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APPLICATION OF OPTICAL SPECTROSCOPY FOR THE ANALYSIS OF PHYSIOLOGICAL CHARACTERISTICS AND ELEMENTAL COMPOSITION OF LICHENS OF THE GENUS *Hypogymnia* WITH DIFFERENT DEGREES OF ANTHROPOTOLERANCE

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The main physiological and biochemical characteristics and elemental composition of three lichen species of the genus Hypogymnia (Nyl.) Nyl. in one habitat were studied using spectroscopic methods. The model species were placed in the following order of decreasing degree of anthropotolerance: H. physodes (L.) Nyl. → H. tubulosa (Schaer.) Hav. → H. vittata (Ach.) Parrique. The contents of chlorophylls a and b, phenolic compounds, pheophytinization quotient, and antiradical activity were determined by a spectrophotometric method. The antioxidant activity was determined by an amperometric method. The physiological and biochemical parameters for each of the three species corresponded to those for background ecotopes. These parameters and the integrity of the system of correlations between the parameters were lower in species with a low degree of anthropotolerance. Twenty-three elements were found in thalli of the model species using atomic emission spectroscopy with inductively coupled plasma. They included macro- and microelements, heavy metals, and metalloids. The maximum concentrations of most elements were found in H. vittata; the minimum concentrations, in H. physodes. An analysis of the interaction between the physiological and biochemical characteristics and the contents of the elements indicated the presence of a complex system of correlations in each species. Differences in this system of correlations may have been due to the specific composition of secondary metabolites, which determine the features of adaptive reactions. The use of various optical spectroscopy methods enabled an evaluation of not only the functional specificity of the studied species but also its connection to their anthropotolerance level. Low resistance to anthropogenic influences was combined with lower coordination of physiological and biochemical characteristics and low integrity of the system of correlations. The most vulnerable species H. vittata had the minimal values of the main functional parameters, a poorer correlation of them with the elemental composition, and higher concentrations of some toxic elements. The use of a complex analysis of the physiological and biochemical characteristics and elemental compositions using various spectral methods was crucial for the bioindication and ecological physiology of lichens.

Keywords: *optical method, spectroscopic method, atomic emission spectroscopy with inductively coupled plasma, biomonitoring, elemental analysis, heavy metal, metalloid, Hypogymnia physodes, Hypogymnia tubulosa, Hypogymnia vittata, epiphytic lichen, indicator species, chlorophyll, phenolic compound, pheophytinization quotient, antioxidant activity, antiradical activity.*

Introduction. Optical spectroscopic methods are becoming increasingly interesting for studying lichen indicators and the ecological physiology of lichens. These methods can produce detailed and accurate information on the physiological-biochemical parameters and elemental composition of lichens [1, 2]. The results of such studies are considered in assessments of environmental pollution levels and revealing the adaptation mechanisms of living organisms to ecotoxins. However, the effects of anthropogenic impacts on ecosystems are highly variable. The pace of transformation of the plant cover and contraction of forested areas is quickening [3], which promotes a cardinal change in microclimate conditions and leads to

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the disappearance of many epiphytic lichens. The species most sensitive to this type of anthropogenic impact are considered indicators of biologically valuable forests (BVFs) [4]. Their physiology and possible reactions to releases of ecotoxins into the environment rarely become the subject of special studies [5–10]. Such research on lichen indicators of terrestrial industrial pollution is rapidly developing and complements ecological monitoring programs.

It seemed interesting to compare physiological features and interaction mechanisms with ecotoxins of various groups of indicator species. The elaboration of this problem presupposes concomitant analysis of comprehensive information on the physiological-biochemical parameters and elemental composition. For this, a complex of spectroscopic methods must used [11]. This direction is associated with detailed studies of some closely related model lichen species with varying anthropotolerance representing various indicator groups [11, 12]. Such research can be performed only with a broad arsenal of spectroscopic methods, the optimal choice of which is an important independent task. The results enable an evaluation of the possible role of optical spectroscopy in the development of lichen bioindication and ecology. They are especially critical for revealing the specifics of compensatory reactions of various lichen groups and adaptation mechanisms to anthropogenic impacts [1, 2].

The search for model taxons and territories creates definite difficulties. The genus *Hypogymnia* (Nyl.) Nyl. was identified with respect to representativeness of various groups of indicator species with varying tolerance to anthropogenic impacts. *Hypogymnia physodes* (L.) Nyl. is a traditional biomonitoring species because of its broad distribution and ability to grow with moderate industrial pollution [13, 14]. *Hypogymnia tubulosa* (Schaer.) Hav. is a rarer species that is used as an indicator of the overall pollution level and anthropogenic disruption of ecosystems [11]. *Hypogymnia vittata* (Ach.) Parrique is the most vulnerable lichen and is often considered a specialized species (indicator) of BVFs [4, 15]. It is included in the Red Book of several regions of European Russia [16–22]. The probability of these three species growing together is extremely low. However, ecosystems in which *H. physodes*, *H. tubulosa*, and *H. vittata* are encountered together do exist in Tver Region within the Central Forest State Nature Biosphere Reserve (CFSNBR) [23]. This enables a comprehensive analysis of the physiological features and elemental compositions of these species.

The aim of the present work was a comparative analysis of the relationship of the physiological characteristics and elemental compositions of three species of the genus *Hypogymnia* with different degrees of anthropotolerance based on data obtained using spectral and amperometric methods. For this, the main physiological-biochemical parameters and their correlations in the model species were determined. The elemental compositions of thalli were found using atomic emission spectroscopy with inductively coupled plasma (ICP-AES). The physiological-biochemical characteristics and elemental contents were analyzed. The nature of the dependence of the degree of anthropotolerance on the functional activity level and specifics of the system of correlations was elucidated.

Experimental. Three lichen species in the genus *Hypogymnia* were studied. These were *H. physodes*, *H. tubulosa*, and *H. vittata*, which differ in the degree of tolerance to anthropogenic transformation of ecotopes and are extremely rarely encountered within a single ecological niche. *Hypogymnia physodes* is most distributed and resistant to pollution and disturbance of biocenosis structure [1, 2, 14]. It is a common species in urban recreational areas. *Hypogymnia tubulosa* is sporadically found, extremely rarely remaining in urban ecosystems, and rather sensitive to anthropogenic successions of forest communities [11, 23]. *Hypogymnia vittata* is a very rare vulnerable species that is extremely sensitive to anthropogenic transformation of natural ecosystems and changes in microclimate conditions [4]. It is distributed exclusively in large integrated forest wetlands with consistently humid air and is included in Red Books of various regions of northwestern European Russia [4, 16–22].

Samples of lichens were collected on August 12, 2020, in Tver Region in the CFSNBR on the boundary of the 63rd and 76th quarters of the South Forest (55°30′06.4″ N, 32°54′55.3″ E). All three species grew in a marshy sphagnum fir forest with birch within a forest-wetland situated on the divide between the sources of the Mezha and Tyuz′ma rivers away from anthropogenic pollution sources. The model biocenosis had an average illumination level and stable high atmospheric humidity. Samples of *H. physodes*, *H. tubulosa*, and *H. vittata* were taken from stump bark of *Betula pubescens* Ehrh. A total of 7–10 samples of each species were studied for a total of ~30 samples.

The physiological-biochemical characteristics were analyzed in the Laboratory of Natural Antioxidants at the education and science cluster of the Institute of Medicine and Science of Life, Immanuel Kant Baltic Federal University (Kaliningrad, Russia) using previously reported standard methods [1, 2, 24]. A spectrophotometric method was used to determine the contents of chlorophylls *a* and *b* (Chl*a* and Chl*b*) and phenolic compounds (PC). The pheophytinization quotient (PQ) was calculated. The antioxidant activity (AOA) was evaluated. The antiradical activity (ARA) was evaluated using an amperometric method.

The elemental composition [macro- and microelements, heavy metals and metalloids (HM)] in lichen samples was determined using an iCAP 6300 Duo ICP-AES spectrometer (Thermo Scientific, USA) using the standard procedure in the CCU Laboratory of Biotechnology Measurements, Tver State University (Tver, Russia) [1, 2, 25]. The results for the element contents were compared to the background values for Tver region [13].

Results were statistically processed. The main parameters [number of samples of a given set, average, standard deviation, coefficients of variation and Pearson correlation, Student *t*-criterion, ANOVA with Tukey criterion (HSD), etc.] were determined using standard mathematical statistics methods and licensed Microsoft Office Excel 2013 and IBM SPSS Statistics 23 programs.

Results and Discussion. *Physiological-biochemical characteristics.* The average total content of chlorophylls (Chl*a* and Chl*b*) in the samples of the studied *Hypogymnia* species varied from 0.61 to 0.87 mg/g of dry mass, which was indicative of background conditions in the ecotope. The Chl*a* content was greater ($p \leq 0.05$) than that of Chl*b* in all samples. This agreed with the literature data [2, 24, 26]. The most labile element of the photosynthetic system (Chl*a*) demonstrated the greatest variability (from 0.38 to 0.54 mg/g of dry mass) (Table 1). The average concentrations of Chl*b* varied less, from 0.23 to 0.33 mg/g of dry mass. The studies species could be placed in the following order of decreasing total chlorophyll contents (Chl*a* + Chl*b*) and the average concentrations of Chl*a* and Chl*b*: *H. physodes* → *H. tubulosa* → *H. vittata*.

The calculated PQ values, which reflected the extent of degradation of the pigment system components, were constant for the model species, indicating that pollution did not have a distinct impact on the photosynthetic system in the collection area. The average PQ values of the lichens corresponded to the background parameters, 0.95–0.98 (Table 1) [2, 24].

Parameters PC, AOA, and ARA were correlated with protective reactions and tolerance to various effects (Table 1) [2]. Specific metabolites of the lichens included aromatic (phenolic) compounds such as physodic acid and depsides and depsidones, high contents of which were characteristic of all *Hypogymnia* species and differentiated them from other lichen taxons [27–32]. Depsidones and anthraquinones possess pronounced AOA and ARA and are involved in protective reactions [32–34]. These reactions protect from the harmful effects of UV radiation and high-intensity light and help to detoxify metals by forming complexes with their cations [35, 36]. The quantitative differences in these parameters reflected the tolerance level of the lichens to various environmental changes, including those associated with pollution [37, 38].

In general, the compositions of secondary metabolites of the model *Hypogymnia* species were similar [27, 28, 31, 32, 39–41]. The upper crust of the thallus contained atranorin and chloroatranorin. The main component in the core was physodic acid. The differences were related to the composition and content of depsidones, primarily secondary ones [27, 28, 31–33, 42]. The characteristic ones for *H. physodes* were physodalic and protocetraric acids. *Hypogymnia tubulosa* and *H. vittata* contained oxyphysodic acid. Paraphysodic acid was specific for *H. tubulosa*; vittatolic acid, for *H. vittata* [10, 27, 28, 42].

The parameters associated with the protection system (PC, AOA, ARA) to a certain extent reflected the anthropotolerance of the model species. The extremely vulnerable and rare lichen *H. vittata*, which is encountered exclusively in large anthropogenically undisturbed forest-wetland areas, had the minimal average PC content (12.94 ± 0.89 mg/kg) (Table 1). This species also had the lowest level of antioxidant protection. The average AOA (0.89 ± 0.04 mg-eq. quercetin/g) and ARA values (14.06 ± 0.95 mg-eq. ascorb. acid/g) were considerably less than for the other studied species. The maximal average PC content was observed in *H. physodes* (21.37 ± 0.15 mg/g of dry mass). This species is most broadly distributed, tolerant to various types of anthropogenic impacts, and stable in urban ecosystems. Its antioxidant potential was significant as compared to the other lichens [29, 31, 43]. The average AOA and ARA parameters for *H. physodes* were almost twice those for *H. vittata*.

Correlation analysis enabled a comparison of the degree of correlation of the physiological characteristics of the model species. *Hypogymnia physodes* showed the greatest number (10) of significant correlations between the physiological-biochemical parameters; *H. vittata*, the least (7) (Fig. 1). Strong direct correlations dominated for *H. physodes*; inverse correlations, often of lesser strength, for *H. vittata*.

The model species had different degrees of correlation of the physiological-biochemical characteristics (Fig. 2). A common feature was the correlation of the protection system parameters AOA–ARA, PC–AOA, and PC–ARA (indirectly through a correlation with AOA for *H. vittata*).

A correlation of the parameters related to protective reactions and the main characteristics of the photosynthetic system components was found (Figs. 1 and 2). All studied species exhibited clear correlations of the protection system parameters and the Chl*a* concentrations, i.e., Chl*a*–PC, Chl*a*–AOA, and Chl*a*–ARA (indirectly through a correlation with AOA for *H. tubulosa*). Significant correlations between the Chl*b* content and the protection system parameters

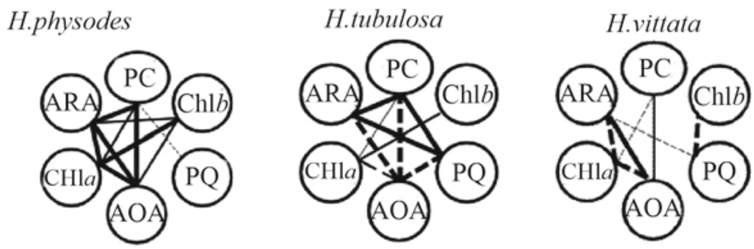

Fig. 1. Correlations of physiological-biochemical parameters of *Hypogymnia* species: direct, solid lines (thick, $r = 0.90–1.0$; medium, $r = 0.80–0.89$; thin, $r = 0.70–0.79$); inverse, dashed lines (thick, $r = –1.0$ to $–0.90$; medium, $r = –0.89$ to $–0.80$; thin, $r = –0.79$ to $–0.70$).

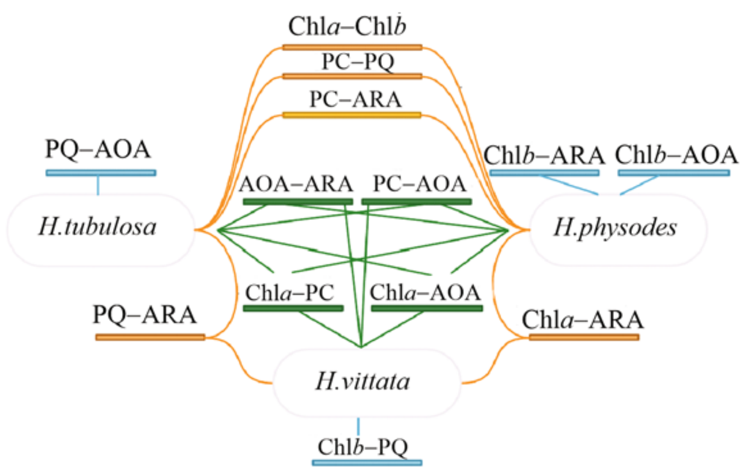

Fig. 2. Significant correlations between physiological-biochemical parameters of *Hypogymnia* species: correlations with $r = 0.70–1.0$ and $r = –1.0$ to $–0.70$ are considered.

TABLE 1. Average Physiological-Biochemical Parameters of Model Species of the Genus *Hypogymnia*

Lichen species	Chl*a*, mg/g of dry mass	Chl*b*, mg/g of dry mass	PQ	PC, mg/g of dry mass	AOA, mg-eq. quercetin/g	ARA, mg-eq. ascorb. acid/g
H. physodes	0.54 ± 0.04	0.33 ± 0.03	0.95 ± 0.02	21.37 ± 0.93	1.66 ± 0.11	23.30 ± 0.69
H. tubulosa	0.51 ± 0.05	0.28 ± 0.03	0.96 ± 0.03	18.49 ± 0.54	1.43 ± 0.02	21.27 ± 0.64
H. vittata	0.38 ± 0.06	0.23 ± 0.03	0.98 ± 0.03	12.94 ± 0.89	0.89 ± 0.04	14.06 ± 0.95

were found only for *H. physodes* (Chl*b*–AOA, Chl*b*–ARA). Similar correlations were not found for *H. tubulosa* and *H. vittata*.

The specifics of the correlation of photosynthetic system characteristics and protection system functioning parameters were responsible for the different adaptation and indicator capabilities of the studied species [2, 24]. Compensatory reactions occurred if the Chl*a* level changed. Adaptation to stress conditions, including environmental pollution, also occurred. Correlations of the Chl*a* concentration and the PC, AOA, and/or ARA content promoted protective reactions. The Chl*b* content depended largely on the microclimate conditions and the features of the ecological niches in which the lichens grew [24]. The lack of significant correlations of the Chl*b* concentration and the protection system parameters with disturbance of natural habitats (e.g., clearcutting) limited the adaptation capabilities of *H. tubulosa* and *H. vittata* and was responsible

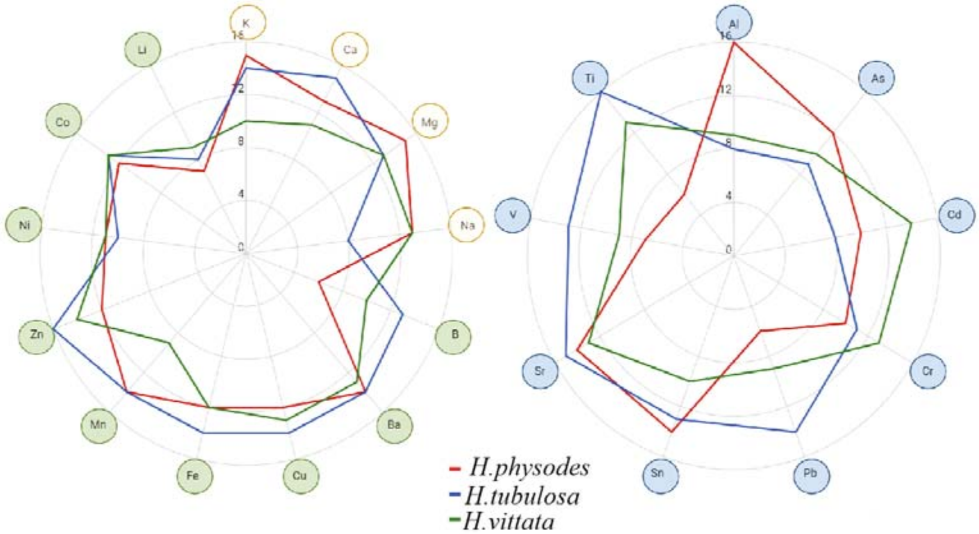

Fig. 3. Total number of significant correlations (r = 0.70–1.0, r = –1.0 to –0.70) between levels of element contents in *Hypogymnia* species: Number of correlations corresponds to a certain radius length: ● HM, ● macroelements, ● microelements.

for their vulnerability. As a rule, *H. tubulosa* was found in undisturbed natural communities. *Hypogymnia vittata* could grow only in large anthropogenically naïve forest-wetland areas with stable atmospheric humidity. The clear correlation of the photosynthetic system (Chl*a* and Chl*b*) and protection parameters of *H. physodes* determined its greater capability to adapt to environmental pollution and high tolerance to a change of microclimate habitat conditions as compared to the other species.

Thus, the values of the main photosynthetic system parameters (Chl*a*, Chl*b*, PQ) of the three *Hypogymnia* species from the same location indicated that it represented a background ecotope. The PQ values confirmed that industrial pollution had no clear effect on the pigment system functioning. The model species could be grouped into the following order according to decreasing photosynthesis (Chl*a* + *b*, Chl*a*, Chl*b*) and protection parameters (PC, AOA, ARA), reduced integrity of their correlations, and degree of anthropotolerance: *H. physodes* → *H. tubulosa* → *H. vittata*. *H. physodes* with the greatest main physiological-biochemical parameters as compared to the other species showed the most coordinated and integral system of strong correlations. These features were combined with a broad adaptation potential and maximal anthropotolerance. *Hypogymnia vittata* had the lowest analogous parameters and least integral system of correlations combined with the greatest sensitivity to variants of anthropogenic effects and extremely high vulnerability. *Hypogymnia tubulosa* occupied a middle position with respect to the degree of manifestation of all these properties. The composition of depsidones characteristic of *H. physodes* and *H. vittata* differed more substantially that its specificity level in *H. tubulosa* relative to these species [27, 28, 42]. This was responsible for the urgency of a more detailed analysis of the physiological and systemic dependence of the internal lichen anthropotolerance mechanisms, which could reach a qualitatively different level for assessing the indicator potential of the various species.

Elemental composition. Twenty-three elements were identified in samples of *Hypogymnia* lichens using ICP-AES analysis. They included macro- (Ca, K, Mg, Na) and microelements (B, Ba, Cu, Fe, Li, Mn, Zn) and heavy and potentially toxic metals and metalloids (HM) (Al, As, Cd, Cr, Co, Ni, Pb, Sn, Sr, Tl, V, W) (Table 2). The spectra of elements were very similar for the model species. However, tungsten (W) was found only in *H. tubulosa* and not in samples of *H. physodes* and *H. vittata*. The concentrations of all elements were within the values for background areas [11, 44]. The model species differed in the levels of element contents. Samples of *H. vittata* had the greatest number of elements (9) with the maximum concentrations. The numbers of these elements were lower for the other species (*H. physodes* 7, *H. tubulosa* 6) although the maximum average concentrations of Cd, Pb, and As were observed. The differences in the HM contents found in the model species indicated they had different absorption capacities for certain metals and metalloids.

Many significant direct and inverse correlations (r = 0.7–1.0, r = –1 to –0.7) were found among the levels of element contents (Fig. 3). The total number of such correlations ranged from 251 (*H. physodes* and *H. vittata*) to 288

TABLE 2. Average Concentrations of Elements in Samples of *Hypogymnia* Species (mg/kg)

Element	*H. physodes*	*H. tubulosa*	*H. vittata*
Heavy metals and metalloids			
Al	252.0 ± 10.6	231 ± 13.1	311 ± 11.1
As	4.22 ± 0.18	4.45 ± 0.07	3.95 ± 0.09
Cd	0.55 ± 0.02	0.46 ± 0.02	0.23 ± 0.02
Cr	3.54 ± 0.13	3.57 ± 0.06	4.12 ± 0.06
Pb	3.95 ± 0.08	2,81 ± 0.03	3.28 ± 0.04
Sn	2.27 ± 0.04	2.17 ± 0.03	2.53 ± 0.03
Sr	6.90 ± 0.18	7.51 ± 0.21	6.9 ± 0.27
V	1.39 ± 0.02	1.38 ± 0.01	1.03 ± 0.01
Ti	14.4 ± 0.44	14.0 ± 0.42	18.4 ± 0.40
W	–	0.61 ± 0.03	–
Macroelements			
K	2495 ± 18	2251 ± 26	1729 ± 32
Ca	4397 ± 64	4493 ± 86	3512 ± 74
Mg	541 ± 11.0	619 ± 14.3	733 ± 16.3
Na	17.14 ± 0.22	20.91 ± 0.27	0.62 ± 0.27
Microelements			
B	1.6 ± 0.02	2.63 ± 0.02	1.03 ± 0.02
Ba	24.0 ± 0.43	23.0 ± 0.81	32.3 ± 0.85
Cu	2.95 ± 0.02	2.25 ± 0.2	2.40 ± 0.2
Fe	280 ± 6.3	218 ± 3.8	328 ± 5.3
Mn	773 ± 13.4	464 ± 16.1	539 ± 15.9
Zn	106 ± 1.7	86 ± 1.85	72 ± 2.42
Ni	1.21 ± 0.02	0.93 ± 0.03	1.55 ± 0.02
Co	0.01 ± 0.00	0.17 ± 0.01	0.11 ± 0.01
Li	0.16 ± 0.01	0.16 ± 0.01	0.23 ± 0.01

(*H. tubulosa*). The maximal number of significant correlations between macroelement concentrations (56) was observed for *H. physodes* as compared to the other species; the minimal, between the contents of microelements and HM (100 and 95). The numbers of significant correlations between the concentrations of microelements and HM were greater for the other species (*H. tubulosa*, 116 and 122; *H. vittata*, 104 and 100).

The maximum levels of correlations with other elements for the macroelement group were noted for Cd, Ca, and Mg (up to 15 correlations); among the microelements, for Ba, Mn, Zn, Fe, and Cu (up to 13–14 correlations); among HM, for Sn, Sb, and Al (up to 12–15 correlations). The most elements with the maximal number of significant correlations occurred in *H. tubulosa* (12 elements). They included only one macroelement (Ca), almost all microelements (7 elements), and half the HM, including toxic Pb. The least elements with the maximal number of significant correlations were found in *H. vittata* (6 elements). They included one macroelement (Na), several microelements (Ni, Co, Li), and HM (Cd, Cr).

Thus, the analysis of the elemental composition of the model *Hypogymnia* species identified 23 elements, including macro- and microelements and HM. The element contents corresponded to values for background ecotopes. The maximum concentrations of most elements were observed in samples of *H. vittata*. The greatest number of significant correlations

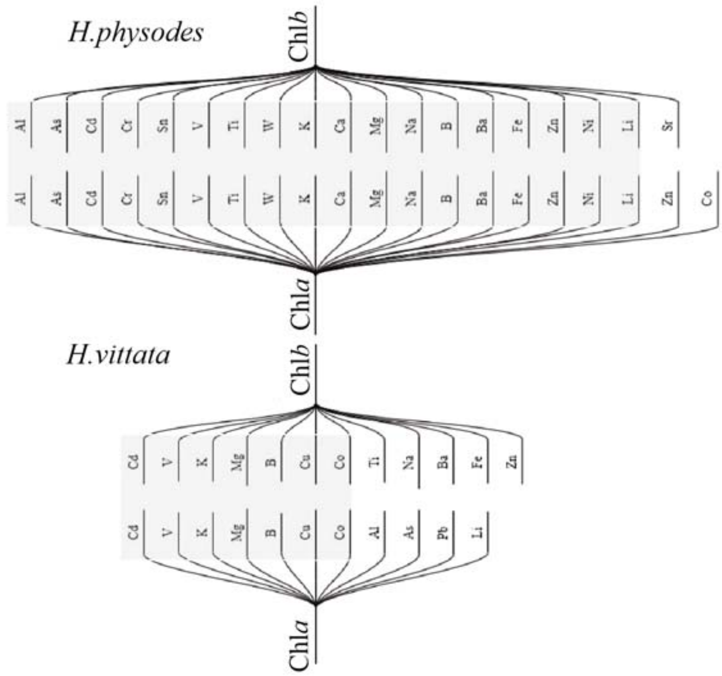

Fig. 4. Composition of elements, the contents of which show significant correlations (r = 0.70–1.0, r = −1.0 to −0.70) with pigment system parameters (Chl*a* and Chl*b*) in model *Hypogymnia* species.

between element contents was found for *H. tubulosa* (288). The species specificity of correlations between elements was indirectly due to the determined degree of anthropotolerance. The maximal number of significant correlations for the macroelements was found for *H. physodes* (56); for microelements and HM, for *Hypogymnia tubulosa* (116 and 122) and *Hypogymnia vittata* (104 and 100).

Correlations of physiological-biochemical characteristics and elemental composition. An analysis of the dependence of the functional parameters and quantitative characteristics of the elemental composition revealed complicated systems of correlations for the model species. The first group of correlations concerned the contents of elements and the photosynthetic system parameters (Chl*a*, Chl*b*, PQ); the second, protection system parameters (PC, AOA, ARA).

The photosynthetic system parameters (Chl*a*, Chl*b*, PQ) were correlated with the contents of various elements (Fig. 4). The Chl*a* concentration had an important coordinating significance and was correlated with the parameters most element contents. The coordinating role of Chl*b* was weaker. The common elements for all model species that exhibited significant correlations with the Chl*a* concentration were K, Mg, B, and Co; with Chl*b*, Na, V, and Cd. The correlations with the content of these elements were explained by their roles in organizing cellular structures and functions and inclusion in the most important chemical compounds [45]. Magnesium was a component of the chlorophyll molecule. Cobalt affected the formation and functioning of the photosynthetic apparatus [46]. Potassium and boron helped to enhance photosynthesis, participating in ATP generation. The effects of Na, V, and Cd on the Chl*b* level have been reported [47].

The degree of correlation of the pigment system characteristics and the elemental composition was species specific, which was responsible for the different tolerances of the species. A reduction in the correlation level was associated with weakened resistance to anthropogenic impacts. Considering this, the model species could be grouped into the following order of decreasing number of significant correlations with elements and degree of anthropotolerance: *H. physodes* (Chl*a* 19, Chl*b* 18) → *H. tubulosa* (Chl*a* 15, Chl*b* 10) → *H. vittata* (Chl*a* 11, Chl*b* 12). Strong correlations of the physiological parameters with the concentrations of the noted macroelements (Ca, K, Na, Mg) were observed only in *H. physodes*. The content of Ca, which plays an important role in reducing HM toxicity, was correlated in *H. physodes* with the Chl*a* and Chl*b* concentrations, which helped to increase the resistance of this species in urban ecosystems [11, 23, 48]. The Ca content in *H. tubulosa* correlated only with the Chl*a* concentration; in *H. vittata*, significant correlations of this element with the pigment system parameters were not found.

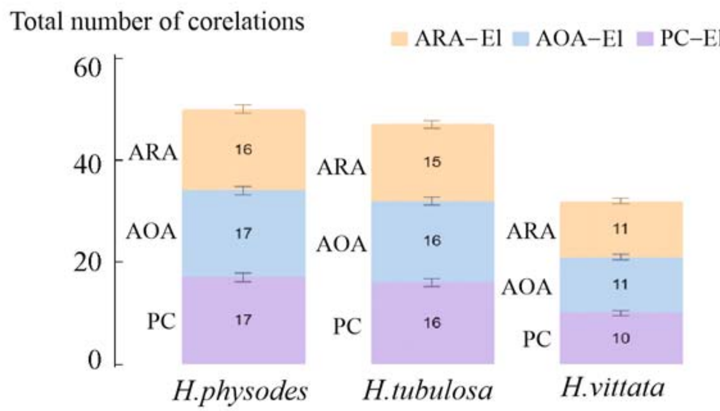

Fig. 5. Total number of significant correlations (r = 0.70–1.0 and r = −1.0 to −0.70) of elemental composition with protection system characteristics (PC, AOA, ARA) of studied *Hypogymnia* species.

The photosynthetic system was highly coordinated with a set of elements significantly like those whose concentrations correlated with the pigment contents. For example, the chlorophyll (Chl*a* and Chl*b*) concentrations in *H. physodes* and *H. tubulosa* showed strong direct correlations, which was due to their similar compositions of elements, the concentrations of which correlated with the contents of these chlorophylls (Fig. 4). The lack of analogous significant correlations between Chl*a* and Chl*b* in *H. vittata* was determined by the different set of elements correlated with these chlorophylls. The PQ, which reflected the degree of degradation of chlorophyll, for the rare and extremely vulnerable species *H. vittata* correlated under the background ecotope conditions with the contents of the maximal number of elements (11). They included most HM (Al, As, Cd, Cr, Pb, Sn, Sr). The number of elements correlated with PQ was minimal (7) for *H. physodes*. The element for which strong correlations with PQ were found for all model species was Pb.

An analysis of the correlations of the elemental composition with the functional parameters characterizing the protective reactions (PC, AOA, ARA) was interesting. The studied species could be grouped according to decreasing number of such significant correlations in the order: *H. physodes* (50) → *H. tubulosa* (47) → *H. vittata* (32) (Fig. 5).

The greatest number of elements were correlated with PC and AOA. The set of common elements, the contents of which correlated with PC, included for all model species toxic HM (Al, As, Cr, V) and macro- and microelements (Ca and Mn). Four common elements, the concentrations of which correlated with the AOA level, were Cr, Sn, K, and Ca. Ca for all species showed significant correlations with all parameters of the protection system (PC, AOA, ARA). Its involvement in protective reactions with environmental pollution has been noted several times. A high Ca content helped to reduce the toxicity of metals [48].

Like for the photosynthetic system, the parameters of the protection system showed a higher degree of coordination if the set of elements was similar to the elements with which they were correlated. *Hypogymnia physodes* had the maximal coordination with the protection system as compared with the other model species [r(PC–AOA) = 0.9, r(PC-ARA) = 1.0, r(AOA–ARA) = 1.0] and was correlated with a similar set for most elements correlating with the parameters of this system (14) (Figs. 1 and 6). *Hypogymnia tubulosa* was also characterized by rather high coordination of parameters associated with protective reactions and similar elements correlated with them. This species also showed significant correlations with Cu and Fe (Fig. 6). *Hypogymnia vittata* with the minimally integrated protection system had the lowest number of elements correlated with it (8). They included primarily toxic HM (Al, As, Cr, Pb, Sn) and only certain macro- and microelements (Ca, Mn, Li).

Differences in the sets of elements correlating with the protection system parameters were connected to the degree of tolerance of the species. All parameters of the protection system (PC, AOA, ARA) of the broadly distributed lichen *H. physodes* correlated with the contents of all macroelements (Ca, K, Mg, Na), most HM (Al, As, Cd, Cr, Sn, Ti, V), and certain microelements (Ba, Li, Zn) (Fig. 6). All parameters of the protection system of *H. tubulosa* correlated primarily with microelements (Ba, Cu, Fe, Mn, Zn), only two macroelements (Ca, K), and a few HM (Sr, Ti, V). *Hypogymnia vittata*, in contrast to the other species, exhibited correlations mainly with toxic HM (Al, As, Cr, Pb, Sn) and significant correlations with very few macro- and microelements (Ca, Mn, Li).

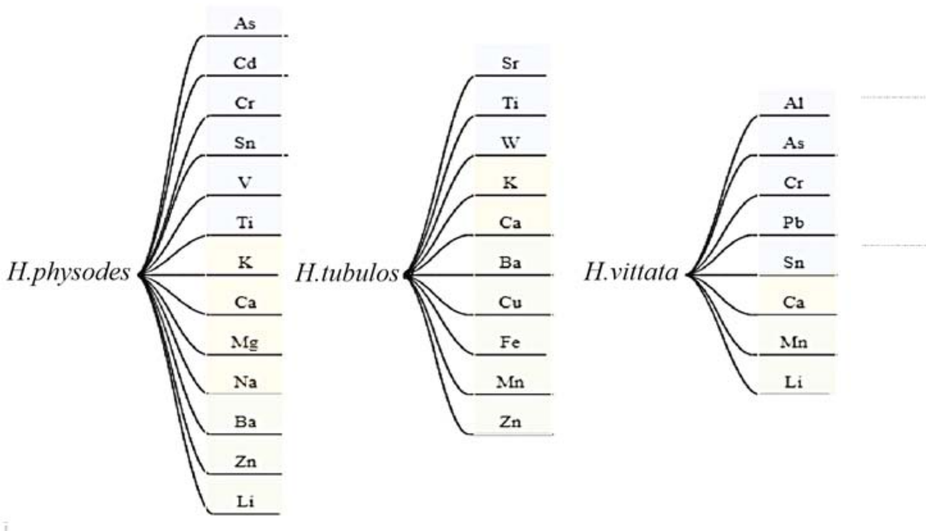

Fig. 6. Composition of total elements, the contents of which show significant correlations (r = 0.70–1.0, r = –1.0 to –0.70) with protection system parameters (AOA, PC, ARA) of *Hypogymnia* species: ● HM, ● macroelements, ● microelements.

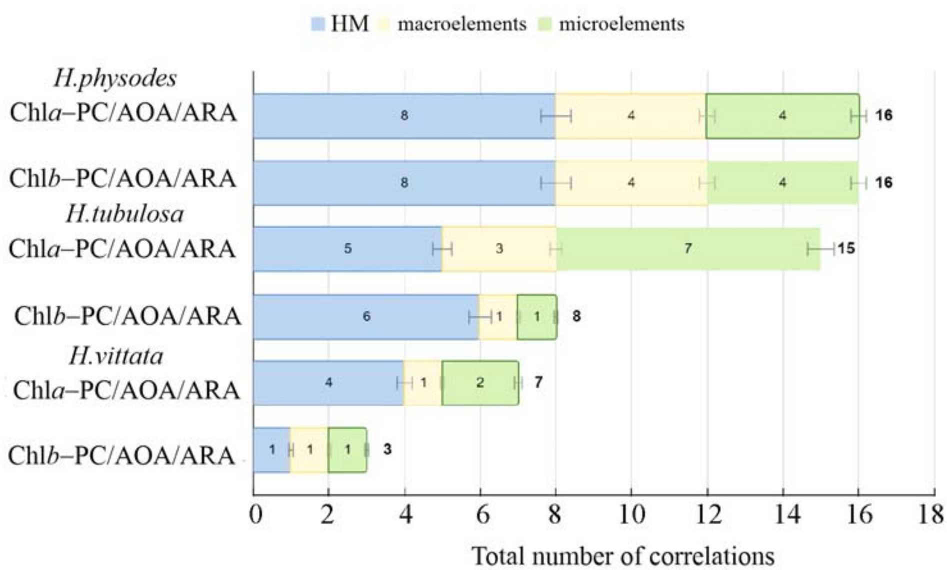

Fig. 7. Number of significant correlations (r = 0.70–1.0, r = –1.0 to –0.70) of the set of total elements combined with pigment contents (Chl*a* and/or Chl*b*) and protection system parameters (PC, AOA, ARA) of *Hypogymnia* species: number of correlations for each groups of elements and their sum is shown.

Thus, the different degrees of anthropotolerance of the model species could be attributed to the correlation of the element-composition components and the parameters of the photosynthesis (Chl*a* and/or Chl*b*) and protection systems (PC, AOA, ARA) related to them (Fig. 7). The high degree of coordination of the functional system of *H. physodes* was connected to a set of elements, the contents of which correlated with the physiological-biochemical parameters. This set included all macroelements (K, Ca, Mg, Na), most HM (all except Pb and Sn), and several microelements (Ba, Zn, Li). A similar dependence appeared in the significant resistance of this species to various anthropogenic impacts, including industrial environmental pollution and transformation of habitat microclimate conditions. The analogous set of elements for *H. tubulosa* had fewer HM (although including Pb, Sn, Sr) and macroelements but many more microelements. The physiological-biochemical characteristics of *H. vittata* were correlated on a lower level with an insignificant number of elements correlating with them. It included primarily toxic HM (Al, As, Pb, V) and several macro- and microelements.

Potential for development of complex analysis of lichens with different tolerances. The results indicated that similar species of the single genus *Hypogymnia* could differ considerably in physiological features and absorption capacity for various elements and in the multifaceted and diverse problems associated with a comprehensive study of various groups of indicator species and the factors and mechanisms responsible for anthropotolerance. These problems involved primarily the physiological and systemic bases of the tolerance of living organisms. Special research on secondary metabolites and different variations of the interaction of lichens with ecotoxins, including absorption and detoxification, is needed for the first aspect. The second aspect is associated with the development of approaches to an evaluation of the degree of functional integrity and coordination of biosystems, including the use of correlation analysis.

Further complex analysis using various spectral and physicochemical methods of the physiological-biochemical characteristics and elemental composition of lichens with different degrees of anthropotolerance is crucial. This direction facilitates a systemic approach to fundamental problems of ecological physiology and bioindication and enables the fusion of interdisciplinary methods that are necessary to achieve a qualitatively different level of understanding of the adaptation mechanisms and internal factors providing anthropotolerance to lichens. Approaches allowing the production of significant volumes of diverse data on various physiological-biochemical parameters and the elemental composition are necessary for effective development of this direction. A broad arsenal of spectroscopic methods could reach a high level of clarity for such complex research. Full use of them would create a potential role for each method and the principles for selecting the optimal combinations of them.

Further comparative studies of various groups of lichen indicator species, i.e., indicators of industrial pollution of territories and BVFs, are advisable. The last group is practically unstudied. The search for model taxons in which several closely related species with different degrees of anthropotolerance, including different types of indicators, could be selected is crucial. In this respect, the family Parmeliaceae Zenker is interesting. The genus *Hypogymnia* is systematically close to other rapidly disappearing representatives of this family, including *Menegazzia terebrata* (Hoffm.) A. Massal. The physiological causes for the vulnerability of BVF indicators should be found based on a detailed analysis using the proposed approach. The adaptation mechanisms should be compared to the anthropogenic stress for various types of indicators. In the future, this could expand the set of indicator species and lead to monitoring considering the response mechanisms of living systems to a change in the natural conditions. Such research in the ecological physiology of lichens would enable on a qualitatively different level studies of the adaptability of physiological systems and processes and assess the integrity of regulatory systems.

The methodical bases for interpreting the complex system of correlations must also be developed. This is an important thrust oriented on a search for a method of adequate handling of results considering the possible unambiguous correspondence of functional and cause-and-effect relationships to the found correlations. Strong correlations may not always be due to physiological features of living organisms. In addition, not all important structural and functional signatures and properties are reflected in a system of correlations. For example, significant correlations of the contents of Mg and Fe with the concentration of photosynthetic pigments are not observed in all instances although Mg is included in chlorophyll and Fe is a component of chlorophyll biosynthesis enzymes. Also, the system of correlations for a certain species may be somewhat variable depending on various factors. Detailed studies of each model species with various physiological activity levels in habitats with contrasting ecotope characteristics and anthropogenic impact levels are needed to assess their relative roles. Apparently, the technogenic transformation of biocenoses is highly significant. Different strengths of correlations for several parameters were reported earlier by us for *H. physodes* and *H. tubulosa* in an urbanized ecotope [2]. The lichen *H. physodes*, which is a very common species and thrives in a wide range of conditions, is especially interesting for further development of correlation analysis in terms of the proposed approach.

Conclusions. Spectral and amperometric methods were used for three species of the genus *Hypogymnia*, two of which were rare and vulnerable species, in the same location to find important differences in the functional activity, nature of relationships of physiological-biochemical parameters, and their connection to the elemental composition and metal absorption level. The activity levels of the photosynthetic system and the parameters related to the functioning of the protection system were different in the studied species. These systems were coordinated and correlated differently among themselves. *Hypogymnia physodes* characteristically had the greatest functional integrity with respect to number and strength of correlations. *Hypogymnia vittata* showed the weakest coordination of physiological characteristics.

Twenty-three elements, including macro- and microelements and HM, were detected in thalli of the model species using ICP-AES. The concentrations of the elements in each of the three species corresponded to the parameters for background ecotopes. An analysis of the correlation of the physiological-biochemical characteristics, elemental composition, and contents of elements could reveal complex systems of correlations for each species that were related to the different degrees of anthropotolerance of the species. Complex analysis of the physiological-biochemical characteristics and elemental compositions using various spectral methods for closely related species with different degrees of anthropotolerance could be used for further development of bioindications and ecological physiology of lichens. Such research results could determine the resistance mechanisms of the species and the reasons for the vulnerability and were interesting for preserving biodiversity.

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