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Detection of Microcystins in Lake Manatee and Lake Washington – Two Florida Drinking Water Systems

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Abstract

Clean, fresh, and safe drinking water is essential to human health and well-being. Occasionally, chemical pollutants taint surface water quality used for consumption. Microcystins (MCs) are toxic heptapeptides produced by freshwater cyanobacteria. These secondary metabolites can reach hazardous concentrations, impairing surface drinking water supplies. Inconsistent screening of MCs is not uncommon in Florida waters as no provisional guidance value is established to protect public health. The occurrence of MCs in Lake Manatee and Lake Washington was monitored over the potential peak algae bloom season (June-August). An indirect competitive enzyme-linked immunosorbent assay (icELISA) quantified total MCs in two drinking water systems. Varied concentrations occurred between June and July, whereas concentrations peaked in August. Overall, MC prevalence was higher in Lake Manatee than Lake Washington. Colorimetric assays measured phosphate and nitrite in environmental water samples. Phosphate and nitrite concentrations strongly correlated with total MCs ($p < 0.01$). The results indicate the intrinsic nature of environmental MCs in surface drinking water supplies and the need to examine hepatotoxin dynamics to preserve drinking water quality in community served areas.

Keywords

Drinking water; Enzyme-linked immunosorbent assay; Harmful algal blooms; Microcystin

INTRODUCTION

Potable water is a top priority in the public health community. Cyanobacteria are photosynthetic microorganisms capable of proliferating as harmful algal blooms (HABs) in eutrophic waters (Kimambo *et al.* 2019). In past decades, the frequency, intensity, and location of HABs have increased worldwide (Anderson 2014; Backer *et al.* 2015). HAB occurrences are widespread in freshwater ecosystems across the United States, including the Great Lakes, small inland lakes, and rivers (Schmale *et al.* 2019). Such phenomena can adversely impact agriculture, drinking water, fisheries, food and real estate industries, recreation, tourism, and water quality (Cheung *et al.* 2013; Watson *et al.* 2015; Carmichael & Boyer 2016). When HABs deteriorate, algal cells emit cyanobacterial toxins (cyanotoxins) into surface waters at environmentally relevant concentrations, posing risks to ecological and human health (Codd *et al.* 2005).

Microcystins (MCs) are heterocyclic cyanotoxins produced by cyanobacteria (Turner *et al.* 2018). Several toxic cyanobacterial species can release MCs, including *Microcystis*, *Anabaena*, *Hapalosiphon*, *Nostoc*, *Oscillatoria*, and *Planktothrix* (Szlag *et al.* 2015). MCs in drinking water is considered a serious health threat (Sakai *et al.*, 2013). Human exposure to MCs

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primarily occurs via accidental ingestion of contaminated drinking water (Funari & Testai 2008). Other relevant MC exposure pathways include aerosol inhalation, consumption of contaminated aquatic foods, and dermal contact to blue-green algae (Drobac *et al.* 2013). The most toxic MC variant is microcystin-LR (MC-LR) (Lone *et al.*, 2015). MC-LR contains the amino acids leucine (L) and arginine (R) at positions 2 and 4, respectively, in the heptapeptide (Carmichael 1992). The recommended guidance value for MC-LR in drinking water is 1 µg/L (WHO 2003).

MC poisonings in livestock, pets, and wildlife are well-documented (Mez *et al.* 1997; Stewart *et al.*; 2006; Backer *et al.* 2013; Wood 2016). Domestic and wild animal and human fatalities have resulted from MC intoxications (Lawton & Robertson 1999). Upon intake, MCs can inactivate serine/threonine phosphatases in hepatocytes (Eriksson *et al.* 1990). Prior animal toxicology experiments revealed abnormal liver changes after oral MC-LR exposure, including altered serum enzyme activities and hepatic injury (Fawell *et al.*; 1999, Heinze 1999; Sedan *et al.* 2015). Lévesque *et al.* 2014 identified a relationship between cyanobacteria contaminated drinking water and potential acute health effects. Epidemiological scholars linked chronic MC exposure to liver cancers or diseases (Zhang *et al.* 2015; Zheng *et al.* 2017). The most renowned human outbreak attributed to MC pollution occurred at a hemodialysis center in Brazil, killing 76 patients (Azevedo *et al.* 2002). The aforesaid studies demonstrate the probable health effects of MC exposure in the vicinity of contaminated waters.

Biochemical assays and chemical methods are conventional methods of MC detection (Shamsollahi *et al.* 2014). Protein phosphatase inhibition assay (PPIA) is based on phosphatase (PP1 or PP2) inhibition. PPIA is efficient at conveniently testing multiple samples without extra analyses. The biochemical assay is simple, feasible, and provides toxicological information on animal and human health (Massey *et al.* 2020). Major limitations of PPIA include false positive results, lack of specificity, and matrix effects (Robillot & Hennion 2004).

High-performance liquid chromatography (HPLC) and liquid chromatography-mass spectrometry (LC-MS) are chemical methods of MC separation and identification (Massey *et al.* 2020). MC analysis via HPLC involves stationary and mobile phases of acetonitrile or methanol. HPLC can verify MC variants in an unknown sample and generate qualitative and quantitative data on toxins. The chemical method is merited for its accurate detection of intracellular and extracellular MCs. Yet, HPLC demands a financial cost, technical expertise, and extensive sample cleanup. LC-MS is an intricate analytic technique used to detect MC variants in the environment. MC structure has been detected in blue-green algae, dietary supplements, human serum, and vegetables (Heussner *et al.* 2014; Parker *et al.* 2015; Qian *et al.* 2017). LC-MS detectors are limited by equipment cost, sample processing time, and technical operation (Ortiz *et al.* 2017; Foss *et al.* 2019).

Water treatment facilities are recommended to use enzyme-linked immunosorbent assay (ELISA) to quantify MCs in raw and treated waters (USEPA 2015). ELISA can track relative changes in MC concentrations and serve as an indication to control algae blooms (Guo *et al.* 2017). Also, the immunoassay is valued for producing reliable, repeatable, and variable MC concentration results (Yu *et al.* 2002; Akter *et al.* 2017). Indirect competitive ELISA (icELISA), a type of ELISA, is widely used to screen cyanotoxins (McElhiney & Lawton 2005). icELISA is an extension of direct ELISA wherein a secondary antibody conjugate binds a primary antibody to detect MCs. Although icELISA cannot differentiate MC variants, it recognizes the Adda moiety on MCs for quantitation (Botha *et al.* 2019; Kleinteich *et al.* 2019). icELISA can analyze

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MC concentrations below the WHO's drinking water guidance value (Baralla *et al.* 2017; Botha *et al.* 2019; Xu *et al.* 2020). The ease of operation, inexpensive cost, fast screening capabilities, and sensitivity of icELISA may represent a critical step in evaluating drinking water quality for the public health community.

Toxic HABs constitute a significant problem to surface drinking water supplies and public health (Burns 2008). Eutrophication is driven by nutrient loading, especially phosphorous and nitrogen (Yang *et al.* 2008). Excess nutrients can stimulate algae proliferation and water blooms (Heisler *et al.* 2003). Former studies examined how anthropogenic nutrient loads of phosphorus and nitrogen influence cyanobacterial algae blooms (Smith *et al.* 2006; Dodds *et al.* 2009; Paerl *et al.* 2011; O'Neil *et al.* 2012). An empirical model supported phosphorus and nitrogen as critical drivers of cyanobacterial abundance and dominance in lakes (McCarthy *et al.* 2009). Monchamp *et al.* 2014 examined minor nitrogenous forms, such as nitrites, on cyanotoxin production. Nitrites and nitrates are key intermediates within the nitrogen cycle before atmospheric evaporation, plant uptake, soil immobilization, and waterway contamination (Xia *et al.* 2018). Nitrite analysis might become more commonplace since it can contaminate drinking water,

Lake Manatee and Lake Washington are two suppliable drinking water sources in Florida. To our knowledge, no study has been conducted to monitor MC occurrence and nutrient forms in Lake Manatee and Lake Washington. Anecdotal reports have suggested the presence of MCs, nutrients, or non-toxic blue-green algae in the lakes. In 2001, Lake Washington had MC concentrations six times the acceptable level of drinking water. Increasing trends in nitrate levels from human activity were documented by the Florida Department of Environmental Protection in Lake Washington and the St. John's River. Blue-green algae in Lake Manatee were blamed for unpleasant drinking water in 2017. Water treatment officials proclaimed the water was safe to drink, albeit worried consumers. Multiple algae blooms were reported in Florida waterways in 2019, particularly in the nearby Manatee River. Thus, we employed an icELISA to monitor the occurrence of MCs in Lake Manatee and Lake Washington over the algae bloom season. We also derived a model of MC prediction via nutrient measurement of phosphate and nitrite.

MATERIALS and METHODS

Study Locations

Lake Manatee, located in Manatee County, Florida (27.4947º N, -82.3479º W, Figure 1 right), has a surface area of 4.75 km^2 and a maximum depth of 3.4 m. Lake Manatee is an artificial reservoir and a major water source for Bradenton and nearby municipalities in Manatee County. The Lake Manatee Water Treatment Plant is located on the western side of Lake Manatee (27.4896º N, -82.3579º W).

Lake Washington, located in Brevard County, Florida, (28.1468º N, -80.7464º W, Figure 1 left) has a surface area of 17.65 km^2 and a surface elevation of 4.9 m. Lake Washington, part of the St. John's River, is the most critical drinking water source in Melbourne. The Melbourne Water Treatment Plant is located on Lake Washington's eastern side (28.1468 º N, -80.7330 º W).

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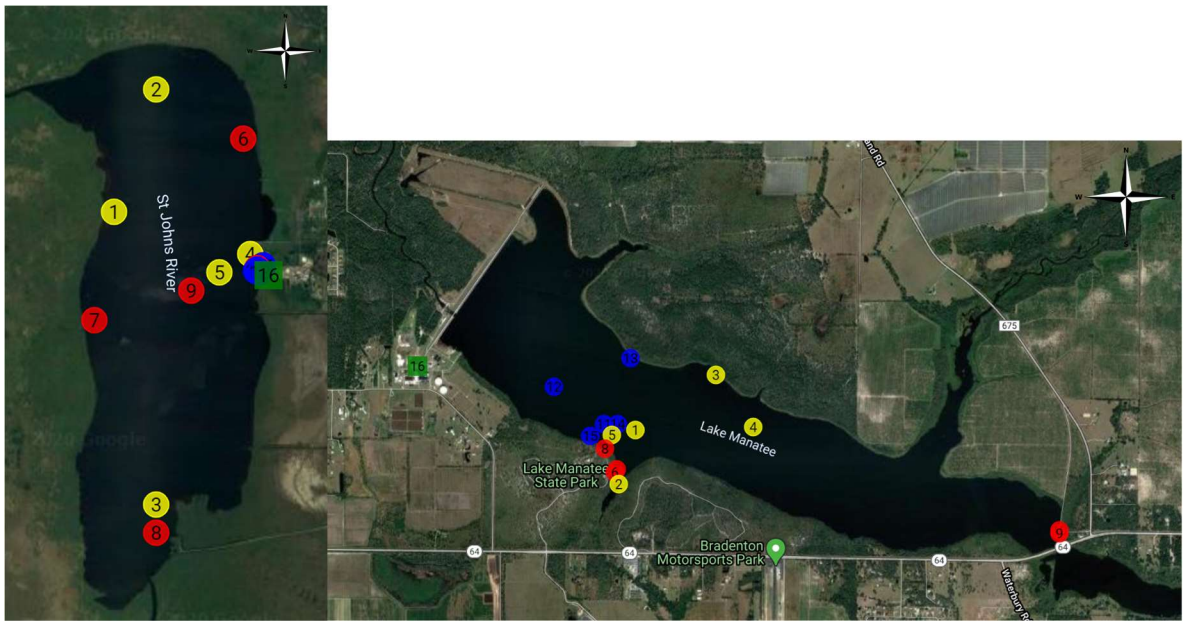
Figure 1. Lake Manatee and Lake Washington Sample Locations



Sample Location ID	Site
LW1-LW5 LM1-LM5	1-5
LW6-LW10 LM6-LM10	6-10
LW11-LW15 LM11-LM15	11-15
Lake Manatee Water Treatment Plant and Melbourne Water Treatment Plant	16

Sample Collection

A total of 120 environmental water were collected across 30 sites in Lake Manatee and Lake Washington between June 22 and August 31, 2019. During each sampling occasion, a series of 4 samples were gathered from five sites to assess the occurrence and distribution of MCs. Site clusters were apparent on days when adverse weather conditions or restricted areas prevented water vehicle usage. Sampling occurred nearshore or closest to water treatment plants during such occasions. Time of collection and field observations were recorded at every site (Table 1A). Samples were filled in 4 fl oz (118.294 mL) glass amber bottles. Pre-rinsed bottles involved submergence within the top 12 inches of the water column. Contents were emptied downstream and re-immersed below the surface water to obtain a sample volume of 100 mL. Samples were stored in labeled bags and transported on ice to Eastern Florida State College (Melbourne, FL). Samples were refrigerated at 4ºC for up to 5 days.

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Sample Preparation

One mL aliquots of sample were prepared in glass vials for MC extraction. The Abraxis QuikLyse System ™ was used to lyse algal cells in water. A volume of 100 µL QuikLyse ™ Reagent A was added to samples, followed by capping and incubating vials at room temperature for 8 minutes. The same process was repeated for QuikLyse ™ Reagent B, except a volume of 10 µL was dispensed into vials.

Microcystin Analysis

The congener-independent detection of intracellular and extracellular MCs and nodularins was determined by Abraxis Microcystins/Nodularins (ADDA) ELISA kit (Microtiter Plate) PN 520011 (Warminster, PA). The limit of detection for the assay, based on MC-LR, was 0.10 µg/L. Appropriate reported concentrations ranged between 0.10 and 5.0 µg/L. Duplicate standards, controls, and samples were plated in a 96-well plate (Hercules, CA). The immunoassay consisted of a secondary conjugated antibody, which bonded to an unlabeled primary antibody. A colored product was formed by reactivity between the secondary conjugated antibody and its substrate. The reaction was halted by 50 µL of stop solution. A Bio-Rad Model 550 microplate reader was used to analyze MCs at 450 nm. The mean absorbance value for each standard was divided by standard zero to calculate $B/B_0\%$. Abraxis standard concentrations plotted against $B/B_0\%$ standards generated a standard curve for total MCs determination. Total MC concentrations were reported in µg/L.

Nutrient Measurement

Colorimetric assays (Phosphate Assay Kit ab65622 and Griess Reagent Kit ab234044) were performed to measure phosphates and nitrites, respectively. Abcam (Cambridge, MA) supplied reagents and standards. The colorimetric assays were optimized for nutrient measurement in biological samples, including algal blooms. They assessed concentrations between 0.001 mM and 1 mM. A fresh set of standards were prepared for every use. All reaction components were increased 5X in 1 mL cuvettes. A Spectronic 200 spectrophotometer was used for duplicate standard and sample readings. Phosphate and nitrite were measured at room temperature at 650 nm and 540 nm, separately. Duplicate readings of standards and samples were averaged and subtracted from standard 0. Corrected absorbances were plotted against standard concentrations. Phosphate or nitrite concentration (mM) in samples was determined by dividing the amount of phosphate or nitrite (nmol/sample) from the standard curve by the sample volume (mL) in a 1 mL cuvette. Final concentrations were converted and reported in µg/L.

Statistical Analysis

Concentration data on total MCs, phosphate, and nitrite were entered in Microsoft Excel. Line graphs in the spreadsheet program were constructed to illustrate the occurrence of total MC and nutrient concentrations in Lake Manatee and Lake Washington. Data were sorted from smallest to largest prior to statistical analysis in Statistical Package for the Social Sciences (SPSS) Version 25. Two samples were excluded from analyses since total MCs concentrations were below the limit of detection (< 0.10 µg/L). Non-detectable phosphate and nitrite concentrations (< 0.01 µg/L) resulted in excluded samples. Two-tailed Pearson correlations measured linear associations between nutrients and total MC concentrations.

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RESULTS

Enzyme-Linked Immunosorbent Assay Calibration

A calibration curve was prepared for every ELISA plate. Sample runs produced consistent R^2 values, which ranged from 0.9797 to 0.9964, acceptable for quantification. The average R^2 statistic of ELISA curves for Lake Manatee was 0.9929, and 0.9872 for Lake Washington. The overall average R^2 statistic for ELISA curves was 0.9901.

Bloom Samples

Blooms samples consisted of visible algal scum or discolored water. Bloom material was observed in 21.18% (25/118) of environmental water samples. The prevalence of bloom samples in Lake Manatee was 8.47% (5/59). A small algae bloom was sighted on June 22, 2019, in a small cove on the north side of Lake Manatee. All Lake Manatee bloom samples were collected from Site 3. The prevalence of bloom samples in Lake Washington was 33.89% (20/59). Lake Washington endured an algae bloom in July 2019, where discolored water was gathered from Sites 6-10. Microscopic examination of a bloom sample in Lake Washington indicated the presence of cyanobacteria.

Microcystin Occurrence and Distribution

Table 2A summarizes total MC concentrations in environmental water samples collected from Lake Manatee and Lake Washington. Reported total MCs (µg/L) included the sum of intracellular and extracellular toxins. From the 120 samples analyzed, 118 (98.36%) contained detectable total MCs, above the limit of detection (0.10 µg/L). Two samples, one from each lake, had undetectable total MCs. A total of 42 (35%) samples across the drinking water systems returned total MCs above 0.20 µg/L.

An upward trend in total MC concentrations was observed in Lake Manatee between June and August (Figure 2). Lake Manatee had a maximum total MC concentration of 0.47 µg/L. The sample was collected from a recreational site (Site 15). Repeated samples from Site 20 contained total MCs of 0.29 µg/L, 0.32 µg/L, and 0.46 µg/L.

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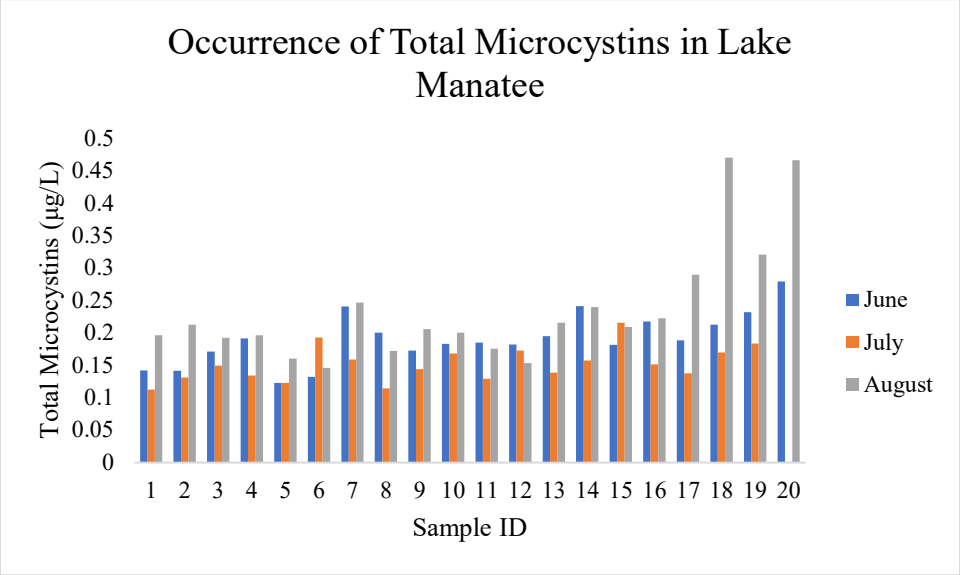
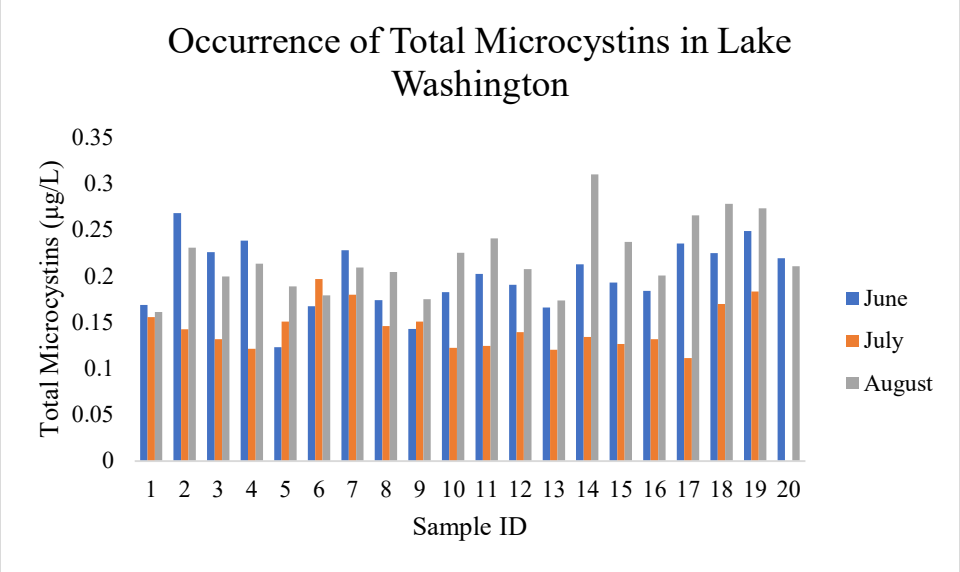
Figure 2. Occurrence of Total Microcystins in Lake Manatee.



In Lake Washington, total MC concentrations were variable and increased over the 10-week sampling period. The maximum total MC concentration in Lake Washington was 0.31 µg/L. The sample was collected from a boat ramp (Site 14). Total MCs of 0.17 µg/L, 0.20 µg/L, and 0.23 µg/L were detected in repeated samples from Site 14.

Total MC concentrations in Lake Manatee and Lake Washington were higher in June than in July. Peak total MCs occurred during August. No sample from either lake exceeded the WHO drinking water guidance value of 1.0 µg/L.

Figure 3. Occurrence of Total Microcystins in Lake Washington.



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Measurement of Nitrites and Phosphates

Of the 120 environmental water samples taken from Lake Manatee and Lake Washington, 60 (50%) were analyzed for nitrites and phosphates (Table 3A). Nitrites were detected in 49 (81.66%) samples and ranged from 0.005 to 0.845 µg/L. Phosphates were detected in 38 (63.33%) samples and ranged from 0.006 to 1.10 µg/L (Table 2).

Table 1. Summary of nutrients in Lake Manatee and Lake Washington

	Samples Analyzed	Recovery Rate of Nitrite	Recovery Rate of Phosphate	Nitrite Concentration Range	Phosphate Concentration Range
Lake Manatee	30	24/30 (80%)	30/30 (100%)	0.005 – 0.702 µg/L	0.098 – 0.52 µg/L
Lake Washington	30	25/30 (83.33%)	18/30 (60%)	0.03 – 0.845 µg/L	0.006 – 1.10 µg/L
Lake Manatee and Lake Washington	60	49/60 (81.66%)	48/60 (80%)	0.005 – 0.845 µg/L	0.006 – 1.10 µg/L

Nitrite concentrations varied considerably, while phosphate concentrations generally increased over the sampling period (Figure 4, Figure 5). Nitrite concentrations remained fairly constant in July and peaked during August. The maximum concentration of nitrite in Lake Manatee and Lake Washington was Lake Washington 0.702 µg/L and 0.845 µg/L, respectively. Lake Washington had a higher concentration of phosphate in August than Lake Manatee. One sample from Lake Washington surpassed a phosphate concentration of 1.0 µg/L.

Figure 4. Measurement of Nitrite in Lake Manatee and Lake Washington

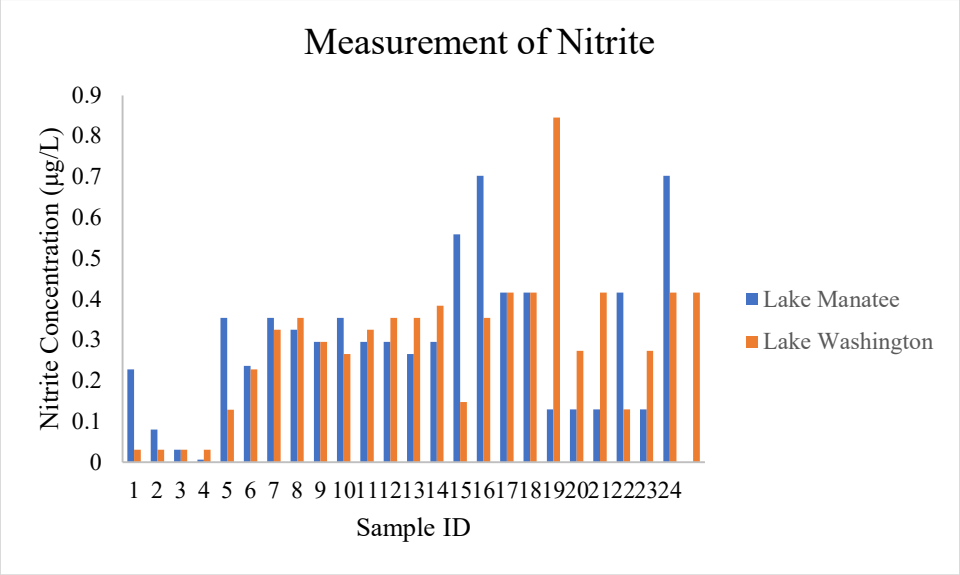

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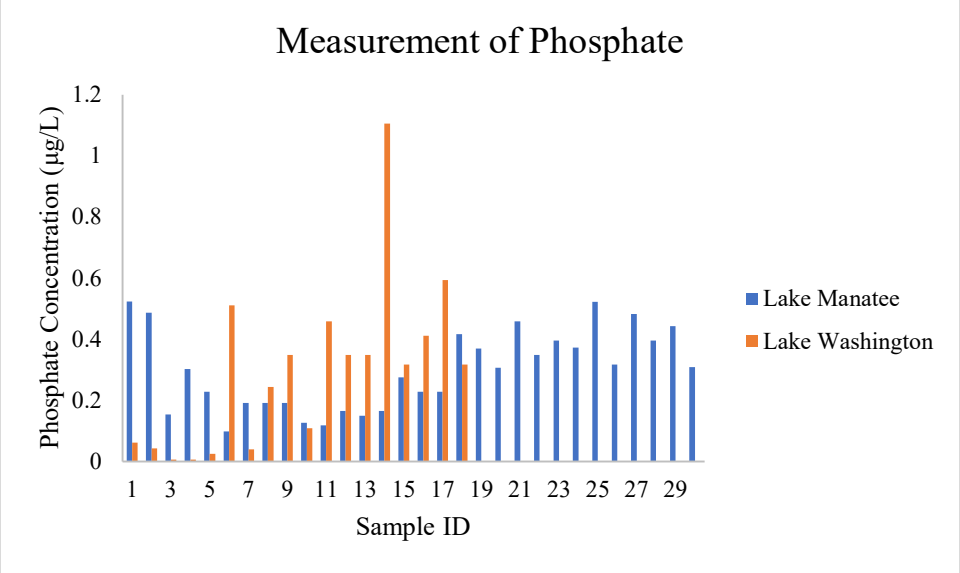
Figure 5. Measurement of Phosphate in Lake Manatee and Lake Washington



Associations Between Nutrients and Total Microcystins

Associations between nutrient and total MC concentrations in Lake Manatee and Lake Washington were measured by two-tailed Pearson correlations in SPSS Version 25. Correlation results indicated strong associations between total MC and nutrient concentrations in the drinking water systems ($p < 0.01$). Correlation coefficients were higher for associations between nutrient and total MC concentrations in Lake Manatee (Table 2).

Table 2. Correlation coefficients for nutrients and total microcystins

		Nitrite	Phosphate
Lake Manatee Total Microcystins	Pearson Correlation	0.955	0.986
	Sample Size	24	30
Lake Washington Total Microcystins	Pearson Correlation	0.876	0.922
	Sample Size	25	18

DISCUSSION

The study implemented an icELISA to monitor the occurrence and distribution of total MCs in Lake Manatee and Lake Washington over the potential peak algae bloom season. Total MC concentration varied across various geographical markers within the lakes. Concentrations peaked during August and ranged between 0.11 µg/L and 0.47 µg/L. A maximum total MC concentration of 0.47 µg/L was detected at a recreational site in Lake Manatee. The concentration was 0.16 µg/L higher than the maximum total MC concentration of 0.31 µg/L in Lake Washington. Environmental factors, including nutrients, pH, and water temperature, probably influenced the maximum total MC concentrations, which have been studied relative to MC production (Oh, H.M., *et al*., 2001). Historically, total MC concentrations may have occurred in the drinking water systems but never documented for the public. Future studies can

regularly test for MCs on a broader scale to evaluate its levels in surface drinking water supplies. Extended longitudinal studies, ranging from six months to a year, can offer valuable information on MC distribution, dynamics, and toxicity.

Findings were consistent with studies analyzing freshwater MCs. Ueno *et al.* (1996) quantified blue-green algal toxins (0.062-0.292 µg/L) in drinking water sources throughout Haimen and Fusui, China. Oliveira *et al.* 2019 monitored MC prevalence in a Brazilian water treatment plant and detected concentrations of 1.5 µg/L, 2.1 µg/L, and 0.60 ug/L between June and August. In 2007, Williams *et al.* (2007) demonstrated annual cyanotoxin production in recreational sites of the St. John's River. Two years later, Bigham *et al.* (2009) determined low MC concentrations in 187 Florida lakes. Neither study reported on MC concentrations in Lake Manatee or Lake Washington, indicating a lack of knowledge or interest in the drinking water systems. The results may extend upon environmental monitoring efforts of MCs in community dependable human systems.

Phosphate and nitrite measurements were taken to examine their association with total MCs. The highest nitrite and phosphate concentration was reported in Lake Washington, where lower total MC concentrations persisted. Strong positive associations occurred between total MCs and nutrients. The observed associations paralleled multiple studies examining the effect of nutrients on cyanobacterial growth (Blomqvist *et al.* 1994; Smith *et al.* 2006; Dodds *et al.* 2009; McCarthy *et al.* 2009; Paerl *et al.* 2011; O'Neil *et al.* 2012). Phosphorus and nitrogen are key nutrient drivers of cyanobacterial blooms (Lu, J. *et al*., 2019). Consequently, algae blooms may deteriorate water quality conditions for aquatic organisms, wildlife, and humans. The results alongside supporting evidence suggest additional studies exploring the influence of nutrient forms on MCs.

CONCLUSIONS

MCs are cyanotoxins produced by cyanobacteria under favorable environmental conditions. Continual development of HABs and their hazardous effects are of ecological and human health concern. The present study explored the distribution and occurrence of MCs in two Florida drinking water systems during potential peak algae bloom season. The results demonstrated an increase in total MC concentrations between June and August. While the study was short-term, long-term studies may provide a comprehensive evaluation of MC prevalence, toxin distribution, and bloom dynamics. Presently, there is no provisional guidance value for MCs in drinking water in Florida. Thus, the results may provide a benchmark for total MCs in drinking water systems. Future studies should regularly measure total MC concentrations and identify the most abundant congeners of the cyanotoxin to monitor lake water quality.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX

Table 1A

Site	Date	Time of Collection	Field Notes
LM1	6/22/2019	14:38	Sampled by canoe. Bloom sighted in a cove.
LM2	6/22/2019	14:52	
LM3	6/22/2019	15:19	
LM4	6/22/2019	15:48	
LM5	6/22/2019	16:20	
LM6	7/13/2019	12:18	Sampled by shore. No visible bloom.
LM7	7/13/2019	12:27	
LM8	7/13/2019	12:50	
LM9	7/13/2019	13:30	
LM10	7/13/2019	13:39	
LM11	8/23/2019	12:28	Sampled by canoe. No visible bloom.
LM12	8/23/2019	12:42	
LM13	8/23/2019	12:59	
LM14	8/23/2019	13:13	
LM15	8/23/2019	13:20	
LW1	6/29/2019	11:17	Sampled by boat. No visible bloom.
LW2	6/29/2019	11:31	
LW3	6/29/2019	11:50	
LW4	6/29/2019	12:11	
LW5	6/29/2019	12:50	
LW6	7/31/2019	11:04	Sampled by boat. Widespread cyanobacterial bloom.
LW7	7/31/2019	11:19	
LW8	7/31/2019	11:28	
LW9	7/31/2019	11:39	
LW10	7/31/2019	11:49	
LW11	8/31/2019	09:57	Sampled by the shore. No visible bloom.
LW12	8/31/2019	10:07	
LW13	8/31/2019	10:13	
LW14	8/31/2019	10:21	
LW15	8/31/2019	10:26	

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Table 2A

Sample ID	Marker	Total Microcystins (µg/L)	Sample ID	Marker	Total Microcystins (µg/L)
LM1	1	0.141659787	LW1	1	0.168673418
LM2	1	0.141255587	LW2	1	0.267971815
LM3	1	0.171060316	LW3	1	0.225682242
LM4	1	0.190952715	LW4	1	0.238199312
LM5	2	0.122624886	LW5	2	0.123009865
LM6	2	0.13170503	LW6	2	0.167299506
LM7	2	0.24033792	LW7	2	0.22753561
LM8	2	0.199885382	LW8	2	0.173713591
LM9	3	0.172286657	LW9	3	0.142519966
LM10	3	0.182941229	LW10	3	0.182450779
LM11	3	0.18477998	LW11	3	0.202255695
LM12	3	0.181639048	LW12	3	0.190377691
LM13	4	0.194810557	LW13	4	0.165936785
LM14	4	0.241025642	LW14	4	0.212428451
LM15	4	0.181379726	LW15	4	0.192885346
LM16	4	0.217154373	LW16	4	0.183949118
LM17	5	0.187975225	LW17	5	0.235102541
LM18	5	0.21255017	LW18	5	0.224577475
LM19	5	0.231574082	LW19	5	0.248548322
LM20	5	0.278837826	LW20	5	0.219134236
LM21	6	< 0.10	LW21	6	< 0.10
LM22	6	0.112150707	LW22	6	0.155592826
LM23	6	0.130753354	LW23	6	0.142417005
LM24	6	0.149196997	LW24	6	0.131496413
LM25	7	0.133788663	LW25	7	0.121061496
LM26	7	0.122580706	LW26	7	0.150705948
LM27	7	0.192591513	LW27	7	0.196523075
LM28	7	0.158688331	LW28	7	0.179620486
LM29	8	0.114261463	LW29	8	0.145760971
LM30	8	0.14373567	LW30	8	0.150487501
LM31	8	0.167817899	LW31	8	0.122119724
LM32	8	0.128706619	LW32	8	0.12426401
LM33	9	0.172206907	LW33	9	0.139351745
LM34	9	0.138474253	LW34	9	0.120186648
LM35	9	0.156877895	LW35	9	0.133999575
LM36	9	0.215389101	LW36	9	0.126262665
LM37	10	0.151135409	LW37	10	0.131496413
LM38	10	0.137484743	LW38	10	0.111293091
LM39	10	0.16926833	LW39	10	0.169495184
LM40	10	0.183424938	LW40	10	0.183039737

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LM41	11	0.196003344	LW41	11	0.160855318
LM42	11	0.212433753	LW42	11	0.230613609
LM43	11	0.192374612	LW43	11	0.199396497
LM44	11	0.196003344	LW44	11	0.213352489
LM45	12	0.159813994	LW45	12	0.188572721
LM46	12	0.145767423	LW46	12	0.178940737
LM47	12	0.246334377	LW47	12	0.209066119
LM48	12	0.171970287	LW48	12	0.204174072
LM49	13	0.205525131	LW49	13	0.17475361
LM50	13	0.200275478	LW50	13	0.224836782
LM51	13	0.175466182	LW51	13	0.240573369
LM52	13	0.153288827	LW52	13	0.207305648
LM53	14	0.215199917	LW53	14	0.173282072
LM54	14	0.239353232	LW54	14	0.309520027
LM55	14	0.209101126	LW55	14	0.236538862
LM56	14	0.222433752	LW56	14	0.200410762
LM57	15	0.289364404	LW57	15	0.265368167
LM58	15	0.470385496	LW58	15	0.277766875
LM59	15	0.320456764	LW59	15	0.273108618
LM60	15	0.466345943	LW60	15	0.210485254

Table 3A

Sample ID	Nitrite (µg/L)	Phosphate (µg/L)	Sample ID	Nitrite (µg/L)	Phosphate (µg/L)
LM1	0.227098473	0.523512905	LW1	0.030435878	0.523512905
LM2	0.079601527	0.486559053	LW2	0.030435878	0.486559053
LM3	0.030435878	0.153974384	LW3	0.030435878	0.153974384
LM4	0.005853053	0.301789792	LW4	0.030435878	0.301789792
LM5	0.353884615	0.227882088	LW5	0.128767176	0.227882088
LM6	0.235923077	0.098543606	LW6	0.227098473	0.098543606
LM7	0.353884615	0.190928236	LW7	0.324394231	0.190928236
LM8	0.324394231	0.190928236	LW8	0.353884615	0.190928236
LM9	0.294903846	0.190928236	LW9	0.294903846	0.190928236
LM10	0.353884615	0.126258995	LW10	0.265413462	0.126258995
LM11	0.294903846	0.118340935	LW11	0.324394231	0.118340935
LM12	0.294903846	0.165378656	LW12	0.353884615	0.165378656
LM13	0.265413462	0.149699416	LW13	0.353884615	0.149699416
LM14	0.294903846	0.165378656	LW14	0.383375	0.165378656
LM15	0.558875556	0.27513334	LW15	0.147451923	0.27513334
LM16	0.702002222	0.228095619	LW16	0.353884615	0.228095619
LM17	0.415748889	0.228095619	LW17	0.415748889	0.228095619

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LM18	0.415748889	0.416246506	LW18	0.415748889	0.416246506
LM19	0.129495556	0.369208784	LW19	0.845128889	0.369208784
LM20	0.129495556	0.306491822	LW20	0.272622222	0.306491822
LM21	0.129495556	0.458634323	LW21	0.415748889	0.458634323
LM22	0.415748889	0.348283459	LW22	0.129495556	0.348283459
LM23	0.129495556	0.395576686	LW23	0.272622222	0.395576686
LM24	0.702002222	0.371930073	LW24	0.415748889	0.371930073
LM25	< 0.01	0.52169196	LW25	0.415748889	0.52169196
LM26	< 0.01	0.31675464	LW26	< 0.001	0.31675464
LM27	< 0.01	0.482280937	LW27	< 0.001	0.482280937
LM28	< 0.01	0.395576686	LW28	< 0.001	0.395576686
LM29	< 0.01	0.442869914	LW29	< 0.001	0.442869914
LM30	< 0.01	0.308872436	LW30	< 0.001	0.308872436

REFERENCES

Akter, S., Vehniainen, M., Meriluoto, J., Spoof, L. & Lamminmaki, U. 2017. Non-competitive ELISA with broad specificity for microcystins and nodularins. *Advances in Oceanography and Limnology* **8**(1), 121–130.

Azevedo, S.M., Carmichael, W.W., Jochimsen, E.M., Rinehart, K.L., Lau, S., Shaw, G.R. & Eaglesham, G.K. 2002. Human intoxication by microcystins during renal dialysis treatment in Caruaru-Brazil. *Toxicology* **181-182**, 441-446.

Anderson, D. 2014. HABs in a changing world: a perspective on harmful algal blooms, their impacts, and research and management in a dynamic era of climatic and environmental change. *Harmful Algae* **2012**(2012), 3-17.

Backer, L.C., Carmichael, W., Kirkpatrick, B., Williams, C., Irvin, M., Zhou, Y., Johnson, T.B., Nierenberg, K., Hill, V.R., Kieszak, S.M. & Cheng, Y. 2008. Recreational exposure to low concentrations of microcystins during an algal bloom in a small lake. *Marine Drugs* **6**(2), 389-406.

Backer, L.C., Landsberg, J.H., Miller, M., Keel, K. & Taylor, T.K. 2013. Canine cyanotoxin poisonings in the United States (1920s-2012): review of suspected and confirmed cases from three data sources. *Toxins* **5**(9), 1597-1628.

Backer, L.C., Manassaram-Baptiste, D., LePrell, R. & Bolton, B. 2015. Cyanobacteria and algae blooms: review of health and environmental data from the harmful algal-bloom related surveillance system (HABISS) 2007-2011. *Toxins* **7**(4), 1048-1064.

Baralla, E., Varoni, M., Sedda, T., Pasciu, V., Floris, A. & Demontis, M. 2017. Microcystins presence in mussels (*M. galloprovincialis*) and water of two productive Mediterranean's lagoons (Sardina, Italy). *BioMed Research International* **2017**, 3769245.

Bigham, D.L., Hoyer, M.V. & Canfield, D.E. 2009. Survey of toxic algal (microcystin) distribution in Florida lakes. *Lake & Reservoir Management* **25**, 264-275.Burns, J. (2008). Toxic cyanobacteria in Florida waters. *Advances in Experimental Medicine & Biology* **619**, 127-137.

Carmichael, W.W. 1992. Cyanobacteria secondary metabolites – the cyanotoxins. *Journal of Applied Microbiology* **72**, 445-459.

Carmichael, W.W. & Boyer, G.L. 2016. Health impacts from cyanobacteria harmful algae blooms: implications for the North American Great Lakes. *Harmful Algae* **54**(2016), 194-212

Chen, L., Chen, J., Zhang, X. & Xie, P. 2016. A review of reproductive toxicity of microcystins. *Journal of Hazardous Materials* **301**, 381-399.

Cheung, M.Y., Liang, S. & Lee, J. 2013. Toxin-producing cyanobacteria in freshwater: a review of the problems, impact on drinking water safety, and efforts for protecting public health. *Journal of Microbiology* **51**(1), 1-10.

Christophoridis, C., Zervou, S., Manolidi, K., Katsiapi, M., Moustaka-Gouni, M., Kaloudis, T., Triantis, T.M. & Hiskia, A. 2018. Occurrence and diversity of cyanotoxins in Greek lakes. *Scientific Reports* **8**(1), 17877.

Codd, G.A., Morrison, L.F. & Metcalf, J.S. 2005. Cyanobacterial toxins: risk management for health protection. *Toxicology & Applied Pharmacology* **203**(3), 264-272.

Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T. & Thornbrugh, D.J. 2009. Eutrophication of U.S. freshwaters: analysis of potential economic damages. *Environmental Science & Technology* **43**(1), 12-19.

Downing, T. G., Meyer, C., Gehringer, M. M., van de Venter, M. 2005. Microcystin content of Microcystis aeruginosa is modulated by nitrogen uptake rate relative to specific growth rate or carbon fixation rate. *Environmental Toxicology* **20**(3), 257–262.

Eriksson, J.E., Grönberg, L., Nygård, S., Slotte, P. & Meriluoto, J.A.O. 1990. Hepatocellular uptake of ^{3}H-dihydromicrocystin-LR, a cyclic peptide toxin. *Biochimica et Biophysica Acta* **1025**, 60-66.

Fawell, J.K., Mitchell, R.E., Everett, D.J. & Hill, R.E. 1999. The toxicity of cyanobacterial toxins in the mouse: I microcystin-LR. *Human & Experimental Toxicology* **18**(3), 162-167.

Foss, A.J., Aubel, M.T., Gallagher, B., Mettee, N., Miller, A. & Fogelson, S.B. 2019. Diagnosing microcystin intoxication of canines: clinicopathological indications, pathological characteristics, and analytical detection in postmortem and antemortem samples. *Toxins* **11**(8), 456.

Funari, E. & Testai, E. 2008. Human health risk assessment related to cyanotoxins exposure. *Critical Reviews in Toxicology* **38**(2), 97-125.

Guo, Y.C., Lee, A.K., Yates, R.S., Liang, S. & Rochelle, P.A. Analysis of microcystins in drinking water by ELISA and LC/MS/MS. *Journal of the American Water Works Association* **109**(3), 13-25.

Heinze, R. 1999. Toxicity of the cyanobacterial toxin microcystin-LR to rats after 28 days intake with the drinking water. *Environmental Toxicology* **14**(1), 57-60.

Heisler, J., Glibert, P., Burkholder, J., Anderson, D., Cochlan, W., Dennison, W., Gobler, C., Dortch, Q., Heil, C., Humphries, E., Lewitus, A., Magnien, R., Marshall, H., Sellner, K., Stockwell, D., Stoecker, D., & Suddleson, M. 2008. Eutrophication and harmful algal blooms: a scientific consensus. *Harmful Algae*, **8**(1), 3–13.

Heussner, A.H., Mazija, L., Fastner, J. & Dietrich, D.R. 2012. Toxin content and cytotoxicity of algal dietary supplements. *Toxicology and Applied Pharmacology* **265**(2), 263-271.

Hu, M., Qu, X., Pan, L., Fu, C., Jia, P., Liu, O. & Wang, Y. 2017. Effects of toxic Microcystis aeruginosa on the silver carp Hypophthalmichyts molitrix revealed by hepatic RNA-seq and miRNA-seq. *Scientific Reports* **7**(10456).

Kimambo, O.N., Gumbo, J. & Chikoore, H. 2019. The occurrence of cyanobacteria blooms in freshwater ecosystems and their link with hydro-meteorological and environmental variations in Tanzania. *Heliyon* **5**(3).

Kleintech, J., Puddick, J., Wood, S.A., Hildebrand, F., Laughinghouse IV, H.D., Pearce, D.A., Dietrich, D.R. & Wilmotte, A. Toxic cyanobacteria in Svaldbard: Chemical diversity of microcystins detected using a liquid chromatography mass spectrometry precursor ion screening method. *Toxins* **10**(4), 147.

Lawton, L.A. & Robertson, P.K. 1999. Physico-chemical treatment methods for the removal of microcystins (cyanobacterial hepatotoxins) from potable waters. *Chemical Society Reviews* **28**, 217-224.

Lévesque, B., Gervais, M.C., Chevalier, P., Gauvin, D., Anassour-Laouan-Sidi, E., Gingras, S., Fortin, N., Brisson, G., Greer, C. & Bird, D. 2014. Prospective study of acute health effects in relation to exposure to cyanobacteria. *Science of the Total Environment* **466-467**, 397-403.

Lone, Y., Koiri, R.K. & Bhide, M. 2015. An overview of the toxic effect of potential human carcinogen microcystin-LR on testis. *Toxicology Reports* **2**, 289-296.

Lu, J., Zhu, B., Struewing, I. & Xu, N. 2019. Nitrogen–phosphorus-associated metabolic activities during the development of a cyanobacterial bloom revealed by metatranscriptomics. *Scientific Reports* **9**, 2480.

Massey, I.Y., Wu, P., Wei, J., Luo, J., Ding, P., Wei, H. & Yang, F. A mini-review on detection methods of microcystins. *Toxins* **12**(10), 641.

McCarthy, M.J., James, R.T., Chen, Y., East, T.L. & Gardner, W.S. 2009. Nutrient ratios and phytoplankton community structure in the large, shallow, eutrophic, subtropical Lakes Okeechobee (Florida, USA) and Taihu (China). *Limnology* **10**, 215-227.

McElhiney, J. & Lawton, L.A. 2005. Detection of the cyanobacterial hepatotoxins microcystins. *Toxicology & Applied Pharmacology* **203**(3), 219-230.

Mez, K., Beattie, K.A., Codd, G.A., Hanselmann, K., Hauser, B., Naegeli, H. & Preisig, H.R. 1997. Identification of a microcystin in benthic cyanobacteria linked to cattle deaths on alpine pastures in Switzerland. *Journal of Phycology* **32**, 111-117.

Monchamp, M.E., Pick, F.R., Beisner, B.E., & Maranger, R. 2014. Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. *PloS one*, **9**(1), e85573.

Oh, H.M., Lee, S.J., Kim, J.H., Kim, H.S., & Yoon, B.D. 2001. Seasonal variation and indirect monitoring of microcystin concentrations in Daechung reservoir, Korea. *Applied and environmental microbiology*, **67**(4), 1484–1489.

Oliveira, E.D.C., Castelo-Branco, R., Silva, L., Silva, N., Azevedo, J., Vasconcelos, V., Faustino, S. & Cunha, A. 2019. First detection of microcystin-LR in the Amazon River at the drinking water treatment plant of the municipality of Macapá, Brazil. *Toxins* **11**(11), 669.

O'Neil, J.M., Davis, T.W., Burford, M.A., Gobler, C.J. 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. *Harmful Algae* **14**. 313-334.

Ortiz, X., Korenkova, E., Jobst, K.J., MacPherson, K.A. & Reiner, E.J. 2017. A high throughput targeted and non-targeted method for the analysis of microcystins and anatoxin-A using on-line solid phase extraction coupled to liquid chromatography-quadrupole time-of-flight high resolution mass spectrometry. *Analytical and Bioanalytical Chemistry* **409**(21), 4959–4969.

Paerl, H.W., Hall, N.S., & Calandrino, E.S. 2011. Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *The Science of the Total Environment*, **409**(10), 1739–1745.

Parker C.H., Stutts W.L. & DeGrasse S.L. 2015. Development and validation of a liquid chromatography-tandem mass spectrometry method for the quantitation of microcystins in blue-green algal dietary supplements. Journal of Agricultural and Food Chemistry **63**(47),10303–10312.

Qian, Z.Y., Li, Z.G., Ma, J., Gong, T.T. & Xian, Q.M. 2017. Analysis of trace microcystins in vegetables using matrix solid-phase dispersion followed by high performance liquid chromatography triple quadrupole mass spectrometry detection. *Talanta* **173**, 101–106.

Rastogi, R.P., Madamwar, D. & Incharoensakdi, A. 2015. Bloom dynamics of cyanobacteria and their toxins: environmental health impacts and mitigation strategies. *Frontier in Microbiology* **6**(1254).

Robillot, C. & Hennion, M.C. 2004. Issues arising when interpreting the results of the protein phosphatase 2A inhibition assay for the monitoring of microcystins. *Analytica Chimica Acta* **512**, 339-346.

Sakai, H., Hao, A., Iseri, Y., Wang, S., Kuba, T., Zhang, Z. & Katayama, H. 2013. Occurrence and distribution of microcystins in Lake Taihu, China. *The Scientific World Journal* **2013**(1), 838176.

Schmale, D.G., Ault, A.P., Saad, W., Scott, D.T. & Westrick, J.A. 2019. Perspectives on harmful algal blooms (HABs) and the cybersecurity of freshwater systems. *Frontiers in Bioengineering & Biotechnology* **7**(128).

Sedan, D., Laguens, M., Copparoni, G., Aranda, J.O., Giannuzzi, L., Marra, C.A. & Andrinolo, D. 2015. Hepatic and intestine alterations in mice after prolonged exposure to low oral doses of microcystin-LR. *Toxicon* **104**, 26-33.

Shamsollahi, H.R., Alimohammadi, M., Nabizadeh, R., Nazmara, S. & Mahvi, A.H. 2014. Measurement of microcystin-LR in water samples using improved HPLC method. *Global Journal of Health Science* **7**(2), 66-70.

Smith, V.H., Joye, S.B., Howarth, R.H. 2006. Eutrophication of freshwater and marine ecosystems. *Limnology and Oceanography* **51**(1), 351-355.

Stewart, I., Webb, P.M., Schluter, P.J. & Shaw, G.R. 2006. Recreational and occupational field exposure to freshwater cyanobacteria – a review of anecdotal and case reports, epidemiological studies and challenges for epidemiological assessment. *Environmental Health* **5**(6).

Szlag, D.C., Sinclair, J.L., Southwell, B. & Westrick, J.A. 2015. Cyanobacteria and cyanotoxins occurrence and removal from five-high-risk conventional treatment drinking water plants. *Toxins* **7**(6), 2198-2220.

Turner, A.D., Dhanji-Rapkova, M., O'Neill, A., Coates, L., Lewis, A. & Lewis, K. 2018. Analysis of microcystins in cyanobacterial blooms from freshwater bodies in England. *Toxins* **10**(1), 39.

Ueno, Y., Nagata, S., Tsutsumi, T., Hasegawa, A., Watanabe, M.F., Park, H.D., Chen, G.C. &

USEPA. 2015. *Recommendations for Public Water Systems to Manage Cyanotoxins in Drinking Water*. Office of Water, U.S. Environmental Protection Agency, Washington, DC. EPA 815-R-15-010

Valerio, E. & Vasconcelos, V., Campos, A. 2016. New insights on the mode of action of microcystins in animal cells – a review. *Mini-Reviews in Medicinal Chemistry* **16**(13), 1032-1041.

Watson, S.B., Whitton, B.A., Higgins, S.A., Paerl., H.W., Brooks, B. & Wehr, J.D. 2015. Harmful algal blooms: In Wehr, J.D., Robert, G., Sheath, R.G. & Kociolek, J.P. (eds.), Freshwater Algae of North America. Academic Press, San Diego, California. 873-920.

Williams, C.D., Aubel, M.T., Chapman, A.D. & D'Aiuto, P.E. 2007. Identification of cyanobacterial toxins in Florida's freshwater lakes. *Lake & Reservoir Management* **23**(2), 144-152.

Wood, R. 2016. Acute animal and human poisonings from cyanotoxin exposure – a review of the literature. *Environment International* **91**, 276-282.

WHO. 2003. *Guidelines for Safe Recreational Water Environments*. Volume 1, Coastal and fresh waters. World Health Organization, Geneva, Switzerland.

Xia, X., Zhang, S., Li, S., Zhang, L., Wang, G., Zhang, L., Wang, J. & Li, Z. The cycle of nitrogen in river systems: sources, transformation, and flux. *Environmental Science: Processes & Impacts* **20**(6).

Yang, X.E., Wu, X., Hao, H.L. & He, Z.L. 2008. Mechanisms and assessment of water eutrophication. *Journal of Zhejiang University Science. B* **9**(3), 197–209.

Yu, S.Z. 1996. Detection of microcystins, a blue-green algal hepatotoxin, in drinking water sampled in Haimen and Fusui, endemic areas of primary liver cancer in China, by highly sensitive immunoassay. *Carcinogenesis* **17**(6), 1317-1321.

Yu, F., Liu, B., Chou, H. & Chu, F. 2002. Development of a sensitive ELISA for the determination of microcystins in algae. *Journal of Agricultural and Food Chemistry* **50**(15), 4176-4182.

Zhang, F., Lee, J., Liang, S. & Shum, C.K. 2015. Cyanobacteria blooms and non-alcoholic liver disease: evidence from a county level ecological study in the United States. *Environmental Health* **14**, 41.

Zheng, C., Zeng, H., Lin, H, Wang, J., Feng, X., Qiu, Z., Chen, J.A., Luo, Y., Huang, Y., Wang, L., Liu, W., Tan, Y., Xu, A., Yao, Y. & Shu, W. 2017. Serum microcystin levels positively linked with risk of hepatocellular carcinoma: a case-control study in southwest China. *Hepatology* **66**(5), 1519-1528.