

Reed – periphyton – mycota interactions in the water of different water bodies

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Abstract: The authors investigated the influence of periphyton that abundantly overgrew stalks of reeds (reed-periphyton) submerged in water [*Phragmites australis* (Cav.) Trin. ex Steudel], especially the old ones, on the occurrence of mycoflora species in different trophic seasons in the water of 4 trophically different water bodies. The seeds and snake exuviae were used as bait. In the spring, diatoms were the predominant group of algae forming the periphyton on old reed stalks, green algae predominated in the summer, and cyanobacteria in the autumn. During the seasons studied, in the water from Lake Blizno equal numbers of mycoflora species were noted inside the control containers and in those with reed-periphyton. In water from the other 3 water bodies completely different results were obtained. In the spring and autumn, a smaller number of mycoflora species grew inside the containers with reed-periphyton. However, during the summer the number of mycoflora species was higher in the Biała river water (control) as compared to the containers with reed-periphyton; in the other 2 water bodies the findings were the opposite.

As known, all algae, including the periphyton constituents, excrete many different organic substances into the environment. Some of them are a medium for various hydrobionts, while others inhibit the growth of aquatic organisms including mycoflora species.

Key words: Reed-periphyton, mycoflora, interactions, water bodies, hydrochemistry

Introduction

The algae occurring in water bodies depending on cells, which may float on the surface or grow on submerged objects and fragments of plants, are divided into 2 groups. Those passively floating on the surface are classified as phytoplankton and the sedentary ones as periphyton (Hutchinson, 1975; Kalff, 2002).

The production of organic matter by periphyton may sometimes be significant (Chetelat et al., 2000), and at times is even higher than that taking place in an aggregation of phytoplankton (Komulaynen, 2000).

Considering the aquatic plants on which the periphyton grow in large amounts, *Phragmites australis* (Cav.) Trin. ex Steudel, which is the main component of the emergent plants, is classified in that group (Lakatos et al., 2001).

We were interested in investigating the interactions between periphytonal aggregations growing submerged on water stalks of reeds and found in water bodies, fungi, and fungus-like organisms, during every season, in different types of reservoir.

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Materials and methods

The experiments were performed using old reeds *Phragmites australis* covered by periphyton (reed-periphyton) taken from the littoral area of Lake Blizno in the Suwalszczyzna region (53°57.6'N-23°04'E, altitude 133.2 m) (Figure 1). Fragments of stalks 15-cm long taken from 3 plants were placed into 1-L capacity containers filled with water from the relevant reservoir. Before the placement in the containers fragments of stalks were fastened with thread to a glass slide, which prevented those fragments from floating on the surface of the containers. The control probes were containers with water from the relevant water bodies, containing 3 fragments 15 cm in length of reeds stalks devoid of periphyton and rinsed several times with distilled water. The water samples were collected from reservoirs in 4 locations:

- River Biała, length 9.8 km, the left-bank tributary of the River Supraśl, flowing through the city of Białystok,

- River Supraśl, length 106.6 km, the right-bank tributary of the River Narew, flowing through the Knyszyńska Forest,

- Lake Blizno, located in the Augustów Forest. Area 238.5 ha, max. depth 28.8 m. South-western shores are surrounded by extensive coniferous woods, while the northern shores of the lake border the village of Ateny. The sampling site was on the northern side of the lake. The bed is muddy.

- Fosa Pond, area 2.5 ha, max. depth 1.75 m. Pond with wild ducks and breeding swans as well as crucian

carp, used by anglers. The pond is surrounded by meadows with linden and elm.

Water samples for the analysis and the experiments were collected from each reservoir at the depth of 15-30 cm at a distance of 0.5 m from the bank. The water was filtered through gauze and then poured into containers. Nineteen parameters were determined for the water using standard methods (Greenberg et al., 1995).

Water samples, 800 mL each, were placed into 1000 mL containers. For each water body, there were 3 containers, containing reed-periphyton. The fourth container served as a control without reed-periphyton. The seeds of buckwheat (*Fagopyrum sagittatum* Gilib.), clover (*Trifolium repens* L.), common vetch (*Vicia sativa* L.), hairy vetch (*Vicia villosa* Roth.), and snake exuviae (*Natrix natrix* L.) were used as bait (in containers from reed-periphyton and controls) in accordance with the general principles of culture (Watanabe, 2002).

All containers were enclosed in petri dishes with the bed turned upside down, to prevent possible airborne contamination in the containers by mycota spores. The containers were stored at 15 ± 2 °C, with access to daylight resembling natural conditions and following the recommended instructions (Seymour & Fuller, 1987). The analyses of water and experiments were carried out in 3 parallel repetitions.

During 1 month of exposure, clusters from the containers' bottoms and side walls and the surface of baits of control samples and with reed-periphyton were examined under the light microscope. Fungal

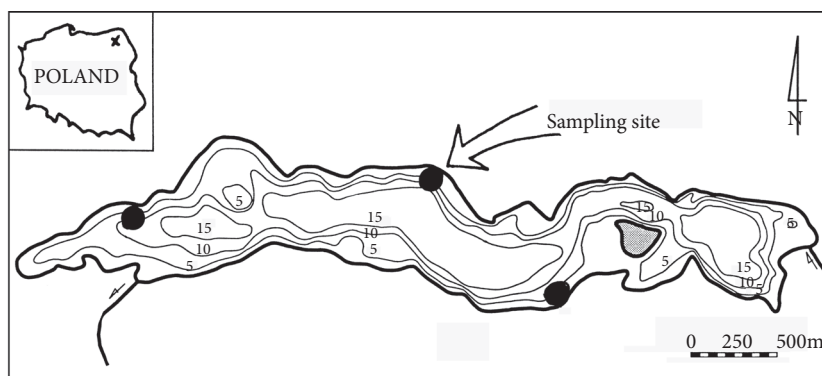


Figure 1. Study area and sampling sites at Lake Blizno.

structure (zoospores, antheridia, and oogonia) of aquatic mycota growing in particular containers was recorded. The baits were observed under the microscope every 3-4 days. The size of the mycota structures was measured using light microscopy at 600×. For determinations of the particular mycota species, the following keys were used: Batko (1975), Karling (1977), Pystina (1998), Watanabe (2002), Johnson et al. (2002, 2005), and by the authors, who were the first to describe the respective species. The systematics of straminipilous organisms were according to Dick (2001) and of fungi according to Blackwell et al. (2006), and of Chytridiomycota according to James et al. (2000).

The effect of reed-periphyton on the number of aquatic mycota species is presented as a ratio of Co/Per – number of cases where the species were found in control (Co) to those in culture with reed-periphyton (Per) (Magurran, 1988).

The results were subjected to statistical analysis (Winer, 1997).

Results

The reservoirs from which water for our experiment was taken varied regarding not only limnology but essentially they contained water with different charges of particular chemical parameters. The water of Lake Blizno contained the most forms of oxygen and the fewest of all forms of nitrogen, phosphates, sulphates, and chlorides (Table 1), whereas the water of Fosa Pond contained the fewest forms of oxygen and the most of all forms of nitrogen, phosphates, sulphates, and chlorides. Concerning the water taken from rivers, the water of the River Biała was more trophically rich than the water of the River Supraśl.

During the spring the predominant group of algae forming the periphyton on the old reeds stalks comprised diatoms, 58.2% of all periphyton; in the summer the predominant group comprised green algae (53.2%), and during the autumn cyanobacteria, 41.5% of all periphyton growing on reeds stalks (Figure 2).

Table 1. Chemical and physical properties of water in particular water bodies.

Specification	River Biała	River Supraśl	Lake Blizno	Fosa Pond
Temperature (°C)	17.4	15.1	13.2	16.2
pH	7.31	7.52	8.02	7.02
O ₂ (mg L ⁻¹)	12.08	11.40	16.42	1.84
BOD ₅ (mg L ⁻¹)	4.82	7.20	2.81	9.22
COD (mg L ⁻¹)	9.02	7.00	4.12	15.12
CO ₂ (mg L ⁻¹)	15.83	8.82	5.32	22.43
Alkalinity in CaCO ₃ (mval L ⁻¹)	4.71	4.34	2.45	5.78
N-NH ₃ (mg L ⁻¹)	0.642	0.232	0.213	0.865
N-NO ₂ (mg L ⁻¹)	0.011	0.008	0.003	0.115
N-NO ₃ (mg L ⁻¹)	0.050	0.030	0.025	0.054
P-PO ₄ (mg L ⁻¹)	1.504	1.204	0.140	3.625
Sulphates (mg L ⁻¹)	68.11	34.97	14.08	23.07
Chlorides (mg L ⁻¹)	40.02	21.02	14.04	45.24
Total hardness (mg Ca L ⁻¹)	92.16	70.56	40.89	79.34
Total hardness (mg Mg L ⁻¹)	22.34	12.47	11.34	26.31
Fe (mg L ⁻¹)	0.90	0.50	0.12	1.08
Dry residue (mg L ⁻¹)	532.0	166.0	182.0	430.0
Dissolved solids (mg L ⁻¹)	496.0	141.0	140.0	369.0
Suspended solids (mg L ⁻¹)	36.0	15.0	42.0	61.0

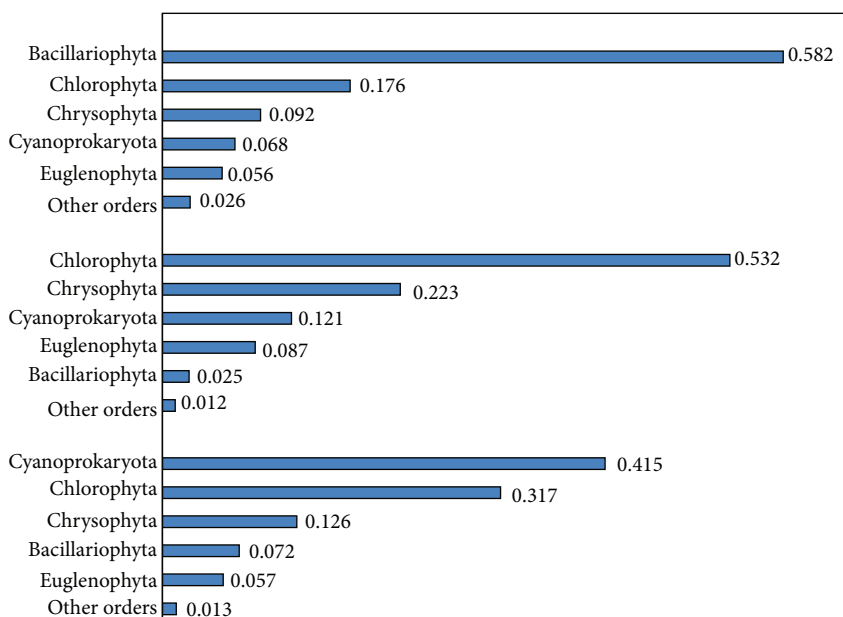


Figure 2. Number of species in particular taxonomic reed - periphyton groups in studied lake.

Spring: Bacillariophyta - *Achnanthes minutissima* Kütz. was dominant and *Cocconeis placentula* Ehr. was subdominant;

Summer: Chlorophyta - *Cladophora glomerata* (L.) Kütz was dominant and *Oedogonium macrandrium* Wottrock was subdominant;

Autumn: Cyanoprokaryota - *Chamaesiphon incrustans* Grunow was dominant and *Rivularia dura* Roth. was subdominant.

During our experiment 49 species of mycoflora grew on the baits used, as follows: 6 species of fungi, 2 species of Plasmodiophoromycetes, and 41 species of fungus-like organisms (Straminipila) (Table 2). The fewest species belonging to Mycota, both in controls and in the containers with the periphyton, grew in water from Lake Blizno, and the most in the water with the most abundant in biogenic compounds - River Biała and Fosa Pond. The great majority of species were noted on the baits only in control containers (20), 14 inside containers with periphyton (significantly different), and 15 species grew both inside control containers and inside containers with periphyton (Table 3). In water from the River Biała, during all 3 seasons, inside the containers with periphyton fewer species of mycoflora were observed than inside the control containers, particularly in the spring and autumn (Table 4). This was also the case the water from the River Supraśl and Fosa Pond

during spring and autumn. However, in the summer, in water from both reservoirs more species of mycoflora grew inside the containers with the periphyton. Considering the water from Lake Blizno during all 3 seasons equal numbers of species of mycoflora were noted inside the containers with periphyton and inside the control containers.

Discussion

The groups of algae composing periphyton are found in all types of water bodies. Abundantly the periphyton appears in rivers in which the river bed is covered with stones of various sizes (Chetelat et al., 2000), and in trophically different types of lakes (Komulaynen, 2000).

As it has been shown in our experiment, generally, inside the containers with periphyton a lower number of species of mycoflora grew than inside the control

Table 2. Aquatic fungi and straminipilous organisms found in water from particular water bodies in the experiment (Co-control, Pe-with periphyton).

Taxa	Control				Periphyton				Co/Per Ratio
	River Biała	River Supraśl	Lake Blizno	Fosa Pond	River Biała	River Supraśl	Lake Blizno	Fosa Pond	
Fungi									
Blastocladiomycota									
Blastocladales									
1. <i>Catenaria anguillulae</i> Sorokin			x						1/0
2. <i>Catenophlyctis variabilis</i> (Karling) Karling	x	x	x		x	x	x		3/3
Chytridiomycota									
Chytridiales									
3. <i>Endochytrium digitatum</i> Karling							x		0/1
4. <i>Phlyctochytrium aureliae</i> Ajello		x							1/0
5. <i>Polyphagus euglenae</i> (Bail) J.Schröt.							x		0/1
Zygomycota									
Entomophthorales									
6. <i>Zoophthora rhizospora</i> (Thaxter) Batko				x				x	1/1
Plasmodiophoromycetes									
Plasmodiophorales									
7. <i>Woronina polycystis</i> Cornu	x	x							2/0
8. <i>W. pythii</i> Goldie-Smith	x								1/0
Straminipila									
Hyphochytriomycetes									
Hyphochytriales									
9. <i>Olpidiopsis saprolegnia</i> (Braun) Cornu	x								1/0
10. <i>O. vexans</i> Barret	x								1/0
Peronosporomycota (Oomycota)									
Leptomitales									
11. <i>Apodachlya pyrifer</i> Zopf								x	0/1
Pythiales									
12. <i>Myzocyttium proliferum</i> Schenk						x			0/1
13. <i>Phytophthora undulata</i> (H.E. Petersen) M.W. Dick				x					1/0
14. <i>Pythium aquatile</i> Höhnk					x			x	0/2
15. <i>P. debaryanum</i> R. Hesse				x					1/0
16. <i>P. diclinum</i> Tokunaga	x							x	1/1
17. <i>P. inflatum</i> V.D. Matthews	x	x							2/0
18. <i>P. oedochilum</i> Drechsler	x								1/0
19. <i>P. rostratum</i> E.J. Butler						x		x	0/2
20. <i>P. tenue</i> Gobi					x				0/1

Table 2. (continued).

Taxa	Control				Periphyton				Co/Per Ratio
	River Biała	River Supraśl	Lake Blizno	Fosa Pond	River Biała	River Supraśl	Lake Blizno	Fosa Pond	
Saprolegniales									
21. <i>Achlya americana</i> Humphrey				x				x	1/1
22. <i>A. apiculata</i> de Bary	x	x		x	x				3/1
23. <i>A. colorata</i> Pringsh.				x					1/0
24. <i>A. crenulata</i> Ziegler						x			0/1
25. <i>A. debaryana</i> Humphrey					x				0/1
26. <i>A. flagellata</i> Coker					x				0/1
27. <i>A. imperfecta</i> Coker	x								1/0
28. <i>A. klebsiana</i> Pieters				x				x	1/1
29. <i>A. polyandra</i> Hildebr.			x			x	x		1/2
30. <i>A. prolifera</i> C.G.Nees	x		x						2/0
31. <i>A. racemosa</i> Hildebr.				x				x	1/1
32. <i>A. treleaseana</i> (Humphr.) Kauffman					x				0/1
33. <i>Aphanomyces irregularis</i> W.W.Scott		x	x	x			x		3/1
34. <i>Aplanes androgynus</i> (W.Archer)Humphrey		x				x			1/1
35. <i>Isoachlya monilifera</i> (de Bary) Kauffman								x	0/1
36. <i>I. torulosa</i> (de Bary) Cejp				x					1/0
37. <i>Pythiopsis cymosa</i> de Bary	x								1/0
38. <i>Saprolegnia anisospora</i> de Bary	x							x	1/1
39. <i>S. ferax</i> (Gruith.) Thur.	x	x	x		x	x	x		3/3
40. <i>S. glomerata</i> (Tiesenh.) A.Lund	x	x	x	x					4/0
41. <i>S. hypogyna</i> (Pringsh.) de Bary				x					1/0
42. <i>S. litoralis</i> Coker	x		x	x			x		3/1
43. <i>S. megasperma</i> Coker		x							1/0
44. <i>S. monoica</i> Pringsh.		x							1/0
45. <i>S. parasitica</i> Coker	x	x	x	x					4/0
46. <i>S. turfosa</i> (Minden) Gäum.	x								1/0
47. <i>S. unispora</i> Coker et Couch		x							1/0
48. <i>Sommerstorffia spinosa</i> Arnaudov						x			0/1
49. <i>Thraustotheca clavata</i> (de Bary) Humphrey	x			x	x				2/1
Total number of species	19	13	9	15	9	8	7	10	56/34

containers. Regarding particular seasons, in the water from Lake Blizno (in our experiment both water and the reeds stalks were always taken from Lake Blizno from the same place) the Co/Per ratio was always 1; this means that equal numbers of species of mycoflora were noted inside the control containers and inside the containers with periphyton. During the seasons examined, inside the containers with the water from

the other 3 water bodies the results were completely different. In the spring and autumn, inside the containers with reed-periphyton a lower number of species of mycoflora grew, during the spring the Co/Per ratio fluctuated between 1.30 and 2.26, and in autumn the Co/Per ratio was from 1.32 to 3.33. However, during the summer a higher number of species of mycoflora was observed in water from the

Table 3. Aquatic fungi and straminipilous organisms found in particular containers.

Specification	Aquatic fungi and straminipilous organisms (see Table 2)	Number of species
Only control	1,4,7,8,13,15,17,18,21,23,27,30,37,40,41,43,44,45,46,47	20*
Only with periphyton	3,5,11,12,14,19,20,24,25,26,32,35,36,48	14*
Control and with periphyton	2,6,9,10,16,22,28,29,31,33,34,38,39,42,49	15

*Asterisks indicate differences significant at the $P \leq 0.05$ level

Table 4. Number of fungi species and straminipilous organisms found in control containers and containers with periphyton in particular seasonal and in particular water bodies (Co- control, Per- container with periphyton).

Water from	Spring			Summer			Autumn		
	Control	Periphyton	Co/Per Ratio	Control	Periphyton	Co/Per Ratio	Control	Periphyton	Co/Per Ratio
River Biała	8.0	4.1*	1.95	7.0	5.2*	1.35	12.0	4.5*	2.67
River Supraśl	9.5	4.2*	2.26	8.0	10.5*	0.76	7.0	5.3*	1.32
Lake Blizno	4.0	4.0	1.00	3.0	3.0	1.00	2.0	2.0	1.00
Fosa Pond	9.2	7.1*	1.30	4.0	5.3*	0.75	7.0	2.1*	3.33

*Asterisks indicate differences significant at the $P \leq 0.05$ level in respect to control (n = 9)

River Biała than inside the containers with periphyton (Co/Per ratio 1.34); in water from the 2 other reservoirs the opposite was found (Co/Per ratio 0.74 and 0.76 respectively). As it has been stated, during the examined seasons representatives from various classes of algae forming periphyton were predominant: during the spring it was a group of diatoms, in the summer green algae, and in the autumn cyanobacteria. As is known, all algae, including those that are compounds of periphyton, excrete into the environment many organic substances (Fogg, 1971). Those are as follows: various organic compounds of carbon, free saccharides (Wartanable, 1980), free amino acids (Carluccio et al., 1984), and even polymeric substances (Hoagland et al., 1993). Among the organic substances excreted into the environment are also many enzymes (Chappell & Goulder, 1994), for example: glycosidase (Corves & Jüttner, 2000) and phosphatase (Pettersson & Kahlert, 2001). Many species of algae excrete into the environment polyphenolics (Serrano & Guisande, 1990; Arnold et al., 1995) and sulphur compounds

(Richmond, 1973), among which the most widespread and inhibiting is alliin (Stoll & Seebeck, 1951). Many of those substances excreted into the environment by algae are a medium for various hydrobionts, especially for micro-organisms (Schriver et al., 1995), and others inhibit the growth of aquatic organisms, what is characteristic for cyanobacteria (Chorus, 2001), and for the representatives of Characeae (Amonkar & Banerji, 1971), and macrophytes (Czeczuga et al., 2005, 2008). Inhibiting substances are excreted into the environment also by numerous species of green algae (Fogg, 1971; Czeczuga & Grądzki, 1974; Debro & Ward, 1979). Inhibiting influence of species of cyanobacteria on the growth of other bacteria, fungi, and fungus-like organisms has been shown in experiments (Czeczuga et al., 2003). It is also known that there is antagonism between bacteria and fungi on decomposing fragments of aquatic plants (Mille-Lindblom & Tranvik, 2003). In rivers, especially in mountain rivers, the aggregations of periphyton overgrow the gravel bed and in lakes aggregations of periphyton

overgrow the submerged in water plants or fragments of plants (Kalff, 2002). Both the periphyton of rivers and periphyton of lakes differ in composition of species; it depends mainly on seasons. The production of an organic matter by periphyton also may differ; sometimes it is equal or is even higher than phytoplanktonal production (Chetelat et al., 2000; Akbulut, 2003). Among macrophytes, especially in rivers from lowlands and in the littoral zones of lakes, the reed *Phragmites australis* is the main plant (Bernatowicz & Wolny, 1969; Hutchinson, 1975), and as it has been shown in experiments (Lakatos et al., 2001) especially fragments of old reeds submerged in water are overgrown by periphyton. It proves that the role of periphyton in the aggregations of reeds is very important for being an organic matter producer

(Wetzel, 1969) and in interactions with other organisms (Kalff, 2002).

Among the 49 species of mycoflora noted during the experiment the most important fact seems to be an occurrence of *Achlya imperfecta* on baits inside the control container with water from the River Biała. It is a new, unknown species in Polish waters. It has been described for the first time in Germany by Minden (1912) as *varietas Achlya de Baryana var. intermedia*; however, Coker (1923) classified that strain as a different species, *Achlya imperfecta*. Apinis (1930) observed the growth of that fungus-like organism in Łatwia in Jugla and Carnicawa, under the meadow soil overgrown by moss predominantly by *Polytrichum* sp.

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