

Original papers

Occurrence of fungi and fungus-like organisms in the Horodnianka River in the vicinity of Białystok, Poland

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ABSTRACT. Studies of fungi and fungus-like organisms in the northeastern Poland have mainly concentrated on running waters in the vicinity of Białystok, including the Horodnianka River. The main objective was to investigate biodiversity of fungi and fungus-like organisms which take part in decomposition of organic matter commonly found in inland waters. To obtain a complete picture of species composition of fungi and fungus-like organisms in running waters we decided to explore representative sites of the Horodnianka River such as Olmonty, Hryniewiczze and Horodniany with close localization of landfill. Fungal species were isolated using baiting technique. Baits of onion skin (*Alium cepa*), hemp-seeds (*Cannabis sativa*), impregnated cellophane and snake skin (*Natrix natrix*) were applied to isolate fungi from water of the Horodnianka River. The fungal community consists of 26 species, 10 species of fungi belonging to class Chytridiomycetes (3), anamorphic fungi (6), and Zygomycetes (1). 16 species belong to fungus-like organisms from class Oomycetes. Most of the recognized species have already been found in other running waters. From all the examined habitats the fungi belonging to 26 species of 18 genera *Achlya*, *Alternaria*, *Aphanomyces*, *Aspergillus*, *Catenophlyctis*, *Dictyuchus*, *Fusarium*, *Karlingia*, *Lagenidium*, *Leptomitius*, *Olpidiopsis*, *Penicillium*, *Phlyctochytrium*, *Pythium*, *Saprolegnia*, *Scoliognia*, *Thraustotheca* and *Zoophagus* were obtained. Certain fungal species like *Aphanomyces laevis*, *Fusarium aqueductum*, *F. moniliforme*, *F. oxysporum*, *Leptomitius lacteus*, *Saprolegnia feax* and *S. parasitica* were found at all the study sites. Among fungi potentially pathogenic and allergenic for humans the genera *Alternaria*, *Aspergillus*, *Fusarium*, *Lagenidium* and *Penicillium* have already been described. However, the species *Lagenidium giganteum* and *Achlya androgyna* are new in the fungal biota of Poland. The greatest number of fungal species occurred in Olmonty (24), the smallest in Horodniany (13). Presence of fungi such as *Leptomitius lacteus*, *Fusarium aqueductum* in the water of the Horodnianka River offers the possibility of using them as indicators of water quality.

Key words: fungal community, Horodnianka River, landfill, vicinity of Białystok, Poland

Introduction

Fungi contribute to the energy flow and productivity of ecosystems through their active role in utilization and biodegradation of organic matter [1–4]. Studies of fungi and fungus-like organisms in the northeastern Poland have mainly related to running waters, including the Horodnianka River.

The main objective of the present research was to analyze biodiversity of fungi and fungus-like organisms which take part in decomposition of organic matter commonly found in inland waters

due to their role in purification process. In order to obtain a complete picture of species composition of fungi and fungus-like organisms in the running waters, we decided to explore representative sites of the Horodnianka River such as Olmonty, Hryniewiczze and Horodniany with close localization of landfill.

Materials and methods

The study was carried out in spring and autumn of 2010. The water used in the experiments was

collected from the Horodnianka River located near Białystok. The Horodnianka River, length of river 27.4 km, catchment area 76 km², depth range from 0.3 to 0.5 depending on the season, average river flow 160 dm³s⁻¹, is the right-bank tributary of the Narew River flowing through the northeastern part of Poland. The Narew River, length 484 km, has its source in Białowieża Primeval Forest in Byelorussia and constitutes the right-bank tributary of the Vistula River [5,6].

The catchment area of the Horodnianka River is a drained agricultural land near Białystok. In the vicinity of the Horodnianka River there are habitations. It flows between arable fields, meadows and pastures. The area is located in the south-western part of Białystok (53°05'N, 23°10'E). Three permanent sampling sites were selected for mycological research: Olmonty – catchment area utilized for agriculture, arable fields, meadows and pastures; Hryniewiczze – meadows and pastures, scattered rural buildings located near landfill and the river spring; Horodniany – compact suburban construction.

Water samples for mycological analyses were collected in two seasons, spring and autumn of 2010, approximately 0.20 m from under water surface with 2l Rutner sampler. Samples of water (500mL) with organic matter (twigs, leaves, etc.) were taken from the surface of the river in sterile plastic bottles.

Fungal species were isolated using baiting technique. Baits of onion skin (*Alium cepa*), hemp-seeds (*Cannabis sativa*), cellophane and snake skin (*Natrix natrix*) were applied to isolate fungi from water as described by Seymour and Fuller [7]. The baits were placed in one-liter containers filled with water from the respective sites. The containers were covered with glass plates, at least partly to protect the water from penetration by bacteria. The samples were stored for approximately one month in the laboratory at the same temperature as measured in the respective water bodies. The light and temperature were regulated to resemble natural thermal and light conditions. Microscopically determined mycelia were removed from the baits and transferred to sterilized Petri plates containing distilled water. The microscopic examination of mycelia was repeated after a few days. Several microscopic preparations were made each time. The identification of zoosporic fungi from class Chytridiomycetes and Oomycetes involved measurement and determination of vegetative

organs – shape and size of the hyphae, asexual reproductive organs – shape of sporangium and spores, and sexual reproductive organs – the structure of the oogonium, oosporangium and antheridium. Identification of anamorphic fungi was based on morphology and biometrical character of mycelium and conidia.

Fungi were identified according to the works of Khulbe [3], Batko [8], Fassatiová [9], and Watanabe [10].

The results were subjected to statistical analysis using t-test to determine the significance of differences in the number of fungi and particular sites of water, and to determine the significance of differences in the number of fungi and their occurrence depending on season. The samples met the criteria at ≤ 0.05 [11].

Results

At all the study sites of the Horodnianka River 26 fungal species were found (Table 1) including 16 fungi-like species (Straminipila) and 10 species of fungi, 3 species belonging to Chytridiomycetes, 6 to anomorphic fungi, 16 to Oomycetes, one to Zygomycetes. However, such species as *Lagenidium giganteum* and *Achlya androgyna* are new in the fungal biota of Poland. From all the covered habitats fungi belonging to 26 species of 18 genera *Achlya*, *Alternaria*, *Aphanomyces*, *Aspergillus*, *Catenophlyctis*, *Dictyuchus*, *Fusarium*, *Karlingia*, *Lagenidium*, *Leptomitius*, *Olpidiopsis*, *Penicillium*, *Phlyctochytrium*, *Pythium*, *Saprolegnia*, *Scoliognia*, *Thraustotheca* and *Zoophagus* were obtained. The greatest number of fungus species occurred at the site Olmonty (24) in autumn (21) and in spring (13), the smallest number at site Horodniany (13) in autumn (12) and in spring (10). Certain fungal species such as *Aphanomyces laevis*, *Fusarium aqueductum*, *F. moniliforme*, *F. oxysporum*, *Leptomitius lacteus*, *Saprolegnia feax* and *S. parasitica* were found at all the study sites. Most of them are classified as saprotrophic but there are also some potential phytopathogens (*Alternaria* sp., *Fusarium* sp., *Karlingia polonica*, *Pythium* sp. and *Thraustotheca clavata*), as well as animal pathogens (*Aphanomyces* sp., *Catenophlyctis variabilis*, *Dictyuchus monosporus*, *Lagenidium giganteum*, *Newbya polyandra*, *Saprolegnia* sp. and *Zoophagus insidians*). Among them potentially pathogenic and allergogenic for humans fungi genera *Alternaria*, *Aspergillus*, *Fusarium*,

Table 1. Fungi and fungus-like organisms found in the Horodnianka River of particular sites (s-spring, a-autumn)

Fungal taxa (kingdom, class, order, species)	Names of sites			
	Olmonty	Hryniewiczze	Horodniany	Total
Straminipila				
Oomycetes				
Leptomitales				
<i>Leptomitus lacteus</i> Agardh	a,s	a,s	a,s	a,s
Pythiales				
<i>Lagenidium giganteum</i> Couch	a	a		a
<i>Pythium debaryanum</i> R. Hesse	a,s		a,s	
Saprolegniales				
<i>Achlya androgyna</i> (W. Archer) T.W. Johnson & R.L. Seym. 2005	a,s	a,s		a,s
<i>Ac. colorata</i> Pringhs	s			s
<i>Ac. debaryana</i> Humphrey	a	a,s		a,s
<i>Aphanomyces laevis</i> de Bary	a,s	a,s	a,s	a,s
<i>A. irregularis</i> W.W. Scott	a,s			a,s
<i>Dictyuchus monosporus</i> Leitg	a			a
<i>Newbya polyandra</i> (Hildebr.) Mark A. Spencer, in Spencer, Vick and Dick 2002	a	a,s		a,s
<i>Saprolegnia ferax</i> (Gruith.) Thur.	a,s	a,s	a,s	a,s
<i>S. parasitica</i> Coker	a,s	a,s	a,s	a,s
<i>S. unispora</i> (Coker & Couch) R.L. Seym.	s			s
<i>Scoliolegnia asterophora</i> de Bary M.W. Dick	a	a	a	a
<i>Thraustotheca clavata</i> de (Bary) Humphrey	a,s	s		a,s
Olpidiopsidales				
<i>Olpidiopsis saprolegniae</i> var <i>indica</i> Dayal & J. Thakur	s	s	s	s
Fungi				
Chytridiomycetes				
Blastocladales				
<i>Catenophlyctis variabilis</i> (Karling) Karling	a	a		a
Chytridiales				
<i>Phlyctochytrium aureliae</i> Ajello	a			a
Spizellomycetales				
<i>Karlingia polonica</i> Hassan	a		a	a
Anamorphic fungi				
<i>Alternaria alternata</i> Fries	a	a	a	a
<i>Aspergillus niger</i> var <i>niger</i> Tiegh.			a,s	a,s
<i>Fusarium aqueductum</i> Rabenh. and Radlk.	a,s	a,s	a,s	a,s
<i>Fusarium moniliforme</i> J. Sheld.	a,s	a,s	a,s	a,s
<i>Fusarium oxysporum</i> Schltdl.	a,s	a,s	a,s	a,s
<i>Penicillium notatum</i> Westling			a,s	a,s
Zygomycetes				
Zoopagales				
<i>Zoophagus insidians</i> Sommerst.	a	a		a
Total	24 92.30% a: 21 91.30% s: 13 72.22%	17 65.38% a: 15 65.21% s: 12 66.66%	13* 50.00% a: 12 52.17% s: 10* 55.55%	26 100% a: 23 100% s: 18 100%

* Differences significant at the $p \leq 0.05$ level.

Lagenidium and *Penicillium* have already been described.

Discussion

Most of the species recognized in the Horodnianka River have already been found in other running waters. As revealed in fungal studies concerning numerous rivers in Poland, especially in the northeastern region, this type of ecosystem, i.e. running waters, promotes the growth of aquatic fungus-like organisms (zoosporic) belonging to class Oomycetes. Species of this class occur worldwide as common aquatic saprobes on organic substance [12,13].

In the present study a larger number of fungus species occurred in autumn as compared to spring. In studies concerning seasonal changes of fungi in springs, rivers and lakes the highest numbers of fungus species were quite frequently noted in the spring and autumn months. Water temperature is a major environmental factor influencing the presence of aquatic fungi in freshwaters. Zoosporic fungi from class Oomycetes and Chytridiomycetes usually prefer colder waters. Many of these species play an important role as parasites on aquatic organisms [3,8].

The genus *Aphanomyces* belongs to the order Saprolegniales (Oomycetes) and comprises ca 30 species. Many species of this group have a saprobiotic mode of life, feeding on decayed animals and plant debris. A few species are detrimental parasites being responsible for economically important diseases affecting agriculture and agriculture crop as well as wildlife populations of freshwater animals [14,15]. Frequently described species *Aphanomyces laevis* has been found in fish attacking both eggs and adult individuals of many economically valuable fish species [16].

Observations of fungus-like organisms revealed species from genus *Pythium*. Nowadays approximately one hundred species from this genus are known, with half of them living in water. These species were considered as soil saprotrophs or parasites of plants. Research has shown that they also grow in different water bodies as phytosaprotroph and most of them were zoosaprotrophs and fish parasites [17,18].

We isolated three species of genera *Achlya* and *Saprolegnia* from the Horodnianka River. Saprolegniales genera, particularly *Achlya* and

Saprolegnia, are generally considered as opportunistic pathogens for fish and eggs [13,19]. In favorable conditions, however, they parasitize weakened and mechanically damaged animals, mainly spawn and fish in their different developmental periods [20–22].

Catenophlyctis variabilis known as a saprotroph, especially zoosaprotroph, was previously isolated from chitin and keratin-containing substrates from human skin, snake exuviae, hair, chips or horn, hoofs, human nails, wool and crustaceans from soil and water [8,23].

Fungi such as *Alternaria alternata*, *Aspergillus niger* and *Penicillium notatum* were found in samples of water from the Horodnianka River. They occur in greater number in waters of increased trophicity [24,25]. The fungi *Aspergillus* and *Penicillium*, which were isolated from body of fish, are etiologically important to fish diseases [26].

However, the most predominant allergogenic and potentially pathogenic fungi belong to the genera *Alternaria*, *Aspergillus*, *Fusarium*, *Lagenidium* and *Penicillium*. These fungi have interminably affected humans in various ways, including plant and animal diseases, superficial and systematic mycoses in humans. Fungi are also involved in many hypersensitivity diseases and toxin-induced health hazards in human. Diseases associated with inhalation of fungal spores include toxic pneumonia and chronic fatigue syndrome. Allergy is one of the most common ailments affecting more than 20% of the industrialized world population. Allergy develops upon sensitization with extraneous proteins from various sources. The fungal allergy usually coexists with allergy to grass pollens, acarids and animal hair [27].

In the Białystok region running waters are exposed to many types of pollutants. The main pollutants are of agricultural, municipal and industrial origin. All types of pollution greatly affect the quality of draining waters in the areas of economic use. The investigated Horodnianka River belongs to such waters. Most of the analyzed hydrochemical parameters classify waters of the Horodnianka River as class IV water quality [6]. Water pollution problem is closely related to animal and human health. On the basis of microbiological and parasitological studies it has been shown that water can be a reservoir of a number of pathogenic bacteria and fungi, protozoan cysts and eggs of worms and other micro-organisms important for sanitary and epidemiological data [28].

Fungi are of particular importance in reducing pollution levels in water reservoirs [8,29].

The sewage fungi *Leptomitius lacteus* and *Fusarium aqueductum* were most frequently encountered in the examined waters. According to literature [29,30], they are typical representatives in mycobiota of waters polluted by municipal wastes and such water contains larger amounts of nitrogen.

The presence of *Leptomitius lacteus* in water of the Horodnianska indicates that the river is heavily polluted with sewage of diversified origin, including waste. *Leptomitius lacteus* is usually reported as a minor component of sewage fungus [31,32]. The sewage fungus is one of only a few aquatic Oomycetes marking saprobe condition of waters. It has been observed in lakes, rivers, springs and ponds of Europe, North America and Africa [29,33]. The frequency of occurrence of different species of fungi is used as a measurement of saprobic index. In biological evolution of water *Leptomitius lacteus* indicates the alpha-mesosaprobe zone, sporadically occurring in cleaner freshwaters [29]. It is important that *Leptomitius lacteus* is taken into account in determination of water quality and its presence suggests significant contamination of the aquatic reservoir. It should be noted that a landfill site is situated near the river in Horodnianska, and waters leaching from the landfill accumulate large quantities of organic matter of diversified origin and flow down with the running waters to the investigated river. Nuttall [34] describes massive growths of *Leptomitius lacteus* in streams receiving drainage from landfills.

Zoophagus insidians, a rotifer-trapping predacious fungus, was found to be a problem in the investigated water. It was detected in sanitary sewage [30, 35]. *Zoophagus insidians* is described as predacious fungus of adults and eggs of rotifers and larvae of molluscs [36].

Fungi and fungus-like organisms found in the Horodnianska River belong to cosmopolitan species observed on other, even distant, continents [37,38]. As shown in numerous fungal studies, the same fungal species are found in waters far in the north [39] and the Subarctic Regions [40]. They also grow in the waters of South America [41], Africa [42] and in aquatic reservoirs of various regions of Asia [43]. Thus, the fungal composition in the respective bodies is determined by its physicochemical properties, whereas climate has a minimal effect.

Conclusions

The presence of fungi such as *Leptomitius lacteus* and *Fusarium aqueductum* in the water of the Horodnianska River offers the possibility of using them as indicators of water quality.

Leptomitius lacteus sewage fungus is one of a few aquatic Oomycetes marking saprobe condition of waters.

However, such species as *Lagenidium giganteum* and *Achlya androgyna* are new ones in the fungal biota of Poland.

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