Nutrient Effects and the Toxicity of Harmful Microcystis Bloom

Submitted by

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Introduction:

Cyanobacteria have existed on Earth for approximately 3.5 billion years and are believed to be the first organisms capable of oxygenic photosynthesis. The phylum Cyanobacteria, formerly known as the blue-green algae, is diverse and occupies virtually all terrestrial and aquatic habitats. It is the *Microcystis* genera however, that are most problematic locally. The Great Lakes basin is a large watershed encompassing vast agricultural areas, large population centers, and heavily industrialized regions making it vulnerable to many different natural and manmade threats. One of the longstanding problems that has increased in severity are the occurrence of harmful algal blooms or HABs in the Western basin of Lake Erie. A HAB occurs when cyanobacteria dominate the lake ecosystem and grow to abnormally high densities. HABs have impacted the region's economy, tourism, recreation, and most importantly, drinking source water. There has been an increase in regulatory attention in recent years to combat these issues to control, mitigate, and abate the blooms and their causes.

To understand the scope of the problem as a whole, an understanding of why the Great Lakes, and especially Lake Erie is important for the Midwest. The Great Lakes contain 95 percent of the nation's fresh water. The lakes support one-seventh of the U.S. population, and two-fifths of U.S. industry (100th Congress). Lake Erie is a social, economic, and environmental hotspot of the Great Lakes Basin. Lake Erie is home to more consumable fish than all the other Great Lakes and supports multi-state and international economies generating over 12 billion dollars annually and over 100,000 jobs.

Lake Erie's Western Basin years has had a higher frequency of HABs in recent. The bloom are comprised primarily up of cyanobacteria, also known as blue-green algae. Cyanobacteria are photosynthetic and can be a pivotal part of the food web in the Great Lake's

ecosystem. Lake Erie provides habitat to many different organisms such as fish, birds, reptiles, including several endangered species. The problem with the HABs in the Western Basin of Lake Erie is that it is dominated by strains of *Microcystis* that are toxin producing (Carpenter). S stated above these HABs are very detrimental to the economy. The recent increase in blooms has negatively affected fishing, recreation, businesses, tourism, health, and most importantly has reduced public confidence in regulatory agencies and drinking water providers. Tuholske and Kilbert).

Some of the reasons why the cyanobacteria are specifically impacting Lake Erie is because of the geomorphology of the lake. Lake Erie is the shallowest and smallest in volume of all the Great Lakes. It was thought that the Great Lakes were so immense that they could assimilate almost any amount of nutrients added to them. The Great Lakes complete a cycle that cleanses themselves of pollutants in a certain period of time known as the residence time. Lake Erie completes this cycle in three years. The problem with Lake Erie is that pollutants are entering and exceeding the levels greater than the cycle can sustain (100th Congress). The nutrients that make their way into the lake create a 'perfect storm' when combined with the right conditions for the HABs to grow. The ideal conditions for bloom growth are water temperatures between 60-80 degrees Fahrenheit, elevated levels of nutrients (nitrogen and phosphorus), low or no flow, no turbulence to disrupt buoyancy, and high rainfall (Carpenter). When considering the Western Basin of Lake Erie these conditions emerge in late

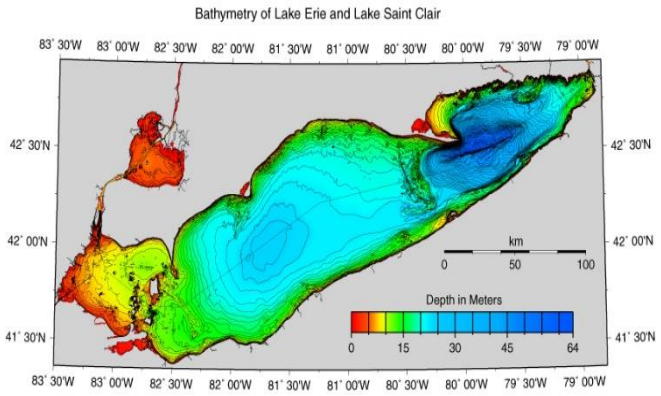


Figure 1: The Bathymetric Map of Lake Erie National Oceanic and Atmospheric Administration

summer when the temperature is in the correct range. The summer thunderstorms increase runoff, which increases the amount of nitrogen and phosphorous from the farms in the area. The Western Basin has little flow out and less mixing compared to other portions of Lake Erie. This makes for a prime environment for the *Microcystis* to grow excessively.

Humans affect the HABs by contributing to climate change, specifically the changing of weather conditions, and the land use change around Lake Erie's Western Basin (Tuholske and Kilbert). Human activities also appear to increase the toxicity of HABs. These toxins are ingested through drinking contaminated water and eating fish causing potential health issues. Skin contact when swimming or other recreational activities in a contaminated lake or stream can also cause health concerns. The target areas affected by the HABs are the liver, nervous system, and gastrointestinal system. The toxicity can either be acute or chronic, and studies have found a linkage between them and liver and colorectal cancers. The human health effects are the driving factor for research and regulation.

The cyanobacteria problem is not a new issue for the ecosystem. These organisms have been in Lake Erie since the last Ice Age, the major problem is the excessive growth and toxicity of the blooms. A major HAB event took place in 1931, when eight thousand people fell ill from drinking water from a tributary of the Ohio River (Carpenter). In the 1970's, Lake Erie was proclaimed dead because eutrophication. The lake had excessive amounts of nutrients from pipe discharges, air pollution, and land runoff causing it to become too fertile which led to excessive algae and *Microcystis* (101st Congress). The *Microcystis sp*. are believed to be native to the Great Lakes. It is hypothesized that nutrient enrichment, invasive zebra and quagga mussels, and elevated lake temperatures have created a "perfect storm" in Western Lake Erie

allowing *Microcystis* to proliferate explosively in late summer and produce high levels of the toxin known as microcystin.

Numerous studies have been conducted on *Microcystis aeruginosa* and *Microcystis viridis* to understand their specific growth and toxicity. Hakanson and associates (2006) studied nutrient uptake and noticed that phosphorous and nitrogen were the main contributors to both growth and toxicity. In 2010 Chen's study (2010) showed that nitrites can increase the toxicity of the cyanobacteria. Another study by Lei (2015) experimented on *Microcystis aeruginosa* by measuring and studying the growth and toxicity impacted by the factors temperature, light, and nitrogen and phosphorus levels. They found that high inputs of nutrients in the environment led to more toxic growth.

Recent researchers have mapped out the mechanism by which microcystin is produced by a non-ribosomal gene cluster consisting of a gene cassette including genes McyA-McyH. It should be noted that microcystin is not believed to be a defensive toxin and is known to be associated with photosynthesis and the thylakoid membrane. The consensus among researchers is that microcystin is produced by the cell to prevent oxidative stress, and that the amount produced is regulated by environmental factors. Polymerase Chain Reaction (PCR) primers have been developed for the genes in the cluster as well as qPCR probes for the McyB and McyE genes (Tillet et al. 2000). These techniques have been used in several field sampling campaigns. The results of these field sampling campaigns have shown that blooms can contain high levels of the McyB and McyE genes but not necessarily produce toxin. This further supports our hypothesis that environmental factors may play a key role in regulating toxin production.

Certain genera of cyanobacteria can proliferate in drinking water sources forming thick scums or blooms that under some conditions produce high concentrations of dangerous cyanotoxins. Recent massive and toxic blooms in Western Lake Erie have overwhelmed some utilities resulting in "Do Not Drink" advisories for the cities of Toledo in 2014 and in the city of Oregon, Ohio in 2013. The key question that must be answered is: what conditions control the concentrations of toxins produced by a *Microcystis* bloom? The 2015 *Microcystis* bloom in Western Lake Erie, covering an even larger spatial extent but resulted in no drinking water advisories because it produced very little toxin.

Three possibilities exist as to why the 2015 bloom appeared to be relatively nontoxic. First, it is possible that the species or strains of *Microcystis* that dominated the bloom lacked the gene cluster and the ability to produce toxin. Second, it is possible that environmental factors such as nitrogen, phosphorous, or the ratio between nitrogen and phosphorous controlled the toxin production by suppressing the gene cluster within each species or strain. Third, environmental factors, such as hydrodynamic mixing and biodegradation, may interact in a highly nonlinear manner and select for nontoxic *Microcystis* strains, or disperse the toxin so that grab sampling does not detect it. Extensive field sampling programs by National Oceanic and Atmospheric Administration (NOAA) have not been able to definitively say which mechanism is controlling and are severely hampered by the limitations of grab sampling when blooms and environmental conditions are spatially and temporally heterogeneous.

Materials and Methods:

Experimental Design:

The planned studies were designed to measure growth rates and biomass production, and microcystin toxin concentration in two phases. In the first phase, we used pure cultures of *Microcystis aeruginosa.* The experiment was described by a sequential series of factorial design experiments that will first, investigate the role of nutrients (nitrogen and phosphorus), the ratio of the nutrients on single species *Microcystis* growth, microcystin toxin production, and the amount of ortho-phosphate and Nitrate/Nitrite within the cultures. The strain of *Microcystis* was purchased through the University of Texas at Austin UTEX algae bank and the sample was a known microcystin producer, UTEX LB 2385. The manipulated factors used in the design were the amount of phosphorous and nitrogen within the media. There were high concentrations and low concentrations of each of the factors resulting in four different artificial environments for the *Microcystis aeruginosa*. We ran these experiments with five replicates, to provide strong statistical power, resulting in a total of 20 microcosms for *M. aeruginosa* for Phase I.

In phase II, we would mirror the Phase I factorial design but substitute a "wild" *Microcystis* consortium collected from a distinct location in the Western Basin of Lake Erie. These cultures were to be grown in autoclaved Z8 media and spiked with high and low levels of nitrogen and phosphorus.

Experimental Methods and Materials:

Inoculum *Microcystis* cultures were grown in Z8 media for 10 days and transferred several times before initiating the factorial experiment to assure healthy cells and that the inoculum was in log phase growth. All microcosms were run simultaneously for Phase I and

shaken, and be transferred over to the new Z8 media groups. Their placement was randomized under the lights to average out any temperature or light intensity gradients.

The sets of samples within the factorial design were labeled to tell what exactly that the culture has. The cultures were either be labeled “A” for *Microcystis aeruginosa* or “V” for *Microcystis viridis*. These two different labeling were necessary to differentiate between species to be able to complete analysis. Throughout this whole process it was crucial to have precise measurements and to use sterile lab techniques. The *Microcystis* are highly sensitive and can be affected enormously by any minimal difference within its environment. Glassware was clean and the solutions and test tubes were autoclaved to create a sterile culture. During this whole process when transferring the cultures, it was important to use sterile pipets and standard lab techniques like the use of gloves and to sanitize hands with ethanol every time there is a transfer of *Microcystis*.

Media Modification:

The Z8 media used to culture the *Microcystis* was manipulated to create different nutrient environments to examine the effects of the environment on the growth and toxicity (Staub 1961). The first environment used the original recipe of the Z8 with no major modifications. Then the next media contained half the amount of phosphorus and nitrogen. This is created by modifying the concentration of NO_3^- to create a solution that is low on nitrogen. To complete this task there needs to be a consideration of NO_3^- from $Ca(NO_3)_2 \times 4H_2O$, which can be counteracted by adjusting the concentration of $NaNO_3$. One of the reasons the $Ca(NO_3)_2 \times 4H_2O$ was not modified within the solution is to maintain the hardness. The modified stock for the half amount of phosphorous required only the K_2HPO_4 to be modified. Each of these modifications to the stock

solutions was only one of the prerequisite solutions to create the final modified Z8 media. Both of those solutions were used to create environment number 2. The third environment only used the half phosphorous solution and then the normal nitrogen solution. The fourth environment was the opposite of the third with solution of low nitrogen and normal phosphorous. Once all the solutions were created the set-up of cultures occurred. Each culture had ten milliliters of the required Z8 media and one milliliter of the previous sample. When transferring cultures, the use of sterile techniques was used including the spraying of ethanol to clean the hood and your gloves. There was use of a Bunsen burner within the hood to flame the test tubes to kill bacteria that might be present. The use of sterile clean pipets for each of the transfers are used. The new cultures were placed in a randomized order with the caps loose under the lights to grow.

Nutrient Analysis:

The first set of test ran on the different samples within the sets was the AQ1 nutrient analyzer ortho-phosphate and nitrate/ nitrite test. The nutrients, N and P, may exist in multiple forms. Nitrogen as ammonia, nitrite, or nitrate will be determined by USEPA methods on an AQ1 discrete analyzer. With the test that were completed did not analyze the ammonia. The nitrate/nitrite test is where a small portion of the sample that is prepared by pulling one milliliter from the sample, vortexing it for five minutes, and then taking the supernatant to run on the AQ1 analyzer. The nitrate was reduced down to nitrite through a chemical reaction and the funneling through of the cadmium coil. With the same sample from the set the ortho-phosphate was calculated. The data received is from the spectrophotometer that transmits light through the chemical reaction with the sample. Both of these methods, ortho-phosphate and nitrate/nitrite

were determined by AQ1 discrete analyzer following USEPA methods. The ortho-phosphate was USEPA Methods 365.2 or 365.3 and the nitrate/nitrite by USEPA Method 353.2Rev. 2.0.

Toxin Analysis:

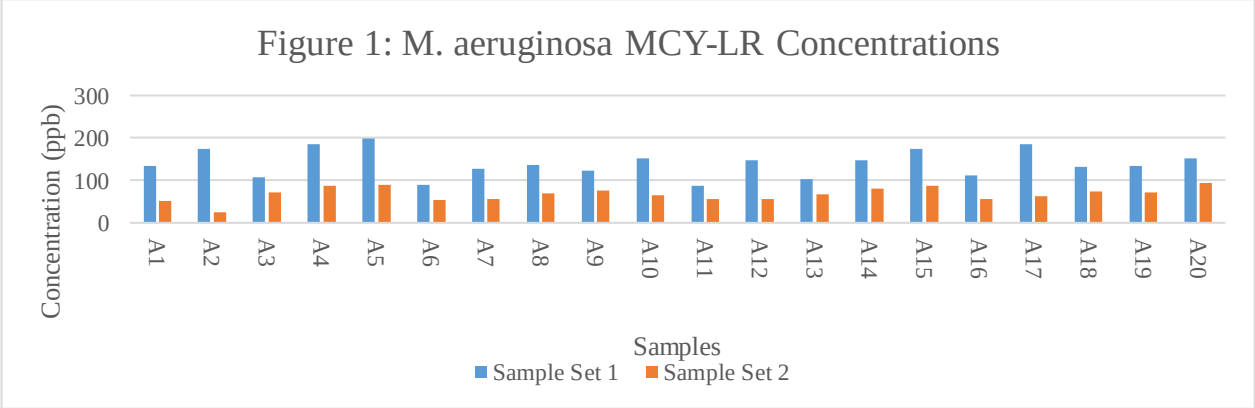
The next test completed on the *Microcystis* samples was run them through the Liquid Chromatograph tandem mass spectrometer (LC/MS/MS) for microcystin analysis. Before LC/MS/MS analysis the sample was freeze dried in a lyophilizer and extracted with 75% methanol. The sample was prepared for this process by taking the remaining sample and transferring it to a fifteen milliliter plastic falcon tube (this is around nine milliliters). The sample was frozen in a -20 $^{\circ}$C freezer, labeled, and placed at an angle in order to increase the surface area. The lyophilizer chambers were covered with bubble wrap covering for insulation. When the lyophilizer was set up, the sample's caps were removed and grouped in 10s, and their openings were covered with Chem wipes. Then the lyophilizer glasses were capped off and placed on the lyophilizer. It is crucial to make sure that both the temperature and the pressure are at the recommended levels.

After the sample were freeze dried, samples were capped off and replaced into the -20 ºC freezer until needed. The solid microcystins were allowed to dissolve into 1 mL of LCMS grade methanol and vortexed for 5 minutes. Samples were then centrifuged for 5 minutes. The supernatant was taken off and placed into a mass spec vial. These were then diluted to ~25% water and ~75% methanol.

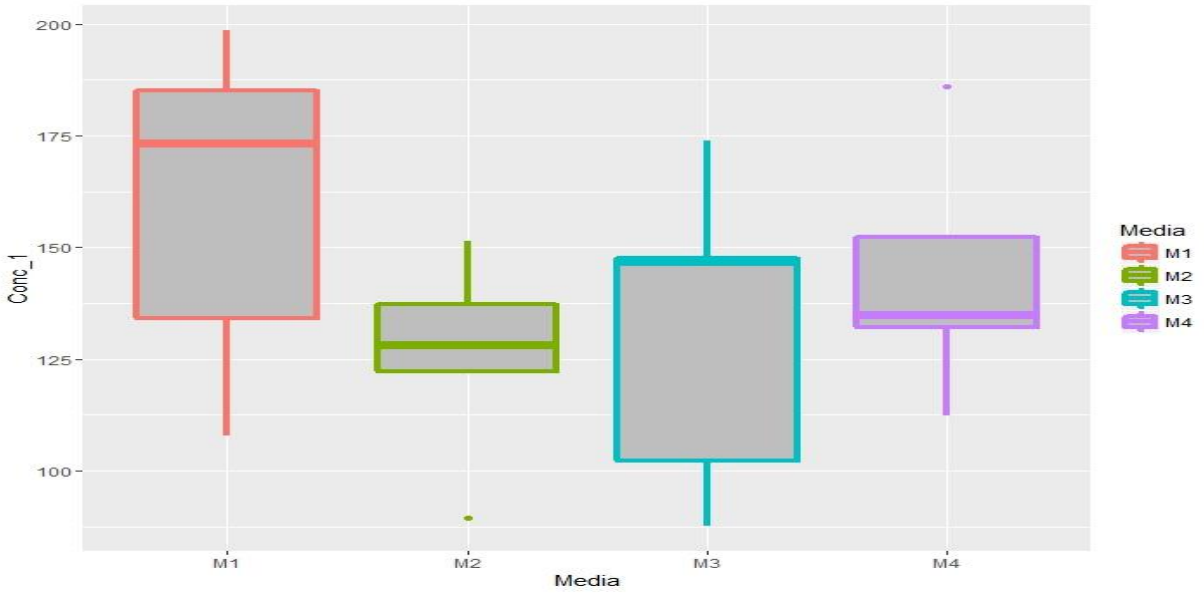
Results:

LC MS/MS Data:

The samples were prepared at Oakland University in the chemistry department but because of technical difficulties with the LC/MS/TOF within the chemistry department the samples were run at Wayne State University. The test used to analyze measured nine different forms of the microcystin. The toxin that is known to be in both *M. aeruginosa* and *M. viridis* is Microcystin LR. It is a common toxin that is harmful to humans and other mammals. When the data was received back, the data showed that there was no Microcystin-LR in the *M. viridis* samples. The data below is for two sample sets from the *M. aeruginosa* samples.



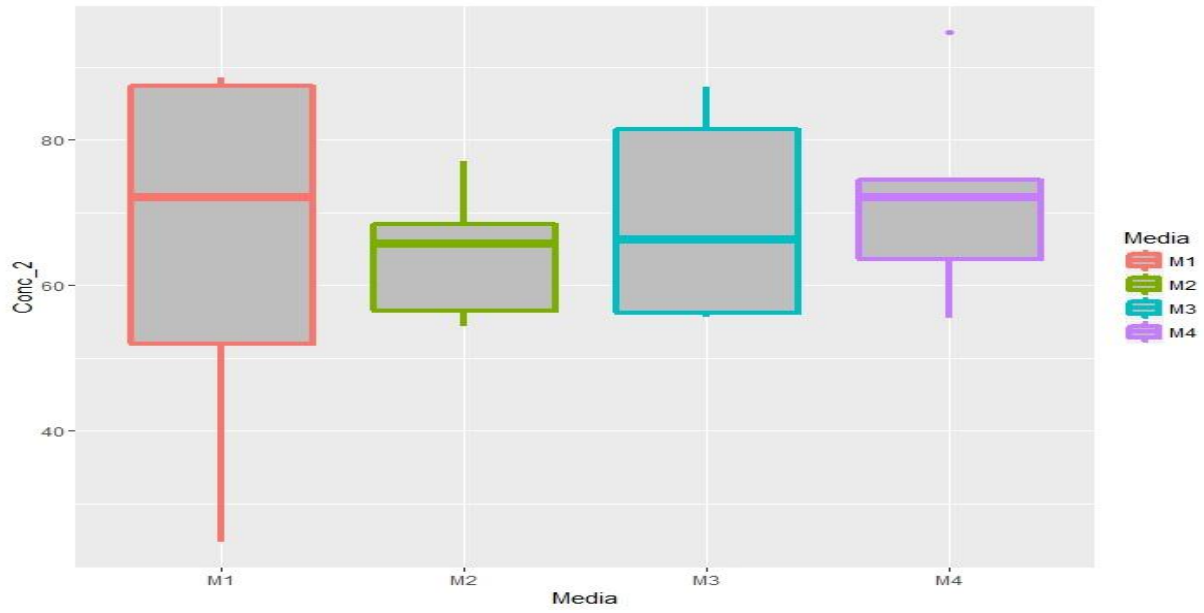
The *M. aeruginosa* MCY-LR concentration in Figure 1 above is the different concentrations of LR within the twenty samples of the two different *M. aeruginosa* sets. The first sample set grew a longer amount of time and the second set grew less than half of the time. This coordinates with the second sets concentrations being around half of the first sets.



M1	M2	M3	M4
HPHN	LPLN	LPHN	HPLN

Boxplot 1: MCY-LR Concentration 1

The boxplot 1 MCY-LR Concentration 1 of the different media types with five duplicates being averaged. As explained before the M1-M4 correspond with the HPHN, LPLN, LPHN, and HPLN in that order. Boxplot 1 shows the data for the first set of samples of *M. aeruginosa* grown for the longer period of time. In this sample set the average for high phosphorous and high nitrogen containing media had a highest concentration of toxins. While the nitrogen poor media extensively reduced the average of microcystin LR produced. In these boxplots the lowest average concentration corresponds to low nitrogen. A couple of outliers were in the media with lower concentrations of nitrogen. These are represented with the dots on either side of the main box with the averages.



Boxplot 2: MCY-LR Concentrations 2

M1	***M2***	***M3***	***M4***
HPHN	*LPLN*	*LPHN*	*HPLN*

Box plot 2, MCY-LR Concentrations 2, shows the data for the second set of samples of *M. aeruginosa* grown for the shorter period of time. In this sample set the averages did not follow any easily found pattern like the first set of samples although media 1 had the highest average again. These averages are in a closer range overall to each other all are in about five parts per billion (ppb). In these boxplots there is only one outlier, it is in the HPLN media to the higher range of concentrations.

Table 1: MCY-LR #1 Concentrations ANOVA

	Df	**Sum Sq**	**Mean Sq**	**F-value**	**P-value**
Conc_2	1	1787	1787	1.698	0.217
Media	3	3513	1170.9	1.113	0.382
Media: Conc_2	3	1430	467.7	0.453	0.72
Residuals	12	12628	1052.3		

This ANOVA for MCY-LR #1 Concentrations is a statistical test completed on Sample Set #1 concentrations of *M. aeruginosa*. The null hypothesis for both of the ANOVAS is that all the samples contain similar toxins levels for each of the different media concentrations and the media type does not affect toxin production. The test compiled the data on the relationship between one set of against the other and the different media types. The two statistics that are evaluated are the F-value and P-value. The concept to the F-value is that it is a measure of variance. The closer the value interpreted is to one then the null hypothesis is true. Sample Set 1 F-values are within 0.6 of one. This means that the null hypothesis is not rejected. The P-values are the calculated probability, in which the data is being tested to analyze what the probability of finding the null hypothesis or an extreme. After gathering the P-values for the three different combinations of statistics all the P-values were not significant at the P=0.05 level. This means that none of the sample sets differed from the null hypothesis.

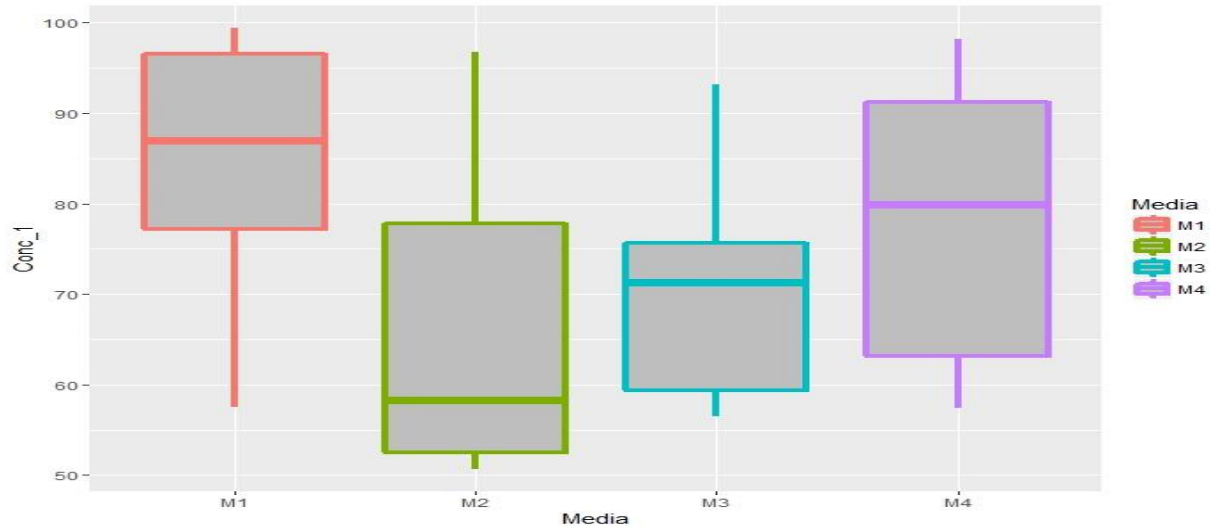
Table 2: MCY-LR #2 Concentrations ANOVA

	Df	Sum Sq	Mean Sq	F-value	P-value
Conc_1	1	476	476.3	1.331	0.271
Media	3	317	105.5	0.295	0.828
Media: Conc_1	3	71	23.7	0.066	0.977
Residuals	12	4296	358		

This ANOVA is for the second concentrations of MCY-LR from above and the categories that are evaluated are the F-value and P-value again with the Sample Set #2 Concentration. The F-values are outstandingly low for this ANOVA test. This means that there is no difference than the null hypothesis. With the P-values for the three different combinations of statistics all the P-values were measured came out not significant. The data points were not in the extremes' range, so this concludes that Sample Set #2 did not differ from the null hypothesis.

After review of the MC-LR data the main conclusion is that there is no statistical difference in toxin production across the four nutrient regimes.

The *M. aeruginosa* produced two other toxin variants or congeners. The two different congeners were D-Asp 3 MCY-LR and MCY-HilR. The same process was completed to analyze these toxins as MCY-LR.



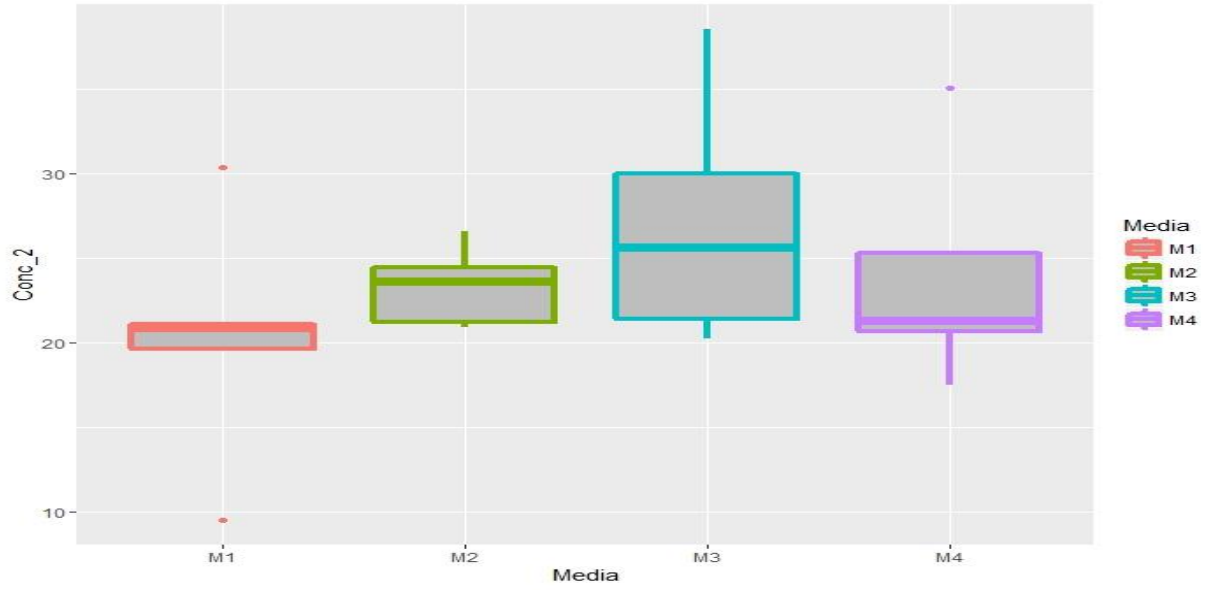
Boxplot 3: D-asp 3 MCY-LR Concentration 1

M1	***M2***	***M3***	***M4***
HPHN	*LPLN*	*LPHN*	*HPLN*

Boxplot 3 is the D-asp 3 MCY-LR concentrations shown as a visual representation of the data for *M. aeruginosa* grown for the longer period of time. As before, the high P and high N had the highest average concentration although the variance in the data does not look statistically

significant. The boxplot that shows a significant difference is the low nitrogen and low phosphorous Z8 media.

In the second sample set the averages for D-asp 3 MCY-LR did not follow any obvious pattern. These averages are in a closer range overall to each other all are in about thirty parts per billion (ppb). In these boxplots there has no outliers.



Boxplot 4:D-asp 3 MCY-LR Concentration 2

M1	***M2***	***M3***	***M4***
HPHN	*LPLN*	*LPHN*	*HPLN*

Boxplot 4 is showing the data for the second set of sample of *M. aeruginosa* grown for the shorter period of time. In this sample set the averages the toxins of the congener D-asp 3 MCY-LR and there was a pattern found. These averages are in a closer range overall to each other all are in about three parts per billion (ppb). In these boxplots there are two outliers, the HPHN and the HPLN media to the higher range of concentrations.

Table 3: D-asp 3 MCY-LR #1 Concentrations ANOVA

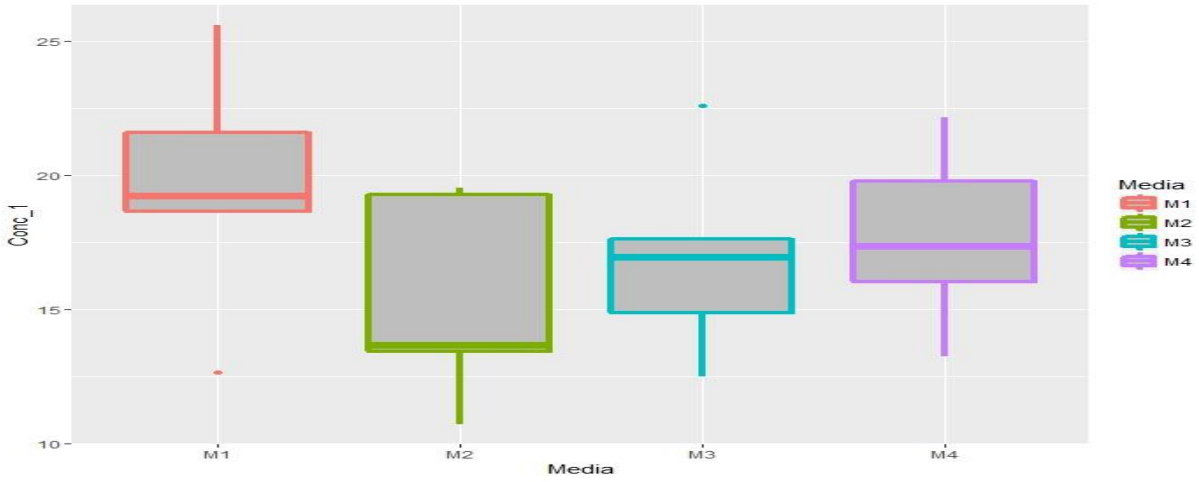
	Df	Sum Sq	Mean Sq	F-value	P-value
Conc_1	1	8	7.5	0.023	0.883
Media	3	890	296.6	0.897	0.471
Media: Conc_1	3	714	238.1	0.720	0.559
Residuals	12	3969	330.8		

The concept to the F-value is that it is a measure of variance. The closer the value interpreted is to one then the null hypothesis is true. F-values are within 0.55 of one. This means that there is no difference than the null hypothesis. After gathering the P-values for the three different combinations of statistics all the P-values were not significant. There is no P-value close to the significant number and this means that there is no difference between the samples.

Table 5: D-asp 3 MCY-LR #2 Concentrations ANOVA

	Df	Sum Sq	Mean Sq	F-value	P-value
Conc_1	1	1.0	1.03	0.023	0.881
Media	3	132.5	44.16	1.003	0.425
Media: Conc_1	3	104.6	34.86	0.792	0.521
Residuals	12	528.2	44.02		

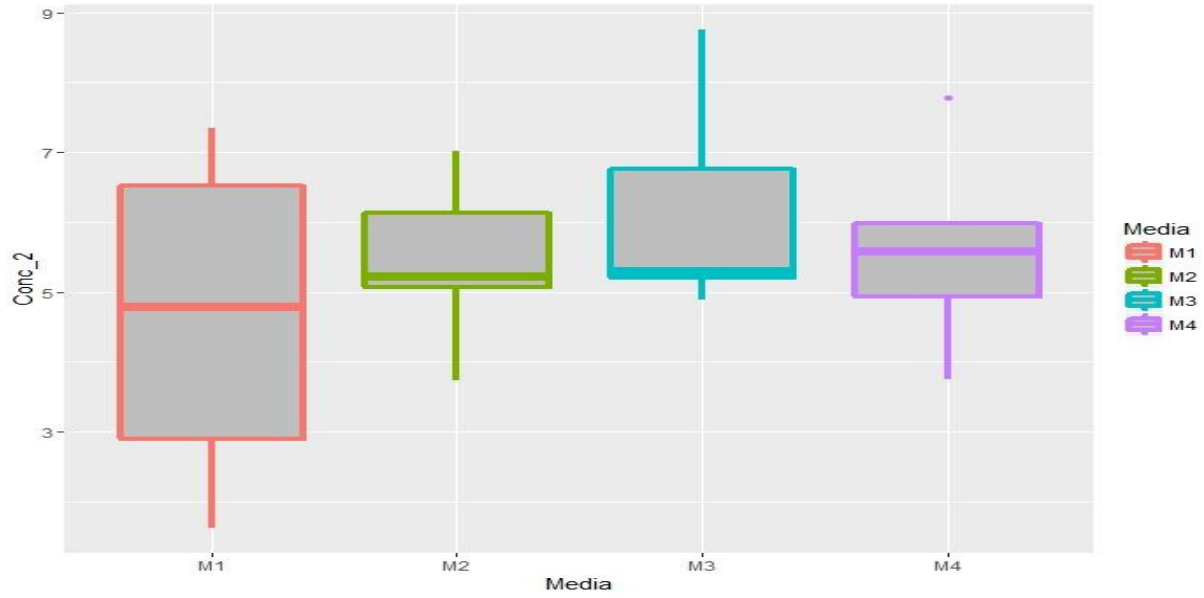
The two categories that are evaluated are the F-value and P-value for the second set of D-asp 3 MCY-LR concentrations. The F-values are extremely low for this ANOVA test. This means that there is no difference from the null hypothesis. With the P-values for the three different combinations none were significant. None of this data set is significant and that means all the samples are similar.



M1	*M2*	*M3*	*M4*
HPHN	*LPLN*	*LPHN*	*HPLN*

Boxplot 4: MCY-HilR Concentration 1

This is the first boxplot for MCY-HilR showing the data for the first set of sample of *M. aeruginosa* grown for the longer period of time. There is no major difference in any of the media. In this sample set the averages did not follow any easily found pattern like the first set of samples. These averages are in a closer range overall to each other all are in about thirty parts per billion (ppb). These boxplots show no outliers.



M1	*M2*	*M3*	*M4*
HPHN	*LPLN*	*LPHN*	*HPLN*

Boxplot 6: MCY-HilR Concentration 2

Boxplot 6, MCY-HilR Concentration 2, shows the data for the second set of sample of *M. aeruginosa* grown for the shorter period of time. In this sample set the averages the toxins are all similar and there was no pattern found. These averages are in a closer range overall to each other and all are within about two parts per billion (ppb).

Table 65: MCY-HilR #1 Concentrations ANOVA

	Df	Sum Sq	Mean Sq	F-value	P-value
Conc_1	1	23.40	23.401	1.350	0.268
Media	3	66.29	22.098	1.275	0.327
Media: Conc_1	3	1.60	0.534	0.031	0.992
Residuals	12	207.94	17.328		

The ANOVA was run on the MCY-HilR concentrations for the longer growth time period to analysis the data looking for trends. There was no significant effect of the media on toxin production

Table 5: MCY-HilR #2 Concentrations ANOVA

	Df	Sum Sq	Mean Sq	F-value	P-value
Conc_1	1	4.25	4.247	1.286	0.279
Media	3	10.17	3.389	1.026	0.416
Media: Conc_1	3	0.27	0.089	0.027	0994
Residuals	12	39.63	3.303		

This is the last ANOVA completed on the MCY-HilR second sample set to evaluate the F-value and P-value. None of this data set is significant and that means all the samples are similar.

Discussion and Conclusion:

The research completed within this project was to help understand the factors affecting production of microcystin. The experiment focused on the different types of microcystin and their correlation with different nutrient levels. This was completed with a factorial design with different types of Z8 media. The MS/MS allowed us to quantify the amount of toxins produced. The two strains that were tested were *Microcystis aeruginosa* and *Microcystis viridis*. All the data analysis indicated that there was no significant difference between the nutrient regimes.

There were a few difficulties with the microcystin analysis test getting ran. The Mass Spectrophotometer at Oakland University broke down in September so the data in this experiment was ran at Wayne State University on their MS/MS machine in January. When the data was received there ended up being no toxins produced by the *M. viridis* in either set. Presumably because of the timing of the testing. Missing the data of toxins being produced in

the *M. viridis* from the MS/MS was a disappointment. Some of the conclusions that could be drawn into why this happened is that the original sample came from a sample from Lake Erie this summer. The toxins could have degraded since then and then that would explain the reason for no results. Another conclusion is that bacteria got into the sample at some point along the process and consumed all of the *M. viridis*. This is a natural occurrence that happens all the time in any environment that has predation within it. This also would cause the loss of toxins within the sample.

There were multiple congeners that were produced within the *M. aeruginosa*. They were all variants of the MCY-LR toxin. The two other congeners were Dasp-3 MCY-LR and MCY-HilR. There is less of the other two toxins within the samples than there is for the original congener LR toxins. As for the LR, there was no significant media affect. There really are no obvious trends with any of the data. There have been experiments were detection of different congeners caused production of harmful toxins while the MCY-LR was determined not significant.

The experiment has not shown any new major breakthroughs to help understand *Microcystis* and how their environment affects the toxin production. However, *Microcystis* is a living organism. In the future there will have to be more research completed to figure out what exactly affects the microcystin and how to reduce the risk on human health. If we can control the algae blooms, then there is a better chance of fewer humans and other organisms affected. Many scientists have predicted an increase of blooms in the future due to climate change and this could be a more substantial problem because it effects water.

Cyanobacteria are a group of microorganisms that naturally exist in both freshwater and marine environments. Their growth increases when there are higher densities of nutrients in

eutrophic or nutrient-enriched water bodies. *Microcystis* are a genera of cyanobacteria that are capable of producing toxins, referred to as microcystins, which can impact human and other organism's health. Under ideal conditions of temperature, light, pH, nutrient availability, and other factors, cyanobacteria can achieve high densities and form a bloom. These blooms are referred to as cyanobacterial HABs. A variety of factors can influence both cyanobacteria proliferation and toxin production, including nutrient (especially nitrogen and phosphorus) concentrations, light levels, temperature, pH, oxidative stressors, and interactions with other organisms. Viruses, bacteria, and competition and food web interactions within the bloom develop, and interact impacting the microcystin concentrations (Human Health…).

Within the HAB there is a complex relationship of environmental factors that elicit spatial and temporal changes in the bloom. This affects the amount of *Microcystis* cells and the cells that produce toxins. The bloom relies on nutrients supplied byhuman activities. Another one of the new interesting ideas being researched is the impact of climate change, including potential warming of surface waters and changes in precipitation, which could cause changes in ecosystem dynamics. Researchers believe this will lead to more frequent formation of HABs and the toxins (Human Health…).

Within the HABs there are approximately 100 different congeners of microcystins, microcystin-LR is the most common. The difference in toxicity of microcystin congeners depends on the amino acid composition. Microcystin-LR's congeners include amino acids leucine and is arginine. The health risks due to exposure to different heterogeneous blooms of microcystin congeners is unknown, and since microcystin-LR is one of the most common congeners it has the majority of toxicological data on adverse health effects. If there is

Microcystin-LR within a bloom, then it is used as an indicator for how toxic they are (D'Anglada).

There are many countries that have regulations for the amount of microcystin within the drinking water. In Brazil, China, the Czech Republic, Denmark, and eleven other countries the limit is 1.0 µg/L microcystin-LR that is based on the World Health Organization (WHO) Provisional Guideline Value. While Australia has 1.3 µg/L microcystin-LR and Canada has 1.5 µg/L microcystin-LR limit. The United States does not have a certain national standard. Some of the states do have limits like Minnesota 0.04 µg/L Microcystin-LR, Ohio 1 µg/L Microcystin, and Oregon 1 µg/L Microcystin-LR within the drinking water (D'Anglada). With no enforced limit in the United States to the concentration of microcystin within the drinking water, there is a lack of data on the presence or absence of microcystin.

One of the recent microcystin events in the United States happened in August 2014 in Toledo, Ohio. The city issued a "do not drink or boil advisory" to nearly 500,000 customers in response to the presence of total microcystins in the city's finished drinking water. The levels were up to 2.50 µg/L. The presence of the microcystins was due to a cyanobacterial bloom near Toledo's drinking water intake located on Lake Erie. The advisory was in place for two days, after treatment was adjusted that led to the reduction of the microcystin concentrations. The concentrations were reduced to below the WHO guideline value of 1 µg/L in in all the drinking water from that treatment plant (D'Anglada).

There is also guidance for recreational water environments. The Environmental Protection Agency has recommended values for microcystin levels. The recreational value is 4 micrograms (µg)/liter (L). Within the past year there has been at least 255 notices that have posted recreational health advisories because the concentrations of microcystin or cyanobacteria

were substantial enough to be hazardous. These advisories included cautions, warnings, public health advisories, and public health warnings, due to the presence of harmful toxins and the microorganisms that produce them. The freshwater HABs that were reported showed microcystin concentrations ranging from below the limit of detection to 392 μg/L. Advisories can last for multiple days or even weeks (Human Health…).

One of the difficulties that come with the detection of HABs in recreational water is their vertical variability. There are four common causes including the sinking of dead/dying cyanobacterial cells and density stratification of the water column, especially nutrient concentrations and light, which affects all aspects of cyanobacteria growth. Also there can be an increase of nutrient supply from organic-rich bottom sediment. Other species-specific factors include surface scums and resting spores. Another one of the problems with microcystin is that they move within water systems or can be transported between systems. All these factor affect the monitoring and the creation of general health advisories (Human Health…).

The problems of measuring the microcystins can affect the amount of health advisories and letting the public know of the blooms. This causes more health risks to humans because they are more likely to come into direct contact or breathe aerosols from the blooms. Children are the most susceptible to microcystin health affects they only need 0.3 ug/L. While most adults would need to ingest about 0.6 ug/L to cause major health issues (Human Health…).

These issues are the reason that research needs to be completed to help understand the effects on human health and to understand the microcystins better.

References

Chen, W., Liu, H., Zhang, Q., & Dai, S. (2011). Effects of nitrite and toxic *Microcystis aeruginosa* PCC7806 on the growth of freshwater rotifer brachionus calyciflorus. *Bulletin of Environmental Contamination and Toxicology, 86*(3), 263-267

Carpenter, Adam. A Water Utility Manager's Guide to Cyanotoxins. *Water Research Foundation*. American Water Works Association.

Dairy Farmers Must Work Together to Address Issues Facing Us and protect the Future of Our Industry.(2015) *Ohio Dairy Producers News & Issues*. Ohio Dairy Producers.

D'Anglada, L. V., & Strong, J. (2015, June 15). Drinking Water Health Advisory for the Cyanobacterial Microcystin Toxins. United States Environmental Agency.

de Tezanos Pinto, P., & Litchman, E. (2010). Interactive effects of N:P ratios and light on nitrogen-fixer abundance. *Oikos, 119*(3), 567-575.

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

(1) Guven, B., & Howard, A. (2006). A review and classification of the existing models of cyanobacteria. *Progress in Physical Geography, 30*(1), 1-24.

(2) Guven, B., & Howard, A. (2006). Modelling the growth and movement of cyanobacteria in river systems. *Science of the Total Environment, 368*(2-3), 898-908.

Hakanson, L., Bryhn, A. C., & Hytteborn, J. K. (2007). On the issue of limiting nutrient and predictions of cyanobacteria in aquatic systems. *Science of the Total Environment, 379*(1), 89-108.

Harmful Algal Blooms and Hypoxia Comprehensive Research Plan and Action Strategy: An Interagency Repot. National Science and Technology Council: Subcommittee on Ocean Science and Technology.

Human Health Recreational Ambient Water Quality Criteria or Swimming Advisories for Microcystins and Cylindrospermopsin. (December 2016) United States Environmental Protection Agency.

Lei, L., Li, C., Peng, L., & Han, B. (2015). Competition between toxic and non-toxic *Microcystis M. aeruginosa* and its ecological implication. *Ecotoxicology*, *24*(7-8), 1411-1418.

Ohio Lake Erie Phosphorus Task Force II Final Report." (November 2013). Lake Erie Commission.

Oversight of EPA and the Great Lakes Water Quality Agreement. (1989) 101st Congress.

Public Law 113–124. (2014) 113th Congress..

Senate Bill Number 1: An Act. (2015) Ohio's 131st General Assembly.

Toxic Pollution in the Great Lakes. (1988)100th Congress.

Daniel Tillett, Elke Dittmann, Marcel Erhard, Hans von Döhren, Thomas Börner, Brett A Neilan, Structural organization of microcystin biosynthesis in Microcystis aeruginosa PCC7806: an integrated peptide–polyketide synthetase system, Chemistry & Biology, Volume 7, Issue 10, October 2000, Pages 753-764, ISSN 1074-5521, http://dx.doi.org/10.1016/S1074-5521(00)00021-1.

Tuholske, J, and Kilbert, K. (2015) Moving Forward: Legal Solutions to Lake Erie's Harmful Algal Blooms. *Lucas County*. Lucas County Government.

U.S. Environmental Protection Environmental Protection Agency Great Lakes National Program Office (GLNPO) Great Lakes National Program Office (GLNPO) Significant Activities Significant Activities Report.(2014) U.S. EPA Great Lakes National Program Office.