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EFFICACY OF THREE ALGAECIDES ON ALGAL BLOOMS IN HYPEREUTROPHIC LAKES

by
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AN ABSTRACT OF THE THESIS OF

Sara Ali Dia for Master of Science, Environmental Sciences
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Title: Efficacy of Three Algaecide on Algal Blooms in Hypereutrophic Lakes

Cyanobacterial blooms are an emerging problem worldwide, affecting many important freshwater systems. The proliferation of these blooms has been linked to public health concerns and to the impairment of the designated uses of several fresh water systems. Several studies have linked the increase in bloom frequency to anthropogenic activities, particularly increased nutrient loading. While basin-level management measures that aim to control nutrient loading are the most effective on the long-term, they are hard to implement given socio-economical constrains. As such, the use of chemical algaecides can provide a viable short-term mitigation measure that aims to control these blooms. In this study, the efficacy of three algaecides, namely Copper Sulfate, Potassium Permanganate, and Diquat, was examined with regards to controlling two toxin-releasing cyanobateria, *Aphanizomenon flos-aquae* and *Microcystis aeruginosa*. The efficacy of each algaecide was quantified under laboratory conditions over a range of selected dosages. The results showed that Diquat and Copper Sulfate were significantly more effective than Potassium Permanganate in controlling *Microcystis*; yet all three algaecides were found to be effective in controlling *Aphanizomenon* blooms. Reduction rates for both cyanobacteria varied temporally, with the largest drop in concentration recorded between 48 and 72 hours of treatment. Following algaecide application, regrowth was observed for *Microcystis aeruginosa* but not for *Aphanizomenon flos-aquae*. The experimental findings are then used to provide recommendations on optimal dosages for each algaecide taking into account the type of cyanobacteria bloom.

Keywords: Algaecides, Copper Sulfate, Potassium Permanganate, Diquat, *Microcystis aeruginosa*. *Aphanizomenon flos-aquae.*

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CHAPTER I

INTRODUCTION

Anthropogenic eutrophication is a global problem affecting many important aquatic systems. Nutrient loading, particularity phosphorus and nitrogen, have increased over the past decades as a result of land management, fossil fuel use, production of fertilizers, and human development (Anderson & Garrison, 1997; Galloway et al., 2008; Schlesinger, 1993; R. A. Smith, Alexander, & Schwarz, 2003; Taylor & Townsend, 2010; Vitousek et al., 1997). Excessive nutrient loading has been one of the main drivers for the proliferation of Harmful Algal Blooms (HABs), hypoxia, fish kills, and the loss of key ecological functions and services (Anderson, Glibert, & Burkholder, 2002; Conley et al., 2009; Leira, Chen, Dalton, Irvine, & Taylor, 2009; Paerl & Scott, 2010; Richardson & Jørgensen, 1996; Turner, Rabalais, Justic, & Dortch, 2003). Nutrient enrichment often affects phytoplankton species composition and favors species that are undesirable, such as cyanobacteria. Additionally, elevated water temperatures appear to have a role in the proliferation of cyanobacterial blooms, accordingly global warming is expected to play an important role in promoting cyanobacteria blooms (Briand, Leboulanger, Humbert, Bernard, & Dufour, 2004; Elliott, 2010; Michalak et al., 2013; Paerl & Huisman, 2008; Paerl & Paul, 2012; Paerl & Scott, 2010; Robarts & Zohary, 1987). Cyanobacteria are the major algae type associated with the proliferation of HABs and impairments of freshwater system (Chorus & Bartram, 1999; Hallegraeff, 1993; Heisler et al., 2008; Hudnell, 2008).

Some cyanobacteria have the ability to release toxins that can cause risks to human health and disrupt aquatic ecosystems (Anderson et al., 2002; W. Carmichael,

1997; W. W. Carmichael, 2001; Falconer, 1999, 2005; Leira et al., 2009; Paerl, Fulton, Moisander, & Dyble, 2001; Paerl & Otten, 2013; R. A. Smith et al., 2003; Wang et al., 2013). *Microcystis*, *Anabaenopsis*, *Aphanizomenon*, *Oscillatoria*, *Nostoc*, and *Planktothrix* are some common species that are known to form blooms and produce cyanotoxins. Blooms of *Micrococystis aeruginosa* are a major concern, given that they can be associated with the release of microcystin, a known hepatotoxin (Falconer, 2005; WHO, 2004). *Micrococystis* blooms have been responsible for the impairment of important lake systems around the world, including Lake Okeechobee in Florida, Lake Erie (the southernmost and shallowest of the Great Lakes of North America), and Lake Taihu in China (Beaver & Havens, 1996; Doblin, Coyne, Rinta-Kanto, Wilhelm, & Dobbs, 2007; Michalak et al., 2013; Paerl & Otten, 2013; Paerl & Scott, 2010). The wide proliferation of *Micrococystis* has been attributed to its ability to regulate its buoyancy, which gives it an advantage over other non-buoyant cyanobacteria (Bormans, Sherman, & Webster, 1999; Humphries & Lyne, 1988; Bastiaan Willem Ibelings, Mur, Kinsman, & Walsby, 1991; Walsby, Hayes, Boje, & Stal, 1997; Walsby, Ng, Dunn, & Davis, 2004). Another pervasive toxin producing cyanobacteria genera is *Aphanizomenon* flos-aquae which is known to release anatoxins that can affect neurological activities and may even cause death, mainly by respiratory paralysis (Paerl et al., 2001; WHO, 2011). Lake Dianchi in China as well as Lake Erie, Utah Lake, and the Upper Klamath Lake in Oregon are all examples of fresh waterbodies negatively affected by *Aphanizomenon* blooms (W. W. Carmichael, Drapeau, & Anderson, 2000; Liu et al., 2006; Strong, 1974). In addition to the release of anatoxins, *Aphanizomenon* is known to form surface scums with high cell densities, which can cause oxygen

depletion and affect the health of aquatic life (Anderson et al., 2002; Chorus & Bartram, 1999).

Several mitigation measures have been developed to deal with excessive algal blooms. These include the application of algaecides, mechanical mixing, food web manipulations, and/or nutrient control (Dokulil & Teubner, 2000; Elser, 1999; Herrero, Muro-Pastor, & Flores, 2001; Huisman et al., 2004; Huisman, van Oostveen, & Weissing, 1999; Bas W Ibelings, Kroon, & Mur, 1994; Jeppesen et al., 1997; V. H. Smith & Schindler, 2009; Visser, Ibelings, Van Der Veer, Koedood, & Mur, 1996). While the latter is expected to be most effective on the long term, particularly when adopted at a river-basin scale by targeting point and non-point sources, they are often socio-economically difficult to implement. As a result, other mitigation measures are often adopted, with the application of algaecides being the most common. In this context, the efficacy of different algaecides has been explored with Copper Sulfate ($CuSO_4$) remaining the most commonly used worldwide (Brand, Sunda, & Guillard, 1986; Jančula & Maršálek, 2011; Le Jeune et al., 2006).

$CuSO_4$ has been in use since the early 1900's, with its first application recorded in the Fairmont lakes (Hanson & Stefan, 1984; Moore & Kellerman, 1905). Its wide use has been promoted by the fact that it is inexpensive, accessible, and highly effective against almost all cyanobacteria (Chorus & Bartram, 1999; Elder & Horne, 1978; Fan, Ho, Hobson, & Brookes, 2013). It interrupts the electron transport through photosystem II and competes with magnesium in the chlorophyll molecule (Garcia-Villada et al., 2004; Jančula & Maršálek, 2011; Pandey, Singh, & Singh, 1992). Another algaecide that has been used since the early 1960s is Potassium Permanganate ($KMnO_4$) (Chen, Yeh, & Tseng, 2009; Hall, Hart, Croll, & Gregory, 2000; Roccaro, Barone, Mancini, &

Vagliasindi, 2007). Its use has been promoted by its low environmental hazard as compared to that associated with copper (Ellis, Bouchard, & Lantagne, 2000; Kemp, Fuller, & Davidson, 1966; Rodríguez, Onstad, et al., 2007). On a cellular level, $KMnO_4$ releases hydrated manganese dioxide MnO_2 that is responsible for stimulating coagulation of surface algae cells (Chen & Yeh, 2005; Dash, Patel, & Mishra, 2009). Several studies have reported on the efficacy of $KMnO_4$ for algae removal (Chen & Yeh, 2005; Cherry, 1962; Knappe, 2004; Rodríguez, Sordo, Metcalf, & Acero, 2007). A less commonly used algaecide is Diquat which has been commercially available since the early 1950s (Clayton & Matheson, 2010; Phlips, Hansen, & Velardi, 1992). It acts directly on photosynthetic tissues and affects electron transport by changing Nicotinamide Adenine Dinucleotide Phosphate (NADP) (Jager, 1983; Zweig, Shavit, & Avron, 1965). It was shown to be highly effective on several species of phytoplankton and cyanobacteria (Bartell, Campbell, Lovelock, Nair, & Shaw, 2000; DeLorenzo, Scott, & Ross, 2001; Melendez, Kepner Jr, Balczon, & Pratt, 1993; Peterson et al., 1994; Phlips et al., 1992; Schrader & Tucker, 2003).

In this study, we assess the efficacy of $CuSO_4$, $KMnO_4$, and Diquat on controlling *Microcystis aeruginosa* and *Aphanizomenon* flos-aquae blooms through a series of inhibition laboratory-based tests, where different algaecide dosages are used. Efficacy is determined in terms of the observed drop in chlorophyll-a concentrations over 96 hours of the inhibition test. Statistical models that best describe the progression of cyanobacteria concentrations over time are developed in an effort to estimate the inhibition rate and quantify any algal regeneration. The results are then used to select the optimal dosage for each algaecide, specific to each of the two cyanobacteria species. Finally, the environmental risks associated with the use of the three algaecides are

assessed by measuring the residual concentration of each at the end of the 4-day experiment.

CHAPTER II

MATERIALS AND METHODOLGY

2.1. Sampling Location / Raw lake water

Surface water samples were collected 10 cm below the water surface of the Qaraoun Reservoir, Lebanon's largest freshwater body (Figure 1). The reservoir is categorized as a hypertrophic aquatic system as a result of the large point and non-point pollutant loads that are delivered to the reservoir by the upper Litani River (ELARD, 2011; Jurdi, Korfali, Karahagopian, & Davies, 2002; USAID, 2005). As a result, the reservoir experiences consistent cyanobacteria blooms throughout the growing season (late spring to early autumn). These blooms have been known to clog sprinkles and irrigation pumps and negatively affect the touristic sector in the area. *Microcystis* blooms in Qaraoun were first observed during the summer of 2009; since then *Microcystis aeruginosa* blooms have been consistently occurring over the summer season (Atoui, Hafez, & Slim, 2013). The reservoir is also known to experience *Aphanizomenon* flos-aquae blooms between late winter and early summer (Atoui et al., 2013).

Cyanobacteria were collected from the reservoir during algal bloom events. Water sampled were collected from sections of the reservoir that had highly concentrated floating algal scum. As is typical of HAB events, algal successions lead to the eventual dominance of a nuisance algal community (Bouvy et al., 2006; Paerl & Tucker, 1995; Tsukada, Tsujimura, & Nakahara, 2006). Collected samples were transported to the lab on ice and observed under the microscope to assess the dominance of the targeted species. Microscopic observations were repeated throughout the

culturing and inhibition tests so as to ensure that no other algae other than the type needed was present. In the event that multiple species were observed under the microscope, the entire batch was discarded and the inhibition tests aborted.

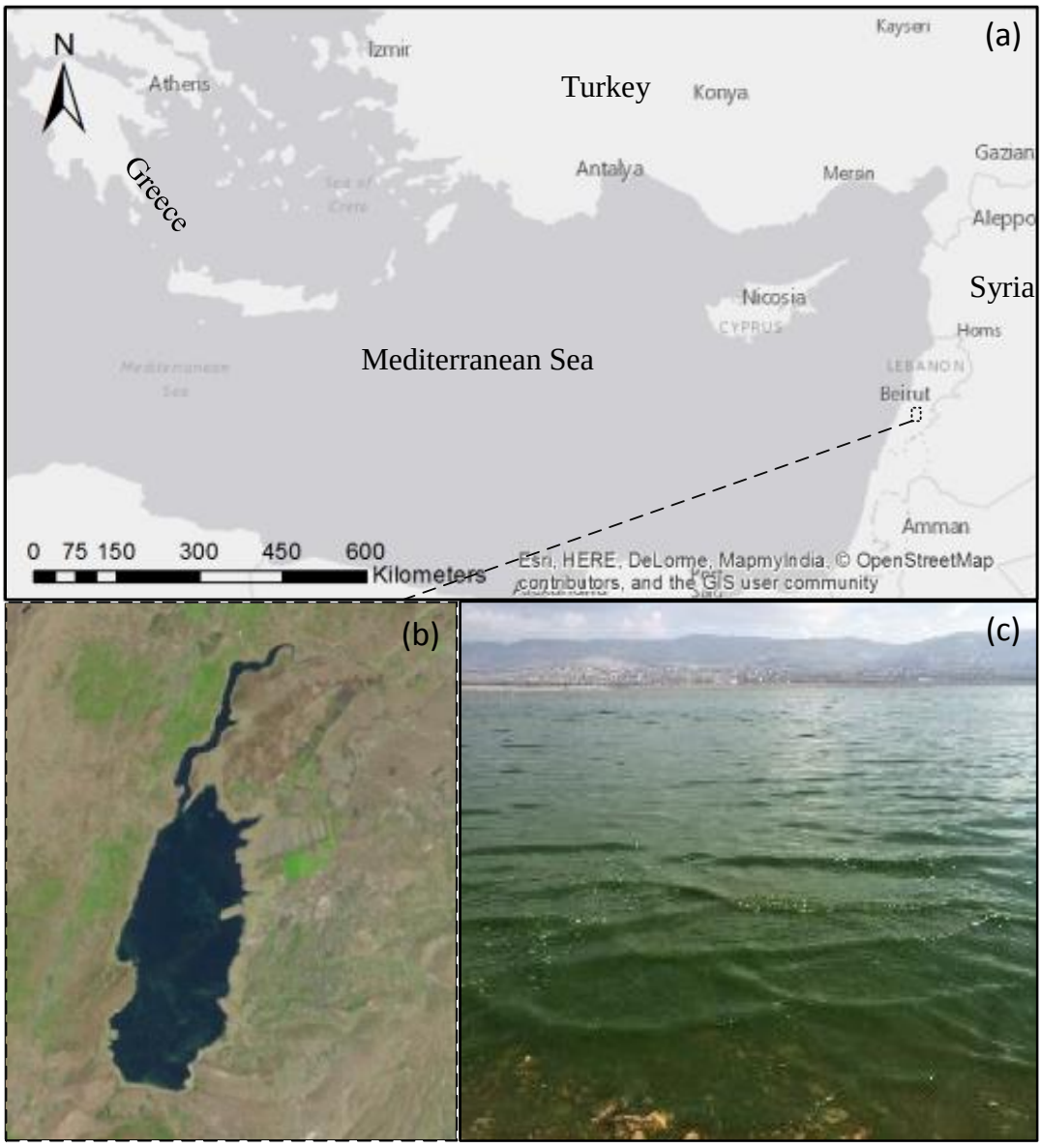

Figure 1 (a) A map showing the location of the Qaraoun reservoir; (b) a Landsat 8 satellite image on July 4, 2013 showing from space a cyanobacteria bloom developing; and (c) an image captured during a *Microcystis aeruginosa* bloom observed on September 22, 2015.

2.2. Cyanobacteria culturing

Several sampling campaigns were conducted at the Qaraoun reservoir between July 2014 and February 2016. Two of the sampling campaigns provided an opportunity to culture the algae in the lab and generate a monoculture of the two targeted cyanobacteria. Samples on October 2015 were collected during a major *Microcystis aeruginosa* bloom, while samples collected on February 2016 were obtained during an equally large *Aphanizomenon* flos-aquae bloom. The collected water samples were transported on ice to the lab. Cell-density, chlorophyll-a concentrations, pH, temperature, nutrients, conductivity, and dissolved oxygen were immediately tested. The samples were cultured under laboratory conditions in 20 L glass beakers. Cultures were provided with a 12:12 light:dark cycle, enriched with nutrients, bubbled with air to ensure no carbon limitation and enhance mixing, and marinated at 25 ^{o}C water temperature for *Microcystis* and 21 ^{o}C for *Aphanizomenon* (USEPA, 2002). In an effort to ensure no nutrient limitation, an F/2 medium based on the Guillard's formulation (1975) of essential nutrients was used. For every liter of water in the cell culturing tanks 0.13 milliliter of Guillard's formulation was added. The formulation used was Proline F/2 Algae Food 239800 produced by Aquatic Eco-systems. To maintain the health of the cyanobacteria, samples were routinely sub-cultured until achieving a cell density $>1.0\times10^{6}$ cells/ml which ensures that the algae remain in the logarithmic growth phase (USEPA, 2002). Samples were taken from the culturing flasks and diluted by adding filtered lake water to reach ~10,000 cells/ml (USEPA, 2002). The cell density was determined by the use of a hemocytometer under a Zeiss Fluorescence microscope (Axiovert 200).

2.3. Experimental procedures

Different concentrations of the three algaecides were prepared from stock solutions to achieve the desired chemical dosages. $CuSO_4$ was supplied from Sigma Aldrich as Copper (II) sulfate pentahydrate (209198 ACS reagents) and used to prepare dosages of 0.2, 0.5, 0.8, and 1 mg/L as $CuSO_4$. $KMnO_4$ was supplied as crystalline solid (Merck and Co. M5080); three dosages (1, 2, and 3 mg/L as $KMnO_4$) were prepared. Diquat was provided from (Supleco N11816 analytical standard) as Diquat dibromide monohydrate. Its efficacy was tested under three concentrations, namely 0.25, 0.5, 1 mg/L as Diquat.

The experimental chambers for inhibition tests were 250 ml Erlenmeyer flasks filled with previously diluted cyanobacteria samples (~10,000 cells/ml). All experiments were conducted according to the EPA method, whereby the flasks were placed on shelves irradiated by 3 florescent lamps that provide 4306 Lux (Lewis et al., 1994) (Figure 2) .For each algaecide-dose combination, triplicate tests were prepared. In addition, algae concentrations were monitored in three control flasks devoid of algaecides.

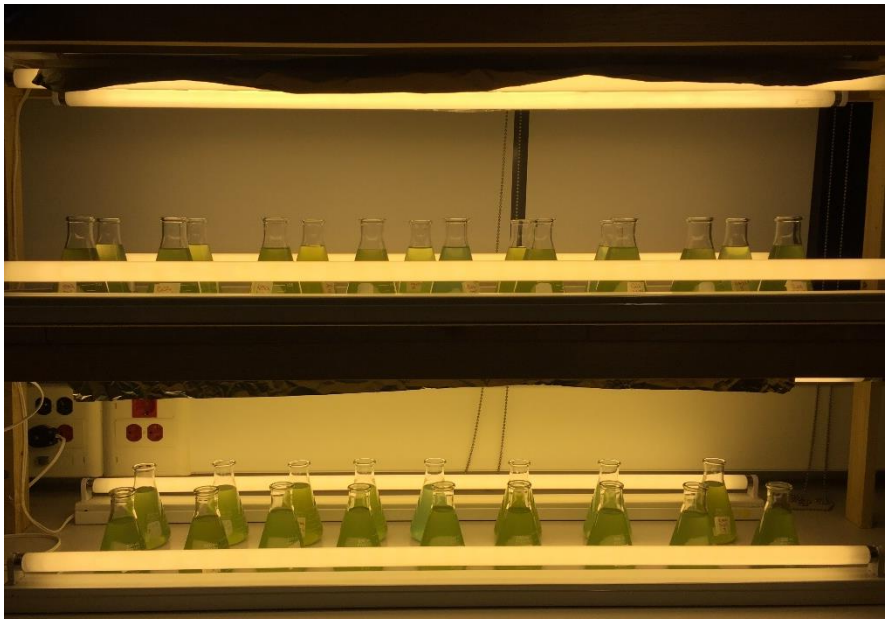

Figure 2. Experimental setup adopted for the inhibition tests

The exposure time of the inhibition test was set to 96h, with daily analysis of 50 ml sample collected at 24h, 48h, 72h, and 96h. The daily analysis included pH, DO, temperature, chlorophyll-a, and cell counts. Across all *Microcystis* inhibition tests, the pH ranged between 7.6 and 9.8, water temperatures between 22 and 25 ^{o}C, and DO levels between 5 and 15 mg/L. As for the *Aphanizomenon* tests, pH levels were between 6 and 7, water temperatures between 21 and 23 ^{o}C, and DO levels between 0.5 and 10 mg/L. Appendix B includes a more detailed daily results. On the last day, nutrients content, hardness, turbidity, and the chemical residuals for each algaecide were also tested (Table 1). A summary of the physio-chemical analysis is shown in Table 4 and 3 for the *Microcystis* and *Aphanizomenon* tests respectively. Copper and potassium permanganate concentrations were tested using graphite furnace atomic absorption spectrophotometry based on USEPA method 200.9 (Creed, Martin, & O'Dell, 1994). High Performance Liquid Chromatography (HPLC) was used to determine Diquat concentrations, according to USEPA method 549.2 (Hodgeson, Bashe, & Eichelberger,

1997) .Chlorophyll-a concentrations were measured by collecting samples with known volumes that were buffered using magnesium carbonate before being filtered through membrane filter papers that were later stored overnight at -20 ^{o}C. Chlorophyll-a was extracted in 90% acetone solution followed by sonification. Extracts were seeped in the acetone solution overnight and then clarified using centrifugation. Chlorophyll-a concentrations were calculated based on absorbance (Standard Method 10200 (HS2)) (Rice, Bridgewater, Water Environment, American Water Works, & American Public Health, 2012) measured on a HACH DR3900 spectrophotometer(Eaton & Franson, 2005). For cell counts, cells of *Aphanizomenon* flos-aquae and *Microcystis aeruginosa* were microscopically monitored using a Zeiss Fluorescence microscope (Axiovert 200), with emphasis on recording whether they were colonial or in unicellular form.

Table 1 Adopted analysis methods used to measure the physio-chemical parameters in the samples (Rice et al., 2012)

	Quality Indicators	**Method**	**Method number**
Physical	pH	Electrometery	4500-H+B
	Turbidity	Nephelometry	2130 B
Chemical	Dissolved Oxygen	Polorgraphic	4500-O.G
	Calcium Hardness	EDTA Titrimetry	3500-CA B
	Total Hardness	EDTA Titrimetry	2340-C
	Nitrate	Colorimetry	4500-NO3-B
	Nitrite	Colorimetry	4500-NO2-B
	Phosphate	Colorimetry	4500-P.B
	Chlorophyll-a	Spectrophotometrically	10200 H.2

Table 2. Physio-chemical analysis conducted at the end of the *Microcystis aeruginosa* experiment
Reading are reported as the average of three readings

Parameter	*Initial*	*End of experiment (96 hrs)*									
		Control	*$CuSO_4$*				*$KMnO_4$*			*Diquat*	
			0.2mg/L	*0.5mg/L*	*0.8mg/L*	*1 mg/L*	*1mg/L*	*2mg/L*	*3mg/L*	*0.25mg/L*	*0.5mg/L*	*1mg/L*
Turbidity (NTU)	130.4	124.3	57.7	30.1	23.9	51.4	230.0	166.3	114	-	29.5	39.2
Calcium Hardness (mg/L)	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6		0.6	0.6
Total Hardness (mg/L)	1.1	1.1	1.1	1.1	1.1	1.2	1.1	1.1	1.1		1.1	1.1
PO_4^{3-} (mg/L)	2.5	1.3	2.2	>2.5	>2.5	>2.5	1.0	1.7	0.3		0.3	0.3
NO^{2-} (mg/L)	0.4	0.1	0.1	0.1	0.1	0.02	0.1	0.1	0.1		0.1	0.1
NO^{3-} (mg/L)	18.8	4.7	17.8	17.7	17.0	15.3	8	13.0	13.5		11.7	17.4

Table 3. Physio-chemical analysis conducted at the end of the *Aphanizomenon flos-aquae* experiment
Reading are reported as the average of three readings

Parameter	*Initial*	*End of experiment (96 hrs)*									
		Control	*$CuSO_4$*				*$KMnO_4$*			*Diquat*		
			0.2mg/L	*0.5mg/L*	*0.8mg/L*	*1 mg/L*	*1mg/L*	*2mg/L*	*3mg/L*	*0.25mg/L*	*0.5mg/L*	*1mg/L*
Turbidity (NTU)	160	16.1	9.0	22.9	27.6	43.8	7.6	20.2	22.7	13.9	12.4	10.0
Calcium Hardness (mg/L)	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Total Hardness (mg/L)	0.7	0.7	0.8	0.7	0.7	0.7	0.7	-	0.7	0.7	0.7	0.7
PO_4^{3-} (mg/L)	11.7	3.2	14.1	5.4	4.4	3.7	3.4	3.0	15.60	2.5	2.4	2.8
NO^{2-} (mg/L)	0.3	1.9	0.2	0.3	0.3	0.4	1.7	4.4	1.3	5.2	8.8	3.4
NO^{3-} (mg/L)	0.8	4.5	1.1	1.3	1.0	2.3	4.1	4.4	2.9	5.8	6.8	4.0

2.4. Statistical Analysis

The effect of dosage and the potential changes in efficiency over time were assessed for the three algaecide across the two cyanobacteria species. Two-way Analysis Of Variance (ANOVA) tests were used to evaluate statistically significant factors. The potential for an interaction between dose and time was also examined through the inclusion of an interaction term between the two factors. In the event that the 2-way ANOVA showed statistical significance, Tukey Honest significant differences multiple comparisons were used to identify significant differences between dosage and time. Note that chlorophyll-a concentration of *Microcystis* and *Aphanizomenon* were square root-transformed to achieve normality. The ANOVA and multiple comparisons analysis were fit using the R software (R Core Team, 2015).

Additionally, multiple linear regression models were developed for each algaecide-dose combination with theaim to predict the rate of change in chlorophyll-a concentrations as a function of time (measured in hours). Two different regression functional forms were considered. The first was based on a second order polynomial model (Equation 1), which can account for algae regrowth. The second attempted to model the drop in chlorophyll-a concentrations as a first order decay process (Equation 2); it does not allow for regrowth. The adjusted R^2 (R^2_{adj}) was used as a measure of model fit. Both models were used to compare efficacy as a function of algaecides across dosages. Models were fit using the lm function in R (R Core Team, 2015).

$$Chl_a = \beta_0 + \beta_1 \times Time_{hrs} + \beta_2 \times Time_{hrs}^2 + \varepsilon;\ \varepsilon \sim Norm(0, \sigma^2) \quad (1)$$

$$Log_e(Chl_a) = \beta_0 + \beta_1 \times Time_{hrs} + \varepsilon;\ \varepsilon \sim Norm(0, \sigma^2)$$
$$Chl_a = C_0 \times e^{\beta_1 \times Time_{hrs}};\ C_0 = e^{\beta_0} \quad (2)$$

Where Chl_a are the measured chlorophyll-a concentrations in µg/L, *Time* is time in hours following the addition of the algaecide, β_0 represents the intercept of the model, β_{1-2} are the slopes on the predictors, ε is the model error terms, and σ is the standard error of the model.

CHAPTER III

RESULTS

3.1. $CuSO_4$

Copper sulfate proved to be significantly effective across all dosages on *Microcystis* and *Aphanizomenon* species as compared to the controls (p-values < 0.05). *Microcystis* colonies appeared to be broken down into individual cells under microscopic observations within the first 24 hrs; however cells started to recover after 72 hrs. Regrowth at the end of 96 hrs remained in unicellular form. Copper sulfate inhibited 55 to 60% of the *Microcystis* cells at the lowest dose (0.2 mg/L) (Figure 3.a). The inhibition rate significantly improved with higher dosages, reaching around 90% with the 0.5 mg/L dose at 48 hrs. At the higher dosages of 0.8 and 1 mg/L, 90% removal efficiency was achieved earlier- after only 24 hrs of treatment. Statistically, the concentrations of chlorophyll-a were significantly different over time; across all dosages with the lowest concentration observed after 48hr. While concentrations appeared to rebound following 48 hrs of treatment, the difference in concentrations between 48 and 72 hrs was not statistically significant (p-value =0.67). *Microcystis* regrowth continued between 72 and 96 hrs with concentrations at 96 hrs similar to those observed at 24 hrs (p-value = 0.99). Regarding the applied dosages, in general higher dosages resulted in better removals (Table 4); yet there was no statistical difference between concentrations measured at 0.8 and 1 mg/L. Nevertheless, the latter suppressed the regrowth of *Microcystis* better after 96 hrs of treatment.

Under microscopic observations, *Aphanizomenon* colonies were found to break down following the first day of treatment. Reductions of 75% in chlorophyll-a

concentration were observed after 48 hrs of contact time even at the lowest applied dose (0.2 mg/L). Concentrations continued to decrease with time, reaching 10% and 6% of their original value 72 and 96 hrs after application respectively. A similar pattern was observed at higher dosages, albeit with increased removal rates. Removals of 86%, 90% and 97% were achieved after 48 hrs of exposure at 0.5, 0.8, and 1 mg/L respectively. No regrowth was evident across all dosages (Figure 3.b). Concentrations of chlorophyll-a dropped significantly over time across all dosages (Table 4); concentrations achieved at 72 and 96 hrs were statistically similar (p-value=0.66), indicating the absence of regrowth under both dosages. Statistically similar concentrations were achieved at the 0.5, 0.8 and 1 mg/L dosages; reductions at these rates were statistically better than those achieved at the 0.2 mg/L (Table 4).

Table 4 Statistical analysis (two-way ANOVA) of *Microcystis* and *Aphanizomenon* reductions following $CuSO_4$ treatment as a function of dose and exposure time

	ANOVA F-static (p-value)			**Pairwise comparison (Tukey)**											
	Dosage	**Time**	**Interaction**	**Dosage (mg/L)**						**Time (hrs)**					
Microcystis	118.47 (<0.05)	112.63 (<0.05)	11.38 (<0.05)		0	0.2	0.5	0.8	1		0	24	48	72	96
				0	／	✔	✔	✔	✔	0	／	✔	✔	✔	✔
				0.2		／	✔	✔	✔	24		／	✔	✔	✖
				0.5			／	✔	✔	48			／	✖	✔
				0.8				／	✖	72				／	✔
				1.0					／	96					／
	ANOVA F-static (p-value)			**Pairwise comparison (Tukey)**											
	Dosage	**Time**	**Interaction**	**Dosage (mg/L)**						**Time (hrs)**					
Aphanizomenon	26.415 (<0.05)	263.779 (<0.05)	2.589 (<0.05)		0	0.2	0.5	0.8	1		0	24	48	72	96
				0	／	✔	✔	✔	✔	0	／	✔	✔	✔	✔
				0.2		／	✖	✔	✔	24		／	✔	✔	✔
				0.5			／	✖	✖	48			／	✔	✔
				0.8				／	✖	72				／	✖
				1.0					／	96					／

✓ significance at the 95% confidence interval (p-value <0.05)
✕ No significance at the 95% confidence interval (p-value >0.05)

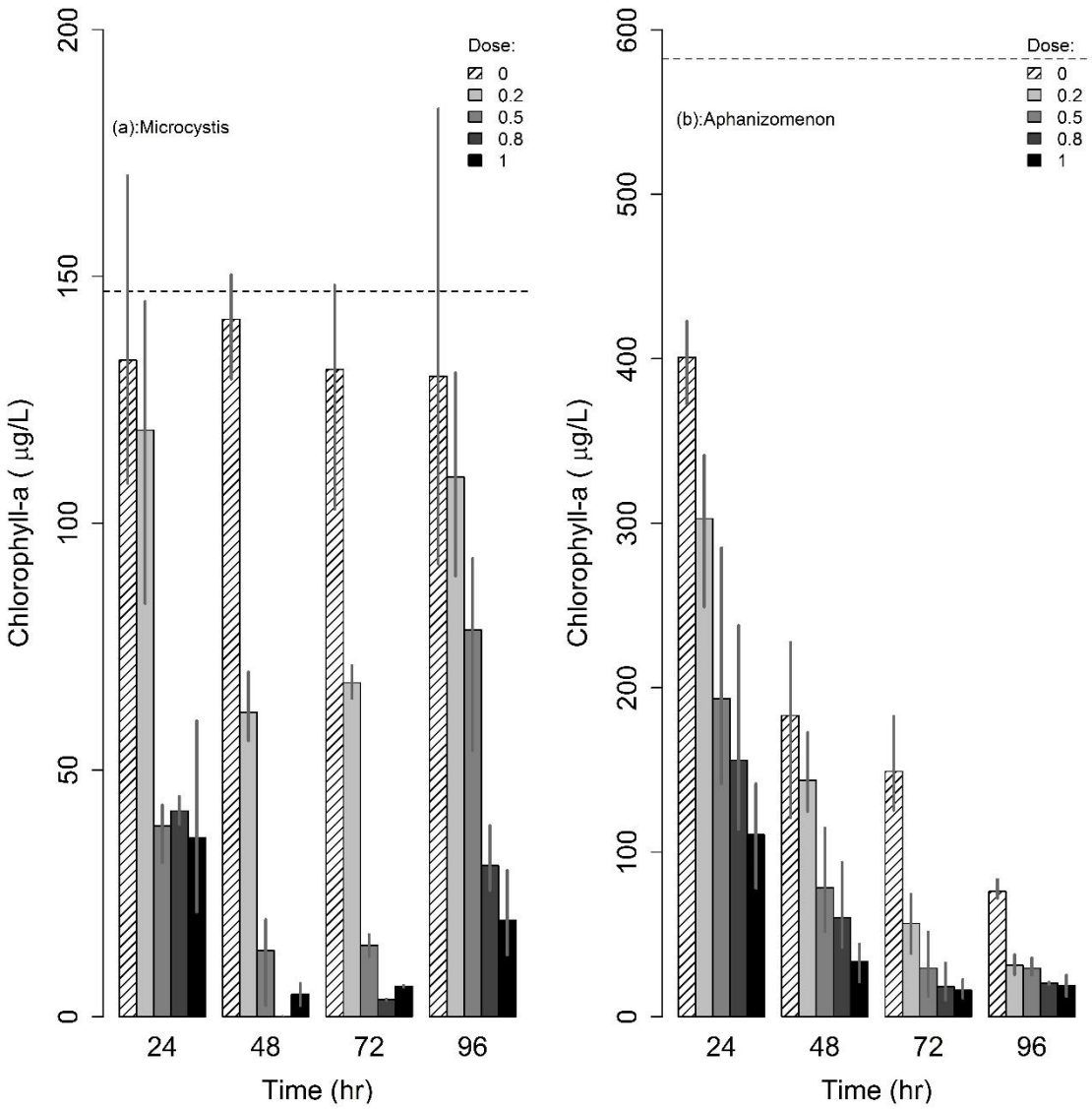

Figure 3 Changes in chlorophyll-a concentrations of (a) *Microcystis* and (b) *Aphanizomenon* following $CuSO_4$ treatment at 4 different algaecide dosages. Concentrations are measured over 96 hrs and compared to the control.

3.2. $KMnO_4$

$KMnO_4$ proved to be the least effective algaecide in controlling *Microcystis*. Overall, the chlorophyll-a concentrations achieved following the application of $KMnO_4$ at the two lower dosages (1 and 2 mg/L) were statistically not-different from the control

(p-values = 0.99 and 0.11 respectively); yet the *Microcystis* concentrations at 48 hrs under the 2 mg/L dose were statistically lower than the control (Table 5). At the highest dosage of 3 mg/L, the removal rate at 48 hrs ranged between 75 and 80%. Concentrations of *Microcystis* at this dosage were statistically lower than the control both after 48 and 72 hrs of treatment (Figure 4.a). Concentrations appeared to rebound after 72 hrs; thus concentrations on day 4 (96 hrs) were statistically similar to those of the control as well as the concentrations achieved at lower dosages (Table 5 and Figure 4.a). The temporal change in average chlorophyll-a concentrations was statistically different from the control after 48 and 72 hrs (p-value<0.05).

In the case of *Aphanizomenon*, $KMnO_4$ proved to be effective, except for the 1 mg/L dose. Significant inhibition was observed for the 2 and 3 mg/L dosages (p-value<0.05). Colonies of *Aphanizomenon* declined significantly even after 24 hrs of treatment across all dosages. The ANOVA results showed statistical significance with dosage; chlorophyll-a concentrations decreased (p-value<0.05) as the $KMnO_4$ dosage increased (Table 5). Reductions in chlorophyll-a concentrations of more than 90% were observed after 48 hrs of treatment with $KMnO_4$ dosages of 2 and 3 mg/L. No statistical difference was observed (p-value=0.77) between these two dosages. Both achieved more than 98% reductions at 96 hrs (Figure 4.b). There were statistical differences in chlorophyll-a levels across all time periods, except for levels recorded at 48 and 72 hrs (p-value = 0.24) and those observed at 72 and 96 hrs (p-value = 0.17). This is an indication of the absence of regrowth.

Table 5 Statistical analysis (two-way ANOVA) of *Microcystis* and *Aphanizomenon* reductions following $KMnO_4$ treatment as a function of dose and exposure time

	ANOVA F-static (p-value)			**Pairwise comparison (Tukey)**											
	Dosage	**Time**	**Interaction**	**Dosage (mg/L)**						**Time (hrs)**					
Microcystis	17.565 (<0.05)	8.061 (<0.05)	4.226 (<0.05)		0	1	2	3			0	24	48	72	96
				0	／	✖	✖	✔		0	／	✖	✔	✔	✖
				1		／	✖	✔		24		／	✔	✖	✖
				2			／	✔		48			／	✖	✖
				3				／		72				／	✖
										96					／
	ANOVA F-static (p-value)			**Pairwise comparison (Tukey)**											
	Dosage	**Time**	**Interaction**	**Dosage (mg/L)**						**Time (hrs)**					
Aphanizomenon	33.740 (<0.05)	179.013 (<0.05)	3.569 (<0.05)		0	1	2	3			0	24	48	72	96
				0	／	✖	✔	✔		0	／	✔	✔	✔	✔
				1		／	✔	✔		24		／	✔	✔	✔
				2			／	✖		48			／	✖	✔
				3				／		72				／	✖
										96					／

✓ significane p-value <0.05

✕ not Significant P-value >0.05

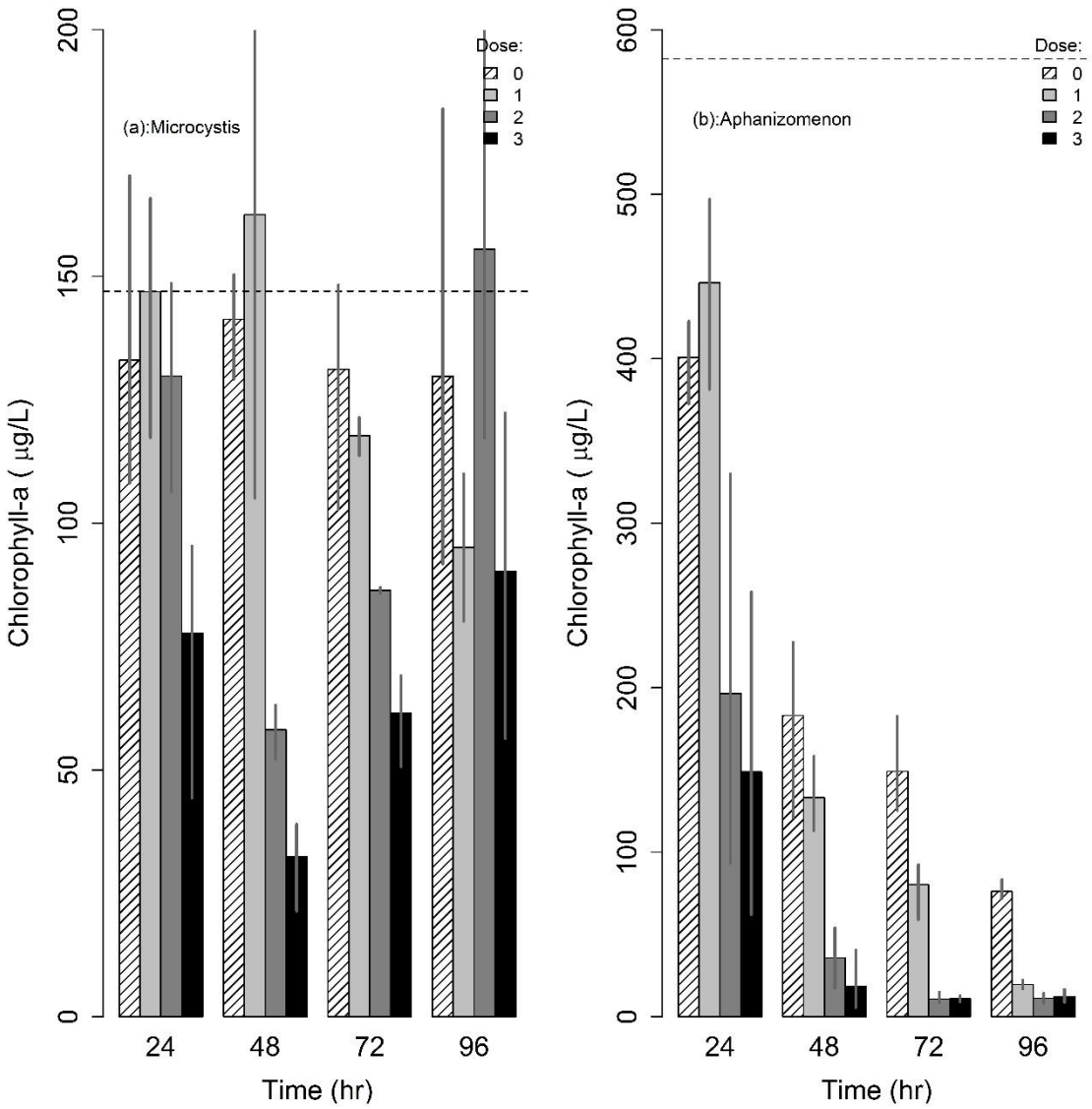

Figure 4 Changes in chlorophyll-a concentrations of (a) *Microcystis* and (b) *Aphanizomenon* following $KMnO_4$ treatment at three different dosages. Concentrations are measured over 96 hrs and compared to the control.

3.3. Diquat

Diquat proved to be highly effective in controlling both cyanobacterial species under all tested dosages as compared to the control (p-value<0.05). No statistically significant difference was detected amongst dosages. During the first 24 hrs,

Microcystis colonies were no longer observed under the microscope following the application of Diquat at 0.5 and 1 mg/L. Unicellular *Microcystis* were also hard to observe after 48 hrs of exposure. No statistical difference was discerned between the two dosages; both were equally effective in reducing *Microcystis* (p-value = 0.95). After 48 hrs, inhibition approached 100%, the highest level achieved among all treatments (Table 6). However, recovery of some unicellular cells was observed on 96 hrs when measured concentrations reached 17 and 11% of the initial concentrations for 0.5 and 1 mg/L respectively (Figure 5.a). With respect to variability across time, the measured concentrations following treatment were statistically different from each other across all times, except for *Microcystis* levels recorded at 48, 72, and 96 hrs. The levels at these intervals were consistently low.

Colonies of *Aphanizomenon* were immediately affected by the Diquat application; colonies disappeared after 24 hrs. More than 95% of *Aphanizomenon* cells were inhibited after 48 hrs, irrespective of dosages (0.25, 0.5, and 1 mg/L). Inhibition rates achieved 99 % reduction after 72 hrs (Figure 5.b). Similar to *Microcystis*, the levels achieved at 48, 72, and 96 hrs were statistically similar, an indication of lack of regrowth (Table 6).

Table 6 Statistical analysis (two-way ANOVA) of *Microcystis* and *Aphanizomenon* reductions following Diquat treatment as a function of dose and exposure time

	ANOVA F-static (p-value)			**Pairwise comparison (Tukey)**											
	Dosage	**Time**	**Interaction**	**Dosage (mg/L)**						**Time (hrs)**					
					0	1	2				0	24	48	72	96
				0	⁄	✔	✔			0	⁄	✔	✔	✔	✔
Diquat				1		⁄	✖			24		⁄	✔	✔	✔
Microcystis	55.17	205.02	25.10	2			⁄			48			⁄	✖	✖
	(<0.05)	(<0.05)	(<0.05)							72				⁄	✖
										96					⁄
	ANOVA F-static (p-value)			**Pairwise comparison (Tukey)**											
	Dosage	**Time**	**Interaction**	**Dosage (mg/L)**						**Time (hrs)**					
					0	0.25	0.5	1			0	24	48	72	96
				0	⁄	✔	✔	✔		0	⁄	✔	✔	✔	✔
Diquat				0.25		⁄	✖	✖		24		⁄	✔	✔	✔
Aphanizomenon	32.47	198.90	3.82	0.5			⁄	✖		48			⁄	✖	✖
	(<0.05)	(<0.05)	(<0.05)	1				⁄		72				⁄	✖
										96					⁄

✓ significane p-value <0.05
✕ not Significant P-value >0.05

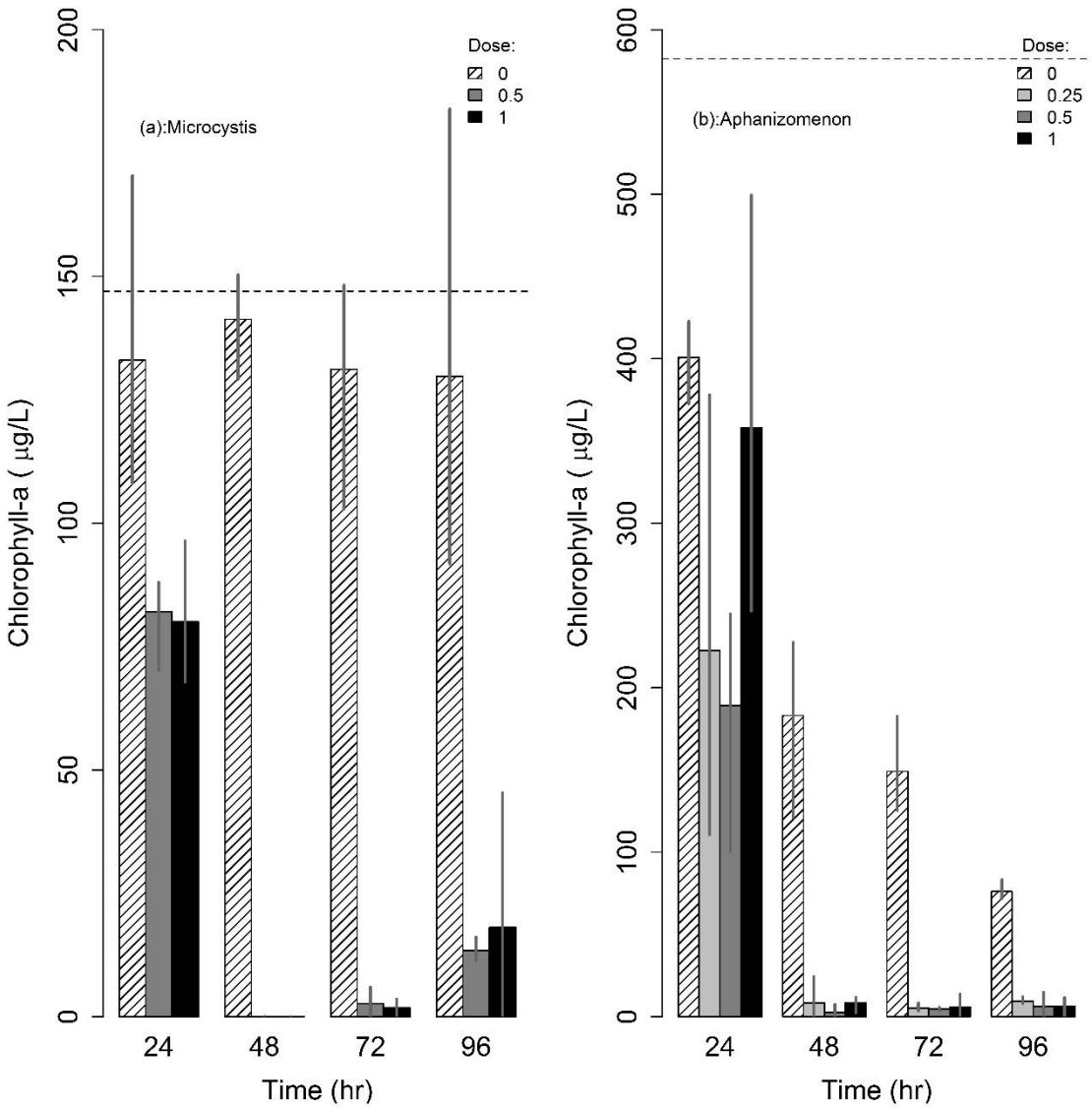

Figure 5 Changes in chlorophyll-a concentrations of (a) *Microcystis* and (b) *Aphanizomenon* following Diquat treatment at different dosages. Concentrations are measured over 96 hrs and compared to the control

CHAPTER IV

DISCUSSION

4.1 Algaecidal effects on the two cyanobacteria

All three tested algaecides had an impact on both cyanobacterial cells. $CuSO_4$ proved to be a potent algaecide, attaining strong inhibition rates on *Microcystis* that reached ~99 % after 48 hrs of exposure at the 1 mg/L dose. This is in agreement with the results reported by Fan et al. (2013), who reported that a 1 mg/L dose resulted in the loss of 98% of *Microcystis* cells after 2 days of treatment. Inhibition continued up until 72 hrs following treatment, beyond that regrowth was evident. Potassium permanganate proved to be a suboptimal algaecide for the control of *Microcystis*; no significant differences were observed between the control and the water treated at 1 mg/L. Reductions that were achieved under the 2 mg/L dose, were marginally better than the control. Under that dose, a modest drop was achieved following 48 hrs of treatment. At 3 mg/L, $KMnO_4$ proved to be effective; inhibition rates reached 75 to 80% whichare similar to those reported by Knappe et al. (2004). Fan et al. (2013)noted that high concentrations of $KMnO_4$ are needed to control *Microcystis*. With regards to the use of Diquat, algae control was most effective in controlling *Microcystis* colonies even at low dosages (0.5 mg/L) which is consistent with results reported by Phlips et al. (1992).

Aphanizomenon flos-aquae proved to be more sensitive to algaecide application as compared to *Microcystis*. Copper sulfate was highly potent in controlling *Aphanizomenon flos-aquae* even at the 0.2 mg/L dose (removal rate ~94% at 72hrs). Higher concentrations resulted in better removals. Peterson et al. (1995)(1995) reported that copper sulfate can be an effective algaecide even at low dosages (as low as

50μg/L). While $KMnO_4$ was largely not effective in controlling *Microcystis*, it showed high inhibition rates when applied to *Aphanizomenon*. Dosages of 2 mg/L and higher achieved removals in excess of 98% at 72hrs. Previous studies reported a higher (5 mg/L) optimal permanganate dosage to control *Aphanizomenon* (Peterson et al., 1995). Similar to *Microcystis*, Diquat proved to be highly effective on *Aphanizomenon* even at a dose as low as 0.25 mg/L. While previous work reported that Diquat can achieve 100% inhibition of *Aphanizomenon* at a dosage of 0.73 mg/L (Peterson et al., 1994), our results indicated that complete inhibition can occur at much lower dosages.

4.2. Algaecidal dynamics: *Microcystis*

One the main features observed in this study is the ability of *Microcystis* to recover and regrow following algaecide application. Recovery rates and timing varied across dosages and algaecide type. The recovery of *Microcystis auerginosa* post algaecide application has been reported in previous studies (Fan, Ho, et al., 2013; Greenfield et al., 2014). Yet, the dynamics of regrowth have not been discussed nor modeled. In an effort to properly characterize regrowth, a polynomial quadratic regression model that predicts concentrations as a function of time and time-squared was adopted. The functional form of such a model allows for a rapid initial decrease associated with the inhibition phase, followed by a recovery phase (Figure 6, Figure 7, and Figure 8). The model also provides an inflection point, beyond which regrowth surpasses any residual algaecidal inhibition. The polynomial quadratic model proved to fit well the dynamics of *Microcystis auerginosa*, especially under high dosages (Figure 6.b, 6.c, 6.d and Figure 8.a, 8.b). An artifact of the quadratic model is its potential to predict concentrations lower than zero at high dosages. This was evident in the case of

$CuSO_4$ and Diquat (Figure 6 and Figure 8). On the other hand, the quadratic model was not supported by data collected from the 1 mg/L $KMnO_4$ application experiments due to low inhibition rates experienced at this dosage. Instead, a simple linear regression model with a modest negative slope proved sufficient (Figure 7.a).

The rates at which *Microcystis* concentrations dropped across the three algaecides under the different dosages are shown in Figure 9. For $CuSO_4$, the model predicted that regrowth will exceed inhibition between 51 and 75 hrs following treatment across the four tested dosages. Overall, higher dosages resulted in slower regrowth. In fact, even though there was no statistical difference between the concentrations of *Microcystis* recorded at 0.8 and 1 mg/L dosages, the predicted inflection point of the latter was delayed by 15 hrs as compared to the former (Figure 9.a). Note that while the inflection point achieved with the 0.2 mg/l dose occurred at 73 hrs, its associated inhibition rate was low, whereby the lowest achieved concentrations were still higher than those recorded on the last day for the higher dosages (Figure 6 and Figure 9.a). Given these findings, the optimal dosage of $CuSO_4$ needed to control *Microcystis* should account for both the achieved removal rates and the retardation of regrowth. As such, the adoption of the 0.5 mg/L dose may require frequent application to counteract the fast regrowth rate achieved by *Microcystis* following 50 hrs from application. Both the 0.8 and 1 mg/L are able to achieve better removals, delay the regrowth phase and reduce its magnitude. As such, the optimal dosage for $CuSO_4$ should be in excess of 0.5 mg/L.

M. aeruginosa exhibited resilience against $KMnO_4$ treatment. The 1 mg/l dose proved to be ineffective and as such it was difficult to separate the inhibition phase from regrowth. This was manifested in the absence of a second order regression term in the

algae dynamics model (Figure 9.b). At the 2 and 3 mg/L dosages, the $KMnO_4$ treatment did show evidence of an initial inhibition followed by a recovery phase (Figure 7 and Figure 9.b). The rates of inhibition and regrowth were largely parallel across the two dosages, with higher inhibition and slower regrowth achieved at the 3 mg/L dose. Clearly, the use of $KMnO_4$ is not suitable for the control of massive *M. aeruginosa* blooms. Yet, under conditions where blooms are starting to develop, its use can prove useful, particularly given its low environmental impact and its ability to reduce concentrations without lysing cells completely which is linked to the release of microcystin (Fan, Daly, Hobson, Ho, & Brookes, 2013).

As outlined above, Diquat proved to be highly effective under both tested dosages with similar significant inhibition rates, delayed recovery, as well as low recovery. As such, the 0.5 mg/L dose is considered to be adequate. Note that the Diquat had the lowest *Microcystis* recovery rate among all tested algaecides, thus requiring the least frequent application (Figure 9.c).

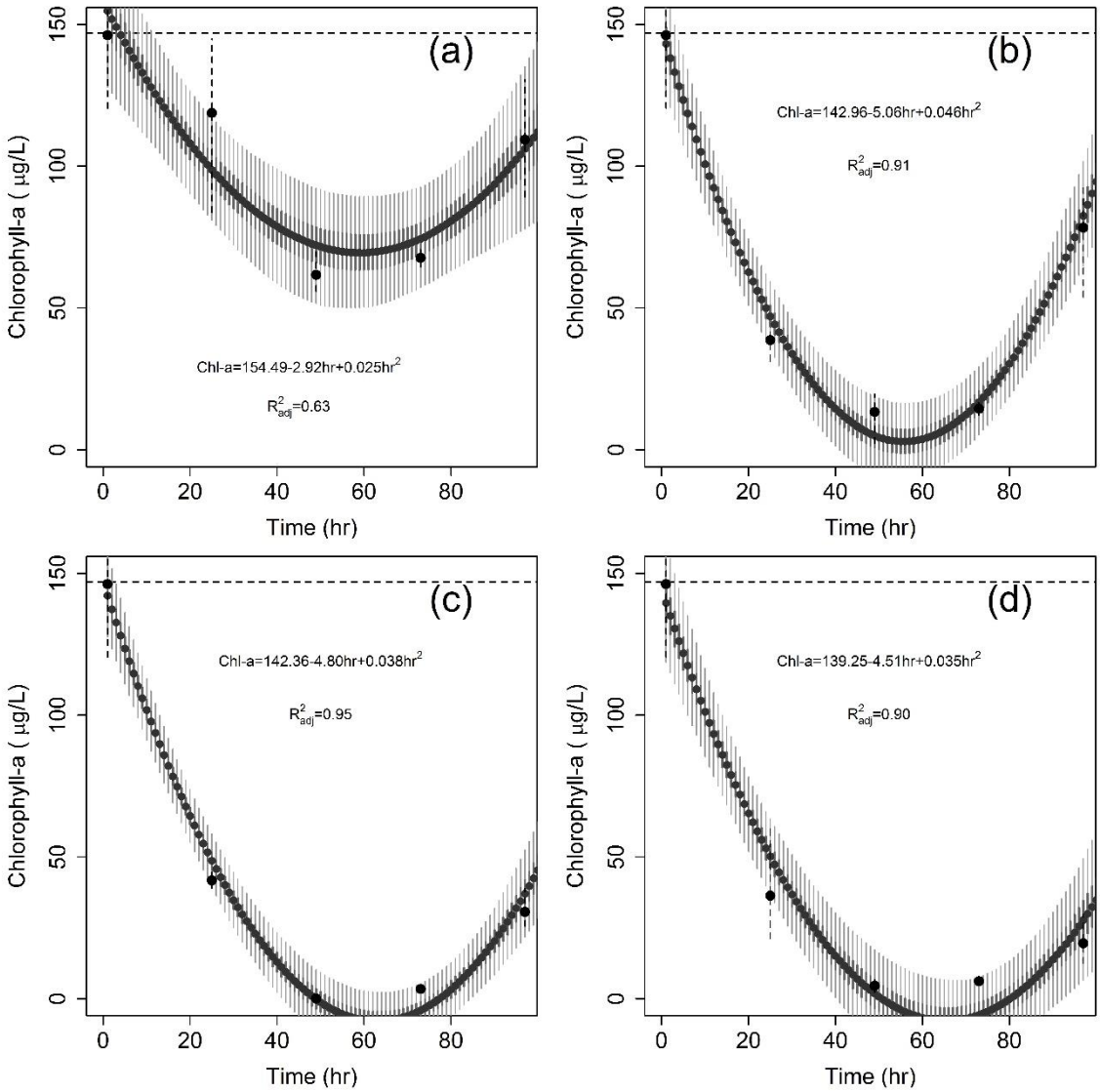

Figure 6 Drop in Chlorophyll-a concentration of *Microcystis* after the application of $CuSO_4$ (a) dosage=0.2 mg/L, (b) dosage=0.5 mg/L, (c) dosage=0.8 mg/L, and (d) dosage=1 mg/L. The dashed horizontal line represents the initial conditions; black solid circles represent average measure concentrations; dashed vertical lines show the range of observed concentration (minimum and maximum). Solid thick vertical grey segments and solid light grey vertical segments represent the 50% and 95% model predicted chlorophyll-a concentrations respectively.

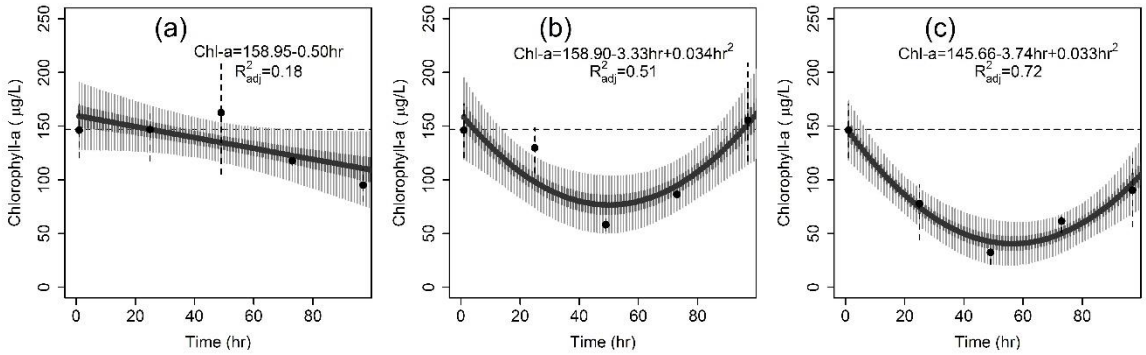

Figure 7 Drop in Chlorophyll-a concentration of *Microcystis* after the application of $KMnO_4$ (a) dosage=1 mg/L, (b) dosage=2 mg/L, and (c) dosage=3 mg/L. The dashed horizontal line represents the initial conditions; black solid circles represent average measure concentrations; dashed vertical lines show the range of observed concentration (minimum and maximum). Solid thick vertical grey segments and solid light grey vertical segments represent the 50% and 95% model predicted chlorophyll-a concentrations respectively.

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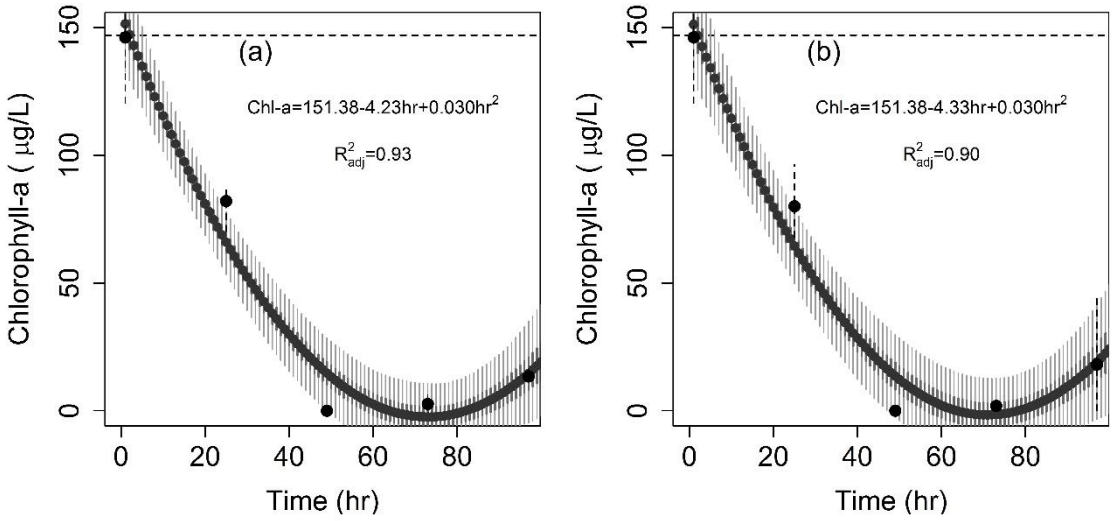

Figure 8 Drop in Chlorophyll-a concentration of *Microcystis* after the application of Diquat (a) dosage=0.5 mg/L, and (b) dosage=1 mg/L. The dashed horizontal line represents the initial conditions; black solid circles represent average measure concentrations; dashed vertical lines show the range of observed concentration (minimum and maximum). Solid thick vertical grey segments and solid light grey vertical segments represent the 50% and 95% model predicted chlorophyll-a concentrations respectively.

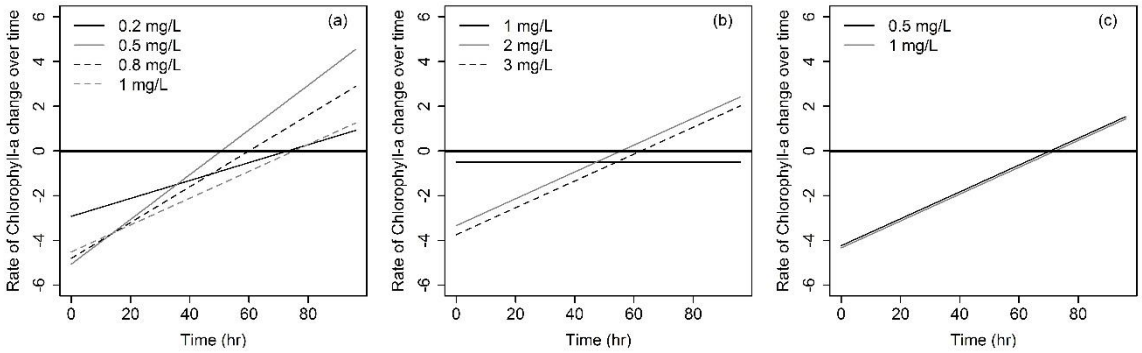

Figure 9 Rates of inhibition and recovery of Microcystis over time for (a) $CuSO_4$; (b) $KMnO_4$; and (c) Diquat. Rates below the solid black horizontal line (y= 0) indicates dominance of inhibition, while the area above the line represents dominant regrowth dynamics

4.3. Algaecidal dynamics: *Aphanizomenon*

Following algaecide application, the temporal dynamics of *Aphanizomenon* was found to be best described with a first order decay model. Unlike *Microcystis*, no evidence of regrowth was observed across all treatments. Overall, concentrations dropped exponentially with time; the rate at which *Aphanizomenon* dropped was found to increase concurrently with algaecide dosage (Figure 10, and Figure 11). Note that the first order decay models explained the behavior of *Aphanizomenon* best when the cyanobacteria was subjected to lower dosage of $CuSO_4$ and $KMnO_4$ (Figure 10 and Figure 11). The model fit partially deteriorated at high dosages, as the initial (first 48 hours) removal rates appear to occur at rates faster than those predicted by the first order decay model. This is evident in Figure 10 and Figure 11, whereby the model consistently over-predicted observed data up to 48 hrs following treatment. Samples treated with Diquat had the same issue of over-prediction across all three applied dosages (Figure 12).

The results show that for $CuSO_4$ there were barely any differences across dosages between concentrations observed on the final day; as such the optimal dose for copper application can be as low as 0.2 mg/L. However, when the time to achieve a major reduction in concentration is critical, *Aphanizomenon* blooms should be treated with higher dosages (0.8 and 1 mg/L). Increasing the $CuSO_4$ dose from 0.2 to 1 mg/L results in reducing the observed concentration after 24 hour by around two thirds (300 to 110 mg/L). For $KMnO_4$, the 1mg/L dose was ineffective in controlling *Aphanizomenon*. The 2 and 3 mg/L were found to be statistically similar and were able to inhibit the growth of *Aphanizomenon* with similar rates (β_1= -0.044). Thus, the 2 mg/L $KMnO_4$ dose is sufficient. In the case of Diquat, similar removal rates were obtained across the three dosages. Hence, the lowest dose of 0.25 mg/L is adequate for use.

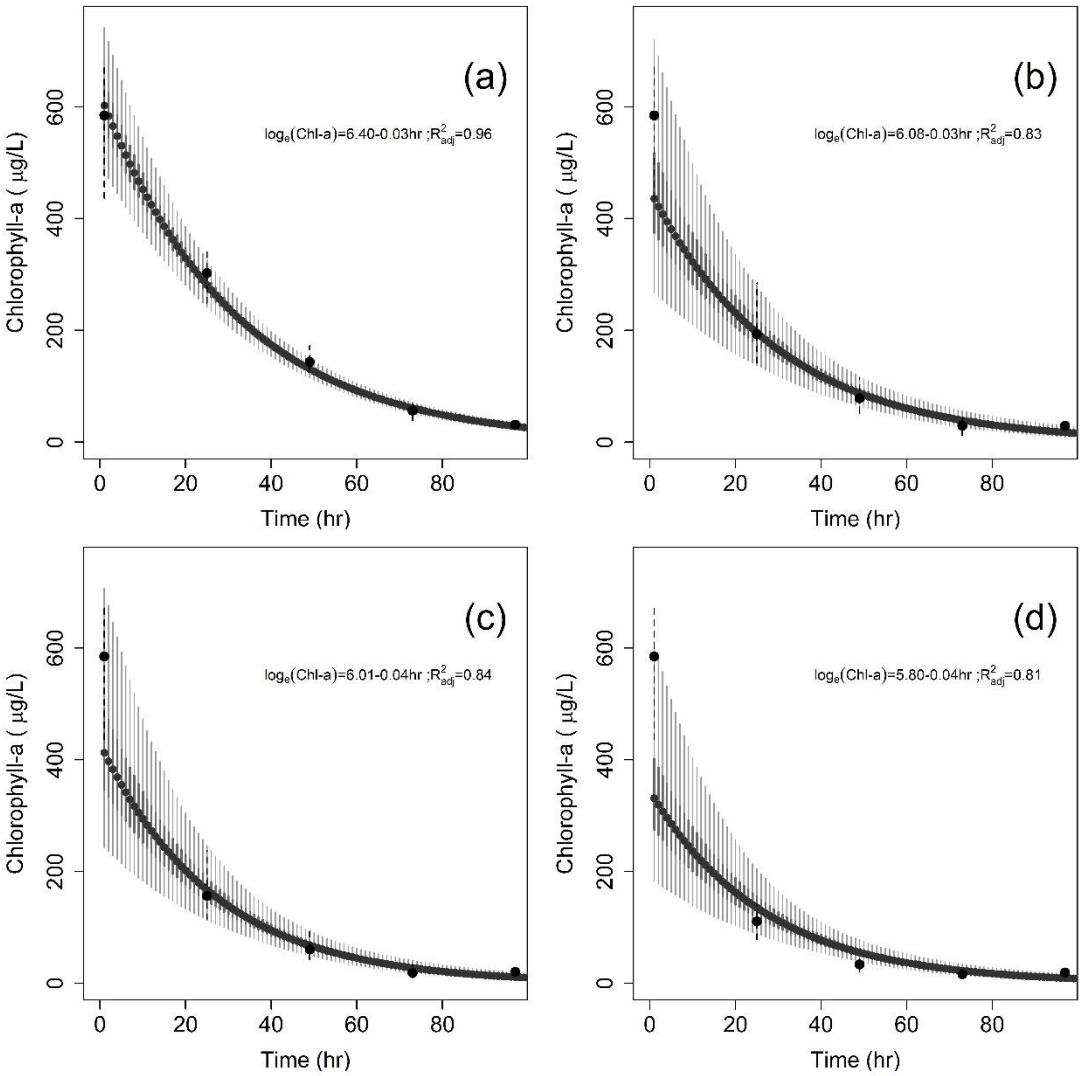

Figure 10 Drop in Chlorophyll-a concentration of *Aphanizomenon* after the application of $CuSO_4$ (a) dosage=0.2 mg/L, (b) dosage=0.5 mg/L, (c) dosage=0.8 mg/L, and (d) dosage=1 mg/L. The dashed horizontal line represents the initial conditions; black solid circles represent average measure concentrations; dashed vertical lines show the range of observed concentration (minimum and maximum). Solid thick vertical grey segments and solid light grey vertical segments represent the 50% and 95% model predicted chlorophyll-a concentrations respectively.

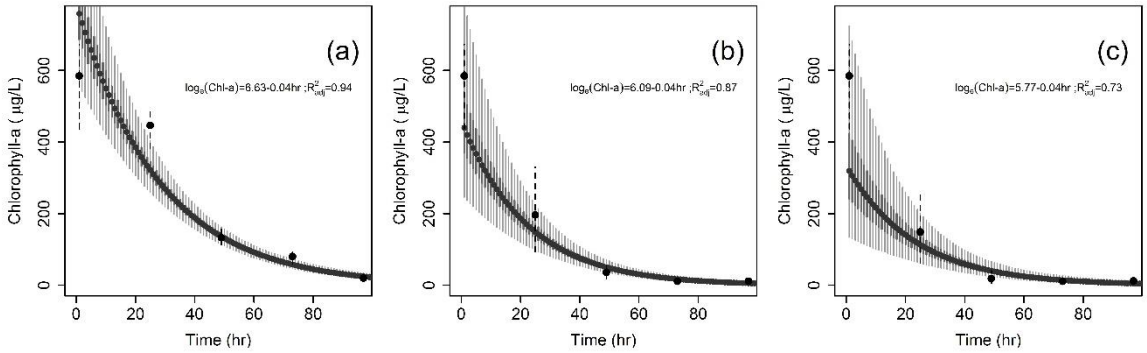

Figure 11 Drop in Chlorophyll-a concentration of *Aphanizomenon* after the application of $KMnO_4$ (a) dosage=1 mg/L, (b) dosage=2 mg/L, and (c) dosage=3 mg/L. The dashed horizontal line represents the initial conditions; black solid circles represent average measure concentrations; dashed vertical lines show the range of observed concentration (minimum and maximum). Solid thick vertical grey segments and solid light grey vertical segments represent the 50% and 95% model predicted chlorophyll-a concentrations respectively.

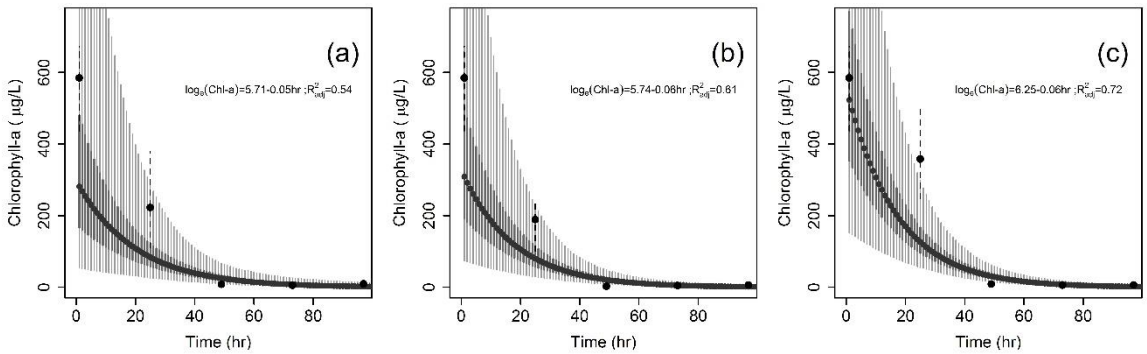

Figure 12 Drop in Chlorophyll-a concentration of *Aphanizomenon* after the application of Diquat (a) dosage=0.25 mg/L, (b) dosage=0.5 mg/L, and (c) dosage=1 mg/L. The dashed horizontal line represents the initial conditions; black solid circles represent average measure concentrations; dashed vertical lines show the range of observed concentration (minimum and maximum). Solid thick vertical grey segments and solid light grey vertical segments represent the 50% and 95% model predicted chlorophyll-a concentrations respectively.

4.4. Management implications

This study established the efficacy of the three tested chemical algaecides in controlling two important nuisance cyanobacteria that are known to form HABs. The results indicate that algaecide efficacy is dependent of the type of cyanobacteria

responsible for the algal bloom. As such, proper characterization of the bloom is critical before application. Moreover, the observed variability in the efficiency of the algaecides across the two cyanobacterial species highlights the potential impact that algaecide application may have on phytoplankton diversity. Changes in diversity can have a cascading effect on the freshwater food web (Jančula & Maršálek, 2011).

While algaecide application proved to be very effective, in the absence of real mitigation measures that aim to reduce the true promoters of algal blooms, repeated application of algaecides may result in a reduced efficacy over time. Several studies have reported the development of resistance strains in some nuisance algal species (Costas et al., 2001; Garcia-Villada et al., 2004). As such, water managers should be made aware that initiating an algaecide application program in a given water body should only be a short-term measure that cannot be a permanent replacement of enacting long-lasting watershed-level management actions. Moreover, the possibility of toxin release upon cell lysis following the use of algaecides is a serious concern that needs attention (Greenfield et al., 2014; Jones & Orr, 1994; Zhou et al., 2013). Note that there is no reported health concerns regarding the residual levels of the algaecides in the treated water. Tests conducted on the residuals levels of Copper, Permanganate, and Diquat after 96 hrs of treatment, showed that all concentrations, irrespective of dose, were within the environmental standards set for drinking water (WHO, 2004).

4.5. Limitation

All inhibition tests were conducted according to the EPA algal inhibition test guidelines that specify that experiments should start with an algae cell density of 10,000 cells/ml (Lewis et al., 1994). Generalizing the findings of our work to observed field

conditions in the Qaraoun reservoir, where algal densities can be more than two orders of magnitude higher (~ 1-2 million cells/ml) than the initial concentrations used in the laboratory inhibition tests, may not be straightforward. There is no consensus in the limnology community on the scalability and transferability of results conducted at the laboratory, mesocosm, and system level (Carpenter, 1996; Pace, 2001; Petersen, Kennedy, Dennison, & Kemp, 2009; Spivak, Vanni, & Mette, 2011). Ultimately, assessing how scale-sensitive our results are will require conducting mesocosm experiments followed by field studies. Yet from an environmental management perspective, application of algaecides should be done at the first sign of an algae bloom. Hypereutrophic conditions are defined when chlorophyll-a concentrations exceed 56 µg/L. Delaying application until concentrations exceed 1,000 µg/L is not recommended. Under such extreme conditions, remedial measures may go beyond algaecide application to include the physical removal of surface scums from the surface water.

From a modeling perspective, the fitted empirical models come with a set of assumptions. The *Microcystis* quadratic regression model structure presumes that the rate of change (slope over time in µg/(L×hrs)) is independent of the initial algal concentrations. As such, algae concentrations (modeled as chl-a) are expected to drop at the same rate over time irrespective of the initial conditions. The model also assumes that the inflection points will not change over time; yet the percent reductions achieved at the inflection point is expected to decrease as initial concentrations increase. Assessing the validity of these assumptions will require additional testing under varying initial concentrations. In the case of the *Aphanizomnenon*, the dynamics following algaecide application were best described with a first order decay model. Under such a model, the rate of change is assumed to be independent of initial concentrations; but

unlike the quadratic regression model, the first order decay model assumes a constant percent drop in chlorophyll-a concentrations over time irrespective of the initial concentration. As such, achieving a particular concentration under a higher initial algal concentration for a given algaecidal dose may still be achieved yet at a longer time period.

It should be noted that this paper did not explore the confounding effects that ambient environmental conditions may have on the effectiveness of the three tested algaecides. In the case of $CuSO_4$, the degree of chelation in the water column can affect its residence time and thus may change its efficacy (Murray-Gulde, Heatley, Schwartzman, & Rodgers Jr, 2002; Salam 2006). In the case of $KMnO_4$, increased hardness of the water has been linked to better algal removal rates (Chen & Yeh, 2005). For Diquat, the presence of high levels of suspended particulates in the water column can cause Diquat to bind to these sediments, rendering it not bioavailable (Clayton & Matheson, 2010; Rodgers Jr, Johnson, & Bishop, 2010).

CHAPTER V

CONCLUSION AND FUTURE WORK

This study is the first to examine concurrently the inhibition that $CuSO_4$, $KMnO_4$, and Diquat have on *Microcystis aeruginosa* and *Aphanizomenon* flos-aquae. The three algaecides proved to be effective in impairing the growth of both cyanobacteria, albeit with significant differences between the two species. Diquat and Copper Sulfate exhibited a strong effect on both targeted cyanobacteria, with Diquat proving to be highly effective on *Microcystis*. Potassium Permanganate was found to be the least effective on *Microcystis*, but was able to effectively control *Aphanizomenon*.

In the future, the following work is proposed:

1. Run the experiments for a longer period of time to better characterize and examine the development of algaecide-resistant cyanobacterial cells (*Microcystis*) that were possibly responsible for the observed regrowth at the end of the treatment. A longer run period will also permit the assessment if the observed regrowth can lead back to the proliferation of dense colonies.
2. Assess the release of cyanotoxins from disrupted cells following the application of the algaecides. This should be conducted through the use of immunochromatographically, phosphatase inhibition, or Enzyme-Linked ImmunoSorbant Assay (ELISA).

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Appendix A

Sample collection and Culturing

Collection

Reservoir sampled used for these experiments were taken from the shores of Qaraoun Lake. During each bloom, a trip was made to collect samples from sections with high algal concentration. Blooms of *Microcytis* were obtained at the beginning of October 2015, while blooms of *Aphanizomenon* were taken later at the end of February 2016. The samples were taken 10 cm below surface of the lake as shown in the figures below (Figures A1 and A2). In the lab the samples were observed under the microscope to ensure that the species responsible for the bloom was the targeted cyanobacteria. The two-targeted cyanobacteria were continuously monitored under the microscope to ensure that a mono-cultural was present.

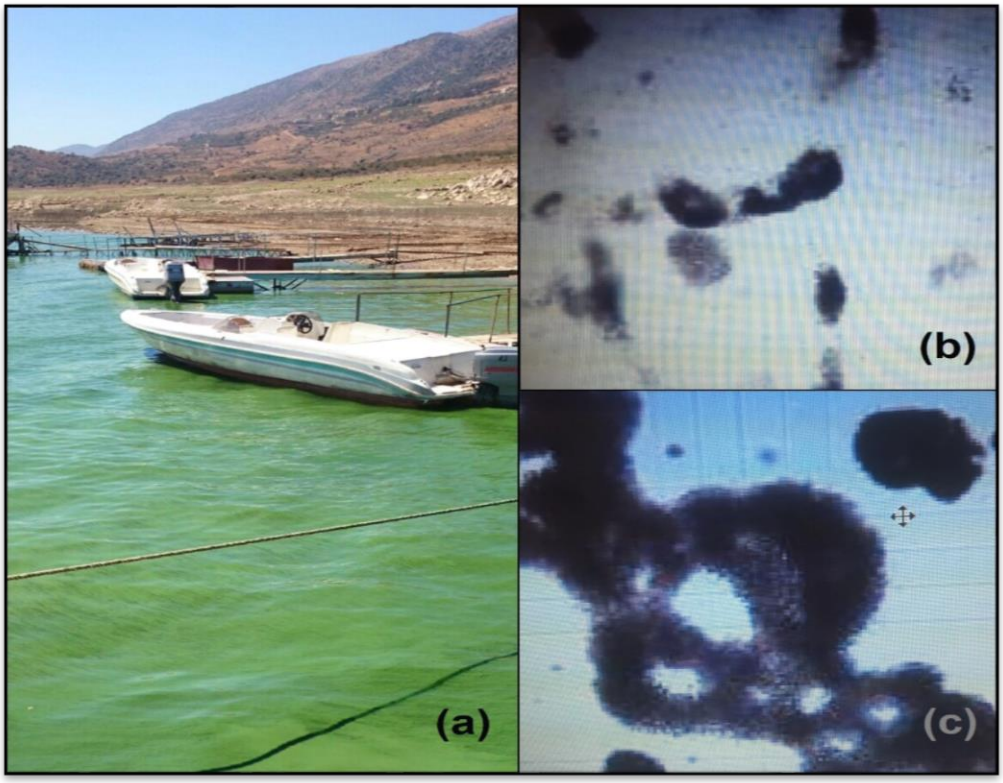

Figure A1 (a) image captured during a *Microcystis* aeruginosa bloom observed at the beginning of October 2015; (b) and (c) are microscopic observations of *Microcystis* colonies

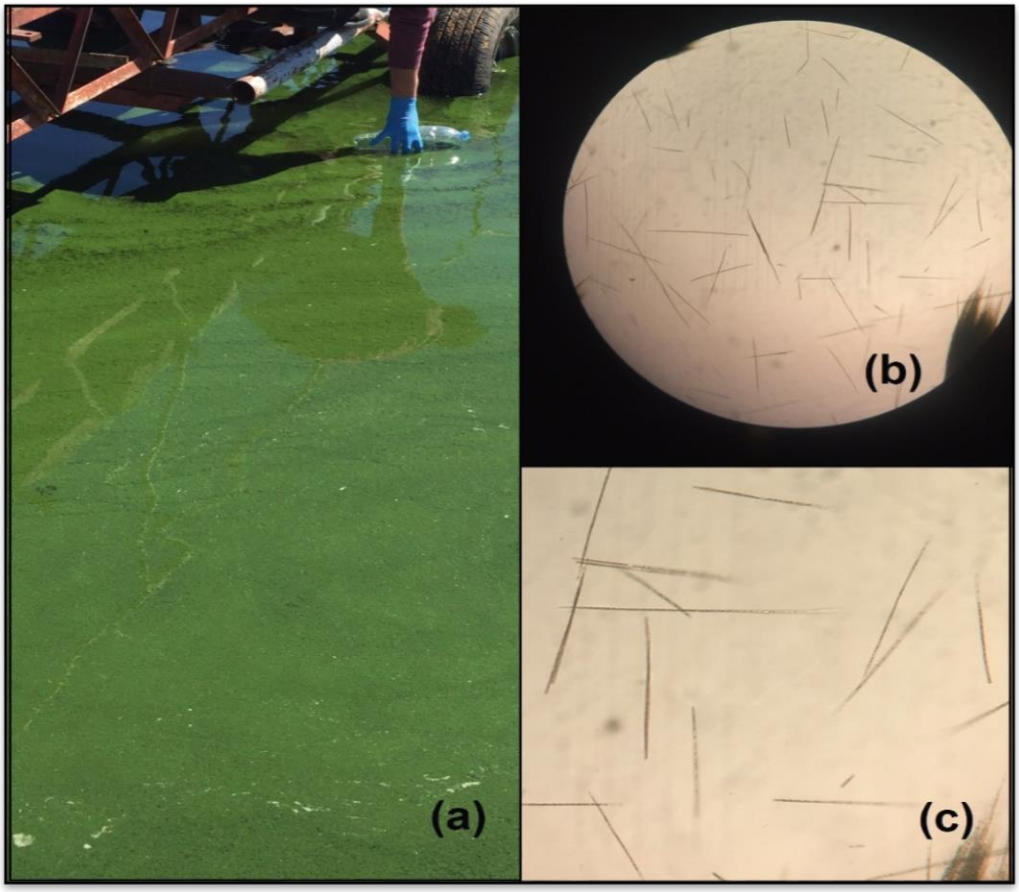

Figure A2 (a) Image captured during an *Aphanizomenon* bloom observed at the end of February 2016; (b) and (c) are microscopic observations of *Aphanizomenon* colonies

Culturing

Samples were grown in 20 L glass beakers that were provided with a 12:12 light:dark cycle, bubbled with air to ensure no carbon limitation and enhance mixing, and 22 ºC , and 25 ºC water temperature were maintained for *Aphanizomenon* and *Microcytis* respectively (Figure A3).

Figure A3. Culturing setup adopted for the cyanobacteria inhibition experiments

Batches were also provided with nutrients, whereby a stock solution of Proline F/2 Algae Food, “A” and “B” for intensive algae culture in fresh or marine water from Aquatic Eco-Systems, was used. The Vitamin and mineral content ratios equal to Guillard's 1975 F/2 formulation (Table A1).

Table A1. Guillard's F/2 Components

Stocks	**per liter**
(1) $NaNO_3$	75g
(2) $NaH_2PO_4.2H_2O$	5.65g
(3) Trace elements (chelated)	
NA_2 EDTA	4.16 g
$FeCl_3.6H_2O$	3.15 g
$CuSO_4.5H_2O$	0.01 g
$ZnSO_4.7H_2O$	0.022 g
$CoCl_2.6H_2O$	0.01 g
$MnCl_2.4H_2O$	0.18 g
$Na_2MoO_4.2H_2O$	0.006 g
(4) Vitamin mix	
Cyanocobalamin (Vitamin B_{12})	0.0005 g
Thiamine HCl (Vitamin B_1)	0.1 g
Biotin	0.0005 g

Medium per liter	
$NaNO_3$	1.0 mL
$NaH_2PO_4.2H_2O$	1.0 mL
Trace elements stock solution	1.0 mL
Vitamin mix stock solution	1.0 mL

To ensure that the algae remained healthy with a mean density > $1.0x10^6$ cells/ml (EPA, 1994), algae samples were sub-cultured in a new vessel and diluted with synthetic water with 3:1 ratio of synthetic to cultured water once the exponential phase was reached. Synthetic water, serving as dilution water, was generated from filtered raw water that was enriched with nutrients and microelements essential for algal growth at the same concentration used in the culture medium (EPA, 2002).

Appendix B

Daily measurements of dissolved oxygen, temperature and pH

Daily measurements of dissolved oxygen, temperature and pH Temperature, pH, and DO were monitored daily throughout the experiment across all inhibition flasks. The readings are shown in details in the table below. The average of the triplicates for each algaecide/dosage combination are shown in Tables B1 and B2.

Table B1 Daily Analysis of Physiochemical parameters of *Microcystis*

Microcystis	**Time (hrs)**	**Temperature (ºC)**	**pH**	**Dissolved Oxygen (ppm)**
Initial	0	22	8.01	-
Control	24	22.93	8.55	11.55
$CuSO_4$ 0.2mg/L	24	22.9	8.53	11.34
$CuSO_4$ 0.5mg/L	24	23.07	8.54	11.25
$CuSO_4$ 0.8mg/L	24	23.1	8.47	11.39
$CuSO_4$ 1 mg/L	24	23.5	8.53	11.31
$KMNO_4$ 1mg/L	24	23.13	8.55	11.81
$KMNO_4$ 2mg/L	24	23.17	8.53	11.5
$KMNO_4$ 3mg/L	24	23.4	8.52	10.71
Diquat 0.5mg/L	24	23.13	8.59	11.42
Diquat 1mg/L	24	23.37	8.6	11.74
Control	48	24.67	9.63	-
$CuSO_4$ 0.2mg/L	48	25.43	8.96	13.39
$CuSO_4$ 0.5mg/L	48	24.77	8.22	8.98
$CuSO_4$ 0.8mg/L	48	24.73	8	8.36
$CuSO_4$ 1 mg/L	48	25.23	7.99	8.13
$KMNO_4$ 1mg/L	48	24.57	9.74	-
$KMNO_4$ 2mg/L	48	24.53	9	15.34
KMNO4 3mg/L	48	24.5	8.64	11.11
Diquat 0.5mg/L	48	24.6	8.33	9.88
Diquat 1mg/L	48	25.13	8.31	9.51
Control	72	24.23	9.54	-
$CuSO_4$ 0.2mg/L	72	24.37	8.41	-
$CuSO_4$ 0.5mg/L	72	24.7	7.73	-
$CuSO_4$ 0.8mg/L	72	23.97	7.64	-

Microcystis	Time (hrs)	Temperature (ºC)	pH	Dissolved Oxygen (ppm)
$CuSO_4$ 1 mg/L	72	24.93	7.67	-
$KMNO_4$ 1mg/L	72	25.07	9.78	-
$KMNO_4$ 2mg/L	72	25.2	9.19	-
$KMNO_4$ 3mg/L	72	24.7	8.67	-
Diquat 0.5mg/L	72	24.43	7.77	-
Diquat 1mg/L	72	24.17	7.91	-
Control	96	23.5	9.26	9.44
$CuSO_4$ 0.2mg/L	96	24.7	7.93	7.76
$CuSO_4$ 0.5mg/L	96	24.67	7.85	6.8
$CuSO_4$ 0.8mg/L	96	24.7	7.81	7.35
$CuSO_4$ 1 mg/L	96	23.5	7.79	7.88
$KMNO_4$ 1mg/L	96	23.7	9.42	8.52
$KMNO_4$ 2mg/L	96	24.17	9.03	8.74
$KMNO_4$ 3mg/L	96	23.8	8.45	8.99
Diquat 0.5mg/L	96	24.8	7.67	8.11
Diquat 1mg/L	96	25	7.66	5.57

Table B2 Daily Analysis of Physiochemical parameters of *Aphanizomenon*

Aphanizomenon	Time (hrs)	Temperature (ºC)	pH	Dissolved Oxygen (ppm)
Initial	0	22.50	6.83	7.40
Control	24	22.10	8.63	8.44
CuSO4 0.2mg/L	24	22.97	7.01	2.26
CuSO4 0.5mg/L	24	23.07	6.60	-
CuSO4 0.8mg/L	24	23.50	6.79	-
CuSO4 1 mg/L	24	23.87	6.54	0.89
KMNO4 1mg/L	24	23.93	9.17	-
KMNO4 2mg/L	24	23.20	8.75	3.38
KMNO4 3mg/L	24	23.10	7.48	5.37
Diquat 0.25mg/L	24	22.80	6.50	-
Diquat 0.5mg/L	24	22.73	6.61	-
Diquat 1mg/L	24	23.20	6.81	-
Control	24	22.80	7.14	0.82
CuSO4 0.2mg/L	48	23.03	6.38	0.96
CuSO4 0.5mg/L	48	23.17	6.44	1.24
CuSO4 0.8mg/L	48	22.83	6.37	1.35
CuSO4 1 mg/L	48	23.03	6.35	1.54
KMNO4 1mg/L	48	23.27	6.20	0.97

Aphanizomenon	Time (hrs)	Temperature (ºC)	pH	Dissolved Oxygen (ppm)
KMNO4 2mg/L	48	23.27	6.18	0.72
KMNO4 3mg/L	48	22.83	6.21	0.78
Diquat 0.25mg/L	48	23.47	6.14	0.60
Diquat 0.5mg/L	48	22.47	5.98	0.46
Diquat 1mg/L	48	22.37	6.03	0.54
Control	48	21.80	7.00	2.71
CuSO4 0.2mg/L	72	22.53	6.45	2.52
CuSO4 0.5mg/L	72	22.63	6.90	3.88
CuSO4 0.8mg/L	72	22.97	6.81	4.20
CuSO4 1 mg/L	72	21.80	6.80	4.10
KMNO4 1mg/L	72	23.47	6.59	2.92
KMNO4 2mg/L	72	23.73	6.60	3.28
KMNO4 3mg/L	72	22.70	6.65	3.15
Diquat 0.25mg/L	72	23.27	6.58	4.36
Diquat 0.5mg/L	72	22.17	6.63	3.76
Diquat 1mg/L	72	22.60	6.72	3.10
Control	96	21.77	7.60	6.85
CuSO4 0.2mg/L	96	22.43	7.49	6.27
CuSO4 0.5mg/L	96	22.37	7.60	7.21
CuSO4 0.8mg/L	96	22.27	7.59	6.81
CuSO4 1 mg/L	96	21.67	7.60	6.86
KMNO4 1mg/L	96	22.67	7.39	6.15
KMNO4 2mg/L	96	22.27	7.34	6.46
KMNO4 3mg/L	96	22.33	7.33	6.48
Diquat 0.25mg/L	96	23.57	7.38	6.30
Diquat 0.5mg/L	96	23.53	7.28	6.51
Diquat 1mg/L	96	22.63	7.22	6.08

Appendix C

Daily Cell Counts

For cell counts, a sample of 0.1 mL was taken from each of the Erlenmeyer flasks and placed on a counting slide (hemocytometer), then covered with a cover slip. The technique of counting cells was adopted from the Marienfeld counting chamber manual. An excel sheet was prepared to make the counting easier based on the following formula:

$$\frac{\text{Number of cells}}{\text{Counted area } (\text{mm}^2) \text{ x chamber depth (mm) x dilution}} = \text{Cells/mL of Cyanobacteria}$$

Daily cell counts averaged over the triplicates are shown in Table C1.

Table C1 Average cell densities of *Microcystis* and *Aphanizomenon* for each dosage across a period of 96 hrs

	Time			
Experiment	24 hrs	48 hrs	72 hrs	96 hrs
Microcystis				
Control	2.17E+04	1.41E+04	1.33E+04	Undetectable
CuSO4 0.2mg/L	1.87E+04	1.37E+04	1.17E+04	Undetectable
CuSO4 0.5mg/L	1.20E+04	9.80E+03	3.90E+03	Undetectable
CuSO4 0.8mg/L	1.07E+04	9.13E+03	4.97E+03	Undetectable
CuSO4 1 mg/L	1.01E+04	9.00E+03	2.80E+03	Undetectable
KMNO4 1mg/L	1.37E+04	9.73E+03	4.67E+03	Undetectable
KMNO4 2mg/L	9.60E+03	8.80E+03	2.27E+03	Undetectable
KMNO4 3mg/L	9.53E+03	7.53E+03	2.13E+03	Undetectable
Diquat 0.5mg/L	2.13E+04	1.83E+04	1.53E+03	Undetectable
Diquat 1mg/L	2.13E+04	1.47E+04	2.87E+03	Undetectable
Aphanizomenon				
Control	5.90E+03	4.45E+03	1.11E+03	2.96E+03
CuSO4 0.2mg/L	4.20E+03	2.33E+03	8.00E+02	4.00E+02
CuSO4 0.5mg/L	-	2.07E+03	1.33E+02	5.00E+01
CuSO4 0.8mg/L	1.90E+03	1.93E+03	7.00E+02	2.00E+02
CuSO4 1 mg/L	6.00E+03	1.27E+03	4.00E+02	1.00E+01
KMNO4 1mg/L	3.01E+03	2.27E+03	6.00E+02	5.13E+01
KMNO4 2mg/L	1.15E+03	3.00E+02	3.33E+01	2.33E+01
KMNO4 3mg/L	-	2.50E+02	3.33E+01	1.23E+01
Diquat 0.25mg/L	1.16E+04	5.20E+03	2.50E+03	9.63E+02
Diquat 0.5mg/L	1.07E+04	1.90E+03	4.00E+02	1.33E+02
Diquat 1mg/L	8.60E+03	4.27E+03	4.00E+02	0.00E+00

Appendix D

Algaecide residual concentrations

The residual levels of Copper and Permanganate was quantified as Cu^{2+} and Mn^{2+} respectively, using graphite furnace atomic absorption spectrophotometry, while High Performance Liquid Chromatography was used for Diquat. All residuals were found to be below the environmental standards set for drinking water (Table D1). The WHO standards for Cu < 2 mg/L. The WHO guideline value for Mn in potable water is 0.4 mg/L. Given that Diquat is strongly adsorbed to soil and is rarely reported in drinking water sources, the WHO proposed an acute health-based values of 20 mg/L.

Table D1 Residual levels of algaecides in samples of *Microcystis* and *Aphanizomenon* after 96 hrs of exposure

		Microcystis	***Aphanizomenon***
Copper Cu2+ (mg/L)	$CuSO_4$ 0.2mg/L	0.197	0.125
	$CuSO_4$ 0.5mg/L	0.449	0.179
	$CuSO_4$ 0.8mg/L	0.662	0.226
	$CuSO_4$ 1 mg/L	0.733	0.245
Permanganate Mn2+ (mg/L)	$KMnO_4$ 1mg/L	0.162	0.154
	$KMnO_4$ 2mg/L	0.309	0.232
	$KMnO_4$ 3mg/L	0.149	0.312
Diquat (mg/L)	Diquat 0.25mg/L	-	0.196
	Diquat 0.5mg/L	0.239	0.284
	Diquat 1mg/L	0.593	0.603

*Values recorded are averages of the triplicates measured on 96 hrs