First publ. in: Toxicology and applied pharmacology 203 (2005), 3, pp. 231-242

Occurrence and elimination of cyanobacterial toxins in drinking water treatment plants☆

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Abstract

Toxin-producing cyanobacteria (blue-green algae) are abundant in surface waters used as drinking water resources. The toxicity of one group of these toxins, the microcystins, and their presence in surface waters used for drinking water production has prompted the World Health Organization (WHO) to publish a provisional guideline value of 1.0 μg microcystin (MC)-LR/l drinking water. To verify the efficiency of two different water treatment systems with respect to reduction of cyanobacterial toxins, the concentrations of MC in water samples from surface waters and their associated water treatment plants in Switzerland and Germany were investigated. Toxin concentrations in samples from drinking water treatment plants ranged from below 1.0 μg MC-LR equiv./l to more than 8.0 μg/l in raw water and were distinctly below 1.0 μg/l after treatment. In addition, data to the worldwide occurrence of cyanobacteria in raw and final water of water works and the corresponding guidelines for cyanobacterial toxins in drinking water worldwide are summarized.

Keywords: Microcystin; Ozonation; Cyanobacteria; TOC; *Planktothrix rubescens*; *Microcystis aeruginosa*

Introduction

The majority of the populations in industrialized countries are dependent on drinking water from public or private water suppliers. These water treatment plants are required to guarantee the drinking water quality according to the respective national drinking water guidelines. These guidelines address microbial (e.g., *E. coli*, coliforme bacteria) and chemical (e.g., cyanides, pesticides) parameters as health-relevant endpoints and indicator parameters (smell, taste, conductivity) as a quality control for the proper functioning of the water treatment plants (Schmitz, 2001). Guidelines for cyanobacterial toxins in water exist in several countries worldwide (Table 1). Most of these countries have a history of problems with cyanobacterial contamination in drinking water reservoirs and they may serve as examples for the rest of the world. In Europe, cyanobacterial toxins are not yet clearly regulated. However, in the European Water Framework Directive (2000) (2000/60/EC), which characterizes high-priority water pollutants, toxin-producing cyanobacteria (blue-green algae) have been specifically highlighted as potential key hazardous pollutants. The harmful potential of cyanobacterial toxins for the population is appreciated in many European countries and has been described in many publications (Funari et al., 2000; Hitzfeld et al., 2000b; Thebault et al., 1995; Vasconcelos, 1999). The fact that cyanobacteria are able to exist even in hot springs in volcanic regions (Ward et al., 1998) and in cold and hot deserts such as Antarctica (Hitzfeld et al., 2000a; Wynn-Williams, 2000) or the Atacama desert (Wynn-Williams, 2000) underlines the omnipresence of these organisms. Cyanobacteria are ubiquitous in surface waters worldwide and many species including *Microcystis*, *Nodularia*, *Cylindrospermopsis*, *Anabaena*, and *Aphanizomenon* are known to produce toxins such as microcystins (MC), nodularins, cylindrospermopsins, anatoxins, and paralytic shellfish poisons (Landsberg, 2002). However, due to a paucity of toxicity data for other

☆ These data were partly presented at the 5th International Conference on Toxic Cyanobacteria (2001), Noosa, Australia, and at the 10th International Conference on Harmful Algal Blooms (2002), St. Pete Beach, USA.

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Table 1
Guideline values for toxic cyanobacterial secondary metabolites

	Microcystins	PSPs	Anatoxin-a	Cylindrospermopsin	
Australia	lifetime exposure: 1.3 µg/l brief period: 10 µg/l	3.0 µg/l (suggested for brief period)	mentioned in guideline (w/o value)	mentioned in guideline (w/o value)	(Fitzgerald et al., 1999; NHMRZ/ARMCANZ, 2001)
Brazil	1.0 µg/l	3.0 µg/l (suggested)	–	15 µg/l (suggested)	(Azevedo, 2001)
Canada	1.5 µg/l	–	–	–	(Health Canada, 2003)
France	1.0 µg/l	–	–	–	(France, 2001)
European Drinking Water Directive, 1998	0.1 µg/l (default value)[a]	0.1 µg/l (default value)[a]	0.1 µg/l (default value)[a]	0.1 µg/l (default value)[a]	(Schmidt et al., 2002)
New Zealand	1.0 µg/l	1.0 µg/l	3.0 µg/l [1.0 µg/l for anatoxin-a(s) + homoanatoxin]	3.0 µg/l	(Ministry of Health, 2002)
Oregon (USA)	1.0 µg/g (health food)	–	–	–	(Gilroy et al., 2000)
WHO	1.0 µg/l (provisional)	–	–	–	(WHO, 1998)

[a] The value of 0.1 µg/l was originally used for pesticides, but nowadays it is applied as a provisional value for other toxins whose character is unknown or as a quality target designed to be well below the concentration derived from health criteria (Schmidt et al., 2002).

toxins including the >80 other MC congeners, the World Health Organization (WHO, 1998) has set a provisional guideline value for MC-LR (L: lysine, R: arginine) of 1.0 µg/l drinking water (Table 1). Microcystins and nodularins have a high acute toxicity with LD50's ranging from 36 to 122 µg/kg in mice and rats i.p. or i.v. (Dawson, 1998; Sivonen et al., 1989) and have also been implicated in tumor promotion in both liver (Ito et al., 1997) and colon (Humpage et al., 2000). Nodularins and MCs are also suspected to induce liver carcinogenesis (Ohta et al., 1994; Zegura et al., 2003). The inhibition of protein phosphatase enzymes seems to be responsible for the toxicity of both substances, but additional mechanisms are likely.

Humans may come into contact with cyanobacterial toxins through ingestion or dermal contact with cyanobacteria and their respective toxins. Possible pathways for exposure are during recreational activities (Chorus et al., 2000; Pilotto et al., 1997), by ingestion of contaminated agricultural products (Abe et al., 1996; Codd et al., 1999; McElhiney et al., 2001), cyanobacterial health foods (Gilroy et al., 2000), or contaminated shellfish (Eriksson et al., 1989) and fish (Ernst et al., 2001). It is becoming increasingly clear that almost every part of the world depending on drinking water from surface waters, has or will encounter problems with toxic cyanobacteria in its drinking water system (Tables 2 and 3), due to the ubiquitous presence in raw water feeding into water treatment plants. Thus, water treatment systems must eliminate cyanobacteria and their toxins from the raw water. Conventional water treatment with only a filtration step (Grützmacher et al., 2002) or with an additional flocculation step (Lambert et al., 1996) has been shown to be ineffective in removing dissolved microcystins from water. Flocculation with an appropriate concentration of flocculent is suitable only for removing cyanobacterial cells from water. However, the possibility of cell lysis could lead to an increase in extracellular toxin concentration, which cannot be eliminated by the methods mentioned. Furthermore, intact cells have been observed in final water after the whole treatment train (Lepistö et al., 1994). In ozonation water treatment processes, both ozone and OH radicals work as oxidizing agents (Staehelin and Hoigné, 1985). Preozonation with 0.5–1.5 mg/l aims to inactivate bacteria (Lee and Deininger, 2000), viruses, and protozoa, and to detoxify harmful compounds such as phenols, polycyclic aromatics, and microcystins. Undesirable taste-and-odor substances are also eliminated. Furthermore, other natural organic matter is modified to products that are more easily adsorbed and filtered (Siddiqui et al., 1997). Subsequent to ozonation two-layer filters (pumice/quartz-sand) remove the majority of the organic substances (e.g., cyanobacterial cells) and thus act as a mechanical rough cleaning step. This function makes backwashing at regular intervals necessary to avoid saturation and clogging of the filters and consequently a breakthrough of cyanobacterial cells. Intermediate ozonation with ~0.5 mg/l is necessary to guarantee the elimination of harmful substances including cyanobacterial toxins, which survive the preceding treatment steps. Furthermore, intermediate ozonation improves particle removal in the subsequent filter system (Becker and O'Melia, 2001). Activated carbon eliminates the surplus ozone, adsorbs hydrophobic compounds, and acts as substrate for bacteria, which mineralize most of the organic by-products (ketones, aldehydes, acids) produced by the ozonation step (Lambert and Graham, 1995; Von Gunten, 1998). Thus, activated carbon filters act as biofilms that potentially metabolize organic compounds; however, they also show a significantly impaired ability to adsorb toxins. Moreover, biodegradation of microcystins by the biofilm does not seem to occur (Falconer et al., 1989; Lambert et al., 1996). The last

step, i.e., the slow sand filter, also functions as a substrate for bacteria, which detoxify and clean the treated water similarly to natural water cleaning conditions.

To verify the efficacy of water treatment with respect to reduction or removal of cyanobacterial toxins, MC concentrations were determined in raw water samples in Switzerland and Germany from surface waters as well as in their respective associated water treatment plants.

Materials and methods

Sample sites

Samples were obtained from drinking water bodies in Germany and Switzerland. The treatment technologies routinely applied at the associated water treatment plants are shown in Table 4.

Cell counting

Cyanobacterial cell densities were only determined in the water treatment plant Lengg/Lake Zurich (Switzerland). A defined volume of sample was filtered through ammonium acetate filter (0.45 μm, Schleicher & Schuell, Germany) and the trichomes measured by using image processing system software (Visiometrics, Konstanz, Germany). The cell counting for Figs. 1 and 2 was carried out by the on-site laboratories of the water treatment plants in Zurich and Siegburg, respectively.

Sampling and sample preparation

Lake water (2 l) containing cyanobacteria was collected in glass bottles from different depths in the lakes (for determination of the MC congeners by HPLC) and between various treatment steps in water treatment plants for quantitative toxin determination by ELISA/PPA/HPLC. A portion of each sample was fixed with formol for phytoplankton/cyanobacteria identification, and the rest was stored in a cooling box for less than 12 h until filtration and storage of the filtered samples at −20 °C. To determine free MC in water, samples were filtered through an ammonium acetate filter (0.45 μm, Schleicher & Schuell) to separate cyanobacterial cells. Solid phase extraction (SPE) of the filtrate was performed using C_{18} end-capped SPE cartridges (Macherey & Nagel, Germany) conditioned with 10 ml methanol and subsequently washed with 10 ml distillated water (H_2O-MQ). Samples were applied to the cartridge and, after washing with 10 ml H_2O-MQ water, eluted with 40 ml 100% methanol. The eluent was dried under a nitrogen atmosphere and resuspended in 1.5 ml H_2O-MQ. The filter (~20 mg sample DW) was extracted 3 times with 1.5 ml 75% methanol in 1.8-ml tubes. Supernatants were collected, dried via vacuum centrifugation, and resuspended in 10 ml H_2O-MQ; and toxins were concentrated by SPE (similar to that described above for the extracellular toxin), dried under a nitrogen atmosphere, and resuspended in 1.5 ml H_2O-MQ.

Determination and quantification of toxins

Radioactive protein phosphatase assay (rPPA). The protein phosphatase assay was performed as described by Fischer and Dietrich (2000) using a phosphatase extracted from rape seed (*Brassicus napus*), ^{32}P-ATP (kindly provided by Prof. Werner Hofer, University of Konstanz), and microcystin-LR (Calbiochem, USA) as a standard. Free ^{32}P in the supernatant was extracted with acid molybdate, scintillation cocktail (Ready Safe, Beckman, Germany) was added and radioactivity counted in a scintillation counter (LS 6500, Beckman). The degree of protein phosphatase inhibition (PPA-inhibiting capacity) was calculated as % phosphatase activity of the control. The PPA-inhibiting capacity of samples was compared to a MC-LR standard-curve. The detection limit of the radioactive PPA used is 0.05 μg MC-LR/l with a derived IC50 of 0.25 μg MC-LR/l. Each sample was analyzed 3 times in triplicate. The mean values of each triplicate yielded the values for calculation of the standard deviation (n = 3).

Adda-ELISA. The ELISA method was performed as described by Fischer et al. (2001). Briefly, ELISA plates (NUNC MaxiSorp, Denmark) were coated overnight with OVA-ADDA-hemiglutaryl (OVA-ADDA-HG) in 0.05 M sodium carbonate buffer pH 9.6 (50 μl/well, 2.5 μg/ml) at 20 °C. Unbound material was removed by aspiration. After washing with PBS, additional binding sites were blocked by incubation with OVA (1% w/v, 200 μl, >2 h, 20–25 °C). Plates were washed 3 times with PBS and used immediately or stored at 4 °C for up to 7 days. In the assay, sample or standard (50 μL) were added to the wells together with antiserum (AB 824 at 1/160 000). After incubation at 20–25 °C for 2 h, wells were washed twice with phosphate buffer solution containing 0.05% Tween™ 20 (PBST) and twice with PBS. Anti-sheep secondary antibody (ICN/Cappel rabbit–anti-sheep–HRP) (100 μl, dilution 1/6000) was then added to the wells and incubated for 2 h. Subsequently, liquid in the wells was aspirated, and the wells washed twice with PBST and twice with PBS. As substrate TMB (100 μl, Sigma, Germany) was added and incubated for 30 min. The reaction was stopped by addition of H_2SO_4 (50 μl, 2 M), and absorbance determined with a microplate reader at 450 nm. The limits of quantitation and detection of the ELISA were 0.2 and 0.05 μg/l, respectively. The samples were analyzed three times in duplicate using a different 96-well plate for each assay. The mean values of each plate yielded the values for calculation of the standard deviation (n = 3).

High-performance liquid chromatography. Toxins were analyzed according to Lawton et al. (1994). External

Tables 2 and 3
Examples for cyanobacteria and cyanobacterial toxins in drinking water treatment plants worldwide

	Water treatment	Cyanobacteria	Raw water	Final water	Removal	Additional information	
Bahía Blanca, Argentina	NR	ANA/MIC	48320–84032 cells/ml	276–2472 cells/ml	NR	–	(Echenique et al., 2001)
San Roque Dam, Argentina	NR	MIC	LD_{50} (mice): 500 µg/kg	MC detectable via HPLC	NR	drinking water supply for Cordoba	(Scarafia et al., 1995)
Malpas Dam, Australia	CS	MIC	NR	NR	NR	effects on human health reported	(Falconer et al., 1983)
Drinking water reservoir, Queensland, Australia	FLSE, PAC, SF, CHL	ANA, MIC	<2200000 cells/ml <8 µg/l (MC), <17 µg/l (PSPs)	<11230 0.0–0.5 µg MC/l, traces (PSPs)	99% 32–100%	–	(Hoeger, 2003)
Lakes, ponds, reservoirs,Bangladesh	NR	MIC	samples MC positive	samples MC positive	NR	–	(Khan et al., 2001)
Itaparica Dam, Brazil	CS	ANA, MIC	NR	NR	NR	effects on human health reported	(Teixera et al., 1993)
Camrose plant, Alberta, Canada	FLSE, SF, CHL, PAC	NR	0.15–0.87 µg/l (n = 14)	0.09–0.18 µg/l (n = 14)	59–97%	–	(Lambert et al., 1996)
Ferintosh plant, Alberta, Canada	FLSE, SE, CHL, GAC	NR	0.27–2.28 µg/l (n = 6)	0.05–0.12 µg/l (n = 6)	7–90%	–	(Lambert et al., 1996)
Czech Republic	NR	NR	≤8.7 µg/l (n = 12)	0.0–7.79 µg/l (n = 12)	11–100%	–	(Blaha and Marsalek, 2001)
China	NR	NR	0.28–35.3 µg/l	≤1.4 µg/l	78–100%	–	(Ling, 2000)
Lake Uelemiste, Estonia	NR	NR	NR	NR	NR	maximum cyanobacterial biomass: 40 mg/l, drinking water for Tallinn	(Olli, 1996)
Finland	BF	PLA/OSC	0.1–1.9 µg/l (n = 8)	0.01–0.1 µg/l (n = 6)	>90%	cyanobacteria observed in drinking water; MCs in 9/58 treated samples	(Lahti et al., 2001)
	RSF	NR			14%		
	RSF, ACF, CHL	NTC	NR		42%	trichomes of *O. agardhii* passed through treatment process in spite of high reduction efficiency in water treatment plant	(Lepistö et al., 1994)
Finland	contact filtration with $Al_2((SO)_4)_3$, ACF, CHL	ANA, APH		NR	99%		
	ACF, FLSE with $Al_2((SO)_4)_3$, SF, CHL	MIC, PLA/OSC			99.9%		
	PAC (20 mg/l)			<1 ng/l	>98%		
	OZ (0.07 mg/l) PAC (40 mg/l)						
Saint-Caprais reservoir, France		APH	63 µg/l			before treatment: 30% of toxin free; after treatment: 100% toxin free	(Maatouk et al., 2002)
	CHL (0.42 mg/l) PAC (20 mg/l)			33.2 ± 8.0 ng/l	45%		
	PAC (40 mg/l)			<1 ng/l	>98%		

Lake Bourget, France	OZ, SF	PLA/OSC	<18 000 cells/ml <5 µg/l	<6000 cells/ml <1 µg/l	40–100% >80%	water intake in 30-m depth	(Humbert et al., 2000)
Weida Reservoir, Dörtendorf, Germany	MS, FLSE, SF	PLA/OSC	7.5–10 µg/l (HPLC) 10–28 µg/l (ELISA)	0–0.1 µg/l (HPLC) 0–0.2 µg/l (ELISA)	>98%	–	(Chorus et al., 2001a)
Warnow River, Rostock, Germany	OZ, FLSE, SF, OZ, ACF	MIC, PLA/OSC	0.4–8.0 µg/l	0.07–0.11 µg/l	93–100%	–	(Kruschwitz et al., 2001)
Radeburg Reservoir, Germany	BF	APH/MIC	2–19 µg/l	≤0.06	75–99%	removal efficiency hard to calculate due to uncertain travel times	(Chorus et al., 2001b)
Lake Kinneret, Israel	FLSE, CHL	APH	≤150 000 cells/ml	NR	cell removal 99.9%	raw water source for the Israeli National Water Carrier	(Banker et al., 1997; Porat et al., 1999)
Lake Simbirizzi, Lake Flumendosa, Lake Mulargia, Italy	NR	PLA/OSC	0.48 and 0.22 mg/g DW	NR	NR	Lake Flumendosa and Lake Mulargia are drinking water source for >400 000 people	(Loizzo et al., 1988; Bruno et al., 1992)
Lakes and reservoirs, Korea	mostly only RSF	MIC (60%), ANA (30%), PLA/OSC (10%)	0.6–171 µg/l (n = 16) 19–1229 µg/g DW (n = 28)	NR	NR	–	(Park et al., 1998)
Lake Mazais Baltezers, Latvia	SF/BF	APH, ANA, MIC	lake: ≤ 0.63 µg/l infiltration basin: ≤0.25 µg/l	≤1.47 µg/l	NR	only dissolved MC investigated, ≤ 5×10^6 cells/ml in lake	(Eynard et al., 2000)
Sulejów Reservoir, Poland	CHLOX, FLSE, PAC, RSF OZ, CHL	MIC	2.1–2.3 µg/l	0.5–0.8 µg/l	62–78%	bloom with between 12–860 µg MCs/g DW, Drinking water reservoir for Lodz	(Nalecz-Jawecki et al., 2000; Tarczynska et al., 2000; Tarczynska et al., 2001)
Crestuma-Lever reservoir, Portugal	NR	APH, MIC	≤12 261 cells/ml (MIC) 4.7 µg STX equiv./g DW (APH)	NR	NR	water supply for Porto and region near Porto (2 million inhabitants)	(Vasconcelos et al., 1993 Ferreira et al., 2001;)
Thailand	partly without treatment	ANA, CYL, MIC	NR	<1.0 µg/l	NR	–	(Peerapornpisal, 2001)
United Kingdom	NR	MIC, PLA/OSC	MC, ANA	NR	NR	part of a water supply network for 1.5 million people	(Codd et al., 1989; Lawton and Codd, 1991; Chaivimol et al., 1994;)
Florida, USA	NR	CYL, MIC	NR	≤90 µg/l	NR	indication of human health effects	(USEPA, 2001; Fleming et al., 2002;)
New York State waters, USA	NR	NR	45 of 191 samples positive for >0.2 µg/l	No toxin detected (>0.2 µg/l)	NR	Lake Ontario and Lake Champlain provide drinking water for>20 million people	(Boyer et al., 2001)

If not named in detail, toxins in raw and final water are microcystins (MCs). NR, not reported. Cyanobacterial genera: ANA, *Anabaena*; APH, *Aphanizomenon*; CYL, *Cylindrospermopsis,* MIC *Microcystis,* NTC no toxic cyanobacteria PLA/OSC *Planktothrix/Oscillatoria*. Water treatment methods: ACF, activated carbon filtration; BF, bank filtration; CHL, chlorination; CS, copper sulfate; FLSE, flocculation/sedimentation; GAC, granular activated carbon; MS, microsieve; OZ, ozonation; PAC, particular activated carbon; RSF, rapid sand filtration; SF, slow filtration.

Table 4
Summary information about the investigated drinking water plants

	Details	Water treatment steps	Predominant cyanobacteria	Analyzed toxins
Lake Zurich (Switzerland)	Water intake in a depth of 30 m, 600 m away from the shore (Kaiser et al., 2000).	Preozonation (1.0 mg/l), rapid sand filtration (pumice/quartzsand), intermediate ozonation (0.5 mg/l), activated carbon filtration (GAC, quartzsand), slow sand filtration.	*P. rubescens*	MCs
Wahnbachtal Dam (Germany)	Water intake depth: 30–40 m, drinking water source for 780 000 people (Bonn and hinterland).	Flocculation (0.8 mg $Al_2(SO_4)_3$/l)/ sedimentation, quartzsand filtration, chlorination of pipeline system (0.15–0.2 mg/l).	*P. rubescens*	MCs

standards were prepared for MC-LR (Alexis, Switzerland), MC-RR (Sigma), MC-YR (Sigma), and desmethyl-MC-LR and -RR (both kindly provided by J. Meriluoto, Turku, Finland). Samples (in 20% methanol) were injected into the HPLC (Diode Array Detector SPD-M10A, Auto Injector SIL-10AD, Column Oven CTO-10AC, System Controller SCL-10A, Liquid Chromatograph LC-10AT, Degasser DGU-14 A, Shimadzu; Column: Grom-Sil ODS-HE, 250 × 4.6 mm, 5 µm) and peaks were compared to standards.

Results

The treatment train used at Zurich/Lengg (Table 4) proved very effective in removing both cyanobacterial cells and toxins. However, this is necessary because Lake Zurich is known for having the toxic cyanobacterium *P. rubescens* as the predominant species throughout the year. Investigations of the water treatment plant at Zurich show the densities of *P. rubescens* at the depth (30 m) of raw water intake (Fig. 1). Cell densities of >3 × 10^4 cells/ml (~180 trichomes/ml) are regularly observed even at this depth. During stratification in September 1999, cell densities of up to 1.5 × 10^5 cells/ml were found in the metalimnion (data not shown). The concentration of microcystins in the raw water on 23/12/1999 (date of spot check) was determined with a chemical (HPLC-DAD), a functional (rPPA) and a structural method (ELISA) (Table 5). The intracellular toxin concentration (IC) varied from 2450 ng MC-RR variant/l (HPLC) to 4152 ± 1357 ng MC-LR equiv./l (rPPA) and 7050 ± 2850 ng MC-LR equiv./l (ELISA). Determination of the cell densities resulted in ~3 × 10^4 cells/ml for raw water, ~6 × 10^3 cells/ml after preozonation, and ~2 × 10^2 cells/ml after fast filtration. Calculated from a density of 30 000 cells/ml in December 1999, the toxin concentration

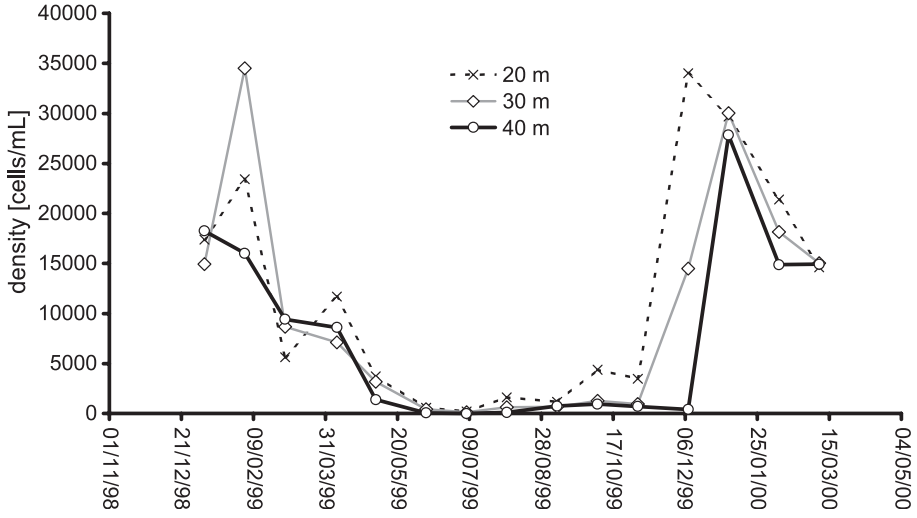

Fig. 1. Densities of *P. rubescens* in raw water of the water treatment plant Lengg/Lake Zurich (raw data provided by S. Gammeter from water treatment plant Zurich/Switzerland).

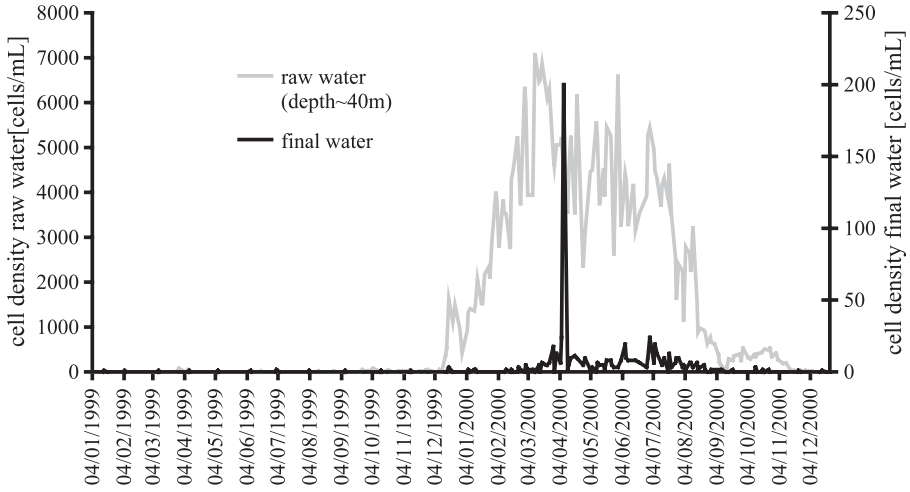

Fig. 2. Densities of *P. rubescens* in raw and final water in the water treatment plant of the Wahnbachtalsperre (raw data provided by J. Clasen from water treatment plant at Wahnbachtalsperre/Germany).

per cell was 0.088 pg (HPLC), 0.18 pg (rPPA), and 0.24 pg (Adda-ELISA). When determined via HPLC or rPPA, the values for the extracellular toxin concentration (EC) in raw water were below 100 ng/l, but 1000 ± 290 ng/l after analysis via Adda-ELISA. After preozonation of the raw water with ~1.0 mg/l, the intracellular concentration of microcystins was below 2% of the initial concentration. In contrast, the EC was in the same range (HPLC, rPPA) or only reduced to 20% (Adda-ELISA) of the initial toxin concentration (Table 5). The sand filtration did further reduce the EC.

Long-term observations by the local authorities at the Wahnbachtalsperre/Siegburg/Germany suggested that there are "*Planktothrix*-years" in this reservoir (personal communication/Wahnbachtalsperre). Almost no *P. rubescens* trichomes were observed in 1999, whereas in 2000, *P. rubescens* predominated from January to November (Fig. 2). Densities of up to 5×10^4 cell/ml were found in the metalimnion of the drinking water reservoir in 2000 (data not shown). The raw water from the intake at a depth of 40 m contained up to 7000 cells/ml (Fig. 2). Even in final water, up to 200 cells/ml could be found. The determined toxin concentration in raw water of this water treatment plant varied with the detection methods (spot check 14/02/2001; Table 6). The values in raw water ranged from 32 ± 28 for IC and 15 ± 16 ng MC-LR equiv./l for EC (rPPA) to 130 ± 15 for IC and 90 ± 60 ng MC-LR equiv./l for EC (Adda-ELISA). After flocculation and sand filtration, the EC was in the same range as in the raw water; only traces of microcystin were detectable in the intracellular toxin sample. HPLC-UV retention time, UV-spectrum, and additional HPLC-MS analysis of a concentrated sample from the Wahnbachtalsperre suggested that a MC-RR variant (mass 1024) was responsible for the ELISA- and rPPA- results (data not shown).

Discussion

The different methods, which were used in the study presented here, yielded often different toxin concentrations. The characteristics of the individual detection methods are subsequently discussed in detail to point out some possible reasons for the observed differences. The Adda-ELISA detects every compound with a free Adda-amino moiety in the sample. The potential biological function of this molecule is unimportant for this method. The microcystin or nodularin molecules must not be intact for this method. Detection of partly degraded products or even completely different molecules with an Adda-moiety is theoretically possible, although, to our knowledge, no evidence exists that molecules other than microcystin/nodularins posses Adda as a module in their peptide chain or ring. The PPA relies on the biochemical interaction of the toxins with the catalytic subunit of the phosphatases (Goldberg et al., 1995). Consequently, the phosphatase-inhibiting capacity

Table 5
Intracellular (IC) and extracellular (EC) toxin concentration (ng MC-LR equiv./l) during the drinking water treatment process at Lake Zurich (Switzerland)

ng MC-LR equiv./l	Raw water			After preozonation			After sand filtration		
	IC	EC	Sum	IC	EC	Sum	IC	EC	Sum
ELISA	7050 ± 2850	1000 ± 290	8050	180 ± 120	190 ± 80	370	50 ± 30	190 ± 120	240
HPLC	2450	90	2540	50	40	90	0	250	250
rPPA	4152 ± 1357	69 ± 36	4220	139 ± 18	66 ± 43	205	43 ± 44	69 ± 60	112

Shown are the results of three different detection methods; for rPPA and Adda-ELISA, each sample was tested three times in triplicate.

Table 6
MC concentration (ng MC-LR equiv./l) in drinking water treatment plant at Wahnbachtalsperre

ng MC-LR equiv./l	Raw water			After flocculation and sand filtration		
	IC	EC	Sum	IC	EC	Sum
ELISA	130 ± 15	90 ± 60	220	20 ± 18	50 ± 14	70
rPPA	32 ± 28	15 ± 16	47	20 ± 21	52 ± 43	72

Shown are the results of rPPA and Adda-ELISA, each sample tested three times in triplicate.

of the investigated sample can be demonstrated by this test system. The IC_{50} concentrations of the over 80 different MCs are variable and for the most part unknown. Variation within the heptapeptide has been shown to influence the ability of the toxins to bind to the phosphatases (An and Carmichael, 1994). Degradation products after ozonation were shown to lose their ability to inhibit protein phosphatases (Hoeger, 2003). Thus, the results yield the balance between phosphatase-inhibiting compounds and phosphatase-activity promoting substances in the sample. The results of this assay and of the Adda-ELISA were compared to an MC standard curve and are specified as MC-LR equivalents in this study; thus, the detection of a specific MC congener with theses two detection system is not possible. If a HPLC-UV is used, a detection and identification of every MC within one run is difficult as the chemical variability in the microcystin family with more than 80 congeners is huge. In this context, "every MC" means detecting every MC, which is available as a standard. Six MC congeners are currently commercially available (MC-LR, -RR, -YR, -LW, -LF, -LA). The spectra of the congeners are all different with absorption maxima roughly between 222 and 240 nm. In addition, the peaks often coelute with other unknown compounds. This makes the identification and quantification difficult and inaccurate. Thus, HPLC tends to underestimate the concentration of the MC cocktail. An international intercomparison exercise for the determination of MCs showed the difficulties involved in the analysis of the same sample with different methods and in different laboratories with different equipment (Fastner et al., 2002). Thus, the detected concentrations must be seen as rough estimates of the real concentrations.

The water treatment system Lengg/Lake Zurich/Switzerland is very effective and efficient in removing microcystin and cyanobacterial filaments from raw water, despite high cell densities and microcystin concentration in the intake water. High cell numbers of *P. rubescens* and toxin concentrations up to 5 μg/l in raw water have also been reported from Lake Bourget/France (Humbert et al., 2000). Similar to Lake Zurich, the raw water in the several water treatment plants at Lake Bourget is taken from a depth of 30 m. An ozonation and sand filtration step at the water works at Lake Bourget are able to reduce the toxin content of the final water to below 1 μg/l, a concentration that must be guaranteed by the water works according to the French drinking water guideline (Table 1). In the water work Lengg at Lake Zurich, the Adda-ELISA system detected an extracellular concentration of ~1.0 μg MC-LR equiv./l, the value determined by HPLC-DAD and rPPA were >10-fold lower (Table 5). This difference is not entirely explainable with the different characteristics of the detection methods. A possible reason could be the degradation products of microcystins after ozonation, with could be detected by the Adda-ELISA but not with the PPA nor with the routine HPLC method. A second possible explanation is the difference in the protein phosphatase inhibiting capacity of different microcystin congeners. Unpublished results form our group provide evidence that the IC_{50} of predominant MC-RR variant of the specific *P. rubescens* strain in Lake Zurich is 4 to 5 times higher than the IC50 of MC-LR. This would result in underestimation of the microcystin content in the sample.

In the water treatment plant Lengg/Zurich, only <5% of the initial toxin concentration could be detected using each of the three detection methods. The elimination of toxin was shown to be efficient during preozonation with ~1.0 mg/l. These results confirm batch experiments in which MCs were effectively removed during ozonation followed by different filtration steps (Rositano et al., 1998; Hoeger et al., 2002). Although the different detection methods yielded slightly variable toxin concentrations, the efficacy of preozonation could be shown. After the fast filtration step, the toxin concentration remained within the same range of 100–200 ng MC-LR equiv./l. However, a shift to a higher percentage of extracellular toxin was recognizable (Table 5). The results demonstrate that the cells did not remain intact during preozonation. After ozonation, the cells appeared more colorless than the cells in raw water, probably indicating damage and consequent release of toxin, which could then be oxidized. This is a plausible explanation for the high cell number and corresponding low toxin concentration detected (Table 5). Geering (1999) suggested that ozone must be applied in such a manner that destruction of algal cells does not occur and hence a negative impact on the remaining water treatment stages is avoided. However, this was not the case in this spot check. Considering the potential health risk for the human population, it is particularly important to eliminate dissolved toxin during the water treatment steps. The ozonation in this water treatment plant seems to result not only in a lysis of the majority of the cyanobacterial cells, but also in a destruction of the dissolved toxins after lysis. While free MCs are also quickly oxidized with low ozone (0.2 mg/l) concentrations (Shawwa and Smith, 2001), this reaction also heavily depends on the DOC, temperature, and pH of the water (Rositano et al., 2001). In the water treatment plant Lengg/Lake Zurich, the subsequent filtration step did not further reduce the toxin concentration (Table 5). This part of the treatment system is intended to decrease the organic load after the preozonation step; however, the dissolved toxins are too small to be

filtered in this treatment step and no toxin degradation by bacteria seems to occur during fast filtration. It can be assumed that with the subsequent intermediate ozonation with 0.5 mg/l, the activated carbon filtration and the slow sand filtration are able to eliminate the remaining toxin. Problems may arise if high densities of *P. rubescens* occur for a longer period in raw water. Unoxidized cyanobacterial toxins and cells could remain in the filters and be washed out with further charges (Hoeger et al., 2002). Furthermore, breakthrough of toxins can occur after filtration of relatively small water volumes (Donati et al., 1994). Regular backwashing of the filter should prevent clogging and accumulation of high organic concentrations in the filter material; however, backwashing has been shown to be unable to remove much biomass from the filter media (Hozalski et al., 1999). The competitive effects of NOM and preloading of organic matter on activated carbon has been determined to cause a reduction in the capacity of activated carbon for MCs (Lambert et al., 1996). The importance of backwashing the sand filters in the case of high cyanobacterial cell densities has also been pointed out by Humbert et al. (2000). In this case, a daily backwashing together with an increased ozone concentration was able to reduce the breakthrough of cyanobacterial cells. The Lengg water treatment plant (Zurich) employs a very effective treatment system. Because the associated lake is known for its annual blooms of *P. rubescens* with cell densities of up to 1×10^5 cells/ml in raw water at a relevant depth, this complex water treatment procedure is necessary to guarantee safe drinking water.

Although only low amounts of microcystins could be detected in raw water of the water treatment work at Wahnbachtalsperre/Siegburg/Germany; toxin elimination was not efficient. Only approximately 60% of the toxicity could be removed through flocculation and filtration. At the time of this particular spot check, only low densities of *P. rubescens* occurred and lysed cells may have already released the toxin. Thus, the percentage of extracellular toxin was high. Treatment systems employing only flocculation and filtration without an additional oxidation step or activated carbon filtration are a disadvantage with respect to the elimination of dissolved toxins. This treatment system is adequate to reduce cyanobacterial cell numbers, but is unable to remove the dissolved toxins (Rositano and Nicholson, 1994). The differences in observed toxin concentrations could be caused by the predominant MC-congener produced by *P. rubescens* as observed in the samples from the water treatment plant Lengg/Switzerland (see above). While no clear and quantifiable peak was detectable via HPLC-analysis, both ELISA and rPPA showed distinct and reproducible signals for the presence of microcystins and proteinphosphatase inhibitors, respectively. The low densities of *P. rubescens* during this spot check make a general risk assessment impossible. Since 2002, new treatment steps have been implemented in this water treatment plant (ultrasonication after flocculation and disinfection with UV). The efficacy of the new treatment to remove cyanotoxins has not yet been tested, but final disinfection with UV could be a useful tool for destroying MCs (Tsuji et al., 1995).

Conclusion

Two completely different ‘‘cleaning’’ methods to eliminate cyanobacterial toxins from raw water were investigated in this study. However, removing these secondary metabolites is not the main objective of water treatment plants. Elimination of color, bad taste and odor, chemicals, and microorganisms should be guaranteed. Thus, it is important to assess the ability of every water treatment plant to remove harmful compounds from cyanobacterial-loaded raw water. In industrialized countries, a maximum of quality should be attained concerning foodstuffs and drinking water. However, the raw water sources offer variable qualities of water from reservoir to reservoir and from day to day. Thus, the drinking water suppliers cannot eliminate the possibility of variations in drinking water quality. Analysis of the results of chemical and microbial investigations requires time. This means that the investigated water charge has already reached the user, before test results become available. A wise compromise could be that water should be as good as necessary not as good as possible (Kaiser et al., 2000). Many lakes and drinking water reservoirs in Germany and Switzerland are predominated by *P. rubescens*. Due to their ‘‘behavior’’ as a metalimnic inhabitant followed by mixing to deeper layers, raw water is often contaminated with high densities of *P. rubescens*. The concentration of MC did not surpass the WHO guideline of 1.0 μg/l in any of the cases presented here; however, contamination of final water during months with MC concentrations below 0.2 μg/l cannot be excluded. Spot checks were taken from water during and after the water treatment plant, but not in the supplied households. Due to chlorination or dilution with uncontaminated water, contamination of tap water with cyanobacterial toxins could actually be distinctly lower. With respect to the possible tumor-promoting activities of MCs, chronic exposure of populations to concentrations below 0.1 μg/l should be avoided. Tables 2 and 3 show the worldwide abundance of toxic cyanobacteria in drinking water reservoirs and the party high concentrations of microcystins. Additionally, cyanobacteria produce a wide variety of other toxins (e.g., saxitoxins, anatoxins, cylindrospermopsins) and molecules with unknown toxic potential (e.g., microviridins, aeruginosins) and demonstrate therefore incalculable difficulties for water treatment works, which are dependent on raw water from surface waters. The existence of other routes of toxin exposure (contaminated food, algal ‘‘health’’ products) emphasizes the need for an improved human toxin exposure assessment. It is thus essential that the potential for cyanobacteria blooms is given careful consideration in the design and operation of drinking water reservoirs and treatment works.

Acknowledgments

This study was supported by the Swiss Federal Office of Public Health (Bern, Switzerland) and a fellowship to S.J.H from the German Federal Environment Foundation (Osnabrueck, Germany). We would like to thank Prof. W. Hofer (University of Konstanz) for generously supplying ^{32}P-ATP and the participating water treatment plants at Lake Zurich (Switzerland) and at the Wahnbachtalsperre (Germany) for supporting the project.

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