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Land use patterns, ecoregion, and microcystin relationships in U.S. lakes and reservoirs: A preliminary evaluation

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ABSTRACT

A statistically significant association was found between the concentration of total microcystin, a common class of cyanotoxins, in surface waters of lakes and reservoirs in the continental U.S. with watershed land use using data from 1156 water bodies sampled between May and October 2007 as part of the USEPA National Lakes Assessment. Nearly two thirds (65.8%) of the samples with microcystin concentrations $\geq$1.0 μg/L (n = 126) were limited to three nutrient and water quality-based ecoregions (Corn Belt and Northern Great Plains, Mostly Glaciated Dairy Region, South Central Cultivated Great Plains) in watersheds with strong agricultural influence. canonical correlation analysis (CCA) indicated that both microcystin concentrations and cyanobacteria abundance were positively correlated with total nitrogen, dissolved organic carbon, and temperature; correlations with total phosphorus and water clarity were not as strong. This study supports a number of regional lake studies that suggest that land use practices are related to cyanobacteria abundance, and extends the potential impacts of agricultural land use in watersheds to include the production of cyanotoxins in lakes.

1. Introduction

Excessive inputs of both nitrogen and phosphorus to aquatic ecosystems are a global concern (Conley et al., 2009; Paerl, 2009; Abell et al., 2010). The potential for increased eutrophication of lakes and reservoirs (hereinafter, lakes) to result in higher incidences of harmful cyanobacteria blooms is recognized (Heisler et al., 2008; Paerl and Huisman, 2008; O'Neil et al., 2012). Land use changes, particularly increased nutrient loading associated with agricultural and urban development, are likely major drivers of degraded water quality in lakes and often result in modified and/or simplified aquatic food webs (Carpenter et al., 2011; Martinuzzi et al., 2013). Temperature is also recognized as a primary structuring factor for the compositional, seasonal, and physiological status of cyanobacteria communities in lakes. Warmer conditions typical of summer favor cyanobacteria in planktonic environments because they can maximize growth rates and exploit buoyancy regulation in a stratified water column (Paerl and Paul, 2012; Carey et al., 2012). In addition to increased nitrogen and phosphorus inputs, the concentrations of dissolved organic carbon (DOC) in lakes have been increasing worldwide (Evans et al., 2005) and may reflect the increased importance of autochthonous carbon in higher productivity plankton environments. The expected impacts of climate change on lakes (e.g., drought and extended stratification) would intensify these competitive adaptations and potentially disproportionately favor toxigenic cyanobacteria (Paerl and Otten, 2013).

Eutrophication of lakes and the associated compositional shifts in phytoplankton communities to increased dominance by cyanobacteria is well documented, but the relationship between watershed land use and microcystin concentrations in lakes remains poorly described. Microcystins are cyclic nonribosomal peptides produced by cyanobacteria which exhibit potent hepatotoxic properties. We used a database consisting of phytoplankton and microcystin analyses from samples collected during the growing season from 1156 lakes located within the 48 contiguous U.S. during the 2007 National Lakes Assessment (USEPA, 2009) to determine whether total microcystin concentrations, a common class of cyanotoxins, and the total abundance of cyanobacteria displayed any relationship to ecoregion, individual watershed land

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use, nutrient concentrations, water temperature, water clarity and DOC. To date, no systematic attempt has been made to relate watershed land use characteristics to microcystin concentrations over such a large geographical area. A recent analysis of this same database indicated that total nitrogen and temperature explained more variance in estimated cyanobacteria biomass than other water quality variables (Beaulieu et al., 2013). Given that a number of regional studies suggest that water column nutrient concentrations and/or phytoplankton community composition are influenced by watershed land use practices (Karatayev et al., 2005; Bremigan et al., 2008; Vanni et al., 2010; Beaver et al., 2012; Katsiapi et al., 2012; Paul et al., 2012; Beaver et al., 2013), we hypothesized that patterns in watershed land use should likewise be related to microcystin concentrations.

2. Methods

2.1. National Lakes Assessment study sites

1156 lakes selected from the USGS/EPA National Hydrography Dataset (NHDPlus) (see Simley and Carswell, 2009) were comprehensively sampled throughout the continental United States in 2007 (USEPA, 2009). Each lake was sampled for water quality, biological condition, habitat conditions, and recreational suitability. Lakes were selected without bias using probability-based selections and constituted a statistically valid representation of lakes in similar regions (USEPA, 2009). Both man-made and natural lakes greater than 4 hectares in size (excluding the Great Lakes) were included as well as some lakes sampled during the National Eutrophication Survey conducted in the 1970s (USEPA, 1975). Our study constitutes the first nationwide assessment of microcystin concentrations in U.S. lakes.

2.2. Sample collection

The study lakes were sampled between May and October 2007 using the protocol developed by the USEPA (USEPA, 2007). Most lakes were sampled near the deepest point which usually was the mid lake area. Samples for total nitrogen (TN) and total phosphorus (TP) analyses were collected from an integrated photic zone sample down to 2 times the Secchi depth or 2 meters, whichever was shallower and were analyzed in accordance with USEPA (2007). Although the vertical temperature profile was usually collected from the deepest part of each lake, for the purposes of this study the mean water column temperature of the top 5 m was used. Approximately ten percent of the lakes were sampled twice during the study period and mean values were used for analyses.

Phytoplankton and microcystin samples were collected from integrated water samples from the euphotic zone of each lake. Phytoplankton samples were preserved with Lugol's iodine and shipped to EcoAnalysts (Moscow, Idaho) for microscopic examination. Microcystin samples were shipped on ice to the USGS Organic Geochemistry Research Laboratory in Lawrence, Kansas.

2.3. Laboratory analyses

A single laboratory determined TP (detection limit 4.0 μg/L), TN (detection limit 20 μg/L), and DOC (detection limit 0.2 mg/L) concentrations (USEPA, 2009). Phytoplankton samples were analyzed with compound microscopes using pre-concentrated samples prepared in Utermöhl sedimentation chambers that had been allowed to sit for a minimum of eight hours. Taxonomists were responsible for the lowest possible taxonomic identification and enumeration of 300 natural algal units and included cell tallies. Although cell densities were determined for all phytoplankton identified, for the purposes of this study only total cyanobacteria abundance was considered. Cyanobacteria cell densities were reduced to taxonomic order (Chroococcales, Nostocales, and Oscillatoriales – see Beaulieu et al., 2013) and summed within each sample prior to statistical analyses. Total microcystin concentrations were determined using the Abraxis Microcystins/Nodularins enzyme-linked immunosorbent assay (detection limit 0.1 μg/L; -ADDA specific) after three freeze-thaw cycles to lyse cyanobacteria cells (USEPA, 2007; Graham et al., 2010).

2.4. Percent land use determination

We assigned watershed land use to 10 categories (Developed, Barren Land, Deciduous Forest, Evergreen Forest, Mixed Forest, Shrub/Scrub, Grassland/Herbaceous, Pasture/Hay, Cultivated Crops, Wetlands). Watersheds were delineated and were grouped into two categories, on-network and isolated lakes. On-network lakes were lake/pond features that are located on the NHDPlus network flowlines (i.e., NHDPlus stream network); whereas isolated lakes were lake/pond features that were lake points in NHDPlus, but not located on the network flowlines (see Simley and Carswell, 2009). For all on-network lake basins, delineations began at the lake outlet as represented by the NHDPlus stream network. The reachcode and measure of the stream network line that represented each lake's outlet was determined and used by the NHDPlus Basin Delineation Tool to derive the drainage basin shapefile. For all isolated lakes, delineations were made using the Watershed Tool, located within Spatial Analyst package of ArcGIS (ESRI, 2011). The input to the Watershed Tool was a rasterized NHDPlus lake polygon and the NHDPlus flow direction grid, and resulted in a basin that was converted into a polygon shapefile. Once the shapefiles were created from the two delineation processes, the NLCS 2001 v1 dataset was applied to each basin shapefile to come up with land use percentages.

2.5. Statistical analyses

A canonical correlation analysis (CCA) was performed using the Canonical Analysis of Principal Coordinates (CAP) function of the PERMANOVA+ add-on in PRIMER 6 (Clarke & Gorley, 2006) between untransformed percentage land use and log-transformed water quality variables. A data matrix was prepared detailing the percentage of each type of land use in the watersheds of the 1156 lakes. A Bray–Curtis resemblance matrix was computed from the percentage land use data matrix prior to analyses. A data matrix was also prepared using five environmental variables including TN (μg/L), TP (μg/L), DOC (mg/L), water temperature (°C), and Secchi depth (m). For CAP analysis, the percentage land use resemblance matrix was analyzed against the sample-matched and log-transformed environmental matrix. Subsequent to the CCA analysis, concentrations of microcystin and cyanobacteria abundance were superimposed over the CCA plot. Additional details on the statistical methodology are provided in Beaver et al. (2012, 2013). Mann–Whitney tests were performed in order to determine significant differences between water quality variables using GraphPad Prism 6.04 software (GraphPad, 2014).

3. Results and discussion

126 samples from the 1156 lakes used in this study (10.9%) contained $\geq$1.0 μg/L of microcystin (Fig. 1). A comparison of mean land use in the high ($\geq$1.0 μg/L) and the low (<1.0 μg/L) microcystin lakes indicated that the mean percentage land use of $\geq$1.0 μg/L and <1.0 μg/L microcystin lakes differed primarily on the proportion of land devoted to agricultural (grassland/herbaceous, pasture/hay, cultivated crops) and forested land uses (Table 1). The mean percentage land use for $\geq$1.0 μg/L microcystin

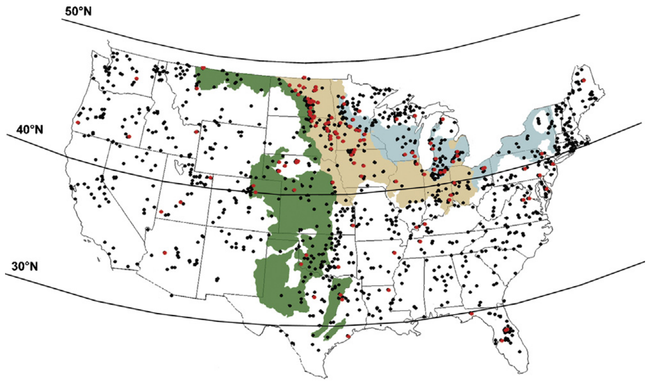

Fig. 1. Locations of the study lakes/reservoirs in the continental United States. Red points indicate sites with microcystin concentrations $\geq$1.0 μg/L, black points indicate lakes with concentrations <1.0 μg/L. Green, yellow, and blue colorations represent the South Central Cultivated Great Plains, Corn Belt and Northern Great Plains, and the Mostly Glaciated Dairy Region, respectively (n = 1156).

lakes devoted to agriculture was 59.4% compared to 32.8% for <1.0 μg/L microcystin lakes (Fig. 2). Comparable values for forested land use in $\geq$1.0 μg/L and <1.0 μg/L microcystin lakes, respectively, were 16.6 and 39.4%.

Of the 126 lakes with $\geq$1.0 μg/L concentration of microcystin, 83 (65.8%) were located within three nutrient and water quality-based ecoregions originally developed to address cultural eutrophication (USEPA, 2001): 36 in the Corn Belt and Northern Great Plains ecoregion (28.6%), 24 in the Mostly Glaciated Dairy Region ecoregion (19.1%), and 23 in the South Central Cultivated Great Plains ecoregion (18.3%). Prominent land uses in these ecoregions include highly productive cropland (Corn Belt and Northern Great Plains ecoregion), dairy and livestock (Mostly Glaciated Dairy Region ecoregion), and cropland and the major winter wheat growing area of the U.S (South Central Cultivated Great Plains ecoregion). All of the samples in these three nutrient-based ecoregions were located in the North-central and Midwestern U.S. (mean latitude 44.2° N). The remaining 43 $\geq$ 1.0 μg/L microcystin lakes were scattered throughout the continental U.S. and were situated in watersheds with less cultivated crops and more forested land use than lakes located in the three major ecoregions. There were no apparent trends in regard to microcystin concentrations when the database was divided into natural versus manmade water bodies. Nutrients and DOC in $\geq$1.0 μg/L microcystin lakes were ~3-fold greater than in <1.0 μg/L microcystin lakes (Table 2). Lakes in the $\geq$1.0 μg/L subset which were located in the 3 major ecoregions displayed higher mean nutrient and DOC concentrations, but greater Secchi disk transparency and total depth when contrasted with lakes in the $\geq$1.0 μg/L subset situated in other ecoregions.

The CCA comparing 10 land use types and 5 water quality variables produced two pairs of canonical axes with moderately significant correlations (Table 3). These two canonical axes explained 40% (first axis) and 21% (second axis) of the variance among correlations between percentage land use and water quality variables. Total nitrogen concentrations and DOC displayed strong positive correlations with the first canonical axis (0.71 and 0.55, respectively), while water temperature showed a strong negative correlation with the second canonical axis (−0.85). Total phosphorus concentrations and Secchi disk transparency were weakly correlated with both canonical axes. Cyanobacteria abundance was correlated with TN, DOC and water temperature (Fig. 3). Robust densities of cyanobacteria were observed between TN and water temperature. Similarly, microcystin concentrations were correlated with TN and DOC, with the highest concentrations strongly related to TN (Fig. 4). A comparison between the two human-controlled land use types (agriculture and developed land) indicated that higher microcystin concentrations were more often associated with lakes situated in watersheds dominated by agriculture with limited developed land when contrasted with microcystin concentrations in watersheds where developed land was an important land use (Fig. 5).

Nutrient concentrations in lakes are strongly correlated to watershed land use patterns in the Midwestern U.S. and other regions (Jones et al., 2004, 2008; Fraterrigo & Downing, 2008; Carney, 2009; Vanni et al., 2010; Nielsen et al., 2012; Carter & Dzialowski, 2012; Cross & Jacobson, 2013; Filbrun et al., 2013). Landscape-lake interactions resulting from watershed land use patterns can be important structuring factors for freshwater phytoplankton communities (Karatayev et al., 2005; Bremigan et al., 2008; Vanni et al., 2010; Stomp et al., 2011; Beaver et al., 2012; Katsiapi et al., 2012; Paul et al., 2012; Beaver et al., 2013) and higher trophic levels (Beaver et al., 2014). Phytoplankton characteristics have been linked to watershed land use patterns, especially the extent of agricultural development, in other regional studies. Katsiapi et al. (2012) reported that agricultural land use was associated with significantly higher phytoplankton biomass. Paul et al. (2012) found that phytoplankton composition in the Rotorua lakes' district, New Zealand was correlated with watershed land use; lakes associated with pasture land use displayed more cyanobacteria than lakes with more forested land use. Ohio reservoir phytoplankton populations were frequently nitrogen-limited in forested watersheds but less nitrogen-limited in agricultural reservoirs, primarily due to underlying land use patterns (Vanni et al., 2010; Beaver et al., 2012). Filbrun et al. (2013) described the long-term eutrophication of Grand Lake St. Marys, Ohio associated with continuing nutrient inputs from a

Table 1
Percent land use comparison of lakes from the USEPA (2007) National Lakes Assessment based on low (<1.0 μg/L) and high ($\geq$1.0 μg/L) microcystin concentrations.

	All samples (n = 1156)		$\geq$1.0 ppb subset (n = 126)	
Land use type	Low microcystin	High microcystin	High microcystin – 3 major ecoregions	High microcystin – other ecoregions
n	*1030*	*126*	*83*	*43*
Developed	8.8	9.2	10.4	7.1
Barren land (Rock/sand/clay)	1.2	0.1	0.0	0.1
Deciduous forest	19.6	11.3	8.1	17.3
Evergreen forest	15.8	3.5	0.3	9.7
Mixed forest	4.0	1.8	0.2	4.8
Shrub/scrub	9.9	4.2	1.3	9.8
Grassland/herbaceous[a]	13.4	19.6	19.5	19.7
Pasture/hay[a]	7.7	10.9	10.7	11.2
Cultivated crops[a]	11.7	28.9	39.1	9.3
Wetlands	5.4	6.3	5.1	8.7

[a] Agricultural land use types.

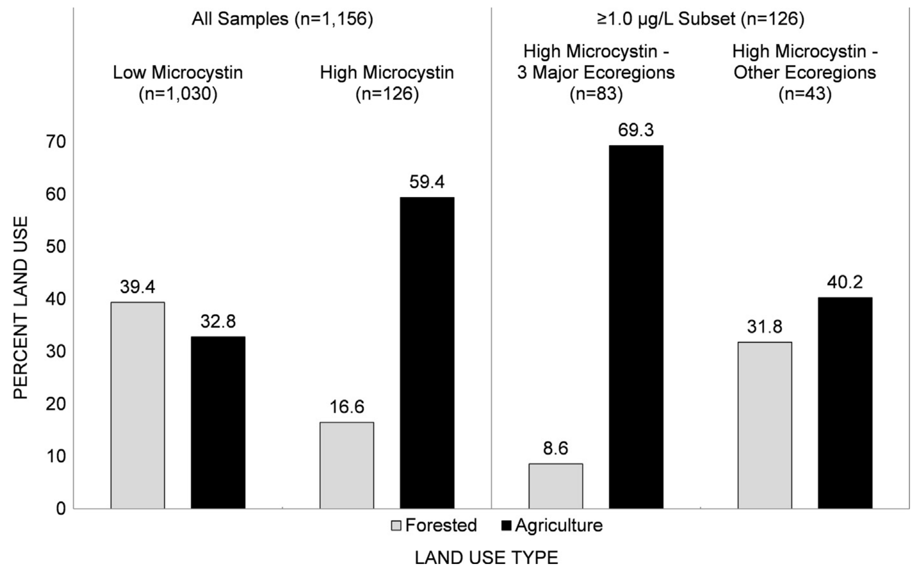

Fig. 2. Mean percent forested and agricultural land use comparison of lakes and reservoirs from the USEPA (2007) National Lakes Assessment based on <1.0 μg/L and ≥1.0 μg/L microcystin concentrations.

Table 2
Mean values for TN, TP, DOC, temperature, Secchi depth and total depth for the USEPA 2007 National Lakes Assessment lakes based on low (<1.0 μg/L) and high (≥1.0 μg/L) microcystin concentrations.

	All samples (n = 1156)		≥1.0 μg/L subset (n = 126)	
Variable	Low microcystin	High microcystin	High microcystin – 3 major ecoregions	High microcystin – other ecoregions
n	*1030*	*126*	*83*	*43*
Microcystin (μg/L)	0.1*	6.6*	7.7	4.4
Total nitrogen (μg/L)	916*	3110*	3281*	2779*
Total phosphorus (μg/L)	89*	256*	291	190
Dissolved organic carbon (mg/L)	7.8*	17.7*	18.7*	15.8*
Temperature (°C)	23.3	23.3	22.8*	24.3*
Secchi depth (m)	2.3	2.0	2.3	1.6
Depth (m)	10.2*	4.6*	5.1	3.6

* Significant differences between low and high microcystin samples (n = 1156) and high microcystin samples from major and other ecoregions (n = 126) (Mann–Whitney test).

watershed dominated by agriculture and eventual sustained high concentrations of microcystin.

Our results are in agreement with other regional surveys of microcystin concentrations in the Midwestern U.S. (Graham et al., 2004, 2010). These studies determined that lakes located primarily in the Upper Corn Belt and Northern Great Plains ecoregion exhibited higher microcystin concentrations (May–September) in watersheds with greater agricultural influence when contrasted with water bodies with less agricultural activity. As in the present study, TN was strongly correlated with microcystin concentrations. Soils in these ecoregions are of recent glacial origin, naturally fertile, and subject to erosion by wind and water; however they are also well suited to agricultural activities and are predisposed to contribute large quantities of nitrogen in runoff to receiving water bodies such as streams and lakes (Karlen et al., 2010; Preston et al., 2011). Another analysis of this database found that both TN and

Table 3
Correlations of water quality variables with their first and second canonical axes in relation to land use (n = 1156).

Variable	CAP1	CAP2
Temperature (°C)	0.430	−0.853
DOC (mg/L)	0.545	0.325
TN (μg/L)	0.713	0.229
TP (μg/L)	0.081	0.336
Secchi depth (m)	−0.054	0.020

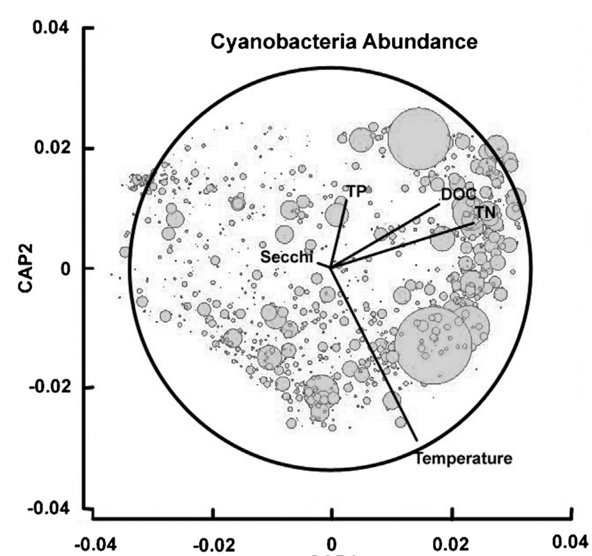

Fig. 3. Water quality CCA bubble diagram for cyanobacteria abundance. Each bubble represents total cyanobacteria abundance (cells/L) in one sample. Bubble size represents the relative magnitude of cyanobacteria abundance in a sample (n = 1156).

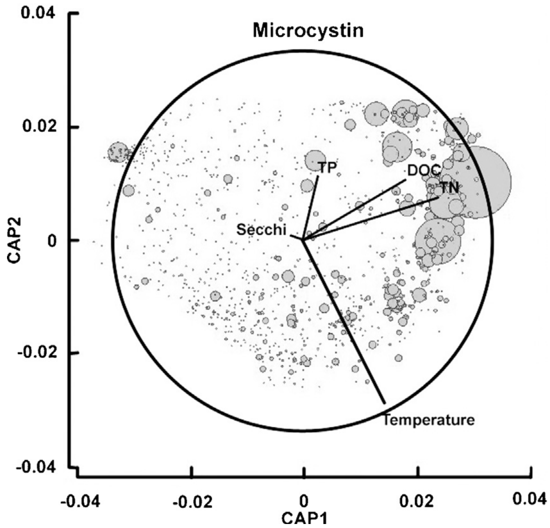

Fig. 4. Water quality CCA bubble diagram for microcystin concentration. Each bubble represents microcystin concentration (μg/L) in one sample. Bubble size represents the relative magnitude of microcystin in a sample (n = 1156).

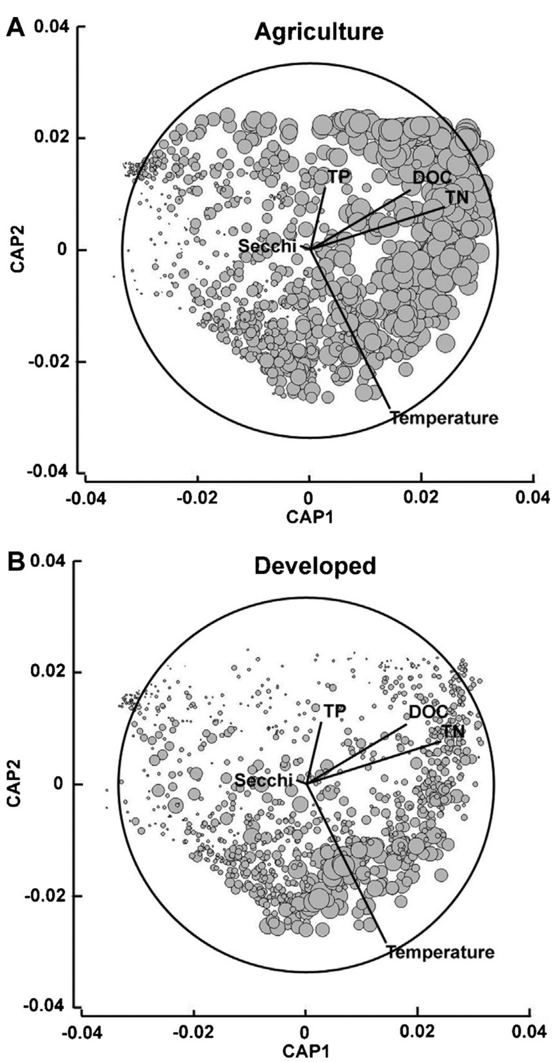

Fig. 5. Water quality CCA bubble diagram for agricultural and developed land uses. Bubble size represents the relative percentage land use in a sample (n = 1156).

temperature were correlated with estimated cyanobacteria biomass (Beaulieu et al., 2013). The mean DOC concentrations in our study were also higher in the lakes with the largest microcystin concentrations as suggested by Paerl and Otten (2013).

The hypothesis that land use characteristics would reflect microcystin concentrations was only partially supported. Whereas lakes located in the agriculturally influenced upper Midwest possessed most of the lakes with microcystin concentrations ≥1.0 μg/L, samples taken from other lakes outside this area with similar watershed land use usually did not reach this threshold. The localization of ≥1.0 μg/L microcystin sites in the upper Midwest may reflect the preponderance of lakes which are highly enriched with nitrogen and phosphorus. There are significant limitations associated with one point sampling events across such a broad latitudinal and geographical range. It is likely that the relationships developed here between land use and microcystin concentrations in lakes located in the contiguous U.S would be improved by more intensive sampling. Nevertheless our study suggests that watersheds strongly influenced by agriculture, particularly those which are located in the Midwest and at higher latitudes, are more likely to display microcystin concentrations (and possibly other less studied cyanotoxins, see Graham et al., 2010) at levels of potential concern for human health. Further study is required to elucidate the regional relationships between watershed land use and the concentrations of microcystin in lakes.

Conflict of interest statement

None declared.

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