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State of Epiphytic Lichen Communities under Anthropogenic Impact: Effect of Abundance Assessment Methods on the Informativity of Indices

I. N. Mikhailova[a], V. S. Mikryukov[a], and I. V. Frolov[b]

[a] *Institute of Plant and Animal Ecology, Ural Branch, Russian Academy of Sciences, ul. Vos'mogo Marta 202, Yekaterinburg, 620144 Russia*

[b] *University of South Bohemia, Českě Budějovice, Branisovska str. 31, 37005 Czech Republic*

e-mail: mikhailova@ipae.uran.ru; vmikryukov@gmail.com; ivfrolov@gmail.com

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Abstract—A comparative analysis has been performed on the informativity of characteristics of the state of lichen cover calculated by two methods for evaluating lichen abundance (frequency and projective cover). A close correlation of the indices obtained by the two different methods was shown, as well as their significant association with the degree of stress, which confirms their equal usefulness in lichenoindication works. However, comparison of the informativity established that the indices based on frequency have higher resolution capability.

Keywords: epiphytic lichens, industrial pollution, urbanization, indices, species diversity, frequency, projective cover, informativity

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INTRODUCTION

Epiphytic lichens have been widely used worldwide for a long time as indicators of atmospheric pollution (Lichens, Bryophytes and Air Quality, 1988; Monitoring with Lichens—Monitoring Lichens, 2002). A wide range of characteristics is used for quantitative assessment of lichen communities, including species diversity, abundance, vital state, and the maximal height of indicator species on the trunk. Many synthetic indices have been suggested, calculated using abundance and diversity, of which the most well-known are the index of poleotolerance *IP* (Trass, 1968), the index of atmospheric purity *IAP* (De Sloover and LeBlanc, 1968), and numerous modifications thereof.

Among the methods for assessing the abundance of lichens, two approaches prevail. The first consists in the exact determination of the species cover (cm^2 or %) using a palette of a certain size (10 × 10, 10 × 20 or 20 × 20 cm) (Martin, 1978; Gorshkov, 1986; Byazrov, 1993; Mikhailova, 1996; Tarasova, 2000; Paukov, 2001). The cover is measured either from certain cardinal points or from the side facing the point source of emissions, or in the place of maximum lichen cover, as well as at the base of a trunk and/or a height of 1.3 m. Sometimes, in further analysis, the projective cover is converted into a numerical score (Trass, 1968). The second approach determines the frequency, i.e., the proportion of cells occupied by lichens within grids of different sizes and configurations. A frame 30 × 50 cm divided into ten squares is often used (Nimis et al., 1990), as well as a grid with a height of 50 cm and a width equal to half of circumference of the described trunk, also divided into ten squares (Herzig and Urech, 1991), and a vertical "ladder" of five consecutive squares 10 × 10 cm in size (Asta et al., 2002).

The large number of lichen bioindication surveys and suggested methods caused comparative studies aimed at finding the most informative indices. In almost every paper, the authors compare the indices used by the degree of labor intensity and subjectivity, as well as by the number of distinguished isotoxic zones and the similarity of their configuration. More often, the informativity of an index is evaluated by the degree of correlation with the results of instrumental measurements of the pollutant content in air or accumulative media. The largest project of this group of works was the Swiss project (Ammann et al., 1987; Herzig and Urech, 1991), in which 20 *IAP* modifications were tested, calculated using different parameters of lichen communities (coverage, frequency, and vitality) in two variants: including all species encountered or with a reduced list (the analysis did not include species that did not correlate with the degree of pollution and species with a single occurrence). Correlation between indices and results of instrumental monitoring was assessed, and it was shown that the concentrations of air pollutants correlate the best with the sum of frequences of species from the reduced list.

Table 1. Materials and method of projective cover determination

Area, phorophyte	Number			Position of palette
	plots*	trees per plot	palettes per tree	
Karabash, *Betula pendula* Roth	9 (3 + 4 + 2)	10	8	N, S, E, W at base of trunk and height of 1.3 m
Kirovgrad, *Abies sibirica* Ledeb	15 (5 + 5 + 5)	10–20	2	From side with maximum cover at base of trunk and height of 1.3 m
Yekaterinburg, *Pinus sylvestris* L.	12 (6 + 6)	5	2	

* The total number of plots and their distribution by pollution zones (background + buffer + impact, background + impact) is given.

However, in many studies, correlations of indices with the content of toxicants are either very weak (e.g., Mikhailova and Vorobeichik, 1995; Scheidegger and Mikhailova, 2000) or not detected at all. Possible reasons for this are: (1) the combined effect of a large array of toxicants on the lichens (not only one used in the analysis); (2) the use of data on toxicants over a short period of time that do not comply with the long-term pollution pattern; and (3) the impact of a variety of habitat factors, which modify the impact of toxicants (Byazrov, 2002). Therefore, conclusions about the informativity of indices cannot be based only on correlation analysis.

The aim of this work is to compare the informativity of the characteristics of the state of lichen cover based on the two methods of evaluating lichen abundance: frequency and projective cover. The analysis was performed for a wide range of conditions (three regions differing in species of phorophytes, the number and arrangement of sampling plots, and the method of determining the projective cover of lichens), thus allowing us to assess the commonality of the findings.

MATERIAL AND METHODS

The analyzed data were collected over the years in order to assess the impact of emissions from nonferrous metallurgical enterprises, namely, the Karabash Copper Smelter (KAR, 2010) and the Kirovgrad Copper Smelter (KIR, 1994), as well as a complex of urban factors (Yekaterinburg, 2010) on epiphytic lichens. KAR is located in the southern Urals, 90 km northwest of the city of Chelyabinsk, in the subzone of preforest–steppe pine and birch forests, and KIR is situated in the Middle Urals, 87 km north of Yekaterinburg, in the southern taiga subzone. The experimental areas were located along transects from emission sources (at distances of 1–40 km to the east of KIR, from 1 to 32 km to the north and south of KAR) and were further grouped into three pollution zones: background, buffer, and impact. The emission sources and transformation patterns of various components of forest ecosystems have been described in detail earlier (Kompleksnaya efologicheskaya otsenka, 1992; Vorobeichik et al., 1996; Scheidegger and Mikhailova, 2000; Purvis et al., 2004; Kozlov et al., 2009; Smorkalov and Vorobeichik, 2011).

When assessing the impact of urban environmental factors, the sample plots were placed in pine stands in Yekaterinburg (six plots) and outside the city (six plots around Lake Chusovskoe and Lake Glukhoe). Accordingly, they were treated as two pollution zones: impact and background. A description of the environmental parameters was given earlier (Zolotarev and Belskaya, 2015; Smorkalov and Vorobeichik, 2015).

The frequency of lichens (on a scale from 1 to 10) were determined using a grid (Herzig and Urech, 1991) at the base of the trunk and a height of 1–1.5 m on the side with the maximum development of lichen cover. The projective cover was determined using a 10 × 10 cm palette with a mesh of 1 cm^2 on the base of the trunk and at a height of 1.3 m. The material is described in detail in Table 1. In the area of KIR, *Scoliciosporum chlorococcum* was not taken into account (species with a high toxitolerance).

From the obtained data, the following pairs of indices were calculated: average number of species per trunk counted using the palette and grid; Shannon diversity index (based on cover and frequency data); total abundance (sum of covers and sum of frequencies); atmospheric purity indices calculated using the (1) cover and (2) frequency values:

$$IAP_c = \sum_1^n Q_i c_i, \quad (1)$$

$$IAP_f = \sum_1^n Q_i f_i, \quad (2)$$

where (Q_i) is the number of species accompanying the *i*th species; (c_i) is the cover of the *i*th species determined using the palette; (f_i) is the frequency of the *i*th species determined using the grid; and (n) is the number of species counted using the grid or palette.

As the measure of informativity of an index, Fischer's *F*-ratio was used (for the entire gradient) and Cohen's effect size *d* (for two contrasting pollution zones: impact and background). To normalize the dis-

Table 2. Coefficients of linear correlation of indices calculated using two methods of lichen recording (a tree is considered as a counting unit)

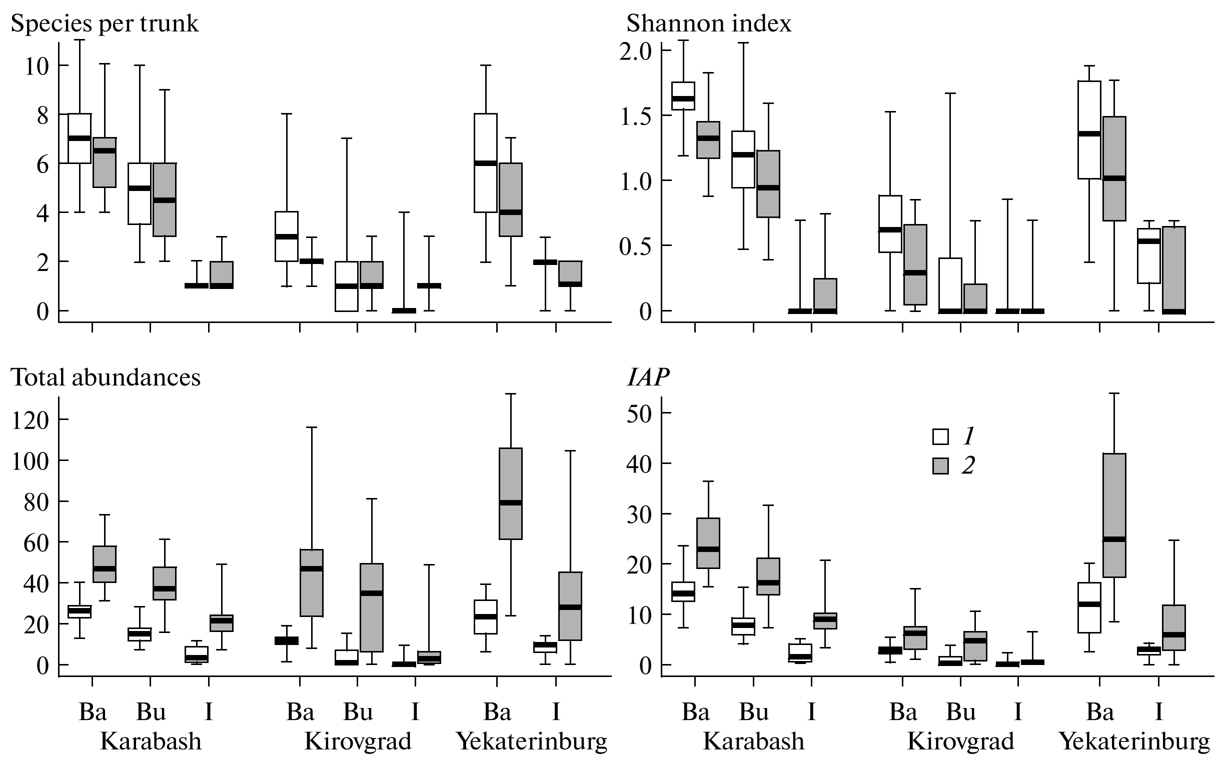
Indices	City		
	Karabash	Kirovgrad	Yekaterinburg
Species per trunk	0.85	0.72	0.84
Total abundance	0.66	0.52	0.81
Shannon index	0.84	0.69	0.73
IAP	0.74	0.65	0.88

The coefficients are statistically significant in all cases ($p < 0.05$).

tributions, data was log-transformed. To verify the hypothesis of equivalence of the counting methods of lichen abundance, i.e., identity in the trends of their changes in the pollution gradient and identical resolution capability, nonparametric ANOVA for adjusted ranks was used (Leys and Schumann, 2010), which tested the significance of "zone of pollution × method" interaction. We used bootstrap (10000 repetitions) to calculate a 95% confidence interval of difference in *d* between the different methods; if the interval did not include zero, it was considered that the effect sizes were significantly different. All listed *p* values include a correction for multiple comparisons (*FDR*, false discovery rate). All calculations were performed in the R program v.2.15.0 (R Core Team, 2014).

RESULTS AND DISCUSSION

In all studied areas, the indices of the state of lichen communities obtained using the two different abundance estimates closely correlate (Table 2). Under the influence of toxic load, degradation of epiphytic lichen communities takes place, which is expressed as a decreasing number of species and their abundance (figure). The pollution effect on all indices is significant with both methods of abundance estimation (Table 3). In all cases, the *F*-ratio is higher for the indi-



Indices of state of lichen communities calculated by counting (*1*) frequency and (*2*) projective cover.

Zones of influence: (Ba) background, (Bu) buffer, and (I) impact. The median (horizontal thick line), first and third quartiles (borders of rectangles), and minimum and maximum values (vertical lines) are given.

Table 3. Informativity of indices of state of epiphytic lichen communities based on determining frequency (*f*) and projective cover (*c*)

Area	Index	*F*-ratio		Effect size *d*	
		f	*c*	*f*	*c*
Karabash ($df = 2$)	Species per trunk	170.71	117.83	4.54	4.05
	Total abundance	106.49	37.16	**3.94**	**2.21**
	Shannon index	**236.64**	**141.91**	7.99	5.06
	IAP	123.22	48.60	3.85	2.52
Kirovgrad ($df = 2$)	Species per trunk	**128.58**	**45.50**	**2.80**	**1.97**
	Total abundance	**190.32**	**93.02**	**5.02**	**2.41**
	Shannon index	**75.37**	**45.22**	2.44	1.65
	IAP	**206.31**	**104.60**	**4.46**	**2.41**
Yekaterinburg ($df = 1$)	Species per trunk	105.65	83.73	2.37	2.24
	Total abundance	**53.20**	**30.07**	2.04	1.76
	Shannon index	84.72	48.56	2.46	1.79
	IAP	**99.00**	**60.14**	2.18	1.99

The *F*-ratio was calculated using one-way ANOVA, and all *F* are significant at $p \leqslant 0.001$; boldface indicates variants in which two-way ANOVA showed significant interaction of factors "pollution zone × method of estimation" ($p < 0.05$) or significant differences in effect size (*d*).

ces calculated based on frequency. The two-way ANOVA revealed significant interaction of "pollution zone × method of estimation" factors in 7 cases out of 12 (Table 3). In most cases, the effect size *d* is also higher when the frequency index is used instead of the coverage index; in 4 cases out of 12, significant differences in the *d* values obtained using different methods of abundance estimation were revealed.

For the majority of indices, the maximum values of both informativity indices were recorded in KAR area, and the most informative of them was the Shannon diversity index, whereas in other areas, traditional lichen bioindication indices proved more informative (total abundance and *IAP* near KIR and number of species per trunk and *IAP* in Yekaterinburg).

By definition, the informativity of a parameter is high when, on the one hand, there is a wide range of values in the stress gradient, and on the other, there is low variability within one stress gradation. The high informativity of indices of the state of the lichen communities near KAR is due to their higher values in the background area (primarily, by the higher species richness: 39 species of lichens on birch compared to 21 species on fir in KIR area and 21 species on pine in Yekaterinburg). A number of indices in the area of Yekaterinburg have similar values or even surpass them (e.g., the total abundance of lichens); however, high variation within one zone reduces their informativity. In the city, the high variation is due to the multidirectional action of multiple environmental factors of urban and suburban forests (fires, eutrophication of bark, degree of habitat fragmentation, etc.).

Since all indices used are associated with the number of recorded species, it is important to pay attention to the differences in the number of species included into the counting area for the different ways of measuring abundance. In the background area, for any variants of placing the palettes, fewer species are counted compared to the grid (figure). In the buffer zone, the difference between methods decreases, and in the impact area, counting with a palette can yield, in some cases, a higher average number of species on the trunk compared with the numbers counted with a grid. This might be related both to the size of the counting area and to specific ways of placing a grid on the trunk. Since the area of the grid is not fixed and depends on the size of the trunk, the frequency is essentially equal to the fraction of the trunk surface on which the species was found. The counting area exceeds the total area of a palette in any case, thus increasing the chance of more species being included in it. In the impact area, lichens mostly inhabit the bases of trunks, including the parts between roots not covered by the grid, but are taken into account by a palette.

Discussing the impact of the size of the counted area on the informativity of indices, it should be noted that when evaluating cover with eight palettes, as was done in the KAR area, no significant interaction of the "pollution zone × method of estimation" factors was found for the number of species per trunk (Table 3); i.e., the method for estimating abundance has no considerable effect on the results. In other areas where the cover was counted using only two palettes on the trunk, the interaction of the factors was significant.

Table 4. Dependence of frequency (*f*) or cover (*c*) of lichen species on degree of pollution (the *F*-ratio for one-way ANOVA is given)

Species	Karabash		Kirovgrad		Yekaterinburg	
	f	*c*	*f*	*c*	*f*	*c*
Cladonia coniocraea	**74.16**	**59.18**	**12.05**	**29.22**	**8.57**	**10.73**
Evernia mesomorpha	**11.71**	1.13	**7.50**	–	**22.23**	2.15
Fuscidea pusilla	**6.37**	5.28	**9.70**	–	1.00	–
Hypocenomyce anthracophila	–	–	–	–	**44.91**	**9.33**
Hypocenomyce caradocensis	0.62	2.30	**7.61**	1.39	**93.66**	**47.89**
Hypogymnia physodes	**73.45**	**45.76**	**291.98**	**180.35**	**175.24**	**122.51**
Lecanora saligna	**39.43**	**34.24**	–	–	1.00	1.00
Lecidea cf. *plebeja*	–	–	–	–	**6.40**	4.03
Lepraria cf. *elobata*	1.00	0.78	**9.17**	**6.71**	**5.69**	2.79
Parmelia sulcata	**17.80**	**6.89**	–	–	–	–
Parmeliopsis ambigua	0.78	4.16	**17.50**	1.61	–	–
Pycnora sorophora	–	–	–	–	**18.28**	**10.76**
Scoliciosporum chlorococcum	**14.29**	**16.57**	–	–	**10.55**	**11.81**
Vulpicida pinastri	**55.38**	**30.43**	4.16	5.21	–	–

The species that demonstrate a significant correlation of abundance with the degree of pollution in at least one case are included; statistically significant values ($p < 0.05$) are given in boldface; a dash indicates that the species was not found in that area or with that method.

In addition to the indices of the state of lichen communities, it is important to compare the "countability" of lichen species by different methods. Of the 48 species identified in the 3 studied areas, only 14 showed a significant dependence of abundance on the degree of pollution in at least one of the areas (Table 4); of them, 3 species (*Cladonia coniocraea, Evernia mesomorpha*, and *Hypogymnia physodes*) showed this dependence in all three areas; and 4 species, in two areas (at least with one estimation method). Other species show a significant dependence on the degree of contamination in only one of the areas, and their composition reflects the specificity of the lichen flora of a particular phorophyte. A group of species can be distinguished for which the presence or absence of a statistically significant association of abundance with toxic stress does not depend on the method of assessing abundance: if a dependence exists, it will be revealed using both methods. This group includes dominants and other species with high projective cover: *C. coniocraea, H. physodes, Hypocenomyce anthracophila, Parmelia sulcata*, *Pycnora sorophora*, and others. However, a group of species exists that show a relationship with the degree of pollution only with one method: counting with a grid (*E. mesomorpha*, *Fuscidea pusilla*, *Hypocenomyce caradocensis* on fir, and *Parmeliopsis ambigua*).

Among the species for which no significant correlation between the abundance and the degree of pollution was found, the main part consists of species with low frequency: the highly sensitive to pollution *Bryoria nadvornikiana*, facultative epiphytes (*Cladonia digitata*, *Cl. fimbriata*, *Cl. macilenta, Placynthiella icmalea*, *P. uliginosa, Trapeliopsis flexuosa*), and species that occur sporadically in the studied area and/or on the studied phorophytes (*Hypogymnia tubulosa*, *Hypocenomyce friesii*, *Physcia* spp., *Pycnora praestabilis*). At the same time, this group includes species that are quite common in the studied area, such as *Hypocenomyce scalaris*, *Chaenotheca ferruginea*, *Lecidella nylanderi*, and *Micarea denigrata*.

CONCLUSIONS

Indices of the state of lichen communities based on the two different methods of assessing abundance—determination of the projective cover using a palette and frequency using a grid—closely correlate with each other and are significantly related to the degree of pollution; consequently, they can be equally legitimately used in lichen bioindication. However, the indices based on counting the frequency with a grid (Herzig and Urech, 1991) are more informative and hence have a higher resolving capability. Owing to the larger coverage of a trunk by the grid, a greater number of species is counted compared to the palettes (at least, in weakly polluted and unpolluted areas), which accounts for the higher informativity of the indices based on frequency. An increase in the counting area when evaluating the projective cover by using a larger number of palettes per trunk increases the informativity of the indices based on the projective cover.

The identified patterns are quite stable: they proved correct for all three areas of research where lichen communities of different phorophytes were studied, with different number and arrangement of plots.

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