PILOT TESTING OF ENVIRONMENTALLY-SAFE ALGAECIDE ON COPCO RESERVOIR WATER – 2011 STUDY RESULTS

A Report for PacifiCorp

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December 10, 2012

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Executive Summary

The effect of algaecide on algal populations from waters collected from Copco reservoir and treated outside the reservoir in discrete containers has been studied over the last several years. In 2008, Watercourse completed a brief review of algaecide applications and conducted a small-scale algaecide experiment on water collected from Copco reservoir to investigate this potential strategy as a component of an overall reservoir management approach for PacifiCorp. In 2009, an algaecide experiment was conducted based on findings from the 2008 pilot experiment, with the objective of determining the efficacy of two distinct algaecides: Algimycin PWF (copper-based) and GreenClean Pro (hydrogen peroxide-based). Another algaecide experiment was conducted in 2011 following from the 2009 experiment, in which the efficacy of GreenClean Liquid at varying doses and its re-application was tested, and a wider suite of water quality parameters examined to further assess potential outcomes that may be observed in an in-reservoir pilot application. Additionally, the 2011 work was intended to address concerns related to potential microcystin release as a result of algaecide application and to assess whether algaecide application was observed to result in an increase in microcystin concentration following treatment. This report focuses on the 2011 experiment. The draft results of the 2011 experiment were presented earlier in 2012 to the Interim Measures Implementation Committee of the Klamath Hydroelectric Settlement Agreement (KHSA) and communicated to the State Water Resources Control Board through a status update on KHSA Interim Measure 11 water quality studies and pilot project underway. This document represents the final report of the 2011 study.

To assess the efficacy of GreenClean Liquid, an experiment was developed and implemented in 2011. Water samples from Copco reservoir were obtained and treated with GreenClean Liquid to determine the impacts on various physical and chemical water conditions (e.g., water temperature, dissolved oxygen, pH, nutrients, microcystin, and algae species). A triplicate study was developed using 55-gallon water samples, which were treated and sampled during a 48-hour period. The experiment examined (1) the efficacy of GreenClean Liquid using the base dosage (manufacturer recommended treatment for a moderate to heavy bloom) and the response of algae after re-application of algaecide, (2) the efficacy of GreenClean Liquid at levels higher than the base dosage, (3) the efficacy of GreenClean Liquid at half of the base dosage, and (4) the water quality response in terms microcystin and nutrients.

Results suggest that GreenClean Liquid at the base dosage can be effective in reducing algae from Copco Reservoir. The 2011 algaecide study was designed based on information from studies conducted in 2008 and 2009. Overall, the study showed that GreenClean Liquid is effective in reducing algae in Copco reservoir water at recommended dosages, as well as at higher dosages. Algaecide treatment appears to increase inorganic nutrient concentrations in the water column, but the fate of these nutrients through time was not investigated. Further, the application of a hydrogen peroxide-based algaecide to water collected from Copco reservoir reduced microcystin levels by approximately 50 percent as well as reducing algae, as measured by chlorophyll *a*. The use of large volumes of water in discrete containers provided a setting to test the efficacy of treatment doses, while maintaining control containers for comparison of

results. The triplicate approach for control and selected elements of the test provided a means to bracket natural variability typical of algae in such settings.

Limitations of the study were identified, including the modest duration of the event (approximately 48 hours), the conditions at the time of the test in which low microcystin levels were present, and the fact that the discrete containers, although useful for providing a means to test several elements of the study, were not representative of an open lake environment.

As such, several recommendations are outlined herein. Identified recommendations include completing experiments at different times during the algae bloom season, assessing longer term condition (in excess of 48 hours), and an in-lake application at Copco Reservoir. Based on the results of the 2011 study work discussed in this report which, among other findings, indicated that microcystin concentrations were not increased as a result of algaecide application, PacifiCorp, in consultation with the Interim Measures Implementation Committee, proceeded with a limited pilot algaecide application (GreenClean Liquid) in Copco reservoir that was conducted on September 6, 2012. The results of that pilot application will be presented in a separate report.

1. Introduction

On February 18, 2010, the United States, the States of California and Oregon, PacifiCorp, Indian tribes, and a number of other stakeholder groups signed the Klamath Hydroelectric Settlement Agreement (KHSA). The KHSA lays out the process for additional studies, environmental review, and a determination by the Secretary of the Interior regarding whether removal of four dams owned by PacifiCorp on the Klamath River (i.e., Iron Gate, J.C. Boyle, Copco 1, and Copco 2 dams) will advance restoration of the salmonid fisheries of the Klamath Basin, and is in the public interest (which includes effects on local communities and tribes).

The KHSA includes provisions and detailed actions for the interim operation of the dams and mitigation activities prior to removal of the dams or the termination of KHSA. One of the measures – titled Interim Measure 11: Interim Water Quality Improvements – emphasizes nutrient reduction projects in the basin to enhance water quality in the Klamath River, while also addressing water quality, algal and public health issues in Klamath Hydroelectric Project (Project) reservoirs and dissolved oxygen in J.C. Boyle reservoir. The purpose of Interim Measure 11 is to improve water quality in the Klamath River during the interim period leading up to potential dam removal.

Prior to the date of the Secretarial Determination, the measure calls for PacifiCorp to fund and implement studies or pilot projects to address four categories of studies specified for Interim Measure 11:

- Development of a Water Quality Accounting Framework
- Constructed Treatment Wetlands Pilot Evaluation
- Assessment of In-Reservoir Water Quality Control Techniques
- Improvement of J.C. Boyle Reservoir Dissolved Oxygen

This study plan addresses one of the proposed activities under Interim Measure 11 over a 2-year time frame (that is, during years 2010 and 2011) to address the third of these four categories of studies.

Watercourse Engineering, Inc. (Watercourse) performed preliminary algaecide experiments in 2008 and 2009, to determine the efficacy of various types of algaecide on water samples from Copco Reservoir. An additional study was performed in 2011 based on the findings from 2008 and 2009. This document contains the details and results from the 2011 study, but also includes earlier work as appropriate.

1.1. Purpose and Objectives

In 2008 and 2009, PacifiCorp retained Watercourse Engineering to initiate effectiveness testing of environmentally-safe sodium carbonate peroxyhydrate (SCP) based algaecide applications in Copco and Iron Gate reservoirs based on controlled bench tests using water withdrawn from the reservoirs. SCP (e.g., GreenClean PRO, GreenClean Liquid) is a hydrogen peroxide-based algaecide approved for use by the U.S. Environmental

Protection Agency (EPA), and is also approved under NSF/ANSI Standard 60 (drinking water treatment chemicals). On February 27, 2006, the California Department of Pesticide Regulation (DPR) registered SCP for aquatic application as an algaecide used to control blue-green algae (see Water Quality Order No. 2004-0009-DWQ, NPDES No. CAG990005, National Pollutant Discharge Elimination System Permit for the Discharge of Aquatic Pesticides for Aquatic Weed Control in Waters of the United States, as amended by adoption of State Water Resources Control Board [State Water Board] Resolution No. 2006-0039).

The purpose of activities proposed under this Study Plan is to conduct selective localized treatments of the environmentally-safe SCP (e.g., Greenclean Pro) using water from Copco reservoir in isolated containers. Based on previous tests conducted by Watercourse Engineering, wherein algaecides were applied to discrete volumes of water collected from Copco reservoir, results indicated that SCP applications may be effective in reducing the algae standing crop and, at certain application rates, reducing microcystin concentrations in water from the reservoirs. This Study Plan extended the experiment to focus on pilot treatment application of algaecides with the principal active ingredient of SCP (e.g., GreenClean Pro, GreenClean Liquid) to reduce algal concentrations and microcystin levels, while assessing the impacts on nutrients, microcystin, chlorophyll *a*, and algae species before and after application.

Water and oxygen are the byproducts of SCP algaecides. Because cyanotoxins are stored intracellularly, algaecides can lead to releases of toxin to surrounding waters (Kenefick *et al*., 1993). Jones and Orr (1994) and Touchette *et al*. (2005) present studies demonstrating microcystins are released when *Microcystis aeruginosa* blooms were treated with algaecides including copper sulfate or SCP. This release following SCP treatment can be quite rapid (e.g., minutes), depending on the algaecide application quantity (Jones and Orr, 1994). Finally, WHO (2007) identifies that more than 95% of the toxin is contained within healthy cells, and that although dying and decaying cyanobacteria release microcystins to the water, biodegradation is typically sufficient to avoid high concentrations of microcystin dissolved in water. However, WHO also states that artificial lysing of the cell may increase dissolved concentrations in the water. For this reason, the fate of *Microcystis* and microcystin was examined in the 2011 study to assess whether microcystin concentrations were increased, decreased, or remained unchanged following application of SCP and to explore the fate of *Microcystis* following treatment.

Discussions with SCP manufacturers, as well as limited testing by Watercourse in 2009, indicate that proper SCP dosing and repeated SCP applications may be effective at reducing microcystin concentrations released from cyanobacteria (either naturally or through application of the algaecide) or otherwise present in the treated waterbody. Antoniou *et al*. (2005) identified that while conventional water treatment processes can result in increased levels of soluble toxin associated with microcystin, chemical oxidation technology using titanium dioxide (an oxidant with similar purposes as hydrogen peroxide, the active chemical in SCP) to enhance photocatalytic oxidation shows promise at reducing those concentrations. Benjamin *et al*. (2000) indicate that such oxidation can be enhanced with hydrogen peroxide, and that, although less effective, hydrogen peroxide

alone can provide treatment. Given these findings, this experiment explored the use of SCP treatment to reduce both algal concentrations and microcystin levels during a blue-green algae bloom period in Copco reservoir.

The purpose of the algaecide studies was to explore the potential benefits and limitations of algaecide as part of an overall algae management approach in Klamath River main stem reservoirs. The 2009 study was focused on comparing the effectiveness of different types of algaecides, while the 2011 study focused in more detail on the outcomes of a single environmentally safe algaecide – GreenClean Liquid, a sodium carbonate peroxyhydrate (SCP) based algaecide. SCP was identified as a preferred algaecide after the 2009 study because it was shown to be effective and its by-products, water and oxygen, are benign.

This study focused on various doses, re-application, and the implications on algal populations, microcystin, and nutrient conditions. Specific objectives of this study were to:

- Identify potential increases in nutrients with SCP treatment. Application of an algaecide leads to algae death and cell disruption or destruction. Because hydrogen peroxide is a fast acting treatment, nutrient (inorganic forms and total) concentrations were sampled prior to and after treatment to ascertain if increases in nutrients occurred with treatment.
- Determine if a hydrogen peroxide-based algaecide such as SCP, which is essentially a strong oxidizer, would lead to an increase, a decrease, or provide no change in microcystin levels. An increase in microcystin would be similar in concept to increases in nutrients related to cell disruption or destruction, and may result from the lysing of cells. Alternatively, because microcystin is an organic molecule, a hydrogen peroxide-based algaecide may oxidize both existing and potentially released microcystin and thereby decrease concentrations.
- Finally, algae species and chlorophyll *a* were included in the investigation to determine the response to algaecide applications, repeat dosing, and increased dosing.

1.2. Background

Copco reservoir has been known to support extensive seasonal algae standing crop for years (USEPA, 1978; PacifiCorp data). The role of algae as a nuisance condition, the algal community present in Copco reservoir, and the role of cyanobacteria are outlined below. Also included herein is a brief summary of algaecides used in these studies as well as the previous studies using Copco reservoir waters. In all cases, waters were collected from Copco reservoir and treated off site, in discrete containers.

1.2.1. Algae Nuisance

Although algae are a key component of aquatic ecosystems, playing a vital role in food webs and producing oxygen through photosynthesis, excessive and/or persistent phytoplankton blooms can pose both a nuisance and an environmental problem. Algae

can cause taste and odor problems in drinking water reservoirs; produce toxins that effect wildlife, livestock, or humans via contact or ingestion; present filter clogging challenges for treatment and, in certain cases, irrigation supplies; and lower the aesthetic appeal and recreational use of surface waters. Algal nuisance becomes more of an issue where eutrophication occurs in reservoirs.

Two of the most common causes of taste and odor problems in drinking water are 2-methylisoborneol (MIB) and geosmin. Blue green algae, such as *anabaena* and *pseudanabaena*, are known to produce these metabolites, respectively. Some of the algae that produce taste and odor problems also produce toxins. (However, the presence or absence of geomsin or MIB is not an indicator of toxicity (NALMS, 2007)). There are three major classes of toxins produced by blue-green algae (cyanotoxins): hepatotoxins (affecting the liver), neurotoxins (affecting the nervous system), and dermatotoxins (affecting the skin). These toxins have the potential to be harmful to human and animal health (SWRCB 2010)

Extensive blooms can reduce aesthetic appeal of reservoirs and lakes, wherein algal growth can form mats or shoreline scums that are unsightly, covering the water surface preventing swimming, and impede boating. Additionally, large algal blooms may produce offensive odors as they decompose along the shorelines. Where toxins are involved, reservoirs and other surface waters may be posted for public health warnings.

1.2.2. The Algal Community and Cyanobacteria in Copco Reservoir

The algal community in Copco reservoir consists of diatoms, golden-brown algae, green algae, dinoflagellates, cryptomonads, microflagellates, and blue-green bacteria (cyanobacteria). Diatoms and cyanobacteria typically make up the vast majority (by biovolume) of the algal community in the reservoir. The seasonal succession of phytoplankton typically progresses from diatoms in the spring (May), followed by cyanobacteria dominance in the summer (July, August, early- to mid-September) (Figure 1). Inter-annual variations are typical, as are the onset and decline of algae blooms (see Raymond 2008, 2009, 2010).

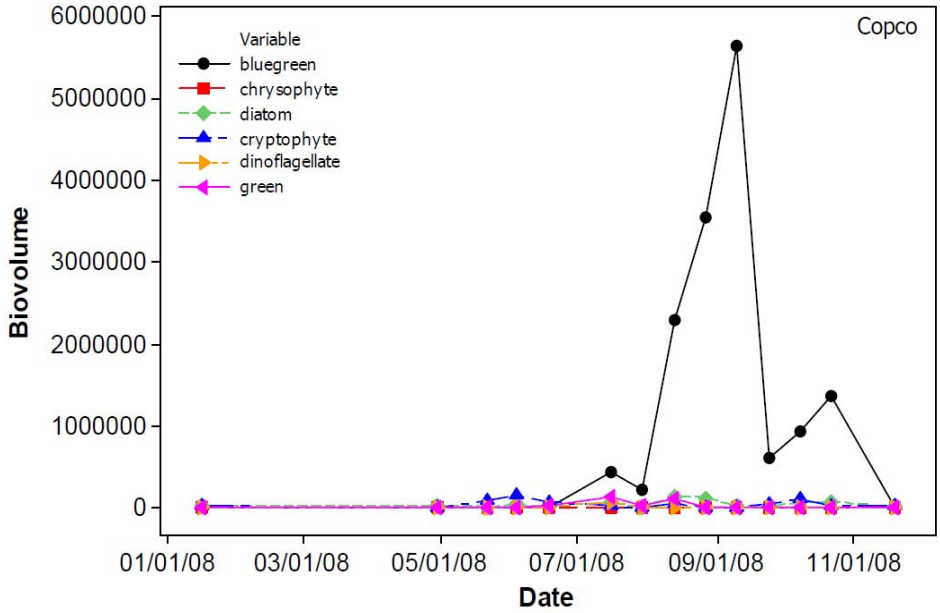

Figure 1. Seasonal changes in biovolume (µm3/mL) of various algal groups in Copco and Iron Gate Reservoir.

Cyanobacteria are of particular concern in reservoir management because they can create nuisance conditions and produce undesirable toxins. A toxin found in the Copco Reservoir is microcystin. Microcystin, a hepatotoxin, primary targets the livers in mammals. The blue-green algae that produce microcystin are *Microcystis*, *Anabaena*, *Planktothrix* (*Oscillatoria*), *Nostoc*, *Hapalosiphon*, and *Anabaenopsis* (WHO, 1999).

Challenges in managing such species include the ability for these species to tolerate elevated temperatures, reproduce at high rates, regulate their buoyancy, and for certain species, the ability to fix nitrogen. These conditions, among others, combine to create a spatially and temporally complex response of cyanobacteria populations. Heterogeneous or "patchy" distributions, accumulation of shoreline mats and scums, wind driven accumulations, variability in toxin production, and other factors contribute to the management challenge of such nuisance species.

1.2.3. History and Methods of Algae Control

Concerns about algae and methods for algae removal and treatment have been present for centuries. The use of chemical biocides can be traced back to Egyptian times, when copper was used to control marine encrustations on ship hulls (Richardson, 1997). In the 1900s, algaecides were developed that use chemicals (usually some form of copper), which are toxic to algae, either as contact or systemic toxins. More recently, oxidizer algaecides which utilize hydrogen peroxide to rupture the cell walls of the algae have been developed (Wagner, 2004).

In recent decades, with increased knowledge of lake and reservoir processes (as well as riverine processes), approaches have expanded to managing algae. In certain systems the physical removal of filamentous and mat-forming algae, along with other weeds and unwanted plants is a strategy. Biological algae control, which includes the use of animals and other plants to control algal growth, has had success, but also faces challenges

regarding invasive species. Cultural algae control includes the minimization of nutrients released into the algal environment due to human activities. Chemical treatments include the application of specific compounds (often referred to as algaecides) to waters to inhibit or directly reduce algal populations. Current typical control methods often include an array of physical (mechanical), biological, cultural, and chemical treatments and strategies. This study focused on chemical treatment, specifically algaecides.

1.3. Algaecides

Algaecides fall into four major categories: natural, copper-based, synthetic organic, and oxidizers. Use of many of these algaecides can provide a rapid removal of algae from the water column, sometimes resulting in dramatic short-term changes in algal standing crop and water clarity. Application frequency is a function of the lake or reservoir management objective (e.g., nuisance, taste and odor issues, toxin management, recreation, etc.). In certain cases, algaecides are applied annually, but more typically are applied several times through the growth season in response to weather, operations, and algal conditions to prevent or reduce algal blooms.

Algaecides kill algae either by direct toxicity or through metabolic interference (Wagner, 2004). There are many types of algaecides produced and available commercially, the two that were tested in the 2009 study were Algimycin PWF (a copper-based algaecide) and GreenClean PRO (an oxidizer). In the 2011 study, a liquid version of GreenClean PRO, called GreenClean Liquid, was tested. Other common algaecides are discussed in Deas *et al*. (2009).

1.3.1. Algimycin PWF

Algimycin PWF is an algaecide based on copper citrate chelates and copper gluconate chelates made by Applied Biochemists (http://www.appliedbiochemists.com/). The algaecide is a solution with 62 grams per liter of copper. Applying different doses of Algimycin PWF can target select types of algae including planktonic, filamentous, and rooted forms. Whether Algimycin PWF can work as an algaestatic (algae inhibitor) is unclear. Algimycin can be toxic to fish and aquatic organisms under certain conditions. Direct application of Algimycin PWF to water may cause a significant reduction in the populations of aquatic invertebrates, plants, and fish. Avoiding the treatment of more than one-half of a lake or pond at one time is recommended to avoid depletion of oxygen levels due to decaying vegetation associated with the treatment. Trout and other species of fish may be killed at application rates recommended for Algimycin PWF, especially in soft or acid waters. Washwater from equipment used to apply Algimycin PWF requires disposal in a safe manner to prevent the contamination of other water sources. Permits may be required before treating public water with Algimycin PWF.

1.3.2. GreenClean PRO

GreenClean PRO is an algaecide produced by Bio Safe Systems, LLC (http://www.biosafesystems.com/). This product consists of granules containing 85% sodium carbonate peroxyhydrate (27.6% hydrogen peroxide). GreenClean PRO can be used as an algaestatic and has no restrictions on water use after treatment (e.g., no restrictions on potable water supply, irrigation water supply, recreation use, livestock

water consumption). The algaecide is applied using a licensed applicator, with appropriate equipment, licenses, a site-specific safety plan, and conducted in accordance with a safety program. If treatment areas include extensive algae mats, a secondary treatment by GreenClean PRO may increase its efficacy. While GreenClean PRO (the granular product) can be toxic to birds, bees, and other beneficial insects at high concentrations, GreenClean liquid applied directly to waters does not have these undesirable effects. GreenClean Liquid is non-persistent and there is no bioaccumulation or sediment accumulation of the product since it degrades into water and oxygen. In addition, it is listed by the U.S. Environmental Protection Agency (EPA) as a biopesticide. EPA defines biopesticides as those "including naturally occurring substances that control pests (biochemical pesticides), microorganisms that control pests (microbial pesticides), and pesticidal substances produced by plants containing added genetic material (plant-incorporated protectants)" (EPA, 2012). EPA further identifies advantages of biopesticides as being inherently less toxic, able to target specific pests, effective in small quantities, and able to decompose quickly to avoid pollution problems caused by typical pesticides (EPA, 2012). Another benefit of SCP-based products such as GreenClean Liquid is the ability to target specific algae species by applying the algaecide at certain doses that adversely affect cyanobacteria, while minimizing effects on other species (e.g., diatoms).

1.3.3. GreenClean Liquid

Similar to GreenClean PRO, GreenClean Liquid is produced by Bio Safe Systems, LLC, and is an alternative to copper-based algaecides or algaecides with other toxic chemicals as their active ingredient. As such, it can be applied in copper restricted areas. There are no known run off or usage restrictions. GreenClean Liquid utilizes a stabilized form of hydrogen peroxide (H2O2) and pairs it with peroxyacetic acid (PAA). These two compounds create a potent oxidation reaction. Working together, PAA and hydroxide (OH) break down algae cell walls. The reaction works quickly (seconds to minutes), reducing the likelihood of mutational resistance (there is no known mutagenicity associated with this algaecide). As the algae-fighting reaction takes place, the hydrogen peroxide and PAA break down into natural compounds: water, oxygen and elements of organic acids. The concentration of PAA is extremely low (on the order of 0.0000033-0.000083 molar for the manufacturer's listed range of application rates (pers. comm. V. Choppakatla)). Like GreenClean PRO, GreenClean Liquid is listed as a biopesticide by EPA.

1.4. Previous Studies and General Treatment Options

A range of studies were completed in the Project area over the past decade to assess methods for addressing algal conditions. These studies address algal population data, response to nutrients, and algaecide. The section concludes with a brief comment about general treatment options for microcystin.

1.4.1. Raymond 2008, 2009, 2010

Three studies were completed by Raymond (2008, 2009, 2010) identifying phytoplankton conditions in the PacifiCorp project area for sampling seasons 2007, 2008, and 2009. These studies covered rivers and reservoirs, and Copco reservoir was included. The

reports include general conditions in the area, species quantification, composition, and succession, as well as potential toxin-producing cyanobacteria.

1.4.2. Moisander *et al*. 2009

In a 2008 study conducted by Moisander *et al*., grab samples at Copco and Iron Gate reservoir were collected and placed into incubation containers treated to determine the nutrients limiting growth. Treatments included: (1) control; (2) daily injections of nutrients; (3) single injection of nutrients. Nutrients included a combination of nitrate (NO3-), ammonia (NH4+), phosphate (PO43-), and urea (Moisander *et al*. 2009). Conclusions from the study suggest phytoplankton biomass growth at Copco Reservoir was limited by nitrogen. Daily injections of nitrogen resulted in increased biomass while additions of phosphorus alone did not increase phytoplankton biomass significantly. The greatest increase in phytoplankton biomass and toxin concentration resulted from the daily addition of nitrogen and phosphorus (Moisander *et al*. 2009).

1.4.3. 2008 Algaecide Experiment

In a 2008 pilot study conducted by Deas *et al*., reservoir samples were collected in 5-gallon containers and tested with three different algaecides; Algimycin PWF (copper), GreenClean Pro (hydrogen peroxide), and PAK-27 (hydrogen peroxide). The samples were analyzed for alkalinity, hardness, total organic carbon (TOC), copper, microcystin, chlorophyll *a*, phaeophytin , and algal species (Deas *et al*. 2009). The report also discusses, in detail, other established algaecides and their respective uses in California. Though the efficacy of the different algaecides was inconclusive, the pilot study provided insight into physical conditions in Copco Reservoir. Conclusions from the study suggest Copco Reservoir had soft, weakly buffered water, which may not suitable for copper-based algaecide treatments (Deas *et al*. 2009).

1.4.4. 2009 Algaecide Experiment

In 2009, another algaecide experiment was conducted based on the outcomes from the 2008 pilot experiment. The main objective of this project was to determine the efficacy of two distinct algaecides: Algimycin PWF (copper based) and GreenClean PRO (peroxide based) and their impacts on the quality of water withdrawn from Copco reservoir. To do so, a bench-top experiment was developed and implemented. The experiment was composed of three principal elements: the efficacy of Algimycin PWF and GreenClean PRO, the efficacy of GreenClean Pro at levels higher than the recommended dosage, and the response of algae after a re-application of algaecide. Results indicated that 1) both Algimycin PWF and GreenClean Pro were effective in reducing cyanobacteria, 2) that GreenClean Pro, at higher dosages, was effective in removing algae, and 3) algaecide re-application, on average, slightly reduced total algae densities, but the efficacy of the re-application on cyanobacteria was difficult to determine because the first application removed most of the cyanobacteria. A small effort was also undertaken in the 2009 study to explore non-chemical means using homogenization via ultrasonic irradiation to damage or destroy algal cells (Tanaka and Deas, 2009). Further water quality studies were recommended to learn more about the relationship between different peroxide dosages and microcystin reductions in water withdrawn from Copco reservoir.

2. Experimental Design

The tasks and work elements that were conducted under this study include planning, field testing and sampling, and reporting. Water quality sampling was carried out consistent with PacifiCorp's Quality Assurance Program Plan and Standard Operating Procedures (SOP).

The experimental design for the 2011 study was intended to extend the information gained in the 2008 and 2009 study. Brief descriptions of the 2008 and 2009 methods are included below for context. In each year attempts were made to include three control and three experimental (i.e., triplicate) samples, as well as selected individual experiments to assess a broader range of treatments. As a pilot projects, these test treatments were aimed to provide a demonstration of the potential application and likely effectiveness of treatments.

2.1. 2008 Study

The 2008 study occurred over a two-day period; August 24 and 25, 2008. The three algaecides included for testing were Algimycin PWF, GreenClean PRO, and PAK-27 (a second hydrogen peroxide based algaecide). Twelve water samples from Copco Reservoir were stored in 5-gallon containers and each algaecide treatment was replicated in three containers. There were three control (no treatment) containers. The containers were placed in groups of three on utility trays on the rubber matting.

A desired dosing of 5 ppm GreenClean PRO or PAK-27 (approximately 348 mg of algaecide per container) and 0.25 ppm Algimycin PWF (approximately 0.08 mL per container) was applied to the appropriate containers (e.g., three containers received the GreenClean PRO treatment).

The containers were sampled before treatment, one hour after treatment, and twenty-four hours after treatment. The water samples were tested for copper, microcystin, chlorophyll *a*, and phaeophytin. Findings were variable and only general trends were identified; however, the approach was deemed feasible and larger volumes of water were recommended.

2.2. 2009 Study

In 2009, 55 gallon high density polyethylene containers were used as discrete containers, and the experiment was completed outside under natural light and temperature conditions to more closely mimic reservoir conditions. Each container was covered with a clear "lid" to preclude dust and to avoid confounding conditions associated with wind. The 2009 study was performed over a 3-day period; September 1to September 3, 2009. The two algaecides included for testing were Algimycin PWF and GreenClean PRO. Twelve water samples (55 gallons each) were obtained from Copco Reservoir on August 31, 2009 and processed immediately.

The 2009 study was comprised of one principal element (algaecide) and two secondary elements (re-application of algaecide and increased algaecide dosing). Three control containers were not treated at any time during the experiment. The principal element

involved dosing three containers with 0.80 mL each of Algimycin PWF and dosing three containers with 3.48 grams each of GreenClean PRO (all dosing presented in this section are based on approximately 50 gallons of water). The secondary study elements included dosing three additional containers with different doses of GreenClean PRO at 1x, 4x, and 8x the minimum dosing level ((3.48 grams, 13.92 grams, and 27.84 grams). Subsequently, these containers were re-dosed 48-hours later (again with 3.48 grams, 13.92 grams, or 27.84 grams, respectively), to form the reapplication study element.

All containers were sampled before treatment, one hour after treatment, 24-hours after treatment, 48-hours after treatment, and 1-hour after re-treatment (if re-treatment occurred). Control containers were sampled at the same frequency. Water samples were tested for copper, microcystin, chlorophyll *a*, and phaeophytin.

The results demonstrated that hydrogen peroxide-based treatment was effective in the following areas:

- reducing only cyanobacteria at the manufacturer recommended doses for cyanobacteria treatment, i.e., cyanobacteria numbers were markedly reduced, but diatoms and green algae were not markedly affected (Figure 2).
- reducing not only cyanobacteria, but other species algal as well at higher doses (Figure 3).

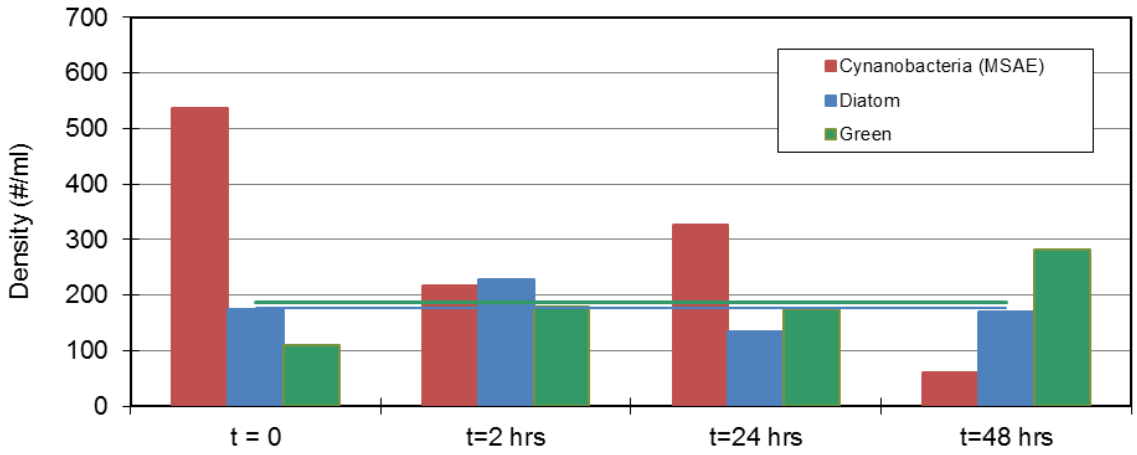

Figure 2. Recommended dosage for Cyanobacteria of GreenClean PRO: 1x recommended dosage (mean for diatoms and green algae included).

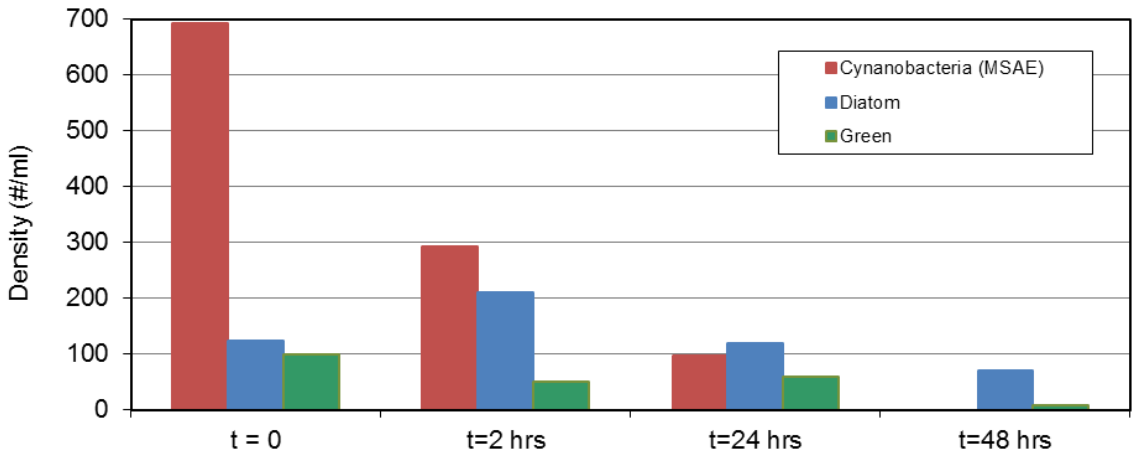

Figure 3. Higher dosage of GreenClean PRO: 4x recommended dosage.

2.3. 2011 Study

Similar to the 2009 experiment design, water samples from Copco Reservoir were obtained and treated in 55-gallon test containers off -site. The water samples were collected on September 12, 2011, and the experiments were performed for a 48-hour period over the next three days (September 13 to September 15, 2011). The copper based treatment was dropped for this experiment and the 2011 study was primarily focused on the effects GreenClean Liquid (versus the granular form GreenClean PRO) on water samples.

Ten test containers were used for the 2011 study. Containers labeled with an "A" were control containers. No algaecide was added to these containers. Containers labeled with a "B" received a dose of 2.5 ml ("base" dosage) of and a re-dosing 24 hours after the initial algaecide application. The calculated base dosage of GreenClean Liquid for cyanobacteria is between 1.8 mL (moderate bloom) and 2.9 mL (heavy bloom) per 50 gallons (all containers were filled to the same level – approximately 50 gallons). Re-application using another 2.5 mL in each of these three test containers was performed 24 hours after the initial dosing. In addition, to study the effects of higher doses of algaecide, containers labeled C1, C2, and C3 received 5 mL (2x the base dose), 7.5 mL (3x the base dose), and 10 mL (4x the base dose) of GreenClean Liquid respectively. The last container, which is labeled D1, received 1.25 mL (0.5x the base dose) of GreenClean Liquid. This last trial was to test the effect of using less than the base dosage. Re-application was not performed for the analyses that involved higher or lower than base algaecide doses (C1, C2, C3, and D1).

The containers were sampled before treatment, one hour after treatment, 24-hours after treatment, 48-hours after treatment, and 1-hour after re-treatment (if re-treatment occurred). The water samples were analyzed for nutrients (total nitrogen, ammonia, nitrate+nitrate, total phosphorus, orthophosphate, dissolved organic carbon), chlorophyll *a*, phaeophytin, and microcystin. Algal species were only sampled pre-treatment and at 48 hours. Additional details of the 2011 experiment design are presented in Appendix A.

3. 2011 Results

The water quality, microcystin, and algae species results from the 2011 study are summarized below. Details on the sampling techniques and dosing procedures are described in Appendix A.

3.1. Water Quality

Grab samples were collected for each of the ten test containers before dosing (t=0), approximately one hour and 24 hours after dosing, after re-dosing (if applicable), and after 48 hours. In order to study the effects of GreenClean Liquid on water quality, these grab samples were analyzed for total nitrogen (TN), nitrate and nitrite (NO3+NO2), nitrite (NO2), ammonia (NH4), total phosphorus (TP), orthophosphate (PO4), and dissolved organic carbon (DOC).

3.1.1. Nitrogen

Total nitrogen (TN) concentrations remained relatively constant throughout the two-day study period (Figure 4). The control containers experienced slight variations in TN concentrations; with container A2 experiencing the most changes (16% reduction after 24 hours and 20% increase from the initial concentrations after 48 hours). After 48 hours, slight increases in TN concentrations were observed in most of the containers (between 1% and 12%, excluding the aforementioned 20% increase in container A2). Containers A1 and C2 were the exceptions, where slight reductions in TN concentrations, 5% and 6% respectively, were observed. All variability was within a relative percent difference (RPD) criteria of 20 percent included in the PacifiCorp QAPP, suggesting that samples were within typical laboratory variability.

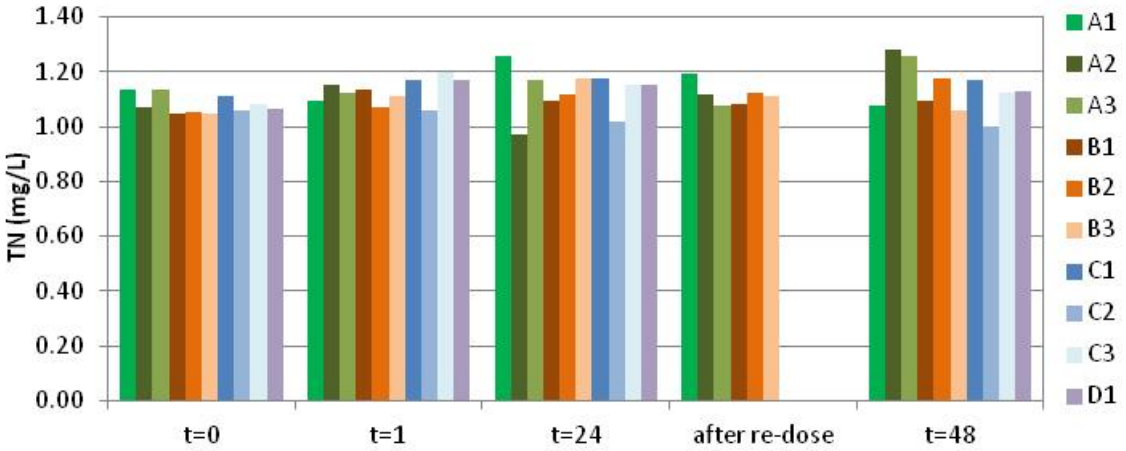

Figure 4. Total nitrogen (TN) concentrations in all test containers at various times.

Nitrite (NO2) levels were too low to be analyzed (i.e., non-detect), and so the combined nitrate and nitrite concentrations (NO3+NO2) are presented herein (Figure 5). NO3+NO2 levels were low at the beginning (at or near the reporting limit of 0.003 mg/L), but increases were seen after the addition of GreenClean Liquid. After about an hour, containers that received base doses of GreenClean Liquid (B1, B2, and B3) saw similar levels of NO3+NO2, while those receiving higher doses (C1, C2, and C3) saw

incremental increases approximately proportional to the amount of algaecide added. Container D1, which received a half-dose of GreenClean Liquid, saw a slight reduction in NO3+NO2 concentrations after one hour. From then on, NO3+NO2 concentrations decreased, with container C3 being the exception. After 48 hours, the NO3+NO2 levels in most of the containers had dropped to levels lower than the initial levels at t=0. Re-dosing appeared to have little effect on concentrations.

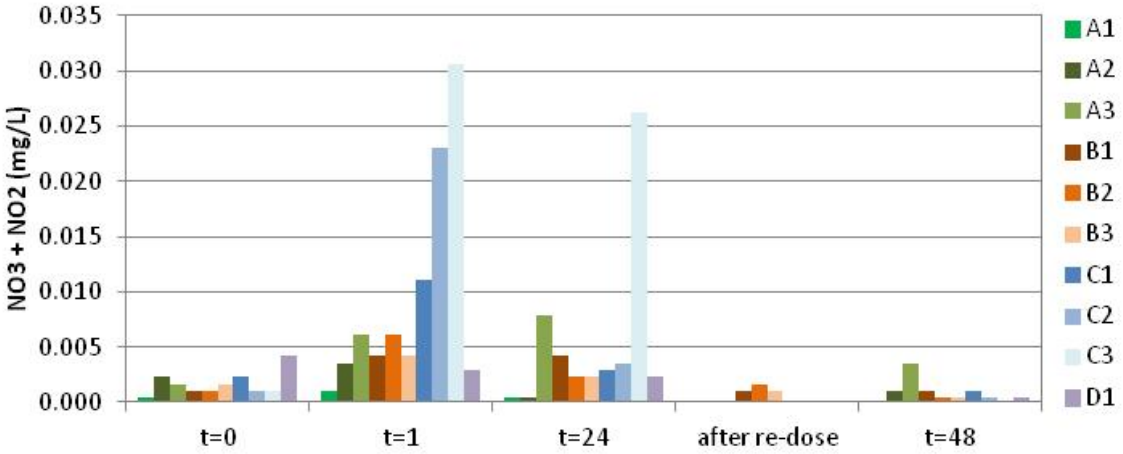

Figure 5. Nitrate and nitrite (NO3 + NO2) concentrations in all test containers at various times.

The concentrations of ammonia (NH4) in the containers were affected by the addition of GreenClean Liquid (Figure 6). The containers with lower doses (B1, B2, B3, and D1) saw increases in NH4 after an hour, but large changes in NH4 levels were only observed in containers with higher doses (C1, C2, and C3) after 24 hours. After 48 hours, an overall trend of increasing NH4 concentrations was observed in all the containers at approximately two to five times the pre-application or control concentration.

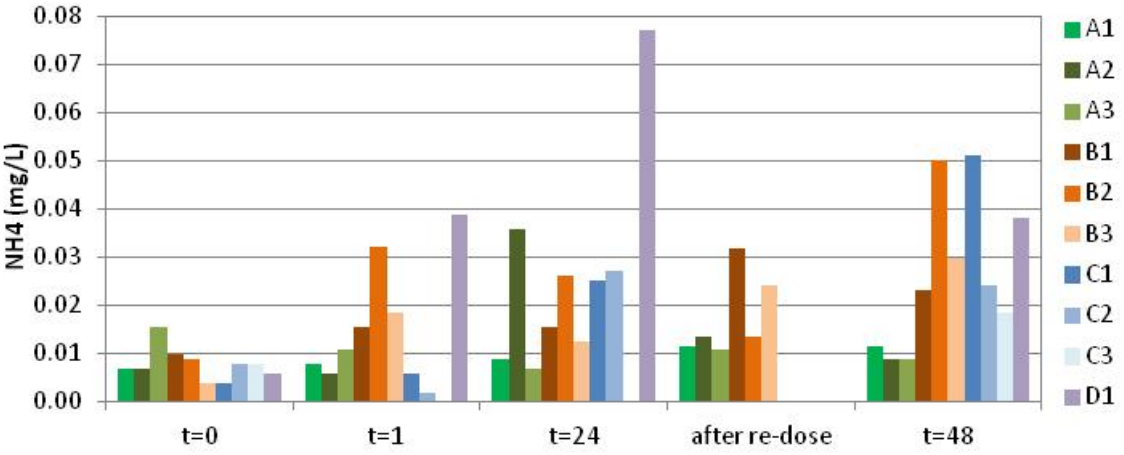

Figure 6. Ammonia (NH4) concentrations in all test containers at various times.

3.1.2. Phosphorus

An increase in total phosphorus (TP) concentrations was observed after about one hour after the addition of the algaecide (ranging from 22% to 102%), with greater increases seen in the samples receiving higher doses of GreenClean Liquid (Figure 7). Changes in

TP concentrations in these samples after 24 hours were minimal. Re-application of GreenClean Liquid in containers B1, B2, and B3 increased the TP concentrations by about the same amount as the first dose. In the control containers, changes in TP concentrations were minimal (below 15% in all cases) throughout the experiment. Discussions with the algaecide manufacturer identified the reason for the increase in TP in the treated containers as due to bound phosphorus in a stabilizer component of the algaecide. TP increases were consistent with estimated values determined by the manufacturer. The manufacturer identified that this phosphorus was, overall, in a largely non-bioavailable form[1] – a finding that is supported by the PO4 data, where increases are an order of magnitude smaller (see Figure 8 below).

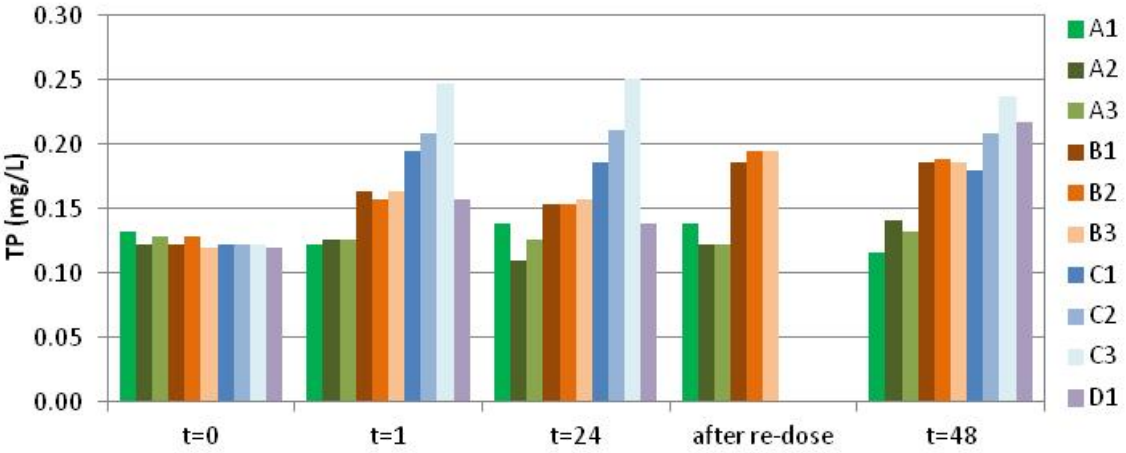

Figure 7. Total phosphorus (TP) concentrations in all test containers at various times.

The pattern of increase in orthophosphate (PO4) is similar to TP (Figure 8), except they are approximately an order of magnitude smaller – on the order of 0.01 to 0.02 mg/L for t=24 versus approximately 0.10 mg/L for TP at t=24. However, the PO4 increases in the containers labeled with "B" were comparable to the containers with higher doses of GreenClean Liquid. For the container receiving a half-dose of GreenClean Liquid (D1), no immediate increase in PO4 was observed, but an increase was seen after 24 hours. The control remained fairly constant for much of the experiment, with small decreases at t=48.

[1] The manufacturer notes that the phosphorus compound used in GreenClean Liquid has high sediment and soil adsorption coefficients, which means that the compound strongly adsorbs to sediments and suspended particles. Also, adsorption appears to be higher at lower concentrations, with a low affinity for desorption. Only small amounts of the phosphorus compound are used in GreenClean Liquid (pers. comm. V. Choppakatla).

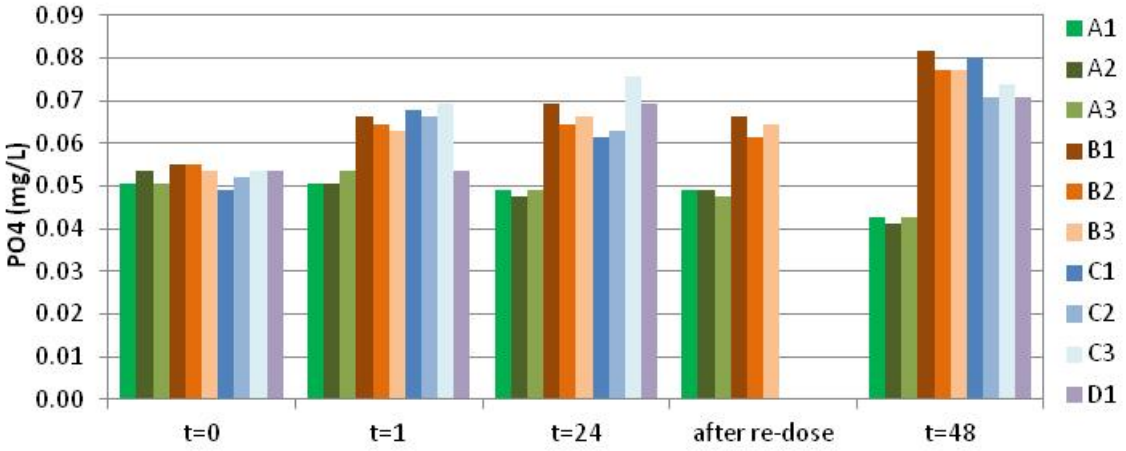

Figure 8. Orthophosphate (PO4) concentrations in all test containers at various times.

3.1.3. Dissolved Organic Carbon

Dissolved organic carbon (DOC) concentrations increased with the addition of GreenClean Liquid between 9% and 30% after the first hour, with the higher doses experiencing a larger increase (Figure 9). However, the rate of increase was offset over time by a general decline back to pre-treatment or control levels. After 48 hours, the DOC levels were about the same as at the beginning of the experiment. DOC in the control containers remained relatively constant (less than 7% variations) throughout the study period.

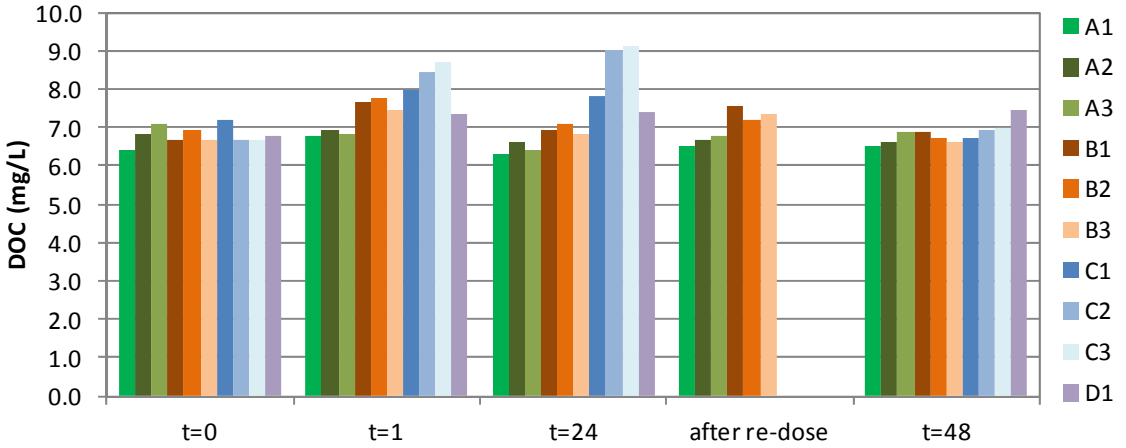

Figure 9. Dissolved organic carbon (DOC) concentrations in all test containers at various times.

3.2. Microcystin

Grab samples were also analyzed for microcystin before dosing, approximately one hour and 24 hours after dosing, after re-dosing (if applicable), and after 48 hours. These results are summarized in Table 1 and Figure 10-13. Microcystin concentrations at the beginning of the study in the containers range between 1.78 and 2.38 micrograms per liter (µg/L), with an average of 2.14 µg/L. After one hour, reductions in microcystin concentrations were approximately proportional to the amount the GreenClean Liquid added. Greater reductions were seen after 24 hours, with proportionality between reductions and dosages

still apparent. After 24 hours, re-dosing was performed on containers B1, B2 and B3, which had already been treated with a standard dose of GreenClean Liquid. In containers B1 and B2, microcystin concentrations increased with the second application of algaecide. The effect of re-dosing in B3 was consistent with the concentrations observed in the containers in which higher doses of algaecide were added. After 48 hours, microcystin concentrations in the control were similar to the beginning of the experiment (two containers experienced moderate decreases in concentration whereas the third container experienced an increase), while all treated containers, with the exception of the 0.5x treatment (D1), indicated microcystin reductions of approximately 50 percent. However, even at half the base dosage (trial D1), microcystin values were reduced on the order of 10 to 20 percent, suggesting that even low doses of algaecide may reduce microcystin to some degree.

Table 1. Microcystin concentrations (µg/L) [all values rounded to the nearest hundredth].

Control	**A1**	**A2**	**A3**
t = 0	2.26	2.12	2.13
t = 1	2.06	2.10	2.37
t = 24	1.81	2.36	0.08
After re-dose*	2.25	2.08	2.02
t = 48	1.43	1.76	2.38
	GreenClean Liquid		
Base dosage	**B1 (1x)**	**B2 (1x)**	**B3 (1x)**
T = 0	2.14	2.09	2.26
T = 1	2.14	1.89	1.89
t = 24	1.09	1.18	1.38
After re-dose	1.23	1.47	0.91
t = 48	1.00	1.33	1.02
Higher dosage	**C1 (2x)**	**C2 (3x)**	**C3 (4x)**
t = 0	2.38	2.05	2.15
t = 1	0.86	0.64	0.66
t = 24	1.52	0.53	0.22
t = 48	1.55	1.14	0.96
Half dosage	**D1 (0.5x)**		
t = 0	1.78		
t = 1	1.62		
t = 24	1.60		
t = 48	1.84		

* This sample was taken at the same time as the re-dosing of B1, B2, and B3, but there was no application or treatment to the control (A1, A2, A3)

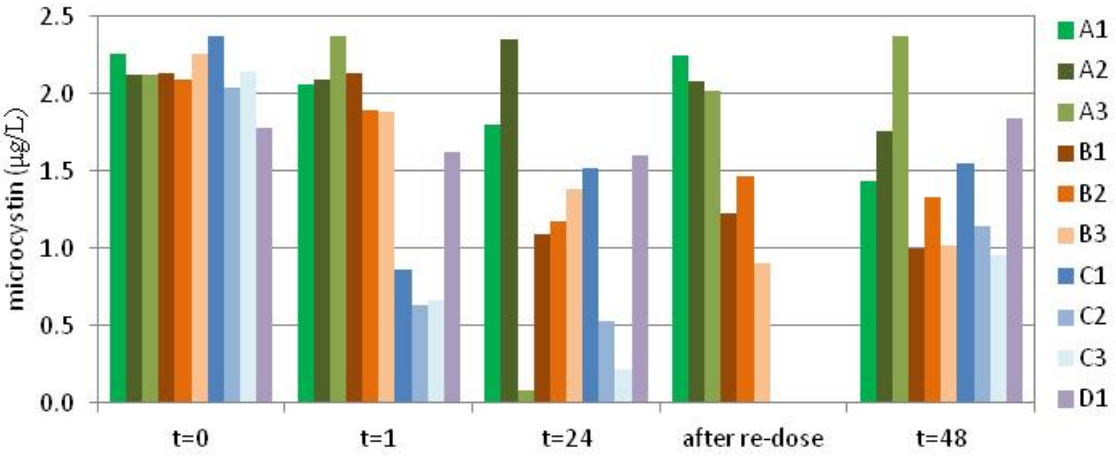

Figure 10. Microcystin concentrations in all test containers at various times.

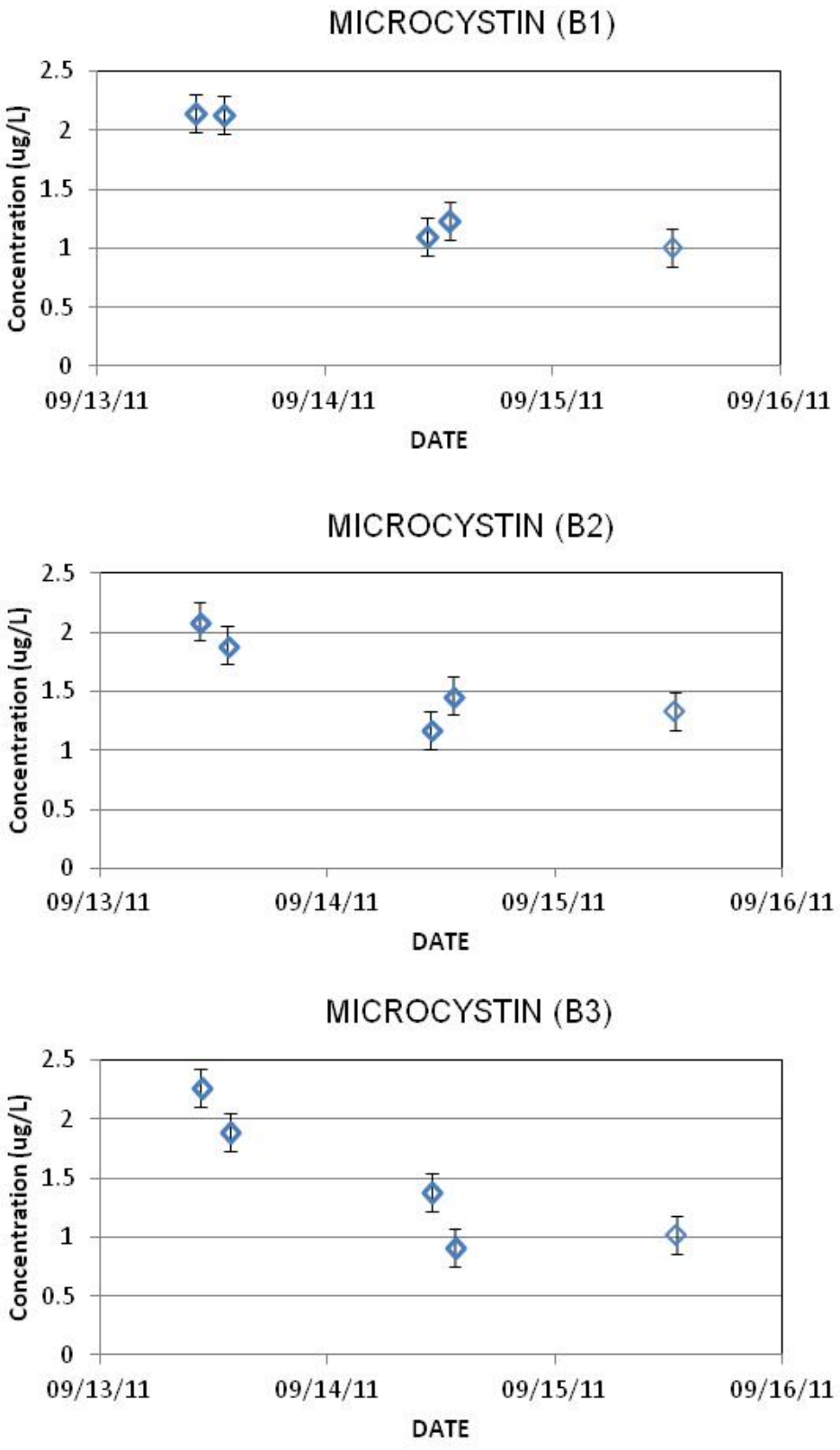

Figure 11. Microcystin concentrations for container B1, B2, and B3, which received the base dose of GreenClean Liquid at the beginning of the experiment and 24 hours after the initial dosage. Error bars are set at the detection limit (0.16 ug/L).

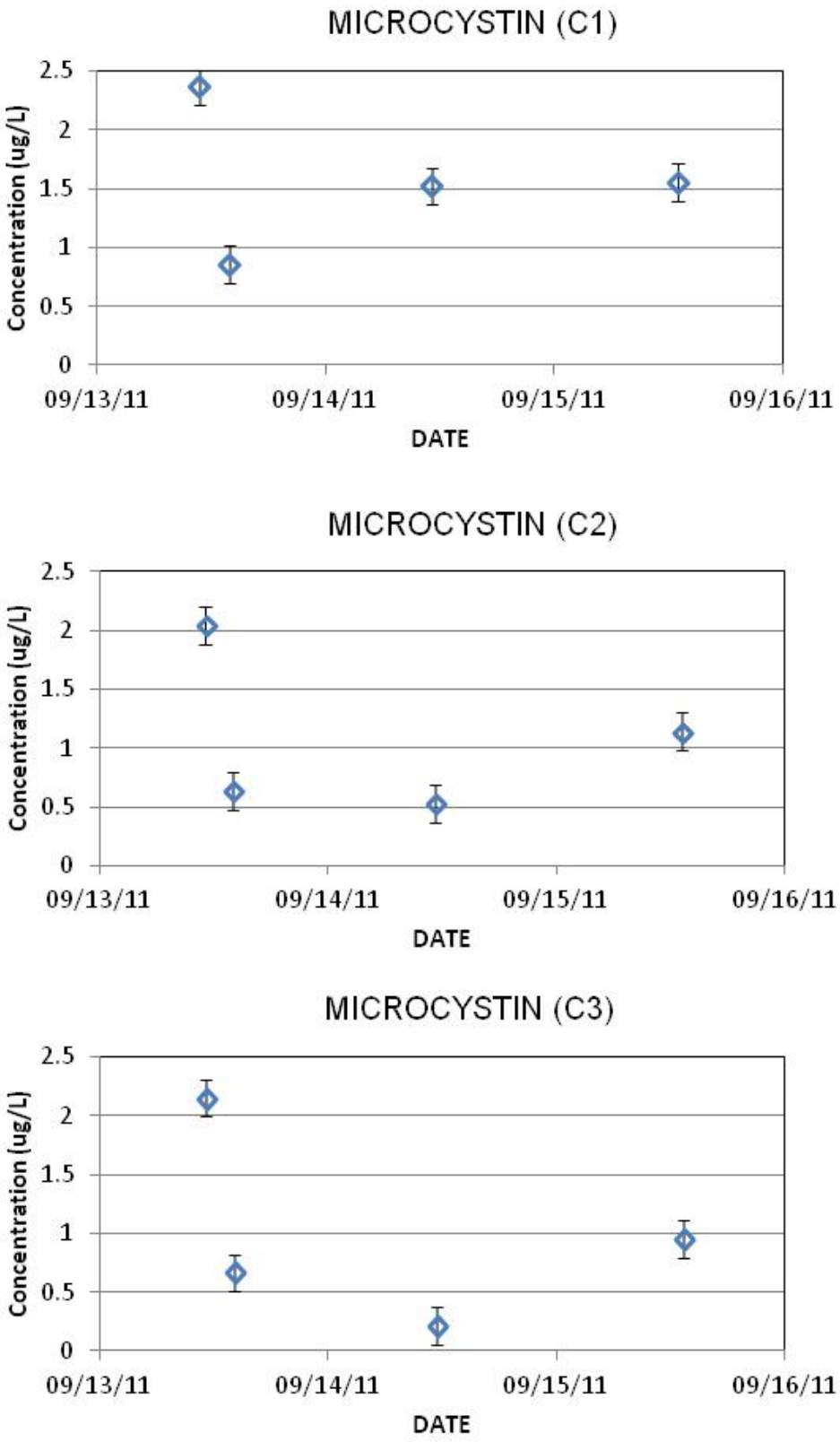

Figure 12. Microcystin concentrations for containers C1, C2, and C3, which received two times, three times and four times of the base dose of GreenClean Liquid, respectively. Error bars are set at the detection limit (0.16 ug/L).

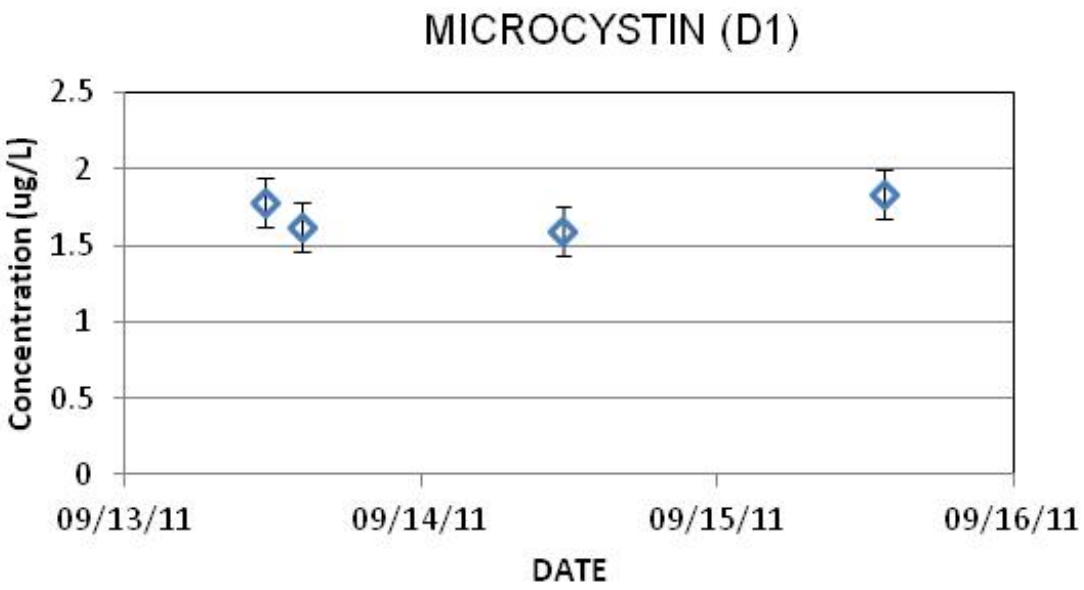

Figure 13. Microcystin concentrations for container D1, which received half of the base dose of GreenClean Liquid. Error bars are set at the detection limit (0.16 ug/L).

3.3. Algae Response

Algae response was measured with two principal methods: chlorophyll *a* (and phaeophytin) and algae species enumeration.

3.3.1. Chlorophyll *a*

Chlorophyll *a* showed marked declines immediately after application and after 24 hours (prior to re-dosing for B1, B2, and B3). Specifically, after an hour, reductions (ranging from 16% to 33%) were observed in most containers, with container B2 being the exception with only 1% reduction (Figure 14). Nevertheless, after 24 hours, chlorophyll *a* concentrations in most of the containers experienced reductions on the order of 90%, except for containers B3 and D1. The effects of re-application appeared relatively minor, which could be because most of the algae have already been broken down with the first dose of GreenClean Liquid. The sample that received half of the base dose did not experience as much reduction as the other samples. In the control containers, there were variations in chlorophyll *a* concentrations that suggest some sampling heterogeneity as well as possible ongoing algal dynamics during the study period. An important consideration is that the manufacturer states that chlorophyll *a* is the best measure of algaecide efficacy – better than algal species enumeration, which is discussed below.

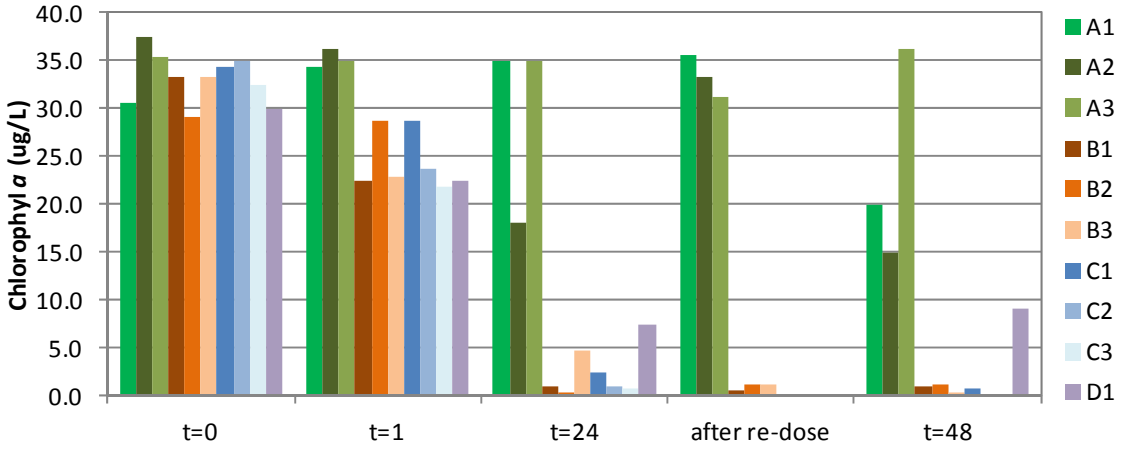

Figure 14. Chlorophyll *a* concentrations in all test containers at various times.

Observations can be made with regards to the phaeophytin concentrations in the containers as well (Figure 15). After an hour, the effects of GreenClean Liquid on phaeophytin not as distinct as with chlorophyll *a*. Reductions were seen after 24 hours, with the exception of container B2, which saw an increase in phaeophytin levels after an hour and no remarkable reduction after 24 hours. After 48 hours, phaeophytin in all containers was reduced to low levels.

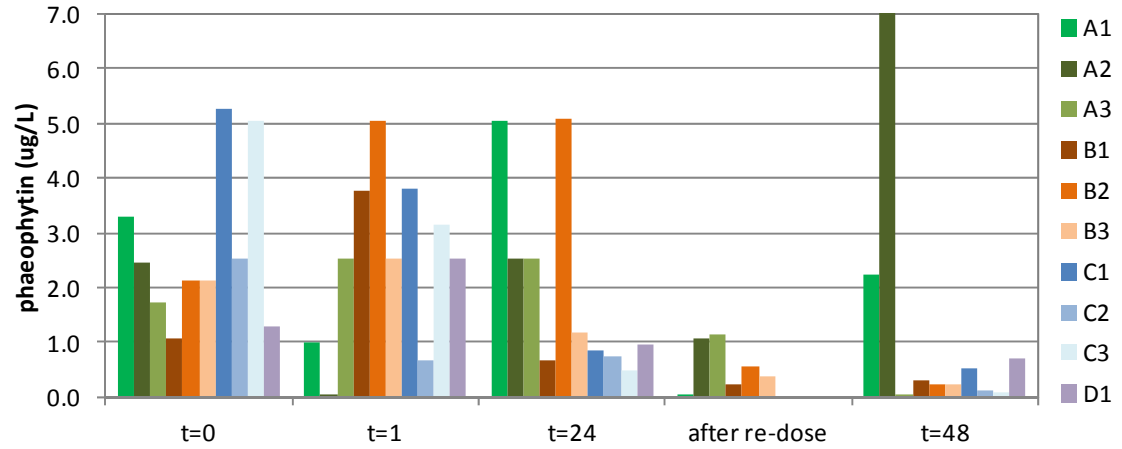

Figure 15. Phaeophytin concentrations in all test containers at various times. Note: at t=48, phaeophytin concentration in A2 is 24.3 ug/L (ppb).

3.3.2. Algal Species Enumeration

Six types of algae species groups were identified within the water samples: blue-green, chrysophyte, cryptophyte, diatom, dinoflagellate, and green. For purposes of comparison, cyanobacteria, diatoms, and greens were explored (with diatoms and cyanobacteria dominating the assemblages).

Algae species counts were collected pre-treatment and at 48 hours after dosing. Species counts for pre-treatment and at 48 hours are shown for *Aphanizomenon* and *Microcystis* in Figure 16.

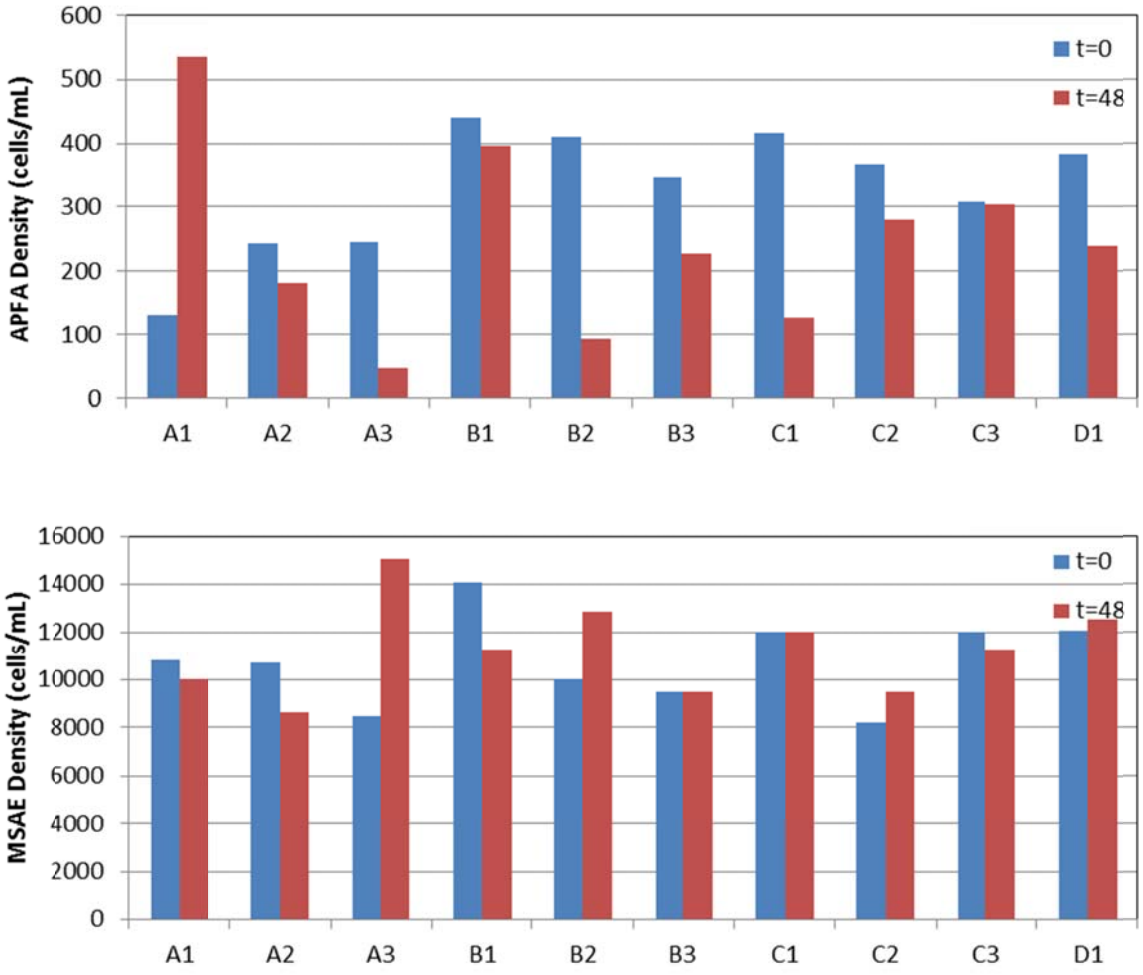

Figure 16. *Aphanizomenon* (APHA) and *Microcystis* (MSAE) density (cells/ml) for all experiments pre-treatment (t=0) and at the end of the experiment (t=48): (A) Control, (B) base dose, (C) increased doses [C1, C2, C3 at 2x, 3x, and 4x, respectively) and (D) half dose.

4. Discussion

The three specific objectives of this algaecide study were:

1. To identify potential increases in nutrients as a result of treatment. Application of an algaecide leads to algae death and cell disruption or destruction. Because hydrogen peroxide is a fast acting treatment, nutrient (inorganic forms and total) concentrations were sampled prior to and after treatment to ascertain if increases in nutrients occurred with treatment.

2. To determine if a hydrogen peroxide-based algaecide, which is essentially a strong oxidizer, would lead to an increase, a decrease, or no change in microcystin levels. An increase in microcystin would be similar in concept to increases in nutrients related to cell disruption or destruction. The key question to be addressed was would algaecide application increase microcystin concentrations as a result of lysing algae cells that may contain microcystin. Alternatively, because

microcystin is an organic molecule, a hydrogen peroxide-based algaecide may oxidize microcystin and decrease concentrations.

3. To investigate the use of algae species and chlorophyll *a* to determine the response to algaecide applications, repeat dosing, and increased dosing.

Overall, these three elements were successfully tested in 2011. Below is a discussion of water quality (objective 1), fate of microcystin (objective 2), and algae response (objective 3).

4.1. Water Quality – Nutrients

The principal findings of the water quality element of the study indicate that inorganic nutrients can increase with the application of GreenClean Liquid. For the base application rate NO2+NO3 increased approximately two to three times, for higher rates (2x, 3x, and 4x) increases were notably higher – approaching an order of magnitude. Background or control concentrations were low, on the order of 0.001 to 0.005 mg/L through the study period, while concentrations for base application rates were typically 0.005 mg/L. For the higher application rates, NO2+NO3 values incrementally increased – up to 0.03 for the 4x application rate. Interestingly, in all treatment cases, NO2+NO3 values decreased to at or below pre-treatment and control concentrations. Reasons for the initial increase are assumed to be associated with the application of GreenClean Liquid. The subsequent decrease in NO3+NO2 levels may be due to algae uptake by viable algae remaining in the container.

Ammonia (NH4) concentrations responded in a similar manner to treatment, with treated waters increasing two to three times above pre-treatment or control conditions. Pre-treatment and control values were on the order of 0.005 to 0.01 mg/L, while post-treatment conditions resulted in concentrations on the order of 0.02 to 0.05 mg/L. However, unlike NO2+NO3, these values remained approximately steady through the end of the experiment. This increase in NH4 is postulated to be associated with algaecide treatment.

Orthophosphate (PO4) concentrations increased slightly with the application of GreenClean Liquid, increasing by up to approximately 15 to 20 percent (from approximately 0.05 mg/L to 0.07 mg/L). Higher doses and redosing appeared to have minimal effect. A systematic decrease in PO4 levels was observed in the control containers during the two-day study period. This may have been due to uptake by remaining, viable algae.

Total nitrogen and phosphorus would be expected to remain constant in all containers. Overall, this was the case with TN. However, TP increased slightly. After discussion with the manufacturer of GreenClean Liquid, the increase was identified as most likely a phosphate stabilizer used in the manufacture of the product. This bound form of phosphorus used in the stabilizer is not bioavailable. Contributions of these TP additions to inorganic forms (that would be bioavailable) seems unlikely as (a) PO4 did not show variability with dosing (as TP did), and (b) the increase in TP was on the order of 0.03 to

over 0.1 mg/L, which was notably larger than the increase in PO4 (approximately 0.02 mg/L). Discussions with the manufacturer identified that the increase in TP was consistent with the dosing used in the experiment (pers. comm. V. Kumar).

Dissolved organic carbon illustrated modest increases for the base dose (approximately 1.0 mg/L), increasing DOC concentrations from approximately 6.5 mg/L to 7.5 mg/L. Slightly higher increases were observed with the higher doses (increases of 1.0 to 2.0 mg/L) with total concentration of 8.0 to 9.0 mg/L.

In general, results suggest increases in inorganic nutrients with algaecide application. There appears to be other processes at work as NO2+NO3 values and DOC values return to pre-treatment levels after 48 hours. The possibility of ongoing algal uptake of nutrients could be one explanation for the decrease in NO2+NO3. However, this trend was not observed in NH4 and PO4.

4.2. Microcystin

The study results show that GreenClean Liquid can be effective in reducing nuisance algae from the water samples (based on chlorophyll *a* results). In addition, GreenClean Liquid also reduced microcystin concentrations. Microcystin levels in the control containers were relatively constant, except for container A3, which exhibit anomalous variability. Microcystin levels in containers A1 and A2 started to decrease after the first day. Given that no algaecide was added to these containers, these changes in microcystin levels suggest that there are other dynamics occurring in the containers (e.g., natural biodegradation), inter-barrel variability, and laboratory sampling uncertainty. The concentration in the third container was more variable, but ultimately increased over the study period.

The results for the base application (B1, B2, and B3) indicated that GreenClean Liquid application reduced microcystin concentrations by about 50%. However, additional application of GreenClean Liquid was not as effective as the initial application since no noticeable additional reduction was observed after re-dosing.

Results from containers C1, C2, and C3 show that while the application of GreenClean Liquid reduced microcystin levels significantly (between 64% and 69% reductions) at the beginning, these lower levels were not sustained. After 48 hours, microcystin levels increased slightly to approximately a 50% reduction as compared to pre-application. The magnitude of increase in microcystin levels after the initial drop in concentration seems to be correlated with the amount of GreenClean Liquid added. This indicates that the presence of algaecide helps to curb the increase in microcystin levels. The exact mechanisms behind these subsequent increases in microcystin concentration after the initial reduction (increases refer to the change from a 66% to 69% reduction at 24 hours to a 50% reduction at 48 hours) in microcystin levels are uncertain. One explanation may be the continued death of damaged or dying algal cells that contribute microcystin through time, when there is little or no hydrogen peroxide remaining to maintain the initial reductions.

The half dose trial (D1) did not show appreciable change in microcystin levels, suggesting that a low dose of GreenClean Liquid may not be adequate to reduce microcystin in the water.

4.3. Algae Response

Algae did respond to treatment as indicated by chlorophyll *a*. Reductions in algal biomass using this metric showed initial reductions after an hour, with considerable reductions after 24 hours. Retreatment impacts were not discernible. The effect of a strong oxidant, such as SCP, on phaeophytin is not widely described in the literature. However, from a biochemical perspective, phaeophytin is structurally similar to a chlorophyll *a* molecule without the central magnesium ion (Mg^{2+}) present. Thus, the reduction in phaeophytin is expected given the strong oxidizing properties of SCP. The use of phaeophytin for assessment of dead algal cells (either alone or in relation to chlorophyll *a*) in this instance cannot be applied in the typical limnological approach of assessing the physiological health of algae populations. Rather, like other organic compounds that are prone to oxidation, phaeophytin showed notable decreases after application.

Interestingly, the application of GreenClean Liquid did not show a noticeable or consistent reduction in cyanobacteria. For all experiments, particularly *Aphanizomenon*, there was variability in the results. However, these results differ from the 2009 results wherein the application of GreenClean Pro (Figure 2 and Figure 3) showed clear reductions. After conferring with the manufacturer (J Kline, V. Kumar, pers. comm.) several reasons were identified. First, the granular product GreenClean Pro includes a bleaching agent. This bleaching agent provides a means to identify cells that have been adversely affected by the treatment. Treatment does not necessarily completely destroy the entire algae cell body, but rather damages the cell such that it is no longer viable. Contrary to the granular product, GreenClean Liquid lacks a bleaching agent, which can make the identification of a damaged cell more challenging. Discussions with Aquatic Analysts (pers. comm. J. Sweet) indicated that the analyst could not readily identify damaged cells, even after a re-examination of the samples. The implications of using a preservative on the species count samples was not investigated, but may also indicate a limitation to using cell counts as a basis for assessing the efficacy of the treatment. Second, the manufacturer also identified that species counts are typically not used to assess efficacy, particularly in the short-term. Rather, the use of chlorophyll *a* is recommended (Figure 14). Algae species typically respond on a longer time period, and the recommendation is that samples be taken five to seven days after treatment. This time span was outside the scope of this project. As such, the use of species data when testing the efficacy of GreenClean Liquid for this experiment is limited.

4.4. Study Limitations

Though the containers were large enough to simulate conditions for a controlled experiment, the containers did not capture the conditions present in Copco Reservoir. The container's lid blocks out dust and reduces wind influences and gives the algae complete access to sunlight. However, in actual conditions, surface accumulations due to wind and advective transport can reduce available sunlight to the water column.

Likewise, diel variations in nutrient availability, temperature, and mixing are different in Copco Reservoir. In future studies, identifying a small portion of Copco reservoir for a pilot test would provide favorable observation conditions and allow for an experiment reflecting actual reservoir conditions.

In addition, the effectiveness of re-application was difficult to ascertain due to the effectiveness of the initial dosing of GreenClean Liquid. Further, microcystin concentrations were relatively low during this experiment – on the order of 2 ug/L. Notably higher concentrations (Interim Measure 15 KHSA monitoring data) have been observed in the reservoir as part of the ongoing public health monitoring under Interim Measure 15. The effectiveness and impacts of algaecide application on reservoir water with higher concentrations of microcystin could not be explored given the conditions observed during the testing and are not known at this time. Thus, further assessment is necessary to demonstrate if the 50 percent reduction illustrated in this experiment is repeated when higher concentrations of microcystin are present. However, these results suggest that algaecide application is effective in reducing microcystin concentrations and not causing microcystin concentrations to increase above the initial concentrations following treatment.

The 48 hour time frame was also a limitation of this study. As identified by the manufacturer, declines in algae species counts may not be seen readily for 5 to 7 days after the application. The use of chlorophyll *a* was identified by the manufacturer as the best metric to measure effectiveness at reducing algae.

5. Summary and Recommendations

The 2011 algaecide study was designed based on information from studies conducted in 2008 and 2009. Overall, the 2011 study showed that GreenClean Liquid is effective in reducing algae in Copco reservoir water at standard dosages, as well as at higher dosages. In this study, algaecide treatment increased inorganic nutrient concentrations in the water column, but the fate of these nutrients through time is not completely understood at this time. Nitrate plus nitrite initially increased, then returned to pre-treatment levels. The case was similar for total dissolved carbon. However, ammonia and orthophosphate both increased and remained higher throughout the study period. Further, the application of a hydrogen peroxide-based algaecide was shown to reduce microcystin levels during the course of the experiment as well as reducing algae. The use of large volumes of water in discrete containers provided a setting to test the efficacy of treatment doses, while maintaining control containers for comparison of results. The triplicate approach for control and selected elements of the test provided a means to bracket natural variability typical of algae in such settings.

Limitations were identified, including 1) the modest duration of the event (approximately 48 hours), 2) the conditions at the time of the test in which overall low microcystin levels were present (on the order of 2-3 ug/L versus levels of one to two orders of magnitude higher during previous periods/years), and the fact that the discrete containers, although useful for providing a means to test several elements of the study, were not truly representative of an open lake environment.

Based on the 2011 findings, as well as previous experiments using Copco Reservoir water, recommendations for future work include:

- Treatment of a portion of the Copco reservoir. Containers have inherent limitation when extrapolating to on-the-ground applications in a lake management setting. An in-situ field-scale test would provide invaluable observations on the natural conditions within the reservoir (e.g., wind factors, advective influences, etc.). A desirable location would be a limited spatial area with minimal water movement and exposure to wind.

- Completing experiments during different times in the algae bloom season. Completing experiments at different period of the summer would provide an opportunity to assess treatment with different algal assemblages present, different levels of algal standing crop, and different levels of microcystin present in the water. These experiments could be completed in discrete containers as used in the 2009 and 2011 studies, or through small pilot applications in isolated areas of Copco reservoir at different times of the year.

- Assessment of longer term conditions. The treatment window of 48 hours provided useful insight into treatment efficacy and outcomes. However, a longer period of assessment (e.g., 96 hours or more) would lend additional insight into the fate of nutrients, remaining viable algae, longer-term response of microcystin, and other elements. These experiments could be completed in discrete containers as used in the 2009 and 2011 studies, or in a small pilot application in an isolated area of Copco reservoir if the location could be isolated to prevent mixing of the area with the larger reservoir. Without effective isolation of a small in-reservoir pilot treatment area from the larger reservoir, it would be difficult to determine whether conditions observed following the treatment were representative of the treatment itself or the effects of wind mixing and advection of algae and microcystin from the larger reservoir into the treated area.

6. References

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Personal Communications

Jeff Kline, BioSafe Systems, LLC. April 29, 2010; July 19, 2012; October 9, 2012

Vijay Kumar Choppakatla, Ph.D, BioSafe Systems, LLC. July 19, 2012; September 11, 2012; October 9, 2012.

Jim Sweet, Aquatic Analysts. January 30, 2012.

Appendix A

Experimental Implementation

A.1. Copco Reservoir Water Collection

Water samples were collected from Copco Reservoir on September 12, 2011 from the boat dock located on the reservoirs southern shore. Overall, the reservoir was calm, with a light breeze coming towards the dock. The cyanobacteria *Aphanizomenon flos aquae* and *Microcystis aeruginosa* were visible in the water during sample collection. There was some near-shore rooted vegetation where the samples were collected.

On-site preparation (setup and environmental rinses) began at approximately 11:00 am and water sample collection began at 11:57 am. A flat-bed truck was used to transport twelve 55-gallon barrels used as containers for the study. To avoid potential accidents and injury, the extension cords and hoses were duct taped to the ground and to the side of the dock. The pump was connected to the hose and lowered about a meter into the lake. The connected hose was left running for ten minutes to flush out any dirt or other residue that was inside the hose. Each of the twelve 55-gallon container was thoroughly washed using water from the lake (i.e., an environmental rinse using about 3 to 5 gallons of water per container).

To obtain containers with similar water quality and algal content, each container was initially filled one-quarter of the way (approximately 12 gallons). Each container was subsequently filled in one-quarter increments until full (about 50 gallons in each). Filling the containers incrementally ensured sample consistency between all containers. When filling the containers, the hose was placed near the edge of the container to avoid splashing or creating disturbances to the algae. In addition to the ten containers needed for the algaecide study, two other containers were filled and these were to be used as environmental rinse water during the experimental phase of the study. (Note: the water in the environmental rinse container will be slightly different from those of the treatment containers after treatment.).

The pump operated at a rate of about 5 to 7 gallons per minute. The range was due to the location and depth of the pump. It took approximately 2 minutes to fill each container one-quarter of the way and about 8 to 10 minutes (cumulatively) to completely fill any one container. Overall, it took approximately two hours to fill all twelve containers. The lids of each container were washed with environmental water from the lake and secured tightly to each barrel after the containers were filled.

A.2. Sampling Setup and Procedures

The twelve containers carrying water samples were unloaded from the flat-bed truck at the testing facility. The containers were placed on plywood sheets on a level patch of ground.

The containers were covered with clear vinyl sheeting to allow sunlight and oxygen to enter into the container, but keep the addition of dust and other materials to a minimum (Figure A-1). The vinyl sheeting was stretched across a 2-foot circular frame for ease of placement. The covers were anchored to the containers using duct tape; this allowed for easy access into each container and avoided forming an air tight seal.

At the start of each sampling period, a YSI Professional Series probe (YSI probe) was used to the measure the water temperature, barometric pressure, dissolved oxygen, pH, and ORP in each container. A Kestrel handheld weather station (model #4000) was used to collect weather data; specifically 30-second averaged air, dew point and wet bulb temperature, relative humidity, barometric pressure, and the average and maximum wind speed. Additionally, a HOBO Water Temp Pro v2 (by Onset Computer Corp) was placed in each container to monitor water temperature every 30-minutes during the study period.

To collect samples, 4-inch, class 125 PVC pipes and rubber stoppers were used to extract samples from the containers. To prevent contamination between samples, each container had its own designated PVC pipe and rubber stopper which were used throughout the study period. Before samples were taken, the PVC pipe was used to stir the content of the container to entrain/re-suspend material that had adhered to the sides of the container or settled to the bottom to ensure a roughly even distribution of material throughout the water column. To extract water samples, the pipe was gently placed down the middle of the container, and pushed into the rubber stopper. The water sample was thus collected on the inside of the pipe and trapped by the rubber stopper. The water contained in the pipe was then removed from the container and poured into an 8-liter churn splitter from which all of the samples were taken (Figure A-2). The clear cover was then placed back on the container and the equipment was washed using distilled water. All samples were stored in ice chests after collection for transport to the appropriate laboratories.

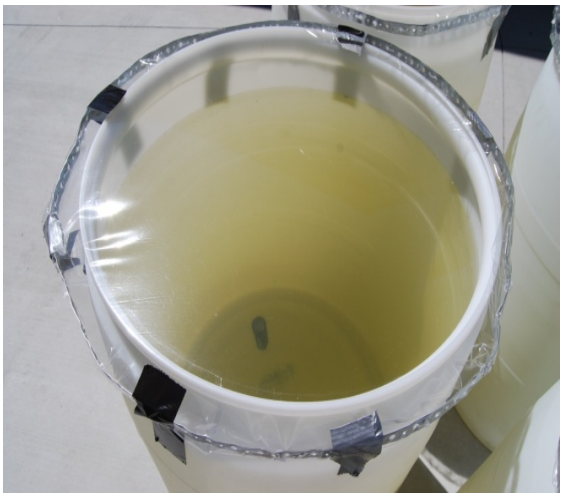

Figure A-1. Close-up of the clear vinyl sheeting cover and suspended logger.

Figure A-2. Pouring water sample into the churn splitter.

A.3. Dosing Information and Calculation

Containers labeled with a "B" were given an initial treatment (base dosage) and then a follow-up dosage approximately 24-hours later. Container(s) labeled with a "C" were given treatment that is higher than the base dosage. The container labeled "D1" was given treatment that is half of the base dosage. No re-application was given for containers labeled with a "C" or "D". Appropriate amounts of GreenClean Liquid for each container were measured using a 5 mL (0.1 mL increment) pipette.

GreenClean Liquid has a target treatment level of 3 to 5 gallons per acre-foot depending on bloom density. As such, for a 50-gallon water sample, the base dosage is between 1.8 mL (moderate bloom) and 2.9 mL (heavy bloom) of GreenClean Liquid (Eqn. 1 and Eqn. 2). For this study, 2.5 mL of GreenClean Liquid was used to represent the base dosage.

$$3\frac{gallons}{acre-ft} = 0.034852\frac{mL}{gallon}\cdot 50\frac{gallons}{drum} = 1.8\frac{mL}{drum} \qquad \text{(Eqn. 1)}$$

$$5\frac{gallons}{acre-ft} = 0.058086\frac{mL}{gallon}\cdot 50\frac{gallons}{drum} = 2.9\frac{mL}{drum} \qquad \text{(Eqn. 2)}$$

A.4. Container Information

A total of ten containers were used for this study (

Table A-1).

Table A-1. Container ID, water temperature logger ID, algaecide treatment type and amount and sample times.

Container ID	Logger ID	Dosing			Sampling Times				
		Type	Amount	Re-Dose	t = 0	t = 1	t = 24	After re-dose	t = 48
A1	1204754	Control	n/a	n/a	x	x	x	x	x
A2	2386756	Control	n/a	n/a	x	x	x	x	x
A3	2045499	Control	n/a	n/a	x	x	x	x	x
B1	2045492	GreenClean Liquid	2.5 mL	Yes	x	x	x	x	x
B2	2045500	GreenClean Liquid	2.5 mL	Yes	x	x	x	x	x
B3	2045509	GreenClean Liquid	2.5 mL	Yes	x	x	x	x	x
C1	2045493	GreenClean Liquid	5.0 mL	No	x	x	x		x
C2	1094918	GreenClean Liquid	7.5 mL	No	x	x	x		x
C3	1204769	GreenClean Liquid	10.0 mL	No	x	x	x		x
D1	2045496	GreenClean Liquid	1.25 mL	No	x	x	x		x

A.5. Sampling Times

Sampling in the control, and first three GreenClean Liquid containers occurred five times over the course of three days. The second set of GreenClean Liquid containers (those that did not receive re-application after 24 hours) were sampled four times during the study period. In total, fifty samples were taken (Table A-2). The sampling tended to occur in the mid- to late-morning.

The water quality nutrient suite (TN, NO3+NO2, NH4, TP, PO4, DOC, chlorophyll *a*, and phaeophytin) were sent to the University of California, Davis Biogeochemistry Laboratory [Randy Dahlgren – PI]. The microcystin analyses were sent to CH2MHill Laboratories in Corvallis, Oregon. The algal species (and density and biovolume) were sent to Aquatic Analysts in Friday Harbor, Washington. The chlorophyll *a*/pheophyton samples were preserved with $MgCO_3$ (magnesium carbonate), and the algal species samples were preserved with Lugols solution (a type of iodine solution); the microcystin samples were not preserved.

Table A-2. Sample IDs, container, sampling date and time, and types of samples taken.

Sample ID	Container	Sampling		Number of Sample Bottles		
		Date	Time	Water Quality	Microcystin	Algal Species
ALG 001	A1	9/13/2011	9:52	1	2	1
ALG 002*	A3	9/13/2011	10:12	1	2	1
ALG 003*	A1	9/13/2011	12:47	1	2	1
ALG 004	B1	9/13/2011	10:20	1	2	1
ALG 005	B2	9/13/2011	10:30	1	2	1
ALG 006	B3	9/13/2011	10:38	1	2	1
ALG 007	C1	9/13/2011	10:44	1	2	1
ALG 008	C2	9/13/2011	11:08	1	2	1

Sample ID	Container	Sampling		Number of Sample Bottles		
		Date	Time	Water Quality	Microcystin	Algal Species
ALG 009	C3	9/13/2011	11:13	1	2	1
ALG 010	D1	9/13/2011	11:20	1	2	1
ALG 011*	A2	9/13/2011	10:07	1	2	1
ALG 012	A2	9/13/2011	12:57	1	2	1
ALG 013	A3	9/13/2011	13:09	1	2	1
ALG 014	B1	9/13/2011	13:20	1	2	1
ALG 015	B2	9/13/2011	13:28	1	2	1
ALG 016	B3	9/13/2011	13:45	1	2	1
ALG 017	C1	9/13/2011	13:53	1	2	1
ALG 018	C2	9/13/2011	14:01	1	2	1
ALG 019	C3	9/13/2011	14:10	1	2	1
ALG 020	D1	9/13/2011	14:19	1	2	1
ALG 021	A1	9/14/2011	10:08	1	2	1
ALG 022	A2	9/14/2011	10:17	1	2	1
ALG 023	A3	9/14/2011	10:28	1	2	1
ALG 024	B1	9/14/2011	10:42	1	2	1
ALG 025	B2	9/14/2011	10:52	1	2	1
ALG 026	B3	9/14/2011	11:01	1	2	1
ALG 027	C1	9/14/2011	11:08	1	2	1
ALG 028	C2	9/14/2011	11:16	1	2	1
ALG 029	C3	9/14/2011	11:24	1	2	1
ALG 030	D1	9/14/2011	11:31	1	2	1
ALG 031	A1	9/14/2011	12:39	1	2	1
ALG 032	A2	9/14/2011	12:47	1	2	1
ALG 033	A3	9/14/2011	12:55	1	2	1
ALG 034	B1	9/14/2011	13:07	1	2	1
ALG 035	B2	9/14/2011	13:15	1	2	1
ALG 036	B3	9/14/2011	13:24	1	2	1
ALG 037	C1	n/a	n/a	n/a	n/a	n/a
ALG 038	C2	n/a	n/a	n/a	n/a	n/a
ALG 039	C3	n/a	n/a	n/a	n/a	n/a
ALG 040	D1	n/a	n/a	n/a	n/a	n/a
ALG 041	A1	9/15/2011	12:06	1	2	1
ALG 042	A2	9/15/2011	12:14	1	2	1
ALG 043	A3	9/15/2011	12:24	1	2	1
ALG 044	B1	9/15/2011	12:33	1	2	1
ALG 045	B2	9/15/2011	12:40	1	2	1
ALG 046	B3	9/15/2011	12:50	1	2	1

Sample ID	Container	Sampling		Number of Sample Bottles		
		Date	Time	Water Quality	Microcystin	Algal Species
ALG 047	C1	9/15/2011	13:00	1	2	1
ALG 048	C2	9/15/2011	13:10	1	2	1
ALG 049	C3	9/15/2011	13:18	1	2	1
ALG 050	D1	9/15/2011	13:26	1	2	1

*At t=0, bottle ALG 011 was filled immediately after ALG 001, and so bottle ALG 002 replaces ALG 003 at t=0, and bottle ALG 003 was used in place of bottle ALG 011 at t=1.

Appendix B

Field Data

Prior to each sampling, field conditions were measured using a Kestrel handheld weather station (model #4000) and YSI Professional Series Probe (Professional Plus), respectively. Weather measurements included 30-second averaged air, dew point and wet bulb temperature (degrees C), relative humidity (percent), barometric pressure (inches of Hg), and the average and maximum wind speed (m/s). The container measurements included water temperature (degrees C), barometric pressure (mmHg), dissolved oxygen (percent and mg/L), pH, and ORP.

The weather condition data is presented in

Table B-1. The container measurements prior to each sampling are presented in Table B-2 through
Table B-6. Finally, the water temperatures in each container (as measured by the HOBO Water Temp Pro v2) are presented in Figure B-1 through Figure B-3.

Table B-1. Weather conditions prior to sampling.

Date	Time	Temperature (°C)			Relative Humidity (%)	Pressure (inHg)	Wind Speed (m/s)
		Air	Dew Point	Wet Bulb			
09/13/11	09:10	21.5	10.3	14.5	49.4	27.00	0.0
09/14/11	10:00	20.9	10.3	14.6	48.8	26.87	0.4
09/15/11	12:00	21.5	10.4	14.7	48.1	26.80	0.4

Table B-2. Container conditions prior to first (t = 0) sampling (09/13/2011).

Container	Time	Water Temperature (°C)	Pressure (mmHg)	Dissolved Oxygen		pH
				(percent)	(mg/L)	(-)
A1	9:10	23.1	27.012	123.1	10.57	9.30
A2	-	22.8	27.011	122.0	10.48	9.34
A3	-	22.2	27.014	117.0	9.90	9.27
B1	-	22.7	27.015	114.8	9.93	9.35
B2	-	22.8	27.018	110.4	9.63	9.33
B3	-	22.8	27.017	116.0	9.92	9.33
C1	-	22.2	27.012	114.0	9.70	9.34
C2	-	22.5	27.015	113.6	9.79	9.35
C3	-	22.2	27.015	113.7	9.86	9.37
D1	-	22.4	27.017	118.5	10.26	9.38

Table B-3. Container conditions prior to the t = 1 sampling (09/13/2011).

Container	Time	Water Temperature (°C)	Pressure (mmHg)	Dissolved Oxygen		pH
				(percent)	(mg/L)	(-)
A1	12:35	24.4	26.99	128.9	10.7	9.40
A2	12:46	23.9	26.98	133.2	10.92	9.39
A3	12:57	23.5	26.98	126.5	10.55	9.42
B1	13:07	23.0	26.97	133.5	10.98	9.35
B2	13:18	24.1	26.97	125.6	10.24	9.33
B3	13:27	24.1	26.97	106.1	8.81	9.36
C1	13:43	24.5	26.96	134.4	11.13	9.38
C2	13:50	24.1	26.95	132.5	10.82	9.31
C3	13:57	25.5	26.95	139.4	11.11	9.26
D1	14:10	26.3	26.95	142.1	11.22	9.36

Table B-4. Container conditions prior to the t = 24 sampling (09/14/2011).

Container	Time	Water Temperature (°C)	Pressure (mmHg)	Dissolved Oxygen		pH
				(percent)	(mg/L)	(-)
A1	10:01	24.2	26.883	128.0	10.76	9.41
A2	10:07	23.6	26.882	124.7	10.51	9.46
A3	10:16	23.0	26.884	120.0	10.00	9.44
B1	10:28	24.9	26.882	117.9	9.58	9.25
B2	10:41	23.2	26.882	112.1	9.27	9.28
B3	10:51	23.8	26.88	111.0	9.35	9.26
C1	10:59	22.7	26.878	115.2	9.77	9.27
C2	11:07	23.0	26.879	112.2	9.57	9.23
C3	11:14	23.8	26.874	116.3	9.72	9.24
D1	11:23	24.1	26.872	108.1	8.83	9.25

Table B-5. Container conditions prior to sampling after re-application (09/14/2011).

Container	Time	Water Temperature (°C)	Pressure (mmHg)	Dissolved Oxygen		pH
				(percent)	(mg/L)	(-)
A1	12:31	25.2	26.829	136.2	11.10	9.46
A2	12:38	24.5	26.825	124.2	10.18	9.47
A3	12:46	24.0	26.824	125.2	10.30	9.45
B1	12:58	25.8	26.817	125.2	10.11	9.23
B2	13:06	24.3	26.817	126.4	10.46	9.24
B3	13:14	24.7	26.816	129.8	10.56	9.22
C1	n/a	n/a	n/a	n/a	n/a	n/a
C2	n/a	n/a	n/a	n/a	n/a	n/a
C3	n/a	n/a	n/a	n/a	n/a	n/a
D1	n/a	n/a	n/a	n/a	n/a	n/a

Table B-6. Container conditions prior to t = 48 sampling (09/15/2011).

Container	Time	Water Temperature (°C)	Pressure (mmHg)	Dissolved Oxygen		pH
				(percent)	(mg/L)	(-)
A1	11:57	23.4	26.824	112.9	9.53	9.53
A2	12:06	22.5	26.821	117.4	10.00	9.57
A3	12:13	22.1	26.824	106	9.12	9.43
B1	12:23	23.5	26.822	102.6	8.63	9.15
B2	12:31	22.4	26.821	102.8	8.98	9.18
B3	12:41	22.8	26.820	107.3	9.11	9.14
C1	12:50	21.3	26.819	112.5	9.87	9.16
C2	13:01	21.8	26.819	126.8	11.70	9.22
C3	13:09	22.8	26.818	141.6	12.09	9.17
D1	13:17	23.2	26.818	90.2	7.68	9.25

The water temperatures in each container were measured using a HOBO Water Temp Pro v2 logger set at 30-minute intervals. (Figure B-1 to Figure B-3)

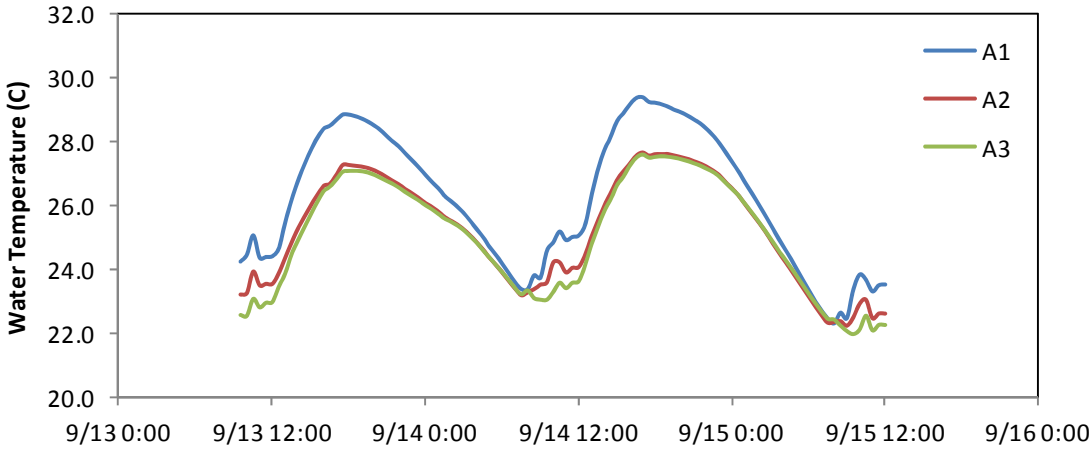

Figure B-1. 30-minute water temperatures in containers A1, A2, and A3 during the study period.

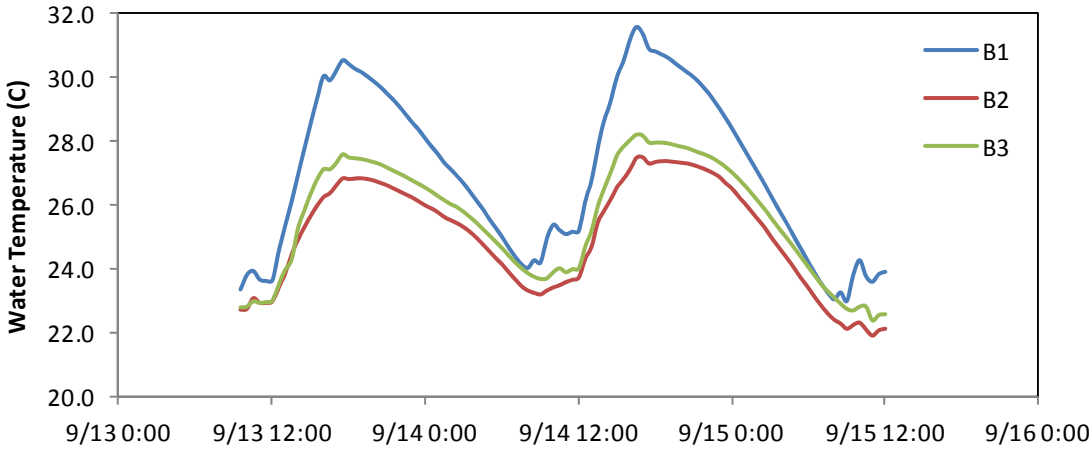

Figure B-2. 30-minute water temperatures in containers B1, B2, and B3 during the study period.

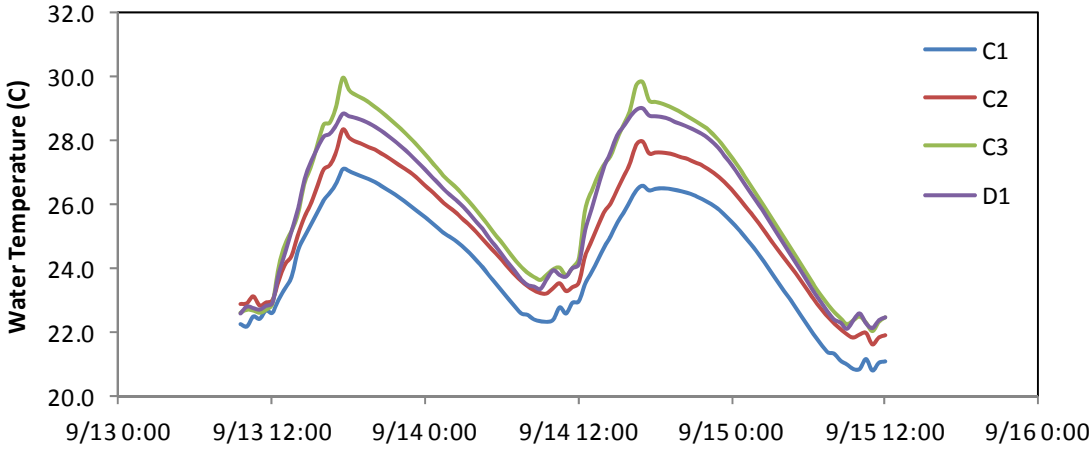

Figure B-3. 30-minute water temperatures in containers C1, C2, C3, and D1 during the study period.