Chapter 7: Cyanobacterial Toxins in New York and the Lower Great Lakes Ecosystems

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Abstract

Toxic cyanobacterial blooms are an increasing problem in the lower Laurentian Great Lakes. To better understand their occurrence and distribution, samples for particulate toxin analysis were collected from more than 140 New York Lakes including Lakes Erie, Champlain and Ontario. Microcystins were of most importance and were detected in nearly 50% of the samples. Anatoxin-a, cylindrospermopsin and the paralytic shellfish toxins occurred much less frequently (0-4%). The implications for the management of cyanobacterial harmful algal blooms are discussed.

Introduction

The North American Great Lakes located between the United States and Canada collectively provide drinking water for more than 22 million people. In addition, numerous smaller lakes provide recreational opportunities for inhabitants and visitors to the northeastern US. Historically, very little is know historically about the occurrence of cyanobacterial toxins in New York State waters, despite the early isolation of a paralytic shellfish toxin (PST)–producing strain (*Aphanizomenon schizoide* aka *Aph. flos–aquae*) from nearby Vermont in the 1960s (Sawyer et al. 1968), and extensive work by Paul Gorham and coworkers at the National Research Council of Canada in the 1950s and 1960s documenting toxic cyanobacterial blooms in the nearby Canadian lakes and ponds (Gorham 1962, Gorham et al.

1964). This situation achieved a much higher awareness following the large outbreak of toxic *Microcystis* bloom in the western basin of Lake Erie in the mid 1990's (Brittain et al. 2000). Additional outbreaks have occurred in subsequent years where concentrations of microcystins have reached levels as high as 20 μg microcystin–LR equivalents ($MC–LR_{eq}$) L^{-1} (Oullette et al. 2005, Rinta Kanto et al. 2005). In 1998 and 1999, several dogs died at public campgrounds located along the shores of Lake Champlain after coming in contact with algal scums washed up along the shoreline (Boyer et al. 2004). Preliminary investigations indicated that the cause of death in these animals was likely due to ingestion of the neurotoxin anatoxin–a. In response to these highly publicized events, the National Oceanographic and Atmospheric Administration (NOAA), through their Monitoring and Event Response for Harmful Algal Blooms (MERHAB) program, the Centers for Disease Control (CDC) and the NOAA's Sea Grant programs initiated extensive field studies to better understand the occurrence and distribution of these toxins in New York waters and the lower Great Lakes. The results from five years of these field studies spanning from 2000 – 2004 are summarized here.

Methods

Sampling methodology has steadily evolved over the 5 years of this study. More than 2500 samples were collected from over 1000 sites at 81 different New York lakes. This includes large lakes such as Lake Erie, Lake Ontario and Lake Champlain, intermediate–sized bodies of water such as the New York Finger Lakes and Oneida Lake, and smaller impoundments and lakes such as Lake Neatahwanta and Labrador Pond. Samples were collected at a variety of times throughout the growing season (June – October) with the bulk of the samples collected in late July and early September, times of peak cyanobacterial abundance. Early in the study (year 2000), 1 liter grab samples were collected from a depth near the surface of the water body and vacuum filtered through a 47mm 945AH glass fiber filter. These filters were immediately frozen on dry ice in the field and returned to the laboratory for extraction and analysis. The aim of these studies was to achieve large geographical coverage and samples were collected from 58 different lakes and over 150 sites, but generally only once during the season in late July and early August. In subsequent years, sampling focused more on single bodies of water (Lake Erie, Lake Ontario, Oneida Lake, Lake Champlain, etc.) with multiple samples taken throughout the growing season. To achieve a greater sensitivity in oligotrophic waters

such as the Great Lakes, the volume filtered in later years was also increased. Samples were collected from 0.5–1 m in depth and rapidly filtered through a 90 mm 934AH glass fiber filter using a peristaltic pump until either 20 L passed through the filter or the filter plugged due to particulate material in the water column. Filters were again frozen immediately in the field and returned frozen to the lab for later toxin analysis. Additional samples were collected for chlorophyll determination, DNA extraction, nutrients and, in selected cases, dissolved toxins. Only the results of the particulate toxin analysis are reported here.

Upon return to the lab, the filters were extracted in 5–10 ml of 50% methanol containing 1% glacial acetic acid using ultrasonic disruption. Control experiments showed that this extraction protocol solubilized more than 95% of the microcystin–LR, –RR, –LF, anatoxin–a (ATX) and the PST saxitoxin from the cells. Microcystins were measured using a combination of assays including inhibition of the protein phosphatase 1A (PPIA, Carmichael and An, 1999), Enzyme linked immunoassays (ELISA) and by high performance liquid chromatography (HPLC) coupled with either photodiode array (PDA) or mass selective (MS) detection (Harada, 1996). Anatoxin–a was determined by HPLC after derivatization with 7–fluoro–4–nitro–2,1,3–benzoxadiazole (NBD–F) (James et al., 1998) and confirmed by HPLC–MS of the free or NBD–derivatized toxin. The PST toxins (saxitoxin, neosaxitoxin, and gonyautoxins 1–4) were measured by HPLC with fluorescent detection after either chemical (PCRS: Oshima 1995) or electrochemical (ECOS: Boyer and Goddard 1999) post–column derivatization. Cylindrospermopsin was measured by HPLC using PDA detection and confirmed by HPLC–MS (Li et al. 2001).

Results

New York Overview

Between 2000 and 2004, more than 1000 samples were collected from 81 different lakes scattered across New York State. The distribution of those samples between lakes and the toxins that they were analyzed for is shown in Table 1. The lower Great Lakes (Lake Erie and Lake Ontario) plus Lake Champlain accounted for a large number of those samples (65%), especially in the later sample years due to the Great Lakes focus of the MERHAB sampling program (Boyer et al. 2004a). Samples from these large lakes consisted of a mixture of open water and “coastal” sites and

were obtained from both sampling cruises as part of the MERHAB–Lower Great Lakes and Microbial Ecology of the Lake Erie Ecosystem (MELEE) scientific programs (i.e. see section on Lake Erie below) on the CCGS Limnos and RV Lake Guardian, as well as from smaller boats and shore samples. Samples collected from the smaller lakes (Oneida Lake, Finger Lakes and other NY Lakes) were either from targeted studies by our own lab (Oneida Lake, Onondaga Lake), from the NY–Department of Environmental Conservation (Finger Lakes) or as part of the broad shotgun survey across NY State conducted in 2000. Only in a few cases were these samples collected in direct response to a reported toxic algal bloom.

Table 1. Sample Distribution between New York Lakes and the combined number of samples tested for the different cyanobacterial toxins during the 2000–2004 sample seasons.

Task	Total #	Lake Ontario	Lake Erie	Lake Champlain	Oneida Lake	Finger Lakes	Other NY Lakes
# Samples collected:	2513	736	308	590	314	138	427
Analyzed for MC's*:	2286	561	293	579	302	137	414
Analyzed for ATX:	2307	589	286	572	315	138	407
Analyzed for PSTs:	1078	258	174	314	163	29	240
Analyzed for CYL:	366	104	79	32	34	0	117

*Abbreviations: MC's = microcystins, ATX = anatoxin–a, PST = saxitoxin + neosaxitoxin, CYL = cylindrospermopsin.

Not all samples were analyzed for all toxins, though more than 90% of them were analyzed for microcystins by at least the PPIA and for anatoxin–a by HPLC–FD. Fewer samples were analyzed for the less common toxins such as the PST toxins and cylindrospermopsin (43% and 15% respectively).

The determination if the sample would be considered toxic was dependant on how "toxic" was defined. Table 2 shows the results for microcystin analysis using three different thresholds for toxicity: the World Health Organization's (WHO) advisory limit of 1 μg MC–LR equivalents L^{-1}, an arbitrary guideline value of 0.1 μg L^{-1}, and roughly the detection limit of the PPIA assay of 0.01 μg MC–LR equiv. L^{-1}. This detection limit is based on collection of a 10 L sample via filtration and its extraction/concentration into 10 ml of solvent. Approximately 14% of the samples collected

statewide exceeded the WHO advisory limit of 1 μg MC–LR_{eq} L^{-1}. As expected, the bulk of these samples (239 or 73%) came from smaller, more eutrophic, water bodies. Samples from the more oligotrophic Great Lakes, Lake Erie and Lake Ontario, exceeded the WHO advisory limit of 1 μg L^{-1} only 15 times during the five year time period. In contrast, samples from Lake Champlain exceeded the 1 μg L^{-1} advisory limit more than 71 times during this same time period. Most of these toxic Lake Champlain samples were collected from the eutrophic Missisquoi Bay region which is characterized by high phosphorus inputs and expansive *Microcystis* blooms during the summer growing season. Similarly, more than 25% of the samples collected from Oneida Lake with its well established cyanobacterial blooms exceeded the WHO advisory limit of 1 μg L^{-1}. Equally informative was the abundance of detectable levels of microcystin–LR_{eq} in the water. Over 50% of the total samples from New York Lakes, including 40% of the samples collected from Lake Erie and 28% of the samples collected from Lake Ontario had easily detectable levels of microcystins that exceeded the 0.01 μg L^{-1}. This dropped to 11% and 29% respectively for Lake Ontario and Lake Erie when that threshold was raised to the more reasonable 0.1 μg L^{-1}.

Table 2. Percentage of samples collected during the 2000 – 2004 sample season that exceeded a 1.0, 0.1 and 0.01 μg L^{-1} threshold for microcystins as measured using the protein phosphatase inhibition assay.

Threshold concentration MC–LR_{eq} L^{-1}	Total # all lakes	Lake Ontario	Lake Erie	Lake Cham–plain	Oneida Lake	Finger Lakes	Other NY Lakes
# Samples Analyzed:	2513	736	308	590	314	138	427
>1.0 μg:	326	4	11	71	71	1	168
(%)	(14%)	(1%)	(4%)	(12%)	(24%)	(1%)	(41%)
>0.1 μg:	829	61	84	190	207	23	264
(%)	(36%)	(11%)	(29%)	(33%)	(69%)	(17%)	(64%)
>0.01 μg:	1223	155	117	296	249	113	293
(%)	(53%)	(28%)	(40%)	(51%)	(82%)	(82%)	(71%)

***** The protein phosphatase inhibition assay is an activity–based technique that calculates toxicity from a microcystin–LR –derived standard curve. Results are given in MC–LR equivalents, e.g. that concentration of microcystin–LR which exhibits the equivalent toxicity to the sample. This is similar, but not identical, to the microcystin–LR equivalents derived from structural–based assays such as ELISA.

The neurotoxic cyanobacteria toxins such as anatoxin–a (ATX) and paralytic shellfish toxins (PSTs) saxitoxin and neosaxitoxin, and hepato-

toxic cylindrospermopsin (CYL) were found much less frequently. Significant numbers of these toxins were detected only when the threshold of analysis was set at or near the detection limit (Table 3). Anatoxin–a was the second most common cyanobacterial toxin observed in New York State lakes with 11 samples (<1%) exceeding the 1 μg L^{-1} threshold, 29 samples (1%) exceeded the 0.1 μg ATX L^{-1} threshold and 74 samples (3%) exceeding the most stringent 0.01 μg L^{-1} threshold. A large proportion of those toxic samples were from either the northern regions of Lake Champlain where animal fatalities from anatoxin–a have been reported in the past (Boyer et al., 2004b), highly eutrophic Lake Agawam on Long Island (Gobler, unpublished), or the eutrophic embayments along the New York coast of Lake Ontario (Yang et al., 2005). High concentrations of ATX were not observed in either Lake Erie or the offshore waters of Lake Ontario. Interestingly, the peak periods of anatoxin–a in Lake Champlain did not coincide with the large microcystin–producing algal blooms. The initial dog intoxication event at Au Sable State Park occurred in June of 1999, a time when the lake was generally cooler and the cyanobacterial biomass was low. In this event, there was very little in the way of surface scums in the water itself, but sizable accumulations did occur along the shore.

Table 3. Occurrence of anatoxin–a, paralytic shellfish toxins and cylindrospermopsin in samples collected during the 2000 – 2004 sample season that exceeded a predefined threshold. Percentage represents the percent of those samples from that particular lake that exceeded the threshold.

Task	Total # all lakes	Lake Ontario	Lake Erie	Lake Champlain	Oneida Lake	Finger Lakes	Other NY Lakes
>1.0 μg ATX L^{-1}:	11	0	0	7	0	0	4
%	(<1%)	(0%)	(0%)	(1%)	(0%)	(0%)	(1%)
>0.1 μg ATX L^{-1}:	29	2	2	12	2	2	9
%	(1%)	(<1%)	(1%)	(2%)	(1%)	(1%)	(2%)
>0.01 μg ATX L^{-1}:	74	14	14	24	4	2	16
%	(3%)	(2%)	(5%)	(4%)	(1%)	(1%)	(4%)
>0.01 μg PST L^{-1}:	2	0	1	0	0	0	1
%	(0%)	(0%)	(1%)	(0%)	(0%)	(0%)	(1%)
>0.01 μg CYL L^{-1}:	8	1	2	0	0	–	5
%	(2%)	(1%)	(3%)	(0%)	(0%)	–	(4%)

The other two classes of cyanobacterial toxins, namely the PSTs and cylindrospermopsin, occurred very rarely if at all. PST toxicity was detected in only two samples of nearly 1100 tested during the 5–year time period with the maximum concentration of only 0.09 μg L^{-1}. At these low concentrations, its identification was tentative and remains to be confirmed. This low occurrence was despite the common occurrence of high biomass blooms of *Aphanizomenon flos–aquae*, and the fact that the original PST–producing strain was isolated from nearby Vermont. Cylindrospermopsin was detected in only 8 samples of 366 tested. Most of those samples (5) occurred during an August bloom on Lake Agawam, Long Island, which coincided with a period of relative nitrogen limitation as evidenced by nutrient addition experiments (Gobler et al. 2006). In all cases, the maximum concentration of these cylindrospermopsin was low (< 0.25 μg L^{-1}). Confirmation of its identification using more advanced (HPLC–MS–MS and the polymerase chain reaction) techniques is in progress and until that time, its identification in New York water should also be considered tentative.

For the toxins other than microcystins, the cyanobacterial species responsible for their production is unknown. However, many of the embayments in Lake Champlain and Lake Ontario with easily measurable anatoxin–a concentrations also had significant co–occurring blooms of *Anabaena* species. The predominate organism responsible for microcystin formation in New York waters is likely to be *Microcystis aeruginosa*. However *Microcystis* is not the only species capable of producing microcystin in these waters. Significant blooms of *Planktothrix* and *Anabaena* species, both known microcystin producers routinely occur in New York State waters. In the absence of physically isolating and culturing the responsible organism from a toxic bloom, molecular techniques are now routinely being used to determine what organism(s) are likely to be responsible for the observed toxicity. For example, the western basin of Lake Erie routinely experiences toxic blooms of *Microcystis aeruginosa*, however *Planktothrix* appears to be the species responsible for microcystin production in the adjacent Sandusky Bay of that lake (Rinta Kanto et al.. 2005, 2007). Blooms of toxic cyanobacteria in Oneida Lake also show very different PCR banding patterns in their microcystin biosynthetic genes, suggesting that genetic differences in microcystin–producing organisms can occur within the same water body (Hotto et al. 2004 unpublished).

Lake Erie and Lake Ontario

With the isolation of toxic *Microcystis* from Lake Erie by Brittain and co-workers, there has been considerable effort to determine the occurrence and distribution of cyanobacterial toxins in Lake Erie and nearby Lake Ontario. Table 4 summarizes the results of five different research cruises on Lake Erie during 2000–2004 that specifically targeted cyanobacterial toxins. Most of the sampling was focused in the highly eutrophic western basin of Lake Erie. Toxicity was highly variable in this basin with some years showing high levels of toxicity (2003, >20 μg L^{-1}) and other years showing little to no toxicity (2002). Part of the explanation for this variability is the time of sampling. The availability of large ships for sampling on Lake Erie is limited and cruise dates were often determined by ship availability rather than optimum time for cyanobacterial blooms. In addition, the timing of the blooms was variable in themselves and occurred as early as July and as late as October. Nor was the western basin of Lake

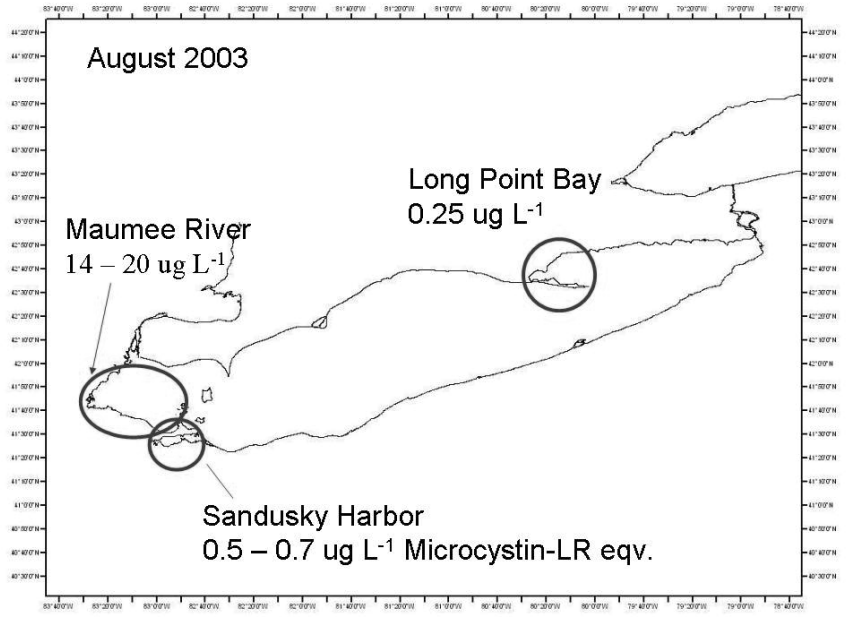

Fig. 1. Distribution of microcystin toxicity during the 2003 research cruises on Lake Erie. Highest concentrations were obtained near the Maumee River in August, but two distinct blooms also occurred near Long Point Bay and in Sandusky Harbor. Toxicity was measured using the PPIA and is expressed in terms of μ $MC–LR_{eq}$ L^{-1}.

Erie the only site of cyanobacterial toxicity. Significant levels of microcystin toxicity were also observed in Sandusky Harbor and near Long Point Bay in the eastern basin (Fig. 1). The source of toxicity in the different regions was quite different. In both the western basin and at Long Point, well defined blooms of *Microcystis aeruginosa* most likely accounted for the observed toxicity. In contrast, Sandusky Harbor often contained a dense cyanobacterial flora that was usually dominated by *Aphanizomenon flos–aquae* and *Anabaena* species. *Microcystis* is present but often a low abundance. Recent molecular analysis has indicated that the source of microcystin toxicity in this system was more likely due to *Planktothrix* species present in the understory of the bloom (Rinta–Kanto and Wilhelm, submitted) rather than *Microcystis* itself. This has important implications for monitoring protocols based on cell abundance. Neither is Lake Erie the only lower Great Lake with demonstrated levels cyanobacterial toxins. In 2003, a large bloom of *Microcystis aeruginosa* formed in the eastern basin of Lake Ontario near Oswego, New York. Microcystin concentration in this bloom near the Onondaga County drinking water intakes approached the WHO advisory limit of 1 μg MC–LR_{eq} L^{-1}.

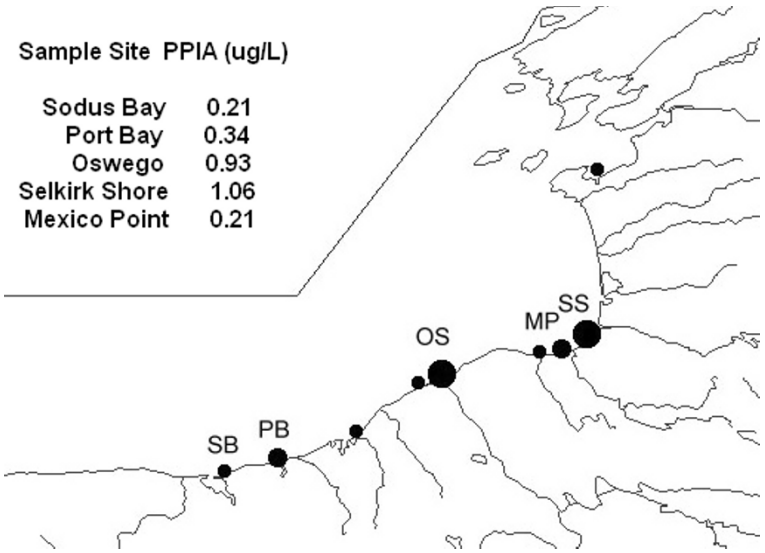

Fig. 2. The amount and distribution of toxicity off Oswego NY during the August 2003 *Microcystis* bloom in eastern Lake Ontario. Toxicity was measured using the PPIA and is expressed in terms of μ MC–LR_{eq} L^{-1}.

Table 4. Recent Occurrence and Distribution of Cyanobacterial Toxins in Lake Erie

Cruise and Date	# samples	% containing toxin	Highest measured value	Comments
Brittain et al. Sept 1996	44	MC's ~10%*	3.4 µg L^{-1} MC	Western basin only
MELEE–VII July 2002	119	MC's 7 % ATX 14% PST's 0 %	0.7 µg L^{-1} MC 0.04 µg L^{-1} ATX	Whole lake survey with highest values at Sandusky, Long Point and Rondeau Bays
MELEE–VIII July 2003	59	MC's 41% ATX 5%	0.65 µg L^{-1} MC 0.11 µg L^{-1} ATX	Whole–lake survey with highest values in the western basin and Sandusky Bay
Lake Guardian & OSU, August 2003	48	MC's 60% ATX 4%	21 µg L^{-1} MC 0.2 µg L^{-1} ATX	Western basin only, Highest values were obtained near the Maumee River
MELEE–IX July 2004	40	MC's 38% ATX 33% CYL 0%	>1 µg L^{-1} MC 0.6 µg L^{-1} ATX	Highest values near the Maumee River and in Sandusky Bay
RV Limnos August 2004	13	MC's 85% ATX 31% CYL 15%	2.4 µg L^{-1} MC 0.07 µg L^{-1} ATX 0.18 µg L^{-1} CYL	Western basin only

*These results are extrapolated from Fig. 2 in Brittain et al. (2000). Abbreviations: MC's = microcystins, ATX = anatoxin–a, PST = saxitoxin + neosaxitoxin, CYL = cylindrospermopsin.

Conclusions and Discussion

Cyanobacteria blooms are common throughout New York State waters however historically, cyanobacteria toxins were not measured. In recent years, several widely publicized animal fatalities have occurred in New York waters due to cyanobacterial toxins. These include dog deaths in Lake Champlain in 1999 due to anatoxin–a and in 2000 due to microcystin toxicity, as well as a dog and water fowl deaths in Lake Neatahwanta in 2004. The presumptive toxin was identified based on the occurrence of toxins in the water, but the causative organisms was not identified and cultured. Several recreation closures due to cyanobacterial toxins have occurred throughout the region and toxic cyanobacterial blooms routinely occur near the water intakes for major drinking water facilities located along Lake Erie, Lake Ontario and Lake Champlain. To date, these toxins have not been observed in the water distribution system. These events have raised the awareness of cyanobacterial toxins in NY waters and as a result, several water treatment facilities and local health departments now sporadically monitor for cyanobacterial toxins.

Detailed analysis of more than 2500 samples indicates that microcystin toxins were the most common toxin encountered in the state with approximately 15% of the samples collected state–wide exceeding the WHO advisory limit of 1 μg L^{-1} and nearly 60% of the samples containing detectable levels of microcystins. These numbers represents a fairly unbiased estimate of the occurrence of cyanobacterial toxins in New York waters as most of the samples were not collected in direct response to a cyanobacterial bloom but rather as part of the ongoing MERHAB or MELEE sampling program whose sample locations were chosen to guarantee broad spatial coverage regardless of the cyanobacterial density. They are also in good agreement with other large regional surveys from the midwestern United States and Europe (see these proceedings). Some bias in sample collection did exist as samples were generally taken in late summer when cyanobacterial species were more likely to predominate and some long–term sampling sample sites such Oneida Lake were chosen for study because of their well established cyanobacterial blooms. In those highly eutrophic systems where cyanobacteria form dense accumulations, the likelihood of finding toxicity was much greater than in the more oligotrophic waters.

Neither were the larger lakes immune from cyanobacterial toxicity. Both Lake Erie and Lake Ontario have well established *Microcystis* popu-

lations that routinely produce toxic blooms that exceed the WHO threshold of 1 μg L^{-1}. Lake Champlain has also experience consist toxic blooms with animal fatalities from both anatoxin–a intoxication and microcystin intoxication occurring in the region. Despite the fact that many of these larger lakes are current serving as drinking water supplies for large metropolitan areas, most of the reported health impacts for cyanobacterial toxins has been due to contact with surface contact with highly toxic scums. It is not uncommon for the wind–borne accumulation of toxic cyanobacteria to yield surface scums that have particulate microcystin concentrations in excess of 500 μg L^{-1} and higher values exceeding 1000 μg L^{-1} have been reported for highly eutrophic embayments such as Missisquoi Bay on Lake Champlain (Watzin and Boyer, unpublished). The health impacts due to recreational contact with the highly toxic surface scums remains to be determined.

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