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Water Research 38 (2004) 4395–4404

WATER RESEARCH

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# Environmental factors influencing microcystin distribution and concentration in the Midwestern United States

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Received 2 March 2004

**Abstract**

During May–September 2000–2001, physicochemical data were collected from 241 lakes in Missouri, Iowa, northeastern Kansas, and southern Minnesota U.S.A., to determine the environmental variables associated with high concentrations of the cyanobacterial hepatotoxin microcystin (MC). The study region represents a south–north latitudinal gradient in increasing trophic status, with total phosphorus (TP) and total nitrogen (TN) values ranging between 2–995 and 90–15870 μg/L, respectively. Particulate MC values, measured by ELISA, ranged from undetectable to 4500 ng/L and increased with increasing latitude. Despite latitudinal trends, environmental variables explained <50% of the variation in MC values. Inspection of MC–TN and MC–Secchi bivariate plots revealed distinctly nonlinear trends, suggesting optima for maximum MC values. Nonlinear interval maxima regression indicated that MC–TN maxima were characterized by a unimodal curve, with maximal (>2000 ng/L) MC values occurring between 1500 and 4000 μg/L TN. Above 8000 μg/L TN all MC values were <150 ng/L. MC–Secchi maxima were characterized by exponential decline, with maximal MC values occurring at Secchi depths <2.5 m. The development of empirical relationships between environmental variables and MC values is critical to effective lake management and minimization of human health risks associated with the toxin. This study indicates MC values are linked to the physicochemical environment; however, the relationships are not traditional linear models.
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*Keywords:* Microcystin; Cyanotoxin; Cyanobacteria; Midwest; ELISA; Nonlinear

## 1. Introduction

Cyanobacteria cause a multitude of water quality concerns, including the potential for toxin production. Implicated in human and animal illness and death in over twenty countries worldwide, the hepatotoxin microcystin is more common than other cyanotoxins.

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doi:10.1016/j.watres.2004.08.004

EXHIBIT 6

Over fifty microcystin variants have been isolated from thirteen cyanobacterial genera, likely contributing to the global occurrence of the toxin (Carmichael, 1997; Chorus, 2001).

Knowledge of microcystin occurrence and understanding factors associated with high concentrations are critical to effective lake management and minimization of health risks associated with the hepatotoxin. Current studies indicate a complex range of physicochemical variables influence microcystin production, and no one variable stands out as an unequivocal link to toxicity. Cyanobacterial blooms are often composed of both toxic and nontoxic strains (Vézie et al., 1998), and laboratory experiments have demonstrated that environmental influences on microcystin production are strain dependent (Sivonen, 1990; Vézie et al., 2002). Environmental variables are therefore likely to influence microcystin concentrations directly, by influencing cellular microcystin production and content (Orr and Jones, 1998; Long et al., 2001), and indirectly, by influencing cyanobacterial species and strain composition (Chorus, 2001; Vézie et al., 2002). Regional studies of microcystin in relation to physicochemical variables provide valuable information on what environmental conditions are most likely to result in high microcystin concentrations (Chorus, 2001); however, relative to the number of regional studies that have been conducted, empirical relationships between microcystin and environmental factors have seldom been developed.

Cyanotoxic incidents have been reported in the Midwestern United States for over a century, but little research has been done in the region (Yoo et al., 1995). Surveys in Wisconsin and Kansas indicate microcystin is common (McDermott et al., 1995; Dodds, 1996), and the toxin has been detected in finished drinking water (Chu and Wedepohl, 1994). Thus, microcystin represents a potential health risk in Midwestern water resources. Lakes in Missouri, Iowa, northeastern Kansas, and southern Minnesota were sampled during summers 2000–2001 to document microcystin occurrence and develop empirical relationships between the physicochemical environment and microcystin concentration.

## 2. Materials and methods

### 2.1. Study area

Lakes ($n=241$) were located within four physiographic provinces: the Ozark Highlands, Osage Plains, Dissected Till Plains, and Western Lake Section (Fenneman, 1938, Fig. 1). Limnological differences among provinces have been well characterized and are associated with geology and land use. Due to rich glacial soils and a landscape dominated by row-crop agriculture the Western Lake Section has nutrient enriched lakes that support high levels of algal biomass. In contrast, the Ozark Highlands, with poor quality soils and little row-crop agriculture, tends to have lakes with low nutrient levels and algal biomass. Lakes in the Osage and Dissected Till Plains are intermediate between lakes

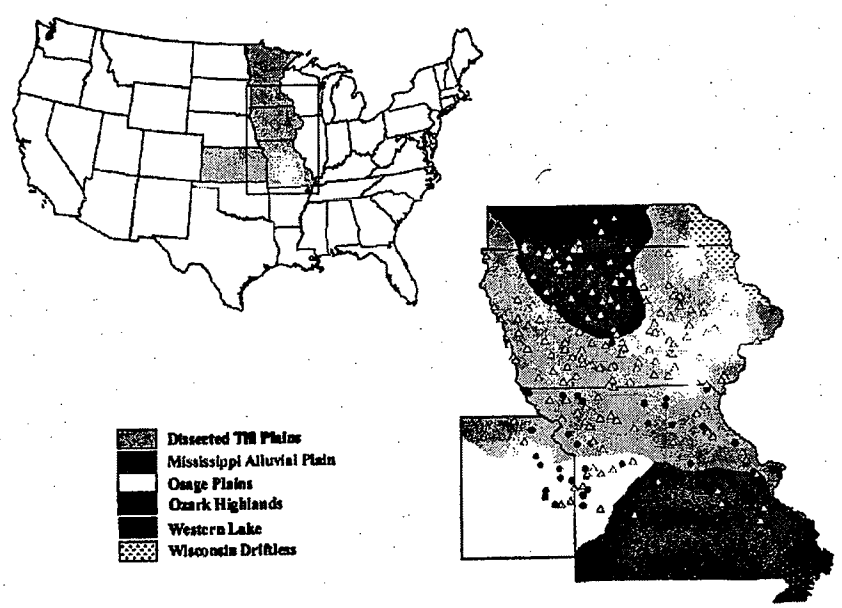


Fig. 1. Physiographic location of lakes sampled and regional trends in microcystin occurrence. Open triangles indicate lakes where microcystin (MC) was detected. Closed circles indicate lakes where MC was not detected.

located in the southern- and northern-most provinces (Jones and Bachmann, 1978; Jones and Knowlton, 1993). Generally, the region represents a south–north gradient in increasing trophic status, with lakes located farther north having many characteristics conducive to cyanobacterial dominance.

### 2.2. Sample collection

A total of 800 lake visits were made during May–September 2000–2001; most lakes were sampled 2–4 times during one or both years. Six University of Missouri (MU) studies and an Iowa State University (ISU) study collected algal samples for particulate microcystin (MC) analysis. All studies measured Secchi transparency and surface temperature (°C) and analyzed composite surface or integrated epilimnetic samples for total phosphorus (TP), total nitrogen (TN), TN:TP ratio, volatile (VSS), nonvolatile (NVSS), and total suspended solids (TSS), chlorophyll (Chl), and Chl:TP ratio. Additionally, ISU (lake $n=132$) determined phytoplankton community structure.

At MU, TP was determined using ascorbic acid (Eaton et al., 1995), and TN by persulfate oxidation (Crumpton et al., 1992). Suspended solids were collected on 1.2 μm Whatman GF/C filters; VSS was calculated by taking the difference between TSS and NVSS (Eaton et al., 1995). Chl was collected on 1.0 μm Pall A/E filters, extracted in heated ethanol and analyzed fluorometrically (Knowlton, 1984; Sartory and Grobbelar, 1986). Detailed ISU methods are given in Downing and Ramstack (2000).

Though produced by cyanobacteria of all size classes, toxic incidents involving MC are most frequently associated with large, surface bloom forming genera (Chorus and Bartram, 1999; Chorus, 2001). Thus, when algal cells > 64 μm were present, 20 L of surface water was concentrated for particulate MC analysis using a 64 μm plankton net (Kotak et al., 2000; Chorus, 2001). Samples were frozen, then lyophilized and stored at −80 °C. ISU sent samples to MU for analysis. The mass of seston > 64 μm (μg/L, dry weight) was measured and MC was extracted from 10 mg sub-samples using deionized water. Envirogard® ELISA kits (detection limit: 100 ng/L) were used to determine MC concentration in seston extracts. MC measured by ELISA includes the variants -LR, -RR, and -YR, as well as nodularian. Particulate MC values were expressed volumetrically by multiplying the seston mass (μg/L d.w.) by the MC content of the seston (μg/g seston d.w., Chorus, 2001); values were re-expressed as ng/L.

### 2.3. Statistical analyses

Relationships between particulate MC values and environmental variables were developed using non-parametric Spearman–Rank correlation ($\alpha=0.05$).

Bivariate plots of the relationships between particulate MC and environmental variables showed non-linear trends that were not linearized through transformation. MC was undetectable across the range of all variables; therefore, the shape of any given relationship was defined by the upper limits created by MC maxima. To characterize the relationships between MC and environmental variables we used interval maxima regression (IMR). Each variable was divided into equal increments, resulting in 6–16 intervals. The maximum MC value and the associated environmental variable value were obtained from each interval and used in nonlinear regression analysis (Blackburn et al., 1992; Scharf et al., 1998).

Nonlinear regression is an iterative fitting analysis in which the form of the relationship between variables must be specified (Snedecor and Cochran, 1989). Inspection of bivariate plots revealed two relationships, which we defined as log-normal 3-parameter

$$y = ae^{[-0.5(\ln(x/x0)/b)\hat{}2]} \quad (1)$$

and inverse first-order polynomial

$$y = y_0 + a/x, \quad (2)$$

where $x$ = the value of the environmental variable and $a$, $b$, $x_0$, and $y_0$ are estimated coefficients. A curve fitter (SigmaPlot® 2001) employing a least-squares method was used to fit equations and estimate coefficients (tolerance = 0.0001, step size = 100, and iterations = 100). This is a parametric analysis and the assumptions of normality and heteroscedasticity were generally met. IMR relationships were considered significant at $\alpha = 0.05$.

## 3. Results

### 3.1. Descriptive limnology

A diverse range of lake conditions was encountered in the study region wherein TP (2–955 μg/L), TN (90–15870 μg/L), and Chl (1–546 μg/L) values spanned 2–3 orders of magnitude (Table 1). Nutrient values were lowest in the Ozark Highlands and increased moving northward through the Osage Plains and into the Dissected Till Plains and Western Lake Section, with median values nearly doubling between each province, respectively (Table 1). The majority of lakes in the Ozark Highlands (73%) were classified as oligo- or mesotrophic, while in all other provinces most lakes (> 85%) were either eu- or hypereutrophic (Nürnberg, 1996).

TN:TP ratios ranged from 1 to 429, and varied widely within provinces (Table 1). Based on TN:TP ratios, the majority of lakes in the study region were potentially P-limited (46%, TN:TP > 17) or co-limited (30%,

**Table 1**
Provincial medians (med) and ranges of limnological variables

| Variable | Ozark Highlands | | | Osage Plains | | | Dissected Till Plains | | | Western Lake Section | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | Med | Range | *n* | Med | Range | *n* | Med | Range | *n* | Med | Range |
| $Z_{mean}$ (m) | 22 | 7.1 | 2.4–20.7 | 25 | 2.9 | 1.9–7.3 | 88 | 3.1 | 0.9–14.9 | 43 | 2.9 | 0.9–11.6 |
| Area (ha) | 22 | 72 | 12–20755 | 18 | 42 | 10–21733 | 132 | 27.9 | 4–7689 | 47 | 153 | 6–2300 |
| TP (μg/L) | 92 | 12 | 2–182 | 111 | 45 | 17–178 | 435 | 79 | 5–721 | 157 | 141 | 12–995 |
| TN (μg/L) | 92 | 405 | 90–1080 | 111 | 730 | 360–2670 | 434 | 1091 | 290–9445 | 158 | 1956 | 236–15870 |
| TN:TP | 92 | 26 | 5–97 | 111 | 16 | 5–35 | 431 | 14 | 3–227 | 157 | 14 | 1–429 |
| Chl (μg/L) | 92 | 5 | 1–105 | 111 | 22 | 3–106 | 431 | 18 | <1–286 | 152 | 37 | 1–546 |
| TSS (mg/L) | 92 | 2.6 | 0.6–17.7 | 111 | 10.0 | 2.5–39.3 | 419 | 8.6 | 0.1–177.9 | 153 | 19.0 | 1.5–295.0 |
| Chl:TP | 92 | 0.4 | 0.1–1.9 | 111 | 0.5 | <0.1–1.1 | 426 | 0.3 | <0.1–2.5 | 159 | 0.3 | <0.1–2.3 |
| NVSS (mg/L) | 92 | 1.1 | 0.1–10.2 | 111 | 5.3 | 0.1–31.7 | 419 | 3.9 | 0.1–151.2 | 153 | 7.9 | 0.1–207.0 |
| VSS (mg/L) | 92 | 1.3 | 0.2–13.3 | 111 | 4.3 | 1.0–16.3 | 419 | 4.0 | 0.1–70.0 | 153 | 9.0 | 0.2–120.0 |
| Secchi (m) | 92 | 2.2 | 0.4–8.6 | 111 | 0.8 | 0.1–3.0 | 437 | 0.8 | 0.1–4.3 | 156 | 0.5 | 0.1–6.3 |
| °C | 92 | 28.0 | 19.9–34.4 | 111 | 27.2 | 8.3–34.3 | 432 | 27.4 | 16.7–32.9 | 153 | 24.6 | 14.1–38.9 |
| CBV ($\mu m^3$/L) | 0 | | | 0 | | | 218 | 5.8e9 | 0–1.0e$^{12}$ | 84 | 7.3e9 | 0–4.7e$^{11}$ |

*n* indicates the number of lake visits in which each variable was measured, with the exception of $Z_{mean}$ and area, where *n* indicates number of lakes. Cyanobacterial biovolume (CBV) was not measured for all lake visits.

17<TN:TP>10) by P and N; only 24% of lakes were potentially N-limited (TN:TP<10, Forsberg and Ryding, 1980). Chl:TP ratios $\ll 1$ indicate algae are not P-limited, while ratios $\cong 1$ are indicative of potential P-limitation (White, 1989). In the study region, Chl:TP ratios ranged from <0.01–2.5 (Table 1); however, only 10% of lakes considered P-limited by the TN:TP ratio had Chl:TP ratios>0.8, suggesting factors other than nutrients may commonly limit algal growth.

Cyanobacterial community structure was assessed for 302 lake visits in the Dissected Till Plains and Western Lake Section. Four genera known to produce MC, *Anabaena*, *Coelosphaerium*, *Microcystis*, and *Oscillatoria* (Yoo et al., 1995), were present in 91% of lake visits. Cyanobacterial biovolume (CBV) was generally dominated (>50% total CBV) by the MC producing genera (81% of lake visits), with *Oscillatoria* dominating most frequently (48%), followed by *Microcystis* (17%), *Coelosphaerium* (11%), and *Anabaena* (5%).

### 3.2. Microcystin occurrence and concentration

Algae>64 μm were collected for particulate MC analysis during 58% of lake visits; of the algal samples collected, 98% had detectable MC. Overall, MC was detected at least once in 78% of lakes sampled. But, in northern provinces there was a greater incidence of MC: 85% of Dissected Till Plain and 100% of Western Lake Section lakes had detectable MC, compared to 26% in the Ozark Highlands and 44% in the Osage Plains (Fig. 1).

**Table 2**
Regional medians and ranges of microcystin values

| Region | Microcystin (ng/L) | | |
|---|---|---|---|
| | *n* | Median | Range |
| Ozark Highlands | 92 | 0[a] | 0–43 |
| Osage Plains | 111 | 0[a] | 0–189 |
| Dissected Till Plains | 439 | 2[b] | 0–2933 |
| Western Lake Section | 158 | 27[c] | 0–4501 |

*n* indicates the number of lake visits in each region. Letters indicate significant differences in median concentrations (Kruskal–Wallis, $p<0.01$).

Particulate MC values ranged from undetectable to 4501 ng/L. Although spanning a wide range, 75% of MC values were <26 ng/L and only 2% of values were>1000 ng/L. Median MC values were significantly greater in northern provinces (Kruskal–Wallis: $H = 237$, $df = 3$, $p<0.01$), increasing from 0 ng/L in the Ozark Highlands and Osage Plains to 27 ng/L in the Western Lake Section (Table 2). Likewise, maximal MC values in the Dissected Till Plains and Western Lake Section were an order of magnitude greater than in the Osage Plains and two orders of magnitude greater than in the Ozark Highlands (Table 2).

### 3.3. Correlation analysis

Particulate MC was significantly correlated with latitude, nutrients, TN:TP ratio, Chl, Chl:TP ratio,

suspended solids, Secchi depth, °C, and $Z_{mean}$ (Table 3). Latitude ($r=0.66$), TN ($r=0.59$), and TP ($r=0.46$) were most highly correlated with MC. In lakes where cyanobacterial biovolume was assessed, MC was positively correlated with CBV ($r=0.32$). Latitude was also significantly correlated with environmental variables (Table 3), suggesting the latitudinal increase in MC values was related to changing environmental conditions.

### 3.4. Interval maxima regression (IMR)

The strong latitudinal gradients in environmental variables and MC presence and concentration, indicate there is a relationship between MC and the physicochemical environment. Despite significant correlations, environmental variables explained <50% of the variation in MC values (Table 3). Correlation analysis measures the closeness of linear relationship between two variables (Snedecor and Cochran, 1989); however, inspection of the maxima in MC–TN and MC–Secchi bivariate plots showed two distinctly non-linear trends.

The MC–TN maxima were characterized by a unimodal curve ($r^2=0.84$, $n=14$, $p<0.01$). Along the TN gradient the greatest MC values (>2000 ng/L) occurred between 1500 and 4000 μg/L; MC values were <150 ng/L above 8000 μg/L TN (Fig. 2a). By comparison, MC–Secchi maxima were characterized by exponential decline ($r^2=0.75$, $n=16$, $p<0.01$)(Fig. 2b). MC values were <150 ng/L above Secchi depths of 2.5 m. The relationships observed along the regional TN and Secchi gradients were reflected in individual lakes. For example, in Beeds Lake, Iowa, TN values ranged from 1800 to 12700 μg/L ($n=8$), but maximal MC values were only observed at TN values ≤4000 μg/L (Fig. 3a). And, in West Okoboji, Iowa, where Secchi values ranged from 0.6 to 6.2m, MC maxima were observed at Secchi depths <2.5 m (Fig. 3b).

IMR analysis was also performed using CBV. CBV–TN ($r^2=0.94$, $n=15$, $p<0.01$), and CBV–Secchi ($r^2=0.76$, $n=11$, $p<0.01$) relationships were strikingly similar to MC relationships. Like MC, the greatest CBV values (>$2^{11}$ μm³/L) occurred within a TN range of 1500–4000 μg/L and at Secchi depths <2.5 m (Fig. 2c and d). The subset of MC values in lakes where CBV was measured show similar upper limit trends as the overall relationship (Fig. 2a and b), but MC and CBV values were not tightly coupled ($r=0.32$).

Other bivariate plots between MC, CBV, and environmental variables had maxima described by either exponential decline or unimodal curves. For example, the TN:TP relationship was characterized by exponential decline. And, the TP relationship could be fitted with a unimodal curve, although the curve was not significant ($p=0.17$). Peak MC and CBV values occurred when TN:TP<50 and within a TP range of 200–600 μg/L (Fig. 4).

While cyanobacterial community composition did not change markedly along the environmental gradients, overall phytoplankton community structure changed substantially. At TN values <8000 μg/L the Cyanophyta dominated (>50% of total phytoplankton biovolume) 71% of lake visits ($n=280$), compared to only 24% at TN values>8000 μg/L ($n=17$). Similar trends were observed along the Secchi and TN:TP gradients, with ~70% of lake visits dominated by Cyanophyta at Secchi <2.5 m ($n=278$) and TN:TP <50 ($n=264$), compared to only ~50% at Secchi>2.5 m ($n=21$) and TN:TP>50 ($n=30$). At TN>8000 μg/L, Secchi>2.5 m, and TN:TP>50 Bacillariophyta and Chlorophyta were the dominant phytoplankton groups. Unlike TN, Secchi, and TN:TP, the Cyanophyta remained dominant along the entire TP gradient.

Table 3
Spearman Rank correlations ($r_s$) between microcystin (MC), latitude (LAT), and environmental variables

| Variable | $n$ | MC | | LAT | |
|---|---|---|---|---|---|
| | | $r_s$ | $p$ | $r_s$ | $p$ |
| Latitude | 800 | 0.66 | <0.01 | — | — |
| TN (μg/L) | 795 | 0.58 | <0.01 | 0.66 | <0.01 |
| TP (μg/L) | 795 | 0.46 | <0.01 | 0.56 | <0.01 |
| VSS (mg/L) | 775 | 0.36 | <0.01 | 0.35 | <0.01 |
| CBV (μm³/L) | 302 | 0.32 | <0.01 | 0.14 | 0.01 |
| Chl (μg/L) | 786 | 0.30 | <0.01 | 0.21 | <0.01 |
| $Z_{mean}$ (m) | 621 | −0.30 | <0.01 | −0.27 | <0.01 |
| TSS (mg/L) | 775 | 0.29 | <0.01 | 0.31 | <0.01 |
| Secchi (m) | 796 | −0.27 | <0.01 | −0.26 | <0.01 |
| NVSS (mg/L) | 775 | 0.17 | <0.01 | 0.23 | <0.01 |
| TN:TP | 791 | −0.15 | <0.01 | −0.20 | <0.01 |
| °C | 788 | −0.10 | <0.01 | −0.19 | <0.01 |
| Chl:TP | 781 | −0.07 | 0.03 | −0.26 | <0.01 |
| Area (Ha) | 716 | 0.02 | 0.52 | 0.07 | 0.06 |

## 4. Discussion

The study region represents a latitudinal gradient in trophic status (Table 1), thereby providing a unique context in which to examine MC concentration with respect to the physicochemical environment. MC presence, and median and maximum values, increased along the trophic gradient (Table 2, Fig. 1), indicating environmental factors play a role in determining MC occurrence and maxima. Nutrient values were greater, and Secchi depths shallower in the Dissected Till Plains and Western Lake Section, where MC was detected most frequently (Table 1, Fig. 1), conditions known to favor cyanobacteria (Reynolds, 1998). MC is

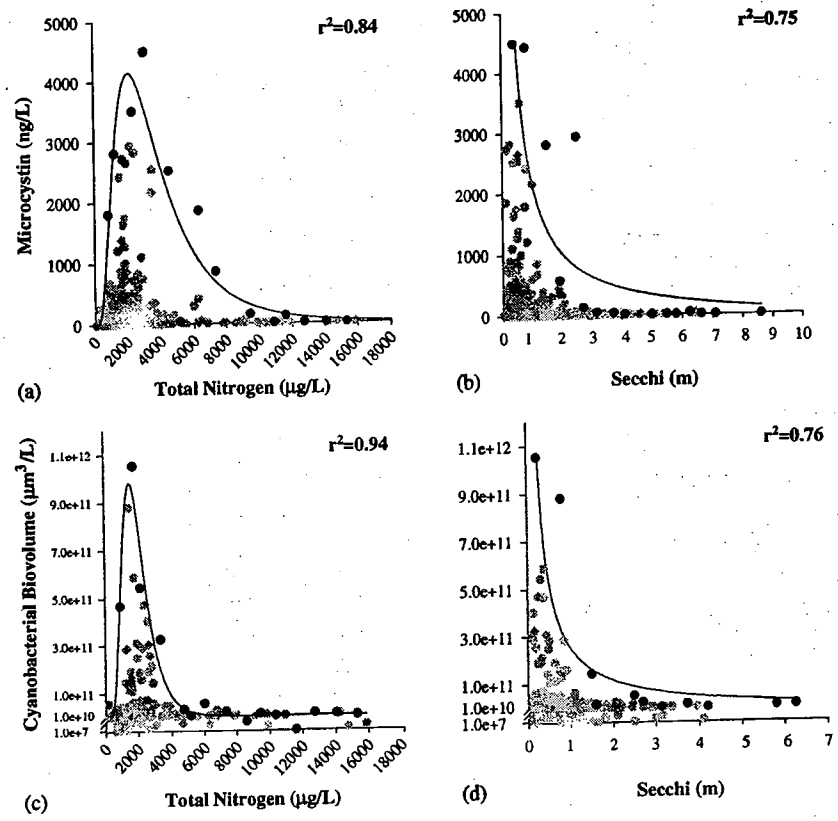


Fig. 2. Microcystin (MC), cyanobacterial biovolume (CBV), total nitrogen (TN), and Secchi bivariate relationships. Curves were estimated using interval maxima regression (IMR). Black points indicate the data used for IMR analysis ($n=11$–16); $r^2$ values are for this fitted line only (all $p<0.01$). (a) The MC–TN relationship ($n=795$), (b) The MC–Secchi relationship ($n=796$), (c) The CBV–TN relationship ($n=299$), (d) The total cyanobacterial biovolume–Secchi relationship ($n=301$).

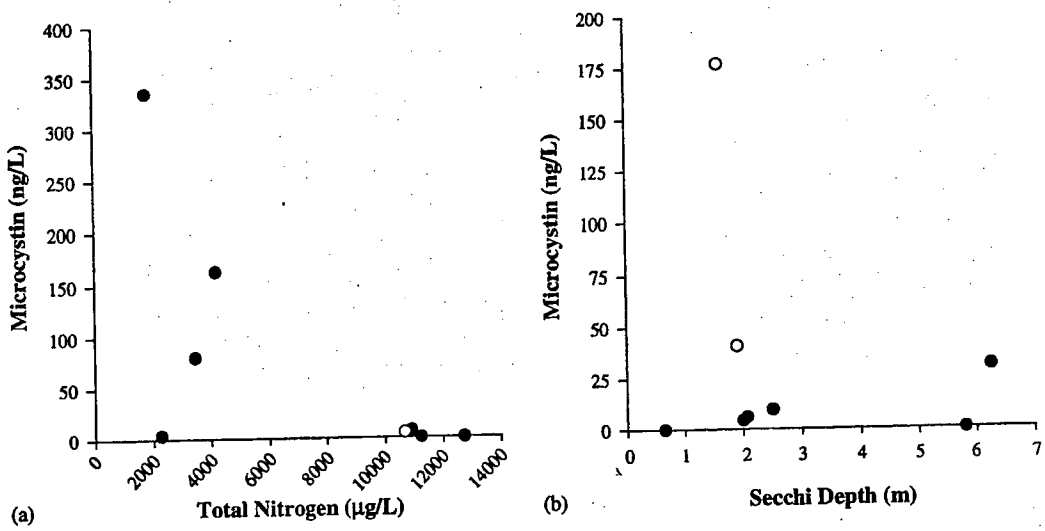


Fig. 3. The microcystin–total nitrogen (a) and microcystin–Secchi (b) relationships in individual lakes. (a) Beeds Lake, Iowa, was sampled 7 times during May–September, 2000 (closed circles), and once in August 2001 (open circle), (b) West Okoboji, Iowa, was sampled 6 times during May–September 2000 (closed circles) and twice (July and September) in 2001 (open circles).

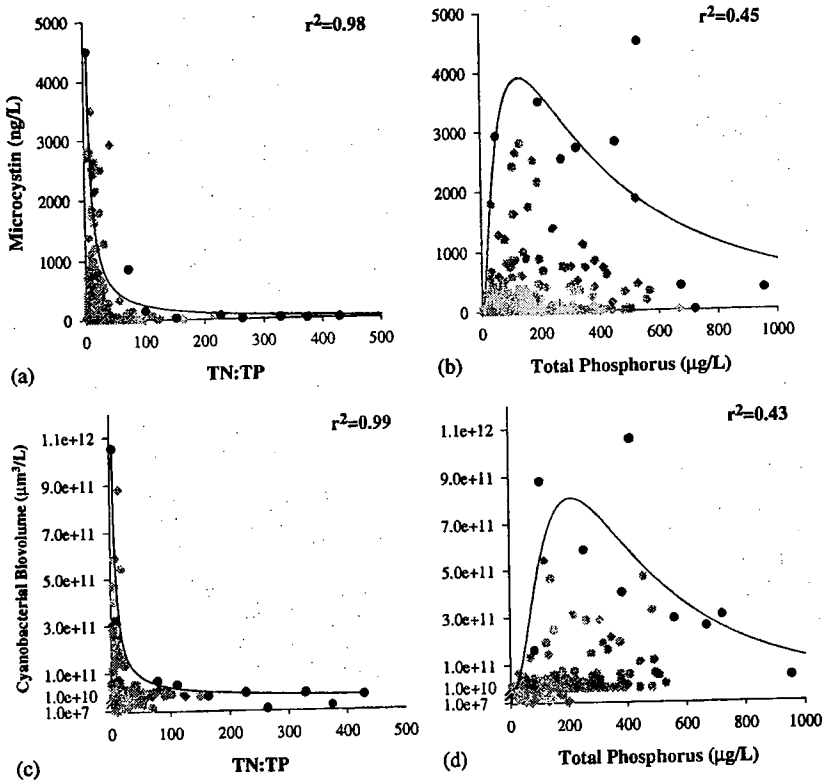


Fig. 4. Microcystin (MC), cyanobacterial biovolume (CBV), and TN:TP and total phosphorus (TP) bivariate relationships. Curves were estimated using interval maxima regression (IMR). Black points indicate the data used for IMR analysis ($n=6$–9); $r^2$ values are for this fitted line only. (a) The MC–TN:TP relationship ($n=791$, IMR $p<0.01$), (b) The MC–TP relationship ($n=795$; IMR $p=0.17$), (c) The CBV–TN:TP relationship ($n=296$; IMR $p<0.01$), (d) The CBV–TP relationship ($n=299$; IMR $p=0.18$).

inextricably linked to cyanobacteria, and factors favoring cyanobacterial dominance are also expected to influence MC presence and concentration. Where data were available, particulate MC was significantly correlated with CBV (Table 3). Similarly, regional studies in Canada and Germany found significant correlations between particulate MC and CBV (Kotak et al., 2000; Chorus, 2001). Relationships between MC and environmental factors, however, are not consistent. For example, in the current study MC was strongly correlated with TN (Table 3), but in Canada and Germany the MC–TN relationship was not significant.

A diverse range of physical, chemical, and biological factors may potentially limit cyanobacterial growth (Reynolds, 1998). In the study region TN:TP and Chl:TP ratios suggest potential TN-, TP-, and nutrient co-limitation as well as limitation by other factors, such as light (Table 1). Field and laboratory studies have demonstrated that the relationship between cyanobacteria, MC concentration, and environmental factors is invariably complex. Cellular MC content is strain dependant, varying by several orders of magnitude between strains (Chorus, 2001). Individual strains also have different environmental optima for growth and MC production, and respond differently to changing environmental conditions (Sivonen, 1990; Vézie et al., 2002). Additionally, many strains may occur simultaneously in an individual lake (Vézie et al., 1998). Observed MC values are therefore the result of interactions between environmental influence on MC production and dominance of individual cyanobacterial strains (Chorus, 2001). Thus, the lack of consistent empirical relationships between MC and environmental variables is not surprising.

Because MC is toxic, maxima are of particular interest. Given the complexity of factors determining observed MC values, focusing exclusively on attributes of mean response along an environmental gradient excludes useful information contained in the maxima (Scharf et al., 1998). Definition of the upper limit of MC values along an environmental gradient represents the potential maximum given all conditions for MC production and MC producing cyanobacteria are optimal. MC values will often fall below the potential maxima because other undetermined abiotic and biotic factors are sub-optimal (Kaiser et al., 1994).

Relationships between particulate MC maxima, CBV maxima, and environmental variables were nearly

identical. Along the TN and TP gradients maxima were characterized by unimodal curves, while maxima along the Secchi and TN:TP gradients were characterized by exponential decline (Figs. 2 and 4). CBV response along these gradients generally take the form expected based on current knowledge of cyanobacterial requirements, limits, and competitive abilities and therefore provide insight into the factors influencing MC values (Chorus, 2001). For example, exponential decline in MC values along the Secchi gradient reflects cyanobacterial adaptation to low light conditions (Chorus, 2001) and superior competitive ability at low nitrogen concentrations along the TN:TP gradient (Blomqvist et al., 1994).

Maximal particulate MC values increased along the TN gradient to a peak between 1500 and 4000 μg/L TN, with low values (< 150 ng/L) above 8000 μg/L (Fig. 2a). Along the TP gradient maximal MC values occurred between 100 and 600 μg/L (Fig. 3b). Lower MC maxima at TN <1500 μg/L and TP <600 μg/L may be due to nutrient limitation of phytoplankton biomass (Chorus, 2001); lower maxima at TN>4000 μg/L and TP>100 μg/L imply either excess nutrients limit MC producers or biological factors influence the shape of the curve. There is no indication nutrient enrichment negatively affects cyanobacterial growth or MC production (Vézie et al., 2002). Although it is commonly accepted that cyanobacteria are abundant in hypereutrophic lakes, cyanobacteria are poor competitors for both nitrogen and phosphorus in nutrient replete systems (Blomqvist et al., 1994; Jensen et al., 1994), even under low light conditions (Huisman et al., 1999). Cyanophytes do not necessarily dominate in hypereutrophic systems, as suggested by increased dominance by Bacillariophytes and Chlorophytes at TN>8000 μg/L; thus, biotic factors such as competition may cause the decline in maximal MC values noted at higher nutrient levels.

IMR is limited by the range of MC values and environmental conditions encountered (Blackburn et al., 1992; Scharf et al., 1998). Although TN, TP, Secchi, and TN:TP values spanned a wide range, data were not uniformly distributed across the range (Figs. 2 and 4). When intervals were created for IMR, the uneven distribution of data resulted in the majority of points falling within 2–3 intervals. For example, TN values ranged between 90 and 15870 μg/L, but 75% of values were <1800 μg/L; 16 TN intervals were created and the number of observations per interval ranged between 0 and 394, with a median of 3.5. Similar trends were noted in TP, Secchi, and TN:TP data and altering interval size did not rectify the uneven spread of the data. The greatest MC values observed within each interval may not have represented true maxima, but in intervals with fewer observations there is a greater chance true MC maxima were not observed (Blackburn et al., 1992; Scharf et al., 1998); thus, the overall shape of the MC-environmental variable relationships may be a result of the low number of observations at the extreme ends of the curves (Figs. 2 and 4). MC upper limits may actually be characterized by other linear or curvilinear relationships. The relationships were, however, consistent when interval maxima regression analysis was conducted using lake means or sub-maximal MC values (i.e., 2nd or 3rd ranking values). Observed regional relationships were also reflected in individual lakes (Fig. 3).

Unlike the TN, Secchi, and TN:TP relationships, the unimodal curve fitted to the TP interval maxima was not significant (Fig. 4b) and cyanobacteria dominated the phytoplankton along the entire TP gradient. In Danish lakes, Cyanophytes have been observed to dominate the phytoplankton at TP values <800 μg/L, while Chlorophytes tended to dominate at TP values>1000 μg/L (Jensen et al., 1994). Although TP values in the study region ranged from 2–995 μg/L, 75% of values were <142 μg/L, and only ten values were>500 μg/L. Therefore, the TP range encountered may not have been large enough to fully characterize the MC–TP relationship.

Despite the limitations imposed by the environmental conditions encountered, the overall trends defined by the IMR relationships are in accord with what is known about cyanobacterial ecology. Furthermore, these empirical relationships are strikingly similar to those noted elsewhere. The range of TN and TP values at which MC maxima were observed is similar to the range noted in Germany (TN: 1500–3000 μg/L, TP: 100–200 μg/L), where MC maxima are several orders of magnitude greater (Chorus, 2001). The German study also measured the $Z_{eu}$:$Z_{mix}$ ratio, an indicator of light availability. Like the Secchi–MC relationship, the outer edge of the MC–$Z_{eu}$:$Z_{mix}$ relationship was characterized by exponential decline (Chorus, 2001). Additionally, Canadian MC–TN:TP, MC–nitrate, and MC–ammonia relationships suggest edges characterized by exponential decline (Kotak et al., 2000).

As noted in many studies (Chorus and Bartram, 1999), MC was common in the study region, but generally detected in low concentrations with only a few high values (Table 2). Little published data from the U.S. is available for comparison; however, observed MC maxima were two orders of magnitude greater than maxima reported from Kansas (22 ng/L, Dodds, 1996) and within the range of maxima reported from Washington (total MC—3800 ng/L, Johnston and Jacoby, 2003) and Alberta, Canada (6200 ng/L, Kotak et al., 2000). Observed maxima were substantially lower than maxima reported from shoreline surface scums in Wisconsin (total MC—200 μg/L, McDermott et al., 1995) and Washington (total MC—43 μg/L, Johnston and Jacoby, 2003), but most lakes in our study were sampled at pelagic locations, thus avoiding extensive surface scums.

All of the lakes sampled are used for recreational purposes, and 45 of the lakes are also used as drinking water supplies. MC was detected in 98% of the algal samples collected; thus, there is a potential MC risk anytime algae > 64 μm are present. Particulate MC values in the study region never exceeded the low risk range (1–10 μg/L) for recreational exposure (Chorus and Bartram, 1999). Similarly, although several drinking water supplies had MC values in raw water near the WHO recommended 1 μg/L limit (Chorus and Bartram, 1999), MC was never detected in finished drinking water (data not presented). Therefore, in the study region, risk of acute MC toxicity appears relatively low and chronic exposure is a greater concern. Effects of chronic MC exposure are currently unknown, but MC is considered to be a tumor promoter (Chorus and Bartram, 1999).

The current study focused on large algae present at the surface; inclusion of subsurface, benthic, and pico-cyanobacteria in future studies would more accurately assess MC presence and concentration. Additionally, MC content of cyanobacteria strains has not been studied in the Midwest, and knowledge of the common MC producers in the region would be of great value in assessing potential maximum MC values. Although maximal values were below levels likely to cause acute toxicity, MC poses a potential chronic health risk in the Midwest. Efforts, such as monitoring programs for recreational lakes and adoption of drinking water guidelines, need to be taken to ensure MC exposure is minimized.

Knowledge of the environmental factors associated with high MC values is critical to effective lake management and minimization of human health risks. Our study demonstrates that MC concentrations are linked to the physicochemical environment; however, the relationships are not traditional linear models. Further empirical work must be conducted to validate the non-linear nature of the relationships between environmental factors and MC maxima. Studies addressing the interaction of biotic and abiotic components will elucidate conditions under which the greatest MC values are produced. Natural systems are characterized by a vast array of biotic and abiotic gradients coupled with multiple species interactions (Reynolds, 1998). As a product of these diverse systems, MC concentration along environmental gradients is similarly complex. Understanding that MC values are not linearly related to a single habitat component, but are rather the complex result of the interaction of many different factors, provides a novel approach to addressing environmental influences on MC concentrations.

## 5. Conclusions

1. Microcystin is common throughout the study region, but both presence and concentration increase moving northward along a gradient of increasing lake trophic status.
2. Microcystin values were typically low, with only 2% of values > 1000 ng/L; but, because microcystin is toxic, maxima are of particular interest.
3. The relationships between microcystin concentration and environmental factors are complex, and to date have largely been explored using traditional linear analyses. The development of nonlinear relationships provides a novel approach to defining the conditions under which high microcystin concentrations are most likely to occur.

## Acknowledgements

Funding for this project was provided by the US EPA opposite ground water and drinking water. This study has not been subjected to EPA administrative review and may not reflect the view of the agency and no official endorsement should be inferred. Funding and support was also provided by the Departments of Fisheries and Wildlife Sciences and Civil and Environmental Engineering at MU, the Department of Ecology, Evolution, and Organismal Biology at ISU, and the USGS Columbia Environmental Research Center. We thank Mark Kaiser for discussing his ideas on nonlinear relationships. We would also like to thank Sarah Panken, Christopher Radcliffe, and Travis Hill for field and laboratory assistance.

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