The Influence of Algal Toxins and Taste-and-Odor Compounds on Water Usage in the Western Basin of Lake Erie

by

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Abstract

The main purpose of this project was to determine if *Microcystis aeruginosa*, a toxic cyanobacteria species, was also a producer of taste-and-odor compounds. This was done using gene sequencing. The secondary purpose was to determine biotic and abiotic factors that contribute to taste-and-odor events during algal blooms. Understanding the factors influencing taste-and-odor production is important because it can have a tremendous effect on how people will continue to use water from Lake Erie. Sampling took place at nine open water areas in the western Lake Erie basin and near the water intake sites of two island communities. High geosmin levels (>> 0.004 ppb detectable limit) were detected at multiple sites but genetic confirmation could not be obtained. Statistical analyses showed a correlation between geosmin, chlorophyll *a*, and total phosphorus and that these factors may also be a cause for geosmin production. Phytoplankton counts also showed that *Anabaena flos-aquae* and *Planktothrix* sp. were present. These are not only known toxin-producers but also producers of taste-and-compounds. This study could not confirm or deny if *Microcystis* is a producer of these compounds as well as algal toxins. However, this phenomenon is occurring throughout the basin at locations where people use water recreationally and for consumption. If western Lake Erie is going to be removed from the Areas of Concern list then this situation must be better understood and this includes further study of whether or not *Microcystis* is a producer of these compounds as well as identifying the contributions made by these known taste-and-odor producers.

Introduction

The ability to provide potable and palatable drinking water to a community is very important, especially in order to ensure the health of the community. However, in Michigan, eutrophication of areas like Saginaw Bay and Western Lake Erie has promoted other problems, such as large blooms of nuisance algae and taste-and-odor problems in drinking water (MDEQ 2008). In the 1960s, Lake Erie was severely eutrophied and cyanobacteria blooms were common. In the 1970s and 1980s, phosphorus levels were reduced beginning with the passing of legislation like the Great Lakes Water Quality Agreement and blooms occurrences decreased. However, in the mid-1990s blooms

composed primarily of *Microcystis* once again increased in frequency (Rinta-Kanto et al., 2009b). These areas have been listed as Areas of Concern because the problems are ongoing and have the potential to lead to serious complications for people in Michigan. According to the Michigan Department of Environmental Quality (2008) a location is considered an Area of Concern when it exhibits a beneficial use impairment (e.g. taste and odor) that sets the area apart from other areas of lesser contamination. Complications such as the increase of nuisance algal blooms, toxins, and taste and odor compounds can have negative impacts on fisheries, water recreation, and overall human health.

A major component of the nuisance algae problem is cyanobacteria, with one species in particular called *Microcystis aeruginosa*. The genus *Microcystis* consists of colony-forming, unicellular cyanobacteria (Rinta-Kanto et al, 2009a). Blooms of this species can look like long tracks of green across the water. If the bloom is dense enough, a bottle of water containing *Microcystis* looks like it is filled with green paint. It is a known producer of the hepatotoxin microcystin. In humans, microcystin toxins have been known to cause gastroenteritis and liver damage (Rinta-Kanto et al., 2009b). Over the last decade, most documented cases of cyanobacteria blooms have been in areas with dense urban shoreline and basin development (Watson et al., 2008). These outbreaks or blooms can also occur near water treatment intakes. Not only is *Microcystis* a concern because of its toxin production, but it may also contribute to taste-and-odor problems in drinking water. Algae and bacteria are two of the most frequently occurring sources of drinking water taste-and-odor (Watson, 2004). Normally, the cyanobacteria usually responsible for producing taste-and-odor compounds are *Anabaena*, *Aphanizomenon*, *Planktothrix*, *Pseudoanabaena*, and *Synechococcus* (Graham et al., 2008). Two of the most common taste-and-odor compounds are geosmin (*trans*-1, 10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (1,2,7,7-tetramethyl-exo-bicyclo-[2,2,1]-heptan-2-01). These compounds are terpene derivatives and may be a form of marine chemical communication. They have extensive olfactory properties with a highly potent earthy or musty aroma (Watson, 2003). These compounds are produced throughout the cell's life cycle, can accumulate within the cell, and can then be released into the environment either through cell leakage or cell lysis (Satchwill et al., 2007). Once in the water, geosmin especially is very slow to break down in water (Dzialowski, et al., 2009). It has

been reported that in the case of *Microcystis*, this genus only produces isopropylthiols and β-cyclocitral, which is generated at cell damage or death (Watson et al, 2008). However, it has been demonstrated that *Microcystis* is capable of producing two major taste-and-odor compounds, geosmin and 2-methylisoborneol (2-MIB) (Huang et al, 2007 and Huang et al., 2008). This is an interesting conflict and could be important in addressing the combined toxin and taste-and-odor issue in Lake Erie.

I studied water collected in the western basin and near water treatment intakes in Western Lake Erie Island communities to determine if *Microcystis* is a producer of taste-and-odor compounds and if the T&O compounds are correlated with other factors such as colony volume, toxin concentration, and nutrient availability. Polymerase Chain Reaction (PCR) analysis based on Giglio et. al. (2008) was used to specifically determine whether *Microcystis* is indeed a T&O producer or if it is only producing toxins.

Methods

Research Area

Samples were collected weekly from the western basin of Lake Erie at nine open water stations and at the water intakes for two island communities (Put-in-Bay and Kelley's Island) from June 2009 to October 2009 (Table 5, Figure 1). Sampling at the open water stations and the water intakes were done one the same day. This was to ensure that both areas were being tested under the same conditions.

Water Collection

In the nine stations, water was only taken from the surface using 4 L bottles. At the two water intakes, water was taken at the surface and the bottom and then at two intermediate depths (approximately 1.5 and 3 m) using a niskin bottle. Once the water was collected, the bottles were placed in a cooler with ice packs. Water was then brought back to the National Oceanic and Atmospheric Administration's Great Lakes Environmental Research Lab (Ann Arbor, MI) and stored at 4°C until filtering and analysis could be done, which was usually within 24 hours.

Filtering and Analysis

Samples were processed to quantify for phytoplankton, chlorophyll a, microcystin, phycocyanin, taste and odor compounds, total suspended solids, and nutrient analyses which included silica, total phosphorus, total dissolved phosphorus, phosphate, particulate carbon, nitrate and ammonia. TP and TDP were determined after digesting 50 mL of unfiltered and filtered sample respectively with potassium persulfate in an autoclave for 30 min (Menzel and Corwin, 1965). Nutrient concentrations were determined using standard automated calorimetric techniques (APHA 1990) on a Technicon Auto Analyzer II, as detailed in Davis and Simmons (1979).

For phytoplankton counts, 100 mL of water was measured into an opaque Nalgene bottle and preserved with 2 mL of Lugol's solution for later microscope enumeration. Phytoplankton counts were performed using Utermohl's sedimentation method (Paxinos and Mitchell, 2000) and an inverted microscope. For microcystin, chlorophyll, and taste and odor samples, water was filtered onto glass fiber filters using a low-pressure vacuum and stored at -20 °C until analysis. Microcystin analysis was performed using a combination of methanol extraction process and Abraxis ELISA kits (Abraxis LLC, Warminster, PA). For T&O analysis at sites with high *Microcystis* densities, raw water was also saved in 1 L bottles to measure the T&O compounds that are not contained within the cells. Samples were sent to Dr. Judy Westrick at Lake Superior State (Sault Ste. Marie, MI) for analysis. They were analyzed using a combination of solid phase micro-extraction and gas chromatography-mass spectrometry (GC-MS). Samples for DNA analysis were filtered onto on 0.8 µm membrane filters (Supor hydrophilic polyethersulfone), then placed in labeled 2 mL tubes and stored in -20 °C freezer until later analysis.

DNA Extraction

The DNA extraction was a two-part process. The first part involved breaking down the colonies as well as the filter. This was done by first adding 1 mL of DNAzol ES (Molecular Research Center, Cincinnati, OH) to the tube with the filter then heating the tube at 90 °C for a total of 2-4 hours. The samples were vortexed every 30 minutes for one and a half hours. After this time, about 0.3 g glass beads (150-200 µm) were

added to each sample tube. The tubes were then placed in a bead beater and beat for three minutes, heat for another hour at 90 °C, and then placed in the bead beater again for three minutes. Next, 1 mL of chloroform was added to each tube. This step removed pigments and insoluble plant debris. The tubes were not vortexed after this step. The tubes sat for five minutes at room temperature and then spun in a centrifuge for ten minutes at speed 14X (rpm). The upper aqueous phase was then transferred to a new tube and then the chloroform step was repeated once more to remove any other organic material. After the second time that the upper phase was transferred to a new tube, the volume of liquid in each tube was recorded. Each volume was multiplied by 0.75 and the resulting number was the volume of 100% EtOH that was added to each tube and then the tubes were inverted about 8-10 times. This step precipitated the DNA in the sample. The samples then sat for 5 minutes at room temperature and then spun for four minutes at speed 6.5X. The supernatant was then decanted. The pellet that is left may not be visible so one must be very careful when disposing of the supernatant. The pellet was then resuspended in 150 µL of sterile deionized water. At this point the samples are allowed to be put in a -20 °C freezer overnight if necessary.

The second step required a Qiagen DNeasy Plant Kit (Qiagen, Valencia, CA) to purify the DNA. These directions were adapted from the directions in the kit. If stored overnight, samples should be allowed to thaw before continuing on. Once thawed, 400 µL of the AP1 buffer was added along with 3 µL of RNase A (100 mg/mL). The samples were then incubated at 65 °C for ten minutes, vortexing occasionally and then AP2 buffer was added (130 µL AP2 per 400 µL of total volume of sample). The samples were then incubated for ten minutes at -20 °C. This step precipitates detergent, proteins, and polysaccharides that may be present. Once spun down the supernatant was removed to a new tube and the volume was measured. This new volume was multiplied by 1.5 and the resulting number was the volume of AP3/EtOH buffer that was added. This was then added to a spin column and spun for one minute at speed of 8X. The filtrate was discarded and the process was repeated until all the lysate had been run through the column. The last step with the spin column involved 500 µL of a wash buffer being run through the spin column twice. The column was then placed in a new tube and 50 µL of sterile deionized water preheated to 65 °C was added to the tubes. The samples sat for

five minutes and then spun for one minute at speed 8X. The extracts were then stored at -20 °C until it was time for further analysis.

PCR, Cloning of PCR Products, and Sequencing

The genes of interest, *mcyB* and geosmin 250F and 971R, were amplified from the extracted DNA by PCR. The reaction mix consisted of 1X buffer, 2.5 mM $MgCl_2$, 200 μM each, dNTP, 5% dimethyl sulfoxide, 300 μM each of forward primer 250F and reverse primer 971R (from paper by Giglio et al., 2008), deionized water, 1U Platinum Taq DNA polymerase (Invitrogen, Carlsbad, CA), and 3 μL of the extracted DNA in a 50 μL volume. This process was repeated using mcyB primers to determine if toxin producers were present at the sites. Reactions were analyzed on a Stratagene Robocycler. Samples were then pipetted into a 1.0% agarose gel for 30 min at 100 volts and stained with ethidium bromide to view PCR products.

When comparing the sample PCR result to the marker ladder, the geosmin result should be at 743 bp and the mcyB result should fall at 685 bp on the ladder. Samples that were at these marks and had strong, clear bands were chosen for sequencing. Similar to the DNA extraction process, cloning and sequencing was a multi-step process. First, was to prepare ligation mixes using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA). The reaction mix consisted of salt solution, sterile water, and the TOPO vector. PCR products for each sample were added individually to a separate tube after the reaction mix was added. This mixture was incubated for 5-10 minutes at room temperature. The next step was to transform the samples. Vials of chemically competent TOP10 cells that had been stored at -80 °C were defrosted on ice and then 25 μL of cells were transferred to pre-chilled 1.5 μL tubes. Then 1 μL of the ligation mix was added and the samples were left to incubate on ice for 30 minutes. After this time, the samples were heat shocked in a water bath at 42 °C for exactly 30 seconds. They were then incubated on ice again for two minutes and then, 125 μL of SOC media were added to each sample. The samples were set to incubate at 37 °C for one hour in a shaker set at 225 rpm. During this incubation period, 35 μL X-gal (50mg/mL stock) was added to LB plates (+ kanamycin) and dried inverted for 1 hr. Samples and plates were done at about the same time.

Lastly, 50-100 µL of cells were added to the plates, spread evenly, and then incubated overnight at 37 °C.

The next day, an isolation step was done and this was only if there were a good amount of cells (> 3 in order to have multiple repetitions for sequencing) in the culture were white in color. In 15 mL centrifuge tubes, 4 mL of LB (+ kanamycin) broth was added and then colonies were picked from the plates using sterilized toothpicks. There was only one colony per tube and there would be multiple tubes allotted for each sample. These tubes were also incubated overnight at 37 °C in a shaker running at 250 rpm. The next day these samples were centrifuged down in a B-22M centrifuge for 15 minutes at 3000 rpm. The LB broth was discarded, isolating a pellet of cells. The next step was to refine the samples into plasmids using QIAprep miniprep kits (Qiagen, Valencia, CA). These samples were stored at -20 °C until they could be checked for the correct sized insert and prepped to be sent off for sequencing. Insert size was checked using EcoRI cuts. This was similar to running the PCR gels. First, a reaction mix was made using 10 X buffer H, sterile water, and EcoRI enzyme (12 U/ µL). Once this was divided into tubes, 5 µL of plasmid was added to each tube, corresponding with each sample. This added up to 10 µL total and the samples were then incubated at 37 °C for at least three hours. The samples were then run on 1.0% agarose gels and stained with ethidium bromide just like the PCR gels. If the bands showed up in the appropriate range, and were strong enough (indicated by brightness of band on photo) then samples could be prepared for sequencing. In 1.5 mL tubes, 2.5 µL of plasmid and 7.5 µL of sterile water were added. Samples were sent to the DNA Sequencing Core at the University of Michigan (Ann Arbor, MI) for DNA sequencing.

Statistical Analyses

Before any statistical tests were run, all data was tested for normality. This was done using the Kolmogorov-Smirnov Test (K-S Test, SPSS 16.0, SPSS Inc.). If data was not normally-distributed, data transformations were done. Transformations of the data alter the distribution of the data, bringing it closer to normality, which is an important assumption in most statistical analyses. The main purpose was to determine which factors contribute to geosmin production. The first step was to see if it correlated with

any other factors. For normally-distributed data, Pearson's Product-Moment Correlation Test was used. Data that was not normally-distributed was analyzed using Spearman's Rank Order Correlation Test. The resulting correlations were used to determine if any of those factors were indeed causes of geosmin production. Multiple linear regression analysis was used since there were multiple independent factors and only one dependent factor.

Results

Genetic Analysis of Geosmin and mcyB

For the DNA analysis of the geosmin-producing gene, the PCR results appeared to be close to the 743 bp mark. However, when the samples were sequenced no consistent results could be found. None of the sequences matched the geosmin sequence shown in the Giglio et al. (2008) paper. For the *mcyB* analysis, the PCR results were close or at the 685 bp mark. When the samples were sequenced there was actually an array of genes found rather than simply showing *mcyB*. The most common genes present were mcyB and mcyC. A phylogenetic analysis of *mcyB* amino acid sequences showed that there were a total of six clusters of sequences. The three major clusters were *mcyC*, *mcyB*, and *anaD* (anabaenopeptin synthetase), another area of toxin-production in other algae species (Figure 2). All *mcy-* clusters could be attributed to *Microcystis* species, while others were mainly attributed to *Planktothrix* species (Table 1).

Biological and Geosmin Analysis

Microscope enumeration showed that the primary cyanobacteria present in the western basin were *Microcystis aeruginosa*, *Microcystis wesenbergii*, *Anabaena flos-aquae*, and *Planktothrix sp.* At Kelly's Island and Put-In-Bay the primary cyanobacteria were *Microcystis aeruginosa*, *Anabaena flos-aquae*, and *Planktothrix*. However, *Planktothrix* was only seen in July and October, the beginning and end of the season, respectively. Though other genera were present, most of the season was dominated by *Microcystis aeruginosa*. The highest colony presence was found along the southern end of the basin at stations 2, 6, 7, and 9 (Table 2). This trend with these sites was seen throughout the season. At the island sites, counts were more variable and occasionally at

Put-in-Bay no cyanobacteria were found in samples at all. Counts for these sites were generally much lower than colony counts from the western basin sites.

For the geosmin analysis, both filter and whole water samples were sent for analysis. The filter samples either had very low concentrations or no T&O compounds were detected. Whole water samples had better results and the sites with high levels of geosmin were stations 6, 9, and PIB-WI. Geosmin was present at sites 6 and 9 throughout the whole season but PIB-WI only had geosmin from August to the beginning of September (Figure 3). For these sites the lowest concentrations was just below 1 parts per thousand (ppth). No linear pattern over time could be determined for any of the sites or for the basin overall.

Statistical Analysis

The K-S test for normality was performed for chl*a*, microcystin, geosmin, total phosphorus (TP), ammonium (NH_4), nitrate (NO_3), and colony count data. Chl*a*, geosmin, and TP were normally-distributed ($p > 0.05$). All the other data were not normally-distributed and needed to be transformed. A square root transformation was done for the colony count data and this brought the data to a normal distribution ($p > 0.05$). A natural log transformation yielded the best results for the microcystin data. The data had improved, however, it was still not normally distributed ($p < 0.05$). No transformations improved the other nutrient data.

Pearson's Product-Moment Correlation was performed on depth, chl*a*, geosmin, TP, and colony data. This analysis revealed that geosmin was significantly correlated with chl*a* ($r = 0.561$, $n = 34$, $p < 0.05$) and TP ($r = 0.499$, $n = 18$, $p < 0.05$). Geosmin was not correlated with the other variables. Spearman's Rank Order Correlation, a non-parametric test, was performed on all the data that was not normally-distributed. Geosmin was not significantly correlated with microcystin, NH_4, or NO_3 data ($p > 0.05$).

A multiple linear regression analysis was then performed on the data that was significantly correlated with geosmin. Geosmin was set as the dependent variable and chl*a* and TP were set as the independent variables. The ANOVA portion of the analysis indicated that the slope of the regression line was significantly different than 0 (Table 3, $p < 0.05$). Overall, the multiple linear regression analysis of dependent variable geosmin

and independent variables chl*a* and TP indicated a significant linear relationship between them ($n = 18$, $r^2 = 0.348$, $p < 0.05$) (Table 4, Figure 4). The equations for the relationships were

$$Geosmin = (0.081*chl\textit{a}) + 1.953$$

$$Geosmin = (0.015*TP) + 1.953$$

Discussion

Though the GC-MS procedure showed high geosmin concentrations at multiple sites in the western basin of Lake Erie, genetic confirmation could not be obtained. Gene sequencing is a complex and nuanced process that often proves problematic. Contamination could have likely occurred between the PCR analysis and the processing for gene sequencing because the PCR results were as expected. There may have also been a problem with the PCR analysis itself. Since it was different cyanobacteria being tested than what was in the Giglio et al. (2008) paper, it is possible that this species did not respond to the PCR method as expected. It is also possible that *Microcystis* in Lake Erie truly does not produce the taste-and-odor compound geosmin. Saadoun et al., (2001) performed an experiment which may be adaptable to test the production of geosmin in *Microcystis*. They isolated *Anabaena* sp. from a bloom and cultured it to determine what factors promoted T&O synthesis. They found that certain temperature, light, and phosphorus levels were important for this genus to produce geosmin. The same kind of experiment could be done for *Microcystis*, which could easily be coupled with further genetic testing.

The colony counts and identification of this study also confirm other results of studies done in the western basin of Lake Erie. A study done in 2003 and 2004 suggested that the microcystin-producing community may not be entirely composed of *Microcystis* (Rinta-Kanto and Wilhelm, 2006). They later found that the most common toxin-producing cyanobacteria were *Microcystis*, *Planktothrix*, and *Anabaena*. That study also found that of those three, *Microcystis* dominant throughout the summer and early fall seasons. Even though the other two species were less abundant than *Microcystis*, their contribution to the situation may be important for further study, especially since they are both toxin and taste-and-odor compound producers.

The statistical analyses did give some positive results in terms of what is causing these T&O events. Initially, the correlation tests showed that geosmin concentrations were positively correlated with chlorophyll *a* and total phosphorus concentrations. This means that as chlorophyll and phosphorus increase, geosmin would increase as well. One interesting result was that colony volume (colony/L) did not correlate with geosmin concentrations. This could be because the colony may be too large of a unit and not every colony may produce geosmin. Further studies may have to use biovolume units of cells per liter. The regression was used to determine if these factors were indeed a cause rather than simply increasing at the same time. The regression showed a positive cause-and-effect relationship between the two independent variables and the dependent geosmin variable. These statistical results confirm with other studies' results showing the positive relationship between geosmin production, chlorophyll (an indicator of biomass), and phosphorus (Rashash et al., 1995; Smith et al., 2002; Wang et al., 2005).

Conclusion

Although this study did not confirm or deny if *Microcystis* in Lake Erie is a producer of taste-and-odor compounds, it did confirm some factors that are producing taste-and-odor compounds. This is a cause for further study. During the summer season, this species of cyanobacteria is easily the dominant species in the western basin. It is a concern because of the scum the colonies form on the water and because of the toxin it produces. If it produces compounds like geosmin as well, then it is impacting viability of drinking water as well as human health. Also, if water treatment facilities are only focusing on removing toxins, then these other compounds could potentially filter through into the main drinking supply. Conversely, future study could also confirm that a different cyanobacterium is responsible for these compounds. This outcome would have to be handled very differently because it would involve handling problems from two or more species rather than one. If Lake Erie is going to be removed from the Areas of Concern list, in regards to nuisance algae and taste-and-odor in drinking water, then further research must be done in this area so that we can fully understand the problem and efficiently and in the best manner possible.

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Table 1. Details of Western Erie *mcyB* sequencing showing date when sample was collected during 2009, site (see Figure 1 for locations), and the gene of interest. Sequence numbers correspond to sample numbers on the phylogenetic tree in Figure 2.

Sequence #	Species	Site & Date	Gene
1621225	M. aeruginosa	WE 9, 7/28/09	amino acid adenylation participated protein
1621228	Microcystis viridis	WE 6, 7/28/09	mcyC
1621230	M. aeruginosa	WE 6-3, 7/28/09	amino acid adenylation participated protein
1621232	Microcystis aeruginosa	WE 7-2, 7/28/09	mcyC
1621233	Microcystis aeruginosa	WE 7-3, 7/28/09	mcyB
1621234	Microcystis botrys	WE 6-1, 8/11/09	mcyC
1621235	Microcystis viridis	WE 6-2, 8/11/09	mcyC
1621236	Microcystis aeruginosa	WE 6-3, 8/11/09	mcyB
1621240	Microcystis viridis	WE 9-1, 8/11/09	mcyC
1621242	Microcystis viridis	WE 9-3, 8/11/09	mcyC
1621554	Planktothrix agardhii	PIB, 8/11/09	ociD (ABC transporter, 99%)
1621555	Microcystis aeruginosa	PIB, 8/11/09	mcyC
1621556	Planktothrix agardhii	PIB, 8/11/10	ociD (ABC transporter, 99%)
1621558	Planktothrix agardhii	WE 6-2, 9/8/09	ociD (ABC transporter, 74%)
1621559	Planktothrix rubescens	WE 6-3, 9/8/09	anaD (anabaenopeptin synthetase - nonribosomal peptide synthetase, 93%)
1621560	Planktothrix rubescens	WE 9-1, 9/8/09	anaD (anabaenopeptin synthetase - nonribosomal peptide synthetase, 93%)
1621561	Planktothrix rubescens	WE 9-2, 9/8/09	anaD (anabaenopeptin synthetase - nonribosomal peptide synthetase, 93%)
1621562	Planktothrix agardhii	WE 9-3, 9/8/09	ociD (ABC transporter, 74%)
1621563	Microcystis sp.	Kelley's Isl, 9/8/09	mcyB (98%) or other peptide synthesis gene
1621564	Microcystis aeruginosa	Kelley's Isl, 9/8/09	mcyA
1621565	Microcystis sp.	Kelley's Isl, 9/8/09	mcyB (98%) or other peptide synthesis gene
1621567	Microcystis viridis	PIB, 9/8/09	mcyB (98%) or other peptide synthesis gene
1621568	Microcystis sp.	PIB, 9/8/09	mcyB (98%) or other peptide synthesis gene

Table 2. Western basin sites that had the highest colony counts. Dominant species found at each site are also given. KIWI = Kelley's Island Water Intake, PIBWI= Put-In-Bay

Date	Site	Total Colonies (# col/L)	Dominant Species
7/21/2009	WE6	1700	*Microcystis aeruginosa*

	WE9	2450	*Microcystis aeruginosa*
	KIWI	1500	*Planktothrix*
8/3/2009	WE2	3550	*Microcystis aeruginosa*
	WE6	3500	*Microcystis aeruginosa*
	WE9	1700	*Microcystis aeruginosa*
8/11/2009	WE2	2500	*Microcystis wesenbergii*
	WE6	5500	*Microcystis aeruginosa*
	WE7	1450	*Microcystis aeruginosa*
	WE8	4250	*Microcystis aeruginosa*
	WE9	7000	*Microcystis aeruginosa*
8/19/2009	WE6	5450	*Microcystis wesenbergii*
	WE8	1000	*Microcystis wesenbergii*
	WE9	4000	*Microcystis aeruginosa*
8/25/2009	WE2	2550	*Microcystis aeruginosa*
	WE8	4150	*Microcystis wesenbergii*
	WE9	2500	*Microcystis aeruginosa*
9/1/2009	WE8	1600	*Microcystis aeruginosa*
9/8/2009	WE2	1500	*Microcystis aeruginosa*
	WE6	3650	*Microcystis aeruginosa*
	WE7	1650	*Microcystis aeruginosa*
	WE8	1400	*Microcystis aeruginosa*
	WE9	3150	*Microcystis wesenbergii*
9/14/2009	WE2	3650	*Microcystis aeruginosa*
	WE8	3300	*Microcystis aeruginosa*

	WE9	2300	*Microcystis aeruginosa*
9/22/2009	WE2	1800	*Microcystis aeruginosa*

Table 3. Multiple linear regression ANOVA (SPSS) to determine slope of regression lines. Results were significant if $p < 0.05$.

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	48.324	2	24.162	4.003	.040[a]
	Residual	90.543	15	6.036		
	Total	138.866	17			

a. Predictors: (Constant), [TP], Chl-A

b. Dependent Variable: Geosmin

Table 4. Coefficient Table for Determining Regression Equations. Constant = y-intercept, chl*a* and TP coefficients = slope of the line.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	Collinearity Statistics	
		B	Std. Error	Beta			Tolerance	VIF
1	(Constant)	1.953	.811		2.408	.029		
	Chl-A	.081	.054	.409	1.513	.151	.593	1.686
	[TP]	.015	.017	.237	.877	.394	.593	1.686

a. Dependent Variable: Geosmin

Table 5. GPS coordinates for western basin sites and water intakes at Kelley's Island and Put-in-Bay for sampling during summer and fall, 2009.

Site	Latitude	Longitude

WE 1	41° 53.130'	83° 19.986'
WE 2	41° 45.224'	83° 19.125'
WE 3	41° 51.567'	83° 15.828'
WE 4	41° 50.035'	83° 11.486'
WE 5	41° 48.262'	83° 15.362'
WE 6	41° 42.269'	83° 23.171'
WE 7	41° 45.852'	83° 24.151'
WE 8	41° 50.076'	83° 21.815'
WE 9	41° 43.059	83° 25.427'
KIWI	41° 35.55'	82° 42.65'
PIBWI	41° 38.763'	82° 48.781'

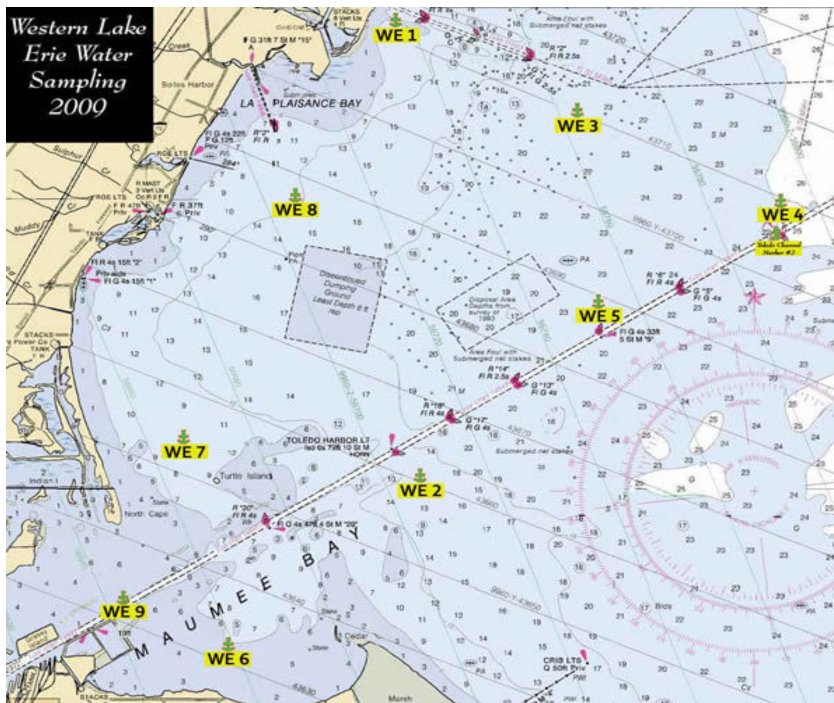

Figure 1. Map of sampling area in western basin of Lake Erie showing the nine stations (WE 1 to WE9) sampled during summer and fall, 2009.

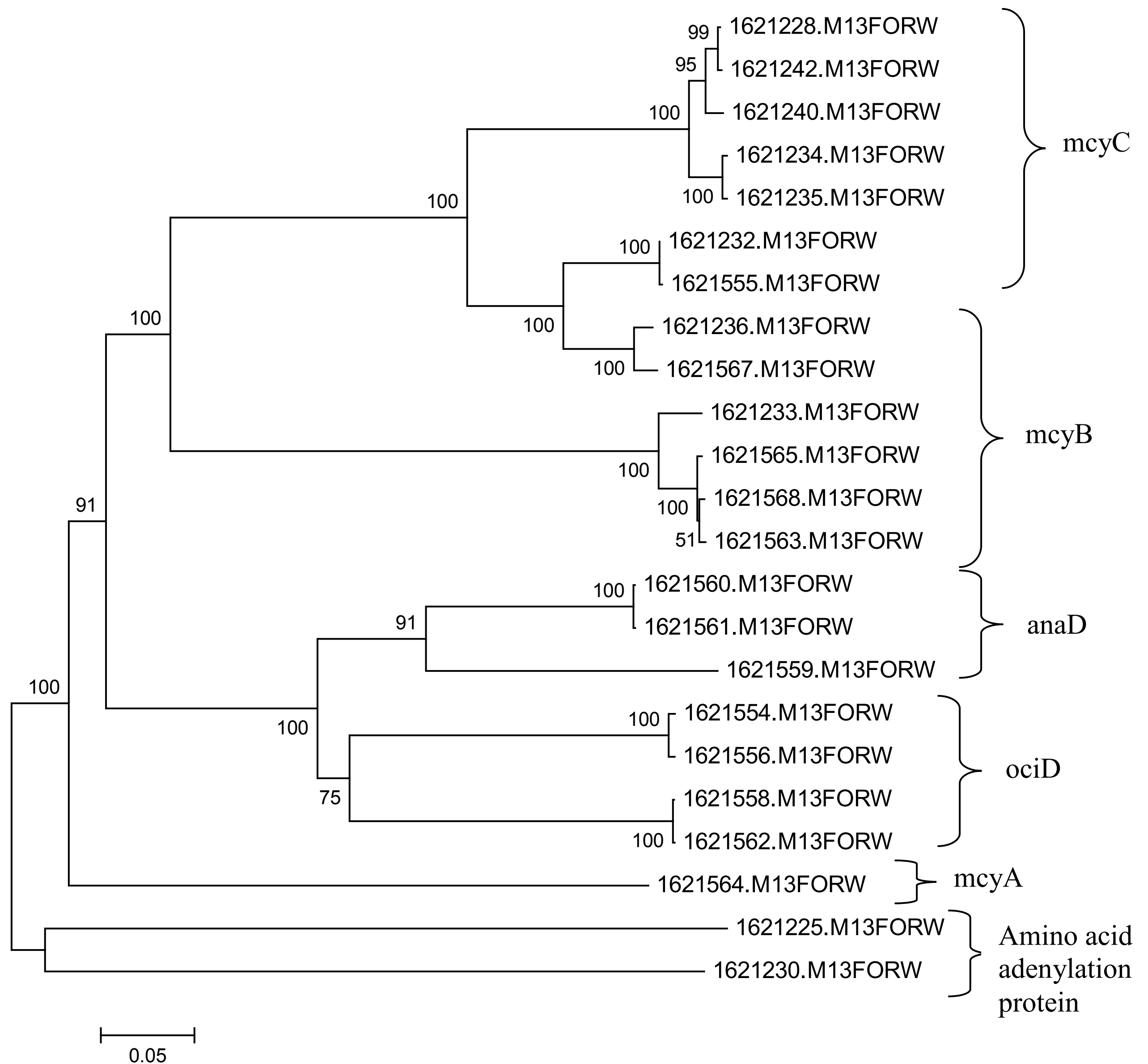

Figure 2. Phylogenetic tree of *mcyB* sequences isolated from western Lake Erie. Each number set stands for one sample. Bootstrap values greater than 50% are indicated at each node.

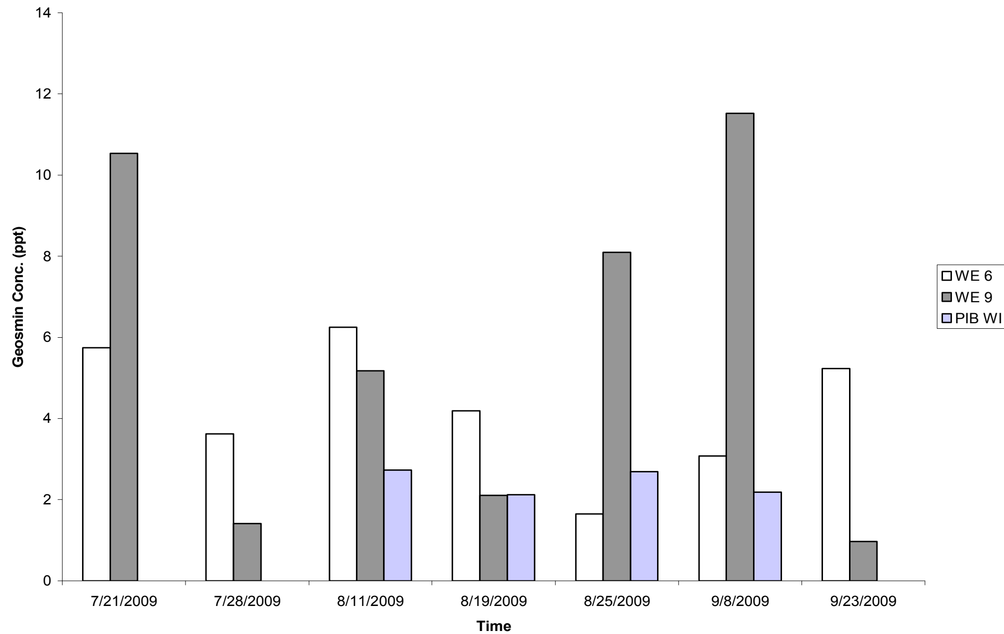

Figure 3. Geosmin trends over the sampling season in the western basin of Lake Erie. Concentrations are from raw water samples and are measured in parts per thousand.

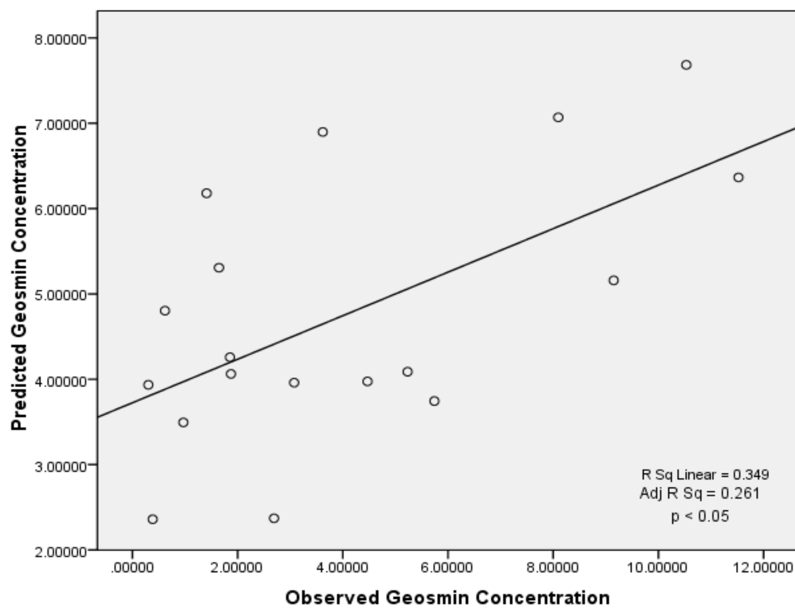

Figure 4. Summary graph of the multiple linear regression. This displays the trend that should result from the formulas, based on the predicted and observed geosmin results of the analysis.