The number and intensity of reported blue-green algae (cyanobacteria) blooms in reservoirs, lakes, ponds, and rivers have increased worldwide. Some species of cyanobacteria are capable of producing microcystins, hepatotoxins that can cause liver damage, shock, and in some cases, death to mammals ingesting contaminated water. In 1998 and 1999, studies were conducted to elucidate the occurrence of microcystin toxins in raw and finished water from five drinking water treatment facilities in Wisconsin. Samples were collected and analyzed for microcystins using an enzyme-linked immunosorbent assay. Microcystin toxins were repeatedly found in the raw water. Conventional water treatment practices used by the facilities effectively removed microcystins by 1–3 logs. The data collected suggest that microcystin toxin removal occurs incrementally at several points in the drinking water treatment train.

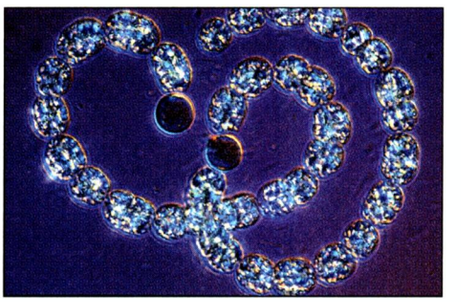


PHOTO: SCIENCE SOURCE

Microcystin algal toxins

IN SOURCE AND FINISHED DRINKING WATER

BY DAWN A. KARNER, JONATHAN H. STANDRIDGE, GREGORY W. HARRINGTON, AND ROBERT P. BARNUM

Surface freshwater is the primary source of drinking water for most of the world's population. The drinking water industry is constantly challenged by surface water contaminants that must be removed to protect public health. One emerging contaminant is associated with cyanobacteria (blue-green algae). Cyanobacteria are common in water systems, and in the presence of enough light and nutrients, they can proliferate quickly to form dense, macroscopic blooms. Certain species of cyanobacteria are capable of producing a variety of potent toxins, including a group of toxins known as microcystins.

BACKGROUND

Microcystins are water-soluble, cyclic hepatopeptides containing seven amino acids. Named in 1988 after the cyanobacteria *Microcystis aeruginosa* (Carmichael et al, 1988), these compounds can also be produced by other cyanobacteria including *Anabaena*, *Planktothrix (Oscillatoria)*, *Nostoc*, *Anabaenopsis*, and terrestrial *Halalosiphon* (WHO, 1999). Approximately 60 variations of microcystins have been isolated from cyanobacterial blooms and cultures (WHO, 1999), with microcystin-LR the most common (WHO, 1999; Rinehart et al, 1994). At near neutral pH, microcystins are very stable and can remain toxic even after being boiled (WHO, 1999).

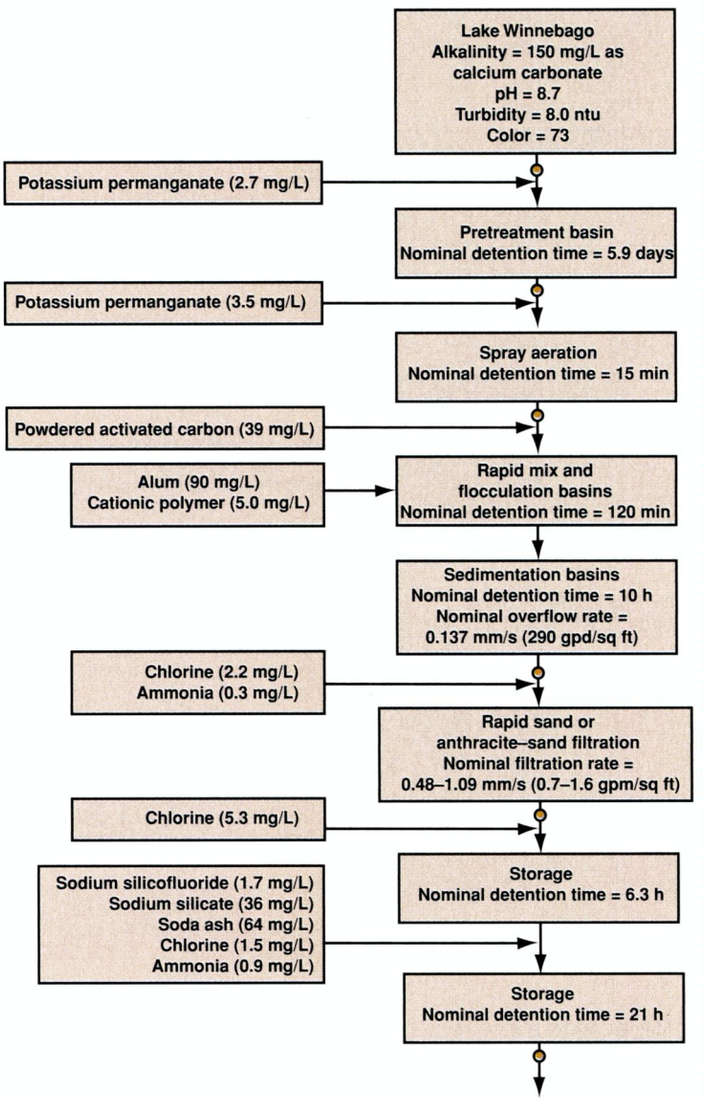
Health risks to mammals. Microcystins are toxins that primarily target the liver in mammals and thus are termed hepatotoxins. Drinking highly contaminated water leads to microcystins accumulating in liver cells, causing the cells to shrink and separate. This results in acute hemorrhaging and can cause extensive

liver damage or even death (WHO, 1999; Carmichael, 1994). Animal deaths were first reported in 1878 after livestock consumed water during a cyanobacterial bloom in Australia (Francis, 1878). Since then, numerous reports have linked cyanobacterial toxins to animal deaths. In 1996, patients receiving dialysis at a medical center in Caruaru, Brazil, experienced severe hepatitis following hemodialysis treatment (Jochimsen et al, 1998). More than 100 of the patients developed acute liver failure, and 50 of them died. Medical and chemical evidence pointed toward insufficient removal of microcystin algal toxins by the hemodialysis water treatment system.

Yu (1995) suggested that long-term chronic exposure to microcystins in drinking water may promote tumor growth or carcinogenesis. In China, rates of liver cancer have been correlated with poor drinking water sources, e.g., eutrophic ditches and ponds, raising concern that drinking from these waters may be a risk factor for primary liver cancer. In these high-endemic areas, microcystins have been detected at high concentrations (Harada et al, 1996; Ueno et al, 1996; Yu, 1995); switching the majority of the affected population to water obtained from deep wells has led to reduced liver cancer rates (Yu, 1995; Yu, 1989).

These health threats have led the World Health Organization (WHO) to establish a maximum allowable guideline value for microcystin-LR of 1,000 ng/L (WHO, 1998). The WHO states that its guideline is for general human consumption and personal hygiene only and should not be used as a standard for high-quality water needs such as dialysis treatment (WHO, 1999). Individual countries are also examining the need for drinking water consumption guidelines for microcystins. Canada is considering a guideline of 1,500 ng/L (Health Canada, 1998), and Australia has a proposed guideline of 1,300 ng/L (NHMRC, 2000). In the United States, the US Environmental Protection Agency has placed algal toxins on its Drinking Water Contaminant Candidate List (USEPA, 1998).

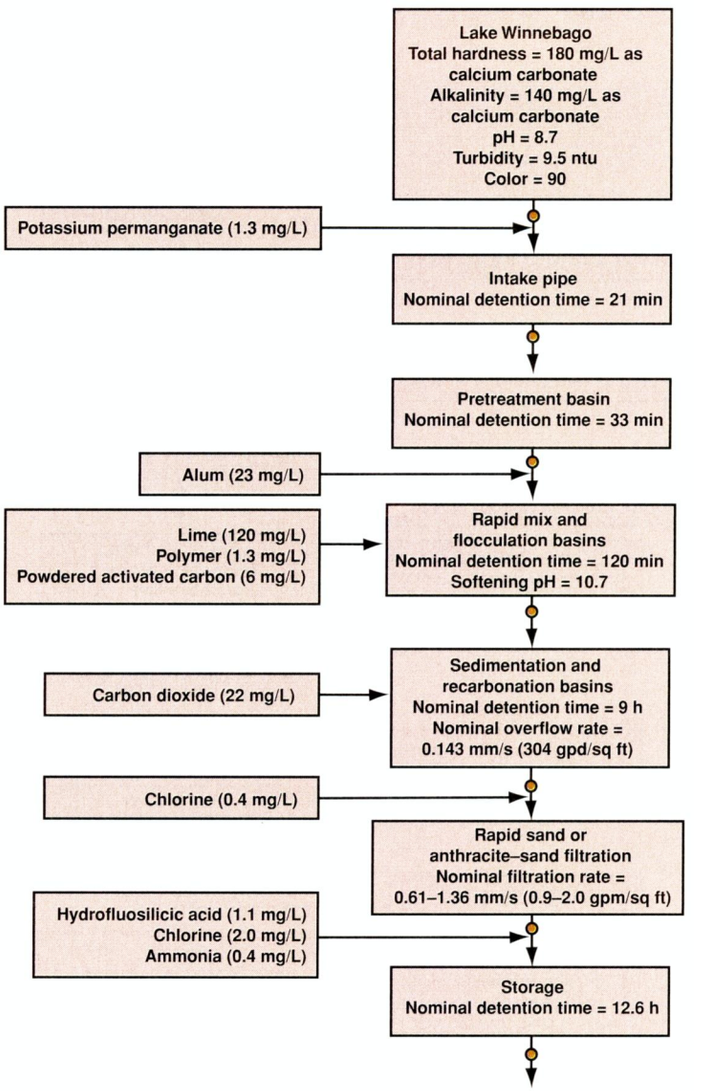
FIGURE 1 Flowchart of treatment processes at the Menasha (Wis.) water treatment plant



Flowchart shows processes and average characteristics from Aug. 2, 1999, through Oct. 13, 1999. Sample locations are shown by yellow circles.

Increased awareness of the health risks associated with algal toxins has spurred the drinking water industry to further investigate the toxins' potential threat. The toxins are found within the algal cell as well as extracellularly after cell lysis caused by natural cell death, algicide treatments, or the rigors of the drinking water treatment process. To safeguard public health, the drinking water community

FIGURE 2 Flowchart of treatment processes at the Neenah (Wis.) water treatment plant



Flowchart shows processes and average characteristics from Aug. 2, 1999, through Oct. 13, 1999. Sample locations are shown by yellow circles.

must determine whether standard drinking water treatment is sufficient to remove both intracellular and extracellular microcystins.

Previous studies. There is some disagreement in the literature regarding the efficacy of conventional drinking water treatment (e.g., coagulation, flocculation, sedimentation, filtration, and disinfection) in removing algal toxins. Some researchers reported that treatment was ineffective (Himberg et al, 1989; Keijola et al, 1988; Hoffman, 1976; Wheeler et al, 1942). Other researchers, however, concluded that conventional treatment may remove algal toxins. In a study of a conventional water treatment pilot plant, the addition of alum at 5.8 mg/L aluminum to a natural water was shown to remove algal cells without significant cell lysis (Chow et al, 1999). Additional studies also confirmed toxin removal but were careful to point out that conventional treatment was not effective at removing extracellular toxin and additional processing must take place to ensure its removal (Hart et al, 1998; Himberg et al, 1989; Hoffman, 1976).

Other researchers reported that appropriate doses of oxidizing agents such as ozone, potassium permanganate, and chlorine effectively removed both the intracellular and extracellular algal toxins (Bruchet et al, 1998; Hart et al, 1998; Rositano et al, 1998; Nicholson et al, 1994). However, Bruchet and colleagues (1998) cautioned that the chlorine dose required was above the tolerance level of most consumers. Chloramination, on the other hand, appeared to cause cell lysis and was ineffective at lowering toxin levels (Hart et al, 1998; Nicholson et al, 1994).

Granular activated carbon (GAC) and powdered activated carbon (PAC), coupled with conventional treatment practices, have been shown to be effective in removing microcystins, particularly extracellular toxins, from water supplies (Bruchet et al, 1998; Hart et al, 1998; Lambert et al, 1996; Himberg et al, 1989; Keijola et al, 1988; Hoffman, 1976). Hart and colleagues (1998) studied the adsorption of microcystins to either PAC or GAC in a drinking water pilot plant. The study showed that to absorb 85% of the microcystins, the PAC dose required was in excess of 20 mg/L (which is on the high end of typical doses and may be impractical). The researchers concluded that addition of PAC to the treatment train at lower doses of 5–20 mg/L may be helpful in reducing microcystin concentrations, but the number of microcystins adsorbed would not be as great. Hart et al also demonstrated that the use of biologically active GAC could be very effective in adsorbing and biodegrading microcystins, provided the contact time was adequate. However, other researchers have cautioned that breakthrough of microcystins may occur if the GAC becomes

saturated during periods of high toxin levels in the water (Bruchet et al, 1998).

CURRENT RESEARCH

Research objectives. To elucidate the conflicts in the literature, an extensive two-year project examined the occurrence of microcystin algal toxins and their removal during drinking water treatment. The first objective (undertaken in a study during the summer of 1998) was to evaluate the occurrence of microcystins in raw and finished drinking water from five water treatment plants in Wisconsin. The second objective (evaluated by a study in the summer of 1999) was to monitor microcystin concentrations in three of the five plants, tracking levels at several points in the drinking water treatment process to determine which treatment steps effectively removed the toxins.

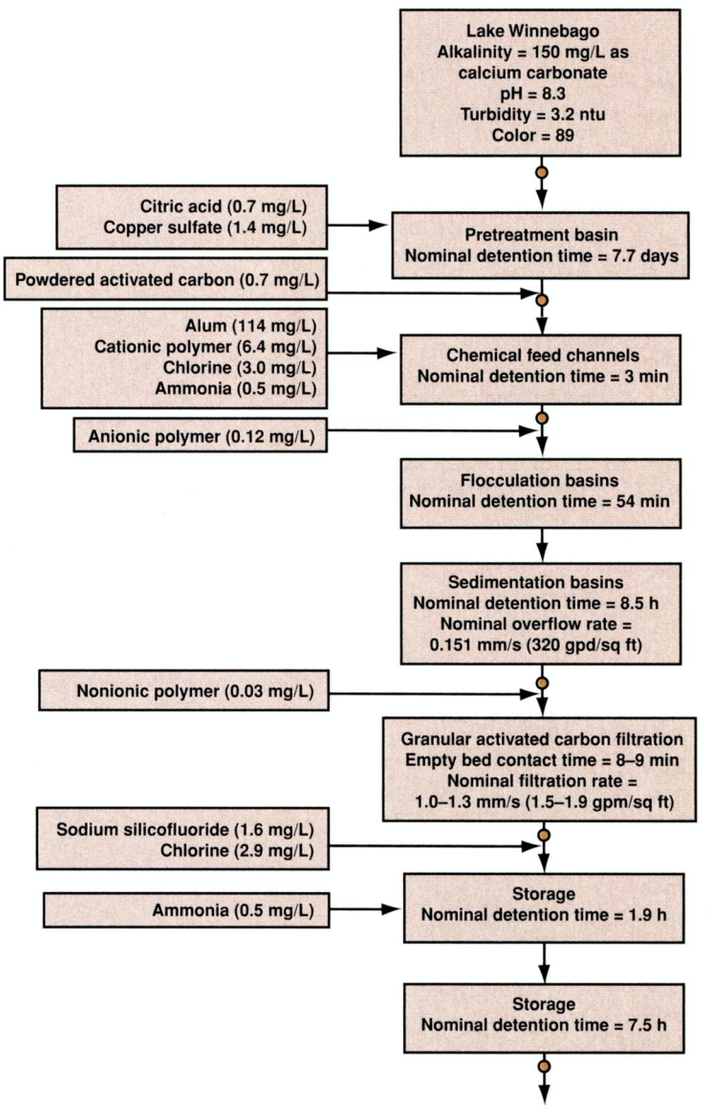
Sampling locations. ***Rainbow Lake.*** Rainbow Lake is one of 22 small lakes in a chain of lakes located in central Wisconsin. Rainbow Lake is a 47 ha (116 acre), mesotrophic, spring-fed lake that receives heavy recreational use, particularly in the summertime. It has an average Secchi depth reading of 3.5 m (11.5 ft) and has been susceptible to blooms of cyanobacteria. The shoreline of Rainbow Lake is almost completely developed, and all development is tied into a sewer system.

The Wisconsin Veterans Home in the town of King is located on the shore of Rainbow Lake and draws water from the lake for its drinking water treatment facility. This treatment facility was included in the 1998 study but not in the 1999 study. In the summer of 1998, the treatment facility used induced draft aeration followed by lime softening. In addition to lime, chemicals added during the softening step included polymer, alum, and PAC. Softening was followed by recarbonation, free chlorination, and rapid sand filtration.

Lake Winnebago. Lake Winnebago is a large, shallow eutrophic lake located in east central Wisconsin. The lake is 48 km (30 mi) long and 16 km (10 mi) wide; it covers 55,444 ha (137,000 acres) at an average depth of 3 m (9.8 ft) and an average Secchi depth reading of 1 m (3.3 ft). The plant community is dominated by algae, rather than macrophytes; dense cyanobacterial blooms are common in July and August. The municipalities of Menasha, Neenah, Oshkosh, and Appleton all use lake water to supply their water treatment facilities. Appleton's treatment plant was included only in the 1998 study; the Menasha, Neenah, and Oshkosh plants were included in both the 1998 and 1999 studies.

During the summer of 1998, the Appleton treatment facility used lime softening followed by filtration with GAC. Other processes at the Appleton plant included potassium permanganate oxidation, PAC adsorption, and chloramine disinfection. Figures 1–3 show flowcharts of the treatment processes at Menasha, Neenah, and

FIGURE 3 Flowchart of treatment processes at the Oshkosh (Wis.) water treatment plant



Flowchart shows processes and average characteristics from Aug. 2, 1999, through Oct. 13, 1999. Sample locations are shown by yellow circles.

FIGURE 4 Microcystin levels for raw and finished waters at the Appleton (Wis.) water treatment plant during the summer of 1998

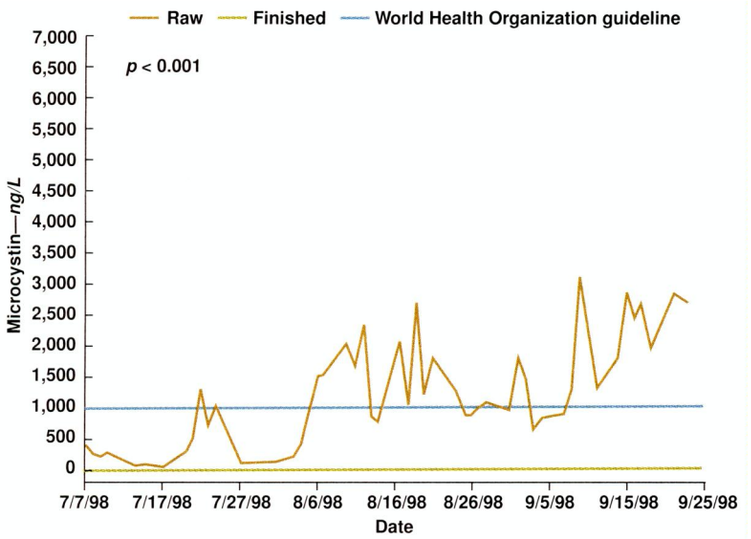
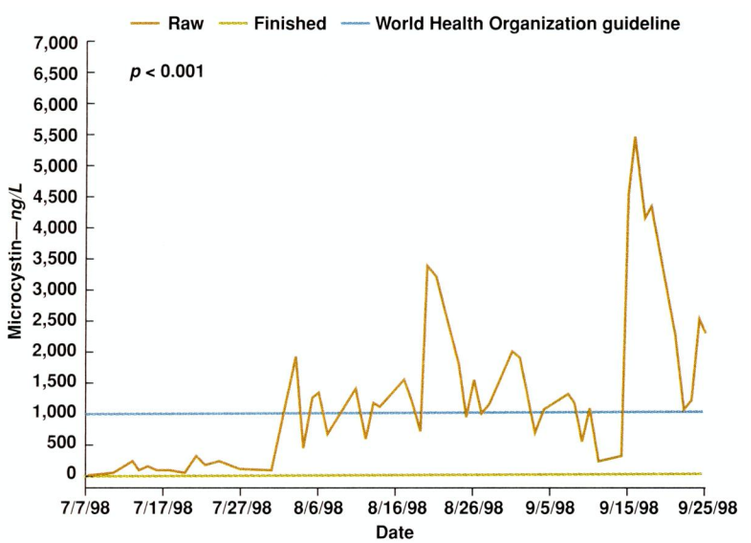


FIGURE 5 Microcystin levels for raw and finished waters at the Menasha (Wis.) water treatment plant during the summer of 1998



Oshkosh, respectively. Because these three treatment plants were sampled more extensively in 1999, the flowcharts show average characteristics for each treatment plant during the 1999 sampling period. The flowcharts also show sample locations for each treatment plant during 1999. Raw water temperatures varied from 25 to 30°C in early August 1999 to 10–15°C in mid-October 1999.

METHODS

Sample collection and preparation. As noted previously, water samples from all five water treatment facilities were collected both before and after treatment during the first year of the study. Samples were collected Monday through Friday, from July 6, 1998, through Sept. 25, 1998. Samples were immediately frozen after collection and were shipped weekly to the Wisconsin State Laboratory of Hygiene for analysis. Upon arrival, samples were thawed at 4°C and subsequently frozen at –20°C. Samples were cycled through this freeze–thaw process three times to lyse the algal cells, releasing intracellular toxins.

The next step was a two-part concentration process to ensure recovery of toxins from both the water and the cellular debris. First, each sample was filtered through a 0.45-µm membrane filter* to concentrate the algal cells. The concentrated cellular material was then rinsed into 20-mL vials using 10 mL of 100% methanol. The filtrate was loaded onto a preconditioned reverse-phase C18 cartridge† and subsequently eluted using 5 mL of 100% methanol. The eluted analyte and the membrane filter rinse were dried under air. Dried samples were resuspended in a known volume of 5% methanol and mixed just prior to analysis. Further dilutions, when necessary, were made using 5% methanol.

During the second year of the project, water samples were taken from the three drinking water treatment plants located in Menasha, Neenah, and Oshkosh every Monday and Wednesday from Aug. 2, 1999, through Oct. 13, 1999. For each sampling event, samples were collected at the locations shown in Figures 1–3. Samples were frozen immediately after collection, shipped to the laboratory, and subjected to the freeze–thaw cycle.

All samples collected after the postsedimentation stage were concentrated as described previously, i.e., using a membrane filter and C18 reverse-phase cartridge. All other samples (raw water samples and samples collected prior to sedimentation) were filtered using a 0.45-µm membrane syringe filter‡ prior to analysis. Microcystin levels in these samples were high enough that concentration using C18 cartridges was unnecessary.

Sample analysis. All samples were analyzed for microcystins using commercially available enzyme-linked immunosorbent assay (ELISA) 96-well plate kits. ELISAs

have become a routinely used laboratory tool that has been well-documented in the literature (NIH, 1996). The ELISA kits used in the 1999 study§ were the commercialized version of the kit system used in the 1998 study,** so all test kits utilized the same antibody originally developed by Chu et al (1990; 1989). All tests were performed according to manufacturers' instructions.

The direct competitive ELISA for algal hepatotoxins was first developed in 1989 and has been reported to have good cross-reactivity with most microcystin variants including microcystin-LR, RR, YR, and Nodularin (Chu et al, 1990; Chu et al, 1989). The commercial version of the kit was recently evaluated and was determined to be a good analytical tool for rapid, onsite detection of these toxins (Rivasseau et al, 1999).

Statistical analysis. To compare raw water and finished water data from each of the five facilities studied in 1998, the authors used a paired, two-tailed Student's *t*-test. To evaluate the significance of microcystin removal between treatment stages in the 1999 study, the authors performed an analysis of variance (ANOVA). A separate ANOVA was conducted for each of the three drinking water facilities sampled in 1999. Percent reductions in microcystin levels were transformed using the arcsin $\sqrt{x}$ to obtain uniform variances. For each ANOVA, post hoc comparisons of paired treatment means were conducted using a Student's *t*-test with a Bonferroni adjustment to account for multiple comparisons.

FIGURE 6 Microcystin levels for raw and finished waters at the Neenah (Wis.) water treatment plant during the summer of 1998

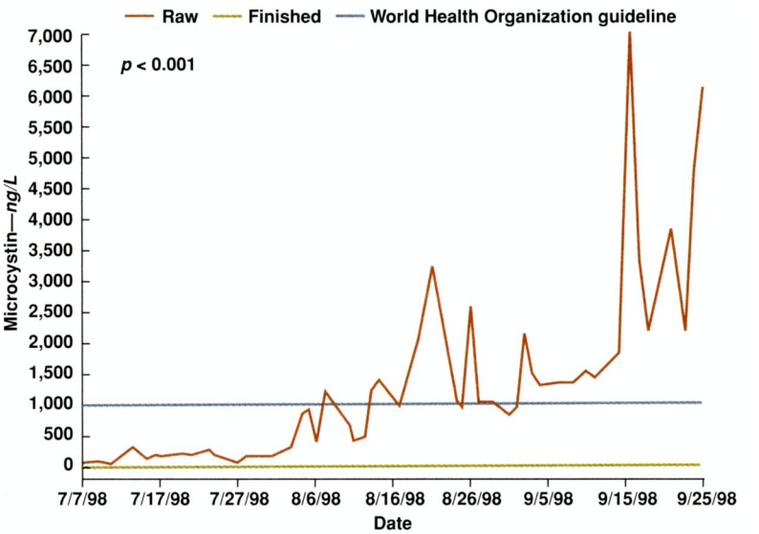
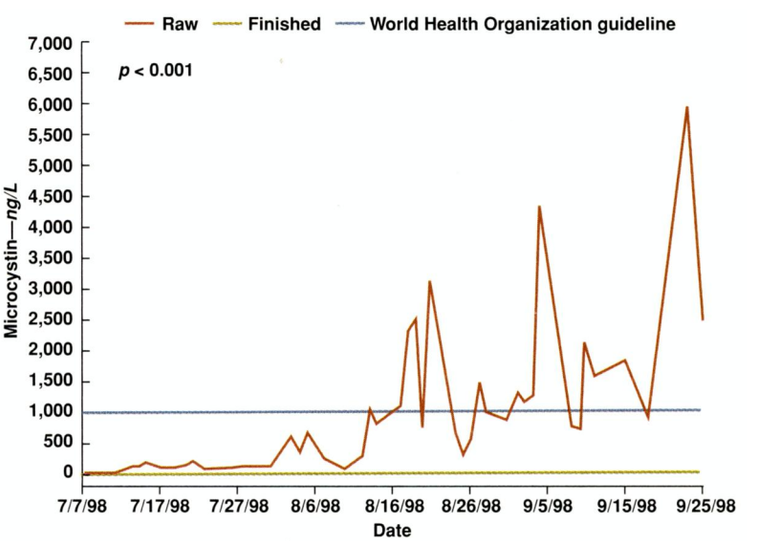


FIGURE 7 Microcystin levels for raw and finished waters at the Oshkosh (Wis.) water treatment plant during the summer of 1998



RESULTS AND DISCUSSION

1998 study. Over the 12-week period from July 6, 1998, through Sept. 25, 1998, 578 raw and finished water samples were collected from the five treatment facilities. Results are shown in Figures 4–8. These figures compare the study results with the WHO drinking water guideline of 1,000 ng/L; the 1,000-ng/L number was chosen as the reference point because it is the most conservative guideline published.

For the first four weeks of the study, toxin levels in the raw water samples generally increased but still remained below the reference level of 1,000 ng/L. At week 5 (Aug. 3–7, 1998), toxin levels approached or exceeded the reference level at the Menasha, Appleton, and Wisconsin Veterans Home facilities. By week 7 (Aug. 17–21, 1998), all the facilities except the Wisconsin Veterans Home were experiencing high toxin concentrations (~2,000 ng/L) in their intake water.

Over the course of the 1998 sampling period, the 1,000-ng/L reference mark was exceeded in 107 of the 289

*Millipore, Bedford, Mass.
†Waters, Milford, Mass.
‡Millex, HV Millipore, Bedford, Mass.
§EnviroGard Mirocystins Plate Kit, Strategic Diagnostics, Newark, Del.
**Chu Laboratories, Madison, Wis.

TABLE 1 Microcystin concentrations for the week of Sept. 21, 1998, at the Neenah (Wis.) water treatment facility

	Microcystins—*ng/L*				
Water Quality	9/21/98	9/22/98	9/23/98	9/24/98	9/25/98
Raw	3,700	2,800	1,900	4,700	6,100
Finished	8.0	9.0	7.7	6.1	9.5

TABLE 2 Microcystin concentrations in raw and finished waters for three Wisconsin drinking water treatment facilities in the summer of 1999

	Microcystins—*ng/L*					
	Oshkosh		Neenah		Menasha	
Date	Raw	Finished	Raw	Finished	Raw	Finished
8/2/99	446	2.6	381	6.1	295	< 2*
8/4/99	422	3.2	190	13.3	578	2.6
8/9/99	477	3.0	410	12.0	NA†	NA
8/11/99	451	3.4	325	8.7	659	< 2
8/16/99	507	2.0	993	16.0	310	< 2
8/18/99	289	8.3	621	21.5	556	< 2
8/23/99	123	2.8	980	9.3	909	< 2
8/25/99	342	2.5	527	2.0	916	< 2
8/30/99	878	2.9	952	9.2	896	2.4
9/1/99	702	2.4	713	7.9	800	< 2
9/6/99	461	2.1	251	4.1	403	< 2
9/8/99	541	2.5	582	10.4	548	< 2
9/13/99	293	2.2	759	5.7	528	< 2
9/15/99	759	2.9	510	7.5	544	< 2
9/20/99	499	2.0	542	7.8	379	< 2
9/22/99	554	2.3	NA	NA	186	< 2
9/27/99	306	3.2	423	7.0	380	< 2
9/29/99	387	4.1	249	12.0	179	< 2
10/4/99	461	2.6	323	10.0	145	< 2
10/6/99	281	3.7	363	10.8	241	< 2
10/11/99	198	2.5	210	6.4	165	< 2
10/13/99	227	3.0	179	6.8	195	2.2

*Values < 2 are below the enzyme-linked immunosorbent assay detection limit.
†NA—not available

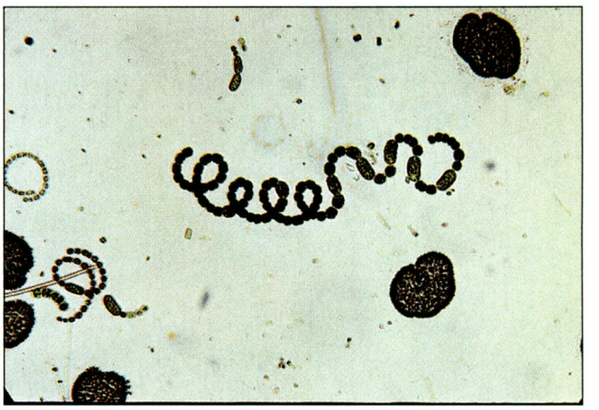
raw water samples (37%). In all cases, treatment processes effectively reduced the toxin to levels ranging from no detection (<2 ng/L) to 17 ng/L in the finished drinking water, well below the WHO standard of 1,000 ng/L.

At the five drinking water facilities, the weekly average microcystin concentration in raw water ranged from 585 to 1,216 ng/L, whereas the finished water weekly average ranged from 2.8 to 5.4 ng/L. As the amount of algal toxins in the raw water increased to levels > 1,000 ng/L, the treatment processes were able to achieve 2–3-log reductions in toxin levels. Statistical analysis showed this reduction in microcystins to be significant ($p < 0.001$) at all five facilities tested in 1998.

The highest toxin concentrations in raw water were found during the final week of sampling, Sept. 21–25, 1998. Table 1 shows daily results from the last week of sampling at the Neenah facility. Variable toxin levels were observed, with the study's highest toxin value of 6,100 ng/L noted on the final sampling day. (Because the end of the sampling effort had been predetermined, samples were not collected after this week.) Even with this high level of microcystin in the raw water, however, all finished water samples remained well below the WHO guideline, ranging from 6.1 to 9.5 ng/L.

1999 study. A total of 448 samples from the three facilities were analyzed over an 11-week sampling period (Aug. 2–Oct. 13, 1999). The raw water and finished water results are summarized in Table 2. The raw water values were lower than those observed in the 1998 study and ranged from 123 ng/L at the Oshkosh facility to a high of 993 ng/L at the Neenah facility. In contrast to 1998 results, microcystin concentrations in the raw water samples collected and analyzed in 1999 never exceeded the reference level of 1,000 ng/L. The difference in the raw water values between the two study years is likely attributable to year-to-year variation in temperature, rainfall, and nutrient loading, all of which influence cyanobacterial growth. Visually, the cyanobacterial blooms in Lake Winnebago in 1999 were not as dense and prevalent as those observed in 1998. As in the 1998 study year, 1999 finished drinking water results were well below the WHO guideline for microcystins and ranged from no detection (<2 ng/L) to 22 ng/L. Figure 9 shows the overall effect of treatment on microcystin removal at the Menasha, Neenah, and Oshkosh facilities. All three facilities demonstrated statistically significant removal of microcystins (overall $p < 0.001$).

Some of the most interesting information obtained during the 1999 study related to the point in the treatment process at which microcystin removal took place. More than 50% of the reduction in microcystins was observed at the pretreatment stage. The Neenah and Menasha plants both used potassium permanganate oxidation



A cyanobacterial bloom is photographed on the shore of Lake Winnebago in Wisconsin (left). Two cyanobacteria (blue-green algae) that can produce the algal toxin microcystin are shown at 100x magnification (right). The "twisted" one is called *Anabaena*. The two circular-looking ones on each side of the *Anabaena* are called *Microcystis*.

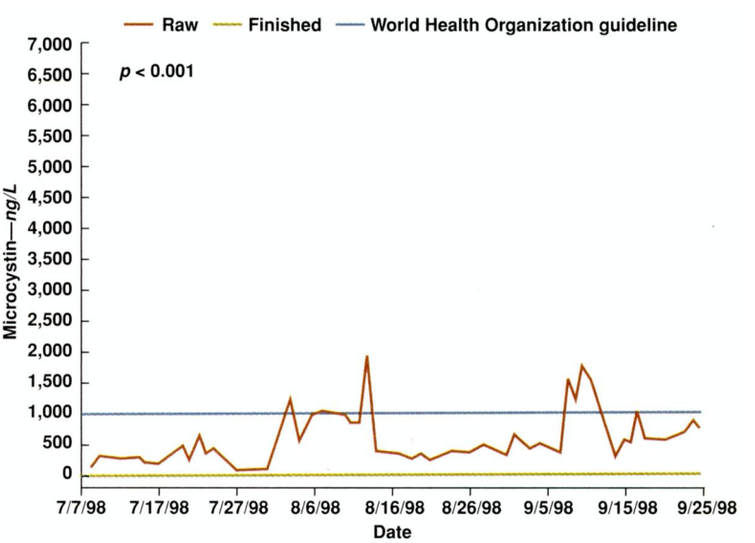
through their pretreatment basins. The Oshkosh facility fed copper sulfate, citric acid, and PAC upstream of the pretreatment sample collection point. The average PAC dose at Oshkosh was considerably smaller than the amount that previous research indicated was required for toxin removal (Hart et al, 1998). Therefore, removal of toxins at Oshkosh was likely attributable to the addition of the copper sulfate algicide and the settling provided in the pretreatment basin.

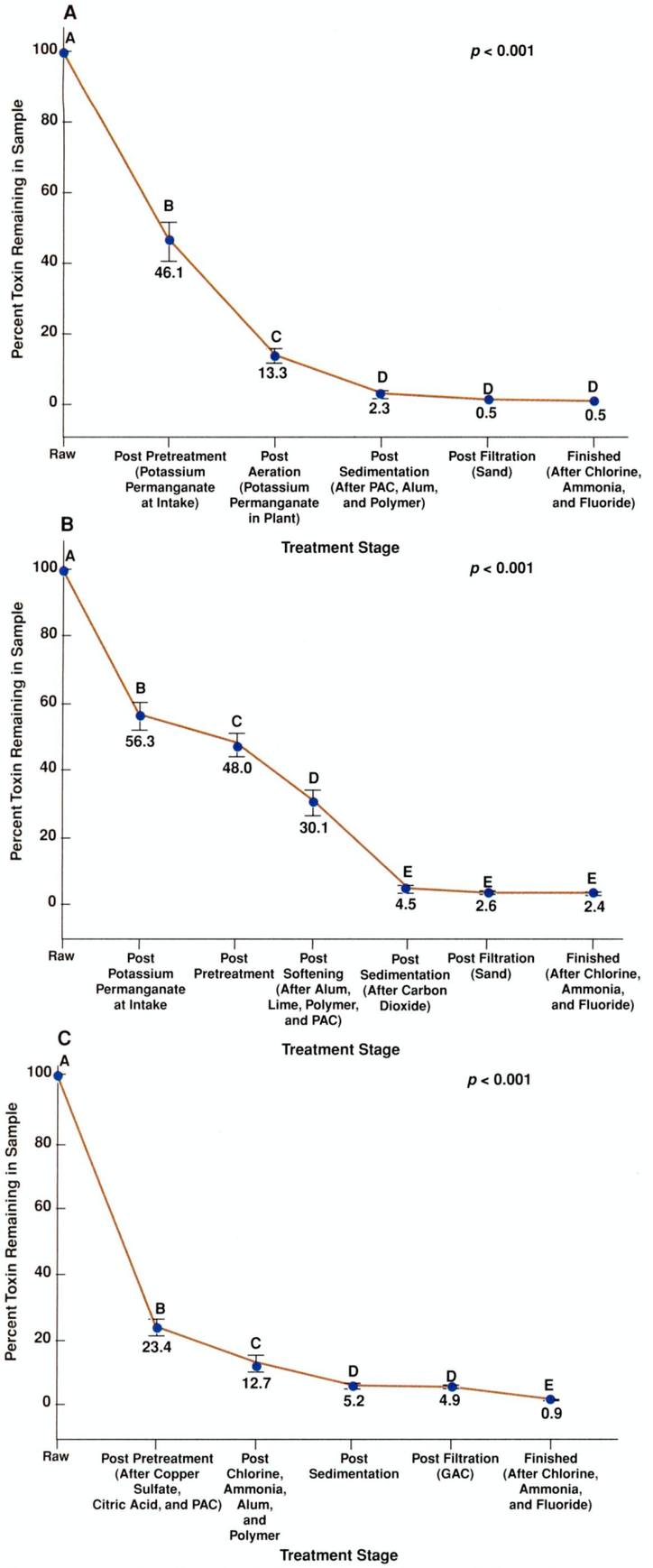
The Neenah and Menasha plants averaged 52% and 54% toxin reductions, respectively, from potassium permanganate pretreatment. The Oshkosh plant averaged 77% microcystin reduction from its pretreatment process. Although this study did not differentiate between removal of intracellular and extracellular toxins, both had the potential to be removed by the pretreatment processes used at the three facilities.

At the Neenah plant, an additional 18% reduction was observed through the softening process and another 25% reduction from sedimentation. Because this study did not differentiate between intracellular and extracellular toxins, the authors cannot determine whether the removal occurred because of adsorption of extracellular toxin to the calcium and magnesium precipitate or because of entrapment of intracellular toxin by the precipitate. At the Menasha plant, 33% reduction was found at oxidation (permanganate and aeration) and another 11% removal at sedimentation. At the Oshkosh plant, an additional 11% removal was observed shortly after the addition of chlorine, ammonia, alum, and polymer, with another 7% after sedimentation. As noted earlier, the processes used at the Menasha and Oshkosh treatment plants were capable of removing both intracellular and extracellular toxins.

Through all treatment processes prior to filtration, microcystin concentrations decreased by 98%, 95%, and 95%, respectively, at the Menasha, Neenah, and Oshkosh plants. After sedimentation, the treatment process generally produced little significant reduction in microcystin levels. At the Oshkosh facility, a statistically significant reduction in toxin levels was observed through storage. Therefore, it appears that chloramination achieved some oxidation of the microcystins, which contradicts the findings of previous research (Hart et al, 1998; Nicholson et al, 1994). As shown in Figure 3, the storage tanks had an average nominal detention time of 9.4 h during the sampling period.

FIGURE 8 Microcystin levels for raw and finished waters at the Wisconsin Veterans Home water treatment plant during the summer of 1998





Plants represented are Menasha (A), Neenah (B), and Oshkosh (C). Data points represent the mean across the sampling period. Points with different letters indicate significantly different levels of microcystins. PAC—powdered activated carbon, GAC—granular activated carbon

FIGURE 9 Microcystin reduction by treatment stage for three Wisconsin drinking water treatment facilities studied in the summer of 1999

The chloramine concentration after storage averaged 1.6 mg/L as Cl_2.

CONCLUSIONS

Microcystin algal toxins commonly occur in untreated surface waters in Wisconsin. Given appropriate conditions, these toxins can exceed the levels deemed safe for human consumption by the WHO. Generally, the concentrations of these algal toxins increase throughout the summer and into the fall season, with the greatest toxin release occurring with cell lysis as cyanobacterial blooms collapse.

This research found 1–3-log reduction (90–99.9%) in the concentration of microcystins through treatment at five Wisconsin drinking water treatment facilities tested during 1998. All finished drinking water samples analyzed from these facilities were well below the WHO microcystin guideline of 1,000 ng/L.

In-depth studies of three of the five drinking water facilities during 1999 elucidated the point in the treatment train at which toxin removal occurred. The additions of potassium permanganate or other pretreatment chemicals (e.g., PAC, citric acid, and copper sulfate) reduced microcystins by an average of 61%. Subsequently, alum coagulation followed by sedimentation or lime softening followed by sedimentation enhanced toxin reduction to an average of 96%. These findings indicate that pretreatment with an algicide such as copper sulfate and preoxidation with potassium permanganate or aeration are beneficial for algal toxin removal.

With increasing eutrophication of surface waters, water suppliers need to become more aware of the emergence of algal toxins and their potential pathway to the public consumer via drinking water. Microcystin algal toxins have been shown to be effectively removed in the drinking water

treatment train at facilities that use conventional treatment processes. The conclusions drawn from this research, however, relate only to microcystin algal toxins; additional studies are needed to address both other classes of cyanobacterial toxins and other treatment processes.

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