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Factors related to water quality and thresholds for microcystin concentrations in subtropical Brazilian reservoirs

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ABSTRACT

Studies of the environmental factors that lead to cyanotoxic blooms in tropical/subtropical reservoirs are limited. While technological advances for water treatment and remediation techniques can mitigate the effects of cyanobacterial blooms, the identification of initial drivers and tipping points for minimizing cyanotoxin concentrations could be more cost effective. We studied factors related to microcystin (MC) concentrations and estimated thresholds to limit MC using a water quality dataset from 6 subtropical Brazilian reservoirs. Reservoirs varied by water chemistry and toxin concentrations (MC yearly means <0.1–17 µg/L). Phytoplankton community composition in most reservoirs was dominated by cyanobacteria, and both nitrogen-fixing and non-fixing genera were common. We found positive correlations ($p < 0.05$, Spearman's rank) between MC and nutrients ($\rho = 0.48$ – 0.74) and chlorophyll *a* (Chl-*a*, $\rho = 0.65$) and a negative correlation between MC and total nitrogen/total phosphorus (TN/TP, $\rho = -0.49$). While no association between MC and water temperature was observed, MC normalized to Chl-*a* values were positively related to higher temperatures and lower wind velocities, indicating warmer and more stable waters had a higher probability of toxic blooms. We found thresholds for MC ≥ 0.1 and ≥ 1 µg/L related to cyanobacterial cell counts of 20 060–136 165 cells/mL, respectively, and for turbidity (5–12 NTU) and TP (0.016–0.028 mg/L), respectively. We considered these MC concentrations (0.1 and 1 µg/L) to account for different levels of toxin production. The thresholds were different from those published for temperate ecosystems but not consistently lower or higher. TN/TP molar ratios favored toxin concentration when ≤ 121 , suggesting that less phosphorus in relation to nitrogen is necessary to allow MC production in subtropical freshwaters. High phosphorus concentrations can increase concentration of cyanotoxins in subtropical reservoirs, and our data confirm that global warming could exacerbate problems associated with toxic algal blooms.

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tipping points

Introduction

Environmental changes induced by population growth and poor management decisions can harm regulating, supporting, provisioning, and cultural services offered by aquatic systems (Dodds et al. 2013). Trophic state enrichment can deplete dissolved oxygen (Doan et al. 2015), impair biological communities (Vařeka et al. 2016), and accelerate algal blooms with potential risks for the ecological balance and water supply (Leigh et al. 2010, Wang et al. 2013). Perhaps the most important negative consequence of eutrophication is the widespread prevalence of toxic cyanobacterial blooms. In recent decades, an increase in intensity and incidence of cyanobacterial blooms has occurred, with consequent ecological, social, economic and health related problems (Merel et al. 2013a, Loftin et al. 2016, Wood 2016). The number of scientific publications per year with the

keywords “cyanobacteria” or “blue-green algae” have increased from 829 in 1998 to 2737 in 2016 (Science Direct Database, Dec 2016, www.sciencedirect.com).

Major concerns about the increased frequency of cyanobacterial blooms follow their potential toxicity through extra and intracellular cyanotoxins (e.g., El-Shehawey et al. 2012, Paerl and Otten 2013, Chen et al. 2016), dominance over competitors (O’Neil et al. 2012), and metabolism plasticity favoring adaptations to environmental shifts and positive relationships to global climate change (Elliott 2012, Paerl et al. 2014, Visser et al. 2016). Research on the toxicity and ecological relevance of cyanotoxins has increased dramatically over recent years (see Merel et al. 2013b for a comprehensive review).

The World Health Organization (WHO 1998) set a guideline of 1 µg/L for microcystin-LR (MC) concentrations in drinking water, assuming a consumption of

2 L/d. To reach such limits, new approaches in water treatment have been introduced where cyanobacterial blooms threaten local water sources, including the use of new adsorption materials (Huang et al. 2007, Lee and Walker 2011), oxidative methods (Zhang et al. 2016b), membranes (Lee and Walker 2008), and biodegradation (Li et al. 2015). However, improved understanding and control of the primary causes of harmful algal blooms may be more cost effective than *post hoc* treatment of water sources for human consumption (Wilhelm et al. 2011).

Mesocosms, laboratory experiments under controlled conditions, and field sampling have been used to identify the environmental drivers that influence toxin concentration (Moisander et al. 2009, Bortoli et al. 2014, Zhang et al. 2016a). Each of these methods provided unique information. Controlled experiments in laboratory and mesocosms can provide mechanistic results but might not scale to whole ecosystems (Schindler 1998). Thus, analyses of statistical relationships across aquatic systems may help elucidate whole system responses.

Nutrient concentrations and nutrient ratios can control MC and toxin quota per cell (Horst et al. 2014),

although information on these relationships in low latitudes is still limited. Lack of information on tropical and subtropical reservoirs could be a missing link in our understanding about cyanobacterial toxin concentrations in lentic aquatic systems because seasonal temperature, precipitation, and insolation cycles and averages are markedly different than those in temperate areas (e.g., Cunha and Calijuri 2011, Cunha et al. 2013). Increased temperature has been associated with higher yields of toxic strains of *Microcystis* spp. and with cells with elevated toxin content (Davis et al. 2009). MC production can be stimulated by low light conditions (Graham et al. 2004), and catchment-scale nutrient loads are sensitive to precipitation timing and intensity.

Several studies have proposed thresholds and alert levels for local environmental conditions associated with MC yields in lakes through a probabilistic approach. Such studies were developed in temperate regions with clear seasonality (e.g., Yuan et al. 2014, Pitois et al. 2016), but considerably less information is available for the tropics, in particular regarding impoundments and reservoirs, which are the dominant lentic waterbodies in many areas without glaciation. Tropical and subtropical reservoirs have different morphological features (e.g., higher mean depth), hydrological conditions (Rossel and De La Fluente 2015), and temperature dynamics than temperate systems, and many of these features coincide with those conditions favorable to increased cyanobacterial growth (Cunha and Calijuri 2011).

Many reservoirs in developing regions have experienced increased eutrophication and cyanobacteria concentrations (Vieira et al. 2003), especially in São Paulo State with >40 million inhabitants (e.g., Sotero-Santos et al. 2006, Moschini-Carlos et al. 2009). Based on the literature, we hypothesized that, with generally high temperatures and modest intraannual temperature variation within reservoirs, other factors such as nutrients and wind mixing would have a stronger relationship to MC concentration in subtropical reservoirs than in temperate. In the present study, we used a 5-year time series of MC concentrations in 6 subtropical reservoirs to identify spatial-temporal variations and the influence of land use, climate, and limnological characteristics. We investigated the main factors related to toxin concentration and estimated thresholds for subtropical reservoirs that could be used to limit toxin production and compared these to studies in temperate regions.

Materials and methods

Study area

Six reservoirs in the São Paulo State, a southeast region of Brazil, were examined (Fig. 1). São Paulo has an average

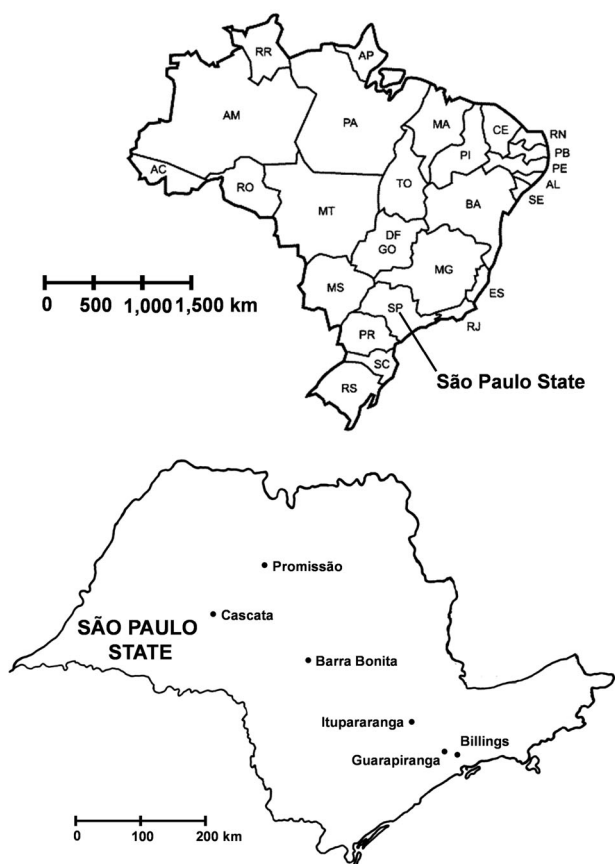


Figure 1. Brazil and São Paulo State map with the approximate location of the analyzed tropical Billings, Cascata, Barra Bonita, Promissão, Guarapiranga, and Itupararanga reservoirs. Adapted from Cunha et al. (2012).

population density of 179 inhabitants/km² and has areas with both tropical and subtropical climates, characterized by dry winters and rainy summers (annual rainfall usually 1500–1900 mm). The study reservoirs had a range of surface areas (<1–936 km²), average depths (6–12 m), and water residence time (<30–629 d; Table 1). Catchment land cover is predominantly urban for Billings, Barra Bonita, Guarapiranga, and Itupararanga reservoirs and mostly agricultural for Cascata and Promissão. The low percentages of original or second-growth vegetation in the watersheds (1.0–12.1%) highlight major anthropogenic pressures. MC occurrence has been previously reported in Billings and Guarapiranga (Carvalho et al. 2007), Barra Bonita (Okumura et al. 2007), and Promissão (Padisák et al. 2000) reservoirs. These waterbodies are used for potable water supplies and other purposes such as irrigation, recreation, and hydropower generation.

According to the São Paulo State Environmental Agency (CETESB) annual reports (CETESB 2011–2015) annual reports, except for Guarapiranga Reservoir, which is dominated by Chlorophyceae, the other 5 reservoirs were mostly dominated by cyanobacteria, including potential MC producers. Commonly observed genera in the remaining reservoirs are (1) *Cylindrospermopsis*, *Microcystis*, *Planktothrix*, *Sphaerocavum*, and *Dolichospermum* (in Billings); (2) *Cylindrospermopsis*, *Microcystis*, *Aphanocapsa*, and *Geitlerinema* (in Cascata); (3) *Microcystis*, *Planktothrix*, and *Dolichospermum* (in Barra Bonita); (4) *Microcystis*, *Planktothrix*, *Pseudanabaena*, and *Sphaerocavum* (in Promissão); and (5) *Cylindrospermopsis* (in Itupararanga; CETESB 2011–2015). Among these organisms, *Cylindrospermopsis*, *Dolichospermum*, and *Geitlerinema* are potentially nitrogen fixers. While these data did not allow a more rigorous analysis, they did allow a qualitative comparison among study reservoirs.

Data collection and analyses

We compiled existing data on MC (µg/L) as determined by the Beacon Analytical Systems Inc. Microcystin plate kit. This kit uses an immuno-assay to detect MCs and MC congeners and has been verified against liquid chromatography/mass spectrophotometry by the US Environmental Protection Agency as giving linear results and a detection limit of 0.15 ppb as MC-LR (<https://archive.epa.gov/nrmrl/archive-etv/web/pdf/p100eexl.pdf>). We also compiled information on cyanobacterial cell density (cells/mL), water temperature (°C), turbidity (NTU), total phosphorus (TP, mg/L), total Kjeldahl nitrogen (TKN, mg/L), nitrate (NO₃, mg/L), nitrite (NO₂, mg/L), chlorophyll *a* (Chl-*a*, µg/L), and

biochemical oxygen demand (BOD, mg/L) from Billings, Cascata, Barra Bonita, Promissão, Guarapiranga, and Itupararanga reservoirs. Surface water samples of each aquatic system were collected by the São Paulo State Environmental Agency (CETESB 2011–2015) once every 3 months and analyzed following standard methods (APHA 2012). Data were available from 2011 to 2015 for all reservoirs on the CETESB database, except for Promissão Reservoir (2011–2014), with at least 4 collections per year. Total nitrogen (TN) concentrations were calculated as the sum of TKN, NO₃, and NO₂. Molar TN/TP ratios were also calculated. Meteorological data from stations closest to each reservoir were obtained from the Brazilian National Institute of Meteorology (INMET – Instituto Nacional de Meteorologia), including annual precipitation (mm), averages of air temperature (°C) and wind velocity (m/s) both as annual averages and for 2 weeks before each sampling period.

Yearly means for all variables were calculated as a conservative method to avoid temporal autocorrelation. Because the data were not normally distributed, Spearman's rank correlations were used to initially assess potential factors related to yearly means of MC, MC/Chl-*a* (concentration of MC per unit total algal mass), and cyanobacteria density. We also tested for factors related to intensity of cyanobacteria blooms by using maximum MC concentration and the coincident water quality conditions. Because the results were similar to those for mean values, however, we do not report them here. The MC/Chl-*a* indicated the relative prevalence of toxic algae.

We used analysis of covariance (ANCOVA) with all ranked data to account for categorical effects (reservoirs) and continuous effects (e.g., time, water temperature, nutrients) on MC and MC/Chl-*a* data (not yearly averaged). The inclusion of time as an independent variable allowed us to account for temporal autocorrelation. ANCOVA approaches are only moderately susceptible to problems with non-normal data, and non-parametric approaches can be too conservative (Feir-Walsh and Toothaker 1974). Therefore, we ran these tests with both parametric and non-parametric ANCOVA methods and obtained the same overall results (data not shown). We used linear regressions to test for significant correlations between yearly means of MC and nutrients (TP and TN) after normalizing data by calculating the difference of the data point and dataset average for each reservoir, divided by the standard deviation in each case. ROC (receiver operating characteristic) analysis was used to estimate environmental thresholds for an MC limit of 1 µg/L (used widely for water quality guidelines) and for MC ≥ 0.1 µg/L. ROC analysis was based on raw data, not yearly means or normalized values, to

Table 1. Surface area (SA, km²), mean depth (Z_m) of the studied reservoirs, geographic coordinates of the sampling sites (GC, latitude/longitude), and calculated water residence time (WRT), as well as land use for natural vegetation (Veg), urban (Urb) and agricultural areas (Agr), and population density (PD, inhabitants (i)/km²) in the watersheds of the studied tropical reservoirs in Brazil.

Reservoir	SA (km ²)	Z _m (m)	GC (lat/long)	WRT (days)	Veg (%)	Urb (%)	Agr (%)	PD (i/km ²)
Billings	127	7	23°43'04"S 46°39'51"W	629	1.0	85.5	13.5	3342
Cascata	<1	6	22°12'4"S 49°55'22"W	<30	5.0	35.5	59.5	28
Barra Bonita	310	10	22°32'39"S 48°26'48"W	100	12.1	49.8	38.1	154
Promissão	530	12	21°17'50"S 49°46'57"W	134	6.1	33.0	60.9	39
Guarapiranga	27	6	23°40'27"S 46°43'40"W	145	1.0	85.5	13.5	3342
Itupararanga	936	8	23°36'47"S 47°24'06"W	184	12.1	49.8	38.1	154

create comparable thresholds that could be useful in a regional alert system. ROC thresholds were estimated based on a minimum sensitivity (true positive rate) of 0.750 and the highest specificity (lowest false positive rate). ROC analysis is commonly used to understand the performance of a binary classifier (Morrison et al. 2003) using the maximum area under the curve (AUC) to compare the explanatory characteristics (specificity and sensitivity) of different environmental characteristics. For statistical procedures, we used IBM SPSS Statistics 21 and Statistica 7. Thresholds were compared to cyanobacteria studies in aquatic systems worldwide.

Results

Hypereutrophic conditions were observed in Billings and Barra Bonita reservoirs, with a maximum TP of 0.64 and 0.27 mg/L, TKN of 4.3 and 1.9 mg/L, and NO₂+NO₃ of 1.2 and 3.8 mg/L, respectively (as yearly averages; Fig. 2).

Table 2. Spearman Rank correlation coefficients among the water and meteorological variables and the microcystin concentrations (MC), microcystin/chlorophyll *a* ratios (MC/Chl-*a*) and cyanobacteria density. Significant correlations were highlighted in bold.

Variables	MC	MC/Chl- <i>a</i>	Cyanobacteria density
MC		0.69*	0.45*
MC/Chl- <i>a</i>	0.69*		−0.01
Cyanobacteria density	0.45*	−0.01	
Water temperature	0.18	0.38*	−0.13
Turbidity	0.68*	0.30	0.62*
Total phosphorus	0.63*	0.03	0.41*
Total Kjeldahl nitrogen	0.74*	0.14	0.56*
Nitrate	0.12	0.09	− 0.37*
Nitrite	0.32	0.01	0.09
Total nitrogen	0.48*	0.01	0.17
Total nitrogen:total phosphorus ratio	− 0.49*	−0.07	− 0.38*
Chlorophyll <i>a</i>	0.65*	0.04	0.75*
Biochemical oxygen demand	0.65*	0.07	0.71*
Annual precipitation	0.16	−0.21	0.11
Annual air temperature (average)	0.23	0.57*	−0.32
Annual wind velocity (average)	−0.24	−0.11	−0.12
Wind velocity for 2 weeks before each sampling period	−0.29	− 0.41*	0.13

*significant at $p < 0.05$

In general, the lowest nutrient concentrations occurred in Itupararanga and Promissão reservoirs (e.g., 0.01 and 0.03 mg/L as the highest yearly TP concentrations). With the exception of a clearly increasing TP trend in Billings Reservoir (0.18 mg/L in 2011 to 0.64 mg/L in 2015), nutrient concentrations were relatively stable across the study period for the other reservoirs (Fig. 2).

Water temperatures were typically subtropical, with yearly means between 21.1 and 26.4 °C (Fig. 2). In general, the among-year temperature variation was higher for the reservoirs with larger surface areas (e.g., 2.5 °C difference for Itupararanga Reservoir), except for small-sized Cascata Reservoir, which presented a 4 °C variation. BOD values were significantly higher in Billings (maximum of 18 mg/L) compared to the other reservoirs (always ≤7 mg/L). Water turbidity values were lower in Promissão, Guarapiranga, and Itupararanga reservoirs (turbidity values <7 NTU). Chl-*a* concentrations were higher in Billings (yearly averages 81–471 µg/L) and consistently low in Promissão Reservoir (≤7 µg/L; Fig. 2).

The highest MC concentrations and interannual variability were observed in Billings (Fig. 3), with concentrations >1 µg/L in Cascata and Barra Bonita, whereas Promissão, Guarapiranga, and Itupararanga maintained concentrations <0.3 µg/L. The cyanobacterial densities were highest in Billings (median of 2.2×10^5 cells/mL; Fig. 3), with yearly means across reservoirs ranging 2 orders of magnitude, from 1.1×10^4 cells/mL (Promissão) to 1.0×10^6 cells/mL (Billings).

Positive significant ($p < 0.05$) correlations between yearly means of MC and cyanobacteria density, TN, turbidity, TP, TKN, Chl-*a*, and BOD and a negative correlation between MC and the TN/TP ratio were found (Table 2). Detailed plots of MC against variables with significant correlations not aggregated by year are shown in the Supplemental Material. Similar to MC, yearly averages of cyanobacteria density had correlations with some water variables (turbidity, TP, TKN, Chl-*a*, BOD, and TN/TP ratio; Table 2) and, by contrast, a

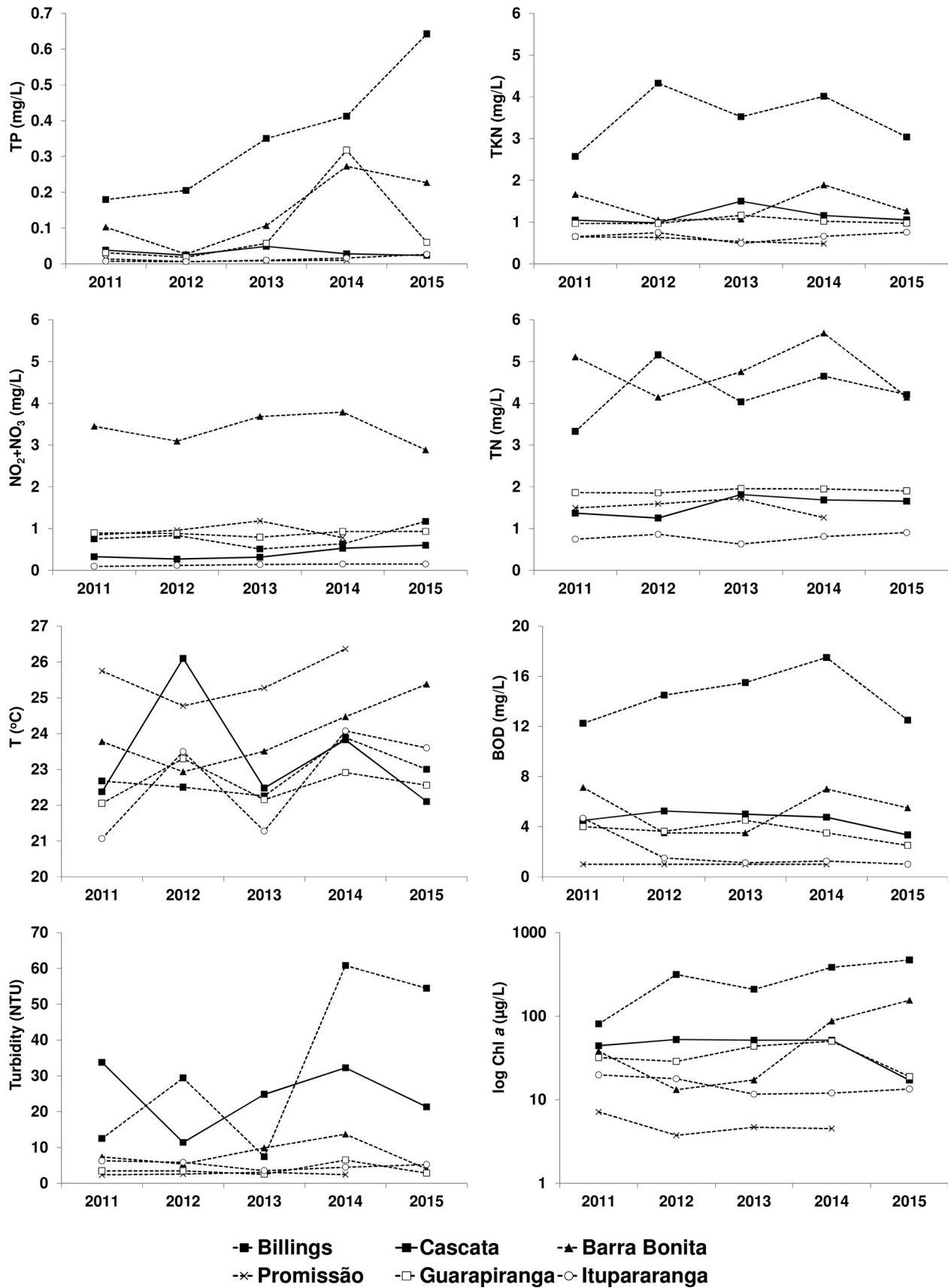


Figure 2. Yearly means of total phosphorus (TP, mg/L), total Kjeldahl nitrogen (TKN, mg/L), nitrite + nitrate (NO₂+NO₃, mg/L of N), total nitrogen (TN, mg/L), water temperature (T, °C), biochemical oxygen demand (BOD, mg/L), turbidity (NTU), and chlorophyll *a* (Chl-*a*, µg/L) in tropical Billings, Cascata, Barra Bonita, Promissão, Guarapiranga, and Itapararanga reservoirs from 2011 to 2015.

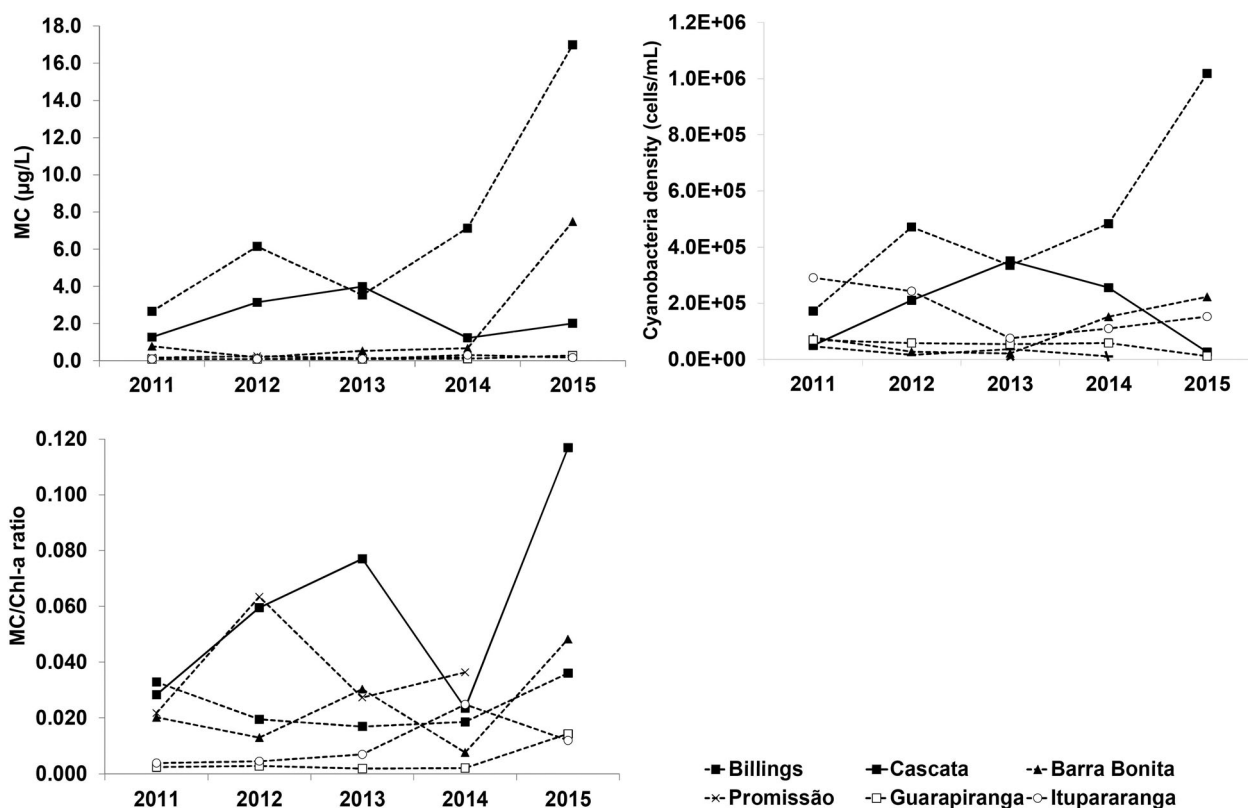


Figure 3. Yearly means of microcystin (MC, $\mu\text{g/L}$), cyanobacteria density (cells/mL) and microcystin/chlorophyll *a* ratio (MC/Chl-*a*) in tropical Billings, Cascata, Barra Bonita, Promissão, Guarapiranga, and Itupararanga reservoirs from 2011 to 2015.

negative correlation with nitrate ($\rho = -0.37$, $p < 0.05$). Yearly means of MC/Chl-*a* ratios were positively related with water temperature ($\rho = 0.38$, $p < 0.05$).

We explored the relationship between short-term and long-term meteorological conditions and MC concentrations to identify possible links. Neither the yearly

mean wind speed nor 2-week averaged wind speed were correlated with MC or cyanobacterial density. Similarly, other meteorological variables (annual precipitation and average annual air temperature) were not significantly correlated with MC or cyanobacteria density (Table 2), indicating that large-scale physical

Table 3. Analysis of covariance (ANCOVA) results with microcystin and microcystin/chlorophyll *a* ratio as the dependent variables and year, reservoir, temperature (T), total nitrogen:total phosphorus ratio (TN/TP), biochemical oxygen demand (BOD), and turbidity (Turb) as predictors. Significant correlations were highlighted in bold ($p < 0.05$).

Dependent variable: microcystin						
Effect	SS	Degrees of freedom	MS	F	<i>p</i>	
Intercept	7096.23	1	7096.228	10.74960	0.001444	
Year	8039.77	1	8039.768	12.17891	0.000726	
Reservoir	23 593.88	5	4718.775	7.14816	0.000010	
T	239.74	1	239.744	0.36317	0.548142	
TN/TP	1556.26	1	1556.263	2.35748	0.127906	
BOD	1023.59	1	1023.590	1.55057	0.216021	
Turb	1142.14	1	1142.138	1.73015	0.191461	
Error	64 693.60	98	660.139			
Dependent variable: microcystin/chlorophyll <i>a</i> ratio						
Effect	SS	Degrees of freedom	MS	F	<i>p</i>	
Intercept	3144.32	1	3144.318	6.06118	0.015565	
Year	4108.65	1	4108.654	7.92009	0.005909	
Reservoir	37 134.63	5	7426.926	14.31660	0.000000	
T	3074.24	1	3074.245	5.92610	0.016728	
TN/TP	469.91	1	469.913	0.90583	0.343564	
BOD	776.05	1	776.054	1.49597	0.224226	
Turb	335.27	1	335.266	0.64628	0.423392	
Error	50 838.81	98	518.763			

variables do not play a dominant role in driving algal biomass. In contrast to MC and cyanobacterial density, however, the wind speed (2-week average prior to the sampling) was negatively related to MC/Chl-*a* ratio ($\rho = -0.41$, $p < 0.05$), and the average annual air temperature was positively related to MC/Chl-*a* ratio ($\rho = 0.57$, $p < 0.05$). Such correlations indicate that the composition of the algal biomass was more sensitive to physical conditions than the overall biomass, and that low wind and high temperature favor the amount of toxin produced per unit algal biomass.

Regarding the correlations among non-MC variables, cyanobacteria densities were moderately correlated ($p < 0.05$) with nutrients ($\rho = 0.41$, $\rho = 0.56$, and $\rho = -0.37$, respectively, for TP, TKN, and NO_3) and significantly correlated to turbidity ($\rho = 0.62$). BOD was positively associated with all forms of nutrients ($\rho > 0.40$) except with NO_3 . MC/Chl-*a* ratios were moderately associated with water temperature ($\rho = 0.27$, $p < 0.05$).

Simple correlation does not allow simultaneous analyses of categorical effects and continuous variables. To reduce the possibility of temporal autocorrelation, we used ANCOVA to account for the influence of time and multiple variables on MC and MC/Chl-*a* ratios. ANCOVA suggested a higher incidence of toxic cyanobacteria (as MC/Chl-*a* ratios) with year and water temperature, with also a significant reservoir effect ($p < 0.05$; Table 3). For MC alone as the dependent variable, results were similar, except for the non-significant effect of temperature ($p > 0.548$; Table 3).

When we considered reservoirs with a pattern of increasing MC concentrations from 2011 to 2015 (Billings, Cascata, and Barra Bonita), the linear regression was significant between standardized MC (sdMC) and TP (sdTP; $p < 0.05$; equation 1) but not with TN (sdTN; $p > 0.05$; equation 2):

$$sdMC = 1.5489sdTP + 0.1674 \quad (R^2 = 0.478, \quad p < 0.05) \quad (1)$$

$$sdMC = -22.22sdTN - 2.1776 \quad (R^2 = 0.002, \quad p > 0.05) \quad (2)$$

sd: standardized concentration:

$$\frac{\text{yearly average} - \text{average across years}}{\text{standard deviation}}$$

Using ROC analyses, 8 of the 11 tested variables provided moderately strong AUCs for $\text{MC} \geq 0.1 \mu\text{g/L}$ (0.610–0.757) with $p < 0.05$ (Table 4). Interestingly, more robust relationships were found for $\text{MC} \geq 1 \mu\text{g/L}$, with higher AUCs (0.709–0.931; Table 4). Using a minimum sensitivity of 0.750, the thresholds for

cyanobacteria density were 20 060 and 136 165 cells/mL, respectively, for $\text{MC} \geq 0.1 \mu\text{g/L}$ and $\text{MC} \geq 1 \mu\text{g/L}$. Turbidity was the variable with the highest specificity for both MC limits, leading to thresholds of 5 NTU ($\text{MC} \geq 0.1 \mu\text{g/L}$) and 12 NTU ($\text{MC} \geq 1 \mu\text{g/L}$). Chl-*a* and BOD provided robust thresholds for $\text{MC} \geq 1 \mu\text{g/L}$ (39.8 and 5 mg/L, respectively). For nutrients, thresholds were identified at 0.016 mg/L (TP), 0.75 mg/L (TKN), 1.57 mg/L (TN), and 156 (TN/TP) for $\text{MC} \geq 0.1 \mu\text{g/L}$, and 0.028 mg/L (TP), 1.32 mg/L (TKN), 1.75 mg/L (TN), and 121 (TN/TP) for $\text{MC} \geq 1 \mu\text{g/L}$. NO_3 and NO_2 did not provide significant thresholds.

Discussion

Land use can influence water quality as well as the quantitative/qualitative aspects of phytoplankton in aquatic ecosystems. Agricultural activities were associated with higher MC concentration in lakes and reservoirs in the United States (Beaver et al. 2014) while the proximity of urban centers or forested areas influenced the distribution of potentially toxic *Microcystis* in Israel (Marmen et al. 2016). Considering the 6 studied Brazilian reservoirs, yearly means of $\text{MC} \geq 1 \mu\text{g/L}$ were more frequently observed in the reservoirs located in predominantly agricultural watersheds; among all occurrences, 50% of the yearly means of $\text{MC} \geq 1 \mu\text{g/L}$ were found at agricultural sites vs. 30% in urban/industrial areas. We recognize, however, that our dataset is too small to detect meaningful statistical associations with land use. Interestingly, reservoirs with the highest absolute MC each had a different dominant land use type (urban Billings Reservoir and agricultural Cascata Reservoir).

When normalized to biomass to remove the autocorrelation that leads to the confounding influence that algae are made up of N and P and cause turbidity, water temperature seemed to favor toxic cyanobacteria dominance (MC/Chl-*a*), with significant spatial (i.e., reservoir) and time (i.e., year) effects (Table 3). Thus, our results indicate that tropical/subtropical waterbodies, which are warmer than their temperate counterparts, can sustain a more stable MC production if nutrient and mixing conditions permit. The temperature effect may also be related to summer stratification in the study reservoirs (i.e., Dec–Mar), presumably leading to more frequent toxic blooms. Thermal stratification can favor buoyant phytoplankton species such as cyanobacteria (Rigosi et al. 2014), and water column stability can be an important variable for forecasting cyanobacteria dominance and toxin production (Persaud et al. 2015). Cyanobacterial blooms can be influenced by thermal stratification in Brazilian lakes and reservoirs (e.g.,

Table 4. Summary of results of the receiver operating characteristic (ROC) analyses for the cases of MC ≥ 0.1 $\mu\text{g/L}$ and ≥ 1.0 $\mu\text{g/L}$ in the studied tropical reservoirs, considering the test variables: cyanobacteria density (Cyano), temperature (T), turbidity (Turb), total phosphorus (TP), total Kjeldahl nitrogen (TKN), nitrate (NO_3), nitrite (NO_2), total nitrogen (TN), total nitrogen:total phosphorus ratio (TN/TP), chlorophyll *a* (Chl-*a*), and biochemical oxygen demand (BOD). For each variable, area under curve (AUC), standard error (SE) and *p* values are shown, as well as the identified thresholds considering minimum sensitivity of 0.750 and the associated (1 – specificity) values. The thresholds for all variables are for values greater than or equal to the numbers presented, except for the TN/TP ratios, for which the thresholds are valid for values less than or equal to the numbers presented.

Test variable for MC ≥ 0.1 $\mu\text{g/L}$	AUC (SE)	<i>p</i>	Threshold	Sensitivity	1 – specificity
Cyano (cells/mL)	0.610 (0.054)	0.048*	$\geq 20\ 060$	0.763	0.720
T ($^{\circ}\text{C}$)	0.525 (0.056)	0.648	≥ 21.0	0.763	0.720
Turb (NTU)	0.757 (0.046)	0.000**	≥ 5	0.763	0.340
TP (mg/L)	0.692 (0.051)	0.001**	≥ 0.016	0.763	0.540
TKN (mg/L)	0.707 (0.049)	0.000**	≥ 0.75	0.763	0.520
NO_3 (mg/L)	0.558 (0.056)	0.298	≥ 0.295	0.763	0.660
NO_2 (mg/L)	0.548 (0.055)	0.390	≥ 0.025	0.763	0.680
TN (mg/L)	0.713 (0.050)	0.000**	≥ 1.57	0.763	0.480
TN/TP	0.647 (0.053)	0.008*	≤ 156	0.750	0.480
Chl- <i>a</i> ($\mu\text{g/L}$)	0.675 (0.052)	0.002**	≥ 13.0	0.763	0.700
BOD (mg/L)	0.681 (0.051)	0.001**	≥ 1	0.780	0.660
Test variable for MC ≥ 1.0 $\mu\text{g/L}$	AUC (SE)	<i>p</i>	Threshold	Sensitivity	1 – specificity
Cyano (cells/mL)	0.833 (0.051)	0.000**	$\geq 136\ 165$	0.750	0.123
T ($^{\circ}\text{C}$)	0.612 (0.065)	0.078	≥ 21.3	0.750	0.667
Turb (NTU)	0.931 (0.023)	0.000**	≥ 12	0.750	0.086
TP (mg/L)	0.748 (0.055)	0.000**	≥ 0.028	0.750	0.494
TKN (mg/L)	0.843 (0.041)	0.000**	≥ 1.32	0.750	0.210
NO_3 (mg/L)	0.439 (0.062)	0.337	≥ 0.255	0.750	0.741
NO_2 (mg/L)	0.555 (0.068)	0.390	≥ 0.015	0.893	0.852
TN (mg/L)	0.709 (0.057)	0.001**	≥ 1.75	0.750	0.481
TN/TP	0.711 (0.055)	0.001**	≤ 121	0.759	0.506
Chl- <i>a</i> ($\mu\text{g/L}$)	0.854 (0.044)	0.000**	≥ 39.8	0.750	0.222
BOD (mg/L)	0.836 (0.042)	0.000**	≥ 5	0.821	0.247

*significant ($p < 0.05$); **significant ($p < 0.01$)

Dantas et al. 2011), although toxicity of these blooms is not commonly measured.

Higher water temperatures can favor greater growth rates of toxic *Microcystis* (Davis et al. 2009), with possible consequences related to global warming (Paerl and

Huisman 2008). Although water and air temperatures were correlated with the relative MC in the water (normalized to Chl-*a*), we found no direct correlation between MC concentrations and temperature. This finding was verified in the lake and reservoir ecosystems

Table 5. Main factors related to microcystin production or microcystin-producing genotype population in different aquatic systems worldwide compared to our study within 6 tropical reservoirs in Brazil. Compared variables included cyanobacteria (Cyano), water temperature (Water T), turbidity or total suspended solids (Turb or TSS), total phosphorus (TP), total Kjeldahl nitrogen (TKN), nitrate (NO_3), total nitrogen (TN), total nitrogen/total phosphorus ratio (TN/TP), chlorophyll *a* (Chl-*a*), biochemical oxygen demand (BOD), and other water variables. \uparrow = positive significant correlation; \downarrow = negative significant correlation; — = no significant correlation; \S = information not available.

Type/location of aquatic system	Correlation of microcystin or microcystin-producing genotype population with:											Reference details
	Cyano ^a	Water T	Turb or TSS	TP	TKN	NO_3	TN	TN/TP	Chl- <i>a</i>	BOD	Others	
Reservoir Kranji, Singapore	\S	—	\uparrow	—	\S	\S	\uparrow	\S	\uparrow	\S	(a)	Te and Gin 2011
Lake Taihu, China	\uparrow	—	\S	—	\S	\S	—	\S	\uparrow	\S	(b)	Ye et al. 2009
Lake Chaohu, China	\uparrow	—	\S	—	\S	\downarrow	\downarrow	\S	\uparrow	\S	\S	Shang et al. 2015 ^b
Two ponds, India	\uparrow	—	\S	\uparrow	\S	\S	\uparrow	—	\uparrow	\S	\S	Singh et al. 2015
241 lakes, United States	\uparrow	\downarrow	\uparrow	\uparrow	\S	\S	\uparrow	\downarrow	\uparrow	\S	(c)	Graham et al. 2004
Nine lakes, United States	\uparrow	—	\S	—	\S	\S	—	\downarrow	—	\S	\S	Jacoby et al. 2015
Lake Erie, United States	\S	—	\S	\uparrow	\S	\downarrow	\downarrow	\downarrow	\uparrow	\S	(d)	Rinta-Kanto et al. 2009
Lake Pamvotis, Greece	\S	\uparrow	\S	\S	\S	\uparrow	\S	\S	\uparrow	\S	(e)	Gkelis et al. 2014
Eight lakes, Australia	—	—	\S	$\uparrow\downarrow$	\S	\S	\S	\S	\S	\S	\S	Sinang et al. 2013
Two reservoirs, South Africa	\uparrow	\uparrow	\S	—	\S	\S	\downarrow	—	—	\S	(f)	Conradie and Barnard 2012
Six reservoirs, Brazil	\uparrow	—	\uparrow	\uparrow	\uparrow	—	\uparrow	\downarrow	\uparrow	\uparrow	\S	This study

^a Abundance, density, biomass, or biovolume of either total cyanobacteria or microcystin producers; ^b Microcystin-LR, RR, and YR were studied. The most frequent correlations for each variable are reported in the table; (a) pH and dissolved oxygen positive; (b) pH positive; (c) mean depth negative, volatile and nonvolatile suspended solids positive; (d) ammonia and pH negative; (e) nitrate, soluble reactive phosphorus, ammonium and dissolved inorganic nitrogen positive; (f) dissolved inorganic nitrogen:dissolved inorganic phosphorus ratio negative. Different correlation tests were considered in each paper used for building this comparative table, including Spearman's rank correlation test, Pearson's correlation and linear or multiple regression.

examined globally (Table 5) but does not necessarily imply that water temperature does not affect MC yields. Lag time periods between water temperature on MC production (days in 2 Canadian lakes studied by Persaud et al. 2015 and months in a Portuguese reservoir evaluated by Teles et al. 2006) should be considered, but unfortunately temporal resolution in the present dataset was insufficient to determine possible lag times. Short-term meteorological variability (days and even hours) can modify cyanobacterial bloom conditions (Huber et al. 2011, Li et al. 2017), and long-term (e.g., yearly) averages would not capture such correlations. Interestingly, wind velocity for 2 weeks before each sampling period negatively affected the MC/Chl-*a* ratio, reinforcing that a stable water column associated with lower wind velocities may have led to a higher rate of toxin production per unit chlorophyll in the study reservoirs.

For most of the cases (Table 5), MC was positively related with abundance, density, and biomass or biovolume of either total cyanobacteria or MC producers. For Lake Chaohu (China) based on an MC guideline of 1 µg/L, safety thresholds for cyanobacteria density varied between 2.1×10^4 and 3.4×10^5 cells/mL, depending on the period (Shang et al. 2015). The associated threshold we determined for the Brazilian reservoirs ($\sim 1.4 \times 10^5$ cells/mL) fell within this range. Our threshold for MC detection (MC ≥ 0.1 µg/L, cyanobacteria density $\geq 20\,060$ cells/mL) can be used as an alert level by local managers concerning water security and public health implications. The 20 000 cells/mL cyanobacterial abundance is also a common alert level used for drinking water sources as a risk indicator of potential cyanobacterial-related treatment issues (e.g., taste and odor problems or cyanotoxins occurrence; Taylor et al. 2006). We recognize, however, that biomass or biovolume estimates can be more accurate than densities because discrepancies between community characterization by counting and biovolume can occur.

Chl-*a* was also directly correlated with MC, and its concentration can be used as an indirect indicator of potential harmful blooms (Table 5). Because cyanobacteria have pigments other than Chl-*a* (e.g., phycocyanin), however, correlations between cyanobacteria and Chl-*a* may vary. In subtropical Lake Taihu, the Chl-*a* threshold related to MC guidelines for drinking water was 12.3 µg/L (Wei et al. 2014). A 50% chance of exceeding 1 µg/L of MC was related with a Chl-*a* threshold of 67.0 µg/L in a large dataset of lakes in the United States (Hollister and Kreakie 2016). Our Chl-*a* threshold for MC ≥ 0.1 µg/L was similar to this Chinese study (13.0 µg/L) while our threshold (39.8 µg/L) for MC ≥ 1 µg/L was much different than the North American study, suggesting that MC yields in relation to biomass (as Chl-*a*) can be sensitive to

different climate regimes, algal, or cyanobacterial community composition and other water quality factors.

The Chl-*a* threshold of 39.8 µg/L for MC ≥ 1 µg/L would match the category of “low risk” to human health according to criteria presented by Hunter et al. (2009). According to Hunter’s decision tree, for C-phycocyanin/Chl-*a* ≥ 0.5 , the upper Chl-*a* limits for no, low, and medium human health risks due to cyanotoxins are 10, 50, and 1000 µg/L, respectively. Therefore, quick estimations of Chl-*a* can also be used in subtropical environments to detect potential toxic blooms and aid in early warning.

The use of satellite imagery for estimating chlorophyll concentrations and surface algal blooms has been used successfully to monitor phytoplankton biomass across a range of aquatic ecosystems (e.g., Tyler et al. 2009), including high turbidity waters (Simis et al. 2005, Zhang et al. 2014). The thresholds we defined for Chl-*a* could be incorporated in remote sensing initiatives for assessing toxin concentration, similar to methods by Shi et al. (2015) in China.

Positive relationships between MC and turbidity were also observed in a reservoir in Singapore and a set of lakes in the United States (Table 5). The estimate of near-surface turbidity is also commonly made through remote sensing (e.g., Potes et al. 2012). Given the significant relationships found in the correlation analysis and ROC analysis (yielding thresholds of 5 and 12 NTU, respectively; Table 4), regular monitoring by authorities or even citizen scientists (Castilla et al. 2015) could be used as a proxy for MC concentrations in these reservoirs, allowing warning alerts for water contamination. Although few studies compare BOD values with MC concentration, the relationships found in the present study were significant ($\rho = 0.65$, $p < 0.05$, Spearman’s; Table 2), not an unexpected finding because algal biomass can contribute to BOD.

The effect of TN on MC is highly variable (Table 5). Earlier studies presented significant positive ($n = 3$), significant negative ($n = 3$), and non-significant ($n = 2$) relationships (Table 5). In our study, we found positive correlations between MC and TN as well as TKN ($p < 0.05$) but no relationship with NO₂ and NO₃ (Table 2). TN/TP ratios (average across reservoirs: 290) indicated P limitation in 90% of the cases (TN/TP > 50 , criteria from Guildford and Hecky 2000). Most investigations reported a negative influence of TN/TP ratios on MC (Table 5), suggesting that an excess of P over N would boost toxin yields. Wang et al. (2010) found greater intracellular and extracellular MC concentrations when TN/TP ratios were < 25 in a shallow subtropical lake in China (Taihu). The presence of MC in temperate lakes in the United States was associated with a similar

value: $TN/TP \leq 25.7$ (Jacoby et al. 2015). High MC also only occurred at low TN/TP and rapidly decreased at higher ratios in Canadian lakes (Orihel et al. 2012). In mesocosm studies, Harris et al. (2014) showed a reduction of cyanobacteria at a TN:TP ratio of 75, similar to our result; however, according to the threshold we calculated for our subtropical reservoirs, $MC \geq 1 \mu\text{g/L}$ cases would be more frequent under $TN/TP \leq 121$ (Table 4), suggesting that in the strongly P-limited Brazilian reservoirs, less P in relation to N is necessary to sustain MC production.

Our thresholds for TN were 1.57–1.75 mg/L as TN and 0.75–1.32 as TKN. The MC–TN relationships can be characterized by a unimodal curve. In North American lakes, maximal MC concentrations were obtained for TN ranging from 1.50 to 4.0 mg/L, with an MC decline for $TN > 8.0$ mg/L (Graham et al. 2004). Runoff (dissolved inorganic and organic N), mixing of ammonium coming from anoxic water layers, biological N_2 fixation, and atmospheric deposition are among the most important N sources to the reservoirs. Although the mechanisms are not yet completely understood, different N forms (e.g., TN, NO_3^- , TKN, NH_4^+) are considered to influence toxin concentrations in accordance with the dominant phytoplankton species, creating a link between available N form and the structure of the cyanobacterial community (Monchamp et al. 2014). This linkage would partly explain why the influence of TN on MC is different across compared waterbodies worldwide (Table 5). Also, considering that other species of cyanobacteria (not only *Microcystis* spp.) can produce MC and that such species might have different responses to N and P availability (as observed by Dolman et al. 2012), the correlations between nutrients and MC might not follow a general rule, similar to patterns for other variables (e.g., temperature; Table 5).

Most studies in the comparative table (Table 5) found no association between MC and TP (56% of the cases). Soluble reactive phosphorus (i.e., a dissolved form) was recognized as the main environmental factor linked to intracellular MC concentrations in a temperate lake (Lee et al. 2015). The lack of response of MC to TP in many studies might be because TP includes particulate P, which is not directly available for phytoplankton (e.g., associated with inorganic suspended sediments). In the 6 reservoirs we studied, however, we found a significant and positive correlation between MC and TP ($p = 0.63$, $p < 0.05$). The relationship between TP and MC is made even more clear when the interannual trend of standardized concentrations were compared, suggesting that MC temporal fluctuations were more sensitive to TP in comparison to TN (equations 1 and 2).

Higher cyanobacterial volumes in UK lakes were associated with retention times > 30 d and $TP > 20 \mu\text{g/L}$ (Carvalho et al. 2011). Most of the study reservoirs had residence times > 100 d (Table 1), and we found TP thresholds between 16 and $28 \mu\text{g/L}$ for MC (Table 4). We recognize, however, the limitation of nutrients' thresholds for toxin concentrations because different genera have potentially toxic cyanobacteria with contrasting environmental requirements and tolerances. Other factors (such as thermal stratification, flushing, grazing by zooplankton, and light deficit) can be concurrent for toxin production because an array of conditions (and not a single factor) stimulate MC synthesis (Pineda-Mendoza et al. 2016).

Conclusions

Toxin production by harmful cyanobacteria is probably the least desirable aspect of eutrophication. In the present study, factors related to MC concentration were analyzed in subtropical reservoirs and thresholds were proposed. While factors related to MC and general trends in correlations with nutrients and nutrient ratios mirrored those from temperate waterbodies, thresholds were substantially different for the subtropical reservoirs studied. In our dataset, P seemed to play a more important role in MC concentration compared to N. Also, based on thresholds for TN/TP ratios, we expected that when P is the limiting factor, relatively less P in relation to N would be required to boost MC concentrations in subtropical compared to temperate reservoirs. This prediction was observed in the TN/TP thresholds that were greater compared to those established for high-latitude aquatic ecosystems.

We propose thresholds for turbidity and Chl-*a* that could be used as a starting point for broad remote sensing-based approaches or ground-based spot monitoring of factors more easily analyzed than MC concentrations. For example, cyanobacterial densities and BOD values can serve as an alert level concerning cyanotoxins in local freshwaters.


Our data suggest that subtropical waterbodies can sustain high MC concentration over time and that toxic cyanobacteria (as MC/Chl-*a* ratios) had higher incidence with greater water temperature. This finding suggests that problems of toxic algae may increase with global warming as waterbodies in these zones become warmer; at lower latitudes this problem could become more widespread. Thus, the distribution and toxicity of cyanobacterial blooms are expected to increase, and tropical and subtropical reservoirs may be especially vulnerable. In summary, our findings indicated that, overall, MC and cyanobacterial density were significantly related

to nutrients and other water quality features (but not temperature and wind speed). The production of toxin per unit biomass of the total algal community showed the opposite, however; the MC/Chl-*a* ratio increased with greater temperature and lower wind speed in the prior 2 weeks (but not nutrients and other water-quality characteristics). This finding suggests that N, P, and other water quality variables probably play an important role in the occurrence of toxic cyanobacteria, but physical factors (e.g., water temperature and meteorological aspects) can enhance and modulate MC production or the relative amounts of toxic cyanobacteria with respect to overall algal biomass.

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