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Harmful Algae Bloom Occurrence in Urban Ponds: Relationship of Toxin Levels with Cell Density and Species Composition

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Abstract

Retention ponds constructed within urban watershed areas of high density populations are common as a result of green infrastructure applications. Several urban ponds in the Northern Kentucky, USA area were monitored for algal community (algae and cyanobacteria) from October 2012 to September 2013. Many of the harmful algal blooms observed during this study were composed primarily of the cyanobacteria genus, *Microcystis.* No correlations were observed between basic water quality parameters (dissolved oxygen, pH, conductivity, temperature, nitrate and soluble reactive phosphate) and the presence of cyanobacteria and/or microcystin cyanobacterial toxin levels. Furthermore, levels of microcystin toxins did not always coincide with high *Microcystis* cell counts. Harmful algal blooms in small urban ponds are common which pose risk to human and ecological health due to proximity of dense human population including pets and wild animals. Because harmful algal blooms were detected throughout the year in this study, adaptation of universal guidelines for the design, construction and maintenance of urban ponds may be necessary to protect watershed aquatic ecosystems, and lower health risks from exposure to such harmful blooms.

Keywords

Cyanobacteria; Harmful Algal Bloom; Microcystin; *Microcystis*; Urban ponds

Introduction

Urbanization can be a major contributor to the eutrophication of aquatic ecosystems within a watershed area. Agricultural runoff, wastewater discharge, and storm water runoff often carry excess nutrients into the urban watershed and create conditions in which algae and

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Ethics Statement
No specific permissions were required for the activities performed at the locations chosen for this study because they are all publicly accessible sites. No field study involved endangered or protected species.

cyanobacteria (both will be referred to as "algae") will thrive [1–3]. Urban ponds containing high levels of algae can lead to "nuisance blooms" which can produce foul odors, disrupt the scenery and develop into harmful blooms containing dangerous toxin (harmful algal blooms, HABs).

HABs readily occur in shallow, warm, eutrophic waters and have been reported world-wide [4]. In the Ohio/Kentucky, USA region especially in the Great Lakes, Grand Lake St. Mary's and the Ohio River, HABs have been commonly reported [5–7]. Although aquatic ecosystems, which are important tourist attractions and/or drinking water sources, receive the most attention, many individuals may also come into almost daily contact with small ponds in their immediate neighborhoods. There is a need for documentation of the occurrence and types of harmful algal and cyanobacterial blooms in small pond ecosystems commonly found in urban watersheds.

Artificial ponds constructed within areas of high density populations are commonplace. A majority of consumers in an urban watershed would pay more for property located in a neighborhood with storm water control structures designed to enhance fish and wildlife use (NAICHI.org). Although many metropolitan areas incorporate local urban storm water ponds in accordance with green infrastructure (GI) applications (which are beneficial in flood control) these same GI sites may pose environmental and human health risks due to biological and chemical contamination, and as breeding grounds for insects and other animals [8].

In one of the few studies concerning small pond ecosystems within an urban watershed, Lewitus et al. [9] reported the occurrence of HABs in a study of 1,500 ponds located along the South Carolina, USA coast. Their study suggested that the incorporation of residential and golf course retention ponds is a common management practice intended to reduce impacts of pollutants (sewage, fertilizer, pesticides, herbicides, etc.) on the urban watershed, but these same local aquatic ecosystems may also inadvertently create an ideal environment for HABs. In another study of 3,500 Dutch eutrophic urban ponds, highly toxic cyanobacterial blooms were reported with microcystin toxin levels as high as 64,000 ppb in scums, and 77 ppb in water [10]. Previous studies have also associated harmful blooms being "triggered" by relatively high temperatures, alkaline pH and nutrient enrichment [11].

Additional studies are needed to determine the extent of exposure to harmful toxins from HABs occurring right in the backyards of residents within an urban watershed. Illnesses caused by exposure to cyanobacteria are difficult to diagnose, and therefore the number of reported cases may be lower than what actually occurs [1,12]. Acute exposure may cause gastrointestinal problems, dermatitis, liver failure, and even death, but chronic exposures could possibly lead to conditions such as asthma, allergic sensitivities, neurological distress, kidney damage or liver tumors [3,13–15].

Abiotic data are often insufficient for predicting the variability often found within cyanobacterial blooms. Biotic factors such as the presence of heterotrophic bacterioplankton and protists are emerging as being highly influential to the make-up of the phytoplanktonic communities giving rise to blooms [16]. It remains difficult to predict the onset, duration and intensity of blooms in both large and small freshwater ecosystems due to the lack of knowledge concerning the processes leading to the dominance of any one cyanobacterial species. There is also a paucity of studies which document all of the cyanobacterial participants before, during and after bloom events which occur in small freshwater ponds [17].

In this study, we report both the occurrence of algal blooms and the percentage of all cyanobacteria genera present in individual algal bloom monitored in five small ponds constructed in urban developments and recreational areas in the Northern Kentucky area. Correlations of water quality parameters with the presence of cyanobacteria and total microcystin toxin levels were also examined. *Microcystis aeruginosa* was found to dominate the communities in the ponds during the study. *Microcystis* can successfully compete against other photosynthetic organisms because of its ability to migrate to different depths within the water column [18]. *Microcystis* is also capable of survival at higher pH and conductivity levels [12] commonly found in waters of the Northern Kentucky area (due to the high mineral concentrations.

Results

During the 32 sampling events reported in this study, 24 (75%) of the samples met World Health Organization (WHO) guidelines for recreational water harmful bloom (cyanobacterial counts ≥ 20,000 cells mL^{-1}) [19]. See (Tables 1 and 2) for a characterization of the 32 sampling events and sites. (Figure 1) shows the sampling sites. For comparison purposes, the sampling events are listed according to the level of microcystin toxin detected and not based on location. Twenty-one (21) of the 32 sampling events (66%) met WHO guidelines for recreational water HABs (microcystin level ≥ 4 ppb) (Table 1).

Twenty-five (25) of the 32 sampling events (78%) were dominated by *Microcystis* spp. (at least 90% of total cells present were *Microcystis* spp.). Of the 25 samples dominated by *Microcystis* spp., 18 occurred with levels of microcystin detected at > 4 ppb (72% would be classified by WHO as HABs, 28% would not be classified as HABs). Sample #21 (Glen Arbor Upper) contained only 34% and 10.7% *Microcystis* and *Anabaena* spp., respectively, but did show a level of > 4 ppb microcystin which would meet the WHO guidelines to be classified as a HAB. The algae communities found in sampling events #24, #26 and #30 had Microcystis spp. levels at 21%, 54% and 41%, respectively. Despite the presence of Microcystis, all of these samples yielded very low levels of microcystin concentrations.

Two sampling events yielded no (zero) *Microcystis* spp. (#4 listed in Table 1, and #28 listed in Table 2). Very low level of micro cyst in toxin (0.4 ppb) was detected in sample #28 although the cyanobacteria *Planktothrix* sp. was also detected in this sample and could have produced toxin. In contrast, sample #4 collected on June 2013 (Valeside Pond) was described as “a scummy and dark green colored water” Pandorina/Volvox were readily observed and dominated in this sample (no *Microcystis* spp. were seen microscopically) with 577.67 ppb microcystin toxin level. Fifteen (15) of the 21 HABs (71%) monitored in the study also met the “High Probability of Acute Health Effects” according to WHO recreational water guidelines due to the microcystin concentrations > 20 ppb. However, during this study there were no samples containing *Microcystis* spp. counts higher than 100,000 cells mL^{-1}, and there was no noticeable evidence of fish or animal deaths in the sampling areas.

The concentrations of microcystins in samples taken from the urban ponds in this survey varied day to day and month to month. Although 13 of 21 (62%) sampling events yielding HAB levels of microcystin toxin (> 10 ppb) were documented in the three summer months of June, July and August, there were no readily discernable patterns exhibited by the microcystin concentrations over time and seasons (Tables 1 and 2). HABs were detected year-round in multiple ponds with the most toxic bloom occurring at Loch Norse in January 2013 (1,286 ppb microcystin) when the water temperature was at 3.3°C. HABs were also detected at Redbud in October 2012 and at Loch Norse on March 2013 during cooler temperatures (Figure 2). In this study, there were no significant correlations found between the levels of microcystin toxin and either conductivity ($R^2 = 0.0436$, Figure 3), or temperature ($R^2 = 0.0000153$, Figure 4). No significant correlations were also noted with *Microcystis* spp. with nitrate (R^2=0.001) and soluble phosphate (R^2=0.0043).

Discussion

The WHO recreational water guidelines [19] for algal blooms do not address the types of species involved in the blooms, and not all algal blooms are toxic. In harmful algal blooms, a mixture of toxic and non-toxic species of cyanobacteria can occur [20]. Documentation of the type(s) of species occurring within a bloom may therefore be necessary to properly inform citizens about the possibility of toxins being present. Taxonomic classification of cyanobacteria is currently undergoing changes. Microscopic identification of the composition of a bloom can be erroneous. WHO states that counts greater than 20,000 cells mL^{-1} are necessary to classify a bloom as low health risk [21]. However, three samples (#s 10, 14 and 21) containing < 20,000 cells mL^{-1} had microcystin levels and general condition of the pond suggesting a HAB. An example of this was seen in the results reported for sampling event #4 (obvious bloom, no *Microcystis* spp. observed microscopically, high levels of microcystin detected). One possibility could be that *Microcystis* cells may have been present previous to the sampling time but had either lysed or migrated away by the time of the sampling. It is also possible that other microcystin-producing genera or species may have been present. Results such as these would suggest that the WHO recreational water guidelines should be expanded to include the monitoring of toxins other than microcystin.

The results of our study suggest that algal blooms are quite common in small ponds constructed in urban development and recreational areas. Seventy-five percent of the sampling events exhibited cyanobacterial cell counts greater than the WHO recreational water guidelines. These blooms were detected throughout the year (even in adverse cold conditions, usually not associated with harmful cyanobacterial blooms). Sixty-six percent of the blooms also exhibited high levels (> 4 ppb) of microcystin toxin).

Because some of the HABs occurred in our study of small urban ponds during periods of cold weather, it is possible that urban HABs could pose a year-round hazard (not just in the warmer summer months). *Microcystis* is known to produce higher concentrations of toxin at lower temperatures in the laboratory [22], but to our knowledge this has not been studied in an environmental setting. Cells of *Microcystis* spp. dominated the blooms observed during the study. However, high microcystin levels were also found in the absence of *Microcystis* dominance.

Unlike larger bodies of water, our results did not establish a correlation between microcystin toxin levels and other water quality parameters measured in this study. There were no significant correlations between toxicity and either pH or dissolved oxygen (data not shown). The natural levels of nitrate in water is < 1 mg L^{-1}. Nitrate levels in these ponds ranged from 0.6 – 70 mg L^{-1} (mean = 2.094, median = 1.2, mode = 1.03) and is not a limiting factor in the growth of cyanobacteria on these ponds. Toxic blooms of *M. aeruginosa* had been reported in nitrogen-limited conditions [23–27]. Soluble reactive phosphate level in these ponds are low (range = 0 – 3.0 μg L^{-1}, mean = 0.21, median 0.20; data not shown) and showed no correlation with *Microcystis* spp. cell count and total microcystin level (R^2 = 0.0043 and 0.0285, respectively; data not shown).

Despite efforts to control phosphate in aquatic environments, harmful blooms can still occur [28]. In this study, soluble phosphate was not associated with the number of cells and microcystin level. *Microcystis* spp. can reabsorb phosphate and store polyphosphate granules as reserve [29]. Previous studies reported contradictory effects of phosphate with *Microcystis* growth and toxin production [30–32]. Water quality parameters including nutrients may not be associated with *Microcystis* spp. and microcystin toxins in small artificial urban ponds since the hydrogeologic conditions (physical-chemical parameters) and biotic conditions are different compared with bigger bodies of water like lakes and rivers. Small urban ponds may not follow the same ecological patterns as larger natural bodies of water [33,34].

The small artificial ponds in our study, unlike lakes and rivers where blooms appear mostly in the summer, do not have much sediment build-up to act as nutrient sinks. The ponds where heavy blooms occurred in our study also contained little to no buffer zones whereas the ponds that had lily pads, trees, grasses and animal life had less severe harmful bloom events. This is most likely due to the plants' effective removal of nutrients from the water.

Harmful algal blooms are common in urban ponds built in urban developments and recreational areas. Our study shows that the ponds have harmful algal blooms year-round, becoming a health hazard and economic strain to residents. Unlike larger natural bodies of water, common water quality parameters used as indicators of harmful algal blooms might need reassessment when applying them to urban ponds. Guidelines about proper depth, plant buffer zones and maintenance procedures for the design/construction of urban ponds may be necessary to protect urban aquatic ecosystems from the possible occurrence of harmful algal blooms [35].

Materials and Methods

Study Sites

Sites were selected by availability to the public, proximity to homes, or suggested by concerned residents. A pond was defined as having surface area less than five acres, or 20,234 m^2 [9]. The study sites are shown in (Figure 1) and briefly described below.

Loch Norse (39°01’52.64’N, 84°27’46.65’W)

Loch Norse is a renovated farm pond in Highland Heights, KY, USA, located in the center of the campus of Northern Kentucky University (NKU). The pond is shallow and aerated by an artificial waterfall. The pond is divided into two sections, with the northern perimeter surrounded by concrete, and the southern perimeter surrounded by cattails, bulrushes and water iris. The pond contains goldfish, and both ducks and geese nest in the surroundings. Loch Norse has been monitored for many years by one of us (MSK) and has recurrent algal blooms. NKU grounds-keeping staff treats the water with copper sulfate and “AquaShade®” pond dye twice a year.

Redbud Pond (39°01’52.64’N, 84°27’46.65’W)

Redbud Pond is a man-made retention pond located in a residential subdivision. Residents routinely report blooms that create foul odors and unsightly conditions in the summer, along the perimeter of the pond and in the residents’ back yards. The perimeter is lined with grasses, cattails and bulrushes. The pond is aerated by three pumps. Grass carp and bluegill are seen frequently in the shallows. There are “no fishing” signs along the shore of the pond.

Glen Arbor Ponds (upper; 39°00’17.58’N, 84°40’06.08’W)

These are two ponds located in a local public golf course. The northernmost pond was designated as “Glen Arbor Upper Pond,” while the southern pond was designated “Glen Arbor Lower Pond”. Golfers routinely reported algae blooms producing foul odors in the Glen Arbor Upper Pond in July 2013. Sampling occurred regularly, with verbal permission from the golf course manager. Other than manicured turf, no plants surround the ponds. The ponds are not aerated, but are connected by a small waterfall.

Valeside Pond (38°58’35.09’N 84°31’22.81’W) and Crystal “Lake” (38°58’59.40”N, 84°31’24.50’W)

These are small retention ponds located in the same residential subdivision. Valeside Pond is surrounded by trees, cattails and various grasses. It slopes off quickly near the shore. Crystal “Lake” is at the entrance of the subdivision. There is very little turf and no other plants surrounding the pond. The ponds are not aerated and the backyards of several homes are aligned along their perimeter.

Monitoring and Plankton Collection

For this study, ponds were sampled from a designated area for each site regardless of whether a bloom was observed or not (Table 1). From October 2012 to September 2013, dissolved oxygen (DO), pH, conductivity, temperature and nitrate were monitored using a YSI Multiparameter Water Quality Instrument (Profession Plus model; Yellow Springs, OH, USA). Phosphate level was also determine using Hach Total Phosphate Kit according to the manufacturer (Hach Company, Loveland, CO, USA). Whole water samples were collected just below the surface using a Van Dorn horizontal 2.2 L water sampler. Approximately 1.0 L of the sample was stored in amber glass bottles and transported back to the laboratory in a cooler for processing.

Cell Counts

Unpreserved whole water samples were immediately examined with a compound microscope for total algae diversity and dominant species. Some *Microcystis* lyse when preserved, so this immediate "Qualitative count" was necessary to assure accuracy when *Microcystis* was present. Lugol's iodine was then added to whole water samples then the samples were allowed to settle overnight. Cell counts were performed using an inverted microscope based on the EPA's Standard Operating Procedure (SOP) for Phytoplankton Analysis [36].

Total Microcystin Analysis

Two mL whole water samples were frozen at −20°C freezer, gently thawed in a warm water bath for 1–2 minutes, and frozen at −2°C again. This process was repeated twice in order to fully lyse the cells. The samples were then tested for the presence of total microcystin toxins (extracellular and intracellular), using the Envirologix™ ELISAkit (Envirologix QuantiPlate™ for Microcystins, Portland, ME, USA). The kit negative control was analyzed in six replicates and standards and test samples in triplicate. Per cent coefficient of variation were < 15%. To quantitate microcystins in a test sample, dilutions were prepared (2–3 dilutions) and analyzed in triplicate. Diluted test samples were within the standard curve linear range. If the optical density (with corresponding microcystin concentration) was below or above the linear range, sample dilution was readjusted and reanalyzed. Samples tested negative were reported as below detection limit. The Molecular Devices SpectraMax M2 spectrophotometer with Software Pro version 4.8 (Molecular Devices Corporation, Sunnyvale, CA, USA) was used to record the absorbance, calculated the mean, *%* CV, R^2 and generated graphs for the standard calibrators.

Determining if an Algal Bloom was a HAB

Although the US EPA has not established guidelines as to what level of cell counts constitute a bloom, the Agency does define a bloom as the "visible coloration of a water body due to the presence of suspended cells, filaments and/or colonies and, in some cases, subsequent surface scums [37]. The World Health Organization's guidelines are based upon both cyanobacterial cell numbers mL^{-1} and toxin levels (WHO 2003). Cell density ≥ 20,000 cells/mL is classified as harmful bloom (low risk), and if the microcystin concentration is ≥ 4 ng/mL (ppb), the bloom is considered a HAB [38,39].

References

1. Pearl HW, Fulton RS, Moisander PH, Dyble J (2001) Harmful fresh-water algal blooms, with an emphasis on cyanobacteria. The Scientific World 1: 76–113.
2. Anderson DM, Glibert PM, Burkholder JM (2002) Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25: 704–726.
3. de Figueiredo DR, Axeitero UM, Esteves SM, Goncalves FJM, Pereira MJ (2004) Microcystin-producing blooms-a serious global public health issue. Ecotoxicology and Environmental Safety 59: 151–163. [PubMed: 15327870]
4. Carmichael WM (2001) Health effects of toxin-producing cyanobacteria: “The CyanoHABs”. Human and Ecological Risk Assessment: An International Journal 7: 1393–1407.
5. Peterson E (2011) Algae bloom may be cause of Ohio River fish kill. WFPL News.
6. Koslow M (29 4 2013) Taken by Storm: How heavy rain is worsening algal blooms in Lake Erie. National Wildlife Federation.
7. Rex E (2014) Harmful algal blooms increase as lake water warms, Scientific American.
8. DeLorenzo ME, Thompson B, Cooper E, Moore J, Fulton MH (2012) A long-term monitoring study of chlorophyll, microbial contaminants, and pesticides in a coastal residential stormwater pond and its adjacent tidal creek. Environmental Monitoring and Assessment 184: 343–359. [PubMed: 21409361]
9. Lewitus AJ, Schmidt LB, Mason LJ, Kempton JW, Wild SB, et al. (2003) Harmful algal blooms in South Carolina residential and golf course ponds. Population and Environment 24: 387–413.
10. Waajen GW, Faassen EJ, Lürling M. (2014) Eutrophic urban ponds suffer from cyanobacterial blooms: Dutch examples. Environmental Science and Pollution Research 21: 9983–9994. [PubMed: 24798921]
11. Rahman S, Jewel AS (2008) Cyanobacterial blooms and water quality in two urban fish ponds. University Journal of Zoology, Rajshahi University 27: 79–84.
12. Guglielmi S, Rice A, Steinitz-Kannan M (2013) Collection and identification of potentially toxic and allergenic cyanobacteria from the Greater Cincinnati area. ASCE.
13. Genitsaris S, Kormas KA, Moustaka-Gouni M (2011) Airborne algae and cyanobacteria: Occurrence and related health effects. Frontiers in Bioscience 3: 772–787.
14. Bernstein JA, Ghosh D, Levin LS, Zheng S, Carmichael W, et al. (2011) Cyanobacteria: An unrecognized ubiquitous sensitizing allergen? Allergy and Asthma Proceedings 32: 106–110. [PubMed: 21439163]
15. Holtcamp W (2012) The emerging science of BMAA: Do cyanobacteria contribute to neurodegenerative disease? Environmental Health Perspectives 120: 110–116.
16. Louati I, Pascault N, Debroas D, Bernard C, Humbert JF, et al. (2015) Structural diversity of bacterial communities associated with bloom-forming freshwater cyanobacteria differs according to the cyanobacterial genus. PLoS ONE 10.
17. Woodhouse JN, Kinsela AS, Collins RN, Bowling LC, Honeyman GL, et al. (2016) Microbial communities reflect temporal changes in cyanobacterial composition in a shallow ephemeral freshwater lake. ISME Journal 10: 1337–1351. [PubMed: 26636552]
18. Brookes JD, Ganf GG (2001) Variations in the buoyancy response of *Microcystis aeruginosa* to nitrogen, phosphorus and light. Journal of Plankton Research 23: 1399–1411.
19. World Health Organization (2003) Guidelines for safe recreational water environments: Coastal and fresh waters. World Health Organization.
20. Neumann U, Campos V, Cantarero S, Urrutia H, Heinze R, et al. (2000) Co-occurrence of non-toxic (cyanopeptolin) and toxic (microcystin) peptides in a bloom of Microcystis sp. from a Chilean lake. Systematic Applied Microbiology 23:191–197. [PubMed: 10930070]
21. Graham JL, Loftin KA, Ziegler AC, Meyer MT (2008) Guidelines for design and sampling for cyanobacterial toxin and taste-and-odor studies in lakes and reservoirs. US Department of the Interior, US Geological Survey.

22. Davis TW, Berry DL, Boyer GL, Gobler CJ (2009) The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. Harmful Algae 8: 715–725.
23. Smith VH (1983) Low nitrogen to phosphorous ratios favor dominance by blue green algae in lake phytoplankton. Science 221: 669–671. [PubMed: 17787737]
24. Fujimoto N, Sudo R, Sugiura N, Inamori Y (1997) Nutrient-limited growth of *Microcystis aeruginosa* and *Phormidium tenue* and competition under various N:P supply ratios and temperatures. Limnology and Oceanography 42: 250–256.
25. Pearl HW, Paul VJ (2012) Climate change: Links to global expansion of harmful cyanobacteria. Water Research 46: 1349–1363. [PubMed: 21893330]
26. Monchamp ME, Pick FR, Beisner BE, Maranger R (2014) Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. PLoS ONE 9.
27. Parrish J (2014) The role of nitrogen and phosphorous in the growth, toxicity, and distribution of the toxic cyanobacteria, Microcystis aeruginosa Master's Project, Paper 8. The University of San Francisco, San Francisco, California, USA.
28. Pearl HW, Otten TG (2013) Harmful cyanobacterial blooms: Causes, consequences, and controls. Microbial Ecology 56: 995–1010.
29. Otten TG, Xu H, Qin B, Zhu G, Pearl HW (2012) Spatiotemporal patterns and ecophysiology of toxigenic *Microcystis* blooms in Lake Taihu, China: Implications for water quality management. Environmental Science and Technology 46: 3480–3488. [PubMed: 22324444]
30. Yoshida M, Yoshida T, Takashima Y, Hosoda N, Hiroishi S (2007) Dynamics of microcystin-producing and non-microcystin-producing *Microcystis* populations is correlated with nitrate concentration in a Japanese Lake. FEMS Microbiology Letters 266: 49–53. [PubMed: 17092296]
31. Ha JH, Hidaka T, Tsuno H (2009) Quantification of toxic *Microcystis* and evaluation of its dominance ratio in blooms using real-time PCR. Environmental Science and Technology 43: 812–818. [PubMed: 19245020]
32. Beversdorf L, Miller TR, McMahon KD (2013) The role of nitrogen fixation in cyanobacterial bloom toxicity in temperate eutrophic lake. PloS ONE 8.
33. Hassall C (2014) The ecology and biodiversity of urban ponds. WIREs Water 1: 187–206.
34. Hill MJ, Biggs J, Thornhill I, Briers RA, Gledhill DG, et al. (2017) Urban ponds as an aquatic biodiversity resource in modified landscapes. Global Change Biology 23: 986–999. [PubMed: 27476680]
35. de la Cruz AA, Hiskia A, Kaloudis T, Chernoff N, Hill D, et al. (2013) A review on cylindrospermopsin: The global occurrence, detection, toxicity and degradation of a potent cyanotoxin. Environmental Science: Processes and Impacts 15: 1979–2003.
36. USEPA. (2010) Standard operating procedure for phytoplankton analysis, revision 5.
37. Fristachi A, Sinclair J (2008) Occurrence of cyanobacterial harmful algal blooms workgroup report, In: Cyanobacterial Harmful Algal Blooms: State of the Science and Research Needs. Hudnell HK, Ed, Springer; New York, NY, USA, pp. 45–103, ISBN 978–0-387–75864-0.
38. Hunt S (2015) Heat, algae kill hundreds of fish at Grand Lake St. Mary's. Columbus Dispatch.
39. NAICHI.org Constructed Wetlands: The economic benefits of runoff controls.

Figure 1:

Study sites in Northern Kentucky with their corresponding GPS location (source: google maps). Water samples were collected on October 2012 to September 2013.

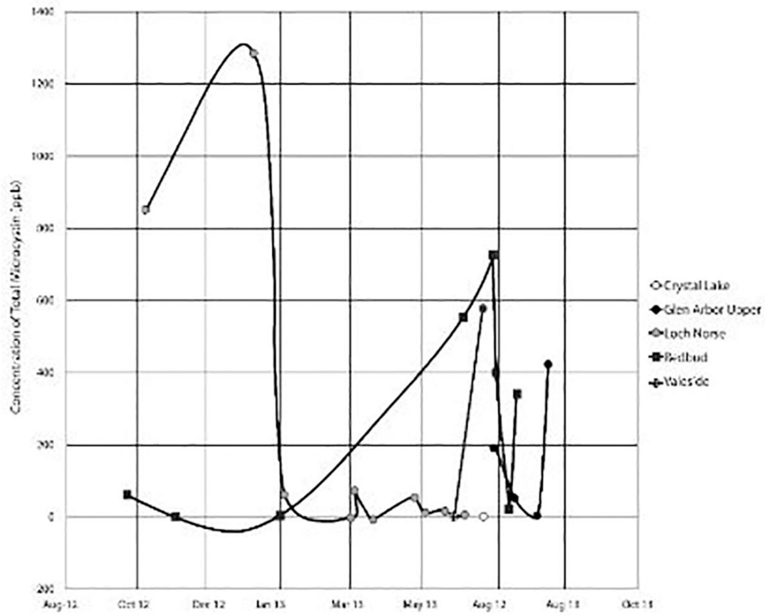

Figure 2:
Total microcystin level trends at five study sites from October 2012 to September 2013. There was no distinct pattern noted at the five different ponds.

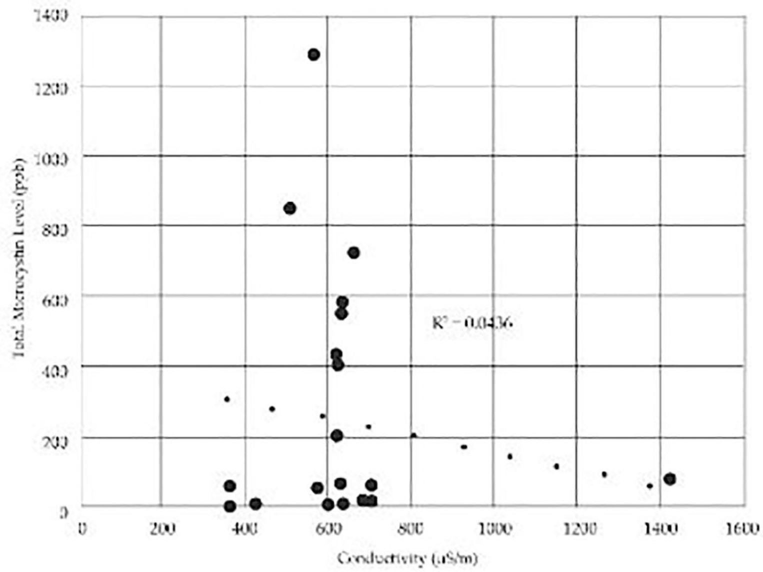

Figure 3:
The relationship of total microcystin level and water conductivity. No correlation was noted between microcystin toxin levels and water conductivity.

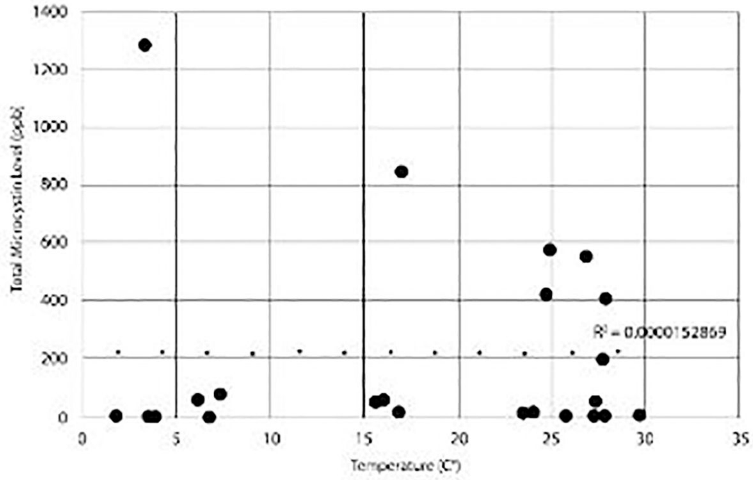

Figure 4:
Relationship of microcystin toxin levels and temperature. The total microcystin levels showed no correlation with the water temperatures.

Table 1:

Urban pond algal blooms in the Northern Kentucky area with high micro cysteine concentrations[a] (HABs).

Srl. No.	***Site***	**Sampling Date**	**Total Microcystin (ppb)**	***Microcystis* sp. Cell Count (cells mL^{-1})**	**%Composition of Species[b]**
1	*Loch Norse*	01-09-13	1286	29,301	97.3% *Microcystis* sp.
2	*Loch Norse*	10/25/2012	851	28,701	97.8% *Microcystis*sp.
3	*Redbud*	6/24/2013	724	57,402	99.4% *Microcystis*sp.
4	*Valeside*	6/18/2013	577.67	0	0.0 % *Microcystis* sp.
5					50.0% *Pandorina* sp.
6					50.0% *Pandorina* sp.
7	*Redbud*	06-05-13	552.55	30805	98.2% *Microcystis* sp.
8	*Glen Arbor U*	08-02-13	423.29	86103	99.3% *Microcystis*sp.
9	*Redbud*	6/26/2013	404.78	28641	97.8% *Microcystis* sp.
10	*Redbud*	07-10-13	340.68	28642	94.8% *Microcystis* sp.
11	*Glen Arbor U*	6/26/2013	195.15	26631	98.2% *Microcystis*sp.
12	*Loch Norse*	3/20/2013	74.79	13362	91.6% *Microcystis* sp.
13	*Loch Norse*	1/30/2013	59.26	29001	86.2% *Microcystis*sp.
14	*Redbud*	10-11-12	57.19	28701	95.7% *Microcystis*sp.
15	*Loch Norse*	4/30/2013	51.33	29001	98.9% *Microcystis*sp.
16	*Glen Arbor U*	07-10-13	48.75	13130	98.1% *Microcystis* sp.
17	*Redbud*	7/26/2013	28.11	28701	98.3% *Microcystis* sp.
18	*Redbud*	08-02-13	15.81	86103	99.3% *Microcystis* sp.
19	*Glen Arbor L*	08-02-13	15.28	29,001	97.0% *Microcystis* sp.
20	*Loch Norse*	5/21/2013	13.84	57402	100.0% *Microcystis*sp.
21	*Loch Norse*	05-07-13	11.81	57402	100.0% *Microcystis* sp.
22	*Loch Norse*	06-05-13	10.31	28701	98.6% *Microcystis* sp.
23	*Glen Arbor U*	7/25/2013	10.03	382	34.3% *Microcystis*sp.
					50.0% *Euglena* sp.
					10.7% *Anabaena*sp.
					4.6% *Aulacoseira* sp.

[a] ≥ 4 ppb microcystin [19], blooms listed according to microcystin concentration

[b] *Blooms usually dominated by* Microcystis*spp., minor components of community not listed unless comprising more than 4.0 % of community.*

Table 2:

Urban pond algal blooms in Northern Kentucky area with low microcystinconcentrations[a].

Srl.No.	Site	Date	Total Microcystin (ppb)	*Microcystis* spp. Cell Count (cells mL^{-1})	%Composition of Species[b]
1	Glen Arbor L	7/25/2013	3.75	28704	97.2% *Microcystis* sp.
2	Loch Norse	41339	2.17	28701	98.6% *Microcystis* sp.
3	Crystal	6/18/2013	1.76	478	21.3% *Microcystis* sp. 13.3% *Fragillaria* sp. 13.3% *Cryptomonas* sp. 4.0 % *Ankistrodesmus* sp. 48.1% Other
4	Redbud	1/28/2013	1.25	57115	98.6% *Microcystis* sp.
5	Valeside	5/29/2013	1.25	470	53.7% *Microcystis* sp. 13.8% *Cyclotella* sp. 13.2% *Aulacoseira* sp. 19.3% Other
6	Redbud	11/15/2012	0.98	86103	98.5% *Microcystis* sp.
7	Crystal	7/25/2013	0.4	0	0.0% *Microcystis* sp. 26.9% *Cyclotella* sp. 15.3%Oocystis sp. 12.1% *Planktothrix* sp. 45.7% Other
8	Loch Norse	3/18/2013	0.15	25387	94.0% *Microcystis* sp.
9	Crystal	5/29/2013	0.02	300	40.6% *Microcystis* sp. 14.3% *Cyclotella* sp. 14.2% *Ankistrodesmus* sp. 30.9% Other
10	Loch Norse	41278	0.01	28701	95.5% *Microcystis* sp.
11	Loch Norse	41222	0	28701	98.1% *Microcystis* sp.

[a] ≤ 4 ppb MC-LR [20], blooms listed according to microcystin concentration

[b] Blooms usually dominated by *Microcystis* spp., minor components of community not listed unless comprising more than 4.0 % of community