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July 22, 2010

Lake Erie Commission
One Maritime Plaza, 4th Floor
Toledo, OH 43604
www.lakeerie.ohio.gov

RE: SG 364-09 "Sediment Contribution to Lake Erie Algal Blooms"

Ohio Lake Erie Commission:

In accordance with final reporting guidance documents provided, I am enclosing 3 paper copies and providing one electronic copy of the Technical Report for SG 364-09 "Sediment Contribution to Lake Erie Algal Blooms." The final accounting form for the grant which expires on 07/31/2010 will be submitted no later than August 15th. We will need this additional time to ensure the final accounting on the grant is correct. This project aimed to determine the potential role for sediment contribution to *Microcystis* bloom formation in the Western Lake Erie Basin. Specifically, we investigated whether or not sediments served as a source and a sink for both *Microcystis* cells and phosphorous. Sediment and Lake Erie water sampling occurred during the summer of 2009 in coordination with the USEPA GLNPO project (GL-00E75701) entitled "Lake Erie Algal Source Tracking (LEAST)" under the direction of Dr. Thomas Bridgeman. Julie Letterhos (Ohio EPA Division of Surface Water and Lake Erie Program Coordinator) served as the agency advisor for this project.

Please feel free to contact me with any questions regarding this submission.

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Technical Report
Ohio Lake Erie Protection Fund
Small Grant SG 364-09 "Sediment Contribution to Lake Erie Algal Blooms"
July 2010

Abstract:

The objective of this research was to determine the potential for sediment contribution to *Microcystis* bloom formation in the Western Lake Erie Basin during summer 2009. It was hypothesized that *Microcystis* will overwinter in sediments and may be transported, along with phosphorus, to the water column when environmental conditions support algal bloom formation. Before, during, and after the *Microcystis* algal bloom, surface water and sediment samples were collected and analyzed from six fixed locations in the Western Lake Erie Basin. Detectable *Microcystis* concentrations were present in the sediment prior to bloom formation at two sites and during and after the bloom at all six sites ($5.5x10^4$ to $2x10^5$ cells per gram of dry sediment). This corresponded with the appearance of detectable *Microcystis* concentration in the lake during and after the bloom at five of the six sites ranging from $5x10^3$ to $7x10^4$ cells per milliliter across the entire water column. However, phosphorus concentrations in the sediment could not be correlated to the *Microcystis* cell density in the lake during or after the bloom. These findings suggest that *Microcystis* colonies deposit into Lake Erie sediments and that these colonies may remain at some sediment sites until bloom formation the following summer.

Activities and Timeline:

Quarter 1 (April – June 2009): Supplies were purchased. Sampling was coordinated. Sampling began on June 9th.

Quarter 2 (July – September 2009): Additional samples were collected in August and September. Sample analyses commenced.

Quarter 3 (October – December 2009): Sample processing continued. Data analysis began. Sediment samples were sent for nutrient analysis. Abstract submitted for OAS.

Quarter 4 (January –March 2010): Participated in Phosphorous Research Forum at the LEC. Physical sediment characterization was completed.

Quarter 5 (April – July 2010): OAS and LEMN presentations were delivered in April. Sample analyses were verified using image analysis during June 2010. Final report was written. Manuscript writing commenced.

Project Deliverables:

MS Thesis entitled "Investigation of *Microcystis* Cell Density and Phosphorus in Benthic Sediment and Their Effect on Cyanobacterial Blooms on Western Lake Erie in the

Summer of 2009" by Erik Lange in the Department of Civil Engineering at the University of Toledo, May 2010.

C. Gruden. February 2010. "Do sediments serve as a source or 'seed' of *Microcytis spp.?*" Phosphorus Research Forum. Sponsored by the Lake Erie Millennium Network of LRRIN, February 11-12, 2010. Lake Erie Center, University of Toledo.

E. Lange and C. Gruden. April 2010. "The Potential Role of Sediments in Algal Bloom Formation in the Western Lake Erie Basin" Platform Presentation, Ohio Academy of Science Annual Meeting, April 9-10, Ohio Northern, Ada, OH.

D. Kane, J. Conroy, D. Bade, W. Edwards, D. Culver, J. Chaffin, K. Wambo, C. Gruden, and T. Bridgeman. April 2010. "Monitoring, mechanisms, and macronutrients: *Microcystis* in the Maumee and Sandusky Systems" Platform Presentation, The 6th Biennial Conference of the Lake Erie Millennium Network, April 27 - April 29, University of Windsor, Windsor, Ontario, Canada.

E. Lange, K. Wambo, and C. Gruden. 2010. "Investigating the potential role of sediments in *Myrocystis spp.* bloom formation in the Western Lake Erie Basin." Under preparation for submission to Water Science and Technology.

Project Background:

Blue-green algal blooms are very common on eutrophic lakes around the world. These blooms are caused by a type of cyanobacteria. Commonly, species from the *Microcystis* genus are either present within the blooms or the dominant cyanobacteria species within the bloom. Some species from the *Microcystis* genus release a harmful toxin known as microcystin. Due to the harmful effects of this toxin, the World Health Organization has set recommended limits for sources of drinking water at 1 microgram per liter. However, many of these cyanobacterial blooms produce microcystin levels that will exceed these limits.

Extensive previous research focused on these cyanobacterial blooms, the species that cause the blooms and the effects of these blooms on eutrophic water systems around the globe. The blooms are commonly considered an indication of the health of the water system. To increase the health of these systems, nutrient control has been the preferred method of limiting these blooms and has previously been successful in Western Lake Erie. Over the last decade; however, harmful algal blooms have returned to the Western Lake Erie Basin. As a result, research focused on these cyanobacterial blooms has increased significantly.

In order to thoroughly understand algal bloom formation, research must focus on not only algae growth in the water column during the bloom but also on the life cycle of *Microcystis* cells including deposition to the sediment, overwintering in the sediment, and reinvasion of the water column. This research project investigated the effect that the sediment in the Western Lake Erie basin has on the cyanobacterial blooms. The first

objective was to investigate whether the sediments serve as a sink or source of *Microcystis* colonies which contribute to *Microcystis* spp. bloom formation. In addition, we investigated if the sediments might serve as a sink or source of phosphorus, promoting *Microcystis* spp. bloom formation.

Methods and Materials:

Sample Collection and Storage. Samples were collected from the Western Lake Erie Basin during the summer of 2009. Samples were collected at each of the sites during three unique sampling events throughout the summer. Sampling on three dates was deemed to be necessary to analyze conditions before, during, and after bloom formation. At each sampling date, six sites within the Western Lake Erie Basin were visited. These sites were GR1, 4P, 8M, 7M, MB18, and MB20 (Figure 1). Table 1 includes pertinent information, including distance from the mouth of the Maumee River and water depth, concerning each of the sampling sites. At each of the six sites, corresponding sediment and lake water samples were collected during each sampling event. Lake water samples were taken as a composite sample over the entire depth of the lake. Duplicate sediment samples were collected using a stainless steel Eckman Dredge at each site.

Figure 1: Map of the Western Lake Erie Basin showing the mouth of the Maumee River and the location of the six sites sampled for this research.

Table 1: Pertinent information of each sampling site

Sampling Sites	Latitude (°)	Longitude (°)	Distance to the Mouth of Maumee River (km)	Water Depth (m)
MB20	41.715	-83.456	2	2
MB18	41.742	-83.402	7	1.5
8M	41.789	-83.356	13	5.5
7M	41.733	-83.297	14	5.7
GR1	41.821	-83.186	26	8.5
4P	41.750	-83.103	30	9.5

Sediment Characterization

Percent Solids. The first test to determine physical characteristics of the sediments that was performed was a moisture density test to determine the percent of solids by weight within the samples. This test was completed in accordance with test P1-A-1 for soil moisture content as set by the Department of Sustainable Natural Resources (AS1289 B1.1). Duplicate samples from each site were combined to create one single composite sample from each of the six sites at each of the six dates (Table 2).

Table 2: The percent of dry solids within each sediment sample.

Site	23-Jun-09	9-Aug-09	14-Sep-09
7M	51.4%	55.2%	49.6%
8M	39.4%	37.2%	40.0%
GR1	44.7%	58.4%	45.1%
4P	31.4%	37.5%	29.5%
MB18	78.0%	75.3%	77.0%
MB20	50.0%	53.8%	48.9%

Sediment samples from site MB18 were noted to be the most coarse-grained, sandy samples. During visual inspection of the soil, samples from the site 4P were conversely noted to be the most fine-grained. Due to the large difference in this physical property of the sediment samples, the percent of dry solids of the samples proved important during analysis of the *Microcystis* cell density in the soils and the phosphorus levels in the sediments. The phosphorus and *Microcystis* cell density value results from each sample analyzed were normalized to gram of dry sediments according to the results of the moisture content test.

Grain Size Distribution of Sediments. A hydrometer analysis for grain size distribution was run for sediment from each sample site. This test was completed in accordance with ASTM D 422. Composite samples were prepared and their grain size distribution determined for each of the six sites sampled. This protocol used a Type 152H hydrometer (Ertco, U.S.A.). Readings, taken from the hydrometer at the liquid surface, were measured in grams of solids per 1000 ml solution.

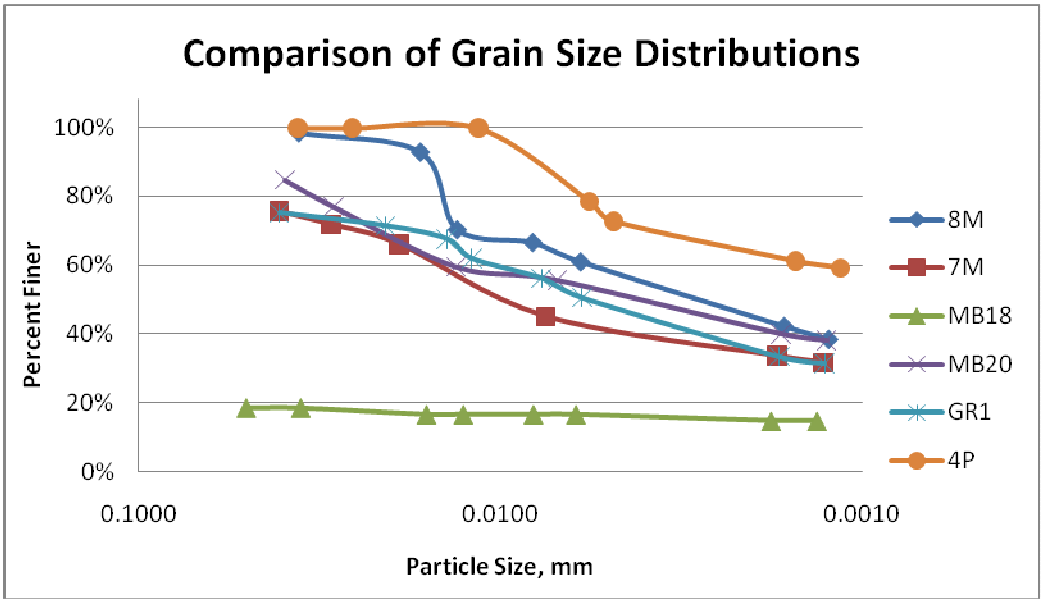

Figure 3: Grain size distribution of composite sediment samples from the each of the six sampling sites.

Visual observations prior to the hydrometer analysis suggested that site MB18 was a sandy soil, as compared to the clayey soils in the samples from the other five sites. Of the other five sites, the sediment from site 8M was noted to be the most coarse-grained of the clayey samples and the sediment from site 4P was noted to be the most fine-grained of the clayey samples.

Table 3: Median grain size of the sediment samples as interpolated or extrapolated from each sampling site taken from the grain size distribution.

Site	MB18	7M	GR1	MB20	8M	4P
Median Grain Size (mm)	.085	.010	.0057	.0051	.0034	.0005

Phosphorus Analysis. The duplicate wet sediment samples were blended into one composite sample. Approximately five grams of each of the eighteen composite samples were transferred to an air-tight falcon tube. These were sent to Jack Kramer at the Heidelberg Water Quality Lab for processing. Each of the eighteen composite samples was processed for concentrations of phosphorus in three forms: soluble phosphorus (SRP), total phosphorus (TP), and iron strip test phosphorus ($FeCl_2P$ or FE P). Results were reported as phosphorus levels per gram dry weight of sediment.

***Microcystis* Analysis in the Sediment.** Each sample was stored with in a plastic Ziploc bag at 4ºC. To begin sediment sample processing, approximately five grams of each duplicate sample was extracted and deposited into a 250 ml graduated cylinder. Laboratory grade water was added to the samples to fill the 250 ml graduated cylinder. The graduated cylinder was then covered and vortexed for one minute. Next, the

mixtures of water and suspended sediment were allowed to settle for a minimum of 48 hours. The buoyancy of the *Microcystis* cells allowed the cells to separate from the other organisms and particles that were within the mixtures.

After settling, 15 ml of the mixture sample was transferred from the top of each graduated cylinder to a single falcon tube and fixed using formalin (10% by volume). In an effort to break apart clumps of *Microcystis* cells for accurate counting, the 1ml subsample underwent sonication (Model 1510, Branson Ultrasonics Corporation, Danbury, Connecticut) for five minutes. After sonication, the samples were ready to be filtered. The 15ml mixture sample was filtered onto a black polycarbonate filter, 0.22 micron, 25 mm (GE Water & Process Technologies, USA). After drying, the polycarbonate filters were mounted on a glass microscope slide using immersion oil and covered with a cover slip. The slides were viewed using a fluorescence microscope (Model BX51, Olympus, Japan) for counting under 400X magnification. The detection limit of this approach was 560 cells/gram wet weight of sediment.

Previous research has indicated that overwintering cells are smaller, non-growing cells, and that the proportion of non-growing cells is the highest during the winter and fall (Bostrom, 1989). Using our method which included buoyancy and 400X magnification, we detected only enlarged (>5µm) cells in the sediment (not smaller overwintering cells) to determine trends of the *Microcystis* cell density in the sediment. Only cells undergoing sedimentation or reinvasion of the water column were targeted to be detected and included in the analysis.

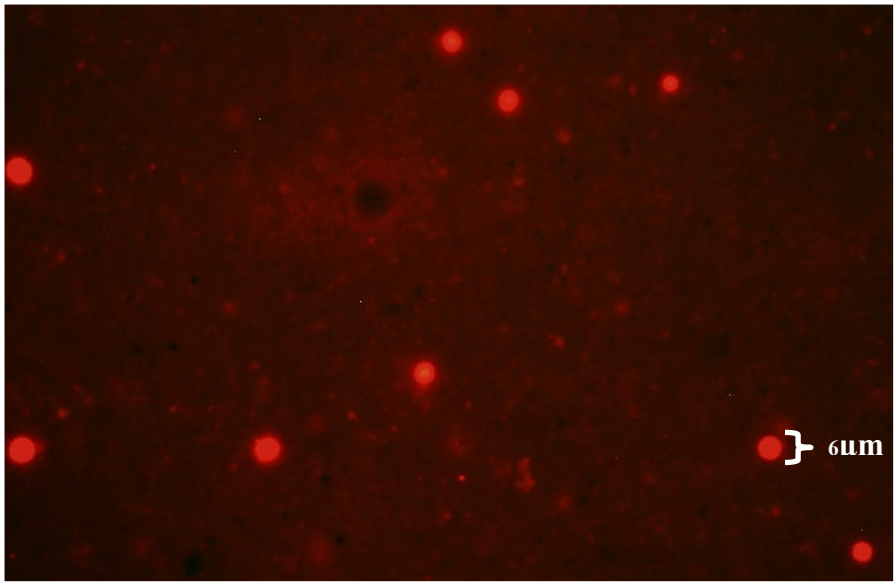

Figure 4: A photograph taken of a field on a slide from the second sampling date of the sediment samples at site 7M (400X).

***Microcystis* Analysis in the Lake Water.** One liter samples from each sampling site were stored at the lab at 4ºC. The samples were allowed to settle in Imhoff cones for a minimum of 48 hours at room temperature. These *Microcystis* spp. samples were

processed as per a method developed in 2008 for determining *Microcystis* cell density in lake water using fluorescence microscopy (Wang, 2008).

Results and Discussion:

Sediment Phosphorus Levels. Results for phosphorus testing from each of the 18 sites were returned from the Heidelberg Water Quality Lab and normalized to account for moisture content. Tables 4 through 6 have the normalized results for soluble phosphorus (SRP) total phosphorus (TP) and iron strip test phosphorus ($FeCl_2P$) concentrations in each of the sediment samples listed as milligrams per gram dry weight of sediment.

Table 4: Soluble phosphorus concentrations in sediments.

	23-Jun-09	6-Aug-09	14-Sep-09
Site	**SRP (mg/g dry weight)**	**SRP (mg/g dry weight)**	**SRP (mg/g dry weight)**
7M	0.0357	0.0356	0.0304
8M	0.0401	0.0157	0.0238
GR1	0.0223	0.0095	0.0196
4P	0.0163	0.0114	0.0063
MB18	0.0048	0.0038	0.0009
MB20	0.0959	0.1167	0.0956

Table 5: Total phosphorus concentration in sediments.

	23-Jun-09	6-Aug-09	14-Sep-09
Site	**TP (mg/g dry weight)**	**TP (mg/g dry weight)**	**TP (mg/g dry weight)**
7M	0.6917	0.7040	0.7310
8M	1.0961	0.6651	0.8827
GR1	0.6038	0.6793	0.7506
4P	0.8023	0.9143	0.8843
MB18	0.0705	0.0357	0.0962
MB20	0.9698	0.9855	0.9456

During the first sampling date, total phosphorus (TP) concentrations in the five fine-grained sediment samples (MB20, 8M, 7M, GR1, and 4P) varied between 0.6038 and 1.0961 mg TP per gram dry weight of sediment. The minimum value was at site GR1, and the maximum value was at site 8M. The total phosphorus concentrations found for each sampling site during the other two sampling dates were also in between this range. In August of 1990, previous research measured a total phosphorus concentration of 1.69 mg per gram dry weight of sediment from the bottom a lake in Sweden with constantly high external phosphorus loading rates averaging between 125 and 340 micrograms per liter (Brunberg, 1992). The concentrations found indicated elevated levels of phosphorus in the sediment. The phosphorus in the sediment is at a high enough concentrations to

have a lingering effect upon the bloom forming *Microcystis* species in the water column. This is especially true if the phosphorus become bioavailable.

Table 6: Iron strip test phosphorus concentration in sediments.

	23-Jun-09	6-Aug-09	14-Sep-09
Site	**$FeCl_2P$ (mg/g dry weight)**	**$FeCl_2P$ (mg/g dry weight)**	**$FeCl_2P$ (mg/g dry weight)**
7M	0.1610	0.1528	0.1739
8M	0.2481	0.1568	0.2055
GR1	0.1412	0.1928	0.2118
4P	0.2159	0.2358	0.2505
MB18	0.0242	0.0162	0.0164
MB20	0.3143	0.3168	0.2869

The iron strip test was an effort to determine the amount of chemically available phosphorus, which was expected to be higher than the amount of soluble reactive phosphorus. Results, in fact, indicated that the $FeCl_2P$ levels were higher than SRP levels. This may be a more accurate representation of the amount of phosphorous that is "available" to contribute to algal bloom formation.

In order to determine if there had been any statistically significant changes in phosphorus levels, comparisons of phosphorus levels were completed from before the bloom to during the bloom (from June to August) and from during the bloom to after the bloom (from August to September). SRP, TP, and $FeCl_2P$ were all compared by the t-tests. For each t-test, there were no statistically significant differences. Previous work has shown that both SRP and TP concentrations in the water column are expected to undergo massive increases due to the phosphorus released from the sediment during *Microcystis* blooms (Xie, 2003). However, microbial activity of *Microcystis* cells undergoing reinvasion of the water column does affect the phosphorus release from the sediment, and that this may not be observed with infrequent sampling (Brunberg, 1992). The location of the site does not seem to have affected the phosphorus concentration. Another physical property of the sediment samples, median grain size, may have had more influence.

***Microcystis* Cell Density in Sediment Samples.** Average *Microcystis* cell density was determined for each of the 18 sediment samples (36 duplicate samples) using all of the analyzed slides. The average levels found are displayed in Table 7. Samples that had cell density values below the detection limit are displayed as 'BDL' in this table. Samples from June 23, 2009 were taken at a time considered to be before the cyanobacterial bloom. Samples from August 6, 2009 were taken at a time considered to be during the cyanobacterial bloom. Samples from September 14, 2009 were taken at a time considered to be after the cyanobacterial bloom.

For each of the six sites, *Microcystis* cell density mean values were above the detection limit during and after the bloom. Only at sites MB18 and GR1 were *Microcystis* cells detectable before the bloom. The cell density mean values during the bloom at sites

MB18 and 8M were slightly greater than after the bloom. The cell density mean values after the bloom at sites MB20, 7M, GR1, and 4P were greater than the cell mean density values during the bloom. At site 4P, the cell mean density values recorded for the third sampling date were greater than the values recorded for the second sampling date.

Table 7: Average values for the *Microcystis* cell density values per gram dry weight of sediment and the associated standard deviations for each sample.

	23-Jun-09	6-Aug-09	14-Sep-09
Site	**(cells/gram dry weight of sediment)**	**(cells/gram dry weight of sediment)**	**(cells/gram dry weight of sediment)**
7M	BDL	$6.66x10^4 \pm 2.21x10^4$	$7.17x10^4 \pm 1.24x10^4$
8M	BDL	$2.07x10^5 \pm 2.67x10^4$	$1.84x10^5 \pm 7.78x10^4$
GR1	$8.60x10^3 \pm 6.01x10^3$	$5.49x10^4 \pm 9.32x10^3$	$8.20x10^4 \pm 2.41x10^4$
4P	BDL	$7.00x10^4 \pm 1.33x10^4$	$1.31x10^5 \pm 4.07x10^4$
MB18	$1.34x10^4 \pm 7.28x10^3$	$6.54x10^4 \pm 2.90x10^4$	$5.69x10^4 \pm 7.47x10^3$
MB20	BDL	$1.04x10^5 \pm 4.41x10^4$	$1.25x10^5 \pm 2.21x10^3$

The cell density mean values at sites MB20, MB18, 8M, 7M, 4P, and GR1 are further represented in Figure 5. The mean cell density values at the site for each date are displayed. The test results indicate that there is a significant increase in the density of these cells in the sediment from before the bloom (June) to during the bloom (August), but there is no significant changes in the concentration of these large cells from during the bloom (August) to after the bloom (September).

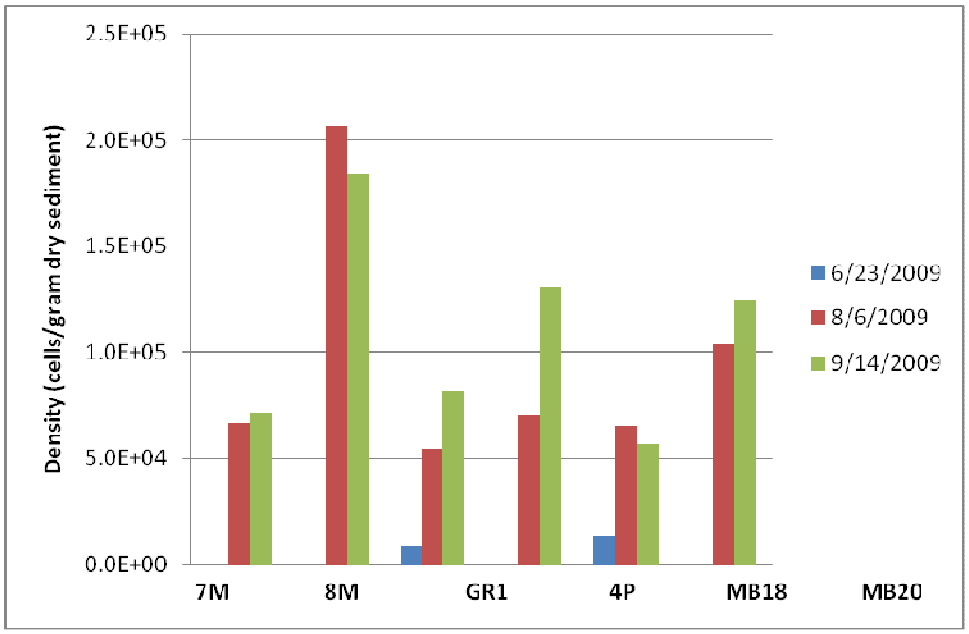

Figure 5: *Microcystis* cell density (cell/mL) in sediment samples as a function of sampling site (MB20, MB18, 8M, 7M, GR1, 4P) and as a function of sampling date (June, August, September).

***Microcystis* Cell Density in Lake Water.** Average *Microcystis* cell density for each of the 18 lake water samples are displayed in Table 8. Samples that had cell density values below the detection limit are displayed as 'BDL' in this table. The results of t-tests indicate that the cell density during the bloom is statistically higher (P=0.05) than the cell density before the bloom for sites MB20, MB18, 8M, and 7M. In addition, the cell density during the bloom is statistically higher than the cell density after the bloom for sites MB20, MB18, and 8M. Finally, the cell density after the bloom is statistically higher than the cell density during the bloom for site 7M. From these results, it was also shown that the *Microcystis* cell density during a cyanobacterial bloom has a negative correlation with the distance from the mouth of the Maumee River and with lake water depth.

Table 8: The average *Microcystis* cell density values and the associated standard deviations found for each of the eighteen samples.

	9-Jun-09	4-Aug-09	14-Sep-09
Site	**(cells/mL)**	**(cells/mL)**	**(cells/mL)**
7M	BDL	$5.78x10^3 \pm 6.11x10^2$	$2.39x10^4 \pm 1.14x10^4$
8M	BDL	$3.06x10^4 \pm 1.03x10^4$	$5.46x10^3 \pm 4.11x10^3$
GR1	BDL	$7.57x10^3$	BDL
4P	BDL	BDL	BDL
MB18	BDL	$6.93x10^4 \pm 2.03x10^4$	$7.42x10^3 \pm 2.69x10^3$
MB20	BDL	$5.48x10^4 \pm 2.81x10^4$	BDL

The objectives of this study included investigating whether the sediments serve as a sink or a source for two suspected contributors to cyanobacterial blooms: *Microcystis* cells and phosphorus. Up to this point, data has been presented concerning levels of *Microcystis* cell density in lake water, levels of *Microcystis* cell density in sediment, and phosphorus concentrations in the sediment. Corresponding data was collected at each of six sites over three sampling periods. Neither *Microcystis* cell density in sediments nor phosphorus concentrations in the sediment was correlated to depth of the lake, distance from the mouth of the Maumee River, or median grain size distribution of the sediment. No statistically significant pattern was determined for changes in phosphorus levels from the first to the second sampling dates or from the second to the third sampling dates. Instead, statistically significant correlations were found linking both total phosphorus concentration and iron strip test phosphorus concentration to the median grain size of the sample. Analysis of the cell density values in lake water showed a statistically significant relationship to both depth of the lake at the site and the distance from the Maumee River.

The highest lake cell density values are at the sites closest to shore (MB20 and MB18). This is consistent with observations in the field that the early cyanobacterial bloom in August was close to the shore. This is also consistent with previous research that has indicated that up to 50% of initial *Microcystis* abundance in surface sediment may reinvade the water at shallow sites (depth of one meter), compared to only 8% at deep sites (depth of seven meters) (Brunberg, 2003).

Previous research on numerous eutrophic lakes has indicated that immediately preceding a cyanobacterial bloom and during the early stage of the bloom, *Microcystis* cells enlarge and reinvade the water column (Ihle, 2005). This reinvasion is driven by the buoyant forces within each cell that rematerialize when the cell is in need of sunlight to create energy. This is the mostly likely cause for the relationship between the increase of *Microcystis* cell density in both the lake and the sediment between the first and second sampling dates. However, additional testing will need to be completed in order to confirm that this is the case. If this is the case, it will be possible to show an increase in density of the detectable cells in the sediment prior to the cyanobacterial bloom, which is indicated by an increase in cells density within the lake water.

Further visual inspection of the *Microcystis* cell density in sediment data reveals a few interesting trends. One interesting trend is the slight decrease in cell density from during the bloom to after the bloom at sites MB18 and 8M. These are two of the three sites that still had measurable cell densities above the detection limit in the lake water samples after the bloom. The third site with a measurable cell density in the lake during the third sampling date is site 7M. At 7M, the cyanobacterial bloom is more prevalent during the third sampling date than the second sampling date as indicated by the statistically significant increase of the cell density in the water between these dates. These two trends may seem to suggest conflicting data. However, the decrease in lake sediment cells density at sites MB18 and 8M could indicate that additional cells reinvaded the water column after the second sampling period. This would account for the remaining detection of cells in the water column at these sites, because after reinvasion, *Microcystis* cells have been shown to undergo growth in the water column (Tsujimura, 2000).

All of the analyzed trends of these larger *Microcystis* cells indicated that the sediment is a source of *Microcystis* cells for the cyanobacterial blooms in the water column. Statistically significant increases in large cells corresponded to statistically significant increases in cell density of the lake. After statistically significant decreases of cell density in the lake, the sediment cell density did not exhibit a significant change.

Conclusions:

This project was undertaken in an effort to determine the source of harmful algal bloom formation. If the source or initial seed can be identified, it may be possible to commence mitigation efforts. This study was completed concurrently with an ongoing USEPA GLNPO project (GL-00E75701) entitled "Lake Erie Algal Source Tracking (LEAST)" under the direction of Dr. Thomas Bridgeman which investigated the possibility of upland surface waters (rivers and streams) providing the seed for bloom formation in the Western Lake Erie Basin. *Microcystis* spp. were detected both in Lake Erie sediments and tributary waters prior to bloom formation in Lake Erie. These findings suggest that there may be more than one location contributing to bloom formation in Lake Erie. Future studies should incorporate sediment analysis in early spring to determine if *Microcystis* spp. are overwintering in tributary sediments as well as Lake Erie sediments. In addition, some genetic analyses should be carried out for source tracking from sediments to overlying waters.

The findings in this study related to sediment phosphorus loads were inconclusive. It is unclear from the data collected if sediments contribute phosphorus directly toward algal formation. However, it was determined that the phosphorus levels in Western Lake Erie Basin sediments are in fact high. Future studies need to incorporate experiments at the bench scale in an effort to verify mechanisms that may occur in the field, including microbially mediated sediment phosphorus release to overlying water. The time scales required to measure phosphorus load changes may be too small to be measured in field studies with a limited number of samples being collected.

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Acknowledgements:

Dr. Thomas Bridgeman and Justin Chaffin, Lake Erie Center, UT

Jack Kramer, Heidelberg Water Quality Laboratory

Amr Zaky, Hui Wang, Erik Lange, Department of Civil Engineering, UT

USEPA GLNPO (GL-00E75701)

This project was funded in part through the Lake Erie Protection Fund. The LEPF is supported by the voluntary contributions of Ohioans who purchase the Erie…Our Great Lake License plate featuring Marblehead Lighthouse.