Freshwater Cyanobacterial Blooms and Cyanotoxin Production in Serbia in the Past 25 Years

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Abstract

Since 1980 cyanobacterial blooms occurred in a large number of reservoirs, lakes and water flows (rivers and channels) in Serbia. Among 83 water ecosystems examined, 58 were found in blooming condition almost every year during last 2 decades. All natural lakes, accumulations, rivers and chanals in Vojvodina province (agricultural part) proved to be sites with frequent cyanobacterial proliferation. During the summer 2005-spring 2006 microcystin-LR survey in Vojvodina, the toxin was permanently present in all examined ecosystems and the highest value of 362.68 $\mu g L^{-1}$ was detected in Ludoš Lake.

The part of Central Serbia is very problematic for ground water supply. For that reason more than 20 reservoirs serve as drinking water suppliers. Significant and persistant cyanobacterial blooms have been recognized in 9 of them. Samples for cyanotoxin analyses were taken during and after blooms in Ćelije Reservoir and in drinking water in Kruševac town 2 days later. Concentration of microcystin-LR was 650 $\mu g L^{-1}$ in the reservoir, while the tap water contained 2.5$\mu g L^{-1}$.

Key words: Cyanobacteria, blooms, cyanotoxins, Serbia

Introduction

During the past decades significantly more frequent occurence of cyanobacterial "blooms" in aquatic systems has been detected and mainly attributed to global warming, increased eutrophication as well as the dispersal of cells through ship traffic (Kahru et al., 1994; Sellner et al., 2003). However, the main factors triggering or controlling cyanobacterial "blooms" have not yet been fully understood (Hense and Beckmann, 2006). In general, low concentrations of dissolved inorganic nitrogen (DIN) and a surplus of phosphorus (DIP) are considered prerequisites for enhanced growth and bloom formation of N_2-fixing cyanobacteria (Kahru et al., 2000). This can explain the occurrence of cyanobacteria after the spring "bloom" of phytoplankton, when DIN is exhausted whereas DIP is still available. In addition to low DIN/DIP ratios several other factors are thought to stimulate bloom formation of cyanobacteria: warm water temperatures under calm weather conditions and the corresponding strong stratification are assumed (but not confirmed) to be necessary. Under severe nutrient depleted conditions, the ability of some cyanobacteria species to take up dissolved organic phosphorus (Huber and Hamel, 1985; Mulholland et al., 2002) and/or to control their buoyancy (Villareal and Carpenter, 2003) may lead to a distinctive advantage over phytoplankton. Other hypotheses for the success of some species include, e.g. a reduced grazing pressure (due to their harmfulness) (Hanazato, 1996; Gobler et al., 2007) or the exclusion of competition (due to shading effects by forming surface scums) (Oliver and Ganf, 2000).

Cyanobacteria are found in freshwater and the sea as well as in all terrestrial environments. They often occur in toxic water blooms and the toxins belong to various classes of substances (Codd, 1995; Chorus and Bartram, 1999). They are responsible for poisoning cases numerous of livestock and other animals, but sometimes they induce serious problems in humans. Beside a long list of toxins, cyanobacteria release other secondary metabolites with cytotoxic or cytostatic activity, some also with tumor promoting activity (Svirčev, 2006).

Cyanotoxins are a very diverse group of toxins. They are either membrane bound or exist free inside the cells. Release of toxins occurs during the cell life, but mostly after cell death through passive flow out of the cellular content. Very interesting point of toxic cyanobacteria is the presence of different toxins within same genus and, on the other hand, the presence of the same toxins in widely different genera. Another specificity of cyanotoxins is the very great variability in toxicity and level of toxicity even between different strains within same species (Dow and Swoboda, 2000). Cyanobacterial toxins include neurotoxic alkaloids (anatoxin-a, anatoxin a(s), saxitoxins), hepatotoxic peptides (microcystins) and the hepatotoxic alkaloid (cylindrospermopsin) (Fitzgerald, 2001).

The presence of toxic cyanobacterial blooms occurring in bodies of water used either as drinking water reservoirs or for recreational purposes may represent serious health risks for the human population. A large number of intoxications not only of cattle (Puschner et al., 1998), dogs (DeVries et al., 1993), and waterfowl (Matsunaga et al., 1999), but also of humans has been reported. The tragic deaths of 76 patients in a hemodialysis clinic in Brazil in 1996 was connected to the presence of cyanobacterial toxins in the water supply (Carmichael et al., 2001) and a high incidence of primary liver cancer in China has been attributed to drinking water contaminated with cyanobacterial toxins (Ueno et al., 1996; Harada et al., 1996).

The presence of cyanobacterial toxins in drinking water supplies poses a serious problem to water treatment facilities since not all technical procedures are able to effectively remove these toxins to below acceptable levels (Hoeger et al., 2002). Despite this, it is highly unlikely that lethal poisonings would occur following con-

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sumption of drinking water contaminated with cyanobacterial toxins. Of much higher concern are low level chronic exposures, since the risks associated with long-term exposure have not been adequately described (Fitzgerald, 2001). Current drinking water treatment practices in Serbia do not regularly monitor or actively remove these toxins from the drinking water since this is a relatively new field of study and would involve extremely expensive measures (Falconer, 1999; Heinze, 1999). Even with treatment, low level chronic exposure to the hepatotoxins, leading to tumor promotion or even carcinogenesis is possible in persons consuming drinking water derived from surface water treatment reservoirs.

The aim of this study was to collect existing results and data bases regarding cyanobacterial "blooms" for Serbia in the past 25 years and to present the first results on cyanotoxins analysis in water ecosystems in Serbia.

Material and Methods

Study area

Mass occurance and blooms of cyanobacteria were studied in all water bodies (rivers, chanals, lakes and accumulations) that had been examined in Serbia during the period 1980-2002.

Data basis for cyanobacterial "blooms"

Mass occurence and "blooming" of potentially toxic cyanobacteria is analysed via informations from relevant data bases (Hydrometeorological Institute of Serbia; Department of Biology and Ecology, Novi Sad; Faculty of Biology, Belgrade; Biology Department, Kragujevac; "Srbija Vode", Belgrade; "Vojvodina Vode", Novi Sad; Institute for Nature Protection, Novi Sad) and published references (Blaženčić et al.,1990; Branković, 1992; Branković et al., 1998; Grašić et al., 2004; Čađo et al., 2003; Čađo et al., 2004a,b; Čomić and Ranković, 1991; Dulić and Mrkić, 1998, 1999, 2001; Đukić, 1991a,b,c,d; Đukić et al.,1994; Đurković et al., 2004; Kalafatić et al., 1982; Karadžić and Subakov-Simić, 2002; Karadžić et al., 2005, 2006; Laušević et al., 1998; Marković and Svirčev, 1998; Martinović-Vitanović and Kalafatić 1990, 1996; Maslać et al., 1992; Milovanović 1963, 1970; 1973; Milovanović and Živković, 1953, 1959, 1963; Miljković et al. 2004; Obušković, 1982a,b, 1983, 1986, 1987, 1989, 1991; Obušković and Kalafatić, 1988; Peršić, 1997; Pujin and Ratajac, 1990; Pujin et al., 1986, 1987, 1999; Ranković and Čomić, 1989; Ranković et al., 1994; Ranković and Simić, 2005; Ristić et al., 1979; Seleši, 1981, 1982; Simeunović et al., 2005; Subakov-Simić, 2004; Svirčev, 1983; Urošević, 1993).

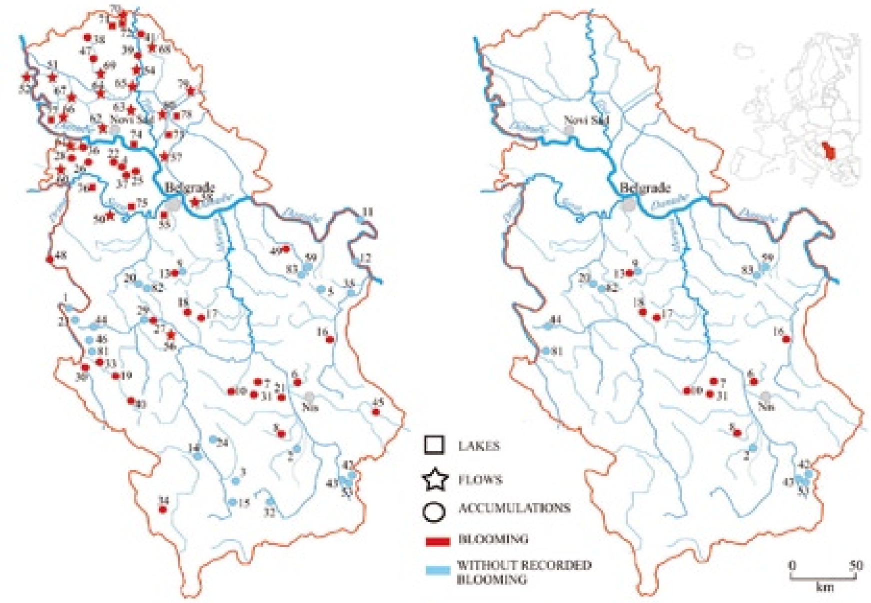

Figure 1 (A) Freshwater ecosystems monitored in the past 25 years in Serbia with detected blooming events; **(B)** Drinking water supply reservoirs in Central Serbia with blooming ones marked by red dots

Legend: *1.Bajina Bašta, 2.Barje, 3.Batlava, 4.Borkovac, 5.Borsko jezero, 6.Bovan, 7.Bresnica, 8.Brestovac, 9.Bukulja, 10.Ćelije, 11.Đerdap I, 12.Đerdap II, 13. Garaši, 14.Gazivode, 15.Gračanka, 16.Grlište, 17.Grošnica, 18.Gruža, 19.Kokin Brod, 20.Kamenica, 21.Krajkovac, 22.Kudoš, 23.Lazić, 24.Lisina, 25.Ljukovo, 26.Međeš, 27.Međuvršje, 28.Moharač, 29.Ovčar Banja, 30.Potpeć, 31.Pridvorica, 32.Prilepnica, 33.Radojina, 34.Radonji, 35.Sokolovica, 36.Sot, 37.Šelovrenac, 38.Tavankut, 39.Tisa, 40.Uvac, 41.Tisa-Novi Kneževac, 42.Vlasina, 43.Vrla 2, 44.Vrutci, 45.Zavoj, 46.Zlatibor, 47.Zobnatica, 48.Zvornik, 49.Pek-Blagojev kamen, 50.Sava Litije-Ostružnica, 51.Kanal Odžaci-Sombor, 52.Dunav-Apatin, 53.Vrla1, 54.Mrtva Tisa-Mol, 55.Rakina bara, 56.Zapadna Morava-Čačak, 57.Opovački Dunavac, 58.Ponjavica, 59.Veliki Zaton, 60.Bosut, 61.Studva, 62.DTD-Novi Sad, 63.Jegrička, 64.DTD-Vrbas, 65.DTD-Bačko Gradište, 66.DTD-Bač, 67.DTD-Srpski Miletić, 68.Zlatica, 69.Krivaja, 70.Kereš, 71.Palić, 72.Ludoš, 73.Carska bara, 74.Koviljski rit, 75.Obedska bara, 76.Zasavica, 77.Provala, 78.Ečka, 79.Stari Begej-Srpski Itebej, 80.Tamiš-Botoš, 81.Ribnica, 82.Divčibare, 83.Pustinjac.*

Microcystin LR measurements

Microcystin monitoring in various fresh water ecosystems in Vojvodina (lakes, accumulations, rivers and channels) has been performed from summer 2005 to spring 2006 on 16 different localities: Borkovac, Palić Lake, Ludoš Lake, Zobnatica, Koviljski rit, DTD (Channel Danube-Tisza-Danube) -Bečej, Krivaja-Srbobran, DTD-Vrbas, DTD-Srpski Miletić, Tavankut, Tisa-Novi Kneževac, Tisa-Bačko Gradište, Begej-Srpski Itebej, Tamiš-Botoš, DTD-Bačko Gradište, by means of standard water sampling methods. Microcystin concentration was measured in only one water supply reservoir in Central Serbia (Ćelije) during the cyanobacterial bloom in July 2004.

Colorimetric protein phosphatase inhibition assay (inhibition of enzyme protein phosphatase 1-PP1) was used for the detection of microcystin-LR concentration in water samples (An and Carmichael, 1994). Samples were concentrated by filtration through 0.45 µm membrane filter and extracted with 75% methanol (Fastner et al.,1998). PP1 activity was determined by mesaurement the rate of color production from the liberation of p-nitrophenol from the substrate p-nitrophenil phosphate, measured at 405 nm using the microtiter plate reader. The assay was carried out at 37°C for 2 hours. Toxin concentrations were determined using standard inhibition curve of microcystin-LR (SIGMA).

Results

Cyanobacterial "blooms" of water ecosystems in Serbia

As far back as 1980, large number of water ecosystems in Serbia was found in "blooming" (Fig.1A). Among 83 water ecosystems examined, 58 were found in blooming condition during last 2.5 decades. All natural lakes, accumulations, rivers and chanals in Vojvodina province (agricultural part) proved to be sites with frequent cyanobacterial proliferation.

Dominant "blooming" cyanobacterial taxa belonged to *Microcystis*, *Aphanizomenon*, *Anabaena* and *Oscillatoria* (*Planktothrix*) genera, represented by the most frequently observed *Microcystis aeruginosa, M. flos-aquae, Aphanizomenon flos-aquae, Anabaena flos-aquae, A. spiroides, Planktothrix agardhii* taxa, all of which are well known toxin producers.

The part of Central Serbia has been found to be very risky for surface reservoirs water supply since more than 20 reservoirs serve as drinking water suppliers, 9 of which were detected in severe and prolonged cyanobacterial "blooming" (Fig.1B).

The first results of microcystin analyses in Serbia

Table 1 represent the very first results on cyanotoxin (microcystin) concentrations detected in water bodies of Vojvodina, Serbia. During 2005 and 2006 the presence of microcystin-LR in water samples was screened using PP1 assay with peak values in the autumn 2005 and spring 2006 at location Ludoš Lake. The concentration of microcystin-LR in the water samples were in the range of 1.25–362.68 µgL^{-1}. The highest concentration of microcystin-LR detected at location Ludoš Lake was 362.68 µgL^{-1} in autumn 2005 and in spring 2006 268.07 µgL^{-1}. In summer 2005 and winter 2006 the highest microcystin concentrations were detected at accumulation Borkovac (165.48 µgL^{-1} and 226.06 µgL^{-1}).

Table 1 *Cyanobacterial toxins (microcystin-LR) present in water ecosystems survey in Vojvodina for summer 2005 - spring 2006 period.*

Locations	**Concentration of microcystin – LR (µgL-1)**			
	summer 2005	**autumn 2005**	**winter 2006**	**spring 2006**
Borkovac	165.48	152.78	226.06	12.90
Palić	40.98	119.43	9.45	136.68
Ludoš	70.59	362.68	4.21	268.07
Zobnatica	10.97	121.07	1.91	18.77
Koviljski rit	85.75	96.32	1.25	10.06
DTD-Bečej	9.73	65.32	1.48	16.85
Krivaja-Srbobran	14.01	79.56	1.43	33.11
DTD-Vrbas	7.99	10.63	4.24	9.78
DTD-Sr. Miletić	4.39	9.01	1.86	13.23
Gornji Tavankut	3.65	8.71	2.76	6.84
Tisa-N. Kneževac	6.87	5.48	8.44	10.37
Tisa-B. Gradište	115.36	25.29	8.37	122.39
Srpski Itebej	9.75	8.74	1.27	11.92
Tamiš-Botoš	7.42	8.84	1.64	5.03
DTD-B. Gradište	104.34	346.85	2.34	102.74

In water samples at 10 locations (Borkovac, Palić, Ludoš, Zobnatica, Koviljski rit, Krivaja-Srbobran, Tisa-B.Gradište, DTD-Vrbas, DTD-Bečej and DTD-B. Gradište) during 2005 and at 12 locations (Borkovac, Ludoš, Palić, Zobnatica,Tisa-B.Gradište, Tisa-N. Kneževac, Koviljski rit, DTD-Bečej, DTD-Sr. Miletić, Krivaja-Srbobran, Begej-Sr. Itebej and DTD-B.Gradište) during 2006, the microcystin concentration was higher than 10 µg L^{-1}. Microcystin concentrations detected in water samples from other locations were below that value.

“Blooming” of potentially toxic cyanobacteria has also been recorded in other reservoirs, like Ćelije (Plate 1). In July 2004, Ćelije reservoir, used as drinking water supply for city of Kruševac and its surroundings, was found “blooming” with taxa belonging to *Aphanizomenon*, *Anabaena* and *Microcystis*. This mass development of toxic cyanobacteria resulted in concentration of microcystin LR 650 µgL^{-1} in water of the reservoir, while in the drinking (tap) water of Kruševac city the concentration was 2.5 µgL^{-1}.

Disscussion

Cyanobacterial “blooms” in Serbia

According to references (listed under 2.2.1.), more than 80 water ecosystems (lakes, rivers, reservoirs) in Serbia have been monitored for “water blooms” in the last 25 years. In this period, “blooms” have been observed in 58 of them, representing 70% of the total number, while all 36 monitored ecosystems in Vojvodina region have been “blooming” in different seasons. Lakes and reservoirs were mostly affected by “blooming” events, cyanobacterial “blooms” detected in all monitored ecosystems in Vojvodina region (100%) and in 22 water bodies in Central Serbia (about 50%).

Eutrophication has been considered as a significant environmental (aquatic) pollution problem in the developed countires of Europe and America. Research so far register 54% of the lakes in Asia and Pacific region as eutrophic, 53% of the lakes in Europe, 48% in North America, 41% in South America, while only 28% registered eutrophic lakes in Africa (Bartram et al., 1999). Having nearly 70% of water ecosystems in Serbia “blooming” during the last 25 years, the eutrophication problem might be much more advanced and place Serbia within countires with the worst surface water quality.

Microcystins in Serbia

Detected levels of microcystin in Vojvodina water ecosystems creates certain questions that need to be elaborated a bit more in detail. Firstly, these results are more or less expected since Simeunović et al. (2005) have shown that water ecosystems in Vojvodina do often “bloom” with potentially toxic and toxic cyanobacteria. Explaining the low, but more or less constant, values of microcystin toxin is more difficult, and can be attributed generally on: i) lack of regular monitoring, ii) lack of coordinated sampling activities during and after “blooming” and iii) degradation of toxins either by bacteria (Takenaka and Watanabe, 1997), UV light (Tsuji et al., 1995) or humic substances (Welker and Steinberg, 1999). The laboratory experiments by Cousins et al. (1996) demonstrated that primary degradation of microcystin-LR in reservoir water occurred in less than one week.

Cyanotoxins can be acutely toxic to humans depending on environmental concentrations to which people might be exposed, e.g. through oral uptake. Microcystin concentrations typically range between 1 and 100 µgL^{-1} in the open water during enhanced cyanobacterial growth, but concentrations up to 25 mgL^{-1} in cell accumulations, e.g. at shorelines, have been found and may be even higher during presence of clones with higher cellular microcystin contents. Chorus and Fastner (2001) calculated that for a averagely sensitive 10 kg child an acutely lethal dose could be reached by ingestion of 1-2 liters of such a cyanobacterial suspension of “pea soup” consistency which contained 25 mgL^{-1} of microcystin (at an LD$_{50}$ of 5 mg per kg body weight or 50 mg for the 10 kg child). Acutely lethal intoxication, particularly of small children, through ingestion of scum material therefore cannot be dismissed as a possibility under circumstances of heavy scum formation with very high microcystin content in the cyanobacterial cells.

On the other hand, it is also very difficult to relate this kind of low toxin levels, although in a prolonged time period, to eventual human health risk. Controversial results in Falconer et al. (1994) test on pigs or Fawell et al. (1999) on mice, that detected only minor changes in liver cell histology and slight increase of liver enzyme values, were not conclusive for any relation to the toxin exposure and contained numerous uncertainty factors. Nevertheless, WHO expert group (Falconer et al., 1999) developed a guideline value (GV) of 20.000 cyanobacterial cells per ml, or 2-10 µgL^{-1} microcystin, as a health hazard value in recreational waters. According to results presented in Table 1, although collected seasonally and outside “blooming” events, this GV is met in several examined water bodies in Vojvodina. For comparison, in two row water supplies of Finnish waterworks microcystins were detected sporadically and the highest microcystin

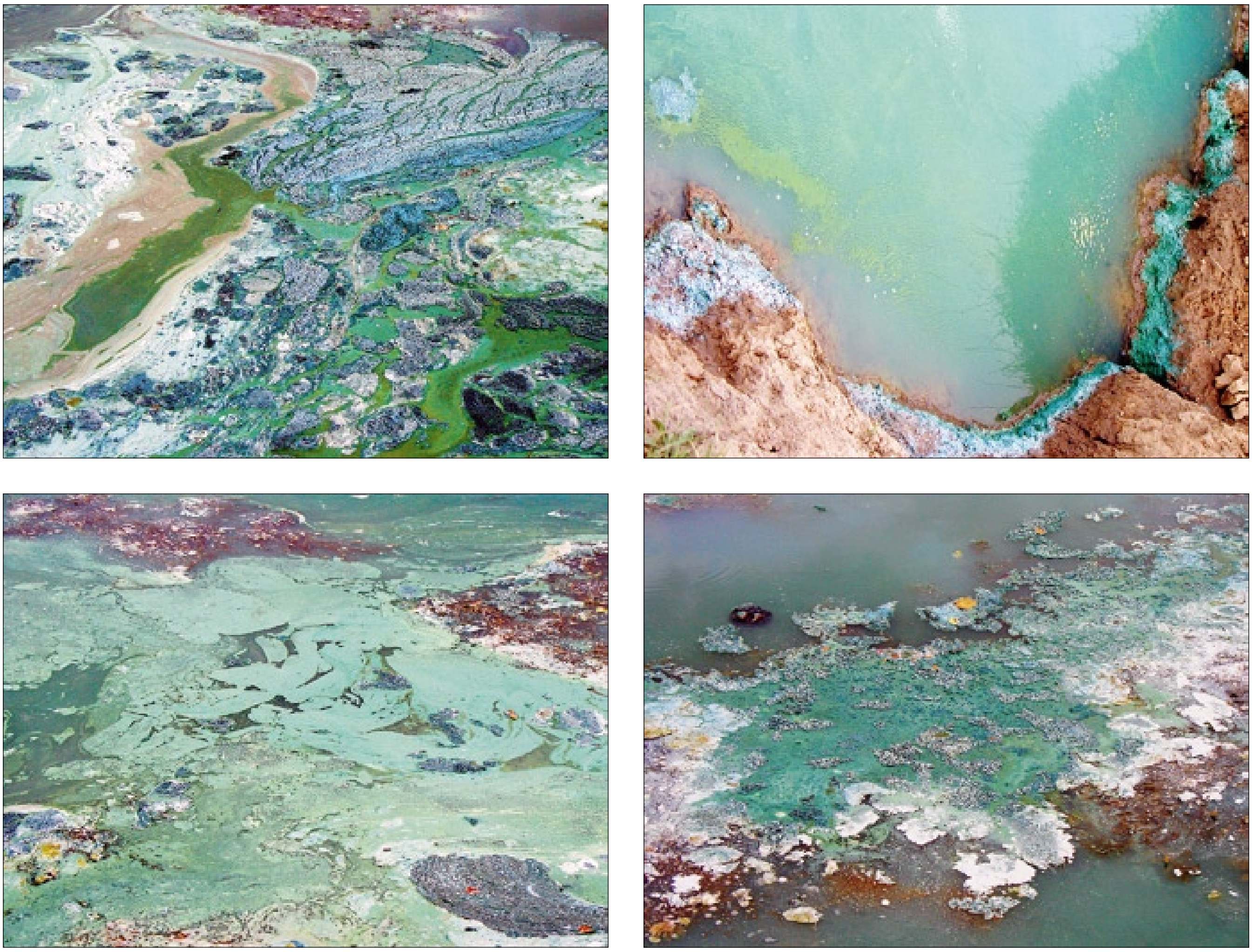

Plate 1 Cyanobacterial "blooming" in Ćelije reservoir in July 2004

concentrations mesaured were approximately 10 µgL^{-1} MC-LR equivalents. Microcystin were detected in 40% of row water samples but usually the concentrations were less than 1 µgL^{-1} (Lahti et al., 2001). The results of concentration of microcystin in Portugal indicate that during the most of the sampling period (1999) cyanotoxin values were below 50 ngL^{-1} and the highest concentration was 173 ngL^{-1} (Caetano et al., 2001). The highest recorded and reported microcistyn concentrations in samples with or without detected "blooms" are: 25.000 µgL^{-1} and 3.300 µgL^{-1} for anatoxin-a(S) in Germany (Chorus et al., 1998); in Japan for 1996 reported microcystin concentrations is 1.300 µgL^{-1} (Ueno et al., 1996), while in 1997 as high as 15.600 and 19.500 µgL^{-1} (Nagata et al., 1997).

There is no regular monitoring of the presence of potentially toxic, toxic cyanobacteria and cyanotoxins in drinking and surface waters in Serbia. Specific regulations and legislation on maximum permitted levels of cyanotoxins in water do not exist. The World Health Organisation (WHO) has addressed health hazards presented by cyanotoxins as a part of the WHO Guidelines for Drinking Water Quality. WHO Guideline Value (GV) for total microcystin-LR in drinking water is 1 µgL^{-1} (WHO, 1998; WHO 1999; Chorus and Bartram, 1999), limit that is already being used in some countries (e.g. Australia, UK) (Codd, 2000).

Regions in Central Serbia mostly have surface drinking water supply reservoirs that are "blooming" during summer months, compared to Vojvodina that has undergound water supply systems. Consequently, the differences in drinking water quality in this regions might be a result of the presence of hepatotoxins as a product of many "blooming" cyanobacteria.

Conclusions

More than 80 water ecosystems (lakes, rivers, reservoirs) in Serbia have been monitored for "water blooms" in the last 25 years. In this period, "blooms" have been observed in 58 of them, representing 70% of the total number, while all 36 monitored ecosystems in Vojvodina region have been "blooming" in different seasons.

During 2005 and 2006 the presence of microcystin-LR in water samples was screened with peak values in the autumn 2005 and spring 2006 at location Ludoš Lake. The concentration of microcystin-LR in the water samples were in the range of 1.25–362.68 µgL^{-1}. The highest concentration of microcystin-LR detected at location Ludoš Lake was 362.68 µgL^{-1} in autumn 2005.

"Blooming" of potentially toxic cyanobacteria has also been recorded in reservoirs for water supply. In July 2004, Ćelije reservoir, used as drinking water supply for city of Kruševac and its surroundings, was found "blooming" with taxa belonging to *Aphanizomenon, Anabaena* and Microcystis. This mass development of toxic cyanobacteria resulted in concentration of microcystin LR 650 µgL^{-1} in water of the reservoir, while in the drinking (tap) water of Kruševac city the concentration was 2.5 µgL^{-1}.

Acknowledgements

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