A 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

Submitted to the Graduate Faculty as partial fulfillment of the requirements for the Master of Science Degree in Civil Engineering

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August 2010

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An Abstract of

Investigation of *Microcystis* Cell Density and Phosphorus in the Sediments and Their Effect on Cyanobacterial Blooms on Western Lake Erie in the Summer of 2009

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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 during and after the bloom at all six sites ranging from $5.5x10^4$ 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 with any significance to the *Microcystis* cell density in the lake during or after the bloom. These findings suggest that *Microcystis* colonies do deposit into Lake Erie sediments and that these colonies may remain at some sediment sites until bloom formation the following summer.

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It is necessary to note that this project was possible due to a grant from the Lake Erie Protection Fund. Continuous funding on topics involving Lake Erie continues to make this region of the world a better place.

Samples were collected by Tom Bridgeman. His work made this thesis possible.

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Table of Contents

Abstract iii

Acknowledgements iv

Table of Contents v

List of Tables vii

List of Figures xiv

1 Introduction 1

2 Literature Review 3

2.1 *Microcystis* Properties 3

2.2 Collecting and Enumerating *Microcystis* 5

2.3 Harmful Effects of Microcystis Blooms 6

2.4 *Microcystis* in Open Lake Water 8

2.5 Sediments and *Microcystis* Recruitment 11

2.6 Lake Erie 14

3 Materials and Methods 19

3.1 Sample Collection 19

3.2 *Microcystis* Analysis in the Lake Water 21

3.3 Percent of Dry Solids of Sediment 25

3.4 Grain Size Distribution of Sediments 27

3.5 Phosphorus Testing of the Sediment 31

3.6 *Microcystis* Analysis in the Sediment 32

3.7 Statistical Analysis of the Data 38

4 Results 44

4.1 *Microcystis* Cell Density in Lake Water ……………………………… 44
4.2 Percent Dry Solids of Sediment ………………………………………. 54
4.3 Grain Size Distribution of Sediment ………………………………….. 56
4.4 Phosphorus Testing of the Sediment ………………………………….. 61
4.5 *Microcystis* Cell Density Values in Sediment ………………………... 72
4.6 Statistical Analysis of the Objectives of this Report …………………. 86

5 Discussion **94**

5.1 Sediments as a Source of Phosphorus Effecting Blooms …………… 94
5.2 Sediments as a Source of *Microcystis* Cells Effecting Blooms ………. 97
5.3 Overview of this Research ……………………………………………. 102

References **105**

Appendices

A Detailed Results of All Statistical Tests **111**

B Detailed Results of Hydrometer Analysis **131**

C Figures Representing Change in Phosphorus Concentration and Change in Lake Cell Density over the Three Sampling Dates **135**

D Figures Indicating Non-Significant Correlations **139**

List of Tables

3-1 Pertinent information of each sampling site. ……………………….……. 21

4-1 The average *Microcystis* cell density values and the associated standard deviations found for each of the eighteen lake water samples. ……...…..... 45

4-2 Test statistics and conclusions drawn for T-tests 4.1.1, 4.1.2, 4.1.3, 4.1.4, 4.1.5, 4.1.6, 4.1.7, and 4.1.8. ……………………………………………... 50

4-3 Test Statistics and Conclusions Drawn for Pearson Correlation Tests 4.1.9 and 4.1.10. ………………………………………………………………… 53

4-4 The percent of dry solids within each sediment sample. ……….………... 55

4-5 Median grain size of the sediment samples as interpolated or extrapolated from each sampling site taken from the grain size distribution. ………..… 61

4-6 Soluble phosphorus concentrations results from the sediment samples from each site. ………………………………………………….……… 62

4-7 Total phosphorus concentration results from the sediment samples from each site. …………………………………………………….……………… 63

4-8 Iron strip test phosphorus concentration results from the sediment samples from each site. ……………………………………………..………… 64

4-9 Test statistics and conclusions drawn for T-tests 4.4.1, 4.4.2, 4.4.3, 4.4.4, 4.4.5, and 4.4.6. ……………………………………………….…….. 66

4-10 Test statistics and conclusions drawn for Pearson correlation T-tests 4.4.7, 4.4.8, and 4.4.9. ………………………………………………….……... 72

4-11 Average values for the *Microcystis* cell density values per gram dry weight of sediment and the associated standard deviations for each sediment sample analyzed. ……………………………..……………… 73

4-12 Test statistics and conclusions drawn for T-tests 4.5.1, 4.5.2, 4.5.3, 4.5.4, 4.5.5, 4.5.6, 4.5.7, 4.5.8, 4.5.9, and 4.5.10. ……………………………… 80

4-13 Test statistics and conclusions drawn for Pearson Correlations tests 4.5.11, 4.5.12, 4.5.13, 4.5.14, 4.5.15, and 4.5.16. ……………………….………….. 83

4-14 Test statistics and conclusions drawn for Pearson Correlations tests 4.5.17, 4.5.18, and 4.5.19. ……………………………………………………….. 85

4-15 Test statistics and conclusions drawn for Pearson Correlations tests 4.6.1, 4.6.2, 4.6.3, 4.6.4, 4.6.5, and 4.6.6. …………………………..………… 89

A-1 Null and alternative hypotheses for t-test Nos. 4.1.1 through 4.1.8. …..…. 111

A-2 Results of t-test No. 4.1.1. ………………………..…………………….. 112

A-3 Results of t-test No. 4.1.2. …………………..………………………….. 112

A-4 Results of t-test No. 4.1.3. ……………………………………..………….. 112

A-5 Results of t-test No. 4.1.4. …………………………………..…………….. 112

A-6 Results of t-test No. 4.1.5. ……………………………………..………….. 112

A-7 Results of t-test No. 4.1.6. …………………………………..…………….. 113

A-8 Results of t-test No. 4.1.7. …………………………………..…………….. 113

A-9 Results of t-test No. 4.1.8. …………………………………..…………….. 113

A-10 Null and alternative hypotheses for Pearson test Nos. 4.1.9 and 4.1.10. …. 113

A-11 Results of Pearson test No. 4.1.9. …………………..…………………….. 114

A-12 Results of Pearson test No. 4.1.10. …………………………..………… 114

A-13 Null and alternative hypotheses for t-test Nos. 4.4.1 through 4.4.6. ……. 115

A-14 Results of t-test No. 4.4.1. …………………………..………………….. 116

A-15 Results of t-test No. 4.4.2. …………………………..………………….. 116

A-16 Results of t-test No. 4.4.3. …………………………..………………….. 116

A-17 Results of t-test No. 4.4.4. ……………………………..……………….. 116

A-18 Results of t-test No. 4.4.5. ……………………………..……………….. 117

A-19 Results of t-test No. 4.4.6. …………………………..………………….. 117

A-20 Null and alternative hypotheses for Pearson test Nos. 4.4.7, 4.4.8 and 4.4.9. ………………………………………………………………… 118

A-21 Results of Pearson test No. 4.4.7. ……………………..………………….. 119

A-22 Results of Pearson test No. 4.4.8. ……………………..………………….. 119

A-23 Results of Pearson test No. 4.4.9. …………………………..………….. 119

A-24 Null and alternative hypotheses for t-test Nos. 4.5.1 through 4.5.10. ...…. 120

A-25 Results of t-test No. 4.5.1. ……………..………………………………….. 121

A-26 Results of t-test No. 4.5.2. ……………………………..……………….. 121

A-27 Results of t-test No. 4.5.3. ……………………………..……………….. 121

A-28 Results of t-test No. 4.5.4. …………………………….……………….. 121

A-29 Results of t-test No. 4.5.5. …………………………….……………….. 122

A-30 Results of t-test No. 4.5.6. ……………………………..…………….. 122

A-31 Results of t-test No. 4.5.7. ……………………………..…………….. 122

A-32 Results of t-test No. 4.5.8. ……………………..……………..……….. 123

A-33 Results of t-test No. 4.5.8. …………………………….……………….. 123

A-34 Results of t-test No. 4.5.10. ………………………………….…..……….. 123

A-35 Null and alternative hypotheses for Pearson test Nos. 4.5.11, 4.5.12, 4.5.13, 4.5.14, 4.5.15, and 4.5.16. ……………………..……………….. 124

A-36 Results of Pearson test No. 4.5.11. ……………………..…………….. 125

A-37 Results of Pearson test No. 4.5.12. ……………………..……………….. 125

A-38 Results of Pearson test No. 4.5.13. ……………………..…………….. 125

A-39 Results of Pearson test No. 4.5.14. ……………………..……………….. 125

A-40 Results of Pearson test No. 4.5.15. ……………………..……………….. 126

A-41 Results of Pearson test No. 4.5.16. ……………………..…………….. 126

A-42 Null and alternative hypotheses for Pearson test Nos. 4.5.17, 4.5.18 and 4.5.19. ………………………….………………………………… 126

A-43 Results of Pearson test No. 4.5.17. …………………………………….. 127

A-44 Results of Pearson test No. 4.5.18. …………………………………….. 127

A-45 Results of Pearson test No. 4.5.19. …………………………………….. 127

A-46 Null and alternative hypotheses for Pearson test Nos. 4.6.1, 4.6.2, 4.6.3, 4.6.4, 4.6.5 and 4.6.6. ……………………………………………….... 128

A-47 Results of Pearson test No. 4.6.1. …………………………..……….. 129

A-48 Results of Pearson test No. 4.6.2. ……………………...…………….. 129

A-49 Results of Pearson test No. 4.6.3. ……………………..……………….. 129

A-50 Results of Pearson test No. 4.6.4. ……………………..……………….. 129

A-51 Results of Pearson test No. 4.6.5. ……………………..……………….. 130

A-52 Results of Pearson test No. 4.6.6. …………………..………………….. 130

B-1 Results of the grain size distribution test run on a composite of samples from site 8M. ……………..……………………………………………... 131

B-2 Results of the grain size distribution test run on a composite of samples from site 7M. ……………………..…………………………………………... 132

B-3 Results of the grain size distribution test run on a composite of samples from site MB18. ……………………………………………………………... 132

B-4 Results of the grain size distribution test run on a composite of samples from site MB20. ……………………………………………………………... 133

B-5 Results of the grain size distribution test run on a composite of samples from site GR1. ………………………..………………………………... 133

B-6 Results of the grain size distribution test run on a composite of samples from site 4P. …………………..……………………………………………... 134

List of Figures

2-1 Cells of the *Microcystis* genus magnified to 800X. ……………………... 4

3-1 Map of the Western Lake Erie Basin showing the mouth of the Maumee River and the location of the six sites from which samples were collected for this research. ……………………………………………………………. 20

3-2 A photograph of a standard Ekman Dredge. …………………………..…. 21

3-3 A photograph taken of a field on a slide from the second sampling date of the lake samples at site MB20. This photograph was taken with the use of a fluorescence microscope at a 400X magnification. ……………..………... 24

3-4 A photograph of a Type 152H Hydrometer within a sedimentation cylinder. ……………………………………………………………………... 28

3-5 A photograph taken of a field on a slide from the second sampling date of the sediment samples at site 7M. This photograph was taken with the use of a fluorescence microscope at a 400X magnification. ………………..... 36

4-1 *Microcystis* cell density (cell/mL) in lake water as a function of sampling site (MB20, MB18, 8M, 7M, GR1, 4P) and as a function of sampling date (June, August, September). ………………….…………………………... 46

4-2 *Microcystis* cell density in the lake water as a function of the sampling date at the site MB20. Error bars represent one standard deviation from the mean in each direction. ………………..……………….…………... 47

4-3 *Microcystis* cell density in the lake water as a function of the sampling date at the site MB18. Error bars represent one standard deviation from the mean in each direction. …………………..………….…………... 47

4-4 *Microcystis* cell density in the lake water as a function of the sampling date at the site 8M. Error bars represent one standard deviation from the mean in each direction. …………………………………….…………... 48

4-5 *Microcystis* cell density in the lake water as a function of the sampling date at the site 7M. Error bars represent one standard deviation from the mean in each direction. …………………………………….…………... 48

4-6 *Microcystis* cell density in the lake water as a function of the sampling date at the site GR1. Error bars are not present on this figure due to the lack of standard deviation values for the data. …………………………... 49

4-7 Graphical representation of the relationship between cell density in the lake water during the cyanobacterial bloom (August 4, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit is included, with its equation, to linearly define the relationship. ... 52

4-8 Graphical representation of the relationship between cell density in the lake water during the cyanobacterial bloom (August 4, 2009) at any given site and the water depth at that site. A line of best fit is included, with its equation, to linearly define the relationship. ……………………………..... 52

4-9 Graphical illustration of the grain size distribution of composite sediment samples from the site 8M. …………………………………...……... 57

4-10 Graphical illustration of the grain size distribution of composite sediment samples from the site 7M. …………………………………….………... 57

4-11 Graphical illustration of the grain size distribution of composite sediment samples from the site MB18. ……………………………….............. 58

4-12 Graphical illustration of the grain size distribution of composite sediment samples from the site MB20. ……………………………...………... 58

4-13 Graphical illustration of the grain size distribution of composite sediment samples from the site GR1. ……………………….………………... 59

4-14 Graphical illustration of the grain size distribution of composite sediment samples from the site 4P. …………………………………….…………….. 59

4-15 Graphical illustration of the grain size distribution of composite sediment samples from the each of the six sampling sites. This figure compares the grain size distributions from each of the sampling sites. The grain size distribution each site was not expected to conform to the grain size distribution from other sites. …………………………………….…………….. 60

4-16 Soluble phosphorus concentrations (mg/gram dry weight of sediment) 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). ... 62

4-17 Total phosphorus concentrations (mg/gram dry weight of sediment) 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)…. 63

4-18 Iron strip test phosphorus concentrations (mg/gram dry weight of sediment) 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) . ………………………………………………………… 64

4-19 Concentrations of phosphorus in the sediment at site 7M. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus. …………………………....… 67

4-20 Concentrations of phosphorus in the sediment at site 8M. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus. ………………………….....… 67

4-21 Concentrations of phosphorus in the sediment at site GR1. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus. …..……...… 68

4-22 Concentrations of phosphorus in the sediment at site 4P. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus. ……………………..……...… 68

4-23 Concentrations of phosphorus in the sediment at site MB18. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus. …………… 69

4-24 Concentrations of phosphorus in the sediment at site MB20. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus. …………… 69

4-25 The relationship of soluble phosphorus (SRP) concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample. The correlation the SRP concentration and the median grain size is represented by the line of best fit, which has an equation shown on this figure. …………………………………….…………………... 70

4-26 The relationship of total phosphorus (TP) concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample. The correlation the YP concentration and the median grain size is represented by the line of best fit, which has an equation shown on this figure. ..……………………………………………………………… 71

4-27 The relationship of iron strip test phosphorus ($FeCl_2P$) concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample. The correlation the $FeCl_2P$ concentration and the median grain size is represented by the line of best fit, which has an equation shown on this figure. ……………………………………………. 71

4-28 *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). ……………………………..…. 74

4-29 *Microcystis* cell density in the sediment samples as a function of the sampling date at the site 7M. Error bars represent one standard deviation from the mean in each direction. ……………………..………...………. 76

4-30 *Microcystis* cell density in the sediment samples as a function of the sampling date at the site 8M. Error bars represent one standard deviation from the mean in each direction. ……………………….…………...………. 76

4-31 *Microcystis* cell density in the sediment samples as a function of the sampling date at the site GR1. Error bars represent one standard deviation from the mean in each direction. …………………………….………… 77

4-32 *Microcystis* cell density in the sediment samples as a function of the sampling date at the site 4P. Error bars represent one standard deviation from the mean in each direction. ……………………..…………...………. 77

4-33 *Microcystis* cell density in the sediment samples as a function of the sampling date at the site MB18. Error bars represent one standard deviation from the mean in each direction. ……………………….………… 78

4-34 *Microcystis* cell density in the sediment samples as a function of the sampling date at the site MB20. Error bars represent one standard deviation from the mean in each direction. ……………………….………… 78

4-35 The comparative look at the Microcystis cell density in the lake water and the Microcystis cell density in the sediment at each site during the first sampling date. The vertical axis is holds values for cell density in both the sediment and lake water. The units on the vertical axis are cells per milliliter for the lake cell density values and cells per gram dry weight sediment for the sediment cell density values. ……………..……….……. 91

4-36 The comparative look at the Microcystis cell density in the lake water and the Microcystis cell density in the sediment at each site during the second sampling date. The vertical axis is holds values for cell density in both the sediment and lake water. The units on the vertical axis are cells per milliliter for the lake cell density values and cells per gram dry weight sediment for the sediment cell density values. ………….………………..…. 92

4-37 The comparative look at the Microcystis cell density in the lake water and the Microcystis cell density in the sediment at each site during the third sampling date. The vertical axis is holds values for cell density in both the sediment and lake water. The units on the vertical axis are cells per milliliter for the lake cell density values and cells per gram dry weight sediment for the sediment cell density values. ………………..…….……. 93

C-1 The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site MB20 over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values. ……………. 135

C-2 The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site MB18 over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values. ……………. 136

C-3 The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site 8M over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values. ……………. 136

C-4 The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site 7M over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values. ……………. 137

C-5 The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site GR1 over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values. ……………. 137

C-6 The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site 4P over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values. ……………. 138

D-1 Graphical representation of the relationship between cell density in the sediment during the cyanobacterial bloom (August 6, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship. …………………………..…………………..…… 139

D-2 Graphical representation of the relationship between cell density in the sediment after the cyanobacterial bloom (September 14, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship. ……………………………………..…. 140

D-3 Graphical representation of the relationship between the change in cell density in the sediment from during the cyanobacterial bloom (August 6, 2009) to after the cyanobacterial bloom (September 14, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship. ………………………………………………… 140

D-4 Graphical representation of the relationship between cell density in the sediment during the cyanobacterial bloom (August 6, 2009) at any given site and the water depth at that site. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship.… 141

D-5 Graphical representation of the relationship between cell density in the sediment after the cyanobacterial bloom (September 14, 2009) at any given site and the water depth at that site. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship. …………………………………………………………..… 141

D-6 Graphical representation of the relationship between the change in cell density in the sediment from during the cyanobacterial bloom (August 6, 2009) to after the cyanobacterial bloom (September 14, 2009) at any given site and the water depth at that site. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship. ... 142

D-7 Graphical representation of the relationship between the cell density in the sediment during the cyanobacterial bloom (August 6, 2009) at any given site and the median grain size distribution of the sediment at that site. A line of best fit, with its defining equation, is included on the figure to linearly define the relationship. ……………….……………………….…. 142

D-8 Graphical representation of the relationship between the cell density in the sediment after the cyanobacterial bloom (September 14, 2009) at any given site and the median grain size distribution of the sediment at that site. A line of best fit, with its defining equation, is included on the figure to linearly define the relationship. ……………………………………..…. 143

D-9 Graphical representation of the relationship between the change in cell density in the sediment from during the cyanobacterial bloom (August 6, 2009) to after the cyanobacterial bloom (September 14, 2009) at any given site and the median grain size distribution of the sediment at that site. A line of best fit, with its defining equation, is included on the figure to linearly define the relationship. ……………………………..…………. 143

D-10 The correlation between the change in *Microcystis* density in the lake water and the change in soluble phosphorus (SRP) concentration in the sediment at each sampling site between the first and second sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in SRP concentration and the change in cell density. ………...…………………... 144

D-11 The correlation between the change in *Microcystis* density in the lake water and the change in soluble phosphorus (SRP) concentration in the sediment at each sampling site between the second and third sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in SRP concentration and the change in cell density. ………………..…..………. 144

D-12 The correlation between the change in *Microcystis* density in the lake water and the change in total phosphorus (TP) concentration in the sediment at each sampling site between the first and second sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in TP concentration and the change in cell density. ………………………………………….. 145

D-13 The correlation between the change in *Microcystis* density in the lake water and the change in total phosphorus (TP) concentration in the sediment at each sampling site between the second and third sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in TP concentration and the change in cell density. ………………………….………..…. 145

D-14 The correlation between the change in *Microcystis* density in the lake water and the change in iron strip test phosphorus ($FeCl_2P$) concentration in the sediment at each sampling site between the first and second sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in $FeCl_2P$ concentration and the change in cell density. ……………………….………. 146

D-15 The correlation between the change in *Microcystis* density in the lake water and the change in iron strip test phosphorus ($FeCl_2P$) concentration in the sediment at each sampling site between the second and third sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in $FeCl_2P$ concentration and the change in cell density. ……………….………..…. 146

Chapter 1

Introduction

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 often been the main method of limiting these blooms. Control methods in place for nutrient limiting to the Western Lake Erie basin have been successful in limiting and eliminating cyanobacterial blooms since extensive biomass growth in the waters of Lake Erie became a concern. Due to the effectiveness that the limitation of the nutrient loading had upon eliminating

cyanobacterial blooms in Lake Erie, research into the blooms has been limited. Over the last decade; however, these blooms have returned to the Western Lake Erie Basin. As the blooms have, again, begun to dominate the Western Lake Erie Basin in the summertime, research focused on these cyanobacterial blooms has begun to increase.

In additional to the growth in the water column during the bloom, the life cycle of *Microcystis* cells includes deposition to the sediment, overwintering in the sediment, and reinvasion of the water column. The topic of this research is determining the effect that the sediment at the bottom of the Western Lake Erie basin has on the cyanobacterial blooms. The first objective is to investigate whether the sediments serve as a sink or source of *Microcystis* colonies that contributes to *Microcystis* spp. bloom formation. The other objective includes investigation as to whether the sediments serve as a sink or source of phosphorus that contributes to *Microcystis* spp. bloom formation.

Chapter 2

Literature Review

2.1 *Microcystis* Properties

Blue-green algae include a gram-negative species from the photosynthetic phylum Cyanobacteria. This phylum originated between 2.8 and 3.5 billion years ago, ranking it as the oldest phylum of oxygenic phototrophs on earth (Yang, 2007). No longer are these organisms classified as algae from the Eukaryota domain as once believed. Instead, members of this phylum are considered to be more closely related to bacteria, and have been included in the Archaebacteria domain. A magnified photo of cells belonging to the *Microcystis* genus is included as Figure 2-1. Many of the dominant bloom-forming species of the Cyanobacteria phylum are of the *Microcystis* genus. *Microcystis novacekii* was found to be a dominant phytoplankton in lake communities over the green alga *Scenedesmus quadricauda* (Takeya, 2004). The dominant *Microcystis* species form colonies of varying size and of varying shape held together by substance similar to mucus (Joung, 2006). These bloom-forming colonies of *Microcystis* float at the surface of eutrophic bodies of water. Individuals within these floating colonies are round and normally between two and three micrometers in diameter.

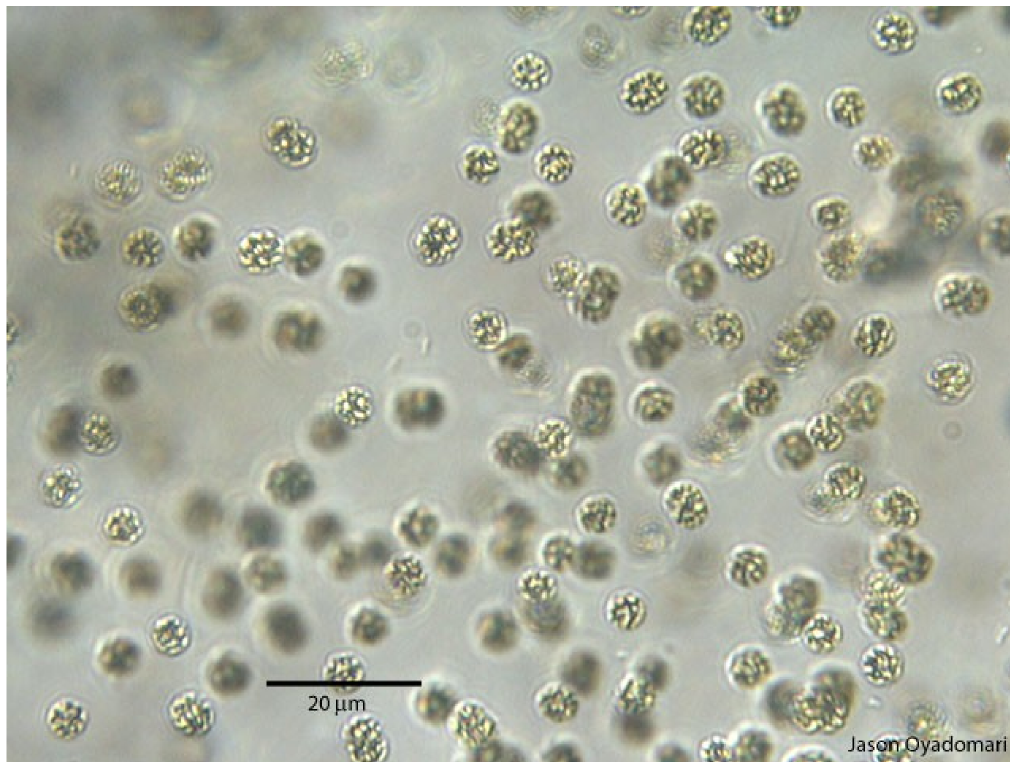

Figure 2-1: Cells of the *Microcystis* genus magnified to 800X.

Colonies composed of *Microcystis* species float at the surface due to the buoyancy of the individual organisms. It has been found that the individual organism is buoyant when the gas content within its vesicle is high. When the organism is exposed to ample amounts of light, buoyancy is lost due to the production and accumulation of polyglucan, which replaces the high gas content vesicles (Kromkamp, 1988). The build-up of polyglucan in the summer causes the colonies to sink to the sediment below in the autumn. At the bottom there is no light, the carbohydrate is utilized by each organism, more gas vesicles are synthesized, and the individual's size may decrease. As this occurs, the colonies regain buoyancy and float back to the surface (Thomas, 1986). Linear relationships have been found and explored that show higher cell densities are associated with more rapid

decrease of those cell densities when no light is available. This correlation has been the basis for successful modeling of the vertical migration of *Microcystis* colonies (Visser, 1997). In addition, this correlation has explained the observed seasonal patterns of emerging and winterizing which these colonies undergo.

2.2 Collecting and Enumerating *Microcystis* spp.

Some research efforts have utilized physical processes to separate *Microcystis* colonies within lake water samples (Davis, 2009). The buoyancy of these populations can be especially useful for separating these colonies from other planktonic cells in large water samples. In addition, the buoyancy of *Microcystis* colonies has been used to separate these colonies from sediments through sedimentation in a 100 ml graduated cylinder (Tsujimura, 2000).

Microcystis cells contain large levels of photosynthetic pigments, which can be helpful in colony identification and counting. These pigments trap light with wavelengths in the range of 400 and 650 nanometers (Glazer, 1977). The photosynthetic pigments are known as phycobiliproteins. These phycobiliproteins capture light energy before it is passed to the chlorophyll organelle during photosynthesis. A very common phycobiliprotein in Cyanobacteria is phycocyanin, which traps light at a maximum wavelength of 620 nanometers and needs red light for production (Takano, 1995). A study on the Vir reservoir in the Czech Republic is one of numerous studies that has taken advantage of this property by using fluorescence microscopy to identify and count *Microcystis* cells. This particular study utilized excitation of the phycocyanin pigments at 485 and 590 nanometers and measured the fluorescence emission at 680 nanometers (Gregor, 2007).

Phycocyanin proteins have varying spectroscopic properties that are influenced by energy distribution and intensity of the incident radiation. The ability of these cells to trap light energy allows studies on *Microcystis* cells to be completed by physical methods such as light microscopy (Glazer, 1977). In addition to difficulties in direct counting, colonies can be very difficult to separate, because the mucus holding these colonies together creates a strong bond. There is no consensus agreement regarding a standard method separating and counting *Microcystis* cells from a colony (Joung, 2006). Division of colonies has been attempted by many methods including vortexing, boiling, titanium dioxide treatment, and sonication. According to a single study that compared these methods, only boiling has been found to completely divide colonies in as little as six minutes (Joung, 2006).

2.3 Harmful Effects of Microcystis Blooms

Massive growths of Cyanobacteria, consisting of large quantities of species from the *Microcystis* genus, are common in shallow eutrophic lakes. Cyanobacteria populations large enough to form algal blooms can create large amounts of cyanotoxins. These toxins can cause health concerns for humans when the water source for both drinking water and recreational use contains Cyanobacteria populations (Rogalus, 2008). Livestock and wildlife poisonings cases due to toxic blooms have been reported as far back as 1878 (Carmichael, 1996). One of the most toxic species from the Cyanobacteria genus is *Microcystis aeruginosa*, which has been proven to be toxic by both oral administering and exposure to skin (Collins, 1978). *M. aeruginosa* produces two toxins, known as slow death factor and fast death factor. Slow death factor causes deaths after an exposure period between four and 48 hours. Fast death factor, producing death in mice in one to

three hours, is alternatively known as microcystin (Collins, 1978). In addition to large dosages of microcystin causing death for mice, mere parts per billion of the toxin has been linked to chronic symptoms for humans, including tumors (Carmichael, 1996). Microcystin is a polypeptide consisting of seven singular amino acids. It is produced by numerous species of *Microcystis* including one third of the *M. aeruginosa* strains (Collins, 1978). Due to the harmful effects, the World Health Organization has set a recommended limit for microcystin in drinking water of 1 microgram per liter (Dyble, 2007).

In addition to adverse health effects from this genus, the species *Microcystis flos-aquae* has been shown to be capable of creating an odor similar to that of natural gas during times of active growth (Jenkins, 1967). These odors can carry over into water supply systems. These problems, in unison with the health concerns, have led to improved management of blooms with use of management models (Arquitt, 2004). To supplement the World Health Organization's limit, Canada, Great Britain, Australia, and other nations have moved toward recommending maximum microcystin levels for healthy drinking water supplies (Carmichael, 1996).

In addition to these concerns, cyanobacteria populations commonly have effects on the aquatic ecosystem. These toxic populations have been found to limit reproduction and growth of native cladoceran communities (de Silva Ferrao-Filho, 2003). These cladoceran communities include the *Daphnia magna*, or water flea, population. *D. magna* are small planktonic crustaceans that are a major food source for aquatic fishes in many lake systems. In many of these systems, the reduction of *D. magna* populations in these lakes can have a devastating effect of large fish populations and the fishing

industry. A significant negative relationship has been found between Daphnia population growth rates and the presence of *Microcystis* populations in their food (Lurling, 2003). In addition, single cells were found to be much more toxic to cladoceran communities than colonies of *Microcystis*, despite similarly high toxin concentrations (de Silva Ferrao-Filho, 2003).

2.4 *Microcystis* in Open Lake Water

Due to the dominance of *Microcystis* in cyanobacterial blooms and the aforementioned negative effects of these organisms, there have been various efforts from various areas of the globe that have yielded results detailing *Microcystis* densities in eutrophic bodies of water. Cyanobacterial blooms have had negative impacts on water systems around the globe. Despite this fact, different studies indicate different dominating *Microcystis* species and variable numbers from lake to lake and from year to year. However, the results have commonly indicated that genetic compositions of these cyanobacterial blooms include multiple species from the *Microcystis* genus.

Between 1998 and 2000, the *M. aeruginosa* community, at population levels between 2.9×10^4 and 2.7×10^6 cells per milliliter, were found to be dominant at the surface in a severely polluted estuary in Turkey (Tas, 2006). Another study, completed in 1993 on a lake in Finland, found that *M. aeruginosa* was only the third most prevalent species of the dominating Cyanobacteria group, trailing both *M. wesenbergii* and *M. viridis* (Lahti, 1997). Additional research has shown that up to 100% of the *Microcystis* population on some water systems can consist of the harmful varieties that produce toxins (Davis, 2009).

Cyanobacteria populations tend to bloom more often in stagnant than in flowing waters. (Takeya, 2004). Nutrients can play a role in *Microcystis* spp. dominance in open lake water. Both phosphorus and nitrogen promote cyanobacterial growth. Ammonia was shown to limit algal growth in general, including *M. novacekii*, under some circumstances (Takeya 2004). However, phosphorus is commonly the limiting nutrient for *Microcystis* spp. in eutrophic systems. In addition, iron has been found to be a limiting factor, because it is a necessary nutrient to create an enzyme essential for nitrogen fixation (Arquitt, 2004).

Lakes with excess of nutrients have long been considered polluted and more susceptible to cyanobacterial blooms. Earlier studies have found that phosphorus can be in excess and does not limit growth (Kappers, 1980). However, this is not the usual case. Increases in phosphorus concentrations yield increases in the growth rate of toxic *Microcystis* communities (Davis, 2009). Due to the tendency of phosphorus to be the limiting growth factor in lakes with low nutrient levels, phosphorus has long been considered the cause of algal growth and cyanobacteria blooms. In addition, it has been shown that eukaryotic plankton growth with decreased *Microcystis* population levels is very common after an improvement in lake water quality.

However, phosphorus control of blooms is a problem that cannot be solved immediately by reducing phosphorus loading to lakes. Another source of phosphorus in lakes is the sediment. It has been shown that the water below the thermocline down to the water overlaying the sediments is enriched with phosphorus released by the sediments (Head, 1999). This gives a distinct advantage to bacterial populations that can travel vertically

across the thermocline. Due to the buoyancy shifts of the individual cells, *Microcystis* colonies can be counted among this advantaged group.

Temperature increases of lake water, in addition to nutrient level increases, have also been shown to encourage *Microcystis* growth. Especially worrying is the that it has been shown that the growth rate in 83% of toxin producing populations had a positive relationship with temperature, while the growth rate in only 33% of non-toxin producing populations increased with elevated temperatures (Davis, 2009). This indicates higher temperatures will yield more blooms consisting of higher percentages of toxic populations. Another study found that temperature itself controls *Microcystis* growth more than the seasonal changes normally associated with bacterial population levels in lakes (Bostrom, 1989). During the summer of 2003, one of the hottest in European history, the conventional method of mixing water to control cyanobacterial blooms was found to be ineffective. This experiment left a conclusion that climate change will cause a greater threat to freshwater pollution effected by Cyanobacteria blooms (Johnk, 2008).

In the fall months, Cyanobacteria biomass has been shown to decrease at the surface of lakes (Lahti, 1997). Despite this evidence, total Cyanobacteria communities in the lakes may not be decreasing in population. In 1994, it was recorded that three dominant species of from the Cyanobacteria genus were recorded moving vertically in a small lake in Southern Scotland (Head, 1999). In the late summer of 2004, a study in the Czech Republic produced reinforcing proof of vertical movement of cells. This study reported populations of both *M. aeruginosa* and *M. wesenbergii* at a depth of ten meters peaked on September 20th at 6.24×10^4 cells per milliliter. At deeper depths, 30 meters and 50 meter, the populations peaked later, on October 11, at 6.6×10^3 and 1.0×10^3 cells per

milliliter, respectively (Gregor, 2006). Shoreline samples have shown higher population densities than offshore samples (Kreider Rogalus, 2007). However, portions of *Microcystis* populations have been observed traveling horizontally from the shallow shorelines to the deeper parts of lakes (Verspagen, 2005).

2.5 Sediments and *Microcystis* Recruitment

In addition to vertical transport through the thermocline, *Microcystis* colonies have been found to overwinter at the bottom of lakes in sediment and return, seasonally, to the water column (Verspagen, 2005). In fact, despite seasonal fluctuations of populations, *Microcystis* colonies normally dominate the microbial community in sediments. The *Microcystis* community was found to compose between 60% and 90% of all surface sediment biomass in a shallow eutrophic lake in central Sweden (Bostrom, 1989). In a different Swedish lake, *Microcystis* colonies were measured at 25.7×10^6 colonies per square meter at 1-2 meter depth and 27.8×10^6 colonies per square meter in the surface sediment during the month of June (Brunberg, 2003). Investigations of *M. aeruginosa* and *M. wesenbergii* populations in the sediments of a shallow lake in Japan revealed gradually decreasing counts from winter to early summer but increasing counts during mid-summer and into autumn (Tsujimura, 2000). More specifically, *Microcystis* colonies populations in sediments undergo four temperature-driven, life-cycle stages: autumnal sedimentation, overwintering, reinvasion, and pelagic growth in the warmest summer months (Ihle, 2005).

The pelagic growth phase is normally indicated by a cyanobacterial bloom at the surface of a lake following *Microcystis* colony recruitment from sediments. During this phase of biomass growth in the water column, there is no noticeable variation of sediment biomass

(Bostrom, 1989). When the temperature falls from 20°C to 15.3°C, 100% of the colonies undergoing pelagic growth enter the autumnal sedimentation phase and begin to sink due to loss of buoyancy resulting from decreased protein synthesis and glycogen accumulation (Visser, 1995). Under these lower temperature conditions, overwintering *Microcystis* colonies that have reached the surface sediment show a resiliency to survive for long periods of time and slowly accrue mass (Tsujimura, 2000). In fact, in the top 2 cm, the biomass of *Microcystis* colonies doubles in late autumn after sedimentation (Bostrom, 1989). It has been simulated that a summer Cyanobacteria bloom would be reduced by more than 64% if the overwintering of pelagic *Microcystis* colonies to the sediment was halted (Verspagen, 2005).

During the reinvasion phase, recruitment of *Microcystis* colonies from shallow sediment to the water column is common prior to a cyanobacterial bloom in the middle or late summer. The overwintered *Microcystis* population in surface sediment at a water depth between one and two meters was found to have a maximum monthly recruitment to the water column during the month of August at an average rate of 2.3×10^5 colonies per square meter per day (Brunberg, 2003). In a deeper basin of a lake (70-90 meter depth); however, *Microcystis* colonies did not return to the water column due to a lack of buoyancy caused by the absence of gas vesicles (Tsujimura, 2000). The difference between recruitment is evident between 6-7 meter depths and 1-2 meter depths, where 8% and 50%, respectively, of colonies in surface sediment returned to the water column (Brunberg, 2003). This large percentage of recruitment from shallow sediments has an immense effect upon Cyanobacteria blooms in a lake. It has been simulated that a summer bloom would be reduced by 50% if recruitment of *Microcystis* colonies from

sediment was halted (Verspagen, 2005). Treatment methods have been explored to limit reinvasion; however, chemical treatment using both aluminum sulfate and sodium aluminate has not been shown to impact recruitment (Perakis, 1996).

An additional method of controlling recruitment may be developed and implemented by understanding the relationship between nutrients and *Microcystis* colony recruitment. In a moderately eutrophic lake in Sweden, the highest recruitment and growth rates corresponded to concentrations of 498 micrograms per liter of dissolved nitrogen and 134 micrograms per liter of total phosphorus (Stahl-Delbanco, 2003). High levels of nutrient addition and low nitrogen to phosphorus ratios are conditions found to most drastically influence the recruitment rate (Stahl-Delbanco, 2003). Growth rates of *Microcystis* colonies in the water column have a significant relationship with the concentration of both nitrogen and phosphorus. Further analysis of this relationship reveals that higher levels of nitrogen and phosphorus in the water column give a competitive advantage to toxic strains of *Microcystis* spp. over the non-toxic strains (Vezie, 2002).

An additional concern is that the levels of the usual limiting nutrient, phosphorus, may increase during recruitment of colonies from the sediment. Concentrations of both total phosphorus and soluble reactive phosphorus in the water have been found to increase during cyanobacterial blooms. This increase is related to a large-scale release of phosphorus from the sediment during *Microcystis* spp. recruitment (Xie, 2003). This is concerning, because expected reductions in *Microcystis* colony populations and resulting cyanobacterial blooms do not occur immediately following reduction of the loading of phosphorus to bodies of water (Head, 1999). In fact, after reducing the total phosphorus loading from 310-340 micrograms per liter in 1981/1982 to 125-130 micrograms per liter

in 1987/1988, a lake in Sweden experienced less than a seven percent decrease of total phosphorus in the sediment from May of 1981 to August of 1990 (Brunberg, 1992). The level of current and past phosphorus loading to lakes has an immense effect on the future of lake water quality due to the fact that sediments have been shown to store the phosphorus for long periods of time after loading has been reduced.

2.6 Lake Erie

Lake Erie is one of five large freshwater lakes in North America that are collectively known as the Great Lakes. The Great Lakes are a vital resource for the surrounding area's agriculture, industry, recreation, and economy. The fishing industry in the lakes has steadily grown since 1820. Each year, 65 million pounds of fish are harvested from the lakes, putting the industry at over a $1 billion per year (Great Lakes Environmental Research Laboratory, 2004). In addition, the Great Lakes provide drinking water for over 40 million people (World Atlas, 1995, Web). In Ohio alone, 530 million gallons per day of water is taken from Lake Erie to supply the 31 lake fed water treatment plants. An additional approximate 3.8 billion gallons per day is taken from Lake Erie for agricultural and other purposes around the state of Ohio (Ohio State University, 1999, Web). In addition, Lake Erie is a major recreational spot for the region. If only the Ohio shoreline is considered, there are over 200 marinas and thousands of public or private beaches (ERC, Web). This is evidence that the people of the region depend on the lake for vacationing and relaxation. With the region's economic, recreational, and industrial future tied to Lake Erie, there has been a variety of studies focusing upon the quality of the lake.

Historically, Lake Erie, especially the Western Basin, has been highly productive (Makarewicz, 1993). By the 1960's, Lake Erie was severely eutrophic and cyanobacterial blooms were common. Following the highly publicized Cuyahoga River burning in 1969, the Great Lakes Water Quality Agreement of 1972, and the Clean Water Act of 1972, reductions in external phosphorus loading greatly reduced the occurrence of cyanobacterial blooms and induced a significant enhancement in the quality of Lake Erie's offshore waters (Makarewicz, 1993). At the time, it was believed that less impactful filamentous bacteria dominated the phytoplankton community in Lake during the 1970's. However, more recent studies have shown that Microcystis cells persisted, and even dominated, throughout this time period (Rinta-Kanto and Saxton, 2009). Sometime in the 1980's, the target levels for phosphorus loading as determined by the Great Lakes Water Quality Agreement were reached. Although phosphorus loading levels have not significantly increased since this time, the Cyanobacteria biomass has increased at all portions of the lake (Conroy, 2005). In fact, a study completed in 2003 and 2004 confirmed that *Microcystis* spp. were the dominant form of Cyanobacteria in the lake (Millie, 2009).

Cyanobacterial blooms had returned by the summer of 2002, when they were observed in both the west and northeastern portions of the lake (Ghadouani, 2005). These blooms persisted through both of the following two summers. During these summers, August bloom levels of *Microcystis* ranged between 4×10^8 cells per liter and 2×10^3 cells per liter in the Western Lake Erie Basin (Rinta-Kanto, 2005). This cyanobacterial population consisted of a mix of toxic and nontoxic *Microcystis* genotypes, with up to 42% of the Cyanobacteria population releasing toxic microcystins (Rinta-Kanto and Konopko,

2009). In fact, microcystin levels during the bloom in western Lake Erie exceeded the World Health Organization's recommendations for drinking water (Rinta-Kanto, 2005 and Dyble, 2007). The colonies of *Microcystis* spp. producing these toxins are not easy to eradicate as they may be stored in sediments from the lake-sediment interface down to twelve centimeters below the surface sediments. Up to five percent of the *Microcystis* cells within the colonies stored in the sediments have been found to produce microcystin (Rinta-Kanto & Saxton, 2009).

Phosphorus loading control has a history of successful control of cyanobacterial blooms in Lake Erie. Phytoplankton abundances in the lake have been linked to phosphorus availability (Millie, 2009). In addition, a strong correlation has been shown between total phosphorus concentrations and abundance of both total and toxic *Microcystis* spp. (Rinta-Kanto, 2009). Resource-based competition, which is effected by both phosphorus levels in water and physical properties such as water temperature and irradiance, can have an effect on the quantity and composition of Cyanobacteria communities (Millie, 2009). However, control methods are not absolute in their suppression of phosphorus loading. Target loading of phosphorus in the lake has not been met in years of high precipitation due to the increased loading from nonpoint sources (Anderson, 2001).

The limitations of phosphorus control are currently being tested by nature, as the cyanobacterial blooms are returning to the lake every summer. As this problem continues, other methods of cyanobacterial bloom control may need to be explored. Controlling environmental conditions may be effective in influencing the resource-based competition. Another method that has been shown successful for limiting accumulation at small scales is water mass movements and mixing (Millie, 2009). However, the time,

energy, and monetary resources needed for large scale application of mass water moving and mixing may be unrealistic.

Source control may be the most promising method of controlling blooms and microcystin concentrations on Lake Erie. Reinvasion of *Microcystis* colonies from the sediments has not undergone noticeable changes since the 1970s (Rinta-Kanto & Saxton, 2009). If the bank of *Microcystis* colonies in the sediments is shown to be a controlling source of colonies and microcystin levels in the open water of Lake Erie, reinvasion control methods may help to control the blooms. The goals of this research work include determining whether or not links exist between the cyanobacterial blooms in western Lake Erie and *Microcystis* biomass in the underlying sediment. Related research is examining the possible existence of similar links between these blooms and *Microcystis* spp. transport from the Maumee River. If links between the blooms and either of the targeted sources are found, methods of source control can be investigated to limit future cyanobacterial blooms in western Lake Erie.

With the goals of this research in mind, two specific hypotheses were created and investigated using the data collected. The first hypothesis was that phosphorus stored in the sediment of the Western Lake Erie Basin directly affects the *Microcystis* spp. cell density in the lake during a cyanobacterial bloom. This hypothesis was examined by collecting measurements for concentrations of total phosphorus (TP) , soluble phosphorus (SRP), and iron strip test phosphorus ($FeCl_2P$) at different sites in the Western Lake Erie Basin in 2009, over the course of the summer. These concentrations may be indicative of high values that may affect the concentration in the water column. In addition, these

sediment phosphorus concentrations were compared to the cell density values of *Microcystis* spp. in the lake water column during a bloom.

The second hypothesis was that *Microcystis* cells stored in the sediment of the Western Lake Erie Basin reinvade the water column and directly affect *Microcystis* cell density in the lake during a cyanobacterial bloom. This hypothesis was examined by collecting data concerning cell density in the sediment at different sites in the Western Lake Erie Basin in 2009, over the course of the summer. The method of cell identification and counting used only identified large cells that were either leaving the sediment to invade the water column or entering the sediment through the process of sedimentation after growth in the bloom. These densities were compared to the cell density values of *Microcystis* spp. in the lake water during a bloom.

Chapter 3

Materials and Methods

3.1 Sample Collection

Samples were collected from the Western Lake Erie Basin during the summer of 2009. Duplicate 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 in order to attempt to allow analysis of conditions before, during, and after bloom formation. Sampling dates could not be set in advance due to uncertainties in the time of occurrence and in the duration of the blooms. Weather conditions, including wind speed, also contributed to uncertainty in possible sampling dates.

At each sampling date, six sites within the Western Lake Erie Basin were visited. These sites have been denoted GR1, 4P, 8M, 7M, MB18, and MB20. Figure 3-1 contains a map locating the six sampling sites in the Western Lake Erie Basin. Table 3-1 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. Sediment samples were collected using a stainless steel Ekman Dredge. Both samples were taken

before the observed bloom, during the observed bloom, and after the observed bloom. The lake and sediment samples were not always taken on the same day. Figure 3-2 includes a photograph of a similar Ekman Dredge. Due to expected high variations of the density of *Microcystis* cells throughout the sediment, duplicate samples were collected at each site. In an effort to allow separate analysis of each location, these two samples were not combined.

Figure 3-1: Map of the Western Lake Erie Basin showing the mouth of the Maumee River and the location of the six sites from which samples were collected for this research

Table 3-1: 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

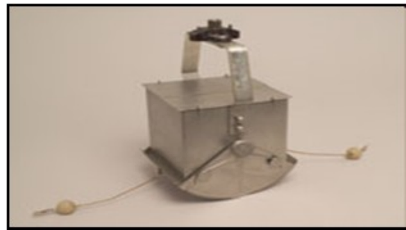

Figure 3-2: A photograph of a standard Ekman Dredge

3.2 *Microcystis* Analysis in the Lake Water

Formaldehyde was added to lake samples prior to transportation to the Environmental Systems Laboratory at the University of Toledo. One liter samples from each sampling site were stored at the lab at four degrees Celsius. Prior to sample analysis, the one liter samples were shaken and distributed evenly between four separate 250 ml graduated cylinders. The samples were allowed to settle in the graduated cylinders for a minimum of forty-eight hours at room temperature. Due to buoyancy of *Microcystis* cells, settling of the samples allowed the *Microcystis* cells to separate from other less buoyant organisms and particles within the lake water sample.

Once the separation of cells in the cylinder was complete, 15 milliliters of sample was transferred from the top of each graduated cylinders to a falcon tube (BD Biosciences, San Jose, California) using a 5ml pipette (Fisherbrand Finnpipette, Fisher Scientific USA). Four falcon tubes of prepared sample were generated from each one liter sample of lake water. The number of falcon tubes that were tested was dependent upon the presence of a sufficient *Microcystis* cells for counting. 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).

Before extracting from a falcon tube, samples were homogenized by vortexing for 30 seconds. Vortexing was completed with the use of a Vortex-Genie 2 (Scientific Industries, Bohemia, New York). Immediately after vortexing was complete, 1 ml of sample was transferred to 1.5 ml graduated microcentrifuge tubes with flat caps (Fisherbrand, Fisher Scientific, USA). Duplicate 1ml subsamples were taken from each falcon tube that was to be analyzed.

Clumping of *Microcystis* cells, as indicated in the literature review, can cause counting of cells in these samples to be impossible or inaccurate. In an effort to break apart clumps of *Microcystis* cells to allow accurate counting, the 1ml subsample underwent sonication (Model 1510, Branson Ultrasonics Corporation, Danbury, Connecticut) for five minutes. After sonication, each 1 ml subsample was ready to be prepared for viewing.

Next, the subsamples were processed through a Model 1225 Sampling Manifold (Millipore Corporation, Billerica, Massachusetts) with the assistance of a sampling pump (Millipore Corporation, Billerica, Massachusetts). The samples were processed through a

black polycarbonate filter, 0.22 micron, 25 mm (GE Water & Process Technologies, USA). The *Microcystis* cells were retained on this filter. Below this filter was a white glass fiber pre-filter (Millipore Corporation, Billerica, Massachusetts). The polycarbonate filter was removed from the sampling manifold and allowed to dry. After drying, the polycarbonate filters were mounted on a glass microscope slide (Fisher Scientific, USA) and covered with a glass cover slip (Fisher Scientific, USA). To induce fluorescence, a drop of Type DF non-drying immersion oil (Cargill Laboratories, Inc., Cedar Grove, New Jersey) conforming to ISO-8036-1 Specification was included with the polycarbonate filter onto the slide.

The prepared slides were viewed using a fluorescence microscope (Model BX51, Olympus, Japan) for counting. Slides with an average of less than one cell per field were considered to be below the detection limit. These slides were not disregarded. Instead it was recorded that these slides were below detection limit and any statistical analysis that used number from these slides assumed a zero cell density.

At a magnification of 400X there are 46,691 total fields per slide. The settled *Microcystis* cells from 250 ml of sample were transferred to a 15 ml falcon tube. As described previously, normal procedures were to process one ml from this falcon tube per slide. Knowing this information and using minimum necessary average of one cell per field, the utilized method's detection limit for counting the density of *Microcystis* cells was determined as follows:

$$Detection\ Limit = C = 46{,}691 \times \left(\frac{1\ cell}{\frac{1\ ml}{15\ ml} \times 250\ ml} \right) = \underline{2{,}800\ cell/ml}$$

The *Microcystis* cells on each slide that averaged more than one cell per field were viewed and counted through the microscope at a magnification of 400X. *Microcystis* cells were identified as round cells that exhibited florescence. The florescence of the microscope was set for excitation between 500-560 nanometers and emittance above 580 nanometers. In accordance with the information provided in the Literature Review section of this report, *Microcystis* cells were best seen and counted at these wavelengths. Cells on each slide were counted and recorded on a minimum of twenty fields. The microscope utilized was packaged with a digital camera (Model DP70, Olympus, Japan). The camera was connected to a computer (Dell, Round Rock, Texas). Photographs were taken of random fields from numerous slides and saved to the computer for future reference. Figure 3-3 is a photograph taken that illustrates a typical field that was viewed and photographed.

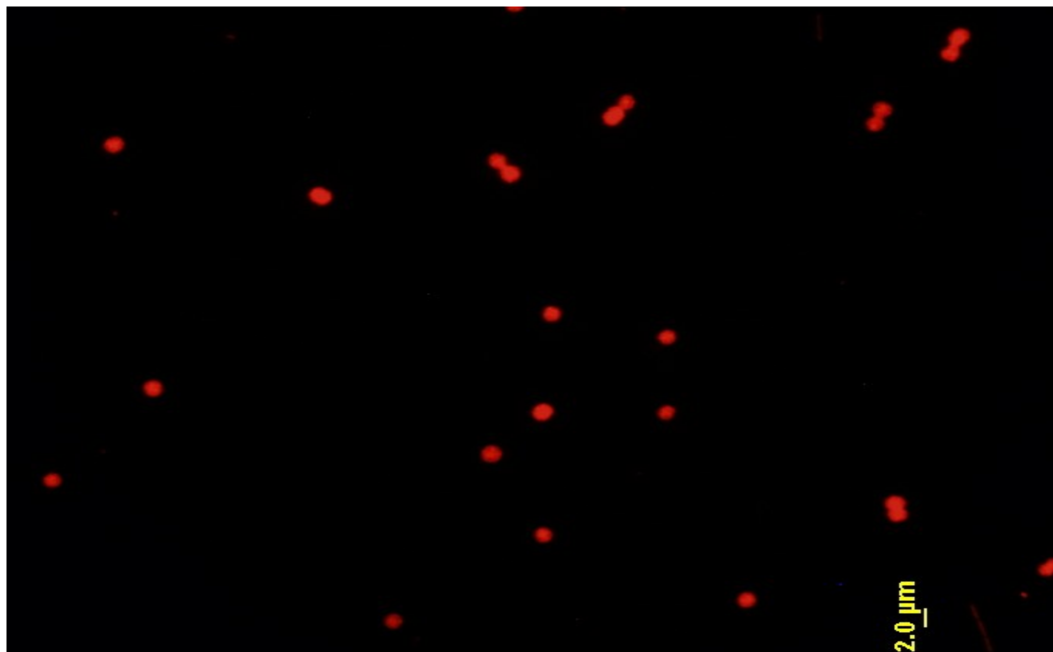

Figure 3-3: A photograph taken of a field on a slide from the second sampling date of the lake samples at site MB20. This photograph was taken with the use of a fluorescence microscope at a 400X magnification.

For these slides, the average and standard deviations of the slides were calculated using these respective functions within Microsoft Excel. Since the slides were viewed at 400X, the average number of cells per field was multiplied by 46,691 fields per slide to give a total number of cells per settled volume, T_L. The following equation was utilized to determine the cell density in total cells per milliliter of sample, C_L:

$$C_L = \frac{T_L}{250\ ml\ settled \times \frac{V_L}{15\ ml\ tube}}$$

where,

V_L = volume of lake water analyzed on the slide from the 15 ml falcon tube

Multiple slides were created for each sample date at each site. The cell density values from each slide were organized and an average was found for each of the eighteen samples. An associated standard deviation was found taking into account difference in cell densities found between the slides for each sample. These values were organized into tables and can be found in the Results section of this report.

3.3 Percent of Dry Solids of Sediment

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 a one single composite sample from each of the six sites at each of the six dates. The eighteen total samples were each

run once to determine moisture content. The mass of eighteen covered aluminum drying cartons was measured and recorded as W_1. Approximately thirty grams of each composite sample was placed on into a drying carton. The mass of the tin and the wet sample was measured and recorded as W_2. The samples were then placed in a solids drying oven (Thelco Model 18, Thermo Scientific, Waltham, Massachusetts) at 110 degrees Celsius for approximately twenty-four hours. When the mass of the sample was constant, the water weight had been fully extracted and the sampled was removed from the drying oven. Then the mass of the dried samples was measured and recorded as W_3. The three recorded masses (W_1, W_2, and W_3) were all found using an electronic scale (Model AE100, Mettler, Toledo, Ohio). Moisture content for each of the eighteen sediment samples was found through use of the following equation:

$$MC\% = \left[\frac{W_2 - W_3}{W_3 - W_1}\right] < \ 00\%$$

For the purposes of this report, the percent of dry solids by weight was more functional for analytical purposes than the moisture content. In order to find the percent of dry solids within each of the eighteen sediments samples, $Solids_{\%}$, the following equations was used:

$$Solids_{\%} = \left[\ - \frac{W_2 - W_3}{W_3 - W_1}\right] < \ 00\%$$

The solids content for each composite sample was found as a percent of dry solids by weight. A table was created to organize these values. This table can be found in the Results section of this report.

3.4 Grain Size Distribution of Sediments

Grain size distribution was another physical property of the sediments samples that was determined. 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. Since the samples from each date were from the same six sampling locations, there should not have been a difference in grain size distribution from date to date at any of the sampling locations. Portions of the samples from each date were composited with other samples from the site. This test was run six times, once for each of the sampling sites. First, the sediment samples were oven-dried at 110 degrees Celsius using a solids drying oven (Thelco Model 18, Thermo Scientific, Waltham, Massachusetts,) ground using mortar and pestle, and passed through a No. 10 sieve (Fisher Scientific Company, USA). Each of the six samples passed 100% of the dried sediment through a No. 10 sieve. Approximately 50 grams of the dried sediment was placed in an evaporating dish. The actual mass was measured and recorded as W_S. Separately, a solution was prepared with distilled water and Sodium Hexametaphosphate (Ele International, Ames, Iowa). The mixing rate for the solution was 40 grams of Sodium Hexametaphosphate per liter of solution. To assure the sediment particle dispersion, 125 milliliters was then poured over the sediments in the evaporating dish to create a slurry. This slurry was covered and allowed to set for 24 hours. Meanwhile, a single 1000 milliliter glass sedimentation cylinder was filled with distilled water. Added to the contents of this cylinder was 125 milliliters of the Sodium Hexametaphosphate solution. This is the control solution and will be used as a zero correction, C_Z, to calibrate the reading from each sample.

After the sediments samples in the evaporating dishes had set for 24, they were transferred to a dispersion cup and mixed for one minute using a mechanical mixer (Hamilton Beach Co., Div. of Scovill Mfg. Co., Racine, Wisconsin). Each mixed slurry sample was transferred to its own glass sedimentation cylinder immediately after mixing. A stopper plug was inserted at the top of the cylinder, which was rotated slowly upside down once, before removing the plug and setting on a level surface. A Type 152H hydrometer (Ertco, U.S.A.) was then inserted into each cylinder to take readings. Figure 3-4 is a photograph of a Type 152H hydrometer in a sedimentation cylinder. Readings, taken from the hydrometer at the liquid surface, were measured in grams of solids per 1000 ml solution. Ideally, nine reading should have been taken: two within the first minute, at two minutes, at five minutes, at 15 minutes, at 30 minutes, at 60 minutes, at 250 minutes, and at 1,440 minutes. The actual time of the reading, the hydrometer reading, and the temperature at the time of the reading were recorded.

Figure 3-4: A photograph of a Type 152H Hydrometer within a sedimentation cylinder.

The zero correction, C_Z, throughout the experiment remained constant at three grams of solids per 1000 ml solution. The slurry temperature, which remained constant at 22°C throughout the experiment, was measured at the time of each reading using an Enviro-Safe thermometer (H-B Instrument Co., U.S.A). This corresponded to a temperature correction factor, C_T, of +0.40. The specific gravity of the soil was taken to be 2.65. Once the procedure for testing each sample was completed, there were two variables that needed to be solved for at each reading. The diameter of the smallest particle that has settled, D, and the percent of soil remaining in the suspension, P, were the two variables of interest.

At any given time, the diameter of the smallest particle that has settled, D, was given by the equation:

$$D = \zeta \times \sqrt{\frac{L}{T}}$$

where,

T = elapsed time, minutes

K = a constant dependent upon temperature and specific gravity, $mm\sqrt{\frac{\min}{cm}}$

L = effective depth of the hydrometer, cm

The constant, K, in this case remained at 0.0133 $mm\sqrt{\frac{\min}{cm}}$ for all measurements (specific gravity = 2.65, temperature = 22°C). The effective depth of the hydrometer represented the distance that the soil has settled. Physically, this was the distance from

the surface of the sediments to the center of the hydrometer bulb. The following equation was used to calculate the effective depth of the hydrometer, L:

$$L = 16.3 - 0.164 \times R$$

where,

R = actual reading on the hydrometer, grams of solids per 1000 ml of solution

The percent of soil remaining in the suspension, P, was equivalent to the percent of soil in the suspension smaller than the size of the smallest settled particle, D. The percentage, P, was calculated using the following equation:

$$P = \left[\frac{R_c \times a}{W_s} \right]$$

where,

R_C = corrected hydrometer reading

a = correction factor for specific gravity

W_S = oven dry mass of the soil samples suspended in the solution, grams

The corrected hydrometer reading was found by subtracting the temperature correction and the zero correction from the actual hydrometer reading, $R_C = R - C_T - C_Z$. Since 2.65 was used for standard gravity of the sediment, the correction factor for specific gravity, a, was 1. The oven dried mass, W_S, was measured prior to creating the slurry.

Once the diameter of the smallest particle that has settled, D, and the percent of soil remaining in the suspension, P, had been determined at each reading, these variables were plotted on a graph for each soil sample. The graphs illustrate the grain size distribution for each sediment sample. The graphs for each of the samples can be found in the Results section of this report.

The median particle size for each sample was taken from the graph as the particle size at which 50% of the sediment is finer than. If the median particle size could not be found from interpolation on the graph, extrapolation was used to estimate the median particle size of the sediment sample. The median particle sizes were collected in a table that can be found in the Results section of this report. The median particle size was used as a continuous variable describing the grain size of the sediment. This value was also used for comparison and statistical analysis.

3.5 Phosphorus Testing of the Sediment

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).

Numerical values, as measured at the Heidelberg Water Quality Lab, for the concentrations of phosphorus in the composite sediment samples had not been standardized to account for the differences in percent of dry solids between the samples.

To standardize these concentration values, each value was changed from raw concentration (milligrams P per liter) to a corrected concentration (milligrams P per gram dry weight of sediment). This corrected concentration was used for analysis in this research. To get this corrected concentration, P_C, the following equation was used:

$$P_C = \frac{(P_m \times 100)}{(W_m \times Solid_{\%})}$$

where,

P_m = measured phosphorus concentration, mg/l

W_m = weight of the sediment analyzed, grams

$Solid_{\%}$ = dry solid percentage within the sediment, %

Tables were created to organize the data for soluble phosphorus concentration, total phosphorus concentration, and iron strip test phosphorus concentration at each sample site on each sampling sate. These three tables of phosphorus levels per gram dry weight of sediment can be found in the Results section of this report.

3.6 *Microcystis* Analysis in the Sediment

The duplicate sediment samples from each sampling site were transported, along with the lake samples, to the Environmental Systems laboratory at the University of Toledo. Each sample was stored with in a plastic Ziploc bag at four degrees Celsius.

To begin sediment sample processing, approximately five grams of each duplicate sample was extracted and deposited into a 250 ml graduated cylinder. Mass of the portion of sample extracted was measured using an electronic balance (Model AE100, Mettler,

Toledo, Ohio). Water purified using a PureLab Ultra System (USFilter, Palm Desert, California) was added to the samples to fill the 250 ml graduated cylinder. The graduated cylinder was then covered and vortexed for one minute. Vortexing was completed with the use of a Vortex-Genie 2 (Scientific Industries, Bohemia, New York). After being vortexed, the sediment was thoroughly mixed with the water.

Next, the mixtures of water and suspended sediment were allowed to settle for a minimum of 48 hours. Due to the amount of suspended solids within the mixtures, settling was a crucial step in the process of analyzing the samples. 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 cylinders to a single falcon tube (BD Biosciences, San Jose, California) using a 5ml pipette (Fisherbrand Finnpipette, Fisher Scientific USA). The samples within the falcon tubes were then fixed by transferring 300 microliters of a formaldehyde solution into each falcon tube using a 150 microliter pipette. The solution used was formaldehyde, 37 wt. % solution in water stabilized with 10-15% methanol (Acros Organics, Belgium).

Similar to the situation encountered when analyzing the lake samples, clumping of *Microcystis* cells can cause counting of cells in these samples to be impossible or inaccurate. 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.

For filtration, the entirety of each 15ml mixture sample was processed through a Model 1225 Sampling Manifold (Millipore Corporation, Billerica, Massachusetts) with the assistance of a sampling pump (Millipore Corporation, Billerica, Massachusetts). The mixture samples were processed through a black polycarbonate filter, 0.22 micron, 25 mm (GE Water & Process Technologies, USA). The *Microcystis* cells were retained on this filter. Below this filter was a white glass fiber pre-filter (Millipore Corporation, Billerica, Massachusetts). The polycarbonate filter was removed from the sampling manifold and allowed to dry. After drying, the polycarbonate filters were mounted on a glass microscope slide (Fisher Scientific, USA) and covered with a glass cover slip (Fisher Scientific, USA). To induce fluorescence, a drop of Type DF non-drying immersion oil (Cargill Laboratories, Inc., Cedar Grove, New Jersey) conforming to ISO-8036-1 Specification was included with the polycarbonate filter onto the slide.

The prepared slides were viewed using a fluorescence microscope (Model BX51, Olympus, Japan) for counting. As noted in the Literature Review section of this report, overwintering cells in the sediment may decrease in size. When viewing sediment samples taken at every sampling site prior to the cyanobacterial bloom, no cells could be located under 400X magnification. When 1000X magnification was used, it was apparent that there were many small overwintering *Microcystis* cells in the sediment. However, even at this high magnification, the cells could not be accurately counted. Only cells large enough to be counted at 400X magnification were included in the *Microcystis* cell density values that were associated with the sediment samples.

Another limitation of this method was the detection limit. The detection limit for cell density values in the sediment was calculated similarly to the detection limit of cell density values in the lake water:

$$Detection\ Limit = DL_S = 46{,}691 \times \left(\frac{1\ cell}{\frac{15\ ml}{15\ ml} \times 5\ wet\ grams} \right)$$

$$= \underline{560\ cell/gram\ wet\ weight\ of\ sed.}$$

Any slides that did not have cells large enough to be counted at 400X magnification or that did not have the minimum cell density of 560 cells per gram wet weight of sediment were recorded as zero values. Multiple slides for some of the samples differed between whether the sample was above or below the detection limit. This allowed some of the average cell densities to be reported, despite being below the detection limit.

It is important to note that the reported cell densities may not be equivalent to the total cell density. Overwintering cells were below the detection size and were not included in the reported cell densities. The cell densities reported included only cell that have increased in size to begin reinvasion into the water column or cells that have recently undergone sedimentation from the water column to the sediments.

When the *Microcystis* cell density level was detectable, the cells on each slide were viewed and counted through the microscope at a magnification of 400X. *Microcystis* cells were identified as round cells that exhibited florescence. The florescence of the microscope was set for excitation between 500-560 nanometers and emittance above 580 nanometers. In accordance with the information provided in the Literature Review section of this report, *Microcystis* cells were best seen and counted at these wavelengths.

Cells on each slide were counted and recorded on a minimum of 20 fields. The microscope used was packaged with a digital camera (Model DP70, Olympus, Japan). The camera was connected to a computer (Dell, Round Rock, Texas). Photographs were taken of random fields from numerous slides and saved to the computer for future reference. Figure 3-5 is a photograph taken that illustrates a typical field that was viewed and photographed.

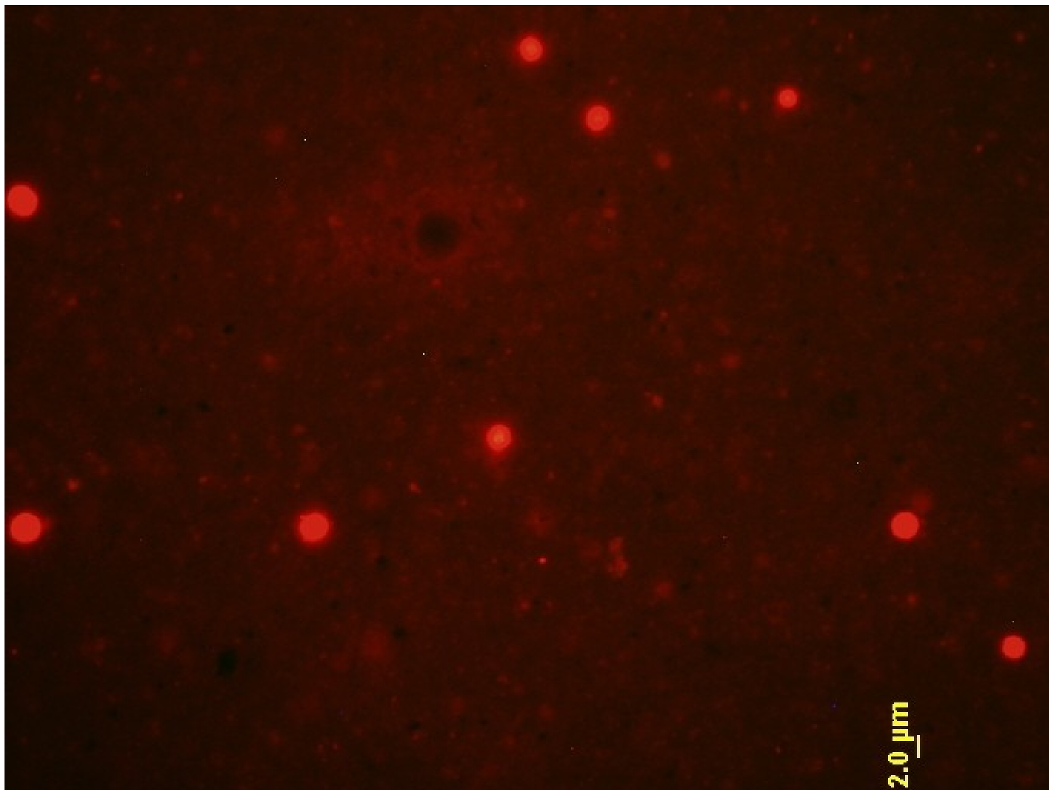

Figure 3-5: A photograph taken of a field on a slide from the second sampling date of the sediment samples at site 7M. This photograph was taken with the use of a fluorescence microscope at a 400X magnification.

Each sample was processed as described in this section. Each duplicate set of samples were processed more than once only if additional data was deemed necessary after initial

analysis. Additional data was considered necessary when the ratio of standard deviation to average cell density was high. For each slide processed, the average cell density value and standard deviations of the slide was calculated using these respective functions within Microsoft Excel. Since the slides were viewed at 400X, the average number of cells per field was multiplied by 46,691 fields per slide to give a total number of cells per analyzed wet weight of sediment, T_S. The following equation was utilized to determine the cell density in total cells per gram wet weight of sediment, C_S:

$$C_S = \frac{T_S}{W_S}$$

where,

W_S = wet weight of sediment analyzed on the slide

The cell densities for the two (or more) slides created for each sample were organized and an average was found. These cell densities were then converted from the measured values, C_{SM}, of cells per gram wet weight to corrected values, C_{SC}, of cells per gram dry weight using the following formula:

$$C_{SC} = \frac{(C_{CM} \times 100)}{(W_m \times Solid_{\%})}$$

where,

W_m = weight of the sediment analyzed, grams

$Solid_{\%}$ = dry solid percentage within the sediment, %

An average cell density was then found from all the corrected values associated with each sample site. An associated standard deviation for each sample was found taking into account the difference between the corrected values for each separately run sample. These values were organized into tables and can be found in the Results section of this thesis.

3.7 Statistical Analysis of the Data

Throughout the analysis of data within this report, different reported values needed to be statistically compared in order to visualize and quantify any correlation between data. In each instance, a hypothesis was created regarding the comparison of the data. Two-sample independent t-tests, dependent samples t-tests, and Pearson correlation tests were used to test these hypotheses. In all cases, these tests resulted in the acceptance or rejection of the stated hypothesis. After either of these tests had been completed and the hypothesis acceptance or rejection was determined, inferences were made regarding the correlation between trends of the data.

The two-sample independent t-test was used to compare a relationship between an independent variable with two values and a continuous dependent variable. Comparison of the *Microcystis* cell density at any given site between two sampling dates is an example of when this test was used. The independent variable was the sampling date (having values of either June 23, 2009 and August 9, 2009 or August 9, 2009 and September 14, 2009). The continuous dependent variable was the *Microcystis* cell density. To begin this test, the hypothesis needed to be stated as a null and an alternative hypothesis. All alternative hypotheses in this report were be set up to create a one-tailed test. This was accomplished by testing against whether values are greater than or less

than other values instead of testing for inequality. Next, criteria for decision making were set. For all two-sample independent t-tests completed in this report, an alpha value of .05 was utilized (corresponding to the 95% significance level). This is a standard value used in statistical analysis of data. A critical value for comparison was taken from a table using the degrees of freedom determined from the quantity of samples analyzed ($df = n_1+n_2-2$), an alpha value of .05, and a one-tailed test. This critical value is reported in the results section of this report for each two-sample independent t-test that was run. Next a test-statistic, t, was found for each hypothesis using the following equation:

$$t = \frac{(\overline{x_1} - \overline{x_2})}{s_{\overline{x_1}-\overline{x_2}}}$$

where,

$\overline{x_1}$ and $\overline{x_2}$ = the mean of values for the continuous variable from each of the compared groups

$$s_{\overline{x_1}-\overline{x_2}} = \sqrt{\left(\frac{{s_1}^2(n_1-1)+{s_2}^2(n_2-1)}{n_1+n_2-2}\right)\left(\frac{1}{n_1}+\frac{1}{n_2}\right)}$$

n_1 and n_2 = the number of readings used to determine $\overline{x_1}$ and $\overline{x_2}$

s_1 and s_2 = the standard deviation of the readings used to determine $\overline{x_1}$ and $\overline{x_2}$

Finally, the test statistic, t, was compared to the critical value. If the test static was greater than the critical value, the null hypothesis was rejected and a significant difference between the compared means was inferred. If the test statistic was less than

the critical value, the null hypothesis could not be rejected and no significant difference was inferred between the compared means with the data presented.

The dependent t-test was another type of statistically test that is used to compare data within this report. This test was used to compare a relationship between an independent variable with two related values and a continuous dependent variable. Comparison of phosphorus concentrations during a bloom and after a bloom at the same site is an example of when this test was used. The independent variable was categorical (during the bloom or after the bloom). The continuous dependent variable was the phosphorus concentration. To begin this test, the hypothesis needed to be stated as a null and an alternative hypothesis. All alternative hypotheses in this report were set up to create a one-tailed test. This was accomplished by testing against whether values were greater than or less than other values instead of testing for general inequality. Next, criteria for decision making were set. For all dependent t-tests completed in this report, an alpha value of .05 was utilized (corresponding to the 95% significance level). This is a standard value used in statistical analysis of data. A critical value for comparison was taken from a table using the degrees of freedom determined from the quantity of samples analyzed (df = n-1), an alpha value of .05, and a one-tailed test. This critical value is reported in the results section of this report for each dependent t-test that was run. Next a test-statistic, t, was found for each hypothesis using the following equation:

$$t = \frac{\overline{D}}{s_{\overline{D}}}$$

where,

$\overline{D}$ = the mean of the difference scores

$$s_{\bar{D}} = \frac{s_D}{\sqrt{n}}$$

S_D = the standard deviation of the difference scores

n = number of difference scores

Finally, the test statistic, t, was compared to the critical value. If the test static was greater than the critical value, the null hypothesis could be rejected and a significant difference between the compared means was inferred. If the test statistic was less than the critical value, the null hypothesis could not be rejected and no significant difference could be inferred between the compared means with the data presented.

The Pearson correlation test was the final statistical tests used within this report to compare data. It was used to compare a relationship between two continuous variables. Comparison of *Microcystis* cell density and distance of the sampling site from the Maumee River was an example of when this test was used. For each set of data to be compared a figure was provided to illustrate the relationship. A linear relationship was assigned to the data in each figure using the line of best fit function on Microsoft Excel. For each Pearson correlation test computed, the slope of the line of best fit was used to indicate whether a negative or positive relationship is inferred. The Pearson correlation test is used to determine whether this inferred relationship was statistically significant for the data that was found.

To begin this test, the hypothesis needed to be stated as a null and an alternative hypothesis. All alternative hypotheses in this report were set up as directional alternative hypotheses (one-tailed test). This was accomplished by stating either a positive or a

negative correlation in the alternative hypotheses. Next, criteria for decision making were set. For all Pearson correlation tests completed in this report, an alpha value of .05 was utilized (corresponding to the 95% significance level). This is a standard value used in statistical analysis of data. A critical value for comparison was taken from a standard table using the degrees of freedom determined from the quantity of samples analyzed (df = n-2), an alpha value of .05, and a one-tailed test. This critical value is reported in the results section of this report for each Pearson Correlation test that was run. Next, a test-statistic, r, was found for each hypothesis. The test statistic, r, represented the ratio of the degree to which the variables vary together to the degree to which the variables vary separately. It was found using the following equation:

$$r = \frac{SP}{\sqrt{SS_X \times SS_Y}}$$

where,

SP = co-variability of the two variables

SS_X and SS_Y = variability of each of the variables

Finally, the test statistic, r, was compared to the critical value. If the test static was greater than the critical value, the null hypothesis could be rejected and a significant correlation between the compared variables was inferred. If the test statistic was less than the critical value, the null hypothesis could not be rejected and no significant correlation could be inferred between the variables with the data presented.

The two sample independent t-test, the dependent t-test, and the Pearson correlation test were used commonly to analyze the research completed as part of this report. Tables A-1, A-10, A-13, A-20, A-24, A-35, A-42, and A-46 define both the null and alternatively hypotheses for each time the test is used. These tables can be found in Appendix A. The results and corresponding analysis from all of the statistical tests described in these tables can be found in the Results section of this report. The test number was assigned based up the section of this report for which the test was carried and the results described.

Chapter 4

Results

4.1 *Microcystis* Cell Density in Lake Water

Average *Microcystis* cell density for each of the 18 lake water samples are displayed in Table 4-1. Samples that had cell density values below the detection limit are displayed as 'BDL' in this table. Samples from June 9, 2009 were taken at a time considered to be before the cyanobacterial bloom. Samples from August 4, 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. The *Microcystis* cell density values before the bloom were below the detection limit at all sites. At site 4P, all sampling dates yielded results below the detection limit. *Microcystis* cell densities at three of the six sites were below the detection limit after the bloom. In addition to the averages of the cell density readings, the standard deviations of these readings were also computed. Table 4-1 also displays all of the standard deviation readings. Also, only one slide was processed for the sample from site GR1 taken during the bloom. With only one slide processed, the standard deviation was not applicable.

Table 4-1: The average *Microcystis* cell density values and the associated standard deviations found for each of the eighteen lake water samples.

Site	9-Jun-09 (cells/mL)	4-Aug-09 (cells/mL)	14-Sep-09 (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

Figure 4-1 displays all of the sample mean cell density values separated by site. Before the bloom, all six sites had cell density values below the detection limit (BDL). For each of the five sites that had detection of *Microcystis* cells, cells density mean values were above the detection limit during the bloom. At sites GR1 and MB20, cell density mean values were only above the detection limit during the bloom. At sites MB18 and 8M, cell density mean values were above the detection limit both during and after the bloom. The cell density mean values during the bloom at these sites were greater than the cell density mean values after the bloom. At site 7M, similar to sites MB18 and 8M, cell density mean values were above detection limit for both during and after the bloom. However, site 7M was the only site at which the cell density mean value increased from August 4 to September 14. This indicates that the cyanobacterial bloom lingered at site 7M later into season than it did at the other sites.

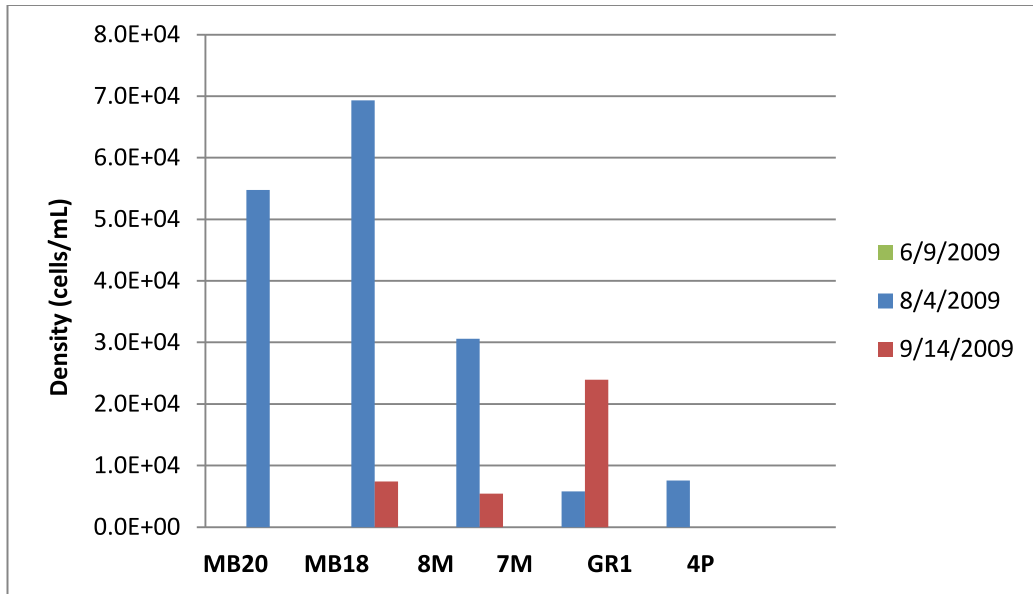

Figure 4-1: *Microcystis* cell density (cell/mL) in lake water as a function of sampling site (MB20, MB18, 8M, 7M, GR1, 4P) and as a function of sampling date (June, August, September).

The cell density mean values at sites MB20, MB18, 8M, 7M, and GR1 are further represented in Figures 4-2, 4-3, 4-4, 4-5, and 4-6. Each one of these figures is specific to one site. Due to the mean cell density values at site 4P never exceeding the detection limit, no figure is supplied for this site. The mean cell density values at the other five sites are displayed for each date. In addition to the mean cell density values, error bars are supplied at each of the values that that exceeded the detection limit. The error bars extend one standard deviation in both directions. The error bars for the mean cell density during the bloom at each site do not extended into the same numerical region as error bar for either the mean cell density before or after the bloom. This indicates that the results show that the *Microcystis* cell densities during a cyanobacterial bloom are not equivalent to the *Microcystis* cell densities before or after a cyanobacterial bloom.

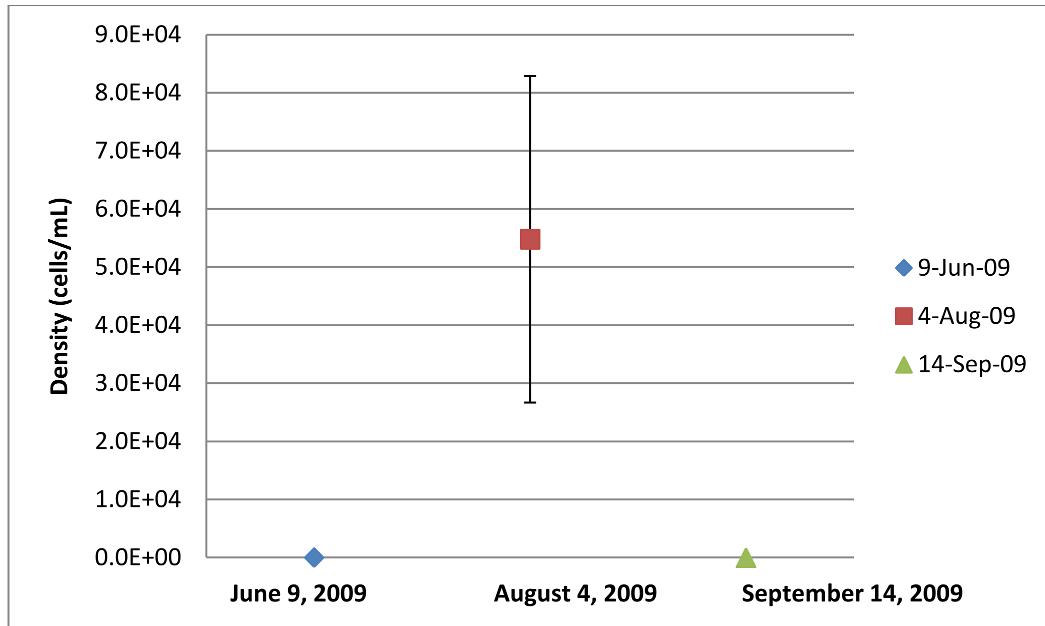

Figure 4-2: *Microcystis* cell density in the lake water as a function of the sampling date at the site MB20. Error bars represent one standard deviation from the mean in each direction.

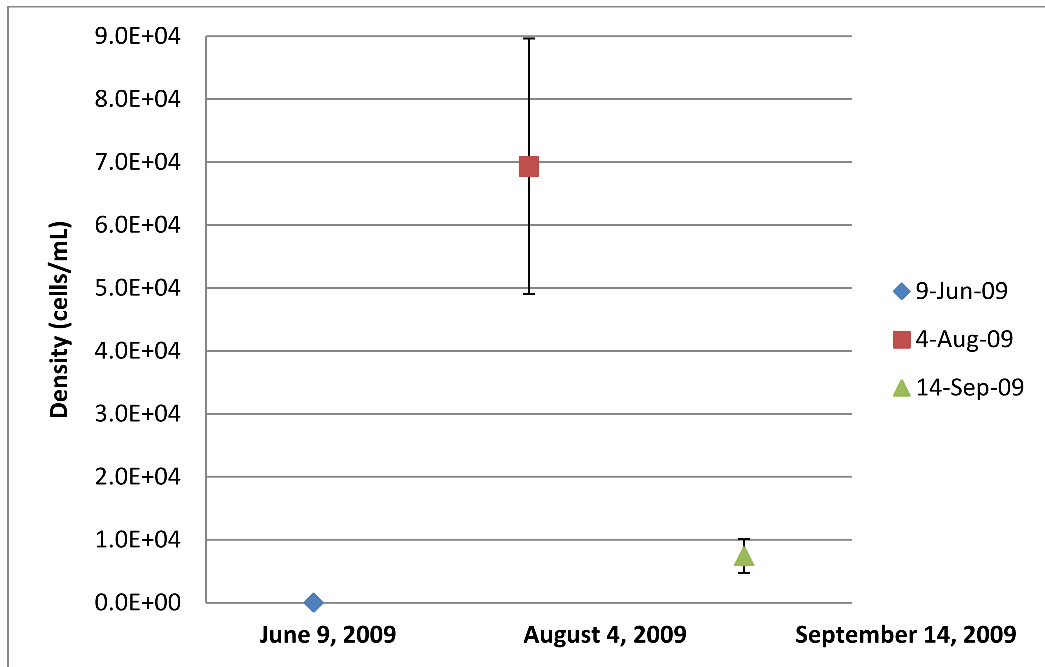

Figure 4-3: *Microcystis* cell density in the lake water as a function of the sampling date at the site MB18. Error bars represent one standard deviation from the mean in each direction.

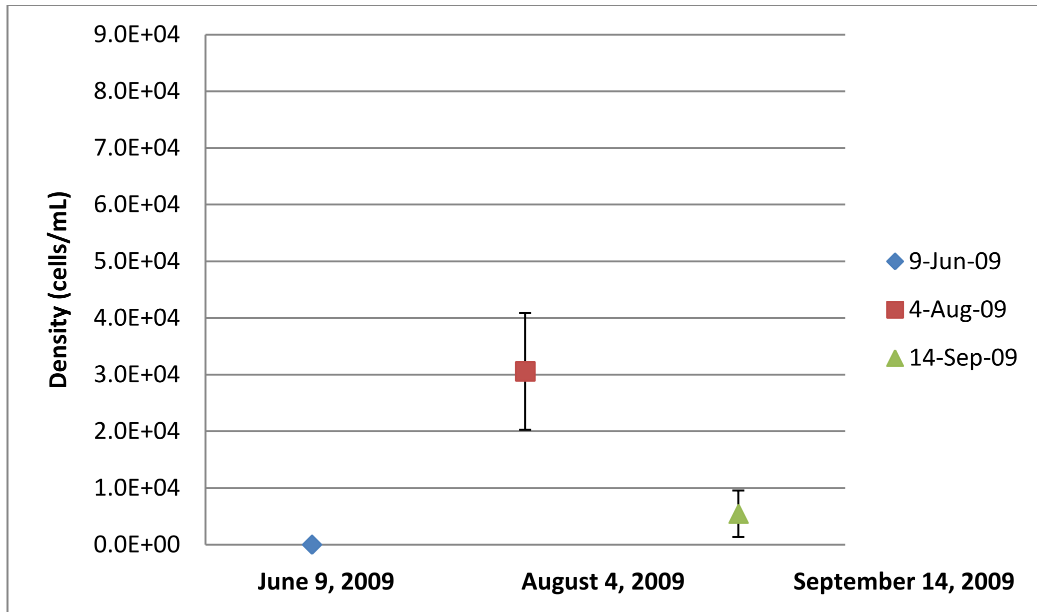

Figure 4-4: *Microcystis* cell density in the lake water as a function of the sampling date at the site 8M. Error bars represent one standard deviation from the mean in each direction.

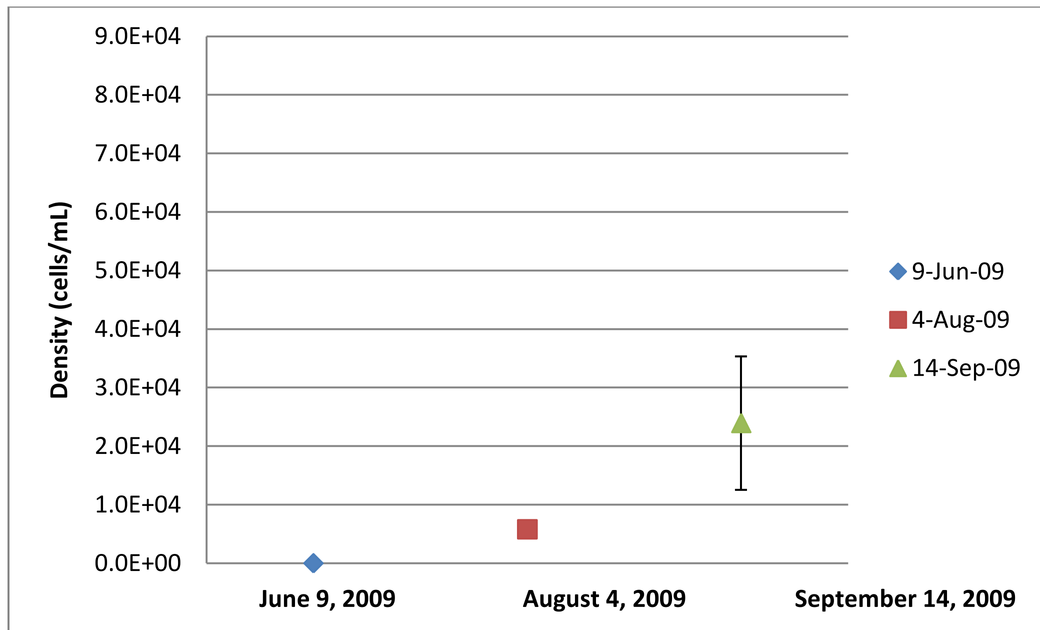

Figure 4-5: *Microcystis* cell density in the lake water as a function of the sampling date at the site 7M. Error bars represent one standard deviation from the mean in each direction.

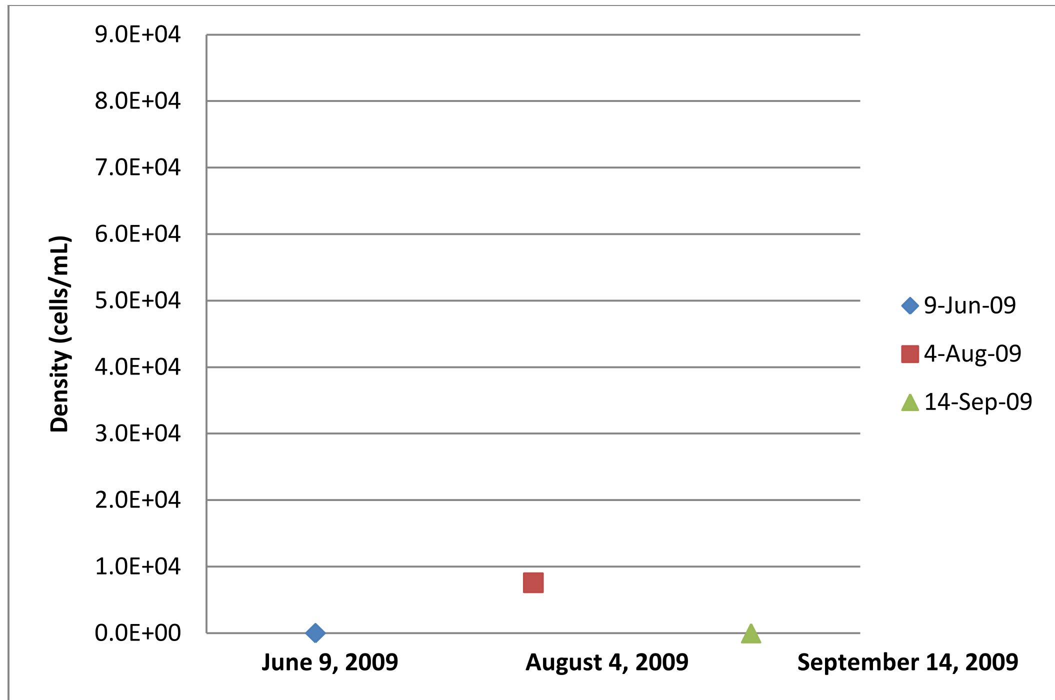

Figure 4-6: *Microcystis* cell density in the lake water as a function of the sampling date at the site GR1. Error bars are not present on this figure due to the lack of standard deviation values for the data.

In order to comment on statistically significant differences between the mean *Microcystis* cell density values; however, the lack of overlap of the error bars is not sufficient. To further investigate these trends, a two-sample independent t-test was run to compare the mean cell density values before and during the bloom and during and after the bloom. T-tests were completed to compare the cell density values for sites MB20, MB18, 8M, and 7M. Due to the lack of cell density values above the detection limit for samples from site 4P, no t-tests were completed. Due to lack of the ability to calculate the standard deviation for samples from site GR1, no tests were completed. Table 4-2 shows the calculated test statistics and the conclusions that can be drawn for these t-tests. Table A-1, which can be found in Appendix A, defines each of the t-tests by listing the null and alternative hypotheses for each examined circumstance. Tables A-2, A-3, A-4, A-5, A-6,

A-7, A-8, and A-9, which can be found in Appendix A, provide the necessary data and calculations that were used during computation of each of these t-tests.

Table 4-2: Test statistics and conclusions drawn for T-tests 4.1.1, 4.1.2, 4.1.3, 4.1.4, 4.1.5, 4.1.6, 4.1.7, and 4.1.8.

Comparison of *Microcystis* Cell Densities Changes at Sites MB18, MB20, 8M and 7M		
T-test No. 4.1.1	Test Statistic	t = 2.616
	Conclusion	At site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.1.2	Test Statistic	t = 3.821
	Conclusion	At site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.
T-test No. 4.1.3	Test Statistic	t = 4.552
	Conclusion	At site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.1.4	Test Statistic	t = 7.601
	Conclusion	At site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom
T-test No. 4.1.5	Test Statistic	t = 3.961
	Conclusion	At site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.1.6	Test Statistic	t = 3.923
	Conclusion	At site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.
T-test No. 4.1.7	Test Statistic	t = 13.378
	Conclusion	At site 7M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.1.8	Test Statistic	t = 2.132
	Conclusion	At site 7M, *Microcystis* cell density after the bloom is statistically greater than *Microcystis* cell density during the bloom.

The results of t-tests 4.1.1, 4.1.3, 4.1.5, and 4.1.7 indicate that the cell density during the bloom is statistically higher than the cell density before the bloom for sites MB20, MB18, 8M, and 7M. In addition, the results of t-tests 4.1.2, 4.1.4, and 4.1.6 indicate that the cell density during the bloom is statistically higher than the cell density after the bloom for sites MB20, MB18, and 8M. Finally, the results of t-test 4.1.8 indicate that the cell density after the bloom is statistically higher than the cell density during the bloom for site 7M. These t-tests suggest that all of the trends in the lake data that have been described are statistically significant.

In addition, cell density numbers from each site of the lake during the bloom can be compared to the distance of the site from the mouth of the Maumee River and to the depth of the lake at the site. Figure 4-7 illustrates the correlation between the *Microcystis* cell density and the distance from the mouth of the Maumee River. The line of best fit suggests a negative correlation for this relationship. Figure 4-8 illustrates the correlation between *Microcystis* cell density and the depth of the lake. The line of best fit suggests a negative correlation for this relationship. Pearson correlation tests 4.1.9 and 4.1.10 were used to determine if either of these negative correlations indicated are statistically significant. Table 4-3 shows the calculated test statistics and the conclusions that can be drawn for these Pearson correlation tests. Table A-10, which can be found in Appendix A, defines each of the tests by listing the null and alternative hypotheses for each examined circumstance. Tables A-11 and A-12, which can be found in Appendix A, provide the necessary calculations and data that were used during computation of each of these Pearson correlation tests.

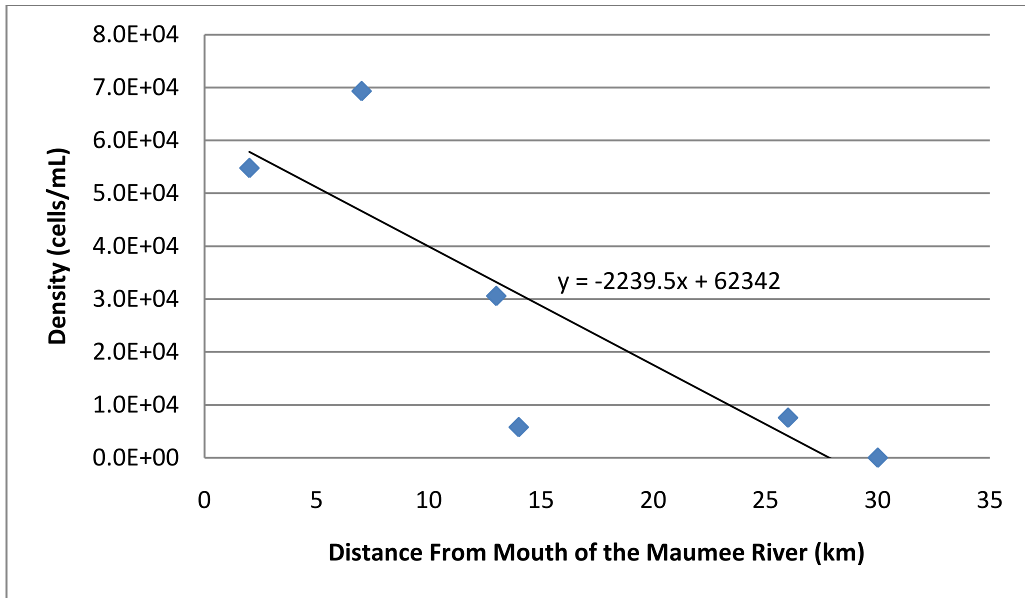

Figure 4-7: Graphical representation of the relationship between cell density in the lake water during the cyanobacterial bloom (August 4, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit is included, with its equation, to linearly define the relationship.

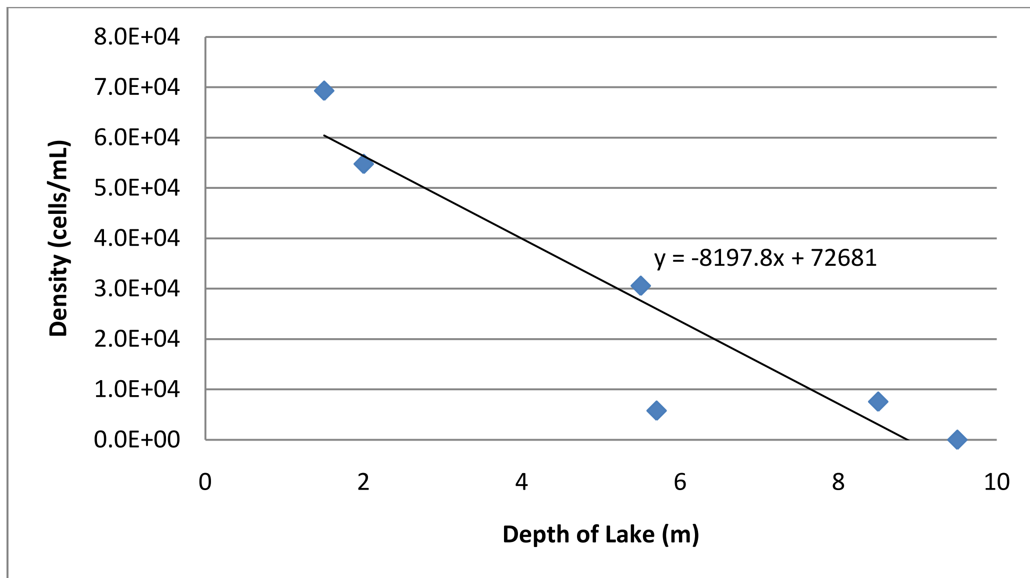

Figure 4-8: Graphical representation of the relationship between cell density in the lake water during the cyanobacterial bloom (August 4, 2009) at any given site and the water depth at that site. A line of best fit is included, with its equation, to linearly define the relationship.

Table 4-3: Test Statistics and Conclusions Drawn for Pearson Correlation Tests 4.1.9 and 4.1.10.

Comparison of *Microcystis* Cell Densities During the Bloom and Location of the Sampling Site		
Pearson Test No. 4.1.9	Test Statistic	r = -.842
	Conclusion	There is a statistically significant strong negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
Pearson Test No. 4.1.10	Test Statistic	r = -.932
	Conclusion	There is a statistically significant very strong negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.

From these results, it is concluded that the *Microcystis* cell density during a cyanobacterial bloom has a statistically significant strong negative correlation with the distance from the mouth of the Maumee River and a statistically significant very strong negative correlation with lake water depth. The closer to the mouth of the Maumee River, the higher the expected *Microcystis* cell density during a bloom would be. The shallower the water depth, the higher the expected *Microcystis* cell density during a bloom would be. These statistically significant correlations hold true for the ranges of the sampling sites on the given sampling date (August 6, 2009). This includes distances between two and thirty kilometers from the mouth of the Maumee River and depths between two meters and nine and a half meters.

In addition, the results of these tests have been negatively affected by the data trends in cell density at site 7M. T-test no. 4.1.8 indicated that the cell density at sampling site 7M was statistically significantly higher for the third sampling date than it was for the second sampling date. It was inferred by this result that the cyanobacterial bloom at site 7M

either lingered longer or occurred later than the bloom at the other sites. Pearson test nos. 4.1.9 and 4.1.10 were run to compare the cell densities during the bloom to the lake depth and site location. However, all of the data used for these tests was from the second sampling date. If the assumed cell density during the bloom from site 7M (data from the third sampling date instead of data from the second sampling date), then the test statistics for Pearson test nos. 4.1.9 and 4.1.10 would have changed to -.912 and -.980, respectively. Both of these changes indicate an increase in the absolute value of the test statistic and increased confidence in the correlation.

This indicates the importance of considering the cell density data from site 7M differently from the other site due to the later bloom. Other trends of data within this report are analyzed using the date of sampling to categorize the data as data from before the bloom, during the bloom, or after the bloom. Data used from the second sampling date of site 7M may be misleading when referred to as during the bloom data. When evaluating the data as it pertains to the objectives of this report, it will be important to consider that the bloom cell density at site 7M may be better represented by the third sampling date than by then second sampling date. This will be taken into account in the Discussion section of this report.

4.2 Percent Dry Solids of Sediment

Visual inspection of the sediment samples from different sites revealed that the samples seemed to have noticeably different moisture contents. Since the sediment samples remained sealed until analysis was done for *Microcystis* cell density and benthic phosphorus, these associated moisture contents were maintained during sample transportation and storage. The water content of the samples was found to range between

22.0% and 70.5%. In order to normalize the samples to dry weight solids content when analyzing the *Microcystis* cell density and benthic phosphorus concentration values, the percent of dry solids of each sample needed to be found. Table 4-4 shows the percent of dry solids for each of the eighteen samples.

Table 4-4: 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%

Sandy soils are capable of holding less moisture than more fine grained soils. Sediment samples from site MB18 were visually 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. These visual notes coincide with the results in Table 4-4. The highest results for percentage of dry solids in the samples came from site MB18. The lowest results for percentage of dry solids in the samples came from site 4P.

Due to the large difference in this physical property of the sediment samples, the percent of dry solids of the samples will prove important during analysis of other results within this research, including the analysis of the *Microcystis* cell density in the soils and analysis of the phosphorus levels in the sediments. The phosphorus and *Microcystis* cell density value results from each sample analyzed will need to be normalized to levels corresponding to levels per grams of dry sediments according to the results of the moisture content test.

4.3 Grain Size Distribution of Sediment

Visual observation from the sites was completed prior to the hydrometer analysis. It was noted that sediment from site MB18 was a sandy soil, as compared to the clayey soils in the samples from the other five sites. Based upon this, the grain size distribution of the sediment from site MB18 should indicate the most coarse-grained soils of all six sites. Of the other five sites, the sediment from site 8M was noted to 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.

Tables B-1 through B-6, found in Appendix B, show all pertinent data and calculations collected during the hydrometer analyses. The data and calculations within these tables are necessary to determine the grain size distribution of the sediment samples from each of the six sites. Graphical representation of the grain size analyses for composite samples from each of the six sampling sites is presented in Figures 4-9 through 4-14. Figure 4-15 is a graphical comparison of the grain size distributions of sediment from each sampling site. It is important to note that each grain size distribution is from a different sampling site. The sediment from these sampling sites is not expected to have similar grain size distributions. It is expected that the grain size distribution from each site, as shown in Figure 4-15, will vary from the others.

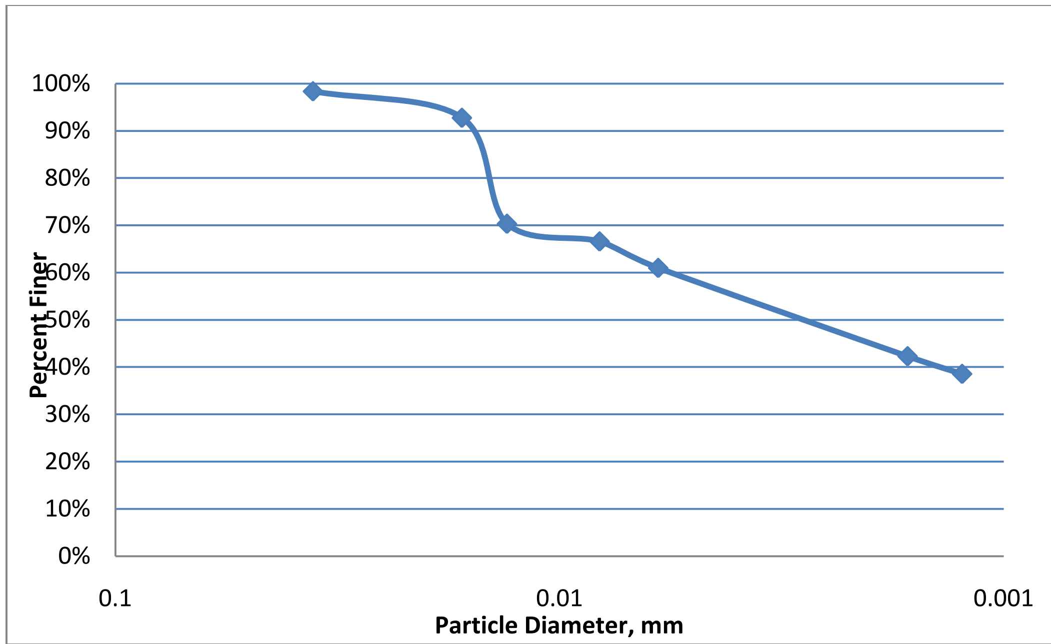

Figure 4-9: Graphical illustration of the grain size distribution of composite sediment samples from the site 8M.

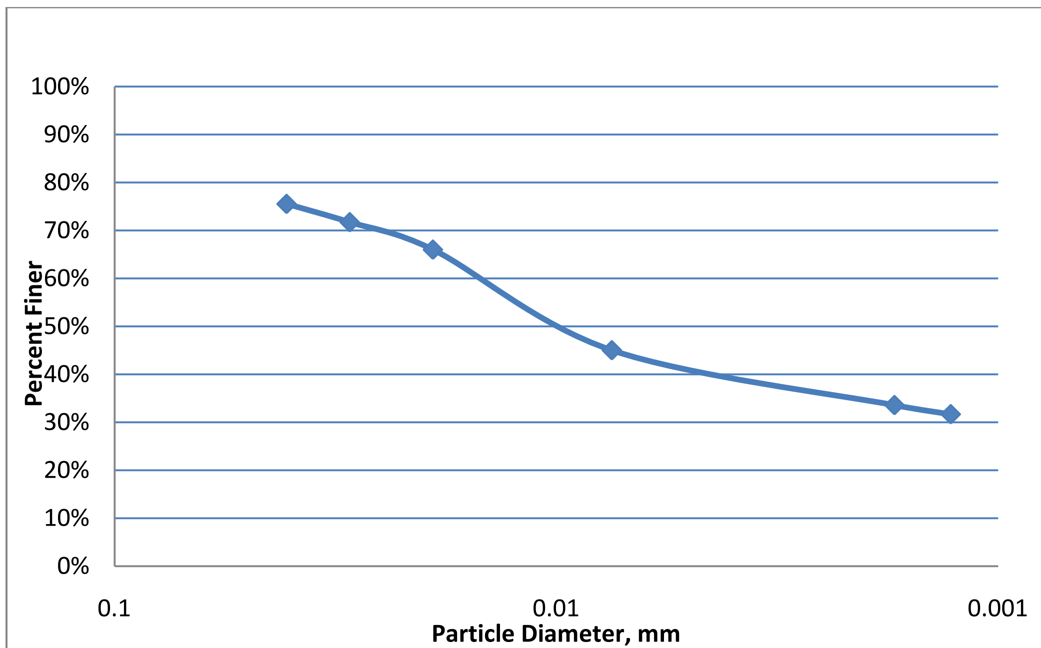

Figure 4-10: Graphical illustration of the grain size distribution of composite sediment samples from the site 7M.

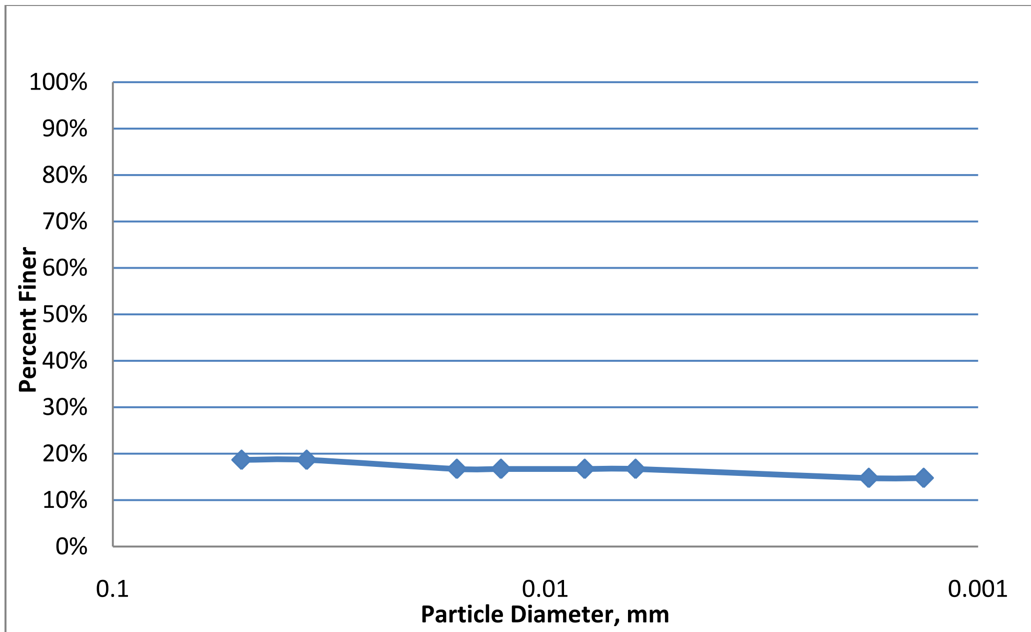

Figure 4-11: Graphical illustration of the grain size distribution of composite sediment samples from the site MB18.

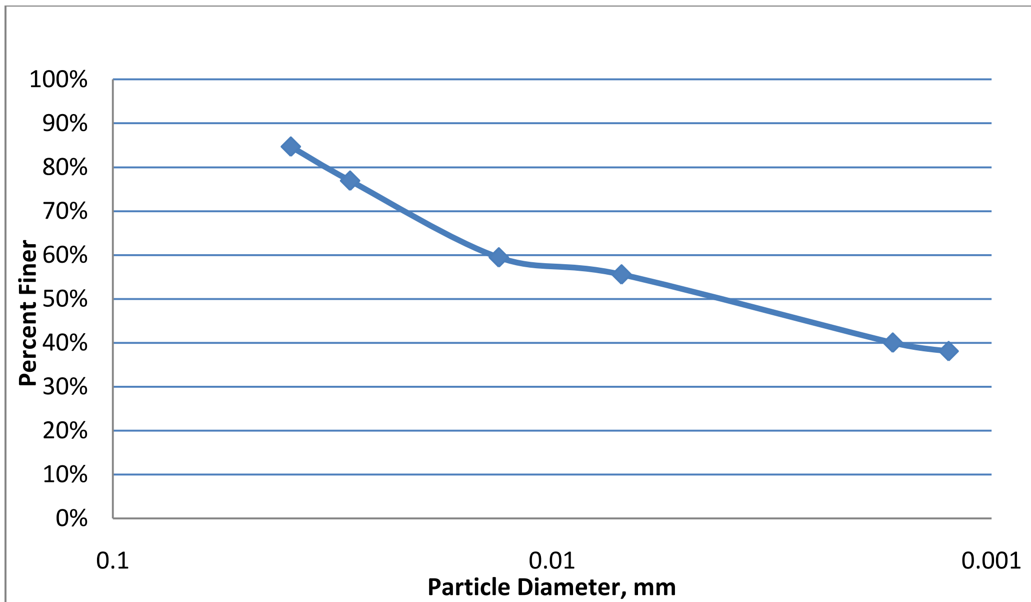

Figure 4-12: Graphical illustration of the grain size distribution of composite sediment samples from the site MB20.

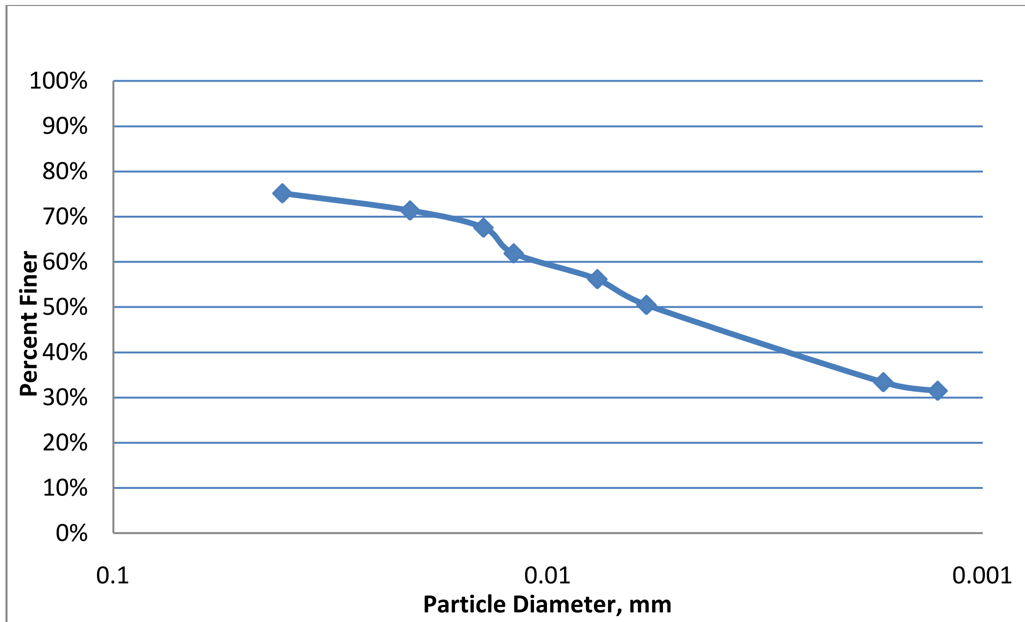

Figure 4-13: Graphical illustration of the grain size distribution of composite sediment samples from the site GR1.

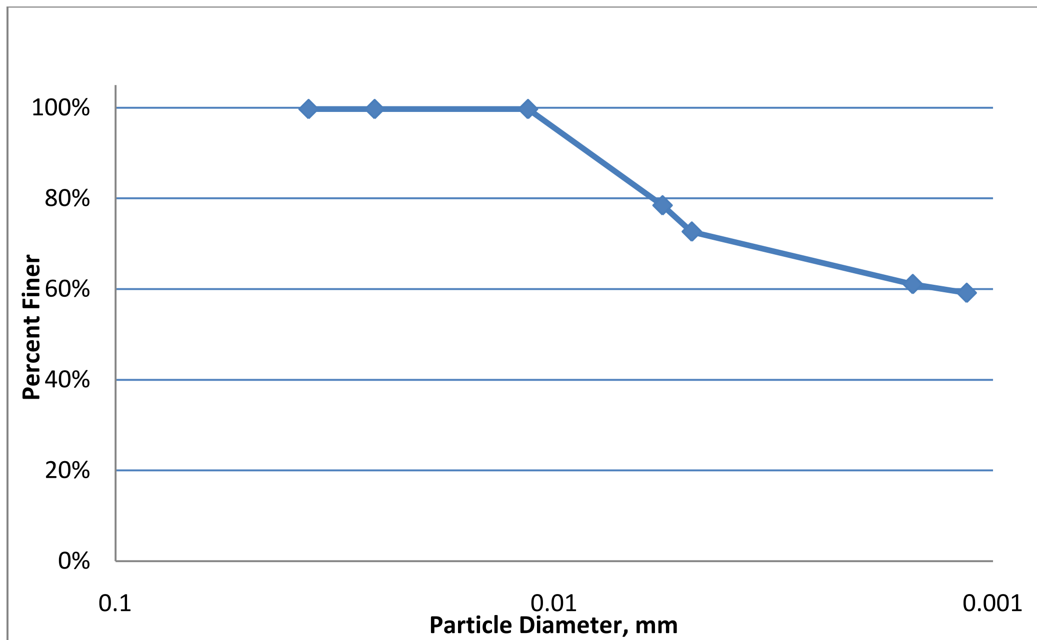

Figure 4-14: Graphical illustration of the grain size distribution of composite sediment samples from the site 4P.

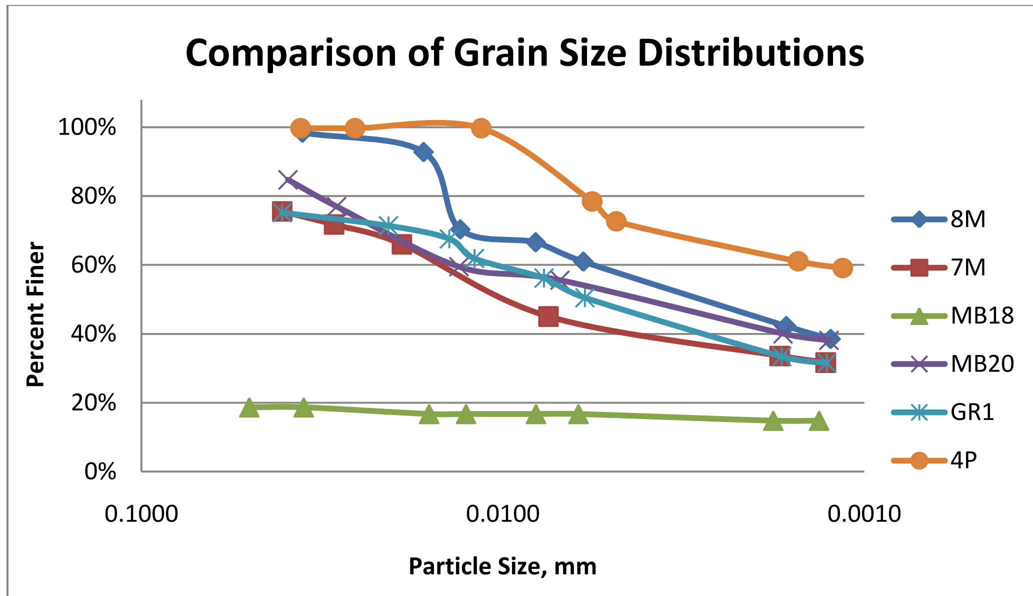

Figure 4-15: Graphical illustration of the grain size distribution of composite sediment samples from the each of the six sampling sites. This figure compares the grain size distributions from each of the sampling sites. The grain size distribution each site was not expected to conform to the grain size distribution from other sites.

The results illustrated in Figure 4-15 confirm that the sandy soil from composite sediment sample from site MB18 is the most coarse-grained of the composite samples from the six sites. Also confirmed is the fact that the composite sample from site 4P is the most fine-grained of the composite samples from the six sites. The clayey soils from the all of the other four composite sediment samples seem to have very similar grain size distributions.

For each of the sediment samples, a continuous variable that well represents each the grain size distribution needs to be determined. This continuous variable will be important to incorporate the grain size distribution of the soils into the statistical analyses examined. In this case, the median grain size of the soil seems to be representative of the entire distribution. The median grain size of the sediment samples from each site is included in Table 4-5. The hydrometer analysis did not determine a range of grain size distribution

including the median for the sediment samples from sites 4P and MB18. The median grain size for the sample from site 4P was estimated using extrapolation of the data. The median grain size for the sample from site MB18 was estimated using extrapolation of the data and the knowledge that 100% on the MB18 soil passed through the No. 10 sieve.

Table 4-5: 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

4.4 Phosphorus Testing of the Sediment

Results for phosphorus testing from each of the eighteen sites were returned from the Heidelberg Water Quality Lab and normalized to account for moisture content. Table 4-6 has the normalized results for soluble phosphorus (SRP) concentrations in each of the sediment samples listed as milligrams per gram dry weight of sediment. Figure 4-16 shows the normalized soluble phosphorus (SRP) concentration results at each site and the SRP variations before, during, and after the bloom. Table 4-7 has the normalized results for total phosphorus (TP) concentrations in each of the sediment samples listed as milligrams per gram dry weight of sediment. Figure 4-17 graphically represents the normalized total phosphorus (TP) concentration results at each site and the TP variations before, during, and after the bloom. Table 4-8 has the normalized results for soluble phosphorus ($FeCl_2P$) concentrations in each of the sediment samples listed as milligrams per gram dry weight of sediment. Figure 4-18 shows the normalized soluble phosphorus

($FeCl_2P$) concentration results at each site and the $FeCl_2P$ variations from before, during, and after the bloom.

Table 4-6: Soluble phosphorus concentrations results from the sediment samples from each site.

	23-Jun-09	6-Aug-09	14-Sep-09
Site	**SRP (mg/ gram dry weight of sediment)**	**SRP (mg/ gram dry weight of sediment)**	**SRP (mg/ gram dry weight of sediment)**
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

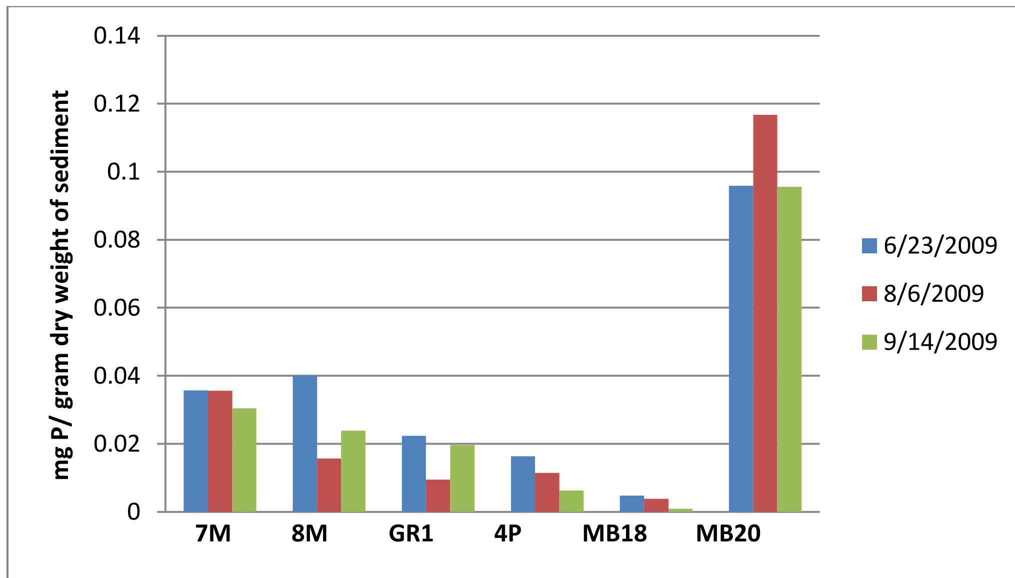

Figure 4-16: Soluble phosphorus concentrations (mg/gram dry weight of sediment) in sediment samples as a function of sampling site (MB20, MB18, 8M, 7M, GR1, 4P) and as a function of sampling date (June, August, September).

Table 4-7: Total phosphorus concentration results from the sediment samples from each site.

	23-Jun-09	6-Aug-09	14-Sep-09
Site	**TP (mg/ gram dry weight of sediment)**	**TP (mg/ gram dry weight of sediment)**	**TP (mg/ gram dry weight of sediment)**
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

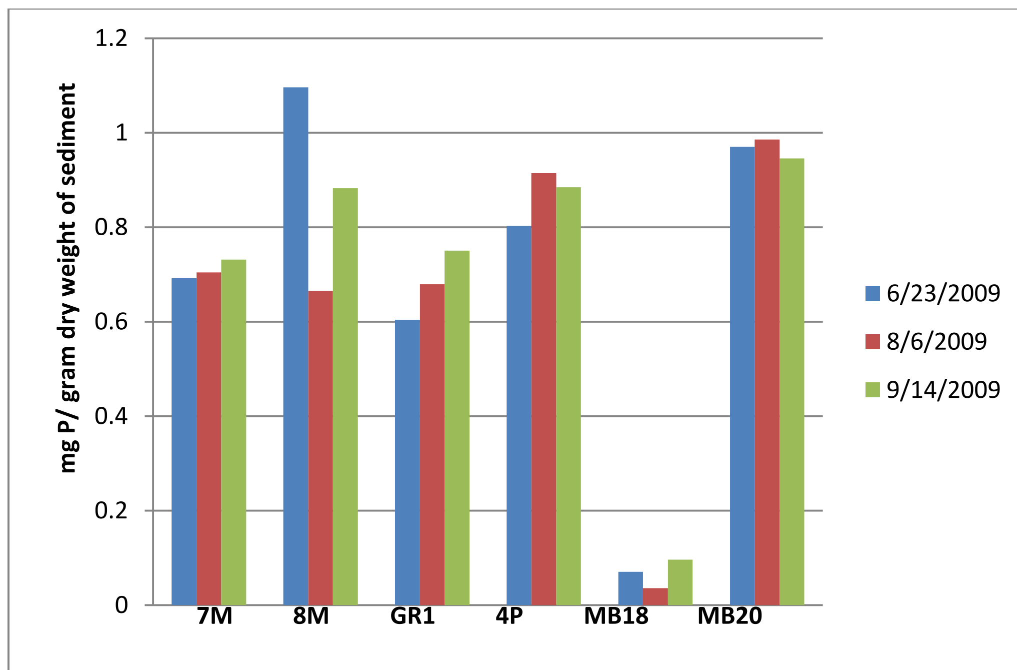

Figure 4-17: Total phosphorus concentrations (mg/gram dry weight of sediment) in sediment samples as a function of sampling site (MB20, MB18, 8M, 7M, GR1, 4P) and as a function of sampling date (June, August, September).

Table 4-8: Iron strip test phosphorus concentration results from the sediment samples from each site.

	23-Jun-09	6-Aug-09	14-Sep-09
Site	**$FeCl_2P$ (mg/ gram dry weight of sediment)**	**$FeCl_2P$ (mg/ gram dry weight of sediment)**	**$FeCl_2P$ (mg/ gram dry weight of sediment)**
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

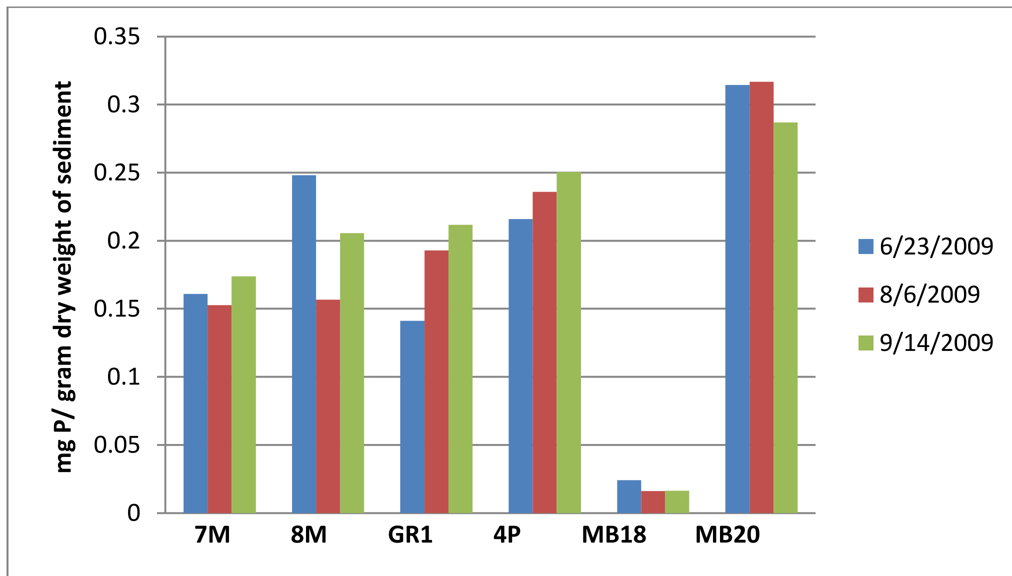

Figure 4-18: Iron strip test phosphorus concentrations (mg/gram dry weight of sediment) in sediment samples as a function of sampling site (MB20, MB18, 8M, 7M, GR1, 4P) and as a function of sampling date (June, August, September).

In order to determine if there had been any statistically significant changes that had occurred, 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). A dependent t-test was utilized in order to determine any statistically significant changes between results at each site. Soluble phosphorus concentrations, total phosphorus concentrations, and iron strip test phosphorus concentrations were all compared by the t-tests. Table 4-9 shows the calculated test statistics and the conclusions that can be drawn for these t-tests. Table A-13, which can be found in Appendix A, defines each of the t-tests by listing the null and alternative hypotheses for each examined circumstance. Tables A-14 through A-19, which can be found in Appendix A, provide the necessary calculations and data that were used during computation of each of these t-tests. For each t-test, it was found that a statistically significant difference could not be found.

Figures 4-19 through 4-24 show the normalized results for each of the three different phosphorus tests. Each of these figures is exclusive for a single sampling site. All three normalized phosphorus test results are included on each of the figures. Despite their proximity in the lake, these tables indicate that phosphorus concentrations at sites MB20 and MB18 are quite different. In fact, for each of the three tests, MB18 sediment samples had the lowest concentrations of phosphorus and MB20 sediment samples had among the highest concentrations of phosphorus.

Table 4-9: Test Statistics and Conclusions Drawn for T-tests 4.4.1, 4.4.2, 4.4.3, 4.4.4, 4.4.5, and 4.4.6.

Comparison of Phosphorus Concentrations In Sediment Samples Before, During, and After the Bloom		
T-test No. 4.4.1	Test Statistic	t = 0.465
	Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, soluble phosphorus (SRP) concentrations in the sediment before the bloom is NOT statistically greater than soluble phosphorus (SRP) concentrations in the sediment during the bloom.
T-test No. 4.4.2	Test Statistic	t = 0.394
	Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, soluble phosphorus (SRP) concentrations in the sediment during the bloom are NOT statistically greater than soluble phosphorus (SRP) concentrations in the sediment after the bloom.
T-test No. 4.4.3	Test Statistic	t = 0.793
	Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, total phosphorus (TP) concentrations in the sediment before the bloom are NOT statistically greater than total phosphorus (TP) concentrations in the sediment during the bloom.
T-test No. 4.4.4	Test Statistic	t = 1.591
	Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, total phosphorus (TP) concentrations in the sediment after the bloom are NOT statistically greater than total phosphorus (TP) concentrations in the sediment during the bloom.
T-test No. 4.4.5	Test Statistic	t = 0.465
	Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment before the bloom are NOT statistically greater than iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment during the bloom.
T-test No. 4.4.6	Test Statistic	t = 0.910
	Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment after the bloom are NOT statistically greater than iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment during the bloom.

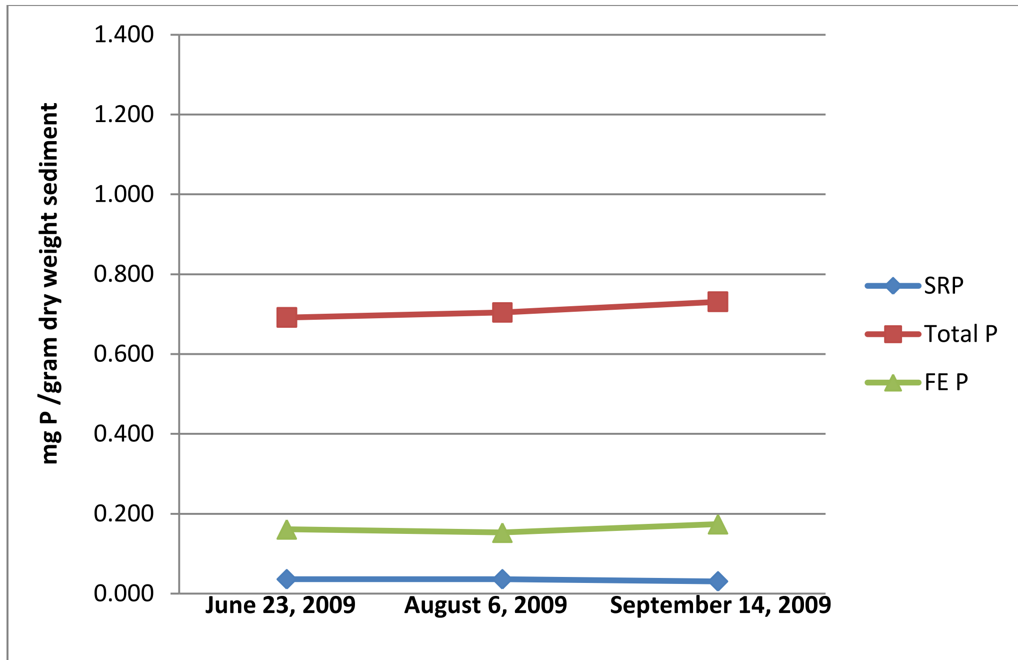

Figure 4-19: Concentrations of phosphorus in the sediment at site 7M. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus.

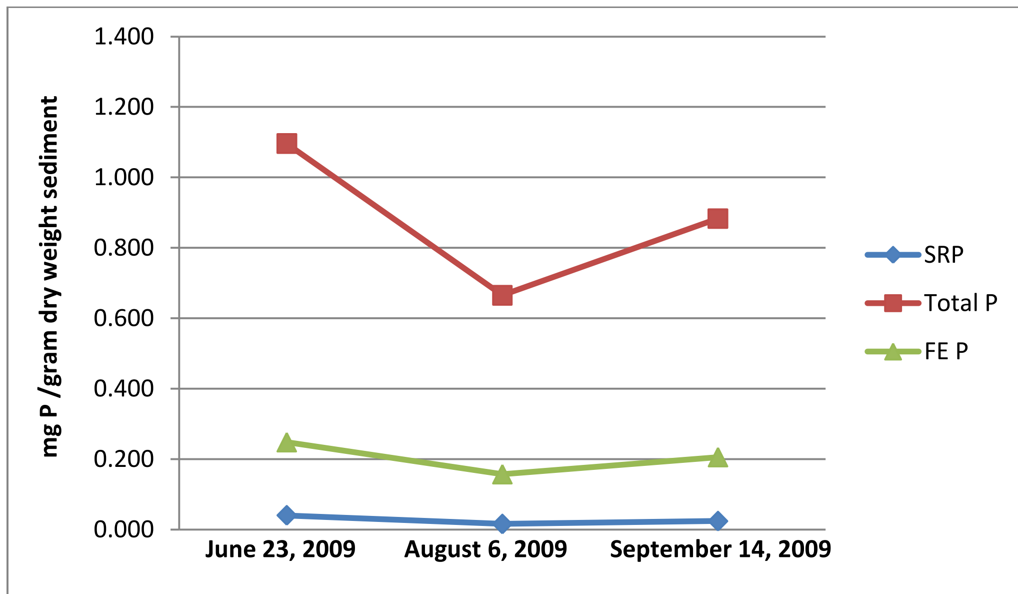

Figure 4-20: Concentrations of phosphorus in the sediment at site 8M. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus.

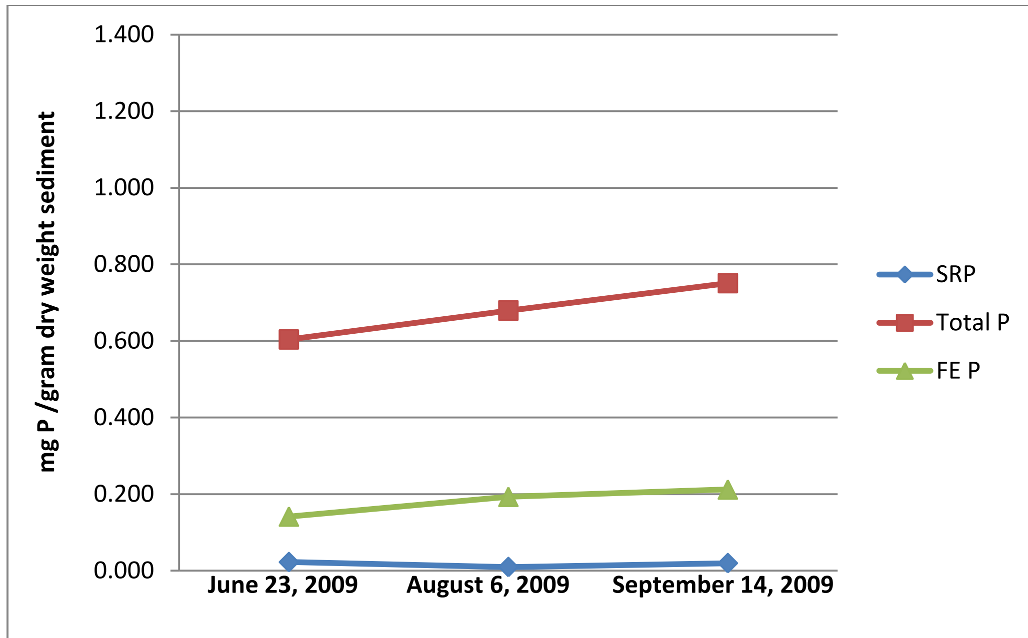

Figure 4-21: Concentrations of phosphorus in the sediment at site GR1. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus.

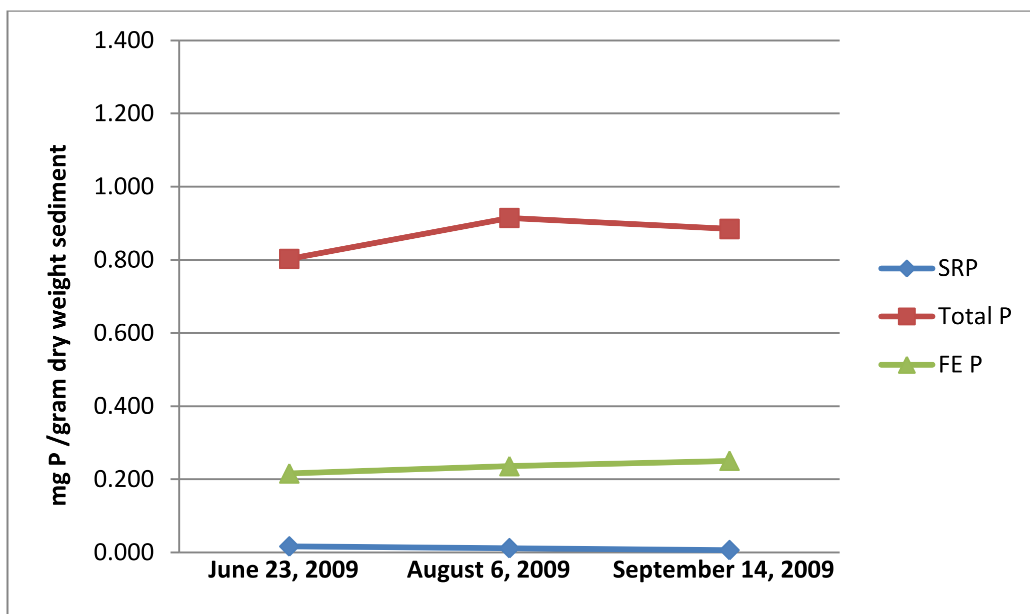

Figure 4-22: Concentrations of phosphorus in the sediment at site 4P. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus.

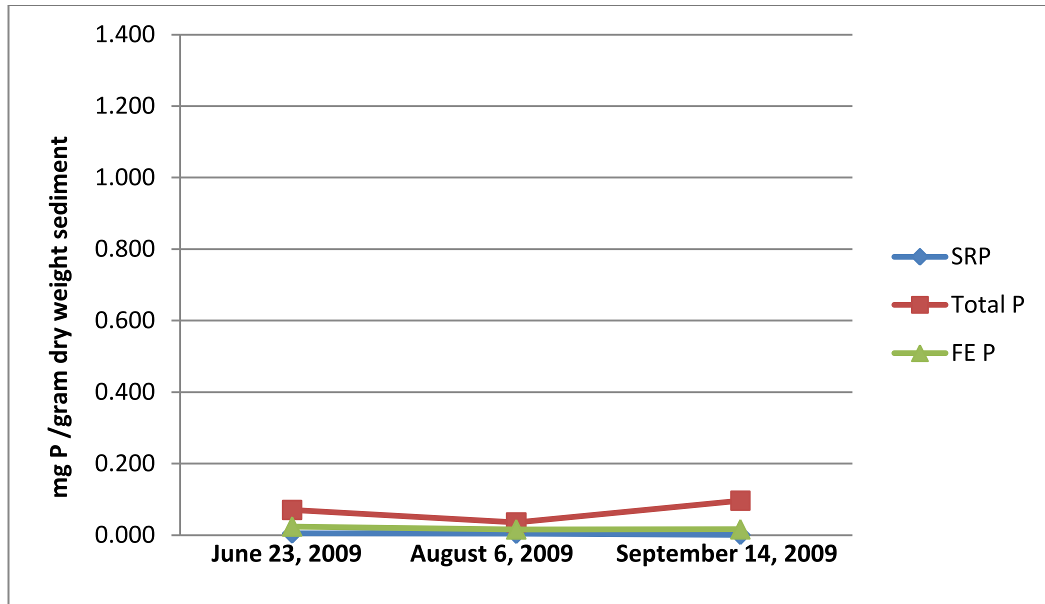

Figure 4-23: Concentrations of phosphorus in the sediment at site MB18. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus.

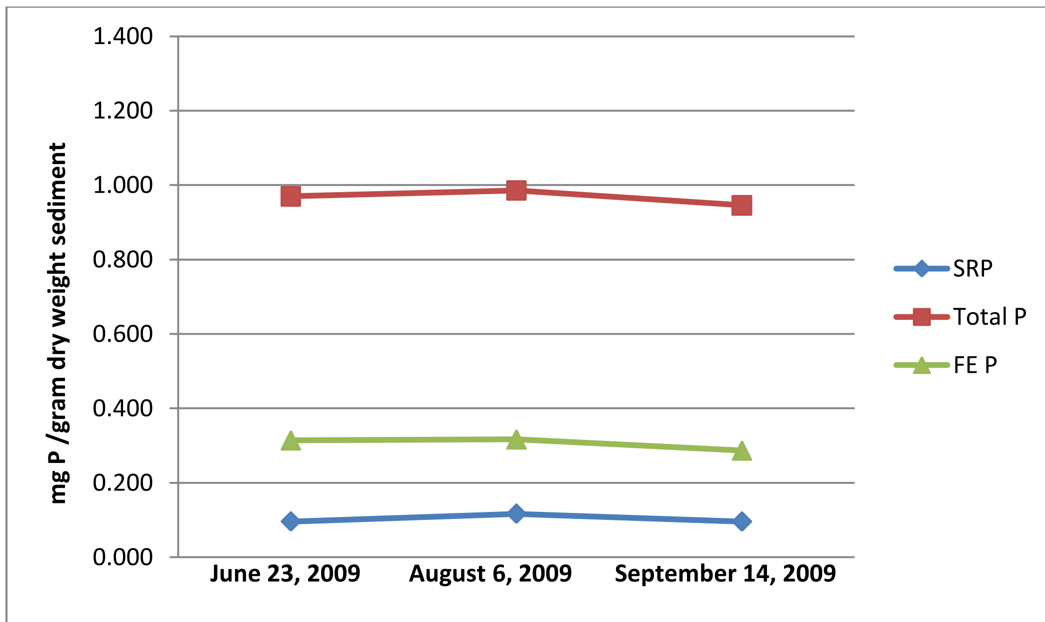

Figure 4-24: Concentrations of phosphorus in the sediment at site MB20. The phosphorus concentration is represented by three forms: soluble phosphorus, total phosphorus, and iron strip test phosphorus.

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. Figure 4-25, Figure 4-26, and Figure 4-27 illustrate the correlation between the median grain size of the sediment sample and the sample's soluble phosphorus, total phosphorus, and iron strip test phosphorus concentrations, respectively. The line of best fit on each of these figures suggests a negative correlation for each relationship. A Pearson correlation test was used to determine if these negative correlations are statistically significant. Table 4-10 shows the calculated test statistics and the conclusions that can be drawn for these Pearson correlation tests. Table A-20, which can be found in Appendix A, defines each of the tests by listing the null and alternative hypotheses for each examined circumstance. Tables A-21 through A-23, which can be found in Appendix A, provide the necessary data and calculations that were used during computation of each of these Pearson correlation tests.

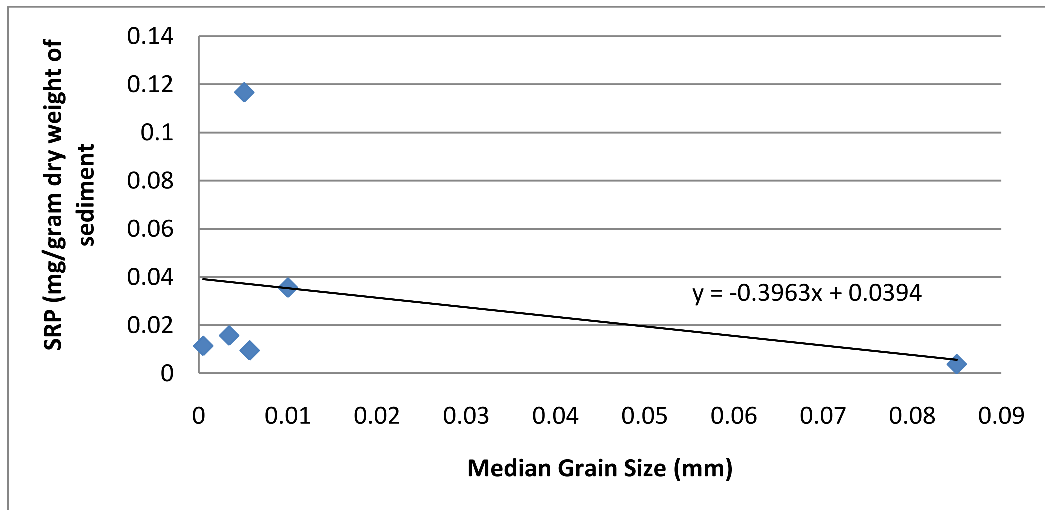

Figure 4-25: The relationship of soluble phosphorus (SRP) concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample. The correlation the SRP concentration and the median grain size is represented by the line of best fit, which has an equation shown on this figure.

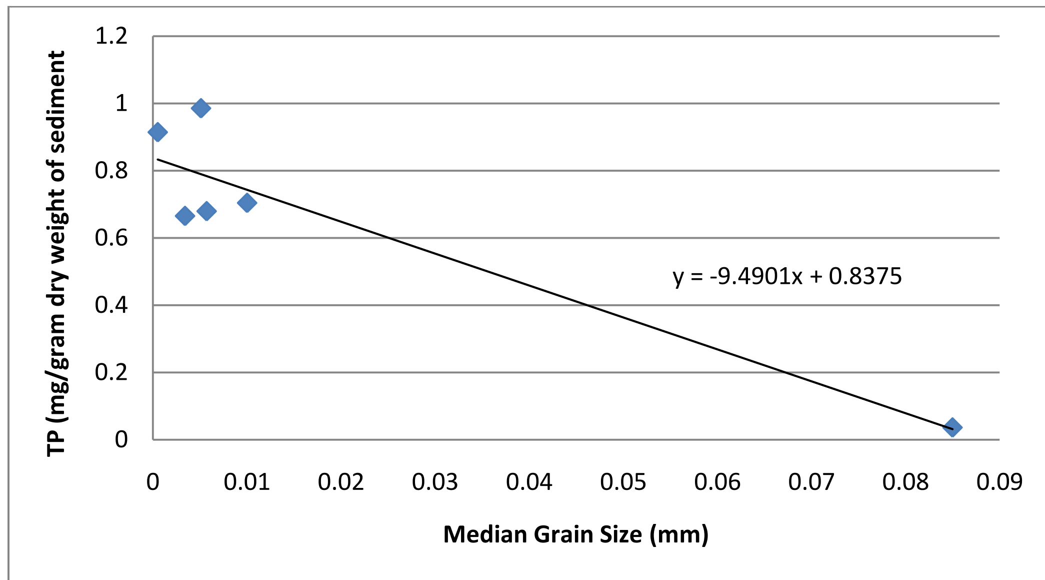

Figure 4-26: The relationship of total phosphorus (TP) concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample. The correlation the YP concentration and the median grain size is represented by the line of best fit, which has an equation shown on this figure.

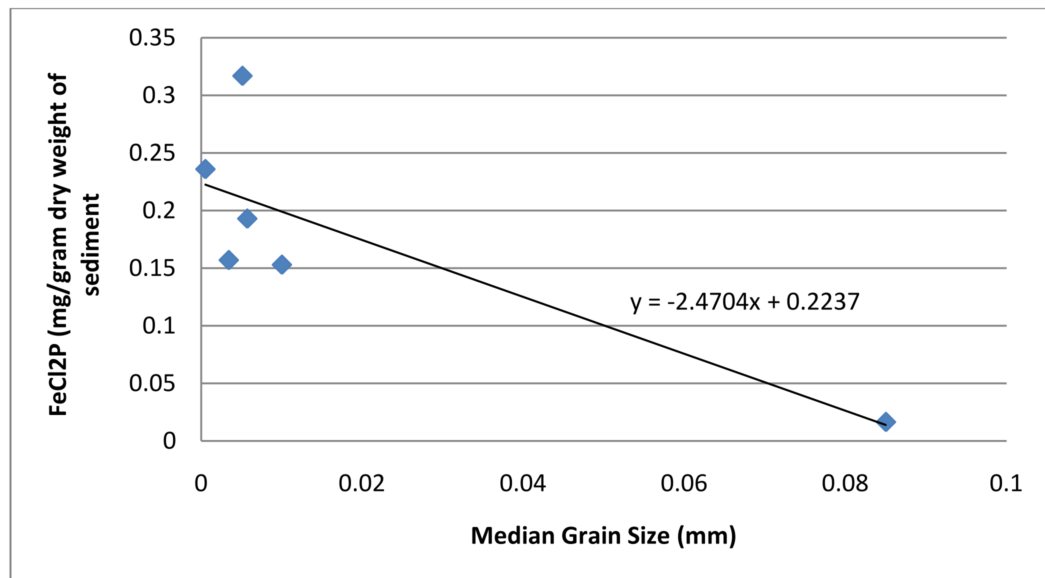

Figure 4-27: The relationship of iron strip test phosphorus ($FeCl_2P$) concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample. The correlation the $FeCl_2P$ concentration and the median grain size is represented by the line of best fit, which has an equation shown on this figure.

Table 4-10: Test Statistics and Conclusions Drawn for Pearson Correlation Tests 4.4.7, 4.4.8, and 4.4.9.

Comparison of Phosphorus Concentrations During the Bloom and Median Grain Size of the Sample		
Pearson Test No. 4.4.7	Test Statistic	r = -.303
	Conclusion	There is NOT a statistically significant negative relationship between soluble phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.
Pearson Test No. 4.4.8	Test Statistic	r = -.929
	Conclusion	There is a statistically significant very strong negative relationship between total phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.
Pearson Test No. 4.4.9	Test Statistic	r = -.810
	Conclusion	There is a statistically significant strong negative relationship between iron strip test phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.

Table 4-10 indicates that there is a statistically significant negative correlation between both total and iron strip test phosphorus concentrations in the sediment and the median grain size of the sediment. However, the data from this report cannot conclude a statistically significant negative relationship between soluble phosphorus and median grain size of the sediment.

4.5 *Microcystis* Cell Density Values in Sediment

Average *Microcystis* cell density was determined for each of the eighteen sediment samples (thirty-six duplicate samples) using all of the analyzed slides. The average levels found are displayed in Table 4-11. 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. The *Microcystis* cell density values before the bloom were below the detection limit at sites 7M, 8M, 4P, and MB20. Based upon historical data from research using different methods of detection, it is believed that there was *Microcystis* biomass at all sites before the bloom. However, the size of overwintering cells in the sediment prior to cyanobacterial blooms is below the detection limit. During the bloom and after the bloom, all sampling sites yielded measurable results. In addition to the averages of the cell density readings, the standard deviations of these readings were also computed. Table 4-11 also displays all of the standard deviation readings. No standard deviations could be computed for samples with cell densities below the detection limit.

Table 4-11: Average values for the *Microcystis* cell density values per gram dry weight of sediment and the associated standard deviations for each sediment sample analyzed.

Site	23-Jun-09 (cells/gram dry weight of sediment)	6-Aug-09 (cells/gram dry weight of sediment)	14-Sep-09 (cells/gram dry weight of sediment)
7M	BDL	$6.66 \times 10^4 \pm 2.21 \times 10^4$	$7.17 \times 10^4 \pm 1.24 \times 10^4$
8M	BDL	$2.07 \times 10^5 \pm 2.67 \times 10^4$	$1.84 \times 10^5 \pm 7.78 \times 10^4$
GR1	$8.60 \times 10^3 \pm 6.01 \times 10^3$	$5.49 \times 10^4 \pm 9.32 \times 10^3$	$8.20 \times 10^4 \pm 2.41 \times 10^4$
4P	BDL	$7.00 \times 10^4 \pm 1.33 \times 10^4$	$1.31 \times 10^5 \pm 4.07 \times 10^4$
MB18	$1.34 \times 10^4 \pm 7.28 \times 10^3$	$6.54 \times 10^4 \pm 2.90 \times 10^4$	$5.69 \times 10^4 \pm 7.47 \times 10^3$
MB20	BDL	$1.04 \times 10^5 \pm 4.41 \times 10^4$	$1.25 \times 10^5 \pm 2.21 \times 10^3$

Figure 4-28 displays all of the sample mean cell density values separated by site. 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 there detectable cells

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. Trends of *Microcystis* cell density values in the sediment cannot be compared to a cyanobacterial bloom, because no bloom was detected in the lake water sample from site 4P. In this report; therefore, *Microcystis* cell density values from the sediment samples taken at site 4P will not be included in analyses comparing data from before, during, and after blooms. Other analyses of the sediment and *Microcystis* cell density vales in the sediment will include this data from sampling site 4P.

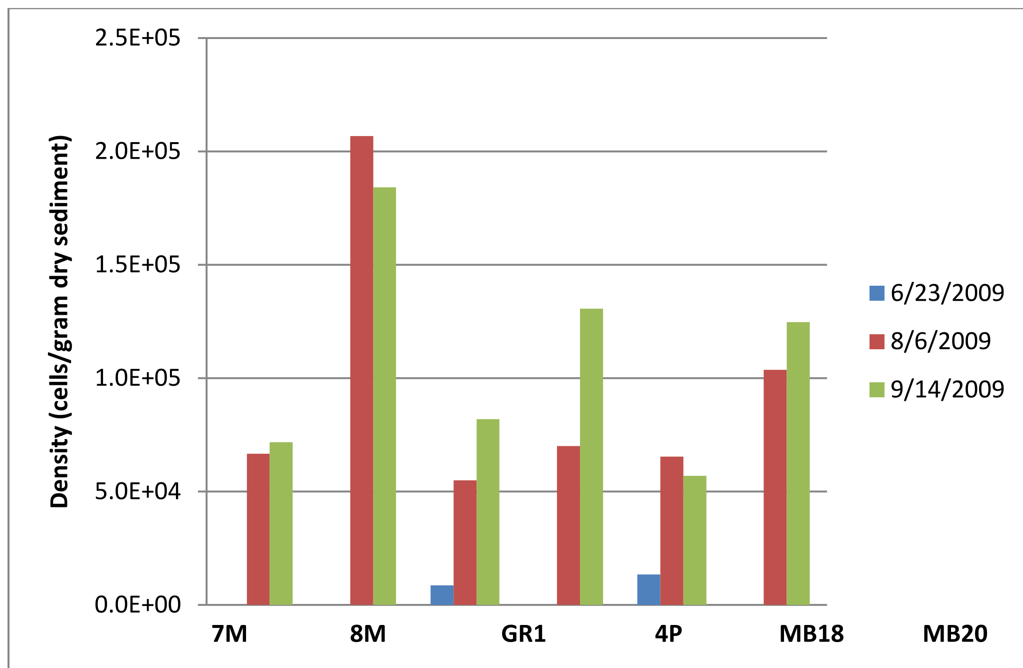

Figure 4-28: *Microcystis* cell density (cell/mL) in sediment samples as a function of sampling site (MB20, MB18, 8M, 7M, GR1, 4P) and as a function of sampling date (June, August, September).

Visual examination of Figure 4-28, reveals no correlation between the two sites that had cell densities above the detection limit before the bloom and the future cell densities at these sites. Also, there is not a visually discernable relationship between the sediment cell density during the bloom and after the bloom. This could be an error due to the limitations of this testing method resulting from only larger and not total biomass occurring within the detection limit. However, this could also be due to trends at each site not visually discernable from this figure. The discussion section of this report further analyzes these trends.

The cell density mean values at sites MB20, MB18, 8M, 7M, 4P, and GR1 are further represented in Figures 4-29 through 4-34. Each one of these figures is specific to a single site. The mean cell density values at the site for each date are displayed. In addition, error bars are supplied for all mean cell density values that were above the detection limit. The error bars extend one standard deviation in both directions. The error bars for the mean cell density during the bloom do extend into the same numerical region as the error bar for either the mean cell density before or after the bloom at some of the sites. This indicates that the results show that the *Microcystis* cell densities during a cyanobacterial bloom are not always dissimilar to the *Microcystis* cell densities before or after a cyanobacterial bloom.

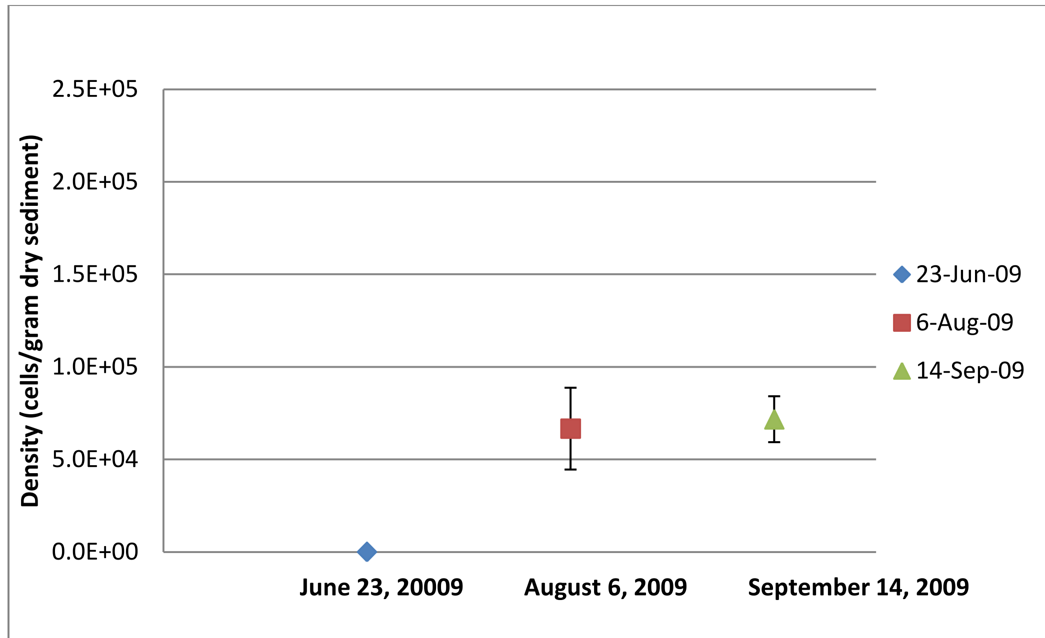

Figure 4-29: *Microcystis* cell density in the sediment samples as a function of the sampling date at the site 7M. Error bars represent one standard deviation from the mean in each direction.

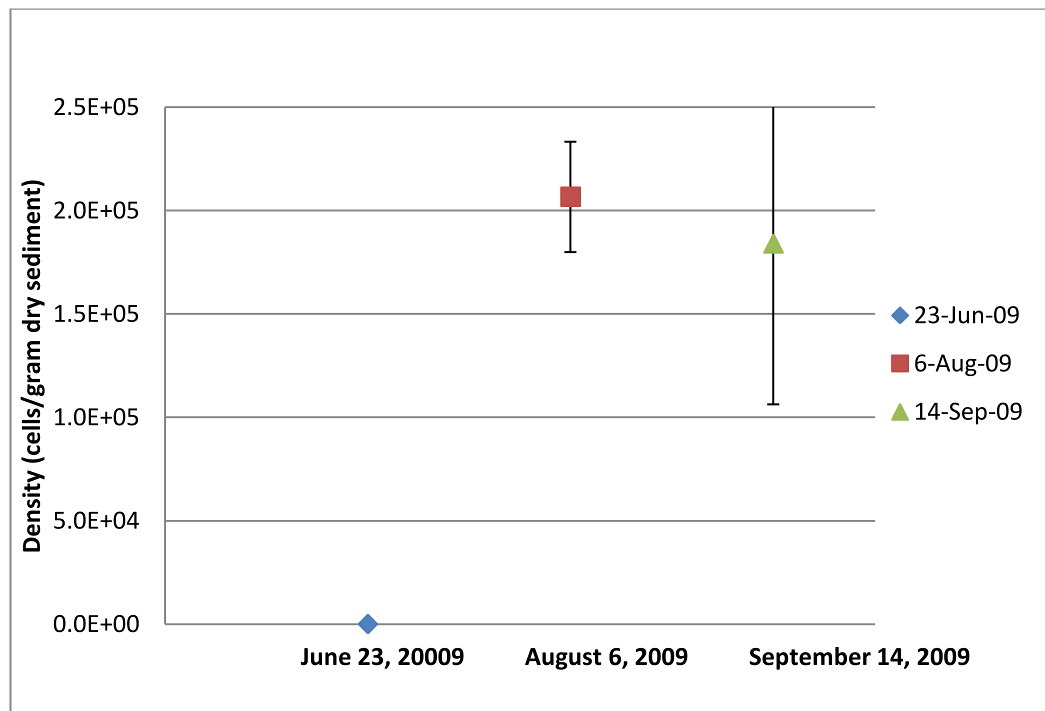

Figure 4-30: *Microcystis* cell density in the sediment samples as a function of the sampling date at the site 8M. Error bars represent one standard deviation from the mean in each direction.

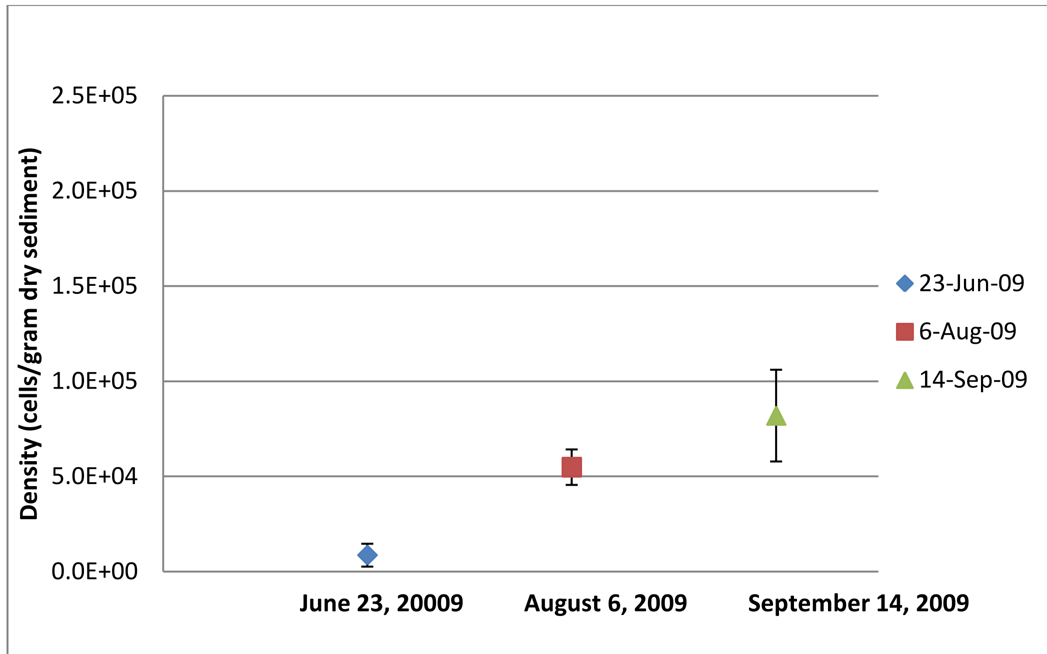

Figure 4-31: *Microcystis* cell density in the sediment samples as a function of the sampling date at the site GR1. Error bars represent one standard deviation from the mean in each direction.

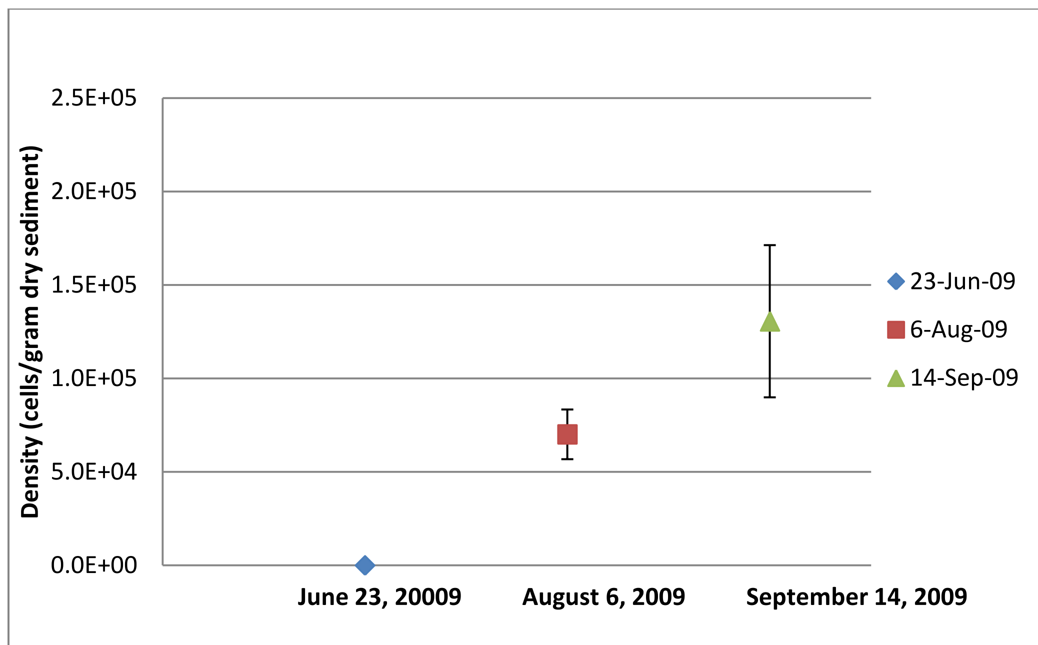

Figure 4-32: *Microcystis* cell density in the sediment samples as a function of the sampling date at the site 4P. Error bars represent one standard deviation from the mean in each direction.

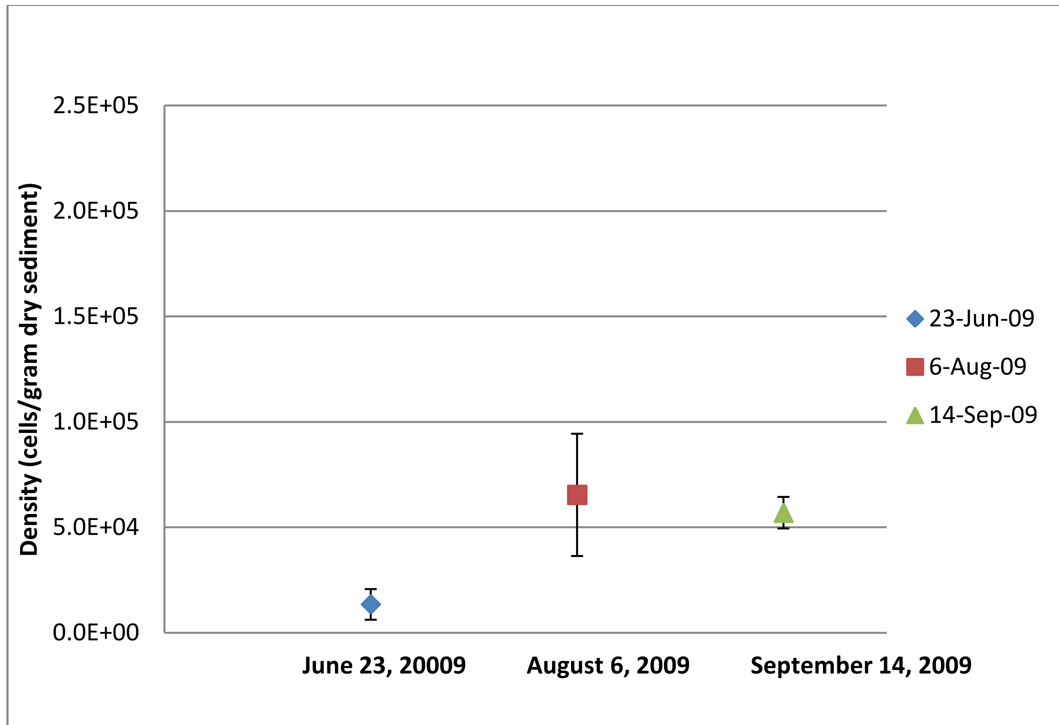

Figure 4-33: *Microcystis* cell density in the sediment samples as a function of the sampling date at the site MB18. Error bars represent one standard deviation from the mean in each direction.

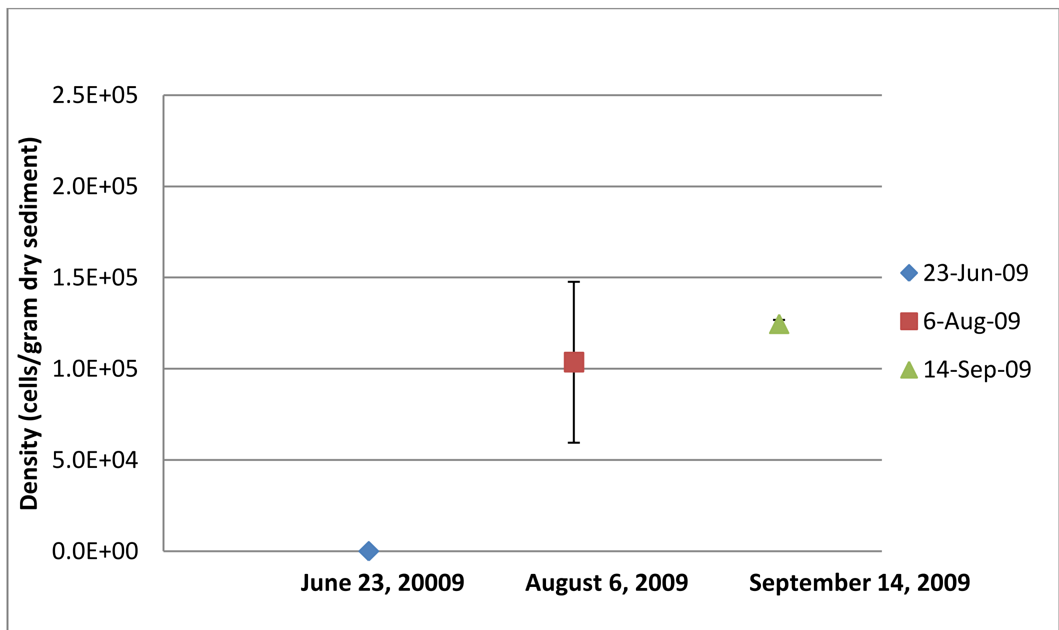

Figure 4-34: *Microcystis* cell density in the sediment samples as a function of the sampling date at the site MB20. Error bars represent one standard deviation from the mean in each direction.

In order to comment on statistically significant differences between the mean *Microcystis* cell density values; however, the lack or presence of overlap of the error bars is not sufficient. To further investigate these trends, a two-sample independent t-test was run to compare the mean cell density values before and during the bloom and during and after the bloom. T-tests were completed to compare the cell density values for sites MB20, MB18, 8M, 7M, and GR1. As mentioned prior, cell densities from the sediment of 4P cannot be compared in this manor due to the lack of a bloom detected by cell density values of the lake samples. Table 4-12 shows the calculated test statistics and the conclusions that can be drawn for these t-tests. Table A-24, which can be found in Appendix A, defines each of the t-tests by listing the null and alternative hypotheses for each examined circumstance. Tables 4-25 through 4-34, which can be found in Appendix A, provide the necessary data and calculations that were used during computation of each of these t-tests.

The results of t-tests 4.5.1, 4.5.3, 4.5.5, 4.5.7, and 4.5.9 indicate that the density of detectable *Microcystis* cells within the sediments during the bloom is statistically higher than the cell density before the bloom for sites MB20, MB18, 8M, 7M, and GR1. In addition, the results of t-tests 4.5.4 and 4.5.6 indicate that the cell density within the sediments during the bloom is not statistically significantly higher than the cell density after the bloom for sites MB18 and 8M. Finally, the results of t-test 4.5.2, 4.5.8, and 4.5.10 indicate that the cell density within the sediments after the bloom is not statistically significantly higher than the cell density during the bloom for sites MB20, 7M, and GR1.

Table 4-12: Test Statistics and Conclusions Drawn for T-tests 4.5.1, 4.5.2, 4.5.3, 4.5.4, 4.5.5, 4.5.6, 4.5.7, 4.5.8, 4.5.9, and 4.5.10.

Comparison of *Microcystis* Cell Densities Changes at Sites MB18, MB20, 8M and 7M		
T-test No. 4.5.1	Test Statistic	t = 3.151
	Conclusion	In the sediment from site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.5.2	Test Statistic	t = 0.636
	Conclusion	In the sediment from site MB20, *Microcystis* cell density after the bloom is NOT statistically greater than *Microcystis* cell density during the bloom.
T-test No. 4.5.3	Test Statistic	t = 2.377
	Conclusion	In the sediment from site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.5.4	Test Statistic	t = 0.388
	Conclusion	In the sediment from site MB18, *Microcystis* cell density during the bloom is NOT statistically greater than *Microcystis* cell density after the bloom.
T-test No. 4.5.5	Test Statistic	t = 10.964
	Conclusion	In the sediment from site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.5.6	Test Statistic	t = 0.387
	Conclusion	In the sediment from site 8M, *Microcystis* cell density during the bloom is NOT statistically greater than *Microcystis* cell density after the bloom.
T-test No. 4.5.7	Test Statistic	t = 4.018
	Conclusion	In the sediment from site 7M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.5.8	Test Statistic	t = 0.355
	Conclusion	In the sediment from site 7M, *Microcystis* cell density after the bloom is NOT statistically greater than *Microcystis* cell density during the bloom.
T-test No. 4.5.9	Test Statistic	t = 5.904
	Conclusion	In the sediment from site GR1, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
T-test No. 4.5.10	Test Statistic	t = 1.483
	Conclusion	In the sediment from site GR1, *Microcystis* cell density after the bloom is NOT statistically greater than *Microcystis* cell density during the bloom.

Due to the magnification used for identification and quantification of *Microcystis* cells in the sediment, the sediment densities tested only represent the larger cells. These larger cells are either preparing to reinvade the water column or have recently re-entered the sediment through sedimentation from the water column. The test results within Table 4-12 indicate that there is a significant increase in the density of these large cells in the sediment from before the bloom (June) to during the bloom (August), but there is no significant changes in the density of these large cells from during the bloom (August) to after the bloom (September).

Also, cell density numbers from each site of the lake can be compared to the distance of the site from the mouth of the Maumee River. Figure D-1 illustrates the correlation between the *Microcystis* cell density during a bloom at each site and the site's distance from the mouth of the Maumee River. Figure D-2 illustrates the correlation between the *Microcystis* cell density after a bloom at each site and the site's distance from the mouth of the Maumee River. Figure D-3 illustrates the correlation between the change in *Microcystis* cell density from during the bloom to after the bloom (the difference average density values from August 6, 2009 and average density values from September 14, 2009) at each site and the site's distance from the mouth of the Maumee River. Each of these three figures can be found in Appendix D. The line of best fit suggests a slightly negative correlation for the data in Figure D-1, a slightly positive correlation for the data in Figure D-2 and a positive correlation for the data in Figure D-3.

In addition, cell density numbers from each site of the lake can be compared to the depth of the lake at the site. Figure D-4 illustrates the correlation between *Microcystis* cell density during a bloom and the depth of the lake. Figure D-5 illustrates the correlation

between *Microcystis* cell density after a bloom and the depth of the lake. Figure D-6 illustrates the correlation between the change in *Microcystis* cell density from during the bloom to after the bloom (the difference average density values from August 6, 2009 and average density values from September 14, 2009) and the depth of the lake. Each of these three figures can be found in Appendix D. The line of best fit suggests a slightly negative correlation for the data in Figure D-4, a slightly positive correlation for the data in Figure D-5 and a positive correlation for the data in Figure D-6.

It was expected that the line of best fit for the data on Figures D-1 and D-2, and on Figures D-4 and D-5 would indicate a similar correlations. If these correlations are significant, this indicates a new pattern that will need to be researched in the future. This importance of determining whether or not there is statistical significance indicates the need for a statistical test.

In order to determine whether or not each of these correlations are statistically significant, a Pearson correlation test was used for each of the six relationships indicated in Figures D-1, D-2, D-3, D-4, D-5, and D-6. Table 4-13 shows the calculated test statistics and the conclusions that can be drawn for these Pearson correlation tests. Table A-35, which can be found in Appendix A, defines each of the tests by listing the null and alternative hypotheses for each examined circumstance. Tables A-36 through A-41, which can be found in Appendix A, provide the necessary data and calculations that were used during computation of each of these Pearson correlation tests.

Table 4-13: Test Statistics and Conclusions Drawn for Pearson Correlations Tests 4.5.11, 4.5.12, 4.5.13, 4.5.14, 4.5.15, and 4.5.16.

Comparison of *Microcystis* Cell Densities in Sediment Samples and the Location of the Sampling Site		
Pearson Test No. 4.5.11	Test Statistic	r = -.287
	Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
Pearson Test No. 4.5.12	Test Statistic	r = .051
	Conclusion	There is NOT a statistically significant positive relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
Pearson Test No. 4.5.13	Test Statistic	r = .639
	Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
Pearson Test No. 4.5.14	Test Statistic	r = -.148
	Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
Pearson Test No. 4.5.15	Test Statistic	r = .196
	Conclusion	There is NOT a statistically significant positive relationship between *Microcystis* cell density in a lake water sample taken after the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
Pearson Test No. 4.5.16	Test Statistic	r = .602
	Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.

From these results, it cannot be concluded that the *Microcystis* cell density during or after a cyanobacterial bloom has a statistically significant correlation with the distance from

the mouth of the Maumee River or a statistically significant correlation with lake water depth. Also, it cannot be concluded that the change in *Microcystis* cell density from during the cyanobacterial bloom to after the cyanobacterial bloom has a statistically significant correlation with the distance from the mouth of the Maumee River or a statistically significant correlation with lake water depth. Despite no statistically significance found, the test statistics corresponding to these changes are within proximity the critical value of 0.729. Therefore, it is possible that if a larger sampling size was used after further testing, a correlation between this change and the distance from the mouth of the Maumee River or a correlation between this change and depth may be revealed. For the results here; however, the change in cell density values in sediment samples cannot be linearly linked to the depth or the location that the sediment samples were taken from.

Despite the fact that no statistically significant correlation was found to indicate that location has an effect on *Microcystis* cell density in the sediment, another physical property of the sediment samples, median grain size, may have more influence. Figure D-7, Figure D-8, and Figure D-9 illustrate the correlation between the median grain size of the sediment sample and the sample's *Microcystis* cell density during the cyanobacterial bloom, the sample's *Microcystis* cell density after the cyanobacterial bloom, and the change between samples in *Microcystis* cell density from before to after the cyanobacterial bloom, respectively. Each of these three figures can be found in Appendix D. The line of best fit on each of these figures suggests a negative correlation for each relationship.

A Pearson correlation test was used to determine if these negative correlations are statistically significant. Table 4-14 shows the calculated test statistics and the

conclusions that can be drawn for these Pearson correlation tests. Table A-42, which can be found in Appendix A, defines each of the tests by listing the null and alternative hypotheses for each examined circumstance. Tables A-43, A-44, and A-45, which can be found in Appendix A, provide the necessary data and calculations that were used during computation of each of these Pearson correlation tests. The results indicated by these tests reveal that no statistically significant correlation can be suggested from the data between median grain size distribution of the sample and *Microcystis* cell density during the cyanobacterial bloom, *Microcystis* cell density after the cyanobacterial bloom, or the change in *Microcystis* cell density from before to after the cyanobacterial bloom.

Table 4-14: Test Statistics and Conclusions Drawn for Pearson Correlations Tests 4.5.17, 4.5.18, and 4.5.19.

Comparison of *Microcystis* Cell Densities in Sediment Samples and Median Grain Size of the Sample		
Pearson Test No. 4.5.17	Test Statistic	r = -.273
	Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the median grain size of the sediment sample.
Pearson Test No. 4.5.18	Test Statistic	r = -.581
	Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the median grain size of the sediment sample.
Pearson Test No. 4.5.19	Test Statistic	r = -.405
	Conclusion	There is NOT a statistically significant negative relationship between change the in *Microcystis* cell density in a sediment sample from during a cyanobacterial bloom to after a cyanobacterial bloom and the median grain size of the sediment sample.

4.6 Statistical Analysis of the Objectives of this Report

The objectives of this report 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. 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 site's distance from the Maumee River. Neither *Microcystis* cell density nor phosphorus concentrations in the sediment were found to be 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. Despite these facts, no conclusions can be drawn between the changes in the lake's *Microcystis* cell density level and the changes in the phosphorus levels. To further investigate any links in this area, which would indicate that the sediment is used as a source or sink of phosphorus for the bloom, the *Microcystis* cell density in the lake needs to be compared to the phosphorus concentration in the sediment. Figures C-1, C-2, C-3, C-4, C-5, and C-6, found in Appendix C, show the lake cell density, soluble phosphorus concentration in the sediment, total phosphorus concentration in the sediment, and iron

strip test phosphorus concentration in the sediment. Each figure illustrates these values for a single site over the three sampling dates.

No trend or relationship between the varying phosphorus concentrations and the varying cell densities become apparent after visual inspection of Figures 4-19 through 4-24. To determine if there is a statistically significant relationship that is not made obvious by these figures, a Pearson correlation test needs to be set up to compare the change in each of these concentrations to the change in the lake cell density. Figure D-10, which can be found in Appendix D, illustrates the correlation between the change in *Microcystis* density in the lake water and the change in soluble phosphorus concentration in the sediment at each sampling site between the first and second sampling sates. A positive correlation is indicated. Figure D-11, which can be found in Appendix D, illustrates the correlation between the change in *Microcystis* density in the lake water and the change in soluble phosphorus concentration in the sediment at each sampling site between the second and third sampling sates. A positive correlation is indicated. Figure D-12, which can be found in Appendix D, illustrates the correlation between the change in *Microcystis* density in the lake water and the change in total phosphorus concentration in the sediment at each sampling site between the first and second sampling sates. A negative correlation is indicated. Figure D-13, which can be found in Appendix D, illustrates the correlation between the change in *Microcystis* density in the lake water and the change in total phosphorus concentration in the sediment at each sampling site between the second and third sampling sates. A negative correlation is indicated. Figure D-14, which can be found in Appendix D, illustrates the correlation between the change in *Microcystis* density in the lake water and the change in iron strip test phosphorus concentration in the

sediment at each sampling site between the first and second sampling sates. A negative correlation is indicated. Figure D-15, which can be found in Appendix D, illustrates the correlation between the change in *Microcystis* density in the lake water and the change in iron strip test phosphorus concentration in the sediment at each sampling site between the second and third sampling sates. A positive correlation is indicated.

Figures D-10, D-11, D-12, D-13, D-14, and D-15 indicated no pattern that seems to repeat between the cell density in the lake and the different phosphorus concentrations. Some indicate positive correlations, while others indicate negative. It was not expected that all of the phosphorus concentrations in the soil would be correlated similarly to the lake cell density values. If the hypothesis that the phosphorus stored in the sediment is a source affecting the cyanobacterial blooms; however, it was be expected that a significant correlation be pulled from at least one of these figures.

For each of these relationships, a Pearson correlation test was used to determine whether these indicated negative or positive correlations are statistically significant. Table 4-15 shows the calculated test statistics and the conclusions that can be drawn for these Pearson correlation tests. Table A-46, which can be found in Appendix A, defines each of the tests by listing the null and alternative hypotheses for each examined circumstance. Tables A-47 through A-52, which can be found in Appendix A, provide the necessary data and calculations that were used during computation of each of these Pearson correlation tests.

None of the Pearson tests 4.6.1 through 4.6.6 could indicate that the relationships indicated on Figures 4-44, 4-45, 4-46, 4-47, 4-48, or 4-49 were significant. Only test

4.6.6 indicates a moderate correlation (indicated by a test statistic greater than .50). Additional testing may need to be completed in order to be more precise about the correlation between these relationships.

Table 4-15: Test Statistics and Conclusions Drawn for Pearson Correlations Tests 4.6.1, 4.6.2, 4.6.3, 4.6.4, 4.6.5, and 4.6.6.

Comparison of Changes of *Microcystis* Cell Densities in Sediment Samples and Phosphorus Concentrations in those Sediment Samples		
Pearson Test No. 4.6.1	Test Statistic	r = .394
	Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change soluble phosphorus concentration in the sediment from sampling period one to sampling period two.
Pearson Test No. 4.6.2	Test Statistic	r = .347
	Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change soluble phosphorus concentration in the sediment from sampling period two to sampling period three.
Pearson Test No. 4.6.3	Test Statistic	r = -.222
	Conclusion	There is NOT a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period one to two and the change total phosphorus concentration in the sediment from sampling period one to two
Pearson Test No. 4.6.4	Test Statistic	r = -.023
	Conclusion	There is NOT a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period two to three and the change total phosphorus concentration in the sediment from sampling period two to three.
Pearson Test No. 4.6.5	Test Statistic	r = -.282
	Conclusion	There is NOT a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period one to two and the change iron strip test phosphorus concentration in the sediment from sampling period one to two.
Pearson Test No. 4.6.6	Test Statistic	r = .550
	Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period two to three and the change iron strip test phosphorus concentration in the sediment from sampling period two to three.

Tests 4.6.1 through 4.6.6 show that the data collected cannot be used to support the hypothesis of this work regarding stored phosphorus. Although this data does not support the hypothesis, it is not indicative of the hypothesis is incorrect. Instead, it indicates that more work will need to be completed. For further investigation into the results of these tests, see the Discussion section of this report.

In addition to investigating whether sedient is the source of the phosphorus that controls the cyanobacterial blooms, this research investigated whether sediment is a source of the *Microcystis* cells that control the cyanobacterial blooms. To further investigate whether the sediment is used as a source or sink of *Microcystis* cells for the bloom, the *Microcystis* cell density in the lake needs to be compared to the *Microcystis* cell density in the sediment. Figure 4-35 shows the cell density values for both the lake and the sediment during the first sampling dates during June, 2009. The values for each sampling site are displayed. The cell density was below the detection limit for all of the lake samples and for four of the six sediment samples. This data indicates that at the time the samples were taken, there was not a *Microcystis* spp. bloom in the Western Lake Erie Basin.

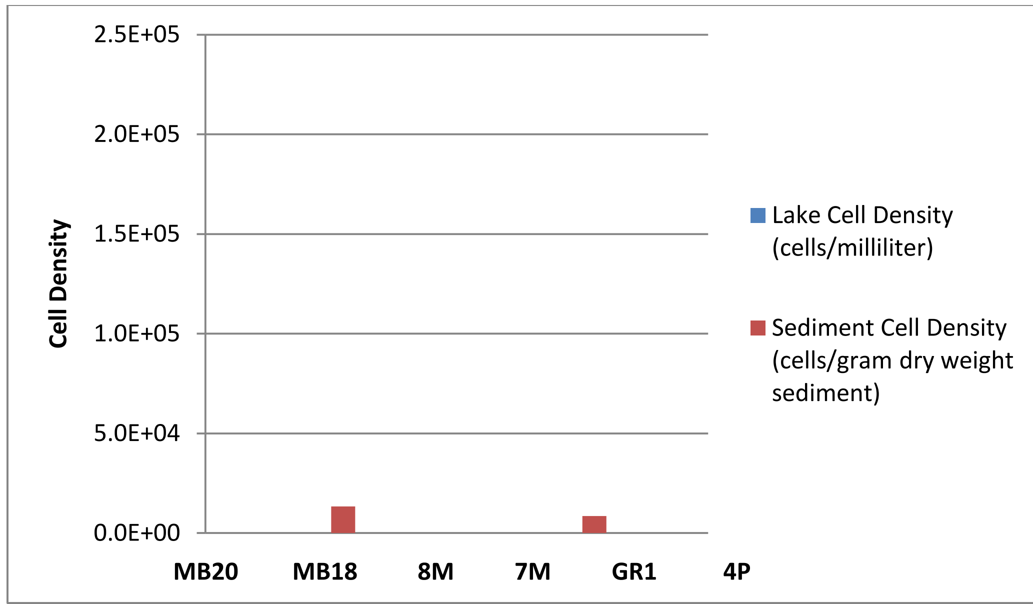

Figure 4-35: The comparative look at the Microcystis cell density in the lake water and the Microcystis cell density in the sediment at each site during the first sampling date. The vertical axis is holds values for cell density in both the sediment and lake water. The units on the vertical axis are cells per milliliter for the lake cell density values and cells per gram dry weight sediment for the sediment cell density values.

Figure 4-36 shows the cell density values for both the lake and the sediment during the second sampling dates during August, 2009. The values for each sampling site are displayed. The cell density was above the detection limit for five of the six lake samples and for all of the sediment samples. This increase shown in the values of the figure in comparison to the values in Figure 4-35 indicates that at the time the samples were taken, there was a *Microcystis* spp. bloom in the Western Lake Erie Basin.

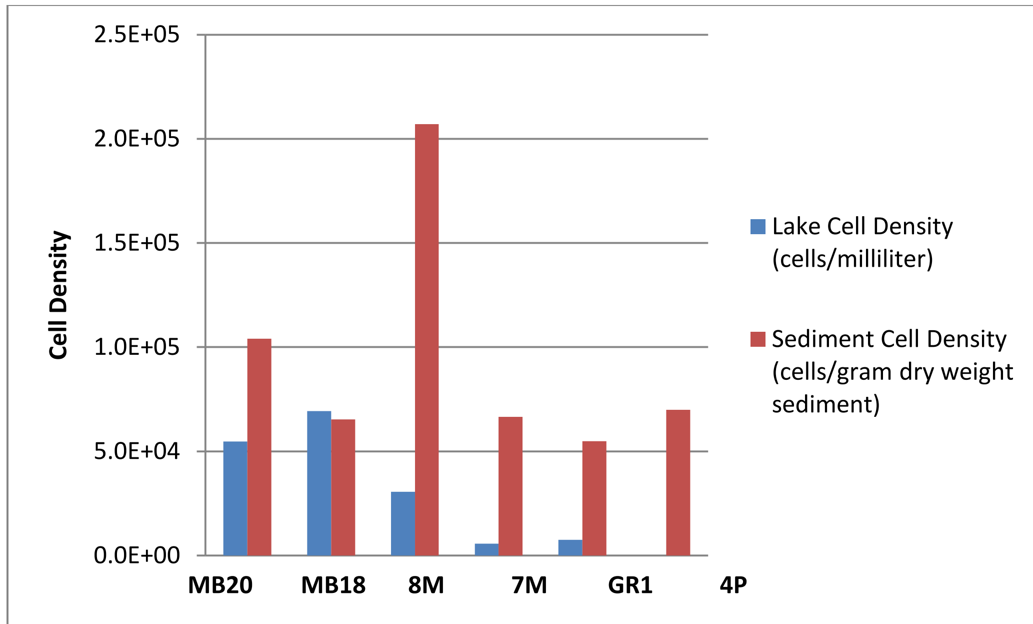

Figure 4-36: The comparative look at the Microcystis cell density in the lake water and the Microcystis cell density in the sediment at each site during the second sampling date. The vertical axis is holds values for cell density in both the sediment and lake water. The units on the vertical axis are cells per milliliter for the lake cell density values and cells per gram dry weight sediment for the sediment cell density values.

Figure 4-37 shows the cell density values for both the lake and the sediment during the third sampling dates during September, 2009. The values for each sampling site are displayed. The cell density was above the detection limit for three of the six lake samples and for all of the sediment samples. The decrease in the lake cell density values in this figure as compared to the values from Figure 4-36, indicate that the bloom has ended or has been reduced at sites MB20, MB18, 8M, and GR1. However, the lake cell density value at site 7M has increased. The sediment cell density values indicated in this figure are not drastically different from those indicated within Figure 4-36.

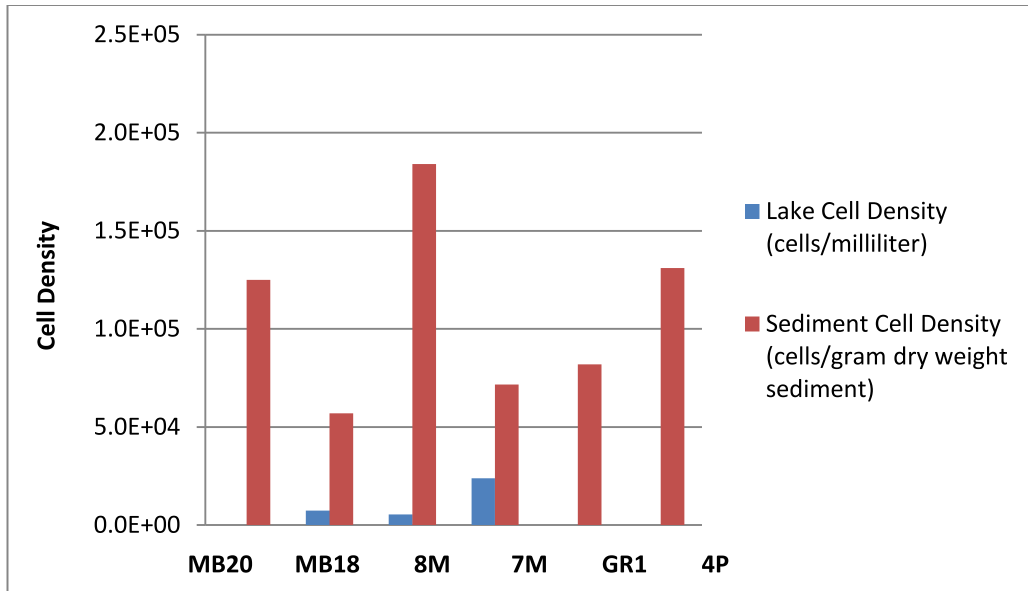

Figure 4-37: The comparative look at the Microcystis cell density in the lake water and the Microcystis cell density in the sediment at each site during the third sampling date. The vertical axis is holds values for cell density in both the sediment and lake water. The units on the vertical axis are cells per milliliter for the lake cell density values and cells per gram dry weight sediment for the sediment cell density values.

Chapter 5

Discussion

5.1 Sediments as a Source of Phosphorus Affecting Blooms

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). This concentration of total phosphorus in the sediment was considered to be extremely high. With values ranging from 35% to 65% of those numbers, the phosphorus found in the fine-grained sediment of the Western Basin of Lake in the summer of 2009 was at levels that would have been expected. However, these levels are not ideal. 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 number become bioavailable.

Pearson tests 4.4.8 and 4.4.9 yielded interesting results. These tests indicated that the negative correlation found between both total phosphorus and iron strip test phosphorus concentration in the sediment samples and the median grain size of those sediment samples are statistically significant. This would explain the exceptionally low total phosphorus and iron strip test phosphorus concentrations found within the coarse-grained sediment samples from site MB18. These results are supported by previous research that has found total phosphorus concentrations in the clays and silts of the sediment from a gulf in the southern Baltic Sea to be significantly higher than corresponding total phosphorus concentrations in sandy sediment (Lukawska-Matuszewska, 2008).

The total phosphorus concentrations in the sediment taken from a gulf in the southern Baltic Sea vary greatly from spring to summer at numerous sites (Lukawska-Matuszewska, 2008). There are slight variations in each of the three phosphorus concentrations at each site between the three test dates. On the other hand, the variations in concentrations between the three sampling dates were quite small, and the results of the dependent t-tests 4.4.1 through 4.4.6 did not indicate any statistically significant trends. Other previous research; however, has displayed similar results through the indication that variations in phosphorus concentrations are not always witnessed. A noticeable consistency of phosphorus in the sediment was noted on the lake in Sweden (Brunberg, 1992). Although small variations were measured between the sampling dates during this research, the consistency indicated by the prior research on the lake in Sweden is similar to the relative consistency measured at each site during this research.

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). Despite this expectation, the results of the Pearson tests 4.6.1 through 4.6.6 in Table 4-15 indicate that no correlation can be found between the change in the phosphorus levels from any test and the change in cell density in the lake.

Although phosphorus concentration levels in the sediment were not found to significantly decrease or increase throughout the summer, this is not an indication that phosphorus from the sediment in the Western Lake Erie basin is not a source of phosphorus to the water column. In fact, previous research has shown that the lack of variation of phosphorus in the sediment indicates that microbial activity of *Microcystis* cells undergoing reinvasion of the water column does affect the phosphorus release from the sediment (Brunberg, 1992). The findings from this research suggested that the lack of correlation found in the results of this research was not an indication that the sediment is not a source of phosphorus to the water column during a cyanobacterial bloom. Microbial activity may be affecting phosphorus in the water column by facilitating phosphorus release from the sediment, despite the lack of correlation found between sediment phosphorus concentrations and cell density.

Even though lack of significant increase or decrease in the phosphorus concentration of the sediment does not indicate lack of phosphorus release from the sediment, future research can further explore this topic by experimenting with water and sediment for the Western Lake Erie basin through an established procedure of observing lake water in

containers both with and without sediment (Xie, 2003). The effect of phosphorus from the sediment on the cyanobacterial bloom in the lake can then be better determined.

A few other hypotheses can be developed that should also be explored. One of these hypotheses is that phosphorus from another source may be supplementing the phosphorus released from the sediment. A second possibility is that another nutrient may be controlling the limitation of *Microcystis* in the lake. Another possibility is that the relatively high phosphorus levels in the sediment of the western Lake Erie Basin may be at a high enough levels that the maximum release rate of phosphorus from the sediment is not being limited. No testing done as part of this research investigated the levels of phosphorus actually being released from the sediment. Actual phosphorus levels in the sediment may be high enough to not limit the release of phosphorus. Further research will need to explore each of these hypotheses to determine the limiting nutrient for *Microcystis* bloom in the lake and how it can be controlled.

5.2 Sediments as a Source of *Microcystis* Cells Affecting Bloom Formations

Median grain size of the sediment does not have statistically significant effect upon the *Microcystis* cell density or the change in cell density between sampling dates two and three. This was indicated by the results of Pearson test 4.5.17, 4.5.18, and 4.5.19. However, previous research has indicated positive correlations between the median grain size and benthic biomass (Cahoon, 1999). The sites from the Western Lake Erie basin used during this research had other variables, most noticeably depth, which changed from

site to site. It may be possible that grain size did have an effect upon the cell density in the sediment. However, this research could uncover no significant correlation.

If the sediment served as a source for *Microcystis* cells for the bloom, there should be a relationship between cell density in the lake and cell density in the sediment. Figure 4-35 shows both the lake and sediment cell density values for each site during the first sampling date. This sampling date has been commonly referred to as the data from before the cyanobacterial bloom. This is evident within Figure 4-35 as there is no detection of *Microcystis* cells within any of the lake water samples. There were very few cells in the sediment large enough to be counted. Only sites MB18 and GR1 had any trace of detectable cells in the sediment.

Figure 4-36 shows both the lake and sediment cell density values for each site during the second sampling date. This sampling date has been commonly referred to as the data from during the cyanobacterial bloom; however, the cell density at site 7M is not at its highest value, which occurs during the third sampling date. As shown on this figure, 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).

At all sites with detectable limits, *Microcystis* cell densities in the lake during the second sampling date were statistically higher than during the first sampling date. In unison with

the appearance of cells in the lake, the sediment cell densities also are statistically higher than during the first sampling date. Since all six sediment samples and all five detectable lake samples statistically significantly increased in cell density between the first and second sampling dates, there is a correlation indicated. The increase in large *Microcystis* cells in the sediment is either proving to be a source for cells in the lake water or proving to be increased due to the bloom.

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. This bloom, or increase in the cell density within the lake water, will be able to be better determined by increasing the quantity of sampling dates.

Figure 4-37 shows both the lake and sediment cell density vales for each site during the third sampling date. The results from this sampling date have been commonly referred to as the data from after the cyanobacterial bloom. This description of the third sampling date is supported by the statistically significant decrease in the cell density of the lake water at all sites within the detection limit except site 7M. As discussed previously, the

cyanobacterial bloom is indicated to have occurred later than at the other sites, and the cell density at site 7M during this sampling date is considered a cell density during the bloom. None of the sites have undergone either a statistically significant decrease or a statistically significant increase in *Microcystis* cell density in the sediment samples.

Previous research on numerous eutrophic lakes has indicated that after a cyanobacterial bloom, *Microcystis* cells move vertically in the water column through the sedimentation process (Ihle, 2005). This is caused by a loss of buoyancy after energy has been created and stored. These cells are still enlarged and would have been detected for this report. Since cells are detected in the sediment after the bloom, it is expected that these are the cells that have undergone sedimentation from the bloom to the sediment. Future research can include more frequent (preferably daily) data as the bloom decreased in an effort to test the correlation between the decrease of the lake cell density following the peak value and the increase of the sediment cell density.

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). Additionally, the detection of cells in the lake water indicates that there may still be cells undergoing sedimentation. If it is correct that there were cells undergoing sedimentation during the third sampling date, the sediment cell density values at these sites should have continued to increase after this date. If samples were taken after the third sampling date, it is predicted that the cell density values would have been increased from the values measured during the second sampling date.

The cell density in the samples from site 7M increased from the second sampling date to the third sampling date. Since this indicates that the bloom at site 7M is persistent at the third sampling date, it is likely that additional cells enlarged due to increased buoyancy between the second and the third sampling dates. This is similar to the increase measured between the first and second sampling dates when the cells are reinvading the water column at all sites. This is consistent with other results.

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 a method that detected only enlarged cells in the sediment (not these smaller overwintering cells) was very helpful to determine trends of the *Microcystis* cell density in the sediment. Only cells undergoing sedimentation or reinvasion of the water column were detected and included in analysis. All of the analyzed trends of these

larger *Microcystis* cells indicate 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. This is as predicted due to the return of cells undergoing sedimentation that have been discussed in previous research (Ihle, 2005). As discussed, this indicates that cells from the sediment are crucial to cyanobacterial blooms and that those cells return to the sediment as the bloom concludes.

5.3 Conclusions and Future Work

Microcystin levels in the water were not measured as part of this research. However, the lake cell density values during the bloom at sites MB18 and MB20 were the highest of all six sites. MB18 and MB20 are the closest sites to shore and best represent one mile from shore, which is the common source of the drinking water supply of the many communities that use Lake Erie's water. Also, recreational activities are highly concentrated to areas nearest the shores. Previous research involving *Microcystis* cell density in the Western Lake Erie Basin discovered a weak correlation between cell density in the lake water and microcystin concentrations (Wang, 2008). This correlation was not found to be statistically significant, so microcystin levels cannot be derived from this relationship. However, the levels that would have been indicated agree with the results of other research that shows that the waters of the Western Lake Erie Basin far exceed the World Health Organization's limits for microcystin levels, one microgram per liter, in sources of drinking water.

Despite the expected trends that were seen, there is always an unknown factor involving cyanobacterial blooms. Environmental conditions can change both recruitment rates and bacteria growth at the water surface. This makes trends difficult to consistently quantify. Also, wind plays a large role in bacterial accumulation at the surface. The wind can cause accumulation of cells at the surface that are not descended from the benthic sediments directly below. In addition, lake data may not be attainable on windy days. This limits the amount of knowledge on the subject matter of wind contribution to the blooms on the Western Lake Erie Basin. These temperature and wind conditions should be reviewed further to determine their role in bloom formation.

In addition to supplemental work to confirm indicated trends that could not be statistically indicated by the data in this report, work must begin to be done to eliminate the *Microcystis* cell densities in the waters of Lake Erie. The harmful effects of the presence *Microcystis* cells and cyanobacterial blooms on water systems have been well documented. Lake Erie is a major source of recreation, industry, and drinking water for the region. Research needs to be done to determine methods which will best begin to limit cyanobacterial blooms on Lake Erie. As this research has indicated, the sediments are a source of *Microcystis* that significantly contributes to the cell density in the lake water during cyanobacterial blooms.

Historically, limiting of nutrient loading has been a major method of control that has been researched and implemented (Chen, 2009). At this point; however, research directed at limiting the recruitment of *Microcystis* from sediment in the Western Lake Erie Basin is necessary due to the findings of this research. After searching for current methods for control of *Microcystis* cell reinvasion, only ineffective methods were uncovered (Perakis,

1996). If future research can target effective methods for limiting reinvasion, future blooms may be limited or prevented in the Western Lake Erie basin.

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Appendix A

The Null and Alternative Hypotheses and the Detailed Results of All Statistical Tests

Table A-1: Null and alternative hypotheses for t-test Nos. 4.1.1 through 4.1.8

T-test No.	Hypotheses
4.1.1	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom at site MB20.
	Alternative Hypothesis: At site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.1.2	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom at site MB20.
	Alternative Hypothesis: At site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.
4.1.3	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom at site MB18.
	Alternative Hypothesis: At site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.1.4	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom at site MB18.
	Alternative Hypothesis: At site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.
4.1.5	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom at site 8M.
	Alternative Hypothesis: At site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.1.6	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom at site 8M.
	Alternative Hypothesis: At site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.
4.1.7	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom at site 7M.
	Alternative Hypothesis: At site 7M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.1.8	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom at site 7M.
	Alternative Hypothesis: At site 7M, *Microcystis* cell density after the bloom is statistically greater than *Microcystis* cell density during the bloom.

Table A-2: Results of t-test No. 4.1.1

Results of T-test No. 4.1.1		
$n_1 = 6$	$n_2 = 2$	Degrees of freedom, df = 6
Critical Value, CV = 1.943	$S_{\bar{x}_1 - \bar{x}_2} = 2.09 \times 10^4$	Computed Test Statistic, t = 2.616
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-3: Results of t-test No. 4.1.2

Results of T-test No. 4.1.2		
$n_1 = 6$	$n_2 = 4$	Degrees of freedom, df = 8
Critical Value, CV = 1.860	$S_{\bar{x}_1 - \bar{x}_2} = 1.43 \times 10^4$	Computed Test Statistic, t = 3.821
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.	

Table A-4: Results of t-test No. 4.1.3

Results of T-test No. 4.1.3		
$n_1 = 4$	$n_2 = 2$	Degrees of freedom, df = 4
Critical Value, CV = 2.132	$S_{\bar{x}_1 - \bar{x}_2} = 1.52 \times 10^4$	Computed Test Statistic, t = 4.552
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-5: Results of t-test No. 4.1.4

Results of T-test No. 4.1.4		
$n_1 = 4$	$n_2 = 6$	Degrees of freedom, df = 8
Critical Value, CV= 1.860	$S_{\bar{x}_1 - \bar{x}_2} = 8.14 \times 10^3$	Computed Test Statistic, t = 7.601
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.	

Table A-6: Results of t-test No. 4.1.5

Results of T-test No. 4.1.5		
$n_1 = 4$	$n_2 = 2$	Degrees of freedom, df = 4
Critical Value, CV = 2.132	$S_{\bar{x}_1 - \bar{x}_2} = 7.73 \times 10^3$	Computed Test Statistic, t = 3.961
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-7: Results of t-test No. 4.1.6

Results of T-test No. 4.1.6		
$n_1 = 4$	$n_2 = 3$	Degrees of freedom, df = 5
Critical Value, CV = 2.015	$S_{\bar{x}_1 - \bar{x}_2} = 6.41 \times 10^3$	Computed Test Statistic, t = 3.923
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.	

Table A-8: Results of t-test No. 4.1.7

Results of T-test No. 4.1.7		
$n_1 = 2$	$n_2 = 2$	Degrees of freedom, df = 2
Critical Value, CV = 2.920	$S_{\bar{x}_1 - \bar{x}_2} = 4.32 \times 10^2$	Computed Test Statistic, t = 13.378
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site 7M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-9: Results of t-test No. 4.1.8

Results of T-test No. 4.1.8		
$n_1 = 2$	$n_2 = 6$	Degrees of freedom, df = 6
Critical Value, CV = 1.934	$S_{\bar{x}_1 - \bar{x}_2} = 8.50 \times 10^3$	Computed Test Statistic, t = 2.132
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	At site 7M, *Microcystis* cell density after the bloom is statistically greater than *Microcystis* cell density during the bloom.	

Table A-10: Null and alternative hypotheses for Pearson test Nos. 4.1.9 and 4.1.10

Pearson Test No.	**Hypotheses**
4.1.9	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
4.1.10	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.

Table A-11: Results of Pearson test No. 4.1.9

Results of Pearson Test No. 4.1.9		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = -.842
Results of Test	$\|r\|$ > CV. Therefore, the null hypothesis is rejected.	
Conclusion	There is a statistically significant strong negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.	

Table A-12: Results of Pearson test No. 4.1.10

Results of Pearson Test No. 4.1.10		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = -.932
Results of Test	$\|r\|$ > CV. Therefore, the null hypothesis is rejected.	
Conclusion	There is a statistically significant very strong negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.	

Table A-13: Null and alternative hypotheses for t-test Nos. 4.4.1 through 4.4.6

T-test No.	Hypotheses
4.4.1	Null Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, there is no statistically significant difference between soluble phosphorus (SRP) concentrations in the sediment before and during the bloom.
	Alternative Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, soluble phosphorus (SRP) concentrations in the sediment before the bloom are statistically greater than soluble phosphorus (SRP) concentrations in the sediment during the bloom.
4.4.2	Null Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, there is no statistically significant difference between soluble phosphorus (SRP) concentrations in the sediment during and after the bloom.
	Alternative Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, soluble phosphorus (SRP) concentrations in the sediment during the bloom are statistically greater than soluble phosphorus (SRP) concentrations in the sediment after the bloom.
4.4.3	Null Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, there is no statistically significant difference between total phosphorus (TP) concentrations in the sediment before and during the bloom.
	Alternative Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, total phosphorus (TP) concentrations in the sediment before the bloom are statistically greater than total phosphorus (TP) concentrations in the sediment during the bloom.
4.4.4	Null Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, there is no statistically significant difference between total phosphorus (TP) concentrations in the sediment during and after the bloom.
	Alternative Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, total phosphorus (TP) concentrations in the sediment after the bloom are statistically greater than total phosphorus (TP) concentrations in the sediment during the bloom.
4.4.5	Null Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, there is no statistically significant difference between iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment before and during the bloom.
	Alternative Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment before the bloom are statistically greater than iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment during the bloom.
4.4.6	Null Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, there is no statistically significant difference between iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment during and after the bloom.
	Alternative Hypothesis: Throughout the sediment samples from sites that experienced a cyanobacterial bloom, iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment after the bloom are statistically greater than iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment during the bloom.

Table A-14: Results of t-test No. 4.4.1

Results of T-test No. 4.4.1		
$n_1 = 5$	Degrees of freedom, df = 4	$s_{\bar{D}}$ = .00752
Critical Value, CV = 1.860	Computed Test Statistic, t = 0.465	
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, soluble phosphorus (SRP) concentrations in the sediment before the bloom is NOT statistically greater than soluble phosphorus (SRP) concentrations in the sediment during the bloom.	

Table A-15: Results of t-test No. 4.4.2

Results of T-test No. 4.4.2		
$n_1 = 5$	Degrees of freedom, df = 4	$s_{\bar{D}}$ = .00559
Critical Value, CV = 1.860	Computed Test Statistic, t = 0.394	
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, soluble phosphorus (SRP) concentrations in the sediment during the bloom are NOT statistically greater than soluble phosphorus (SRP) concentrations in the sediment after the bloom.	

Table A-16: Results of t-test No. 4.4.3

Results of T-test No. 4.4.3		
$n_1 = 5$	Degrees of freedom, df = 4	$s_{\bar{D}}$ = .0913
Critical Value, CV = 1.860	Computed Test Statistic, t = 0.793	
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, total phosphorus (TP) concentrations in the sediment before the bloom are NOT statistically greater than total phosphorus (TP) concentrations in the sediment during the bloom.	

Table A-17: Results of t-test No. 4.4.4

Results of T-test No. 4.4.4		
$n_1 = 5$	Degrees of freedom, df = 4	$s_{\bar{D}}$ = .0423
Critical Value, CV = 1.860	Computed Test Statistic, t = 1.591	
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, total phosphorus (TP) concentrations in the sediment after the bloom are NOT statistically greater than total phosphorus (TP) concentrations in the sediment during the bloom.	

Table A-18: Results of t-test No. 4.4.5

Results of T-test No. 4.4.5		
$n_1 = 5$	Degrees of freedom, df = 4	$s_{\bar{D}}$ = .0230
Critical Value, CV = 1.860	Computed Test Statistic, t = 0.465	
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment before the bloom are NOT statistically greater than iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment during the bloom.	

Table A-19: Results of t-test No. 4.4.6

Results of T-test No. 4.4.6		
$n_1 = 5$	Degrees of freedom, df = 4	$s_{\bar{D}}$ = .0130
Critical Value, CV = 1.860	Computed Test Statistic, t = 0.910	
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	Throughout the sediment samples from sites that experienced a cyanobacterial bloom, iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment after the bloom are NOT statistically greater than iron strip test phosphorus ($FeCl_2P$) concentrations in the sediment during the bloom.	

Table A-20: Null and alternative hypotheses for Pearson test Nos. 4.4.7, 4.4.8 and 4.4.9

Pearson Test No.	Hypotheses
4.4.7	Null Hypothesis: There is no statistically significant relationship between soluble phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.
	Alternative Hypothesis: There is a statistically significant negative relationship between soluble phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.
4.4.8	Null Hypothesis: There is no statistically significant relationship between total phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.
	Alternative Hypothesis: There is a statistically significant negative relationship between total phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.
4.4.9	Null Hypothesis: There is no statistically significant relationship between iron strip test phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.
	Alternative Hypothesis: There is a statistically significant negative relationship between iron strip test phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.

Table A-21: Results of Pearson test No. 4.4.7

Results of Pearson Test No. 4.4.7	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.303
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant negative relationship between soluble phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.

Table A-22: Results of Pearson test No. 4.4.8

Results of Pearson Test No. 4.4.8	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.929
Results of Test	$\lvert r \rvert$ > CV. Therefore, the null hypothesis is rejected.
Conclusion	There is a statistically significant very strong negative relationship between soluble phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.

Table A-23: Results of Pearson test No. 4.4.9

Results of Pearson Test No. 4.4.9	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.810
Results of Test	$\lvert r \rvert$ > CV. Therefore, the null hypothesis is rejected.
Conclusion	There is a statistically significant strong negative relationship between soluble phosphorus concentration from a sediment sample taken during a bloom and the median grain size of that sediment sample.

Table A-24: Null and alternative hypotheses for t-test Nos. 4.5.1 through 4.5.10

T-test No.	Hypotheses
4.5.1	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom in the sediment from site MB20.
	Alternative Hypothesis: In the sediment from site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.5.2	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom in the sediment from site MB20.
	Alternative Hypothesis: In the sediment from site MB20, *Microcystis* cell density after the bloom is statistically greater than *Microcystis* cell density during the bloom.
4.5.3	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom in the sediment from site MB18.
	Alternative Hypothesis: In the sediment from site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.5.4	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom in the sediment from site MB18.
	Alternative Hypothesis: In the sediment from site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.
4.5.5	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom in the sediment from site 8M.
	Alternative Hypothesis: In the sediment from site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.5.6	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom in the sediment from site 8M.
	Alternative Hypothesis: In the sediment from site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density after the bloom.
4.5.7	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom in the sediment from site 7M.
	Alternative Hypothesis: In the sediment from site 7M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.5.8	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom in the sediment from site 7M.
	Alternative Hypothesis: In the sediment from site 7M, *Microcystis* cell density after the bloom is statistically greater than *Microcystis* cell density during the bloom.
4.5.9	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density before and during the bloom in the sediment from site GR1.
	Alternative Hypothesis: In the sediment from site GR1, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.
4.5.10	Null Hypothesis: There is no statistically significant difference between *Microcystis* cell density during and after the bloom in the sediment from site GR1.
	Alternative Hypothesis: In the sediment from site GR1, *Microcystis* cell density after the bloom is statistically greater than *Microcystis* cell density during the bloom.

Table A-25: Results of t-test No. 4.5.1

Results of T-test No. 4.5.1		
$n_1 = 5$	$n_2 = 2$	Degrees of freedom, df = 5
Critical Value, CV = 2.015	$S_{\bar{x}_1 - \bar{x}_2} = 3.30x10^4$	Computed Test Statistic, t = 3.151
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	In the sediment from site MB20, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-26: Results of t-test No. 4.5.2

Results of T-test No. 4.5.2		
$n_1 = 2$	$n_2 = 5$	Degrees of freedom, df = 5
Critical Value, CV = 2.015	$S_{\bar{x}_1 - \bar{x}_2} = 3.30x10^4$	Computed Test Statistic, t = 0.636
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	In the sediment from site MB20, *Microcystis* cell density after the bloom is NOT statistically greater than *Microcystis* cell density during the bloom.	

Table A-27: Results of t-test No. 4.5.3

Results of T-test No. 4.5.3		
$n_1 = 5$	$n_2 = 2$	Degrees of freedom, df = 5
Critical Value, CV = 2.015	$S_{\bar{x}_1 - \bar{x}_2} = 2.19x10^4$	Computed Test Statistic, t = 2.377
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	In the sediment from site MB18, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-28: Results of t-test No. 4.5.4

Results of T-test No. 4.5.4		
$n_1 = 5$	$n_2 = 2$	Degrees of freedom, df = 5
Critical Value, CV = 2.015	$S_{\bar{x}_1 - \bar{x}_2} = 2.19x10^4$	Computed Test Statistic, t = 0.388
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	In the sediment from site MB18, *Microcystis* cell density during the bloom is NOT statistically greater than *Microcystis* cell density after the bloom.	

Table A-29: Results of t-test No. 4.5.5

Results of T-test No. 4.5.5		
$n_1 = 2$	$n_2 = 2$	Degrees of freedom, df = 2
Critical Value, CV = 2.920	$S_{\bar{x}_1 - \bar{x}_2} = 1.89 \times 10^4$	Computed Test Statistic, t = 10.964
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	In the sediment from site 8M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-30: Results of t-test No. 4.5.6

Results of T-test No. 4.5.6		
$n_1 = 2$	$n_2 = 4$	Degrees of freedom, df = 4
Critical Value, CV = 2.132	$S_{\bar{x}_1 - \bar{x}_2} = 5.95 \times 10^4$	Computed Test Statistic, t = 0.387
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	In the sediment from site 8M, *Microcystis* cell density during the bloom is NOT statistically greater than *Microcystis* cell density after the bloom.	

Table A-31: Results of t-test No. 4.5.7

Results of T-test No. 4.5.7		
$n_1 = 4$	$n_2 = 2$	Degrees of freedom, df = 4
Critical Value, CV = 2.132	$S_{\bar{x}_1 - \bar{x}_2} = 1.66 \times 10^4$	Computed Test Statistic, t = 4.018
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	In the sediment from site 7M, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-32: Results of t-test No. 4.5.8

Results of T-test No. 4.5.8		
$n_1 = 3$	$n_2 = 4$	Degrees of freedom, df = 5
Critical Value, CV = 2.015	$S_{\bar{x}_1 - \bar{x}_2} = 1.44x10^4$	Computed Test Statistic, t = 0.355
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	In the sediment from site 7M, *Microcystis* cell density after the bloom is NOT statistically greater than *Microcystis* cell density during the bloom.	

Table A-33: Results of t-test No. 4.5.9

Results of T-test No. 4.5.9		
$n_1 = 2$	$n_2 = 2$	Degrees of freedom, df = 2
Critical Value, CV = 2.920	$S_{\bar{x}_1 - \bar{x}_2} = 7.84x10^3$	Computed Test Statistic, t = 5.904
Results of Test	t > CV. Therefore, the null hypothesis is rejected.	
Conclusion	In the sediment from site GR1, *Microcystis* cell density during the bloom is statistically greater than *Microcystis* cell density before the bloom.	

Table A-34: Results of t-test No. 4.5.10

Results of T-test No. 4.5.10		
$n_1 = 2$	$n_2 = 2$	Degrees of freedom, df = 2
Critical Value, CV = 2.920	$S_{\bar{x}_1 - \bar{x}_2} = 1.83x10^4$	Computed Test Statistic, t = 1.483
Results of Test	t < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	In the sediment from site GR1, *Microcystis* cell density after the bloom is NOT statistically greater than *Microcystis* cell density during the bloom.	

Table A-35: Null and alternative hypotheses for Pearson test Nos. 4.5.11, 4.5.12, 4.5.13, 4.5.14, 4.5.15, and 4.5.16

Pearson Test No.	Hypotheses
4.5.11	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
4.5.12	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant positive relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
4.5.13	Null Hypothesis: There is no statistically significant relationship between change the in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant negative relationship between change the in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.
4.5.14	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
4.5.15	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant positive relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
4.5.16	Null Hypothesis: There is no statistically significant relationship between change the in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.
	Alternative Hypothesis: There is a statistically significant negative relationship between change the in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.

Table A-36: Results of Pearson test No. 4.5.11

Results of Pearson Test No. 4.5.11		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = -.287
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.	

Table A-37: Results of Pearson test No. 4.5.12

Results of Pearson Test No. 4.5.12		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = .051
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant positive relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.	

Table A-38: Results of Pearson test No. 4.5.13

Results of Pearson Test No. 4.5.13		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = .639
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant positive relationship between change the in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the distance from the mouth of the Maumee River at the site that the sample was taken from.	

Table A-39: Results of Pearson test No. 4.5.14

Results of Pearson Test No. 4.5.14		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = -.148
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a lake water sample taken during the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.	

Table A-40: Results of Pearson test No. 4.5.15

Results of Pearson Test No. 4.5.15		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = .196
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant positive relationship between *Microcystis* cell density in a lake water sample taken after the cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.	

Table A-41: Results of Pearson test No. 4.5.16

Results of Pearson Test No. 4.5.16		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = .602
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant positive relationship between change the in *Microcystis* cell density in a sediment sample from during the cyanobacterial bloom to after a cyanobacterial bloom and the depth of the Lake Erie at the site that the sample was taken from.	

Table A-42: Null and alternative hypotheses for Pearson test Nos. 4.5.17, 4.5.18 and 4.5.19

Pearson Test No.	**Hypotheses**
4.5.17	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and median grain size of the sediment sample.
	Alternative Hypothesis: There is a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the median grain size of the sediment sample.
4.5.18	Null Hypothesis: There is no statistically significant relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the median grain size of the sediment sample.
	Alternative Hypothesis: There is a statistically significant positive relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the median grain size of the sediment sample.
4.5.19	Null Hypothesis: There is no statistically significant relationship between change the in *Microcystis* cell density in a sediment sample from during a cyanobacterial bloom to after a cyanobacterial bloom and the median grain size of the sediment sample.
	Alternative Hypothesis: There is a statistically significant negative relationship between change the in *Microcystis* cell density in a sediment sample from during a cyanobacterial bloom to after a cyanobacterial bloom and the median grain size of the sediment sample.

Table A-43: Results of Pearson test No. 4.5.17

Results of Pearson test No. 4.5.17	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.273
Results of Test	$|r|$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken during the cyanobacterial bloom and the median grain size of the sediment sample.

Table A-44: Results of Pearson test No. 4.5.18

Results of Pearson test No. 4.5.18	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.581
Results of Test	$|r|$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant negative relationship between *Microcystis* cell density in a sediment sample taken after the cyanobacterial bloom and the median grain size of the sediment sample.

Table A-45: Results of Pearson test No. 4.5.19

Results of Pearson test No. 4.5.19	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.405
Results of Test	$|r|$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant negative relationship between change the in *Microcystis* cell density in a sediment sample from during a cyanobacterial bloom to after a cyanobacterial bloom and the median grain size of the sediment sample.

Table A-46: Null and alternative hypotheses for Pearson test Nos. 4.6.1, 4.6.2, 4.6.3, 4.6.4, 4.6.5 and 4.6.6

Pearson Test No.	Hypotheses
4.6.1	Null Hypothesis: There is no statistical significant relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change soluble phosphorus concentration in the sediment from sampling period one to sampling period two.
	Alternative Hypothesis: There is a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change soluble phosphorus concentration in the sediment from sampling period one to sampling period two.
4.6.2	Null Hypothesis: There is no statistical significant relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change soluble phosphorus concentration in the sediment from sampling period two to sampling period three.
	Alternative Hypothesis: There is a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change soluble phosphorus concentration in the sediment from sampling period two to sampling period three.
4.6.3	Null Hypothesis: There is no statistical significant relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change total phosphorus concentration in the sediment from sampling period one to sampling period two.
	Alternative Hypothesis: There is a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change total phosphorus concentration in the sediment from sampling period one to sampling period two.
4.6.4	Null Hypothesis: There is no statistical significant relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change total phosphorus concentration in the sediment from sampling period two to sampling period three.
	Alternative Hypothesis: There is a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change total phosphorus concentration in the sediment from sampling period two to sampling period three.
4.6.5	Null Hypothesis: There is no statistical significant relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change iron strip test phosphorus concentration in the sediment from sampling period one to sampling period two.
	Alternative Hypothesis: There is a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change iron strip test phosphorus concentration in the sediment from sampling period one to sampling period two.
4.6.6	Null Hypothesis: There is no statistical significant relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change iron strip test phosphorus concentration in the sediment from sampling period two to sampling period three.
	Alternative Hypothesis: There is a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change iron strip test phosphorus concentration in the sediment from sampling period two to sampling period three.

Table A-47: Results of Pearson test No. 4.6.1

Results of Pearson test No. 4.6.1	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = .394
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change soluble phosphorus concentration in the sediment from sampling period one to sampling period two.

Table A-48: Results of Pearson test No. 4.6.2

Results of Pearson test No. 4.6.2	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = .347
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change soluble phosphorus concentration in the sediment from sampling period two to sampling period three.

Table A-49: Results of Pearson test No. 4.6.3

Results of Pearson test No. 4.6.3	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.222
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change total phosphorus concentration in the sediment from sampling period one to sampling period two.

Table A-50: Results of Pearson test No. 4.6.4

Results of Pearson test No. 4.6.4	
n = 6	Degrees of Freedom, df = 4
Critical Value, CV = .729	Test Statistic, r = -.023
Results of Test	$\lvert r \rvert$ < CV. Therefore, the null hypothesis cannot be rejected.
Conclusion	There is NOT a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change total phosphorus concentration in the sediment from sampling period two to sampling period three.

Table A-51: Results of Pearson test No. 4.6.5

Results of Pearson test No. 4.6.5		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = -.282
Results of Test	$\|r\|$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant negative relationship between the change in *Microcystis* cell density in the lake from sampling period one to sampling period two and the change iron strip test phosphorus concentration in the sediment from sampling period one to sampling period two.	

Table A-52: Results of Pearson test No. 4.6.6

Results of Pearson test No. 4.6.6		
n = 6		Degrees of Freedom, df = 4
Critical Value, CV = .729		Test Statistic, r = .550
Results of Test	$\|r\|$ < CV. Therefore, the null hypothesis cannot be rejected.	
Conclusion	There is NOT a statistically significant positive relationship between the change in *Microcystis* cell density in the lake from sampling period two to sampling period three and the change iron strip test phosphorus concentration in the sediment from sampling period two to sampling period three.	

Appendix B

Detailed Results of the Hydrometer Analyses

Table B-1: Results of the grain size distribution test run on a composite of samples from site 8M.

Hydrometer Analysis				February 1, 2010		
Sediment Description		Sample from Site 8M (visually observed to be coarser-grained)				
Oven Dry Soil Mass, W_S		53.47 grams				
Zero Correction, C_Z		2				
Elapsed Time (min.)	Hydrometer Reading, R	Temperature (°C)	Temperature Correction, C_T	D (mm)	Corrected Reading, R_C	Percent Finer, P
1	55	22	+.40	0.0359	52.6	98.4%
5	52	22	+.40	0.0166	49.6	92.8%
10	40	22	+.40	0.0131	37.6	70.3%
27	38	22	+.40	0.0081	35.6	66.6%
52	35	22	+.40	0.0060	32.6	61.0%
797	25	22	+.40	0.0016	22.6	42.3%
1441	23	22	+.40	0.0012	20.6	38.5%

Table B-2: Results of the grain size distribution test run on a composite of samples from site 7M.

Hydrometer Analysis				February 1, 2010		
Sediment Description			Sample from Site 7M			
Oven Dry Soil Mass, W_S			52.42 grams			
Zero Correction, C_Z			2			
Elapsed Time (min.)	Hydrometer Reading, R	Temperature (°C)	Temperature Correction, C_T	D (mm)	Corrected Reading, R_C	Percent Finer, P
1	42	22	+.40	0.0408	39.6	75.5%
2	40	22	+.40	0.0294	37.6	71.7%
5	37	22	+.40	0.0190	34.6	66.0%
38	26	22	+.40	0.0075	23.6	45.0%
783	20	22	+.40	0.0017	17.6	33.6%
1426	19	22	+.40	0.0013	16.6	31.7%

Table B-3: Results of the grain size distribution test run on a composite of samples from site MB18.

Hydrometer Analysis				February 1, 2010		
Sediment Description			Sample from Site MB18 (visually observed to be sandy)			
Oven Dry Soil Mass, W_S			51.44 grams			
Zero Correction, C_Z			2			
Elapsed Time (min.)	Hydrometer Reading, R	Temperature (°C)	Temperature Correction, C_T	D (mm)	Corrected Reading, R_C	Percent Finer, P
1	12	22	+.40	0.0504	9.6	18.7%
2	12	22	+.40	0.0356	9.6	18.7%
10	11	22	+.40	0.0160	8.6	16.7%
16	11	22	+.40	0.0127	8.6	16.7%
39	11	22	+.40	0.0081	8.6	16.7%
67	11	22	+.40	0.0062	8.6	16.7%
811	10	22	+.40	0.0018	7.6	14.8%
1455	10	22	+.40	0.0013	7.6	14.8%

Table B-4: Results of the grain size distribution test run on a composite of samples from site MB20.

Hydrometer Analysis				February 1, 2010		
Sediment Description			Sample from Site MB20			
Oven Dry Soil Mass, W_S			51.47 grams			
Zero Correction, C_Z			2			
Elapsed Time (min.)	Hydrometer Reading, R	Temperature (°C)	Temperature Correction, C_T	D (mm)	Corrected Reading, R_C	Percent Finer, P
1	46	22	+.40	0.0394	43.6	84.7%
2	42	22	+.40	0.0289	39.6	76.9%
11	33	22	+.40	0.0132	30.6	59.5%
41	31	22	+.40	0.0070	28.6	55.6%
786	23	22	+.40	0.0017	20.6	40.0%
1430	22	22	+.40	0.0013	19.6	38.1%

Table B-5: Results of the grain size distribution test run on a composite of samples from site GR1.

Hydrometer Analysis					February 1, 2010	
Sediment Description				Sample from Site GR1		
Oven Dry Soil Mass, W_S				52.69 grams		
Zero Correction, C_Z				2		
Elapsed Time (min.)	Hydrometer Reading, R	Temperature (°C)	Temperature Correction, C_T	D (mm)	Corrected Reading, R_C	Percent Finer, P
1	42	22	+.40	0.0408	39.6	75.2%
4	40	22	+.40	0.0208	37.6	71.4%
9	38	22	+.40	0.0141	35.6	67.6%
13	35	22	+.40	0.0120	32.6	61.9%
33	32	22	+.40	0.0077	29.6	56.2%
58	29	22	+.40	0.0059	26.6	50.5%
804	20	22	+.40	0.0017	17.6	33.4%
1447	19	22	+.40	0.0013	16.6	31.5%

Table B-6: Results of the grain size distribution test run on a composite of samples from site 4P.

Hydrometer Analysis				February 1, 2010		
Sediment Description				Sample from Site 4P (visually observed finer-grained)		
Oven Dry Soil Mass, W_S				51.75 grams		
Zero Correction, C_Z				2		
Elapsed Time (min.)	Hydrometer Reading, R	Temperature (°C)	Temperature Correction, C_T	D (mm)	Corrected Reading, R_C	Percent Finer, P
1	54	22	+.40	0.0363	51.6	99.7%
2	54	22	+.40	0.0257	51.6	99.7%
10	54	22	+.40	0.0115	51.6	99.7%
51	43	22	+.40	0.0057	40.6	78.5%
73	40	22	+.40	0.0049	37.6	72.7%
817	34	22	+.40	0.0015	31.6	61.1%
1461	33	22	+.40	0.0011	30.6	59.1%

Appendix C

Figures Representing Change in Phosphorus Concentration and Change in Lake Cell Density over the Three Sampling Dates

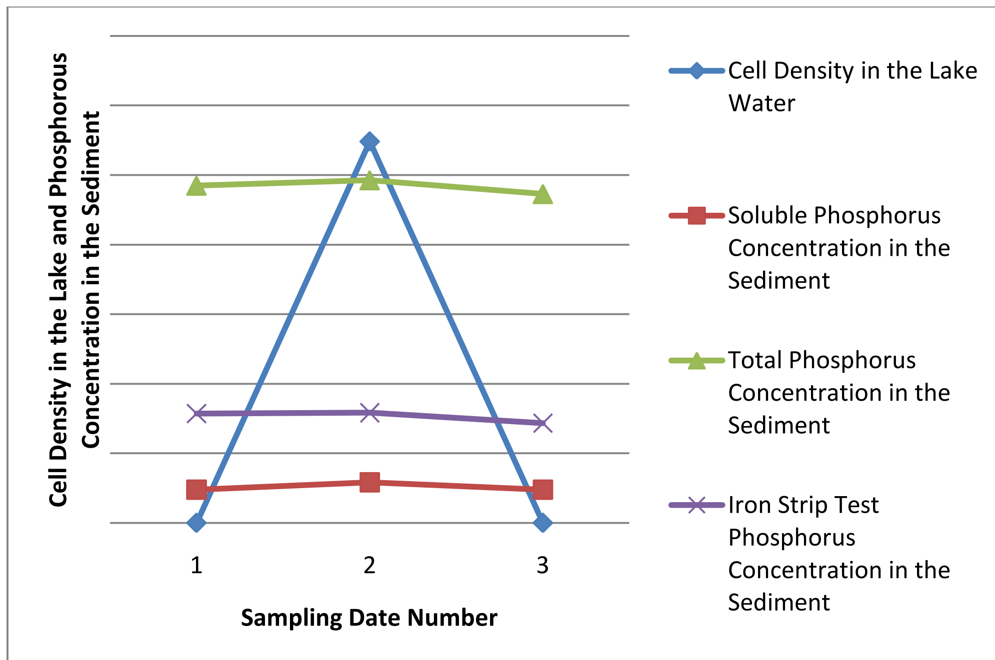

Figure C-1: The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site MB20 over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values.

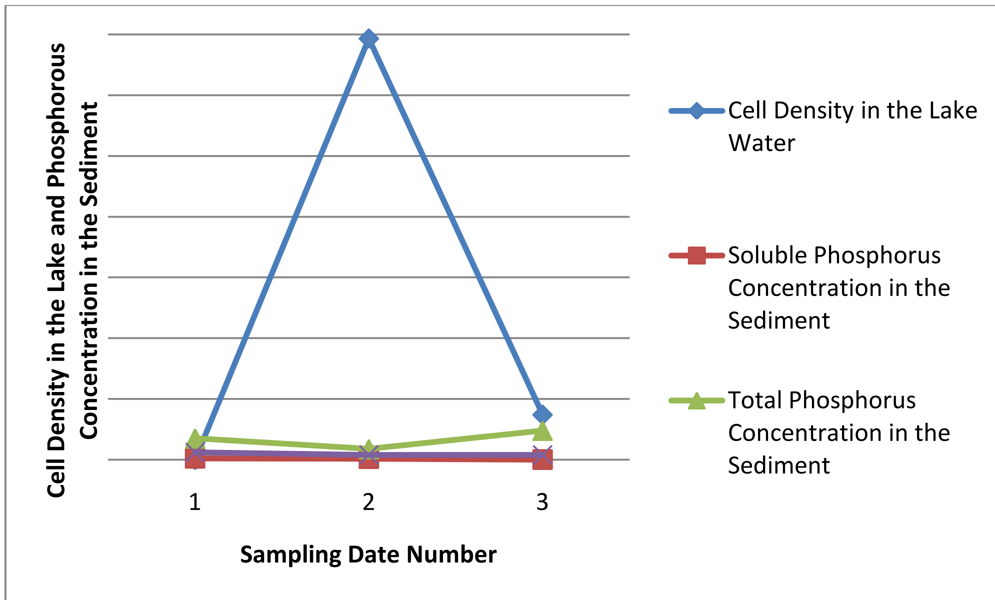

Figure C-2: The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site MB18 over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values.

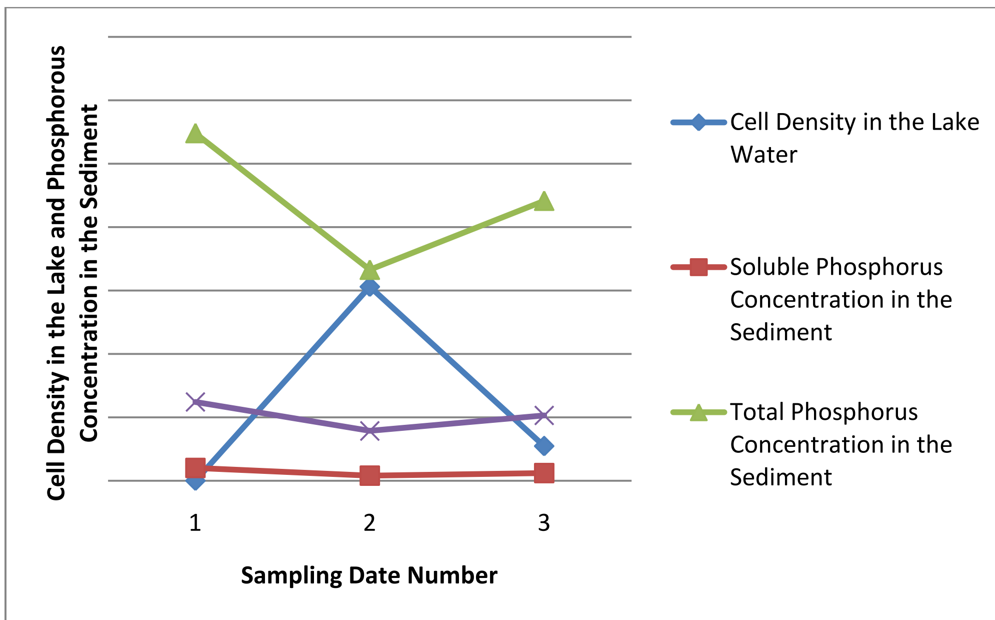

Figure C-3: The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site 8M over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values.

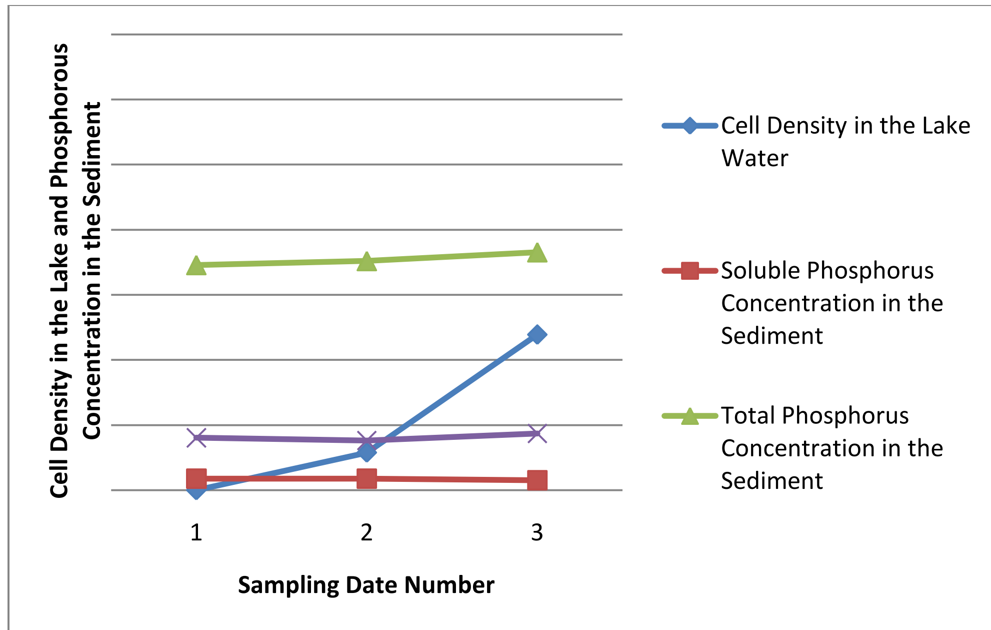

Figure C-4: The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site 7M over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values.

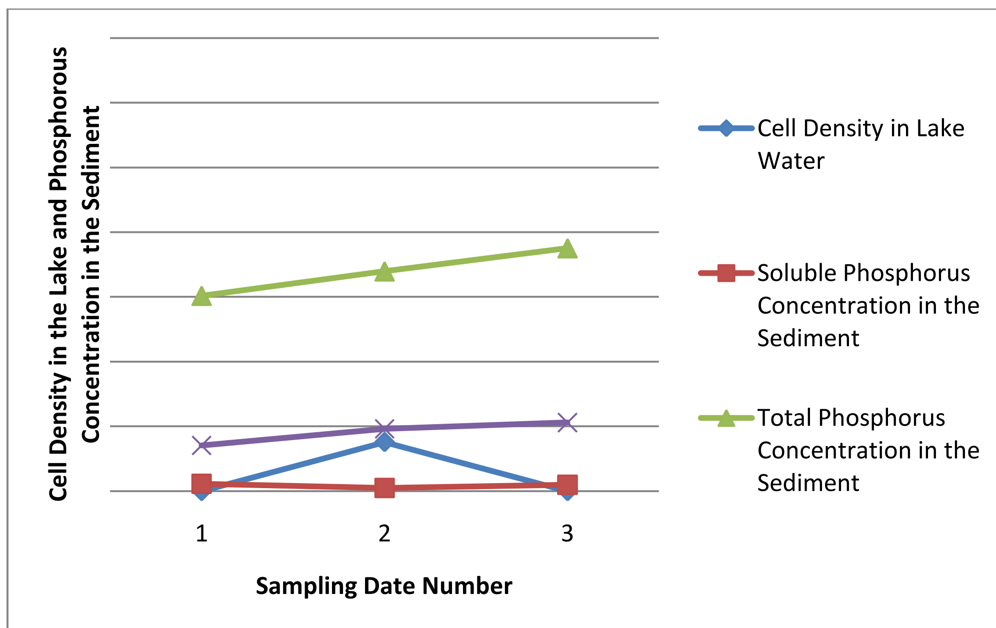

Figure C-5: The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site GR1 over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values.

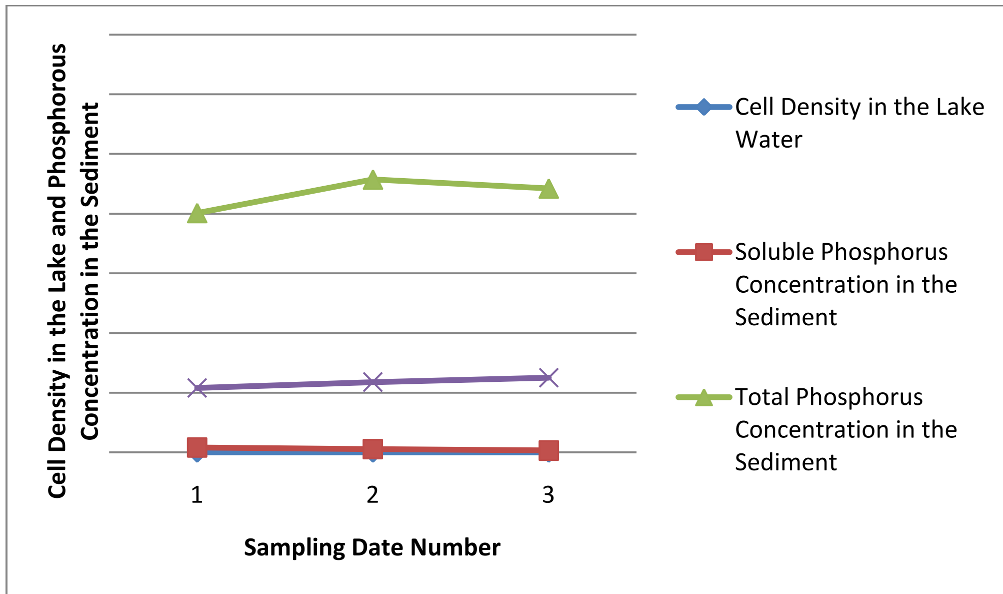

Figure C-6: The values for cell density in the lake, the soluble phosphorus concentration in the sediment, the total phosphorus concentration in the sediment, and the iron strip test phosphorus concentration in the sediment at site 4P over all three sampling dates. The horizontal axis indicates scaled trends in for each set of data instead of actual values.

Appendix D

Figures Indicating Non-Significant Correlations

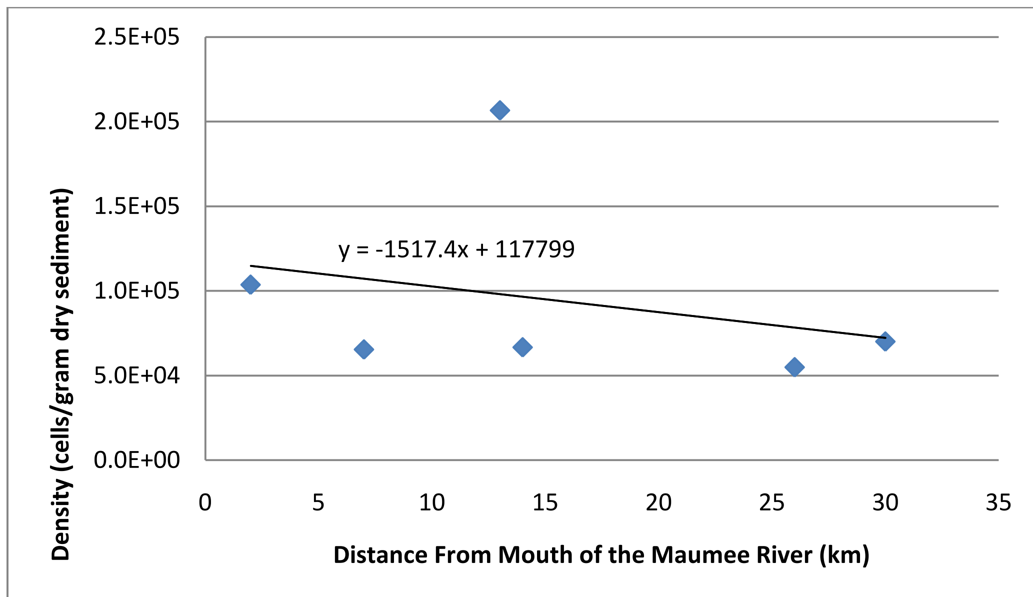

Figure D-1: Graphical representation of the relationship between cell density in the sediment during the cyanobacterial bloom (August 6, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship.

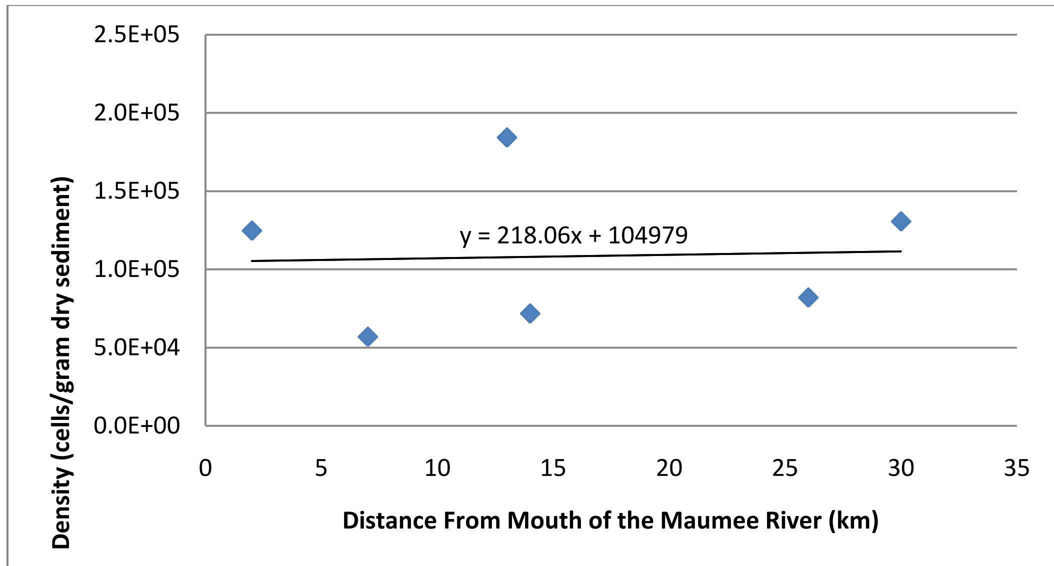

Figure D-2: Graphical representation of the relationship between cell density in the sediment after the cyanobacterial bloom (September 14, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship.

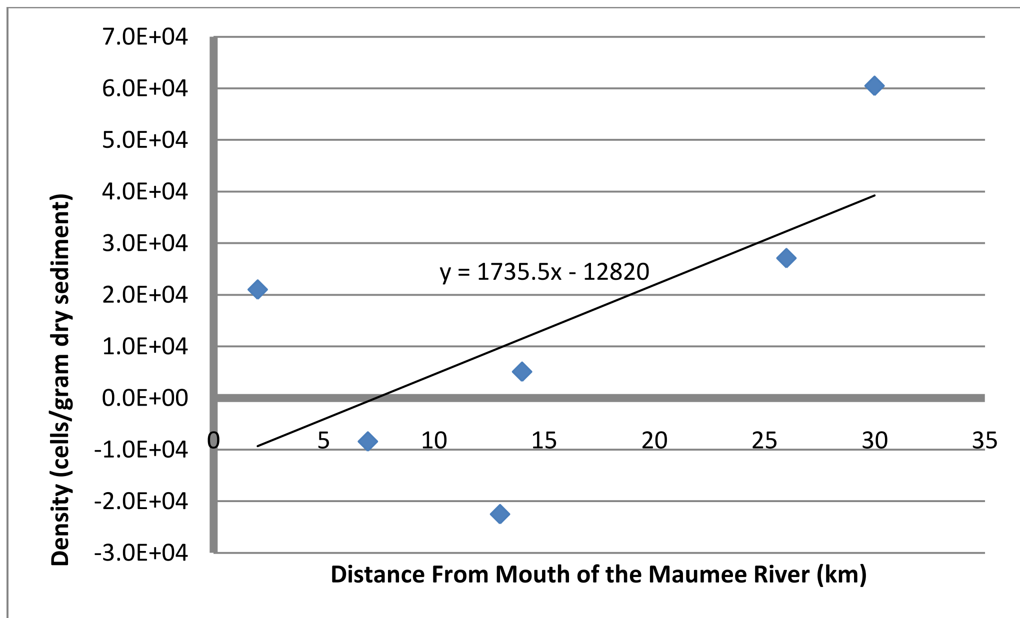

Figure D-3: Graphical representation of the relationship between the change in cell density in the sediment from during the cyanobacterial bloom (August 6, 2009) to after the cyanobacterial bloom (September 14, 2009) at any given site and the site's distance from the mouth of the Maumee River. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship.

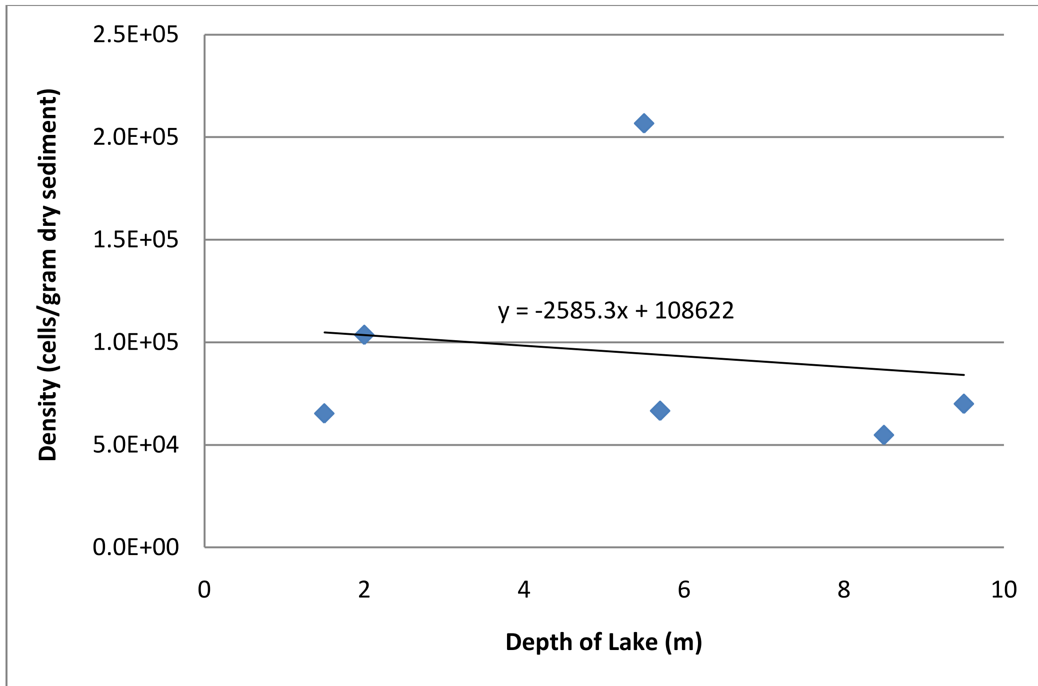

Figure D-4: Graphical representation of the relationship between cell density in the sediment during the cyanobacterial bloom (August 6, 2009) at any given site and the water depth at that site. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship.

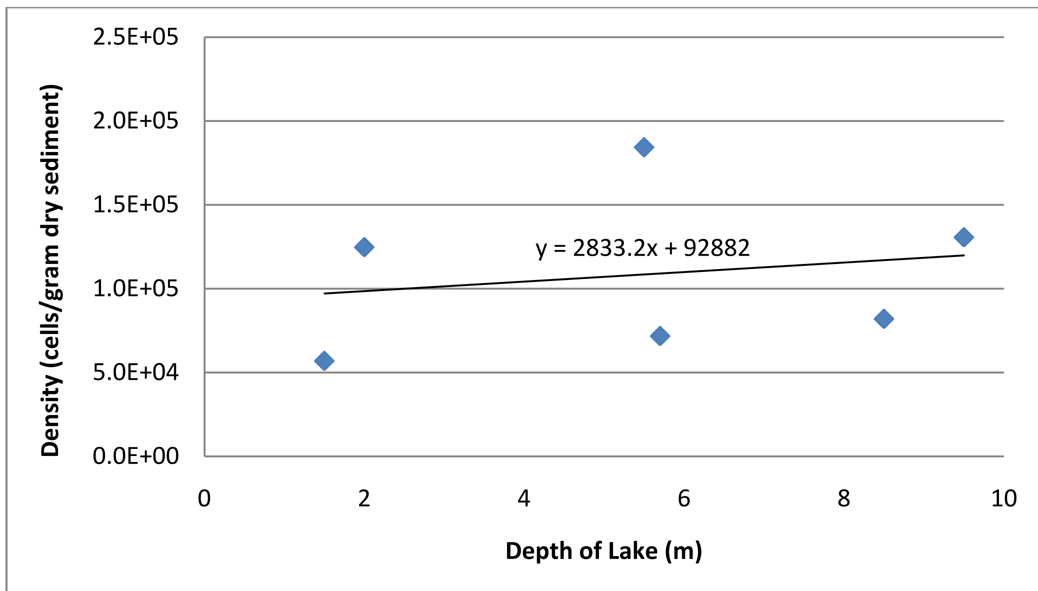

Figure D-5: Graphical representation of the relationship between cell density in the sediment after the cyanobacterial bloom (September 14, 2009) at any given site and the water depth at that site. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship.

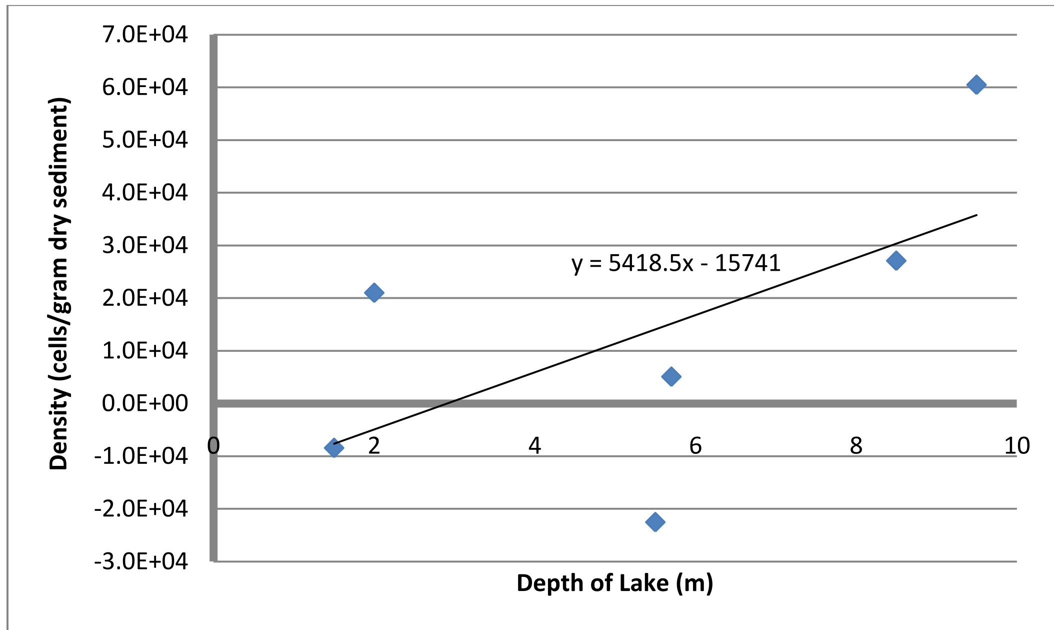

Figure D-6: Graphical representation of the relationship between the change in cell density in the sediment from during the cyanobacterial bloom (August 6, 2009) to after the cyanobacterial bloom (September 14, 2009) at any given site and the water depth at that site. A line of best fit, with its defining equation, is included within the figure to linearly define the relationship.

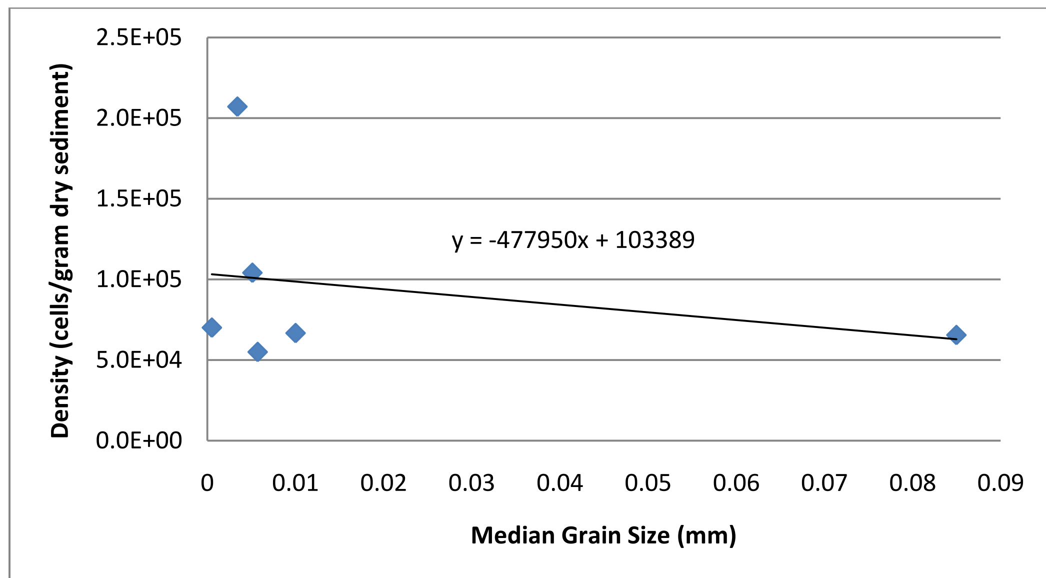

Figure D-7: Graphical representation of the relationship between the cell density in the sediment during the cyanobacterial bloom (August 6, 2009) at any given site and the median grain size distribution of the sediment at that site. A line of best fit, with its defining equation, is included on the figure to linearly define the relationship.

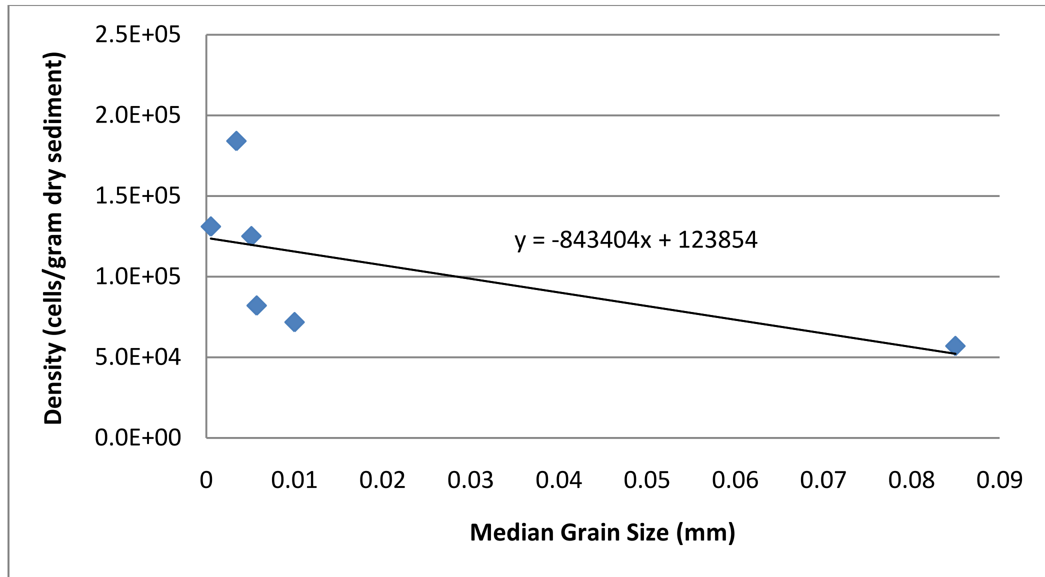

Figure D-8: Graphical representation of the relationship between the cell density in the sediment after the cyanobacterial bloom (September 14, 2009) at any given site and the median grain size distribution of the sediment at that site. A line of best fit, with its defining equation, is included on the figure to linearly define the relationship.

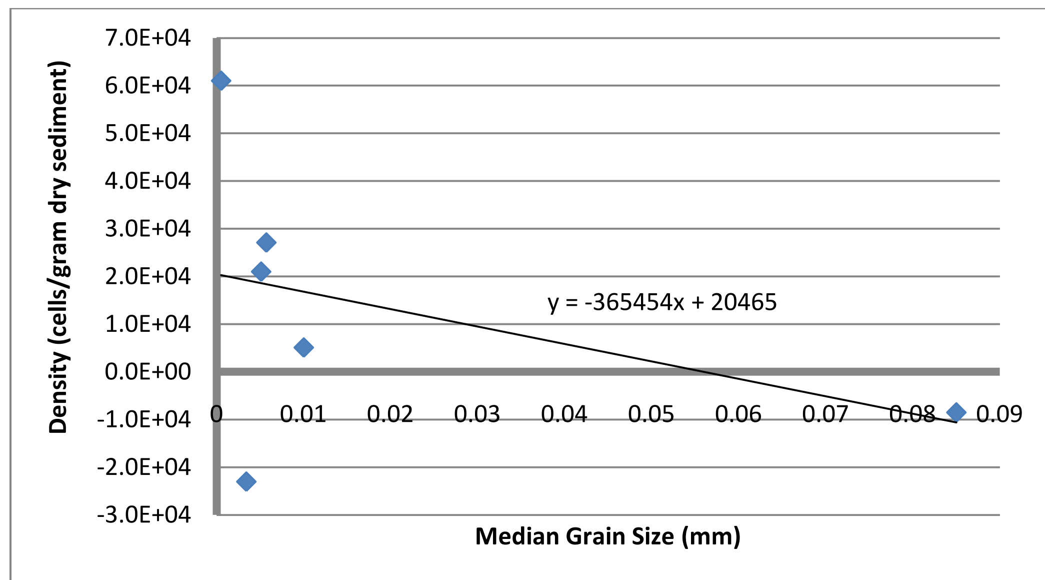

Figure D-9: Graphical representation of the relationship between the change in cell density in the sediment from during the cyanobacterial bloom (August 6, 2009) to after the cyanobacterial bloom (September 14, 2009) at any given site and the median grain size distribution of the sediment at that site. A line of best fit, with its defining equation, is included on the figure to linearly define the relationship.

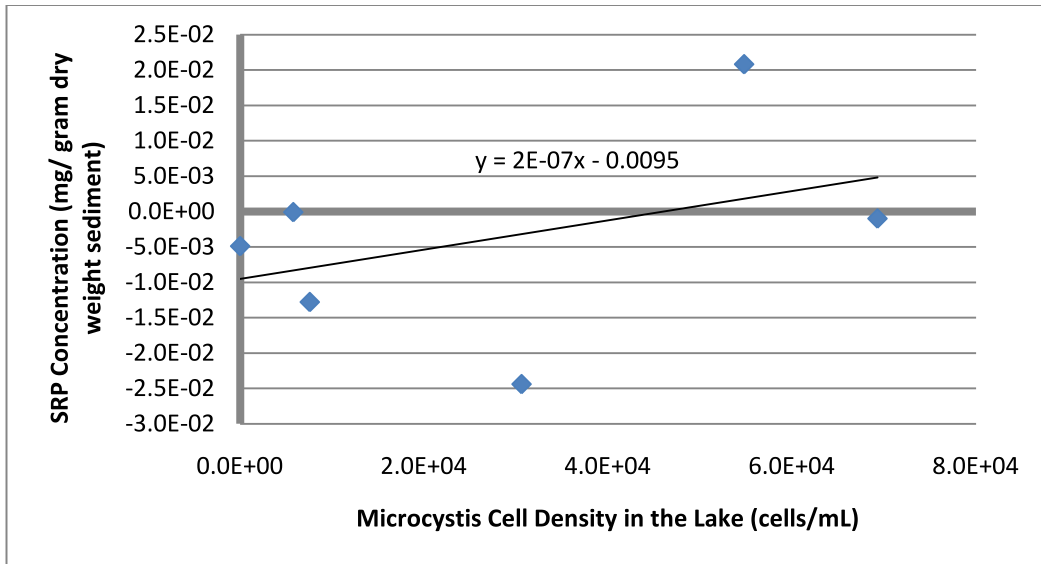

Figure D-10: The correlation between the change in *Microcystis* density in the lake water and the change in soluble phosphorus (SRP) concentration in the sediment at each sampling site between the first and second sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in SRP concentration and the change in cell density.

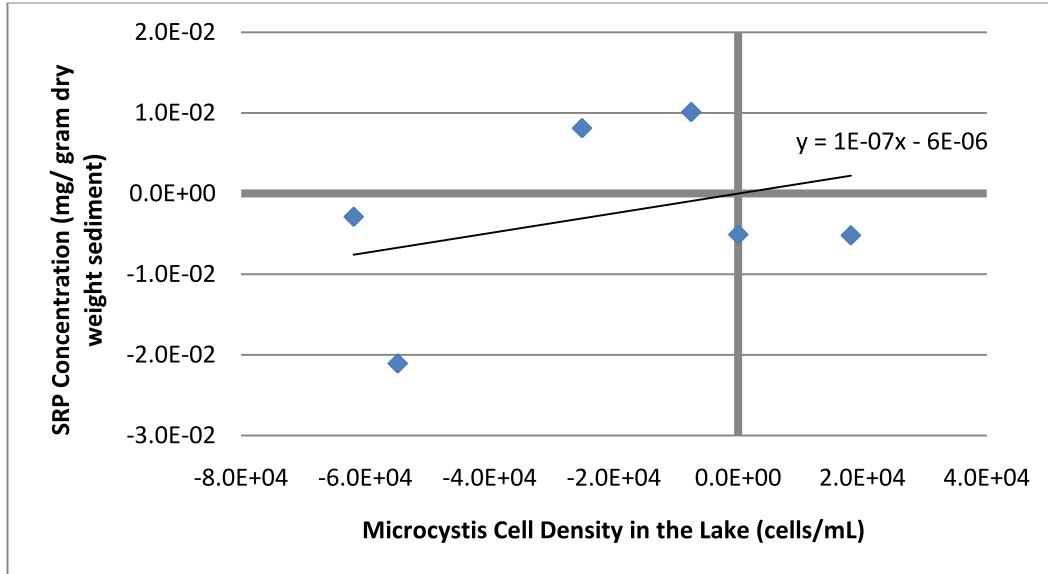

Figure D-11: The correlation between the change in *Microcystis* density in the lake water and the change in soluble phosphorus (SRP) concentration in the sediment at each sampling site between the second and third sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in SRP concentration and the change in cell density.

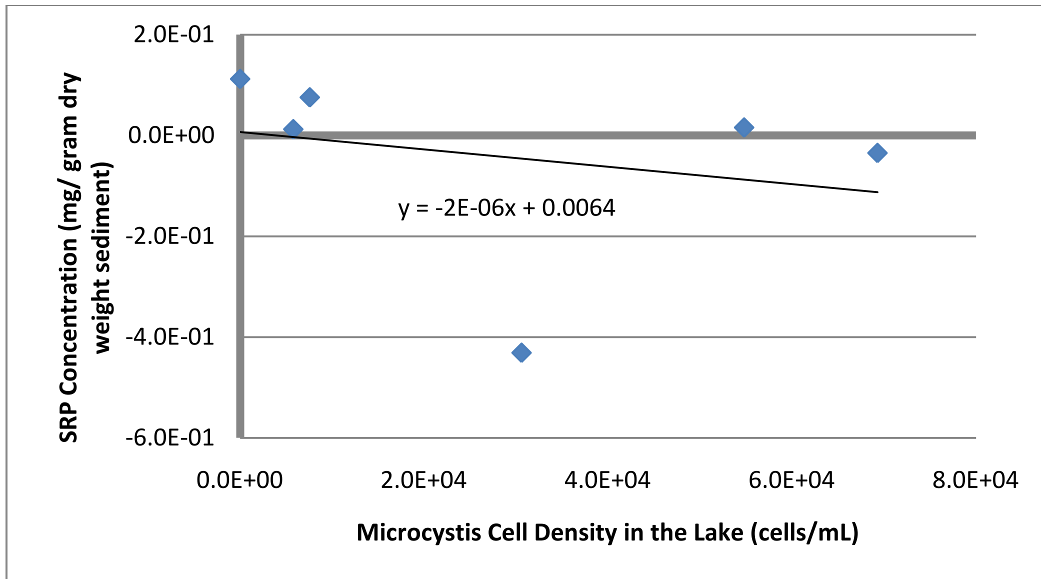

Figure D-12: The correlation between the change in *Microcystis* density in the lake water and the change in total phosphorus (TP) concentration in the sediment at each sampling site between the first and second sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in TP concentration and the change in cell density.

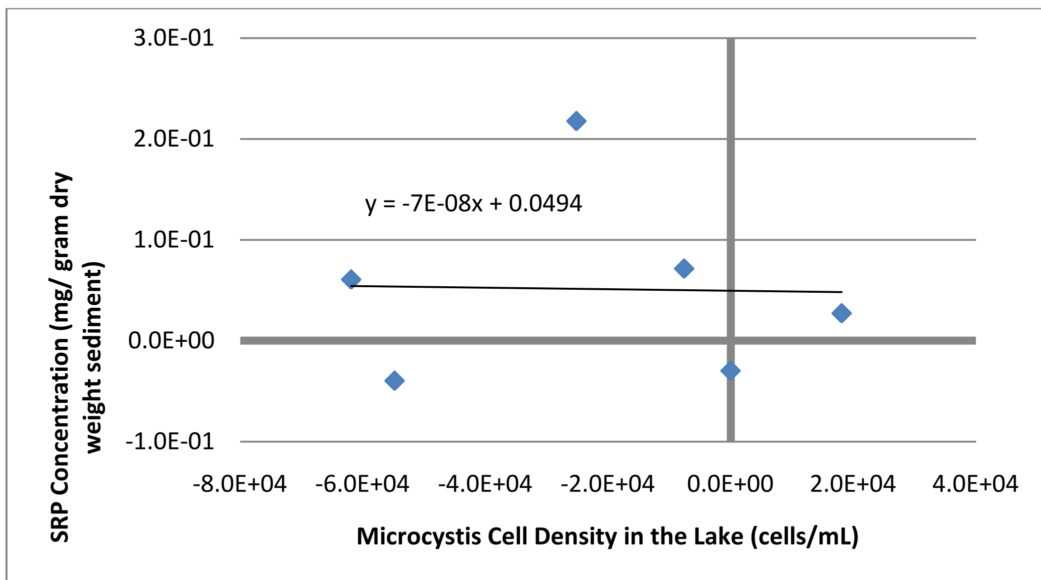

Figure D-13: The correlation between the change in *Microcystis* density in the lake water and the change in total phosphorus (TP) concentration in the sediment at each sampling site between the second and third sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in TP concentration and the change in cell density.

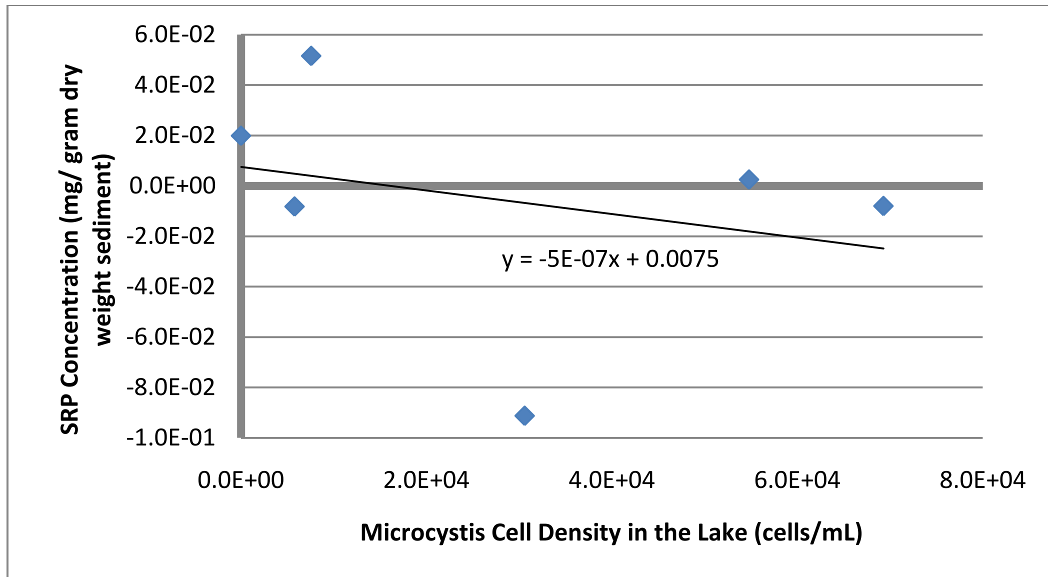

Figure D-14: The correlation between the change in *Microcystis* density in the lake water and the change in iron strip test phosphorus ($FeCl_2P$) concentration in the sediment at each sampling site between the first and second sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in $FeCl_2P$ concentration and the change in cell density.

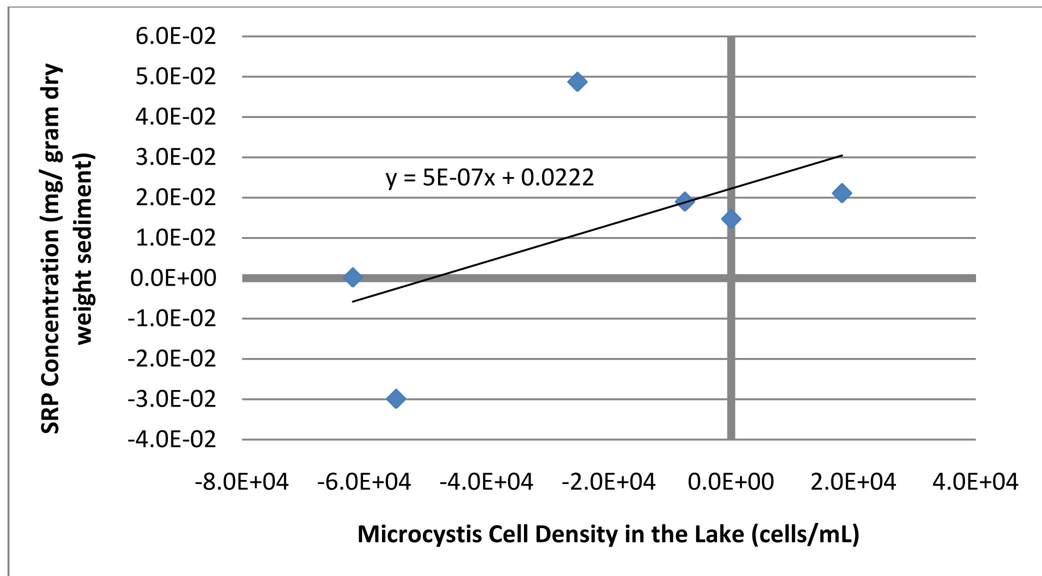

Figure D-15: The correlation between the change in *Microcystis* density in the lake water and the change in iron strip test phosphorus ($FeCl_2P$) concentration in the sediment at each sampling site between the second and third sampling sates. The line of best fit, with its defining equation, is included on the figure to illustrate the correlation between the changes in $FeCl_2P$ concentration and the change in cell density.