# Oceanological and Hydrobiological Studies

International Journal of Oceanography and Hydrobiology

Volume 40, Issue 4

ISSN 1730-413X	(30-37)	~
eISSN 1897-3191	2011	VERSITA

DOI: 10.2478/s13545-011-0038-z Original research paper

Blooms of toxin-producing Cyanobacteria – a real threat in small dam reservoirs at the beginning of their operation

Barbara Pawlik-Skowrońska<sup>1,2</sup>, Magdalena Toporowska<sup>1</sup>

<sup>1</sup>Department of Hydrobiology, University of Life Sciences in Lublin, ul. Akademicka 13, 20-950 Lublin, Poland<sup>2</sup> <sup>2</sup>Polish Academy of Sciences, Centre for Ecological Research in Dziekanów Leśny, Experimental Station, ul. Niecała 18, 20-080 Lublin, Poland

Key words: anatoxin-a, microcystins, dam reservoir, Microcystis, Planktothrix, Anabaena

#### Abstract

Large and harmful cyanobacterial blooms appeared in two newly-built artificial reservoirs shortly after being filled with water. Taxonomic composition of cyanobacterial communities was highly variable in both water bodies and fast species replacement was observed. In the first year of the operation of the smaller Konstantynów Reservoir, the mass development of *Anabaena flos-aquae* and *Planktolyngbya limnetica* (48.7 and 53.6% of the cyanobacterial abundance) occurred in summer, while in autumn the dominance of *Planktothrix agardhii* (99.9%, 14.95 × 10<sup>6</sup> ind. dm<sup>-3</sup>) was noted. The surface scum developed in summer consisted of *An. flos-aquae* that contained high amounts of anatoxin-*a* (1412.4 µg AN-a dm<sup>-3</sup> of scum) and smaller amounts of microcystins (10 µg eq. MC-LR dm<sup>-3</sup> of scum). In the larger Kraśnik Reservoir, *Aphanizomenon flos-aquae* occurred in high abundance in spring and summer, however, it was replaced *Received: Accepted:* 

February 10, 2011 September 22, 2011

by different species of *Microcystis*  $(1.3 \times 10^7 \text{ ind. dm}^{-3})$  which created thick surface scum. Simultaneously, a hazardous increase in the total concentration of microcystins (from 13.6 to 788.5 µg eq. MC-LR dm<sup>-3</sup> of water with scum) and anatoxin-*a* (from 0.03 to 43.6 µg dm<sup>-3</sup>) was observed.

## INTRODUCTION

In shallow eutrophic lakes and dam reservoirs, toxin-producing species of cyanobacteria often create water blooms and become an increasing problem in many countries (Sivonen et al. 1990, Dokulil and Teubner 2000, Pawlik-Skowrońska et al. 2004, Tonk et al. 2005, Kurmaver and Christiansen 2009, Toporowska et al. 2010). Mass development of toxigenic Cyanobacteria may be a real hazard in water bodies used as drinking water supplies and for recreational or fishery purposes (Malbrouck and Kestemont 2006, Palus et al. 2007, Grabowska and Pawlik-Skowrońska 2008). Hepatotoxins, neurotoxins and other secondary metabolites produced by cyanobacteria, are dangerous for humans, domestic animals and wildlife (Carmichael 1992, Mazur-Marzec 2006, Welker and von Dohren 2006). Hepatotoxic microcystins (MC), which occur in about 80 different isoforms, inhibit the eukaryotic enzyme protein phosphatase 1 and 2A. Among the former, microcystin-LR (MC-LR) is the most common and toxic one. Neurotoxic anatoxin-a (ANpotent a) is а post-synaptic depolarizing neuromuscular blocking agent (Carmichael 1992, Van Apeldoorn et al. 2007). Although there are several potential exposure routes for cyanotoxins (oral consumption, inhalation, or skin absorption), the most common is the ingestion of water by drinking and/or by an accidental recreational intake (Kabziński and Kabziński 2006). The World Health Organization established a provisional guideline limiting MC-LR to 1 µg dm-3 in drinking water (Chorus and Bartram 1999) and to 10 µg dm-3 in

<sup>1</sup> Corresponding author: pawlik@poczta.umcs.lublin.pl

Copyright © of Institute of Oceanography, University of Gdansk, Poland www.o andhs.org

recreational waters (WHO, 2008). The anatoxin-a database is limited in quantitative and qualitative studies on effects following the oral exposure to sublethal levels, however, neurotoxicity and death were observed in acute, short-term and sub-chronic oral animal studies (USEPA, 2006). Anatoxin-a halflife in natural blooms in eutrophic lakes was estimated at about 24 hours while under laboratory conditions – at about five days, therefore 450 µg ANa dm-3 was recommended by the Washington State Department of Health (USA) as a provisional shortterm recreational guideline value and 75 µg dm-3 as a sub-chronic one (Hardy 2008). According to the Directive of UE 2006/7/WE, assessment of recreational water quality should be based on the abundance of bloom-forming cyanobacteria capable of toxin production. Recently, a tendency for construction of small, water retention reservoirs on rivers has been increasing in Poland.

The aim of this paper was to study the structure and development dynamics of cyanobacteria communities, as well as threats posed by their toxins in newly-built, multipurpose dam reservoirs.

# MATERIALS AND METHODS

## Study area

Samples of surface water (0 - 0.5 m) were collected (August – October, 2007) near the shore (3 - 4 m) of the small, lowland Reservoir Konstantynów (2 ha, mean depth 1.9 m, max. depth 2.9 m) located (N 52° 12', E 23° 5') on the Czyżówka

River, and in May - September, 2008, 4-5 m far from the shore of the larger, upland Reservoir Kraśnik (39.1 ha; mean depth 2.5 m, max. depth 6 m) located (N 50° 56', E 22° 11') on the Wyżnica River (both in the Lublin province, Eastern Poland). The Konstantynów Reservoir was built in countryside and it was filled with water in May, 2007. The reservoir is mainly used for retention and fishing purposes. Its agricultural catchment area has no sewage system on most of the area. The Kraśnik Reservoir, located at the border of Kraśnik city, was filled with water in 2006 and since then it has been affected by mass development of cyanobacteria. It is used for retention and recreational (swimming, angling) purposes. The reservoir is enriched with nutrients contained in water of the Wyżnica River and its catchment area.

# Physicochemical and biological analyses

Physicochemical and biological characteristics of water from both reservoirs are presented in Table 1. Biogenic nutrients were determined according to Golterman (1971) and chlorophyll-a – according to PN-ISO 10260 (2000). The Carlson Trophic Status Index (TSI) based on chlorophyll-a and SD values was also calculated (Carlson 1977). The samples of water (VIII b) with thick scum (Kraśnik R.) were not taken into account for the TSI calculation. The quantitative qualitative and structure of phytoplankton communities was analyzed by means of an inverted microscope (Utermöhl 1958), and

				Phys	ic-chemical parar	neters							
Parameters		Konstantynów R. Kraśnik R.											
Parameters		Months											
	VIII <sup>a</sup>	VIII <sup>b</sup>		IX	X	V		VII					
Temperature (°C)	19.8	21.5		17.0	10.0	nd.		20.7	15.9				
pH	7.6	7.6		7.4	6.7	7.7		8.1					
Conductivity (µS cm <sup>-1</sup> )	398	372		479	562	421		nd.					
Transparency - SD (m)	0.33	0.30		0.41	0.55	1.73		0.65					
Oxygen conc. (mg dm <sup>-3</sup> )	9.9	nd.		8.6	9.0	nd.	nd.		6.9				
$P-PO_4$ (mg dm <sup>-3</sup> )	nd.	nd.		0.073	0.133	0.059		0.085	0.090				
N-NH <sub>4</sub> (mg dm <sup>-3</sup> )	nd.	nd.		0.106	0.053	0.301	0.301		0.043				
N-NO <sub>3</sub> (mg dm <sup>-3</sup> )	nd.	nd.		nd.	nd.	0.076		0.069	0.786				
				В	iological paramet	ers							
		Konstantynów R. Kraśnik R.											
		Months											
	VIII <sup>a</sup>	VIII <sup>b</sup>	IX	X	V	VII	VIII <sup>a</sup>	VIII <sup>b,c</sup>	IX				
Chlorophyll-a (µg dm <sup>-3</sup> )	140.4	212.6	122.8	69.9	9.7	63.2	132.8	1600.0	29.9				
Trophic status		TSI =	= 78		TSI = 68								
Phytoplankt. biomass (mg dm <sup>-3</sup> )	35.6	66.8	37.4	26.1	4.9	16.3	21.1	273.0	10.5				
Total MCs (μg equiv. MC-LR dm <sup>-3</sup> )	10.0 <sup>c</sup>	nd.	nd.	nd.	nd.	nd.	13.6	788.5	nd.				
AN-a (µg dm <sup>-3</sup> )	1412 <sup>c</sup>	nd.	nd.	nd.	nd.	nd.	0.03	43.6	nd.				

taxonomical identification was based on Van den Hoek et al. (1995) and Komárek and Anagnostidis (1999/2000, 2005). For all Oscillatoriales and Nostocales with straight filaments 100 µm was set as one individual. One curve of curved *Anabaena* spp. and one colony of *Microcystis* spp. were recognized as individuals. Phytoplankton biomass was estimated by cell volume measurement (Kawecka and Eloranta 1994).

# Analyses of cyanotoxins

For determination of cyanotoxin concentrations, surface water (0.5 - 1.0 dm-3) containing cvanobacterial biomass was concentrated on Whatman GF/C filters to 1 - 1.5 cm<sup>-3</sup> and extracts of the biomass were prepared in 75% (v/v) methanol (Merck, pure p.a.) using ultrasonication (3 times for 5 min., 50W, ultrasonic homogenizer Sonoplus, Bandelin).

Total concentration of microcystins (MC) in determined extracts was using gas chromatography/mass spectrometry (GC/MS, Varian) according to Kaya and Sano (1999) and the modified procedure described by Pawlik-Skowrońska et al. (2008). The method is based on oxidation of Adda (a specific amino acid present in MCs) to MMPB (2-methyl-3-methoxy-4-phenylbutyric acid) and determination of MMPB as a methyl ester. The oxidation was carried out for 4 hours with 99.8% NaIO<sub>4</sub> and 0.024 M KMNO<sub>4</sub>. For derivatization, 14% BF<sub>3</sub> -methanol was used. As a modification, phenylbutyric acid (PB) was used as an internal standard step after oxidation. Derivatized samples were dissolved in n-hexane and subjected to GC/MS analysis (Saturn 2000, Varian). In EI-MS mode, the

identification and quantification of MMPB methyl ester was based on ions at m/z 91, 131 and 190; for PB methyl ester 91, 104 and 146 m/z were used. The identification of MMPB and PB was confirmed by CI-MS at m/z 191 and 147, respectively. Total microcystin concentrations were expressed as equivalents of MC-LR, which was used as a standard (MC-LR, Alexis)

Anatoxin-*a* (AN-*a*) in extracts was determined using liquid chromatography (HPLC, Beckman) with fluorescence detection (Shimadzu) according to James et al. (1998) and Furey et al. (2005). For AN-*a* derivatization 10% NBD-F (4-fluoro-7nitrobenzofuran; Fluka) was used. The detector parameters were as follows: excitation wavelength 470 nm, emission wavelength 530 nm. For identification and quantitative determinations, the standard AN-*a* (Tocris, Bioscence) was used.

# RESULTS

The mass development of cyanobacteria in both reservoirs was observed shortly after being filled with water. Hydro-morphological and chemical parameters (Table 1) supported this negative phenomenon. Low water transparency in summer (0.30 - 0.55 m and 0.40 - 0.65 m in Konstantynów and Kraśnik, respectively) and high chlorophyll-a concentrations were a consequence of mass development of phytoplankton. Also the total phytoplankton biomass was very high (>20 mg dm-3) in the surface water of both reservoirs (Table 1). In terms of species richness, cyanobacteria (after Chlorophyceae and Bacillariophyceae) were the third most important components of phytoplankton (Fig. 1). Both the number of cyanobacteria taxa and their



Fig. 1. The contribution of the number of taxa (%) from particular taxonomic groups in phytoplankton recorded in the reservoirs of Konstantynów (A) and Kraśnik (B). The total number of phytoplankton taxa was set as 100%. Parentheses shows number of taxa from particular taxonomic groups.



percentage contribution in the total number of phytoplankton taxa were similar (Fig. 1), however, different cyanobacteria taxa dominated quantitatively in different study periods (Table 2). In total, 18 taxa of cyanobacteria from 11 genera were found in the Konstantynów Reservoir. Fourteen of them (Table 2) were potential toxin producers and 11 were bloomforming species. In the Kraśnik Reservoir 19 taxa of cvanobacteria from 11 genera were identified. Twelve of them were potential toxin producers (Table 2) and 10 were able to create water blooms. In the Konstantynów Reservoir the total abundance of particular taxonomic groups of cyanobacteria ranged within 6.59 - 19.7 ind.  $\times 10^{6}$  dm<sup>-3</sup> (Fig. 2A), and their biomass ranged within  $22.0 - 29.9 \text{ mg dm}^{-3}$  (Fig. 3A) accounting for 45 - 62% of the total phytoplankton biomass. In both reservoirs, the species composition of bloom-forming cyanobacteria communities varied significantly during the study periods (Table 2). At the beginning of August (Figs 2A, 3A) mass development Nostocales of (52.4%) of the cyanobacteria abundance and 94.5% of the cyanobacteria biomass) with the dominant filamentous Anabaena flos-aquae Bréb. ex Born. et Flah. (48.7% of the cyanobacteria abundance and 89.9% of the cyanobacterial biomass) was observed in Konstantynów R. Two weeks later Oscillatoriales (Planktolyngbya limnetica (Lemm.) Kom.-Legn. et Cronberg) dominated quantitatively (10.6 ind.  $\times$  10<sup>6</sup> dm-3; 54.0%), though the abundance of An. flos-aquae was still high (2.6 ind.  $\times$  10<sup>6</sup> dm<sup>-3</sup>; 7.3%) and this



Fig. 2. Comparison of the total abundance of Chroococcales, Nostocales and Oscillatoriales in the studied reservoirs: A – Konstantynów (a – the beginning of August, b – the middle of August), B – Kraśnik. \* - abundance in water with *Microcystis* scum.

species dominated in the biomass of blue-green algae (20.3 mg dm<sup>-3</sup> and 67% of cyanobacteria biomass; Fig. 3A, Table 3). In early autumn, a strong increase in the density of *Planktothrix agardhii* (Gom.) Anagn. et Kom. (Oscillatoriales) occurred (Fig. 2A, 3A,

## Table 2

The list of bloom-forming ( $\mathbf{\nabla}$ ) and potential toxin-producing (\*) taxa of Cyanobacteria in the phytoplankton of the reservoirs and their contribution (%) in the total abundance of phytoplankton.

	Konstantynów R.					Kraśnik R.					
Таха	Month										
	VIII <sup>a</sup>	VIII <sup>b</sup>	IX	Х	V	VII	VIII <sup>a</sup>	VIII <sup>b</sup>	IX		
Anabaena flos-aquae (Lyngb.) Bréb. ex Born. et Flah. *▼	++++	++	+		+						
An. lemmermannii Richt. * 🔻	+		+								
An. planctonica Brunnth.*		+	+								
An. solitaria Kleb.* 🔻	++	++				+					
An. spiroides Kleb. * 🔻	+	+	+		+						
Aphanizomenon flos-aquae (L.) Ralfs ex Born. et Flah. *▼					+++	+++++	++++	+			
Aph. gracile (Lemm.) Lemm. * 🔻			+								
Aph. Issatschenkoi (Usač.) ProškLavr.*	+	+	+								
Limnothrix redekei Van Goor 🔻			+								
Lyngbya sp. *			+								
Microcystis aeruginosa (Kütz.) Kütz. * 🔻		+	+			+	++++	+++++	+		
M. flos-aquae (Wittr.) Kirch. *▼		+				+	+++	++++	+		
M. wesenbergii (Kom.) Kom.in Kondr.*▼						+	+	++	+		
M. viridis (A. Br. in Rabenh.) Lemm. *▼						+	+		+		
Oscillatoria sp. *			+								
Planktolyngbya sp.*					+						
P. limnetica (Lemm.) KomLegn. et Cronb. *▼	++	++++	+++	+	+	+			+		
Planktothrix agardhii (Gom.) Anagn. et Kom. *▼		++	++	+++++		+	+		+		
Snowella lacustris (Chod.) Kom. et Hind.*	+	+							+		
Woronichinia sp. * 🔻	++		+								
+ - (< 1%); ++ - (1 - 10%); +++ - (10 - 25%); ++++ - (25 - 50%); +++++ - (>50%)											





**Fig. 3.** Comparison of the total biomass of Chroococcales, Nostocales and Oscillatoriales in the studied reservoirs: A – Konstantynów (a – the beginning of August, b – the middle of August), B – Kraśnik. In September the biomass <0.08 mg dm<sup>-3</sup>; \* - biomass in water with *Microcystis* scum.

## Table 3

Abundance and biomass of the dominant, bloom-forming species of Cyanobacteria.

Abundance (×10 <sup>6</sup> ind. dm <sup>-3</sup> )										
	К	onstan	tynów	Kraśnik R.						
Таха				Month	ths					
	VIII <sup>a</sup>	VIII <sup>b</sup>	IX	Х	VII	VIII <sup>a</sup>	VIII <sup>b,c</sup>			
An. flos-aquae	3.21	2.60	1.02	—	—	—	—			
Aph. flos-aquae	—	—	—	—	9.66	6.00	0.03			
Microcystis spp.	—	—	—	—	0.01	0.20	13.00			
Pl. limnetica	0.91	10.58	5.22	0.92	—	—	—			
P. agardhii	0.27	3.32	3.43	14.95	—	—	—			
Biomass (mg dm <sup>-3</sup> )										
	Konstantynów R. Kraśnik R.									
Таха	Months									
	VIII <sup>a</sup>	VIII <sup>b</sup>	IX	Х	VII	VIII <sup>a</sup>	VIII <sup>b,c</sup>			
An. flos-aquae	25.05	20.33	7.94	—	—	—	—			
Aph. flos-aquae	—	—	—	—	11.19	12.91	0.72			
Microcystis spp.	—	—	—	—	0.01	7.80	272.80			
Pl. limnetica	0.03	1.08	0.78	0.12	—	—	—			
P. agardhii	0.28	4.45	4.51	19.63	—	—	—			
<sup>a</sup> – beginning of August, <sup>b</sup> – middle of August, <sup>c</sup> – per dm <sup>3</sup> of water with cyanobacterial scum										

Table 3), and its dominance  $(14.95 \times 10^6 \text{ ind. dm}^{-3}; 99.9\% \text{ of the cyanobacterial abundance and 19.6 mg dm}^{-3}; 99.99\% \text{ of the cyanobacterial biomass}) was observed even at low water temperature.$ 

Copyright© of Institute of Oceanography, University of Gdansk, Poland www.oandhs.org

Cvanobacterial scum, which appeared on the water surface at the beginning of August, consisted mainly of An. flos-aquae and contained high amounts of cvanotoxins i.e. 1412.4 µg of anatoxin-a dm-3 and 10.0 µg eq. MC-LR dm-3 of water with scum (Table 1). In the Kraśnik Reservoir other species of cyanobacteria (Nostocales and Chroococcales) created a large water bloom (Table 2). In summer months, the total abundance of particular taxonomic groups of Cyanobacteria in the surface water ranged within  $6.2 - 13.0 \times 10^6$  ind. dm<sup>-3</sup> (Fig. 2B), while the biomass - within 11.2 - 272.2 mg dm-3 (Fig. 3B) due to a big change in the species composition. In spring and the beginning of summer (Fig. 2B, 3B) the bundle-forming cyanobacterium Aphanizomenon flosaquae (L.) Ralfs ex Born. et Flah. (Nostocales) dominated. Heavy water blooming of this species  $(9.67 \times 10^6 \text{ ind. dm}^{-3}; 82\% \text{ of the cyanobacterial})$ abundance, 99% of the biomass) occurred in July and also at the beginning of August (Tables 2, 3), whereas about 3 weeks later, Chroococcales comprising different species of Microcystis (Microcystis aeruginosa, M. flos-aquae, M. wesenbergii and M. viridis) replaced Aph. flos-aquae and other cvanobacteria. Only during 19 days of August, a strong increase in the total abundance of *Microcystis* spp. occurred – from 0.2.  $\times$  $10^6$  to  $13 \times 10^6$  colonies per dm<sup>-3</sup> (Tables 2, 3). The biomass of Microcystis spp. in the surface water layer covered with scum increased within this period about 35 times. At the same time, an increase in the total concentration of microcystins (from 13.6 to 788.5 µg eq. MC-LR dm<sup>-3</sup>) and anatoxin-a (from 0.03 to 43.6 µg dm-3) was recorded in the water containing the scum (Table 1). The heavy water blooming, formed by *Microcystis* spp., disappeared already in the early autumn when the water temperature decreased to about 15°C.

## DISCUSSION

Intense toxic cyanobacterial blooms and their consequences are the most detrimental effects of eutrophication in various water bodies (Paerl 1988, Smith 2003, Burchardt and Pawlik-Skowrońska 2005). In the two newly-built, water reservoirs located on small rivers, water blooms caused by different species of cyanobacteria appeared shortly after filling with water (also in Kraśnik R. already in summer 2006, Pawlik-Skowrońska, personal communication). The values of the trophic status index (TSI = 68 and 78), estimated according to Carlson (1977), showed that both dam reservoirs are



highly eutrophicated. Their shallowness enabled a rapid increase in the water temperature in spring/summer, supporting thus the mass development of different cyanobacteria species, which seriously reduced the water quality by lowering the water transparency and formation of toxincontaining cyanobacterial scum on the water surface. Interestingly, in spite of the similar reservoir depth and high trophic status, the qualitative and quantitative structures of cyanobacteria communities in the studied reservoirs were completely different. Similar, high species variability of bloom-forming communities and species succession were also stated in other artificial eutrophic water reservoirs in Poland (Pawlik-Skowrońska et al. 2004, Grabowska and Pawlik-Skowrońska 2008). In both studied reservoirs, N2-fixing filamentous Nostocales Anabaena flos-aquae and Aphanizomenon flos-aquae created water blooms first, being competitive with both Oscillatoriales and Chroococcales, which are dependent on other nitrogen sources (Dokulil and Teubner 2000, Wilkand Mazurkiewicz-Boroń 2003). The Woźniak observed replacement of Nostocales by Chrooococcales (in the larger Kraśnik Reservoir) or by Oscillatorialles (in the smaller Konstantynów Reservoir) might be caused by an increase in supplies of other forms of nutrients and/or a change in temperature, as well as light and water mixing conditions during mass proliferation of Cyanobacteria (Paerl 1996). Interestingly, no mass development of Limnothrix redekei (Van Goor) Meffert, typical of other hypertrophic, polymictic reservoirs (Rücker et al. 1997, Wiśniewska et al. 2007) was observed. In the Konstantvnów Reservoir, the cold-adapted abundance of the Ρ. agardhii (Oscillatoriales) was similar to that found in the eutrophic dam reservoir Zemborzycki near Lublin (Pawlik-Skowrońska et al. 2004). However, at the same water temperature (10°C), it was four times lower than in a small hypertrophic lake (Wiśniewska et al. 2007). The dominance of P. agardhii observed in autumn, could result from a higher nutrient level in the studied reservoir. Probably, also the lower water temperature, the declining intensity of photosynthetically active radiation and stronger water mixing in the very shallow reservoir enhanced its development (Scheffer et al. 1997, Pawlik-Skowrońska et al. 2004, Toporowska et al. 2010). P. agardhii may develop even under the ice cover and create perennial blooms (Seip and Reynolds 1995, Polučkowa et al. 2004, Wiśniewska et al. 2007, Burchardt et al. 2009) dangerous for aquatic

ecosystems due to its capability of microcystin production (Tonk et al. 2005, Akcaalan et al. 2006, Pawlik-Skowrońska et al. 2008). For example, the replacement of Chroococcales and Nostocales by P. agardhii in water of a large dam reservoir in Eastern Poland resulted in an essential increase in microcystin concentrations (Grabowska and Pawlik-Skowrońska 2008). In the case of the Kraśnik Reservoir, an extremely high increase in the total levels of microcystins and anatoxin-a in the surface water laver was a consequence of strong development of Microcystis spp. and/or their sub-populations with higher capability of MC production. In large water bodies with poor vertical mixing (Jöhnk et al. 2008) and high temperatures (>20°C), the abundance of Microcystis can increase rapidly within a few days. Such a phenomenon was also observed earlier, in several large dam reservoirs in Poland (Wilk-Woźniak 1996, Grabowska 2005, Mankiewicz-Boczek et al. 2006). The upward floating of Microcystis supports the formation of dense cyanobacterial blooms at the water surface (Visser et al. 1996). It seems that in both reservoirs apart from high nutrient concentrations, also their size, water retention time and water mixing intensity could be essential factors determining the differences in species dominance of cyanobacteria.

In the Konstantynów Reservoir the bloomforming An. flos-aquae produced considerable amounts of AN-a, however, MCs were also detected. Apart from Anabaena spp, other species i.e. M. aeruginosa, M. flos-aquae, P.aghardii are known as MCproducers (Carmichael 1992, Kurmayer and Christiansen 2009). Also anatoxin-a producing Microcystis sp. was previously reported (Van Apeldoorn et al. 2007). It must be emphasized that within the same species of cyanobacteria their subpopulations of diverse capability of producing different isoforms and amounts of cyanotoxins develop under natural conditions (Kurmayer et al. 2004, Kardinaal et al. 2007). Coccoid Microcystis spp, filamentous Anabaena spp, Aph. flos-aquae, P. agardhii, and Pl. limnetica, which created water blooms in the reservoirs we studied, are the most common bloomforming cyanobacteria in freshwaters (Bucka and Wilk-Woźniak 2007, Pawlik-Skowrońska et al. 2004). The nineteen taxa of cyanobacteria found in both reservoirs during the study period make up almost half of about 40 species of freshwater blue-green algae, which are so far known as potential toxin producers (Burchardt and Pawlik-Skowrońska 2005). Their successive mass development may cause a



serious human and animal health risk (Dawson 1998, Orr et al. 2001, Osswald et al. 2007). Consumption of fish living in water reservoirs affected by cyanobacterial blooms is hazardous due to accumulation of cyanotoxins in edible fish muscles (Malbrouck and Kestemont 2006, Toporowska and Pawlik-Skowrońska 2009) and possible transfer to human bodies (Chen et al. 2009). The collapse of a cyanobacterial bloom causes a release of cyanotoxins directly to water and sediment, where some of them, especially microcystins, can persist for a long time (Pawlik-Skowrońska et al. 2010) and affect aquatic biocenoses and reservoir users.

Prevention of the excessive development of cyanobacteria in small dam reservoirs is extremely desirable, although difficult or even impossible in cases when they are filled with waters from nutrientpolluted rivers or when their catchment areas are not properly managed. In eu/hypertrophic water reservoirs, where nutrient limitation does not play any role, the population dynamics of different cyanobacteria species are driven by light availability, temperature and water mixing processes.

## **CONCLUSIONS**

Our studies showed that the mass development of toxin-producing Cyanobacteria in the newly-built dam reservoirs may appear shortly after the commencement of their operation and become a serious ecological and social problem. The species richness and composition of cyanobacterial communities in two small nutrient-rich water bodies were significantly different and highly variable within one season, with successive quantitative dominance of species producing both hepatotoxins and neurotoxins. The obtained results suggest that there is a strong need for environmental expertise concerning both the water quality in rivers and the management of their catchment areas before each decision has been taken on the construction of small dam reservoirs.

## REFERENCES

- Akcaalan R., Young F.M., Metcalf J.S., Morrison L.F., Albay M., Codd G.A., 2006, Microcystin analysis in single filaments of Planktothrix spp. in laboratory cultures and environmental blooms, Wat. Res., 40: 1583-90
- Bucka H., Wilk-Woźniak E., 2007, Pro- and eukaryotic algae in reservoirs of Southern Poland, Kraków, pp. 352 (in Polish)
- Burchardt L., Goździcka-Józefiak A., Messyasz B., Gąbka M., Dondajewska R., et al., 2009, The influence of temperature gradient and trophic status on phytoplankton structure of Lake Góreckie

Copyright© of Institute of Oceanography, University of Gdansk, Poland www.oandhs.org

(Wielkopolski National Park) during winter ice cover, In: Wielkopolski National Park in Natural Studies, Eds. Walna B., Kaczmarek L., Lorenc M., Dondajewska R., Poznań-Jeziory, pp. 11-26 (in Polish)

- Burchardt L., Pawlik-Skowrońska B., 2005, Blue-green algal blooms interspecific competition and environmental treat, Wiad. Bot., 49(1/2): 39-49 (in Polish)
- Carlson R.E., 1977, A trophic state index for lakes, Limnol. Oceanogr., 22: 361-369
- Carmichael W.W., 1992, Cyanobacteria secondary metabolites the cyanotoxins, J. Appl. Bacteriol., 72: 445-459
- Chen J., Xie P., Li L., Xu J., 2009, First identification of the hepatotoxic microcystins in the serum of a chronically exposed human population together with indication of hepatocellular damage, Toxicol. Sci., 108(1): 81-89
- Chorus I., Bartram J., 1999, *Toxic Cyanobacteria in Water: a Guide to Public Health Significance.* [in] *Monitoring and Management*, E & FN Spon /Chapman & Hall, Londyn, pp 416
- Dawson R.M., 1998, The toxicology of microcystins. Toxicon, 37: 953-962
- Directive of UE 2006/7/WE, 2006.
- Dokulil M.T., Teubner K., 2000, Cyanobacterial dominance in lakes, Hydrobiologia, 438; 1-12
- Furey A., Crowley J., Hamilton B., Lehane M., James K.J., 2005, Strategies to avoid the mis-identyfication of anatoxin-a using mass spectrometry in the forensic investigation of acute neurotoxic poisoning, J. Chromatogr., 1082: 91-97
- Golterman H.L., 1971, Methods for chemical analysis of fresh waters. Blackwell, IBP Handbook no 3, Oxford – Edinburgh, pp. 166
- Grabowska M., 2005, Cyanoprocaryota blooms in the polyhumic Siemianówka dam Reservoir in 1992-2003, Oceanol. Hydrobiol. Stud., 24(1): 73-85
- Grabowska M., Pawlik-Skowrońska B., 2008, Replacement of Chroococcales and Nostocales by Oscillatoriales caused a significant increase in microcystin concentrations in a dam reservoir, Oceanol. Hydrobiol. Stud., 37(4): 23-33
- Hardy J., 2008, Washington State Recreational Guidance for Microcystins (Provisional) and Anatoxin-a (Interim/Provisional). Final Report, Washington State Department of Health. Washington, pp 14
- James K.J., Furey A., Sherlock I.R., Stack M.A., Twohing M. et al., 1998, Sensitive determination of anatoxin-a, homoanatoxin-a and their degradation products by liquid chromatography with fluorometric detection, J. Chromatogr. A., 798: 147-157
- Jöhnk K., Huisman J., Sharples J., Sommeijer B., Visser P.M., Strooms J.M., 2008, Summer heatwaves promote blooms of harmful cyanobacteria. Glob. Change Biol., 14: 495-512
- Kabziński K.M., Kabziński K.A., 2006, Toxic cyanobacterial blooms. Medical effects of contact with cyanobacterial blooms, part VI, Bioskop, 1: 13-20 (in Polish)
- Kardinaal W.E.A., Tonk L., Janse I., Hol. S., Slot P. et al., 2007, Competition for light between toxic and nontoxic strains of the harmful cyanobacterium Microcystis, Appl. Environ. Microbiol., 73: 2939-46
- Kawecka B., Eloranta V.P., 1994, *Outline of ecology of algae from aquatic and terrestrial habitats*. PWN, Warszawa, pp 252 (in Polish)
- Kaya K., Sano T., 1999, Total microcystin determination using erythro-2methyl-3-(methoxy-d<sub>3</sub>)-4-phenylbutyric acid (MMPB-d<sub>3</sub>) as the internal standard, Anal. Chim. Acta, 386: 107-112
- Komárek J., Anagnostidis K., 1999, 2000, Süßwasserflora von Mitteleuropa. T1: Chroococcales, Spektrum Akademischer Verlag, GmbH, Heidelberg – Berlin, pp. 548



- Komárek J., Anagnostidis K., 2005, Süßwasserflora von Mitteleuropa. T2: Oscillatoriales. Spektrum Akademischer Verlag, Elsevier GmbH, München, pp.759
- Kurmayer R., Christiansen G., 2009, *The genetic basis of toxin* production in Cyanobacteria, Freshwater Reviews, 2: 31-50
- Kurmayer R., Christiansen G., Fastner J., Börner T., 2004, Abundance and active and inactive microcystin genotypes in populations of the toxic cyanobacterium Planktothrix spp, Environ. Microbiol., 6: 831-841
- Malbrouck C., Kestemont P., 2006, Effects of microcystins on fish, Environ. Toxicol. Chem., 25(1): 72-86
- Mankiewicz-Boczek J., Urbaniak M., Romanowska-Duda Z., Izydorczyk K., 2006, Toxic cyanobacteria strains in lowland dam reservoir (Sulejów Res. central Poland): amplification of MCY genes for detection and identification, Pol. J. Ecol., 54: 171-180
- Mazur-Marzec H., 2006, *Characterization of phycotoxins produced by Cyanobacteria*, Oceanol. Hydrobiol. Stud., 35: 85-109
- Orr P.T., Jones G.J., Hunter R.A., Berger K., De Paoli D.A., Orr C.L.A., 2001, Ingestion of toxic Microcystis aeruginosa by dairy cattle and the implications for microcystin contamination of milk. Toxicon, 39: 1847-1854
- Osswald J., Rellan S., Carvalho A.P., Gago A., Vasconcelos V., 2007, Acute effects of an anatoxin-a producing cyanobacterium on juvenile fish – Cyprinus carpio L. Toxicon, 49: 693-698
- Palus J., Dziubałtowska E., Stańczyk M., Lewińska D., Mankiewicz-Boczek J. et al., 2007, Biomonitoring of cyanobacterial blooms in Polish water reservoir and the cytotoxicity and genotoxicity of selected cyanobacterial extracts, Int. J. Occup. Med. Environ. Health., 20(1): 48-65
- Pawlik-Skowrońska B., Kornijów R., Pirszel J., 2010, Sedimentary imprint of cyanobacterial blooms – a new tool for insight into recent bistory of lakes, Pol. J. Ecol., 58(4): 663-670
- Pawlik-Skowrońska B., Pirszel J., Kornijów R., 2008, Spatial and temporal variation in microcystin concentrations during perennial bloom of Planktothrix agardhii in a hypertrophic lake, Ann. Limnol.-Int. J. Lim., 44(2): 63-68
- Pawlik-Skowrońska B., Skowroński T., Pirszel J., Adamczyk A., 2004, Relationship between cyanobacterial bloom and anatoxin-a and microcystin occurrence in the eutrophic dam reservoir (SE Poland), Pol. J. Ecol., 52(4): 379-390
- Paerl H.W., 1988, Nuisance phytoplankton blooms in coastal, estuarine and inland waters, Limnol. Oceanogr., 33: 823-847
- Paerl H.W., 1996, A comparison of cyanobacterial bloom dynamics in freshwater, estuarine and marine environments, Phycologia, 35: 25-35
- PN-ISO 10260, 2002, Water quality. Measurement of biochemical parameters. Spectrophotometric determination of chlorophyll-a, PWN, Warszawa, pp. 11 (in Polish)
- Polučkova A., Hašler P., Kitner M., 2004, Annual cycle of Planktothrix agardhii (Gom.) Anagn. and Kom. nature population, Internat. Rev. Hydrobiol., 89: 278-288
- Rücker J., Wiedner C., Zippel P., 1997, Factors controlling the dominance of Planktothrix agardii and Limnothrix redekei in eutrophic shallow lakes, Hydrobiology, 342/343: 107-115
- Scheffer M., Rinaldi S., Gragnani A., Mur L.R., Van Nes E.H., 1997, On the dominance of filamentous Cyanobacteria in shallow, turbid lakes, Ecology, 78(1): 272-282
- Seip K.L., Reynolds C.S., 1995, *Phytoplankton functional attributes* along trophic gradient and season, Limnol. Oceanogr., 40: 589-597
- Sivonen K., Niemelä S.I., Niemi R.M., Lepistö L., Luoma T.H., Räsänen L.A., 1990, *Toxic Cyanobacteria (blue-green algae) in Finnish fresh coastal waters*, Hydrobiologia, 190: 267-275
- Smith V.H., 2003, Eutrophication of freshwater and coastal marine ecosystems: A global problem, Environ. Sci. Pollut. Res., 10: 1-14

- Tonk L., Visser P.M., Christiansen G., Dittmann E., Snelder E.O.F.M., Wiedner C., Mur L.R., Huisman J., 2005, The microcystin composition of the Cyanobacterium Planktothrix agardhii changes toward a more toxic variant with increasing light intensity, Appl. Environ. Microbiol., 71: 5177-5181
- Toporowska M., Pawlik-Skowrońska B., 2009, Microcystins produced by Cyanobacteria and their accumulation in ichthyofauna of a hypertrophic lake, [in:] Abstracts of the 21st Conference of Polish Hydrobiologists, Lublin, pp 165
- Toporowska M., Pawlik-Skowrońska B., Krupa D., Kornijów R., 2010, Winter versus summer blooming of phytoplankton in a shallow lake: effect of hypertrophic conditions, Pol. J. Ecol. 58(1): 159-168
- USEPA, 2006, Toxicological Reviews of Cyanobacterial Toxins: Anatoxin-a (External Review Draft), U.S. Environmental Protection Agency, Washington, DC, NCEA-C-1743
- Utermöhl H., 1958, Zur Vervollkommung der quantitative Phytoplanktonmethodik, Mitt. Internat. Verein. Limnol., 2: 1-38
- Van Apeldoorn M.E., Van Egmond H.P., Speijers G.J.A., Bakker G.J.I., 2007, *Toxins of Cyanobacteria*, Mol. Nutr. Food Res., 51: 7-60
- Van Den Hoek C., Mann D.G., Jahns H.M. 1995, Algae. An introduction to phycology, Cambridge Univ. Press, Cambridge, pp. 623
- Visser P.M., Ibelings B.W., Van Der Veer B., Koedood J., Mur L.R., 1996, Artificial mixing prevents nuisance blooms of the Cyanobacterium Microcystis in Lake Nieuwe Meer, The Netherlands, Freshwat. Biol., 36: 436-450
- Welker M., Von Dohren H., 2006, Cyanobacterial peptides nature's own combinatorial biosynthesis, FEMS Microbiol. Rev., 30: 530-563
- WHO. 2008, *Guidelines for Drinking-water Quality*, Third Edition, Incorporating the first and second Addenda, Volume 1, Recommendations, Geneva
- Wilk-Woźniak E., 1996, Changes in the biomass and structure of phytoplankton in the Dobczyce Reservoir (soutern Poland), Acta Hydrobiol., 38: 125-131
- Wilk-Woźniak E., Mazurkiewicz-Boroń G., 2003, The autumn dominance of cyanoprokaryotes in a deep meso-eutrophic submontane reservoir, Biologia, Bratislava, 58(1): 17-24
- Wiśniewska M., Krupa D., Pawlik-Skowrońska B., Kornijów R., 2007, Development of toxic Planktothrix agardhii (Gom.) Anagn. et Kom. and potentially toxic algae in the hypertrophic Lake Syczyńskie (E. Poland), Oceanol. Hydrobiol. Stud., 34: 173-179