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The content of proteinaceous nitrogen, micro- and macroelements, and the condition of the photobiont *Trebouxia* in *Hypogymnia physodes* from the Knyszyn Forest

Zawartość azotu białkowego makro- i mikroelementów oraz kondycja fotobionta *Trebouxia* w *Hypogymnia physodes* z Puszczy Knyszyńskiej

SUMMARY

The paper presents results of research on the content of proteinaceous nitrogen, micro-and macroelements as well as on the condition of the photobiont *Trebouxia* in *Hypogymnia physodes*, the most common lichen species in the Knyszyn Forest. The 24-hour accumulation values of the analysed elements by *H. physodes* have been assessed. The content values of micro- and macroelements in the examined samples are lower than those from the southern region of Poland, except for the values of manganese. The condition of the photobiont *Trebouxia* in *H. physodes* was evaluated on the basis of the number of undamaged cells and those devoid of chromatophors. No correlation was found between the accumulation of the examined elements and the number of cells excluded from photosynthesis. In the samples collected from the cultivated lands the number of photobiont damaged cells in the thalli collected from wooden constructions ranged from 1.43% to 14.43%, with a definitely higher number of cells with undamaged chromatophor. The signalled increase in the number of damaged cells on cultivated lands is probably caused by local anthropogenic factors, such as the use of fertilisers and pesticides, as well as coal containing sulphur used in household fire places, whereas a higher number of photobiont cells noted in each sample is the result of an easy mycobiont access to the organic remains.

STRESZCZENIE

W pracy przedstawiono wyniki badań zawartości azotu białkowego, makro- i mikroelementów oraz kondycji fotobionta *Trebouxia* w *H. physodes*, najpospolitszego gatunku porostów w Puszczy Knyszyńskiej. Podano szacunkowo dobową akumulację analizowanych pierwiastków przez *H. phy-*

sodes. W badanych próbach zawartości metali ciężkich są niższe w stosunku do obszarów w południowej Polsce, z wyjątkiem zawartości manganu. Kondycję fotobionta *Trebouxia* w *H. physodes* oceniano ilością komórek nieuszkodzonych i pozbawionych chromatoforów. Nie stwierdzono zależności między akumulacją badanych pierwiastków a liczbą komórek wyłączonych z fotosyntezy. W próbach pobranych z obszarów użytkowanych rolniczo liczba uszkodzonych komórek fotobionta w plechach zebranych z konstrukcji drewnianych wahała się od 1,43% do 14,43% przy zdecydowanie większej liczbie komórek o nieuszkodzonym chromatoforze. Sygnalizowany wzrost komórek uszkodzonych w obszarach użytkowanych rolniczo jest powodowany prawdopodobnie lokalnymi czynnikami antropogenicznymi, m.in. stosowaniem nawozów i środków ochrony roślin, zasiarczonego węgla w paleniskach domowych, a odnotowywana w każdej próbie większa liczba komórek fotobionta — łatwym dostępem do resztek organicznych dla mykobionta.

Key words: *Hypogymnia physodes*, proteinaceous nitrogen, micro- and macroelements, *Trebouxia*, Knyszyn Forest.

INTRODUCTION

Lichens have been many times used as bioindicators of atmospheric pollution. Lichenindication in the cities was carried out earlier (2, 9, 11, 13, 14, 17, 21–23, 28). The bibliography related to the reaction of lichens to industrial emissions is abundant. Changes in the morphology and anatomy of thalli, the activity of metabolic processes, cumulation of sulphur, heavy metals and radioactive elements were described. Lichen indicator scales were worked up (12); they were modified to the local conditions (14–15) and completed with a list of *Bryophyta* indicator species in lichen- and bryoindicator scales (5–6). Lichen-indicating regional maps are drawn and compared with real distribution of sulphur and heavy metals concentration values.

Hypogymnia physodes has been regarded as the best biotesting lichen. Lichenindication with the use of this species was carried out on many levels (1, 8, 16, 18, 19, 26). The thalli collected in the area of studies or transported from other areas were analysed. Methods of transplantation (2, 4, 24) as well as methods of examining photobiont reaction in *H. physodes* (4, 24) were worked out.

The paper aimed at analysing the accumulation of proteinaceous nitrogen and several elements in *H. physodes* in the areas of low anthropopression. At the same time, the condition of the examined specimens collected in forest biocoenoses and in the inhabited areas of the Knyszyn Forest, was determined; it was measured on the basis of changes in *Trebouxia* photobiont cells chromatophors.

MATERIAL AND METHODS

Hypogymnia physodes collected in several dozen stands of the Knyszyn Forest were the research material. The thalli selected for the studies had no visual damage in the form of brown spots on the cortex surface, and they had numerous soralia at the end of their segments. Thalli samples of 3- and 4-year spruce branches collected in July and August 1999 were subject to a chemical analysis. The latter was preceded by examinations (stands 1–11). The photobiont condition in the *H. physodes* thalli was also analysed on the basis of specimens successively collected (1998–1999) from the tree cortex in various forest communities, from wayside trees and mouldering wooden fences on cultivated lands (stands 12–41).

The dried up thalli were ground in a disk-grinder, then thoroughly mixed and dried up again to the constant mass. 1-gram samples were wet-mineralised in the mixture of H_2SO_4/H_2O_2 (27).

The obtained remaining part was treated with NH_3 solution until pH 3,0 was reached, and was completed with deionized water up to 100 cm^3. Cadmium, lead, copper and zinc were analysed by means of anode inversion voltamperometry (746 VA Trace Analyser Metrohm equipped with measurement stand 747 VA Stand). Arsenic was determined by means of hydride technique, whereas nickel, manganese, ferrum, calcium, magnesium and potassium — by means of flame technique of nuclear absorption spectrometry (Solaar 929 Unicam with a countershaft for hydrides VP 90 Vapour System). Mercury was analysed directly in the initial, dry and comminuted material by means of mercury analyser (AMA 254 Altec Czech Republic).

Thallus samples for the analysis of photobiont condition were collected with injection needle no 10 and were ground in the weak solution of potassium lye (photobiont cells are visible in KOH [4]). The *Trebouxia* cells were counted in microscopic preparations (magn. 400×). The means (counted for each test) from four independent repetitions, were drawn in the table.

RESULTS AND DISCUSSION

The content of 11 elements and proteinaceous nitrogen in *Hypogymnia physodes* was examined in 11 samples collected from spruce branches in different stands of the Knyszyn Forest (Tab. 1). The average content of the accumulated elements was: 0.143 μg Hg/g dry mass, 0.384 μg Cd/g dry mass, 1.94 μg As/g dry mass, 3.39 μg Cu/g dry mass, 9.96 μg Pb/g dry mass, 5.28 μg Ni/g dry mass, 526.0 μg Fe/g dry mass, 2890.0 μg K/g dry mass, 573.0 μg Mg/g dry mass and 3390 μg Ca/g dry mass. These values are lower (except for manganese) than those recorded in south and south-western Poland. Bielecki et al. (1) examined the content of copper, lead, zinc, ferrum and manganese in *H. physodes* collected from pine and oak at different distances from the pollutant (Tab. 2). They drew attention to a distinctly inverse relationship between the level of accumulation and the distance from the sources of emission. Fabiszewski et al. (7) examined heavy metals and sulphur content in the thalli transplanted from different distances from the emittor, and they observed the similar correlation. Differences between the control stand and the stands most exposed to emissions were very large and amounted to: 45–4148 μg/g of dry mass for Pb and 20–8500 μg/g of dry mass for Cu. Kiszka (15) reported a higher content of the examined elements in *H. physodes* collected in Biała Wisełka, Czarna Wisełka and on the Barania Góra (Mt.) than in those recorded in the Knyszyn Forest. The content values of some elements in *Hypogymnia physodes* collected in various regions of Poland are compared in Table 2. The latter also compiled the data related to above species thalli collected in 1975–1989 at tens of stands in western Finland (10) and from the cultivated lands in Finland (16).

In the course of studies on the *Trebouxia* condition in *Hypogymnia physodes* it was observed that the number of the cells devoid of chromatophors or with a damaged chromatophor amounted to 1.43–14.43% in the examined samples

Table 1. Content of proteinaceous nitrogen, micro- and macroelements in *Hypogymnia physodes* in the Knyszyn Forest

	N	Hg	Pb	Cd	Cu	Zn	Ni	As	Fe	Mn	Ca	Mg	K
	%					µg/g s.m.							
1	0.955	0.129	7.510	0.311	3.700	118.10	16.810	1.630	423	193	3000	526	2770
2	0.939	0.157	6.460	0.253	3.680	79.50	8.920	2.020	402	159	2340	498	2070
3	0.879	0.126	12.830	0.486	3.430	93.26	5.720	1.690	379	299	4560	543	3000
4	1.352	0.168	9.460	0.605	3.790	94.67	3.080	1.980	878	228	2550	726	3920
5	1.059	0.129	10.190	0.279	2.490	84.97	3.250	1.780	586	180	4420	784	2110
6	1.183	0.168	9.820	0.388	2.180	102.50	4.190	1.960	511	174	4100	448	2670
7	1.266	0.159	10.210	0.263	2.250	90.16	6.140	2.110	626	202	3260	435	2560
8	1.023	0.090	8.460	0.219	4.840	81.22	6.030	1.340	485	151	3300	780	3170
9	0.962	0.180	13.310	0.451	3.910	86.81	4.900	2.430	555	202	3670	483	3480
10	0.903	0.130	10.590	0.393	3.620	87.89	3.120	2.560	427	185	3310	490	2590
11	1.269	0.139	9.660	0.573	3.400	136.00	1.870	1.880	515	248	2800	594	3460
*	1.072±	0.143±	9.960±	0.384±	3.390±	95.90±	5.820±	1.940±	526±	202±	3390±	573±	2890±
**	0.0008–	0.001	0.00011–	0.00014	0.008–	0.010	0.0003–	0.0004	0.0026–	0.0030	0.075–	0.999–	0.0045–
	0.0061–	0.0015–	0.0020	0.410–	0.549–	0.158–	0.210–	2.648–	3.531–	0.447–	0.596–	2.257–	3.010–

* The mean from 11 tests, ** 24-hour accumulation.

Table 2. Comparison between the results of own examinations of micro-macroelements and proteinaceous nitrogen in *Hypogymnia physodes* from the area of the Knyszyn Forest, and the data from other regions of Poland and Finland

Places	Pb	Cu	Fe	Ni	Mn	Zn	Ca	Mg	K	N
					µg/g s.m.					
Knyszyn Forest (own examinations)	9.90	3.39	526	5.82	202	95.9	3390	573	2890	1.07
Huta Miedzi Lubin: Pinus Bielecki and al. (1979)	31–99	8–418	905–1443	–	10–33	36–71	–	–	–	–
Huta Miedzi Lubin: Quercus Bielecki and al. (1979)	33–78	1–242	887–1342	–	10–92	13–59	–	–	–	–
Bory dolnośląskie (control)	45	20	1171	–	20	67	–	–	–	–
15 km from emitor										
1 km from emitor	100–4148	6–8500	1391–3469	–	20–70	80–4825	–	–	–	–
Fabiszewski et al. (1983).										
Finland, 1975	15.6	5.63	860	0.41	56.9	81.9	2517	358	1829	7.61
Halonen and al. (1993)										
Finland, 1983										
Halonen et al. (1993)	16.4	6.22	–	0.53	56.7	74.7	1276	344	1820	1.24
Finland, 1989										
Halonen et al (1993)	9.9	5.63	750	1.91	67.3	85.8	1554	400	1850	1.34
Finland, 1985–86.										
Kubin (1990)	18.0	7.3	540	2.6	131.0	86.0	3340	400	2750	1.30
Biała Wisełka										
Kiszka (1998)	20.1	8.63	2223	1.63	23.78	147.7	–	–	–	–
Czarna Wisełka										
Kiszka (1998)	13.11	7.10	1774	1.67	19.16	124.6	–	–	–	–
Barania Góra										
Kiszka (1998)	23.91	5.74	829	2.87	60.48	86.2	–	–	–	–

(Tab. 3) The smallest number of damaged photobiont cells was noted in the samples from the reservation Czołnowo (1.36%), Cieliczanka (2.01%) and in the samples from Katrynka collected within the forest inspectorate buildings: on a fence (1.91%) and on a poplar (1.43%). The highest percentage of dead cells was observed in the thalli from deforested areas, in specimens growing on fences and on balustrades (Trzciane 14.43%, Sokółka 13.68%, Supraśl 12.25%, Krasne village 11.88%), and on wayroad trees in the villages: Rozedranka (13.50%), Chraboły (10.62%), Kłodziewo (8.74%), Supraśl (5.60%). At the same time, the number of the *Trebouxia* photobiont cells in the majority of this species thalli collected around the mouldering wooden buildings, was higher than in the thalli collected in forest communities. The condition of *H. physodes* in the Knyszyn Forest in per cent of photobiont cells, either capable of photosynthesis or necrotic, is comparable with the state recorded in other areas of Poland (the Białowieża Forest, the Wigry National Park, the Roztocze National Park). In the areas with a high concentration of industrial and communal emissions, where *H. physodes* does not grow any longer, the analysis of photobiont condition is possible only on the transplanted thalli. For example, after 40-day exposition of this species thalli in the traffic centre and in residential districts of Lublin (Bystrek, examinations in progress) and around Nitrogen Plants in Puławy (20), the percentage of damaged photobiont cells started to increase together with the increase of anthropogenic factors. A distinctly inverse correlation between the number of damaged photobiont cells in the *H. physodes* and the anthropopression increase can be observed, just as in the case of heavy metals accumulation signalled by Fabiszewski et al. (8).

Applying micro- and macroelements accumulation measurements in lichens as an indicator of atmospheric pollution is very advantageous, since in lichens it is a rhythmic process, not disturbed by absorbing pollution from the substratum. In *H. physodes* collected in the Knyszyn Forest no significant differences were found between the examined stands as regards the amount of accumulated elements. The examined elements accumulation is slight (except for manganese, potassium and calcium). Assuming that the examined thalli were 3–4 years old and the examined elements accumulation lasted approximately 320 days in a year, the 24-hour heavy metals accumulation in 1 g of lichen dry mass amounted to 0.00 012–0.00 014 μg Hg, 0.0003–0.0004 μg Cd, 0.008–0.01 μg Pb, 0.075–0.099 μg Zn, 2.648–3.531 μg Ca, 2.257–3.01 μg K. Accumulation of Fe, Zn, Ca, K and proteinaceous N in *H. physodes* thalli, lower than in southern Poland and approximating that of cultivated lands in Finland as well as good condition of the thalli testify to low anthropopression in the examined area.

The number of the photobiont *Trebouxia* in *H. physodes* collected in cultivated lands, larger than that collected in forest communities probably depends on the

Table 3. Condition of *Hypogymnia physodes* in the Knyszyn Forest

Nr			Condition of photobiont cells			
			1	2	3	4
1.	*Stara Dębina	*Pa*	140	135	5	3.57
2.	*Międzyrzecze	*Pa*	126	123	3	2.38
3.	*Pieszczanki	*Pa*	118	115	3	2.54
4.	*Grzybowiec	*Pa*	122	119	3	2.46
5.	*Surażkowo	*Pa*	126	122	4	3.17
6.	*Chraboły	*Pa*	132	121	11	8.33
7.	*Katrynka	*Pa*	113	107	6	5.21
8.	*Krzemienne Góry	*Pa*	126	120	6	4.76
9.	*Dąbrowa	*Pa*	133	129	4	3.00
10.	*Lacka Buda	*Pa*	131	123	8	6.10
11.	*Klin *prope* Krynki	*Pa*	134	125	9	6.72
12.	*Krzemianka.	*Pa*	128	121	7	5.47
13.	Podsupraśl	*Bp*	165	152	13	7.87
14.	Cieliczanka	*Bp*	149	146	3	2.01
15.	Machnacz	*Bp*	134	127	7	5.22
16.	Machnacz	*Ps*	152	148	4	2.63
17.	Budzisk	*Bp*	148	143	5	3.38
18.	Karczmisko	*Ps*	151	147	4	2.65
19.	Karczmisko	*Pa*	145	141	4	2.76
20.	Woronicze	*Ps*	128	121	7	5.47
21.	Woronicze	*Bp*	139	130	9	6.44
22.	Czołnowo	*Bp*	147	145	2	1.36
23.	Krasne	*Bp*	176	165	11	6.25
24.	Katrynka**	epx	209	195	4	1.91
25.	Krasne	epx	202	178	24	11.88
26.	Chraboły	epx	200	189	11	5.50
27.	Katrynka**	*Populus sp*	210	207	3	1.43
28.	Waliły	epx	200	194	6	3.00
29.	Wasilówka	*Populus sp*	200	195	5	2.50
30.	Kłodziewo	*Populus sp*	183	167	16	8.74
31.	Rozedranka	epx	200	189	11	5.50
32.	Rozedranka	*Populus sp*	200	173	27	13.50
33.	Czarna Białostocka	*Populus sp*	153	147	6	3.92
34.	Supraśl	*Populus sp*	159	150	9	5.60
35.	Supraśl	*Fe*	143	137	6	4.20
36.	Kopna Góra	epx	200	193	7	3.18
37.	Chraboły	*Populus sp*	160	143	17	10.62
38.	Supraśl	epx	204	179	25	12.25
39.	Krynki	*Ps*	160	147	13	8.12
40.	Sokółka	epx	190	164	26	13.68
41.	Trzciane	epx	194	166	28	14.43
	Białowieża Forest	epif.	167	164	3	1.79
	Wigry National Park	epif.	141	133	9	5.38
	Roztocze National Park	epif.	181	178	3	1.65

Explanation: 1 — number of photobiont cells in the range of microscopic view (400×), 2 — number of live photobiont cells, 3 — number of damaged photobiont cells, 4 — % of damaged photobiont cells, ** the seat of forest inspectorate, * the examined organic nitrogen, micro- and macroelements. *Pa — Picea abies, Bp — Betula pendula, Ps — Pinus sylvestris, Fe — Fraxinus excelsior,* epx — wood, epif. — cortex.

access of mycobiont to organic remains. However, a higher percentage of damaged cells in several thalli in relation to the forest area thalli probably results from using sulphurized mineral coal in household fire-places, pesticides and mineral fertilisers.

The obtained results referring to the thalli condition and the cumulated elements enable comparative examinations evaluating the influence of communal and traffic emissions on the natural habitat.

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