New interpretative scales for lichen bioaccumulation data: the Italian proposal

Elva Cecconi [1§], Lorenzo Fortuna [1§], Renato Benesperi [2], Elisabetta Bianchi [2], Giorgio Brunialti [3], Tania Contardo [4], Luca Di Nuzzo [2], Luisa Frati [3], Fabrizio Monaci [4], Silvana Munzi [5], Juri Nascimbene [6], Luca Paoli [7], Sonia Ravera [8], Andrea Vannini [4], Paolo Giordani [9*], Stefano Loppi [4*], Mauro Tretiach [1*]

[1] Department of Life Sciences, University of Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy; elva.cecconi@phd.units.it; lfortuna@units.it; tretiach@units.it
[2] Department of Biology, University of Firenze, Via La Pira 4, 50121 Firenze, Italy; renato.benesperi@unifi.it; e.bianchi@unifi.it; luca.dinuzzo@stud.unifi.it
[3] TerraData environmetrics, Spin Off of the University of Siena, Via L. Bardelloni 19, 58025 Monterotondo Marittimo (GR), Italy; brunialti@terradata.it; frati@terradata.it
[4] Department of Life Sciences, University of Siena, via Mattioli 4, 53100 Siena, Italy; tania.contardo2@unisi.it; fabrizio.monaci@unisi.it; andrea.vannini@unisi.it; stefano.loppi@unisi.it
[5] Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal; ssmunzi@fc.ul.pt
[6] Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; juri.nascimbene@unibo.it
[7] Department of Biology, University of Pisa, via L. Ghini, 13, 56126 Pisa, Italy; luca.paoli@unipi.it
[8] Department of Biosciences and Territory (DiBT), University of Molise, C.da Fonte Lappone, 86090 Pesche (Isernia), Italy; sonia.ravera@unimol.it
[9] DIFAR, University of Genova, Viale Cembrano 4, 16148 Genova, Italy; giordani@difar.unige.it

§ These authors contributed equally to this work
* Correspondence: giordani@difar.unige.it +39-010-3532645 (P.G.); stefano.loppi@unisi.it +39-0577-233740 (S.L.); tretiach@units.it, +39-040-5583885 (M.T.)

Supplementary material

METHODS S1

TABLES S1-S6

FIGURES S1-S3

METHODS S1

In order to assess whether *B* and *EU* ratios were substantially unaffected by inter-specific differences, their median values were tested for significant differences using Mann-Whitney's U test for independent samples. Median *B* ratios were tested using the column vector reported in Data S1.
Median *EU* ratios were tested before outlier removal (overall *EU* ratio data), and separately in each *EU ratio* sub-dataset after outlier removal (exposure time spans of 4, 8 and 12 weeks; Data S2-S4). Statistical significance was tested in all cases at $\alpha = 0.05$ (Table S1).

SUPPLEMENTARY TABLES

Table S1. Results of statistical testing (Mann-Whitney U test for independent samples) for differences between median values of *B* ratios (native lichens) in *Flavoparmelia caperata* and *Xanthoria parietina*, as well as between median values of *EU* ratios (lichen transplants) in *Evernia prunastri* (*Ep*) and *Pseudevernia furfuracea* (*Pf*) (either reported for the overall data or separately for each exposure time span). Data counts (n) are reported in brackets in the headers.

***B* ratio** (*Flavoparmelia caperata*, n = 3005; *Xanthoria parietina*, n = 768)		
U	**Z**	***p*-value**
1˙120˙389	1.245	0.213

***EU* ratio** (*Evernia prunastri*, n = 150; *Pseudevernia furfuracea*, n = 670)			
Exposure time span	**U**	**Z**	***p*-value**
Overall	49˙992	0.098	0.922
4 weeks (*Ep*, n = 77; *Pf*, n = 92)	3406	-0.428	0.669
8 weeks (*Ep*, n = 24; *Pf*, n = 306)	3164	1.128	0.259
12 weeks (*Ep*, n = 48; *Pf*, n = 240)	5406	0.671	0.502

Table S2. Lichen Background Element Concentration values (BECs, μg g^{-1} DW) reported by Bargagli [2], Bennett [9] and Cecconi et al. [13], with indication of target lichen species, reference areas and number of sampling sites (in brackets, when available). Data reported by Bargagli refer to element concentration ranges [2].

Element	**Bargagli** [1]	**Bennett** [1]		**Cecconi et al.** [2]			
	Foliose species	***Hypogymnia physodes***		***Pseudevernia furfuracea***			
	Multiple areas	**Multiple areas**		**Eastern Alps** (12)	**Central Alps** (13)	**Western Alps** (11)	**Apennines** (18)
Al	150 ÷ 300	448	(32)	688	1024	835	1600
As	0.7 ÷ 2.0	1.33	(7)	0.611	0.605	0.398	0.407
Cd	< 0.1 ÷ 0.3	0.562	(37)	0.159	0.204	0.155	0.251
Cr	1 ÷ 4	2.11	(33)	5.26	5.93	7.02	8.33
Cu	4 ÷ 10	5.96	(37)	4.5	7.36	4.79	4.95
Hg	< 0.1 ÷ 0.2	0.253	(15)	-	-	-	-
Ni	1 ÷ 3	1.72	(33)	1.17	1.83	4.46	1.77
Pb	1 ÷ 8	19.5	(39)	2.77	4.49	2.35	4.6
Ti	5 ÷ 35	26.4	(5)	43.4	60.2	55	106
V	< 1 ÷ 3	16.9	(8)	1.2	1.88	1.41	2.8
Zn	20 ÷ 90	73	(43)	38	64.4	40	31.4

[1] Review-based BECs.

[2] Field-assessed BECs.

Table S3. Percentile-based, 7-class, naturality/alteration scale for bioaccumulation data provided by Nimis and Bargagli [14] (the abbreviations in brackets are the same used in Table S5). Data refer to percentile thresholds, corresponding element concentration values (μg g^{-1} DW) for the 11 elements included in the *N* dataset after the methodological data filtering (Sect. 2.2 and 3.1), and the colours suggested by the authors [14] (data counts are reported in brackets below each element).

	Class (abbreviation)		Percentile	Al (626)	As (435)	Cd (626)	Cr (654)	Cu (656)	Hg (606)	Ni (655)	Pb (699)	Ti (138)	V (416)	Zn (699)	Color
1	Very high naturality	(V.h.n)	< 20th	< 350	< 0.2	< 0.2	< 1.2	< 7	< 0.07	< 1	< 4	< 13	< 0.63	< 30	Blue
2	High naturality	(H.n.)	20th - 50th	350-600	0.2-0.6	0.2-0.4	1.2-2.2	7-10	0.07-0.13	1-2	4-10	13-27	0.63-1.7	30-40	Dark green
3	Middle naturality	(M.n.)	50th - 75th	600-1000	0.6-1.2	0.4-0.8	2.2–4.0	10-15	0.13-0.20	2-3	10-25	27-70	1.7-3.1	40-65	Pale green
4	Low nat./alteration	(L.a.)	75th - 90th	1000-1600	1.2-1.9	0.8-1.4	4.0–6.0	15-25	0.20-0.29	3-5	25-55	70-97	3.1-5.1	65-94	Yellow
5	Middle alteration	(M.a.)	90th - 95th	1600-2500	1.9-2.4	1.4–2.0	6.0–9.0	25-34	0.29-0.42	5-6	55-80	97-113	5.1-6.7	94-115	Orange
6	High alteration	(H.a.)	95th - 98th	2500-3200	2.4–3.0	2.0-2.6	9.0–16.0	34-53	0.42-0.74	6-8	80-108	113-150	6.7-9.3	115-155	Red
7	Very high alteration	(V.h.a.)	> 98th	> 3200	> 3.0	> 2.6	>16.0	> 53	0.74	> 8	> 108	> 150	> 9.3	> 155	Crimson

Table S4. Accumulation/loss scale provided by Frati et al. [25] with 5 *EC* ratio classes (the abbreviations in brackets are the same used in Table S6).

Accumulation/loss (abbreviation)		***EC* ratio**
Severe loss	(S.l.)	0 – 0.25
Loss	(Loss)	0.25 – 0.75
Normal	(N.)	0.75 – 1.25
Accumulation	(Acc.)	1.25 – 1.75
Severe accumulation	(S.a.)	> 1.75

Table S5. Results of the comparative application of the naturality/alteration scales by Nimis and Bargagli [14] (nat./alt. class) and the bioaccumulation scale in Table 4 (B. class) to a real case study. Data refer to three elements (As, Cd, Cr) measured in samples of *Flavoparmelia caperata* and *Xanthoria parietina* collected in 40 sampling sites (Figure 3). Abbreviations for classes of naturality/alteration and bioaccumulation scales are reported in Table S3 and Table 4. The attribution of classes is based on absolute element concentration expressed in μg g^{-1} DW (Elem. conc.) for naturality/alteration scales, whereas it is based on the *B* ratio for the bioaccumulation scale.

Site	*Flavoparmelia caperata*												*Xanthoria parietina*											
	As				Cd				Cr				As				Cd				Cr			
	Elem. conc.	Nat./alt. class	*B* ratio	B. class	Elem. conc.	Nat./alt. class	*B* ratio	B. class	Elem. conc.	Nat./alt. class	*B* ratio	B. class	Elem. conc.	Nat./alt. class	*B* ratio	B. class	Elem. conc.	Nat./alt. class	*B* ratio	B. class	Elem. conc.	Nat./alt. class	*B* ratio	B. class
A4	0.23	2 (H.n.)	1.65	2 (L)	0.11	1 (V.h.n.)	0.81	1 (A)	0.84	1 (V.h.n.)	0.99	1 (A)	0.85	3 (M.n.)	8.12	5 (S)	0.07	1 (V.h.n.)	1.24	2 (L)	1.40	2 (H.n.)	1.17	2 (L)
A6													0.28	2 (H.n.)	2.67	3 (M)	0.07	1 (V.h.n.)	1.31	2 (L)	1.10	1 (V.h.n.)	0.92	1 (A)
A7													0.20	2 (H.n.)	1.91	2 (L)	0.09	1 (V.h.n.)	1.72	2 (L)	0.86	1 (V.h.n.)	0.72	1 (A)
A8													0.22	2 (H.n.)	2.10	3 (M)	0.10	1 (V.h.n.)	1.92	2 (L)	1.41	2 (H.n.)	1.17	2 (L)
B3	0.27	2 (H.n.)	1.94	2 (L)	0.18	1 (V.h.n.)	1.33	2 (L)	1.61	2 (H.n.)	1.91	2 (L)	0.25	2 (H.n.)	2.34	3 (M)	0.12	1 (V.h.n.)	2.21	3 (M)	1.75	2 (H.n.)	1.46	2 (L)
B4													0.12	1 (V.h.n.)	1.15	2 (L)	0.05	1 (V.h.n.)	0.87	1 (A)	1.10	1 (V.h.n.)	0.92	1 (A)
B5													0.32	2 (H.n.)	3.06	3 (M)	0.22	2 (H.n.)	4.06	4 (H)	1.30	2 (H.n.)	1.08	2 (L)
B6													0.29	2 (H.n.)	2.77	3 (M)	0.12	1 (V.h.n.)	2.22	3 (M)	0.92	1 (V.h.n.)	0.77	1 (A)
C2													0.20	2 (H.n.)	1.91	2 (L)	0.07	1 (V.h.n.)	1.22	2 (L)	2.30	3 (M.n.)	1.92	2 (L)
C3	0.18	1 (V.h.n.)	1.29	2 (L)	0.17	1 (V.h.n.)	1.23	2 (L)	0.67	1 (V.h.n.)	0.79	1 (A)	0.29	2 (H.n.)	2.72	3 (M)	0.09	1 (V.h.n.)	1.62	2 (L)	1.45	2 (H.n.)	1.21	2 (L)
C4													0.24	2 (H.n.)	2.29	3 (M)	0.28	2 (H.n.)	5.17	5 (S)	1.60	2 (H.n.)	1.34	2 (L)
C5													0.21	2 (H.n.)	2.01	2 (L)	0.09	1 (V.h.n.)	1.64	2 (L)	0.97	1 (V.h.n.)	0.81	1 (A)
C6	0.31	2 (H.n.)	2.22	3 (M)	0.45	3 (M.n.)	3.32	3 (M)	2.20	3 (M.n.)	2.60	3 (M)	0.25	2 (H.n.)	2.39	3 (M)	0.64	3 (M.n.)	11.82	5 (S)	2.00	2 (H.n.)	1.67	2 (L)
C7	0.35	2 (H.n.)	2.51	3 (M)	0.24	2 (H.n.)	1.77	2 (L)	1.50	2 (H.n.)	1.77	2 (L)												
C8	0.27	2 (H.n.)	1.94	2 (L)	0.12	1 (V.h.n.)	0.89	1 (A)	1.10	1 (V.h.n.)	1.30	2 (L)												
D1	0.20	2 (H.n.)	1.43	2 (L)	0.18	1 (V.h.n.)	1.33	2 (L)	0.92	1 (V.h.n.)	1.09	2 (L)												
D2	0.21	2 (H.n.)	1.51	2 (L)	0.27	2 (H.n.)	1.96	2 (L)	0.77	1 (V.h.n.)	0.90	1 (A)												
D3	0.28	2 (H.n.)	2.01	2 (L)	0.23	2 (H.n.)	1.66	2 (L)	1.38	2 (H.n.)	1.63	2 (L)												
D4	0.16	1 (V.h.n.)	1.15	2 (L)	0.11	1 (V.h.n.)	0.81	1 (A)	1.10	1 (V.h.n.)	1.30	2 (L)	0.18	1 (V.h.n.)	1.69	2 (L)	0.05	1 (V.h.n.)	1.00	1 (A)	1.27	2 (H.n.)	1.06	2 (L)
D5	0.51	2 (H.n.)	3.66	4 (H)	0.18	1 (V.h.n.)	1.33	2 (L)	2.10	2 (H.n.)	2.48	3 (M)	0.26	2 (H.n.)	2.48	3 (M)	0.07	1 (V.h.n.)	1.29	2 (L)	0.69	1 (V.h.n.)	0.58	1 (A)
D6	0.30	2 (H.n.)	2.15	3 (M)	0.15	1 (V.h.n.)	1.11	2 (L)	3.45	3 (M.n.)	4.08	4 (H)												
D7													1.00	3 (M.n.)	9.55	5 (S)	0.75	3 (M.n.)	13.85	5 (S)	5.10	4 (L.n.)	4.26	4 (H)
E1	0.35	2 (H.n.)	2.47	3 (M)	0.37	2 (H.n.)	2.69	3 (M)	1.45	2 (H.n.)	1.71	2 (L)												
E2	0.20	2 (H.n.)	1.40	2 (L)	0.20	2 (H.n.)	1.44	2 (L)	0.80	1 (V.h.n.)	0.94	1 (A)												
E3	0.23	2 (H.n.)	1.65	2 (L)	0.24	2 (H.n.)	1.73	2 (L)	0.71	1 (V.h.n.)	0.84	1 (A)												
E4	0.24	2 (H.n.)	1.72	2 (L)	0.24	2 (H.n.)	1.77	2 (L)	0.56	1 (V.h.n.)	0.66	1 (A)												
E5	0.33	2 (H.n.)	2.37	3 (M)	0.20	2 (H.n.)	1.48	2 (L)	1.60	2 (H.n.)	1.89	2 (L)												
E6	0.61	3 (M.n.)	4.34	4 (H)	0.19	1 (V.h.n.)	1.37	2 (L)	3.65	3 (M.n.)	4.31	4 (H)												
F1	0.21	2 (H.n.)	1.51	2 (L)	0.20	2 (H.n.)	1.48	2 (L)	0.84	1 (V.h.n.)	0.99	1 (A)												
F2	0.18	1 (V.h.n.)	1.25	2 (L)	0.19	1 (V.h.n.)	1.37	2 (L)	0.59	1 (V.h.n.)	0.69	1 (A)												
F3	0.26	2 (H.n.)	1.89	2 (L)	0.44	3 (M.n.)	3.22	3 (M)	0.57	1 (V.h.n.)	0.67	1 (A)												
F4	0.19	1 (V.h.n.)	1.33	2 (L)	0.24	2 (H.n.)	1.77	2 (L)	0.53	1 (V.h.n.)	0.62	1 (A)												
F5	0.26	2 (H.n.)	1.86	2 (L)	0.67	3 (M.n.)	4.95	5 (S)	1.00	1 (V.h.n.)	1.18	2 (L)												
F6													0.31	2 (H.n.)	2.96	3 (M)	0.10	1 (V.h.n.)	1.77	2 (L)	3.10	3 (M.n.)	2.59	3 (M)
G1	0.23	2 (H.n.)	1.65	2 (L)	0.16	1 (V.h.n.)	1.14	2 (L)	1.20	2 (H.n.)	1.42	2 (L)	0.20	2 (H.n.)	1.91	2 (L)	0.06	1 (V.h.n.)	1.16	2 (L)	2.50	3 (M.n.)	2.09	2 (L)
G2	0.21	2 (H.n.)	1.51	2 (L)	0.24	2 (H.n.)	1.75	2 (L)	0.68	1 (V.h.n.)	0.80	1 (A)												
G3	0.20	1 (V.h.n.)	1.41	2 (L)	0.27	2 (H.n.)	1.99	2 (L)	0.45	1 (V.h.n.)	0.53	1 (A)												
G4	0.31	2 (H.n.)	2.22	3 (M)	0.29	2 (H.n.)	2.10	3 (M)	1.02	1 (V.h.n.)	1.21	2 (L)												
G5	0.23	2 (H.n.)	1.62	2 (L)	0.21	2 (H.n.)	1.53	2 (L)	0.97	1 (V.h.n.)	1.15	2 (L)												
G6	0.48	2 (H.n.)	3.44	4 (H)	0.33	2 (H.n.)	2.40	3 (M)	4.10	4 (L.n.)	4.85	4 (H)												

Table S6. Results of the comparative application of the accumulation/loss scale by Frati et al. [25] (Acc./loss classes) and the bioaccumulation scale in Table 5 (B. class) to a real case study. Data refer to three elements (As, Cd, Cr) measured in samples of *Pseudevernia furfuracea* collected in 30 transplant sites (Figure 4). Abbreviations for naturality/alteration and bioaccumulation classes are reported in Table S4 and Table 5. The attribution of classes is based on the *EU* ratio (former *EC* ratio) for both scales.

Site	As				Cd				Cr			
	Element concentration	*EU* ratio	Acc./loss class	B. class	Element concentration	*EU* ratio	Acc./loss class	B. class	Element concentration	*EU* ratio	Acc./loss class	B. class
A1	0.26	1.06	N.	2 (L)	0.23	1.21	N.	2 (L)	1.74	1.35	Acc.	2 (L)
A2	0.26	1.02	N.	2 (L)	0.46	2.44	S.a.	3 (M)	1.59	1.23	N.	2 (L)
A3	0.32	1.26	Acc.	2 (L)	0.23	1.22	N.	2 (L)	2.35	1.82	S.a.	3 (M)
A4	0.25	0.98	N.	2 (L)	0.21	1.10	N.	2 (L)	1.53	1.19	N.	2 (L)
A5	0.37	1.47	Acc.	2 (L)	0.46	2.44	S.a.	3 (M)	2.38	1.84	S.a.	3 (M)
A6	0.27	1.10	N.	2 (L)	0.25	1.34	Acc.	2 (L)	1.85	1.44	Acc.	2 (L)
B1	0.31	1.24	N.	2 (L)	0.25	1.30	Acc.	2 (L)	1.46	1.13	N.	2 (L)
B2	2.34	9.35	S.a.	5 (S)	0.17	0.90	N.	1 (A)	1.46	1.14	N.	2 (L)
B3	0.28	1.12	N.	2 (L)	0.22	1.14	N.	2 (L)	1.85	1.43	Acc.	2 (L)
B4	0.32	1.28	Acc.	2 (L)	0.26	1.35	Acc.	2 (L)	1.35	1.05	N.	2 (L)
B5	0.29	1.16	N.	2 (L)	0.30	1.60	Acc.	2 (L)	2.02	1.57	Acc.	2 (L)
B6	0.27	1.07	N.	2 (L)	0.23	1.21	N.	2 (L)	1.40	1.09	N.	2 (L)
B7	0.33	1.31	Acc.	2 (L)	0.28	1.48	Acc.	2 (L)	1.63	1.26	Acc.	2 (L)
B8	0.33	1.31	Acc.	2 (L)	0.24	1.29	Acc.	2 (L)	2.09	1.62	Acc.	2 (L)
C1	0.37	1.48	Acc.	2 (L)	0.28	1.49	Acc.	2 (L)	2.37	1.83	S.a.	3 (M)
C2	0.35	1.38	Acc.	2 (L)	0.24	1.29	Acc.	2 (L)	2.09	1.62	Acc.	2 (L)
C3	0.22	0.88	N.	1 (A)	0.21	1.13	N.	2 (L)	1.16	0.90	N.	2 (L)
C5	0.31	1.26	Acc.	2 (L)	0.30	1.60	Acc.	2 (L)	1.60	1.24	N.	2 (L)
C6	0.29	1.15	N.	2 (L)	0.20	1.07	N.	2 (L)	1.81	1.41	Acc.	2 (L)
D1	0.21	0.84	N.	1 (A)	0.20	1.03	N.	2 (L)	1.65	1.28	Acc.	2 (L)
D2	0.22	0.90	N.	1 (A)	0.21	1.10	N.	2 (L)	1.33	1.03	N.	2 (L)
D3	0.29	1.15	N.	2 (L)	0.44	2.31	S.a.	3 (M)	2.03	1.57	Acc.	2 (L)
D4	0.23	0.93	N.	2 (L)	0.21	1.11	N.	2 (L)	1.19	0.92	N.	2 (L)
D5	0.37	1.50	Acc.	2 (L)	0.40	2.09	S.a.	3 (M)	2.06	1.60	Acc.	2 (L)
D6	0.24	0.97	N.	2 (L)	0.25	1.33	Acc.	2 (L)	1.69	1.31	Acc.	2 (L)
E1	0.24	0.95	N.	2 (L)	0.27	1.41	Acc.	2 (L)	1.75	1.36	Acc.	2 (L)
E2	0.25	1.02	N.	2 (L)	0.22	1.15	N.	2 (L)	1.63	1.26	Acc.	2 (L)
E3	0.25	1.01	N.	2 (L)	0.27	1.41	Acc.	2 (L)	1.54	1.20	N.	2 (L)
E4	0.22	0.90	N.	1 (A)	0.22	1.14	N.	2 (L)	1.76	1.37	Acc.	2 (L)
E5	0.22	0.90	N.	1 (A)	0.19	0.99	N.	2 (L)	1.28	0.99	N.	2 (L)

SUPPLEMENTARY FIGURES

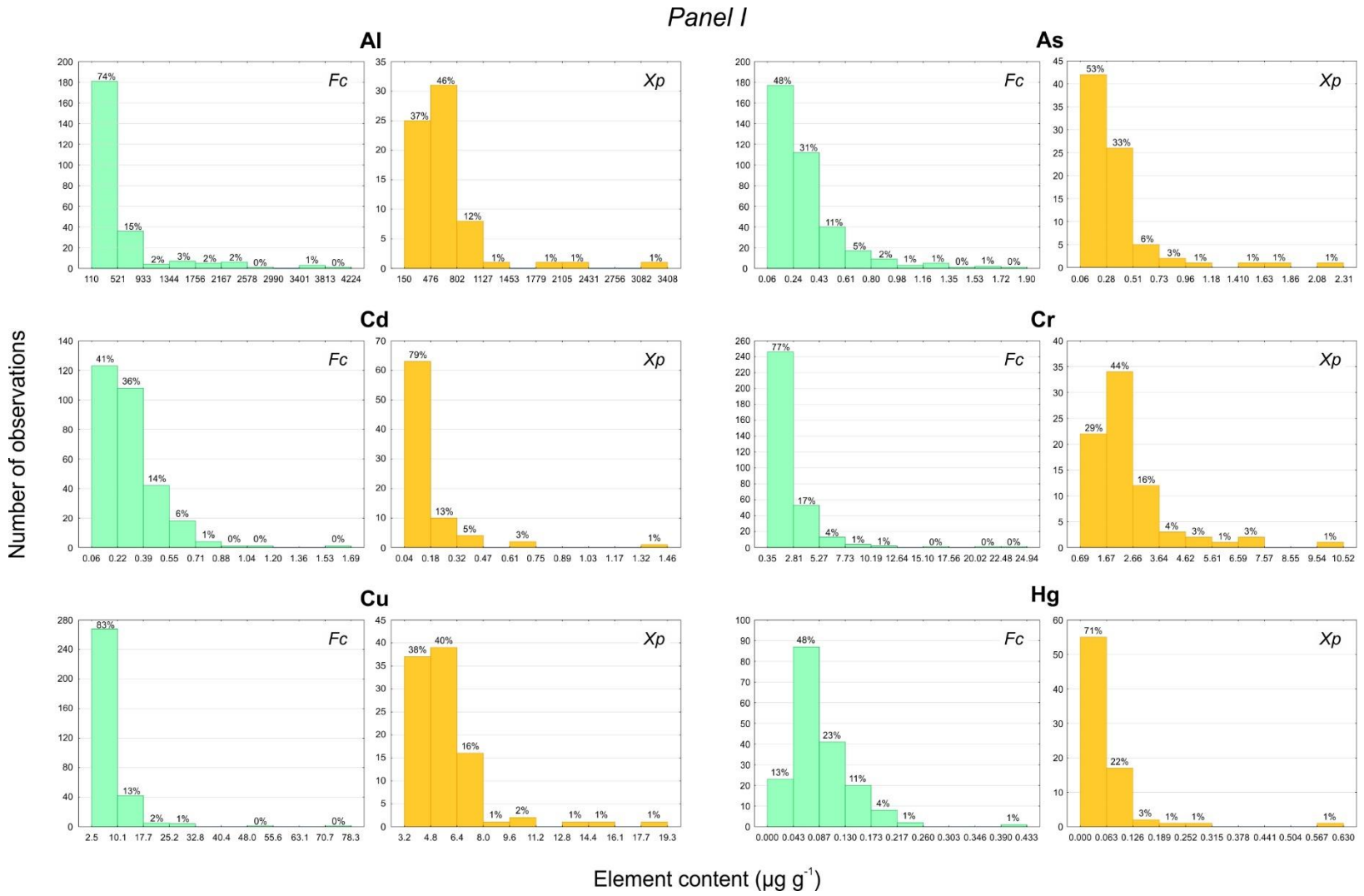

Figure S1. Distributions of element concentration data for *Flavoparmelia caperata* (*Fc*: pale green bars) and *Xanthoria parietina* (*Xp*: orange bars) in the dataset *N* (panel I: Al, As, Cd, Cr, Cu, Hg; panel II: Ni, Pb, Ti, V, Zn).

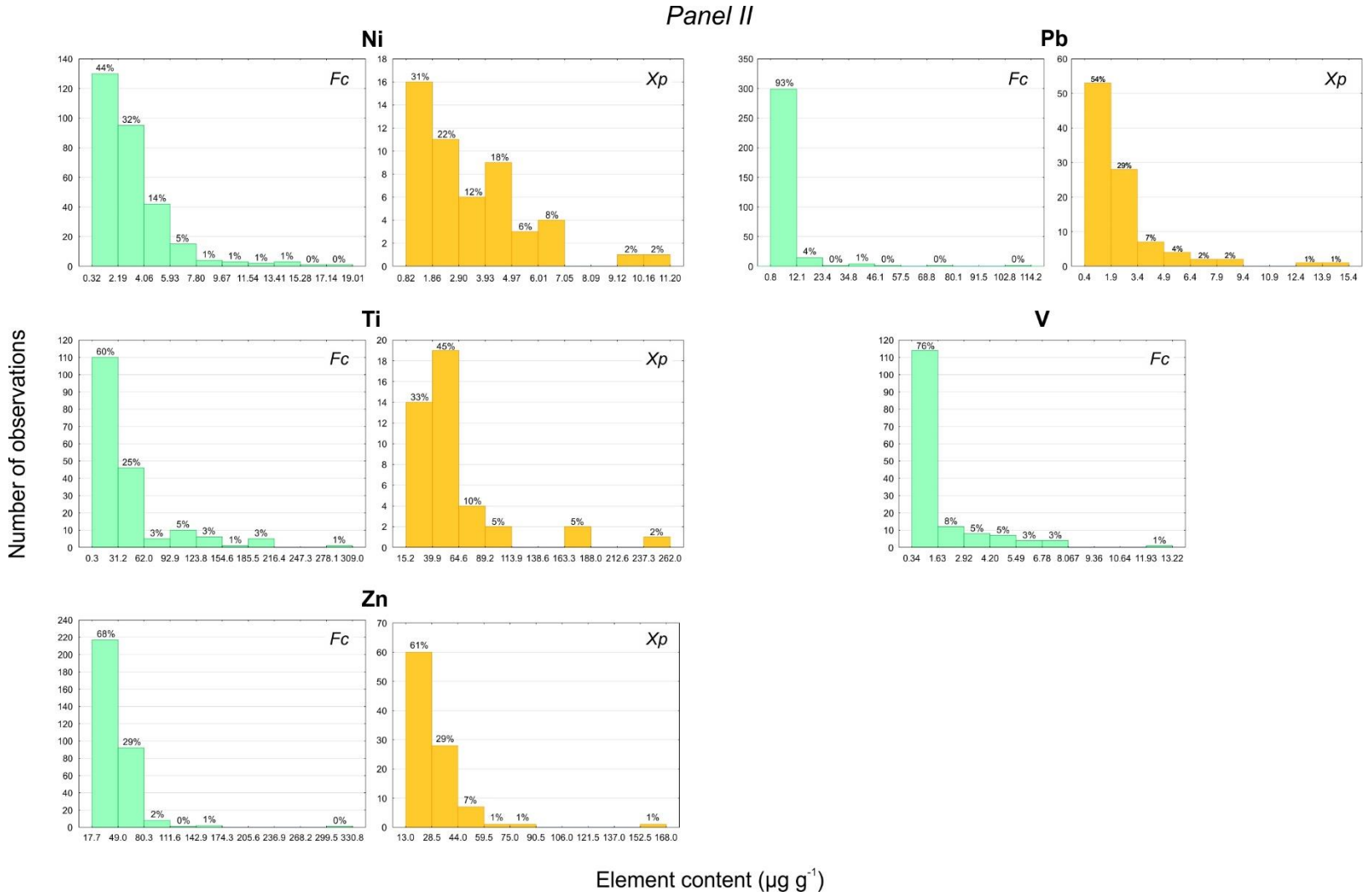

Figure S1. Distributions of element concentration data for *Flavoparmelia caperata* (*Fc*: pale green bars) and *Xanthoria parietina* (*Xp*: orange bars) in the dataset *N* (panel I: Al, As, Cd, Cr, Cu, Hg; panel II: Ni, Pb, Ti, V, Zn).

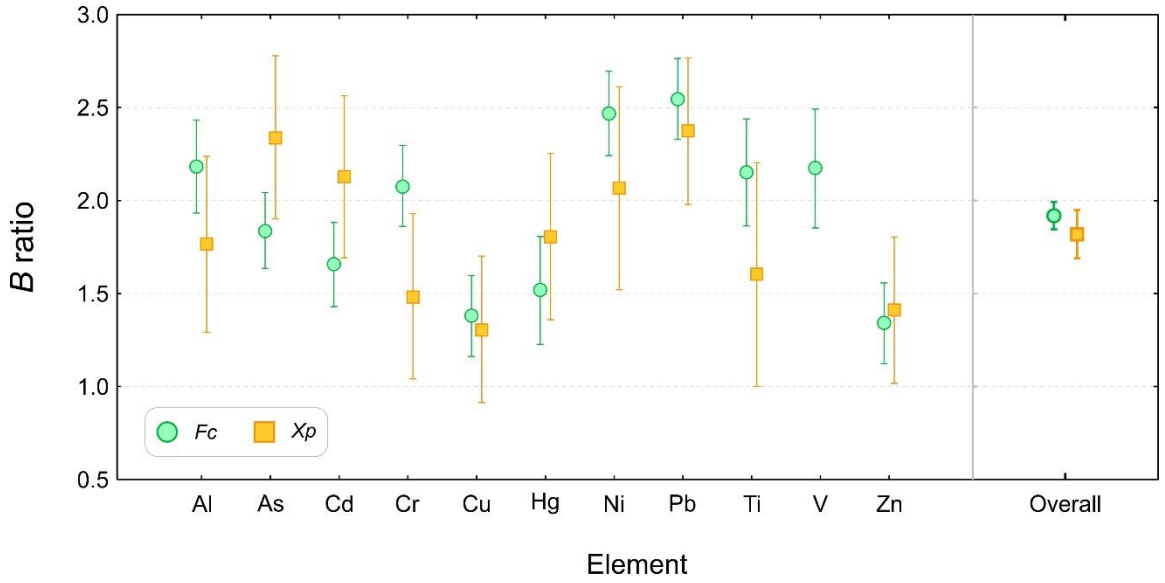

Figure S2. *B* ratio data, separately reported for 11 elements or not (overall), in the lichen species *Flavoparmelia caperata* (*Fc*: pale green) and *Xanthoria parietina* (*Xp*: orange). Data are shown as means and 95% confidence intervals.

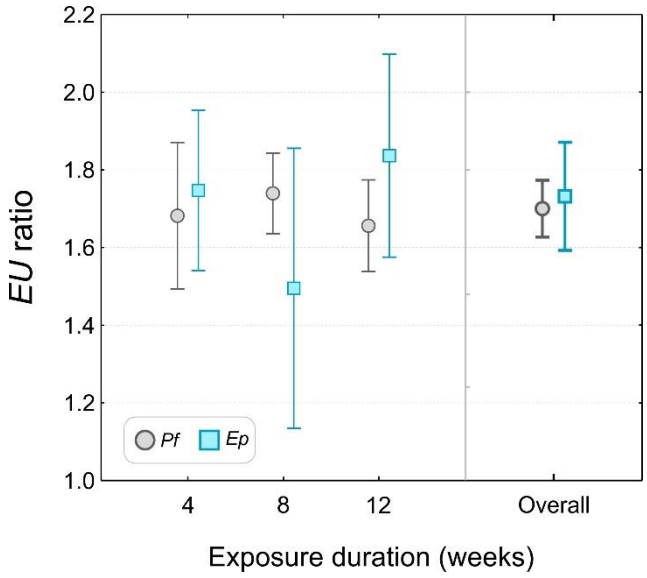

Figure S3. *EU* ratio data, separately reported for three exposure time spans (4, 8 and 12 weeks) or not (overall), in the lichen species *Evernia prunastri* (*Ep*: pale blue) and *Pseudevernia furfuracea* (*Pf*: grey). Data are shown as means and 95% confidence intervals.