Summer Changes in Cyanobacterial Bloom Composition and Microcystin Concentration in Eutrophic Czech Reservoirs

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ABSTRACT: In mid-July and August 2003 and 2004, 18 reservoirs in the Czech Republic were sampled for phytoplankton species composition and concentration of intracellular microcystins (MCs). As a consequence of high nutrient loading, most of the reservoirs experienced cyanobacterial blooms of various intensities, with the prevalence of cyanobacteria increasing markedly in August, along with a conspicuous shift in species composition toward dominance of *Microcystis* spp. Microcystins were detected in 90% of the samples, and their amount also increased considerably in August, reflecting the cyanobacterial biomass. In *Microcystis*-dominated samples, a significantly higher amount of MCs ($p < 0.001$) occurred than in samples in which other taxa prevailed. Microcystins were positively correlated with chlorophyll *a* and cyanobacterial biovolume ($p < 0.05$, $R^2 = 0.61$ and 0.66, respectively), with the strongest correlation found for *Microcystis* spp. biovolume ($p < 0.001$, $R^2 = 0.87$). This taxon was the most important producer of MCs in Czech reservoirs. The main structural variants of MCs were MC-LR, MC-RR, and MC-YR. This study's data also indicate that the relative share of MC variants (MC-LR and MC-RR) varies considerably with time, most likely as a consequence of different species and strain compositions during the summer. This study clearly demonstrates a high prevalence of MC-producing cyanobacteria in Czech reservoirs. Therefore, regular monitoring of these reservoirs is highly desirable in an effort to minimize potential health risks to the human population. Environ Toxicol 21: 236–243, 2006.

Keywords: reservoirs; cyanobacteria; microcystins; bloom; *Microcystis*

INTRODUCTION

Each summer an excessive growth of cyanobacteria takes place in many Czech reservoirs as a consequence of high nutrient loading, particularly of phosphorus. Recently, problems associated with cyanobacterial blooms in

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freshwaters and their ecological consequences have been intensively studied (Chorus and Bartram, 1999; Chorus, 2001), documenting the detrimental effects of cyanobacteria in recreational, domestic, and industrial areas. A high cyanobacterial biomass contributes to aesthetic problems and impairs recreational use (because of scums and unpleasant odors) and affects the taste of treated drinking water, and some cyanobacterial species produce toxic compounds (Chorus and Bartram, 1999; Chorus, 2001; Codd et al., 2005).

Hepatotoxic microcystins (MCs) belong to the most commonly encountered cyanotoxins (Chorus and Bartram, 1999) and are cyclic heptapeptides containing an unusual C20 amino acid: 3-amino-9-methoxy-2,6,8-trimethyl-10-phenyl-4,6-decadienoic acid, abbreviated Adda (Sivonen and Jones, 1999). Microcystins inhibit eukaryotic protein phosphatases 1 and 2A, resulting in excessive phosphorylation of cytoskeletal filaments and causing serious liver damage. Cases of such poisoning have been reported in wild animals and domestic livestock (Carmichael, 1992). Microcystins were also involved in fatal intoxication of renal hemodialysis patients at a clinic in Caruaru, Brazil (Pouria et al., 1998). Although there are several potential exposure routes for MCs (oral consumption, inhalation, or skin absorption), the most common is ingestion of water by drinking and/or accidental recreational intake.

Microcystins have been isolated and characterized in the most common freshwater planktonic cyanobacterial genera, including *Microcystis, Anabaena,* and *Planktothrix* (Sivonen and Jones, 1999). As a consequence of the widespread occurrence of cyanobacteria-producing MCs, the World Health Organization (WHO) established a provisional guideline limiting MC-LR to 1 μg L^{-1} in drinking water (WHO, 1998, 2003). Recently, this guideline was introduced in the Czech legislation as well (Decree 252/2004). Although no human poisoning incidents from cyanobacteria and little data on toxic cyanobacterial blooms in Czech reservoirs have been published, cyanobacterial blooms related to high nutrient concentration in freshwaters have been observed repeatedly. Prior to the present study, the only other survey took place during 1993–1998, when Maršálek et al. (2001) collected 65 samples from Czech reservoirs. Microcystins were detected in 90% of the samples analyzed. Bláha and Maršálek (2003) also found MCs in drinking-water reservoirs and in raw and treated drinking water in the Czech Republic. In the present survey, the occurrence and species composition of cyanobacterial blooms as well as the amounts of MCs in the reservoirs were investigated for two consecutive summers. The main study objectives were: (1) to assess phytoplankton composition and MC concentration in several Czech reservoirs and (2) to evaluate changes in the concentrations of cell-bound MCs in relation to cyanobacterial assemblage composition and biomass over two consecutive summers.

MATERIAL AND METHODS

Description of Study Sites

The Czech Republic has only a few small natural lakes, but it is a country with a large number of man-made reservoirs, built by damming rivers and creeks to accumulate water for power, drinking water, recreation and/or flood control, and water regime regulation. Most studied localities are dimictic, stratified canyon-shape reservoirs. Only a few are shallow impoundments where the temporary stratification may be easily disrupted by wind. Their size varies from less than 1 km^2 to almost 50 km^2, with maximum depths to 74 m and volumes of up to hundreds of millions of cubic meters. Mean retention time ranges from a few days to several months. As a consequence of high nutrient concentrations in the water, many reservoirs experience occasional or recurring cyanobacterial blooms of varying intensities, mainly in late summer. For this study a total of 18 eutrophic, recreational, and/or drinking-water reservoirs were chosen for sampling.

Sampling and Limnological Analyses

For a 1-week period during both mid-July and August in 2003 and 2004, 18 reservoirs were sampled in the southern and western parts of the Czech Republic. Samples were taken near dams using a Friedinger sampler at a depth of 0.5 m. Transparency (Secchi disk), temperature, and pH (WTW 330i meter, Weilheim, Germany) were measured in the field.

All measurements were done in duplicate. Samples for chemical measurements were frozen immediately in the laboratory at $-20^{\circ}C$ and analyzed later. Total phosphorus (TP) and soluble reactive phosphorus (SRP) were determined spectrophotometrically according to Kopáček and Hejzlar (1993) and Murphy and Riley (1962), respectively. Samples for NH_4^+ and NO_3^- were filtered through glass-fiber filters (Macherey Nagel GF5, Düren, Germany, porosity of 0.4 μm) and analyzed by ion chromatography (Dionex IC25, Sunnyvale, CA, USA); the sum of both ions was assumed equal to total inorganic nitrogen (TN_{inorg}).

Phytoplankton species were identified by light microscope observation of living and Lugol-preserved samples according to commonly used monographs on cyanobacteria (Anagnostidis and Komárek, 1988; Komárek and Anagnostidis, 1989, 1999). Phytoplankton samples for biovolume estimation were also preserved with a Lugol solution. Phytoplankton species were enumerated employing the Utermöhl sedimentation method on an inverted microscope (Olympus IMT-2, Tokyo, Japan; Lund et al., 1958). Samples containing dense cyanobacterial blooms were diluted 1:10 with distilled water to achieve optimal cell density in the sedimentation chamber. The mean cell dimensions were obtained for biovolume calculation using the approximation of cell to geometrical solids method. Chlorophyll *a* concentration was determined spectrophotometrically after acetone extraction (Lorenzen, 1967).

TABLE I. Main physico-chemical parameters measured at reservoirs studied

	July		August	
	Median	Range	Median	Range
pH	8.9	7.4–9.9	9.2	7.2–10.3
Secchi depth (m)	1.2	0.1–3.1	1	0.1–2.7
Chlorophyl *a* (μg L^{-1})	24	4–230	35	5–918
SRP (μg L^{-1})	7.6	2–233	4.1	2–338
TP (μg L^{-1})	62	16–310	83	12–671
TN_{inorg} (mg L^{-1})	0.96	0.03–6.06	0.63	0.02–4.05
N/P ratio in water	1.33	3–955	42	1–1623

Microcystin Analyses

Cyanobacterial biomass for cell-bound microcystins (MCs) estimation was retained on glass-fiber filters (Whatman GF/C, Brentford, UK, 1.2 μm porosity). The volume of filtered water ranged from 0.1 to 1 L according to the bloom density. Filters were dried in desiccator for 24 h under laboratory temperature until they were a constant weight and then stored at -20°C. High-performance liquid chromatography (HPLC) with a diode array detector was employed for analyses of intracellular MCs according to a procedure described previously (Jurczak et al., 2004).

Microcystins in the cyanobacterial extracts were identified against MCs standards (dmMC-RR, MC-RR, MC-YR, MC-LR, MC-LY, MC-LW, MC-LF). MC-YR was a commercial product from Calbiochem (La Jolla, CA, USA); the other microcystin standards were purified at Abo Akademi University from *Microcystis* PCC 7820 (Institute Pasteur, Paris, France) and *Anabaena* strain 90 (culture collection of Professor Kaarina Sivonen, University of Helsinki, Finland; Spoof et al., 2001). Although MC concentrations are usually expressed in units of weight per unit dry weight, more appropriate volumetric units (μg L^{-1}) obtained by filtering samples of a defined volume of water were used in this survey.

Statistical Evaluation

Data were log-transformed and tested for normality by the chi-square test (Statistica 6.0). The two-tailed Student's *t* test was performed to examine the differences between both annual seasons and between July and August samples, with differences accepted as significant at $p < 0.05$. The nonparametric Mann–Whitney test was used for comparison of MC cell contents of *Microcystis* spp. and other species. Linear correlation analysis was made to relate the amount of MCs to the parameters measured.

RESULTS

Physicochemical Parameters

The physical conditions of the Czech reservoirs were characterized by high water temperatures and relatively low transparency throughout both summer seasons. Because most of the reservoirs are in canyons, stable thermal stratification was achieved during both summers. The majority of the reservoirs are also eutrophic to hypertrophic because of the high concentrations of both phosphorus and nitrogen.

Water temperature was significantly higher in July than in August ($p < 0.01$, mean 23.6°C and 21.7°C, respectively). Secchi depth varied around 1 m, and only on rare occasions was it higher than 2 m (Table I). Chlorophyll *a* ranged from several to hundreds of micrograms per liter, the latter in reservoirs where dense cyanobacterial blooms were observed. Both chlorophyll *a* and TP were significantly higher in August than in July ($p < 0.05$). In August the amount of N_{inorg} in water significantly decreased ($p < 0.01$), whereas SRP concentration remained stable. Despite high TP concentrations, low SRP concentrations indicated that phytoplankton growth might occasionally be phosphorus limited in several of the reservoirs. This was supported by a high N/P ratio in the water (N_{inorg}/SRP) as well. However, at several sites nitrogen limitation appeared to occur.

Phytoplankton Biovolume and Composition

During both seasons cyanobacteria were the most abundant among phytoplankton, particularly in August, when their prevalence and biovolume grew markedly (Fig. 1). Cyanobacterial blooms composed of several colonial and filamentous genera (*Microcystis, Woronichinia, Anabaena, Aphanizomenon,* and *Planktothrix*) occurred in different proportions. Heavy cyanobacterial blooms were always attributed to *Microcystis* spp. dominance (Table II), with only a few species coexisting. *Microcystis* spp. biovolume was significantly higher in August than in July ($p < 0.05$). In total,

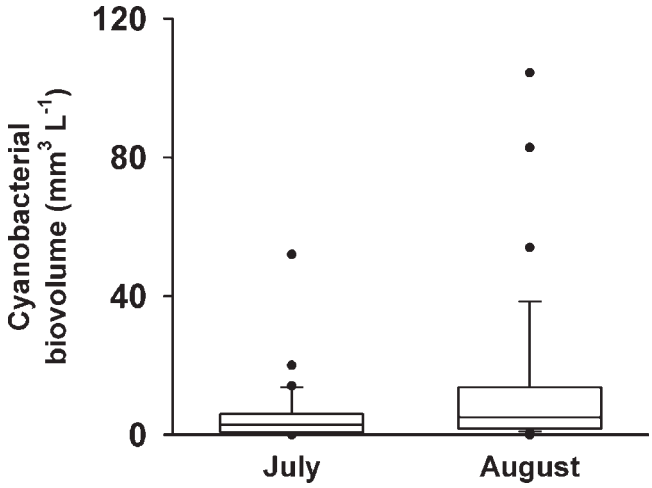

Fig. 1. Box-whiskers plot of cyanobacterial biovolume. Boxes show medians and 25th and 75th quartiles, bars indicate 10th and 90th percentiles, and dot symbols represent outliers.

TABLE II. Number of samples and biovolumes of particular dominant cyanobacterial genera

	No. of samples	Median	Range
Microcystis	40	7.5	0.8–103
Anabaena	16	2.3	1.1–18
Aphanizomenon	6	0.8	0.3–6
Woronichinia	4	0.6	0.1–5

Microcystis spp. prevailed in 40 samples and contributed on average to 50% of the total cyanobacterial biovolume. The most widespread species was *Microcystis aeruginosa* (Kütz.) Kütz., whereas *M. viridis* (A. Braun) Lemm., *M. ichthyoblabe* (Kütz.) Kütz., *M. flos-aqua*e (Wittr.) Kirchn., and *M. wesenbergii* (Lemm.) Lemm. were subdominant or dominated only in a few of the sampled reservoirs. Another colonial species, *Woronichia naegeliana* (Ung.) Elenk., was common as well; however, its abundance was generally low (Table II).

Filamentous cyanobacteria prevailed less frequently than *Microcystis,* and their biovolumes were generally much lower (Table II). Among those species, *Anabaena* was the most important genus. It dominated in 16 samples, accounting for 21% of the total cyanobacterial biomass. *Anabaena* tended to dominate, particularly in July; nonetheless, the decrease in its biovolume as the summer progressed was not significant. Seven *Anabaena* species were identified: *A. lemmermannii* Richt., *A. flos-aquae* (Lyngb.) Born. et Flah., *A. crassa* (Lemm.) Kom.-Legn. et Cronb., *A. circinalis* Rabenh. ex Born. et Flah, *A. spiroides* Kleb., *A. mendotae* Trel., and *A. planctonica* Brunn. Frequently, one or a few *Anabaena* species dominated in a particular reservoir. *A. lemmermannii* was the most abundant species in 50% of *Anabaena*-dominated samples, whereas the rest of the samples differed markedly in which *Anabaena* species occurred.

Subdominant populations of *Aphanizomenon* commonly occurred; however, it dominated in only six samples (Table II). Four species of *Aphanizomenon* were identified (*Aph. klebahnii* (Elenk.) Pech et Kal., *Aph. yezoense* M. Wat., *Aph. flos-aquae* (Linnn.) Ralf. ex Born. et Flah., and *Aph. issatschenkoi* Pros.-Lavr.), accounting for 11% of cyanobacterial biovolume. However, there was only one species per reservoir. *Planktothrix agardhii* (Gom.) Anagn. and Kom. occurred only sparsely. Although the species was dominant in three samples, no heavy bloom formation was observed.

Microcystins Concentration

Microcystins were detected in 90% of the samples. Despite the presence of potentially microcystin-producing taxa, MCs were not detected in seven samples. The highest concentrations of MCs were always associated with either the formation of or the accumulation of dense blooms at the sampling sites. Microcystin concentrations were significantly higher in August ($p < 0.05$), apparently as a result of the development of an intense cyanobacterial bloom in most reservoirs [Figs. 1 and 2(a)]. MC content in cells (MCs per cyanobacterial biovolume) followed the same trend as MCs per liter of water [Fig. 2(b)]. That is, cyanobacteria contained considerably more MCs in August than in July ($p < 0.05$). Microcystin content in cells was highly variable. Significant differences were found between samples dominated by *Microcystis* spp. with those dominated by other species ($p < 0.001$). Because a limited number of samples were dominated by taxa other than *Microcystis* spp., a statistical comparison of MC content of *Microcystis* spp. versus content of other species was made using the

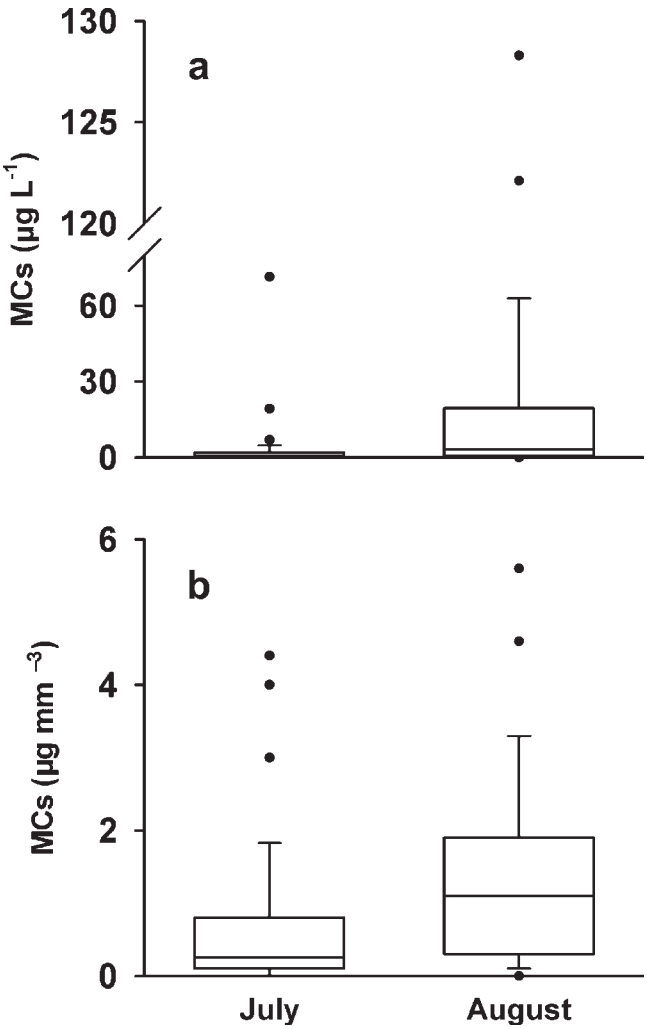

Fig. 2. Box-whiskers plots of intracellular MCs in (a) water and (b) cyanobacterial biomass. Boxes show medians and 25th and 75th quartiles, bars indicate 10th and 90th percentiles, and dot symbols represent outliers.

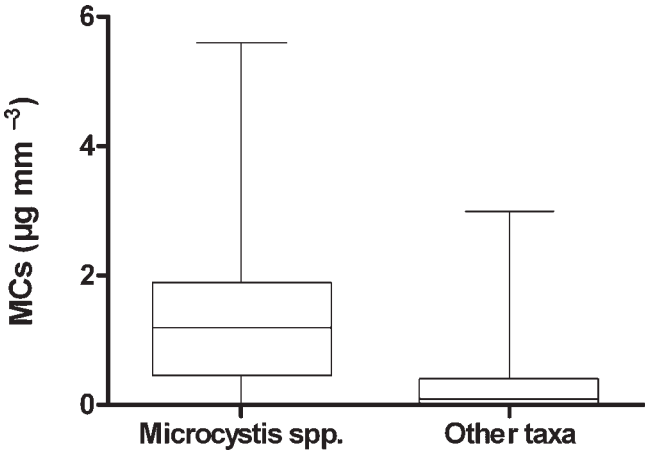

Fig. 3. Box-whiskers plot of intracellular microcystin content in samples dominated by *Microcystis* spp. and other taxa. Boxes show medians and 25th and 75th quartiles; bars indicate maximum and minimum.

Mann–Whitney test. The *Microcystis* spp. population showed the highest absolute (5.6 μg mm^{-3}) and the highest median (1.2 μg mm^{-3}) MC cell contents (Fig. 3).

A correlation analysis was performed to relate the amount of MCs in water to physicochemical parameters measured, but no significant correlation was found. However, MCs were positively correlated with chlorophyll *a* and cyanobacterial biovolume ($R^2 = 0.61$ and 0.66, respectively; $p < 0.05$), with the strongest correlation ($R^2 = 0.87$) with a *Microcystis* spp. biovolume of ($p < 0.001$). Therefore, *Microcystis* spp. is the most important microcystin-producing taxon in the Czech reservoirs.

The main variants of MCs in Czech reservoirs were MC-LR, MC-RR, and MC-YR, regardless of whether the bloom was dominated by *Microcystis* or other taxa. Other variants of MCs accounted in total for less than 1% of the MCs in the reservoirs studied, and their share was only rarely higher than 10% in a reservoir. Changes in MC composition in the summers were both quantitative and qualitative. The concentration of all appointed variants of MCs (MC-LR, MC-RR, MC-YR) was markedly higher in August, which corresponded with a considerable increase in MCs. However, the proportion of the main MC variants changed noticeably, whereas no changes were observed in the percentage of MC-YR. The share of MC-RR considerably grew in August ($p < 0.05$), whereas the share of MC-LR was significantly lower than in July ($p < 0.05$).

DISCUSSION

In this survey, cyanobacteria formed dense blooms in August rather than in July. However, this increase in cyanobacterial biomass cannot be related to increasing nutrient concentrations, as no such increase was observed in SRP concentration, and the amount of N_{inorg} in the water significantly decreased in August. Although nutrient concentrations are considered fundamental for the development of cyanobacterial blooms, many other variables are involved in their ecological success (Dokulil and Teubner, 2000).

The August increase in the cyanobacterial biomass was followed by changes in dominants. Although filamentous *Anabaena* spp. prevailed mainly in July, when the concentration of N_{inorg} was markedly higher, in August, *Microcystis* spp. (especially *M. aeruginosa*) tended to dominate. Inorganic nitrogen depletion in the water was shown to support the development of filamentous N2-fixing cyanobacteria rather than *Microcystis* (Hyenstrand et al., 1998). On the other hand, Ferber et al. (2004) showed that cyanobacterial nitrogen acquisition through fixation was low despite a pronounced nitrogen limitation of phytoplankton growth in a eutrophic shallow pond. According to Johnston and Jacoby (2003), who studied the migration of *Microcystis* in mesotrophic Lake Sammamish (WA), *Microcystis* occurrence was associated with a stable water column, increased total phosphorus concentration, surface temperatures greater than 22°C, high total nitrogen-to-phosphorus ratios, and increased water transparency. Johnston and Jacoby (2003) also noted the importance of *Microcystis* migration as a factor in the development and maintenance of the *Microcystis* bloom. Although some of these factors may also contribute to the pronounced development of *Microcystis* in the Czech reservoirs, others are difficult to accept and are not necessarily the same in all reservoirs (Dokulil and Teubner, 2000).

Attempts have been made to use field data to correlate environmental factors with MCs; however, the results remain inconclusive. Kotak et al. (1995) found a positive correlation between total and total dissolved P and MC concentrations in water dominated by *Microcystis aeruginosa*. Other research found that the amount of MCs in water positively correlated with water temperature (Amé et al., 2003). Unfortunately, these results could not always be duplicated (Kotak et al., 1995). Chorus (2001) demonstrated in a large set of data that MC concentrations in water are determined primarily by the biomass of MC-producing cyanobacteria, even though the influence of environmental conditions cannot be ruled out. Although the cellular content of MCs is nearly constant over a wide range of growth factors (Orr and Jones, 1998), temperature, light intensity, nutrient concentration, pH, and trace metals were found to influence the MC production of cyanobacteria under laboratory conditions (Sivonen and Jones, 1999; Duy et al., 2000; Chorus, 2001).

Most samples are composed of several codominant species and additional subdominant species. Thus, it is difficult to attribute MC production to one particular species. Nonetheless, a strong positive correlation between MC concentration and *Microcystis* spp. biovolume suggests that

Microcystis, especially most the common taxon, *M. aeruginosa*, was the major MC-producing cyanobacterium in the Czech reservoirs. Similar positive correlations in *Microcystis*-dominated samples were found in other studies (Kotak et al., 1995; Welker et al., 1999). *Microcystis* spp. has been reported as a dominant MC producer worldwide; however, *Anabaena* spp. and *Planktothrix* are also common MC-producing taxa in Finnish and German lakes, respectively (Sivonen et al., 1989; Chorus, 2001). A weak correlation between *Microcystis* biovolume and MC content in cells reflects remarkable differences in microcystin content of a respective bloom. These differences are caused by the numerous morphologically identical (indiscernible) but genetically different strains of the same species (Vezie et al., 1998; Kurmayer et al., 2001; Via-Ordorika et al., 2004). These strains may or may not produce various microcystins according to the presence or absence of microcystin-encoding genes. Some strains do not produce microcystins, even though they contain genes for their biosynthesis (Via-Ordorika et al., 2004). Only 16 of 98 strains of *Microcystis aeruginosa* isolated from a shallow eutrophic lake during a 5-month study contained MCs (Vezie et al., 1998). However, a considerably higher share of microcystin-producing strains (70%) was found by other studies (Kurmayer et al., 2001; Via-Ordorika et al., 2004). In addition to different strain compositions of blooms, MC concentrations in water can vary substantially among reservoirs because of buoyancy and windblown cell accumulation along shorelines.

To date, more than 70 structural variants of microcystins have been isolated and characterized from cyanobacterial blooms and cultures (Sivonen and Jones, 1999). Most of these variants were found only in isolated strains or in small amounts in field samples. Microcystin-LR, -RR, and -YR were the primary microcystins in the Czech reservoirs. Pronounced prevalence of these variants is often reported to be characteristic of *Microcystis*-dominated blooms, even though the variants do not always concur and their relative shares are variable (Vezie et al., 1997; Sivonen and Jones, 1999; Chorus, 2001; Kurmayer et al., 2001; Via-Ordorika et al., 2004). In contrast, microcystin composition in *Anabaena* spp. populations was found to be more diverse. Of note is that monospecific populations of the genus *Planktothrix* exclusively contain demethylated variants of MCs (Henriksen, 1996; Fastner et al., 2001). Few authors reported that temporal variability of the main microcystins variants as well as their relative shares in *Microcystis* spp. dominated samples was low (Fastner et al., 1999; Welker et al., 2003). In contrast, the data in the present study indicate that the percentages of main MC variants (MC-LR and MC-RR) vary considerably with time. This is likely a consequence of different cyanobacterial species and strain compositions during the summer. In addition, MC content in cyanobacterial cells increased considerably in August, indicating a pronounced prevalence of toxic strains. This finding is in contradiction to the study of Welker et al. (2003), which showed that *Microcystis* spp.–dominated blooms in shallow Lake Müggelsee (Germany) contained less MCs when biomass was high. However, MC content in cells spanned the same range in this study and that of Welker et al. (2003).

Microcystins are supposed to be retained mainly in cyanobacterial cells and not released in huge amounts into ambient water except for cell lysis (Sivonen and Jones, 1999). In many cases, no extracellular MCs were detected, although the total amount of MCs reached levels of several hundred micrograms per liter (Ueno et al., 1996). However, high concentration of extracellular MCs may occur during the senescence and decomposition of the cyanobacterial bloom at the end of summer. Accordingly, the total concentrations of both dissolved and intracellular MCs might be even higher than values reported in this survey.

As a consequence of the frequent cyanobacterial occurrence in Czech reservoirs, almost all samples contained MCs even when cyanobacteria were subdominant. The amount of MCs found in this survey was similar to that described for natural blooms worldwide (Kotak et al., 1995; Chorus, 2001). The frequency of microcystin detection (90%) on a par-sample basis is the same as that found previously by Maršálek et al. (2001), who studied Czech reservoirs in 1993–1998. Although the frequency of MC-positive samples is one of the highest among similar surveys worldwide (Chorus, 2001), MC concentrations were usually below 10 μg L^{-1} and only rarely exceeded 100 μg L^{-1}. On the other hand, Bláha and Maršálek (2003) reported a considerable concentration of MCs in those Czech reservoirs, which are a source of drinking water. Moreover, MC concentrations in raw water and treated drinking water occasionally exceeded the derived WHO guideline of 1 μg L^{-1} (WHO, 1998). A threat to public health in recreational reservoirs occurs primarily through formation of scum and accumulation of biomass. This was repeatedly observed along the shores, where public baths and campsites are located. As a consequence of the high incidence of toxic cyanobacterial blooms, it is highly desirable to regularly monitor the reservoirs studied in order to minimize potential health risks to the human population.

CONCLUSIONS

This study has shown high prevalence of MC-producing cyanobacteria in Czech reservoirs. The most common cyanobacterium, *Microcystis aeruginosa*, which tends to dominate the phytoplankton principally in August, is presumably the major producer of MCs. This study's survey showed that the threat associated with MCs in water rises in late summer, when significantly higher MC concentrations were measured. As MC concentrations in water were coupled to the biovolume of cyanobacteria, an estimate of potential

health risk could be accomplished by following the population dynamics of cyanobacteria, especially *Microcystis* spp.

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