JOINT INSTITUTE
FOR NUCLEAR RESEARCH

BioMAP
Biomonitoring of Atmospheric Pollution

BACKGROUND ELEMENT CONTENT OF THE LICHEN *PSEUDEVERNIA FURFURACEA*: A COMPREHENSIVE OVERVIEW. FROM THE SUPRANATIONAL STATE OF ART TO A NEW METHODOLOGICAL FRAMEWORK FOR THE ASSESSMENT OF REGIONAL BENCHMARKS

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Società Lichenologica Italiana

Background

BEC values: **Background Element Content** values

Key issue of **bioaccumulation studies:**
interpretation of pollutant contents in terms of deviation from a pre-existing, "unaltered" condition.

Among possible approaches to quantitatively assess such deviation:

→ 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).

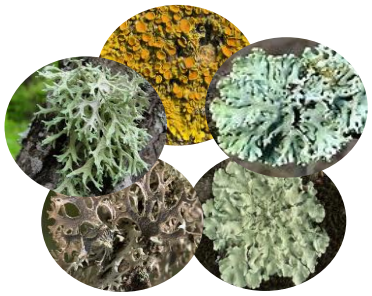
"*The **knowledge of background content** of persistent chemicals is a **fundamental pre-requisite for the correct evaluation of pollution phenomena***" (Reimann and Garrett, 2005).

Background

BEC values: **Background Element Content** values

Essential pre-requisite for the correct assessment of pollution levels

Previous BEC values for lichens…

	Bibliographic survey	Field sampling
Single species	Bennet, 2000 (*Hypogymnia physodes*)  	Bargagli et al., 1999 (*Umbilicaria decussata*, Antarctica) Monaci et al. 2012 (*Nephroma sp., Usnea* sp., Patagonia)  
Species pools	Bargagli, 1998  	Bergamaschi et al., 2004 (various mountain areas)  

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	Bibliographic survey	Field sampling
Single species	Bennet, 2000 (*Hypogymnia physodes*) Methodological different studies	Bargagli et al., 1999 (*Umbilicaria decussata*, Antarctica) Monaci et al. 2012 (*Nephroma sp., Usnea* sp., Patagonia) • Species not used in common biomonitoring application • Fairly low number of sites
Species pools	Bargagli, 1998	Bergamaschi et al., 2004 (various mountain areas)

Different species can have different bioaccumulation performance

Background

BEC: **Background Element Content** values

Essential pre-requisite for the correct assessment of pollution levels

Overcoming issues… A combined approach:

Background

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Overcoming issues… A combined approach:

- ✓ Targeting a single lichen species;

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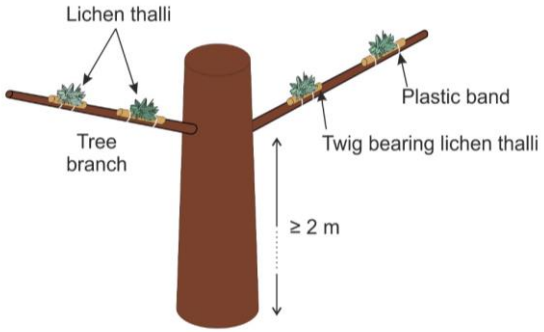
✓ Targeting a single lichen species;

***Pseudevernia furfuracea* (L.) Zopf**

- Widely used in biomonitoring application, especially in ***transplants***.
- Widely distributed (in Italy: mountain environments).
- Targeted in studies addressing methodological aspects in biomonitoring.
- Lichen standard reference material for multi-element determination (CRM 482; Quevauviller et al., 1996).



Certified reference material of lichen (CRM 482) for the quality control of trace element biomonitoring

Ph. Quevauviller*[a], R. Herzig[b], H. Muntau[c]

Background

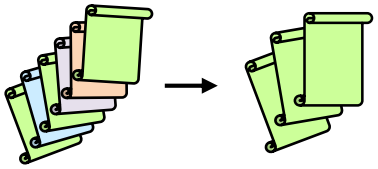
BEC: **Background Element Content** values

Essential pre-requisite for the correct assessment of pollution levels

Overcoming issues… A combined approach:

- ✓ Targeting a single lichen species;

- ✓ Literature review + methodological «filter»:
  - Single species;
  - Sample pre-processing/processing.



- ✓ Lichen material collection from ad hoc sampling campaign:

  Shared protocols, from the collection to the analytical determination.

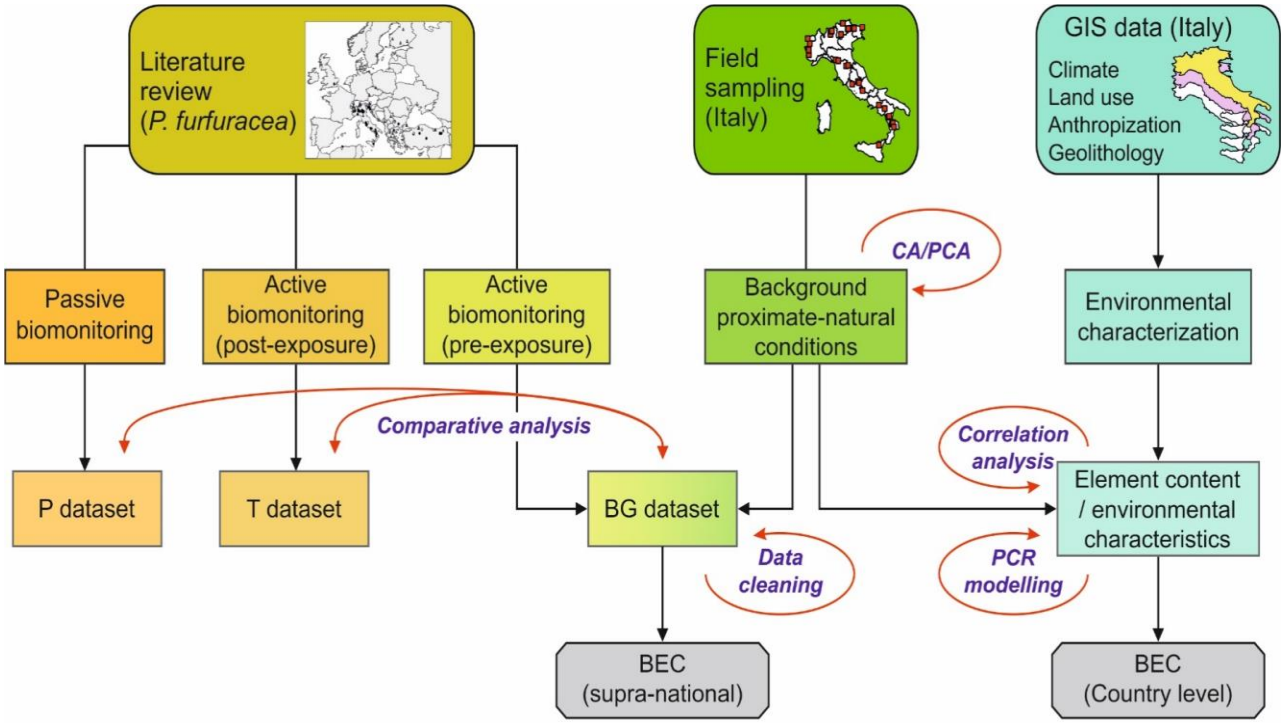
Objectives

1a To assess methodological differences among biomonitoring studies targeting *Pseudevernia furfuracea* (literature survey).

1b To provide preliminary broad reference on BEC for biomonitoring application at supra-national scale.

2a To explore BEC pattern at national level, in relation to environmental variables, assessed by a GIS-based environmental characterization of the sampling sites.

2b To test the predictivity of target environmental descriptors on BEC, using multiple regressive modelling.



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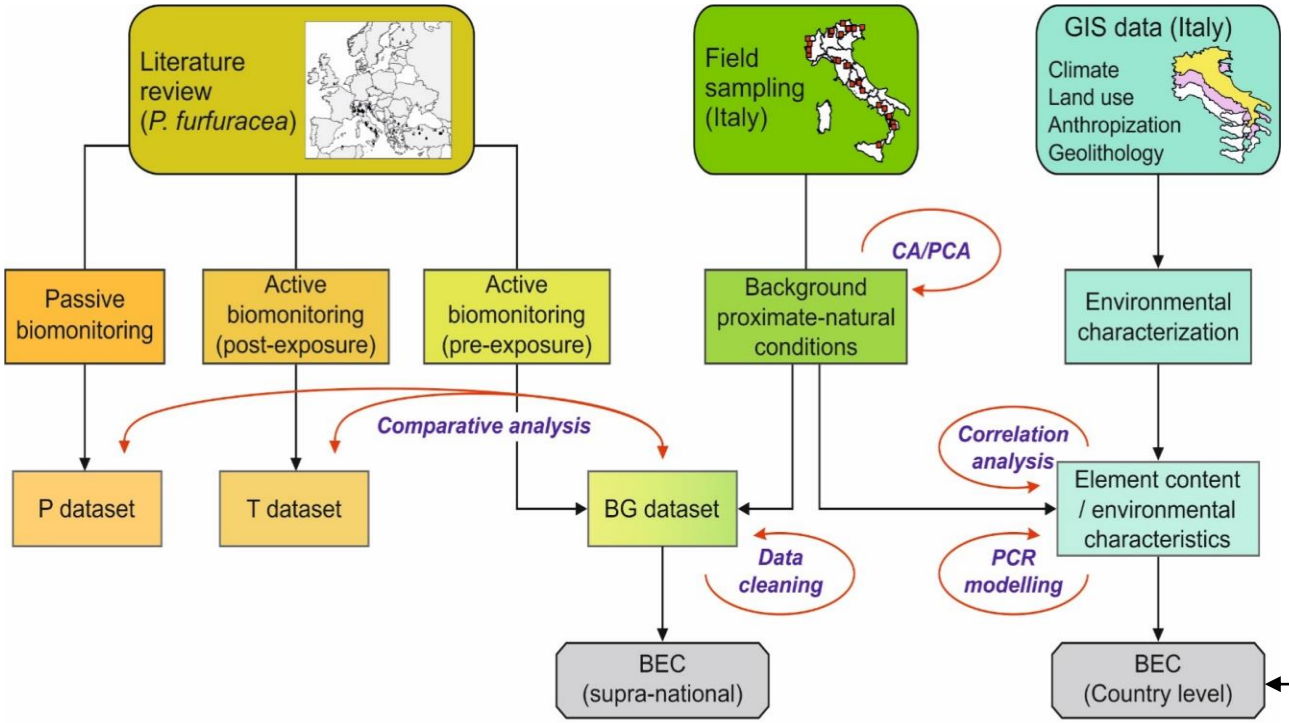
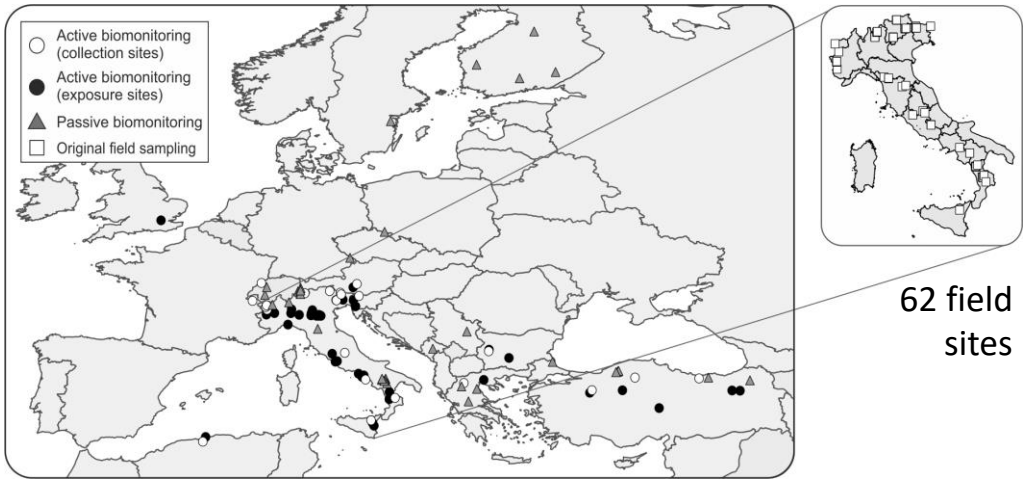


c To produce 2 sets of BECs based on different acid sample digestion.

Partial digestion

Total digestion

1a. Literature survey - *Results*



62 field sites

62 papers, 16 countries

Active biomonitoring: 69%; Passive biomonitoring: 29%

Acid mixtures for sample digestion

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%)

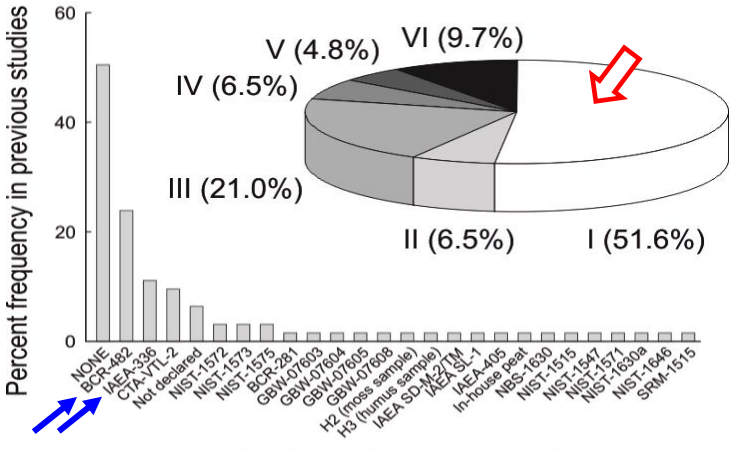
- HNO_3, H_2O_2, HF
- HNO_3, HCl, HF
- HNO_3, HF

Not reported (31%)

QC procedures (Certified Reference Materials)

Detail level Provided

I **Not reported (51.6%)** ←
II (6.5%) CRM?
III (21.0%) CRM, but NO recovery %
IV (6.5%) CRM, "OK" recovery %
V (4.8%) CRM, range %
VI (9.7%) CRM, metrics for each element



Lichen materials:

BCR 482 '*Pseudevernia furfuracea*' (Quevauviller et al., 1996)

IAEA-336 '*Evernia prunastri*' (Stone et al., 1995; Schmeling et al., 2007)

1b. Preliminary supranational BEC - *Methods*

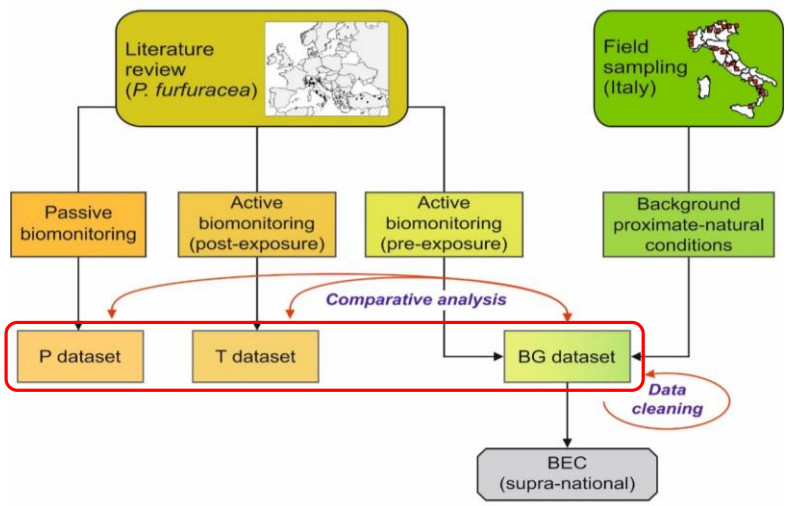
Data cleaning

BG dataset

Field data + pre-exposure control data from active biomonitoring studies

Subjected to **data cleaning:** removal of…

- Methodologically conflicting records
- Extreme outliers for each element (Tukey's method);
- Elements with low sample size (n < 30).



1b. Preliminary supranational BEC - *Methods*

Dataset comparison

BG dataset

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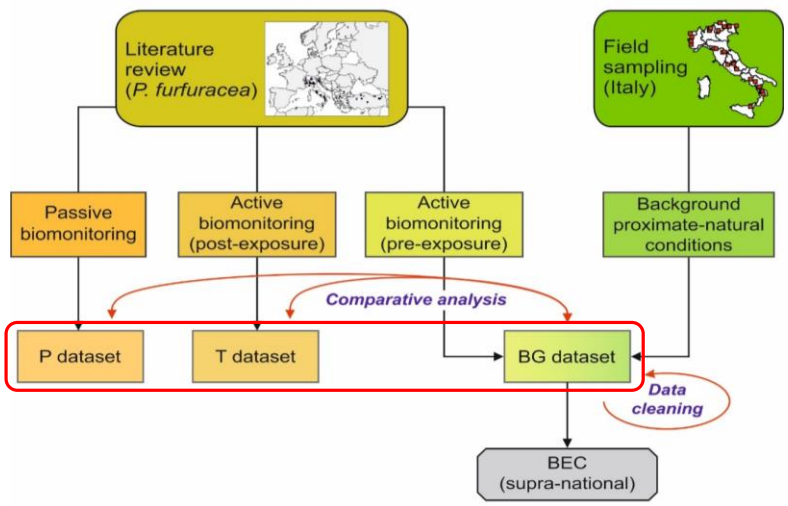


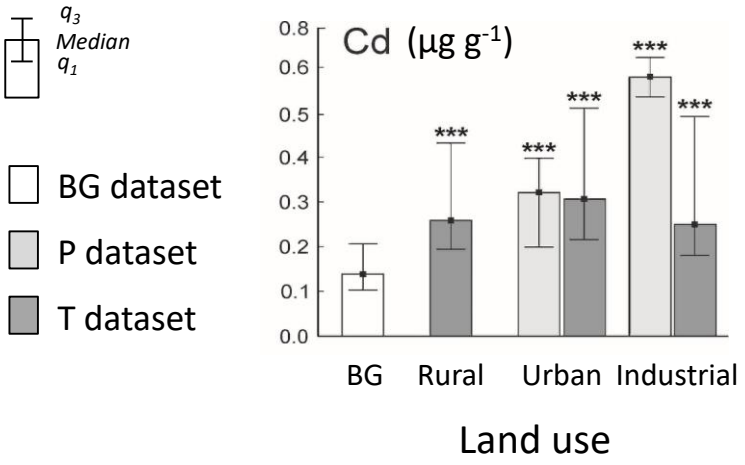
Comparison datasets:

P dataset

Data from **P**assive biomonitoring studies referring to autochthonous thalli.

T dataset

Data of **T**ransplanted samples from active biomonitoring studies exposed in polluted areas.



→ **Preliminary supranational BEC reference for 25 elements**

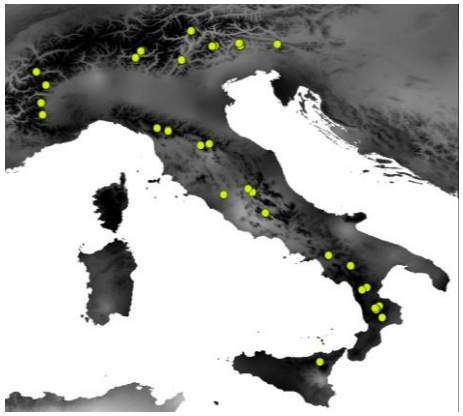
2a. BEC pattern at national level - *Methods*

Lichen element composition in remote areas reflects local environmental conditions

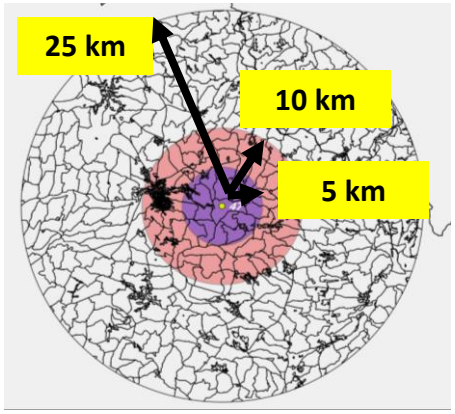
- among controlling factors: meteoclimate (Garty 2001), lithology (Agnan et al. 2014, 2015) -

GIS environmental characterization of the 62 Italian collection sites

Climate Bioclim



Anthropization - census units



Population density (inhabitants km^{-2}) $\rho = \frac{\sum_i c_i n_i}{A}$

c_i: relative census unit area included in the buffer, ci ϵ (0, 1]

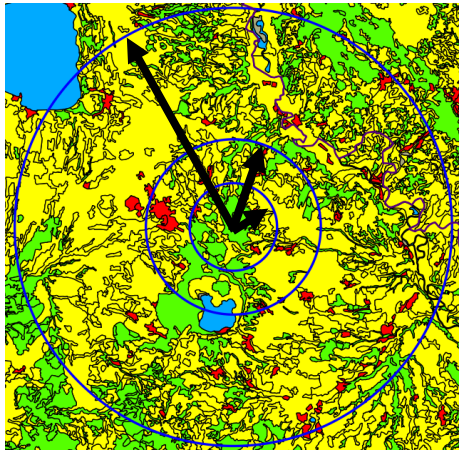
n_i: inhabitants of the ith census unit in the buffer

A: area of the buffer

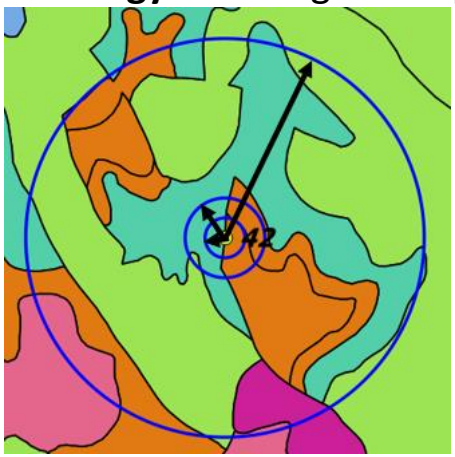
Built-up area cover for:

(1) residential, (2) productive, (3) scattered buildings

Land use CLC 2012



Lithology - lithological maps

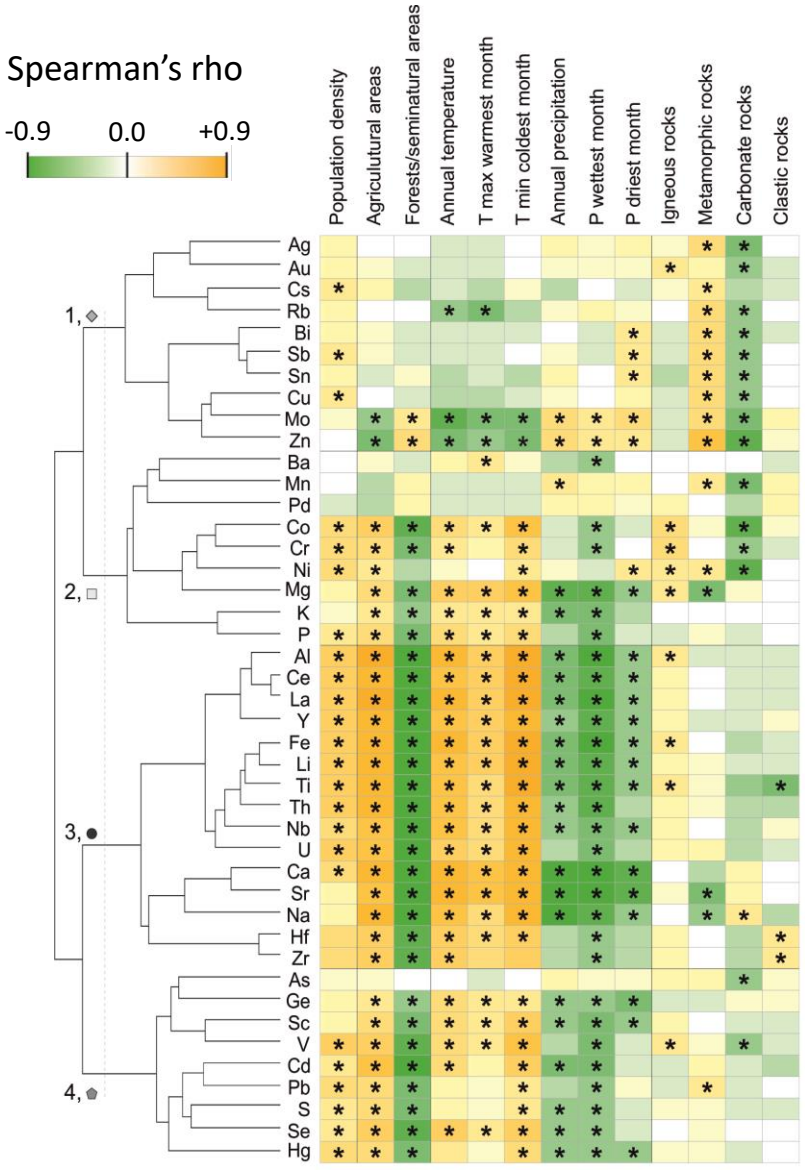


First macrocategory assessment:

1. Metamorphic rocks
2. Igneous rocks
3. Sedimentary carbonate rocks
4. Sedimentary clastic rocks

2a. BEC pattern at national level - *Methods*

Correlation analysis



Anthropization (buffer radius 25 km)

1. Population density (km^{-2})

Land use (buffer radius 25 km)

2. Agricultural areas (% coverage)
3. Forests and seminatural areas (%)

Climate (grid cell 1 km)

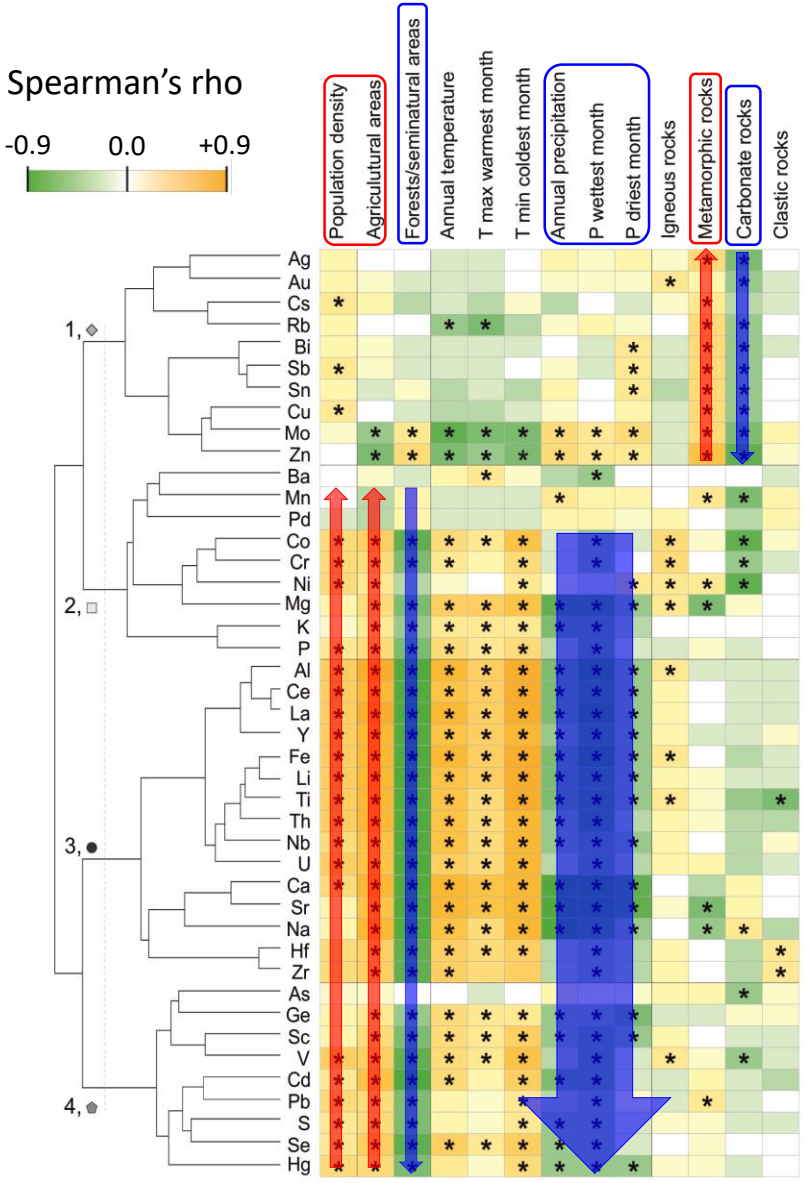
4. Annual temperature (°C)
5. Min T of coldest month (°C)
6. Max T of warmest month (°C)
7. Annual P (mm)
8. P of wettest month (mm)
9. P of driest month (mm)

Lithology (buffer radius 0.5 km)

10. Igneous rocks (% coverage)
11. Metamorphic rocks (%)
12. Carbonate rocks (%)
13. Clastic rocks (%)

2a. BEC pattern at national level - *Methods*

Correlation analysis



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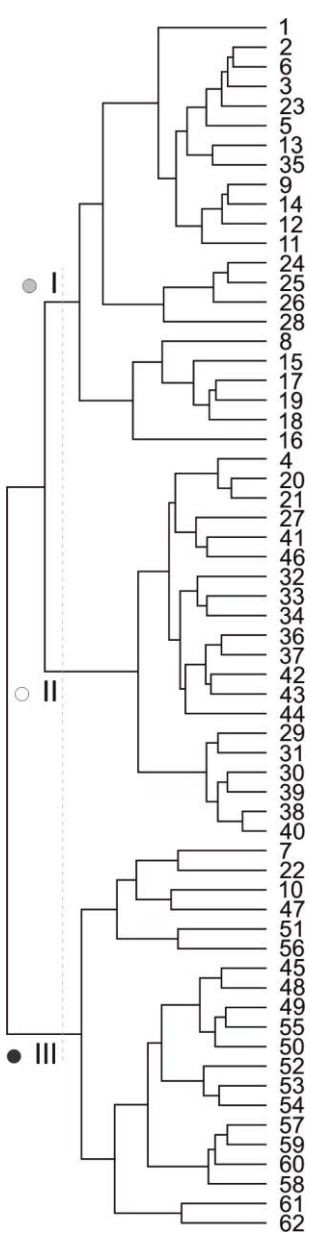

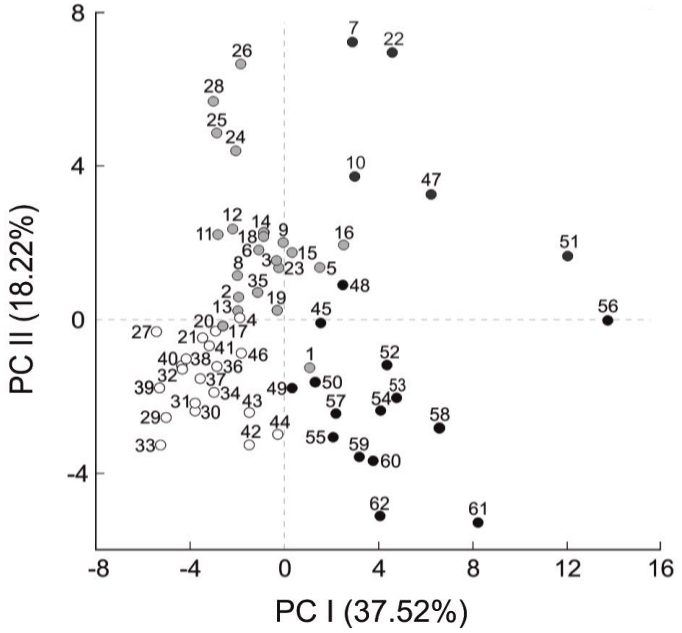

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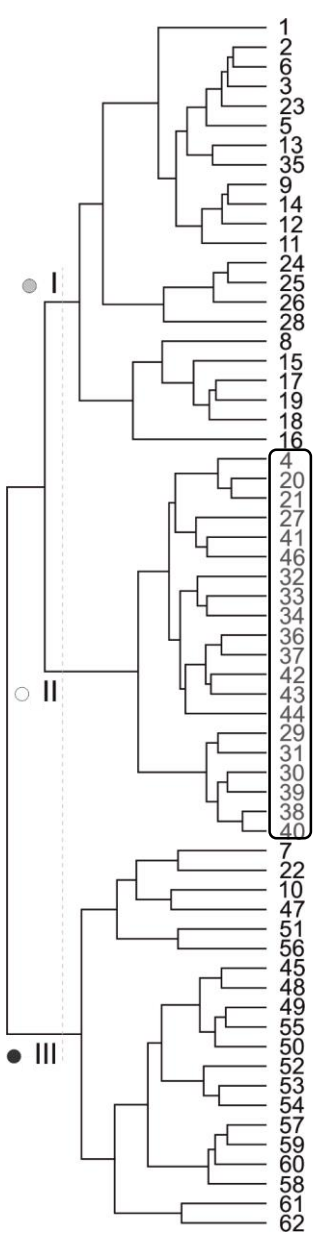

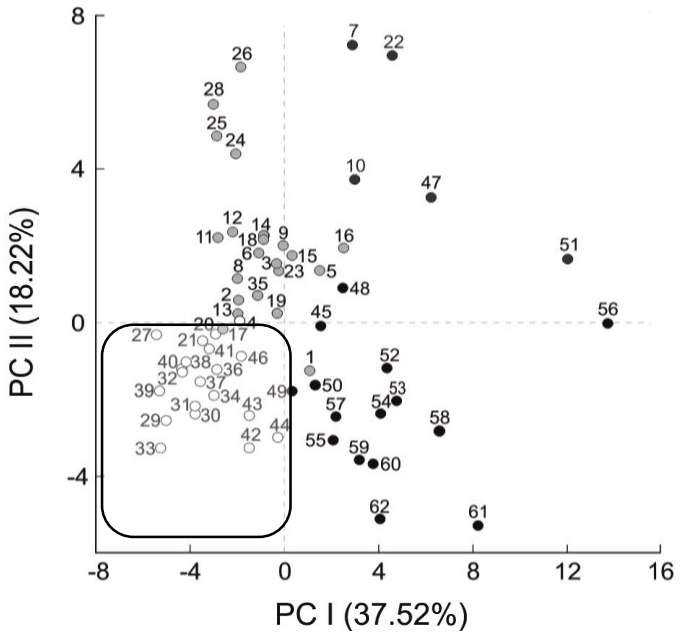

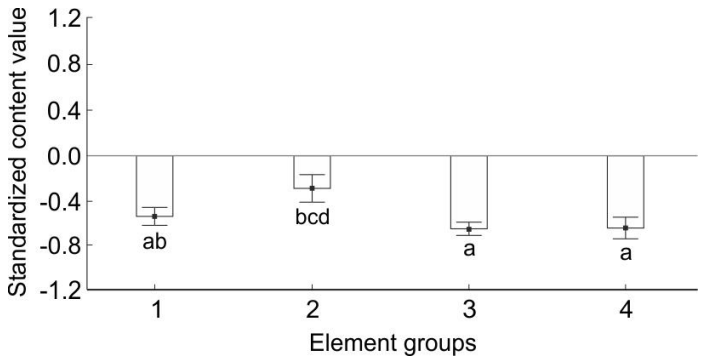

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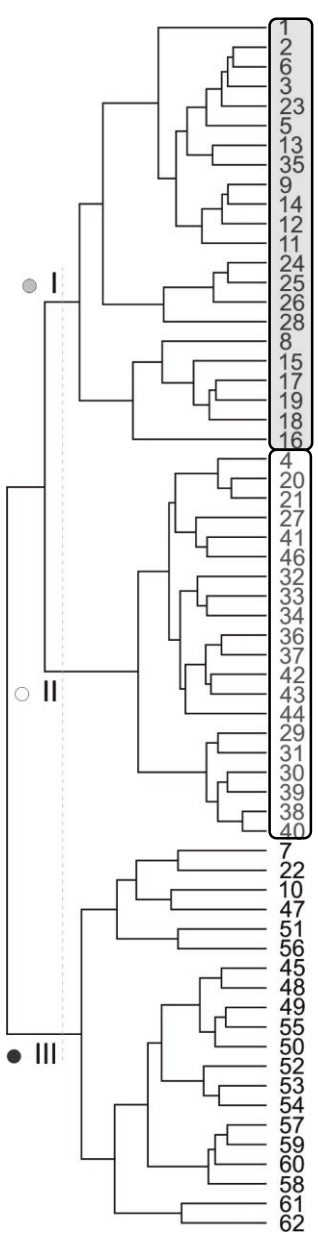

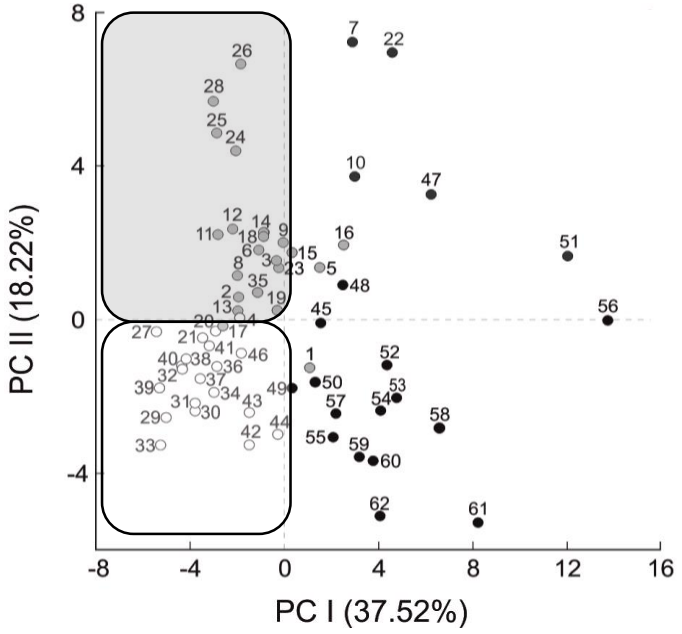

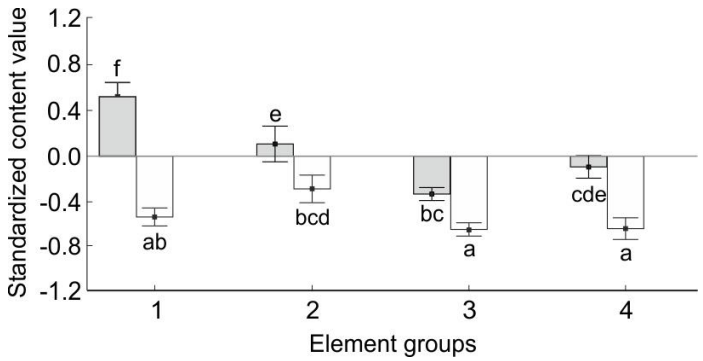

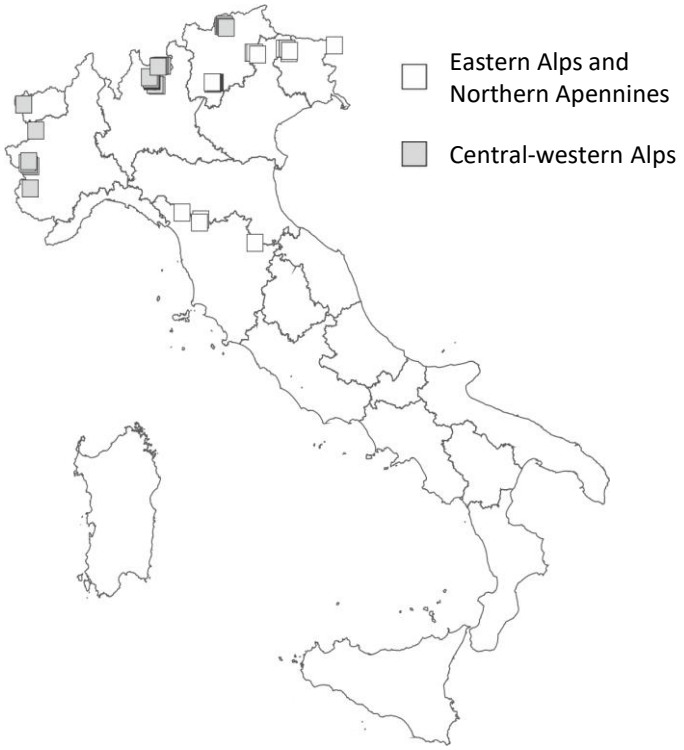

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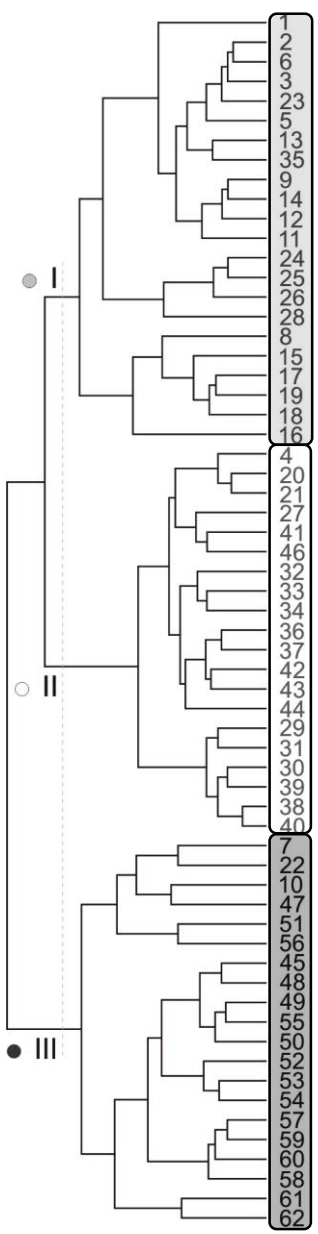
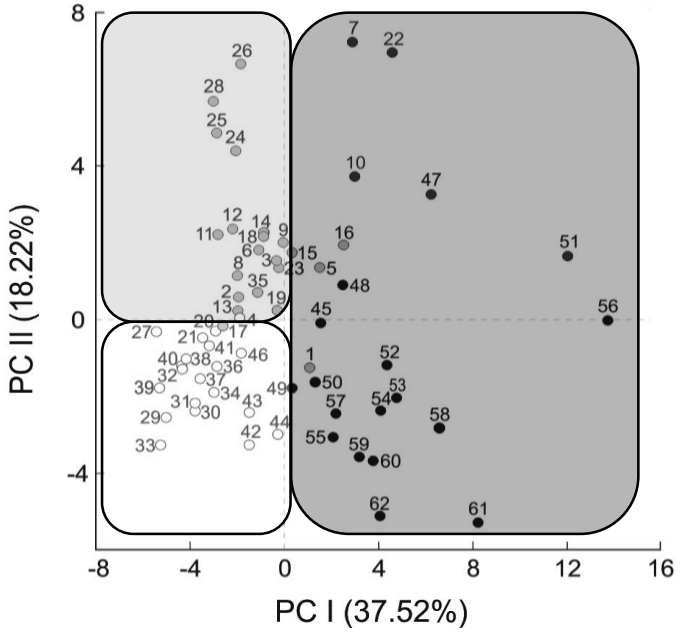
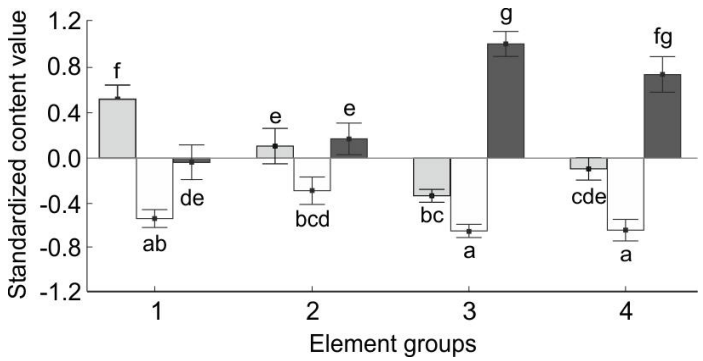
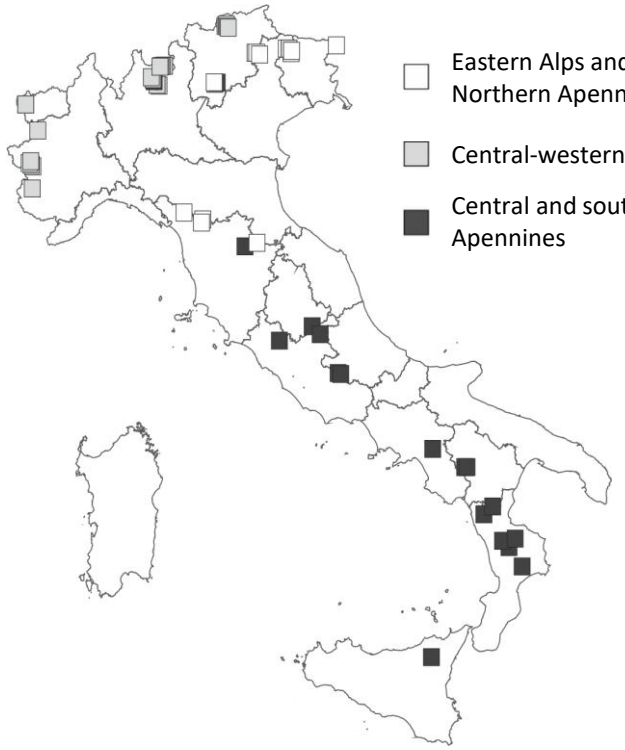






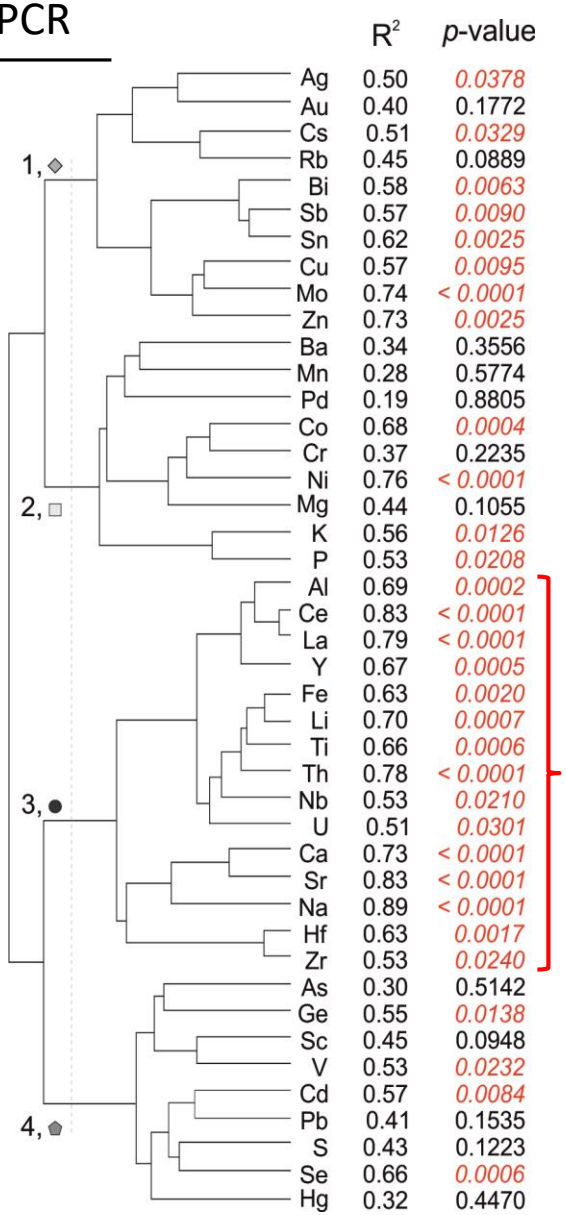


National BECs: (43 elements)

	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	MAD	98th %ile	Mean ± SD	Median	MAD	98th %ile	Mean ± SD	Median	MAD	98th %ile	p-Valu
Ag	0.025 ± 0.008	0.023[b]	0.003	0.052	0.016 ± 0.007	0.014[a]	0.002	0.040	0.020 ± 0.006	0.020[ab]	0.003	0.003	*0.0001*
Al	359 ± 71	366[a]	58	500	310 ± 78	300[a]	48	500	758 ± 214	640[b]	120	1200	$<10^{-4}$
As	0.221 ± 0.081	0.207[b]	0.042	0.408	0.143 ± 0.072	0.113[a]	0.023	0.320	0.220 ± 0.091	0.220[b]	0.060	0.450	*0.0015*

2b. Predictivity of environmental descriptors on BEC - *Results*

PCR

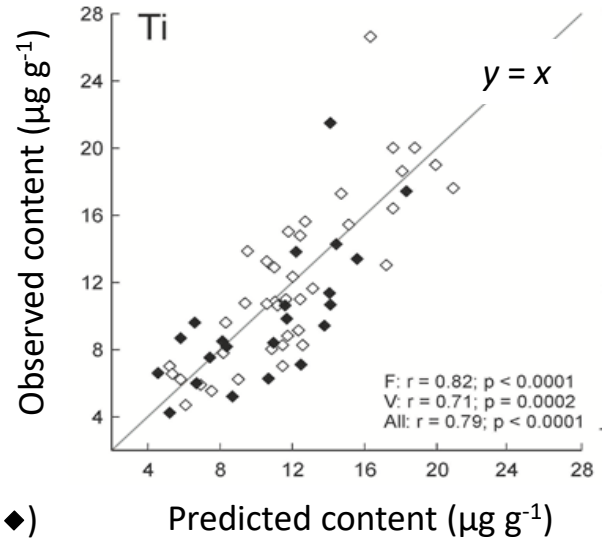


Predictivity of target environmental descriptors on BEC was tested by Principal Component Regression (PCR; Jolliffe, 2002).

Linear combinations of environmental predictors significantly associated with lichen BEC (31 out of 43 elements).

PCR models fitted on data from 40 randomly selected sites (Fitting dataset), and tested on data from remaining 22 sites (Validation dataset).

PCR models significantly predictive for the validation datasets for most elements of group 3.



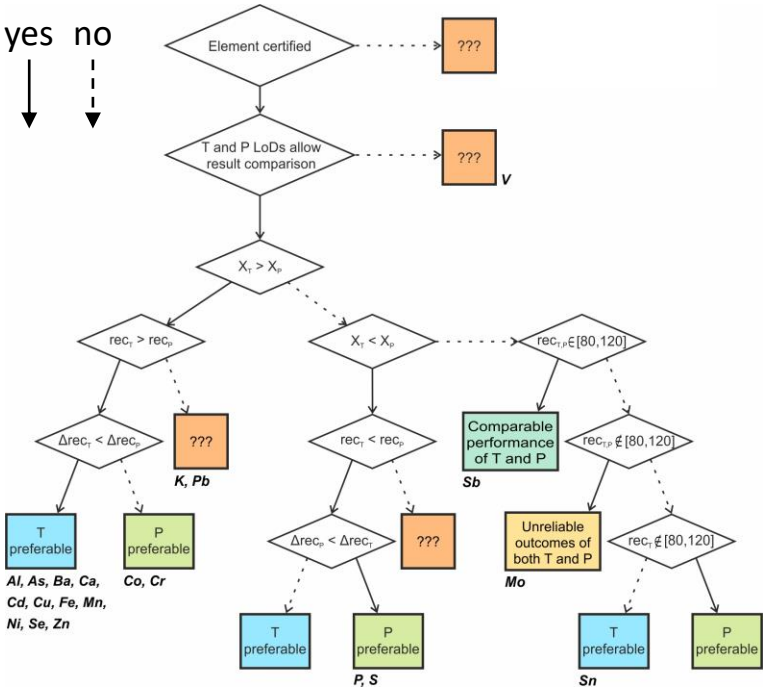
F: fitting dataset (◇)
V: validation dataset (◆)

c. Comparison of digestion performance - *Results*

Acid sample digestion: integral part of most multi-element measurement techniques (Gaudino et al., 2007).

The effects of different mineralization protocols were addressed by methodological studies of environmental chemistry targeting several biological matrices (Rodushkin et al., 1999; Tuncel et al., 2004; Rashid et al., 2016).

Previous results on *P. furfuracea* BECs ← partial (i.e, *aqua regia*) sample digestion;
The same pipeline for BEC assessment was followed after a total (HF-based) digestion of paired samples replicates.



Procedure used to assess the relative performance of total (T) and partial (P) digestion methods:

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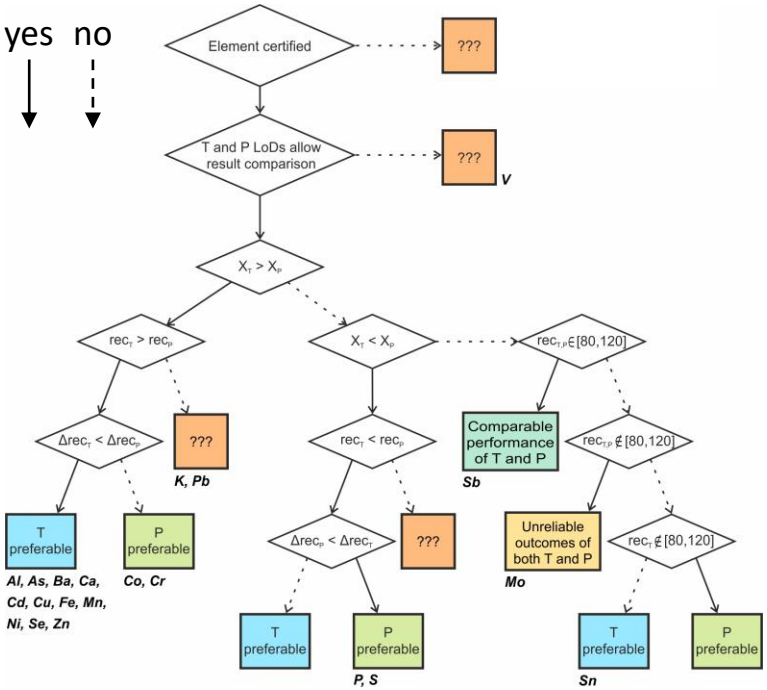

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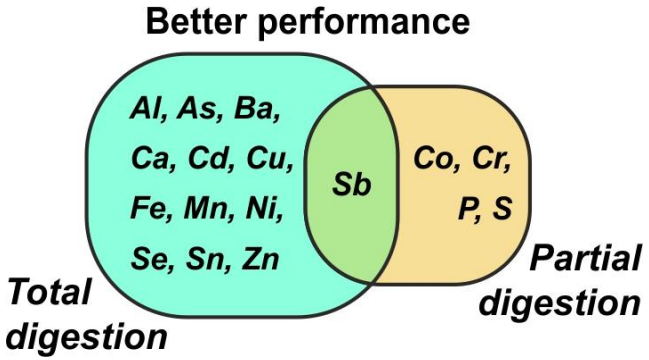

c. Comparison of BECs obtained with two digestion methods - *Results*

Total digestion (HF-based)

Partial digestion (*aqua regia*)

Few differences, mostly at the Alpine level, but rather conservative BEC pattern

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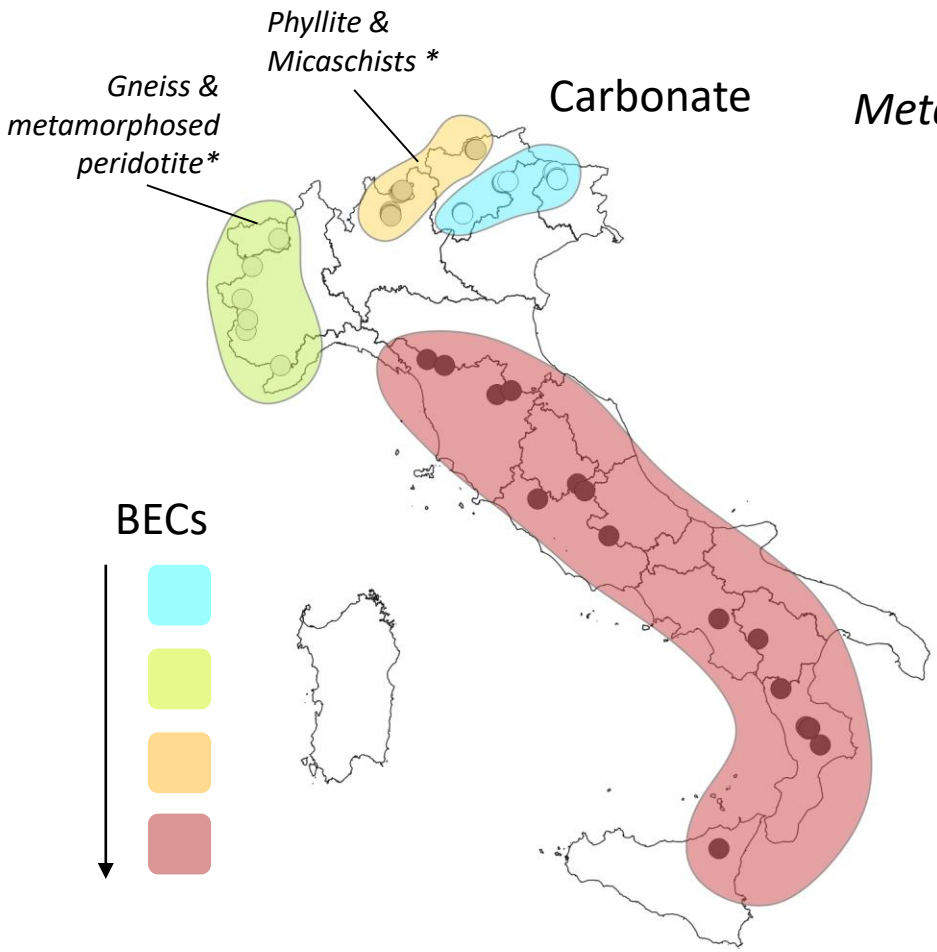
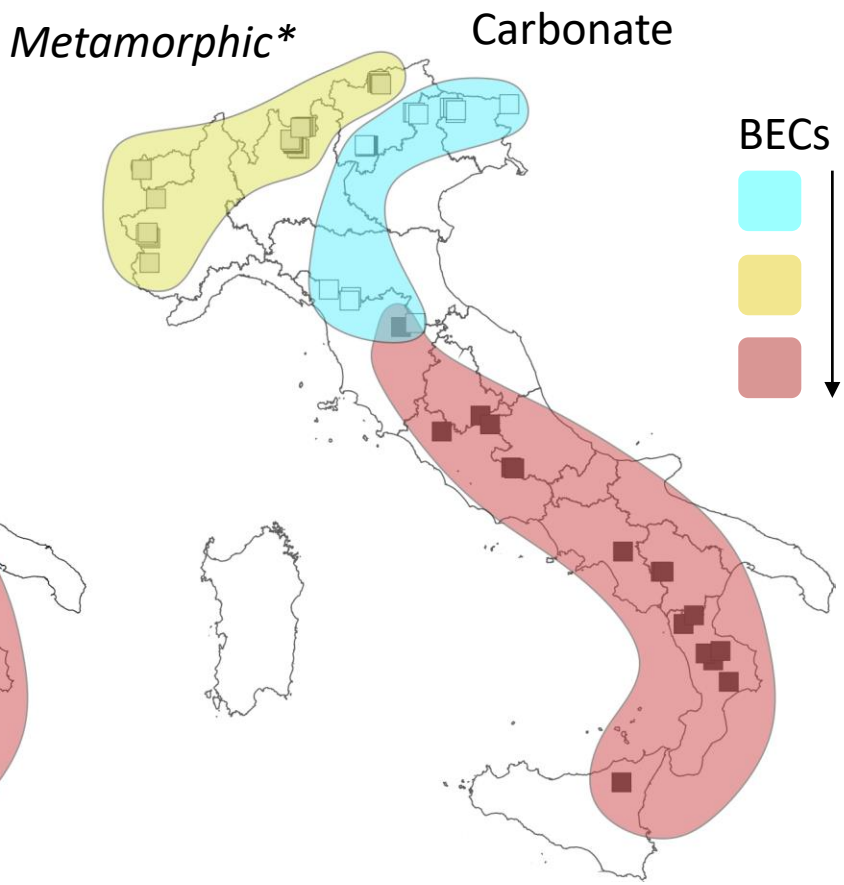
Total digestion (HF-based)



Partial digestion (*aqua regia*)

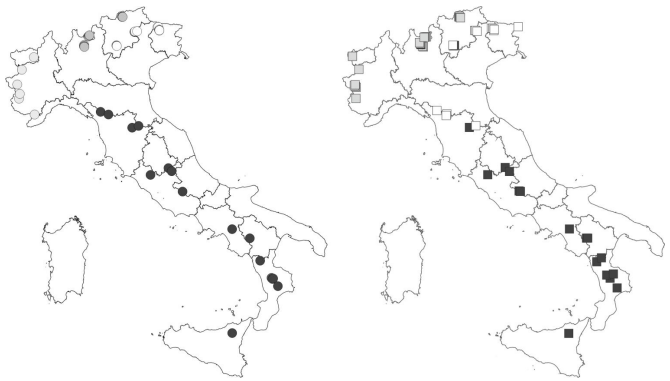


Few differences, mostly at the Alpine level, but rather conservative BEC pattern

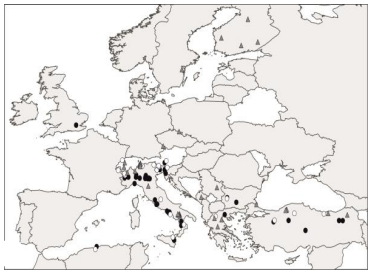
Conclusions

Framework for the assessment of regional BEC values →

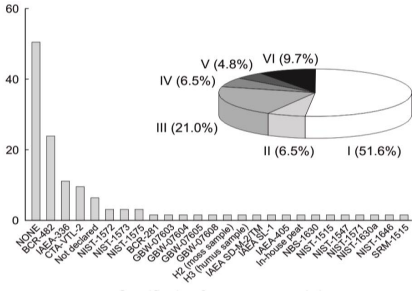
P. furfuracea BECs (43 elements) *for* ***homogeneous, geographically separated contexts in Italy,*** *based on* ***partial*** *and* ***total sample digestions*** *to be used as a* ***reference for biomonitoring application***.



Deficit of methodological uniformity among biomonitoring studies with *P. furfuracea*: *sample pre-processing*, *digestion*, *QA/QC assessment.*



Element	n	Mean ± SD	Median	IQR
1 Al	81	457 ± 236	380	300 ÷ 535
2 As	63	0.205 ± 0.096	0.180	0.130 ÷ 0.270
3 Ba	63	12.0 ± 5.5	11.0	8.1 ÷ 13.6
4 Ca	74	7615 ± 4092	6185	4680 ÷ 10000
5 Cd	87	0.183 ± 0.088	0.160	0.120 ÷ 0.240
6 Co	65	0.255 ± 0.094	0.240	0.170 ÷ 0.310
7 Cr	80	2.73 ± 0.77	2.69	2.43 ÷ 3.12
8 Cu	91	5.40 ± 2.09	4.99	3.78 ÷ 6.63
9 Fe	79	516 ± 251	480	348 ÷ 620
10 Hg	74	0.199 ± 0.059	0.180	0.160 ÷ 0.250
11 K	74	3305 ± 616	3258	2867 ÷ 3740



Need for shared standard procedures.

Thank you for your attention

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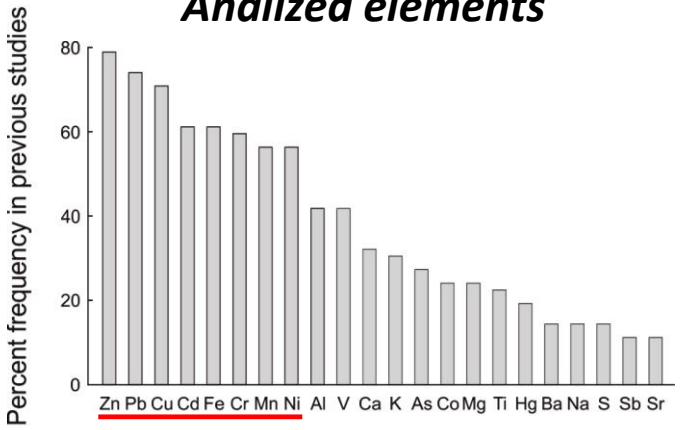
1a. Literature survey - *Results*

Analized elements



Analytical technique for element content determination

- Not reported (4.8%)
- Atomic absorption spectrometry: AAS (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%)
- Neutron activation analysis: NAA (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%)

Sample pre-processing

- Not reported (3%)
- Debris removal (57%)
- Washing (21%)
- Oven-drying (11%)
- Other (8%)

Acid mixtures for sample digestion

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

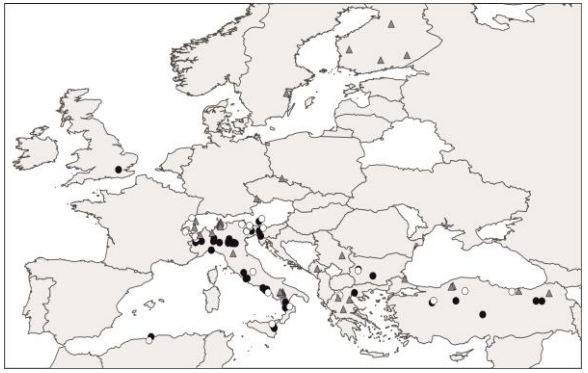
Not reported (31%)

1b. Preliminary supranational BEC - *Results*

25 elements (μg g^{-1})

	BG				
	n	Mean ± SD	Median	MAD	IQR
Al	81	457 ± 236	380	90	300–535
As	63	0.205 ± 0.096	0.180	0.068	0.130–0.270
Ba	63	12.0 ± 5.5	11.0	2.7	8.1–13.6
Ca	74	7615 ± 4092	6185	2416	4680–10,000
Cd	87	0.183 ± 0.088	0.160	0.050	0.120–0.240
Co	65	0.255 ± 0.094	0.240	0.070	0.170–0.310
Cr	80	2.73 ± 0.77	2.69	0.36	2.43–3.12
Cu	91	5.40 ± 2.09	4.99	1.25	3.78–6.63
Fe	79	516 ± 251	480	132	348–620
Hg	74	0.199 ± 0.059	0.180	0.043	0.160–0.250
K	74	3305 ± 616	3258	442	2867–3740
Mg	72	766 ± 171	725	96	642–847
Mn	90	56.5 ± 30.8	50.4	18.5	34.2–74.3
Mo	65	0.249 ± 0.143	0.200	0.082	0.130–0.340
Na	73	77.3 ± 67.4	40.0	16.0	30.0–134.0
Ni	87	1.72 ± 0.90	1.42	0.51	1.03–2.18
Pb	85	4.46 ± 2.94	3.44	1.36	2.38–5.51
S	65	1534 ± 237	1540	140	1371–1650
Sb	63	0.093 ± 0.052	0.083	0.031	0.054–0.118
Se	59	0.276 ± 0.095	0.270	0.051	0.220–0.300
Sn	62	0.335 ± 0.166	0.300	0.098	0.210–0.410
Ti	64	11.1 ± 4.7	10.6	3.2	7.5–13.8
U	63	0.022 ± 0.013	0.020	0.008	0.011–0.028
V	73	2.12 ± 0.47	1.96	0.13	1.90–2.20
Zn	92	41.4 ± 17.4	39.8	13.3	27.0–53.5

Overview of the BEC ranges for a single highly performing lichen biomonitor at supranational scale.



BG dataset is not exhaustively representative of the "European" BEC.

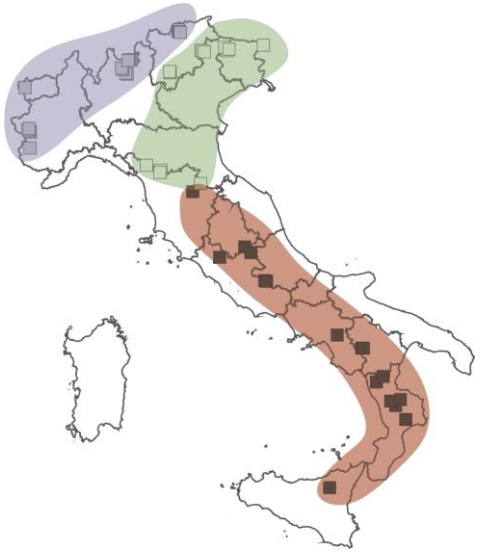
HOWEVER

It summarizes the current state of data availability at supranational scale;

It provides a novel methodological frame for the analysis of further biomonitoring data.

2a. BEC pattern at national level - *Results*

Environmental descriptors	Western Alps	Eastern Alps + northern Apennines	Central and Southern Apennines
Anthropization (km^{-2})			
Population density	68.2 ^{ab}	30.0 ^{a}	90.4 ^{b}
Land use (% coverage)			
Agricultural areas	10.2 ^{a}	6.8 ^{a}	32.7 ^{b}
Forests and seminatural areas	87.9 ^{b}	91.9 ^{b}	65.0 ^{a}
Climate			
Annual temperature (°C)	3.6 ^{a}	4.0 ^{a}	7.7 ^{b}
Min T of coldest month (°C)	-7.4 ^{a}	-8.7 ^{a}	-2.4 ^{b}
Max T of warmest month (°C)	16.3 ^{a}	17.9 ^{a}	20.5 ^{b}
Annual P (mm)	1003 ^{b}	915 ^{b}	833 ^{a}
P of wettest month (mm)	128 ^{b}	125 ^{b}	104 ^{a}
P of driest month (mm)	48 ^{b}	45 ^{b}	31 ^{a}
Geolithology (% coverage)			
Igneous rocks	0 ^{a}	0 ^{a}	10 ^{a}
Metamorphic rocks	89 ^{b}	0 ^{a}	20 ^{ab}
Sedimentary carbonate rocks	0 ^{a}	100 ^{b}	80 ^{b}
Sedimentary clastic rocks	0 ^{a}	0 ^{a}	0 ^{a}



National BEC (N = 43 elements)

	Siliceous metamorphic western Alps			Sedimentary eastern Alps and northern Apennines			Central and southern Apennines			K-W Anova
	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}*