

PREVALENCE OF POTENTIALLY PATHOGENIC FUNGI IN THE BATHING SITES OF THE SULEJÓW RESERVOIR¹

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ABSTRACT. Progressive degradation of water ecosystems may contribute to the propagation of fungi pathogenic to humans and animals. The aim of this study was a quantitative evaluation and identification of species of potentially pathogenic fungi in the littoral zone of the Sulejów Reservoir used as recreation site. We studied samples of surface water and sediments collected from 6 sampling sites of the reservoir in the years 2000 and 2001. In 2000, fungi were isolated from 82.7% of samples, while in 2001 from as many as 95.4%. There were 28 species representing genera: *Candida*, *Cryptococcus*, *Geotrichum*, *Kloeckera*, *Rhodotorula*, *Saccharomyces*, and *Trichosporon*. The most common species were *Rhodotorula glutinis* and *Candida guilliermondii*. The density of the population of identified species depended on the season and sampling site and ranged from 80 to 328000 cells/dm³ (also uncountable growth). The waters of the Sulejów Reservoir may be a convenient place of anamorphic developmental forms, for the 15 cultured species which can also propagate in humans and other mammals.

Key words: pathogenic fungi, Sulejów Reservoir, water pollution, water quality

INTRODUCTION

Aggravation of physical, chemical, and biological parameters of water quality as a result of progressive degradation of the environment has been observed in many aquatic ecosystems. Contaminations, especially municipal sewage, may be a source of many microorganisms, including fungi potentially pathogenic to humans.

Ecology of inland water fungi is one of the least developed fields of mycology and hydrology.

The Sulejów Reservoir was built in 1973 when the Pilica River was dammed up on its 138.9 km. It is situated between two ravined parts of the river, in the towns Smardzewice and Sulejów (Ambrożewski 1977, 1993). The principal task of the reservoir is accumulation of drinking water for the city of Łódź. Furthermore, the reservoir retains floodwaters, plays a recreational role, and produces energy.

The aim of the present study was a quantitative evaluation and identification of potentially pathogenic fungi species in the littoral zone of the Sulejów Reservoir, where many recreation sites are located.

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MATERIALS AND METHODS

The surface samples of water (pelagic zone) and sediments were studied. The samples were collected from 6 sampling sites of the Sulejów Reservoir every week during April-September 2000 and every two weeks during April-October 2001. The description of sampling sites is given in Table 1.

Table 1. Description of sampling sites on Sulejów Reservoir

Sampling site	Localization and description of sampling site
Tresta-bay	Lower part of the reservoir; used as a bathing place; mean depth 3 m
Tresta	Lower part of the reservoir; frequently used for water sports; mean depth 6 m
Borki	Lower part of the reservoir, bay close to a tourist centre Borki; used as a bathing place; mean depth 2 m
Bronisławów	Middle part of the reservoir, close to water intake for Łódź in Bronisławów; mean depth 6 m
Lubanów	Middle part of the reservoir, close to a bank with forest, not exposed to strong anthropopressure; mean depth 2 m
Zarzęcin	Upper part of the reservoir; bank exposed to strong anthropopressure due to numerous summer houses; mean depth 3 m

Samples of 250 cm³ surface water were filtered with 45 µm Wathman GF/F filters. To isolate fungi strains the filters were washed out with 20 cm³ of 0.9% NaCl sterile solution. Subsequently, 1 cm³ of the obtained suspension, as well as the samples of collected sediments, were inoculated onto a solid Sabouraud medium with streptomycin and gentamycin, incubated for 24 h at 37°C, then left at 20-25°C. After 3-5 days the fungal colonies were counted and the fungi were transferred onto the Sabouraud medium without antibiotics. The obtained axenic strains of fungi were evaluated macroscopically, microscopically and the biochemical features were determined: fermentation and ability to assimilate carbohydrates with API 20 C and API 20 C AUX tests (bioMérieux), ability to assimilate nitrogen compounds nitrogen auxanogram (Lodder 1971, Kurnatowska 1995).

To evaluate the numbers of particular species, the numbers of grown colonies in 1 cm³ of water sample or sediment were counted per 1 dm³.

RESULTS

Our study covered fungi, which in past were described as yeast-like fungi and are classified presently as *Ascomycota* or *Basidiomycota* (De Hoog et al. 1996).

In 2000 the fungi were found in 82.7% of the investigated samples and in 2001, the fungi were more often isolated, in up to 95.4% of samples. The numbers of the organisms in pelagic water samples and sediments are shown in Fig. 1.

In both seasons investigated there were significant fluctuations of the total fungi

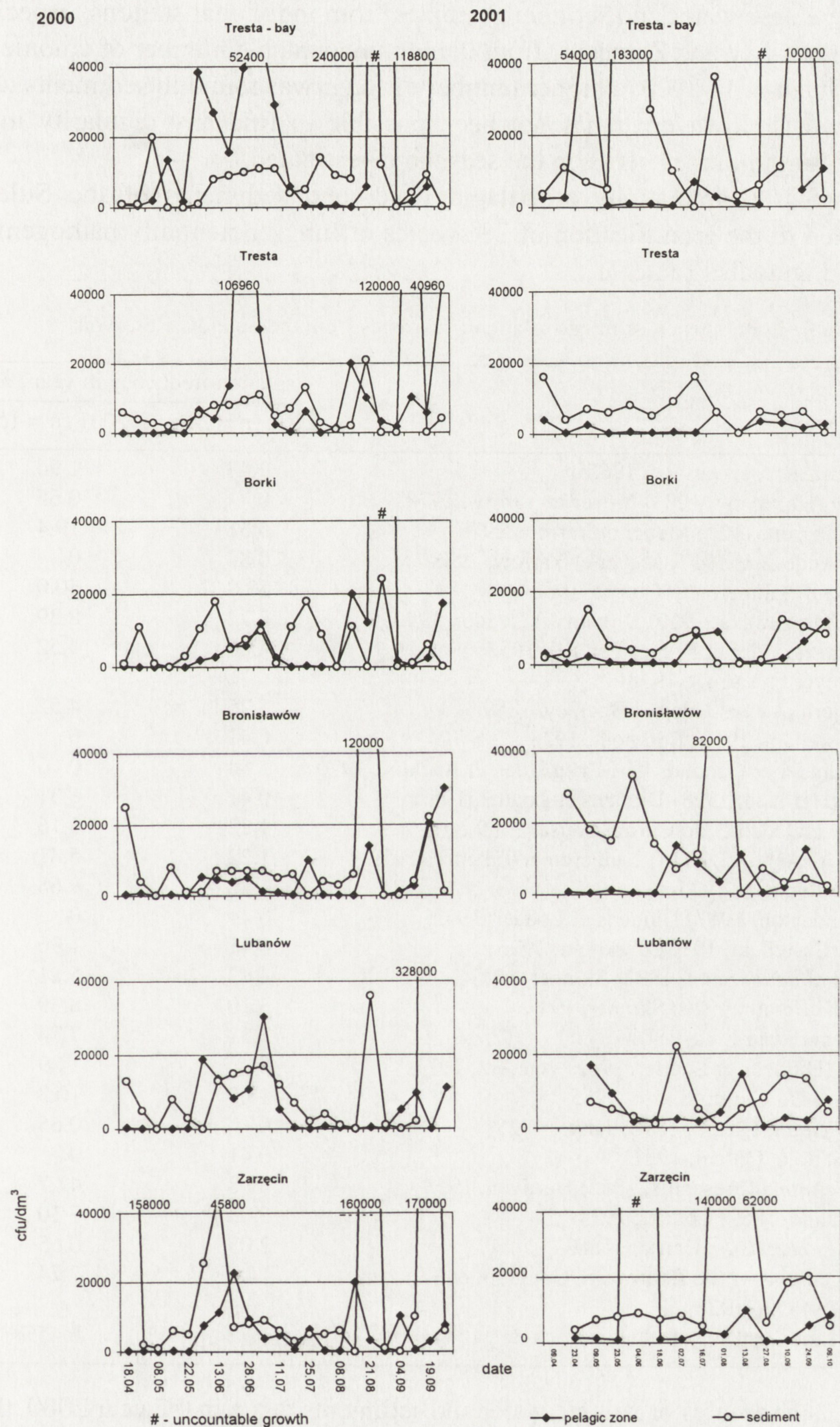


Fig. 1. Total number of fungi (cfu – colony forming units) in samples from Sulejów Reservoir sampling sites in 2000 and 2001

number in pelagic water and sediment samples from individual stations, especially from the Tresta-bay and Zarzęcin (from 0 up to uncountable number of colonies on diagnostic media). In 2000, a higher number of fungi was found in sediments taken in mid August. However, it has not been possible to find any regularity in the changes of fungi number between the seasons investigated.

Mycological examinations of pelagic water and sediments of the Sulejów Reservoir led to the identification of 28 species of fungi, potentially pathogenic to humans and animals (Table 2).

Table 2. Frequency of fungi isolation in samples from the Sulejów Reservoir

Species	Isolation frequency in year (%):	
	2000 (n = 246)	2001 (n = 155)
<i>Candida ciferrii</i> Kreger-van Rij, 1965	2.43	1.96
<i>C. colliculosa</i> (Hartmann, 1903) Meyer et Yarrow, 1978	0	0.65
<i>C. famata</i> (Harrison, 1928) Meyer et Yarrow, 1978	5.31	19.4
<i>C. glabrata</i> (Anderson, 1917) Meyer et Yarrow, 1978	0.82	0
<i>C. guilliermondii</i> Langeron et Guerra, 1938	13.8	40.0
<i>C. humicola</i> (Daszewska, 1912) Diddens et Lodder, 1942	7.72	8.39
<i>C. inconspicua</i> (Lodder et Kreger-van Rij, 1952) Meyer et Yarrow, 1978	4.06	4.52
<i>C. kefyr</i> (Beijerinck) van Uden et Buckley, 1889	3.25	4.52
<i>C. krusei</i> (Castellani, 1910) Berkhout, 1923	0.41	0
<i>C. lambica</i> (Linder et Genoud, 1913) van Uden et Buckley, 1970	2.84	0
<i>C. lipolytica</i> (Harrison, 1928) Diddens et Lodder, 1952	0.41	1.31
<i>C. lusitaniae</i> van Uden et do Carmo-Sousa, 1959	1.22	5.16
<i>C. parapsilosis</i> (Ashford, 1928) Langeron et Talience, 1959	1.22	5.81
<i>C. pelliculosa</i> Redaelli, 1925	1.22	9.68
<i>C. rugosa</i> (Anderson, 1917) Diddens et Lodder, 1942	0.41	0
<i>C. tropicalis</i> (Castellani, 1910) Berkhout, 1923	0.41	1.96
<i>Cryptococcus albidus</i> (Saito, 1922) Skinner, 1947	2.43	5.81
<i>C. laurentii</i> (Kufferath, 1920) Skinner, 1947	6.10	8.39
<i>Geotrichum candidum</i> Link, 1809	7.32	7.74
<i>G. capitatum</i> (Diddens et Lodder, 1942) von Arx, 1977	0	14.9
<i>G. penicillatum</i> Do Cormo-Sousa, 1965	18.3	10.3
<i>Kloeckera apiculata</i> (Reess, 1870) Janke, 1928	0	0.65
<i>K. japonica</i> Saito et Ohtani, 1931	0.41	0
<i>Rhodotorula glutinis</i> (Fresenius, 1852) Harrison, 1928	39.8	47.7
<i>R. rubra</i> (Demme, 1889) Lodder, 1934	2.03	7.10
<i>Saccharomyces cerevisiae</i> Hansen, 1883	2.03	0.65
<i>Trichosporon cutaneum</i> (de Beurmenn, Gougerot et Vaucher, 1909) Ota, 1926	14.6	7.74
<i>T. pullulans</i> (Lindner, 1895) Diddens et Lodder, 1942	7.72	6.45%

Among 245 samples of pelagic water and sediments taken in the year 2000, there were 356 fungi strains, representing 25 species linked to *Ascomycota* or *Basidiomycota*, while in the year 2001, in 153 samples – a total of 342 fungi strains

representing 23 species. The dominant species among fungi found in the year 2000 was *Rhodotorula glutinis* which was found in 87 pelagic water samples (out of 123 investigated) and in 11 sediment samples (out of 123). The second most prevalent species was *Geotrichum penicillatum*, followed by *Trichosporon cutaneum* and *Candida guilliermondii*. *R. glutinis* was again the dominant in 2001 and it was present in 56 out of 77 pelagic samples and in 18 out of 78 sediment ones. The next in frequency of occurrence was *C. guilliermondii* – over twice as frequently isolated from pelagic than sediment samples as in previous year. The next in the row were: *C. famata*, *G. capitatum*, and *G. penicillatum*. The frequency of other species did not exceed 10% (Table 2). Changes in the number of *R. glutinis* (cfu/dm³) from all sampling sites in both years are shown in Fig. 2 and the number of the most often isolated species from genus *Candida* – *C. guilliermondii* in Fig. 3.

Candida famata, the second most prevalent species of the genus *Candida* in the Sulejów Reservoir, in 2000 was isolated from 13 samples examined, more often at the end of September with the numbers from 240 to 128000 cfu/dm³ both from the pelagic zone and the sediment. In 2001 it was found in 30 samples during all seasons, from sediments and also from pelagic zone.

Candida humicola is a species which in the year 2000 was isolated from 7.72% of the water samples examined, whereas in 2001 – in 8.39%. In the year 2000 it occurred mostly in the spring and summer, whereas in 2001 – in the spring and autumn in pelagic water.

Candida kefyr was cultured from the sites: Tresta-bay, Tresta, Borki, and Zarzęcin from samples obtained in different months of the year 2000, and in the year 2001 only in the period from May to June from Borki, Bronisławów, and Zarzęcin.

Candida inconspicua was cultured in the year 2000 mainly from sediment samples obtained in all examined stations in April and in the beginning of May. This species was not detected in subsequent months. However, in 2001 it occurred in May in Tresta-bay and in August at all sites, except for Bronisławów and Zarzęcin.

In 2000, *Candida pelliculosa* was cultured only twice from the sediments in June and one time from pelagic water in September, but in 2001 – from 15 pelagic and sediment samples from July to September.

Candida parapsilosis was found three times in September 2000 from samples obtained in Tresta-bay and Borki and in 2001 – from Tresta, Lubanów and Zarzęcin in 9 examined samples taken in different months.

Candida lusitaniae was isolated three times in September 2000 only from pelagic water, whereas in 2001 – in 8 examined samples of water and sediments.

The other fungi species from the *Candida* genus occurred with low frequency, only in some sites. Also fungi from genus *Kloeckera* and *Saccharomyces* were found in single samples.

Cryptococcus albidus was isolated from individual samples of water and sedi-

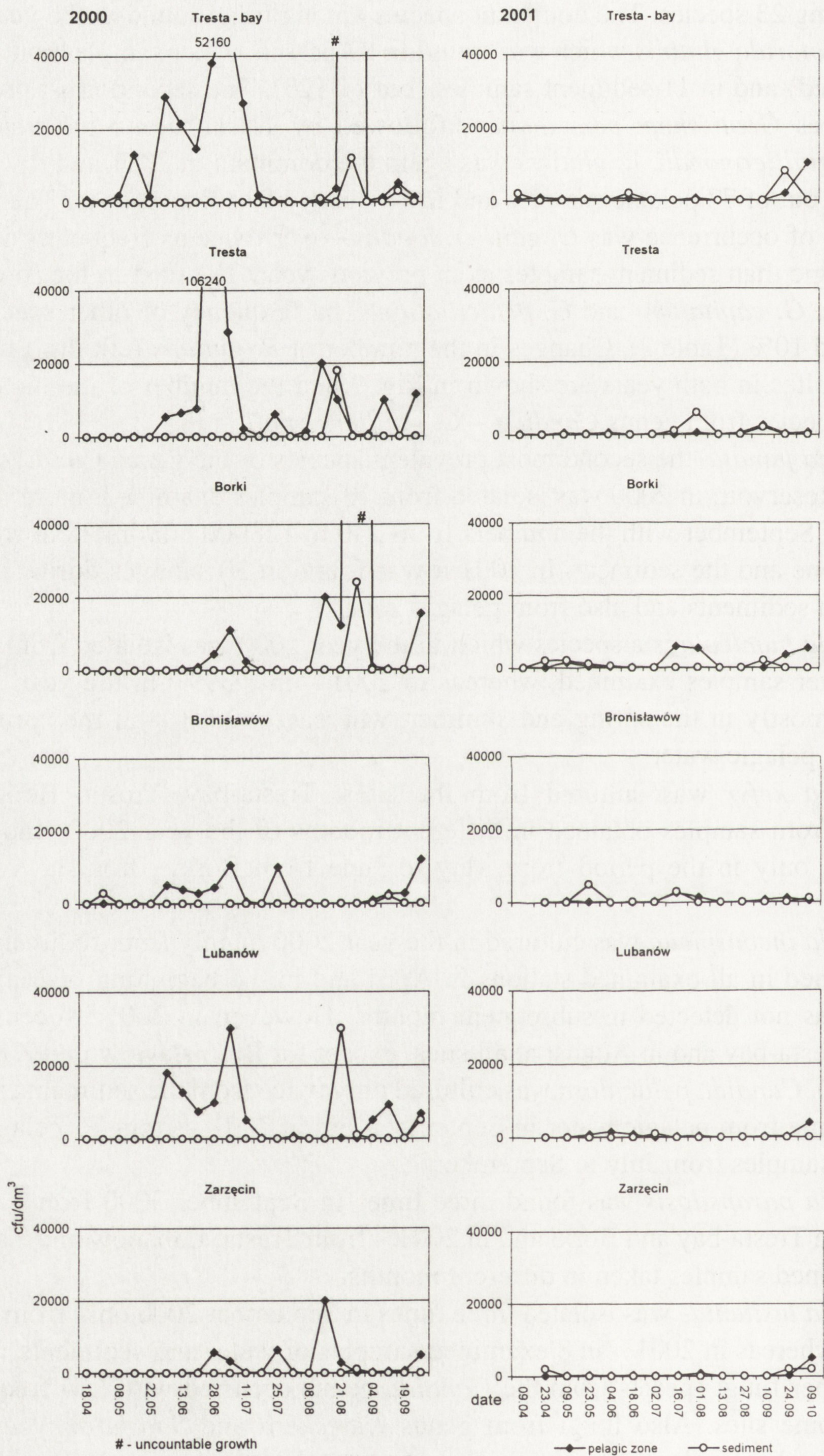


Fig. 2. Number of *Rhodotorula glutinis* cfu in samples from Sulejów Reservoir sampling sites in 2000 and 2001

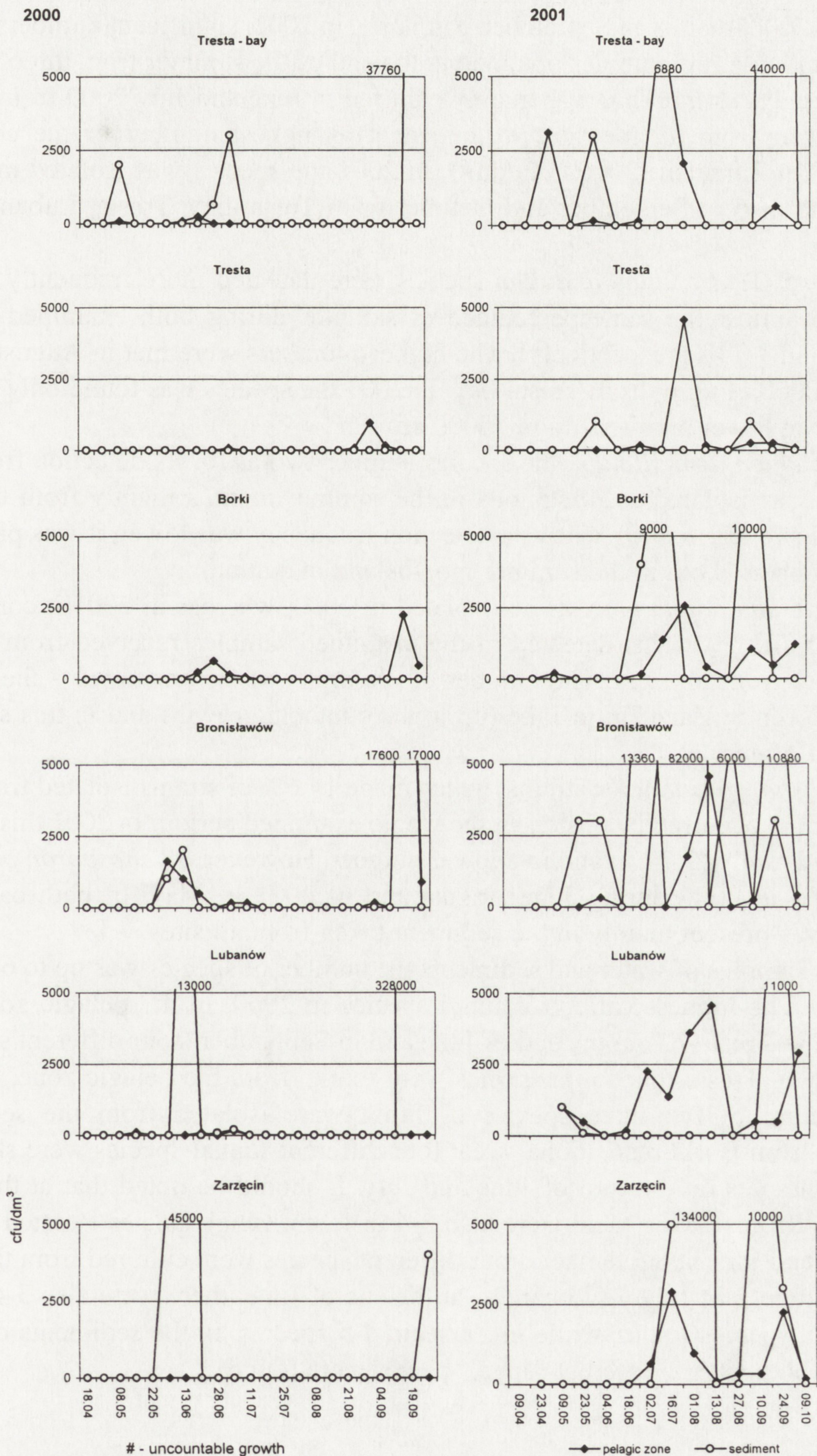


Fig. 3. Number of *Candida guilliermondii* cfu in samples from Sulejów Reservoir sampling sites in 2000 and 2001

ments in 2000, mainly in September. Similarly, in 2001, significant numbers of cells were found in a few samples obtained in the end of the summer from three stations.

Cryptococcus laurentii was mainly cultured in June and July 2000 from pelagic water taken from all the sites examined. The highest number of the cells was observed in Zarzęcin (20000 cfu/dm³). In 2001 the species was isolated mainly in April, and also in September and October from Tresta-bay, Tresta, Lubanów, and Zarzęcin.

Cells of *Geotrichum candidum* species were detected more frequently in sediments in almost the same percentage of samples during both examined seasons (7.32% and 7.74%, respectively). The highest numbers were met in August 2000 – up to 240000 cfu/dm³ from Tresta-bay. In 2001 the species was found only in April and May in lower numbers up to 15000 cfu/dm³.

Geotrichum penicillatum, the species number two as for its detection frequency in 2000, was isolated at all stations in the summer months mainly from the sediments. However, in 2001 when its detection frequency was lower, it was present in lower numbers, both in the summer months and in autumn.

Geotrichum capitatum was not isolated in 2000, whereas in 2001 it constituted 14.9% of all the strains detected in the examined samples received from May to September from all sites. The number of cfu/dm³ was differentiated – the highest rate was seen in Zarzęcin in June (up to uncountable growth) and in this station it was most often met.

Trichosporon cutaneum strains, which made 14.6% of strains isolated from samples in 2000, occurred in all sites in the whole examined period. In 2001 this species was found rarely (7.74%) and in a lower number. However, *Trichosporon pullulans* was found in 2000 during June-August and in 2001 in May. In both cases this species was present mainly in the sediment taken from all sites.

In the samples of water and sediments the number of species was up to 6 (Tables 3 and 4). The highest variety of fungi species in 2000, in the pelagic zone, was observed in Tresta-bay at the end of June and in September (four different species). Similarly, in Tresta four fungi species were found, from the pelagic zone, in June. A maximum of two-three species of fungi were isolated from the sediments obtained from Borki recreational area; four different fungal species were shown in pelagic samples on the turn of June and July. It should be noted that at the water intake in Bronisławów there were also 3-4 different fungi species isolated in June and July and four in September. Four different species were cultured from the same site (sediment), in July. In Lubanów, at the end of June, there were also 3-4 different fungal species found, while in Zarzęcin 4-5 species; in the sediments obtained from the both sites 2 different fungal species were found.

Table 3. Number of fungi species isolated from sampling sites of the Sulejów Reservoir in 2000

Date	Sampling site											
	Tresta-bay		Tresta		Borki		Bronisławów		Lubanów		Zarzęcin	
	p	o	p	o	p	o	p	o	p	o	p	o
18.04.	1	1	0	2	3	1	3	3	1	2	2	2
25.04.	1	1	0	2	0	2	2	2	1	1	2	1
8.05.	2	2	0	2	0	1	1	0	0	0	0	1
15.05.	3	0	2	2	0	0	1	1	0	1	2	1
22.05.	1	0	0	1	0	1	1	1	2	1	0	1
4.06.	2	1	2	*	*	*	2	1	2	0	*	*
13.06.	*	1	*	1	*	2	*	2	*	1	*	2
20.06.	3	2	3	2	3	1	3	2	3	2	4	2
28.06.	4	*	4	*	4	*	4	*	4	*	5	*
3.07.	2	2	3	2	4	1	3	2	2	2	2	1
11.07.	*	2	2	3	3	1	3	1	3	*	2	3
17.07.	3	2	1	2	0	3	2	4	2	3	2	2
25.07.	1	1	1	1	2	2	1	0	0	1	1	1
1.08.	1	2	0	1	0	1	1	2	1	2	1	1
8.08.	1	1	1	1	0	0	1	1	1	1	2	1
15.08.	0	3	1	1	1	2	0	1	0	0	1	0
21.08.	2	1	1	2	1	0	1	1	2	2	2	1
29.08.	2	1	2	0	1	1	0	0	1	1	1	1
4.09.	1	0	2	0	2	0	2	0	1	0	2	0
12.09.	2	2	2	1	1	1	1	2	3	1	1	3
19.09.	3	3	1	0	1	1	2	2	0	1	3	2
25.09.	4	0	3	1	4	0	4	1	3	3	4	2

p – pelagic zone samples, o – sediment samples, * – not counted

Table 4. Number of fungi species isolated from sampling sites of the Sulejów Reservoir in 2001

Date	Sampling site											
	Tresta-bay		Tresta		Borki		Bronisławów		Lubanów		Zarzęcin	
	p	o	p	o	p	o	p	o	p	o	p	o
9.04.	3	2	3	2	2	1	*	*	*	*	*	*
23.04.	3	3	1	2	2	2	2	2	*	*	4	3
9.05.	4	4	2	2	4	3	1	3	4	4	3	2
23.05.	1	3	1	2	2	3	2	5	*	*	4	3
4.06.	4	2	1	3	*	*	2	2	1	1	2	3
18.06.	2	5	1	2	2	2	2	4	*	1	4	2
2.07.	4	2	4	3	1	2	3	1	1	3	5	2
16.07.	1	0	1	2	3	1	2	2	2	1	2	1
1.08.	2	1	3	1	3	0	2	1	2	0	3	2
13.08.	2	1	1	0	2	0	2	0	3	1	2	2
27.08.	2	1	2	2	1	1	1	2	1	2	1	2
10.09.	2	2	2	3	3	2	4	2	3	2	3	4
24.09.	5	4	4	1	3	2	3	2	3	2	4	6
9.10.	2	1	4	0	4	3	2	2	3	1	3	1

p – pelagic zone samples, o – sediment samples, * – not counted

In 2001, 4 different species were cultured in May, June and July and 5 in September from Tresta-bay pelagic zone. Similarly, four different species were isolated from the sediments in the same months, while in June – as many as five. From Tresta, four different species were cultured in July, at the end of September, and in October: from sediments – up to three different fungi species were found. In May and in October, four different species were isolated from the pelagic zone in Borki, while in the sediment the highest variety was three species, also in May and October. In Bronisławów, the highest variety of species was demonstrated in the sediments, in May and June (3-5 species). The number of fungal species cultured in May from Lubanów was 4. The highest number of species as compared to the other sites was detected in Zarzęcin: 4-5 species in pelagic water and up to 6 in sediments.

We noticed that along with the variety of fungi species, the total number of fungi cells is significant. But it must be emphasized that in both years studied, with the observed uncountable growth on medium, the number of species was not high, usually not exceeding one or two species.

DISCUSSION

The fungi occurring in freshwater basins are the elements of the food chain that plays an important role in the process of basal metabolism in aquatic biocoenoses. With the increasing pollution and progressive degradation of the environment, fungal species are being isolated from waters of various trophic nature. They can be used as an indicator of purity or contamination of water. Another problem is the detection of fungi strains, which can potentially be pathogenic to humans and animals (Czeczuga and Woronowicz 1991-1992; Czeczuga 1994, 1995, 1996; Kornilłowicz 1994; Dynowska 1995, 1997; Dynowska and Biedunkiewicz 2001).

We cultured a total of 16 fungal species of the genus *Candida*, two of each: *Cryptococcus*, *Rhodotorula* and *Trichosporon*, and three of *Geotrichum* from the sediment samples, taken from the areas of the Sulejów Reservoir with intensive recreational use. All the fungal species isolated from the reservoir have been detected in different human ontocenoses, as asymptomatic infections as well as symptomatic ones (Kurnatowska 1995, 1997, 2001; Baran 1998). *Candida albicans* has most often been isolated in different ontocenoses of patients treated at the Centre for Treatment of Parasitic and Fungal Diseases, Division of Biology and Medical Parasitology, Medical University of Łódź. In our earlier studies on the Sulejów Reservoir the fungi cells were isolated twice (Wójcik and Tarczyńska 2000). Presently, they have not been cultured. However, the species most frequently isolated was *Candida guilliermondii* – the species that has also been isolated very frequently from the patients with general- or multifocal mycoses, with fungemia (Kurnatowska 2001). *C. guilliermondii* shows a significant biochemical activity, it can utilize carbon from many sugars, also nitrogen from ammonium sulphate,

asparagine, urea, and peptone, thus playing an important role in the process of self-purification of waters. It is notable that this species has not been found in the waters of the Tucholski Landscape Park (Rózga et al. 1999) or in earlier surveys of the Sulejów Reservoir (Wójcik and Tarczyńska 2000) and in the waters of the Olsztyn lakes (Dynowska 1995). It seems to be possible that such a massive presence of *C. guilliermondii* in the waters of the reservoir is linked to the flood that took place on the turn of July and August 2000 and twice in 2001: at the end of April and July.

This is worth to notice that *C. fama* was quite often isolated from different sites. This fungus has a low sugar-fermentation potential but can assimilate carbon from many compounds. It is rarely found in the biological materials in patients.

Another species isolated from the waters of the Sulejów Reservoir – *C. inconspicua* – was recorded by Rózga et al. (1999) in the lakes of the Tucholski Landscape Park but has not hitherto been recorded from any other Polish lake. There were also strains of the genus *Geotrichum*. They may be human pathogens and cause bronchial, pulmonary, and gastro-intestinal infections, as well as skin and nail lesions. The *Cryptococcus* species, which we detected in samples from the Sulejów Reservoir, are rarely isolated in patients but may cause, among other conditions also cerebrospinal meningitis (Kurnatowska 1995, Baran 1998).

The results obtained from different aquatic ecosystems indicate that the fungi occur mostly in autumn (Korniłowicz 1994, Dynowska 1995). However, a high number of their populations is visible also in the period from March to May, for instance in strongly eutrophic Trackie Lake (1200 cells/dm³) (Dynowska 1995). In the waters obtained from different sites of the Szczecin Lagoon, the number of fungi amounted to 2095 cells/dm³; significant presence was observed in June, July, and October (Dąbrowski et al. 1998). It seems that the variety of species and the number of fungal cells is linked predominantly to the water trophy. The most important nutrients for fungi, which are accessible in eutrophic waters, include nitrogen and phosphorous compounds. These compounds significantly support the waters of the Sulejów Reservoir. The overabundance of organic compounds, biogenic substances, and other pollution surely promotes the fungi development and expansion. Dynowska (1997) proposed *Rhodotorula glutinis*, *Candida albicans* and *Trichosporon cutaneum* as mycological indicators of the contamination. The species most frequently found in water samples obtained from the Odra River estuary and the Szczecin Lagoon were *C. colliculosa* and *C. glabrata* (cf. Dąbrowski et al. 1998). It seems that all the fungi strains, obtained from the waters of the Sulejów Reservoir, may be considered as bioindicators of human influence and impact on the environment. Their presence indicates the possibility of spreading many pathogenic fungi in aquatic ecosystems.

CONCLUSIONS

(1) The frequency of positive inoculations (selective medium) for the presence of fungi was 82.7 % and 95.4 % in 2000 and 2001, respectively and was higher in the pelagic zone than in the sediment.

(2) The density of a detected fungi population depended on the season and the site from which the samples were obtained (pelagic zone, sediment, sampling station) and showed a wide range (80-328000 cells/dm³ up to uncountable).

(3) A total of 28 fungal species were found, representing seven genera, associated with Ascomycota or Basidiomycota.

(4) For 15 species the waters of the reservoir may be the proper environment for the development of anamorphic forms, which can also be produced in humans and mammals.

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