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PHOTOSYNTHETIC PIGMENTS IN *Hypogymnia Physodes* WITH DIFFERENT METAL CONTENTS

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Chlorophyll a and b contents in Hypogymnia physodes specimens collected from various economic areas and natural complexes of Tver Region were found to differ substantially using a spectrophotometric method, showing that the lichen photosynthetic system is highly adaptable. The chlorophyll b content was linked primarily to adaptation to specific environmental features in various plant communities. The chlorophyll a content changed to provide the necessary compensatory responses under technogenic stress. A total of 15 metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Ti, V, and Zn) were detected in H. physodes samples using inductively coupled plasma atomic-emission spectroscopy (ICP AES). The most widespread of them were Fe, Al, and Ti. Significant correlations among the concentrations of these metals and the chlorophyll a content were revealed.

Keywords: *photosynthetic pigments, epiphytic lichens, Hypogymnia physodes, inductively coupled plasma atomic-emission spectroscopy, spectrophotometric method, bioindication, metals, ecosystems, anthropogenically transformed areas, Tver Region.*

Introduction. Spectroscopic methods have recently been widely applied in bioindication and monitoring [1–5]. They complement considerably traditional methods for assessing the environmental quality and enable biological subjects and various natural components to be investigated [6, 7]. They can be used for comprehensive studies of the impacts of pollutants on the functioning of living systems. A joint analysis of the metal and photosynthetic pigment contents in living systems is critical to the development of this area.

Metals are one of the most widely distributed pollutant types. Several of them are toxic to biological subjects. However, information on their mechanisms of action on physiological processes is fragmented [8–10]. Accurate data on the metal and photosynthetic pigment concentrations can be obtained by using several spectral methods [2, 4]. Lichens are especially interesting for such investigations. They can absorb various pollutants to a greater extent than higher plants [11, 12]. The lichen photosyntheticsystem was confirmed to be sensitive to external influences by studying specimens collected in various regions and ecotopes [13–20]. The lichen *Hypogymnia physodes* (L.) Nyl. is often used as a model species [5, 14, 17, 21–24]. The impact of N-containing pollutants was analyzed using mostly using this species [25].

Tver Region is a convenient model area within the Central Federal District of the Russian Federation for comprehensive studies of lichens using spectroscopic methods [26]. It has a significant area (84,200 km^2), highly varied physical geography and flora, and unique natural communities [27]. The region also has a complicated business infrastructure that can be divided into five business districts (Volga-Tver, Eastern, Northern, Volga, and Western) [28]. The significant heterogeneity of Tver Region enables the selection of model ecosystems with various levels of anthropogenic impacts, including preserved natural areas, undisturbed natural complexes, and industrial zones.

The goal of the present work was a joint analysis of metal and photosynthetic pigment contents in the indicator lichen *H. physodes* collected in ecosystems with various anthropogenic impacts. The tasks included finding the specifics of the selected model territories (MTs) and collecting material for analysis; assessing the metal and photosynthetic pigment contents in the lichen samples; revealing the dependence of the parameters for chlorophylls *a* and *b* on the habitat type, level of ecosystem anthropogenic impact, and degree of metal contamination; and evaluating the bioindicative role of these data.

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TABLE 1. Characteristics of CSs of *Hypogymnia physodes*

MT	Municipal districts and population points	CS	CS type	Area, ha	CV	TPC	Degree of AD
Volga–Tver District							
1	Kalininskii, Tver	1	Komsomol′skaya Grove	498	N	D	moderate
		2	Bobachevskaya Grove	14.9	N, R	P	strong
		3	Berezovaya Grove	12	N, R	D	moderate
		4	Pervomaiskaya Grove	50	N	P	moderate
2	Konakovskii, Konakovo	5, 6	Konakovskii Forest Park	300	N	F	moderate
		7, 8				F-P	
Western District							
3	Penovskii, Peno	9–11	recreational zones	12	R	D	moderate
4	Selizharovskii, Selizharovo	12–15	recreational zones	15	R	D	D
5	Andreapol′skii, near Donskoe	16–19	forest-bog area near lake	500	N	F	undestroyed unique
6	Ostashkovskii, near Zvyagino	20–23	fragments of forest communities on lake shore	280	N	D	undestroyed unique
7	Toropetskii, near Dubinino	24–27	forest area	400	N	D–F	undestroyed unique

Note. MT, model territory; CS, collection site; CV, character of vegetation; N, natural; R, replanted (trees); TPC, type of plant community; F, fir; F–P, fir with pine; P, pine; D, deciduous forest; D–F, deciduous forest with fir; AD, anthropogenic destruction of ecosystems.

Experimental. Specimens of the epiphytic lichen *H. physodes* that were collected in the summer of 2016 were studied. The MTs were selected based on analyses of the regional industrial infrastructure, characteristics of the natural systems, and previous results [26]. The MTs were situated in seven municipal districts of Tver Region (Kalininskii, Konakovsii, Andreapol′skii, Penovskii, Ostashkovskii, Selizharovskii, and Toropetskii) that represented two business districts [Volga–Tver (VTD) and Western (WD)] (Table 1). The VTD and WD differed in industrial development, transportation network coverage, and types of basic urbanized areas [28]. The VTD was located in the southeast of the region. It was crisscrossed by key railroads and federal highways. The largest regional industrial centers were concentrated there. The district was thickly populated. The flora was significantly impacted. The area was very thinly forested. In contrast, the WD had practically no industrial sites, was dominated by small villages, preserved the flora, and was thickly forested with forests and bogs occupying large areas.

The selected MTs represented three levels of anthropogenic impacts on the flora, i.e., highly destroyed, moderately destroyed, and undisturbed (in several instances, unique with respect to biodiversity and preservation of plant communities) natural systems (Table 1). Each MT contained 3–5 collection sites (CSs). The total number of CSs in all MTs was 27. Samples (5–7) were collected at each CS to give a total of >170 samples.

The concentration of photosynthetic pigments was analyzed in the Laboratory of Natural Antioxidants of the Institute of Living Systems at I. Kant Baltic Federal University. The contents of chlorophylls *a* and *b* in the *H. physodes* samples were determined on an SF-2000 spectrophotometer at $\lambda = 665$ and 648 nm according to literature method [29]. The extractant was DMSO solution (99%). The pigment concentration in the extract (mg/L) was calculated using the formulas:

$$C_{\mathrm{Chl}\ a} = 14.85D_{665} - 5.14D_{648};\ C_{\mathrm{Chl}\ b} = 25.48D_{648} - 7.36D_{665}\ ,$$

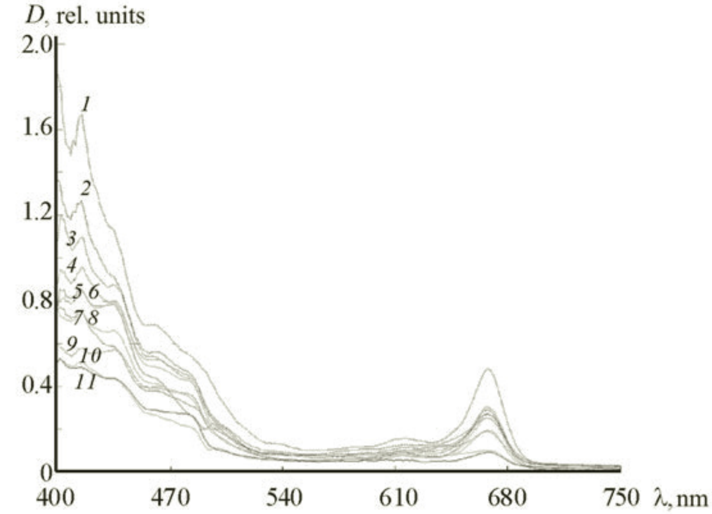

Fig. 1. Absorption spectra of pigments in DMSO extracts from *Hypogymnia physodes*: 1) Bobachevskaya Grove (CS 2); 2) Pervomaiskaya Grove; 3) Komsomol′skaya Grove (CS 1); 4) Berezovaya Grove (CS 3); 7) Konakovskii Forest Park (CS 5 and 6); 9) Konakovskii Forest Park (CS 7 and 8); 5) Selizharovo village (CS 12–15); 6) Peno village (CS 9–11); 8) near Dubinino (CS 24–27); 10) near Donskoe (CS 16–19); 11) near Zvyagino (CS 20–23).

where D_{665} and D_{648} are the extract optical densities at $\lambda = 665$ and 648 nm.

The pigment contents (mg/g of air-dried raw material) in lichen thalli were:

$$F = (VC)/P\ ,$$

where V is the extract volume (L); C, pigment concentration (mg/L); and P, weight of lichen raw material (g).

The metal contents in *H. physodes* samples were determined using an iCAP 6300 Duo ICP-AES spectrometer (Thermo Scientific, USA) at the Tver State University CCU and the standard procedure [5, 26]. Concentrations of the identified metals were compared with the background values for metal contents in Tver Region [5]. Global background values for metals and the MPC and APC of metals in soil were also considered [5, 26].

Data were processed statistically and parameters (number of samples in an actual set, average, standard deviation, variation and correlation coefficients, Student *t*-criteria, etc.) were determined using standard mathematical statistics and licensed Microsoft Office Excel 2013 programs.

Results and Discussion. Substantial differences were found in the chlorophyll *a* and *b* contents in *H. physodes* samples ($p = 0.05$) (Fig. 1). The amount of principal photosynthetic pigment (chlorophyll *a*) was greater than that of the other (chlorophyll *b*) in all samples, which agreed with the literature [16]. The average content of chlorophyll *a* was 2.9 times greater than that of chlorophyll *b* (1.40 and 0.49 mg/g of dry raw material). The average ratio of chlorophylls *a* and *b* (*a*/*b*) was 3.2. Similar average values were found for other lichen species [4, 14, 24].

The differences between the maximum and minimum chlorophyll contents were significant. The chlorophyll *a* concentration was more variable than that of chlorophyll *b* (standard deviations 0.60 and 0.16 mg/g of dry raw material). The maximum content of chlorophyll *a* was five times greater than the minimum (2.77 and 0.52 mg/g of dry raw material) with a difference of 2.25 mg/g. The analogous values for chlorophyll *b* were 2.4 times greater (0.74 and 0.31 mg/g of dry raw material) with a difference of 0.43 mg/g.

The different results obtained by the researchers indicated that the contents of chlorophylls *a* and *b* were affected by various factors. Chlorophyll concentrations were found to depend on the environmental quality of the habitats [14], which was related to the degree of ecosystem anthropogenic impact, primarily air pollution [17, 19, 30]. The ratio of chlorophyll concentrations *a*/*b* and their relative contents depended on these same factors. However, various factors determined the contents of chlorophylls *a* and *b*.

The chlorophyll *b* content depended largely on the microclimate characteristics of certain plant communities and the specifics of the lichen habitat. These factors determined the different physiological activities of the lichens and their dynamics. The chlorophyll *b* concentration was greatest (0.74 mg/g of dry raw material) in firs with very dense crowns

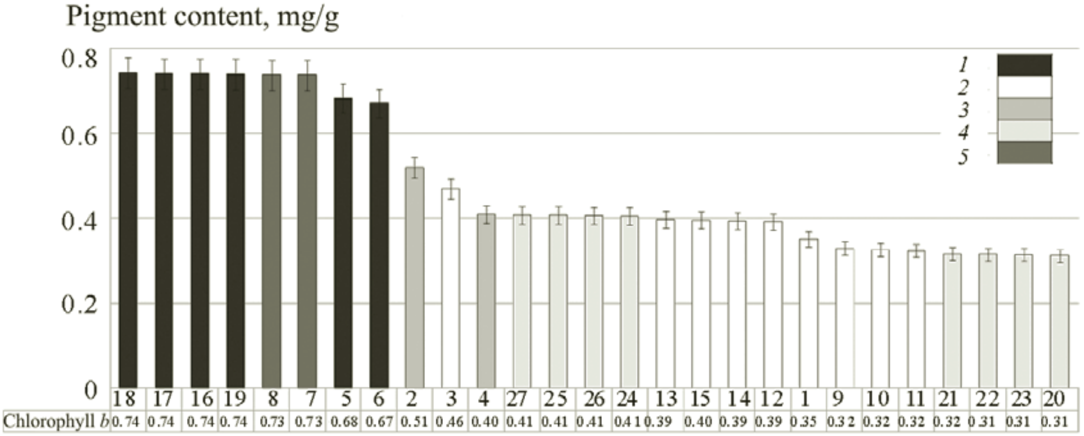

Fig. 2. Chlorophyll *b* content in *Hypogymnia physodes* samples (CS 1–27); types of plant communities in which material for analysis was collected: 1) fir; 2) deciduous forest; 3) pine; 4) deciduous forest with fir; 5) fir with pine.

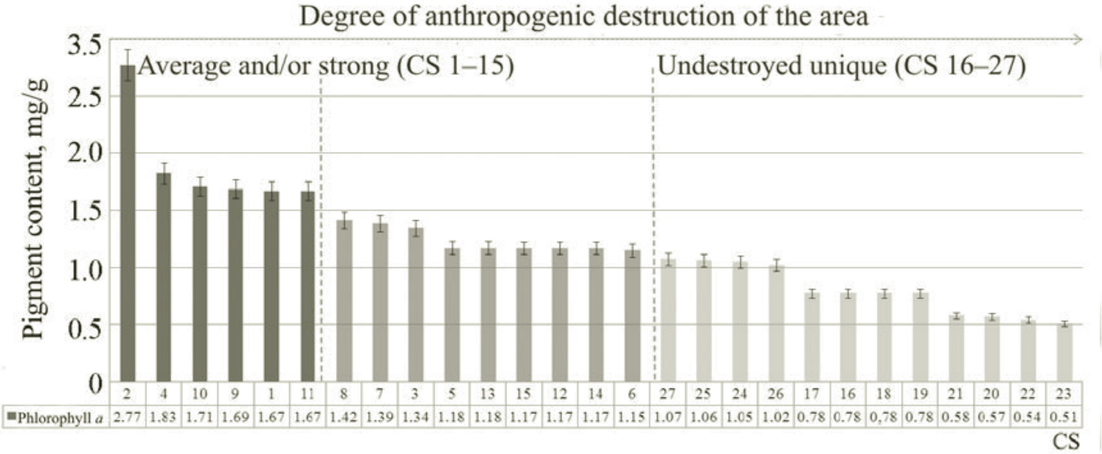

Fig. 3. Chlorophyll *a* content in *Hypogymnia physodes* samples from MT with various degrees of anthropogenic impact on the flora (CS 1–27).

(CS 16–19) (Fig. 2). Lichens under these conditions are usually physiologically active because the air is more humid than in other communities and the humidity is more consistent. The increased chlorophyll *b* content also ensures efficient functioning of the photosynthetic system in the dim lighting under the fir canopy [31]. The chlorophyll *b* contents are lower (0.31 mg/g of dry raw material) in plant communities with predominantly deciduous species (CS 20–23) than in firs. The physiological activity parameters in these communities are more variable because of the lower humidity and its inconsistency. The illumination level in microenvironments of deciduous and pine plant communities in which *H. physodes* are found is greater than that in firs.

The chlorophyll *a* contents were less dependent on the type of plant community and the lichen habitat than those of chlorophyll *b*. However, the chlorophyll *a* content increased considerably as the anthropogenic impact on the plant community increased (Fig. 3). This was especially evident in forest park zones associated with urban ecosystems. The microclimate conditions were highly unstable in such habitats, often due to increased levels of metal pollution. The chlorophyll *a* contents in samples collected in ecosystems with different levels of anthropogenic impacts (from urban industrial sites to natural areas with practically no impacts) increased from 0.51 to 2.77 mg/g of dry raw material) (Fig. 3). The chlorophyll *a* content was greatest in samples collected in the most industrially developed business district (VTD). The greatest chlorophyll *a* content (2.77 mg/g) of the MT in this business district was found in samples from Tver city (CS 2, Bobachevskaya Grove). High levels of air pollution by metals were reported previously in that area [26]. The

TABLE 2. Average Metal Concentrations (mg/kg) in *Hypogymnia physodes* Samples and Their Correlation with Chlorophyll *a* and *b* Contents

CS	As	Pb	Cd	Zn	Co	Cu	Mo	Ni	Sb	V	Al	Cr	Fe	Mn	Ti
1	1.25	3.62	0.69	89.30	0.00	30.91	0.00	0.66	0.00	2.03	828.80	4.37	1485.00	274.00	50.67
2	2.57	27.01	1.06	110.35	3.38	30.46	0.77	4.29	1.36	4.76	1292.00	7.59	2188.00	93.39	76.56
3	1.43	12.70	1.28	160.20	0.16	13.26	0.00	2.36	0.76	1.36	454.40	0.00	753.70	397.90	31.74
4	1.52	11.31	0.97	220.25	0.00	13.61	0.34	2.03	0.00	2.05	774.67	1.25	1188.00	175.90	44.90
5–6	2.04	12.98	0.94	216.00	0.50	6.66	0.00	1.91	1.84	5.22	352.53	0.00	540.30	80.45	18.23
7–8	1.36	9.89	0.58	203.40	0.10	5.09	0.00	1.41	1.16	4.76	243.46	0.00	334.40	479.80	11.44
9–11	0.78	12.68	0.88	200.80	0.00	4.76	0.12	0.00	0.00	2.22	736.00	3.32	1075.80	274.00	49.44
12–15	1.24	3.38	0.64	92.26	0.00	3.00	0.08	0.00	0.00	2.18	536.00	2.58	912.80	329.00	31.96
16–19	0.00	0.36	0.40	45.50	0.00	2.30	0.00	0.00	0.00	0.80	171.92	0.70	213.20	80.22	11.02
20–23	0.76	4.10	0.22	55.94	0.00	1.16	0.16	0.00	0.00	1.18	219.00	0.70	238.80	79.62	11.32
24–27	0.74	2.60	0.56	95.66	0.00	2.06	0.10	0.00	0.00	0.92	167.48	0.52	189.42	248.60	9.26
max. value	2.57	27.01	1.28	220.25	3.38	30.91	0.77	4.29	1.84	5.22	1292.00	7.59	2188.00	479.80	76.56
min. value	0.74	0.36	0.22	45.50	0.10	1.16	0.08	0.66	0.76	0.80	167.48	0.52	189.42	42.72	9.26
av. value	1.24	9.15	0.75	135.42	0.38	10.30	0.14	1.15	0.47	2.50	525.11	1.91	829.04	225.01	31.50
r (chlorophyll *a*)	0.76	0.84	0.64	0.35	0.75	0.78	0.78	0.76	0.33	0.51	0.93	0.79	0.92	0.04	0.91
r (chlorophyll *b*)	0.36	0.22	–0.17	0.21	0.14	–0.18	0.02	0.26	–	0.55	–0.28	–0.31	–0.29	–0.025	–0.35

lowest chlorophyll *a* concentrations were found in samples from the WD. Samples collected in undisturbed forest areas of Andreapol′skii (CS 16–19), Ostashkovskii (CS 20–23), and Toropetskii districts (CS 24–27) had especially notable values. These districts were unique with respect to preservation of old-growth forest communities that were vulnerable and protected biodiversity components.

Metal pollution was considered as a type of technogenic impact on the ecosystems in which *H. physodes* raw material was collected. The broad spectrum of metals detectable by ICP-AES enabled joint analysis of metal contents in *H. physodes* samples and chlorophyll *a* and *b* concentrations. The collected material was examined for a total of 15 metals, i.e., Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Ti, V, and Zn (VTD, 15; WD, 13 metals) (Table 2). Metals were characterized according to three environmental hazard classes as highly toxic (As, Cd, Pb, and Zn), moderately toxic (Co, Cu, Mo, Ni, Sb, and V), and slightly toxic (Al, Cr, Fe, Mn, and Ti) [26]. Correlations between their contents and those of chlorophyll *a* were found for most of them. A statistically significant relationship between the chlorophyll *b* content and particular metals or the whole spectrum of metals was not found.

Correlations of chlorophyll *a* with various metals varied in nature and strength. Several slightly toxic metals (Al, Fe, Ti) gave strong direct correlations ($0.9 < r < 1$) with the chlorophyll *a* content (Table 2). Correlations of the content and occurrence were established for these same metals. The pairs Fe–Al, Fe–Ti, and Ti–Al ($r = 0.99$) were identified. As a rule, concentration changes for the elements of each pair were linked.

A direct relationship at the next significance level ($0.7 < r < 0.9$) was found for the chlorophyll *a* concentration and Pb, Cr, Mo, Cu, Ni, As, and Co. This group had various toxicities, i.e., highly toxic (As and Pb), moderately toxic (Co, Cu, Mo, and Ni), and slightly toxic (Cr). Strong correlations ($0.7 < r < 0.9$) were found in this group of metals for the pairs Cr–Fe, Cr–Al, Cr–Ti, Cr–Co, Cr–Cu, Cu–Fe, Cu–Al, Cu–Ti, Mo–Ti, Mo–Co, Mo–Pb, Co–Pb, Co–Ni, Co–As, As–V, As–Pb, Pb–Ni, As–Ni, Sb–V, and Sb–Ni.

Significant chlorophyll *a* concentrations were observed in ecosystems with the maximum concentrations and a broad spectrum of the detected metals. The ecosystems included Bobachevskaya Grove (CS 2), where the greatest number of metals

(Al, As, Co, Cr, Fe, Mo, Ni, Pb, and Ti) with average contents above background was found [26]. The photosynthetic system adapts to elevated levels of technogenic pollution primarily by increasing sharply the chlorophyll *a* content. Such changes in the photosynthetic system properties can be viewed as a compensatory response to environmental stress. Such changes in the chlorophyll concentration could also be due to air pollution by N-containing pollutants such as ammonia, nitrogen oxides, ammonium and nitrates as dissolved species in air and as solids that were mostly immobile and transformed into each other [25, 32]. Similar results for chlorophyll contents in lichens were reported from Russia and central Europe [33, 34].

The results indicated that lichens were more tolerant than vascular plants to the principal technogenic pollutants and that the lichen photosynthetic system was more adaptable and could provide distinct compensatory responses. The photosynthesis intensity and chlorophyll concentration in vascular plants fell sharply under stress caused by various ecotoxins, including metals [7]. Lichens could sustain for a long time elevated photosynthetic activity under stress caused by ecotoxins, including highly toxic and moderately toxic metals.

Several differences in adaptation strategies related to chlorophyll *a* and *b* concentration changes were found. Compensatory responses involving a change of chlorophyll *b* content occurred mainly in slightly impacted natural ecosystems. They maintained the required photosynthesis intensity with the insufficient humidity and illumination typical of several plant communities with certain types of trees. Chlorophyll *a* is the most labile component of the photosynthetic system. It provides the required compensatory responses and adaptation to stress associated with technogenic factors. Special investigations of the methods for adapting to high concentrations of ecotoxins that exceed considerably the regulatory values (MPC, APC, etc.) would be interesting.

The joint interdependence of the contents of chlorophyll *a* and several metals indicated that Fe, Al, and Ti played special roles ($r = 0.99$). These metals had a definite influence on the condition of the lichen photosynthetic system components and somewhat similar fundamental properties. The relationships found for the photosynthetic system parameters and the metal contents were definitely interesting for bioindication and biomonitoring. However, further joint analysis of the contents of chlorophylls *a* and *b* and metals in lichen specimens from various MT are required to assess fully the potential of this approach.

Conclusions. The contents of chlorophylls *a* and *b* in samples of the indicator lichen *H. physodes* collected in various business districts and natural areas of Tver Region differed considerably according to spectrophotometric results. Microclimate conditions and specifics of the lichen habitats had the greatest influence on the chlorophyll *b* content. The chlorophyll *a* concentration depended on the anthropogenic impact on the ecosystems. Chlorophyll *a* provided the necessary compensatory responses and adaptation to technogenic pollution associated with the distribution and effects of metals. The most widely distributed of the 15 metals (Al, As, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, Ti, V, and Zn) detected using ICP-AES in *H. physodes* samples were Fe, Al, and Ti. Significant correlations between the concentrations of these metals and the chlorophyll *a* content were observed. The results could be interesting for bioindication. Further research on the joint analysis of chlorophylls *a* and *b* and metal contents in lichen specimens from various MT is advisable.

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