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Research Article

# TOXIC CYANOBACTERIAL BLOOMS IN THE KOCIEWSKIE LAKES (NORTHERN POLAND)

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## Abstract

The presence of toxic cyanobacterial blooms has been frequently reported in natural and artificial water bodies from different regions of the world. The toxic compounds (mainly hepato- and neurotoxins) produced by a number of cyanobacterial species have caused severe problems for animals and humans. Studies were carried out in the Kociewskie District on Lakes Słone, Czarne Południowe, and Kałębie, which are used for recreational purposes. Based on microscopic analyses the most frequently occurring cyanobacterial species were determined in samples collected every ten days from May until the end of September, 2004. They belonged to the genera: *Pseudanabaena*, *Phormidium*, *Woronichinia, Planktothrix, and Anabaena*. Picoplankton cyanobacteria were also recorded in the samples. HPLC was used to identify and quantify cyanobacterial toxins present in the lakes. In each lake microcystin-RR and -LR were found. The highest concentrations of Mcyst-RR measured in Lakes Słone, Czarne Południowe and Kałębie amounted to 6.65, 14.5, and 20.2 µg $l^{-1}$, respectively. Mcyst-LR was present only in trace amounts.

## INTRODUCTION

Cyanobacteria are photosynthetic prokaryotes which can live as a single cells or form colonies, simple and branched filaments. Cyanobacterial blooms have been frequently reported in eutrophicated fresh and brackish water bodies worldwide. The occurrence of cyanobacterial blooms under natural conditions is determined by a combination of physical and chemical factors such as temperature, pH, light intensity, and nutrients concentrations, especially nitrogen and phosphorus level (Skulber *et al*. 1984, Codd *et al*. 1989). At high biomass, cyanobacteria can have negative effects on aquatic ecosystem. They may cause oxygen depletion, decrease in biodiversity and increase in primary production. Some species of cyanobacteria produce toxic compounds which can pose a serious threat to animals and humans (Kuiper-Goodman *et al*. 1999). Up until now, many toxic metabolites with various biological activities have been identified and isolated from cyanobacteria; they include: neurotoxic alkaloids (anatoxin-a, anatoxin-a(s), saxitoxins; Dow and Swoboda 2000) and hepatotoxic cyclic peptides (microcystins and nodularin; Rinehart *et al*. 1994, Sivonen *et al*. 1989). The most commonly occurring and potentially harmful genera of cyanobacteria include *Microcystis*, *Anabaena*, *Aphanizomenon,* and *Planktothrix*.

Beside the typical scum- and bloom-forming cyanobacteria, there are also some species of very small cell size (typically 0.2-2 μm), which usually do not form scum and are called picocyanobacteria (Meada *et al*. 1992). They are known to produce several toxic substances, including microcystins. Cytotoxic and immunotoxic effects of picocyanobacterial strains were reported by Bláha and Maršálek (1999).

The aim of this work was to determine the species composition of cyanobacteria occurring in the Kociewskie Lakes. It was also important to examine if any toxins are produced by the cyanobacteria present in the lakes.

## MATERIAL AND METHODS

Samples were collected from three lakes: Słone, Kałębie, and Czarne Południowe situated in the Kociewie Lake District (northern Poland) (Fig. 1). The lakes range in are from 980 $km^2$ (Lake Czarne Południowe) to 4660 $km^2$ (Lake Kałębie) (Tab. 1). Their mean depth is 2.4-2.8 m (Jańczak 1999). The surface water samples with phytoplankton were collected once every ten days from May until the end of August, 2004. Sub-samples for qualitative and quantitative analyses of cyanobacterial species were immediately preserved in Lugol solution. Inverted microscope (400 x) was used to determine the

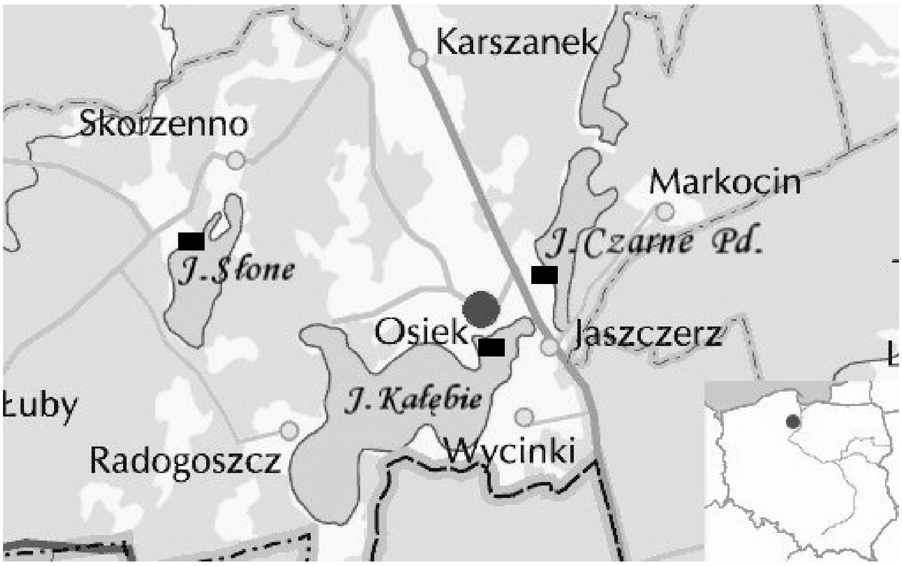


**Fig. 1.** Location of sampling stations.

**Table 1**

Area and depth of the Kociewskie Lakes (Jańczak 1999).

| Lake | Area [km$^2$] | Depth max. [m] | Depth average [m] |
|---|---|---|---|
| Kałebie | 4660 | 6.4 | 2.4 |
| Słone | 1190 | 5.3 | 2.7 |
| Czarne Południowe | 980 | 7.6 | 2.8 |

dominating cyanobacterial taxa and their abundance. Water samples were filtered onto 25-mm GF/C glass fibre filter discs and frozen at –20 ºC until further analyses. After thawing, the material was homogenized by ultrasonication and extracted in 2.0 ml 90% methanol. The samples were centrifuged for 15 min at 6,140 x *g*. Chlorophyll *a* (Chla) concentration in the supernatant was determined spectrophotometrically by absorbance measurements at 665 ($A_{665}$) and 750 nm ($A_{750}$) using the equation: $C_{chla} = 13.42 (A_{665} - A_{750})$ [µg ml$^{-1}$]. Toxin concentration was analyzed by high-performance liquid chromatography (HPLC) (Waters, Milford, MA, USA) with a PDA 966 photodiode array detector set at 238 nm. The analyses were conducted using a RP-18 column (MERCK, 5 µm, 250 x 4.0 mm) at a flow rate of 1.0 ml min$^{-1}$. Isocratic elution was used with (A) 10% acetonitrile in 0.05% TFA and (B) 100% acetonitrile in 0.05% TFA (53: 47). Concentrations of the toxins were quantified by calibrating them against the standard microcystins MCYST-LR, -RR, -YR (Sigma). HPLC gradient grade solvents and MilliQ water were used for all analyses.

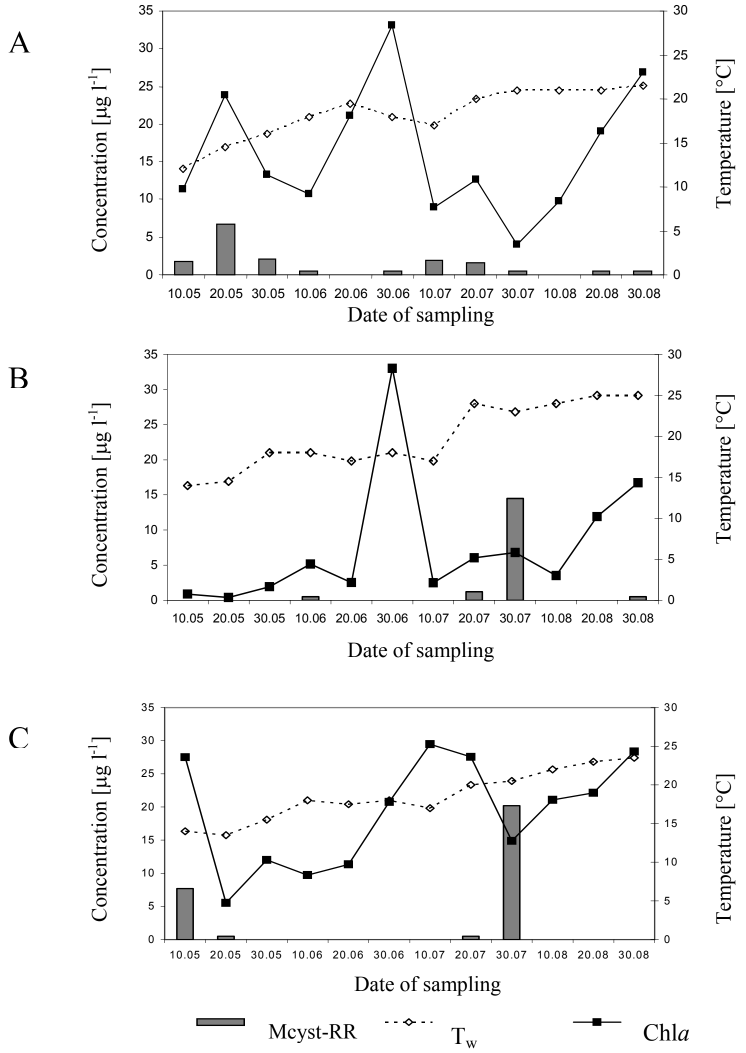


**Fig. 2.** Microcystin-RR and chlorophyll *a* concentration in samples and water temperature in the Kociewskie Lakes: A – Słone, B – Czarne Południowe, C – Kałębie.

## RESULTS AND DISCUSSION

In these studies phytoplankton samples were collected from Kociewskie Lakes (Pomorskie Province, northern Poland) which are used for recreation or fishing. The samples with higher concentration of chlorophyll *a* or the toxins were subjected to more detailed microscopic analyses. During the studies a constant increase in lake water temperature from about 12.0-14.0 °C in May to 21.5-25.0 °C at the end of August was observed. As temperature around 25 °C is optimal for cyanobacterial growth, it can be concluded that most favourable conditions for bloom development prevailed at the end of the sampling period. In all samples, with the exception of the one collected on June 30 from Lake Słone, cyanobacteria constituted 80-99% of phytoplankton community.

A dynamic change in phytoplankton composition was observed in Lake Słone (Fig. 2A). At the end of May, mass occurrence of cyanobacteria of the genera *Anabaena* and *Pseudanabaena* was recorded. In June *Chlorophyta* were the dominating phytoplankton organisms (84 %), while in samples collected on August 30, *Pseudanabaena* sp. prevailed (99%, 21262.6 μgC $l^{-1}$). This situation was rather unusual since *Pseudanabaena* sp. is not the typical bloom-forming species. The highest concentration of Mcyst-RR (6.6 μg $l^{-1}$) was measured on May 20, when *Anabaena* was most abundant.

In Lake Kałębie, picocyanobacteria of the genera *Aphanocapsa*, *Aphanothece,* and *Cyanodiction* were present throughout the complete sampling period (Tab. 2). Some *Microcystis* species were present at the beginning of May and reached the biomass of 8964.7 μgC $l^{-1}$. At that time, concentration of Mcyst-RR was 7.7 μg $l^{-1}$. Despite the fact that on July 30, Chla concentration reached only 12.8 μg $l^{-1}$, the highest concentration of Mcyst-RR (20.2 μg $l^{-1}$) was measured on that day (Fig. 2B). The opposite situation was observed on August 30 when mass occurrence of *Anabaena* and *Aphanizomenon* was observed, but no toxins were detected. In Lake Kałebie, trace amounts of Mcyst-LR were detected on July 10.

In most samples collected from Lake Czarne, picocyanobacteria constituted more than 60% of the organisms present (Tab. 2). They mainly belonged to *Aphanocapsa, Aphanothece* and *Cyanodiction* genera. The highest concentration of Mcyst-RR (14.5 μg $l^{-1}$) was determined on July 30, when *Anabaena* biomass was 300.3 μgC $l^{-1}$ (Fig. 2C).

In Polish water bodies, the presence of cyanobacterial toxins has been reported by some authors. Several microcystin analogues were detected in phytoplankton samples from Lake Sulejów, Jeziorsko, and Włocławek (Kabziński *et al*. 2000). In most of the water bodies, as in many other areas of the world, Mcyst-LR was the main cyanotoxin found (85-95%). The same toxin

**Table 2**

Biomass of cyanobacteria and concentration of microcystins in the Kociewskie Lakes.

| ***Cyanobacteria*** | **Biomass of cyanobacteria [μgC $l^{-1}$]** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | ***Lake Kałębie*** | | | | ***Lake Slone*** | | | ***Lake Czarne*** | | |
| | **10 May** | **10 Jul.** | **30 Jul.** | **30 Aug.** | **20 May** | **30 Jun.** | **30 Aug.** | **30 Jun.** | **30 Jul.** | **30 Aug.** |
| Microcystis | 8964.7 | 12.5 | 462.0 | 52.4 | - | - | - | 27.9 | 25.0 | - |
| *Anabaena* | - | 96.1 | 283.6 | 1286.6 | 7078.7 | - | 305.1 | 2.0 | 300.3 | 58.5 |
| *Planktothrix* | - | - | - | - | - | 445.5 | 151.6 | 33.3 | 96.4 | 19.0 |
| *Woronichinia* | - | - | 4.9 | - | - | - | - | - | 3.4 | 9.4 |
| *Aphanizomenon* | - | - | - | 108.0 | - | - | - | - | - | - |
| *Pseudanabaena* | 33.8 | - | - | - | 1989.8 | 60.1 | 21262.6 | - | - | 15.4 |
| picocyanobacteria | 4.3 | 256.3 | 196.6 | 491.5 | - | - | - | 21.7 | 36.2 | 5.4 |
| Other | 5.9 | 14.2 | 34.5 | 112.6 | - | 3.5 | 49.6 | 1.8 | 5.9 | 19.6 |
| TOTAL | 9008.7 | 379.1 | 981.3 | 2051.1 | 9068.5 | 509.1 | 21768.9 | 86.7 | 467.2 | 127.3 |
| **Concentration of microcystins [μg $l^{-1}$]** | 7.7 Mcyst-RR | Trace Mcyst-LR | 20.2 Mcyst-RR | n.d. | 6.9 Mcyst-RR | Trace Mcyst-RR | Trace Mcyst-RR | Trace Mcyst-LR | 14.5 Mcyst-RR | Trace Mcyst-RR |

dominated in Lake Barlewice where *Aphanizomenon flos-aquae* (L.) Ralfs ex Born. et Flath. 1888 (80%), *Microcystis aeruginosa* (Kütz.) Kütz. 1846 (10%) and *Planktothrix agardhii* (Gom.) Anagh. et Kom. 1985 (10%) were identified (Codd *et al.,* 2004). Jurczak *et al*. (2004) detected 10 microcystins analogues in 36 cyanobacterial bloom samples from 8 different water bodies in Poland. Mcyst-LR, Mcyst-RR, and Mcyst-YR were the major microcystins present. In most of the samples *Microcystis aeruginosa* dominated; in two reservoirs *Aphanizomenon flos-aquae* or *Nostoc* spp. were present. Analyses of samples collected from Kashubian Lakes (northern Poland) showed that in this part of Poland Mcyst-RR was at least as common as Mcyst-LR (Mazur *et al*., 2003). In the Kashubian Lakes, numerous cyanobacterial species were found; they belonged to the genera *Microcystis, Anabaena, Aphanizomenon, Planktothrix,* and *Phormidium*. The measurements carried out in this work showed that Mcst-RR was the major cyanobacterial toxin present in the Kociewskie Lakes. In this work, production of hepatotoxins by cyanobacteria was proved in 22 out of 36 bloom samples (*i.e*. in over 61 %) collected from the Kociewskie Lakes. In each lake Mcyst-RR and -LR were detected, however, the Mcyst-RR analogue, not LR was the major cyanobacterial toxin present. Temporarily the concentration of the toxin was relatively high (6.6-20.2 μg $l^{-1}$) and exceeded the safety limit of 1 μg $l^{-1}$ required for drinking water. Mcyst-LR occurred only in trace amounts. The dominating canobacteria genera identified in phytoplankton samples were: *Microcystis, Anabaena, Planktothrix, Aphanizomenon, and Pseudanabaena.* In Lakes Kałębie and Czarne, a contribution of picocyanobacteria *Aphanocapsa, Aphanothece,* and *Cyanodiction* was significant. According to Bláha and Maršálek (1999), the presence of small unicellular cyanobacteria should be monitored in water blooms, since these micro organisms are potential toxin producers, as well.

Based on studies carried out in different parts of the world it can be assessed that 25-90% of cyanobacterial blooms are toxic. However, it is still not clear, why certain cyanobacteria produce toxins when others do not. Even within the same species, both toxic and not toxic strains can be found. Since the bloom samples analysed in this work contained several cyanobacterial taxa each, it was rather difficult to determine which of them are the real toxin-producers.

## CONCLUSION

Chlorophyll *a* concentration or phytoplankton biomasses are not reliable parameter for the assessment of cyanobacterial bloom toxicity. Chlorophyll *a* is present in all photosynthesizing organisms. Additionally, there is no clear correlation between the abundance of potentially toxic or toxic cyanobacteria and the concentration of toxins in phytoplankton samples. The simultaneous application of chemical methods and biological assays are indispensable to determine the concentration of toxins and toxicity of sample.

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