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Pathogenic fungi in the waters of selected lakes in the "Bory Tucholskie" National Park

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The occurrence of potentially pathogenic fungal strains in the 7 lakes of the Struga Siedmiu Jezior and in three lobelia lakes situated in the central part of the "Bory Tucholskie" National Park was investigated. Ten fungal species belonging to 4 genera: *Candida* (*C. humicola, C. famata, C. guilliermondii*), *Cryptococcus* (*C. neoformans, C. laurentii, C. albidus, C. uniguttulatus*), *Rhodotorula* (*R. rubra* and *R. glutinis*), and *Trichosporon* (*T. cutaneum*), were recorded and analysed in the summers of 2001 and 2002.

Key words: pathogenic fungi, aquatic fungi, "Bory Tucholskie" National Park

INTRODUCTION

A growing number of Polish lakes is subject to excessive eutrophication. Only some water reservoirs in forest areas that are exposed to anthropogenic pressure are not significantly affected. They are usually contained within special national reserves and can thus resist the effects of the process. Such lakes undoubtedly include those situated in the "Bory Tucholskie" National Park.

Of species occurring in the hydrosphere, those pathogenic to humans as well as those that may be regarded as indicators of water purity or contamination attract particular attention (Niewolak 1976; Małyszko, Lewonowska and Januszko 1978; Czeczuga and Woronowicz 1991; Czeczuga 1994, 1995, 1996; Korniłowicz 1994; Dynowska 1993, 1995; Kurnatowska 1997; Dąbrowski, Bogusławska-Wąs and Daczkowska-Kozon 1998; Kiziewicz and Czeczuga 2001). The composition of species and their number depend primarily

lly: the number of fungal colonies was calculated. The cultures were transferred onto the Sabouraud solid medium without antibiotics. Mycological analysis was based on morphological and biochemical features. Morphological features were evaluated on the basis of macroscopic assessment of the incubated colonies (colour, shape, surface structure and borders). Microcultures on slides covered with a layer of Sabouraud agar, incubated for 24-48 hours, were used for microscopic examination.

The type, size and manner of the distribution of spores was evaluated. Biochemical features and fermentation as well as the ability to assimilate carbohydrates were determined with the API 20C test and API 20C AUX according to the rule of numerical identification (Analytical Profile Index, bioMérieux Lyon 1990), while the ability to assimilate nitrogen was evaluated with nitrogen auxanogram (Lodder 1971; Kreger-van Rij 1984; Kurnatowska 1995).

RESULTS AND DISCUSSION

Water samples from the surface (p) and bottom (d) from 12 sites in the 7 lakes of the Struga Siedmiu Jezior and 3 lobelia lakes were analysed. In the summer 2001, 10 fungal species potentially pathogenic to humans were isolated. The genus *Candida* was represented by: *Candida humicola* (Paszewska) Diddens et Lodder, *Candida famata* (Harrison) Meyer et Yarrow and *Candida guilliermondii* Langeron et Guerra; the genus *Cryptococcus: Cryptococcus neoformans* (Sanfelice) Vuillemin, *Cryptococcus laurentii* (Kufferath) Skinner, *Cryptococcus albidus* (Seito) Skinner and *Cryptococcus uniguttulatus* Phaff et Fell; the genus *Trichosporon* by: *Trichosporon cutaneum* (de Beurmann, Gougerot et Vaucher) Ota, and the genus *Rhodotorula* by: *Rhodotorula glutinis* (Frasenius) Harrison, *Rhodotorula rubra* (Demme) Lodder. In 2002, only 6 species were cultured. The genus *Candida* was represented by: *Candida famata* and *Candida guilliermondii*, the genus *Cryptococcus* by: *Cryptococcus neoformans* and *Cryptococcus albidus* and the genus *Rhodotorula* by: *Rhodotorula glutinis* and *Rhodotorula rubra*. The results are presented in Tables 1 and 2.

Three species belonging to the genus *Cryptococcus: C. laurentii, C. albidus* and *C. neoformans*, were obtained from the samples collected in the two sites in Zielone lake. They occurred numerously only in the samples from the lake bottom. Fusing growth of mycelium was observed on the Sabouraud medium. *R. glutinis* occurred abundantly both in the samples from the surface and the bottom of the lake. *R. glutinis* was isolated also in all the sites in the samples collected in 2002. The numbers of fungi belonging to this species ranged between 92.5 cells/dm^3 of water and fusing growth on media, impossible to count. In 2002, *C. guilliermondii* was also isolated from two surface sites. Strains of fungi from this species are isolated from various forms of clinical candidosis in humans, from multifocal infections and generalized infections with fungaemia and endocarditis. *Pichia guilliermondii* Wickerham, which forms sacs, is the perfect state of this *Candida* species. The cells of this fungus are resistant to high osmotic pressure of 5 to 13% NaCl in their growth environment. *C. guilliermondii* is characterised by a significant activity: it can assimilate carbon from many sugars and nitrogen from ammonium sulfate, asparagine, urea and peptone, and it therefore plays an important role in the processes of self-purification of waters (Kurnatowska 1995; Van Hoog and Guarro 1995; Baran 1998).

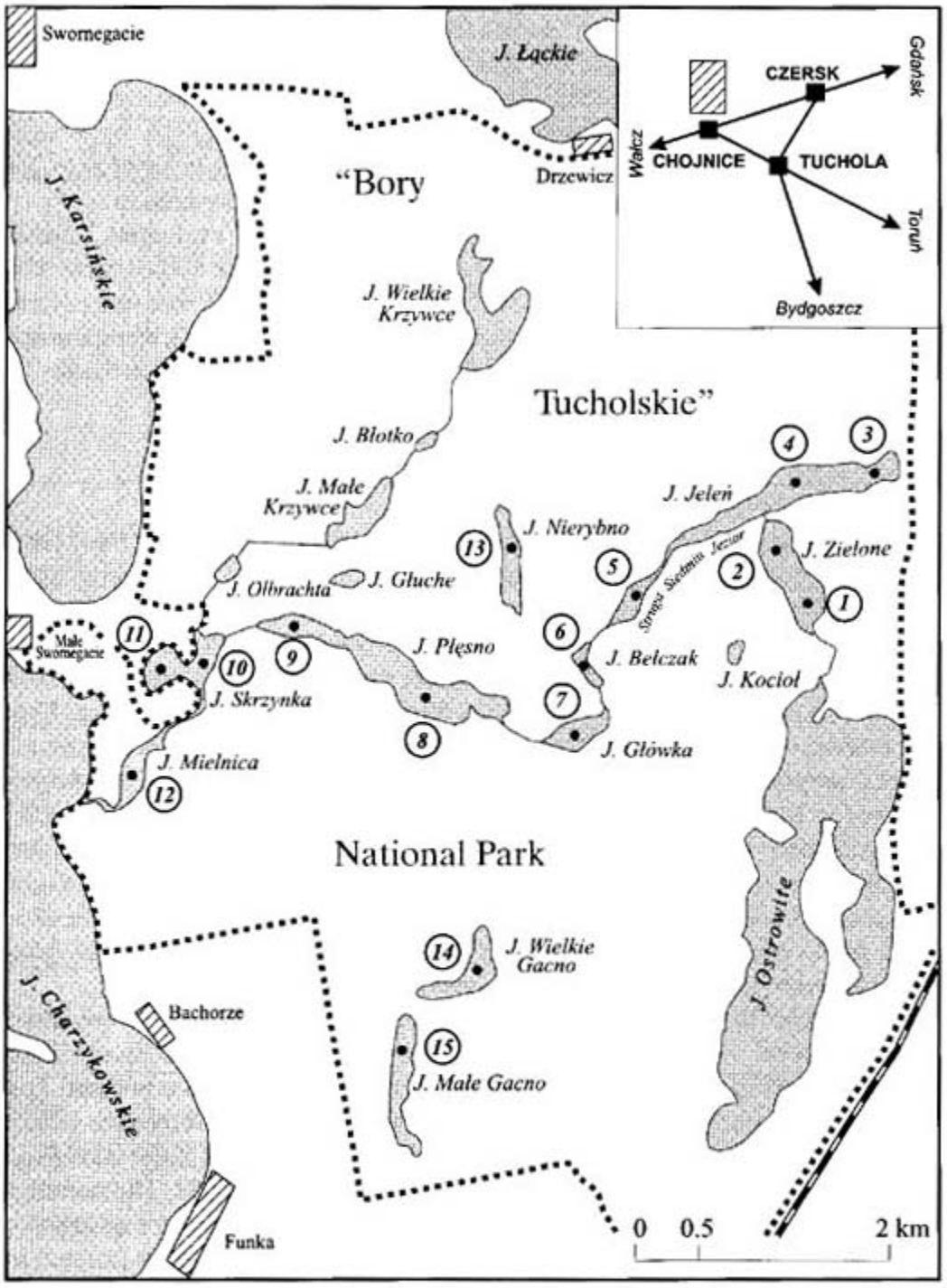

Fig. 1. Localization of lakes in the catchments of the Struga Siedmiu Jezior.

each, were collected from each sampling site and centrifuged for 30 min. at 3000 rpm. The sediment was diluted to 25 cm^3 with 0.9% NaCl and centrifuged again. The sediment was diluted once more to 1 cm^3 with 0.9% NaCl and was transferred onto the solid Sabouraud medium with: 4% glucose, 1% peptone, 2% agar, 0.025% streptomycin, 0.025% chloromycetin, 0.1% chloramphenicol. Cultures were incubated for 48 hours up to two weeks at 25°C, and evaluated macroscopically and microscopica-

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Table 1
Pathogenic fungi in the water of Struga Siedmiu Jezior and lobelias lakes in the National Park "Bory Tucholskie" - 2001 year

Locality - lake	Species	No of site - abundance of colony/dm^3
Zielone	*Rhodotorula glutinis*	1d – poor growth, 2p – 2.50
	Cryptococcus laurentii	1d – poor growth
	Cryptococcus albidus	2d – poor growth
	Cryptococcus neoformans	2d – poor growth
Jeleń	*Rhodotorula glutinis*	3p – 2.50
		3d – 245
		4p – poor growth
	Rhodotorula rubra	5p – 2.50
Bełczak	*Candida guilliermondii*	6p – 7.50
	Rhodotorula glutinis	6p – 1.25
		6p – 5.00
Główka	*Cryptococcus neoformans*	7p – 3.75
	Candida guilliermondii	7d – 7.50
	Rhodotorula glutinis	7p – 1.25
		7d – poor growth
Płęsno	*Rhodotorula glutinis*	8d – poor growth
		9p – 6.25
		9d – 17.50
		8p – 3.75
		9p - 6.25
		9d - 17.50
	Cryptococcus albidus	8p - 3.75
Skrzynka	*Cryptococcus uniguttulatus*	10p – 12.50
	Cryptococcus albidus	10p – 12.50
	Candida guilliermondii	11p – 3.75
Mielnica	*Trichosporon cutaneum*	12p – 2.50
	Rhodotorula glutinis	12p – 2.50
Gacno Wielkie	*Rhodotorula glutinis*	14p – poor growth
		14d – poor growth
	Cryptococcus neoformans	14p – 5.00
	Trichosporon cutaneum	14p – 6.25
	Candida famata	14d – 1.25
Gacno Małe	*Candida humicola*	15p – 2.50
	Cryptococcus neoformans	15d – 6.00
	Rhodotorula rubra	15p – 1.25
	Rhodotorula glutinis	15d – 3.75
Nierybno	*Rhodotorula glutinis*	13p – 26.75
		13d – poor growth

Table 2
Pathogenic fungi in the water of Struga Siedmiu Jezior and lobelias lakes in the National Park "Bory Tucholskie" - 2002 year

Locality - lake	Species	No of site - abundance of colony/dm^3
Zielone	*Rhodotorula glutinis*	1p – 32.5
		1d – 200
		2p – poor growth
		2d – 45.0
	Candida guilliermondii	1p – 17.5
		1d – 5.00
		2p – 45.0
Jeleń	*Rhodotorula glutinis*	3p – poor growth
		3d – 170
		4p – 18.8
		4d – poor growth
	Rhodotorula rubra	5p – poor growth
		5d – poor growth
	Cryptococcus neoformans	3p – 8.75
		5d – poor growth
	Cryptococcus albidus	5p – 6.25
	Candida famata	3d – 151
Bełczak	*Rhodotorula glutinis*	6p – poor growth
		6p – poor growth
Główka	*Rhodotorula glutinis*	7p – 47.5
		7d – 65.0
Płęsno	*Rhodotorula glutinis*	8p – poor growth
		8d – 110
		9p – 28.8
		9d – poor growth
	Cryptococcus albidus	9d – poor growth
Skrzynka	*Rhodotorula glutinis*	10p – poor growth
Mielnica	-	-
Gacno Wielkie	*Rhodotorula glutinis*	14p – 57.5
		14d – 55.0
	Cryptococcus albidus	14d – 27.5
	Candida guilliermondii	14p – 30.0
Gacno Małe	*Cryptococcus neoformans*	15p – 650
		15d – 60.0
	Rhodotorula rubra	15p – 600
		15d – poor growth
Nierybno	*Rhodotorula glutinis*	13d – 10.0

Pathogenic fungi belonging to this species have not been isolated from fresh water surface waters so far (Dynowska 1995; Czeczuga 1991, 1994, 1996; Dąbrowski et al. 1998; Rózga et al. 1999, 2000, 2002), apart from the waters of the Sulejowski Reservoir (Wójcik and Tarczyńska 2000; Kurnatowski et al. 2001, 2002) where it was the most frequently isolated species belonging to the genus *Candida*. *C. albidus* is isolated in the cerebrospinal fluid and sputum in patients with meningitis, respiratory system infections as well as skin and nail infections; *Filobasidium floriforme* Olive forming basal spores is its perfect state (Kurnatowska 1995; Baran 1998).

This species was sporadically isolated in the lakes in the Olsztyn area (Dynowska 1995) and in the Sulejowski Reservoir (Wójcik et al. 2000; Kurnatowski et al. 2002), both in water samples and in sediments, mainly in autumn. *C. laurentii* was described in lung abscesses in humans. The perfect state of this fungus is not known. The species was also cultured from the waters of the Sulejowski Reservoir in the samples from all the sites collected in June and July, while in August it was isolated from the samples collected only in one bay. The third *Cryptococcus* species occurring in Zielone lake, *C. neoformans*, is the etiological factor of cryptococcosis of the skin, subcutaneous tissue and lungs. It can spread from these foci of primary infection and cause generalised infection of the dental nervous system and internal organs. Its perfect state, *Filobasidiella neoformans* Kwon-Chung, multiplies by basal spores: basidiospores (Kurnatowska 1995; Baran 1998). The species has not been reported in surface waters in Poland so far. Various bird species are the natural reservoir of the fungus, which may possibly explain its occurrence in some lakes of the Struga Siedmiu Jezior (Pal 1989; Hubalek 1994; Nice 1994; Pinowski, Pinowska and Haman 1999).

R. glutinis and *R. rubra* were cultured in the samples collected in the waters of Jeleń lake in both periods studied. In 2001, their numbers ranged form 2.5/dm^3 to fusing growth on the Sabouraud medium, while in 2002 the number of colonies in a sample could not be calculated. *R. rubra* was particularly numerous, both in the surface and bottom samples. In 2002, *C. neoformans* was also isolated in two sites, and it was especially numerous in the bottom samples. *C. albidus,* which occurred in small numbers (6.25 cells/dm^3 in a single surface sampling site), was also especially numerous in the bottom samples. *C. famata* (151.25 cells/dm^3 of water) occurred in one bottom sampling site that year. It is isolated in patients with mycoses of sexual organs, oral cavity and gastrointestinal tract, as well as in cases of generalised candidosis with fungaemia. *Debaromyces hansenii* Lodder et Kreger van Rij forming spore sacs is the perfect state of this fungus. The species tolerates high osmotic pressure 5-13% NaCl in culture (Kurnatowska 1995; Baran 1998).

In Bełczak lake in 2001, *C. guilliermondii* was present in small numbers in one surface sample, while *R. glutinis* occurred in both periods studied.

While *R. glutinis* was isolated both in 2001 and 2002 from the sample from Główka lake, small numbers of *C. neoformans* and *C. guilliermondii* were cultured only in 2001 collected in the site.

In the surface samples of Skrzynka lake, C. *albidus* and *C. uniguttulatus* were cultured in small numbers: 12.5 cells/dm^3 and single cells of *C. guilliermondii* in 2001. Only *R. glutinis* occurred numerously in 2002.

The following fungi were observed in Mielnica lake in 2001: *R. glutinis* and *T. cutaneum*. The latter is isolated in patients with skin and nail lesions (Baran 1995), as well as from soil and water (Dynowska 1993, 1995, 1996; Slavikowa and Vadkertiova 2000). The perfect state of this fungus has not been described.

Fungi belonging to the genera *Cryptococcus, Rhodotorula, Trichosporon* were found in faeces, on decomposing plants and organic wastes of various origin, in particular those containing cellulose (Cooke et al. 1960; Cooke 1965; Meyers, Ahearn and Cook 1970). Fungi belonging to the genus *Rhodotorula* play an important role in the process of self-purification of waters as they provide carbon and nitrogen obtained from numerous organic and inorganic compounds (Katayama-Hirayama, Tobita and Hirayama 1994). They also produce urease decomposing urea. The perfect state of *Rhodotorula, Rhodosporidium*, is commonly isolated from the air, soil and water, fruit juices and diary products (Kurnatowska 1995; Baran 1998). It should be noted, however, that *R. glutinis* and *R. rubra* can cause mycosis in humans, in particular in immunocompromised persons (Warnock and Richardson 1991).

Apart from Zielone and Jeleń lakes, the greatest variety of species was observed in lobelia lakes: Wielkie Gacno and Małe Gacno. The following fungi were present in the waters of Wielkie Gacno in 2001: *R. glutinis* (fusing growth on media), *C. neoformans* (5 cells/dm^3 of water), *T. cutaneum* (6.25 cells/dm^3 of water), and *C. famata* (1.25 cells/dm^3 of water). In the samples from Małe Gacno, small numbers of *R. glutinis, R. rubra, C. neoformans* and *C. humicola*, species isolated from the oral cavity in patients contacting dentists, patients with candidosis of sexual organs and gastrointestinal tract and multifocal fungal infections, were recorded. The perfect state of this fungus is not known.

In Wielkie Gacno lake in 2002, 3 species were observed: *R. glutinis, C. albidus* and *C. guilliermondii*, while only two species occurred in Małe Gacno: *R. rubra* and *C. neoformans*. Only *R. glutinis* was cultured from the waters of the third lobelia lake, Nierybno, in both years.

The density of the populations of species of fungi isolated from the waters of the Struga Siedmiu Jezior ranges between several cells per dm^3 of water and fusing growth where the number of colonies cannot be calculated. As many as 10 species of fungi belonging to *Ascomycota* or *Basidiomycota* were found. *C. neoformans* is the species most pathogenic to humans among them. Lake waters provide the best environmental conditions for the development of anamorphic forms of only some fungal species (*Rhodotorula, Trichosporon, Cryptococcus*) that can also occur in humans and other mammals. Some species may multiply sexually in water environments (in telemorphic stage), which determines their spread and the formation of reservoirs of pathogenic fungi. It seems that all species of fungi detected in waters that had previously been isolated from humans as etiologic factors of mycosis should be taken into consideration in the assessment of water quality and in the discussion on the possibility of spread of pathogenic fungi in water ecosystems.

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Grzyby chorobotwórcze w wodach wybranych jezior Parku Narodowego „Bory Tucholskie"

Streszczenie

Zbadano występowanie potencjalnie patogennych szczepów grzybów w 7 jeziorach Strugi Siedmiu Jezior i w 3 jeziorach lobeliowych leżących w centrum Parku Narodowego „Bory Tucholskie". Latem 2001 i 2002 roku wyizolowano 10 gatunków grzybów z 4 rodzajów związanych z workowcami (*Ascomycota*) lub podstawczakami (*Basidiomycota*): *Candida humicola, C. famata, C. guilliermondii, Cryptococcus neoformans, C. laurentii, C. albidus, C. uniguttulatus, Rhodotorula rubra* i *Rh. glutinis* oraz *Trichosporon cutaneum.*

Gęstość populacji wykrywanych w wodach Strugi Siedmiu Jezior gatunków grzybów zawierała się w szerokich granicach, od kilku komórek/dm^3, aż do niepoliczalnego, zlewnego wzrostu.

Wydaje się, że wszystkie gatunki grzybów wykrywane w wodach, wcześniej opisane u człowieka jako czynniki etiologiczne grzybic, powinny być brane po uwagę przy ocenie jakości wód oraz możliwości rozprzestrzeniania się grzybów chorobotwórczych w ekosystemach wodnych.