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Original Research

Water Blooms and Cyanobacterial Toxins in Lakes

Natalia Jakubowska*, Paweł Zagajewski, Ryszard Gołdyn

Department of Water Protection, Faculty of Biology, Adam Mickiewicz University in Poznań, Umultowska 89, 61-614 Poznań, Poland

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Abstract

In summer 2007 water samples were collected in three lakes situated in the region of the Great Mazurian Lakes (northeastern Poland) displaying different types of catchment area: Lake Niegocin, Lake Piłwąg and Lake Rekąty. The main objective of this study was to analyze the difference in species composition of cyanobacteria and to determine the concentration of cyanotoxins. Potentially toxic species of cyanobacteria were found to be the dominant species in each sample. *Microcystis aeruginosa* was dominant in Lake Niegocin, *Limnothrix redekei* and *Planktolyngbya limnetica* in Lake Piłwąg, and *Planktothrix agardhii* in Lake Rekąty. Furthermore, the occurrence of an invasive cyanobacteria species, *Cylindrospermopsis raciborskii*, was detected in two lakes, Rekąty and Piłwąg. The toxin concentration in all of the samples did not exceed the guideline value of 5 $\mu g \cdot l^{-1}$ recommended by the World Health Organization for recreational waters, which may indicate the dominance of non-toxic strains.

Keywords: cyanobacteria, toxins, Niegocin, Piłwąg, Rekąty

Introduction

An increasing appearance of algal blooms is the best indicator of progressive eutrophication of water bodies. In many cases, intensive human activities have caused rapid changes in natural ecosystems. Contamination of lakes by pollutants, both directly discharged from point sources and by surface runoff from catchment areas, as well as recreational use of lakes – leads to population changes [1]. As a result, a massive occurrence of cyanobacteria (mostly species of the genera *Microcystis*, *Aphanizomenon*, *Dolichospermum*, and *Limnothrix*) has been observed in water bodies [2]. The presence of cyanobacterial toxins poses a serious problem because of their capacity for secondary metabolite production [3]. Microcystins (MC), the most common hepatotoxins in freshwater environments, can poison both animals and humans. Thus, toxic cyanobacterial blooms are not only an aesthetic problem but also a potential health risk [2, 4-8].

*e-mail: jakubowskan@gmail.com

The present study was done in order to assess problems associated with the appearance of cyanobacterial blooms in lakes differing in morphometric features and their catchment areas, on the basis of three lakes situated in the region of the Great Mazurian Lakes (northeastern Poland). Hitherto, no data concerning the presence of cyanotoxins in this lakeland was available, hence the main objective of this study was to analyze concentrations of the most frequently detected microcystins in connection with the species composition of cyanobacteria in those lakes.

Material and Methods

Niegocin Lake is the third largest lake of the Great Mazurian Lakes, situated in the central part of the region. Lake Niegocin is connected with Lake Grajewko in the east, and in the south with lakes Wojnowo and Boczne [9]. It also is connected with Lake Kisajno via the Łuczański Canal and with Lake Tajty via the Niegocin Canal in the north.

Although the town Giżycko is situated on the northern bank of Lake Niegocin, urban and rural areas represent only 14% of the direct catchment area. The remaining 86% are the following: fallow lands (48.4%), forest (23.7%), arable land (13.3%), and grassland (0.5%) [10]. The shoreline is moderately developed. Due to the close proximity of the city and villages Wilkasy, Strzelce, Rydzewo, and Bystry, the lake is threatened by tourist activities and the presence of the local wastewater treatment plant, as well as the District Dairy Cooperative and the PFM agro-industrial plant in Ruda Village [10, 11].

The other two lakes are smaller and do not have direct connections with human agglomerations. Rekąty Lake is located close to the village of Stare Juchy, northwest of Ełk. The lake has two tributaries: a watercourse that connects Lake Rekąty and Lake Garbas, and the Gawlik River, which is connected with Lake Ułówki and, further, with Lake Łaśmiady by a thin isthmus. Around 61% of the direct catchment area of Lake Rekąty is covered by forest, while the remaining part comprises wasteland (21%) and arable land (15%). Although the lake is not used for tourism and recreational purposes, sewage from the municipal wastewater treatment plant in Stare Juchy released into Gawlik River is a major threat to the water. The lake is very susceptible to degradation due to its polymictic character and shallow depth [12].

Lake Piłwąg is the third studied water body (Table 1), located in the middle of Borecka Forest. The Czarna Struga River flowing through the eastern part of the lake connects it with lakes Szwałk Wielki and Łaźno. Lake Piłwąg is a nonstratified lake with diverse shoreline habitats, free from anthropogenic pollution. There are no human settlements or recreational areas near the lake. The direct catchment area is made up of multi-species broadleaved forests growing on dry or wet soil, which are part of the Mazurian Landscape Park [12].

The analyzed water bodies differ considerably in terms of the morphometric properties of their basins and catchments, which is reflected in the assessment of their susceptibility to degradation (Table 1).

Water samples for phytoplankton identification and cyanobacterial toxin analyses were taken at the surface layer of lakes Piłwąg and Rekąty during mid-August 2007, in accordance with the criteria described in Regulation (2002) [14]. The surface water of Lake Niegocin was sampled in July 2007, and then 4 times at the turn of August and September 2007.

The water for biological and physicochemical analysis (determination of the content of chlorophyll-a and seston dry weight) was sampled to 3-litre cans and transported to the laboratory. Seston dry weight ($mg \cdot l^{-1}$) was measured by the gravimetric method, according to PB 062-06-WS Standard. The chlorophyll-a concentration ($\mu g \cdot l^{-1}$) was analyzed spectrophotometrically according to Polish Standard PN-86/C-05560/02.

The preparation of samples for toxin analyses was done according to ISO/CD 20179 Standard. 0.5 litre of each water sample was filtered through glass fiber filters Whatman GF 75. The concentrated material and water samples were frozen and then sonicated in the Bandeline sonificator before solid phase extraction (SPE) with C-18 sorbent. SPE preparation was carried out according to the procedure proposed by Jurczak et al. [15]. The sample was redissolved in 0.5 ml of 75% methanol before high performance liquid chromatography (HPLC) analysis. All toxin samples were analyzed with the use of HPLC/MS - Agilent 1100S with mass spectrometry (binary pump, autosampler, ionization mode API-ES, Agilent 1100/1200 thermostated column). The calibration curve was constructed using microcystin-RR (MC-RR), microcystin-YR (MC-YR) and microcystin-LR (MC-LR) from Calbiochem (La Jolla, USA) as standards.

Directly after sampling, the samples for phytoplankton were preserved with Lugol's iodine (modified after Utermöhl 1958) and stored in brown glass bottles. The qualitative and quantitative compositions of phytoplankton were determined microscopically. Enumeration of cyanobacterial and algae cells was done using a 0.65 ml Sedgwick-Rafter counting chamber.

Table 1. Morphometric characteristics of lakes Niegocin, Piłwąg, and Rekąty [10, 12, 13].

Name of lake	Niegocin	Piłwąg	Rekąty
Surface area (ha)	2,600.0	135.1	53.4
Volume (thousands m^3)	258,521.6	2,025.3	1,232.2
Average depth (m)	9.9	1.5	2.3
Maximum depth (m)	39.7	3.6	5.5
Direct catchment area (ha)	481.0	73.1	86.6
Total catchment area (km^2)	403.5	82.0	174.0
Susceptibility of lake to degradation (category)	II a), b)	Outside cat.	Outside cat. b)
Water quality classes	III	III	Outside classes

a) The presence of point sources of pollution that discharge sewage directly to the lake.
b) The presence of point sources of pollution that discharge sewage into the lake inlet.

Results and Discussion

The research was conducted in summer, which provided highly favourable conditions for the growth of cyanobacteria. Water temperatures ranging from ca. 20 to 30°C during the period of investigation provided good conditions for blue-green algae growth [16, 17]. These observations were confirmed by biological analyses showing the occurrence of cyanobacteria in water samples at above 1,000 cyanobacterial cells per 1 ml of water, which is stated as a characteristic value for "water bloom" [18]. Other criteria also indicated the presence of a cyanobacterial water bloom. In seven water samples chlorophyll-a

Table 2. The concentration of microcystins in waters of the monitored lakes (μg·l^{-1}).

Sample	MC-LR	MC-RR	MC-YR	Sum of micro-cystins content
Niegocin 20. 07. 2007	< 0.001	< 0.003	< 0.006	< 0.010
Niegocin 23. 08. 2007	< 0.010	< 0.010	< 0.010	< 0.030
Niegocin 29. 08. 2007	< 0.010	< 0.010	< 0.007	< 0.027
Niegocin 7. 09. 2007	< 0.010	< 0.010	< 0.010	< 0.030
Niegocin 12. 09. 2007	0.037	0.048	0.074	0.159
Piłwąg 22. 08. 2007	< 0.010	< 0.010	< 0.010	< 0.030
Rekąty 21. 08. 2007	< 0.010	< 0.010	< 0.010	< 0.030

concentrations were between 9 and 720 mg·m^{-3}. Seston content also was high, from 4 up to 169 mg·l^{-1}. The increase of chlorophyll-a and seston content was directly proportional; a large deviation was visible only in a sample from 7 September, when the seston weight definitely did not correspond to lower chlorophyll-a concentration (Fig. 1).

The presence of all the analyzed microcystins (MC-LR, MC-RR and MC-YR) was observed in lake water samples, but the obtained values did not exceed the guideline of 5 μg·l^{-1} recommended by the World Health Organization (WHO) for recreational waters [4]. The concentration of MC in water samples ranged generally from 0.01 to 0.03 μg·l^{-1}. A higher MC concentration (0.159 μg·l^{-1}) was found only in one sample (Table 2).

Neither did MC concentration exceed acceptable levels in biomass. Measurements showed similar results with small variability. The obtained values ranged from 0.115 to 1.814 μg·g^{-1} of dry weight, i.e. from 0.0007 to 0.3062 μg in 1 litre of water (Table 3) and were much lower than the values obtained in the collected water samples.

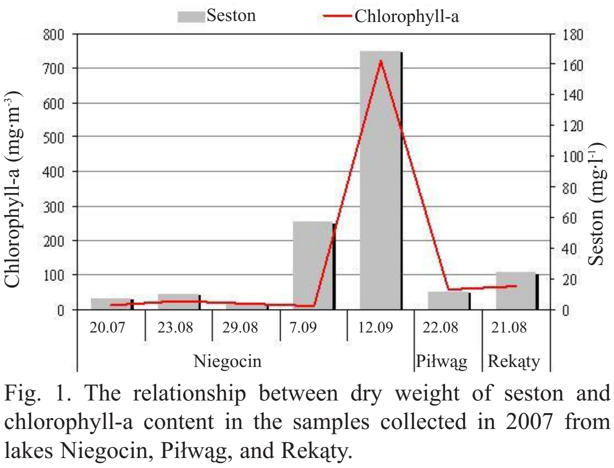

Fig. 1. The relationship between dry weight of seston and chlorophyll-a content in the samples collected in 2007 from lakes Niegocin, Piłwąg, and Rekąty.

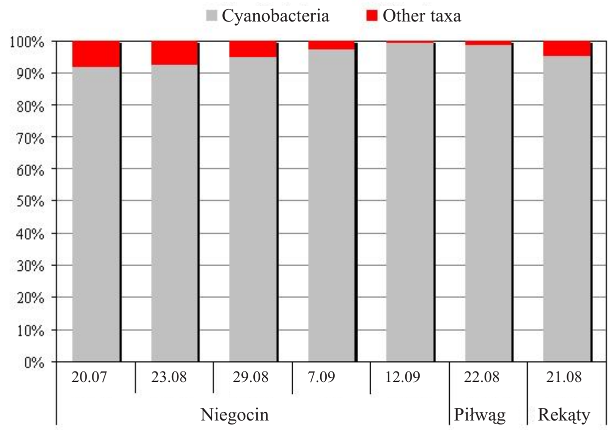

Fig. 2. Percentage share of cyanobacteria and other phytoplankton taxa in the studied lakes.

The dominant species in all the collected samples were cyanobacteria, with a relative abundance ranging between 91.78% and 99.46% of the total phytoplankton number (Fig. 2). A total of 17 taxa of cyanobacteria were recorded, most of which belonged to potentially toxic species (Table 4).

In Lake Niegocin, the most common species throughout the study period was the potentially hepatotoxic *Microcystis aeruginosa* (Kützing) Kützing 1846, which had already been noted there previously [11, 19]. It was also observed in other lakes, but in smaller quantities. Besides this species, *Chroococcus minimus* (Keissler) Lemmermann 1904 and *Chroococcus turgidus* (Kützing) Nägeli 1849 appeared in large quantities in all the water bodies; these species are also announced as toxic [5, 20-23]. In Lake Niegocin there were also potentially toxic *Aphanizomenon gracile* (Lemmermann) Lemmermann 1907, *Dolichospermum affine* (Lemmermann) Wacklin, Hoffmann et Komárek 2009, *Dolichospermum spiroides* (Kleb.) Wacklin, and Hoffmann et Komarek 2009, as well as nontoxic *Microcystis wesenbergii* (Komárek) Komárek 1968 [24, 25]. Potentially toxic *Pseudanabaena limnetica* (Lemmermann) Komárek 1974 and *Anabaenopsis* sp. were observed in two lakes, but *Anabaenopsis elenkini* Miller 1923 only in Lake Rekąty [22, 25, 26].

It is worth emphasizing that a relatively large number of cyanobacteria can produce neurotoxins that were not identified in this study. These species include: *Aphanizomenon flos-aquae* Ralfs ex Barnet et Flahault 1888, *Cuspidothrix issatschenkoi* (Usačev) Rajaniemi et al. 2005, *Dolichospermum flos-aquae* (Brébisson ex Bornet et Flahault) Wacklin, Hoffmann and Komárek 2009, *Limnothrix redekei* (van Goor) Meffert 1988, *Planktolyngbya limnetica* (Lemmermann) Komárková-Legnerová et Cronberg 1992, and *Planktothrix agardhii* (Gomont) Anagnostidis et Komárek 1987 [4, 5, 26-28].

The occurrence of the cyanobacterium *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya and Subba Raju 1972 in Lake Piłwąg (2,879 cells·ml^{-1}) and Lake Rekąty (1,095 cells·ml^{-1}) seems to be noteworthy as it is characteristic for tropical and subtropical areas. This cyanobacterium also has recently begun to appear in Poland

Table 3. Concentrations of microcystins in the phytoplankton biomass of the monitored lakes ($\mu g \cdot l^{-1}$).

Sample	MC – LR ($\mu g \cdot g^{-1}$ d.w.)	MC – RR ($\mu g \cdot g^{-1}$ d.w.)	MC – YR ($\mu g \cdot g^{-1}$ d.w.)	Total microcystins ($\mu g \cdot g^{-1}$ d.w.)	Total microcystins ($\mu g \cdot l^{-1}$)
Niegocin 20. 07. 2007	0.021	0.027	0.071	0.119	0.0009
Niegocin 23. 08. 2007	0.011	0.028	0.076	0.115	0.0012
Niegocin 29. 08. 2007	0.019	0.064	0.103	0.186	0.0007
Niegocin 7. 09. 2007	0.032	0.071	0.102	0.205	0.0117
Niegocin 12. 09. 2007	0.259	0.528	1.027	1.814	0.3062
Piłwąg 22. 08. 2007	0.321	0.103	0.071	0.495	0.0057
Rekąty 21. 08. 2007	0.204	0.034	0.028	0.266	0.0064

Table 4. Species composition and numbers of cyanobacteria vs. the rest of phytoplankton quantity in the studied lakes ($cells \cdot ml^{-1}$).

Species	Niegocin					Piłwąg	Rekąty
	20. 07	23. 08	29. 08	07.09	12. 09	22. 08	21. 08
Anabaenopsis elenkinii	-	-	-	-	-	-	504
Anabaenopsis sp.	-	-	-	246	-	-	+
Aphanizomenon flos-aquae	-	2,573	4,873	164	1,695	38,000	24,528
Aphanizomenon gracile	-	-	-	-	+	-	-
Chroococcus minimus	3,460	2,278	3,285	3,936	14,761	2,566	964
Chroococcus turgidus	-	2,540	3,460	1,968	12,176	2,566	1,818
Cuspidothrix issatschenkoi	-	-	-	492	931	-	-
Cylindrospermopsis raciborski	-	-	-	-	-	2,879	1,095
Dolichospermum affine	-	-	-	-	+	-	-
Dolichospermum flos-aquae	-	6,329	5,760	902	232,403	10,298	3,066
Dolichospermum spiroides	-	679	-	-	2,278	-	-
Limnothrix redekei	-	3,559	-	-	438	234,001	24,309
Microcystis aeruginosa	35,347	36,091	16,447	21,910	419,845	26,715	19,973
Microcystis wesenbergii	-	-	-	-	12,702	-	-
Planktolyngbya limnetica	-	3,833	2,902	3,444	25,952	113,670	30,386
Planktothrix agardhii	-	602	548	1,025	329	8,636	52,122
Pseudanabaena limnetica	-	-	-	-	+	-	+
Abundance of Cyanobacteria	38,807	58,484	37,274	34,087	723,510	439,331	158,765
Abundance of other phytoplankton species	3,477	4,752	2,081	869	3,964	5,831	7,753

and in other countries of Europe [29] although it requires temperatures of up to 25ºC for optimal growth [30]. *Cylindrospermopsis raciborskii* is a dangerous species as it can produce such toxins as cylindrospermopsin, saxitoxin, anatoxin-a, and paralytic shellfish poisons (PSP) [26, 30-33].

Potentially toxic species of cyanobacteria were dominant in all samples. However, the hepatotoxin levels found in both water and biomass samples were not as high as expected on the basis of abundance of those species. This was probably due to the presence of not only toxic but also nontoxic strains of those species and the latter were surely the dominants. Such toxic and nontoxic strains of cyanobacteria species occur frequently together in one water body [3, 34, 35].

Conclusions

1. Potentially toxic species of cyanobacteria were dominant in all samples. *Microcystis aeruginosa* were dominant in Lake Niegocin, *Limnothrix redekei* and *Planktolyngbya limnetica* in Lake Piłwąg, and *Planktothrix agardhii* in Lake Rekąty.
2. A large number of potentially neurotoxic species have been identified, including *Planktolyngbya limnetica*, *Aphanizomenon flos-aquae*, *Limnothrix redekei,* and *Dolichospermum flos-aquae*.
3. The occurrence of an invasive Cyanobacteria species – *Cylindrospermopsis raciborskii* – was detected in two lakes: Rekąty and Piłwąg. This is a particularly dangerous invasive species due to its ability to produce such toxins as cylindrospermopsin, saxitoxin, anatoxin-a, and PSP.
4. In no sample did the MC concentration exceed the guideline value of 5 $\mu g \cdot l^{-1}$, recommended by the WHO for recreational waters.
5. Relatively low levels of hepatotoxins found in samples may indicate the coexistence of non-toxic strains.

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