

## Keratinophilic fungi in various types of water bodies<sup>1</sup>

BAZYLI CZECZUGA, ELŻBIETA MUSZYŃSKA

Department of General Biology, Medical Academy, Kilifiskiego 1,  
15-230 Białystok, Poland

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The keratinophilic fungi in various types of water bodies (slough, pond, beach pool, two lakes and two rivers) were studied. Samples of water were collected every other month for hydrochemical analysis and once a month (1989-1990) in order to determine the fungus content. Human hair, snippings of finger-nails, chips of hoofs, feathers and snake exuviae were used as bait.

Twenty-five species of keratinophilic fungi were found in various types of water bodies. *Hyphochytrium catenoides*, *Aphanomyces stellatus*, *Leptolegniella caudata* and *Achlya oligacantha* represent new records as keratinophilic fungi.

### INTRODUCTION

From the physiological standpoint, fungi occurring in an aquatic environment constitute a diverse group of organisms. Some of them grow on living plant and animal organisms such as parasites, others play an important role in the decomposition of dead plant or animal remains thus constituting the phyto- or zoosaprophyte groups (S p a r r o w, 1968). Within this latter group, there are a number of species on growing animal substrate which is not easily hydrolyzed such as feathers, hair, skin, horns or hoofs formed, from keratin. The remains of these parts of animals, whose bodies had already decayed on the land, were washed by streams of rain water into a body of water or water-course. Frequently a considerable amount of the animal remains containing keratin comes from municipal or industrial wastes.

The aim of the present study was to determine those species of fungi the aquatic fungi which are capable of hydrolyzing keratin proteins which are in various types water bodies with reference to the chemism of water.

<sup>1</sup>Part 31 in the series "Studies of Aquatic Fungi"

## STUDY AREA

The following types of water bodies were investigated study: a slough, 1 pond, 1 pool, 2 lakes and 2 rivers. The characteristics of these waters and the sites from which samples were taken are presented below:

— the slough – this is a mirey puddle, 1 x 0.5 m in dimensions, situated in the lower part of the Branicki Palace Park in which water overflowing from the fountains in the middle of the park collects. Rain water from the palace roofs also collects here.

— the pond in the Palace Park (2.5 ha max, depth of 1.75 m), in which swans are bred and where wild ducks also come. In addition, crucian carp and tench are bred for anglers.

— the beach pool (27.2 ha, max. depth 2.5 m), is located in the Dojlidy (district Białystok) and serves as a swimming pool in summer for the inhabitants of the city and for water sports. The samples were collected from the west side of this pool which the inhabitants use as a beach.

— Lake Białe (485 ha, max. depth 30 m), is surrounded by extensive coniferous woods of Augustów Forest. The western part of the lake is adjacent to Augustów Forest. The site for sampling was on the western part of the lake next to the Military Recreation Centre.

— Lake Necko (area 518 ha, max. depth 25 m). The northern shores of the lake adjoin Augustów Forest while the south-western shores border with the town of Augustów. For this reason most of the municipal and industrial wastes of the town are drained into the lake. The sampling site was on the eastern side of the lake next to the Polish Tourist Country-Lovers' Association Centre; the shore is sandy for 1.5 m.

— the River Biała (length 9.8 km) – a left-bank tributary of the Supraśl River flowing through Białystok City. Three sites differing in the degree of pollution were chosen:

Site I – the upper course of the Biała River, the water was the least polluted,

Site II – middle of the river, the site situated in the centre of Białystok – at this site numerous drains empty the municipal and industrial wastes into the river,

Site III – lower course of the Biała River below the city just where the Fish Processing Plant drains wastes rich in keratin into the river.

— the River Supraśl (length 106.5 km) – this is the right-bank tributary of the middle part of the Narew River, flowing through the Knyszyn Forest. The river is polluted with municipal wastes from the towns of Gródek, Michałowo, Supraśl and above all, from Białystok city (lower course). Along a stretch of 1 km of the Supraśl River, 3 sites were chosen:

Site I – above the municipal swimming pool at the sluice of an arm of the Supraśl River flowing just through the town of Supraśl,

Site II – situated several score meters below Site I at the municipal swimming pool and the junction of two arms of the river above the main drain of Supraśl town,

Site III – below the main drain of Supraśl town about 500 m away from Site II.

## MATERIALS AND METHODS

In order to determine to species composition of keratinophilic fungi were collected once a month (for fungi) in the years 1989-1990. From each site one sample was taken for hydrochemical analysis (every other month) and two samples for the mycological studies. Water was collected in 5-litre Ruttner bucket from the depth at which the bucket was immersed. In the water, the temperature was measured and the following determinations were carried out: the pH, CO<sub>2</sub>, dissolved oxygen, oxidability of water and its alkalinity, hardness of water calculated in Ca and Mg, amonium, organic nitrogen, nitrates, phosphate, chlorides, iron and sulphate concentrations, dry residue, substances dissolved in water and suspensions in water (Table 1). For the determinations of the different chemical elements in the water the methods recommended by Standard Methods (G o l t e r m a n, C l y m o, 1969) were employed; details of these methods were described in a previous paper (C z e c z u g a, P r ó b a, 1980). For mycological studies the water samples from each of the sites were transported in sterile glass containers of 1.5 litres capacity. Subsequently, in the mycological laboratory, they were placed in sterilized beakers (capacity of 0.6 l), to which the appropriate baits were added in accordance with the general principles of culture (F u l l e r, J a w o r s k i, 1986) and those of the To-Ka-Va method for keratinophilic fungi (V a n b r e u s e g h e m, 1952; B e d e n e k, 1972). Human hair, snippings of finger-nails, chips of hoofs, feathers and snake exuviae were used as bait. The above materials were previously cut into small pieces, washed carefully, boiled in a weak seap solution, rinsed thoroughly and then boiled several times. The samples thus prepared from each site were kept in the laboratory for 3-6 months and precautions were taken to ensure that the thermo-lighting conditions were as close as possible to those prevalent outside the laboratory. The fungi found were determined by their morphological features, measurements being made of the shreds oogonia, and oospores by means of an ocular microscope. Species of the keratinophilic fungi were determined from the mycological keys (S k i r g i e l l o, 1954; S p a r r o w, 1960; B a t k o, 1975).

In order to determine the relation between the number of species of fungi at a given site and the various factors in the aquatic environment, statistical calculations were made. For this purpose the multiple correlation coefficient was used; details of these methods were described in a paper of C z e c z u g a and P r ó b a (1987). The regression programme with a choice of variables was applied on a O D R A - 1 2 0 4 digital computer.

## RESULTS

As regards the water from the slough, pond, pool and lakes it was found that in respect of such properties as oxidability and the content of various forms of nitrogen and phosphorus, the slough contained water nearest to the eutrophic type, whereas

the others can be classified in descending order of trophicity as follows: the beach pool, the palace pond, Lake Necko and Lake Biale. The highest mean concentration of calcium and magnesium was noted in the slough that, of sulphates in the palace pond, and that of iron – in the beach pool. In the running water of the rivers Biała and Supraśl River, the former was richer in biogens particularly at Sites II and Sites III. A Site I, the Biała River had lower values of nearly all the properties. The mean values noted in the water from the indicated that Site I was rather richer in biogens, having greater oxidability and alkalinity and a higher concentration of calcium and chlorides.

In the investigated water bodies 25 species of keratinophilic fungi were noted, most of which have previously been found as keratinophilic fungi (B a t k o, 1975). However, some new species of keratinophile fungi were also reported i.e.: *Hyphochytrium catenoides*, *Aphanomyces stellatus*, *Leptolegniella caudata* and *Achlya oligacantha*. Among these species 12 belonged to *Chytridiomycetes*, 1 to *Hyphochytridiomycetes*, 10 to *Oomycetes* and 2 to *Endomycetes* (Table 2). During the two year study the lowest number of species occurred in Lake Necko (8) whereas the highest number of species was noted in the rivers, particularly in the Supraśl River (23 species). Such species as *Rhizophydium keratinophilum*, *Blastocladiopsis parva*, *Aphanomyces irregularis* and *Achlya megasperma* were found in all the water bodies, whereas *Catenaria sphaerocarpa*, *Catenophlyctis variabilis* and *Mitochytridium regale* occurred only in the rivers. *Aphanomyces keratinophilus* was reported only from the slough and from the palace pond whereas *Leptolegniella caudata* occurred in the palace attractive substrate was found to be the snake exuviae on which 18 fungus species grew whereas the lowest number of species (only 4) developed on feathers. The development of *Leptolegniella piligena* was observed in these waters in which this fungus grew only on feathers (Table 3).

Seasonal changes in the occurrence of the various keratinophilic fungus species in the investigated waters are presented in Tables 4-6. The data show that in each water body some species were presented throughout most of frequently species occur in the slough: *Blastocladiopsis parva* and *Aphanomyces irregularis*, in the palace pond – *Blastocladiopsis parva*, *Aphanomyces irregularis*, *Leptolegniella keratinophila*, *Aphanodictyon papillatum* and *Achlya oligacantha*, and in the beach pool – *Chytriomycetes annulatus* and *Blastocladiopsis parva*. The most frequently noted species in Lake Biale were *Aphanomyces irregularis*, *Achlya oligacantha* and in 1989 *Rhizopodium keratinophilum*, *Chytriomycetes poculatus* and *Blastocladiopsis parva* while during a six months' period only *Rhizopodium keratinophilum* was reported from Lake Necko occurrence.

In the investigated rivers, the frequency of occurrence of the various species differed at various sites and in the years of investigations. In 1989 the species following were most frequently encountered at all three Sites in the Biała River. *Blastocladiopsis parva* and *Aphanomyces irregularis* whereas, in 1990, only *Aphanomyces irregularis* was found. In the Biała River such species as *Hypochytrium catenoides* and *Trichosporon cutaneum* did not appear at Site I during the whole period of the study but were quite frequently encountered at the other two Sites.

Table 1

Chemical composition of the water (mean from 12) of the investigated bodies of water (in mg l<sup>-1</sup>)

Specification	Slough	Pond	Beach Pool	Lake Biata	Lake Necko	River Biala			River Supradl		
						I	II	III	I	II	III
Temperature °C	5.56	7.68	8.35	10.17	10.71	12.49	11.05	12.15	8.70	9.13	9.66
pH	7.59	7.32	6.75	6.99	7.34	7.42	7.22	7.22	7.62	7.32	7.59
Oxidability	8.14	15.91	9.04	7.52	10.21	8.47	9.92	9.92	9.55	8.69	8.29
CO <sub>2</sub>	19.11	21.52	18.45	4.68	2.35	22.60	28.26	27.81	14.91	10.69	14.66
Alkalinity in CaCO <sub>3</sub> (in mval l <sup>-1</sup> )	3.95	4.53	3.35	2.42	3.43	4.31	4.56	4.78	3.90	3.61	3.60
N(NH <sub>3</sub> )	0.26	0.25	0.38	0.03	0.043	0.61	1.77	1.97	0.27	0.27	0.31
N(NO <sub>2</sub> )	0.009	0.009	0.066	0.101	0.015	0.04	0.06	0.13	0.02	0.02	0.02
N(NO <sub>3</sub> )	0.16	0.038	0.062	0.035	0.011	0.18	0.26	0.31	0.29	0.08	0.20
PO <sub>4</sub>	1.57	1.03	0.59	0.045	0.113	0.45	1.35	1.21	0.96	0.85	1.27
Cl	49.25	89.38	63.50	40.75	38.88	71.75	74.87	92.00	52.63	51.62	38.00
Total hardness in Ca	91.89	82.39	65.87	41.23	54.99	90.86	100.25	100.70	68.24	63.85	66.24
Total hardness in Mg	35.87	29.19	19.19	30.77	14.07	22.11	21.62	56.04	15.26	13.88	17.51
SO <sub>4</sub>	67.00	85.27	48.13	19.22	21.66	51.64	55.45	60.36	22.28	24.49	22.35
Fe	0.83	1.11	1.68	0.097	0.10	1.23	1.07	1.23	1.41	1.18	1.59
Dry residue	474.00	649.63	341.38	910.13	255.75	488.12	523.50	540.37	297.00	317.00	326.38
Dissolved solids	414.75	637.25	299.88	830.75	238.25	356.87	442.50	468.00	272.87	259.88	219.62
Suspended solids	59.25	11.13	14.50	80.38	17.50	133.25	85.25	72.38	24.25	56.00	106.37

Table 2

Keratinophilic fungi found in particular of the investigated bodies of water

Fungi	Slough	Pond	Beach pool	Lake Biata	Lake Necko	River Biata	River Supraßl
<b>Chytridiomycetes</b>							
<i>Rhizophyidium apiculatum</i> Karl.	.	X	.	X	.	X	X
<i>R. keratinophilum</i> Karl.	X	X	X	X	X	X	X
<i>R. nodulosum</i> Karl.	X	X	X	X	.	X	X
<i>R. piligensum</i> Oo. et Kob.	.	.	.	X	.	X	X
<i>Chytromyces anisulzeus</i> Dogma	.	X	X	X	X	X	X
<i>C. poculatus</i> Willough. et Town.	.	X	.	X	X	X	X
<i>Catenaria anguillicolae</i> Sor.	X	X	.	X	X	X	X
<i>C. sphaerocarpa</i> Karl.	.	.	.	.	.	X	X
<i>C. verrucosa</i> Karl.	.	.	X	X	.	X	X
<i>Catenophlyctis variabilis</i> (Karl.) Karl.	.	.	.	.	.	X	X
<i>Blastocladiopsis parva</i> (Whiffen) Spar.	X	X	X	X	X	X	X
<i>Mitrochrysidium regale</i> Haasian	.	.	.	.	.	X	X
<b>Hypochytridiomycetes</b>							
<i>Hypochytrium catenoides</i> Karl.	.	.	.	.	.	X	.
<b>Oomycetes</b>							
<i>Lagenidium humanum</i> Karl.	.	.	X	.	.	X	X
<i>Aphanomyces irregularis</i> Scott	X	X	X	X	X	X	X
<i>A. keratinophilus</i> (Oo. et Kob.) Seym. et John.	X	X	.	.	.	.	.
<i>A. scilicatus</i> de Bary	.	.	X	X	.	.	X
<i>Leptolegnia caudata</i> de Bary	X	X	.	.	.	.	X
<i>Leptolegnia keratinophila</i> Hun.	X	X	.	.	.	.	X
<i>L. piligena</i> Oo. et Kob.	.	X	X	X	X	.	X
<i>Aphanodictyon papillatum</i> Hun.	X	X	X	X	.	X	X
<i>Achlya megasperma</i> Humpb.	X	X	X	X	.	X	X
<i>A. oligacantha</i> de Bary	X	X	.	X	X	X	X
<b>Ectomycetes</b>							
<i>Canidia albicans</i> (Robin) Berk.	.	.	X	X	.	X	X
<i>Trichosporon cutaneum</i> (de Beurn. Goug. et Vauch.) Ota	X	.	X	X	.	X	X
Number of species	10	15	14	17	8	21	23

Table 3

Attractive substrate for particular of the keratinophilic fungi (1 - feathers, 2 - human hairs, 3 - hoofs, 4 - finger-nails, 5 - snake exuviae)

Fungi	Slough	Pond	Beach pool	Lake Biata	Lake Necko	River Biata	River Supradl
<b>Chytridiomycetes</b>							
<i>Rhizoglyphidium apiculatum</i> Karl.	.	5	.	5	.	5	5
<i>R. keratinophilum</i> Karl.	1, 2, 3, 4, 5	5	5	5	2, 5	5	5
<i>R. nodulosum</i> Karl.	3, 5	1, 3, 5	3, 4, 5	2	.	2, 3, 4	3, 4, 5
<i>R. piligenum</i> Oo. et Kob.	.	.	.	2, 3	.	3	3, 4
<i>Chytromyces annulatus</i> Dogma	.	5	5	.	.	5	5
<i>C. proclivus</i> Willough. et Town.	.	5	.	5	5	5	5
<i>Caenaria anguilulae</i> Sor.	5	5	.	5	5	5	5
<i>C. sphaerocarpa</i> Karl.	.	.	.	5	.	5	5
<i>C. verrucosa</i> Karl.	.	.	5	5	.	5	5
<i>Cateanophlyctis variabilis</i> (Karl.) Karl.	.	.	.	.	.	5	5
<i>Blastocladiopsis parva</i> (Whiffen) Spar.	5	5	5	5	5	5	5
<i>Mitochytridium regale</i> Hassan	.	.	.	.	.	5	5
<b>Hypochytridiomycetes</b>							
<i>Hypochytridium caenoides</i> Karl.	.	.	5	5	.	5	.
<b>Oomycetes</b>							
<i>Lagenidium humanum</i> Karl.	.	.	3, 5	.	.	3, 5	3, 5
<i>Aphanomyces irregularis</i> Scott	2, 3, 4, 5	3, 5	3, 5	3, 5	3, 5	3, 5	3, 5
<i>A. keratinophilus</i> (Oo. et Kob.) Seym. et John.	2, 3	3	.	.	.	.	.
<i>A. stellatus</i> de Bary	.	.	2, 3, 4	2, 3, 4	.	.	2, 3, 4
<i>Lepidogonia caudata</i> de Bary	.	3, 5	.	.	.	.	3, 5
<i>Lepidogonia keratinophila</i> Hun.	5	5	.	.	.	5	5
<i>L. piligena</i> Oo. et Kob.	.	1	1	1	1	.	.
<i>Aphanodictyon papillatum</i> Hun.	2, 3, 4	3	1, 2, 3	3	3	3	3
<i>Achlya mesasperma</i> Humpb.	2, 3, 4	3	3	3	3	3	3
<i>A. oligacantha</i> de Bary	2, 3, 4	3	.	3	3	3	3
<b>Endomycetes</b>							
<i>Candida albicans</i> (Robin) Berk.	.	.	3, 5	3, 5	.	3, 5	3, 5
<i>Trichosporon cutaneum</i> (de Beaurm. Goug. et Vauch.) Ota	3, 5	.	3, 5	3, 5	.	3, 5	3, 5

Table 4

Seasonal changes in the occurrence of the various keratinophilic fungus species in the stagnated water

Fungi	Slough		Pond		Beach pool		Lake Biiale		Lake Necko	
	1989	1990	1989	1990	1989	1990	1989	1990	1989	1990
<b>Chytridiomycetes</b>										
<i>Rhizophyllum apiculatum</i> Karl.										
<i>R. keratinophilum</i> Karl.	II, XII		II, VI, X, XI	VI	VI, VIII	III, VIII	I, II, IX, X	I, II, IX, X	I, V, IX, X	
<i>R. nodulosum</i> Karl.	IX	VIII	IV, VIII, IX	VIII, IX	I	I	I, V, IX, X	IV, VIII		
<i>R. piligenum</i> Oo. et Kob.			II	II	V-XI	V-X	I, II, IX, X	I, II		
<i>Chytrionyces annulatus</i> Dogma			I				I, V, IX, X	IX, X	IX, X	
<i>C. poculatus</i> Willough. et Town.			VIII-X	V, VIII-X			I, II, IX, X	IX, X	IV, V, IX, X	
<i>Catenaria anguiliferae</i> Sor.		III			I	I	I, II, IX, X	I, II, IX, X		
<i>C. verrucosa</i> Karl.			I-III, VI-	I, II, VI, X	VI-X	VI-IX	I, II, VII-X	I, II, IX, X	IV-VIII	VII-X
<i>Blastocladiopsis parva</i> (Whiffen) Spar.	I-XII	I-VI, VIII-XII								
<b>Hypochytridiomycetes</b>										
<i>Hypochytrium catenoides</i> Karl.					III-V, IX	IV, X	IX, X			
<b>Oomycetes</b>										
<i>Aphanomyces irregularis</i> Scott	I, III-VII, IX, XI-XII	I-VII, IX, XII	I-IV, VI, VII, IX-XII	I, II, IV, VII, X, XII	VIII-X	VIII-X	I-V, VII-X	IV-X	VII-X	VII, VIII
<i>A. keratinophilus</i> (Oo. et Kob.) Seym. et John.	VI		VI							
<i>A. stellatus</i> de Bary					II, IX	II, X	I, II, IX, X	IX, X		
<i>Lagenidium humanum</i> Karl.			XI	IX, X	V, VIII-X	X				
<i>Leptogonia caudata</i> de Bary			I-III, VI-							
<i>Leptogoniella keratinophila</i> Hun.		VIII	VIII, IX-XI							
<i>L. piligena</i> Oo. et Kob.			I-VII, IX-XI	I, II, IV-VI, X, XI	VI, IX, X	VI	I, II, IX, X	IV, V, IX, X	IX, X	
<i>Aphasodictyon papillatum</i> Hun.			I, II, XI, XII	IX-XII	X	VIII	I, V	VII-VIII		
<i>Achlya megasperma</i> Humpb.	I, XI	I, XI	I, II, XI, XII	I, XI, XII	I, XI, XII	I, XI, XII	I, II	I, II	I, II	
<i>A. oligacantha</i> de Bary	V, VII, IX, XII	V, X, XII	VI, VII, IX-XII	IV, VI, X			I, V, IX, X	IV-X	IV-VIII	IV-VIII
<b>Endomycetes</b>										
<i>Candida albicans</i> (Robin) Berk.	V				IX, X	IX, X	IX, X	IV, V, IX, X		
<i>Trichosporon estantenum</i> (de Beaurm. Goug. et Vauch.) Ota					V, VI, IX	VI, VII	VII, VIII	IX, X		



Table 5

Seasonal changes in the occurrence of the various keratinophilic fungus species at particular sites of the running water

Fungi	River Biaba						River Supradí						
	Site I		Site II		Site III		Site I		Site II		Site III		
	1989	1990	1989	1990	1989	1990	1989	1990	1989	1990	1989	1990	
<b>Chytridiomycetes</b>													
<i>Rhizophyidium apiculatum</i> Karl.	VI, XII	IV, XII	VII	IV	I, III, IX, XI	I, III, IX, XII	III, IX	IV, IX	I-III	III-VI, IX	III, IX	III, IX	
<i>R. keratinophilum</i> Karl.	I, II, VIII	VI, VIII	I, XI	III, IV, XI	II-VIII	IV, IX, XII	I, II, X	IV-VI, X, XI	IX, X	IX	VIII	III, IX	
<i>R. nodulosum</i> Karl.	.	.	I	.	I, V, IX	V-VII, IX	.	IV	IV, V	.	.	.	
<i>R. piligenum</i> Oo. et Kob.	.	.	IX, XI	XI	VII-XI	X, XII	.	VIII	.	.	.	.	
<i>Chytriumyces annulatus</i> Dogma	.	.	II, VII, X	III-V, VII, VIII-X	I-III, VII, VIII-X	III, XII	VIII	.	.	.	III, IV	.	
<i>C. pocolatus</i> Willough. et Towa.	V, VI, X	VI	I, V, IX-XII	IX-XII	I, V, XI, XII	I, V, IX	.	I, XII	.	.	.	.	
<i>Catenaria anguilulatae</i> Sor.	XI	XI	X	IV	VI-X	III-X	.	III-IV	.	.	XI	XI	
<i>C. sphaerocarpa</i> Karl.	VI	VI	IX	III, VII	II, III, VII-X	VII, IX	.	.	.	X	X	X	
<i>C. verrucosa</i> Karl.	.	.	II-IV, VII, IX	IX	I, III, IX-XI	IX	.	.	XI	XI	.	.	
<i>Catenophlyctis variabilis</i> (Karl.) Karl.	.	IV	IV, IX-XI	IV, VIII-XI	II, III, IX-XI	II, VII, IX	IV-XII	III, VIII-XII	IV	III, VII-XII	V-XII	IV-VI, VIII-X, XII	
<i>Blastocladiopsis parva</i> (Whiffen) Spar.	I, IV, VIII-XII	II, III, VIII-XII	I, IV, VI, VII, XII	I, IV, VI, VII, XII	I, II	I, VI, XII	I, VIII, XII	.	.	.	.	.	
<i>Mitocytridium regale</i> Haasan	.	.	XI	VI, XI	V, VI, X	V, VI, X	.	.	III	III	.	.	
<b>Hyphochytridiomycetes</b>													
<i>Hyphochytrium catenoides</i> Karl.	.	.	II	.	II, V, VIII-XIII, V, VIII-XII	.	.	.	.	.	.	.	
<b>Oomycetes</b>													
<i>A. stellatus</i> de Bary	I-XII	VI	II-V, VIII-XII	II-V, IX, XII	I, IV, VI, IX, XII	I, IX, XII	VII, VIII, IX, XII	VIII, IX, I-VI, IX, XII	V-IX, I, VI, XI, XII	III, V, IX, I, VI, XI, XII	I-VI, IX, I, VI, XI, III-VII	.	
<i>Lagenidium humanum</i> Karl.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>Aphanomyces irregularis</i> Scott	.	.	.	.	.	.	.	.	.	.	.	.	
<i>A. stellatus</i> de Bary	.	.	.	.	.	.	.	.	.	.	.	.	
<i>Leptolegnia caudata</i> de Bary	.	.	.	.	.	.	.	.	.	.	.	.	
<i>Leptolegnia keratinophila</i> Hun.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>L. piligena</i> Oo. et Kob.	.	.	.	.	.	.	.	.	.	.	.	.	
<i>Aphanodicyyon papillatum</i> Hun.	VIII, IX, XII	I, XII	VIII, IX, XII	I, XII	II, III, XII	II, III, XII	I, II, XI, XII	I-III, XII	I-III, XI, XII	I-III, XI, XII	I, II, XII	.	
<i>Achlya megasperma</i> Humpff.	VI, VII, IX	VI-IX	VI-XI	VI, IX, XII	VI, IX, XII	V-XI	VI, VII, IX, XII	VI, VII, IX, XII	VI, VII, X, XI	VI, VII, X, XI	VI, X, XI	XI	
<i>A. oligarcantha</i> de Bary	.	.	.	.	.	.	.	.	.	.	.	.	
<b>Endomycetes</b>													
<i>Candida africana</i> (Robin) Berk.	.	.	I, IV-XI	IV-IX, XI	X, I-IV, IX, XII	VI, VII, IX, XII	VIII-XI	IX	VI, XI	III, VIII	III, VIII	III, V, IX, X	
<i>Trichosporon cutaneum</i> (de Beurm. Goug. et Vauch.) Ota	.	.	.	.	.	.	I, IX, X	VI	V-IX	I-V, XII	IX	I, III	

Table 6

The coefficients of correlation between the values of the environmental factors and the number of keratinophilic fungus species in particular of the investigated bodies of water (level of significance 0.04)

Specification	Slough	Pond	Beach Pool	Lake Blade	Lake Nescko	River Biala	River Supradl
Temperature °C	0.4597	0.0673	0.3688	-0.3491	0.2201	-0.1601	-0.0345
pH	0.3548	0.1802	0.3414	0.2856	0.5664	-0.3550	-0.2350
Oxidability	-0.1659	-0.2222	-0.2362	0.4150	0.2036	0.0661	0.2932
CO <sub>2</sub>	0.2611	0.7347*	0.1818	0.5385	0.3966	0.0693	-0.0175
Alkalinity in CaCO <sub>3</sub> (in mval l <sup>-1</sup> )	-0.3987	0.0114	-0.2839	0.5867	0.2435	0.1654	-0.0912
N(NH <sub>3</sub> )	0.0529	0.7702*	-0.1197	0.8336*	0.4316	0.1173	-0.0353
N(NO <sub>2</sub> )	0.0305	0.2315	-0.1223	0.6678	0.3341	0.3163	0.0214
N(NO <sub>3</sub> )	0.6281	0.7071*	0.1500	-0.1716	0.4082	0.0680	0.0936
PO <sub>4</sub>	0.7608*	0.4717	0.5136	-0.0473	0.3494	0.1763	0.2079
Cl	-0.1149	0.2017	-0.6136	0.3961	0.0692	-0.1425	-0.4531*
Total hardness in Ca	-0.2564	-0.7114*	-0.4976	0.3377	0.4252	0.0430	0.0473
Total hardness in Mg	-0.2056	0.2117	-0.0337	0.6722	0.1925	0.0169	0.0568
SO <sub>4</sub>	-0.2483	-0.3504	-0.1822	-0.6453	0.5858	-0.1084	0.0274
Fe	0.0449	0.5594	0.2905	0.6429	0.5303	0.0225	0.0492
Dry residue	-0.2701	-0.3541	0.3178	0.1831	0.5331	0.1911	-0.1976
Dissolved solids	-0.3629	-0.3415	0.1840	0.1810	0.2691	0.2803	0.1652
Suspended solids	0.3961	-0.4540	0.2927	0.2466	0.4352	-0.1354	-0.2652

\* - statistically credible

We also observed that some species appeared only in one month during the two years study. In the river Supraśl, *Catenaria spaerocarpa* occurred only in October of 1989 and 1990. *Mitothyridium regale* was noted only in March. On the other hand, there were some species which appeared during the two-year study in some of the waters studied only once whereas in the other water bodies they were found quite often. Within this group of species the following species noted: *Aphanomyces keratinophilus*, *Leptolegniella keratinophila* and *Trichosporon cutaneum* in the slough, *Chytriomycetes poculatus* in the Palace Park pond, *Lagenidium humanum*, in the Biala River, *Catenophlyctis variabilis* (Site II) and *Leptolegniella keratinophila* (Site III) in the Supraśl River.

## DISCUSSION

The studies showed, that the investigated water bodies differed not only morphologically but also to a greater or lesser extent, in the chemism of the water; there were marked differences between the various sites in the Biala River. The comparison of the degree of pollution of the water (due to municipal wastes at Site II and municipal wastes and garbage from the Fish Processing Plant at Site III) and the number of keratinophilic fungus species, showed that number of species and their regularity of appearance increased with the degree of pollution. This problem has been studied from a similar aspect by Simordova and Hejtmanek (1969) and Ulfig (1990). A larger or greater variety of species of keratinophilic mycoflora and their frequency of occurrence in the various seasons was also noted in the investigated water bodies. This physiological group of fungi grew on substrates containing keratin in an aquatic environment, the chemical composition of which varied in each body of water in time and space. Statistical data confirmed the relation between the number of species and the values of some hydrochemical parameters. These correlations differed greatly in the various water (Table 4, 5, 6). The studies conducted by Fischer and Werner showed (1955, 1958 a, b) that the attraction of the same substrate for the zoospores of representatives of the *Saprolegniales* depends not only on the age of the zoospores but above all on the chemism of water and concentration and appropriate proportions of chlorides, sodium, potassium, calcium and magnesium. The presence of minute quantities of amino acids enhances the attraction of the substrate for the zoospores of some *Saprolegniales* representatives. Furthermore, the zoospores and planozygotes of some species of *Allomyces* react differently to certain amino acids water (Machlis, 1958; Carlile, Machlis, 1965 a, b). This applies not only to zoosporophytes but also to phytopathogenic fungi (Hickman, Hi, 1966). It is beyond doubt that temperature exerts a decisive effect on the development of some keratinophilic fungi (Couch, 1935; Karling, 1948; Cooke, 1961; Irneo, Dogma, 1969). In our case, this applied to *Achlya megasperma*; this fungus occurred in all the water bodies only in the autumn and winter. It should be noted that the use of substrate containing keratin

by one and the same species of fungus differed in the various water bodies. *Rhizophydium keratinophilum* grew only on snake skin in the ponds, rivers and in Lake Białe whereas in Lake Necko it also grew on human hair and in the slough it was noted on snake skin, hair, feathers, hoofs and finger-nails. Karling (1946 a, b) isolated *Rhizophydium keratinophilum* on hair only. The same results were in the case of *Aphanomyces irregularis* and both species of *Achlya*. In the slough, *Aphanomyces irregularis* grew on hair, hoofs, finger-nails and snake skin but in all the remaining water bodies it developed only on hoofs and snake skin. Similarly, both *Achlya* species grew on the chips of hoofs in all the water bodies but in the slough it grew on hair and finger-nails in addition to hoof chips. It should be noted that the slough has the lowest volume of water (circa 1.5 m<sup>3</sup>), the lowest mean temperature of water and the highest mean value of pH, phosphorus and sulphates. It is possible that one of these factors alone or perhaps all of these factors together make these substrates attractive to these fungi.

The investigation showed that, the most frequently encountered fungi were *Blastocladiopsis parva* and *Aphanomyces irregularis* whereas *Catenaria sphaerocarpa*, *Catenophlyctis variabilis* and *Mitochytridium regale* occurred only in the rivers, and *Aphanomyces keratinophilus* in the slough and the palace pond (they are situated next to each other) while *Leptolegnia caudata* was found only in the water of the palace pond and the Supraśl River.

*Blastocladiopsis parva* is a species which occurs as saprophyte in north-eastern Poland in various types of waters ranging from springs (Czczuga et al., 1989) to rivers (Czczuga, 1991 a, b; Czczuga, Brzozowska, Woronowicz, 1990) and in many lakes of various types. *Aphanomyces irregularis*, on the other hand, is a well known keratinophilic fungus quite frequently found on substrates containing chitin (Sparrow, 1968). It was a common keratinophilic fungus in Lake Allahabad throughout the year (Dyal, Tandon, 1962). It has also been found in the water of Iceland (Johnson, 1968). As regards *Aphanomyces keratinophilus*, this fungus has been described so far as a representative of soil mycoflora (Okubo, Kobayashi, 1955; Sparrow, 1965; Karling, 1968; Seymour, Johnson, 1973). Karling (1968) isolated it on snake exuviae. *Catenaria sphaerocarpa* was first isolated by Karling (1938). Rothwell (1956) stated that this fungus, like other keratinophilic species of the genus *Catenaria* (Ichida, 1968; Nolon, 1970), grows best with a slightly alkaline pH and an optimum temperature of 25°C. In our case, the species of genus *Catenaria* also developed in low temperature. *Catenophlyctis variabilis* was found in a ditch containing very little water (Karling, 1947). It was isolated on such bait as human skin, hair, chips or horn, hoofs, human nails and wool from the soil of Brazil and the USA (Karling, 1951). In our study it occurred only in the rivers and was isolated only on snake skin. Booth and Barrett (1971) cultured *Catenophlyctis variabilis* on snake exuviae even in water from Arctic ice. Couch (1945), who studied the taxonomy and life cycle of *Catenaria* spp., concluded that the earlier descriptions of this species under

the name *Catenaria anguillulae* Sorokin represented more than one species. Sparrow (1960), Karling (1970) and Singh (1989) gave similar comments on the above fungus owing to its morphological variation. *Mitochytridium regale* also occurred only in the investigated rivers. This fungus was first isolated and described by Hassan (1986 b) who found it in a small pool in the Łazienki Park in Warsaw. The rivers in which we found this species would, therefore be the second place of occurrence of this fungus to be reported. *Leptolegniella caudata* is considered to be an aquatic and soil saprophyte or a parasite of the pelagic crustacean, *Leptodora kindtii*, frequently causing the complete destruction of populations of this crustacean (Peterson, 1910). It is also included in the group of acid water fungi (Batkó, 1975). Another finding worthy of note was the presence of the only representative of the *Hydrochytridiomycetes*, *Hyphochytrium catenoides* in the water of the beach pool, Lake Białe and the Biała River. This fungus is considered to be more common in soil than in water (Barr, 1970).

Its development was observed in water on the remains of *Chara* sp. and on higher plants. It is also known to be a parasite of *Charales* of the *Nitella* genus. In addition, this fungus has been isolated from waters of varying degrees of purity. Several authors (Harder, Persiel, 1962; Willoughby, 1971; Ellis-Evans, 1985) isolated this fungus from pure waters of the Antarctic whereas in our earlier studies we found it in the littoral area of Lake Niegocin, one of the most polluted lakes in the Masurian Lake District (Czeczuga, Woronowicz, 1992). It was also isolated from a small pond near Warsaw (Hassan, 1986 a). Karling was the first to describe this species (1939) and it has been found to be a cosmopolitan species occurring in soil, air and water in USA (Karling, 1939), Australia (Persiel, 1960 a, b), Iceland (Hohnk, 1960), India (Karling, 1966) and New Zealand (Karling, 1967).

The investigated lakes surprisingly differed in the number of species. In the water of Lake Necko the presence of 8 species of keratinophilic fungus was determined, while in the nearby Lake Białe (divided from the former lake by 100 m of land and connected to it by a canal for inland navigation) as many as 17 were noted. When comparing the hydrochemical analysis of the water of these two lakes, it was found that the mean values of pH, oxidability, alkalinity, phosphorus, calcium, sulphates and iron were higher in Lake Necko than in Lake Białe.

On the other hand, the mean values of such parameters as carbon dioxide, nitride and nitrate nitrogen, chlorides, magnesium, dry residue and substances dissolved in the water were far higher in Lake Białe. Furthermore, the localization of the spots for sampling differed. While the sampling site on Lake Białe was near the west shore where it was not affected by waves, the site on Lake Necko was near the east shore which is markedly affected by waves due to the north-west winds blowing in this part of Poland. As we know, a strong movement of water masses limits the development of hydromycoflora (Sparrow, 1968).

It may therefore, be assumed that the species composition of keratinophilic fungi in a given body of water is the resultant of the relations between the morphology of the water bodies, availability of substrate and chemism of water. The relations between these factors differ in time in the various water bodies. That is why, as is the case with plant saprophytes (Czeczuga, Próba, 1987), the factors limiting the number of keratinophilic fungus species differ in the various water bodies and also in different years in the same water body.

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