Pinto Lake Pilot Treatment Project

Watsonville, California

Final Report

Prepared for the City of Watsonville

Agricultural and Natural Resources Biogeochemistry Laboratory

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Acknowledgements

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1. Introduction

Freshwater cyanobacterial harmful algal blooms (CHABs) pose threats to humans and other mammals via direct ingestion and bioaccumulation of cyanotoxins. Pinto Lake, in Watsonville, CA, develops CHABs in the late spring through warm autumn months. Besides reducing the recreational and aesthetic value of the lake, the seasonal CHABs at Pinto Lake also produce cyanotoxins that present a health hazard to visitors and wildlife (Figure 1). In the period between 2007 and 2011, late summer to early autumn levels of microcystins averaged 183 ppb, with dense accumulation areas spiking as high as 2,893,000 ppb.

Figure 1. The Pinto Lake picinic area (left), the surface of the water during a cyanobacterial bloom (center), and an undewater photo the density of cyanobacteria in the water column (right).

To understand potential treatment alternatives, California State University Monterey Bay (CSUMB) graduate students conducted preliminary research and a thorough literature review about freshwater CHAB and cyanotoxin filtration and removal techniques through a series of internships with the City of Watsonville. Based on their findings and recommendations, a pilot study of the remediation of the Pinto Lake CHAB effluent was designed to assess the feasibility, procedures, cost, and the scope of a full scale effluent remediation of Pinto Lake CHABs. The resulting Pinto Lake CHAB remediation pilot study was carried out in 2012 to assess the efficacy of filtration and removal of cyanobacteria and the associated microcystin cyanotoxin.

In the course of the Pinto Lake CHAB remediation pilot study, several rounds of treatments of lake water were conducted. Treatment modalities included a settling tank with alum injection, slow sand filtration, filtration through granular activated carbon, ozonation, and filtration through polymorphic resin beads. Samples were collected before and after successive treatments and returned to CSUMB laboratories for analysis of reduction in chlorophyll *a*, cyanobacterial cell density, and microcystin content. Treatments were compared for both efficacy and feasibility for large scale remediation projects at Pinto Lake. With a history of dense seasonal CHABs and high microcystin levels, the target concentration of microcystin in the effluent of lake water after treatment in the pilot removal project was 4 ppb, the WHO-recommended recreational exposure limit.

2. Methods

2. 1 Treatments

A field laboratory was constructed in and around the boathouse adjacent to Pinto Lake in Watsonville to carry out the Pinto Lake effluent treatment pilot study (Figure 2). The field laboratory, including a workbench, water-proof counter space, and a sink draining into the municipal sewer system, were constructed in the interior of the boathouse. A two inch PVC line was installed within a trench from the boathouse to the lake, with a water intake approximately three-quarters of the way down a jetty located next to the boat launch area.

Figure 2. The Pinto Lake Field Laboratory and the small-scale treatment facility, located at the boathouse at Pinto Lake in Watsonville, CA.

Lake water was pumped through this line to a 550 gallon water storage tank, and a submersible pump within the storage tank was used to ensure circulation of the water in the storage tank during experimental runs (Figure 3). Water from the storage tank was then pumped to the treatment components, with samples taken before and after each component.

Figure 3. Water was pumped from the lake to a storage tank located outside the field laboratory for each experimental run. Water within the tank was circulated with a submersible pump during each experimental run to ensure uniform bacterial distribution throughout the run.

2. 1.1 Settling Tank with Alum Injection

A settling tank consisting of a mixing tank to mix the lake water with aluminum sulfate (alum), an inlet manifold, a settling trough, a laminar baffle, two surface skimming baffles, and a standpipe drain were constructed using marine grade plywood. Water from the storage tank was pumped into the alum mixing tank where a predetermined concentration of alum was added using a metering peristaltic pump (Figure 4).

Figure 4. The settling tank, showing the inlet manifold, the settling trough, the laminar baffle, the two surface skimmers, and the standpipe drain (left). Lake water from the storage tank was pumped into the alum mixing tank where a predetermined concentration of alum was added using a paristaltic metering pump (right).

Alum is a widely used flocculating agent in water treatment, and causes solids and other contaminants within treatment water to adhere to one another, gain mass and settle to the bottom of the water column (Figure 5).

Figure 5. Sample jars showing raw lake water (left), and lake water treated with alum and allowed to settle (right).

The settling tank was originally constructed with only the laminar flow baffle to ensure proper mixing of treatment water in the settling trough and to decrease channelization of water through the settling tank. During early test runs with various alum concentrations, we observed that a majority of the cyanobacteria were flocking on the surface of the water and traveling down the standpipe drain. To prevent the movement of the flocculated cyanobacteria from the settling tank to other treatment components, we

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added surface skimming baffles to the settling tank. The surface skimming baffles successfully sequestered floating cyanobacterial flock within the settling tank and prevented movement of the flock down the standpipe drain to other treatment components.

Various concentrations of alum were used during the course of the pilot study to determine appropriate alum concentrations for differing water conditions. Water flowed from the alum mixing tank and through the inlet manifold to the settling trough. Water flowed through the settling tank and out of the tank through the standpipe drain to a 25 gallon intermediate storage and sample collection tank.

2. 1.2 Slow Sand Filtration

We built a passive slow sand filter consisting of a water inlet manifold, water filtering media (sand), a back-flushing system, and an outflow manifold using marine grade plywood. Water was pumped from a storage tank to the water inlet manifold which sat upon a bed of sand approximately two feet deep. Assisted by gravity, water percolated through the two feet of sand to a bed of gravel at the base of the sand filter, depositing bacterial cells in the bed of sand along the way (Figure 6).

Figure 6. Interior of the slow sand filter, showing the outflow manifold (left), the bed of gravel (center), the inflow manifold and the bed of sand used to filter the lake water (right).

Water then flowed out of the slow sand filter through the outflow manifold and into a 25 gallon intermediate storage and sample collection tank. A back-flushing system was installed and operated regularly to clean the sand after repeated experimental runs, both to ensure proper water flow through the sand, and to remove bacterial cells and the associated toxins remaining from previous runs that might confound results (Figure 7).

The slow sand filter was originally constructed with the outflow drain covered in 2 inch base rock and a layer of fine washed sand (125 to 250 um) on top of the base rock. Fine sand is used widely in water filtering applications because the small size of the sand decreases interstitial spaces between grains, preventing small particulates and biological matter from penetrating the layer of sand. Conversely, 2 inch base rock was used to surround the sand filter drain to increase interstitial spaces between the rocks and facilitate the flow of water out of the filter after treatment. During repeated test runs of the slow sand filter it was observed that the fine sand was becoming clogged with cyanobacterial biomass causing the

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treatment water to channelize through the sand, transporting the sand through the channels into the interstitial spaces between the base rock, preventing efficient draining of the water out of the filter after treatment. Additionally, the high velocity channelized water was transporting the fine sand through the drain and into the connecting plumbing where it fouled valves and settled in areas of lower water velocities, preventing the movement of water to other treatment components.

Figure 7. Intermediate storage tanks used for lake water collection (left) and the back-flushing manifold used to clean the filtering sand using fresh water (right). The back-flushing manifold was placed on permeable cloth above the layer of gravel (Figure 5).

To reduce fouling and channelization of the sand, and to prevent transport of the sand into the base rock and down the filter drain, the sand was removed from the filter and a layer of fine filter cloth was installed between the base rock and the layer of sand. The filter cloth was installed as a single sheet that extended up the walls of the sand filter above the level of the filter sand to prevent sand from channelizing and being transported around the edges of the filter. Additionally, while the drain and the base rock were exposed, a back flushing and plumbing "blowout" system were installed within the filter. The first component of the system was a 1/2 inch pipe installed vertically through the top of the drain pipe, plumbed to the high pressure fresh water supply at the lab. This "blowout" component allowed the injection of high velocity water through the drain system and associated plumbing to remove fine sand that made it through the filter cloth and settled within the pipes.

The second part of the system was a back flushing component; a 1/2 inch manifold installed on top of the filter cloth and below the layer of sand. The manifold was plumbed to the high pressure fresh water supply, and had a series of water jets directed vertically into the layer of sand. High pressure water from the manifold jets mixed the sand layer from below, eliminating channels in the sand carved from above during water filtration. Additionally, the high pressure water from below removed the cyanobacterial biomass from the layer of sand and carried the biomass out of the sand filter through a drain installed on the side of the filter two inches above the mean high water mark of treatment water during active filtration. After the back flushing system was in place, there was a substantial decrease in cyanobacterial fouling of the filter sand, and reduced channelization and transport of the sand. Although the complete

prevention of sand transport into plumbed filtration components was not accomplished, the "blowout" was able to effectively remove sand from pipes within the filter system downstream of the slow sand filter.

2. 1.3 Granular Activated Carbon Filtration

A passive granular activated carbon (GAC) filtration tank consisting of a water inlet, water filtering media, and a water outlet was constructed using a 5 gallon PVC bucket. GAC is a commonly used water treatment and filtration technique, because molecules within treatment water have a high affinity for the highly reactive carbon molecules.

Water was gravity fed to the bottom of the GAC tank and allowed to percolate up through a bed of GAC, and out the water outlet at the top of the GAC tank. The GAC was contained within a fine mesh bag to prevent carbon from flowing out of the GAC tank, and to facilitate periodic washing of the carbon to remove bacterial cells and the associated toxins remaining from previous runs (Figure 8).

Figure 8. Passive Granular Activated Carbon (GAC) filtration tank (left) and GAC contained within a fine mesh bag.

After treatment water passed through the sand filter, it was gravity fed into the GAC tank. The GAC used during the course of the experiment was fine ground to approximately a cubic millimeter in size. The ground GAC had a high powder content that was transported with the treatment water during early system testing, potentially resulting in incorrect laboratory analysis of the treated water. To prevent the transport of GAC powder within the water during actual filtration testing, the GAC was thoroughly washed with fresh water and contained within a fine mesh bag. While the increased surface area of the ground GAC may have been beneficial during water treatment, the required washing and containment of the ground GAC may have decreased its effectiveness. The fine mesh bag was able to contain the GAC, but it also fouled with cyanobacteria easily, preventing water from making contact with the GAC and making frequent cleaning necessary, possibly further diminishing the effectiveness of the GAC.

2. 1.4 Ozonation

A passive ozonation tank consisting of a water inlet, aeration tubes, and a water outlet was constructed using a 5 gallon PVC bucket. An ozone generator situated in the lab was used to generate ozone, which was pumped through an air tube to the ozonation tank and through a set of aeration tubes. Water was gravity fed to the bottom of the ozonation tank and allowed to percolate up through a stream of ozone bubbles, and out the water outlet at the top of the ozonation tank (Figure 9).

Figure 9. Passive ozonation tank and the aeration tubes (left and center), and the ozone generator located within the field laboratory (right).

The ozonation of the treatment water required the use of expensive and delicate electrical equipment, which presented some logistical challenges. The first ozone generator delivered to the laboratory was faulty and required replacement, delaying the implementation of ozonation until late in the experimental duration. The ozone generator was located within the field laboratory, making it a health concern and therefore proper ventilation within the laboratory was necessary. Additionally, the generator required an external air pump to deliver the generated ozone outside the building and into the ozonation tank. A simple aquarium pump was used to pump the generated ozone, but the efficiency of the pump and the rate of ozone delivery from the pump were difficult to quantify.

2. 1.5 Polymorphic Resin Beads

We filtered the untreated lake water through five different compositions of polymorphic resin beads, each having different chemical structures and affinities for the microcystin toxin. To prevent clogging of the beads by large amounts of bacterial cells, water that had already been filtered by other components was pumped into an intermediate storage tank for the experimental runs. Water was pumped through the five bead types simultaneously using a filtration manifold containing six filter columns, and a six channel programmable peristaltic metering pump (Figure 10). We collected water samples before and after filtration through the five bead filtration columns and a single control column with no beads.

Additionally, the resin beads from each experimental run were collected to assess how much toxin was absorbed by each type of bead.

Figure 10. Polymorphic bead filtration manifold with five bead types (2-6) and a single control tube (1), and programable six channel paristaltic metering pump.

We performed the resin bead treatment inside the field laboratory using a subsample of treatment water. The resin beads were very small, about the same size as the filtration sand (125 to 250 um) and required the use of mechanically filtered water to prevent clogging. Typically, we subsampled treatment water after mechanical filtration by the settling tank and the slow sand filter before treatment with the resin beads. The resin beads require activation before use, and after repeated use they lose their affinity for the toxins within the treatment water. The polymorphic resin beads must be reactivated when they stop attracting toxin molecules using methanol, an extremely flammable liquid. Because of the dangers associated with recharging the resin beads with potentially dangerous chemicals, during the course of the pilot study at the field lab, resin beads were used only once and not recharged for future reuse.

2.2 Water Sample Testing

We collected water samples during two sampling periods, July to September 2012, and October to December 2012. During the first sampling period, we focused on determining the optimal concentration of alum for the reduction of chlorophyll *a*, cyanobacteria, and microcystin. During the second sampling period, we focused on comparing the different treatments and the analyte reductions associated with each.

2.2.1 Chlorophyll a analysis

We vacuum filtered 250 ml subsamples of pre- and post- treatment water using glass fiber filter paper (Figure 11). We weighed each filter before the filtration and weighed them again after drying to determine the biomass within the sample. The chlorophyll *a* concentration (micrograms per liter of water) was extracted in 90% acetone and measured with fluorometry.
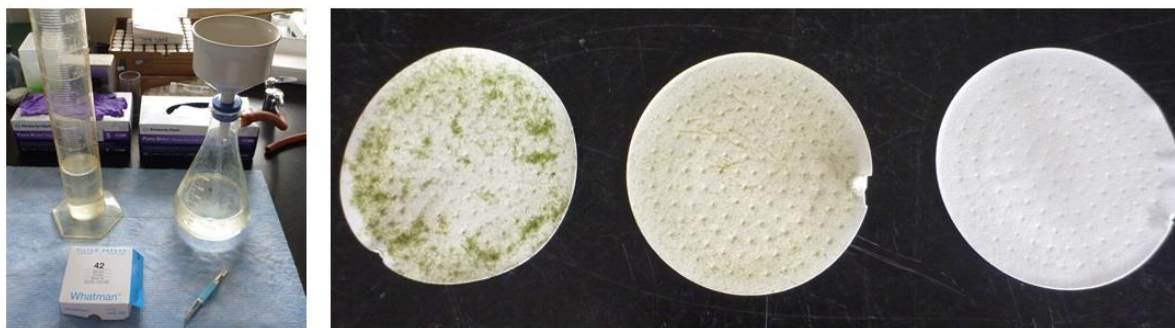
Figure 11. Assessment of chlorophyll *a* was performed using vacuume filtration (left). The filter paper to the left is raw lake water, at center is water after the settling tank, and to the right after the slow sand filter (right).

2.2.2 Cyanobacteria cell density analysis

We performed cyanobacterial cell counts for all pre- and post- treatment water samples using light microscopy. A 50 ml aliquot of each sample was withdrawn, preserved with Lugol's iodine, and allowed to settle in the refrigerator. We examined a 1ml aliquot of the sample concentrate (after settling) above an inverted microscope at 100x, and cyanobacterial cells were identified to genus. We estimated the cell counts based on the "natural unit method" using measuring tools in a microscope image capture and analysis software environment (Figure 12).
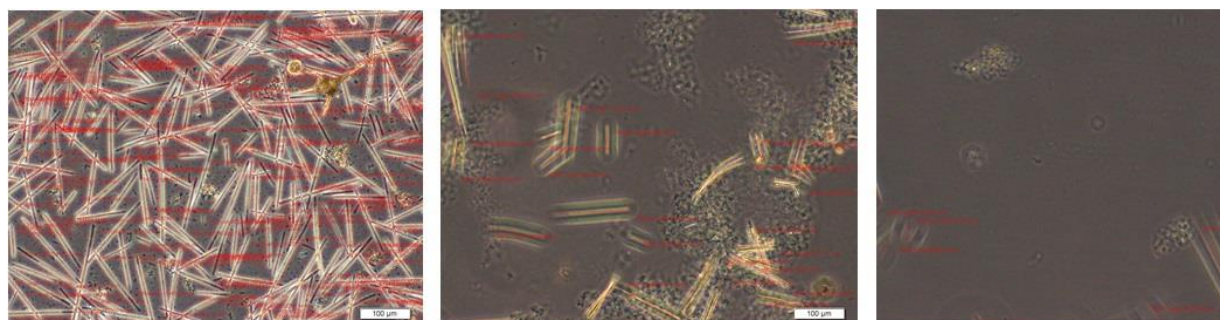
Figure 12. Examples of cell counts using a microscope and computer counting program from raw lake water (left), after the settling tank with alum injection (center), and after the slow sand filter (right).

2.2.3 Microcystin analysis

We vacuum filtered pre- and post- treatment waters samples onto 0.70 µm glass fiber filters, stored them at -20°C, and then macerated and extracted the samples for analysis in a 50% methanol solution. To quantify microcystins in the samples, we performed enzyme-linked immunosorbent assays (ELISA) on the samples collected between July and September. For the samples collected between October and December, microcystins were quantified using liquid chromatography tandem mass spectrometry

(LC-MS/MS) at the University of California, Santa Cruz laboratories. The toxin results based on ELISA are presented in microcystin-LR equivalents and the LC-MS/MS results are presented in total microcystins.

2.3 Statistical Analyses

In the sampling period between July and September, we tested four different concentrations of alum, 50 mg/L, 75 mg/L, 100 mg/L, and 200 mg/L, and we observed the changes in chlorophyll *a* concentration, cyanobacterial cell density, and microcystin associated with each concentration. Pre-treatment lake water samples were compared to post- settling tank and post-sand filtration samples after being treated with all four different alum concentrations. We conducted a multi-model analysis to determine the relationship between changes in alum concentrations and analyte values. We compared four models representing different patterns that the analyte measurement might follow as alum concentrations changed:

$$M0 = \text{Null}; \ Y \sim 1$$
$$M1 = \ Y \sim X$$
$$M2 = \ Y \sim X^2$$
$$M3 = \ Y \sim 1/X$$

where *Y* was the analyte measurement and *X* was the alum concentration. We compared the models based on the Akaike's Information Criterion (AIC), a statistical method that was chosen due to its suitability for multi-model analysis. To infer the optimal alum concentration, we conducted an analysis of variance (ANOVA) to test the significance of the difference between the means of each analyte before and after treatment, with a probability of ≤ 0.05 being considered significant.

In the October through December sampling period, after determining the optimal alum concentration in the settling tank, we focused on three treatment components: slow sand filtration (SF), granular activated carbon (GAC), and ozonation (O3). Samples collected before and after each treatment were tested for chlorophyll *a* content (μg/L), cyanobacteria abundance (cells/ml), and intracellular microcystins (μg/L). Additionally, we tested the microcystin removal efficiency of a fourth treatment component: resin beads (RES). We compared five types of resin, Resin 1: WA21J; Resin 2: PA308; Resin 3: HPA25L; Resin 4: WA30; and Resin 5: SP207-05L to a control to determine if the resins provided any additional removal after other treatments.

We conducted an analysis of variance (ANOVA) to test the significance of the difference between the mean concentration of each analyte before and after each treatment, with a probability of ≤ 0.05 being considered significant. The statistical program R (R Development Core Team 2010) was used for all the statistical analyses. See Appendix D for the R code used.

3. Results

3. 1 Optimal alum concentration

3.1.1 Post- Settling Tank

Higher concentrations of alum were associated with higher removal efficiencies for all measured constituents (Figure 13).

Figure 13. Percent reduction of Chlorophyll *a* (A), Cyanobacterial Cell Density (B), and Microcistyn (C) associated with four different concentrations of alum (50, 75, 100, and 200 mg/L) after lake water samples were treated in the settling tank.

From the multi-model analysis, we found the M3 model to be the best fit for chlorophyll *a* and microcystin (ΔAIC = 0.0; see appendix B for AIC tables), indicating that as the alum concentration increased, the amount of analyte in the samples decreased. For cyanobacteria, M0 represented the best fit model (ΔAIC = 0.0; see appendix B for AIC tables), indicating that the alum concentration did not affect the removal efficiency of the settling tank for this analyte.

The two highest alum concentrations tested, 100 and 200 mg/L, were associated with the highest removal of all analytes, but we found no significant differences between the two, making 100 mg/L the recommended concentration for the settling tank treatment component.

3.1.2 Post Sand Filtration

Changes in alum concentration did not seem to affect the removal efficiency of the sand filter (Figure 14).
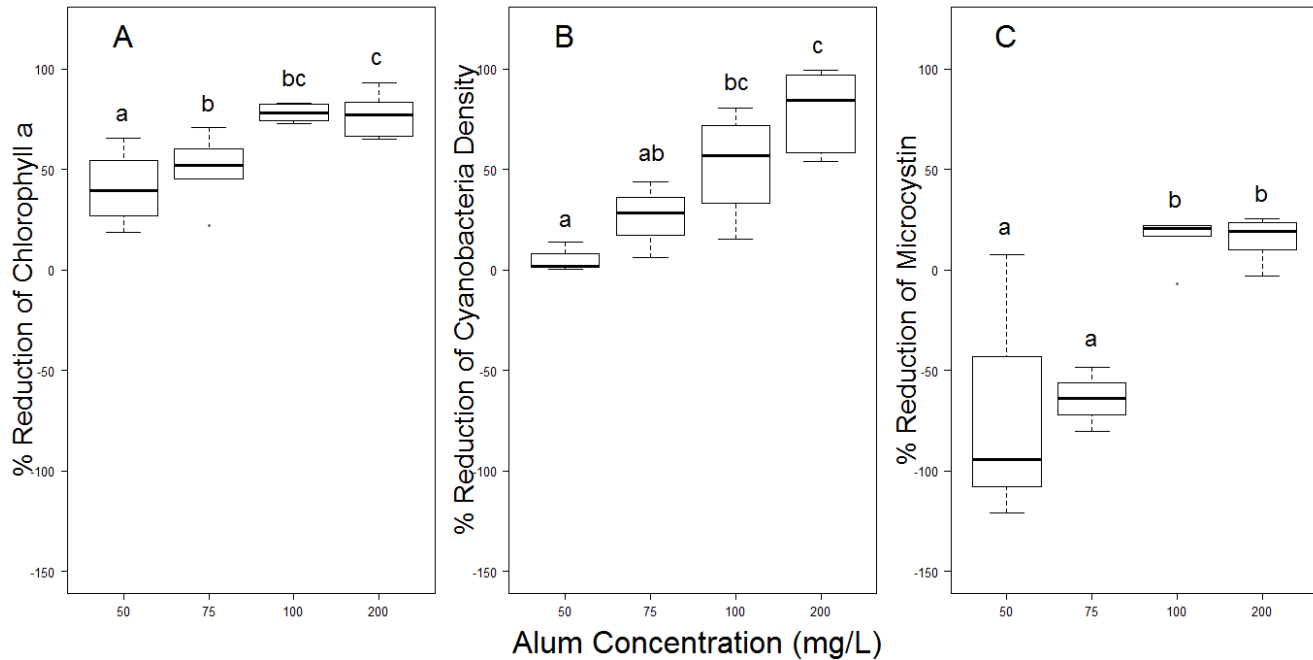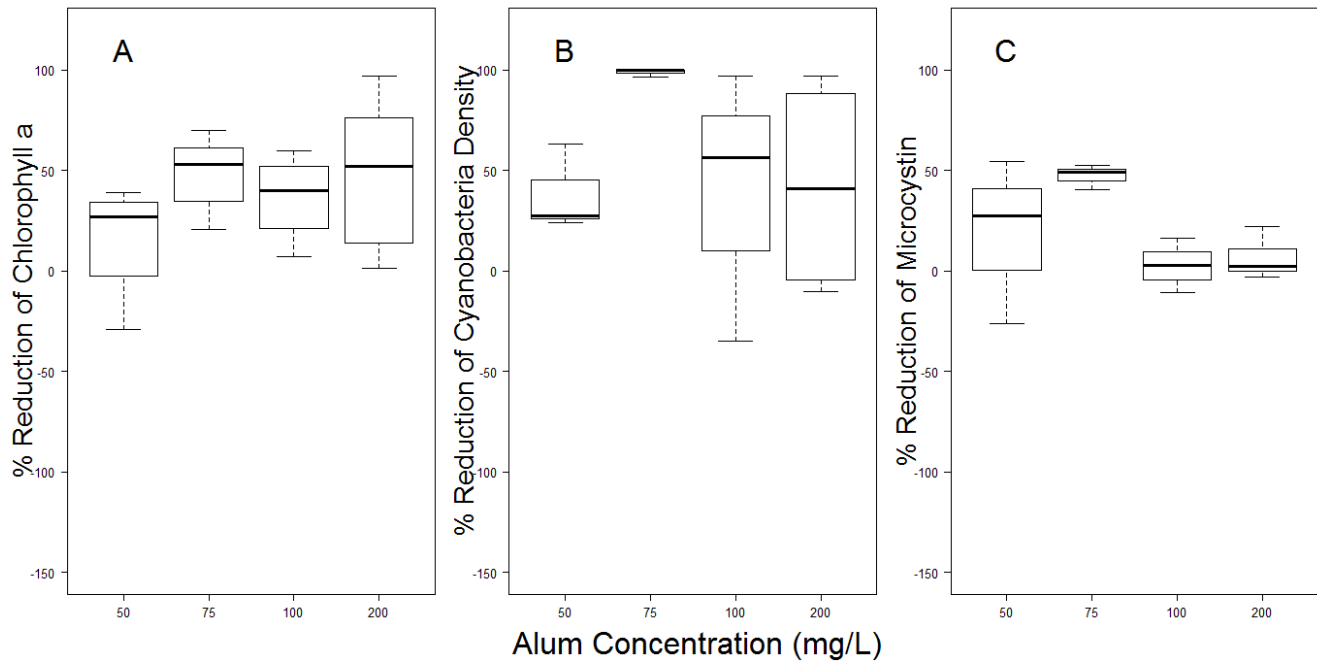

Figure 14. Percent reduction of Chlorophyll *a* (A), Cyanobacterial Cell Density (B), and Microcistyn (C) associated with four different concentrations of alum (50, 75, 100, and 200 mg/L) after lake water samples were treated in the sand filter.

From the multi-model analysis, we found the M0 model to be the best fit model for all analytes (ΔAIC = 0.0; see appendix B for AIC tables), indicating no relationship between the concentration of alum and the changes in analyte values. Since variations in alum concentration between 50 to 200 mg/L did not seem to affect the removal efficiency of the sand filter, we chose 100 mg/L of alum for the remaining experiments based on the results of the post-settling tank experiments.

3.2 Comparison of treatments

We found no significant difference between the removal efficiency of the GAC, Sand Filter, and Ozone treatments for chlorophyll *a* and cyanobacteria (Figure 15 and 16). For microcystins (Figure 17), we found that while the removal efficiencies associated with the GAC and Ozone treatments were not significantly different from each other, microcystin abundance was higher after the sand filter, which may be due to the rupture of the cyanobacterial cells and release of microcystins. The average microcystin measured in the homogenized sample in the time was 55 ug/L (min = 7.8 ug/L, max =213 ug/L), indicative of the higher toxin levels associated with higher cyanobacterial biomass in the beginning of the

autumn and lower cyanobacterial biomass associated with late season lower cyanobacterial biomass. We did not find a signifficant reduction of microcystin after the treatment with any of the resin beads when compared with the control (Figure 18) (see Appendix A for all results) .
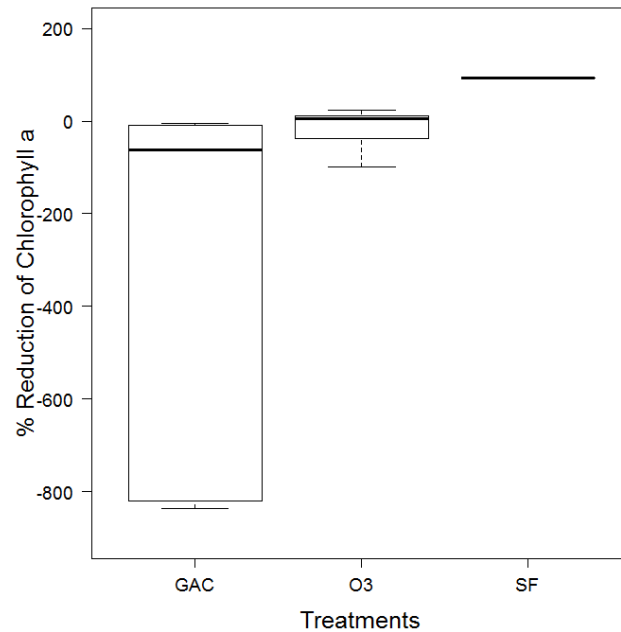
Figure 15. Percent reduction of Chlorophyll *a* associated with granular activated carbon (GAC), ozone (O3), and sand filtration (SF) in the sampling period between October and December 2012.
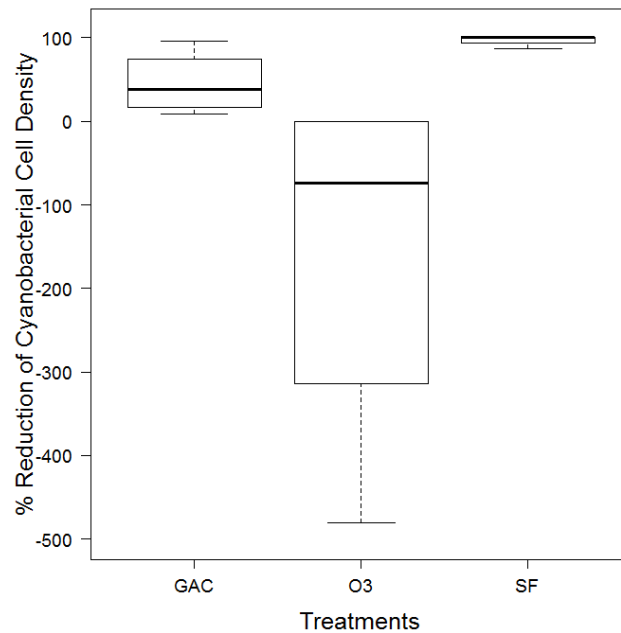
Figure 16. Percent reduction of cyanobacterial cell density associated with granular activated carbon (GAC), ozone (O3), and sand filtration (SF) in the sampling period between October and December 2012.
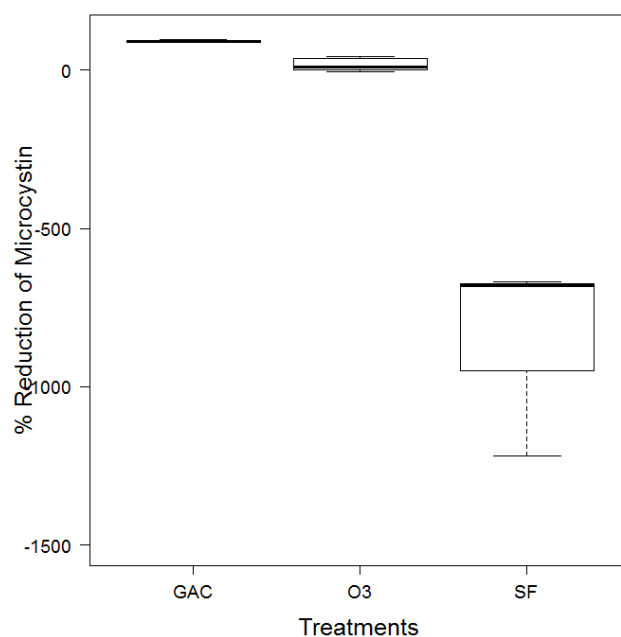
Figure 17. Percent reduction of microcystin associated with granular activated carbon (GAC), ozone (O3), and sand filtration (SF) in the sampling period between October and December 2012.
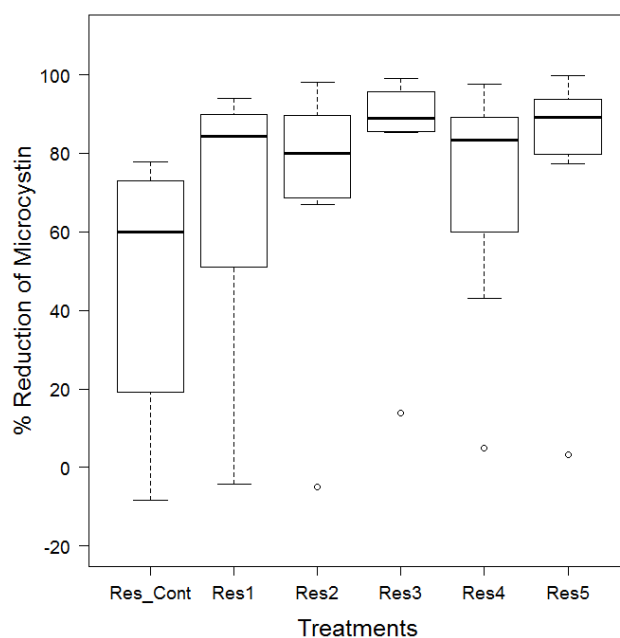
Figure 18. Percent reduction of microcystin associated with five types of resin and a control in the sampling period between October and December 2012.

We found that different treatments were effective for the removal of different analytes; GAC provided the highest removal of cyanobacteria and microcystins, while the sand filter provided the highest removal of chlorophyll *a*. Thus, we recommend a treatment system that includes these two elements, preceded by the alum treatment and the settling tank.

4. Scale-up Considerations and Costs

We recommend a treatment train that starts with alum addition in a concentration of 100 mg/L, an alum mixing tank, a slow sand filter, and ending with granulated activated carbon, as our results suggest that this sequence of treatments may be the most effective for the removal of chlorophyll *a*, cyanobacteria, and microcystins. We recommend a follow up study using these treatment components under different seasonal conditions to verify their removal efficiency in series, at peak toxicity levels. The current capacity of the treatment plant allows for an average flow of 0.25 L/s or 0.009 cfs. The cost of construction materials and construction labor for the three recommended treatment components in the current lab was just under $6,500 (Table 1).

Table 1. Laboratory Construction Costs. Current treatment capacity of 0.25 L/s or 0.009 cfs

All plumbing and wood materials	$3,411.77
Aluminum Sulfate	$60.00
Sand and gravel for Sand Filter	$52.50
Granular Activated Carbon	$15.00
Ozone Generator	$2,874.00
Polymer Resin Beads	$434.00
Construction Labor	$2,910.00
Total Construction Costs with all treatments	$9,757.27
Total Construction Costs with 3 recommended treatments	$6,449.27

Using the discharge measured at the outflow point, the average December through April flow that would have to be treated would be approximately 3.5 cfs. To scale up our current lab to treat 3.5 cfs would cost around $2.5M for the startup costs and an estimated $60,000 per year for operation and maintenance costs, not including hazardous waste disposal, which was not examined in this study. It is important to consider, nonetheless, that a threshold-based adaptive treatment plan that was informed by actual microcystin abundance will not likely require treating all of the winter effluent, since cyanobacteria toxicity is lowest in the winter months. In the summer and fall, when toxicity is the highest, the effluent discharge is minimal to null (Table 2).

Table 2. Startup cost projections based on current lab processing capacity and costs

	Flow (cfs)	Startup Cost (USD)
Current flow capacity of treatment plant	0.01	6,449.27
Average outflow during microcystin peak (September - October)	0.00	0.00
December - April average outflow	3.51	2,562,409.22
Highest outflow registered in past 3 years (April, 2011)	19.81	14,471,045.67

We estimate that a large scale treatment facility will require approximately 20 personnel hours a week to operate and maintain under normal conditions. Waste disposal represents a recurring and potentially high cost associated with the operation and maintenance of the facility. The cyanobacterial biomass collected as slurry from the surface of the settling tank will require disposal at a waste management facility. Because the slurry contains water, it will be heavy in large quantities, and because it will be a concentration of cyanobacterial biomass it is potentially extremely toxic. During the operation of the experimental treatment facility, approximately 67 cubic feet of lake water (~500 gallons) yielded approximately 0.5 cubic foot of slurry (~3.75 gallons), or approximately 30 pounds of slurry. Both the volume and the weight of the slurry could be reduced by drying the slurry, but the possibility of the toxin becoming an aerosolized human health hazard during drying must be taken into consideration during drying site identification and designation. We did not determine the appropriate disposal protocols or estimate the disposal costs of the slurry because they were outside of the scope of this study. However, we note there is no legal or experimental data that can be used to promote one option over others, e.g. land fill, bioreactors, or sewer treatment facility, and this is in part because limited research has been done to evaluate the nature of the toxin and slurry once harvested from a bloom.

Overall, the investment to treat the lake for the cyanobacteria seems high compared to investments that might be made in the watershed itself to reduce loading. However, without more extensive monitoring of surface water and ground water sources, the relative importance of various source reduction activities is highly uncertain. Our project did not include a benefit cost estimate of various options; however, we would be hesitant to recommend a treatment course without a full cost analysis based on a detailed characterization of the watershed and surrounding watersheds for comparison.

5. Appendices

Appendix A – Results

Analyte measurements before and after treatments.

Abbreviation Key:
Cyano: Cyanobacteria
Microc.: Microcystin
Alum Conc.: Alum Concentration
ST: Settling Tank
SF: Sand Filter
GAC: Granular Activated Carbon
O3: Ozone
Res.: Resin
Res. Cont.: Resin Control

Table 1. Alum was added in four different concentrations, 50, 75, 100, and 200 mg/L to untreated lake water. Chlorophyll *a* (μg/ml) was measured in untreated lake water (before alum addition), and after two treatment components, in series, the settling tank (ST), and the slow sand filter (SF). Data from July – September 2012.

Date	Alum Conc. (mg/L)	Chlorophyll *a* Untreated (μg/ml)	Chlorophyll *a* ST (μg/ml)	Chlorophyll *a* SF (μg/ml)
7/12/2012	200	80.44	15.45	13.31
7/13/2012	200	7.41	2.48E+00	2.45
7/18/2012	200	44.74	3.03	0.96
7/27/2012	200	78.27	2.73E+01	0.74
7/30/2012	50	109.44	70.66	53.43
8/1/2012	100	108.19	29.09	18.75
8/3/2012	200	112.35	2.98E+01	7.08
8/6/2012	50	83.25	28.61	36.97
8/8/2012	200	96.44	15.77	10.05
8/13/2012	100	35.86	6.58	2.64
8/15/2012	75	45.99	18.14	11.8
8/17/2012	50	39.83	22.51	13.76
8/20/2012	100	46.32	11.51	6.4
8/24/2012	75	42.07	22.94	6.83
8/26/2012	75	44.52	12.85	4.95
9/10/2012	100	84.56	14.31	13.29
9/12/2012	75	164.18	78.35	61.97
9/15/2012	50	253.08	205.27	144.08
9/17/2012	75	282.63	220.35	103

Table Alum was added in four different concentrations, 50, 75, 100, and 200 mg/L to untreated lake water. Cyanobacteria cell density (cells/ml) was measured in untreated lake water (before alum addition), and after two treatment components, in series, the settling tank (ST), and the slow sand filter (SF). Data from July – September 2012.

Date	Alum Conc. (mg/L)	Cyano Untreated (cells/ml)	Cyano ST (cells/ml)	Cyano SF (cells/ml)
7/12/2012	200	9.79E+04	9.87E+03	1.03E+04
7/13/2012	200	2.18E+05	1.50E+03	1.66E+03
7/18/2012	200	2.17E+04	6.84E+02	7.89E+01
7/23/2012	100	2.94E+04	5.63E+03	1.66E+02
7/27/2012	200	8.43E+03	3.87E+03	1.14E+02
7/30/2012	50	2.34E+04	2.01E+04	1.52E+04
8/1/2012	100	3.20E+04	1.57E+04	6.66E+03
8/3/2012	200	2.05E+04	8.52E+03	1.81E+03
8/6/2012	50	7.77E+03	7.72E+03	5.60E+03
8/8/2012	200	1.46E+03	3.04E+02	2.93E+02
8/15/2012	75	7.37E+02	5.29E+02	1.79E+01
8/17/2012	50	1.56E+03	1.53E+03	5.62E+02
8/20/2012	100	3.28E+02	2.77E+02	1.24E+02
8/24/2012	75	3.50E+02	3.28E+02	0.00E+00
8/26/2012	75	1.22E+02	6.85E+01	0.00E+00
9/10/2012	100	1.11E+04	4.12E+03	5.55E+03

Table 3. Alum was added in four different concentrations, 50, 75, 100, and 200 mg/L to untreated lake water. Microcystin cell density (cells/ml) was measured in untreated lake water (before alum addition), and after two treatment components, in series, the settling tank (ST), and the slow sand filter (SF). Data from July – September 2012.

Date	Alum Conc. (mg/L)	Microc. Untreated (ug/ml)	Microc. ST (ug/ml)	Microc. SF (ug/ml)
7/12/2012	200	1.28E+01	9.82E+00	1.01E+01
7/13/2012	200	1.08E+01	9.72E+00	9.73E+00
7/18/2012	200	1.15E+01	9.39E+00	9.36E+00
7/23/2012	100	1.35E+01	1.07E+01	9.69E+00
7/27/2012	200	1.46E+01	1.09E+01	9.70E+00
7/30/2012	50	1.46E+01	3.23E+01	1.46E+01
8/1/2012	100	1.40E+01	1.50E+01	1.25E+01
8/3/2012	200	1.54E+01	1.58E+01	1.23E+01
8/6/2012	50	1.59E+01	1.46E+01	1.85E+01
8/8/2012	200	1.68E+01	1.35E+01	1.28E+01
8/13/2012	100	1.40E+01	1.16E+01	1.22E+01
8/15/2012	75	1.27E+01	2.29E+01	1.16E+01
8/17/2012	50	1.28E+01	2.48E+01	1.80E+01
8/20/2012	100	1.36E+01	1.06E+01	1.03E+01
8/24/2012	75	1.32E+01	2.17E+01	1.03E+01
8/26/2012	75	1.26E+01	1.87E+01	1.11E+01
9/10/2012	100	1.48E+01	1.00E+01	1.11E+01

Table 4. Chlorophyll *a* (μg/ml) was measured in untreated lake water, and after three treatment components: slow sand filter (SF), granular activated carbon (GAC), and Ozone (O3). The samples were processed under two different treatment trains; treatment train "A" started with GAC followed by Ozone, while treatment "B" started with the sand filter followed by GAC and then Ozone. Data from October – December, 2012.

Date	Treatment Train	Chlorophyll *a* Untreated (μg/ml)	Chlorophyll *a* SF (μg/ml)	Chlorophyll *a* GAC (μg/ml)	Chlorophyll *a* O3 (μg/ml)
10/17/2012	A	2.92E+02		3.27E+02	3.08E+02
10/27/2012	A	3.97E+02		4.15E+02	4.02E+02
11/10/2012	A	7.94E+02		8.71E+02	6.62E+02
11/17/2012	B	2.39E+02	1.82E+01	3.86E+01	7.70E+01
11/28/2012	B	4.40E+02	3.01E+01	2.76E+02	2.45E+02
12/8/2012	B	1.28E+02	8.03E+00	7.53E+01	1.04E+02

Table 5. Cyanobacteria cell density (cells/ml) was measured in untreated lake water, and after three treatment components: slow sand filter (SF), granular activated carbon (GAC), and Ozone (O3). The samples were processed under two different treatment trains; treatment train "A" started with GAC followed by Ozone, while treatment "B" started with the sand filter followed by GAC and then Ozone. Data from October – December, 2012.

Date	Treatment Train	Cyano Untreated (cells/ml)	Cyano SF (cells/ml)	Cyano GAC (cells/ml)	Cyano O3 (cells/ml)
10/17/2012	A	7.28E+04		6.60E+04	6.60E+04
10/27/2012	A	5.45E+04		2.37E+03	5.85E+03
11/10/2012	A	1.22E+04		9.34E+03	9.37E+03
11/17/2012	B	4.81E+02	6.36E+01	2.93E+01	1.70E+02
11/28/2012	B	6.04E+01	0.00E+00	NA	NA
12/8/2012	B	1.40E+03	0.00E+00	NA	NA

Table 6. Microcystin cell density (cells/ml) was measured in untreated lake water, and after four treatment components: slow sand filter (SF), granular activated carbon (GAC), Ozone (O3), and five types of resins. The samples were processed under three different treatment trains: treatment train "A" started with GAC followed by Ozone, and then Resin; treatment "B" started with SF followed by GAC and then Ozone; treatment "C" only had resins. Data from October – December, 2012.

Date	Treat. Train	Microc. Untreated (ug/ml)	Microc. SF (ug/ml)	Microc. GAC (ug/ml)	Microc. O3 (ug/ml)	Microc. Res. Cont. (ug/ml)	Microc. Res. A (ug/ml)	Microc. Res. B (ug/ml)	Microc. Res. C (ug/ml)	Microc. Res. D (ug/ml)	Microc. Res. E (ug/ml)
10/17/2012	A	1.81E+01		5.92E+00	3.70E+00	2.99E+00	3.85E+00	3.88E+00	3.19E+00	3.52E+00	3.58E+00
10/27/2012	A	2.13E+02		1.63E+02	1.59E+02	8.46E+01	7.07E+01	5.24E+01	1.67E+00	3.88E+00	3.74E+00
11/10/2012	A	2.99E+01		2.38E+01	2.36E+01	9.70E+00	1.16E+01	4.69E+00	3.42E+00	1.34E+01	3.40E+00
11/10/2012	C	2.99E+01				6.66E+00	1.55E+01	6.58E+00	4.39E+00	1.20E+01	6.04E+00
11/17/2012	B	2.20E+01	1.72E+02	3.44E+00	3.60E+00	3.90E+00	5.01E-01	1.13E+00	4.30E-01	5.98E-01	3.82E-01
11/17/2012	C	2.20E+01				8.63E+00	2.23E+00	2.30E+00	2.35E+00	3.64E+00	2.41E+00
11/28/2012	B	3.82E+01	2.94E+02	3.73E+01	2.14E+01	5.79E+00	2.18E+00	1.06E+00	9.09E-01	1.63E+00	1.35E+00
11/28/2012	C	3.82E+01				1.04E+01	2.27E+00	7.49E-01	1.21E+00	8.76E+00	8.55E-02
12/8/2012	B	7.83E+00	1.03E+02	9.62E+00	7.83E+00	7.23E+00	1.36E+00	1.58E+00	9.20E-01	1.03E+00	1.78E+00
12/8/2012	C	7.83E+00				2.02E+00	8.36E-01	8.25E-01	4.01E-01	8.41E-01	8.03E-01

Appendix B – AIC Results

Table 1. AIC table showing the linear models and the results of model comparisons. This comparison was used to determine the best-fit model for the change in chlorophyll *a* from pre- to post- settling tank treatment as alum concentration increased.

Model	df	AIC	AICc	ΔAIC	AICw
M0	2	338.44	338.89	3.65	0.10
M1	3	338.72	339.64	4.41	0.07
M2	4	336.19	337.79	2.55	0.18
M3	3	334.31	335.24	0.00	0.65

Table 2. AIC table showing the linear models and the results of model comparisons. This comparison was used to determine the best-fit model for the change in cyanobacteria abundance pre- to post- settling tank treatment with increasing alum concentration.

Model	df	AIC	AICc	ΔAIC	AICw
M0	2	421.76	422.62	0.00	0.37
M1	3	421.64	423.49	0.87	0.24
M2	4	421.92	425.25	2.63	0.10
M3	3	421.22	423.07	0.45	0.29

Table 3. AIC table showing the linear models and the results of model comparisons. This comparison was used to determine the best-fit model for the change in microcystins from pre- to post- settling tank treatment with increasing alum concentration.

Model	df	AIC	AICc	ΔAIC	AICw
M0	2	121.82	122.62	5.96	0.04
M1	3	118.92	120.63	3.97	0.10
M2	4	116.43	119.51	2.84	0.17
M3	3	114.95	116.67	0.00	0.70

Table 4. AIC table showing the results of the model comparisons. This comparison was used to determine the best-fit model for the change in chlorophyll *a* (µg · L^{-1}) from pre-treatment lake water samples to "SF" treatment with increasing alum concentration.

Model	df	AIC	AICc	ΔAIC	AICw
M0	2	336.57	337.03	0.00	0.50
M1	3	338.56	339.52	2.49	0.14
M2	4	337.99	339.66	2.63	0.13
M3	3	337.70	338.66	1.64	0.22

Table 5. AIC table showing the results of the model comparisons. This comparison was used to determine the best-fit model for the change in cyanobacteria abundance (cells · ml^{-1}) from pre-treatment lake water samples to "SF" treatment with increasing alum concentration.

Model	df	AIC	AICc	ΔAIC	AICw
M0	2	421.41	422.27	0.00	0.37
M1	3	421.36	423.20	0.93	0.23
M2	4	421.53	424.86	2.59	0.10
M3	3	420.94	422.78	0.51	0.29

Table 6. AIC table showing the results of the model comparisons. This comparison was used to determine the best-fit model for the change in Microcystins (µg · L^{-1}) from pre-treatment lake water samples to "SF" treatment with increasing alum concentration.

Model	df	AIC	AICc	ΔAIC	AICw
M0	2	97.40	98.20	0.00	0.38
M1	3	99.14	100.86	2.66	0.10
M2	4	96.65	99.72	1.52	0.18
M3	3	96.72	98.44	0.24	0.34

Appendix C– ANOVA Results

Optimal Alum Concentration

We added four alum concentrations (50, 75, 100, and 200 mg/L) to the mixing tank and we observed the associated changes in Chlorophyll *a*, Cyanobacteria and Microcystin. We followed a null hypothesis testing approach, and we conducted an analysis of variance (ANOVA) to test the significance of the difference between the mean reductions associated with each treatment with a probability of ≤0.05 being considered significant. For chlorophyll *a*, we found a significant difference between the mean reductions associated with each treatment (df= 3, F= 7.76, p= 0.00233); for cyanobacteria, we found a significant difference between the mean reductions associated with each treatment (df= 3, F= 10.32, p= 0.00233); and for microcystin, we also found a significant difference between the mean reductions associated with each treatment (df= 3, F= 10.39, p= 0.000923).

Comparison of treatments

We compared pre- and post- treatment values for all analytes for Sand Filtration, Ozonation, and Granular Activated Carbon. We also compared microcystin reductions associated with five types of resins. We followed a null hypothesis testing approach, and we conducted an analysis of variance (ANOVA) to test the significance of the difference between the mean reductions associated with each treatment, with a probability of ≤0.05 being considered significant. For chlorophyll *a*, we found no significant difference between the mean reductions associated with each treatment (df= 2, F= 2.735, p= 0.105); for cyanobacteria, we found no significant difference between the mean reductions associated with each treatment (df= 2, F= 3.333, p= 0.0886); and for microcystin, we did find a significant difference between the mean reductions associated with each treatment (df= 2, F= 41.02, p= 3.00E-05). Additionally, we compared the mean abundances of microcystin after treatment with five resins and one control (no resin) and found no significant difference between the reductions associated with the resins and the control (df= 5, F= 1.866, p= 0.116).

Appendix D – R Code

Code for all statistical analyses for the R statistical analysis software:

```
#Analysis of Variance
#to determine if there are differences associated
#with four concentrations of alum (50, 75, 100, 200 ug/L)
#after the settling tank ("ps", post-settling) and after the sand filter ("sf")
# in pre and post treatment sample means of
#chlorophyll a, cyanboacteria, and microcystin reductions
#data from 07-09, 2012


##Load multcomp and multcompView packages
##Import Chlorophyll a data
ch<-read.csv(file.choose(), header=TRUE); ch


##Import Cyanobacteria Data
cy<-read.csv(file.choose(), header=TRUE); cy


##Import Microcystin Data
mi <-read.csv(file.choose(), header=TRUE); mi


#Check that alum conc is a factor for all datasets
        str(ch)
        str(cy)
        str(mi)
#If alum conc is not a factor, convert (and change the name to "alum"):
        ch$alum <- as.factor(ch$Alum_Conc_mgL);str(ch)
        cy$alum <- as.factor(cy$Alum_Conc_mgL); str(cy)
        mi$alum <- as.factor(mi$Alum_Conc_mgL); str(mi)


##Generate boxplots for all analytes
#Set up box plot characteristics
        par(mfrow = c(1, 3), mar=c(5.5,5,1,1), las = 1, cex.axis=1.2)
#mfrow is rows, columns; mar is for margins in lines from bottom, left, top, and right; las = 1 is for all labels to be parallel to x
axis


#Chlorophyll a, After ST
boxplot( Chla_PercChang_PS~alum, data=ch,
   xlab = "",
```

```
  ylab = "",

  ylim = c(-150, 120),

  cex.lab = 2)

  mtext ("% Reduction of Chlorophyll a", side = 2, line = 2.7, las=0, cex = 1.75)

  text(110, "A", cex=3)



#Cyanobacteria, After ST

boxplot( Cyano_PercChang_PS~alum, data=cy,

  xlab = "",

  ylab = "",

  ylim = c(-150, 120),

  cex.lab = 2)

  mtext ("% Reduction of Cyanobacteria Density", side = 2, line = 2.7, las=0, cex = 1.75)

  mtext(" Alum Concentration (mg/L)", side=1, line=4, las=0, cex=2)

  text(110, "B", cex=3)


#Microcystin, After ST

boxplot( MC_PercChang_PS ~alum, data=mi,

  xlab = "",

  ylab = "",

  ylim = c(-150, 120),

  cex.lab = 2)

  mtext ("% Reduction of Microcystin", side = 2, line = 2.7, las=0, cex = 1.75)

  text(110, "C", cex=3)



##boxplots for changes associated with varying alum concentrations AFTER SAND FILTER for Chl a, Cyano density, and Microc
for data from 07-09, 2012


#Chlorophyll a, After SF

boxplot( Chla_PercChang_SF~alum, data=ch,

  xlab = "",

  ylab = "",

  ylim = c(-150, 120),

  cex.lab = 2)

  mtext ("% Reduction of Chlorophyll a", side = 2, line = 2.7, las=0, cex = 1.75)

  text(110, "A", cex=3)
```

```
#Cyanobacteria, After SF
boxplot( Cyano_PercChang_SF~alum, data=cy,
    xlab = "",
    ylab = "",
    ylim = c(-150, 120),
    cex.lab = 2)
    mtext ("% Reduction of Cyanobacteria Density", side = 2, line = 2.7, las=0, cex = 1.75)
    mtext(" Alum Concentration (mg/L)", side=1, line=4, las=0, cex=2)
    text(110, "B", cex=3)


#Microcystin, After SF
boxplot( MC_PercChang_SF ~alum, data=mi,
    xlab = "",
    ylab = "",
    ylim = c(-150, 120),
    cex.lab = 2)
    mtext ("% Reduction of Microcystin", side = 2, line = 2.7, las=0, cex = 1.75)
    text(110, "C", cex=3)



####ANOVA and Multiple Comparisons
#######Post Settling Tank#######

####Chlorophyll a


##ANOVA
                    ch.aov <- aov( Chla_PercChang_PS~alum, data=ch); ch.aov
                    summary(ch.aov)
                    par(mfrow=c(2,2))
                    plot(ch.aov)

        ##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different
                    par(mfrow=c(1,1))
                    TukeyHSD(ch.aov)
                    Tukey.ch.aov <- glht(ch.aov, linfct=mcp( alum = "Tukey"))
                    plot(Tukey.ch.aov)
```

```
multcompBoxplot(Chla_PercChang_PS~alum, data=ch)
```

####Cyanobacteria

##ANOVA
```
cy.aov <- aov( Cyano_PercChang_PS~alum, data=cy); cy.aov
summary(cy.aov)
par(mfrow=c(2,2))
plot(cy.aov)
```

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different
```
par(mfrow=c(1,1))
TukeyHSD(cy.aov)
Tukey.cy.aov <- glht(cy.aov, linfct=mcp( alum = "Tukey"))
plot(Tukey.cy.aov)
multcompBoxplot(Cyano_PercChang_PS~alum, data=cy)
```

#Microcystin

##ANOVA
```
mi.aov <- aov( MC_PercChang_PS~alum, data=mi); mi.aov
summary(mi.aov)
par(mfrow=c(2,2))
plot(mi.aov)
```

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different
```
par(mfrow=c(1,1))
TukeyHSD(mi.aov)
Tukey.mi.aov <- glht(mi.aov, linfct=mcp( alum = "Tukey"))
plot(Tukey.mi.aov)
multcompBoxplot(MC_PercChang_PS~alum, data=mi)
```


#######Post Sand Filter#######

####Chlorophyll a
##ANOVA

```
sf_ch.aov <- aov( Chla_PercChang_SF~alum, data=ch); sf_ch.aov

summary(sf_ch.aov)

par(mfrow=c(2,2))

plot(sf_ch.aov)
```

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different

```
par(mfrow=c(1,1))

TukeyHSD(sf_ch.aov)

Tukey.sf_ch.aov <- glht(sf_ch.aov, linfct=mcp( alum = "Tukey"))

plot(Tukey.sf_ch.aov)

multcompBoxplot(Chla_PercChang_SF~alum, data=ch)
```

####Cyanobacteria

#ANOVA

```
sf_cy.aov <- aov( Cyano_PercChang_SF~alum, data=cy); sf_cy.aov

summary(sf_cy.aov)

par(mfrow=c(2,2))

plot(sf_cy.aov)
```

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different

```
par(mfrow=c(1,1))

TukeyHSD(cy.aov)

Tukey.sf_cy.aov <- glht(sf_cy.aov, linfct=mcp( alum = "Tukey"))

plot(Tukey.sf_cy.aov)

multcompBoxplot(Cyano_PercChang_SF~alum, data=cy)
```

####Microcystin

##ANOVA

```
sf_mi.aov <- aov( MC_PercChang_SF~alum, data=mi); sf_mi.aov

summary(sf_mi.aov)

par(mfrow=c(2,2))

plot(mi.aov)
```

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different

```
par(mfrow=c(1,1))

TukeyHSD(sf_mi.aov)

Tukey.sf_mi.aov <- glht(sf_mi.aov, linfct=mcp( alum = "Tukey"))

plot(Tukey.sf_mi.aov)

multcompBoxplot(MC_PercChang_SF~alum, data=mi)
```

#Analysis of Variance, Pinto Lake Data (10-12, 2012)

#Load multcomp and multcompView packages

#Import Chlorophyll a data

ch2 <-read.csv(file.choose(), header=TRUE); ch2

#Import Cyanobacteria Data

cy2 <-read.csv(file.choose(), header=TRUE); cy2

#Import Microcystin Data

mi2 <-read.csv(file.choose(), header=TRUE); mi2

Chlorophyll a analysis

#Check that Treatment is a factor

str(ch2) #if not a factor, convert using "as.factor"

##ANOVA

ch2.aov <- aov(Chla_PercChang~Treatment, data=ch2); ch2.aov

summary(ch2.aov)

par(mfrow=c(2,2))

plot(ps_ch.aov)

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different

par(mfrow=c(1,1))

TukeyHSD(ch2.aov)

Tukey.ch2.aov <- glht(ch2.aov, linfct=mcp(Treatment = "Tukey"))

plot(Tukey.ch2.aov)

multcompBoxplot(Chla_PercChang~Treatment, data=ch2)

#Cyanobacteria analysis

#Check that Treatment is a factor

str(cy2) #if not a factor, convert using "as.factor"

##ANOVA

```
cy2.aov <- aov( Cyano_PercChang~Treatment, data=cy2); cy2.aov
summary(cy2.aov)
par(mfrow=c(2,2))
plot(cy2.aov)
```

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different

```
par(mfrow=c(1,1))
TukeyHSD(cy2.aov)
Tukey.cy2.aov <- glht(cy2.aov, linfct=mcp( Treatment = "Tukey"))
plot( Tukey.cy2.aov)
multcompBoxplot(Cyano_PercChang~Treatment, data=cy2)
```

#Microcystin analysis

#Check that Treatment is a factor

```
str(mi2) #if not a factor, convert using "as.factor"
```

##ANOVA

```
mi2.aov <- aov( MC_PercChang ~Treatment, data=mi2); mi2.aov
summary(mi2.aov)
par(mfrow=c(2,2))
plot(mi2.aov)
```

##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different

```
par(mfrow=c(1,1))
TukeyHSD(mi2.aov)
Tukey.mi2.aov <- glht(mi2.aov, linfct=mcp( Treatment = "Tukey"))
plot( Tukey.mi2.aov)
multcompBoxplot(MC_PercChang ~Treatment, data=mi2)
```

##Resins analysis, microcystin
##Load Microcystin Resin Data
mi_res <-read.csv(file.choose(), header=TRUE); mi_res

#Check that Treatment is a factor

```
str(mi_res) #if not a factor, convert using "as.factor"
```

#ANOVA

```
mi_res.aov <- aov( MC_PercChang ~Treatment, data=mi_res); mi_res.aov
```

```
        summary(mi_res.aov)
        par(mfrow=c(2,2))
        plot(mi_res.aov)


##Multiple Comparison; only nec. if ANOVA p<= 0.05, to determine which means are different
        par(mfrow=c(1,1))
        TukeyHSD(mi_res.aov)
        Tukey.mi_res.aov <- glht(mi_res.aov, linfct=mcp( Treatment = "Tukey"))
        plot( Tukey.mi_res.aov)
        multcompBoxplot(MC_PercChang ~Treatment, data=mi_res)


####Box plots for chlorophyll a, cyanobacteria and microcystin for three treatments, GAC, O3, and SF
        # close graph device used for previous graphs/reset parameters
dev.off()
        #Set new graph parameters
par(mar=c(4.1,4.5,.5,.51), las = 1, cex.axis=1.2, cex.lab=1.6)


## Chlorophyll a boxplot
        boxplot( Chla_PercChang~Treatment, data=ch2,
                xlab = "Treatments",
                ylab = "% Reduction of Chlorophyll a",
                ylim = c(-900, 110))
        ## Cyanobacteria boxplot
        boxplot( Cyano_PercChang~Treatment, data=cy2,
                xlab = "Treatments",
                ylab = "% Reduction of Cyanobacterial Cell Density",
                ylim = c(-500, 110))



    ## Microcystin boxplot
        boxplot( MC_PercChang~Treatment, data=mi2,
                xlab = "Treatments",
                ylab = "% Reduction of Microcystin",
                ylim = c(-1500, 110))


##Resins, Microcystin
        boxplot( MC_PercChang~Treatment, data=mi_res,
                xlab = "Treatments",
```

```
                    ylab = "% Reduction of Microcystin",

                    ylim = c(-20, 110))


--

######### R code for AIC analysis of differences in analytes compared to alum concentrations

## this code produces AIC tables and saves them as .csv, as well as plots with best-fit model lines

######## Pinto Lake Project - 9/29/13 Megan Gehrke

##############################################################################################

# import data and rename file

dat=PL_Data_R_130423 # dat=filename

dat=read.csv(file.choose())

# reshape data into long format using melt function from reshape package

library(reshape)

MD=melt.data.frame(dat, id.vars=c("Date","Sample","Treatment.Type","Alum.Conc","Treatment"),na.rm=T)

md=MD[order(MD$Alum.Conc,MD$Date),] # order data by alum concentration and date

analy=unique(md$variable[md$variable!="Notes"]) # create list of each analyte name

alum=na.omit(unique(md$Alum.Conc)) # create list of each alum concentration

treat=unique(md$Treatment) # create list of each treatment

##############################################################################################

# this loop will create an AIC table and a plot with best-fit line for differences

# between analyte levels for each alum concentration and BETWEEN each treatment level

# (Homo>PS, PS>SF, SF>Car, Car>Ozone)

for (k in 1:length(analy)){

 for (l in c(2:5)){ # calls out treatments 2:5 to compare with each other

  dat=subset(md,md$variable==analy[k]) # subset data by analyte name

  dat=dat[order(dat$Alum.Conc,dat$Date),] # order subsetted data by alum concentration and date

  dates=intersect(dat$Date[dat$Treatment==treat[l]],dat$Date[dat$Treatment==treat[l-1]]) # list of dates where each
treatment was performed

  Y=numeric() # empty vectors to store loop outputs in

  date=character() # date and conc vectors are for visual verification if needed
```

```
conc=numeric()

for (j in 1:length(dates)){ # for each date

 for (i in 1:length(alum)){ # and each alum concentration

  d=subset(dat,dat$Alum.Conc==alum[i]&dat$Date==dates[j]) # subset data for each date and alum conc

  if (length(d$value)==0){ # if there's no data, move on to next iteration

   next

  } else if (is.na(d$value)||length(d$value)==0L){ # if values are NA or non-existent, move on to next iteration

   next

  } else {

  d$value=as.numeric(d$value) # make sure values are in numeric format

  val=(d$value[d$Treatment==treat[l]])-(d$value[d$Treatment==treat[l-1]]) # Y = difference in analyte levels between the two
treatments being compared

  Y=append(Y,val,after=length(Y)) # put value of Y into a vector for use later

  date=append(date,rep(dates[j],length(val)),after=length(date)) # same for date and alum conc

  conc=append(conc,rep(alum[i],length(val)),after=length(conc))

  }

 }

}

df=data.frame(date=date,conc=conc,y=Y) # new data frame with y = differences in analyte levels for each date and alum conc

# head(df)

x=df$conc # for the AIC models, the x variable is defined as alum conc

y=df$y # the y variable is defined as difference in analyte levels between the two treatments being compared

if (length(y)==0L){ # when there are no values, move on to next iteration

 next

}else if(length(unique(x))>4){ # sets a minimum number of values necessary for AIC analysis

# define models for AIC

m0=lm((y)~1, data=dat)

m1=lm((y)~x, data=dat)

m2=lm(y~poly(x,2), data=dat)
```

```r
m3=lm(y~poly(1/x),data=dat)

aic=AIC(m0,m1,m2,m3)

n=length(df[,1])

AICtable <- function( aic, n) {

 K <- aic$df

 AICc <- aic$AIC + 2 * K * (K+1) / ( n - K - 1 )

 delAIC<- AICc - min( AICc )

 AICw <- exp(-0.5*delAIC) / sum( exp(-0.5*delAIC))

 #This is the AIC table:

 data.frame( aic, AICc, delAIC , AICw)}

AICtab=AICtable(aic,n)

# write AIC table to csv and save in working directory

write.table(AICtab,paste("AICtable_",analy[k],"_",treat[l-1],">",treat[l],"_",

     ".csv"),sep=",",qmethod="double")

AICnew=as.data.frame(AICtab) # convert AIC table to data frame format for plotting purposes

# plot and add best-fit line

xlab="Alum Concentration (ppm)"

ylab=""

models=list(m0,m1,m2,m3)

modelName=c("m0","m1","m2","m3")

quartz(paste("PL_AIC_",treat[l-1],">",treat[l],analy[k],".png"),5.5,5)

par(mar = c(3.5, 4, 2, 0.5), oma = c(0,0,0,0))

par(mgp=c(2.3,0.8,0))

cex=0.8

par(cex=cex)

plot(x,y, xlab=xlab, ylab=ylab,cex.lab=cex, ylim=c(min(y),max(y)),cex.main=0.7,

 main=paste("Difference between ",treat[l],"and",treat[l-1]," for ",analy[k]))

lines(x,predict(models[[which(AICnew$delAIC==0)]]), col="black")

mtext(paste("Model = ",modelName[which(AICnew$delAIC==0)]),side=3,line=0,cex=0.6)
```

```
 }

 }

}




##########################################################################

# this loop is the SAME AS ABOVE, but will produce outputs for analyte levels at all treatments

# in comparison to pre-treatment ("Homo") levels

# (Homo>SF, Homo>Car, Homo>Ozone)

for (k in 1:length(analy)){

 for (l in 3:5){ # will call out treatments 3:5 and compare to treatment 1 (Homo)

 dat=subset(md,md$variable==analy[k]) # subset data by analyte name

 dat=dat[order(dat$Alum.Conc,dat$Date),] # order subsetted data by alum concentration and date

 dates=intersect(dat$Date[dat$Treatment==treat[1]],dat$Date[dat$Treatment==treat[l]])

 Y=numeric()

 date=character()

 conc=numeric()

 for (j in 1:length(dates)){

  for (i in 1:length(alum)){

   d=subset(dat,dat$Date==dates[j]&dat$Alum.Conc==alum[i])

   if (length(d$value)==0){

    next

   } else if (is.na(d$value)||length(d$value)==0L){

    next

   } else {

   d$value=as.numeric(d$value) # make sure values are in numeric format

   val=(d$value[d$Treatment==treat[1]])-(d$value[d$Treatment==treat[l]]) # Y = difference in analyte levels between the two
treatments being compared

   Y=append(Y,val,after=length(Y))
```

```
date=append(date,rep(dates[j],length(val)),after=length(date))

conc=append(conc,rep(alum[i],length(val)),after=length(conc))

 }

 }

}

df=data.frame(date=date,conc=conc,y=Y)

x=df$conc

y=df$y

if (length(y)==0L){

 next

}

if(length(unique(x))>4){

 # choose models for AIC

 m0=lm((y)~1, data=dat)

 m1=lm((y)~x, data=dat)

 m2=lm(y~poly(x,2), data=dat)

 m3=lm(y~poly(1/x),data=dat)

 # make AIC table

 aic=AIC(m0,m1,m2,m3)

 n=length(df[,1])

 AICtable <- function( aic, n) {

 K <- aic$df

 AICc <- aic$AIC + 2 * K * (K+1) / ( n - K - 1 )

 delAIC<- AICc - min( AICc )

 AICw <- exp(-0.5*delAIC) / sum( exp(-0.5*delAIC))

 #This is the AIC table to be published:

 data.frame( aic, AICc, delAIC , AICw)}

 AICtab=AICtable(aic,n)

 AICtab
```

```r
# write AIC table to csv and save in working directory

write.table(AICtab,paste("AICtable_",analy[k],"_",treat[1],">",treat[l],".csv"),sep=",",qmethod="double")

AICnew=as.data.frame(AICtab)

# plot and add best-fit line

xlab="Alum Concentration (ppm)"

ylab=""

models=list(m0,m1,m2,m3)

modelName=c("m0","m1","m2","m3")

cex=0.8

quartz(paste("PL_AIC_","Homo >",treat[l],analy[k],".png"),5.5,5)

par(mar = c(3.5, 4, 2, 0.5), oma = c(0,0,0,0))

par(mgp=c(2.3,0.8,0))

cex=0.8

par(cex=cex)

plot(x,y, xlab=xlab, ylab=ylab,cex.lab=cex, ylim=c(min(y),max(y)),cex.main=0.7,

  main=paste("Difference between ",treat[l],"and",treat[1]," for ",analy[k]))

lines(x,predict(models[[which(AICnew$delAIC==0)]]), col="black")

mtext(paste("Model = ",modelName[which(AICnew$delAIC==0)]),side=3,line=0,cex=0.6)

 }

}

}

############################################################
```