Cent. Eur. J. Biol. • 5(2) • 2010 • 231–239
DOI: 10.2478/s11535-010-0009-1

VERSITA

Central European Journal of **Biology**

Phytoplankton community of the drinking water supply reservoir Borovitsa (South Bulgaria) with an emphasis on cyanotoxins and water quality

Research Article

Ivanka Teneva[1], Rumen Mladenov[1], Detelina Belkinova[1],
Ivanka Dimitrova-Dyulgerova[1], Balik Dzhambazov[2,*]

[1]*Department of Botany, Faculty of Biology,*
University of Plovdiv,
BG-4000 Plovdiv, Bulgaria

[2]*Department of Medical Biochemistry and Biophysics,*
Karolinska Institute,
SE-17177 Stockholm, Sweden

Received 21 September 2009; Accepted 07 January 2010

Abstract: The phytoplankton diversity, algal biomass, and selected physicochemical parameters were investigated in the drinking water reservoir (Borovitsa) located in the Kardzhali region, Bulgaria. Particular attention was given to Cyanoprokaryota and presence of cyanotoxins in the water samples. Twenty-nine species belonging to six divisions (Cyanoprokaryota, Chlorophyta, Zygnemophyta, Dinophyta, Euglenophyta and Bacillariophyta) were identified. The microscopic examination of the phytoplankton samples showed the dominance of *Ankyra judayi, Oocystis lacustris* (Chlorophyta) and *Aphanizomenon flos-aquae* (Cyanoprokaryota) in July 2006, and *Microcystis pulverea, Synechococcus elongatus* (Cyanoprokaryota), *Radiococcus planktonicus* (Chlorophyta) and *Melosira varians* (Bacillariophyta) in September 2006. A blooming event due to *Aphanizomenon flos-aquae* was observed in July 2006. The reservoir exhibits a tendency to shift from an oligotrophic environment to a state of mesotrophy. Presence of cyanotoxins such as anatoxin-*a*, microcystins and saxitoxins were analyzed by HPLC and ELISA methods. Our results demonstrated the presence of anatoxin-a and microcystins (0.09 μg/L) in the raw water samples from July 2006, and saxitoxins (2.5 μg/L) and microcystins (0.18 μg/L) in the raw water samples from September 2006. The study underlines that permanent monitoring programs of Cyanoprokaryota in the reservoirs used as sources of drinking water and toxicity assessments should be implemented. Indirect exposure and transfer of cyanotoxins through food chains must also be considered.

Keywords: *Borovitsa reservoir • Cyanoprokaryota • Drinking water • Microcystins • Phytoplankton • Saxitoxins*

1. Introduction

Blue-green algae (Cyanoprokaryota) are among the oldest autotrophic life forms on Earth. Nowadays they have cosmopolitan distribution as inhabitants of fresh-, brackish-, and marine waters, as well as terrestrial environments. Most commonly, they are known as planktonic members of marine and freshwater habitats.

Fluxes in environmental conditions may allow an increased proliferation of the phytoplankton, particularly of bloom-forming species of Cyanoprokaryota. Blooms are mainly caused by a complex interaction of high concentration of nutrients, sunlight, warm temperature, turbidity, pH, conductivity, salinity, carbon availability, and slow-flowing or stagnant water [1]. Cyanoprokaryota blooms are often associated with the eutrophication of freshwater systems and can cause

---

* *E-mail: Balik.Dzhambazov@ki.se*

severe changes in water quality including a decrease in water transparency, heavy fluctuation of oxygen levels, and release of cyanotoxins. The release of cyanotoxins into reservoirs used for drinking-, recreation-, and irrigation-water constitute a potential risk for public health and the environment [2]. The most common and well-studied producers of cyanotoxins are *Microcystis aeruginosa, Aphanisomenon flos-aquae, Anabaena flos-aquae, Cylindrospermopsis raciborskii, Planktothrix agardhii, Lyngbya majuscula, Nodularia* and *Oscillatoria* [3-5]. Cyanotoxins cause direct intoxications of animals and humans by contact with bloom water or indirect poisoning due to consumption of contaminated food [6-8]. Generally, cyanotoxins are divided into hepatotoxins (e.g. microcystins, nodularins, cylindrospermopsins), neurotoxins (e.g. anatoxin-a, homoanatoxin-a, saxitoxins) and dermatotoxins (e.g. lyngbyatoxins, aplysiatoxins). Hepatotoxins are inhibitors of serine/threonine specific protein phosphatases (PP1 and PP2A), neurotoxins block the neuronal transmission by binding to the voltage-gated Na^+ channels in nerve cells, and dermatotoxins cause mainly skin irritations, allergic reactions, and gastroenteritis. During the last years, the frequency and global distribution of toxic algae appear to have increased, and human intoxications from novel algal sources have occurred (reviewed in [2]). Cyanotoxins are responsible for almost all known cases of fresh- and brackish water intoxications. The problem is that toxin production appears highly variable, both within and between blooms. This production and potency can also vary with time for an individual bloom [9], which makes the prediction of toxic blooms and assessment of the potential risk difficult, if not impossible [1,10]. Therefore, water used for human consumption should be regularly monitored for cyanotoxins.

Yet, Cyanoprokaryota, often detected in the Bulgarian freshwater systems, are not studied in toxicological aspects. On the other hand, most cities in the country use surface water as a source of drinking water. Such lakes and reservoirs are used also as fishponds and for water sports. In fact, so far the research has been focused mainly on the phytoplankton diversity, while very little effort has been made to look at the toxin production of determined Cyanoprokaryota species. There is only one report about microcystin contamination of water samples from 15 Bulgarian reservoirs and lakes [11]. Data showed that the concentration of total microcystins (MC-LR, MC-RR and MC-YR) in the biomasses ranged from 8 to 1070 µg/g (d.w.). Presence of dissolved microcystins was obtained as well in one water sample with a concentration of total microcystins 1.64 µg/L. The World Health Organization has calculated a provisional guideline value of 1 µg/L for the total concentration of all microcystin variants in drinking water [12]. The same guideline value (1 µg/L) has been proposed for the total concentration of anatoxins as well [2,12].

Figure 1. Location map of Borovitsa Reservoir, with the sampling station (41°45'N, 25°08'E).

The aim of our study was to evaluate the diversity, distribution and quantitative development of the phytoplankton as well as the presence of cyanotoxins in the public reservoir Borovitsa, which supplies drinking water to the inhabitants of Kardzhali, Momchilgrad, Dzhebel and 22 additional villages in the region.

2. Experimental Procedures

2.1 Site description and physicochemical water quality analysis

Borovitsa Reservoir is located in the Kardzhali region of South Bulgaria (geographic coordinates 41°45'27''N and 25°08'36''E, altitude 495 m) (Figure 1). It is of great importance, since it supplies drinking water to more than 80,000 inhabitants. Borovitsa dam is 69 m high and it was completed in 1988. It is one of three big dams that have been built along the valley of Arda River. The morphometric characteristics of the reservoir are: surface area 814 000 m^2, crest length 235 m, water holding capacity 31 000 000 m^3, volume of water during the study period 27 300 000 m^3, maximum depth of 44 m and mean depth of 35 m.

Physicochemical water quality parameters, including water temperature, pH, transparency, saturation, electrical conductivity, turbidity, dissolved oxygen, ammonia nitrogen, nitrate nitrogen, nitrite nitrogen, total phosphorus and orthophosphate were measured in the field by a portable photometer pHotoFlex® (WTW GmbH, Weilheim, Germany) or in the laboratory.

2.2 Sample collection and phytoplankton analysis

For qualitative analyses of the phytoplankton, samples were collected twice (July 26th, 2006 and September 26th, 2006) from the surface layer (0.5 m) with a 20 µm mesh size net at a fixed collection station (Borovitsa Dam) and preserved in 4% formaldehyde. For phytoplankton counting, samples were collected with Meyer bottles of 1 L and preserved with Lugol solution. Phytoplankton analyses were performed on both fresh and preserved (in 4% formaldehyde) samples, with an inverted microscope (PZO Poland) according to Lund *et al.* [13], using sedimentation chambers for phytoplankton identification and cell density estimation.

Water samples for chemical and toxicological analysis were collected at the same time as the phytoplankton samples from the same sampling point. These samples are referred to as "raw water samples". A second water sampling point was set up at the outlet of the treatment station, which is referred as "treated water". All water samples were filtered immediately through a 0.22 µm Millipore filter and transported on dry ice to the laboratory. Samples were stored at -20°C until analyzed (within 2 weeks) for presence of cyanotoxins. Samples for determining chlorophyll-*a* concentration were passed through 0.45 µm Whatman GF/F glass microfiber filters (25 mm diameter) with a suction pressure of ~ 0.3 10^5 N/m^2. The filters were stored in darkness (plastic tubes covered with aluminium foil to prevent light entering) and transported on ice to the laboratory. Chlorophyll-*a* was extracted in acetone:dimethylsulfoxide solution (1:1, v/v) and later analysed by the international standard method [14].

The trophic state index (TSI) which is an indicator of the biological productivity was calculated according to Carlson [15]:

$TSI = 10(6 - \ln SD/\ln 2)$

where SD is Secchi depth (Secchi disk transparency) in meters. The following classification was used: oligotrophic (<38), mesotrophic (38-48), eutrophic (49-61) and hypertrophic (>61).

2.3 Analysis of cyanotoxins by High Performance Liquid Chromatography (HPLC)

HPLC Super gradient acetonitrile was purchased from Lab-Scan Analytical Sciences (Dublin, Ireland) and ammonium acetate from Scharlau Chemie S.A. (Barcelona, Spain). Water used for HPLC was purified with a Milli-Q plus PF system (Millipore, Molsheim, France). Anatoxin-a (AnTx-a) was purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany), microcystin-LR (MC-LR) was from BIOMOL GmbH (Hamburg, Germany) and saxitoxin (STX) was from R-Biopharm GmbH (Darmstadt, Germany).

Chromatography was performed with an ÄKTA explorer 100 Air system and the UNICORN software (Amersham Pharmacia Biotech AB, Uppsala, Sweden) as previously described by Teneva *et al.* [16]. Briefly, two hundred microliters of filtered through a 0.22 µm Millipore filter water samples were injected for HPLC analysis using an analytical column Discovery® C18 (5x4 mm I.D., 5 µm) from Supelco (Bellefonte, PA, USA). The mobile phase consisted of a mixture of solvent A (10 mM ammonium acetate, pH 5.5) and solvent B (10 mM ammonium acetate–acetonitrile, 80:20, v/v) as follows: 0% of B at 0 min, 100% of B at 45 min to 65 min using a linear gradient. Flow-rate was 0.8 mL/min and UV detection was performed at 238 nm. The patterns of substances in the samples were compared with the standards based on retention times and peak areas.

2.4 Analysis of cyanotoxins by Enzyme-Linked ImmunoSorbent Assay (ELISA)

2.4.1 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).

2.4.2 Microcystins and nodularins

The analysis of the water samples for presence of microcystins and nodularins was performed using the Microcystins ELISA (Abraxis LLC, Warminster, PA). 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 limit of detection of the Microcystins ELISA is 0.10 ppb (µg/L).

Parameter	July	September
Water temperature (°C)	26.4	20.2
pH	8.56	7.32
Secchi disk transparency (m)	3.0	3.0
Saturation (%)	113.0	115.4
Electrical conductivity (μS/cm)	78.1	61
Dissolved oxygen (mg/L)	8.56	9.74
Total nitrogen (mg/L)	2.32	3.48
Ammonia nitrogen (mg/L)	0.3	0.3
Nitrate nitrogen (mg/L)	0.64	1.35
Nitrite nitrogen (mg/L)	0.002	0.019
Total phosphorus (mg/L)	0.029	0.025
Orthophosphate (mg/L)	0.02	0.01
Total nitrogen / Total phosphorus	80	139.2
Chlorophyll-a (μg/L)	2.07	2.2
Carlson's trophic state index (TSI)	44	44

Table 1. Water quality analysis of samples collected from Borovitsa Reservoir in July and September 2006.

3. Results

3.1 Physicochemical and biological parameters

The environmental variables of the investigated reservoir are given in Table 1. The water temperature at the surface ranged from 26.4°C in July to 20.2°C in September and pH values were between 8.56 and 7.32, respectively. Mean water clarity (transparency) was 3 m and did not show variations during the studied period. The reservoir was well aerated during the sampling time with dissolved oxygen levels of 8.56 mg/L in July and 9.74 mg/L in September. Total nitrogen (TN) concentrations varied from 2.32 mg/L in July to 3.48 mg/L in September. Nitrogenous compounds were presented mainly by the nitrate ions with an increased concentration (1.35 mg/L) in September. Nitrite and ammonium ions present only 0.5-0.6% and 8-12% of the total mineral nitrogen, respectively. Total phosphorus (TP) concentrations were always relatively low (from 0.029 to 0.025 mg/L). 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 of N limitation [17-19]. P has been shown to be the principal limiting factor in freshwater environments, while N is commonly limiting in marine ecosystems [19]. In Borovitsa reservoir, the TN/TP ratio was 80 in July and 139.2 in September, which indicate phosphorus limitation and absence of nitrogen limitation. P is most likely the limiting nutrient responsible for the relatively low phytoplankton abundance.

Chlorophyll-*a* concentrations varied within a range of 2.07 to 2.2 μg/L. Carlson's trophic state index (calculated from the seccki-disk transparency) had a mean value of 44, according to which the studied reservoir is classified as mesotrophic. Also, the mesotrophic status of Borovitsa reservoir correlated well with the total phosphorus and chlorophyll-*a* concentrations.

3.2. Taxonomic composition and structure of the phytoplankton community

In this study, 29 taxa of algae were identified belonging to six divisions (Cyanoprokaryota, Chlorophyta, Zygnemophyta, Dinophyta, Euglenophyta and Bacillariophyta), with a total of 21 species in July and 21 species in September (Table 2). The most representative division in terms of species richness was Chlorophyta (11 species with 8 in July and 8 in September), followed by Cyanoprokaryota (7 species, with 4 in July and 5 in September). Figure 2 presents the relative abundances of the phytoplankton groups during the studied period. During both months, July and September, Chlorophyta represented 38.1% of the total phytoplankton, followed immediately by Cyanoprokaryota (19% in July and 23.8% in September) and Bacillariophyta (19%). Dominated species in July were the green algae *Ankyra judayi* and *Oocystis lacustris*, and the cyanoprokaryote *Aphanizomenon flos-aquae*, while in September the dominance was shifted to the species *Microcystis pulverea* (Cyanoprokaryota), *Synechococcus elongatus* (Cyanoprokaryota) and *Radiococcus planktonicus* (Chlorophyta), which were not present in July, and the bacillariophyte *Melosira varians* (Table 2).

The total phytoplankton density in the reservoir ranged from 3.3x10^{6} cells/L to 5.6x10^{6} cells/L during July and September, respectively (Table 3). In July, Cyanoprokaryota represented 55.5% of the total phytoplankton density dominated by the most frequent species being the potentially toxic cyanoprokaryote *Aphanizomenon flos-aquae* (48%) with a blooming concentration of 1.59x10^{6} cells/L. During our survey, this species always co-occurred with *Anabaena flos-aquae*. Although the highest density, *Aphanizomenon flos-aquae* had a biomass value of 0.13 mg/L, which forms only 9.2% from the total phytoplankton biomass (1.41 mg/L) of the reservoir. In contrast to the density, relatively high percentage of the biomass was observed for Chlorophyta (62.4%), followed by Bacillariophyta (24.8%) and then Cyanoprokaryota (10%).

In September, the phytoplankton density and biomass were rapidly increased (5.6x10^{6} cells/L and 3.91 mg/L, respectively) with dominance of the groups Chlorophyta, Cyanoprokaryota and Bacillariophyta (Table 3). In September, a dominance of Chlorophyta density was observed (3.5x10^{6} cells/L), followed by Cyanoprokaryota (1.4x10^{6} cells/L), while in July the

Taxon	July	September
Cyanoprokaryota		
Anabaena flos-aquae Born. et Frauh.	+	+
Anabaena spiroides Klebahn	+	
Aphanizomenon flos-aque (L.) Ralfs	+ (D)	+
Chroococcus minutus (Kütz.) Näg.	+	
Gomphosphaeria lacustris Chod.		+
Microcystis pulverea (Wood) Forti em. Elenk.		+ (D)
Synechococcus elongatus (Näg.) Näg.		+ (D)
Chlorophyta		
Ankyra judayi (G.M. Smith) Fott	+ (D)	+
Chlamydomonas sp.		+
Elakatothrix genevensis (Leverd.) Hind.	+	+
Eudorina elegans Ehr.	+	+
Eutetramorus planktonicus (Korš.) Bourr.	+	+
Kirchmeriella sp.	+	
Monoraphidium minutum (Näg.) Kom.-Legn.	+	
Oocystis lacustris Chod.	+ (D)	+
Pandorina morum (O.F.Müll.) Bory	+	
Radiococcus planktonicus Lund.		+ (D)
Volvox aureus Ehr.		+
Zygnemophyta		
Closterium acutum Bréb. in Ralfs	+	+
Gonatozygon pilosum Wolle		+
Staurastrum gracile Ralfs var. gracile	+	+
Dinophyta		
Ceratium hirundinella (O.F.M.) Bergh.	+	+
Gymnodinium sp.	+	
Euglenophyta		
Colacium vesiculosum Ehr.	+	
Bacillariophyta		
Asterionella formosa Hass.	+	+
Fragilaria crotonensis Kitt.	+	+
Melosira varians Ag.	+	+ (D)
Stephanodiscus hantzschii Grun.		+
Synedra ulna (Nitzch.) Ehr.	+	

Table 2. Phytoplankton species observed in Borovitsa Reservoir during July and September, 2006. The dominant species are indicated in brackets (D).

Divisio	Density cells/L	%	Biomass mg/L	%
July				
Cyanoprokaryota	1 833 000	55.5	0.140	10.0
Chlorophyta	1 046 200	31.7	0.880	62.4
Zygnemophyta	3 000	0.1	0.003	-
Dinophyta	41 900	1.3	0.040	2.8
Bacillariophyta	377 100	11.4	0.350	24.8
Total:	3 301 200	100.0	1.410	100.0
September				
Cyanoprokaryota	1 423 211	25.4	0.060	1.5
Chlorophyta	3 541 737	63.3	0.400	10.2
Zygnemophyta	44 272	0.8	0.040	1.1
Bacillariophyta	590 290	10.5	3.410	87.2
Total:	5 599 510	100.0	3.910	100.0

Table 3. Quantitative development of the phytoplankton in Borovitsa Reservoir during July and September, 2006.

density of Cyanoprokaryota was dominant. The density of Bacillariophyta was 0.59x10^6 cells/L, but this group represented 87.2% of the total phytoplankton biomass, which is due particularly to the mass growth of the large species *Melosira varians*. During this period, Cyanoprokaryota represented 1.5% from the total phytoplankton biomass of the reservoir.

3.3 Analyses of water samples for presence of cyanotoxins

Collected raw and treated water samples from the reservoir were analyzed for presence of cyanotoxins by HPLC and commercially available ELISA kits for saxitoxins and microcystins. Figure 3A shows the HPLC chromatogram of a standard mixture including anatoxin-a, saxitoxins and microcystin-LR. The chromatogram of the raw water sample collected in July (Figure 3B) revealed the presence of anatoxin-a (7.39 min) and peaks having characteristic spectra of microcystin's isomers with retention time 43.71–46.58 min. ELISA tests detected maximum

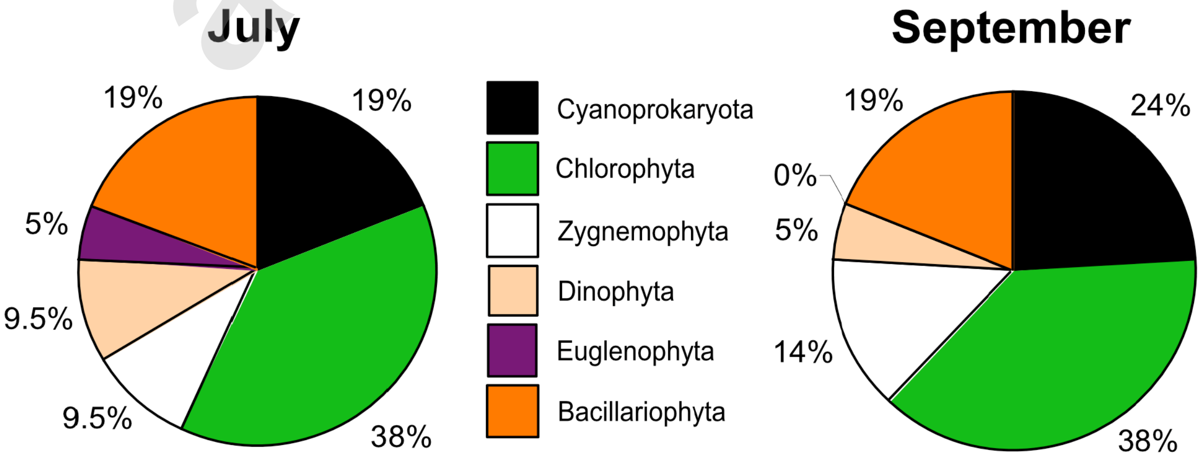

Figure 2. Relative abundance of the phytoplankton taxa (%) in the surface water of Borovitsa Reservoir in July and September 2006.

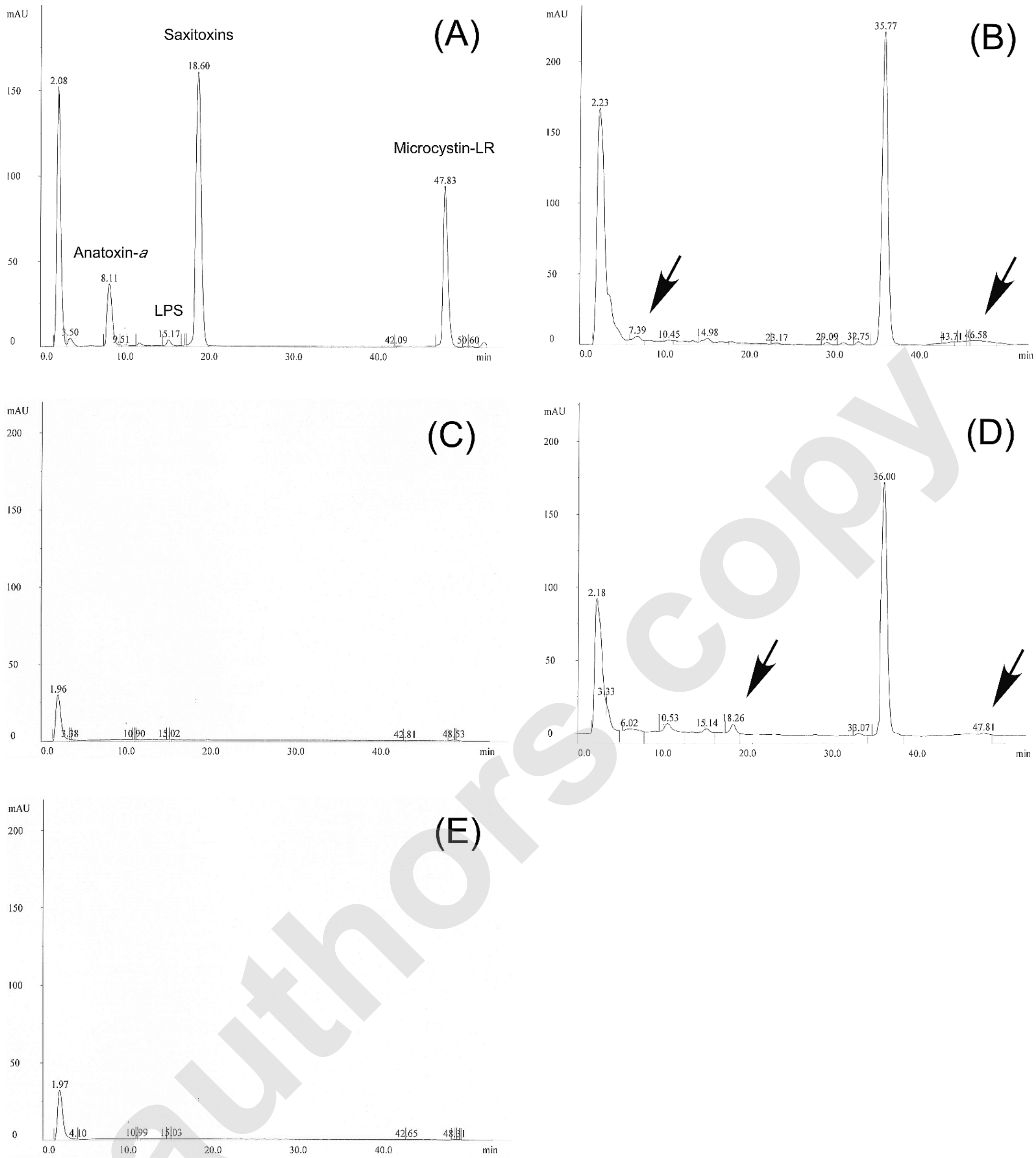

Figure 3. HPLC chromatograms of: (A) a mixture of standard cyanotoxins; (B) raw water collected in July 2006; (C) treated water collected in July 2006; (D) raw water collected in September 2006; (E) treated water collected in September 2006. A volume of 200 μl of each sample was used for HPLC analysis under the conditions described in Experimental Procedures.

values of 0.09-0.12 µg/L of microcystins/nodularins in the examined sample. HPLC and ELISA analyses showed no presence of saxitoxins in the raw water sample collected in July.

Measurable levels of saxitoxins and microcystins, but not anatoxin-a, were detected in the raw water sample collected in September. HPLC analysis of this sample assigned the peaks with retention time 18.26 min and 47.81 min as saxitoxins and microcystins, respectively (Figure 3D). The other detected peaks require more detailed identification steps. According to the saxitoxin ELISA, which has cross reactivity to decarbamoyl saxitoxin, gonyautotoxins II, III, B1, C1 and C2, the raw water sample collected in September contained 2.5 µg/L of this group of cyanotoxins. The total microcystins/nodularins concentration in this sample detected by ELISA, which cross-reacts with microcystin LR, LA, RR, YR and nodularin, was 0.18 µg/L.

In the treated water samples collected in July and September, neither group of cyanotoxins was detectable by HPLC (Figure 3C,E) or ELISA. This indicates that the local water purification process (including coagulation, decantation, filtration, ozonation and chlorination) is quite efficient.

4. Discussion

In this study, we have investigated the phytoplankton diversity and abundance in a drinking-water reservoir with an emphasis on the presence of cyanotoxins and water quality. Although the toxic algal species are object of intensive research worldwide, the aspect of microalgal toxicity is still poorly investigated in Bulgaria. Toxic species are encountered among the dinophytes, chrysophytes and mainly Cyanoprokaryota (blue-green algae). Representatives of these taxonomic groups are frequently encountered in Bulgarian reservoirs, including those used for drinking water supply. In addition, according to the European Union (EU) requirements, the state of different types of lakes within the European Union needs to be established [20].

Borovitsa reservoir showed relatively low phytoplankton diversity with a total of 29 taxa distributed into 6 divisions. Species richness within the divisions showed small fluctuation during the studied periods (Table 2). The dominant group in July was Chlorophyta, as the most frequent species were *Ankyra judayi* and *Oocystis lacustris*. Cyanoprokaryota, such as *Aphanizomenon flos-aquae*, dominated in July, but in September, the phytoplankton was dominated by two coexisting cyanoprokaryotic species (*Microcystis pulverea* and *Synechococcus elongatus*) and one chlorophyte (*Radiococcus planktonicus*).

Reynolds *et al.* [21] suggested a functional classification of the freshwater phytoplankton based on the major abundant species of temperate lakes. Thirty-one functional groups (associations) with typical representatives and specific ecological characteristics were outlined. We were able to assign the phytoplankton species from Borovitsa reservoir to 3 of the 31 assemblages during July and 3 of the 31 assemblages during September.

In July, the first codon X1 assemblage was formed by the chlorophytes *Ankyra judayi* (dominant species) and *Monoraphidium minutum*. This group usually occurs in shallow mixed layers and nutrient enriched lakes [21]. The codon assemblage F was formed by the colonial chlorophytes *Oocystis lacustris* (dominant species), *Eudorina elegans* and *Pandorina morum*, which are known to be represented in the plankton of a wide spectrum of lakes (mainly mesotrophic), but they are sensitive to carbon deficiency. H1 assemblage formed by the dominant cyanoprokaryote *Aphanizomenon flos-aquae*, which bloomed during this period, and the subdominants *Anabaena flos-aquae* and *Anabaena spiroides*, showed the highest density in July. This is a typical dinitrogen-fixing and bloom-forming cyanoprokaryotic group.

In September, L_M and Z assemblages, formed by *Microcystis pulverea*, *Ceratium hirundinella* and *Synechococcus elongatus*, respectively, and the P assemblage formed by *Melosira varians* and *Fragilaria crotonensis*, became dominant groups in Borovitsa reservoir. Albay and Akçaalan [22] showed that in Turkey (Ömerli reservoir, Istanbul), a neighboring country with similar climatic conditions, cyanoprokariotic blooms in September were always dominated by the genus *Microcystis*.

In lakes, the appearance of cyanoprokaryotic blooms is one of the major consequences of eutrophication [23]. Phytoplankton diversity and biomass are related to light, pH values, temperature, mixing and water-level fluctuation, but most significantly to the concentration of nutrients as total nitrogen and total phosphorus [22,24]. Our data showed some differences between the studied periods (July and September) in terms of the phytoplankton density and biomass (Table 3), and environmental factors (Table 2). The surface water temperature of Borovitsa reservoir was decreased with 6.2°C in September (20.2°C) compared to July (26.4°C). No big periodic difference in the water temperature-depth profiles was observed. The values during the sampling periods were between 13.7-13.9°C at a depth of 10 m and 5.7-6.0°C at a depth of 25 m. The strong temperature-depth gradient within the reservoir might facilitate intense vertical water mixing, which can induce changes in the nutrient concentrations in the surface layer [25]. We observed an increase in the concentrations of NO_2^--N and NO_3^--N in September compared to July, accompanied by an increase in the dissolved oxygen concentration (Table 1). These observations are in agreement with the data reported from Yamamoto and Nakahara [25].

The present study reveals that anatoxin-*a* and microcystins appeared during the same period as the blooms of *Aphanizomenon flos-aquae* (July), indicating the predominance of potentially toxin-producing species in the reservoir. Both cyanoprokaryotic species from the assemblage H1, *Aphanizomenon flos-aquae* (dominant) and *Anabaena flos-aquae* (subduminant), observed during this period are potential producers of detected toxins [26]. Maximum values of 2.5 µg/L of saxitoxins and 0.18 µg/L of microcystins/nodularins were found in the samples of raw water when *Microcystis pulverea* occurred as a dominant species (September). On the other hand, microcystins are not released above trace amounts into water until cell lysis and death [27], suggesting that detected cyanotoxins in Borovitsa reservoir could be released after degradation of the cyanoprokaryotic species bloomed during the previous months (mainly *Aphanizomenon flos-aquae*).

Although cyanotoxins were detected only in the raw water and their concentrations were lower than the recommended levels for drinking water, there is an urgent need from monitoring programs of Cyanoprokaryota and their toxins in the reservoirs used as sources of drinking water.

Acknowledgments

This study was financially supported by a Marie Curie European Reintegration Grant to Ivanka Teneva (Contract No. MERG-CT-2007-210514).

References

[1] Van Apeldoorn M.E., Van Egmond H.P., Speijers G.J., Bakker G.J., Toxins of cyanobacteria, Mol. Nutr. Food Res., 2007, 51, 7-60

[2] Funari E., Testai E., Human health risk assessment related to cyanotoxins exposure, Crit. Rev. Toxicol., 2008, 38, 97-125

[3] Chorus I., Bartram J., (Eds.), Toxic Cyanobacteria in water: a guide to their public health consequences, monitoring and management, WHO, E & FN Spon, London, 1999

[4] Hitzfeld B.C., Lampert C.S., Spaeth N., Mountfort D., Kaspar H., Dietrich D.R., Toxin production in cyanobacterial mats from ponds on the McMurdo ice shelf, Antarctica, Toxicon, 2000, 38, 1731-1748

[5] Lakshmana Rao P.V., Gupta N., Bhaskar A.S., Jayaraj R., Toxins and bioactive compounds from cyanobacteria and their implications on human health, J. Environ. Biol., 2002, 23, 215-224

[6] Jochimsen E.M., Carmichael W.W., An J.S., Cardo D.M., Cookson S.T., Holmes C.E., et al., Liver failure and death after exposure to microcystins at a hemodialysis center in Brazil, N. Engl. J. Med., 1998, 338, 873-878

[7] Falconer I., An Overview of problems caused by toxic blue-green algae (cyanobacteria) in drinking and recreational water, Environ. Toxicol., 1999, 14, 5-12

[8] Ito E., Satake M., Ofuji K., Kurita N., McMahon T., James K., et al., Multiple organ damage caused by a new toxin azaspiracid, isolated from mussels produced in Ireland, Toxicon, 2000, 38, 917-930

[9] Duy T.N., Lam P.K., Shaw G.R., Connell D.W., Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water, Rev. Environ. Contam. Toxicol., 2000, 163, 113-185

[10] Pitois S., Jackson M.H., Wood B.G.B., Problems associated with the presence of cyanobacteria in recreational and drinking waters, Int. J. Environ. Health Res., 2000, 10, 203-218

[11] Pavlova V., Babica P., Todorova D., Bratanova Z., Marsalek B., Contamination of some reservoirs and lakes in Republic of Bulgaria by mycrocystins, Acta Hydrochim. Hydrobiol., 2006, 34, 437-441

[12] World Health Organization, Guidelines for Drinking-water Quality, 3rd Ed., Vol.1, Recommendations, World Health Organization, Geneva, 2008

[13] Lund J.W.G., Kipling C., Le Cren E.D., The inverted microscope method of estimating algal numbers and the statistical basis of estimations by counting, Hydrobiologia, 1958, 11, 143-170

[14] ISO 10260, Water Quality - Measurement of Biochemical Parameters - Spectrophotometric Determination of Chlorophyll Concentration. Geneva, Switzerland, ISO, 1992, (E), 1-6

[15] Carlson R.E., A trophic state index for lakes, Limnology and Oceanography, 1977, 22, 361-369

[16] Teneva I., Dzhambazov B., Koleva L., Mladenov R., Schirmer K., Toxic potential of five freshwater Phormidium species (Cyanoprokaryota), Toxicon, 2005, 45, 711-725

[17] Hecky R.E., Kilham P., Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment, Limnol. Oceanogr., 1988, 33, 796-822

[18] Downing J.A., McCauley E., The nitrogen: phosphorus relationship in lakes, Limnol. Oceanogr., 1992, 37, 936-945

[19] Yang X.E., Wu X., Hao H.L., He Z.L., Mechanisms and assessment of water eutrophication, J. Zhejiang Univ. Sci. B, 2008, 9, 197-209

[20] EC, Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy, Off. J. Eur. Communities, 2000, L375, 1-8

[21] Reynolds C.S., Huszar V., Kruk C., Naselli-Flores L., Melo S., Towards a functional classification of the freshwater phytoplankton, J. Plankton Res., 2002, 24, 417-428

[22] Albay M., Akçaalan R., Factors influencing the phytoplankton steady state assemblages in a drinking-water reservoir (Ömerli reservoir, Istanbul), Hydrobiologia, 2003, 502, 85-95

[23] Conley D.J., Paerl H.W., Howarth R.W., Boesch D.F., Seitzinger S.P., Havens K.E., et al., Ecology. Controlling eutrophication: nitrogen and phosphorus, Science, 2009, 323, 1014-1015

[24] Sun L., Jin X.C., Zhong Y., Zhi Y.L., Li H., Zhou Q.X., et al., Seasonal dynamics of phytoplankton in relation to key aquatic habitat factors in a polluted urban small water body in Tianjin, China, Bull. Environ. Contam. Toxicol., 2009, 82, 543-548

[25] Yamamoto Y., Nakahara H., Seasonal variations in the diel vertical distribution of phytoplankton and zooplankton in a shallow pond, Phycol. Res., 2006, 54, 280-293

[26] Falconer I., Cyanobacterial toxins of drinking water supplies: cylindrospermopsins and microcystins, CRC Press, 2005

[27] Codd G.A., Cyanobacterial toxins: occurrence, properties and biological significance, Water Sci. Technol., 1995, 32, 149-156