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Content of Heavy Metals in Thalli of the Lichen *Hypogymnia physodes*: Sources of Heterogeneity

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Abstract—Size and reproductive traits of thalli of the epiphytic lichen *Hypogymnia physodes* (L.) Nyl. and concentrations of heavy metals (Cu, Fe, Cd, Zn, Pb) in them were determined in the region of the Middle Ural Copper Smelting Plant, in two contrast zones of atmospheric pollution. The nonuniformity of populations in both size and reproductive traits and metal concentration was demonstrated. A high heterogeneity of subpopulations growing on different phorophytes was revealed. It is recommended to optimize data collection by taking samples from a larger number of trees (20–30) at a smaller number of thalli (10–20) from one phorophyte.

Keywords: epiphytic lichens, populations, heterogeneity, accumulation of pollutants, lichen indication, industrial pollution, copper smelting plant, heavy metals, Middle Urals

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When performing works on accumulative lichen indication, the attention of researchers has repeatedly been paid to the high variation in the concentrations of toxicants in thalli [1]. A number of authors account for it by a different income of pollutants owing to the spatial heterogeneity of a different scale: altitudes of a locality [2, 3], local topography and structure of vegetation [4, 5], and position of thalli on a tree trunk [6, 7]. The following sources of variation are mentioned much rarely—“individual” differences such as age of thalli and their parts and their morphological and anatomic peculiarities determining the ability to capture and retain pollutants. For example, a higher content of elements in the old parts of the same thallus was revealed for several species of foliose lichens [8–10]. However, even under the standard methods for sampling from relatively homogenous populations, a high unexplained variation in the concentration of elements in thalli was found [11].

To solve methodical problems of accumulative lichen indication, ranking the importance of effects determining the accumulation of metals in thalli is necessary. The choice of an experimental design, i.e., the sampling scheme and number of samples, depends on the factor which determines accumulation— is it a peculiarity of a phorophyte or a thallus. The goal of the study is to compare several sources of variation in the content of metals in the epiphytic lichen *Hypogymnia physodes* (L.) Nyl. under the contrast toxic load.

The investigations were carried out in the area of atmospheric pollution from the Middle Ural Copper Smelting Plant (MUCSP), located near the city of Revda in Sverdlovsk oblast. The major ingredients of the emissions are SO_2 and heavy metals. The species *H. physodes* dominating in the epiphytic lichen communities of the region was chosen as a model object. The sample plots were selected in fir (Abies) forests in the background (30 km to the west from the MUCSP) and impact (5 km) areas. Thalli were collected from fir trunks at a height of 5 to 50 cm. In the background area, all thalli were collected from a quadrate of 20 × 20 cm from each of five trees (from 8 to 57 per tree, 124 thalli in total). In the impact area, where the density of lichens is much lower, all thalli (from 1 to 49 per tree) were collected from 12 trees (in total, 158). The age states of 282 thalli of *H. physodes* were determined on the basis of the quantity and degree of development of soralia (ordinal variable): (1) *AS*—asorediate; (2) *CS*—cryptosorediate; (3) *S*1—hyposorediate; (4) *S*2—mesosorediate; (5) *S*3—hypersorediate [12]. Thalli of all age states were noted in each of the populations under investigation, but not on every phorophyte.

After determining an age state, thalli were cleaned of bark fragments, dried at 40°C for 48 h, and weighed on an analytical balance with an accuracy of 0.1 mg. Samples were decomposed in microwave laboratory oven (Ural-Gefest, Russia). The total content of Cu, Fe, Cd, Zn, and Pb was determined individually in each thallus using an AAS Vario 6 atomic absorption spectrometer (Analytik Jena AG, Germany) with the flame or electrothermal atomization. The analytical laboratory is accredited for technical competence (certificate POCC.RU0001.515630).

Since individual thalli are not simple independent replications but grouped by a phorophyte, the mixed effects model of analysis of variance (ANOVA) was used. The populations of thalli from contrast pollution areas were considered as two levels of fixed effect factor "Area", and subpopulations (population within a single tree) were considered as random levels of the "Phorophyte" nested into the levels of the first one [13, 14]. In order to estimate potential influence of the size and reproductive traits of thalli on metal content, analysis of covariance was used separately for each population. In order to normalize the response and stabilize its intragroup variance, the logarithm log10(y) was used, and the descriptive statistics (median, geometric mean, and 95% confidence interval, CI) presented after the inverse transformation. To assess the robustness of statistical inference, we used also nonparametric Mann-Whitney test (Z-approximation) and double-sided Exact Fisher test (EFT) as an analogue of two groups Median test. To estimate a heterogeneity in age structure of two thalli populations, a standardized deviations $r_{st} = (N_{observed} - N_{expected})/(N_{expected})^{0.5}$; $r_{st} \approx N(0;1)$ as components of the Pearson statistics $X^2 = \Sigma(r_{st})^2$ were analyzed [16]; the odds ratio (OR) was estimated after collapsing of homogenous age groups [14].

RESULTS AND DISCUSSION

Average metal concentrations for the subpopulations of *H. physodes*, individual phorophytes, are shown in Table 1. As was expected, the average metal concentrations calculated using the samples of thalli or subpopulations (the average values for separate phorophytes) are a factor of 2–6 higher in the impact population. However, the statistical inference is robust for the copper, lead, and zinc concentration, but not for the iron and cadmium content (Tables 1, 2). The iron concentration has a polymodal distribution, and a significant heterogeneity is typical of the populations of both background and impact areas. A high heterogeneity between the subpopulations of the impact area was revealed for cadmium.

According to ANOVA results (Table 2) and estimates of intraclass correlation, a significant contribution to the accumulation of metals is made not only by the distance from the emission source but also by the properties of phorophytes ($\rho = 0.15 - 0.65$), that evidently reflects both the spatial heterogeneity of metal fallout on a scale of meters to tens of meters and characteristics of individual trees (the size and shape of the crown, structure of the bark, age, etc.). Our experimental design does not allow for differentiating the effects of the indicated sources of variation.

The subpopulations of different phorophytes are heterogeneous in the age spectra of thalli: *age* × *phorophyte* $X^2(64) = 185.19$. The structure of the subpopulations of the two contrast polluted areas is also heterogeneous: *age* × *area* $X^2(4) = 55.56$. The analysis of standardized deviations shows a relative excess of hypersorediate ($n_{background}/n_{impact} = 40/16$; $r_{st}^{background}/r_{st}^{impact} = 3.10/-2.74$) and mesosorediate (34/12; 3.06/–2.71) thalli in the background area and their deficiency in the impact area; and vice versa, a deficiency/excess of asorediate (17/63; –3.06/2.72) thalli. After collapsing of the last two and first three classes of age states $\{S2 + S3\}/\{AS + CS + S1\}$ (for the background population, 130/50; for the impact population, 28/74; $X^2(1) = 52.97(p(\text{EFT}) = 2.5\text{E} - 13)$, the estimates of odds of hyper- and mesosorediate $\{S2 + S3\}$ thalli in the background population by 6.9 (95% CL 4.0 – 11.8) higher than in the impact one. This result is in accordance with the earlier data [12] on the shift of the age spectrum in the impact territory toward less fertile and/or sterile thalli.

The impact population is characterized by a 3–5 times smaller average thallus weight (see table 1). The thallus weight and reproductive state of the thallus are not independent variables, which is evidenced by the high values of pairwise correlation coefficients for both the background ($r = 0.76$) and impact ($r = 0.70$) populations. The effect of the size and reproductive traits of thalli on the content of heavy metals was estimated using the analysis of covariance ("phorophyte" as a random factor) only for the subpopulations with sufficient sample size for all age states. The difficulties related to the collinearity of predictors were overcome by their being included in turn in the model and/or by model selection. Adding the logarithm of the thallus weight to a set of predictors increased the explained variance (R^2) by 5% only for the cadmium content in the impact population. The absence of an unambiguous dependence between the accumulation of metals and the thallus weight is unexpected: thus, in two subpopulations of the impact area, larger thalli were characterized by a higher cadmium content, and, in the third one, its decrease was observed. Based on ANOVA results, we can conclude that the effect of the size and reproductive traits of thalli on the content of heavy metals is weaker than the effects of the distance from an emission source and peculiarities of subpopulations.

CONCLUSIONS

Thus, the content of heavy metals in thalli of epiphytic lichens under atmospheric pollution is influenced both by the distance from an emission source and properties of phorophytes. The latter appear to be related to the spatial variation of metal fallout on the scale of tens of meters and features of trees (tree age, size and shape of the crown, structure of the bark, etc.), that explains the similarity ($\rho_{average} = 0.48$) of metal content in thalli within the subpopulation. The weak effects of the size and reproductive characteristics can be neglected, since they influence the content of heavy metals to a lesser extent than the distance

Table 1. Descriptive statistics: thallus weight (mg) and metal concentration (μg/g) in thalli of *H. physodes*

Population	Number of trunks (thalli)*	Median	Geometric mean	95% CL	
		Thallus weight			
Background (Z** = 1.79)	5	21.3	14.2	3.6	56.4
Impact	12	4.3	4.7	3.0	7.4
Background ***(Z = 5.79)***	*(124)*	*14.4*	*12.0*	*9.6*	*15.0*
Impact	*(158)*	*3.8*	*4.5*	*3.8*	*5.5*
		Cu			
Background **(Z = 3.16)**	5	37.0	35.0	24.3	50.3
Impact	12	222.9	202.0	151.8	268.6
Background ***(Z = 13.44)***	*(122)*	*37.8*	*36.4*	*34.6*	*38.3*
Impact	*(147)*	*126.1*	*132.9*	*122.0*	*144.7*
		Fe			
Background (Z = 1.58)	5	103.4	171.3	38.7	758.9
Impact	12	464.5	367.0	210.7	639.3
Background ***(Z = 4.31)***	*(123)*	*586.8*	*283.8*	*223.5*	*360.4*
Impact	*(145)*	*749.9*	*543.2*	*459.4*	*642.4*
		Pb			
Background **(Z = 3.16)**	5	25.2	25.8	15.6	42.6
Impact	12	88.8	93.1	73.9	117.3
Background ***(Z = 12.48)***	*(110)*	*28.7*	*25.1*	*21.6*	*29.3*
Impact	*(152)*	*93.9*	*93.2*	*86.1*	*100.8*
		Zn			
Background (Z = 2.95)	5	103.3	114.9	56.1	235.4
Impact	12	296.0	314.1	249.6	395.3
Background (Z = 5.19)	*(115)*	*146.7*	*145.5*	*124.6*	*169.9*
Impact	*(136)*	*258.2*	*244.1*	*219.9*	*271.0*
		Cd			
Background (Z = 1.69)	5	0.370	0.440	0.245	0.778
Impact **p(EFT)$^{\#}$ = 0.03**	12	1.836	1.100	0.466	2.580
Background ***(Z = –3.06)***	*(117)*	*0.509*	*0.523*	*0.447*	*0.612*
Impact	*(146)*	*0.270*	*0.279*	*0.211*	*0.368*

Note: *Italic marks the comparison of means without consideration for their grouping by a phorophyte, which represents the situation of so-called "pseudoreplications" [15, 13]; ** Z-approximation of the Mann-Whitney statistic for comparing the populations of two areas according to the mean values of subpopulations; bold type indicates $p < 0.05$. # EFT was used as an analogue of the median test. Ignoring the dependence of observations causes biased estimates — "paradoxical" values (underlined).

from an emission source and the differences between phorophytes.

The available methodical literature does not give clear indications of the number of sampled trees needed to obtain reliable results. Some authors [17] recommend collecting thalli from at least three trees. Our results showed that the number of phorophytes must be greater at least by 5–10. Variance structure of the content of heavy metals revealed permits us to recommend optimizing the data collection for accumulative lichen indication by taking samples from a larger number of trees (20–30) at a smaller number of thalli (10–20) from one phorophyte to enhance the power of a statistical inference at a fixed or even lower cost. The equality between the number of samples and also the balance between different size and reproductive traits of thalli and consideration for their effects are desirable but less important for a correct statistical inference.

Table 2. Results of the mixed effects nested ANOVA and estimates of intraclass correlation (ρ)

Source of variation	*SS*	Effect		Residual		*F*	*p*≤	ρ
		df	*MS*	*df*	*MS*			
Log_{10} [Age]								
Area	**11.24**	**1**	**11.241**	**19.1**	**1.058**	**10.62**	**0.004**	
Phorophyte (Area)	**28.14**	**15**	**1.876**	**264**	**0.241**	**7.77**	**0.000**	**0.325**
Log_{10} [Thallus weight]								
Area	**5.68**	**1**	**5.675**	**17.4**	**1.233**	**4.60**	**0.046**	
Phorophyte (Area)	**34.35**	**15**	**2.290**	**264**	**0.178**	**12.88**	**0.000**	**0.458**
Log_{10} [Cu]								
Area	**13.86**	**1**	**13.862**	**16.7**	**0.172**	**80.71**	**0.001**	
Phorophyte(Area)	**4.73**	**15**	**0.316**	**252**	**0.018**	**17.32**	**0.001**	**0.547**
Log_{10} [Fe]								
Area	2.7	1	2.702	16.1	1.526	1.77	0.202	
Phorophyte(Area)	**42.39**	**15**	**2.826**	**250**	**0.108**	**26.06**	**0.001**	**0.652**
Log_{10} [Pb]								
Area	**7.32**	**1**	**7.322**	**25**	**0.148**	**49.49**	**0.001**	
Phorophyte (Area)	**3.36**	**15**	**0.224**	**244**	**0.069**	**3.27**	**0.001**	**0.148**
Log_{10} [Zn]								
Area	**4.31**	**1**	**4.307**	**20.6**	**0.257**	**16.74**	**0.001**	
Phorophyte (Area)	**6.36**	**15**	**0.424**	**233**	**0.079**	**5.4**	**0.001**	**0.262**
Log_{10} [Cd]								
Area	3.81	1	3.809	16.5	1.836	2.07	0.168	
Phorophyte (Area)	**50.87**	**15**	**3.391**	**245**	**0.182**	**18.63**	**0.001**	**0.574**

Note: Bold type indicates $p < 0.05$.

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