Original Research

Biomass and Abundance of Aquatic Fungi in a Polyhumic Dam Reservoir

Anna Pietryczuk, Andrzej S. Górniak*, Adam Więcko, Adam Cudowski

Institute of Biology, University of Białystok,
Świerkowa 20B, 15-950 Białystok, Poland

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Abstract

The aim of this study was to determine the biomass and abundance of fungi in the waters of the shallow and polyhumic reservoir located in northeastern Poland. It was shown that fungal biomass in water positively correlated with abundance (CFU/ml, colony-forming unit/ml) and negatively with the number of morphotypes. The largest biomass of fungi was recorded in October (0.55 μg/dm^3) and the lowest in winter (0.22-0.28 μg/dm^3), when fungal colonies had greater phenotypic diversity. The main river supplying the reservoir had greater average fungal biomass and abundance than the reservoir.

Keywords: dam reservoir, aquatic fungi, biomass, CFU, humus substances

Introduction

Aquatic fungi play a major role in the functioning of water environment and actively participate in the microbial decomposition of organic matter, especially of plant origin, preparing it for colonization by other organisms, mainly bacteria or invertebrates. Fungi are a phylogenetically diverse group of organisms, with four classes:

(i) *Hyphomycetes* sensu Ingold, which occur mainly on decaying leaves in water
(ii) aquatic *Ascomycetes* occurring on submerged wood
(iii) *Chytridiomycetes*
(iv) *Oomycetes,* responsible mainly for diseases in humans and animals, and the decomposition of organic substances that do not contain cellulose [1-3].

Aquatic fungi are actively involved in mitigating the effects of anthropogenic stress as they participate in the biotransformation of organic xenobiotics in the aquatic environment and therefore may improve water quality [4, 5]. These characteristics indicate the previously ignored role of this group of organisms in the process of water purification and their potential application in biotechnology.

*e-mail: hydra@uwb.edu.pl

There are numerous literature data on the taxonomic diversity of *Hyphomycetes*, yeast-like and zoosporic fungi in water [6-8]. However, there is a lack of quantitative data (biomass and abundance) of fungi in the water column, especially in water reservoirs closely related to river ecosystems. The specific environment of a dam reservoir is influenced by the effect of accumulation minerals, organic compounds, and contaminants of different origin, which determine the quality of water and the species diversity of flora and fauna.

Organic compounds in natural water may be of soil origin like a detritus, humus-clay complexes, and humus acids in microcolloids or dissolved forms [9]. Fresh water can contain wide concentration values of dissolved organic carbon (DOC) as well as humus substances (HS) up to 50-70 mgC/dm^3. The HS role in lakes and rivers have been presented elsewhere, but the HS effect on freshwater mycoflora are rarely shown. The freshwater fungi in non-acidic waters can utilize HS as a source of energy for physiological processes or as an inhibiting substance in fungi growth, causing specific HS components like polyphenols [10]. The limiting role of fungi in algae development was shown in the case of high blooms of diatoms and green algae [11]. The interactions between fungi, cyanobacteria, and humus substances have not yet been discussed.

The existence of these three elements in freshwater have a place in the Siemianówka Dam Reservoir, where long-time limnological monitoring is provided [12]. Our study aims were to determine fungal biomass and abundance in the reservoir non-acidic waters with high concentrations of DOC and intensive cyanobacteria abundance in a situation lacking artificial water pollution.

Materials and Methods

Study Area

The Siemianówka Dam Reservoir (SDR) (52º55'N and 23º49'E) was created in 1977-90 by damming the Narew River near the border with the Republic of Belarus. The reservoir catchment is forested with large areas of wetlands that are rich sources of organics for reservoir water [12]. SDR maximum area is 32.5 km^2 with a capacity of 79.5 million m^3. The length of the reservoir is 11 km, width ranges from 0.8 km in the vicinity of the dam up to 4-5 km in its middle part, the mean depth is 3.5 m, and the maximum depth is more than 10 m at the dam. SDR morphometry, their direction (parallel to dominant wind direction) caused intensive water mixing to the bottom in the ice-free season.

Physico-Chemical Analyses of Water

The study was performed at two sites of the Narew River – above and below SDR and at the three sites on the reservoir (Fig. 1). Water samples for chemical and mycological analyses were collected starting from July to December 2011.

Basic physiochemical water parameters, including electrolytic conductivity, temperature and pH, were measured in the field by means of a HQD 9200 meter by Hach Lange. Chlorophyll *a* concentration was measured by spectrophotometric measurement after homogenization of the suspension remaining on a GF/F filter and extraction with boiling ethanol [13, 14]. Dissolved organic carbon (DOC) was determined by the method of high-temperature catalytic combustion in a Shimatzu TOC-5050A analyzer [15]. The total phosphorus concentration was determined by spectrophotometric measurement according to the method described by Hermanowicz [16].

Ergosterol and CFU Estimation

Unfiltered samples of water (250 µl), diluted to proportions 1:10 and 1:100, were cultured directly onto Petri plates containing chloramphenicol-enriched (POCh) Sabouraud agar. The plates were incubated at a temperature of 25ºC for 5 days. After the incubation period the total number of colonies and different morphotypes of fungi within the colonies were determined [17].

The biomass of suspended fungi in water was calculated based on chromatographic quantitative analyses of ergosterol extracted from seston remaining on the filter of glass fiber GF/G with a pore size of 0.7 microns after filtration of 1 dm^3 of water using the HPLC method [18-20]. The chromatographic set consisted of the following modules: System Gold 125 Solvent Module, 166 Detector, Autosampler 502 made by the Beckman Company, and a computer equipped with the System Gold Personal™ Chromatograph program. Chromatographic analyses was done at 30ºC on a Beckman C18 Ultrasphere ODS 5µ 4.6 mm × 25 cm column with the isocratic flow of methanol and distilled water solution at 98:2 volume proportions, set at the 1.5 ml/min level. Ergosterol was determined using a UV detector with wavelength of 282 nm after elution time lasting approximately 11 minutes. Taking into account the fact that very small amounts of ergosterol were present in the river samples being analyzed, the standard addition method was used. To

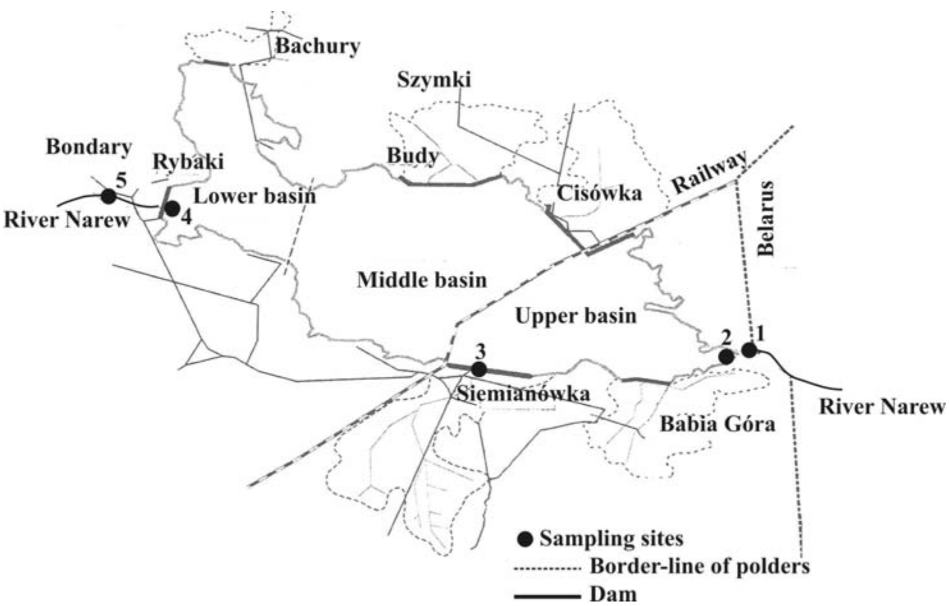

Fig. 1. Map of Siemianówka Dam Reservoir and sampling sites.

Table 1. Physico-chemical parameters of water in the Siemianówka Dam Reservoir in July-December 2011 (average values ±SD).

Site	Conductivity [µS/cm]	pH	Temperature [ºC]	Chlorophyll *a* [µg/dm³]	Total phosphorus (TP) [mgP/dm³]	DOC [mgC/dm³]
1.	313 (±45.18)	7.31 (±0.8)	11.62 (±7.30)	3.22 (±2.52)	0.12 (±0.04)	19.76 (±4.35)
2.	328 (±48.43)	7.64 (±0.60)	13.27 (±9.02)	41.01 (±26.72)	0.16 (±0.07)	18.07 (±0.60)
3.	279 (±27.51)	7.68 (±0.6)	13.58 (±8.88)	47.35 (±34.39)	0.20 (±0.05)	18.81 (±4.03)
4.	293 (±15.07)	7.77 (±0.69)	13.48 (±8.70)	50.22 (±23.95)	0.16 (±0.05)	19.80 (±2.53)
5.	314 (±55.52)	7.54 (±0.74)	12.92 (±8.58)	49.85 (±22.19)	0.14 (±0.06)	19.11 (±2.44)

convert ergosterol to biomass of aquatic fungi a coefficient of 5.5 mg of ergosterol in 1 g of aquatic fungi and 35% carbon content was accepted [20]. Method validation revealed that the reclamation of ergosterol released from carancules and propagules of the fungi is 91±3%, and the method error does not exceed 0.1 ng/dm³.

Statistical analyses were done in SPSS 19 software. The Kruskal-Wallis test was used to estimate the difference between means. The significance of the correlation was estimated by the Pearson coefficient. Differences of $p<0.01$ were considered as statistically significant.

Results and Discussion

The Siemianówka Dam Reservoir is a typical polymictic and polyhumic reservoir. Average water pH ranges from 6.5 to 8.7, with the lowest pH recorded in the Narew River above and below the reservoir, i.e. where the greatest fungal biomass was recorded. It was observed that with an increase in pH of the water, fungal biomass decreases (Fig. 2). These sites, i.e. Narew River above the reservoir (No. 1) and in the reservoir near the railway embankment (No. 2), also had the highest conductivity, 313 and 328 µS/cm, respectively, which then decreased along the reservoir to the level 279-293 µS/cm. A renewed increase in conductivity was recorded in the Narew River below the reservoir. In this paper we also analyzed changes in total phosphorus (TP) and dissolved organic carbon (DOC). The average content of total phosphorus at different sites ranged from 0.12 to 0.20 mg/dm³, with the highest values observed in the reservoir (compared to the Narew River above and below the reservoir). There were no statistically significant differences in DOC concentrations between the sites. The concentration at all the analyzed sites was high and ranged from 18.1 to 19.8 mgC/dm³ (Table 1).

It is known from previous studies that the Siemianówka reservoir has a great abundance of algae. With about 256 identified taxa, the average biomass of phytoplankton ranges from 0.16 to 50.3 mg/dm³, reaching the highest values from June to September, with a distinct dominance of cyanobacteria in summer and early autumn [21]. In our study, the highest concentration of chlorophyll *a* was in summer and early autumn, and then began to decline, reaching the lowest values in November and December. The Narew River above the reservoir had very low concentrations of chlorophyll *a*, at 3.22 µg/dm³ (Table 1).

Fungal biomass in the Siemianówka Dam Reservoir significantly depended on the temperature (Fig. 3) and month in which the samples were collected. In the summer months (July to September), fungal biomass in the reservoir was within the range 0.31-0.50 µg/dm³. The greatest biomass of fungi, up to 55 µg/dm³, was recorded in October, and then decreased, reaching the lowest values in December, i.e. 0.22-0.28 µg/dm³ (Fig. 4). The average biomass of fungi was closely positively correlated with their abundance determined by colony forming units (CFU) (Fig. 5). The CFU ratio was the highest in October, within a broad range of 4000-7800, and reached the lowest values in December (1500-3400).

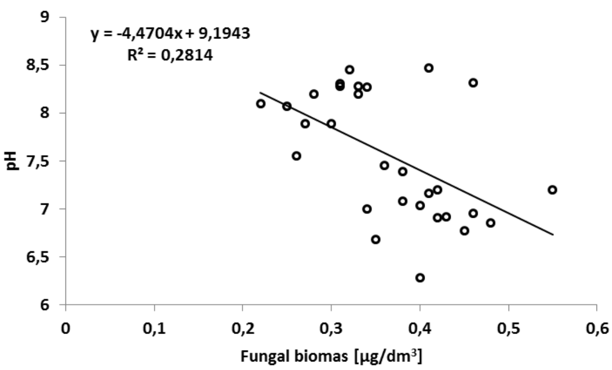

Fig. 2. Significant relationships between average fungal biomass and average pH in the water of Siemianówka Dam Reservoir ($p<0.01$).

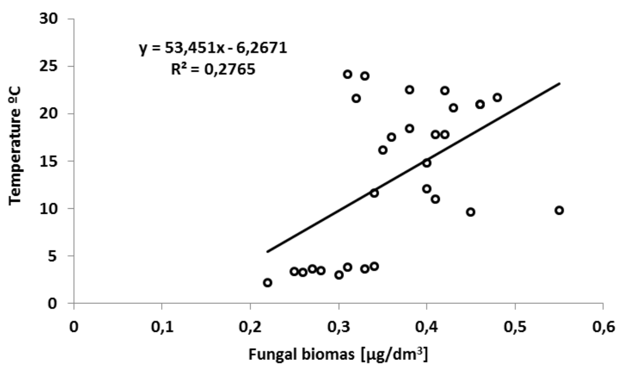

Fig. 3. Significant relationships between average fungal biomass and average temperature in the water of Siemianówka Dam Reservoir ($p<0.01$).

A greater number of species of aquatic *Hyphomycetes* in autumn, compared to other seasons, also was noted previously. Eight different species of *Hyphomycetes* fungi have been identified in the Narewka River located 20 km from upper Narew. Autumn species richness was two times higher than in the spring [22]. The greater abundance of fungi in autumn compared to summer may be caused by the abundance of plant detritus, especially leaves falling from trees into the river. Other studies [23] showed that the biomass of aquatic fungi may reach 90-95% of the total biomass of microorganisms in the water. Maximum number of fungi in autumn also was recorded in the waters of a eutrophic Bartąg Lake and its drainage basin [24]. However, availability of nutrients such as nitrogen and phosphorus, and of organic matter are not the only factors that influence the diversity of fungal biomass and abundance of aquatic fungi – pH and temperature of water also are of great importance [3].

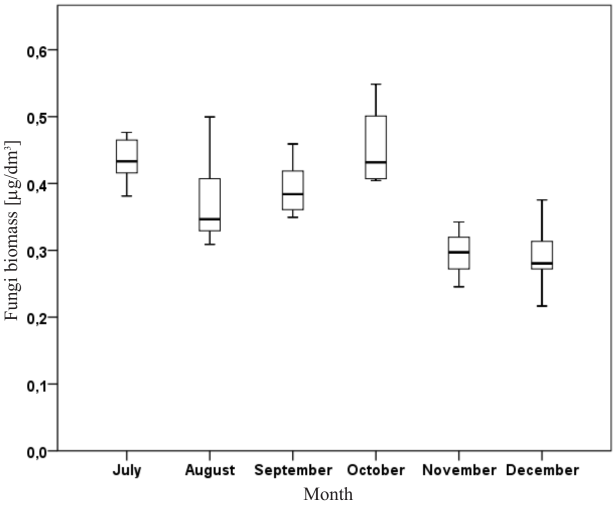

Fig. 4. Monthly changes in the biomass of fungi in the Siemianówka Dam Reservoir (minimum, maximum, and median value of fungi biomass in each month).

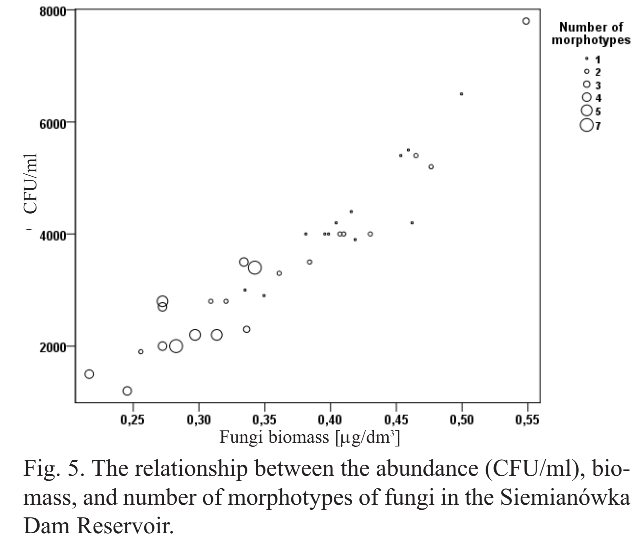

Fig. 5. The relationship between the abundance (CFU/ml), biomass, and number of morphotypes of fungi in the Siemianówka Dam Reservoir.

There are as yet very few literature data on the biomass of fungi in temperate waters, especially in reservoirs. So far only taxonomic research has been carried out. For example, 87 species of parasitic fungi have been identified in the water of Siemianówka Reservoir, most of which were in the Narew River above the reservoir, while the reservoir itself had the fewest species [7]. The authors also found season-related differences in species structure in the reservoir. The smallest number of species was found in summer (June and July), although low species diversity also was observed in September and October. In this study on the SDR the largest biomass and abundance of fungi (CFU/ml) was observed in October. Therefore, in autumn the abundance of fungi in the investigated reservoir was the greatest, but the number of their species significantly decreased. This is confirmed by a negative correlation between biomass and CFU/ml and a number of different morphotypes (Fig. 5).

The higher the mean biomass of fungi and CFU, the smaller the morphological diversity of fungal colonies. The seasonal occurrence of aquatic fungi results directly from differences in temperature [25]. Studies on the effects of temperature on fungi showed that some species occur throughout the year, while there are also some that are typical for a particular season. For example, seasonal occurrence of individual species has been shown in a study on the Indian Dudhawa Dam Reservoir, where *Aspergillus niger* occurred most frequently in winter, rainy season, while in summer this species did not occur or was reported only occasionally [26]. The growth of *Hyphomycetes* fungi and of many other species of yeast is inhibited by temperatures below 5ºC, so in winter these fungi disappear from water, while the *Oomycetes* are the most abundant or occur exlusively in colder periods.

The increased biomass and abundance of fungi in the summer and autumn also can be associated with a greater concentration of dissolved carbohydrates in the water. Carbohydrates are synthesized mainly by algae, cyanobacteria and other plants. It is widely known that aquatic *Hyphomycetes* and other heterotrophic microorganisms can satisfactorily use a wide range of carbohydrates as a major source of carbon [27, 28]. Research conducted on the Siemianówka Reservoir Dam in 1991-2001 showed that the highest average concentrations of dissolved carbohydrates occurred in autumn (October) and summer (August), and varied in the range 2.21-3.57 mgC/dm³. However, the lowest concentration of this parameter was observed in March and from December to January (1.32-2.28 mgC/dm³) [Górniak et al., unpublished data].

In this study it was found that the average biomass of fungi was the highest in the upper part of the reservoir near the railway embankment, which then decreased steadily along the reservoir. An increase of the average biomass of fungi was observed at site 5 (Narew below the reservoir), but was lower than in the Narew River above the reservoir (Fig. 6). An earlier study shows that flowing waters contains a greater number of fungal species than typical limnic waters [1]. On the other hand, a smaller number of fungi in the reservoir, especially in summer, may be explained by cyanobacteria blooms (which secrete cytotoxic substances) and strong eutrophication of the reservoir and the resulted oxygen deficit. Growth of many species of fungi is then inhibited or even completely impossible.

Only certain species of aquatic fungi have evolved mechanisms for surviving in a eutrophic environment in which cyanotoxins are present [7, 29, 31]. The polymictic character of SDR affects high summer water temperature where water fungi development is moderate.

It was shown that average fungal biomass increases with increasing content of TP in the Siemianówka Dam Reservoir (Fig. 7). There are various literature reports on the effects of eutrophication on the structure of aquatic fungi. On one hand, the enrichment of water with nutrients, especially phosphorus and nitrogen, stimulates the growth and development of mycoplankton [32]. On the other hand, we have seen declining species diversity of fungi in highly eutrophic reservoirs, which indicates that some fungal species are better adapted to the increased concentration of nutrients in the water than other species [33]. This is probably related to an increase in the concentration of organic and inorganic toxins closely linked to the eutrophication of the reservoir, reducing the growth of fungal species that have not evolved resistance mechanisms to these toxins [34].

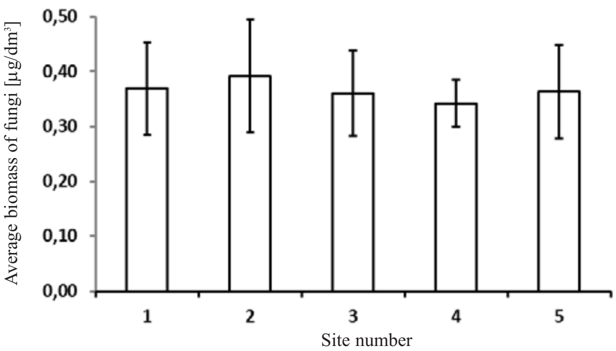

Fig. 6. Changes in the fungal biomass content at individual sampling sites (July-December 2011) (average biomass ± SD).

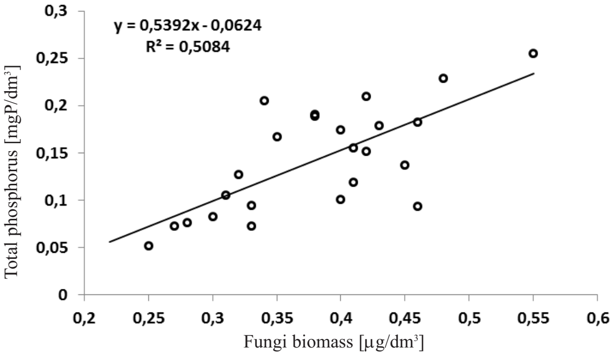

Fig. 7. Significant relationships between average fungal biomass and average total phosphorus concentration [mg P/dm³] in the water of Siemianówka Dam Reservoir ($p<0.01$).

It has been shown that humic substances (HS) increase the availability of nutrients in water environment by retaining trace elements in solution and thus preventing their precipitation. In this way the nutrients are available to plants, especially phytoplankton. In low concentrations, HS stimulate the growth of green algae, while in large quantities these compounds reduce the availability of light, thus inhibiting photosynthesis (blocking the photosystem II) and the synthesis of chlorophyll *a*, which leads to the inhibition of green algae growth. Only cyanobacteria exhibit increased resistance to HS and have a much higher content of chlorophyll *a* compared to eukaryotic green algae [35].

As a result, the more humic substance in the water, the greater the advantage of cyanobacteria over green algae, due to their better adaptation to reduced light intensity in the alkaline polyhumic waters of the Siemianówka reservoir [21]. Cyanobacterial blooms, especially in summer and early autumn, significantly reduce the diversity of fungal species because, as previously mentioned, only a few species of fungi are able to co-exist with cyanobacteria and have become resistant to their toxins.

In our research, the greatest diversity of fungal morphotypes occurred in late autumn and winter, when the biomass of cyanobacteria declined in the reservoir. The highest abundance and biomass of fungi was observed at sites 1 and 2, with the lowest average chlorophyll biomass (Table 1). In addition, sites with the highest average conductivity (1, 2, and 5) had also the largest biomass of fungi. In light of results of Grabowska et al. [21], who observed that the biomass of cyanobacteria negatively correlated with conductivity, this confirms the hypothesis that cyanobacteria significantly reduce growth and species diversity of aquatic fungi.

Conclusion

The biomass of aquatic fungi are affected by many biotic and abiotic factors, but in the polyhumic water, such as Siemianówka Dam Reservoir, the content of HS is the most important. The more HS and DOC, the smaller the biomass and species diversity of fungi, probably due to increased cyanobacterial blooms and the toxins they produce. In our study, fungal biomass in the Narew River above and below the reservoir was greater in comparison to the Siemianówka reservoir itself, characterized by a high content of chlorophyll *a* and strong summer cyanobacterial blooms. The structure of aquatic fungi also seemed considerably influenced by temperature – it contributed to the seasonal occurrence of aquatic fungi.

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