Background element content in the lichen *Pseudevernia furfuracea*: a comparative analysis of digestion methods

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Abstract In bioaccumulation studies, the interpretation of pollutant contents in the target biomonitor has to be performed by assessing a deviation from an unaltered reference condition. A common strategy consists in the comparison with background element content (BEC) values, often built up by uncritically merging methodologically heterogeneous data. In this respect, the acid digestion of samples was identified as a major step affecting BEC data. Here, the analytical outcomes of two acid mixtures were compared on a set of matched paired samples of the lichen *Pseudevernia furfuracea*, a widely used biomonitor for which BEC values based on partial digestion were previously provided. The standard reference material BCR 482 (*P. furfuracea*) was used to validate analytical procedures consisting of either a HF total mineralization or an *aqua regia* partial one, both associated to ICP-MS multi-element analysis. In particular, the performance of the procedures was evaluated by comparing analytical results of field samples with the accuracy obtained on BCR aliquots (measured-to-expected percentage ratio). The total digestion showed a better performance for Al, As, Ba, Ca, Cd, Cu, Fe, Mn, Ni, Se, Sn, and Zn, whereas the opposite was found for Cr, Co, P, and S. Moreover, new BEC values were provided for *P. furfuracea* using a consolidated statistical approach, after a total sample digestion with hydrofluoric acid. The multivariate investigation of the

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background variability of 43 elements in 57 remote Italian sites led to the identification of geographically homogeneous areas for which BEC values are provided for use as reference in biomonitoring applications.

Introduction

Bioaccumulation of trace elements from atmospheric depositions is widely evaluated in lichens, symbiotic organisms with peculiar morphological and physiological traits, which allow their elemental content to reflect the chemical composition of the air (Bargagli 1998; Nash III 2008). In bioaccumulation studies, the interpretation of the pollutant contents in the target biomonitor has to be performed by assessing a deviation from an unaltered reference condition. A common strategy consists in the comparison with background element content (BEC) values, i.e., element concentration values measured in samples collected in remote areas, far distant from known emission sources (Bargagli 1998).

In a recent contribution (Cecconi et al. 2018), the review-based procedure used to assess lichen BEC values was extensively discussed, and several methodological criticalities were highlighted within so-obtained reference values. Such criticalities were further confirmed by an extensive survey of lichen biomonitoring literature spanning over 50 years of "established" practices. A remarkable variability of methods concerned almost all the steps of biomonitoring procedures, ranging from the processing of lichen material to the digestion and quality assurance procedures. Unexpectedly, details on acid digestion were often missing in the biomonitoring literature, possibly introducing a bias in BEC values built up on highly heterogeneous sample digests, as affecting accuracy for specific elements. The acid digestion is actually an integral part of most multi-element measurement procedures (Gaudino et al. 2007). Accordingly, the effects of different mineralization protocols were often addressed in the context of methodological studies of environmental chemistry concerning soil (e.g., Carvalho Vieira et al. 2005; Yafa and Farmer 2006; da Silva et al. 2014), sediments and sludges (e.g., Sastre et al. 2002) and biological matrices from plant materials (Rodushkin et al. 1999; Tuncel et al. 2004;

Rashid et al. 2016), lichens included (Baffi et al. 2002), to fish tissues (Ashoka et al. 2009).

Acid mixtures for wet sample digestion are traditionally subdivided in two main groups, according to the presence/absence of hydrofluoric acid (HF) (Gaudino et al. 2007). "Total" digestions include HF, whereas "partial" digestions (also referred as "strong acid digestion" or "aqua regia") do not (Niazi et al. 1993; Cook et al. 1997; Perez-Santana et al. 2007). Furthermore, aqua regia mixtures are sometimes referred as "acid leaching" procedures rather than "acid digestions," since often they do not permit the determination of total analyte contents (Castilho et al. 2012). By contrast, it is generally agreed that total, HF-based digestions produce more accurate results for materials containing resistant mineral phases (e.g., Sandroni and Smith 2002), because they ensure a more performing dissolution of aluminosilicates (Yafa and Farmer 2006). Nevertheless, several drawbacks were also highlighted in relation to the use of HF when digesting airborne particulate matter samples. For instance, its use may determine the formation of volatile fluorides, eventually lost during sample preparation (Castilho et al. 2012), or insoluble calcium fluoride (CaF_2), which can co-precipitate metals, hence lowering element recoveries (Rönkkömäki et al. 2008). Most importantly, digestions with HF pose health concerns for operators (Le Fèvre and Pin 2005), have higher costs (Goddard and Brown 2014), and can damage the instrumentation (Silva et al. 2014). On these bases, alternative acid mixtures consisting of fluoroboric acid (HBF_4), nitric acid (HNO_3), and aqua regia were also developed for the analysis of particulate matter collected by quartz filters (Castilho et al. 2012).

The selection of a proper digestion approach should rely on element speciation form, matrix character of the sample, and evaluation of possible interferences among elements (Kalembkiewicz and Sitarz-Palczak 2001). In the majority of cases, this does not fall within the bounds of practical feasibility for biomonitoring surveys, with their numerous, low-biomass sample batches, often analyzed by external accredited laboratories. In these conditions, it is often not practically and economically viable to select different digestions techniques for the analysis of different sets of elements. As a matter of fact, the use of HF-based digestions to analyze lichen samples is becoming more common in the last years, as a painstaking analysis of lichenological literature revealed (Cecconi et al. 2018). However, an aqua regia partial digestion coupled with ICP-MS determination was used

to define BEC values of the epiphytic lichen *Pseudevernia furfuracea*, one of the most widely used, highly performing biomonitor, on the basis of samples from 62 remote Italian sites (Cecconi et al. 2018). Baffi et al. (2002) obtained different results for *P. furfuracea* (in the form of reference material BCR 482; see Quevauviller et al. 1996), by using "total" vs. "partial" digestion mixtures. Since no solubilization procedure meets the needs of all analytical techniques or solve all type of problems for trace element determination (Bettinelli et al. 2002), in this work, new BEC values are proposed for this important biomonitor, based on a "total" sample digestion instead of a "partial" one, as a further contribution to the methodological standardization of lichen biomonitoring techniques (Incerti et al. 2017; Cecconi et al. 2018, 2019) aimed at enhancing proper data comparison.

Materials and methods

The target species

P. furfuracea is a fruticose, meso-xerophilous, and photophilous lichen, occurring on acidic, non-eutrophicated bark, with optimum in cool-temperate/boreal areas (Rikkinen 1997; Smith et al. 2009). *P. furfuracea* has two morphologically undistinguishable varieties (var. *furfuracea* and var. *ceratea*), with richly branched thalli often densely covered by finger-shaped outgrowths (isidia), which considerably increase particle entrapment (Riga-Karandinos and Karandinos 1998; Bargagli and Mikhailova 2002). The large thallus size and the easy identification in the field ensure fast and easy sampling and preparation. In addition, the availability of very flourishing and abundant populations definitely limits the risk of population impoverishment by dedicated sampling campaigns. For these reasons, *P. furfuracea* is frequently used in biomonitoring surveys of trace elements (e.g., Tretiach et al. 2011a; Gallo et al. 2017); it has already been used for the production of a standard reference material (Quevauviller et al. 1996) and targeted for the application-driven determination of BEC values (Cecconi et al. 2018).

Lichen sampling and processing

Thalli of *P. furfuracea* without distinction of the two varieties (Incerti et al. 2017) were collected at 57 remote sites of the main Italian mountain ranges (Supplementary Table S1). The source lichen material was largely the same used in a previous study (Cecconi et al. 2018), with the addition of lichen thalli sampled in previously unexplored areas (Supplementary Table S1).

Details on lichen sampling are provided in Incerti et al. (2017). In the laboratory, the lichen material was dried out at room temperature until reaching constant mass. All thalli were carefully cleaned from fragments of tree bark and other lichens and mosses using powder-free gloves and plastic tweezers. Terminal lobes homogenous in size (15–25 mm), shape, and isidia density (visually assessed) were selected, separated from the thallus using ceramic scissors, and pulverized through a planetary ball mill (Retsch PM100), with milling cycles of 4 min at 550 rpm. Once processed, the lichen material was sealed under vacuum and stored at − 20 °C until analytical determination. Overall, 171 analytical replicates (3 replicates × 57 sites) underwent a total digestion; in 20 cases out of 57, the number of replicates was doubled in order to perform also a partial sample digestion (additional 3 replicates × 20 sites; Supplementary Table S1).

Analytical procedures

All analytical replicates were submitted to multi-element analysis at Bureau Veritas Mineral Laboratories (BVML, former ACME Analytical Laboratories, Vancouver, Canada), a widely acknowledged high-quality data provider for element content in different matrices, including lichens (e.g., Tretiach et al. 2011b; García-Ordiales et al. 2016). The element concentrations were determined for all the samples after a multi-acid digestion with perchloric and HF. In addition, the coupled set of analytical replicates (60 replicates from 20 sites) were subjected to a HF-free, partial digestion. Regarding the total digestion, samples were analyzed at BVML according to the MA250 analytical packet (protocol for Ultra Trace ICP-MS chemical analysis of multi-acid digested samples). Replicate aliquots of 0.25 g were heated in HNO_3-$HClO_4$-HF solution (volume ratio 1:1:2) until fuming and then dried; the resulting residue was dissolved in 50% HCl solution and heated using a mixing hot block. After cooling, the solutions were transferred to test tubes and brought to volume using HCl. The use of strong oxidizing acids (HNO_3 and $HClO_4$) ensures the removal of organic matter, while the use of HF enables the dissolution of silicates, allowing the near-total dissolution of the mineral fraction (Yafa and Farmer 2006). Regarding the partial

digestion, samples were analyzed according to the VG101-EXT analytical packet (protocol for dry plant material analysis). Replicates aliquots of 1.0 g were cold leached with HNO_3 for 1 h, then digested in a hot water bath. After cooling, an *aqua regia* solution (HCl-HNO_3, volume ratio 3:1) was added to each sample to leach in a heating block of boiling water bath. Samples were made up to volume with dilute HCl and then filtered.

The content of 59 (MA250 packet) and 53 elements (VG101-EXT packet) was analyzed through ICP-MS with a Perkin Elmer Elan 6000 ICP mass spectrometer. All the reagents used were at least ACS-grade. Values were expressed on a dry mass basis ($\mu g\ g^{-1}$).

BVML quality assurance/control (QA/QC) protocol includes a sample-prep blank carried through all stages of preparation and analysis as the first sample, a pulp duplicate to monitor analytical precision, two reagent blanks to measure background, and aliquots of in-house standard reference materials. In-house standards V16 and CDV-1 (plant leaves) were used to monitor accuracy in the case of partial digestion protocol on 38 and 44 elements, respectively, each with 11 replicates. OREAS25A-4A and OREAS45E (soil) were used in the case of total digestion on 39 and 57 elements, respectively, each with 5 replicates. The standard reference material CRM 482 "lichen" *P. furfuracea* was also sent to BVML to be blindly analyzed with both packets. Quality control was expressed in terms of mean recovery percentages, calculated as the percentage ratio between the measured and the expected values for the lichen standard BCR 482 and for the internal standards (Table 1, Supplementary Table S2). For BCR 482, mean recoveries were calculated for elements with either certified or indicative expected values (Quevauviller et al. 1996). Accuracy was deemed satisfactory when mean recovery percentage was between 80 and 120% of the expected value.

Data analysis

The median values of the 47 elements analyzed by ICP-MS after partial and total digestion methods were tested for significant differences using Wilcoxon's-matched pair test, considering all 60 analytical replicates for each digestion method. In order to assess which digestion method had the better performance, a comparative analysis was conducted taking into account the results of analytical determination on both field samples and the

standard BCR 482 replicates blindly included in the batch, following the flow illustrated in Fig. 1.

The pipeline followed to obtain the BECs for totally digested samples of *P. furfuracea* at the national level is the same used in Cecconi et al. (2018). First, extreme outliers (OLs) were removed for each element, as possibly affected by unexpected anthropogenic contributions at the sampling sites. Extreme OLs were identified according to the Tukey's method (i.e., values higher than the 3rd quartile of the distribution plus three times the interquartile range), which makes no distributional assumptions, thus being applicable to skewed or non-bell-shaped data distributions (Hoaglin et al. 1986). Then, the elements with at least 50% of field values below the lower limit of detection (LoD) were excluded from further data analysis. Finally, the content data matrix of 57 collection sites × 43 elements, with data standardized for each element to account for different content magnitudes in different elements, was submitted to cluster analysis (CA, with Euclidean distance as distance measure and Ward's method as grouping algorithm) and principal component analysis (PCA). Both CA and PCA were separately performed for elements and collection sites. For the resulting clusters of sites, the mean standardized content of each group of elements was calculated, and a complete series of basic descriptive statistics of element content was separately provided for each element, after the outlier removal. In detail, mean, standard deviation, median, median absolute deviation (MAD; Reimann et al. 2005), and 98th percentile were calculated for each element.

All data analyses and graphics were performed with the software packages Statistica v. 10 (StatSoft Inc., Tulsa, OK) and R (R Core Team 2013). Statistical significance was tested at $\alpha = 0.05$ in all cases.

Results and discussion

Accuracy of the analytical procedures

When compared with certified or indicative values (Quevauviller et al. 1996; Table 1), mean recoveries in the lichen standard BCR 482 were either below 80% or above 120% limited to 3 (i.e., Cr, Mo, and S) and 5 (Al, Ba, Mo, Se, Sn) elements out of 21, for totally and partially digested replicates, respectively (Table 1). Total

Table 1 Element content ($\mu g\ g^{-1}$) of the epiphytic lichen *Pseudevernia furfuracea* as resulted from the chemical analyses of 20 samples either subjected to total or partial digestion (MA250 and VG101-EXT packets, respectively)

Element	Total digestion (MA250)			Partial digestion (VG101-EXT)			Wilcoxon		T/P	T/P recovery
	Mean ± SD	LoD	BCR 482 recovery (95% C.I.)	Mean ± SD	LoD	BCR 482 recovery (95% C.I.)	z	p value		
Ag[b]	0.033 ± 0.008	0.02	–	0.021 ± 0.008	0.002	–	6.57	≤10⁻⁶	1.57	–
Al[c]	1038 ± 387	200	99.7 (99.7 ÷ 99.7)	455 ± 166	100	36.3[e] (36.3 ÷ 36.3)	6.74	≤10⁻⁶	2.28	2.75
As	0.555 ± 0.346	0.2	84.7 (40.4 ÷ 129.0)	0.333 ± 0.222	0.1	80.0 (73.5 ÷ 86.5)	6.74	≤10⁻⁶	1.67	1.06
Ba[bc]	16.2 ± 6.6	1	95.3 (91.6 ÷ 99.0)	12.2 ± 5.7	0.1	65.4[c] (63.7 ÷ 67.0)	6.74	≤10⁻⁶	1.33	1.46
Be[ba]	1.0 ± 0.0	1	–	0.1 ± 0.0	0.1	–				–
Bi	0.056 ± 0.026	0.04	–	0.047 ± 0.027	0.02	–	4.25	2.1 · 10⁻⁵	1.19	–
Ca	7467 ± 3410	100	102.5 (101.5 ÷ 103.6)	7237 ± 3221	100	86.1 (81.9 ÷ 90.4)	3.52	4.3 · 10⁻⁴	1.03	1.19
Cd	0.203 ± 0.066	0.02	95.0 (91.0 ÷ 99.0)	0.160 ± 0.059	0.01	94.3 (91.9 ÷ 96.7)	6.57	≤10⁻⁶	1.27	1.01
Ce	1.61 ± 0.74	0.02	–	1.08 ± 0.51	0.01	–	6.74	≤10⁻⁶	1.49	–
Co[b]	0.287 ± 0.085	0.2	115.6 (98.3 ÷ 133.0)	0.268 ± 0.077	0.01	88.8 (86.6 ÷ 90.9)	2.42	0.016	1.07	1.30
Cr[bc]	5.90 ± 1.13	1	150.5[c] (125.3 ÷ 175.7)	2.98 ± 0.53	0.1	101.5 (97.0 ÷ 105.9)	6.74	≤10⁻⁶	1.98	1.53
Cs[b]	0.175 ± 0.111	0.1	–	0.132 ± 0.108	0.005	–	6.65	≤10⁻⁶	1.33	–
Cu[b]	5.68 ± 2.22	0.1	97.9 (94.4 ÷ 101.4)	5.56 ± 2.1	0.01	94.0 (90.4 ÷ 97.6)	2.78	0.005	1.02	0.99
Fe[b]	618 ± 216	100	97.5 (94.7 ÷ 100.3)	557 ± 198	10	90.3 (86.8 ÷ 93.8)	5.99	≤10⁻⁶	1.11	0.97
Ga[b]	0.277 ± 0.11	0.02	–	0.127 ± 0.045	0.1	–	6.66	≤10⁻⁶	2.18	–
Hf[b]	1.356 ± 0.569	0.02	–	0.064 ± 0.035	0.001	–	6.74	≤10⁻⁶	21.19	–
In[a]	0.01 ± 0.00	0.01	–	0.02 ± 0.00	0.02	–				–
K	3470 ± 617	100	96.4 (94.7 ÷ 98.2)	3057 ± 593	100	103.6 (100.0 ÷ 107.2)	6.27	≤10⁻⁶	1.14	0.93
La[b]	0.818 ± 0.407	0.1	–	0.496 ± 0.247	0.01	–	6.74	≤10⁻⁶	1.65	–
Li[b]	0.642 ± 0.286	0.1	–	0.369 ± 0.139	0.01	–	6.49	≤10⁻⁶	1.74	–
Mg[b]	832 ± 148	100	–	805 ± 143	10	–	3.01	0.003	1.03	–
Mn	61.2 ± 27.2	1	93.3 (90.2 ÷ 96.5)	58.0 ± 27.1	1	92.1 (87.8 ÷ 96.4)	4.82	≤10⁻⁶	1.06	1.01
Mo[c]	0.306 ± 0.207	0.05	42.8[c] (39.5 ÷ 46.2)	0.307 ± 0.209	0.01	44.7[c] (38.6 ÷ 50.8)	0.16	0.870	1.00	0.85
Na	142.0 ± 84.6	10	–	84.3 ± 85.4	10	–	6.74	≤10⁻⁶	1.68	–
Nb	0.175 ± 0.067	0.04	–	0.046 ± 0.019	0.01	–	6.74	≤10⁻⁶	3.80	–
Ni	1.96 ± 1.01	0.1	102.0 (95.5 ÷ 109.0)	1.57 ± 0.87	0.1	89.1 (84.0 ÷ 94.1)	6.55	≤0⁻⁶	1.25	1.27
P	558 ± 194	10	89.0 (86.9 ÷ 91.0)	580 ± 188	10	98.0 (93.1 ÷ 102.8)	3.73	1.9 · 10⁻⁴	0.96	0.91
Pb	3.87 ± 1.98	0.02	89.6 (87.3 ÷ 91.8)	3.49 ± 1.83	0.01	89.7 (86.3 ÷ 93.0)	6.62	≤10⁻⁶	1.11	1.00
Rb	12.26 ± 5.13	0.1	–	10.91 ± 4.7	0.1	–	6.74	≤10⁻⁶	1.12	–
Re[a]	0.002 ± 0.000	0.002	–	0.001 ± 0.000	0.001	–				–
S[c]	1073 ± 288	400	71.8[c] (69.1 ÷ 74.5)	1178 ± 308	100	96.0 (85.0 ÷ 107.1)	3.61	3.1 · 10⁻⁴	0.91	0.75
Sb	0.123 ± 0.075	0.02	88.0 (72.3 ÷ 103.7)	0.125 ± 0.074	0.02	83.4 (77.1 ÷ 89.8)	1.17	0.240	0.98	1.06
Sc	0.182 ± 0.093	0.1	–	0.340 ± 0.069	0.1	–	6.18	≤10⁻⁶	0.54	–
Se[c]	0.507 ± 0.189	0.3	104.0 (94.9 ÷ 114.0)	0.357 ± 0.145	0.1	70.0[c] (60.7 ÷ 79.3)	4.98	≤10⁻⁶	1.42	1.52
Sn[c]	0.413 ± 0.227	0.1	106.9 (89.1 ÷ 124.6)	0.411 ± 0.231	0.02	156.8[c] (92.3 ÷ 221.3)	0.35	0.723	1.00	0.68
Sr	16.50 ± 7.52	1	–	14.62 ± 6.10	0.5	–	6.07	≤10⁻⁶	1.13	–
Ta[ba]	0.100 ± 0.000	0.1	–	0.002 ± 0.001	0.001	–				–
Te	0.094 ± 0.046	0.05	–	0.020 ± 0.001	0.02	–	6.74	≤10⁻⁶	4.70	–
Th[b]	0.167 ± 0.073	0.1	–	0.104 ± 0.039	0.01	–	6.57	≤10⁻⁶	1.61	–
Ti[b]	66.17 ± 26.43	10	–	11.68 ± 3.36	1	–	6.74	≤10⁻⁶	5.67	–
Tl[a]	0.052 ± 0.006	0.05	–	0.020 ± 0.000	0.02	–				–
U[ba]	0.100 ± 0.000	0.1	–	0.024 ± 0.013	0.01	–				–

Table 1 (continued)

Element	Total digestion (MA250)			Partial digestion (VG101-EXT)			Wilcoxon		T/P	T/P recovery
	Mean ± SD	LoD	BCR 482 recovery (95% C.I.)	Mean ± SD	LoD	BCR 482 recovery (95% C.I.)	z	p value		
V	1.90 ± 0.77	1	85.6 (70.7 ÷ 100.4)	2.35 ± 0.48	2	101.6 (86.8 ÷ 116.5)	4.23	$2.4 \cdot 10^{-5}$	0.81	0.84
W	0.118 ± 0.047	0.1	–	0.117 ± 0.046	0.1	–	0.42	0.673	1.00	–
Y[b]	7.068 ± 4.09	0.1	–	0.636 ± 0.281	0.001	–	6.74	$\leq 10^{-6}$	11.11	–
Zn	43.4 ± 20.9	0.2	96.7 (94.3 ÷ 99.1)	39.6 ± 19.3	0.1	90.1 (88.6 ÷ 91.6)	6.18	$\leq 10^{-6}$	1.10	1.03
Zr[b]	58.51 ± 24.5	0.2	–	2.83 ± 1.36	0.01	–	6.74	$\leq 10^{-6}$	20.67	–

[a] Elements with null variance (all data below the LoD) for either a single analytical packet or both

[b] Elements with LoDs differing for at least one order of magnitude between the analytical packets VG101-EXT and MA250

[c] Mean recoveries either below 80% or above 120%

Data refer to mean ± standard deviation and instrumental lower limit of detection (LoD) for the 47 elements analyzed with both analytical packets. Mean recovery percentages and 95% confidence interval (95% C.I.) are reported for the standard reference material BCR 482 "lichen" *P. furfuracea*; mean recoveries and confidence interval were calculated on five analytical replicates. Results of statistical testing for differences between element content of samples subjected to total (*T*) and partial (*P*) digestion are reported (Wilcoxon-matched pair test, significant *p* values in italic). The ratio between mean element content values and mean recovery percentages from total (*T*) and partial (*P*) digestion is also reported (*T/P* and *T/P* recovery, respectively). Wilcoxon test results and *T/P* ratios were not reported for elements with null variance

and partial recovery medians in BCR 482, as tested for significant differences using Wilcoxon's-matched pair test, were significantly higher ($p < 0.05$) in partially digested replicates for K, P, and S, while Al, Ba, Ca, Co, Fe, Ni, Se, and Zn showed the opposite trend.

For the total digestion, recoveries obtained on the in-house standard OREAS25A-4A were always included between 80 and 120% for all the 39 tested elements, while those obtained on the standard OREAS45E were either below 80% or above 120% limited to 4 cases out of 55 (Cd, Tb, Te, and Tl). When considering the partial digestion, recoveries obtained on the standard CDV-1 and V16 exceeded the 80–120% range in 8 cases out of 44: Ge, S, Se, V (CDV-1) and Au, Ga, La, S (V16) (Supplementary Table S2).

Comparison of analytical results after total and partial digestion of samples

Overall, the elemental content of totally digested samples was higher than that of matched partially digested ones. Indeed, in 33 cases out of the 41 tested elements (80.5%), totally digested samples had significantly higher median content values, whereas the opposite situation was highlighted for P, S, Sc, and V. For Mo, Sb, Sn, and W, the two digestions did not produce significantly different results. Among the 33 elements

showing significantly higher values in totally digested samples, Al, Ga, Hf, Nb, Te, Ti, Y, and Zr showed the most pronounced differences (total/partial ratio, Table 1), with mean element content values of totally digested samples ranging from c. 2 times (Al, Ga) to c. 20 times (Hf, Zr) the value of matched partially digested samples. For the elements showing the opposite trend, the differences were far less pronounced, with mean values of totally digested samples ranging from 0.54 times (Sc) to 0.96 times (P) the value of matched samples (Table 1).

Following the flow illustrated in Fig. 1 and considering the results of analytical determination on field samples and BCR 482 (Table 1), it was possible to evaluate the relative performance of the two digestion methods. In particular, the HF digestion showed a better performance for Al, As, Ba, Ca, Cd, Cu, Fe, Mn, Ni, Se, Sn, and Zn; partial digestion showed a better performance limited to the lichen macronutrients P and S and for Cr and Co. The two digestion methods showed comparable performance for Sb, whereas for Mo both procedures produced comparable but very low recoveries, hence unreliable. It was not possible to draw conclusive results for V (due to the differences in the LoDs associated to the two analytical packets and to the generally low median values characterizing both sets; Table 1, Fig. 1) as well as for all the elements with missing certified or indicative values.
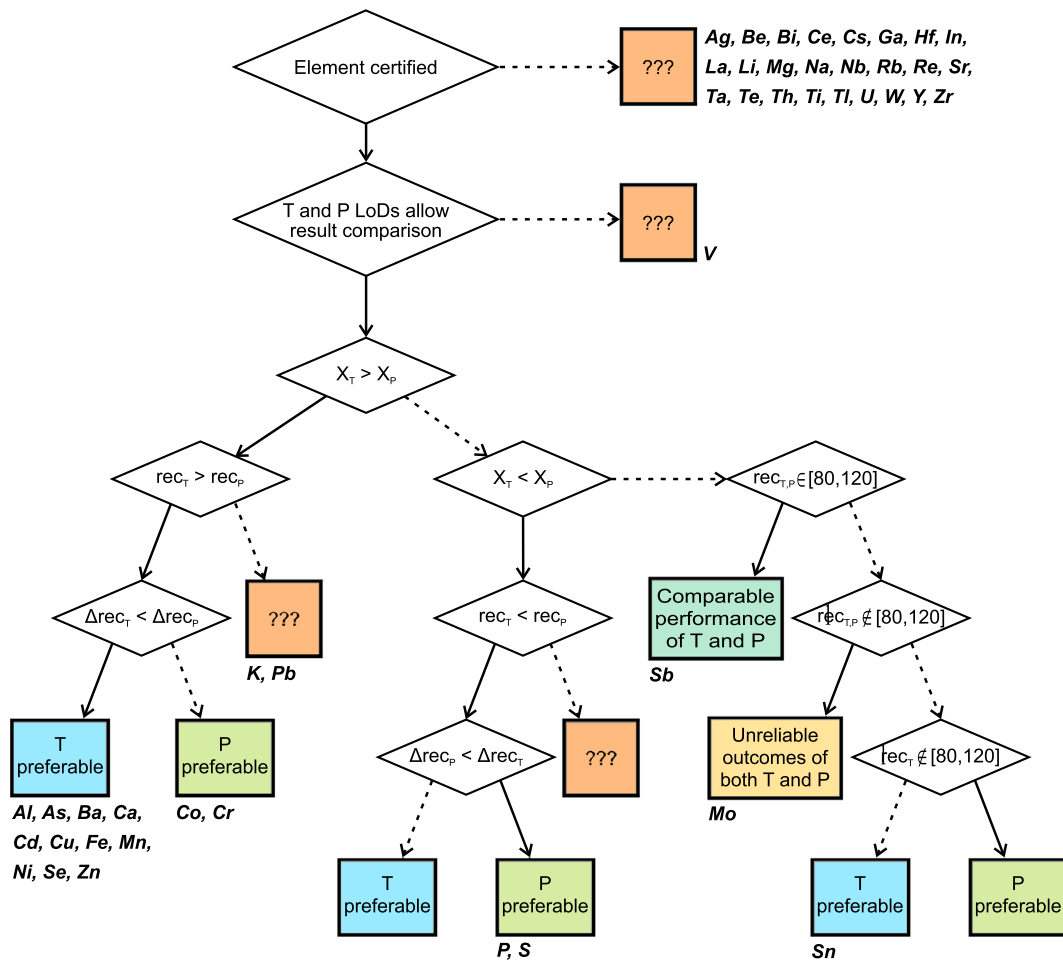
Fig. 1 Flow chart illustrating the procedure used to assess the relative performance of total (*T*) and partial (*P*) digestion methods. In the flow chart, full and dotted arrows indicate negative and positive statements, respectively; X_T and X_P refer to the median values of a generic chemical element X, similarly; rec_T and rec_P indicate the recovery percentages obtained after total and partial digestion of samples. Δrec_T and Δrec_P indicate the offset from the 100% recovery ($\Delta rec_T = |100 - rec_T|$, $\Delta rec_P = |100 - rec_P|$). Boxes with question marks indicate the impossibility to draw conclusive results due to (i) the lack of certified values, (ii) differences in LoDs having repercussions on results comparison, and (iii) inconsistent results between element content values and recoveries

Evaluation of the performance of total and partial digestion methods

The acid attack is an integral part of the analytical measurement, and it is widely acknowledged as a factor influencing the elemental content results (Gaudino et al. 2007). Indeed, the effects of the use of different acid mixtures for the mineralization of environmental and biological samples were frequently investigated over the last 20 years (e.g., Chen and Ma 1998; Rodushkin et al. 1999; Baffi et al. 2002; Sastre et al. 2002; Ashoka et al. 2009).

A total digestion scheme must include the use of HF to completely release the trace elements included in the aluminosilicate phase (Sastre et al. 2002); however, the use of HF consistently enlarges the timing and costs of the procedure, requiring the adoption of safety measures and highly trained personnel (Le Fèvre and Pin 2005). Nonetheless, HF-free digestions, although generally safe and cheap, may lead to the underestimation of the content of environmental-concerning trace elements held at aluminosilicate sites (Tam and Yao 1999).

In order to reliably compare our results on the relative performance of digestion methods to those obtained in the context of different studies on the same lichen matrix, the use of a comparable technique for multi-element determination should be considered as a fundamental constraint. In this study, the results on field

samples and certified material aliquots were analyzed through ICP-MS, an analytical technique used also by Baffi et al. (2002). These authors showed that the digestion in microwave oven with HF-HNO$_3$-H$_2$O$_2$ was more suitable than the digestion with HNO$_3$-H$_2$O$_2$ for Al, Ba, Cr, Cu, Fe, Mn, V, and Zn. Our results are in substantial agreement with such findings: in particular, the total digestion better performed for Al, Ba, Cu, Fe, Mn, and Zn. On the other hand, the content of Cr in totally digested samples was importantly overestimated (150%, Table 1), apparently making the HF-free digestion preferable for the quantification of this element. Interestingly, Rönkkömäki et al. (2008) pointed out that the formation of low solubility chromyl fluoride (CrO$_2$F$_2$) may compromise Cr recovery. By contrast, several authors besides Baffi et al. (2002) reported better recoveries with total digestions for Cr (e.g., Kackstaetter and Heinrichs 1997; Chen and Ma 2001), although the same authors noticed the occurrence of spectral interference during the ICP-MS determination of ^{52}Cr, which possibly affected the results. As a matter of fact, ICP-MS techniques may suffer from interferences caused by polyatomic ions formed from precursors having different sources (such as the sample matrix, reagents used for plasma gases preparation, and entrained atmospheric gases; May and Wiedmeyer 1998).

Limited to V, our results cannot be considered fully conclusive since the LoDs obtained by the analytical packets VG101-EXT and MA250 were limiting for the interpretation of the results (Table 1). Indeed, the LoDs obtained with total and partial digestion packets were 1 and 2 µg g^{-1}, respectively (Table 1), and the overall number of records below or equal to total and partial LoDs was 35 and 65%, therefore affecting median values as well as the outcome of statistical testing. As far as K and Pb, the median values obtained by total digestion were higher than those obtained by partial digestion; however, the opposite situation was found for the recovery percentages on the certified lichen replicates (Fig. 1, Table 1), suggesting that the source of measurement bias could be unrelated to the acid attack. Nonetheless, it should also be considered that the lichen CRM is generally characterized by a low content of terrigenous particulate and may not always give fully consistent indications about the degree of mineralization obtained on real samples (Bettinelli et al. 2002) with respect to some elements mainly held at aluminosilicate sites, such as Pb (Tam and Yao 1999).

Interestingly, for the lichen macronutrients P and S, as well as for Co (Nash III 2008), the partial digestion

provided the best accuracy. Limited to P and S, our results were in contrast with those of Chen and Ma (1998) on soil samples. However, the relative abundance of organic and inorganic P and S is acknowledged as a source of variability for analytical results (Sastre et al. 2002; Hamilton et al. 2015), along with the major interferences affecting the most abundant isotopes of P and S, possibly resulting from nitrogen, oxygen and hydrogen (and their molecular ions: e.g., ^{14}N^{16}O^{1}H$^+$, ^{15}N^{16}O$^+$, ^{14}N^{17}O$^+$, ^{13}C^{18}O$^+$, ^{12}C^{18}O^{1}H$^+$; May and Wiedmeyer 1998) during ICP-MS determination (Bandura et al. 2002). Furthermore, it must be considered that volatilization during fuming may result in some loss of S during the total MA250 digestion (BVML technical information package; Castilho et al. 2012), which could potentially clarify the observed pattern for this element.

Geographical pattern of BEC

The CA based on the element content data from the field sampling produced four main clusters of sites (Figs. 2a and 3) separated for geographical location. Cluster I included 14 sites located in the eastern Alps (with the exception of site 8, located in the western Alps, and site 41, located in the northern Apennines), generally characterized by sedimentary carbonate substrates; cluster II included 11 sites, located in the western Alps, exclusively over metamorphic siliceous rocks; cluster III included 13 sites located in the central Alps, mostly characterized by metamorphic substrates. Finally, cluster IV included 19 sites located in the Apennines (with the exception of site 5 located in the western Alps) over heterogeneous lithological substrates. When removing from clusters the few sites inconsistent for geographic location (i.e., sites 8 and 41 from cluster I and site 5 from cluster IV), four geographically homogeneous sets of lichen samples were obtained (Supplementary Fig. S1). A clear pattern of lichen element composition emerged from the PCA (Fig. 2b): in particular, elements of group 1 (Ag, As, Bi, Cs, Cu, Mo, Rb, S, Sb, Se, Sn, Zn) were consistently placed at high scores of the second principal component (PC II), inversely related to longitude and positively to latitude. Differently, elements of group 2 (Al, Cd, Ce, Fe, Ga, Gd, La, Li, Na, Nb, Pb, Sc, Sm, Th, Ti, V), including several elements of lithogenic origin, were placed at negative scores of the first axis (PC I), positively related to longitude and inversely to latitude. Elements of group 3 (Ba, Ca, Co, Cr, Hf, K, Mg, Mn, Nd, Ni, P, Sr, Te, Y, Zr) were placed at negative scores of

Fig. 2 Results of multivariate analysis of element content in *Pseudevernia furfuracea* samples collected at 57 remote field sites in Italy. **a** Factorial scores of field sites, symbolized according to CA results of Fig. 3. **b** PCA plot showing loading vectors of the elements symbolized according to the cluster analysis (CA) and their relationship with geographical location of the field sites (i.e., longitude and latitude) plotted as supplementary variables (Legendre and Legendre 1998)
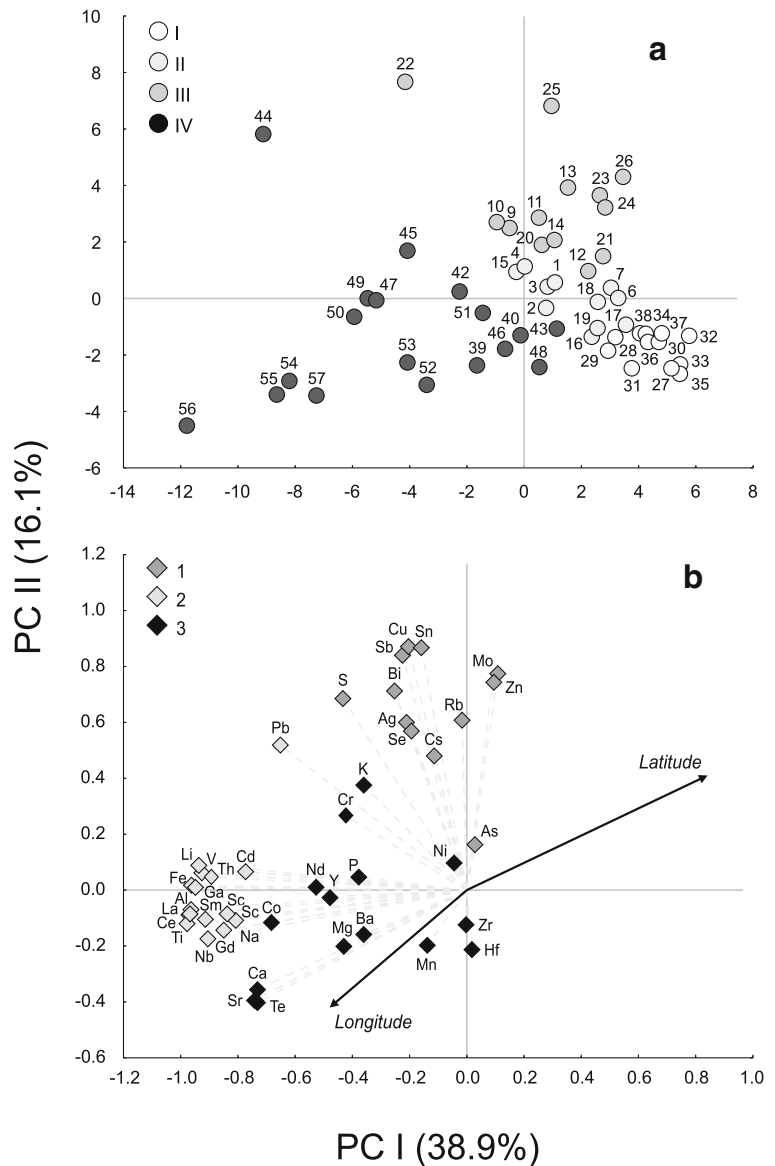
PC I, hence negatively related to latitude, but scattered along PC II. Such geographical correspondence is better noticeable in the plot of collection sites in the ordination space (Fig. 2a). Indeed, the sampling sites were distributed in the ordination space according to element content gradients. In particular, Apennine sites of cluster IV were placed at negative scores of PC I, differently from the clusters of Alpine sites (i.e., clusters I–III), which were instead placed at positive scores of PC I. The latter clusters slightly segregated along PC II. Indeed, these were characterized by increasing averaged scores along this axis (− 1.64, − 0.17, and 3.32, for sites of cluster I,

II, and III, respectively). Consistently, the clusters of sites at different geographic locations showed significant differences of standardized content of the three groups of elements (Fig. 3). Eastern Alpine sites of cluster I showed the lowest standardized element content of all the three element groups. Namely, cluster I had the lowest content of 26 elements out of 43 (Table 2). Western Alpine sites of cluster II showed negative standardized content of element groups 1 and 2 and positive content of element group 3; differently, central Alpine sites of cluster III showed the highest levels of elements of group 1, but negative element content of groups 2 and 3. In
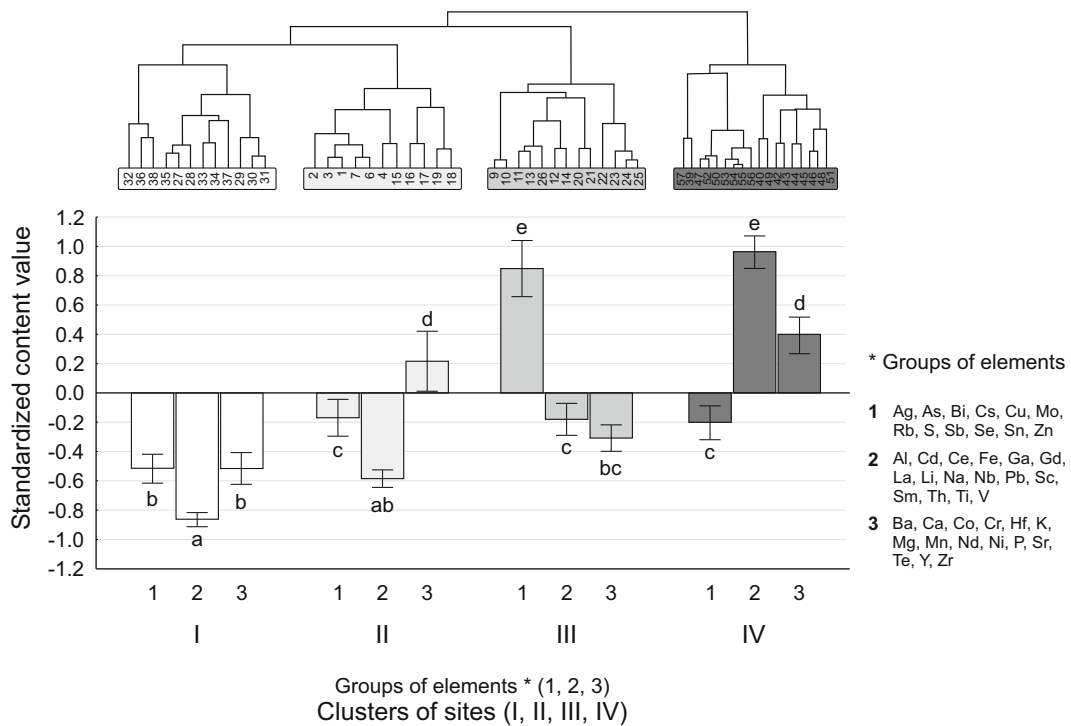
Fig. 3 Content of element groups in the site clusters I–IV: data are separately standardized for each element and showed as mean and 95% confidence interval for three different element groups, as resulting from CA. Letters above bars indicate significant pairwise differences (Tukey's HSD test for unequal sample size, $p < 0.05$)

particular, cluster II exhibited lower elemental content than cluster III for 19 elements (either significantly or not), including As, Bi, Cu, Rb, S, Sb, Se, Sn, Zn (group 1), and Pb (group 2); the opposite situation was highlighted limited to 5 ,elements, including Co, Cr and Ni (group 3) (Table 2). Finally, Apennine sites of cluster IV showed negative values of elements of group 1, but the highest standardized content of elements of groups 2 and 3 (Fig. 3) also exhibits the highest content of 22 out of 43 elements (Table 2).

BEC of *P. furfuracea*: final remarks

Previous results on *P. furfuracea* BEC, as obtained by Cecconi et al. (2018) by *aqua regia* digestion, indicated a pattern of association between *P. furfuracea* BECs and the geographical location of Italian remote sites. The results based on total digestion here presented clearly confirm the same general pattern. Consistently, elemental content generally increases moving southwards, with increasing averaged levels of population density and agricultural landcover in the surroundings of the sampling sites, and contextual decreasing mean annual rainfall and forest landcover. Among the Alpine sites, elemental content decreases moving eastwards (with increasing precipitation levels), whereas western sites (with prevalent siliceous and/or metamorphic bedrocks) showed higher background levels with respect to the easternmost ones (with prevalent sedimentary bedrocks) (Cecconi et al. 2018). The single cluster obtained for Apennine sites is likely the result of site under-sampling in such a large geographical domain; however, the utmost differences still observed between Alpine and Apennine *P. furfuracea* BECs are certainly not a sampling artifact. Indeed, Apennine sites showed higher content of lithogenic (Al, Ca, Fe, Li, Th, and Ti) and rare earth elements (REE: Ce, La, Sc, Y), as related to higher soil susceptibility to erosion and influence by agricultural areas (Capozzi et al. 2016; Cecconi et al. 2018).

Compared with BECs based on partially digested samples, some interesting differences arose. In particular, the Alpine sites segregated into three (instead of two) clusters, substantially differing in elemental content (Table 2, Fig. 3): (i) the eastern sites of cluster I, showing the lowest BEC values; (ii) the western Alpine sites, with intermediate BEC values; and (iii) the central sites of cluster III, located in Valtellina (province of

Table 2 Background element content (BEC) values (μg g^{-1}) in the epiphytic lichen *Pseudevernia furfuracea* in Italy

Element (group)	Cluster I (Eastern Alps, n = 12)			Cluster II (Western Alps, n = 11)			Cluster III (Central Alps, n = 13)			Cluster IV (Apennines, n = 18)			K-W p value
	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	
Ag (1)	0.030 ± 0.009	0.027^a (0.004)	0.052	0.030 ± 0.004	0.032^{ab} (0.003)	0.035	0.037 ± 0.010	0.034^b (0.002)	0.057	0.032 ± 0.008	0.030^{ab} (0.004)	0.049	0.0724
As (1)	0.611 ± 0.220	0.533^b (0.117)	1.000	0.398 ± 0.131	0.433^a (0.083)	0.567	0.605 ± 0.252	0.633^b (0.200)	1.000	0.407 ± 0.21	0.367^a (0.100)	0.967	0.0083
Bi (1)	0.046 ± 0.013	0.040^a (0.000)	0.090	0.046 ± 0.008	0.040^a (0.000)	0.060	0.066 ± 0.026	0.060^b (0.017)	0.137	0.053 ± 0.025	0.040^{ab} (0.000)	0.123	0.0118
Cs (1)	0.120 ± 0.041	0.100^a (0.000)	0.200	0.163 ± 0.165	0.100^{ab} (0.000)	0.600	0.286 ± 0.241	0.200^b (0.100)	0.800	0.154 ± 0.096	0.100^a (0.000)	0.500	0.0085
Cu (1)	4.50 ± 1.28	4.18^a (0.47)	8.37	4.79 ± 0.85	4.50^a (0.37)	6.00	7.36 ± 1.92	6.72^b (1.00)	12.70	4.95 ± 1.66	4.53^a (0.53)	10.97	< 10^{-4}
Mo (1)	0.223 ± 0.193	0.160^{ab} (0.035)	0.828	0.340 ± 0.188	0.273^{bc} (0.043)	0.787	0.423 ± 0.139	0.373^c (0.090)	0.710	0.141 ± 0.058	0.140^a (0.037)	0.277	< 10^{-4}
Rb (1)	7.36 ± 4.65	6.57^a (1.35)	18.77	10.11 ± 3.00	11.70^a (2.20)	13.90	19.97 ± 9.04	17.92^b (4.73)	43.00	10.49 ± 5.57	7.90^a (3.13)	21.00	0.0001
S (1)	797 ± 190	800^a (117)	1283	972 ± 170	1000^{ab} (100)	1167	1176 ± 256	1200^c (200)	1767	1077 ± 216	1000^{bc} (133)	1600	0.0003
Sb (1)	0.070 ± 0.039	0.057^a (0.020)	0.147	0.094 ± 0.029	0.097^a (0.020)	0.153	0.163 ± 0.066	0.140^b (0.015)	0.343	0.088 ± 0.039	0.077^a (0.017)	0.207	< 10^{-4}
Se (1)	0.408 ± 0.068	0.400^a (0.050)	0.533	0.431 ± 0.054	0.433^a (0.033)	0.500	0.586 ± 0.134	0.583^b (0.133)	0.767	0.478 ± 0.158	0.467^a (0.117)	0.867	0.0055
Sn (1)	0.276 ± 0.110	0.267^a (0.067)	0.500	0.376 ± 0.167	0.333^a (0.100)	0.750	0.540 ± 0.213	0.517^b (0.117)	1.100	0.311 ± 0.153	0.267^a (0.067)	0.833	0.0002
Zn (1)	38.0 ± 12.2	38.7^a (8.2)	65.0	40.0 ± 9.5	37.1^a (2.3)	62.7	64.4 ± 18.3	61.8^b (12.1)	101.2	31.4 ± 10.3	32.2^a (8.6)	52.2	< 10^{-4}
Al (2)	688 ± 279	600^a (100)	1333	835 ± 190	800^{ab} (200)	1100	1024 ± 402	983^b (217)	2067	1600 ± 485	1633^c (433)	2533	< 10^{-4}
Cd (2)	0.159 ± 0.037	0.153^a (0.015)	0.235	0.155 ± 0.032	0.153^a (0.030)	0.192	0.204 ± 0.059	0.200^{ab} (0.023)	0.367	0.251 ± 0.098	0.227^b (0.042)	0.537	0.0003
Ce (2)	1.07 ± 0.49	0.92^a (0.18)	2.04	1.10 ± 0.28	1.20^a (0.20)	1.47	1.47 ± 0.58	1.34^a (0.30)	2.91	2.70 ± 0.91	2.79^b (0.87)	4.19	< 10^{-4}
Fe (2)	417 ± 173	400^a (100)	800	537 ± 146	500^{ab} (100)	800	612 ± 213	600^b (117)	1067	868 ± 247	900^c (200)	1300	< 10^{-4}
Ga (2)	0.188 ± 0.067	0.183^a (0.041)	0.350	0.221 ± 0.047	0.230^{ab} (0.042)	0.280	0.286 ± 0.111	0.258^b (0.065)	0.523	0.398 ± 0.119	0.387^c (0.067)	0.637	< 10^{-4}
Gd (2)	0.113 ± 0.025	0.100^a (0.000)	0.183	0.104 ± 0.011	0.100^a (0.000)	0.133	0.126 ± 0.030	0.133^a (0.033)	0.200	0.189 ± 0.069	0.167^b (0.067)	0.300	0.0003
La (2)	0.520 ± 0.249	0.433^a (0.133)	0.967	0.557 ± 0.158	0.600^a (0.117)	0.833	0.733 ± 0.31	0.667^a (0.133)	1.433	1.407 ± 0.484	1.467^b (0.467)	2.200	< 10^{-4}

Table 2 (continued)

Element (group)	Cluster I (Eastern Alps, $n = 12$)			Cluster II (Western Alps, $n = 11$)			Cluster III (Central Alps, $n = 13$)			Cluster IV (Apennines, $n = 18$)			K-W p value
	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	
Li (2)	0.383 ± 0.230	0.300[a] (0.100)	0.900	0.463 ± 0.143	0.433[ab] (0.100)	0.733	0.655 ± 0.276	0.633[b] (0.117)	1.433	0.944 ± 0.27	0.967[c] (0.233)	1.400	< 10⁻⁴
Na (2)	82.4 ± 32.7	70.0[a] (6.7)	155.0	86.1 ± 20.4	80.0[a] (13.3)	126.7	108.8 ± 40.6	101.7[a] (21.7)	203.3	227.6 ± 54	233.3[b] (20.0)	323.3	< 10⁻⁴
Nb (2)	0.122 ± 0.042	0.127[a] (0.040)	0.198	0.155 ± 0.035	0.150[a] (0.027)	0.223	0.159 ± 0.054	0.158[a] (0.042)	0.270	0.286 ± 0.093	0.287[b] (0.083)	0.463	< 10⁻⁴
Pb (2)	2.77 ± 0.98	2.71[a] (0.44)	4.79	2.35 ± 0.57	2.38[a] (0.19)	3.15	4.49 ± 1.83	4.22[b] (0.35)	10.11	4.60 ± 1.28	4.46[b] (0.64)	7.86	< 10⁻⁴
Sc (2)	0.166 ± 0.070	0.167[a] (0.050)	0.350	0.133 ± 0.024	0.133[a] (0.000)	0.167	0.195 ± 0.081	0.167[a] (0.05)	0.333	0.294 ± 0.103	0.300[b] (0.067)	0.467	0.0002
Sm (2)	0.114 ± 0.027	0.100[a] (0.000)	0.183	0.106 ± 0.012	0.100[a] (0.000)	0.133	0.140 ± 0.051	0.100[a] (0.000)	0.233	0.218 ± 0.083	0.200[b] (0.067)	0.367	< 10⁻⁴
Th (2)	0.120 ± 0.045	0.100[a] (0.000)	0.233	0.126 ± 0.040	0.100[a] (0.000)	0.200	0.164 ± 0.067	0.167[a] (0.067)	0.300	0.277 ± 0.122	0.233[b] (0.067)	0.633	< 10⁻⁴
Ti (2)	43.4 ± 17.5	40.0[a] (10.0)	76.7	55.0 ± 12.0	56.7[a] (10.0)	73.3	60.2 ± 21.2	58.3[a] (13.3)	103.3	106.6 ± 34.2	106.7[b] (26.7)	173.3	< 10⁻⁴
V (2)	1.20 ± 0.41	1.00[a] (0.00)	2.00	1.41 ± 0.43	1.33[ab] (0.33)	2.00	1.88 ± 0.59	2.00[b] (0.33)	3.00	2.80 ± 0.68	3.00[c] (0.33)	4.33	< 10⁻⁴
Ba (3)	16.8 ± 8.6	15.7[a] (5.0)	44.0	17.3 ± 7.8	17.3[a] (5.7)	32.0	15.6 ± 3.1	17.2[a] (2.0)	18.7	20.7 ± 9.1	17.0[a] (4.7)	43.0	0.4639
Ca (3)	6248 ± 3889	4567[a] (808)	15,700	4902 ± 1331	4600[a] (967)	6967	6481 ± 3069	5550[a] (700)	14,967	14,306 ± 6902	12067[b] (3300)	33,367	< 10⁻⁴
Co (3)	0.227 ± 0.062	0.200[a] (0.000)	0.400	0.402 ± 0.094	0.400[b] (0.067)	0.567	0.269 ± 0.077	0.250[a] (0.050)	0.400	0.362 ± 0.119	0.333[b] (0.067)	0.600	< 10⁻⁴
Cr (3)	5.26 ± 0.94	5.00[a] (0.33)	8.17	7.02 ± 1.58	6.00[b] (0.33)	10.00	5.93 ± 0.96	5.67[ab] (0.50)	8.33	6.04 ± 0.77	6.00[b] (0.50)	8.00	< 10⁻⁴
Hf (3)	1.51 ± 0.57	1.43[b] (0.21)	2.74	1.44 ± 0.36	1.40[ab] (0.30)	1.88	1.11 ± 0.39	0.95[a] (0.15)	2.09	1.33 ± 0.39	1.32[ab] (0.18)	2.41	0.0513
K (3)	2934 ± 410	2800[a] (167)	3767	3444 ± 622	3167[b] (367)	4700	3536 ± 397	3567[b] (83)	4667	3696 ± 478	3633[b] (233)	4633	0.0005
Mg (3)	688 ± 90	700[a] (83)	833	1056 ± 217	933[c] (167)	1433	731 ± 83	717[a] (67)	900	950 ± 130	933[b] (67)	1267	< 10⁻⁴
Mn (3)	61.5 ± 27.6	52.7[a] (16.7)	126.5	77.4 ± 25.8	75.0[a] (18.7)	123.3	64.9 ± 21.4	63.0[a] (18.7)	105.0	62.1 ± 50.4	38.3[a] (16.7)	178.0	0.1244
Nd (3)	0.443 ± 0.203	0.400[a] (0.067)	0.850	1.048 ± 1.685	0.533[ab] (0.100)	5.533	0.629 ± 0.252	0.583[ab] (0.083)	1.233	1.122 ± 0.365	1.067[b] (0.300)	1.800	< 10⁻⁴
Ni (3)	1.17 ± 0.63	0.97[a] (0.11)	2.67	4.46 ± 2.34	4.57[b] (1.47)	9.17	1.83 ± 0.54	1.70[a] (0.27)	2.90	1.77 ± 0.34	1.80[a] (0.17)	2.57	< 10⁻⁴

Table 2 (continued)

Element (group)	Cluster I (Eastern Alps, n = 12)			Cluster II (Western Alps, n = 11)			Cluster III (Central Alps, n = 13)			Cluster IV (Apennines, n = 18)			K-W p value
	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	Mean ± SD	Median (MAD)	98th %ile	
P (3)	416 ± 88	427[a] (50)	580	587 ± 255	543[ab] (180)	1077	526 ± 106	495[ab] (53)	717	670 ± 209	620[b] (113)	1233	*0.0005*
Sr (3)	14.3 ± 7.5	12.7[a] (4.2)	36.3	12 ± 2.9	11.3[a] (2.0)	16.3	12 ± 4	10.8[a] (2.0)	21.0	32.2 ± 14.4	27.3[b] (4.3)	75.0	*< 10⁻⁴*
Te (3)	0.079 ± 0.039	0.063[a] (0.013)	0.165	0.066 ± 0.019	0.060[a] (0.003)	0.103	0.076 ± 0.03	0.073[a] (0.018)	0.140	0.190 ± 0.101	0.153[b] (0.023)	0.483	*< 10⁻⁴*
Y (3)	6.55 ± 4.08	4.90[ab] (0.47)	18.97	5.02 ± 0.74	5.33[a] (0.60)	5.93	5.67 ± 1.54	5.50[a] (0.98)	8.33	7.90 ± 3.47	6.50[b] (1.70)	17.17	*0.0466*
Zr (3)	66.1 ± 26.5	62.6[a] (8.5)	128.9	57.1 ± 9.7	56.7[a] (7.4)	68.3	51.2 ± 16.1	49.0[a] (13.6)	76.7	55.4 ± 18.4	52.5[a] (7.8)	109.4	*0.2392*

BECs are reported for sites with similar lichen element content (site clusters according to CA results; Figs. 2a and 3). Data refer to mean ± standard deviation, median, median absolute deviation (MAD), and 98th percentile for 43 elements listed according to their group (according to CA results; Figs. 2b and 3). Between-groups differences (Kruskal-Wallis ANOVA; K-W, and significant p values in italic) are reported for each element. Different letters above the median values indicate significantly different groups within each row (Dunn's post hoc test at $p < 0.05$)

Sondrio) and Adige Valley (provinces of Trento and Bolzano), with the highest content of some elements of anthropogenic origin (e.g., Zn; Li et al. 2017), long-range transported (i.e., As, Pb; Bargagli 1998), or occurring in several mineralogical assemblages of metamorphic substrates (i.e., Cu, Mo, Rb, and Sb; Salminen et al. 2005; Kuleshov 2016). Interestingly, the metamorphic substrates of site clusters II and III were substantially different, with sites of cluster II mostly laying over metamorphosed peridotite and gneiss, and in proximity of serpentinized ultramafic rocks (likely explaining the high contents of Ni and Mg; Aziz et al. 2015), and those of cluster III over phyllite and micaschists. Besides highlighting finer lithological differences, such pattern also reflects differences in anthropization in the Alpine ranges. Indeed, the BEC revealed in the three clusters well matches with differences in population density in the surroundings of the study sites, when calculated from national census data within circular buffers of 25-km radius centred in the sampling sites, as described in Cecconi et al. (2018). As a matter of fact, population density showed utmost between-cluster differences, with a minimum in cluster I (24.2 ± 10.0 inhabitants km^{-2}), a maximum in cluster III (95.9 ± 21.2 inhabitants km^{-2}), and intermediate values in cluster II (67.2 ± 16.4 inhabitants km^{-2}), also reflecting the impact of relevant winter tourism in the surveyed areas.

The limited discrepancies between the two sets of BEC values can be explained by the interplay of several factors, including the acid digestion and different sets of biological replicates and chemical elements analyzed. However, despite the above-mentioned local differences, the overall large-scale pattern emerged from the multivariate analysis of totally digested samples definitively confirmed the robustness of the BEC assessment of Cecconi et al. (2018), based on extensive, purposely devoted field sampling.

Conclusions

The selection of a specific acid digestion to be applied in a biomonitoring survey can be a thorny issue, which should not be underrated, as possibly leading to under-/overestimation of the content of several elements. As such, this analytical step should always be taken into account as a possible source of bias in the interpretation of bioaccumulation results, especially for elements of particular environmental concern, such as As, Ba, Cd,

Cu, Ni, and Zn. Depending on the specific aim of the biomonitoring survey and the target set of chemical elements, different mineralizations may produce satisfactory recoveries. The acid digestion with HF was proved to produce more accurate results for the majority of tested elements, especially for Al, Ba, Cu, Fe, Mn, and Zn. In this light, when addressing these elements in biomonitoring applications, a partial digestion should be disregarded in favor of a digestion with HF. Moreover, when a specific digestion method is adopted in routine biomonitoring studies, this should be clearly stated, and accuracy results should be fully provided, in order to allow easier and more robust cross-study comparisons.

Finally, the background levels were explored for 43 elements after total mineralization of samples of *P. furfuracea*. BEC patterns assessed at very large scale, and based on a large amount of field samples, they were proved to be rather conservative. The context-dependency of national BEC values was confirmed by identifying geographically separated site clusters for which further BEC values are now available. In this way, a major methodological gap in biomonitoring procedures was filled by providing a complete set of BEC values based on different acid digestions and one of the most performing analytical techniques for multi-element determination, to be alternatively used as a reference according to the selected mineralization procedure.

Compliance with ethical standards

Conflict of interest   The authors declare that there is no conflict of interest.

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