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Review of 130 years of research on cyanobacteria in aquatic ecosystems in Serbia presented in a Serbian Cyanobacterial Database

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ABSTRACT

The presence of toxic cyanobacteria in aquatic ecosystems in the territory of the Republic of Serbia was surveyed over a period of several decades. Increasing attention is being paid to some negative consequences that may be caused by these microorganisms. Information from available literary sources regarding the distribution and frequency of cyanobacteria and their toxins over a period of 130 years, together with the effects on humans and wildlife in aquatic ecosystems, were gathered and incorporated into a Serbian Cyanobacterial Database created for the CYANOCOST Action. This database encompasses information on 65 aquatic ecosystems, including rivers, lakes, ponds, canals, irrigation reservoirs, reservoirs used for drinking water supply and reservoirs used for other purposes. Cyanobacterial blooms were found in almost 80% of the investigated aquatic ecosystems. The analysis of the research showed the presence of more than 70 species, including blooms of 24 species from 13 genera. Five species of cyanobacteria: *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*, *Planktothrix agardhii*, *Microcystis flos-aquae* and *Planktothrix rubescens* frequently formed blooms in the investigated waterbodies and cyanotoxins were also detected in some of them, which had certain negative effects. Here, we present an overview of data contained in the Serbian Cyanobacterial Database, concerning cyanobacterial distribution, cyanotoxin production and associated biological effects in different types of water bodies from the Republic of Serbia. Also, recent important and major cases of cyanobacterial blooming in reservoirs used for drinking water supply at Vrutci and Ćelije, the Aleksandrovac irrigation reservoir, the Ponjavica River and Lake Palić, including systematic research on the Lake Ludoš and few fishponds are further described. It can be concluded that cyanobacteria and cyanotoxins are omnipresent in different water bodies throughout the Republic of Serbia. For these reasons it is imperative to continue with the monitoring of cyanobacteria and cyanotoxins, as well as to continuously supplement the established database with new information. The Serbian Cyanobacterial Database represents a treasury of information on cyanobacteria and their toxins, and serves as a model for other countries in the region and beyond.

Key words: Cyanobacterial blooms; cyanotoxins; database; historical overview.

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INTRODUCTION

Research of algae in Serbia began over 130 years ago, with the publication of 'Fragmenta Phycologiae Bosniaco-Serbicae' by Scharschmidt (1883), who listed 46 algal species. The species were determined in samples of washed silt from herbarium specimens of the waterwheel plant (*Aldrovanda vesiculosa* (L.)) collected by the famous lecturer and botanist, Doctor Josif Pančić (Blaženčić, 1986). Over 50 species, mostly belonging to the green algae and cyanobacteria, were identified by Magnus, Simić and Katić during the late 19th and early 20th Centuries (Milovanović, 1949). Another notable name in this initial period of algal research was Nedeljko Košanin (Blaženčić, 1986).

World War I prevented further investigations of algae and cyanobacteria in Serbia, however, during the 1930s and 1940s exploration continued in the form of complex hydrobiological studies. A bibliographic review of the few Serbian algological studies until 1947 was presented by Milovanović (1949). This period is also important because it can be seen as the cradle of almost all of the subsequent trends in algal research (Blaženčić, 1986). After World War II, favorable conditions for more advanced, and continuous studies in this scientific area were created. During this period, the spatial distribution, seasonal dynamics and ecology of algae and cyanobacteria were investigated in springs, streams, rivers, ponds, swamps, canals, lakes and mineral waters, *i.e.*, in most aquatic ecosystems in Serbia, which resulted in a wealth of data (Blaženčić, 1986).

Since the 1970s the field of research has been expanding as methods and techniques have been modernized. An increasing number of hydrobiologists, microbiologists and botanists are providing their contributions to the study of cyanobacteria in Serbia (Blaženčić, 1985). Undoubtedly the greatest contribution to the knowledge of algae and cyanobacteria has been given through the meticulous work of Prof. Jelena Blaženčić who performed systematic analysis and assessment of numerous aquatic ecosystems in Serbia.

Most recently, available data on the presence of cyanobacteria and their toxins in aquatic ecosystems in the Republic of Serbia were gathered and incorporated into the Serbian Cyanobacterial Database (SCDB) (https://cloud.pmf.uns.ac.rs/index.php/s/v6tErVCV-cAuaXQN), as a result of the CYANOCOST Action. The objective of the present paper was to analyze, present and supplement available data from SCDB.

DATA OVERVIEW

Data on cyanobacterial occurrence, cyanotoxin production and the possible impact on humans and aquatic ecosystems in the Republic of Serbia were evaluated in over 70 data sources (Tab. 1). A comprehensive review of research articles, project reports, conference abstracts, dissertations, books, annual and news reports was performed, and collected data were entered into the SCDB. The analysis of the research showed that 65 different aquatic ecosystems had been investigated during the last 130 years. The microalgal and cyanobacterial research summarized in the SCDB consisted of over 250 analyses, of which most (about 150) were conducted since the year 2000. These analyses included determination of cyanobacteria, cyanotoxin detection and the documentation of associated biological effects and health incidents (Svirčev *et al.,* 2014a).

Qualitative determination of cyanobacteria showed the presence of more than 70 species in the investigated aquatic ecosystems, with 24 species from 13 genera being recorded in blooms, the most frequent being *Microcystis aeruginosa* (Kützing), *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahaul, *Planktothrix agardhii* (Gomont) K. Anagnostidis & J. Komárek, *Microcystis flos-aquae* (Wittrock) Kirchner and *Planktothrix rubescens* (de Candolle ex Gomont) K. Anagnostidis & J. Komárek (Svirčev *et al.,* 2014a). All the Latin genera and species names are taken from the original publications. For the currently accepted nomenclature of the species cited in this paper, refer to the Supplementary Tab. 1. Cyanobacterial blooms were found in almost 80% of the investigated aquatic ecosystems. This paper summarizes their distribution, cyanotoxin production and associated biological effects in different types of waterbodies from the Republic of Serbia.

Canals

The canals in the Vojvodina region (Kralja Aleksandra canal and Kralja Petra canal) were explored during the 1930s, when a bloom of *M. aeruginosa* was recorded (Protić, 1935). The cyanobacterium *Anabaena flos-aquae* (*Dolichospermum flos-aquae* (Brébisson ex Bornet & Flahault) P. Wacklin, L. Hoffmann & J. Komárek) also formed a mass occurrence in the Kralja Petra canal (Protić, 1936). Recently, most research was done on the Canal Danube-Tisa-Danube system where the presence of a common cyanotoxin, microcystin (MC), was detected. The highest concentrations (up to 347 µg L^{-1} MC-LR equivalents) were found in the autumn of 2006 at the locality of Bačko Gradište where the cyanobacterial species *A. flos-aquae*, *Aph. flos-aquae*, *M. aeruginosa*, *M. flos-aquae* and *Oscillatoria agardhii* (*Planktothrix agardhii*) occurred (Simeunović, 2009).

Ponds

Very limited data are available on the occurrence of cyanobacteria and cyanotoxins in marsh-wetland ecosystems (Jakovljević and Stanković, 1931-1932; Milovanović, 1970; Pujin *et al.,* 1987; Maslać *et al.,* 1992; Subakov-Simić *et al.,* 2004; Fužinato *et al.,* 2010; Cvijan and Fužinato, 2011, 2012). Recent data from 2006 showed the first ocurrence of the invasive and potentially toxic cyanobacterial species *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju in Slatina, a salt marsh pond (Cvijan and Fužinato, 2012). The presence of the cyanobacterial species *M. aeruginosa* in Rakina bara (Jakovljević and Stanković, 1931-1932), *Aph. flos-aquae* in Carska bara (Pujin *et al.,* 1987) and *Arthrospira*

Tab. 1. Overview of the data in Serbian Cyanobacterial Database (1930-2012).

Location	Cyanobacteria	Cyanotoxins	Biological effects	Reference
Canals (4) Ponds (7) Rivers (13) Fishponds (8) Reservoirs for irrigation (11) Lakes (6) Reservoirs for drinking water supply (12) Reservoirs with other purposes (4)	Over 70 species found, frequently blooming: - *Microcystis aeruginosa* - *Aphanizomenon flos-aquae* - *Planktothrix agardhii* - *Microcystis flos-aquae* - *Planktothrix rubescens*	Microcystin (MC) Analyses: - PPI - ELISA - HPLC Detection in: - Water - Soil -Plant tissues - Fish tissues	- *Artemia salina* bioassay - Fish histopathology - Animal mortality - Epidemiological survey	Over 70 literature sources: - Peer-reviewed papers (24) - International documents (english) (10) - National documents (non-english) (40) - Newspaper/internet reports (3) - Own document (1)

fusiformis (Voronikhin) J. Komárek & J.W.G. Lund in Slatina (Fužinato *et al.,* 2010) causes some concerns as these species are known cyanotoxin-producers.

Rivers

Interestingly, there are a large number of data on cyanobacterial blooming in river ecosystems. One of the most publicized incidents happened in 2009 when the mortalities of fish and hundreds of cows and pigs which drank water from Ponjavica, the river near the town of Pančevo, occurred. The detected cyanobacterial species belonged to the genera *Anabaena*, *Aphanizomenon*, *Aphanocapsa*, *Aphanothece*, *Chroococcus*, *Cylindrospermopsis*, *Geitlerinema*, *Jaaginema*, *Limnothrix*, *Microcystis*, *Phormidium*, *Planktothrix* and *Raphidiopsis.* During 2008 and 2009 in the Ponjavica river, MCs (concentrations given as MC-LR equivalents in ELISA) were detected in the water (up to 4.84 mg L^{-1}), sediment (5.7 mg per 100 g), macrophytes (up to 5.0 mg per 100 g) and fish (up to 3.3 mg per 100 g), and the dominance of the invasive cyanobacterium *Cyl. raciborskii* was found (Karadžić, 2011; Natić, 2012; Karadžić *et al.,* 2013). MCs may have contributed to the deaths of animals, however, the exact cause of death has not been determined.

MCs were also found in rivers Krivaja, Tamiš, Tisa and Begej, with the maximum concentrations of 80, 33, 32 and 22 µg MC-LR equivalents L^{-1} respectively, where *Aph. flos-aquae*, *M. flos-aquae* and *O. agardhii* bloomed (Simeunović, 2009). These and other bloom-forming cyanobacteria, such as *A. flos-aquae*, *M. aeruginosa* and *Oscillatoria rubescens* (*Planktothrix rubescens*), were recorded in many rivers throughout the Republic of Serbia (Obušković, 1982, 1987, 1989, 1991; Sedmak and Svirčev, 2011).

Fishponds

Research on the occurrence of cyanobacteria in fishponds began with the investigations of the Ečka, Kolut and Živača fishponds where *M. aeruginosa* formed blooms (Milovanović and Živković, 1953, 1959; Milovanović, 1963), while *Anabaena* was found blooming in the Futog I fishpond (Ristić *et al.,* 1979). In Kapetanski rit the presence of the invasive cyanobacterial species *Cyl. raciborskii* was noted (Ćirić *et al.,* 2010).

In the last five years, more attention has been turned to possible effects of cyanobacteria and their toxins on the quality of fish meat and cyanotoxin accumulation in fish tissues, that could consequently endanger the health of consumers. In fishponds with the code BO during 2010 and 2011, mass occurrences of cyanobacterial species *Aph. flos-aquae, M. aeruginosa, Phormidium foveolarum* (*Leptolyngbya foveolara* (Gomont) Anagnostidis & Komárek), *Jaaginema subtilissimum* (Kützing ex Forti) Anagnostidis & Komárek*, Pseudanabaena limnetica* (Lemmermann) Komárek and *Geitlerinema amphibium* (C. Agardh ex Gomont) Anagnostidis were recorded. Toxicity in an *Artemia salina* (L.) bioassay was detected in two of the six fishponds, when the maximum concentration of MCs in water amounted to 52 MC-LR equivalents L^{-1} in protein phosphatase inhibition (PPI) assay and 18 µg L^{-1} in an enzyme-linked immunosorbent assay (ELISA). Besides water, MCs were found in the muscle and liver of fish, as well as in aquatic plants and sludge (World Bank Report, DM 4307 2011).

During the summer of 2011 in another fishpond, encoded MU (Fig. 1A), mass occurrences of *G. amphibium*, *J. subtilissimum*, *M. aeruginosa*, *O. agardhii* and *Phor. foveolarum* were recorded (World Bank Report, DM 4307 2011). The toxicity of water samples from the fishpond was confirmed by *A. salina* bioassay, and the presence of MCs and saxitoxin(s) in water was identified by a PPI assay and ELISA, respectively (Tokodi *et al.,* 2013, 2014; Drobac, 2015; Drobac *et al.,* 2016). The highest concentrations of MCs (181 µg MC-LR equivalents L^{-1} in the PPI assay) were detected in a water sample from September 2011 (Tokodi *et al.,* 2014). The variant MC-RR was also detected in the muscle of fish *Cyprinus carpio* (L.) grown in the fishponds where high cyanobacterial occurrence was detected (Drobac, 2015; Drobac *et al.,* 2016). Additionally, pathological alterations in the fish tissues of liver, kidneys, gills, intestine and muscle were also observed (Drobac, 2015; Drobac *et al.,* 2016).

The observed adverse effects and accumulation of cyanotoxins in fish tissues show that cyanobacteria and their toxins in fishponds could be hazardous to fish quality, the economy, the consumers' health and the environment in general.

Reservoirs used for irrigation

Cyanobacteria and MCs were recorded in reservoirs in Serbia which are used for irrigation. Of 13 blooming species, the most frequently observed were *Aph. flos-aquae* (Borkovac, Bukulja, Manđelos, Mrtva Tisa, Pavlovci, Provala, Zobnatica), *O. agardhii* (Borkovac, Mrtva Tisa, Pavlovci, Zobnatica), *A. flos-aquae* (Bukulja, Jegrička, Pavlovci) and *M. flos-aquae* (Koviljski rit, Pavlovci) (Đukić *et al.,* 1991a, 1991b; Simeunović, 2009; Karadžić *et al.,* 2010; Sedmak and Svirčev, 2011; Svirčev *et al.,* 2013a). The highest concentration of 280 µg L^{-1} MC-LR equivalents in water was recorded in 2007 in Mrtva Tisa (Simeunović, 2009) when *Aph. flos-aquae* and *O. agardhii* were abundant (Fig. 1B).

Cyl. raciborskii was detected in September 2010 in the Aleksandrovac reservoir which is used for irrigation (Simić *et al.,* 2011). An extensive fish mortality in Aleksandrovac occurred on 20 December 2012 and was associated with the presence of *Cyl. raciborskii* blooming a few weeks before the incident. Almost the entire fish population was killed (over 1.7 tonnes) including the species *C. carpio*, *Silurus glanis* (L.), *Ctenopharyngodon idella* (Valenciennes), *Hypophthalmichthys molitrix* (Valenciennes), *Abramis brama* (L.), *Carassius gibelio* (Bloch), *Aspius aspius* (L.), and *Squalius cephalus* (L.). *A. salina* bioassay showed high toxicity of water samples from Aleksandrovac. However, the most common cyanotoxins (MCs, cylindrospermopsin, and saxitoxin) were not detected. It is possible that some other unknown or undetected toxic metabolites of this cyanobacterium were present and were a potential cause of the fish mortality in Aleksandrovac (Drobac, 2015; Svirčev *et al*., 2016a).

Information about cyanotoxins in reservoirs used for irrigation is important because it is known that irrigation from water sources containing cyanobacteria and cyanotoxins may affect agricultural plants and lead to accumulation of these toxins. Therefore, the health risks to people and animals due to the consumption of agricultural products irrigated with cyanotoxin-containing water must be taken seriously (Codd *et al.,* 1999; Crush *et al.,* 2008; Saqrane *et al.,* 2009; Chen *et al.,* 2010; Drobac, 2015).

Lakes

In Gazivode Lake, Sjeničko Lake and Veliki Zaton Lake *Anabaena circinalis* (*Dolichospermum circinale* (Rabenhorst ex Bornet & Flahault) P. Wacklin, L. Hoffmann & J. Komárek), *A. flos-aquae* and *O. rubescens* were observed in mass occurrences (Shllaku and Landner, 1992; Miljković *et al.,* 2004; Sedmak and Svirčev, 2011). Detection of MCs was performed only in the lakes Palić and Ludoš.

Research from 2005 to 2007 shows the presence of MCs in Lake Palić, and the highest MC concentration, 389 µg MC-LR equivalents L^{-1}, was detected in the autumn of 2006. The most frequently blooming species was *M. aeruginosa*, followed by *Anabaena spiroides* (*Dolichospermum spiroides* (Klebhan) P. Wacklin, L. Hoffmann & J. Komárek), *A. circinalis*, *M. flos-aquae* and *Microcystis wesenbergii* (Komárek) Komárek ex Komárek (Simeunović, 2009). In Lake Palić, fish mortality was observed by the author Seleši (1982), and this recurred in later years as well. In 2009 there was an extensive mortality of fish with loss of over 12 tonnes of fish stocks. Reasons cited included the lack of dissolved oxygen due to an excessive production of algae followed by their decay (http://www.zjzs.org.rs/page.php?id=286). Furthermore, in 2012 the invasive *Cyl. raciborskii* was also found in this lake (Institute of Public Health of Serbia, 2013).

In Lake Ludoš, MC concentrations reached up to 604

Fig. 1. Cyanobacterial blooming in fishpond (code MU) (A); reservoir used for irrigation (Mrtva Tisa) (B); lake (Lake Ludoš) (C); and reservoir used for drinking water supply (Vrutci) (D).

μg MC-LR equivalents L^{-1} in the summer of 2006, and the species that bloomed during the study period were *Aph. flos-aquae*, *M. aeruginosa*, *M. flos-aquae*, *M. wesenbergii* and *P. agardhii* (Simeunović, 2009). Research on Lake Ludoš during 2011 indicated not only MCs in the water, but also their accumulation in macrophytes (*Phragmites communis* Trin., *Typha latifolia* (L.) and *Nymphaea elegans* Hook.) and in the tissues (intestine, muscles, kidney, gills and gonads) of Prussian carp (*C. gibelio*). Histopathological changes in different organs of Prussian carp (liver, kidney, gills and intestines) from Lake Ludoš were associated with the high abundacies of potentially toxic cyanobacterial species *Limnothrix redekei* (van Goor) Meffert and *P. limnetica* that were found in the center of the lake (Fig. 1C). Given that Lake Ludoš is a Ramsar site, the stability of this aquatic ecosystem is of great and global importance (Tokodi, 2016).

Reservoirs used for drinking water supply

Unlike the Vojvodina region, where groundwater is used for water supply, central Serbia has a large number of surface reservoirs used for drinking water supply. There are more than 20 reservoirs used as sources of drinking water, and constant mass occurrences of cyanobacteria have been observed in nine of them (Svirčev *et al.,* 2007).

In the following Serbian drinking water supply reservoirs: Bovan, Bresnica, Garaši, Grlište, Grošnica, Gruža, Krajkovac and Pridvorica, the emergence of the bloom-forming cyanobacteria *Anabaena solitaria* (*Dolichospermum solitarium* (Klebahn) P. Wacklin, L. Hoffmann & J. Komárek), *Aph. flos-aquae*, *Gomphosphaeria lacustris* (*Snowella lacustris* (Chodat) Komárek & Hindák), *Gomphosphaeria aponina* Kützing, *P. limnetica* and *M. aeruginosa* has been documented, as has the presence of cyanotoxins in some of the reservoirs (Sedmak and Svirčev, 2011; Svirčev *et al.,* 2014a). In Ćelije, the reservoir used for drinking water supply for the city of Kruševac, a bloom of *A. circinalis*, *Aph. flos-aquae* and *M. aeruginosa* was observed in 2004. MC was found in water samples from the reservoir (650 μg MC-LR L^{-1}) and in the tap water (2.5 μg L^{-1}) (Svirčev *et al.,* 2009). In addition to the mentioned species, *Aphanizomenon issatschenkoi* (*Cuspidothrix issatschenkoi* (Usachev) P. Rajaniemi, Komárek, R. Willame, P. Hrouzek, K. Kastovská, L. Hoffmann & K. Sivonen) (2001) and *J. subtilissimum* were detected in Ćelije (2007) (Svirčev *et al.,* 2009; Sedmak and Svirčev, 2011). In Gruža, along with a bloom of *Aph. flos-aquae*, ultrastructural, apoptotic and necrotic changes in the liver of perch (*Perca fluviatilis* (L.)) were observed, as well as an impact on antioxidant biomarkers (Perendija *et al.,* 2011).

Recently, cyanobacterial blooms of *P. rubescens* have occurred in Vrutci reservoir used for the water supply of the city of Užice (Fig. 1D), where 70.000 inhabitants were potentially exposed to cyanotoxins in December 2013. Based on the number of cells per mL and concentration of MCs, according to World Health Organization (WHO, 1999), water from reservoir Vrutci could be classified as a high-risk water for recreation and drinking water abstraction purposes. The results from *A. salina* bioassay showed significant toxicity of the cyanobacterial biomass. Modest fish mortality was observed during the cyanobacterial bloom, and MCs were detected in fish, including the muscle of frozen fish from 2013 which could indicate the presence of cyanobacteria even before the confirmed bloom. Furthermore, a questionnaire and epidemiological results showed that health problems possibly related to cyanotoxins (diseases of digestive system, skin and subcutaneous tissue) occurred already at least two years prior to the incident. This might be a sign that the population of the city of Užice could have been exposed to the cyanobacteria and cyanotoxins even two years before the observed bloom in 2013 (Svirčev *et al.,* 2016b).

Chronic exposure to cyanotoxins (*e.g.* MCs) from drinking water could present a risk factor for primary liver cancer and possibly even other types of cancer (Svirčev *et al.,* 2010; Drobac, 2015). Epidemiological studies conducted in Serbia have revealed a significant correlation between an increased incidence of several cancers (brain; heart, mediastinum and pleural; ovarian; testicular; gastric; colorectal; retroperitoneal and peritoneal; leukemia; malignant skin melanoma; and primary liver cancer) and cyanobacterial blooms in reservoirs used for drinking water supply (Svirčev *et al.,* 2009, 2013b, 2014b; Drobac, *et al.* 2011; Drobac, 2015).

Reservoirs used for other purposes

Reservoirs used for hydropower generation have been poorly investigated. Three cyanobacterial species were noted: *M. aeruginosa*, *Aph. flos-aquae* and *O. rubescens*. Only *O. rubescens* formed mass occurrences in investigated reservoirs (Milovanović, 1973; Obušković, 1983; Sedmak and Svirčev, 2011).

CONCLUSIONS

Based on the reviewed data from our SCDB it can be concluded that cyanobacteria and cyanotoxins are omnipresent in different waterbodies throughout the Republic of Serbia. A systematic review and meta-analyses of the available literature is useful for an understanding of cyanobacterial biodiversity in Serbian waters. Some information is also available concerning the impact of cyanotoxins on other organisms, including humans. As a set of systemized data unique in the Balkan Peninsula, the database represents a possible model for other countries in the region and beyond. Such databases encopassing all previous research (including monitoring and case reports), as well as continuous supplementation with the new available data are valuable in order to provide a timely and adequate reaction to toxic and noxious cyanobacteria, and thus prevent potential negative consequences.

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