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Original Research

Fungi and Straminipilous Organisms Found in Ponds in Białystok

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Abstract

Our study involved the quantitative analysis of species composition of fungi and straminipilous organisms in four ponds situated in Białystok, Poland. The observations performed in April and November 2006 with respect to hydrochemical conditions revealed the occurrence of 48 species, including 9 fungi and 39 straminipilous organisms. Among species of fungi and straminipilous organisms, we found such pathogens of crustaceans and fish as *Achlya dubia, Ac. oblongata, Ac. polyandra, Aphanomyces bosminae, Ap. laevis, Dictychus monosporus, Pythium jirovecii, Py. undulatum, Saprolegnia ferax, S. parasitica, S. pseudocrustosa* and *Thraustotheca clavata*. The human pathogens *Aspergillus niger, Candida tropicalis* and *Catenophlyctis variabilis* as well as plant pathogens *Pythium butleri* and *Py. debaryanum* were also found. Such phytosaprophytes as *Achlya klebsiana, Karlingia rosea, Nowakowskiella elegans, N. macrospora, Pythium akanense, Py. aquatile, Py. elongatum, Py. inflatum* and *Py. intermedium* were relatively common.

Most species of fungi and straminipilous organisms were found to grow in Dojlidy Pond (27), the fewest in Akcent Pond (14). The hydrochemical analysis of water showed that Dojlidy was the least burdened with organic matter, whereas Akcent was the most abundant in biogenic compounds.

Keywords: fungi, straminipilous organisms, ponds, hydrochemistry, Białystok

Introduction

Fungi constitute a large diverse and common group of organisms in aquatic ecosystems. They can be found in various types of water reservoirs, in fresh, sea, stagnant, surface and underground waters. Fungi are a biotic component of hydroecosystems and an important link in the chain of trophic transformations in aquatic biocenosis. Most of them belong to saprothrophs that take part in mineralization of dead organic matter, both of plant and animal origin, either produced in reservoirs or carried from the outside. They thus act as destruents, contributing to water self-purification and naturally preventing eutrophization [1, 2]. Therefore, according to many authors, aquatic fungi can be a good biological index of water purity and pollution [3-6].

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The progressing pollution of surface waters with various chemical compounds as the antropopressure or natural factors has contributed to a growing interest in the studies of aquatic fungi and straminipilous organisms.

Some of them are plant, animal or human parasites. In favourable conditions, fungi acting as saprobionts can assume pathogenic properties, being a potential source of infection [7].

Until now, mycological studies have mainly referred to running waters of streams and rivers, to a smaller extent concerning stagnant waters of lakes. Fungus species diversity in ponds has been investigated sporadically.

The current study objective was to establish the diversity of fungal biota in several ponds situated in Białystok and to demonstrate the effect of physico-chemical factors on the growth of aquatic fungi.

Material and Methods

The experiments were performed in April and November 2006. Water samples were collected from four ponds within the town of Białystok:

- Dojlidy Pond, situated in Białystok: area 34.2 ha, max. depth 2.85 m, its south shores border with coniferous woods and its western part with the town of Białystok. The samples were collected from the western end of this pond, which is used by town inhabitants as a beach.
- Pond at Mickiewicza Street, an artificial basin: area 1.27 ha, max. depth 1.0 m, located at Mickiewicza Street in Białystok. This reservoir is fed with surface and rain waters during rainfalls; their excess is channelled to the river Biała. The banks of the pond are grown with herbaceous plants and surrounded by single trees. The basin is used to breed crucian carp, carp, tench, roach and amura. Ducks and swans appear there. Its bed is muddy.
- Fosa Pond, located in the Palace Park of Białystok: area 2.5 ha, max. depth 1.75 m. Habitat of wild ducks, breeding swans, crucian carp and tench - used by anglers. The pond is surrounded by meadows.
- Akcent Pond: area 0.45 ha, depth 1.5 m, located in the Municipal Park, is a habitat of wild ducks and breeding swans. The sampling site is surrounded by single trees. The bed is muddy.

For the analysis of fungi and straminipilous organisms 3 samples were collected from each sampling site. The water collected from the respective reservoir was poured in sterile conditions into beakers, 0.6 l capacity, and placed in the laboratory in conditions resembling those of the natural environment. Baiting method described by Fuller and Jaworski [8], Kiziewicz and Czeczuga [9] was used to isolate the fungi from the water. The following baits were used: crustacean *Gammarus pulex*, snake skin *Natrix natrix*, clover seeds of *Trifolium repens*, hemp seeds *Cannabis sativa* and buckwheat seeds *Fagopyrum esculentum*, and onion skin *Alium cepa*. All the substrates, prior to being added to water samples, were boiled and rinsed with distilled water a few times. The baits were successively observed under an optic microscope (100 and 400x magnification) every 3-5 days, starting from day 3 of the culture. Next, several microscope preparations were prepared from each sample. The samples were stored for about a month to detect fungal physiology associated with sexual and asexual reproduction.

Fungi were identified, taking into consideration the following morphological features: the shape and size of the thallum, the shape of the sporangium and spores, the structure of the oogonium, gametangium and oospora. Works of many authors were used to determine the fungi [1, 7, 10-16].

Water samples for physicochemical analyses were collected at a distance of approximately 2m from the shore and 50cm depth by means of a Ruttner apparatus (2l capacity). Hydrochemical analysis was done in the laboratory. Nineteen water parameters were investigated in each water reservoir (Table 1). Physicochemical investigations of water were conducted according to Standard Methods [17].

Results

The hydrochemical analysis of water samples collected for analysis from the respective ponds within Białystok showed distinct differences in the study parameters. In all study reservoirs, water temperature was higher in April than in November 2006. In April 2006, the lowest noted value was 10.5ºC (Dojlidy) and the highest – 13ºC (Mickiewicza Street and Akcent). Oxygen content in the four study reservoirs was higher in November as compared to April 2006. In November the oxygen content ranged from 2.50 (Akcent) to 8.40 mg l^{-1} (Dojlidy). However, the content of carbon dioxide in all the ponds was higher in April than in November 2006, ranging from 14 (Dojlidy) to 27.5 mg l^{-1} (Akcent). Water oxidability in these four ponds was higher in autumn than in spring of 2006. The lowest value was noted in November 2006 in Dojlidy (14.50), the highest in Akcent (32.56 mg l^{-1}). The lowest contents of nitrogen (all the three forms) and phosphates were observed in Dojlidy. At the same time the highest contents were found in Akcent. Dojlidy water was the poorest in sulphates; their highest levels were noted in Akcent. The content of chlorides was higher in spring than in autumn of 2006, ranging between 54 (pond Dojlidy) and 67.5 mg l^{-1} (pond Fosa). Pond water in Dojlidy was characterized by the lowest content of dry remains and dissolved substances, with the highest level noted in Akcent. Relatively high concentrations of the suspension and dissolved substances were also detected in the pond at Mickiewicza Street and in Fosa (Table 1).

The study conducted in the four ponds in Białystok showed the occurrence of 48 species, including 39 straminipilous organisms belonging to the Peronosporomycetes and 9 species of fungi proper belonging to the Chytridiomycetes (6), Saccharomycetes (1), Ascomycetes (1) and Zygomycetes (1) (Table 2). The largest number of species was found in Dojlidy (27), the smallest in Akcent (14) (Table 2, Fig. 1, 2). Taxons identified in all the ponds included *Myzocytium zoophthorum, Saprolegnia ferax, S. parasitica, Thraustotheca clavata* and *Catenophlyctis variabilis*.

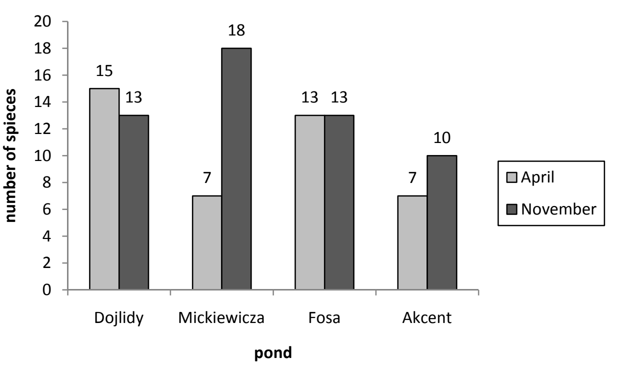


Fig. 1. Number of fungus species and straminipilous organisms recovered from the ponds in April and November.

Table 1. Physical and chemical proporties of water in particular water sites (mean of 3 samples).

Specification	April				November			
	Dojlidy Pond	Mickiewicza Str. Pond	Fosa Pond	Akcent Pond	Dojlidy Pond	Mickiewicza Str. Pond	Fosa Pond	Akcent Pond
Temperature (ºC)	10.5	13.0	12.0	13.0	9.5	6.5	9.0	9.5
pH	7.34	7.20	7.54	7.60	7.46	7.40	7.64	7.90
O_2 (mg l^{-1})	6.80	2.25	2.40	1.50	8.40	4.45	4.50	2.50
BOD_5 (mg l^{-1})	1.90	1.20	0.10	1.30	2.70	1.40	0.20	1.90
COD (mg l^{-1})	10.5	17.12	20.3	27.1	14.5	22.16	25.24	32.56
CO_2 (mg l^{-1})	14.0	12.8	20.5	27.5	8.0	4.3	4.7	7.6
Alkalinity in $CaCO_3$ (mval l^{-1})	3.20	3.50	3.90	6.80	3.80	4.30	4.70.	7.60
N-NH_3 (mg l^{-1})	0.355	0.510	0.440	2.915	9.175	0.620	0.530	4.115
N-NO_2 (mg l^{-1})	0.004	0.007	0.006	0.010	0.006	0.009	0.008	0.014
N-NO_3 (mg l^{-1})	0.030	0.834	0.770	0.530	0.080	0.962	1.020	0.720
P-PO_4 (mg l^{-1})	0.17	2.68	2.21	14.32	0.07	1.26	1.13	11.12
Sulphates (mg l^{-1})	19.24	48.0	45.0	72.56	42.28	96.0	93.0	105.78
Chlorides (mg l^{-1})	54.0	63.7	67.5	65.0	32.0	40.5	36.7	33.2
Total hardness (mg Ca l^{-1})	61.21	61.13	49.16	112.23	59.65	55.12	63.06	162.73
Total hardness (Mg l^{-1})	12.10	12.65	11.60	25.17	10.92	10.19	11.20	18.49
Fe (mg l^{-1})	0.43	0.61	0.51	0.58	0.26	0.48	0.38	0.46
Dry residue (mg l^{-1})	360.0	610.0	520.0	820.0	190.0	350.0	360.0	450.0
Dissolved solids (mg l^{-1})	341.0	596.0	509.0	786.0	171.0	336.0	349.0	416.0
Suspended solids (mg l^{-1})	19.0	14.0	11.0	34.0	19.0	14.0	11.0	34.0

Discussion

Fungi constitute the biological component of hydroecosystems, considerably affecting the natural environment and contributing to its modification.

The current study allowed determination of species diversity of aquatic fungi and straminipilous organisms in four ponds of Białystok. Most species (27) were isolated from Dojlidy Pond water. As shown by hydrochemical analysis, this reservoir was the least eutrophized, i.e. its water had high O_2 content, the lowest oxidability and low levels of ammonium, organic nitrogen, phosphates, chlorides and sulphates. According to literature data, less polluted water exhibits greater fungus species diversity, whereas an increase in pollution is accompanied by a decrease in the number of species [1, 18-19]. The finding of *Apodachlya pyrifera* only in pond Dojlidy seems to indicate a relatively low content of organic substances in this reservoir as compared to the three others. This taxon is known to grow in pure waters in cool seasons of the year [1].

However, waters in the pond at Mickiewicza Street and in Fosa Pond showed only slight physicochemical differences. Both reservoirs had relatively high content of organic substances and biogenic compounds. From the hydrobiological point of view, these ponds can be referred to as the eutrophized reservoirs. According to literature reports, elevated levels of organic compounds in water frequently delimit the occurrence of fungi [1]; hence, in comparison with Dojlidy, the pond at Mickiewicza Street contained 22, whereas Akcent had 19 zoosporic fungus species.

A comparison of physicochemical parameters revealed that the water in pond Akcent was the most highly eutrophized of all and therefore showed the smallest number of aquatic fungi and straminipilous organisms. The data could indicate a decrease in the number of fungus species under the influence of high content of biogenic compounds, which seems consistent with observations reported by other authors [18-21].

Of the species identified in the current study, the most numerous belonged to the genus *Pythium* (13), *Achlya* (8), *Saprolegnia* (8) and *Aphanomyces* (4). Similar species of straminipilous organisms of these genera have also been found to grow in pond water in Egypt, on seeds of *Cannabis sativa* [22].

Table 2. Fungi and straminipilous organisms found in ponds in Białystok in April and November, in 2006.

Classification (kingdom, class, order and species)	Water sites							
	Dojlidy		Mickiewicza Str.		Fosa		Akcent	
	April	November	April	November	April	November	April	November
Fungi								
Ascomycetes								
Eurotiales								
1. *Aspergillus niger* Thieghem		x				x		
Chytridiomycetes								
Blastocladiales								
2. *Catenophlyctis variabilis* (Karling) Karling		x		x	x		x	x
Chytridiales								
3. *Karlingia rosea* (de Bary et Woronin) Johanson		x	x	x				
4. *Nowakowskiella elegans* (Nowak.) Schr.	x		x		x			
5. N. macrospora Karling						x		
6. *Phlyctochytrium aureliae* Ajello					x			x
7. *Septochytrium variabile* Berdan							x	
Saccharomycetes								
Saccharomycetales								
8. *Candida tropicalis* Castellani (Berkhot)						x		
Zygomycetes								
Mucorales								
9. *Mucor hiemalis* Wehmer		x						
Straminipila (Chromista)								
Peronosporomycetes								
Leptomitales								
10. *Apodachlya pyrifera* Zopf	x							
Pythiales								
11. *Myzocytium zoophthorum* Sparrow	x		x	x	x	x	x	
12. Pythium akanense Tokunaga				x				
13. *Py. aquatile* Höhnk							x	
14. *Py. aristosporum* Vanterpool		x						
15. *Py. butleri* Subramaniam	x							
16. *Py. debaryanum* Hesse	x							
17. *Py. elongatum* Matthews							x	
18. *Py. helicandrum* Drechsler	x							
19. *Py. hemmianum* Takahashi			x					
20. Py. inflatum Matthews				x				
21. *Py. intermedium* de Bary								x
22. *Py. jirovecii* Cejp	x						x	
23. *Py. rostratum* Butler				x	x	x		x
24. *Py. undulatum* Petersen				x				

Table 2. Continued.

Classification (kingdom, class, order and species)	Water sites							
	Dojlidy		Mickiewicza Str.		Fosa		Akcent	
	April	November	April	November	April	November	April	November
Saprolegniales								
25. *Achlya americana* Humphrey	x				x			
26. *Ac. diffusa* Harvey	x							
27. *Ac. dubia* Coker						x		x
28. *Ac. klebsiana* Pieters		x		x	x			
29. *Ac. oblongata* de Bary	x							
30. *Ac. orion* Coker			x			x		
31. *Ac. polyandra* Hildebrand		x		x	x	x		
32. *Ac. treleaseana* (Humphr.) Kauffm.		x			x			
33. *Aphanomyces bosminae* Scott		x						
34. *Ap. irregularis* Scott					x		x	x
35. *Ap. laevis* de Bary	x			x				
36. *Ap. stellatus* de Bary								x
37. *Cladolegnia unispora* (Coker et Couch) Johannes			x					
38. *Dictyuchus sterilis* Coker					x			
39. *Isoachlya monilifera* (de Bary) Kauffm.						x		
40. *S. anisospora* de Bary		x		x				
41. S. asterophora de Bary				x				
42. *S. delica* Coker	x			x				
43. *S. ferax* (Gruith) Thuret	x			x	x	x		x
44. *S. glomerata* (Tiesenh.) Lund	x	x		x		x		
45. *S. litoralis* Coker		x						
46. *S. parasitica* Coker		x	x	x	x	x		x
47. S. pseudocrustosa Lund				x				
48. *Thraustotheca clavata* (de Bary) Humphrey	x			x		x		x
Total number of species in April and November 2006	15	13	7	18	13	13	7	10
Total number of species	7		22		19		14	

Most of them are phyto- and zoosaprotrophs with outstanding enzymatic ability to decompose dead organic matter of plant and animal origin. Therefore, aquatic fungi, apart from bacteria, are known to mineralize organic matter and prevent eutrophization to a remarkable extent [23-24].

Straminipilous organisms belonging to the above mentioned genera can also be the parasites of animals, plants and humans, although they were initially classified as saprobionts.

Of the 48 species of fungi and straminipilous organisms found in the four ponds, 12 commonly inhabit crustaceans and freshwater fish. They induce mycotic infections called aphthae [25-29], thus causing substantial losses in hatcheries, pond fish farms, lakes and rivers. Mycelia grow on injured fish tissues and may also appear on spawn [30]. Among the fish and crustacean parasites worthy of note are *Achlya dubia, Ac. oblongata, Ac. polyandra, Aphanomyces bosminae, Ap laevis, Dictyuchus monosporus, Saprolegnia ferax, S. parasitica, S. pseudocrustosa* and *Thraustotheca clavata. Achlya dubia* grows frequently on the fish gill, fins and skin. This taxon was found, among other places, in the waters of Egypt, where it caused great losses in fish farming [31]. *Achlya polyandra* has been known to cause mycotic infections of economi-

Table 3. Occurrence of fungi and straminipilous organisms on animal and plant substrates.

Animal and plant substrates	Fungi	Number of fungi
buckwheat seeds (*Fagopyrum esculentum* Moench L.)	21, 22, 34, 39	4
clover seeds (*Trifolium repens* L.)	3, 4, 6, 7, 10, 11, 14, 15, 16, 19, 20, 22, 23, 29, 31, 32, 34, 37	18
crustacean (*Gammarus pulex* L.)	1, 2, 8, 13, 14, 16, 17, 18, 19, 21, 22, 23, 24, 25, 28, 29, 30, 31, 33, 34, 35, 36, 37, 38, 39, 44, 46	27
hemp seeds (*Cannabis sativa* L.)	19, 22	2
onion skin (*Allium cepa* L.)	5, 12, 41, 42, 43, 44	6
snake skin (*Natrx natrix* L.)	2, 6, 9, 14, 25, 26, 27, 29, 33, 34, 37, 40, 47, 48	14

cally valuable fish species and their eggs [32]. Substantial losses of the spawn and fish breeding are also caused by *Aphanomyces laevis*, *Dictyuchus monosporus* and *Thraustotheca clavata* [32-34]. *Ahanomyces laevis,* commonly considered a fish parasite, has also been detected on amphibians [1].

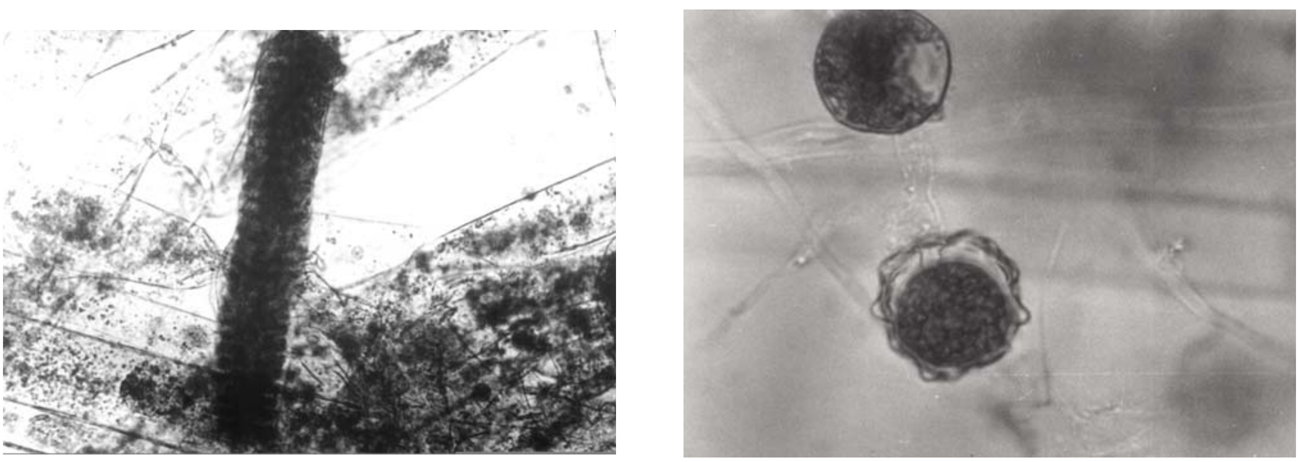
However, it is *Saprolegnia ferax* and *S. parasitica* that cause the greatest losses in hatcheries, pond fish farms and lakes [28, 35-39]. These fungi are known to have caused the death of over half of the *Salmo trutta* population in England [40].

Worthy of note is identification of two species of straminipilous organisms previously described as saprobionts, namely *Pythium jirovecii* and *Py. undulatum*. The former was isolated in the spring months in Dojlidy and Akcent only on the crustacean *Gammarus pulex*. The latter was found to grow in the spring period at Mickiewicza Street only on *Trifolium repens* seeds. According to literature data, the two species can parasite on crustaceans and cause mycotic infections in fish. *Pythium jirovecii* was first described by Cejp (1959) in Czechoslovakia as a parasite of the crustacean *Daphnia pulex* embryos in Żoldanka Pond near Blatana in Bohemia. [41]

Interesting is also the finding of two species of straminipilous organisms that induce plant diseases *Pythium butleri* – a parasite of tobacco and potatoes, and *Py. debaryanum* – a soil pathogen causing decay of cotton, pea, cabbage, tobacco and sugar beet [1]. These phytopathogens were found to grow mainly on seeds *Trifolium repens* in pond Dojlidy only in spring months.

Of saprobionts that can be pathogenic to humans, *Catenophlyctis variabilis* was found in all the ponds investigated in the current study. This species has been described as a saprobiont occurring on cellulose and keratin substrates and on human fibrin sheet [1, 42]. Keratinophilic pathogenic fungi growing on human skin, nails, hair, on animal fur and bird feathers have been observed in various types of reservoirs [42-49]. In the investigated ponds, *Catenophlyctis variabilis* was always identified only on snake skin *Natrix natrix.*

Moreover, the finding of fungi that are pathogenic to humans, namely *Candida tropicalis* and *Aspergillus niger*, in the aquatic reservoirs investigated in the current study should be emphasized. The former was noted only in autumn in pond Fosa, the latter in pond Dojlidy and pond Fosa also in autumn. Both taxons belong to potential yeast-like fungi



A B

Fig. 2. Some straminipilous organisms found in ponds in Białystok.
A - *Thrausthoteca clavata* - sporangium.
B - *Saprolegnia asterophora* - oogonia.

inhabiting human skin, alimentary tract and genitourinary system [45, 50-52]. *Aspergillus niger* produces one of the most toxic poisons - aphlatoxin, rich in hepatocarcinogens, which induces aspergiliosis or "lung disease" [53]. It occurs in all human body fluids and tissues. *Candida tropicalis* is the cause of blastomycosis or aphthae, especially of the sexual organs.

Worthy of note is also the presence of numerous phytosaprobionts that show distinct enzymatic capability of splitting cellulose and thus mineralizing dead plant fragments, most commonly seeds, fruits, flower petals, leaves and stalks. These include *Achlya klebsiana, Karlingia rosea, Nowakowskiella elegans, N. macrospora, Pythium akanense, Py. aquatile, Py. elongatum, Py. inflatum* and *Py. intermedium*. Fungi and straminipilous organisms growing on various plant substrates in the waters of north-eastern Poland have been investigated by Czeczuga et al. [54-56].

The study revealed that in ponds with lower load of organic matter and biogenic compounds the number of fungus species was higher. However, when pollution exceeded the tolerable norms, it became a limiting factor for the occurrence of the respective species.

Conclusions

The hydrochemical analysis of water showed trophic diversity of the ponds within Bialystok, exerting an effect on the occurrence and growth of straminipilous organisms and aquatic fungi. High content of organic matter and elevated physico-chemical parameters of water were the likely factors limiting the occurrence of aquatic fungi and straminipilous organisms. This mainly referred to such biogenes as various forms of nitrogen, phosphate and sulphates. High oxygen content and low oxidability had a stimulatory effect on the growth of fungi.

The least eutrophized was Dojlidy, which contained the largest number of straminipilous organisms and fungus species (27). The Ponds at Mickiewicza Street and Fosa were characterized by a relatively high content of organic substances and biogenic compounds, and therefore the numbers of fungus species found were lower (Mickiewicza 22, Fosa 19). The most eutrophic was Akcent, with only 14 species found there.

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