumulation are widely acknowledged in the literature (e.g. Garty, 2001; Nimis, 2001; Sorbo et al., 2008; Agnan et al., 2014), but they were neglected in the build-up of BEC values and interpretative scales (Nimis and Bargagli, 1999).

In our national dataset, elemental content generally increases with increasing population density, temperatures, cover of agricultural areas and metamorphic substrate (hence, moving southwards), whereas it decreases with increasing precipitation, forests and carbonate substrates cover (hence, moving northwards, and eastwards within the Alps). Sites of central-southern Italy showed consistently higher content of lithogenic (Al, Ca, Fe, Li, Th, and Ti) and rare earth elements (REE: Ce, La, Sc, Y), as related to higher levels of anthropization agricultural landcover, and soil susceptibility to erosion (Jones et al., 2012), as well as lower precipitations. REE and lithogenic elements are often considered tracers of geochemical transport processes (Aubert et al., 2001; Laveuf and Cornu, 2009). Therefore, central and southern sites, often located along slopes prone to upward blowing winds from relatively arid rural lowlands, may be affected by important depositions of windblown dust. Moreover, bedrock weathering and soil erosion, in areas characterized by crystalline and marine clay rich sediments outcrops as well as by a high incidence of rural activities, may be major sources of REE. Indeed, some REE such as Ce, La and Y are also known for being included in phosphate fertilizers and insecto-fungicides (Sadeghi et al., 2013; Carpenter et al., 2015; Di Palma et al., 2017), which could explain higher background levels in samples from sites subjected to influence by agricultural areas. This is further supported by the higher background levels of P in central and southern sites, compared to northern ones. These evidences suggest that local background of central and southern Italy is likely affected by medium-long-range depositions. In this view, local precipitation regimens would have contrasting effects, either enriching lichen element content under lower precipitation conditions (Bargagli, 1995), or washing lichen thalli subjected to higher precipitation (Knops et al., 1991).

When considering the northern Italian sites, we observed a general pattern of higher vs lower background levels at western vs eastern sites, respectively, for 25 out of 43 elements, while no element showed the opposite trend. Such pattern, clearly reflects the main lithological traits of local substrates, with western sites laying over siliceous metamorphic rocks, and eastern sites over sedimentary carbonates, respectively. Although the traceability of rare elements may be questionable, this pattern is particularly evident for Ag, Au, Bi, Cu, Mo, Rb, Sb, Sn and Zn, which occur into different metamorphic substrate types, as in the cases of Cu, Rb and Sb (Aubert and Pinta, 1980; Bargagli et al., 1999; Salminen et al., 2005; Kuleshov, 2016), and Mo into sulphides pyrite, galena and sphalerite (Salminen et al., 2005). Consistently, previous observations on element content in mosses showed that differences at regional scale are associated to the main geochemical traits (Bargagli, 1995).

Our PCR modelling of element background levels at national scale showed reliable results for most lithogenic elements and REE (Al, Ca, Hf, La, Li, Nb, Sb, Th, Ti, Zr) and for Na, with models for the fitting dataset being also predictive of the validation datasets. In the case of Na, the scatterplot of observed vs predicted values clearly separated Alpine from Apennine sites, with the latter consistently showing higher Na levels. Besides environmental predictors included in the PCR model, an effect of long-range transport of marine aerosol by prevailing south-westerly winds from the Tyrrhenian sea (Zecchetto and Cappa, 2001) should not be excluded. On the other hand, the common pattern observed for lithogenic and REE elements reinforce our hypothesis on the common source and transport phenomena for such elements. Finally, PCR models for As, Au, Bi, Co, Cs, Mg, Pd, and V showed the worst performance. Therefore, further investigation is recommended to explore the relationships between the BEC of these elements and other environmental factors.

4.4 Background element content as an interpretative tool in biomonitoring application

The knowledge of chemical background is an essential pre-requisite for the assessment of pollution levels and possible biological effects (Bargagli, 1998). Our results on BEC in *P. furfuracea* at supra-regional scale provide a main advance in this direction, highlighting significant differences among three Italian macroregions for most elements due to a remarkable context-dependency of BEC values. Our new approach overcomes previous assessments based on limited datasets, bibliographic surveys, species not used in biomonitoring application or data pooled for different sites and groups of species (Bargagli, 1998; Bargagli et al., 1999; Bennet, 2000; Bergamaschi et al., 2004; Monaci et al., 2012). As such, the BEC values reported in Table 3 should be regarded as reference datasets for biomonitoring application with *P. furfuracea* in Italy as well as in areas with the same combinations of environmental conditions at continental level. In particular, the use of our data is recommended at the interpretation stage of biomonitoring results. In the case of passive biomonitoring application, the comparison of enriched samples with our geographically-consistent background estimate is suggested in place of commonly used multi-species interpretative scales (Nimis and Bargagli, 1999). In the case of transplants, element content in unexposed samples should be carefully compared to our background estimates for the appropriate collection area, in order to exclude natural enrichment in control samples prior to arbitrarily calculate EC ratios (Frati et al., 2005). On the other hand, further collection of element content data in native lichen samples, based on highly standardized methodological protocols, could help to build up other reliable interpretative tools, such as single-species-interpretative scales (Nimis et al., 2001; Tretiach and Baruffo, 2001a), which were not presented in this contribution due to sample size constraints. Indeed, it could be possible to compare the element content data in native lichen samples, with the distribution of total element concentration in top-soils of Europe (Salminen et al. 2005; De Vos et al., 2006), while the collection of new data at very local scale coupled with environmental datasets at high spatial resolution should allow to provide accurate BEC estimates for more restricted and environmentally homogenous contexts.

4.5 Methodological issues in transplant studies: the urgent need for standardization

Several studies used *Pseudevernia furfuracea* to face specific methodological issues of the transplant technique, such as the effects of exposure on sample vitality (Tretiach et al., 2007), the position of samples on host tree species on element composition (Adamo et al., 2008), sample pre-treatment (Adamo et al., 2003), and exposure techniques (Giordano et al., 2013) on bioaccumulation capacity. Interspecific comparisons were also frequently reported (e.g. Bergamaschi et al., 2007; Tretiach et al., 2007; Adamo et al., 2008). Recently, element content in transplanted *P. furfuracea* thalli was compared to their magnetic properties, tracing different particulate matter sources in complex environments (Kodnik et al., 2017), and to element concentration in bulk deposition and leachates, showing either enrichment or washing effects by rainfall for different elements (Gallo et al., 2017). However, although generally based on the same target species, also these works are affected by a surprising deficit of methodological uniformity, possibly due to the lack of standard procedures for bioaccumulation techniques at the EU level. Therefore, in order to provide reliable data, allow appropriate assessment of data quality, and implement unbiased comparative analyses, it would be strongly advisable to share a European standard norm for bioaccumulation with lichens. Among the key issues to address, a key point is the selection of appropriate background areas for sample collection. On the basis of our results, we recommend to carefully characterize the collection areas in terms of land use, vegetation, climate, altitude and lithology.

5. Conclusions

The extensive review of active and passive bioaccumulation studies centered on the epiphytic lichen *P. furfuracea* revealed a huge methodological variability in sample pre-treatment, digestion, analytical technique, and QC assessment. By compiling and comparatively analysing methodologically homogeneous datasets, however, we could provide a first, comprehensive overview of element content data at supra-continental spatial scale, showing potential background ranges for 25 chemical elements. Limited to original field data from Italy, we explored the background levels for 43 elements in relation to environmental conditions at the sampling sites, finding high predictivity of anthropization, land use, climate and lithological variables for most elements. We also identified three homogeneous and geographically separated contexts (western Alps, eastern Alps plus northern Apennines, central and southern Apennines), for which specific BEC values are now available as reference datasets for biomonitoring applications.

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TABLES

Table 1. Methodological steps applied in the 62 surveyed studies to assess element content in lichen samples, with types of technical procedures and percent frequency of application.

Sample pre-processing	**Acid mixture for sample digestion**	**Analytical technique for element content determination**	**QC procedures**
Not reported (3%) Debris removal (56%) Washing (21%) Oven-drying (11%) Washing + oven-drying (5%) Other (3%)	Not reported (31%) Partial digestion (42%) – HNO_3 – HNO_3-H_2O_2 – HNO_3-HCl – HNO_3-H_2O_2-HCl – HNO_3-$HClO_4$-HCl – HNO_3-$HClO_4$ – HNO_3-$HClO_4$-H_2SO_4 Total digestion (27%) – HNO_3-H_2O_2-HF – HNO_3-HCl-HF – HNO_3-HF	Not reported (4.8%) Atomic absorption spectrometry: CVAAS, ETAAS, FAAS, GAAS, ZETAAS (33.3%) Mass emission spectrometry: ICP-MS (29.6%) X-ray fluorescence: XRF (11.8%) Optical emission spectrometry: ICP-OES (5.6%) Atomic emission spectrometry: AES (4.3%) Instrumental neutron activation analysis: INAA (3.8%) Flow injection mercury system: FIMS (1.9%) Flash combustion elemental analyser (1.6%) Isotope-excited X-ray spectrometry (1.6%) γ-ray Spectrometry (1.6%)	I Not reported (52%) II CRM used, but neither CRM type nor recovery percentages specified (6%) III CRM type specified, but no information reported about recovery (21%) IV CRM type specified and satisfactory quality of recovery data generically claimed (6%) V CRM type specified, and range of recovery percentages reported (5%) VI CRM type specified and descriptive statistics of recovery percentages reported for each element (10%).

Table 2. Element content (µg g^{-1}) of the epiphytic lichen *Pseudevernia furfuracea* in the datasets BG, P and T. Data refer to descriptive statistics (counts, mean ± standard deviation, median and interquartile range for BG data; counts, median and interquartile range for the elements with data count ≥ 10 in either dataset P or T). For each element, results of statistical testing for differences from the BG data are also reported (Mann-Whitney U test for independent samples; M-W, significant *p*-values in italic).

	BG				**P**			**M-W (BG vs P)**	**T**			**M-W (BG vs T)**
	n	**Mean ± SD**	**Median**	**IQR**	**n**	**Median**	**IQR**	***p*-value**	**n**	**Median**	**IQR**	***p*-value**
Al	81	457 ± 236	380	300 ÷ 535					208	1274	847 ÷ 1710	$< 10^{-10}$
As	63	0.205 ± 0.096	0.180	0.130 ÷ 0.270	10	0.435	0.330 ÷ 0.500	$3.7 \cdot 10^{-5}$	145	0.480	0.370 ÷ 0.830	$< 10^{-10}$
Ba	63	12.0 ± 5.5	11.0	8.1 ÷ 13.6					29	21.9	14.5 ÷ 28.8	$6.8 \cdot 10^{-7}$
Ca	74	7615 ± 4092	6185	4680 ÷ 10000					97	15870	10315 ÷ 23310	$< 10^{-10}$
Cd	87	0.183 ± 0.088	0.160	0.120 ÷ 0.240	23	0.618	0.400 ÷ 0.706	$3.4 \cdot 10^{-10}$	272	0.330	0.230 ÷ 0.556	$< 10^{-10}$
Co	65	0.255 ± 0.094	0.240	0.170 ÷ 0.310					110	0.59	0.48 ÷ 0.73	$< 10^{-10}$
Cr	80	2.73 ± 0.77	2.69	2.43 ÷ 3.12	48	3.44	2.73 ÷ 15.00	$6.9 \cdot 10^{-6}$	263	4.16	2.73 ÷ 6.00	$1.4 \cdot 10^{-10}$
Cu	91	5.40 ± 2.09	4.99	3.78 ÷ 6.63	40	6.60	4.47 ÷ 22.00	*0.002*	329	11.00	6.33 ÷ 22.50	$< 10^{-10}$
Fe	79	516 ± 251	480	348 ÷ 620	57	965	612 ÷ 1560	$5.7 \cdot 10^{-9}$	204	868	630 ÷ 1333	$< 10^{-10}$
Hg	74	0.199 ± 0.059	0.180	0.160 ÷ 0.250					59	0.200	0.170 ÷ 0.290	*0.043*
K	74	3305 ± 616	3258	2867 ÷ 3740					91	3417	2370 ÷ 4540	0.733
Mg	72	766 ± 171	725	642 ÷ 847					91	1185	895 ÷ 1819	$< 10^{-10}$
Mn	90	56.5 ± 30.8	50.4	34.2 ÷ 74.3	43	41.7	26.0 ÷ 71.9	0.269	300	50.0	32.7 ÷ 74.1	0.964
Mo	65	0.249 ± 0.143	0.200	0.130 ÷ 0.340					91	0.620	0.270 ÷ 1.664	$< 10^{-10}$
Na	73	77.3 ± 67.4	40.0	30.0 ÷ 134.0					33	575	300 ÷ 918	$< 10^{-10}$
Ni	87	1.72 ± 0.90	1.42	1.03 ÷ 2.18	38	3.55	1.97 ÷ 20.00	$4.0 \cdot 10^{-9}$	253	4.50	2.70 ÷ 6.88	$< 10^{-10}$
Pb	85	4.46 ± 2.94	3.44	2.38 ÷ 5.51	51	8.70	4.70 ÷ 43.00	$<10^{-10}$	336	21.00	11.95 ÷ 38.90	$< 10^{-10}$
S	65	1534 ± 237	1540	1371 ÷ 1650	11	1260	900 ÷ 1422	*0.002*	49	3170	2660 ÷ 4340	$< 10^{-10}$
Sb	63	0.093 ± 0.052	0.083	0.054 ÷ 0.118					53	0.220	0.170 ÷ 0.480	$< 10^{-10}$
Se	59	0.276 ± 0.095	0.270	0.220 ÷ 0.300					17	0.220	0.150 ÷ 0.350	0.458
Sn	62	0.335 ± 0.166	0.300	0.210 ÷ 0.410					38	0.495	0.320 ÷ 0.720	$1.4 \cdot 10^{-6}$
Ti	64	11.1 ± 4.7	10.6	7.5 ÷ 13.8	23	98.2	47.3 ÷ 161.2	$<10^{-10}$	88	51.2	21.9 ÷ 96.5	$< 10^{-10}$
U	63	0.022 ± 0.013	0.020	0.011 ÷ 0.028	23	0.102	0.082 ÷ 0.185	$<10^{-10}$				
V	73	2.12 ± 0.47	1.96	1.90 ÷ 2.20					181	3.78	2.9 ÷ 5.7	$< 10^{-10}$
Zn	92	41.4 ± 17.4	39.8	27.0 ÷ 53.5	57	65.0	31.4 ÷ 118.0	$1.3 \cdot 10^{-5}$	336	81.1	49.3 ÷ 129.9	$< 10^{-10}$

Table 3. Background element content (μg g^{-1}) in the epiphytic lichen *Pseudevernia furfuracea* in Italy. These include sites with similar lichen element content according to CA results (Fig. 3A). Data refer to descriptive statistics (mean ± standard deviation, median and inter-quartile range) for 25 elements. Between-groups differences (Kruskal-Wallis ANOVA; K-W, and significant *p*-values in italic) are reported for each element. Different letters indicate significantly different groups within each row (Dunn's post hoc test at $p < 0.05$).

	Siliceous metamorphic western Alps (n=20)			**Sedimentary eastern Alps and northern Apennines (n=18)**			**Central and southern Apennines (n=17)**			**K-W**
	Mean ± SD	**Median**	**IQR**	**Mean ± SD**	**Median**	**IQR**	**Mean ± SD**	**Median**	**IQR**	***p*-value**
Ag	0.025 ± 0.008	0.023^{b}	0.020 ÷ 0.028	0.016 ± 0.007	0.014^{a}	0.013 ÷ 0.017	0.020 ± 0.006	0.020^{ab}	0.015 ÷ 0.020	*0.0001*
Al	359 ± 71	366^{a}	300 ÷ 400	310 ± 78	300^{a}	269 ÷ 360	758 ± 214	640^{b}	620 ÷ 820	$< 10^{-4}$
As	0.221 ± 0.081	0.207^{b}	0.177 ÷ 0.270	0.143 ± 0.072	0.113^{a}	0.090 ÷ 0.156	0.220 ± 0.091	0.220^{b}	0.156 ÷ 0.270	*0.0015*
Au	0.180 ± 0.101	0.164^{b}	0.109 ÷ 0.216	0.070 ± 0.062	0.055^{a}	0.034 ÷ 0.106	0.130 ± 0.088	0.100^{ab}	0.080 ÷ 0.160	*0.0004*
Ba	11.8 ± 4.6	11.6^{a}	9.3 ÷ 12.6	12.7 ± 6.8	10.8^{a}	6.9 ÷ 17.4	13.3 ± 7.7	9.3^{a}	7.6 ÷ 16.0	0.8592
Bi	0.054 ± 0.017	0.051^{b}	0.040 ÷ 0.063	0.032 ± 0.010	0.030^{a}	0.024 ÷ 0.039	0.040 ± 0.018	0.040^{ab}	0.024 ÷ 0.050	*0.0005*
Ca	4840 ± 962	4798^{a}	4350 ÷ 5178	6071 ± 2864	5909^{a}	3820 ÷ 6237	11901 ± 4213	10487^{b}	9050 ÷ 14040	$< 10^{-4}$
Cd	0.134 ± 0.031	0.137^{a}	0.115 ÷ 0.158	0.114 ± 0.033	0.099^{a}	0.089 ÷ 0.144	0.190 ± 0.053	0.170^{b}	0.168 ÷ 0.210	$< 10^{-4}$
Ce	0.79 ± 0.19	0.80^{a}	0.65 ÷ 0.93	0.67 ± 0.22	0.63^{a}	0.57 ÷ 0.75	1.98 ± 0.66	1.82^{b}	1.60 ÷ 2.19	$< 10^{-4}$
Co	0.288 ± 0.131	0.261^{b}	0.213 ÷ 0.322	0.166 ± 0.041	0.159^{a}	0.138 ÷ 0.170	0.340 ± 0.090	0.310^{b}	0.278 ÷ 0.410	$< 10^{-4}$
Cr	3.41 ± 1.59	2.85^{b}	2.56 ÷ 3.62	2.48 ± 0.18	2.46^{a}	2.35 ÷ 2.56	3.12 ± 0.46	3.00^{b}	2.82 ÷ 3.18	$< 10^{-4}$
Cs	0.152 ± 0.108	0.098^{b}	0.080 ÷ 0.171	0.073 ± 0.040	0.062^{a}	0.052 ÷ 0.073	0.120 ± 0.065	0.100^{b}	0.080 ÷ 0.160	*0.0007*
Cu	7.05 ± 2.26	6.79^{b}	5.42 ÷ 8.17	4.23 ± 1.01	3.88^{a}	3.74 ÷ 4.32	4.69 ± 1.51	4.24^{a}	3.52 ÷ 5.49	$< 10^{-4}$
Fe	468 ± 112	481^{b}	372 ÷ 532	352 ± 90	348^{a}	319 ÷ 398	830 ± 246	762^{c}	691 ÷ 880	$< 10^{-4}$
Ge	0.012 ± 0.004	0.012^{a}	0.010 ÷ 0.013	0.012 ± 0.002	0.011^{a}	0.010 ÷ 0.013	0.020 ± 0.004	0.020^{b}	0.013 ÷ 0.020	*0.0002*
Hf	0.053 ± 0.024	0.049^{b}	0.039 ÷ 0.063	0.036 ± 0.015	0.032^{a}	0.026 ÷ 0.040	0.080 ± 0.025	0.070^{c}	0.057 ÷ 0.080	$< 10^{-4}$
Hg	0.208 ± 0.040	0.200^{b}	0.176 ÷ 0.233	0.171 ± 0.059	0.164^{a}	0.134 ÷ 0.180	0.240 ± 0.036	0.250^{b}	0.220 ÷ 0.260	$< 10^{-4}$
K	3309 ± 427	3360^{a}	2990 ÷ 3675	3235 ± 500	3255^{a}	2754 ÷ 3740	3676 ± 645	3550^{a}	3240 ÷ 3988	0.0717
La	0.354 ± 0.098	0.340^{a}	0.283 ÷ 0.417	0.279 ± 0.103	0.261^{a}	0.230 ÷ 0.306	0.90 ± 0.311	0.840^{b}	0.714 ÷ 0.920	$< 10^{-4}$
Li	0.293 ± 0.071	0.291^{a}	0.238 ÷ 0.343	0.215 ± 0.063	0.212^{a}	0.182 ÷ 0.236	0.540 ± 0.154	0.500^{b}	0.444 ÷ 0.570	$< 10^{-4}$
Mg	778 ± 274	661^{a}	605 ÷ 919	753 ± 138	721^{ab}	682 ÷ 810	878 ± 152	830^{bc}	774 ÷ 970	*0.0153*
Mn	64.2 ± 18.3	62.8^{bc}	49.3 ÷ 77.5	58.6 ± 30.2	46.7^{ab}	33.2 ÷ 80.5	50.7 ± 45.9	35.1^{a}	23.5 ÷ 47.0	*0.0083*
Mo	0.380 ± 0.129	0.358^{b}	0.294 ÷ 0.429	0.165 ± 0.049	0.165^{a}	0.129 ÷ 0.190	0.150 ± 0.062	0.120^{a}	0.116 ÷ 0.190	$< 10^{-4}$
Na	34.9 ± 24.3	30.0^{a}	23.7 ÷ 35.8	65.9 ± 50.9	48.1^{a}	32.3 ÷ 72.0	179.9 ± 38.5	174.0^{b}	158.0 ÷ 190.0	$< 10^{-4}$
Nb	0.033 ± 0.007	0.034^{b}	0.030 ÷ 0.038	0.026 ± 0.019	0.022^{a}	0.018 ÷ 0.029	0.070 ± 0.022	0.070^{c}	0.052 ÷ 0.080	$< 10^{-4}$
Ni	2.06 ± 1.06	1.52^{b}	1.20 ÷ 3.16	0.85 ± 0.31	0.76^{a}	0.67 ÷ 1.00	1.31 ± 0.33	1.26^{b}	1.06 ÷ 1.37	$< 10^{-4}$
P	550 ± 145	512^{ab}	474 ÷ 579	518 ± 161	467^{a}	388 ÷ 610	668 ± 191	622^{b}	556 ÷ 724	*0.0101*
Pb	3.15 ± 1.902	3.36^{b}	2.39 ÷ 3.85	2.19 ± 0.57	2.12^{a}	1.88 ÷ 2.58	3.66 ± 1.65	3.05^{b}	2.59 ÷ 4.51	*0.0002*
Pd	0.0033 ± 0.0016	0.0027^{a}	0.0022÷0.0038	0.0026 ± 0.0009	0.0023^{a}	0.0019÷0.0026	0.0024 ± 0.0007	0.0022^{a}	0.0019÷0.0026	0.1070
Rb	15.6 ± 7.6	14.0^{b}	10.8 ÷ 19.3	7.5 ± 4.2	6.1^{a}	4.5 ÷ 8.0	10.0 ± 5.8	7.1^{a}	5.3 ÷ 13.5	*0.0018*
S	1508 ± 15	1535^{ab}	1420 ÷ 1610	1370 ± 189	1340^{a}	1280 ÷ 1500	1640 ± 210	1600^{b}	1540 ÷ 1700	0.0006
Sb	0.119 ± 0.039	0.115^{b}	0.093 ÷ 0.145	0.055 ± 0.016	0.054^{a}	0.044 ÷ 0.061	0.080 ± 0.046	0.070^{a}	0.050 ÷ 0.090	$< 10^{-4}$
Sc	0.342 ± 0.032	0.337^{a}	0.323 ÷ 0.360	0.324 ± 0.041	0.316^{a}	0.300 ÷ 0.338	0.410 ± 0.050	0.400^{b}	0.375 ÷ 0.440	$< 10^{-4}$
Sr	9.7 ± 2.1	9.9^{a}	8.2 ÷ 11.1	13.9 ± 5.9	12.7^{a}	9.9 ÷ 15.1	26.4 ± 9.7	24.7^{b}	22.0 ÷ 29.4	$< 10^{-4}$
Se	0.259 ± 0.044	0.268^{b}	0.218 ÷ 0.284	0.209 ± 0.053	0.218^{a}	0.180 ÷ 0.238	0.410 ± 0.139	0.420^{c}	0.287 ÷ 0.540	$< 10^{-4}$
Sn	0.425 ± 0.142	0.408^{b}	0.326 ÷ 0.495	0.246 ± 0.055	0.257^{a}	0.198 ÷ 0.282	0.270 ± 0.164	0.230^{a}	0.164 ÷ 0.330	$< 10^{-4}$
Th	0.085 ± 0.027	0.081^{b}	0.060 ÷ 0.105	0.056 ± 0.015	0.055^{a}	0.050 ÷ 0.062	0.170 ± 0.068	0.160^{c}	0.138 ÷ 0.190	$< 10^{-4}$
Ti	10.4 ± 2.4	10.1^{b}	8.5 ÷ 12.0	7.0 ± 1.7	6.8^{a}	6.0 ÷ 7.8	16.8 ± 4.1	17.2^{c}	13.4 ÷ 19.0	$< 10^{-4}$
U	0.020 ± 0.009	0.020^{b}	0.014 ÷ 0.023	0.011 ± 0.003	0.010^{a}	0.009 ÷ 0.012	0.030 ± 0.012	0.030^{c}	0.022 ÷ 0.040	$< 10^{-4}$
V	2.23 ± 0.51	2.04^{b}	1.94 ÷ 2.22	1.94 ± 0.12	1.90^{a}	1.90 ÷ 1.92	2.60 ± 0.65	2.50^{b}	2.00 ÷ 2.83	$< 10^{-4}$
Y	0.470 ± 0.153	0.448^{a}	0.346 ÷ 0.584	0.363 ± 0.109	0.349^{a}	0.318 ÷ 0.379	0.930 ± 0.309	0.900^{b}	0.745 ÷ 970	$< 10^{-4}$
Zn	48.5 ± 14.9	43.9^{b}	37.3 ÷ 54.5	30.4 ± 9.4	27.3^{a}	22.1 ÷ 39.7	24.5 ± 6.5	24.36^{a}	18.6 ÷ 28.4	$< 10^{-4}$
Zr	2.29 ± 0.90	2.15^{b}	1.66 ÷ 2.99	1.53 ± 0.51	1.35^{a}	1.18 ÷ 1.74	3.13 ± 1.00	2.74^{b}	2.59 ÷ 3.33	$< 10^{-4}$

FIGURE CAPTIONS

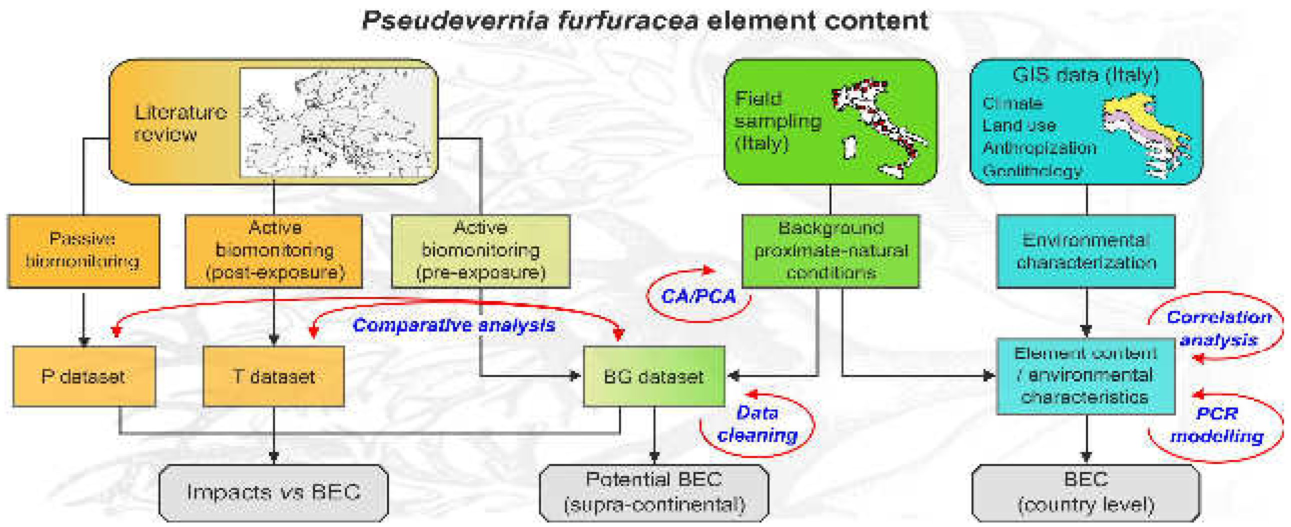
Figure 1. Map of *Pseudevernia furfuracea* collection and exposure sites, symbolized according to source study type.

Figure 2. Comparative analysis of *Pseudevernia furfuracea* element content ($\mu g\ g^{-1}$) in background vs impacted conditions. Data refer to median and quartiles of element content distribution in the datasets BG (white bars), P (light grey), and T (dark grey), the two latter separately calculated according to the land use at the collection/exposure sites: (BG: background areas; R: rural areas; U: urban areas; I: industrial areas). Data from the dataset P are not shown for Co, Cd, Hg, and V, due to limited sample size. Asterisks above bars indicate significant differences with respect to background conditions (Mann-Whitney U test, *: $0.01 < p < 0.05$; **: $0.001 < p < 0.01$; ***: $p < 0.001$).

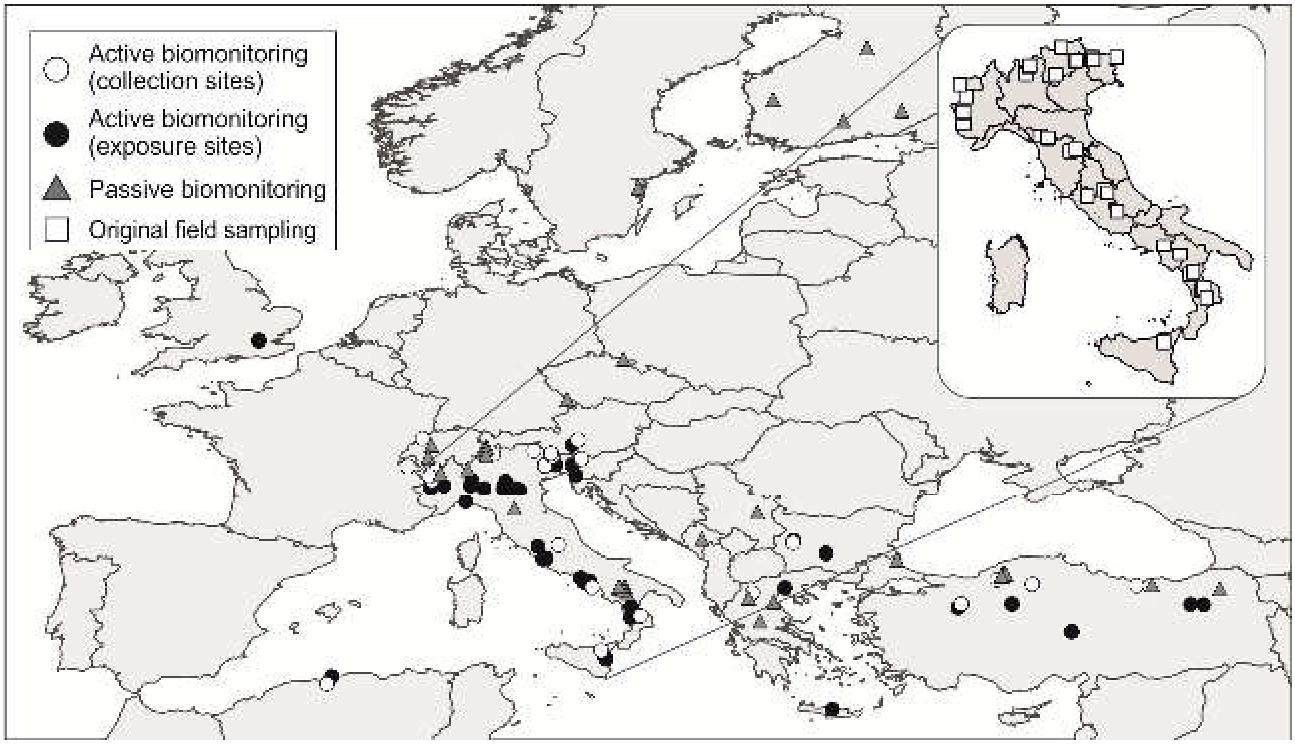
Figure 3. Results of multivariate analysis of element content in *Pseudevernia furfuracea* samples collected at 62 remote field sites in Italy. (A) Dendrogram of field sites from CA, with three main clusters (I: ●; II: ○; III: ●); (B) factorial scores of field sites, symbolized according to CA results; (C) PCA plot showing loading vectors of the elements symbolized according to the CA of Fig. 5 and their relationships with environmental descriptors characterizing the field sites, plotted as supplementary variables following Legendre and Legendre (1998).

Figure 4. Content of element groups in the site clusters I-III of Fig. 3: data are separately standardized for each element and showed as mean and 95% confidence interval for 4 different element groups, as resulting from CA (Fig. 5). Letters above bars indicate significant pair-wise differences (Tukey's HSD test for unequal sample size, $p < 0.05$).

Figure 5. Heatmap showing the association between *P. furfuracea* element content and environmental descriptors at the field sites. Dendrogram of the elements based on content values in the lichen at 62 field sites is also shown. Asterisks indicate significant rank correlation values (Spearman's *rho*, $p < 0.05$), either positive (orange) or negative (green). For each element, the predictivity (multiple-R^2 and associated *p*-values) of a Principal Component Regression model based on the set of environmental descriptors is also shown.



Graphical abstract



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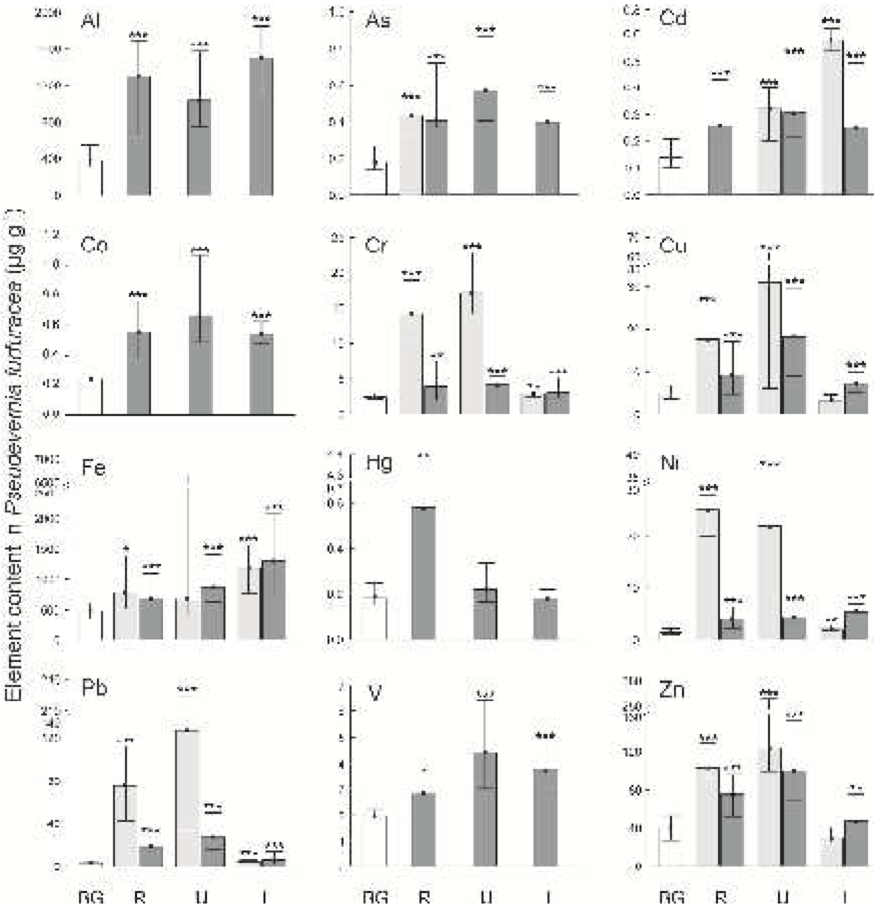

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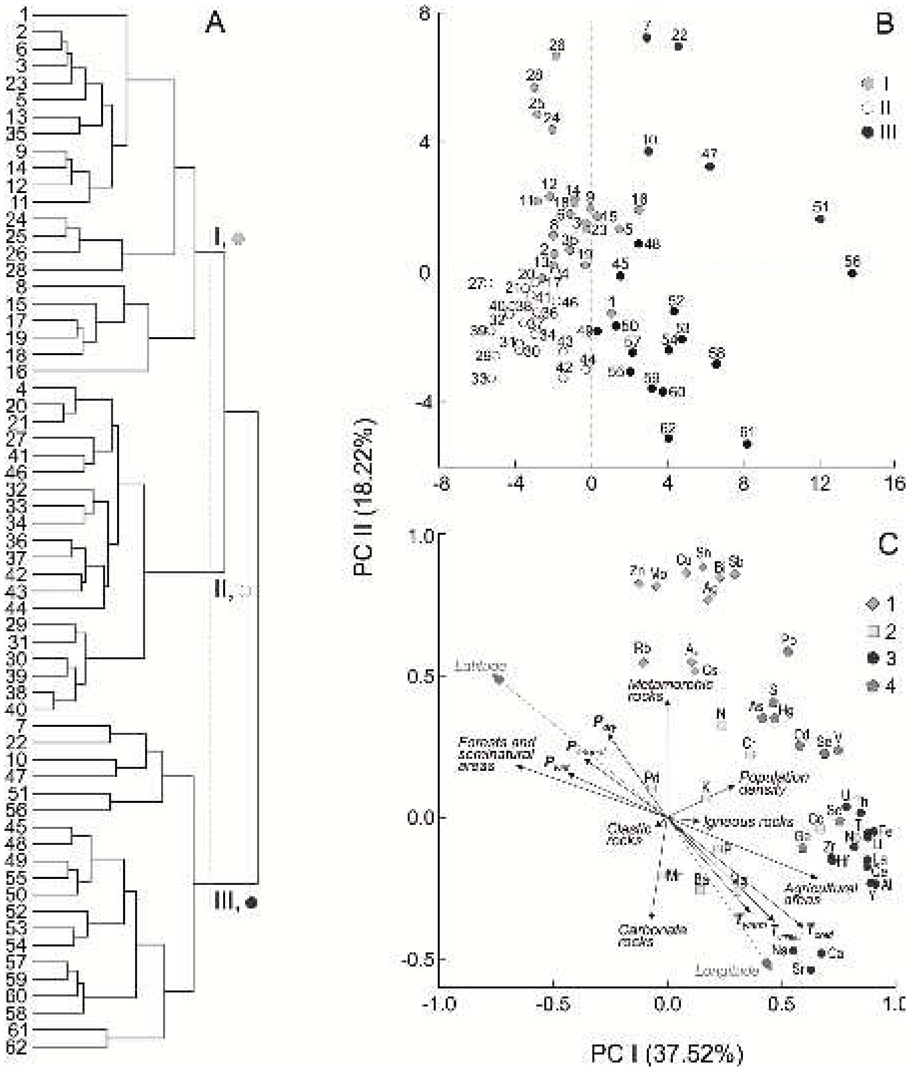

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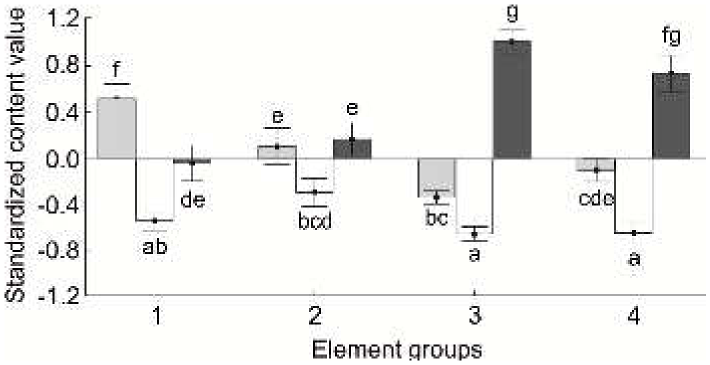

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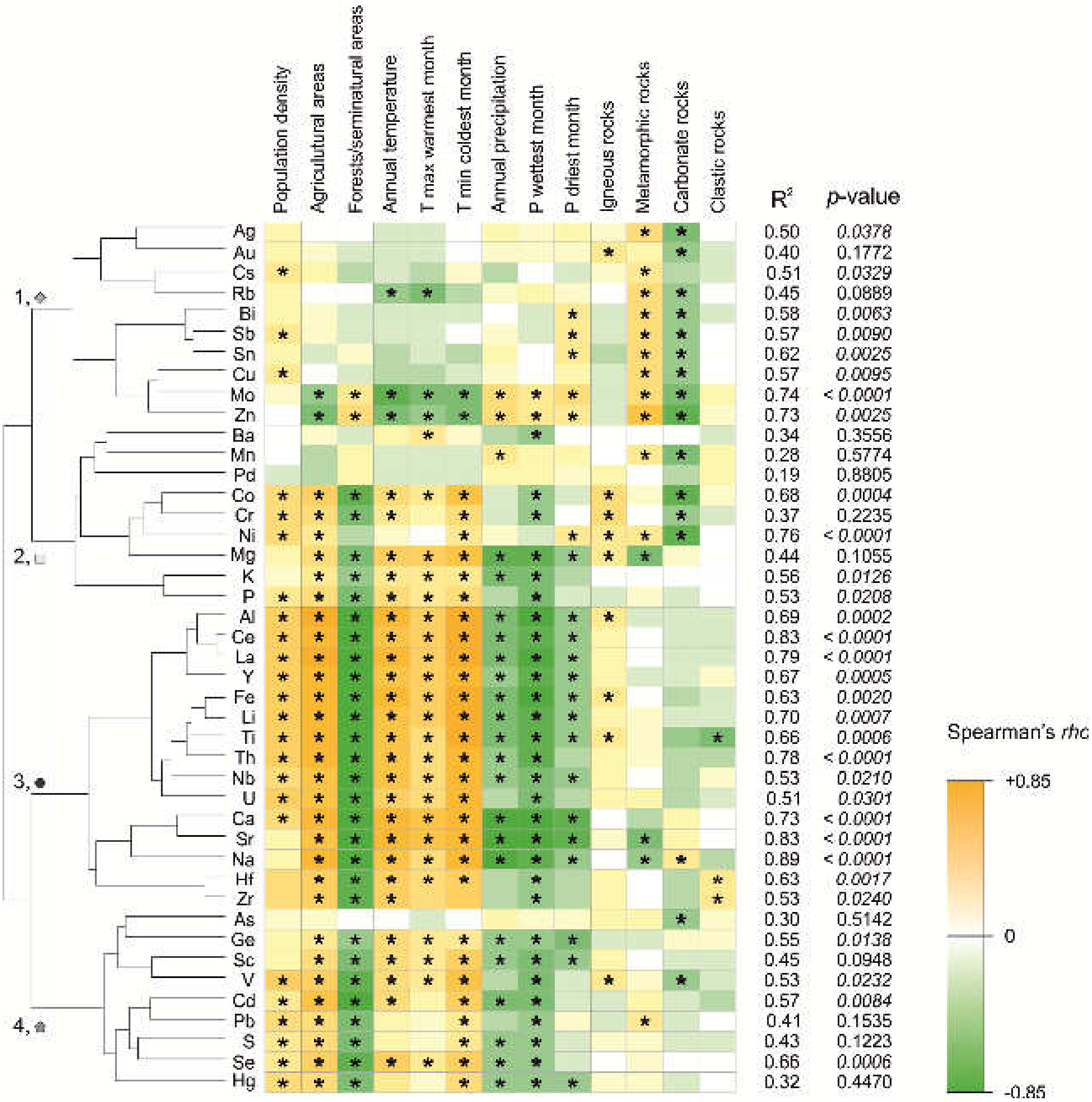

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