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Development of toxin-producing cyanobacteria during the water level manipulation in a shallow heavily modified lake

by

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Abstract

The development of cyanobacteria and microcystin variation was studied during two years in response to periodical water-level regulation in modified Lake Tomaszne. Before the water entrance from a canal, the biomass of toxigenic cyanobacteria was 0.001-0.33 mg dm^{-3}, the microcystin concentration was below 1 µg dm^{-3}, and three variants of microcystins were detected. After the water entrance from the canal, the biomass of cyanobacteria increased to 1.3-7.1 mg dm^{-3} with the dominants *Aphanizomenon gracile* and *Dolichospermum planctonicum*. After the water discharge from the lake, *Planktothrix agardhii* reached the highest biomass (2.3-6.6 mg dm^{-3}). During the mass development of toxigenic cyanobacteria, the total microcystin concentrations were mostly higher than 5 µg dm^{-3} and the number of MC variants increased. Both *Pl. agardhii* and *D. planktonicum* accounted for microcystin production. The higher NH_4-N concentrations supplied with water from the canal supported the biomass increase of Nostocales, whereas *Pl. agardhii* mass development was due to the low light intensity and high TP concentrations. Our study revealed that the use of nutrient-rich waters for the water management in the hydromorphologically modified lake supported the persistent development of cyanobacteria species leading to increased amounts of MC and a higher number of their structural variants.

Key words: hydromorphologically modified lake, water level manipulation, toxigenic cyanobacteria, microcystins variants

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Introduction

The proliferation of cyanobacteria in eutrophic waters often leads to the formation of nuisance blooms. This problem is global and the cases of blooms are reported in many countries (Graham et al. 2004, Carvalho et al. 2011, Kobos et al. 2013). Usually, *Microcystis* spp., *Planktothrix agardhii*, *Dolichospermum* spp., *Cylindrospermopsis raciborskii* and *Aphanizomenon flos-aque* create multispecies blooms that are observed in the surface water, sometimes in the form of scum (Carmichael 2001). The excessive biomass of cyanobacteria can cause adverse effects in the aquatic environment, such as transparency reduction, enhanced productivity, a decrease in biodiversity and potentially oxygen depletion (Pearl & Otten 2013).

Freshwater cyanobacterial blooms are a serious problem because some species/populations are able to produce toxins that are hazardous to human health, animals and plants (Lahrouni et al. 2012, Merel et al. 2013). Cyanobacterial toxins are secondary metabolites which usually are classified as hepatotoxins (microcystins, cylindrospermopsins), neurotoxins (anatoxin-a, saxitoxin) or dermatotoxins (lyngbyatoxins) (Dittman et al. 2013). The most commonly occurring toxins are cyclic heptapeptide microcystins (MCs) (Zurawell et al. 2005). Members of several cyanobacterial genera, such as *Microcystis*, *Dolichospermum*), *Planktothrix*, *Oscillatoria*, *Pseudanabaena* and *Woronichinia*, are responsible for the production of microcystins (Dietrich & Hoeger 2005). Microcystins are a heterogonous group of chemical compounds. To date, nearly 80 variants of these hepatotoxins have been isolated which vary in their peptide sequence of L-amino acids, degree of methylation and toxicity (Meriluoto & Codd 2005, Gągała & Mankiewicz-Boczek 2012). Contact with microcystins occurs most frequently through ingestion of drinking water during bathing in water with blooms. Cyanotoxins can cause various symptoms of poisoning, including skin irritation, allergic rash, diarrhoea, gastrointestinal disturbances, and liver and kidney damage (Codd et al. 2000).

Recently, intensive cyanobacterial blooms have been observed in waterbodies such as dam reservoirs or lakes with a regulated water level (Grabowska & Pawlik-Skowrońska 2008, Solis et al. 2009, Pawlik-Skowrońska et al. 2013). Such altered lakes, in accordance with the requirements of the Water Framework Directive (2000/60/EC, EU), can be classified as Heavily Modified Water Bodies, i.e. "bodies of surface water which as a result of physical alterations by human activity are substantially changed in character." In those waterbodies, the water level is controlled with regards to flood protection, recreation, irrigation and consumption. The level often varies irregularly and the amplitude of its fluctuation is much higher than in natural lakes (Zohary & Ostrovsky 2011). The development of cyanobacteria in freshwater ecosystems are stimulated by excessive nutrient loading (Carey et al. 2012). The water-level changes can affect nutrients balance; the low water level promotes internal loading and indirectly contributes to an increase in cyanobacteria biomass (Haldna et al. 2008). In waterbodies with a regulated water level, the inflow/outflow of water usually reduces the increase of cyanobacteria biomass, mainly by shortening the water residence time and enhancement of flushing (Olding et al. 2000, Paerl et al. 2011). The higher biomass of cyanobacteria occurs rather during isolation from the supply with water (isolation phase), when water horizontal flushing is strongly reduced (Padisak et al. 1993).

In the two-years study, we focused on the shape of toxigenic cyanobacteria assemblages in a heavily modified lake with periodically regulated water levels. We speculated that manipulation of the water level and volume for a short period (few days) might affect the development of cyanobacteria in the isolation phase, especially microcystin producers, and account for its persistence during a year.

Study area

Lake Tomaszne (51°28'27"N, 23°0'7"E) is a shallow ($depth_{max.}$ = 3.1 m, $depth_{aver.}$ = 1.8 m) and polymictic waterbody that is connected with the large Wieprz-Krzna Canal drainage system (Eastern Poland) (Fig. 1). The lake is heavily modified due to the enlargement of the surface (area = 0.95 km^2) and the embankment of the shoreline, with a length of 3.65 km. The lake was designed to retain water (maximal volume = 2.21 × 10^6 m^3), and the water volume is regulated by two sluices. One sluice is used for the entrance of water from the canal, and

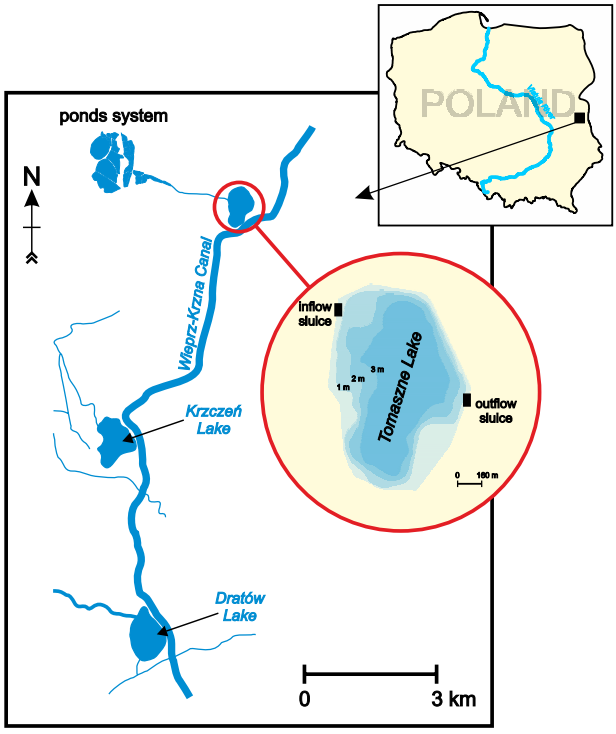

Figure 1
Location of the studied lake

the second sluice is used for the discharge of water from the lake for fish-pond alimentation and/or the irrigation of vegetable crop cultivation. The lake is also used recreationally, and for fish farming and fishing.

Materials and methods

Biweekly sampling was performed between May and September 2010 and 2011. Integrated water samples were collected with a 2 dm^3 Ruttner sampler (Hydrobios) in the central part of the lake at half-meter intervals, up to the lower limit of the euphotic zone (depth of 1.5–2.5 m in the studied lake). In situ, water temperature, electrolytic conductivity and pH were measured with CC-401, CP-411 and CC-411 meters (Elmetron, Poland), respectively.

Light intensity in the range of Photosynthetic Active Radiation (PAR) was measured with a quantum sensor (LI-190-SA, Li-250, LiCor) at a 0.25-m interval. The mean light intensity in the range of PAR in the mixed water column (taken as the mean depth of the lake) was calculated based on an attenuation coefficient and the sum of global radiation according to Behrendt & Nixdorf (1993). Data of global radiation were obtained from the meteorological station in Włodawa, situated approximately 40 km from the lake.

Concentrations of PO_4-P, NH_4-N and NO_3-N were determined by ion-exchange chromatography from water subsample (250 cm^3). The total content of P and N (after prior mineralisation) was determined spectrophotometrically (APHA 1995).

Fresh cyanobacterial material was used for species identification under a light microscope (Nikon 80i). The abundance was assessed in preserved samples (with Lugol's solution) using the sedimentation method of Utermöhl (1958) under an inverted microscope (Zeiss Axiovert 135). In a 5 cm^3 chamber, a minimum of 200 individuals were counted, assuming that single cells, colonies or filaments (100 µm long units) were individuals. Cyanobacterial biomass was estimated from the approximate geometric volumes for each species. The mean dimensions of cells or a colony (30-50 individuals) were used for the calculations of cell volume (Hillebrand et al. 1999). The chlorophyll-*a* concentration was determined spectrophotometrically according to the Polish norm PN-ISO 10260:2002.

The intracellular MCs concentrations were determined from the extracts of fresh cyanobacterial biomass (previously concentrated from 0.5-1 dm^3 of water on Whatman GF/C filters) which were prepared in the acidified (0.002 M HCl) 50% methanol using ultrasonication (3 times for 5 min, 50 W, ultrasonic homogenizer Sonoplus, Bandelin). MCs identification and quantification was carried out using a high liquid chromatography (HPLC) system (Shimadzu) equipped with a Diode Array Detector (DAD) operating at 238 nm. Extracts were separated on the LiChroCART 125-3 Purospher RP-18 column (125 × 3 mm, 5 µm, Merck). The gradient (30-100%) of aqueous acetonitrile (Merck) acidified with 0.05% trifluoroacetic acid, at a flow rate of 0.7 ml min^{-1}, was used according to Lawton et al. (1994). MC-LR, -RR, -LA, -LY, -LW, -LF and -WR (Alexis Biochemicals) standards were used for calibration curve preparation.

The water level in the lake was read at the watermark. The volumes of water introduced or discharged into/from the lake were estimated based on the differences in the water level and volumetric curves. Loads of nutrients that entered into the lake were determined from the ratio of nutrient

concentrations in the canal's water and volume of entered water.

The statistical analyses were performed to determine significant differences between the mean values with the Kruskal-Wallis test (K-W), whereas relationships between variables were established by the nonparametric Spearman rank correlation. The Statistica 7.0 software package was applied.

Results

Throughout the study period, the water level in the lake was regulated twice each year due to the need for alimentation of nearby fish-ponds. In June/July, a water volume of 192 × 10^3 m^3 in 2010 and 306 × 10^3 m^3 in 2011 entered the lake (within 8-13 days), causing an increase in the lake's volume by 12 and 15%, respectively. The loads of dissolved phosphates (40.3 and 56.4 kg), ammonia nitrogen (21.9 and 11.6 kg) and nitrate nitrogen (173.3 and 345.0 kg) were then introduced in both years, respectively. In the first half of August, the volume of water discharged from the lake (within a few days) was 192 × 10^3 m^3 (4% of the lake' volume) in 2010 and 176 × 10^3 m^3 (1.5% of the volume) in 2011. Although the changes in the mean water level were rather small (16-25 cm), three hydrological periods have been identified (P_{K-W} = 0.003): I – the lower water level with a smaller water volume, before the entrance of water from the canal (May – June); II – the higher water level and the water volume, after the entrance of the water (July – mid-August); and III – the decreased water level and the volume, after the water discharge from the lake (mid-August – September) (Fig. 2).

The physicochemical characteristics of the lake's water are presented in Table 1. The water temperature, which is an essential factor responsible for the cyanobacterial growth, varied from 15.0 to 26.7°C. The lowest mean temperature was noted in period III and the highest mean temperature in period II. The water reaction was always alkaline (pH=8.0-8.8) and the electrolytic conductivity (EC) ranged from 295 to 413 µS cm^{-1}. The mean light intensity (I_{mix}) in the mixed zone ranged from 2.8 to 17.5 E m^{-2} d^{-1}. Both total and dissolved nutrient concentrations indicated a strong eutrophication of the lake. Phosphorus compounds and ammonia concentrations displayed a high dynamics over the three hydrological periods. The significant increases in PO_4-P, NH_4-N and TP (in 2010) concentrations were found after the entrance of water from the canal (period II). However, the highest TP concentrations were noted after the water discharge from the lake (period III). The mean concentrations of total nitrogen and nitrate nitrogen showed no specific trend in changes over the hydrological periods.

The chlorophyll-*a* concentrations (Chl-*a*), indicating a total biomass of phytoplankton in

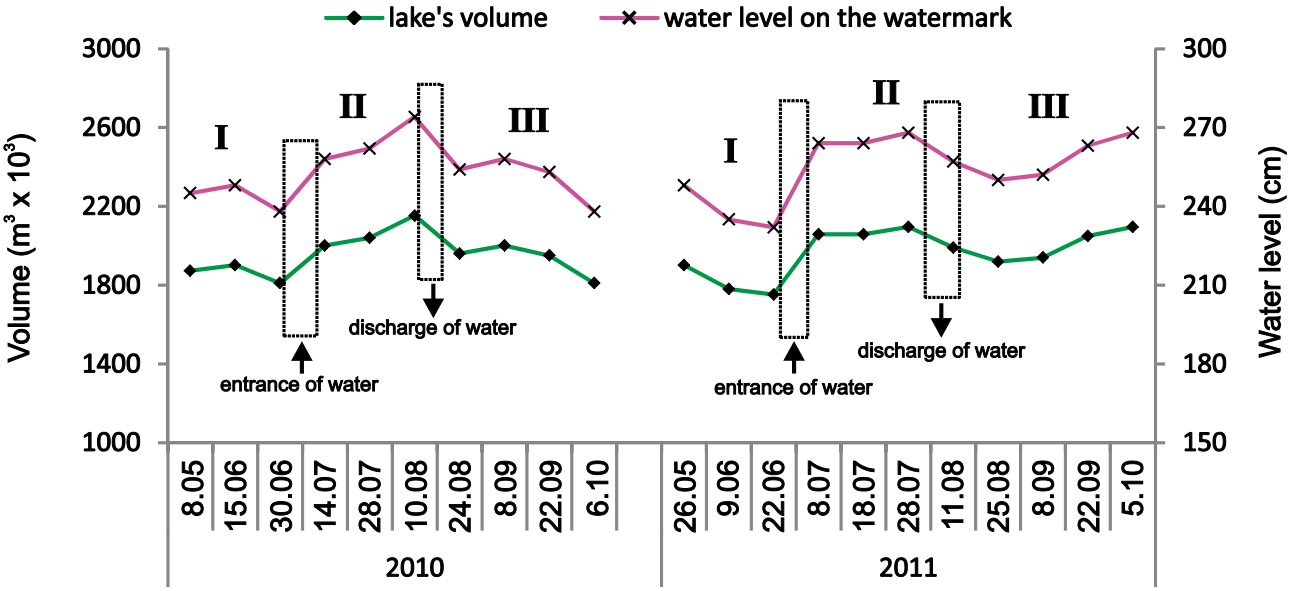

Figure 2

Changes in the water level and volume in Lake Tomaszne. I, II, III – identified hydrological periods

Table 1

Physical-chemical parameters of water over three hydrological periods

Parameter	Unit	2010			2011			P_{K-W}
		I	II	III	I	II	III	
T	°C	20.6 (±4.4)	24.4 (±2.3)	17.9 (±5.0)	22.9 (±2.8)	22.5 (±1.6)	19.9 (±2.1)	0.148
I_{mix}	E m^{-2} d^{-1}	15.4 (±6.3)	4.0 (±1.4)	2.8 (±0.6)	17.5 (±5.1)	6.8 (±2.8)	3.1 (±1.7)	0.014*
pH		8.4 (±0.1)	8.3 (±0.3)	8.6 (±0.1)	8.4 (±0.0)	8.3 (±0.2)	8.6 (±0.2)	0.959
EC	µS cm^{-1}	413 (±4.9)	343 (±34.5)	295 (±8.7)	385 (±22.0)	319 (±23.9)	307 (±16.1)	0.026*
TP	mg dm^{-3}	0.054 (±0.019)	0.117 (±0.008)	0.137 (±0.013)	0.087 (±0.042)	0.095 (±0.040)	0.300 (±0.123)	0.095*
TN		2.26 (±0.74)	4.31 (±0.58)	3.67 (±0.74)	4.72 (±1.35)	4.52 (±0.51)	6.72 (±3.43)	0.043*
PO_4-P		0.007 (±0.003)	0.019 (±0.006)	0.024 (±0.007)	0.032 (±0.009)	0.053 (±0.013)	0.027 (±0.007)	0.036*
NH_4-N		0.11 (±0.02)	0.33 (±0.28)	0.37 (±0.26)	0.10 (±0.02)	0.32 (±0.32)	0.18 (±0.20)	0.044*
NO_3-N		1.60 (±0.61)	1.86 (±0.78)	0.69 (±0.58)	0.33 (±0.34)	0.87 (±1.48)	1.11 (±0.58)	0.173

WV - water volume, T - temperature, I_{mix} - light intensity (PAR) in the mixing water column, EC - electrolytic conductivity. The values are the means ± standard deviations. Differences are identified with the Kruskal-Wallis test (P_{K-W}). * - significant differences

the lake, varied between 34.06 and 178.28 µg dm^{-3} throughout the study period (Fig. 3).

Altogether 22 species of cyanobacteria, primarily *Microcystis* spp., *Dolichospermum* spp., *Aphanizomenon* spp,. *Planktothrix* spp., *Planktolyngbya* spp., and *Limnothrix* spp. were found (Table 2), whose total biomass ranged from 0.001 to 8.1 mg dm^{-3} (Fig. 3). The potentially toxigenic cyanobacteria (TCb) contributed always >99% to the cyanobacterial biomass. The biomass of TCb was low (<0.1 mg dm^{-3}) in May-June (I period) (Fig. 3) with dominants *Planktothrix agardhii*, *Aphanizomenon gracile* or *Dolichospermum planctonicum*. The biomass of TCb increased significantly to 1.3-7.1 mg dm^{-3} (P_{K-W}: = 0.0435) after the water entrance into the lake (period II) due to the mass development

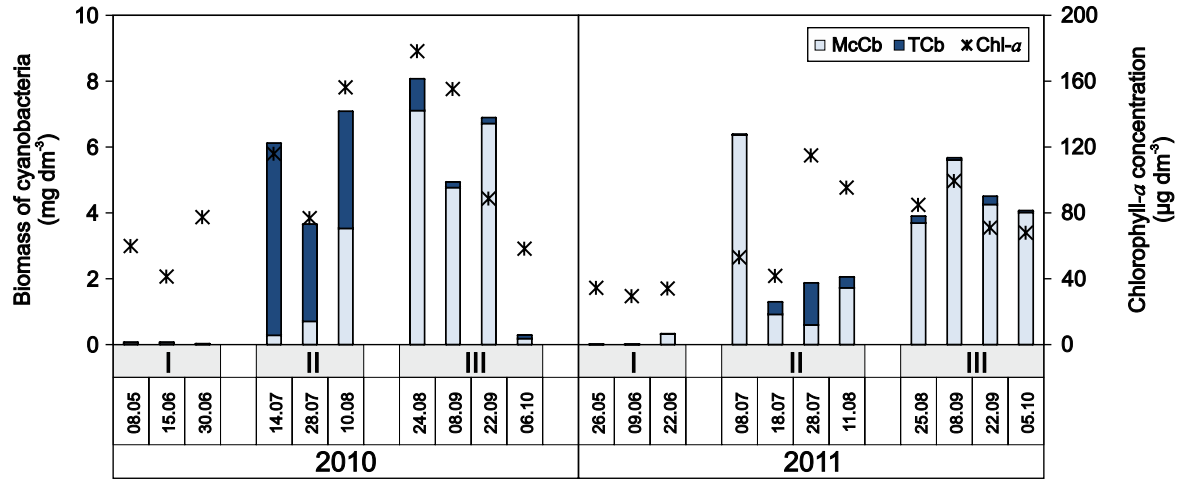

Figure 3

The biomass of potentially toxigenic cyanobacteria (TCb – Aph. gracile up to 95%), including microcystins producers (McCb – *D. planctonicum* up to 86%; *Pl. agardhii* up to 96%) and chlorophyll-*a* concentrations over the three hydrological periods

Table 2

Cyanobacterial species and their contribution in the total biomass of cyanobacteria that developed over the hydrological periods

		2010			2011		
		I	II	III	I	II	III
Snowella lacustris (Chod.) Kom. Hindák	*	++	+				
Woronichinia naegeliana (Unger) Elen.	*				+	+	+
Microcystis aeruginosa (Kütz.) Kütz.	*		+		+		+
M. flos-aque (Witt.) Kirch.	*	+					
M. viridis (Braun) Lemm.	*						+
M. wesenbergii (Kom.) Kom. ex Kom.	*	+	+			+	+
Chroococcus minutus (Kütz.) Näg.							+
Pseudanabaena limnetica (Lemm.) Kom.	*	+		+		+	+
Limnothrix redekei (Van Goor) Meffert		+		+	+		+
L. planctonica (Woł.) Meffert		+		+			+
Jaaginema spp.		+	+				
Planktolyngbya limnetica (Lemm.) Kom.-Legn. et.Cron.	*	+	+	+	+	+	+
Planktothrix agardhii (Gom.) Anagn. et Kom.	*	++++	++	++++	++++	+++	++++
Aphanizomenon gracile (Lemm.) Lemm.		+++	++++	+++	+	+++	+
Cuspidothrix elenkini Kisselev			+				
C. issatchenkoi (Usacev) Prosh.-Lav.		+	+	+			
Anabaenopsis elenkini V.V. Miller	*	+	+	+			
Dolichospermum circinale (Raben. ex Bornet & Flahault)	*		+				
D. flos-aque (Bréb. ex Bornet & Flahault)	*		+				
D. lemmermanii (Ricter)	*		+				
D. planctonicum (Brunnth.)	*		++	+++	+++	+++	+
D. spiroides (Kleb.)	*	+				+	
Number of species		15	14	9	7	8	12

* - potential producers of microcytins, + < 5%, ++ 5-10 %, +++ 10-50 %, ++++ > 50 %

of Nostocales, including the most abundant representation of *Dolichospermum planctonicum* or *Aphanizomenon gracile* in the cyanobacterial biomass. The development of *D. planctonicum* in 2010 was associated with *Aph. gracile*, a potential producer of neurotoxins and cylindrospermopsins, which reached the high biomass from 2.9 to 5.8 mg dm^{-3} (50-95% of cyanobacterial biomass). In the following year, *D. planctonicum* formed one high biomass peak (6.39 mg dm^{-3}, 86% of cyanobacterial biomass) in period II. The biomass of TCb ranged between 3.9 and 8.1 mg dm^{-3} after the water discharge (period III) and *Pl. agardhii* reached the highest biomass (2.3 and 6.6 mg dm^{-3}) (P_{K-W} = 0.0159) (Fig. 3, 4). In other periods, its biomass was very low, <0.1 mg dm^{-3} in I period or up to 1.68 mg dm^{-3} in period II.

Out of the 15 species of the potential microcystins' (MCs) producers, two of them dominated – *Pl. agardhii* and *D. planctonicum* – within the 2-year study (Table 3), contributing 20-93% to the cyanobacterial biomass. Other potential

Table 3

Correlations between the biomass of potentially toxigenic cyanobacteria, potential microcystin producers including the dominants and MCs concentrations including their several variants. In bold are marked significant correlations ($p < 0.05$) (Spearman rank coefficient).

	MCs	MC-RR	MC-LR	MC-LY	MC-LW	MC-LF	MC-WR	MC-LA
TCb	**0.80**	**0.53**	**0.62**	**0.47**	0.45	**0.67**	0.14	-0.07
TB_{MCprod}	**0.75**	**0.72**	**0.63**	0.28	0.44	0.45	0.14	-0.07
$TB_{Pl.\ ag.}$	**0.81**	**0.87**	**0.57**	0.20	**0.50**	0.45	0.27	-0.14
$TB_{Dol.}$	0.33	-.012	0.41	**0.46**	0.20	**0.46**	-0.42	0.38

TCb - biomass of potentially toxigenic cyanobacteria, TB_{MCprod} - biomass of potential microcystin producers, $TB_{Dol.}$ - biomass of *Dolichospermum planctonicum*, $TB_{Pl.\ ag.}$ - biomass of *Planktothrix agardhii*

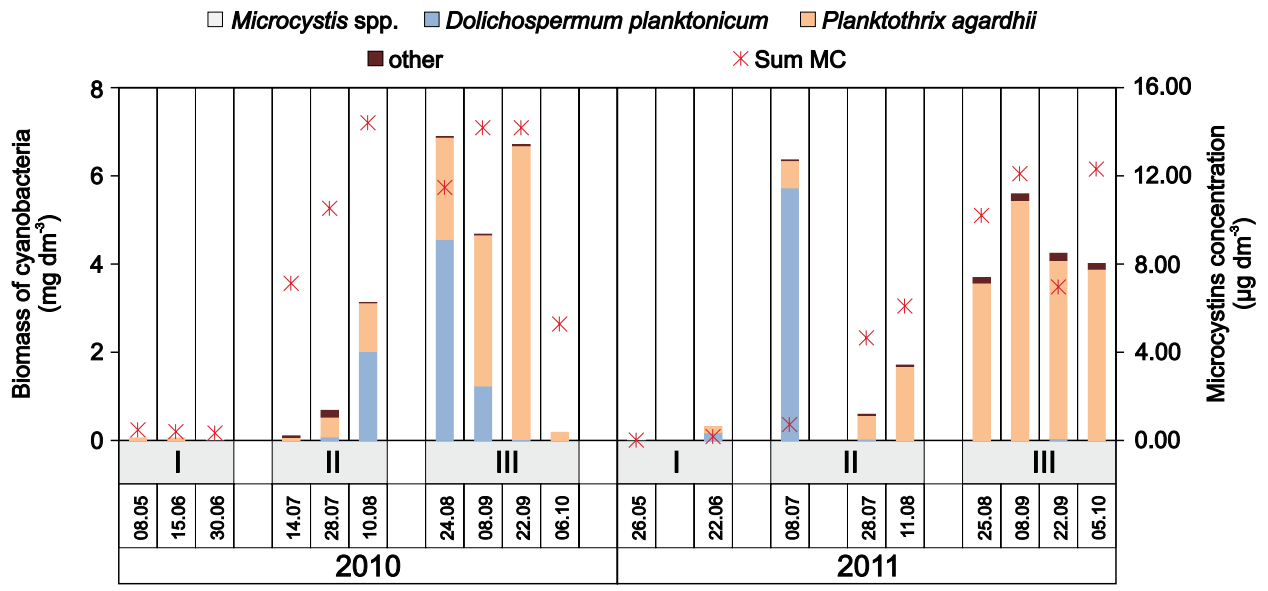

Figure 4

Variability in the contribution of potential MCs-producers in the cyanobacterial biomass and the concentrations of microcystin over the three hydrological periods

producers of microcystins were less abundant; their biomass did not exceed 0.5 mg dm^{-3} (Fig. 4). Throughout the study period, the intracellular MCs concentrations in water varied from an undetectable level to 14.43 µg dm^{-3} (Fig. 4). In period I, three variants of MCs (-LF > -LA > -RR) were detected in the cyanobacterial biomass and their combined concentrations were less than 1 µg dm^{-3} (Fig. 5). As a result of the development of MCs-producing species in periods II and III, the MCs concentrations significantly increased (P_{K-W} = 0.044), being mostly greater than 5 µg dm^{-3}. In addition, the higher number of MCs variants (-LF > -RR > -LR > -LY > -LW > -LA > -WR) were found. During the same period, the extracellular MCs concentrations ranged from 0.71 to 1.74 µg dm^{-3} (Pawlik-Skowrońska and

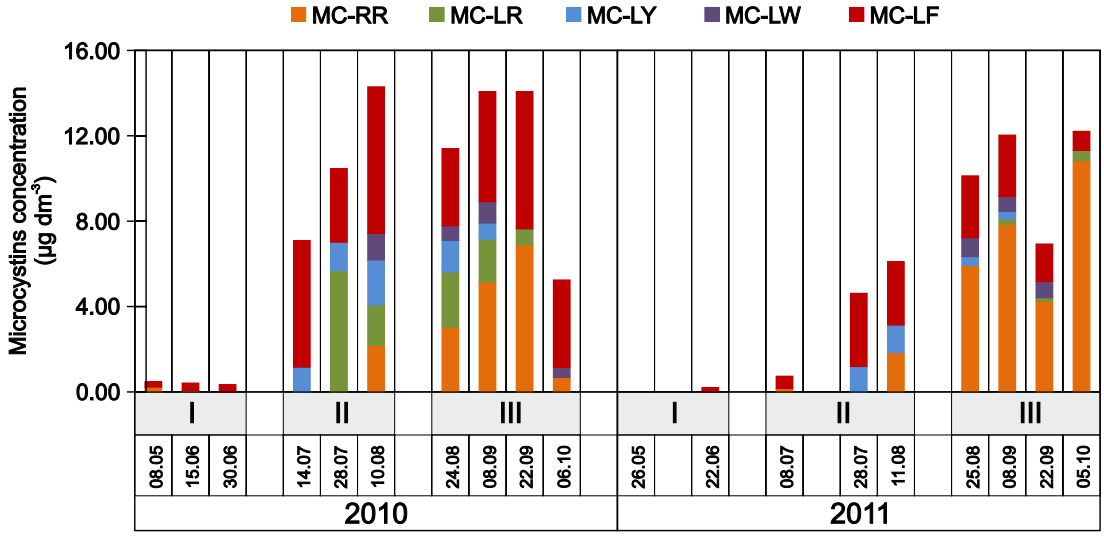

Figure 5

Changes in concentrations of the main detected MCs variants during the three hydrological periods

Kalinowska, unpublished data). The MC-RR was produced in the highest amounts (3.04-10.93 μg dm^{-3}) during the mass development of *Pl. agardhii* in III period. In 2010, when *D. planctonicum* had the highest biomass, high concentrations of MC-LF (3.47-6.93 μg dm^{-3}) and -LR (1.91-5.74 μg dm^{-3}) were found. The concentrations of other MC variants were always below 2 μg dm^{-3}.

The total MCs concentration positively correlated with the total biomass of 18 toxigenic cyanobacteria and *Pl. agardhii* (Tables 2, 3). Considering the relationships with several MCs variants, the biomass of *Pl. agardhii* positively and significantly correlated with -RR, -LR, -LW, whereas *D. planctonicum* biomass correlated significantly with -LF, -LY (Table 3).

The correlation analysis with environmental parameters revealed that the total biomass of toxigenic cyanobacteria, the potential producers of microcystins and *Pl. agardhii* are all significantly correlated with the light intensity (negative correlations with I_{mix}) and TP concentrations (positive correlations) (Table 4). The biomass of Nostocales, which is mostly represented by *Dolichospermum planctonicum* and *Aphanizomenon gracile*, was positively correlated with the NH_4-N concentrations. Considering the relationships with several MCs variants, the -RR, -LR and -LY were negatively correlated with the I_{mix}, while the -RR, -LW and -LF were positively correlated with the TP. Two variants, -LY and -LF, were correlated with ammonia concentrations (positive correlations) (Table 4).

Discussion

Cyanobacterial blooms are a frequent phenomenon in artificial water bodies such as dam reservoirs in which the inflow of fertile river waters enhances the process of eutrophication (Grabowska & Mazur-Marzec 2011, Tang et al. 2012). The present study was performed in a transformed lake that was periodically supplied with fertile river water via a canal. The entrance of these waters affected the balance of phosphorus and nitrogen in the studied lake and it supported the development of phytoplankton (Solis 2012). Intensive blooms of potentially toxigenic cyanobacteria (mainly *Pl. agardhii*) were observed earlier in this lake (2004-2005), and other lakes, connected with the Wieprz-Krzna Canal system, although the blooms were formed mainly by *Microcystis aeruginosa*, *M. wesenbergii* and *M. viridis* (Solis et al. 2009).

The water level and volume changes in lakes and artificial reservoirs can affect the structure of cyanobacteria assemblages. Nöges et al. (2003) reported that natural fluctuations of water levels caused the dominant *Planktolyngbya limnetica* (at the lower level) to replace *Limnothrix redekei/L. planctonica* (at the higher level) in the shallow

Table 4

Correlations between biomass of potentially toxigenic cyanobacteria, potential microcystin producers, including the dominants, MCs concentrations including their several variants, and environmental parameters. In bold are marked significant correlations ($p < 0.05$) (Spearman rank coefficient).

	T	I_{mix}	TP	PO_4-P	TN	NO_3-N	NH_4-N
TCB	0.18	**-0.57**	**0.49**	0.12	0.25	-0.03	0.18
TB_{MCprod}	-0.13	**-0.64**	**0.56**	0.23	0.27	-0.15	0.16
$TB_{Nost.}$	0.40	-0.16	0.09	0.28	-0.08	-0.21	**0.50**
$TB_{Pl.\ ag.}$	-0.35	**-0.73**	**0.69**	0.19	0.33	-0.02	0.05
MCs	-0.14	**-0.78**	**0.60**	0.00	0.24	0.11	0.25
MC-RR	-0.44	**-0.59**	**0.57**	0.02	0.25	-0.10	0.09
MC-LR	-0.09	**-0.80**	0.40	-0.21	0.02	0.22	0.17
MC-LY	0.43	**-0.50**	0.45	0.07	0.41	0.10	**0.48**
MC-LW	-0.06	-0.45	**0.73**	0.20	0.27	-0.23	0.34
MC-LF	0.03	**-0.52**	**0.52**	0.06	-0.02	0.09	**0.47**
MC-WR	-0.55	-0.41	-0.27	-0.41	-0.14	0.41	-0.55
MC-LA	0.56	-0.05	0.10	0.08	-0.14	0.15	0.01

T - temperature, I_{mix} - light intensity (PAR) in the mixed column, TCB - biomass of potentially toxigenic cyanobacteria, TB_{MCprod} - biomass of potential microcystin producers, $TB_{Nost.}$ - biomass of Nostocales, $TB_{Pl.\ ag.}$ - biomass of *Planktothrix agardhii*

eutrophic Võrtsjärv lake (Estonia). The intensive bloom of *Microcystis* spp. in the artificial, deep reservoir Arancio (Italy) was associated with a sudden decrease in the water level due to the abstraction of water (Naselli-Flores & Barone 2005). In the studied lake, the water level manipulation supported the development of Nostocales (mainly *Aph. gracile* and *D. planctonicum*) and Oscillatoriales (mainly *Pl. agardhii*). The mass development of the toxigenic *Aphanizomenon gracile* (Kokociński et al. 2013) and the microcystin-producing *Dolichospermum planctonicum* (Pawlik-Skowrońska et al. 2013) occurred after an increase in NH_4-N and PO_4-P concentrations in the lake's water due to the introduction of fertile water from the canal. The larger growth of the Nostocales population in 2010 could be a result of the higher ammonia load that entered from the canal (21.9 kg in 2010 and 11.6 kg in 2011). Experimental studies revealed (Chaffin & Bridgeman 2014) that Nostocales have the higher assimilation rate for NH_4^+ and prefer this ion over NO_3^- (Jacoby et al. 2000). *Pl. agardhii* was the most abundant cyanobacterium when the water was discharged from the lake and its level decreased. The higher biomass of *Pl. agardhii* was significantly correlated with the low light intensity and the high total phosphorus concentrations. The low-light adapted *Pl. agardhii* (Kokociński et al. 2012) is frequently associated with turbid waters. It commonly occurs in polymictic, wind-exposed lakes and reservoirs (Wiśniewska et al. 2007, Pawlik-Skowrońska & Toporowska 2011) that have intensive sediment/water interactions and short-term nutrient pulses. Released phosphates from the bottom sediment are efficiently taken up by *Pl. agardhii* and incorporated into its biomass (Nixdorf et al. 2003). The supply of phosphates from the bottom of the studied lake could be enhanced by water discharge followed by a decrease in the water level. Nagid et. al. (2001) reported that even a small decrease in the water level fluctuations (16 cm) increased the probability of sediment resuspension in a shallow waterbody.

The water temperature, which is commonly attributed as an important factor stimulating the cyanobacteria growth (Wagner & Adrian 2009, Carey et al. 2012), was not significantly correlated with cyanobacterial biomass in the studied lake. It suggests that the range of temperature (19-26°C) was optimal for cyanobacteria development throughout the study period.

Our study revealed that 15 species that occur in the studied lake could potentially produce microcystins (Carmichael 2001). The two species, *Pl. agardhii* and *Dolichospermum planctonicum*, which conspicuously dominated, have been reported as dominants in some toxic water blooms (O'Neil et al. 2012, Pawlik-Skowrońska et al. 2013, Grabowska et al. 2014). The mass development of toxigenic cyanobacteria was closely associated with the increase in concentrations of intracellular microcystins that were primarily produced in the periods when the water volume regulations were performed. The amount of MCs was typical of the range reported during blooms in other shallow lakes (Kobos et al. 2013) and dam reservoirs (Grabowska & Pawlik Skowrońska 2008, Pawlik-Skowrońska et al. 2013). It was also found that the number of detected MCs variants in the studied lake (-RR, -WR, -LA, -LR, -LY, -LW, -LF) was much higher than in several shallow Pomeranian lakes (-LR, -RR, -YR, -LA) with blooms of *Pl. agardhii* (Mankiewicz et al. 2005) and in the shallow highly eutrophic Taihu lake in China (- LR, RR, YR), with intensive blooms of *Microcystis aeruginosa* (Liu et al. 2008). However, in a shallow, highly eutrophic dam reservoir with toxic, mixed blooms formed by planktic species of *Anabeana* (syn. *Dolichospermum*), *Aphanizomenon* spp., *Microcystis* spp. and *Pl. agardhii*, the number of detected variants was similar (-RR, -YR, -LR, -WR, -LA, -LY, -LW, -LF) (Pawlik-Skowrońska et al. 2013).

In the studied lake, the variation in microcystin concentrations and two their variants (-RR and –LR) was mostly associated with the variation of *Planktothrix agardhii* biomass, although *Pl. agardhii* can produce up to 25 isoforms of microcystins (Mbedi et al. 2005) and one strain can even produce seven isoforms (Tonk et al. 2005). Studies of *Pl. agardhii* toxic strains revealed that -RR, -LR and -YR are usually the principal variants in extracts of its biomass (Yéprémian et al. 2007); however, -LR occurs in much lower amounts than -RR (Luukkainen et al. 1993). Different populations of *Pl. agardhii* are known to produce also other MC variants, which can be identified only by more advanced techniques LC-MS/MS (Grabowska et al. 2014). The content of -RR and -LR in the biomass can be regulated by light intensity. The amount of

the more toxic variant -LR is greater when the light intensity increases (Tonk et al. 2005). The biomass of *Dolichospermum planctonicum* correlated (strongly or weakly) with the MC-LF, -LY, -LR and -LA variants. Strains of planktic *Anabeana* spp. may produce three to seven different microcystins (Sivonen et al. 1992). The microcystins -LA, -LY, -LF were most abundant during *Anabeana planctonica/affinis* blooms in a shallow dam reservoir (Pawlik-Skowrońska et al. 2013), however, the -RR and -LR variants were detected in extracts of planktic *Anabeana* sp. biomass by Meriluoto et al. (2004).

In this study, the same environmental factors influenced the variability of *Pl. agardhii* biomass and the variability of microcystin amounts. Their intracellular concentrations tended to increase when the light intensity decreased and the TP concentrations increased. However, this potential impact on MCs production appears to be indirect and a result of *Pl. agardhii* proliferation. As suggested by Orr & Jones (1988), the rate of cell division most often determines the production of microcystins providing an explanation for the impact of many environmental factors. The variation in the cell density of *Pl. agardhii* is the most important factor that may regulate the frequency of strains with the mcyA gene, which is involved in the microcystin production (Briand et al. 2008).

However, several studies revealed that the light might be an important factor regulating the microcystin content in the cyanobacterial biomass. In the field research conducted in a shallow lake, the population of *Pl. agardhii* occurring in the illuminated surface water contained double the amount of microcystins compared to the population with a similar abundance that occurred near the lake bottom (Pawlik-Skowrońska et al. 2008). Experimental studies revealed that the production of microcystins by *Microcystis* might increase due to an increase in light intensity (LeBlanc Renaud et al. 2011). In turn, phosphates could influence, to lesser extent, the proportion of toxic and non-toxic strains of *Pl. agardhii*; this impact is rather indirect, however, being a result of the population growth (Briand et al. 2008). By contrast, the production of microcystins is a process involving a large energy demand and, therefore, a large amounts of phosphorus is required (Vézie et al. 2002).

In general, the amounts of microcystins detected in the summer period (average 10.24 μg dm^{-3}) were typical of the range indicating a moderate health risk (WHO 2003). It may be a risk for people who use contaminated water for recreational purposes. Furthermore, toxins can often accumulate in consumable fish tissues (Pawlik-Skowrońska et al. 2013) or in plants irrigated with water contaminated by toxins (Peuthert et al. 2007).

Our results indicate that due to the water level manipulation in shallow lakes, various cyanobacterial species of different toxigenic potential may develop and create very harmful conditions for aquatic biocenoses and users of water.

Conclusions

The mass development of particular species of toxigenic cyanobacteria and, as a consequence, the microcystin production can be strongly prompted by a periodical manipulation of water levels, even in a small range, in waterbodies transformed by man. Both the supply of fertile waters via a canal and water discharged from lakes could support the development of cyanobacteria producing hepatotoxins, and consequently, an increase in the amount and diversity of toxins in water may occur. The proper management of water and cyanobacterial monitoring is particularly necessary in modified lakes.

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