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# Cyanobacterial Blooms and their Toxicity in Vojvodina Lakes, Serbia

**Svirčev, Z.[1], Simeunović, J.[1], Subakov-Simić, G.[2], Krstić, S.[3], Pantelić, D.[1] and Dulić, T.[1*]**

[1] University of Novi Sad, Department of Biology and Ecology, Faculty of Sciences, Trg Dositeja Obradovića 2, 21000 Novi Sad, Serbia

[2] University of Belgrade, Faculty of Biology, Studentski trg 16, 11000 Belgrade, Serbia

[3] University "Ss.Cyril and Methodius", Faculty of natural Sciences and Mathematics, Arhimedova 5, 1000 Skopje, Macedonia

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**ABSTRACT:**The presence of cyanobacteria (blue-green algae) is a significant indicator of water quality, having in mind that several genera have the quality of producing cyanotoxins, which are harmful to animals, plants and humans. Seven lakes in Vojvodina (Republic of Serbia) were examined in May and September 2007 for cyanobacterial presence and cyanotoxins content, Chl *a* concentration and index of phosphatase activity as the indicators of water quality. In the spring period, cyanobacteria species were not dominant in only 2 of 14 water samples. Microcystins were detected in all of the lakes examined. The concentrations of microcystins ranged from 6.66 μg/L (Provala Lake) to 199.9 and 238 μg/L (Palić and Ludaš Lake respectively).The investigations conducted on 7 water ecosystems in Vojvodina, regarding the basic parameters of eutrophication, Chl *a* concentration and phosphatase activity index, have rendered the examined ecosystems immensely endangered due to significant water quality deterioration. It was determined that the intensification of eutrophication processes has been linked to the increased presence and abundance of cyanobacteria as well as significant toxin production.

**Key words:** Cyanobacteria,Cyanobacterial Blooms, Microcystins, Chlorophyll *a,* Phosphatase activity, Vojvodina, Serbia

## INTRODUCTION

Multiple ecological factors (ex. temperature, light, nutrient concentration, etc.) influence the development of cyanobacteria and algae, their physiological activity and hence the production and release of toxins (Whitton and Potts, 2000). In every case where one or several cyanobacterial/algal species proliferate and reach concentrations of above 10.000 cells per milliliter, the event is termed "water bloom" (Falconer, 1998; Falconer *et al.,* 1999; Fleming *et al.,* 2002). Research also confirmed that cyanobacterial presence even in concentrations of above 1.000 cells per milliliter is potentially hazardous and the toxin concentration is possibly harmful to the biota. Having that in mind, chlorophyll *a* concentration values may represent an additional significant indicator of possible toxin presence in the case of cyanobacterial dominance in the ecosystem (Falconer, 1998). Cyanobacteria are especially abundant in shallow, warm, nutrient rich or polluted water low in oxygen, and can grow to form thick scum that could color the water, creating blooms (Stotts *et al.,* 1993). Blooms of cyanobacteria usually follow enrichment by nutrients such as phosphates and nitrates in the water (Oberholster *et al.,* 2004). Most of these nutrients are derived from human wastes such as sewage and detergents, industrial pollution, run-off of fertilizers from agricultural land, and the input of animal or bird wastes from intensive farming (Bell and Codd, 1994; Baker, 2002). Toxic cyanobacteria are found worldwide, and cyanotoxins have been described for most of the countries where surveys have been made (Kuiper-Goodman *et al.,* 1999; Sivonen and Jones, 1999; Stoyneva, 2003; Boauru *et al.,* 2006; Pavlova *et al.,* 2006). Research shows that approximately half of cyanobacteria that cause water bloom also have toxic effects (Rapala and Lahti, 2002). To date, there are more than 50 identified species of cyanobacteria able to produce toxins. In Europe, the most frequently observed genera in fresh waters during blooms are *Microcystis*, *Anabaena, Aphanizomenon, Oscillatoria, Planktothrix, Nodularia* and *Nostoc*

*Corresponding author E-mail:waterlifea@gmail.com

(Sivonen and Jones, 1998). Cyanobacterial blooms produce secondary metabolites potentially toxic to secondary consumers, including zooplankton, fish and mammals that use affected waters as a habitat, and humans for drinking and recreational purposes (Chorus and Bartram, 1999). They could induce death of humans and animals or different health problems after accidental intaking (ingestion or after taking toxic sea food), inhalation and dermal contact (Granéli and Turner, 2006). There is also a fear that some of these toxins can contribute to the development of cancer when present in the water supply at very low levels (Carmichael, 1986; Svirčev *et al.,* 2010). 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 (Stewart *et al.,* 2006). 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 and Serbia has been attributed to drinking water contaminated with cyanobacterial toxins (Yu, 1989; Harada *et al.,* 1996; Ueno *et al.,* 1996; Svirčev *et al.,* 2009b).

Cyanotoxins are a very diverse group of toxins (Robertson *et al.,* 1997). They are either membrane bound or exist free inside the cells. The release of toxins occurs during cell life, but mostly after cell death through passive flow out of the cellular content. A very interesting point of toxic cyanobacteria is the presence of different toxins within the same genus and, on the other hand, the presence of the same toxins in widely different genera. Another specificity of cyanotoxins is the 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 World Health Organization has drawn up guideline values for microcystin in drinking water and recommendations for recreational waters (WHO, 1998a; 1998b; 1999). Proliferation of cyanobacteria in recent years has resulted in the deterioration of the recreational water quality and the production of good quality potable water in Serbia. These were the main reasons for initiating systematic investigations of drinking water and recreational reservoirs, including a detailed epidemiological study, in the last 15 years. The main purpose of this article is to present the primary results on cyanobacteria and cyanotoxins presence in the same type of recreational water ecosystems in Vojvodina.

## MATERIALS & METHODS

Vojvodina is a typical lowland region in northern Serbia, located in the southeastern part of the Pannonian Basin, and encompassing the confluence area of large European rivers - the Danube, Sava and Tisa (Marković *et al.,* 2008).Due to the gemorphological, hydrological and climatic conditions, the majority of the water bodies in the area covered by the research are shallow and nutrient-rich (Svirčev *et al.,* 2008; 2009a). The occurrence of toxic cyanobacteria water blooms in freshwater ecosystems in the Vojvodina region was studied in 7 natural and artificial lakes used for recreation and irrigation in May and September 2007. Mrtva Tisa and Provala are fluvial lakes in the floodplains of the Danube and Tisa rivers. Palić and Ludaš are natural lakes located in the contact of sand cover area and loess plateau near Subotica, a town close to the Hungarian border. Zobnatica, Borkovac and Kudoš are artificial reservoirs accumulated in the loess valleys surrounded by intensively cultivated land (Dolinaj *et al.,* 2011).

Expert and correct sampling is a major precondition for successful and high quality biological analyses, as well as the proper transport, storage and preparation of the samples (Petrović *et al.,* 1998). All samples from the investigated ecosystems in Vojvodina were collected by means of standard sampling methods, using suitable sterile equipment for each sample. The samples were transported in a short time period, at low temperature (4°C). The sampling was performed seasonally, in May for spring and in September for the autumn aspect, in 2007 on 7 selected localities in Vojvodina (Fig. 1) in order to obtain the seasonal variations of the samples from the same localities.

The conducted research included lakes (Mrtva Tisa, Palić, Ludaš) and reservoirs (Zobnatica, Borkovac, Pavlovci and Provala) (Table 1). Many of these ecosystems are used as important water resources in irrigation, aquaculture and for recreational purposes, but were also found blooming at least once in the last 25 years (Svirčev *et al.,* 2007).

The surface of the lakes covered by this study measured between 420.000 $m^2$ and 5.600.000 $m^2$, while that of the reservoirs measured between 360.000 $m^2$ and 3.400.000 $m^2$, with a maximum depth of 5 to 10 m and a maximal volume of several million cubic meters. Water samples were collected from the surface water layer within littoral zone. Samples measuring 200 mL were collected for phytoplankton analysis and preserved in a 4% solution of formaldehyde according to the European standard EN 15204 (EN 15204, 2006). Samples for quality phytoplankton analysis were

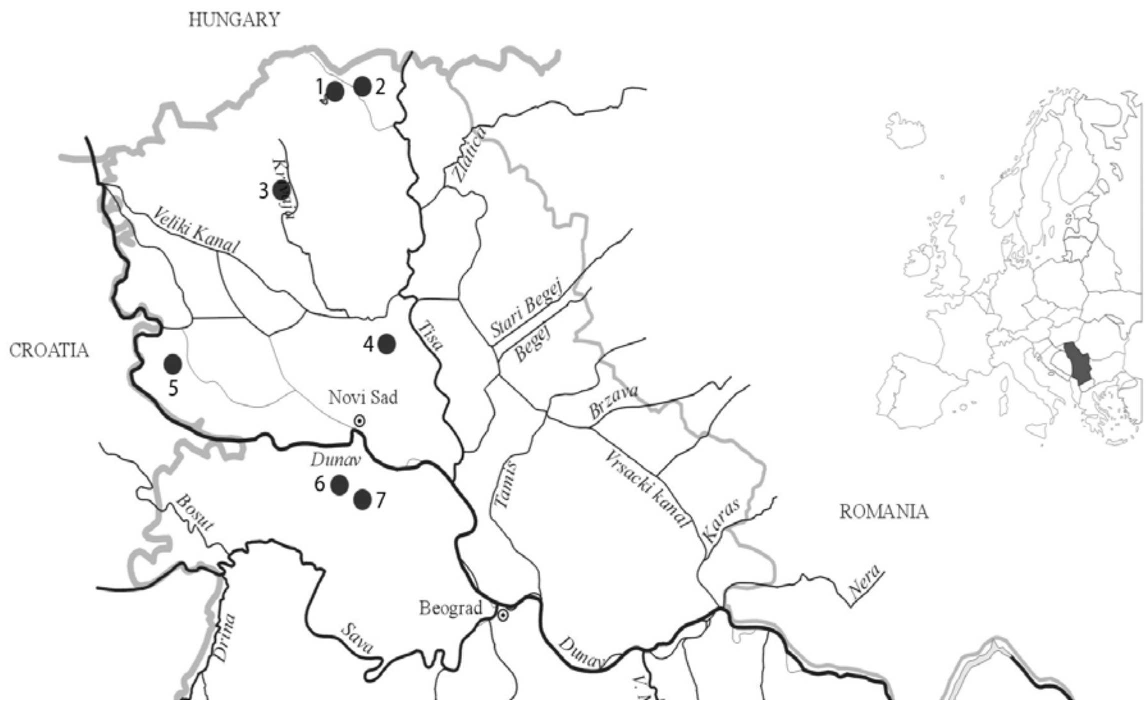


**Fig. 1. Investigated water ecosystems in Vojvodina**

Legend **Palić; Ludaš**
**Zobnatica** **MrtvaTisa**-B. Gradište
**Pavlovci (Kudoš)** **Borkovac** 7. **Provala**

**Table 1. Characteristics of the studied lakes (Stanković, 2005; Svirčev *et al.,* 2008; Dolinaj *et al.,* 2011)**

| Lakes | Lake area (ha) | Lake volume ($10^3$ $m^3$) | Average depth (m) | Maximum depth (m) |
|---|---|---|---|---|
| Mrtva Tisa | 300 | 6000 | 2 | 12 |
| Provala | 4.2 | 286 | 7 | 19 |
| Zobnatica | 340 | 5500 | 1.6 | 6 |
| Palić | 560 | 9000 | 1.6 | 2.5 |
| Ludaš | 328.5 | 3285 | 1 | 2.25 |
| Borkovac | 36 | 900 | 2.5 | 5 |
| Kudoš-Pavlovci | 64.5 | 3235 | 5 | 9 |

collected by sweeping the plankton net (netframe 25 cm ø, mesh net 22 µm) from the bottom to the surface. All samples were preserved at once in a 4% solution of formaldehyde. All samples were kept cool and in a dark place. Quantitative analyses of phytoplankton were made using Utermöhl method (EN 15204, 2006) with a Leica inverted microscope and it is expressed as number of cells per mL. Water samples were collected for chl *a* determination from the 0.3 m water depth, mixed and concentrated by filtering 0.5 L of water through a 0.45 µm membrane filter. Filters were wrapped in clean 15 mL centrifuge tubes and chlorophyll *a* was extracted with 90% acetone overnight at 4°C. Extracts were centrifuged at 1500g for 10 minutes and measured spectrophotometrically (Clesceri *et al.,* 1996). Measurements were done in duplicate and results are expressed as mean values. Water quality assessment by this parameter was used for the trophic state determination according to Felfoldy (1980) and the evaluation of eutrophication of the investigated water ecosystems. Phosphatase activity (APA) in the water samples was measured using the spectrophotometric method (Matavulj and Flint, 1987). Enzyme activity was measured as the rate of hydrolysis of a phosphatase substrate p-nitrophenylphosphate (p-NPP), by detecting the released product, p-nitrophenol. Phosphatase activity (activity of acid and alkaline phosphatases) was determined by adding 0.3 mL of

5% p-nitrophenylphosphate (p-NPP Sigma in 1 M Tris buffer at pH 5 for acid phosphatases and pH 9 for alkaline phosphatases) into 2.4 mL of water sample and incubated at 30°C for 1h. The reaction was interrupted directly by adding 10 M NaOH and the enzyme activity was measured at 420 nm with a spectrophotometer (Beckman 25). Samples were processed in the laboratory immediately after field sampling, without previous refrigeration. Detection of microcystins was done by colorimetric protein phosphatase inhibition assay (An and Carmichael, 1994). The activity of recombinant protein phosphatase 1 enzyme expressed in *Escherichia coli* (Sigma-Aldrich) was determined by measuring the rate of color production from the liberation of p-nitrophenol from substrate p-nitrophenyl phosphate (Fluka), at 405 nm using the microtiter plate reader MULTISCAN EX (Thermo Labsystems). The assay was carried out at 37ºC for 2 hours. The values of microcystins calculated as microcystin-LR equivalents were determined according to a standard inhibition curve with pure microcystin-LR (Sigma-Aldrich). The water samples for toxin analyses were collected from the surface layer (0.3 m depth), mixed well and concentrated by filtration through a 0.45 µm membrane filter. Filters were dried at 45ºC overnight and extracted with 75% (v/v) aqueous methanol (Fastner *et al.,* 1998). Extracted samples were sonicated (3 times for 1 minute) to ensure cell lyses and release of toxins into the solution. Extracts were centrifuged at 10000g for 10 minutes and triplicate aliquots of each sample were then analyzed by the PP1 inhibition assay for microcystin concentrations. Results are presented as mean values of measurements.

## RESULTS & DISCUSSION

Intensive algal blooms caused mainly by cyanobacteria were noted in all of the lakes observed. Of the 30 different species of cyanobacteria encountered *Aphanizomenon flos-aquae* Bréb., *Planktothrix* spp., *Gomphosphaeria* sp., *Pseudanabaena limnetica* (Lemm.) Kom., *Microcystis* spp., *Merismopedia tennuissima* Lemm and *Anabaena spiroides* Kleb were the most dominant (Table 2). Cyanobacterial species from the following genera were also observed: *Anabaena*, *Anabaenopsis*, *Limnothrix*, *Gloeocapsa* and *Spirulina*. Mass development of different cyanobacterial taxa was observed in 6 out of the 7 localities (Borkovac, Palić, Ludaš, Zobnatica, Mrtva Tisa, and Pavlovci) during 2007. Cyanobacterial genera or species composition varied depending on the season. In the spring of 2007 intensive blooms by *Microcystis* and *Anabaena* were recorded in two localities: Palić and Ludaš. During the autumn season, the blooms were recorded in 3 localities: Palić, Ludaš and Mrtva Tisa, with the mass development of *Planktothrix, Microcystis* and *Merismopedia* taxa. The share of cyanobacteria in total phytoplankton abundance was as high as 99.95% in the Mrtva Tisa Lake, 98.85% in the Palić Lake and 98.65% in Ludaš Lake. In some lakes (Borkovac and Kudoš) cyanobacterial dominance was not registered in May, but in September the presence of *Aphanizomenon flos-aquae* in more than 50% was observed in both lakes (Table 2).

In spring, the highest concentration of microcystin-LR was detected in Ludaš (238.00 µg/L) and Palić (199.87 µg/L) lakes (Table 2). Significant concentration was also recorded in Mrtva Tisa (63.44 µg/L), while Pavlovci, Provala and Zobnatica showed low microcystin presence (6.36 µg/L, 6.66 µg/L and 29.12 µg/L respectively); locality Borkovac was free of microcystins. During the autumn period, elevated concentrations of toxins were recorded on 3 localities (Table 2). The highest value was recorded in Mrtva Tisa (112.09 µg/L). In Palić and Ludaš lakes a significant decrease in toxin concentrations in relation to spring samples was recorded, with Ludaš having 55.81 µg/L, and Palić 49.30 µg/L. The lowest concentration of toxins was recorded in the Pavlovci Lake, of only 1 µg/L. The rest of examined localities, Zobnatica, Provala and Borkovac, had toxin concentrations below 35 µg/L (34.65, 23.12 and 12.98 respectively).In order to monitor the process of eutrophication and to detect the trophic level of the examined ecosystems, the chlorophyll *a* measurements, as the most indicative parameter of the process, were performed. During the period over which the examinations were performed in 2007, similar variations of the Chl *a* were recorded in all investigated localities (Fig. 2). On the basis of Chl *a* measurements in the spring samples, the Provala locality was labeled as an oligotrophic system, and Pavlovci as an oligo-mesotrophic one. Borkovac and Zobnatica were revealed to be mesotrophic systems, Lake Palić was labeled as eutrophic (77.43 mg/m$^3$), whereas Lake Ludaš was the only one revealed to be a eu-polytrophic system (124.60 mg/m$^3$).

In the autumn samples, an increase of Chl *a* in concentration in all the examined ecosystems was recorded (Fig. 2). Lake Ludaš had the highest Chl *a* concentration of 224.28 mg/m$^3$. Also, Lake Palić showed high Chl *a* values (103.83 mg/m$^3$) thus having an eu-polytrophic character. Zobnatica (71.75 mg/m$^3$) and Mrtva Tisa (66.75 mg/m$^3$) localities were revealed to be eutrophic systems, while Pavlovci reservoir was meso-eutrophic. The last two of the examined localities, Borkovac and Provala, were revealed to be mesotrophic and oligo-mesotrophic systems respectively. It being

**Table 2. Phytoplankton abundance (cell/mL) and microcystins in the studied lakes (μg/L)**

| Lakes | Date | Sum of Cyanobacteria (number of cells per mL) | Total number of phytoplankton (number of cells per mL) | Percentage of Cyanobacteria | Microcystins (μg/L) | Dominant species |
|---|---|---|---|---|---|---|
| **Mrtva Tisa** | May | 54155 | 54246 | 99.83 | 63.44 | *Aphanizomenon flos-aquae* |
| | September | 1544808 | 1545516 | 99.95 | 112.09 | *Planktothrix* sp. |
| **Provala** | May | 2907 | 5416 | 53.68 | 6.66 | *Gomphosphaeria* sp. |
| | September | 15468 | 16012 | 96.60 | 23.12 | *Gomphosphaeria* sp. |
| **Zobnatica** | May | 62103 | 65341 | 95.04 | 29.12 | *Pseudanabaena limnetica* |
| | September | 90992 | 93348 | 96.28 | 34.65 | *Planktothrix agardhii* |
| **Palić** | May | 747440 | 1158520 | 64.52 | 199.87 | *Microcystis aeruginosa* |
| | September | 2222080 | 2247936 | 98.85 | 49.30 | *Merismopedia tennuissima* |
| **Ludaš** | May | 2469600 | 3865920 | 63.88 | 238.00 | *Anabaena spiroides* |
| | September | 435210 | 441160 | 98.65 | 55.81 | *Microcystis* spp. |
| **Borkovac** | May | 300 | 19600 | 1.53 | n.o. | *Pediastrum* spp. |
| | September | 2690 | 4904 | 54.85 | 12.98 | *Aphanizomenon flos-aquae* |
| **Kudoš-Pavlovci** | May | 1152 | 15600 | 7.38 | 6.36 | *Coelastrum microporum* |
| | September | 8960 | 10560 | 84.85 | 1.1 | *Aphanizomenon flos-aquae* |

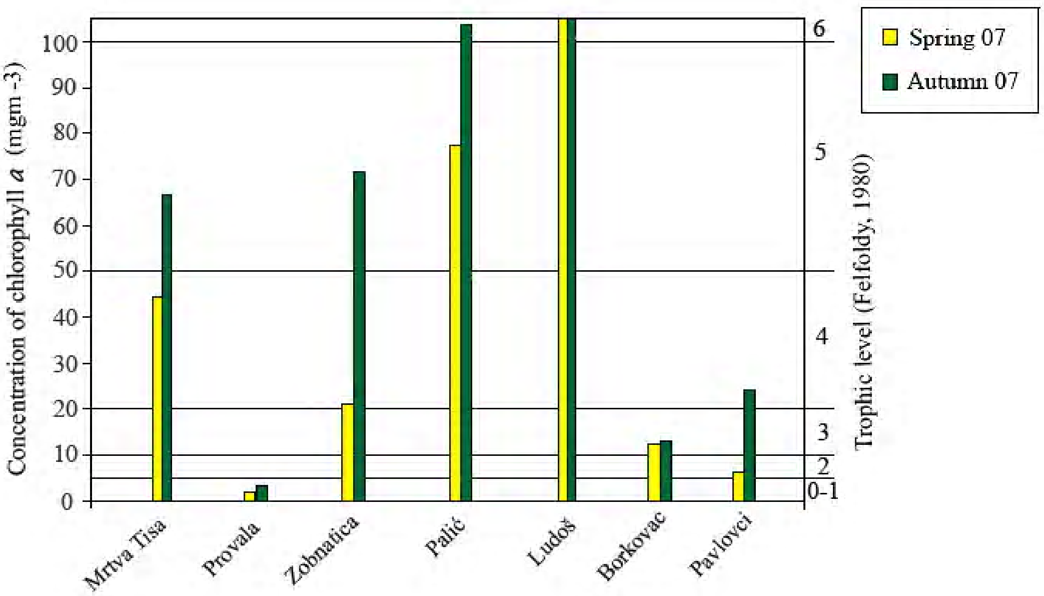


**Fig. 2. Chlorophyll a concentrations in water ecosystems in Vojvodina during 2007**

universally present in all aquatic microorganisms, the phosphatase enzyme complex is regarded as a useful biochemical parameter in water quality investigations. The water phosphatase activity is expressed as a phosphatase activity index (IPA) (Matavulj, 1986), whose level reflects the state of the overall organic load in the ecosystem.

Seasonal variations of the phosphatase activity in the ecosystems examined were clearly recorded during the analyzed period (Fig. 3). Increased phosphatase activity was recorded in the majority of ecosystems in spring samples. The exceptions were recorded in Mrtva Tisa with twice as much activity in the autumn period and Zobnatica with almost the same level of activity in both periods. During spring sampling, the detected values were in the range of 3 µmoL/pNPs/dm$^3$ in Zobnatica (IIIA class, polluted water) up to 11.83 µmol/pNPs/dm$^3$ in Lake Ludaš (IVA class, highly polluted water). Values of phosphatase activity indicative of highly polluted waters (IIIB class) were also recorded in Palić (5.86 µmoL/pNPs/dm$^3$) and Mrtva Tisa (5.89 µmoL/pNPs/dm$^3$). The majority of localities (Borkovac, Zobnatica, Provala and Pavlovci) had phosphatase activity typical of IIIA class (polluted waters).

In the autumn samples, the highest value of phosphatase activity was detected in locality Mrtva Tisa (11.33 µmoL/pNPs/dm/$^3$) representing the lowest water quality (IVA class). High enzyme activity typical for IIIB water class was detected in Lake Ludaš (6.94 µmoL/pNPs/dm$^3$). Based on the detected enzyme activities Borkovac, Palić and Zobnatica were labeled as IIIA class (polluted waters). Provala and Pavlovci showed phosphatase activity characteristic of moderately polluted waters (II-III class).

Since 1980, a large number of water ecosystems in Serbia was found with cyanobacterial blooms (Svirčev *et al.*, 2007). Among the 83 water ecosystems examined, 58 were found in blooming condition over the last 2.5 decades. All natural lakes, reservoirs, rivers and channels in the Vojvodina province (agricultural part) proved to be sites with frequent cyanobacterial proliferation (Simeunović *et al.*, 2005). Dominant "blooming" cyanobacterial taxa belonged to *Microcystis*, *Aphanizomenon*, *Anabaena* and *Planktothrix* genera, represented by the most frequently observed *Microcystis aeruginosa, M. flos-aquae, Aphanizomenon flos-aquae, Anabaena flos-aquae, A. spiroides, Planktothrix agardhii*, all of which are well known toxin producers (Simeunović *et al.*, 2005).

Our studies showed higher number of cyanobacteria in the phytoplankton of all lakes compared to some other shallow and nutrient-rich water bodies (Kasperoviciene *et al.*, 2005; Komarzewska and Glogowska, 2005; Szelag-Wasielewka, 2005; Honti *et al.*, 2007; Zagajewski *et al.*, 2007; 2009).

In the Mrtva Tisa and Zobnatica lakes, low-light adapted filamentous cyanobacteria, *Planktothrix agardhii* dominated in autumn. Mass occurrence of *P.*

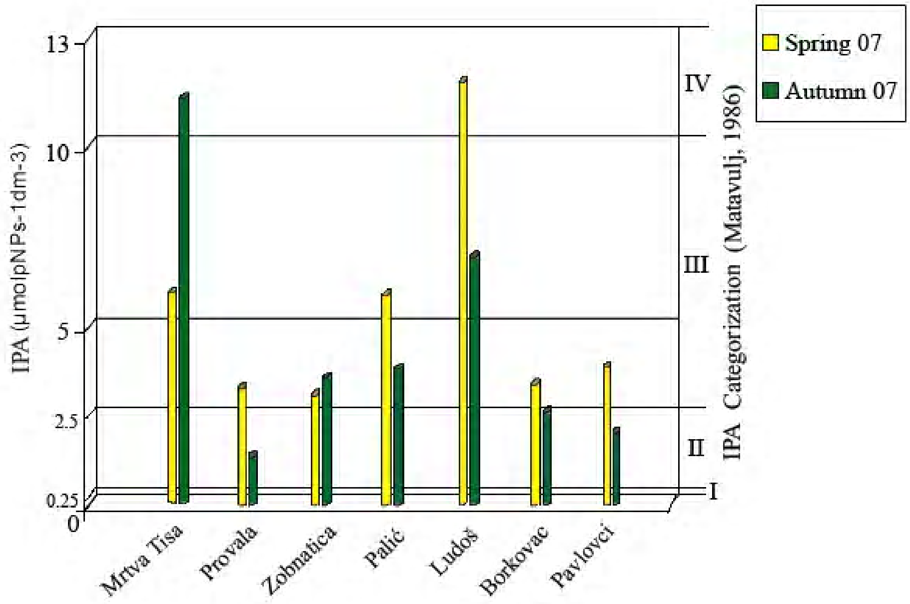


**Fig. 3. Phosphatase activity in water ecosystems in Vojvodina during 2007**

*agardhii* was commonly observed in other turbid, hypertrophic or eutrophic lakes and reservoirs (Kohler and Hong, 2000; Pawlik-Skowronska *et al.,* 2004; 2008; Grabowska and Mazur-Marzec, 2011). The mass development of *P. agardhii* was concomitant with higher toxin concentrations.

Merismopedia tenuissima was recorded with other cyanobacteria species Pseudanabaena limnetica and *Merismopedia glauca* in Lake Palić. The share in phytoplankton abundance was as high as 98.85%. This species is an epiphytic cyanobacterium that develops on submerged macrophytes. The higher values of microcystins in water samples can be explained by the presence of epiphytic species in phytoplankton. Zakaria and Al Shehri (2010) showed that *M. tenuissima* can produce microcystins. But high microcystin concentration might also be the result of metabolic activity connected to development of *Microcystis aeruginosa* during spring and warm summer months (Table 2). It means that very high autumn concentration of cyanotoxins sometimes might be explained by the blooming of present cyanobacteria (like Mrtva Tisa in September), but also by mass development and blooming of cyanobacteria during the spring months (like Palić in September when nontoxic *Merismopedia* was found to be in intensive blooming). Evidence might also be very significant in order to elucidate the origin of microcystins: the high concentration of extracellular microcystins in Palić during September (Četojević-Simin, 2009) most probably originated in former *Microcystis* blooms than *Merismopedia* activity. Examinations inevitably show that almost half of the bloom forming cyanobacteria exhibits toxic production abilities (Rapala and Lahti, 2002). On the other hand, toxin production may or may not be related to water bloom events, since natural communities are almost always composed of both toxin and non-toxin producing forms. Cyanotoxins are usually liberated from dead or injured cells and remain in water in an unchanged state for a short period of time. Their concentration decreases by natural decomposition processes in the period of several days-weeks-months, depending on the conditions.

Based on the results obtained on the total value of microcystins, it is clear that they were not recorded only in the Borkovac reservoir in May, when *Pediastrum* spp. was detected as dominant and the cyanobacteria presence was only 1.5% (Table 2). In September, on the same locality, cyanobacterial abundance rose to 50% with the toxic *Aphanizomenon flos-aquae* as dominant, thus resulting in cyanotoxins occurrence (12.98 g/L). Apart of Borkovac, relatively low microcystin concentrations were also detected in localities with a low total number of phytoplankton and cyanobacterial cells (Provala and Pavlovci). This is an expected result since the lack of microcystins would be a consequence either of a small number of cells or the absence of toxic cyanobacterial strains, and also without preceding the massive bloom with high toxin production. With the increase of cell abundance and cyanobacterial taxa participation, the concentrations of cyanotoxins in samples also increase

(Table 1). In works reported by Maatouk et al. (2002), Shen et al. (2003) and according to our observations, microcystin concentrations are not related to seasons of the year, since they were detected in June as well, and not exclusively in autumn, when they were to be expected.

After Četojević-Simin (2009), ELISA analyses also showed positive results and similar trends in all water ecosystems examined. The concentration of microcystins, measured as extracellular Microcystin-LR, ranged from 0.04 ?g/L to 12.37 µg/L. The highest concentration of microcystins was detected in Lake Mrtva Tisa in September. The concentration of saxitoxins ranged from 0.015 µg/L to 0.035 µg/L. The highest concentration of saxitoxins was measured in Mrtva Tisa Lake samples in September.

The detected values for microcystin concentrations in our examinations (Table 2) highly surpass the limit values for recreational waters (10-20 µg/L) given by the World Health Organization (WHO, 2004). All water ecosystems with high concentrations of microcystins that are above the critical values represent a serious health hazard and what is therefore recommended is a restriction on their utilization as potable water, water for recreational use, irrigation and aquaculture resources. In our examinations, the majority of samples had microcystin concentrations from 1 to 100 µg/L, a range typical for surface waters. Nevertheless, the maximum detected values in specific

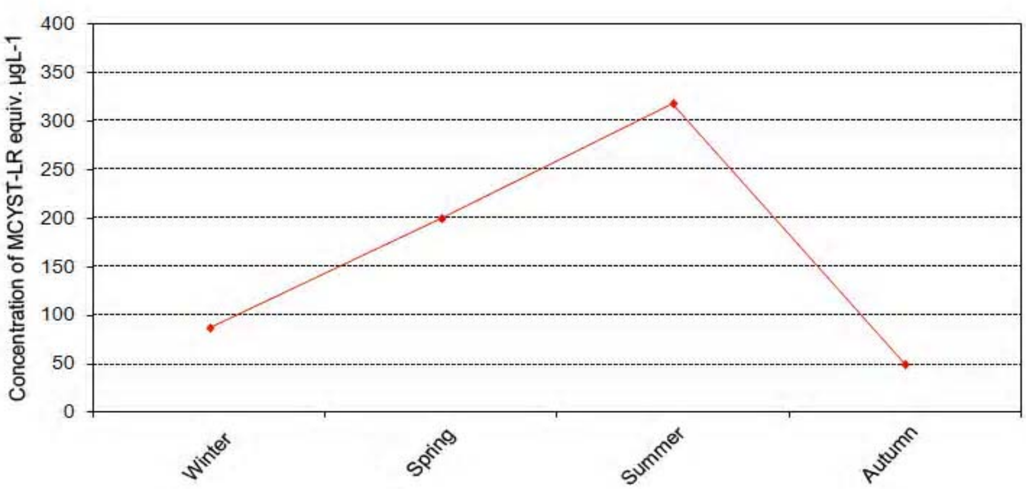


**Fig. 4. Microcystin concentration dynamics in Lake Palić in 2007 (Simeunović, 2009)**

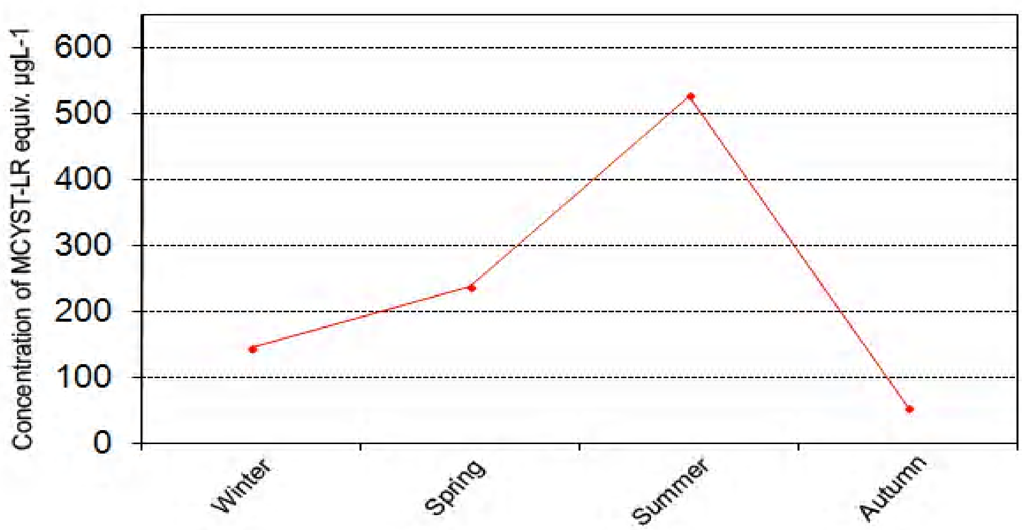


**Fig. 5. Microcystin concentration dynamics in Lake Ludaš during 2007 (Simeunović, 2009)**

localities (Palić and Ludaš lakes) are well beyond the stated range. During the set monitoring over several years, Simeunović (2009) reports a very detrimental trend of microcystin concentration increase in Lakes Palić and Ludaš during summer period (Fig. 4 and 5).

Research conducted in Finland revealed the highest microcystin concentration of app. 10 µg/L MCYST-LR equivalents in two drinking water reservoirs, with *Planktothrix agardhii* as the dominant species. Microcystins in this case were present in 40% of samples, but the majority of the concentrations were below 1 µg/L (Lahti *et al.,* 2001). Investigations of water ecosystems in The Czech Republic revealed the maximum cyanotoxins concentrations of 36 µg/L (Bláhová *et al.,* 2007). Out of 29 examined samples in Portugal, 28 were positive for microcystins, with the maximum concentration of 37 µg/L in a drinking water reservoir and approximately 35 µg/L in a recreational water body (Vasconcelos, 1999). Investigations on cyanobacterial occurrence and blooms in Poland during 2003-2004 revealed very low concentrations with maximum of 9.4 µg/L, while the lakes in Central Italy had a maximum of 7.6 µg/L microcystins (cit. u Chorus, 2005). Water quality monitoring on two lakes in Turkey revealed microcystin concentrations of 2.43 and 3.65 µg/L (Meric *et al.,* 2003). Testing of water samples from blooming surface waters in Southeast Brazil using a PP1 assay revealed microcystin concentrations from n.d. to 100 µg/L (Almeida *et al.,* 2006). Jordan et al. (2001) detected microcystin concentration in surface waters of Germany in the range of 0.14-119 µg/L, while Shen et al. (2003) detected microcystin concentrations in the range of 24.5-97. 3 µg/L in 6 out of 100 examined samples in China. Waters of Lake Balaton in Hungary had the highest microcystin concentration of 260 µg/L, while the analyses of 48 samples in Holland revealed microcystin concentration in the range of 0.15-147 µg/L, with significantly higher concentrations of 390 in Lake Zwemlust (Chorus, 2005). Some studies in Bulgaria (Pavlova *et al.,* 2006) indicate the presence of three microcystins (-LR, -RR and –YR) in lakes Vaya, Mandra and Pchelina as a result of the high level of eutrophication in these lakes. Krstić (2011) reports a maximum value of 288 µg/L microcystins detected in Dojran Lake during the one year surveillance monitoring of several natural and man made ecosystems in Macedonia.

The maximum concentrations of microcystins detected in the water ecosystems of Vojvodina are significantly higher in relation to the above stated values, especially regarding the concentrations reported for Finland, Czech Republic, Portugal, Poland and Italy. In the majority of thewater ecosystems of Vojvodina the detected concentrations were bordering the range reported for Germany (Chorus, 2001; Jordan *et al.,* 2001), Holland (Chorus, 2005), Czech Republic (Znachor *et al.,* 2006), Romania (Boaru *et al.,* 2006), Bulgaria (Pavlova *et al.,* 2006), Hungary, China and Brazil. On the other hand, there are many reports from different countries in the world where the detected concentrations are markedly high in water samples with or without blooms. The highest concentration of microcystins reported for Greece was recorded in Lake Kastoria of 3.186 µg/L (Chorus, 2005), while in Australia one of the highest ones was 1.800 µg/L (Jones and Orr, 1994). Analysis of several samples from France showed the concentration of microcystins ranging from a few to 100 µg/L, but, with blooming scum, this number was much higher, 1.000-10.000 µg/L (Chorus, 2005). Numerous investigated water ecosystems in Japan had a range of microcystin concentrations between 15.600 and 19.500 µg/L (Nagata *et al.,* 1997), while in Germany and Norway it was as high as 25.000 µg/L (Berg *et al.,* 1987; Chorus and Bartram, 1999), in Denmark 25.856 µg/L (Christoffersen, 2000), while one report on the investigations on Lake Oubeira in East Algeria points to microcystin concentrations of 3 to 29.163 µg/L (Nasri *et al.,* 2004).

As mentioned, chlorophyll *a* concentrations are used as an important indicator parameter for microalgae biomass production in water ecosystems. In our research, cyanobacterial blooms occurred in those ecosystems where Chl *a* concentrations were higher than 50 mg/m$^3$ (Table 2 and Fig. 2). By analysing the results of the trophic status and bloom occurrence in the surface waters in Vojvodina covered by the research, several ecosystems can be distinguished according to vulnerability. Lakes Palić and Ludaš are the most vulnerable ones according to Chl *a* concentrations (Fig. 2); they were also found to have the worst water quality during the several years of research (Simeunović, 2009). A high level of vulnerability was also detected in the case of Zobnatica and Mrtva Tisa. Conversely, with the Provala reservoir, blooming was not observed during the period of the research and has retained a good water quality status over the years (Simeunović, 2009).

Presented results point towards yet another important trend, i.e. the correlation between microcystin concentrations in water and phosphatase activity (Table 2 and Fig. 3). Organic matter increase in waters represents a major indicator of accelerated eutrophication processes in the ecosystem (Chrost and

Suida, 2002). Many studies have documented the usefulness, apart from Chl *a* measurements, of other parameters such as the enzymatic activity. There exist numerous papers emphasizing the highly positive correlation of the enzymatic activity with the concentration of dissolved organics in waters (Matavulj *et al.,* 1990; Chrost and Suida, 2002). Namely, the enzymatic activity exponentially increases in relation to trophic gradient and has a significant positive correlation to index of the trophic status of waters (Matavulj and Flint, 1987).

Our research shows precisely this type of correlation as well. Phosphatase enzimatic activity was the highest in waters found to be blooming, and especially high levels of enzimatic activity were detected in those samples where the concentration of microcystins reached its highest point. In that respect, it can be said that the measurements of enzymatic activity of aquatic microorganisms represent a practical and effective tool for rapid detection of the trophic status of water ecosystems, but also an additional parameter and an indicator in the estimation of microcystin presence in the environment.

Microalgae blooming, as a global phenomenon, has become ever more frequent over the previous decades, as a more intensive and widespread event in surface waters of all continents (Kahru *et al.,* 1994; Codd *et al.,* 1999; Anderson *et al.,* 2002). Calm and warm waters, high nutrients concentration, high pH, low levels of avaliable $CO_2$, are among the most favorable conditions for cyanobacteria development and blooming. Robarts and Zohary (1987) have also shown that the optimal temperature for cyanobacterial blooming is arround 25º C. Corroborative evidence are the numerous statements of warning that climate change, or global warming, may affect the functioning of aquatic environments, forcing changes in distribution of phytoplankton members over time and space in direction of more frequent occurence of invasive cyanobacteria species in the temperate climate regions (Vasconcelos, 2006).

Consequently, in many European countries, as well as America and Australia regular monitoring activities regarding the state and blooming of cyanobacteria have been established(Metcalf and Cood, 2004). Unfortunatelly, there is no monitoring of mass development of cyanobacteria and cyanotoxins production in different water environments in Serbia. Even more importantly, there are no legal regulations (by-laws, act standards) on the maximum permissible levels of cyanotoxin concentrations present in waters used for drinking, recreation, aquaculture or irrigation.

## CONCLUSION

Cyanobacterial taxa are usually dominant among the phytoplankton communities of the examined water ecosystems in Vojvodina in 2007. Their communities are dominated by genera known to encompass numerous toxin producing taxa: *Aphanizomenon flos–aquae, Pseudanabaena limnetica, Planktothrix agardhii, Microcystis aeruginosa* and *Anabaena spiroides.* Using the optimized protein phosphatase inhibition essay 1 (PP1) the presence of hepatotoxin microcystin was detected in water ecosystems in Vojvodina. Detected microcystin concentrations have reached up to 238 μg/L. The highest toxin concetrations were closely connected to blooming events, and cyanobacterial biomass concentration, detected in Lakes Palić and Ludaš. The research performed on 7 water ecosystems in Vojvodina, based on the results obtained after analysed eutrophication parameters, chlorophyll *a* concentrations and phosphatase activity index, has revealed that the investigated ecosystems are highly threatened and under constant anthropogenic influence, which leads to deterioration of the ecological condition and water quality, and manifests itslef in the acceleration of eutrophication processes. Based solely on seasonally performed research, it is very difficult to determine with absolute certainty whether the risk of microcystins exposure is present only in the month of June or September, or if it is constant throughout the summer season or even throughout the year. This estimation is only possible after the continual year-long monitoring of microcystin concentrations, even with multiple monthly samplings, during the warmer period (June to October). It is, therefore, necessary to design a monitoring program that will impose, apart from the relevant water quality parameters enacted by existing laws, the monitoring of cyanobacteria and cyanotoxins as obligatory parameters. With regards to the real health risks resulting from the occurrence of toxic cyanobacterial blooms in Vojvodina, it is imperative for the permissible concentrations of cyanotoxins to be established and legally regulated in accordance with the WHO recommendations.

***Abbreviations***:
**Chl *a***-chlorophyll *a* concentration;
**ELISA**-Enzyme Linked Immunosorbent Assay;
**IPA**-index of phosphatase activity;
**MCYST-LR**-microcystin-LR;
**WHO-**World Health Organization.

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