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Species Composition and Toxic Potential of Cyanobacteria in Some Western Rhodopes Dams

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Abstract. Cyanobacteria are photosynthetic prokaryotes with cosmopolitan distribution. They are major producers of primary biomass and free oxygen in most of the freshwater and marine biomes on the planet. Under certain conditions of the environment, these organisms can evolve massively and cause the so-called "water blooms". Very often these blooms are toxic due to the ability of some cyanobacteria to produce dangerous toxins (cyanotoxins) with hepatotoxic, neurotoxic or dermatotoxic effects. This determines the cyanobacteria as an ecological risk for the aquatic ecosystems as well as a threat for the health of animals and humans. Climate changes also lead to an increase in the percentage of cyanobacterial blooms. Therefore, the investigation of the species composition of this group in the Bulgarian reservoirs, the tracking of the blooms frequencies as well as the assessment of their toxic potential is of great importance. Unfortunately, such data at this stage are scarce. This study presents data on the species composition and toxic potential of Cyanobacteria during the summer months of 2017 in five reservoirs (Batak, Dospat, Shiroka polyana, Golyam Beglik and Krichim dams) in the Western Rhodope Mountain. During the investigation period, except the representatives of Cyanobacteria, seven algal phyla (Chlorophyta, Bacillariophyta, Euglenophyta, Dinoflagellata, Cryptophyta, Chrysophyta and Xantophyta) were also found in the dams. The frequency and percentage of cyanobacteria were reported. A taxonomic list of the determined cyanobacterial species is given for each water body. In addition, a quantitative characteristic for each species (density and biomass) was performed. By using ELISA tests for cyanotoxins we have analyzed the presence of microcystins/nodularins (MCs/Nod) and saxitoxins (STXs). Cyanotoxins were found in four of the studied water basins - Dospat Dam, Batak Dam, Shiroka polyana Dam and Krichim Dam.

Key words: Cyanobacteria, cyanotoxins, blooms, Western Rhodopes dams, phytoplankton.

Introduction

Cyanobacteria are a unique group of organisms adapted to colonize almost every habitat on our planet. Their cosmopolitan distribution is mostly due to their long evolutionary history (about 2.5-2.7 billion years). They are distributed in freshwater and saltwater habitats, in soils, in hot thermal springs or in the molten water layer on the snow surface of the poles. Cyanobacteria are the only prokaryotes capable of photosynthesis and they are responsible for up to 20-30% of the photosynthetic biomass production of the

Earth (Anagnostidis & Komárek, 1985; Honda *et al.*, 1999). Their light harvesting systems may change according to the available light, and many cyanobacteria can change their position in the water basin, varying in depth. Some of the cyanobacteria are able to fix atmospheric nitrogen, which makes them preferred partners for symbiosis with animals, higher plants or mushrooms (Carpenter & Foster, 2002; Janson, 2002).

Cyanobacteria are producers of compounds with antifungal, antitumour, antiviral, immunosuppressive and immunostimulating activity, as well as substances acting as hormones (Namikoshi & Rinehart, 1996). In addition, they can produce as secondary metabolites several toxic substances called "cyanotoxins". The mechanisms of action of the cyanotoxins are associated with manifestation of hepato-, neuro-, gastro- and dermatotoxicity (van Apeldoorn *et al.*, 2007). There are a number of reports for death of fish, domestic animals and even humans after using of cyanotoxin-contaminated water (Jochimsen *et al.*, 1998). Cyanotoxins can reach high and dangerous concentrations in the waterbodies after rapid growth of toxin-producing cyanobacterial strains known as "water blooms" (Funari & Testai, 2008). These blooms are most commonly caused by members of the genera *Aphanizomenon, Cylindrospermopsis, Anabaena, Dolichospermum, Microcystis, Nodularia, Planthotrix* (Huisman *et al.*, 2018). The massive development of cyanobacteria in the waterbodies leads to many problems related to the water quality. Depletion of the oxygen (hypoxia and anoxia) accompanying the "water blooms" causes death of fish and benthic invertebrates. It was considered that over the last few decades the eutrophication, increased levels of carbon dioxide and global warming are the main reason for the observed increase in the frequency, intensity and duration of the cyanobacterial blooms in many aquatic ecosystems across the planet (Huisman *et al.*, 2018).

In Bulgaria, cyanobacteria and their toxins have been actively investigated since 2009 (Teneva *et al.*, 2009; 2010a, b; 2011; 2014; Pavlova *et al.* 2014; 2015). Summarized information was provided by Teneva *et al.* (2015); Stoyneva-Gärtner *et al.* (2017) and Descy *et al.* (2018). The monitoring of the status of the waterbodies is important since the expansion of the "cyanobacterial blooms" can leads to negative consequences for the biodiversity and the functioning of the aquatic food chains, and threatens the use of the affected waters for drinking, bathing, fishing and other recreation activities.

Studies on the phytoplankton in Bulgarian dams in the recent years indicated an increase in the frequency of Cyanobacteria. Together with diatoms (Bacillariophyta) and green algae (Chlorophyta), cyanobacteria are very often dominant or subdominant phyla in the phytoplankton structure at a number of large dams, including Dospat and Batak (Teneva *et al.*, 2009; Dochin & Stoyneva, 2016; Dochin *et al.*, 2018). Phytoplankton, which is a part of each aquatic ecosystem, is an important indicator for assessing the ecological status of standing waters. It is included in the control and operational monitoring programs and in most cases is used as the main biological element for assessment of the surface standing water bodies quality. Species composition, frequency of the blooms and phytoplankton abundance are the main normative defined indicators used for monitoring and assessment of the water quality. In terms of species composition, the emphasis is placed on the ratio between the main taxonomic groups as well as on the proportion of cyanobacteria. The abundance is measured with the indicators biomass, chlorophyll-*a* and trophic indices. In Bulgaria, this comprehensive approach for assessment of the water quality started to apply after 2009, and despite the active work in this direction, the data for some water basins are still incomplete.

The current study, conducted in 2017 and covering 5 dams in the Western

Rhodopes (Batak, Dospat, Shiroka Polyana, Golyam Beglik and Krichim), contributes to a great extent for replenishment and enrichment of the available information. This study provides for the first time detailed information on the species composition and toxic potential of Cyanobacteria in the Golyam Beglik, Shiroka Polyana and Krichim dams. Also, for the first time was assessed the toxic potential of the cyanobacterial species in Dospat and Batak dams during the summer period.

Materials and Methods

Dams description and physicochemical water quality analysis

TSubject of this study are 5 dams: Golyam Beglik, Batak, Shiroka Polyana, Dospat and Krichim. Three of the dams (Golyam Beglik, Batak and Shiroka Polyana) are part of the Batak water Cascade (Batashki vodnosilov pat), which is one of the most complicated hydrocomplexes built with the intent to exploit the rich raining outflow of the Western Rhodopes for production of electricity and irrigation. Golyam Beglik and Batak dams are the main reservoirs, and Shiroka Polyana Dam, which is part of the first stage of the cascade, serves as an additional reservoir. The other two dams (Dospat and Krichim) are part of the water cascade Dospat-Vacha, which consists of three hydro-stations: Dospat-Teshel, Vacha and Krichim. Location data and morphometric characteristics of the dams are presented in Fig. 1 and Table 1.The physicochemical parameters, including water temperature and pH were measured in situ, total nitrogen and phosphorus in laboratory following the adopted standards.

Sample collection and phytoplankton analysis

Phytoplankton was sampled three times (July, August and September, 2017). The taxonomic composition of the phytoplankton was determined by using a light microscope Amplival (400x) on live and formalin-preserved specimens. Counting of the phytoplankton was performed by using an inverted microscope (UTERMÖHL, 1958). For numerous species, at least 100 specimens were counted (LUND *et al.*, 1958). The biomass was calculated by using the formulas for geometric shapes (HILLEBRAND *et al.*, 1999). Water samples for chemical and toxicological analysis were collected at the same time as the phytoplankton samples from the same sampling points.

Analysis of cyanotoxins by ELISA

Saxitoxins

The water samples were analyzed by the Ridascreen™ saxitoxin ELISA kit (R-Biopharm, Darmstadt, Germany). This is a competitive ELISA for the quantitative analysis of saxitoxin and related toxins based on the competition between the free toxins from samples or standards and an enzyme-conjugated saxitoxin for the same antibody. The mean lower detection limit of the Ridascreen™ saxitoxin assay is about 0.010 ppb (µg/L).

Microcystins and nodularins

The analysis of the water samples for presence of microcystins and nodularins was performed using a Microcystins/Nodularins (ADDA), ELISA Kit (Abraxis LLC, Warminster, PA, USA). As for the saxitoxin ELISA, this is a quantitative, competitive immunosorbent assay that allows the congener-independent detection of microcystins and nodularins in water samples. The detection limit of the kit is 0.10 ppb (µg/L).

Results and Discussion

Physicochemical parameters

The environmental variables of the investigated reservoirs are given in Table 2. In 2017, the water temperature at the surface ranged from 17°C (July, in Krichim Dam) to 24°C (August, in Batak Dam). Values of pH were between 4.5 (September, in Shiroka polyana Dam) and 7.4 (July, in Krichim Dam). Total nitrogen (TN) concentrations varied from 0 mg/L to 4.1 mg/L. Total phosphorus (TP) concentrations were from 0 to 0.22 mg/L. In the surveyed reservoirs, the TN/TP ratio was in the range from 0 to 130. The ratio of TN/TP (Redfield ratio) is an important indicator for nutrient limitations of the phytoplankton growth. It was suggested that an TN/TP ratio >16 indicates P limitation on a community level, while an TN/TP ratio <14 is indicative for N limitation (HECKY & KILHAM, 1988; DOWNING & MCCAULEY 1992; YANG *et al.*, 2008). Phosphorus has been shown to be the principal limiting factor in freshwater environments, while N is commonly limiting in marine ecosystems (YANG *et al.*, 2008). The TN/TP ratio data in the present study indicate phosphorus limitation on a community level in Shiroka polyana Dam during September and Krichim Dam during July and August. Interesting is the shifting from phosphoros to nitrogen limitation in Krichim Dam during summer months. Phosphorus is most likely the limiting nutrient responsible for the relatively low phytoplankton abundance.

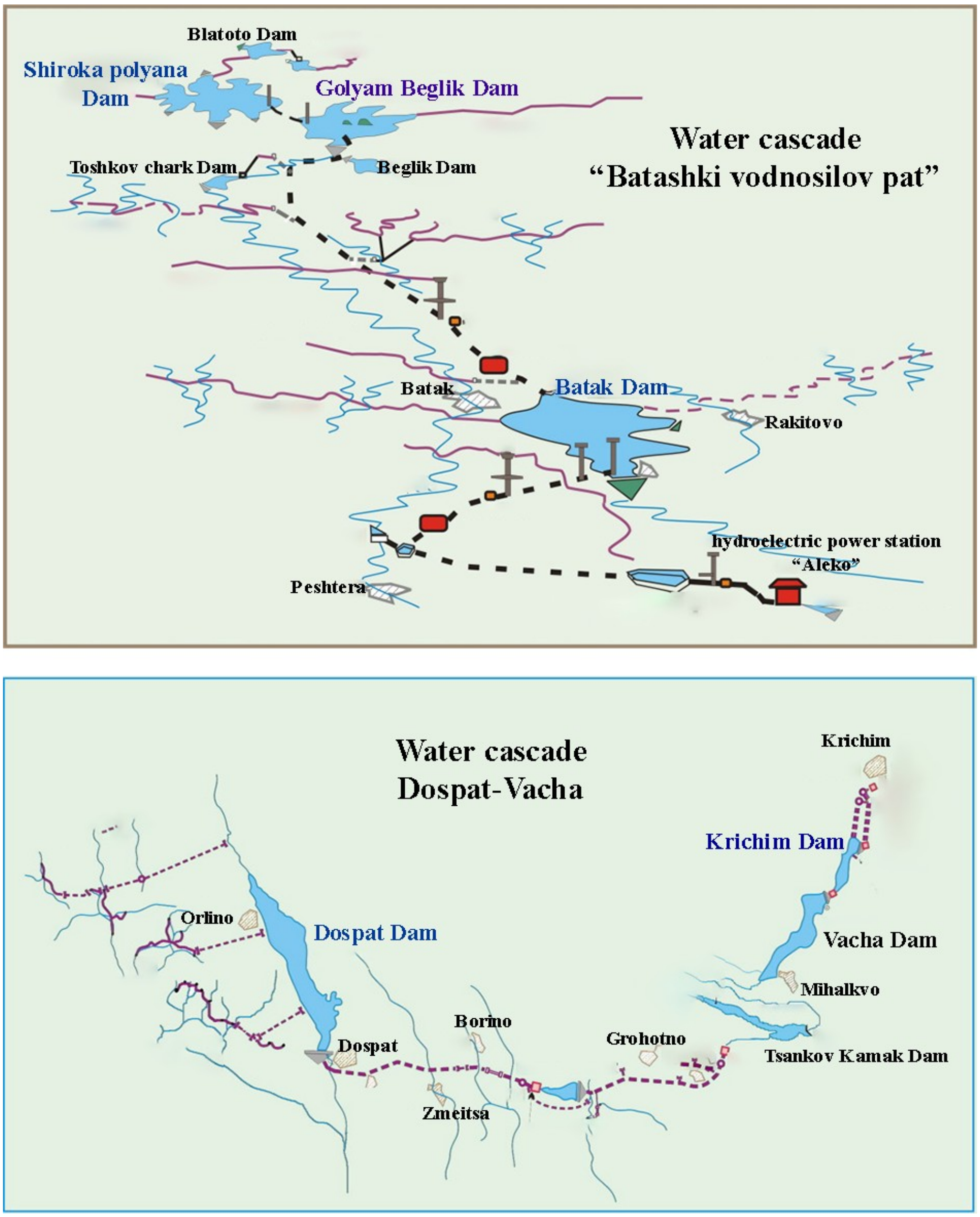

Fig. 1. Indicative maps of the studied dams.

Table 1. Morphometrical characteristics of the investigated dams.

Dam name	Golyam Beglik	Shiroka polyana	Dospat	Batak	Krichim
Altitude (m)	1528	1500	1200.5	1107.8	418
Water volume (m^3)	62 110 000	24 000	449 220 000	310 000 000	20 260 000
Source	Kriva River	Kerelova River	Dospat River	Matnitsa River	Vacha River
Built (year)	1951	1963	1969	1959	1972
Tributary (m^3/s)	4.19	NA	249.07	NA	564.59
Area (km^2)	4.8	20	18.3	22	0.8
Catchment area (km^2)	331	81	432.3	463.29	29.3
GPS coordinates	41°49.007'N 024°07.442'E	41°46.038'N 024°08.493'E	41°38.367'N 024°09.086'E	41°56.385'N 024°10.550'E	41°59.361'N 024°28.044'E
specified use	production of electricity	production of electricity	production of electricity	complex	production of electricity
National lake type	L3	L3	L11	L3	L11

Taxonomic composition and structure of the phytoplankton community

In this study, phytoplanktonic organisms belonging to eight phyla – Cyanobacteria, Bacillariophyta, Chlorophyta (incl. Zygnemaphyceae), Xantophyta, Euglenophyta, Dinoflagellata, Cryptophyta and Chrysophyta were identified (Fig. 2). During the studied period, in Dospat Dam as well as at the wall of Batak Dam the highest percentage of the total phytoplankton was represented by Chlorophyta, followed by Bacillariophyta and Cyanobacteria. The green algae (Chlorophyta) were represented by the highest percentage in the Krichim dam as well, followed by Bacillariophyta. Other phyla were almost equally represented. Diatoms (Bacillariophyta) dominated in the phytoplankton of the Shiroka polyana Dam, followed by Chlorophyta. In this dam, the other phyla were again equally represented. Similar trend was observed also in Batak (tail) and Golyam Beglik dams, where the third position after Bacillariophyta and Chlorophyta was occupied by Cyanobacteria (Batak Dam - the tail) or shared between Cyanobacteria and Dinoflagellata (Golyam Beglik Dam).

Data showed that during the studied period Chrysophyta were presented in all the studied dams with exception of Dospat Dam. Also, representatives of Cryptophyta were not found in Dospat Dam. In 2011, in Dospat Dam was found only one species belonging to Cryptophyta (DOCHIN & STOYNEVA, 2016). Representatives of Euglenophyta have not been identified in Krichim and Golyam Beglik dams, as well as in the tail of Batak Dam. Xantophyta were presented only in the phytoplankton of Dospat Dam (in August, 5%) and Shiroka Polyana Dam (in September, 3.1%). During the summer months in all five dams Cyanobacteria had a dominant role in the phytoplankton structure along withBacillariophyta and Chlorophyta. Similar data were reported also in other studies on the phytoplankton in Dospat and Batak dams (DOCHIN & STOYNEVA, 2016; DOCHIN *et al.*, 2018).

An increase in the percentage of cyanobacteria presented in the phytoplankton of the investigated dams was observed from July to September, as this percentage was highest in September (Fig. 3). A similar trend was reported by DOCHIN *et al.* (2018) studying the phytoplankton of Batak Dam.

Data from the present study showed that the most abundant of cyanobacterial species is Dospat Dam in September (30.4%), followed by Batak Dam and Golyam Beglik. Cyanobacteria were relatively less represented in Shiroka Polyana and Krichim dams.Twenty four cyanobacterial species from 12 genera have been identified in the five studied dams. Among them we identified known producers of cyanotoxins such as *Aphanizomenon flos-aquae*, *Dolichospermum spiroides* and *Dolichospermum sciremetieviae* (Table 3). Four cyanobacterial species were found in Krichim Dam, 7 in Dospat Dam, 13 in Batak Dam, 5 in Shiroka polyana Dam and 7 in Golyam Beglik Dam. Determined cyanobacteria belong to some of the richest of species genera represented in the Bulgarian ponds such as *Aphanocapsa*, *Dolichospermum*, *Aphanizomenon*, *Anabaena*, *Pseudanabaena*, *Oscillatoria* and *Chroococcus* (STOYNEVA-GÄRTNER *et al.*, 2017). The obtained data (in terms of total biomass) do not show blooming concentrations of cyanobacterial species. Higher values of total biomass (near to first degree of blooming) were recorded in July and September in Dospat Dam.

Analyses of water samples for presence of cyanotoxins

Collected water samples from the investigated dams were analyzed for presence of cyanotoxins by commercially available ELISA kits for saxitoxins and microcystins/nodularins. The data are presented in Fig. 4. Performed ELISA test for microcystins/nodularins showed presence of these toxins in the water samples collected in July from Dospat Dam (0.09 ng/mL) as well as in the water samples collected in August from Batak Dam (0.27 ng/mL), where *Aphanizomenon flos-aquae* (L.) Ralfs was identified as a dominant species. Saxitoxins with a concentration of 0.05 ng/mL were detected in the water samples collected in September from Shiroka polyana and Krichim dams where as dominant taxa were determined *Anabaena sp.* and *Aphanocapsa sp*.

Table 2. Physicochemical variables of the surface water.

Reservoir name/ Parameters	Golyam Beglik			Shiroka polyana			Dospat			Batak			Krichim		
	VII	VIII	IX	VII	VIII	IX	VII	VIII	IX	VII	VIII	IX	VII	VIII	IX
Water temperature (°C)	20	21.5	19	20	21.5	18	21.5	23	21	23	24	22	17	19	18.5
pH	6.5	5.5	5.5	6.5	5.5	4.5	6.5	5.5	5.0	6.6	5.5	6	7.4	6	7
Total nitrogen (mg/L)	0	0	0.1	0.3	2.2	4.1	0.2	0	0	0.4	0.4	0.9	2.6	3.1	0.1
Total phosphorus (mg/L)	0.09	0.19	0.02	0	0.19	0.04	0	0.14	0	0.22	0.03	0.12	0.02	0.03	0.01
TN/TP	0	0	5	0	11.6	102.5	0	0	0	1.82	13.3	7.5	130	103	10
Water color *	RB	BG	RB	RB	GB	RB	SG	SG	SG	RG	BG	BG	OG	RG	OG

* OG - oil green color, RG - rifle green color, RB- rusty brown color, BG -brown green color, SG - sage green color, GB - greenish brown.

Table 3. Species composition and quantitative characteristics of the cyanobacteria in studied reservoirs.

Months	Species composition	Density (cells/L)	Biomass (mg/L)	Total density	Total biomass
	Krichim Dam (wall).				
July	*Aphanothece clathrata* W. et G.S. West	25 800	0.002	37 200	0.003
	Lyngbya sp.	11 400	0.001		
August	*Aphanocapsa delicatissima* W. et G.S. West	591 250	0.001	662 200	0.002
	Aphanothece clathrata W. et G.S. West	70 950	0.001		
September	*Aphanocapsa delicatissima* W. et G.S. West	903 000	0.001	1 444 800	0.006
	Aphanothece clathrata W. et G.S. West	541 800	0.005		
	Synechococcus elongatus (Näg.) Näg.	-	-		

	Dospat Dam (wall)				
July	*Aphanizomenon flos-aquae* (L.) Ralfs	2 531 410	0.203		
	Woronichinia naegeliana (Ung.) Elenk.	-	-	2 531 410	
	Dolichospermum spiroides Kleb.	-	-		
August	*Aphanizomenon flos-aquae* (L.) Ralfs	145 125	0.012		
	Aphanothece clathrata W. et G.S. West	145 125	0	290 250	
	Woronichinia naegeliana (Ung.) Elenk.	-	-		
	Dolichospermum spiroides Kleb.	-	-		
September	*Anabaena variabilis* Kütz.	361 200	0.072		
	Aphanizomenon flos-aquae (L.) Ralfs	2 528 400	0.202		
	Aphanocapsa planctonica (G.M. Smith) Kom. et Anagn.	-	-		
	Aphanothece clathrata W. et G.S. West	90 300	0	2 988 930	
	Oscillatoria mougeotii (Kütz.) Forti	9 030	0.013		
	Woronichinia naegeliana (Ung.) Elenk.	-	-		
	Batak Dam (tail end).				
July	*Anabaena sp.*	116 000	0.023		
	Aphanizomenon flos-aquae (L.) Ralfs	-	-		
	Aphanothece clathrata W. et G.S. West	-	-	116 000	0.023
	Merismopedia tenuissima Lemm.	-	-		
	Pseudanabaena catenata Laut.	-	-		
	Woronichinia naegeliana (Ung.) Elenk.	-	-		
August	*Aphanothece clathrata* W. et G.S. West	215 000	0.001		
	Merismopedia tenuissima Lemm.	-	-	247 250	0.001
	Snowella lacustris (Chod.) Kom. et Hind.	32 250	0		
September	*Dolichospermum scheremetieviae* Elenk.	-	-		
	Aphanizomenon flos-aquae (L.) Ralfs	618 754	0.050		
	Aphanothece clathrata W. et G.S. West	-	-		0.08
	Oscillatoria tenuis Ag.	12 212	0.016	915 919	
	Synechococcus capitatus Bail.-Watts et Kom.	-	-		
	Woronichinia naegeliana (Ung.) Elenk.	284 953	0.014		
	Batak Dam (wall).				
July	*Aphanizomenon flos-aquae* (L.) Ralfs	210 800	0.02		
	Aphanothece minutissima (W. West) Kom.-Legn. et Cronb.	128 600	0.001	354 400	0.021
	Woronichinia naegeliana (Ung.) Elenk.	15 000	-		
August	*Dolichospermum spiroides* Kleb.	-	-		
	Aphanizomenon flos-aquae (L.) Ralfs	-	-		
	Aphanothece minutissima (W. West) Kom.-Legn. et Cronb.	224 675	0.000	228 223	0.001
	Pseudanabaena limnetica (Lemm.) Kom.	3 548	0.001		
	Woronichinia naegeliana (Ung.) Elenk.	-	-		
September	*Dolichospermum scheremetieviae* Elenk.	968	0.004		
	Aphanizomenon flos-aquae (L.) Ralfs	207 045	0.02		
	Aphanothece minutissima (W. West) Kom.-Legn. et Cronb.	41 603	0.000	327 016	0.028
	Synechococcus elongatus (Näg.) Näg				
	Woronichinia naegeliana (Ung.) Elenk.	77 400	0.004		

		Shiroka polyana Dam			
July	*Anabaena sp.*	1 183	0	1 183	0
August	*Anabaena sp.*	35 475	0.007	59 125	0.007
	Aphanothece clathrata W. et G.S. West	23 650	0		
	Oscillatoria sp.	-	-		
September	*Anabaena sp.*	77 400	0.02	77 400	0.02
	Dolichospermum spiroides Kleb.	-	-		
	Aphanizomenon flos-aquae (L.) Ralfs	-	-		

		Golyam Beglik Dam			
July	*Aphanothece clathrata* W. et G.S. West	48 375	0	106 425	0.005
	Woronichinia naegeliana (Ung.) Elenk.	-	-		
	Aphanizomenon flos-aquae (L.) Ralfs	58 050	0.005		
August	*Dolichospermum scheremetieviae* Elenk.	129 000	0.001	279 500	0.009
	Aphanothece clathrata W. et G.S. West	150 500	0.008		
	Chroococcus limneticus Lemm.	-	-		
	Woronichinia naegeliana (Ung.) Elenk.	-	-		
	Aphanizomenon flos-aquae (L.) Ralfs	-	-		
September	*Merismopedia glauca* (Ehr.) Kütz.	63 855	0.001	63 855	0.001
	Pseudanabaena catenata Laut.	-	-		
	Woronichinia naegeliana (Ung.) Elenk.	-	-		

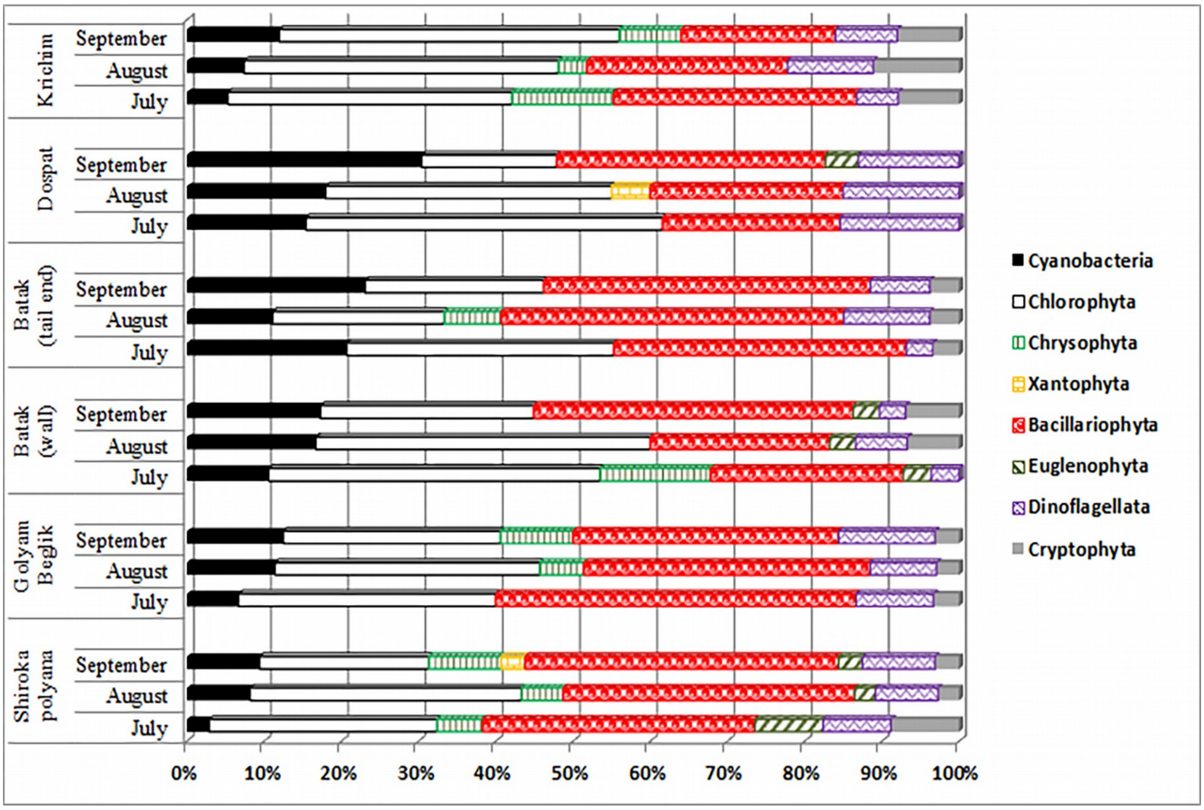

Fig. 2. Relative abundance of the phytoplankton taxa (%) in the surface water of studied reservoirs in July, August and September 2017.

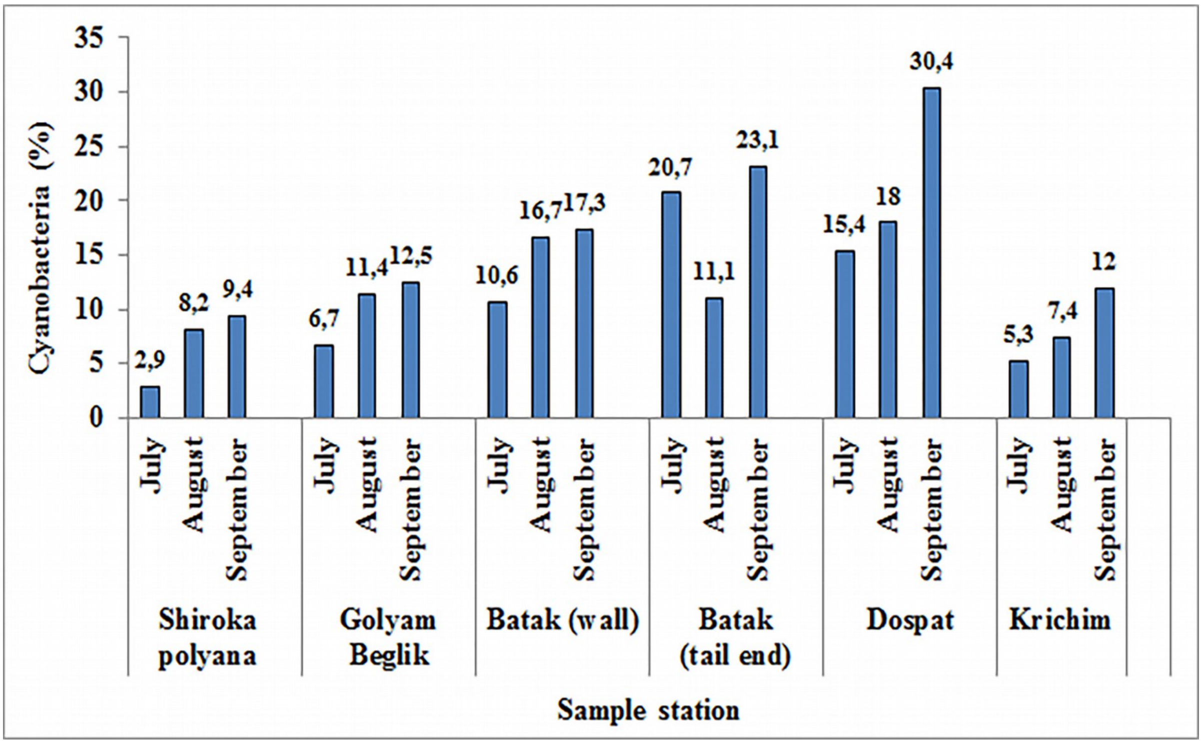

Fig. 3. Relative abundance of Cyanobacteria (%) in the surface water of studied reservoirs.

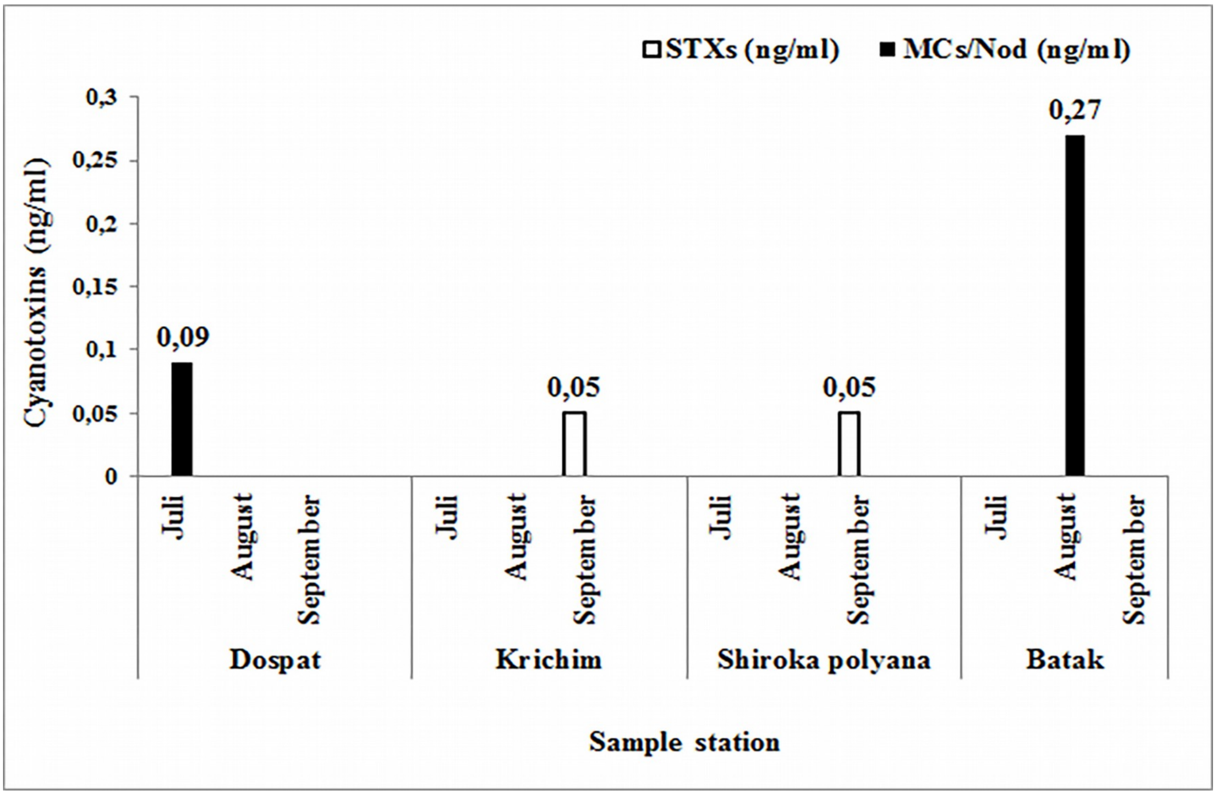

Fig. 4. Presence of cyanotoxins (MCs/Nod, STXs) in the water samples tested by ELISA.

Conclusions

Data from the physico-chemical parameters measured in this study have determined the phosphorus as a limiting factor for the biodiversity and abundance of the phytoplankton in two of the studied dams - Shiroka Polyana and Krichim.

Aphanothece clathrata and *Aphanizomenon flos-aquae* were found in each of the studied reservoirs. Among the 24 cyanobacterial species identified in the present study, we found proven producers of cyanotoxins such as *Aphanizomenon flos-aquae*, *Dolichospermum spiroides* and *Dolichospermum sciremetieviae*.

Our data showed a positive correlation between the species composition of Cyanobacteria in the surveyed reservoirs and the presence of cyanotoxins detected by the ELISA test.

This study underlines the need of analyses for cyanotoxins to assess the risk of water contamination. Although cyanotoxins were detected in concentrations lower than the recommended levels for drinking water, there is an urgent need from monitoring programs of Cyanoprokaryota and their toxins in the dams.

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