Arch. Biol. Sci., Belgrade, 67(2), 355-366, 2015 DOI:10.2298/ABS141023024P

Diversity of Cyanobacteria in the Zasavica River, Serbia

Dragana Predojević[1,*], Slađana Popović[1], Željko Kljajić[2], Gordana Subakov-Simić[1], Ana Blagojević[1], Jelena Jovanović[1] and Milojko Lazić[2]

[1] *Faculty of Biology, Institute of Botany and Botanical Garden "Jevremovac", University of Belgrade*, 11000 Belgrade, Serbia

[2] *Faculty of Mining and Geology, Department of Hydrogeology, University of Belgrade*, 11000 Belgrade, Serbia

***Corresponding author**: delfin0230@yahoo.com

Abstract: Cyanobacteria are ancient organisms that are capable of colonizing different habitats in various climatic zones due to their plasticity and rapid accommodation. They are a widely studied group of microorganisms due to the presence of many potentially toxic and invasive species. The aim of this research was a diversity exploration of the freshwater Cyanobacteria in the Zasavica River, which is part of the Special Nature Reserve "Zasavica" in Serbia. Organisms were sampled once a month at two study sites during one year. Phytoplankton and metaphyton analysis showed the presence of 50 freshwater cyanobacterial taxa, of which 12 are new taxa for Serbia. Three invasive and potentially toxic species (*Cylindrospermopsis raciborskii*, *Sphaerospermopsis aphanizomenoides* and *Raphidiopsis mediterranea*) were recorded only in metaphyton in April at one site. It can be expected that, if conditions change, this species can migrate and form phytoplankton blooms.

Key words: Cyanobacteria; Zasavica; toxic; invasive

Received October 23, 2014; **Revised** January 20, 2015; **Accepted** January 22, 2015

INTRODUCTION

Cyanobacteria, also known as blue-green algae, are ancient, cosmopolitan inhabitants of fresh, brackish and marine environments, of soil and moist surfaces. Their plasticity and rapid accommodation make them very successful organisms that are able to colonize different habitats in various climatic zones. Cyanobacteria are a natural component of phytoplankton in most of the world's surface waters. They fulfill key roles in the biogeochemical cycling of matter and in the structure, maintenance and biodiversity of microbial and higher organism communities (Codd et al., 2005). However, high cyanobacterial biomass has long been recognized mainly as a cause of taste and odor problems in freshwater bodies, but it also contributes to aesthetic problems, reduces recreational use, etc.

The increase in human population and the consequent intensification of agriculture and industry along with deficient water management lead to the enhancement of eutrophication in many freshwater bodies (De Figueiredo et al., 2004). The occurrence of phytoplanktonic blooms, caused by the appearance of cyanobacteria in high biomass, is also becoming more frequent worldwide. Many cyanobacterial species produce toxins that cause mortality or illness in freshwater organisms, livestock and even humans (Chorus at al., 2000; Codd, 2000; Duy et al., 2000; Hitzfeld et al., 2000; Carmichael, 2001; De Figueiredo et al., 2004; Svirčev et al., 2009; Karadžić, 2011). The presence of algal toxins in the aquatic environment (Svirčev at al., 2007, 2009, 2013) has been recognized as a serious problem and is of important socioeconomic concern in many places worldwide. Knowledge about the function of these species, their toxins and the distribution of particular compounds that cyanobacteria of different genotypes, morphotypes and ecology produce, is important for hydrobiology and water quality monitoring, as well as theoretical studies about diversity and chemotaxonomy (Hrouzek, 2010). Noteworthy is that not all cyanobacterial blooms are toxic, neither are all strains within one species. Toxic and non-toxic strains show no predictable difference in appearance (Spoof et al., 2003). Environmental conditions such as higher temperature

Table 1. Results of physicochemical analysis during the study period (from December 2012 to November 2013) at both study sites.

	Month	Temperature (°C)	Transparency (m)	Conductivity (μs cm^{-1})	Depth (m)	pH	Thickness of ice (m)	Dissolved oxygen (mg L^{-1})
M	XII	0.6	1	250	1	8.42	0.07	15.1
	I	4.5	1.4	420	1.4	8.38	0	12.6
O	II	6.7	0.9	470	1.4	8.07	0	10.5
	III	15.2	1.2	780	1.2	8	0	12.1
L	IV	19.6	1.4	700	1.4	7.92	0	10.2
	V	18.7	1.7	760	1.7	7.77	0	3.9
O	VI	22.6	1.3	710	1.4	8.1	0	9
	VII	28.3	0.95	585	1.1	7.81	0	9.27
	VIII	20.9	0.95	500	0.95	7.75	0	0.24
	IX	13.2	0.95	595	0.95	7.6	0	3.45
	X	14.7	1.1	680	1.1	7.88	0	6.45
	XI	0.4	1.2	438	1.2	8.45	0	13.12
M	XII	2.1	0.8	480	0.9	8.82	0.1	14.1
	I	3.3	1.35	462	1.35	8.4	0	15.2
O	II	6.2	1.3	500	1.3	8.08	0	12
	III	14.4	1.3	820	1.3	7.87	0	11.5
S	IV	18.8	1.3	745	1.3	7.8	0	7.5
	V	18.1	1.7	725	1.7	7.79	0	1.92
T	VI	21.2	1.3	720	1.3	7.6	0	1.59
	VII	25	0.2	600	1	7.56	0	7.85
I	VIII	19.4	0.48	472	0.48	8	0	2.16
	IX	12.6	0.75	485	0.75	8	0	5.6
Ć	X	11	0.4	490	0.4	8.14	0	5.98
	XI	3.9	0.9	598	0.9	8.23	0	11.9

and pH values, low turbulence and high nutrient inputs (particularly phosphorus, as well as nitrogen) favor the development of planktonic cyanobacteria. Some advantageous characteristics, such as lower nutrient requirements and buoyancy regulation in the water column for achieving better light and nutrient level conditions, make some species dominant at the surface water layer (De Figueiredo et al., 2004).

There are numerous papers from the previous floristic, taxonomic and ecological studies in Serbia that contain information about taxa from Cyanobacteria division. The presence of Cyanobacteria in Serbia is first mentioned in a hydrobiology study by Schaarschmidt (1883). The aim of this research was to explore the diversity of freshwater cyanobacteria in the River Zasavica with special attention to the presence of potentially toxic species.

MATERIALS AND METHODS

The Zasavica River is the center of the Special Nature Reserve "Zasavica" in Serbia (Predojević et al., 2014; Predojević et al., 2015) which has very high biodiversity. This river can be characterized as both flowing and standing water according to its properties.

The samples for physicochemical and biological analyses from the Zasavica River were taken

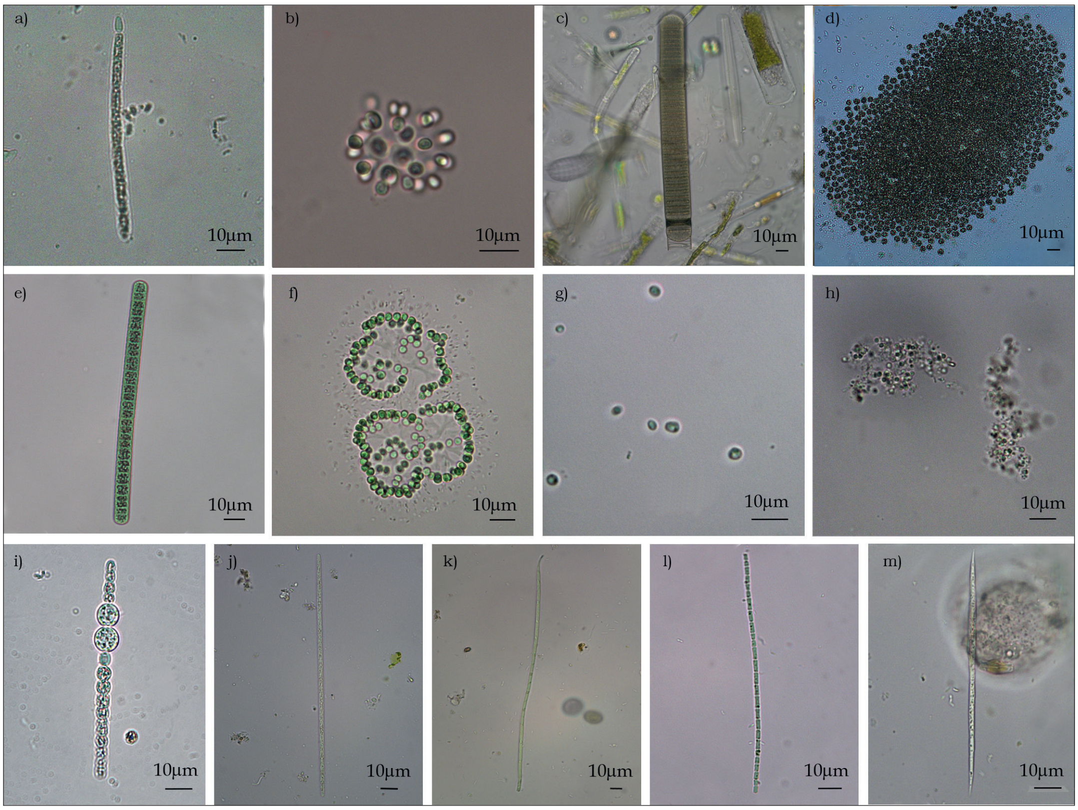

Fig 1. Photographs of potentially toxic Cyanobacteria taxa in the Zasavica River during the study period: (a) *Cylindrospermopsis raciborskii*; (b) *Woronichinia naegeliana*; (c) *Oscillatoria limosa*; (d) *Microcystis aeruginosa*; (e) *Planktothrix isothrix*; (f) *Snowella lacustris*; (g) *Synechocystis aquatilis*; (h) *Aphanocapsa holsatica*; (i) *Sphaerospermopsis aphanizomenoides*; (j) *Aphanizomenon klebahnii*; (k) *Geitlerinema acutissimum*; (l) *Pseudanabaena catenata*; (m) *Raphidiopsis mediterranea*

once a month from December 2012 to November 2013 at two study sites, “Molo” (44°57’26.14” N and 19°31’37.58” E) and “Mostić” (44°56’59.26” N and 19°29’38.84” E).

The main physical parameters were measured using the following standard analytical methods and instruments: a multi digital thermometer for measuring the temperature, ECTestr 11+ multi range for electrical conductivity and WTW multi 3430 multiparameter meter for measuring pH value and dissolved oxygen. The transparency of the river was measured by Secchi disc.

The samples for qualitative phytoplankton analysis were collected at these two study sites by towing a plankton net (pore diameter 22 μm) through the open water. In the field, phytoplankton samples were placed in a plastic bottle (0.1 L) and preserved with Lugol’s solution. One more sample that was taken for qualitative analysis was not preserved immediately, but after exploration on the same day of sampling. The metaphyton samples were collected by towing a plastic bottle (0.1 L) through the water near the bank, among the submerged vegetation. These samples were fixed with Lugol’s solution immediately.

Qualitative analysis of algological material was performed using a Carl Zeiss AxioImager M1 microscope and digital camera AxioCam MRc5 with AxioVision 4.8 software. Taxonomic identifications of Cyanobacteria were made according to widely used taxonomic keys (Komárek, 2013; Komárek and Anagnostidis, 1998, 2005).

Phytoplankton samples for quantitative analysis were collected using a Ruttner bottle (1 L volume), after which they were put in 1 L plastic bottles and fixed with Lugol’s solution. Phytoplankton quantitative analysis was performed with a Leica inverted microscope using the Utermöhl method (Utermöhl, 1958). The results were expressed as the number of cells mL^{-1} and number of individuals mL^{-1}.

All samples were analyzed and stored in the Institute of Botany and Botanical Garden “Jevremovac” at the Faculty of Biology, University of Belgrade.

Table 2. The list of all identified Cyanobacteria taxa from December 2012 to November 2013 in the phytoplankton and metaphyton of the Zasavica River. The new taxa for Serbia are marked with an asterisk.

Cyanobacteria taxa	**Phytoplankton**		**Metaphyton**	
	"Molo"	**"Mostić"**	**"Molo"**	**"Mostić"**
Chroococcales				
**Aphanocapsa conferta* (W. et G.S. West) Komárkova-Legnerová et Cronberg	+	+		
Aphanocapsa holsatica (Lemmermann) Cronberg et Komárek	+	+		
**Aphanocapsa nubilum* Komárek et Kling	+			
Chrococcus minutus (Kützing) Nägeli	+	+		
**Chrococcus subnudus* (Harsgirg) Cronberg et Komárek		+		
Chrococcus turgidus (Kützing) Nägeli	+	+		+
**Cyanobiumplancticum* (Drews) Komárek et al.	+	+		
Cyanothece Komárek sp.	+			
Merismopedia glauca (Ehrenberg) Kützing	+		+	
**Merismopedia hyalina* (Ehrenberg) Kützing	+	+		

Cyanobacteria taxa	**Phytoplankton**		**Metaphyton**	
	“Molo”	**“Mostić”**	**“Molo”**	**“Mostić”**
Merismopedia tenuissima Lemmermann		+	+	
Microcystis aeruginosa (Kützing) Kützing	+	+		
Microcystis firma (Kützing) Schmidle	+			
**Pannus planus* Hindák		+		
Snowella lacustris (Chodat) Komárek et Hindák	+	+		
**Synechococcus sigmoideus* (Moore et Carter) Komárek			+	
**Synechocystis aquatilis* Sauvageau		+		
Woronichinia compacta (Lemmermann) Komárek et Hindák	+	+	+	+
Woronichinia naegeliana (Unger) Elenkin	+	+		
Woronichinia ruzickae Komárek et Hindák	+	+		+
Oscillatoriales				
Arthrospira jenneri Stizenberger ex Gomont	+			
**Geitlerinema acutissimum* (Kufferath) Anagnostidis	+	+	+	+
Geitlerinema amphibium (Agardh ex Gomont) Anagnostidis	+	+	+	+
Limnothrix planctonica (Woloszyńska) Meffert	+	+	+	
Oscillatoria limosa Agardh ex Gomont	+	+	+	+
Oscillatoria tenuis Agardh ex Gomont	+			
Phormidium chalybeum (Mertens ex Gomont) Anagnostidis et Komárek		+	+	
Phormidium chlorinum (Kützing ex Gomont) Anagnostidis	+	+	+	
**Phormidium chlorinum* var. *perchlorina* Lauterborn	+	+		
Phormidium granulatum (Gardner) Anagnostidis	+			+
Phormidium Kützing ex Gomont sp.		+		
Phormidium tergestinum (Kützing) Anagnostidis et Komárek	+	+	+	+
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová et Cronberg	+	+		
Planktothrix isothrix (Skuja) Komárek et Komárková	+	+		+
Planktothrix cryptovaginata (Schkorbatov) Anagnostidis et Komárek	+	+		
**Pseudanabaena articulata* Skuja	+			
Pseudanabaena catenata Lauterborn	+	+	+	+
Pseudanabaena limnetica (Lemmermann) Komárek	+	+	+	+
Pseudanabaena papillaterminata (Kiselev) Kukk	+	+		+
Pseudanabaena Lauterborn sp.				+
**Schizothrix vaginata* (Nägeli) Gomont	+	+	+	+
Spirulina Turpin ex Gomont sp.		+		
Nostocales				
Anabaena Bory De Saint-Vincent ex Bornet & Flahault sp. 1	+	+	+	+
Anabaena Bory De Saint-Vincent ex Bornet & Flahault sp. 2	+	+		+
Aphanizomenon klebahnii Elenkin ex Pechar			+	
Cylindrospermopsis raciborskii (Woloszyńska) Seenayya et Subba Raju			+	
Cylindrospermum stagnale (Kützing) ex Bornet et Flahault	+	+		
Dolichospermum viguieri (Denis et Frémy) Wacklin et al.	+	+	+	+
Raphidiopsis mediterranea Skuja			+	
Sphaerospermopsis aphanizomenoides (Forti) Zapomelová et al.			+	

RESULTS

Physicochemical analysis

Results of physicochemical analysis during the study period are given in Table 1. From these results it can be concluded that the Mostić site is characterized by fewer temperature fluctuations. At both sites, the higher the temperature, the greater the abundance of Cyanobacteria. Water level was low, but variable. According to values of pH of the water, it can be concluded that the water of the Zasavica River is slightly alkaline. The values of water conductivity of the Zasavica River can be characterized as medium-high.

Biological analysis

Exploration of all samples of phytoplankton and metaphyton from both study sites showed the presence of about 450 taxa from seven divisions: Cyanobacteria, Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Euglenophyta and Pyrrophyta. From the total number of taxa, there are 50 cyanobacterial taxa (Table 2) in the Zasavica River. From the total number of recorded taxa, 12 are new for Serbia and they are marked with an asterisk in Table 2. Some of the photographs of cyanobacterial taxa are given in Fig. 1.

At the Molo site, cyanobacterial abundance ranged from a minimal value of 0 individuals mL^{-1} in January and April, to maximal value of 302 individuals mL^{-1} in August (Fig. 2a). Cyanobacterial abundance expressed as number of cells mL^{-1} on the same site shows the same pattern, minimal value of 0 cell mL^{-1} in January and April and maximal value of 6 838 cell mL^{-1} in August (Fig. 2b). At the Mostić site, the number of individuals mL^{-1} ranged from 0.8 individuals mL^{-1} in December, to 528 individuals mL^{-1} in July (Fig. 2a). Minimal abundance at Mostić expressed as the number of cells mL^{-1} was in December (11.2 cell mL^{-1}) while maximal abundance was in August (13 888 cell mL^{-1}) like at the Molo site (Fig. 2b). The percentage of Cyanobacteria in the phytoplankton of the Zasavica River compared to the other divisions (Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Euglenophyta and Pyrrophyta) from December 2012 to November 2013, when the abundance is expressed as a number of individuals mL^{-1}, showed the highest values during summer months (Fig. 3). The percentage of Cyanobacteria (when the abundance is expressed as the number of cells mL^{-1}) in the phytoplankton of the Zasavica River compared to the other divisions (Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Euglenophyta and Pyrrophyta) during the study period showed the same pattern, but higher values (Fig. 4).

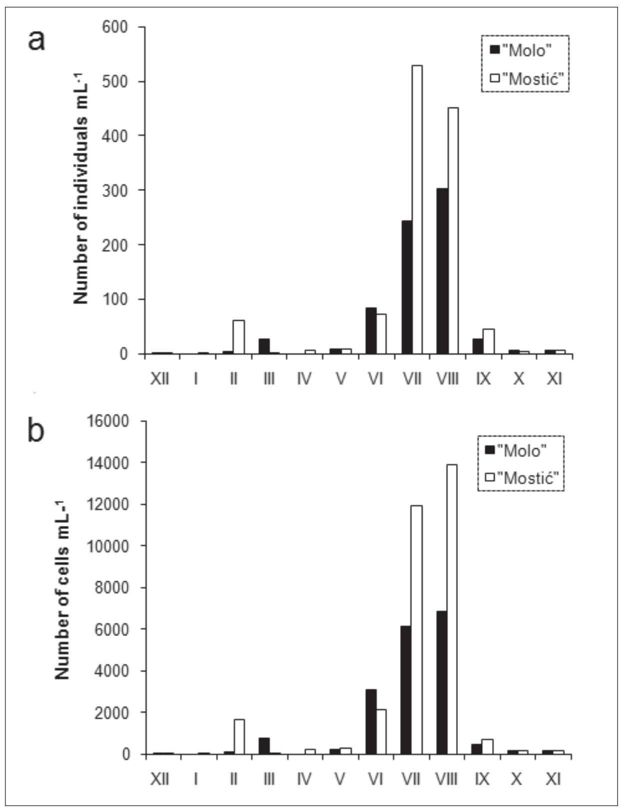

Fig. 2. Cyanobacterial abundances expressed as (a) number of individuals mL^{-1} and (b) number of cells mL^{-1}, for both sites during the study period.

A dense development of submerged (*Ceratophyllum* sp.) and floating (*Salvinia natans*) plants was noticed from April to July at both study sites. In addition, when examining the phytoplankton and metaphyton samples a lot of grazing zooplankters were noticed per sample.

DISCUSSION

According to published data (Obušković et al., 1994; Cvijan and Blaženčić, 1996; Urošević, 1996, 1997a, b, 1998; Urošević and Savić, 1996; Simić, 1996; Veljić and Cvijan, 1997; Obušković and Obušković, 1998; Pujin et al., 1998; Jurišić et al., 1999; Nikitović and Laušević, 1999; Makovinská et al., 2002; Nemeth et al., 2002; Simić, 2002; Ržaničanin et al., 2003; Simić et al., 2004; Subakov Simić et al., 2004, 2006, 2007; Ranković et al., 2006; Fužinato et al., 2007; Anđelković, 2008; Svirčev et al., 2008; Vučković and Mirjačić-Živković, 2008; Ljaljević Grbić et al., 2010; Cvijan and Fužinato, 2011; Karadžić, 2011; Ćirić, 2013; Đorđević and Simić, 2014; Simić et al., 2014; Svirčev et al. 2014), a total number of 327 cyanobacterial taxa is present in Serbia, among which are both freshwater as well as terrestrial cyanobacteria.

A total of 50 freshwater cyanobacterial taxa from 27 genera belonging to the orders Chroococcales, Oscillatoriales and Nostocales were recorded during this research. Since this is almost one-sixth of all taxa recorded in Serbia found in Zasavica River, it could be concluded that this is a locality with high diversity of freshwater cyanobacteria. However, it is documented that there are localities with even higher cyanobacterial diversity in Serbia (for example the River Ponjavica with 76 cyanobacterial taxa detected (Karadžić et al., 2013)). The River Zasavica, which is characterized both as flowing and standing water, provides a suitable habitat for the development of phytoplankton. In addition, during the study period transparency was very high, often to the bottom, which also favored phytoplankton development.

Algae not recorded earlier in published data represent new taxa of freshwater cyanobacteria for Serbia. A total of 12 new taxa of freshwater cyanobacteria in the River Zasavica can be added

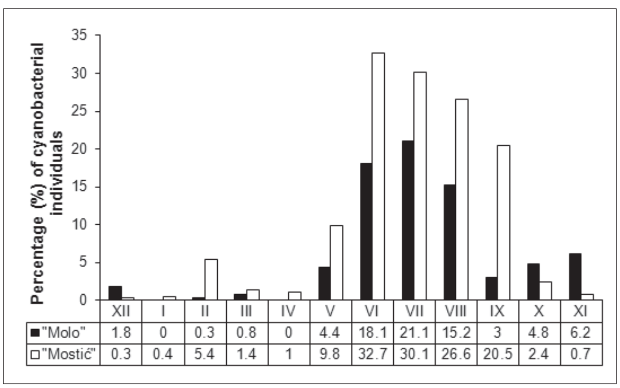

Fig. 3. The percentage of the Cyanobacteria division in the phytoplankton of the Zasavica River compared to the other divisions (Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Euglenophyta and Pyrrophyta) from December 2012 to November 2013, when the abundance was expressed as a number of individuals mL^{-1}.

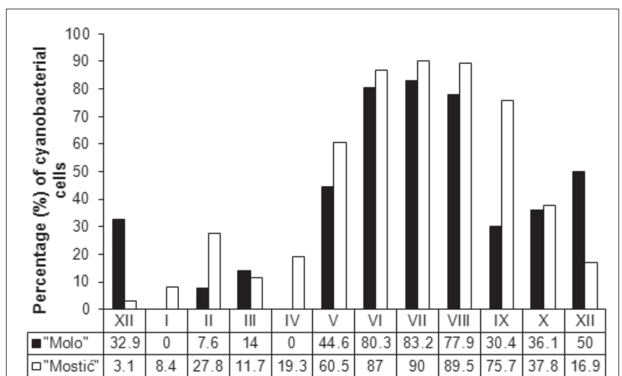

Fig. 4. The percentage of the Cyanobacteria division in the phytoplankton of the Zasavica River compared to the other divisions (Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Euglenophyta and Pyrrophyta) during the study period, when the abundance was expressed as the number of cells mL^{-1}.

to the former list of cyanobacteria, which makes a total number of 339 cyanobacterial taxa documented in Serbia so far. In an earlier exploration of the phytoplankton of the River Zasavica, a total of 31 cyanobacterial taxa were recorded, of which seven are not documented in this research: *Nostoc* Adanson sp., *Planktothrix rubescens* (De Candolle ex Gomont) Anagnostidis and Komárek (recorded as *Oscillatoria rubescens* (D.C.) Gomont), *Anabaena solitaria* Klebahn, *Chroococcus limneticus* Lemmerman, *Phormidium inundatum* Kützing, *Phormidium formosum* (Bory de Saint-Vincent ex Gomont) Anagnostidis and Komárek (recorded as *Oscillatoria formosa* Bory) and *Phormidium autumnale* (Agardh) Gomont. With their addition to the list of cyanobacterial taxa in the River Zasavica, the total number of cyanobacteria in the phytoplankton is 57.

About 13 taxa of cyanobacteria (*Aphanocapsa holsatica*, *Microcystis aeruginosa*, *Snowella lacustris*, *Synechocystis aquatilis*, *Woronichinia naegeliana*, *Geitlerinema acutissimum*, *Oscillatoria limosa*, *Planktothrix isothrix*, *Pseudanabaena catenata*, *Aphanizomenon klebahnii*, *Cylindrospermopsis raciborskii*, *Raphidiopsis mediterranea* and *Sphaerospermopsis aphanizomenoides*), which are potentially toxic according to Carmichael (1992), Domingos et al. (1999), Sivonen and Jones (1999), De Hoyos et al. (2004) and Bláha et al. (2009), were present in the River Zasavica. Invasive and potentially toxic taxa *Cylindrospermopsis raciborskii*, *Sphaerospermopsis aphanizomenoides* and *Raphidiopsis mediterranea* were recorded only in metaphyton at the Molo site in April. For the first time in Serbia, *Cylindrospermopsis raciborskii* was recorded in Kapetanski rit and after that in Slatina near Opovo (Cvijan and Fužinato, 2011). A *C. raciborskii* bloom has very high toxic potential and may cause adverse effects on human health (Briand et al., 2004). It is known that this species can produce saxitoxin, anatoxin-a and cylindrospermopsin (Chorus and Bartran, 1999). For the first time in Serbia, a bloom of this cyanobacteria was detected in the River Ponjavica in 2008 (Karadžić et al., 2013) and later on in the Aleksandrovac reservoir (Simić et al., 2011; Simić et al., 2014; Đorđević and Simić, 2014). So far, *C. raciborskii* has been detected in many European countries: Hungary (Padisák, 1997), Slovakia (Hindák, 1998; Maršálek et al., 2000), Germany (Stüken et al., 2006; Fastner et al., 2007), Portugal (Saker et al., 2003a, b), France (Briand et al., 2004), Austria (Dokulil and Mayer, 1996), Czech Republic (Dvořák and Hašler, 2007), Italy (Manti et al., 2005), Greece (Vardaka et al., 2005; Moustaka-Gouni et al., 2009), Spain (De Hoyos et al., 2004) and Poland (Stefaniak et al., 2005). *Raphidiopsis mediterranea* was detected for the first time in Serbia in 1949 at Obedska bara (Milovanović, 1949) and again in 1996 (Martinović-Vitanović, 1996). Later, this cyanobacterium was documented in the River Ponjavica (Karadžić et al., 2013). *R. mediterranea*, which produces homoanatoxin-a and anatoxin-a (Namikoshi et al., 2003; Watanabe et al., 2003; Mohamed, 2007) rarely exists in high abundance and its bloom was not detected in Serbia. *Sphaerospermopsis aphanizomenoides,* cyanobacteria that produce microcystins, was recorded for the first time in Serbia in the River Ponjavica (Karadžić et al., 2013). This invasive and potentially toxic taxon was found in many other European countries too: Hungary (Kaštovský et al., 2010), Slovakia (Hindák, 2000), Great Britain (John et al., 2002), Greece (Vardaka et al., 2005), Poland (Stefaniak et al., 2005), Germany (Stüken et al., 2006), France (Brient et al., 2009) and Czech Republic (Kaštovský et al., 2010). During this research, these three species were not abundant and just a few individuals of each taxon were recorded.

These three invasive and potentially toxic species did not cause any bloom although conditions were favorable, which is probably due to the stable state among phytoplankton and submerged vegetation. Submerged vegetation was well developed from April to July as were grazing zooplankters. Grazing zooplankters that are especially numerous within the submerged vegetation probably go out at night to graze on any developing phytoplankton. This keeps the water clear and provides good light conditions for the submerged vegetation. It seems that the submerged vegetation provides refuge for the animals against fish predation and allows large and effective grazer communities to coexist with the fish. In the places where such refuges are absent, for example, in the turbid basin, fish remove most of the grazers. This allows the development of phytoplankton algae, which reduce the light that is needed for developing plants (Moss, 1998). Finally, plant-dominated communities that have important roles in this river can be lost if the artificial eutrophication becomes higher and more rapid, because then the phytoplankton will be dominant. The thick layer of sludge at the bottom of the river and the depth of the river of about 1.1 m at medium water level, which is lower than the depth recorded about 10 years ago (2.5 m) (Stanković, 2006), provide good proof for the natural process of eutrophication. In an alternative stable state with phytoplankton dominance, it can be expected that potentially toxic and invasive cyanobacteria taxa from metaphyton will form blooms. Because of this, further monitoring is recommended.

The seasonal dynamics of Cyanobacteria are in accordance with their ecology (Komárek, 2013; Komárek and Anagnostidis, 1998, 2005). They had the highest abundance and highest percentage compared to taxa from other divisions (Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Euglenophyta and Pyrrophyta) during the summer months. When the abundance was expressed as the number of cells mL^{-1}, the percentage of Cyanobacteria as compared to other divisions (Bacillariophyta, Chlorophyta, Cryptophyta, Chrysophyta, Euglenophyta and Pyrrophyta) was much higher than when the percentage of abundance was expressed as the number of individuals mL^{-1}. This is due to the morphological properties of cyanobacterial taxa. Most of the recorded cyanobacterial taxa are trichal or colonial, which is the reason why the number of cells per individual is sometimes very high. Their high abundance is in positive correlation with low values of dissolved oxygen and high water temperature.

The diversity of cyanobacteria in the River Zasavica has changed over the last twenty years. Some of the taxa have disappeared while some new, potentially toxic and invasive, have appeared. The ecosystem is under self-control for now. If the anthropogenic impact in the future remains insignificant, changes in cyanobacterial diversity and abundance in the phytoplankton can be considered a consequence of the natural process of succession and aging of this ecosystem.

Acknowledgments: This research was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, Project No. ON 176020.

Authors' contribution: All authors contributed equally. Physicochemical analyses were done by Željko Kljajić and Milojko Lazić, while phytoplankton analyses were done by Dragana Predojević, Slađana Popović, Ana Blagojević, Jelena Jovanović and Gordana Subakov-Simić.

REFERENCES

Anđelković, A. J. (2008). Biological wastewater treatment by biodisc as contribution to an efficient and sustainable water exploitation. 37th Annual Conference of Yugoslav Water Pollution Control Society „Water 2008", Conference Proceedings, Mataruška Banja, 375-380.

Bláha, L., Babica, P. and *B. Maršálek* (2009). Toxins produced in cyanobacterial water blooms toxicity and risks. *Interdisc. Toxicol.* **2(2)**, 36-41.

Briand, J.F., Leboulanger, C., Humbert, J.F., Bernard, C. and *P. Dufour* (2004). *Cylindrospermopsis raciborskii* (cyanobacteria) invasion at mid-latitudes: selection, wide physiological tolerance, or global warming? *J. Phycol.* **40**, 231-238.

Brient, L., Lengronne, M., Bormans, M. and *J. Fastner* (2009). First occurence of Cylindrospermopsin in freshwater in France. *Environ. Toxicol.* **24(4)**, 415-420.

Carmichael, W. W. (1992). Cyanobacteria secondary metabolites – the cyanotoxins. *J. Appl. Bacteriol.* **72**, 445-459.*Carmichael, W. W.* (2001). A mini-review of cyanotoxins: toxins of cyanobacteria (blue-green algae), In: *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium* (Eds. , W. J. De Koe, R. A. Samson, H. P. Van Egmond, J. Gilbert and J. M. Sabino), 495-504. Ponsen & Looyen, Wageningen.

Chorus, I. and *J. Bartram* (1999). *Toxic Cyanobacteria in Water: A Guide to the Public Health Consequences, Monitoring and Management*. E & FN Spon, London.

Chorus, I., Falconer, I.R., Salas, H.J. and *J. Bartram* (2000). Health risks caused by freshwater cyanobacteria in recreational waters. *J. Toxicol. Env. Heal. B* **3**, 323-347.

Codd, G. A. (2000). Cyanobacterial toxins, the perception of water quality and the prioritization of eutrophication control. *Ecol. Eng.* **16**, 51-60.

Codd, G.A., Morrison, L.F. and *J.S. Metcalf* (2005). Cyanobacterial toxins: risk management for health protection. *Toxicol. Appl. Pharm.* **203 (3)**, 264-272.

Cvijan, M. and *J. Blaženčić* (1996). *Flora algi Srbije, Cyanophyta 1*. "Naučna knjiga", Beograd.

Cvijan, M. and *S. Fužinato* (2011). The first finding of *Cylindrospermopsis raciborskii* (Woloszińska) Seenayya et Subba raju, 1972 (cyanoprokaryota) in Serbia. *Arch. Biol. Sci.* **63(2)**, 507-510.

Ćirić, M. (2013). *Uticaj različitih tipova dodatne hrane u poluintenzivnoj proizvodnji mlađi šarana (Cyprinus carpio) na strukturu i dinamiku ribnjačkog ekosistema*. Doktorska disertacija, Poljoprivredni fakultet, Univerzitet u Beogradu, Beograd.

De Figueiredo, R.D., Azeiteiro, M.U., Esteves, M.S., Fernando, J.M., Gonçalves, J.M.F. and *J.M. Pereiraa* (2004). Microcystin-producing blooms – a serious global public health issue. *Ecotox. Environ. Safe.* **59**, 151-163.

De Hoyos, C., Negro, A.I. and *J.J. Aldasoro* (2004). Cyanobacteria distribution and abundance in the Spanish water reservoirs during thermal stratification. *Limnetica* **23(1-2)**, 119-132.

Dokulil, M.T. and *J. Mayer* (1996). Population dynamics and photosynthetic rates of a *Cylindrospermopsis-Limnothrix* association in a highly eutrophic urban lake, Alte Donau, Vienna, Austria. *Algol. Studies* **83**, 179-195.

Domingos, P., Rubim, T.K., Molica, R.J.R., Azevedo, S.M.F.O. and *W.W. Carmichael* (1999). First report of microcystin production by picoplanktonic cyanobacteria isolated from a northeast Brazilian drinking water supply. *Environ. Toxicol.* **14(1)**, 31-35.

Duy, T.N., Lam, P.K.S., Shaw, G.R. and *D.W. Connell* (2000). Toxicology and risk assessment of freshwater cyanobacterial (blue-green algae) toxins in water. *Rev. Environ. Contam. Toxicol.* **163**, 113-186.

Dvořák, P. and *P. Hašler* (2007). Occurrence and morphological variability of *Cylindrospermopsis raciborskii* (WOLOSZ.) SEENAYYA et SUBBA RAJU (Cyanophyta, Nostocales) near Olomouc in 2006. *Fottea* **7(1)**, 39-42.

Đorđević, N. and *S. Simić* (2014). Cyanobacterial blooms in oligosaline, alkaline microaccumulation before and after rehabilitation. *Pol. J. Environ. Stud.* **23(6)**, 1975-1982.

Fastner, J., Rücker, J., Pastüken, A., Preuβel, K., Nixdorf, B., Chorus, I., Köhler, A. and *C. Wiedner* (2007). Occurrence of the Cyanobacterial Toxin Cylindrospermopsin in Northeast Germany. *Environ. Toxicol.* **22(1)**, 26-32.

Fužinato, S., Fodora, A., Subakov-Simić, G. and *J. Krizmanić* (2007). Algological research of the Novo Ilje I and the Novo Ilje II near Melenci (Vojvodina, Serbia). Zbornik radova 36. godišnje konferencije o aktuelnim problemima korišćenja i zaštite voda „Voda 2007", Tara, 141-146.

Hindák, F. (1998). Planktic species of two related genera *Cylindrospermopsis* and *Apnabaenopsis* from Western Slovakia. *Arch. Hydrobiol. Suppl.* **80**, 283-302.

Hindák, F. (2000). Morphological variation of four planktic nostocalean cyanophytes- members of the genus *Aphanizomenon* or *Anabaena*? *Hydrobiologia* **438**, 107-116.

Hitzfeld, B.C., Hoger, S.J. and *D.R. Dietrich* (2000). Cyanobacterial toxins: removal drinking water treatment and human risk assessment. *Environ. Health Persp.* **108**, 113-122 (Supplement).

Hrouzek, P. (2010). *Cytotoxicity and secondary metabolites production in cyanobacteria*. Ph. D. Thesis, University of South Bohemia, Faculty of Science, České Budějovice, Czech Republic.

John, M.D., Whitton, A.B. and *J.A. Brook* (2002). The freshwater algal flora of the British Isles: an identification guide to freshwater and terrestrial algae. The Natural History Museum, Cambridge.

Jurišić, I., Kalinić, S., Jovanović, M. and *N. Purić* (1999). How pollutants affect the Zapadna Morava watercourse on the area of Cacak. The 28th Annual Conference of Yugoslav Water Pollution Control Society "Water Pollution Control 1999", Conference Proceedings, Soko Banja, 207-212.

Karadžić, V. (2011). *Eutrofikacija i njene posledice na primeru reke Ponjavice (opština Pančevo).* Doktorska disertacija, Biološki faultet, Univerzitet u Beogradu, Beograd.

Karadžić, V., Subakov-Simić, G., Natić, D., Ržaničanin, A., Ćirić, M. and *Z. Gačić* (2013). Changes in the phytoplankton community and dominance of *Cylindrospermopsis raciborskii* (Wolosz.) Subba Raju in a temperate lowland river (Ponjavica, Serbia). *Hydrobiologia* **711**, 43-60.

Kaštovský, J., Hauer, T., Mareš, J., Krautová, M., Bešta, T., J. Komárek et al. (2010). A rewiew of the alien and expansive species of freshwater cyanobacteria and algae in the Czech Republic. *Biol. Invasions* **12**, 3599-3625.

Komárek, J. (2013). Cyanoprokaryota. 3. Teil: Heterocytous Genera, In: *Süßwasserflora von Mitteleuropa* (Eds. B. Büdel, G. Gärtner, L. Krienitz and M. Schagerl), 1-1130. Springer Spektrum Verlag, Heidelberg, Berlin.

Komárek, J. and *K. Anagnostidis* (1998). Cyanoprokaryota, 1. Teil: Chroococcales, In: *Süßwasserflora von Mitteleuropa* (Eds. H. Ettl, G. Gärtner, H. Heynig and D. Mollenhauer), 1-548. Spektrum Akademischer Verlag, Heidelberg, Berlin.

Komárek, J. and *K. Anagnostidis* (2005). Cyanoprokaryota. 2. Teil: Oscillatoriales, In: *Süßwasserflora von Mitteleuropa* (Eds. B. Büdel, G. Gärtner, L. Krienitz and M. Schagerl), 1-759. Spektrum Akademischer Verlag, Berlin.

Ljaljević-Grbić, M., Vukojević, J., Subakov-Simić, G., Krizmanić, J. and *M. Stupar* (2010). Biofilm forming cyanobacteria, algae and fungi on two historic monuments in Belgrade, Serbia. *Arch. Biol. Sci.* **62(3)**, 625-631.

Makovinská, J., Hindákova, A. and *F. Hindák* (2002). Phytobenthos. Joint Danube Survey, Technical Report of the International Commission for the Protection of the Danube River, ICPDR, Vienna, 65-77.

Manti, G., Mattei, D., Messineo, V., Melchiorre, S., Bogialli, S., N. Sechi et al. (2005). First report of *Cylindrospermopsis raciborskii* in Italy. *Harmful Algal News* **28**, 8-9.

Maršálek, B., Bláha, L. and *F. Hindák* (2000). Review of toxicity of cyanobacteria in Slovakia. *Biologia* **55(6)**, 645-652.

Martinović-Vitanović, V. (1996). *Ekološka studija Obedske bare*. Javno preduzeće za gazdovanje šumama "Srbija šume", "Geokarta", Beograd.

Milovanović, D. (1949). *Sezonski sastav alga u jednom oknu Obedske bare*. Glasnik Prirodnjačog muzeja Srpske Zemlje, Serija **B**, knjiga **1-2**, 127-162.

Mohamed, Z. A. (2007). First report of toxic *Cylindrospermopsis raciborskii* (Cyanoprokaryota) in Egyptian fresh waters. *Fems. Microbiol. Ecol.* **59**, 749-761.

Moss, B. (1998). *Ecology of fresh waters: man and medium, past to future*, 3rd edn. Blackwell Publishing, Oxford.

Moustaka-Gouni, M., Kormas, K., Vardaka, E., Katsiapi, M. and *S. Gkelis* (2009). *Raphidiopsis mediterranea* Skuja represents non-heterocytous life-cycle stages of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju in Lake Kastoria (Greece), its type locality: Evidence by morphological and phylogenic analysis. *Harmful Algae* **8**, 864-872.

Namikoshi, M., Murakami, T., Watanabe, M.V. and *T. Oda* (2003). Simultaneous production of homoanatoxin-a, anatoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium *Raphidiopsis mediterranea* Skuja. *Toxicon* **42**, 533-538.

Nemeth, J., Boyanovski, B. and *I. Traykov* (2002). Phytoplankton. Joint Danube Survey, Technical Report of the International Commission for the Protection of the Danube River, ICPDR, Vienna, 102-122.

Nikitović, J. and *R. Laušević* (1999). Benthic algae in the river Vlasina (Serbia, Yugoslavia). *Ekologija* **34(1-2)**, 19-26.

Obušković, LJ., Mastić, M., Cakić, P. and *D. Jakovčev* (1994). Hidroekološko istraživanje nekih voda na području Stare planine. Konferencija o aktuelnim problemima zaštite vode „Zaštita voda '94", zbornik radova, Igalo, 188-192.

Obušković, LJ. and *M. Obušković* (1998). Ispitivanje kvaliteta vode mikroakumulacije na Gvozdačkoj reci (sliv Ibra) na osnovu alga kao bioindikatora. Konferencija o aktuelnim problemima zaštite vode "Zaštita voda '98", zbornik radova, Kotor, 309-318.

Padisák, J. (1997). *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju, an expanding, highly adaptive cyanobacterium: worldwide distribution and a review of its ecology, *Arch. Hydrobiol. Suppl.*, Monograph Studies 4, **107**, 563-593.

Predojević, D., Kljajić, Ž., Kovačević, E., Milosavljević, J., Papić, P., Lazić, M. and *G. Subakov Simić* (2014). Diversity of Chrysophyceae (Heterocontophyta) in the Zasavica River (Serbia). *Arch. Biol. Sci.* **66(3)**, 1195-1204.

Predojević, D., Subakov Simić, G., Kovačević, E., Papić, P., Ćuk, M., Kljajić, Ž. and *M. Lazić* (2015). Diversity of the Euglenophyta division in the Zasavica River, Serbia. *Bot. Serb.* **39(1)**, 23-29.

Pujin, V., Stojković, S. and *A. Drobac* (1998). Fitoplankton i zooplankton kao indikatori kvaliteta vode u reci Tamiš, In: *Naš Tamiš* (Eds. S. Marković and Z. Svirčev), 65-74. Univerzitet u Novom Sadu, Prirodno-matematički fakultet, Institut za Geografiju, Novi Sad.

Ranković, B., Simić, S. and *D. Bogdanović* (2006). Phytoplankton as indicator of water quality of Lakes Bubanj and Šumarice during autumn. *Kragujevac J. Sci.* **28**, 107-114.

Ržaničanin, A., Cvijan, M., Krizmanić, J. and *G. Subakov-Simić* (2003). Phytoplankton community in river Tisa near Bečej after cyanide spill. Godišnja konferencija o aktuelnim problemima zaštite voda „Voda 2003", zbornik radova, Zlatibor, 227-232.

Saker, M.L., Nogueira, I.C.G., Vasconcelos, V.M., Neilan, B.A., Eaglesham, G.K. and *P. Pereira* (2003a). First report and toxicological assessment of the cyanobacterium *Cylindrospermopsis raciborskii* from Portuguese freshwaters. *Ecotox. Environ. Safe.* **55**, 243-250.

Saker, M.L., Nogueira, I.C.G. and *V.M. Vasconcelos* (2003b). Distribution and toxicity of *Cylindrospermopsis raciborskii* (cyanobacteria) in Portuguese freshwaters. *Limnetica* **22 (3-4)**, 129-136.

Schaarschmidt, J. (1883). Notification of Cluj-Napoca, M. Royal. University Institute for botany. XXXIII, Fragmenta Phycologiae Bosniaco-Serbicae I, Hungary botany books, seven, Vol. No. 75. Budapest.

Simić, S. (1996). Algae from the Trgoviški Timok river (Serbia, Yugoslavia). *Glasnik instituta za Botaniku i Botaničke Bašte univerziteta u Beogradu* Tom XXX, 107-118.

Simić, S. (2002). Distribution of blue-green algae (Cyanophyta) in streams of Mt. Stara Planina (Serbia). *Arch. Biol. Sci.* **54(3-4)**, 141-148.

Simić, S., Komárek, J. and *N. Đorđević* (2014). The confirmation of the genus *Glaucospira* (Cyanobacteria) and the occurrence of *Glaucospira laxissima* (G.S. West) comb. nova in Serbia. *Cryptogam. Algol.* **35(3)**, 259-267.

Simić, S., Miščević, M., Đorđević, N. and *N. Popović* (2011). Cijanobakterije u Aleksandrovačkom jezeru - pre i posle sanacije. 16. Studenička akademija, zbornik abstrakata, Novi Sad, 17-18.

Simić, S., Simić, V., Cvijan, M. and *B. Ranković* (2004). Contribution to the knowledge of bioindicating features of some algal species in streams of Serbia. Proceedings of the 35th IAD Conference, Novi Sad, Serbia and Montenegro, 355-360.

Sivonen, K. and *G. Jones* (1999). Cyanobacterial toxins, In: *Toxic Cyanobacteria in Water: a Guide to Public Health Significance, Monitoring and Management* (Eds. I. Chorus and J. Bertham). The World Health Organization, ISBN 0-419-23930-8. E and FN Spon, London, UK.

Spoof, L., Vasterkvist, P., Lindholm, T. and *J. Meriluoto* (2003). Screening for cyanobacterial hepatotoxins, microcystins and nodularin in environmental water samples by reversed-phase liquid chromatography-electrospray ionisation mass spectrometry. *J. Chromatogr. A* **1020**, 105-119.

Stanković, M. (2006). Vodič kroz prirodu u specijalnom rezervatu prirode Zasavica. Pokret gorana Sremske Mitrovice, Sremska Mitrovica, 1-219.

Stefaniak, K., Kokocinski, M. and *B. Messyasz* (2005). Dynamics of *Planktothrix agardhii* (Gom.) Anag. et Kom. blooms in polymictic lake Laskownickie and Grylewskie (Wielkopolska region) Poland. *Oceanol. Hydrobiol. Stud.* **XXXIV(3)**, 125-136.

Stüken, A., Rücker, J., Endrulat, T., Preußel, K., Hemm, M. and B. Nixdorf et al. (2006). Distribution of three alien cyanobacterial species (Nostocales) in Northeast Germany: *Cylindrospermopsis raciborskii*, *Anabaena bergii* and *Aphanizomenon aphanizomenoides*. *Phycologia* **45(6)**, 696-703.

Subakov-Simić, G., Karadžić, V., Fužinato, S., Fodora, A. and *M. Cvijan* (2006). Algological research of the Slatina and the Pečena Slatina near Opovo (Vojvodina). 35. godišnja konferencija o aktuelnim problemima zaštite voda "Voda 2006", zbornik radova, Zlatibor, 139-144.

Subakov-Simić, G., Karadžić, V. and *J. Krizmanić* (2007). Phytoplankton production of the Velika slatina near Opovo. 36. godišnja konferencija o aktuelnim problemima zaštite voda „Voda 2007", zbornik radova, Tara, 137-140.

Subakov-Simić, G., Plemić, N., Karadžić, V., Cvijan, M. and *J. Krizmanić* (2004). Qualitative and quantitative phytoplankton composition of the Slatina near Opovo. Godišnja konferencija o aktuelnim problemima zaštite voda "Voda 2004", zbornik radova, Borsko jezero, 327-330.

Svirčev, Z., Cetojević-Simin, D., Simeunović, J., Karaman, M. and *D. Stojanović* (2008). Antibacterial, antifungal and cytotoxic activity of terrestrial cyanobacterial strains from Serbia. *Sci. China Ser. C.* **51(10)**, 941-947.

Svirčev, Z., Krstić, S., Miladinov-Mikov, M., Baltić, V. and *M. Vidović* (2009). Freshwater Cyanobacteria Blooms and Primary Liver Cancer Epidemiological Studies in Serbia. *J. Environ. Sci. Heal. C* **27**, 36-55.

Svirčev, Z., Simeunović, J., Subakov-Simić, G., Krstić, S., Pantelić, D. and *T. Dulić* (2013). Cyanobacterial blooms and their toxicity in Vojvodina lakes, Serbia. *Int. J. Environ. Res.* **7(3)**, 745-758.

Svirčev, Z., Simeunović, J., Subakov-Simić, G., Krstić, S. and *M. Vidović* (2007). Freshwater cyanobacterial blooms and cyanotoxin production in Serbia in the past 25 years. *Geographica pannonica* **11**, 32-38.

Svirčev, Z., Tokodi, N., Drobac, D. and *G. Codd* (2014). Cyanobacteria in aquatic ecosystems in Serbia: effects on water quality, human health and biodiversity. *System. Biodivers.* **12(3)**, 261-270.

Urošević, V. (1996). Peryphyton Algae in the System of Djeravica Lakes on the Spring Brauch of Erenok. *Univ. Thought Nat. Sci.* **3(2)**, 11-21.

Urošević, V. (1997a). Overgrowth of algae of upper Veljinbeško Lake on Šutman (Šar-planina). Proceeding of the 5th Meeting of the Flora of south-eastern Serbia, Zaječar, 8-18.

Urošević, V. (1997b). Peryphyton Algae in two small lakes on the Spring Brauch of Crnkamenska reka river on Šar-planina Mt. *Univ. Thought Nat. Sci.* **4(1)**, 15-21.

Urošević, V. (1998). The Yugoslav Part of Šar-Planina Mt. Lake Eco-system's peryphyton algae. *Univ. Thought Nat. Sci.* **5(2)**, 43-57.

Urošević, V. and *A. Savić* (1996). Algae of the Lepenac Springs on the Šar-Planina Mt. *Univ. Thought Nat. Sci.* **3(1)**, 23-32.

Utermöhl, H. (1958). Zur vervolkmmung der quantitativen phytoplankton methodik. *Mitt. Int. Ver. Limnol.* **9**, 1–38.

Vardaka, E., Moustaka-Gouni, M., Cook, M.C. and *Z. Lanaras* (2005). Cyanobacterial blooms and water quality in Greek waterbodies. *J. Appl. Phycol.* **17**, 391-401.

Veljić, M. and *M. Cvijan* (1997). Qualitative analysis of Algae of the confluences and the middle course of the Kolubara river. *Arch. Biol. Sci.* **49(1-2)**, 43-49.

Vučković, M. and *B. Mirjačić-Živković* (2008). Phytoplankton of reservoir "Grliste". 37. godišnja konferencija o aktuelnim problemima zaštite voda "Voda 2008", zbornik radova, Mataruška Banja, 153-156.

Watanabe, M.F., Tsulimura, S., Oishi, S., Niki, T. and *M. Namikoshi* (2003). Isolation and identification of homoanatoxin-a from a toxic strain of the cyanobacterium *Raphidiopsis mediterranea* Skuja isolated from lake Biwa, Japan. *Phycologia* **42**, 364-369.