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Temporal and spatial variability of cyanobacterial toxins microcystins in three interconnected freshwater reservoirs

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Abstract: In spite of substantial research on health and the ecological risks associated with cyanobacterial toxins in the past decades, the understanding of the natural dynamics and variability of toxic cyanobacterial blooms is still limited. Herein, the results of long term monitoring 1998–1999/2001–2008 of three reservoirs (Vír, Brno and Nové Mlýny, Chech Republic), where toxic blooms develop annually, are reported. These three reservoirs provide a unique model because they are interconnected by the Svratka River, which allows possible transfer of phytoplankton as well as toxins from one reservoir to another. The frequency of the occurrence and dominance of the major cyanobacterial taxa *Microcystis aeruginosa* did not change during the investigated period but substantial variability was observed in the composition of other phytoplankton. Although absolute concentrations of the studied toxins (microcystins) differed among the reservoirs, there were apparent parallel trends. For example, during certain years, the microcystin concentrations were systematically elevated in all three studied reservoirs. Furthermore, the concentration profiles in the three sites were also correlated (parallel trends) within individual seasons based on monthly sampling. Microcystin-LR, a variant for which the World Health Organization has recommended a guideline value, formed only about 30–50 % of the total microcystins. This is of importance, especially in the Vír reservoir that serves as a drinking water supply. The maxima in the cell-bound microcystins (intracellular; expressed per dry weight biomass) generally preceded the maxima of total microcystins (expressed per volume of water sample). Overall, the maximum concentration in the biomass (all three reservoirs, period 1993–2005) was 6.1 mg g^{-1} dry weight and the median values were in the range 0.065–2.3 mg g^{-1} dry weight. These are generally high concentrations in comparison with both Czech Republic and worldwide reported data. The present data revealed substantial variability of both toxic cyanobacteria and their peptide toxins that should be reflected by detailed monitoring programs.

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INTRODUCTION

Anthropogenic contamination of surface waters with non-toxic nutrients (phosphorus and nitrogen) has resulted in massive cyanobacterial water blooms worldwide. Especially the production of cyanobacterial secondary metabolites (cyanotoxins) has attracted the attention of both scientists and public health authorities, since serious adverse health effects in both domestic animals and humans have been reported.[1]

Microcystins (MCs), unusual cyclic heptapeptides produced by several planktonic species of cyanobacteria, are the most frequently studied cyanotoxins. Based on toxicological data, the World Health Organization (WHO) recommended a provisional guideline value for MC-LR in drinking water of 1 μg L^{-1} (WHO, 1998). Preliminary surveys conducted in Europe (including the Czech Republic) showed that cyanobacterial blooms occur in about 80 % of large reservoirs.[2] About 90 % of the water blooms in the Czech Republic contained MCs (MC-LR, the toxin considered by the WHO, was present in 98 % of the positive samples) with median and maximum concentrations in the biomass of 0.7 and 5.8 mg g^{-1} dry weight (dw), respectively.[3] There are also some preliminary indications on the occurrence of toxic cyanobacteria in less explored areas of South and East Europe, *e.g.*, Serbia.[4,5] However, to the best of our knowledge, the highly important issue of cyanobacterial toxins in surface waters and their impacts on ecosystem quality and the health of the population in this region have not yet attracted sufficient scientific attention and remain to be explored in more detail.

In this paper, analyses of long-term data (1998–2008) on toxic cyanobacteria and their toxins in three large reservoirs, which are connected by a river in the region of South Moravia, the Czech Republic, are presented The Reservoir Vír (the most upstream) serves as an important supply of drinking water for the city of Brno (population 400,000), while other two reservoirs (Brno and Nové Mlýny) were built to help regulate water regimes, and were supposed to be also used for recreational purposes. The unique long-term data enabled the natural variability in the occurrence of dominant toxic cyanobacteria and the levels of cyanotoxins microcystins in these reservoirs to be studied, as there is a possible transfer of toxic blooms among reservoirs *via* the Svratka River (Fig. 1).

In spite of extensive research on the health and ecological risks associated with cyanobacterial toxins, a general understanding of the natural dynamics and variability of toxic cyanobacterial blooms is still limited. The presented analytical data provide some new insights into the relationships between the concentrations of MCs and the dominant cyanobacteria in water blooms.

Fig. 1. Map of the studied reservoirs in the Czech Republic (Vír, Brno and Nové Mlýny) interconnected by the River Svratka.

EXPERIMENTAL

Samples

Samples were collected annually from the Reservoirs Vír, Brno and Nové Mlýny (Fig. 1) during the vegetation seasons (during several seasons, repeated samplings were performed to obtained a more detailed picture about the intra-seasonal variation). The covered period includes the years 1998–2008; the results from the year 2000 are not presented as no monitoring of the Brno Reservoir was performed this year. Water bloom samples were collected using a plankton net (20 μm diameter); they were stored frozen at –20 °C, freeze-dried and stored for extraction and analyses by HPLC (described below). Samples of raw waters (for analyses of total microcystins, μg L^{-1}) were collected from the upper water layers (≈0.5 m depth) at each locality and stored at –20 °C until analysed by Elisa. Detailed observations at the studied reservoirs were compared with the results of the National Monitoring Program in the Czech Republic, which was organized by the authors of this paper. The same methods of sampling and microcystin analyses were employed for all samples.

Taxonomical determination

A portion of the cyanobacterial biomass collected in the plankton net was fixed with 4 % (v/v) formaldehyde for taxonomical identification, and the dominant phytoplankton species were identified and quantified according to current literature.[6] For evaluation purposes, the occurrence of each group of organisms was categorized according to their dominance.

Elisa analyses (total microcystin content)

Prior to MCs determination, the samples were thawed, sonicated, centrifuged (10,000 g, 5 min) and the supernatant analysed for MCs by direct competitive Elisa according to Zeck *et al.*[7] and as described in detail in a recent survey.[8] Briefly, high protein binding microplates (NUNC, Roskilde, Denmark) were pre-incubated with anti-mouse anti-Fc-IgG (ICN) following incubation with monoclonal antibodies developed against microcystin-LR (ALEXIS, Lausen, Switzerland). To visualize the reaction, MC-LR conjugated with horseradish peroxidase (HRP), prepared according to Zeck *et al.*[7] and TMB substrate (Sigma, Prague, Czech Republic), was used. The absorbance (420 nm with reference 660 nm) was determined using a GENios microplate reader (Tecan, Mannerdorf, Switzerland). The samples were analyzed in three replicates and compared with a 0.125–2 mg L^{-1} calibration curve constructed using a MC-LR standard (Alexis).

HPLC Analyses

Cyanobacterial blooms were extracted with 50 % methanol (100 mg dw mL^{-1}), and analysed as described previously[9] using an HPLC Agilent 1100 Series instrument (Agilent Technologies, Waldbronn, Germany) on Supelcosil ABZ+ Plus, 150×4.6 mm, 5 µm column (Supelco, Bellefonte, USA) at a temperature of 30 °C. The binary gradient mobile phase consisted of (A) H_2O + 0.1 % trifluoroacetic acid (TFA) and (B) acetonitrile + 0.1% TFA (linear increase from 20 % B at 0 min to 59 % B at 30 min); the flow rate was 1 mL min^{-1}. The chromatograms at 238 nm were recorded with an Agilent 1100 Series PDA detector (Agilent Technologies) and the MCs were identified by the retention time and characteristic UV-absorption spectra (200–300 nm) of individual peaks. Quantification was based on external calibrations of MC-RR, -YR and -LR.

Statistics

Relationships between the occurrence of phytoplankton and the concentrations of MCs were evaluated by χ^2 in Statistica for Windows 8.0 (StatSoft, Tulsa, OK, USA); *P*-values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

The concentrations of the cyanobacterial toxins microcystins in the three studied reservoirs together with the summary statistics from the Czech National Monitoring Program are presented in Table I. As calculations of the arithmetic mean are affected by the log-normal distribution (a few extreme concentrations), the median values, as another statistical parameter, are also presented (Table I). The concentrations of MCs in the dry biomass (expressed in µg g^{-1} dry wt), which reflects the actual toxic potential of the respective water bloom (sum of the dominant variants MC-LR, -RR, -YR is presented in Table I) were also investigated. Furthermore, the MC concentrations were also analysed directly in the raw water samples (µg L^{-1}), which corresponds to the total microcystin content. These values might be translated to the actual health risks resulting, for example, from consumption of contaminated drinking water, *etc.*[10,11]

Regarding the water bloom biomass, detectable concentrations of microcystins were found in all the collected biomass samples at the studied localities (no values the detection limit). The median values ranged from 25 µg g^{-1} dry weight

(2006 – Nové Mlýny) up to more than 6000 μg g^{-1} (1999 – Brno). The latter value from Brno Reservoir was the highest concentration determined in 1999 in the all Czech National Monitoring Program (Table I). In general, very high microcystin concentrations in the biomass were observed in the Vír Reservoir (see for example the years 2003–2005), which is the drinking water supply for the city of Brno with a population of about 400,000.

Table I. Summary statistics of microcystins concentrations in the reservoirs Vír, Brno and Nové Mlýny in 1998–2008 (*N*-number of samples, mean, median, maximum) in comparison with the overall data from the Czech National Monitoring Program. Concentrations in biomass (mg g^{-1} d.w.) and total water concentrations (μg L^{-1})

Year	Czech Republic (total)				Vír Reservoir				Brno Reservoir				Nové Mlýny Reservoir			
	Concentrations in biomass, μg g^{-1} d.w.															
	N	Mean	Med	Max	*N*	Mean	Med	Max	*N*	Mean	Med	Max	*N*	Mean	Med	Max
1998	21	452	98	3793	1	91	91	91	4	473	333	1130	2	157	157	289
1999	83	1049	588	6171	3	1914	2608	3134	14	1655	846	6171	9	1328	1315	2931
2001	43	979	830	3027	1	81.5	81.5	81.5	15	937	1090	1638	10	2087	2185	3027
2002	52	790	760	2438	2	1212	1212	1529	25	911	936	1371	2	818	818	1138
2003	84	505	318	3335	5	2075	2231	3335	41	236	154	1109	11	807	716	1489
2004	210	534	348	3945	2	1528	1528	2760	24	627	597	1303	13	820	850	1797
2005	198	467	183	3673	2	2929	2929	3213	30	434	343	1291	5	751	616	2082
2006	151	395	153	3954	3	238	112	542	2	1129	1129	1354	1	24.9	24.9	24.9
2007	161	347	81	2759	3	318	250	955	3	324	341	632	7	872	176	2609
2008	98	422	51	3312	2	2566	2566	3312	3	202	77	528	6	1588	1496	2665
	Concentrations in water, μg L^{-1}															
2004	285	0.99	0.22	36.9	2	5.26	5.26	9.18	56	0.81	0.15	4	12	1.21	1.03	2.63
2005	229	0.74	0.2	18.7	1	0.62	0.62	0.62	13	0.49	0.21	1.3	8	0.37	0.18	1.14
2006	208	1.29	0.5	24.8	3	0.86	1.27	1.32	2	0	0	0	3	0.5	0.19	1.17
2007	322	1.23	0.57	29.7	4	0.68	0.59	1.30	4	1.79	1.08	4.5	14	5.22	2.19	29.7
2008	197	1.05	0.3	20.1	3	0.40	0	1.2	2	0.38	0.38	0.49	5	2.85	1.49	9.4

The measured concentrations from the Czech Republic are comparable (and in many cases higher) in comparison with literature data from Europe. For example, the maximum reported from Poland was 1687 μg g^{-1}.[12] In Germany, the maxima ranged from 1100μg g^{-1} d.w. [13] to 5595μg g^{-1}.[14] The highest value of 2565 μg g^{-1} was detected during 1994–2000 in Greece.[15] In Portugal, very high concentration of microcystin, 7100 μg g^{-1}, was detected.[16]

The concentrations of microcystins in water samples (μg L^{-1}, analysed by Elisa) were analysed in the years 2004–2008. When considering the national statistics, the mean values were in general about two-times higher than the median. A similar trend was also observed at the three localities studied in detail. However, the differences were not highly pronounced with respect to the lower number of values. In some cases, concentrations above the WHO guideline value of 1 μg L^{-1} were observed (including the Vír Reservoir) but most of the values were

lower even in the studied raw (untreated) water as also discussed elsewhere.[10] On the other hand, extreme concentrations (> 10 μg L^{-1}) were observed in several cases and the maximum value 29.7 μg L^{-1}, observed in Nové Mlýny during 2007, was the highest within the whole country.

The observed extreme concentrations (9–36 μg L^{-1}) are comparable with those given in the literature. For example, Zhang *et al.*[17] reported a maximum of 8 μg L^{-1} in China during the 2002 season. Very high concentrations were found in surface cyanobacterial scum in Germany[18] (up to 120 μg L^{-1}) and Algeria[19] (711.8 μg L^{-1}) but these represent a different type of sample and also a different analytical method (protein-phosphatase assay inhibition) was employed[19] than that used in the present study. Lower maximum concentrations were reported from Finland 0.21 μg L^{-1},[20] where water blooms are often dominated by different cyanobacterial species than *Microcystis* sp., which is the most common in the Czech Republic and Central Europe.[10,8,21]

As is apparent from Table I, there was no clear temporal trend in the microcystin concentrations in the biomass (decrease or increase) during the studied years in the studied localities, which might be attributed to specific environmental or meteorological factors.[22,23] During the present study, the summers of the years 1998, 2006–2007 were unusually cold, which might explain the lower incidence of toxic cyanobacterial blooms as well as the decrease in the microcystin concentrations in the biomass.[24,25]

Detailed seasonal variations in both biomass-bound MCs (Fig. 2A) and MCs in water (Fig. 2B) are shown in Fig. 2. As is apparent, there was no uniform trend within each of the investigated seasons. For the biomass concentrations (Fig. 2A), there seemed to be an increase in the Vír and Nové Mlýny Reservoirs, but a slight decrease during all the studied seasons was observed in Brno. On the other hand, these trends were not in all cases confirmed by the MC concentrations in water (Fig. 2B), where a slight increases could be observed also in the Brno reservoir (for example during 2004–2005). The observations at Vir and Nove Mlyny, in general, correspond to some previously published studies that demonstrated an increase in the production of MCs in the biomass during the vegetation season.[26] On the other hand, some studies reported two peaks in the concentrations of MCs (early summer *vs*. end of the season).[27,28]

The observed differences might be related to the biochemistry of microcystin production and its excretion, which are known to be influenced by a number of environmental factors. For example, different seasonal profile of temperature and other parameters can be expected in the Vír Reservoir, which is situated upstream of the Svratka River in an area of highlands, while the other two reservoirs (Brno and Nové Mlýny) are lowland reservoirs. MCs are mostly accumulated inside the cyanobacterial cells, while extracellular (dissolved) toxins form usually up to 10 % of the total MCs in water.[18] However, at the end of the season – following the

collapse of the water bloom – the ratio of the extracellular MCs significantly increases up to 96 % of the total content, as documented by Nasri *et al.*[19] During the short periods, dissolved MCs concentrations can rise to 100 μg L^{-1} and these concentrations might remain in the water for several days to weeks.[18] On the other hand, MC bound to particulate matter is known to be more rapidly degraded.[20]

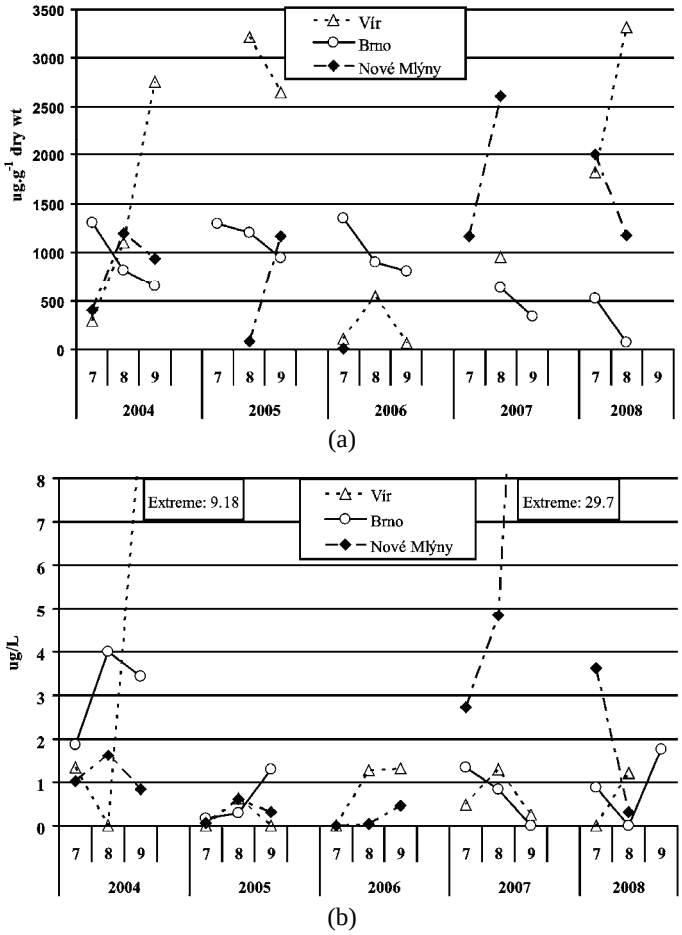

Fig. 2. Detailed seasonal variations in the concentrations of microcystin (MCs) in the biomass (upper panel, concentrations in microgram per gram biomass dry weight) and water (bottom panel, microgram per litre) in three reservoirs (Vír, Brno, Nové Mlýny) during July, August and September (7, 8, 9) of the years 2004–2008.

The present study, as well as literature data, indicates that under certain environmental situation, high concentrations of MCs may be observed during all vegetation seasons, and this should be carefully considered during monitoring studies.

In addition to the MCs concentrations, the composition of the water blooms in the studied reservoirs was also studied. In all three studied reservoirs, *Microcystis aeruginosa* was the most dominant species, which also correspond to the general situation in Central Europe.[8,12,13,21] However, during the studied period (1998–2008), an increase in the occurrence of other species, such as *Anabaena* sp., *Aphanizomenon* sp. or *Planktothrix* sp, were also observed. However, the issue of the occurrence and toxicity of less-studied species, including the tropical *Cylindrospermopsis raciborskii* , in Europe will require further research attention.[29]

CONCLUSIONS

The present report provides further insight into the occurrence and dynamics of toxic cyanobacterial metabolites – microcystins. The analyses showed that the appearance of dominant cyanobacterial taxa (*M. aeruginosa*) did not significantly change during the investigated period but there was an increase in the occurrence of other species belonging to genus Aphanizomenon, Anabaena and Planktothrix. Although absolute concentrations of the studied toxins microcystins differed between the reservoirs, there were apparent parallel trends. For example, during certain years, the microcystin concentrations were systematically elevated in all the studied reservoirs. Furthermore, based on monthly sampling, the concentration profiles in the three sites were also correlated (parallel trends) within individual seasons. Microcystin-LR, a variant for which the WHO has recommended a guideline value, formed only about 30–50 % of the total microcystins. In addition, a shift between the peaks of the cell-bound microcystins (expressed per biomass dry weight) was determined that seemed to precede the maxima of microcystins in water. The overall maximum toxin concentration in the biomass (all three reservoirs, period 1998–2008) was about 6000 microgram per gram dry weight with median values ranging from 65 to 2300. The concentrations of MCs in the raw waters only occasionally exceed the WHO limit of 1 microgram per litre but they occurred throughout the season and cannot be easily predicted. In summary, the obtained data revealed substantial variability of both toxic cyanobacteria and their peptide toxins that should be reflected in detailed monitoring programs.

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ИЗВОД

ПРОСТОРНО–ВРЕМЕНСКА ВАРИЈАБИЛНОСТ ЦИЈАНОБАКТЕРИЈСКИХ ОТРОВА МИКРОЦИСТИНА У ТРИ ПОВЕЗАНА РЕЗЕРВОАРА СВЕЖЕ ВОДЕ

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Упркос значајном обиму истраживања ризика по здравље и животну средину који су повезани са цијанобактеријским токсинима последњих деценија, схватање природне динамике и варијабилности токсичних цијанобактеријских цветања још увек је ограничено. Овде су приказани резултати дугорочног мониторинга 1998–1999/2001–2008. три резервоара (Vír, Brno и Nové Mlýny, Република Чешка) где се токсична цветања јављају сваке године. Ови резервоари представљају јединствен модел, зато што су повезани реком Svratka, што омогућава трансфер фитопланктона, као и токсина, између резервоара. Фреквенција појављивања и доминације већинских таксона *Microcystis aeruginosa* није се мењала током испитиваног периода, али значајна варијабилност је уочена у саставу осталог фитопланктона. Иако су се апсолутне концентрације проучаваних токсина (микроцистина) разликовале између резервоара, паралелни трендови су били очигледни. На пример, током неких година, концентрације микроцистина су систематски расле у сва три резервоара. Осим тога, концентрациони профили на све три локације су такође били корелисани (паралелни трендови) у оквиру појединачних сезона, на основу месечних узорковања. Микроцистин-ЛР, варијанта за коју је Светска здравствена организација препоручила оквирну референтну вредност, чинила је само 30–50 % укупних микроцистина. Ово је важно нарочито за резервоар Vír, који служи за снабдевање питком водом. Максимуми микроцистина везаних за ћелије (интрацелуларни, изражени по тежини суве биомасе) генерално се јављају пре максимума укупних микроцистина (изражених по запремини узорка воде). Општи максимум концентрације у биомаси (сва три резервоара, период 1993–2005.) био је 6,1 mg g^{-1} тежине суве материје; вредности медијане су биле у распону 0,065–2,3 mg g^{-1} тежине суве материје. Опште узевши, ово су високе концентрације у поређењу са подацима објављеним за Републику Чешку и свет. Подаци указују на значајну варијабилност како токсичних цијанобактерија тако и њихових пептидних токсина, што би требало да има одраза на детаљне програме мониторинга.

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