Environmental Science
Water Research & Technology

QUANTIFYING THE EFFICACY OF DIQUAT DIBROMIDE IN CONTROLLING MICROCYSTIS AERUGINOSA AND APHANIZOMENON FLOS-AQUAE IN COMPARISON TO COPPER SULFATE AND POTASSIUM PERMANGANATE

Journal:	*Environmental Science: Water Research & Technology*
Manuscript ID	EW-ART-08-2018-000532.R2
Article Type:	Paper
Date Submitted by the Author:	26-Nov-2018
Complete List of Authors:	Dia, Sara; American University of Beirut Faculty of Engineering and Architecture, Civil and Environmental Engineering Alameddine, Ibrahim; American University of Beirut Faculty of Engineering and Architecture, Civil and Environmental Engineering El-Fadel, Mutasem; Amer Univ Beirut, Civil and Environmental Engineering

The results showed that Diquat dibromide was more effective than commonly used algaecides in controlling toxin forming *Microcystis aeruginosa* and *Aphanizomenon flos-aquae*. Irrespective of the algaecide used and the dosage applied, *Microcystis aeruginosa* was able to reestablish itself over time.

QUANTIFYING THE EFFICACY OF DIQUAT DIBROMIDE IN CONTROLLING *MICROCYSTIS AERUGINOSA* AND *APHANIZOMENON FLOS-AQUAE* IN COMPARISON TO COPPER SULFATE AND POTASSIUM PERMANGANATE

Sara Dia[1], Ibrahim Alameddine[1*], Mutasem El-Fadel[1]

[1]: Department of Civil & Environmental Engineering, Maroun Semaan Faculty of Engineering & Architecture, American University of Beirut, Beirut, Lebanon.

*: Corresponding author: Department of Civil & Environmental Engineering, Maroun Semaan Faculty of Engineering & Architecture, American University of Beirut, Beirut, Lebanon. Email: ia04@aub.edu.lb

ABSTRACT

Cyanobacterial harmful algal blooms (HABs) are an emerging problem worldwide, affecting many important freshwater systems. The use of chemical algaecides can provide an effective short-term mitigation measure to control HABs. In this study, the efficacy of Diquat dibromide was examined under laboratory conditions to control two problematic toxin-releasing cyanobacteria, namely *Aphanizomenon flos-aquae* and *Microcystis aeruginosa.* Its performance was then compared to that of Copper sulfate ($CuSO_4$) and Potassium permanganate ($KMnO_4$), two commonly used algaecides. The results suggest that while all three algaecides were effective in controlling *Aphanizomenon,* the highest inhibition rates achieved were associated with the application of Diquat dibromide. *Aphanizomenon* exhibited a half-life of 0.48 days with Diquat dibromide dosages of 0.5 and 1 mg/L. Both Diquat dibromide and $CuSO_4$ exhibited more effectiveness in controlling *Microcystis aeruginosa* as compared to $KMnO_4$. Reductions achieved by applying 0.5 mg/L of Diquat dibromide or 1 mg/L of $CuSO_4$ exceeded 95% after 48 hrs of treatment. Nevertheless, Diquat dibromide suppressed the net regrowth of *Microcystis aeruginosa* up to 70 hrs, while suppression with $CuSO_4$ did not exceed 64 hrs even with the highest applied dosage. Irrespective of the algaecide and the application dosage, regrowth was observed for *Microcystis aeruginosa* but not for *Aphanizomenon flos-aquae*. Statistical models were proposed to simulate inhibition rates and estimate net algal regeneration.

Keywords: Algaecide, cyanobacteria, HAB, Diquat dibromide, Copper Sulfate, Potassium Permanganate, *Microcystis aeruginosa*, *Aphanizomenon flos-aquae*

1 INTRODUCTION

Anthropogenic eutrophication is a global problem affecting many important aquatic systems. Excessive nutrient loading has been a main driver for Harmful Algal Blooms (HABs) proliferation [1-3]. Cyanobacteria are the major algae type associated with the proliferation of HABs and impairments of freshwater systems [4-7]. *Microcystis*, *Anabaenopsis*, *Aphanizomenon*, *Oscillatoria*, *Nostoc*, and *Planktothrix* are some common genera 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 [8, 9]. *Micrococystis* blooms have been responsible for the impairment of important lake systems around the world, such as Lake Okeechobee in Florida [10], Lake Erie [11, 12], and Lake Taihu in China [1, 13]. Another pervasive toxin producing cyanobacteria genera is *Aphanizomenon flos-aquae*. It is known to release cylindrospermopsins, a hepatoxin that can cause gastrointestinal damage [9, 14, 15], along with anatoxins and saxitoxin, which are known to affect neurological activities and can lead to death through respiratory paralysis [16, 17]. Several freshwater bodies in the Mediterranean region along with Lake Dianchi in China, Lake Erie, Utah Lake, and the Upper Klamath Lake in Oregon have been negatively affected by *Aphanizomenon* blooms [14, 18-20].

Mitigation measures have been tested to control excessive algal blooms including algaecides applications, mechanical mixing, food web manipulations, and/or nutrient regulation [21-30]. Nutrient enrichment has been strongly linked to increased eutrophication and the stimulation of harmful algal blooms [3]. Examples of the success of nutrient management in reducing HAB events include the decline of HAB events in Lake Washington and the Potomac River following

the removal of sewage discharges into these systems [31, 32]. Similar successes have also been reported in Lake Erie, where improved wastewater treatment and the ban on phosphate detergents in the early 1980s significantly reduced HAB events [33]. In 2003, the US Environmental Protection Agency concluded that managing nutrient inputs to the watershed can lead to significant reduction in HABs [34]. Nevertheless, there is evidence to show that the overall effect of nutrient load reductions on harmful algal proliferation tends to be species specific [3]. While controlling nutrients emerging from point and non-point sources at the river-basin level remains the most effective approach on the long term, effective implementation is often hindered by socio-economic constraints and/or lack of regulatory enforcement. As a result, short-term mitigation measures are needed. Of these measures, algaecides application remains the most commonly adopted.

Copper Sulfate ($CuSO_4$) remains to date the most commonly used algaecide in freshwater systems [35-37]. It has been in use since the early 1900's, with early application recorded in the Fairmont lakes [38, 39]. Its wide use was promoted by the fact that it is inexpensive, accessible, and effective against cyanobacteria [5, 40-44]. It acts by interrupting electron transport through photosystem II, increasing oxidative stress, and competing with magnesium in the chlorophyll molecule [35, 45-47]. Potassium Permanganate ($KMnO_4$) is another commonly used algaecide that was promoted by its low environmental hazard [48-53]. Its use has been documented across many freshwater systems including storm-water retention lakes in Canada, fish ponds in the United States, Lake Biwa in Japan, and to lakes and ponds in China [54-58]. $KMnO_4$ is an oxidant that releases hydrated manganese dioxide (MnO_2), which stimulates oxidation-enhanced coagulation of surface algae cells [59-62]. A less commonly used algaecide is Diquat dibromide (IUPAC name: 6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium dibromide), which has been commercially

available since the early 1950s [63, 64]. It was registered with the United States Environmental Protection Agency (USEPA) in 1986 [65] and shown to be effective on a wide spectrum of cyanobacteria [64, 66-70]. It disrupts the electron transport in photosynthetic tissues by changing Nicotinamide Adenine Dinucleotide Phosphate (NADP) [71, 72].

In this study, we examined the efficacy of Diquat dibromide in controlling *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* blooms through a series of laboratory-based inhibition tests conducted under different dosages. Efficacy was determined in terms of the measured decrease in chlorophyll-a concentrations over 96 hours of the inhibition test. The performance of Diquat dibromide was then compared to that of $CuSO_4$ and $KMnO_4$. Mathematical decay models were then developed to quantify the net change in cyanobacteria over time for the three algaecides using the measured chlorophyll-a concentrations. The adopted decay models permit a temporally variable rate of change. A positive net change value indicates a net regrowth of the algae, while a negative value represents a net drop in their levels. The residual concentrations of the three algaecides at the end of the 4-day experiment were measured and compared to the World Health Organization drinking water quality standards [8, 16].

2 MATERIALS AND METHODOLGY

2.1 Sample collection

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 hypertrophic due to excessive point and non-point pollution loading [73, 74]. *Microcystis* blooms have been consistently occurring over the summer season, while *Aphanizomenon* blooms tend to occur between late winter and early summer [75]. Cyanobacteria were collected from the reservoir during algal bloom events.

Blooms are often characterized by the dominance of a single nuisance algal community [76-78]. 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 (2 weeks) and inhibition tests (5 days) to ensure that no other algae type other than the one targeted was observable under the microscope. In the event that multiple species were observed, the entire batch was discarded and the inhibition tests were aborted.

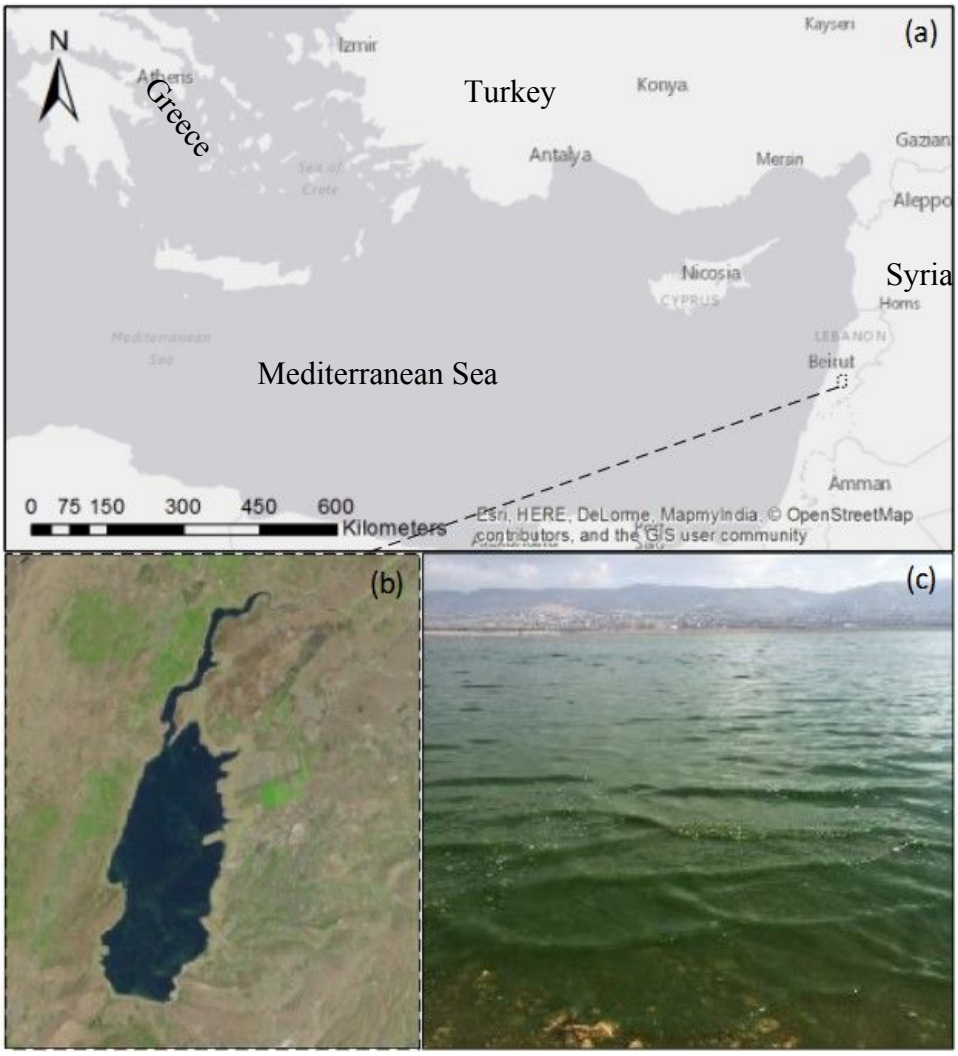


Figure 1 (a) Geographical location of the Qaraoun reservoir; (b) a Landsat 8 satellite image on July 4, 2013 showing from space a developing cyanobacteria bloom; and (c) an image captured during a *Microcystis aeruginosa* bloom observed on September 22, 2015.

2.2 Cyanobacteria culturing

Collected cyanobacteria water samples were cultured under laboratory conditions in 20 L glass containers under a 12:12 light:dark cycle with a light intensity ~74 Lux. Cultures were enriched with nutrients, air bubbled to enhance mixing and ensure no carbon limitation, and maintained at 25 ^{o}C water temperature for *Microcystis* and 21 ^{o}C for *Aphanizomenon* [79]. In an effort to ensure no nutrient limitation, an F/2 medium based on the Guillard's formulation [80] of essential nutrients was used. Samples were routinely sub-cultured to achieve a cell density $>1.0\times10^{6}$ cells/ml; this ensures that the algae maintains the logarithmic growth phase [79]. For the algaecide inhibition experiments, the algae were extracted and diluted by adding filtered lake water to reach ~10,000 cells/ml [79]. The cell density was determined with a hemocytometer using a fluorescence microscope. Note that the culturing of the cyanobacteria under laboratory conditions guaranteed that: 1) all experiments were run with monocultures; 2) the algae were acclimatized and able to achieve a logarithmic growth phase prior to exposing them to the algaecides; and 3) that all experiments were started with the same initial cell density.

2.3 Experimental procedures

Selected algaecide concentrations represented concentrations previously used for algae control. All tested algaecide concentrations were prepared using reagent grade quality and ultra-pure water freshly provided by a water filter system (MilliQ, Millipore). Diquat dibromide[1] was prepared from analytical standard grade Diquat dibromide monohydrate (SUPELCO N11816). Its efficacy was tested under two concentrations, namely 0.5 and 1 mg/L as Diquat. High-

[1] Diquat dibromide will be referred to as Diquat in the remaining of the manuscript

performance liquid chromatography (HPLC) with a C18 Column provided by SUPELCO (Z226033), based on USEPA method 549.2, was used to measure Diquat concentrations (Hodgeson et al. 1997). When Diquat concentrations were below the detection limit (<0.72μg/L), a solid-phase extraction [81] was carried out with C_8 (500 mg)–6 ml cartridges obtained from SUPELCO (52714-U). All solvents for the mobile phase and extraction procedure were HPLC grade. For $CuSO_4$ (Sigma Aldrich 209198 ACS reagents) and $KMnO_4$ (Merck and Co. M5080), stock solutions were used to achieve the desired chemical dosages (0.2, 0.5, 0.8, and 1 mg/L as $CuSO_4$ and 1, 2, and 3 mg/L as $KMnO_4$) following USEPA method 200.9 [82].

The experimental chambers for inhibition tests were 250 ml Erlenmeyer flasks with a cyanobacteria density of ~10,000 cells/ml. All experiments were conducted according to the USEPA method with all chambers irradiated by florescent lamps providing 4306 Lux [83]. Triplicate tests were run for each algaecide dose. In addition, cyanobacterial concentrations were monitored in three control flasks. The duration of the inhibition test was set to 96h, with the analysis of 50 ml samples collected every 24h. Daily analysis included for pH, DO, temperature, chlorophyll-a, and cell counts. The physio-chemical analysis for the *Microcystis* and *Aphanizomenon* tests are summarized in Tables S1 and S2 in the Supplemental Material.

Chlorophyll-a concentrations were measured by collecting samples with known volumes buffered using magnesium carbonate before being filtered through membrane filter papers. They were stored overnight at -20 ^{o}C. Chlorophyll-a was extracted in liquid chromatography grade acetone (90%) solution followed by sonification. Extracts were seeped in the acetone solution overnight and then clarified using centrifugation. Chlorophyll-a concentrations were evaluated based on absorbance readings (Standard Method 10200 (HS2)) [84] on a HACH DR3900 spectrophotometer [85]. For cell counts and cell density, cells of *Aphanizomenon flos-aquae* and

Microcystis aeruginosa were microscopically monitored using a Zeiss Fluorescence microscope (Axiovert 200) with an improved hemocyatometer (Marienfeld), with emphasis on recording whether they were colonial or unicellular. Chlorophyll-a concentrations were used as the response endpoint to test algaecide potency. Calomeni and Rodgers [86] identified chlorophyll-a as a suitable surrogate to assess algal viability.

2.4 Statistical Analysis

The effect of dosage and potential changes in efficiency over time were assessed across the two cyanobacteria species. Two-way Analysis Of Variance (ANOVA) tests were used to evaluate the potential for an interaction between dose and time. In the event that the 2-way ANOVA showed statistical significance, Tukey Honest significant differences multiple comparisons were used. Note that chlorophyll-a concentration of *Microcystis* and *Aphanizomenon* were square root-transformed to achieve normality. ANOVA and multiple comparisons analysis were conducted in the R software [87].

Dose-dependent algaecide specific cyanobacterial decay models were developed to predict the rate of change in chlorophyll-a concentrations as a function of time (measured in hours) for the two cyanobacteria. Two decay model structures were considered for this purpose. The first modeled the drop in chlorophyll-a concentrations as a first order exponential decay (Equation 1), while the second was based on a second order polynomial model (Equation 2). Note that the later model structure allows for the occurrence of an inflection point beyond which regrowth surpasses the residual algaecidal inhibition. The adopted quadratic concave formulation has been reported to account for decreasing proportional pressure, which can ultimately lead to net regrowth [88]. The exponential decay model was fist linearized and both models were fit using

linear regression that minimizes the sum of squared errors. The adjusted R^2 (R^2_{adj}) was used as a measure of model fit. Models were fit using the linear model lm function in R [87].

$$Chl_a = C_0 \times e^{\beta_1 \times Time_{hrs}} \quad (1)$$

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

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

Where Chl_a is the measured chlorophyll-a concentration in µg/L over time, C_0 is the initial chlorophyll-a concentration at time t=0, *Time* is time in hours following algaecide application, β_0 represents the intercept of the model, β_{1_2} are the slopes on the predictors, ε is the model error term, and σ is the standard error of the model. The predictive model uncertainties were quantified using a Monte Carlo simulation in the "*arm*" package in R [89]. Note that the use of Monte Carlo simulations helps address re-transformation biases [90].

3 RESULTS

3.1 Descriptive statistics

3.1.1 Diquat

Diquat was highly effective in controlling both cyanobacterial species under the tested dosages. During the first 24 hrs, *Microcystis* colonies were no longer visible under the microscope for both the 0.5 and 1 mg/L applications. Yet after the first 24 hrs, chlorophyll-a concentrations were still around 50 to 65 % of their original levels for both dosages. After 48 hrs of treatment, unicellular *Microcystis* became hard to observe and inhibition approached 100%. Recovery of some unicellular cells was observed after 96 hrs, with measured chlorophyll-a concentrations

reaching 17 and 11% of their initial concentrations for the 0.5 and 1 mg/L doses respectively (Figure 2.a). Measured chlorophyll-a concentrations at 48, 72, and 96 hrs were found to be statistically similar indicating that the net regrowth of *Microcystis* was slow.

Similarly, the effect of Diquat on *Aphanizomenon* colonies was immediate with colonies disappearing after 24 hrs; the drop in chlorophyll-a concentrations ranged between 40% and 68% in the first 24 hrs. Nevertheless, the inhibiting action of Diquat accelerated in the next 24 hrs whereby chlorophyll-a concentrations dropped by more than 95 to 99% after 48 to 72 hrs of treatment (Figure 2.b). Chlorophyll concentrations achieved at 48, 72, and 96 hrs were statistically similar indicating a lack of net regrowth. Note that while previous work reported 100% inhibition of *Aphanizomenon* at Diquat dosages in excess of 0.75 mg/L [66], our results indicated that complete inhibition can occur at a lower dosages (0.5 mg/L).

3.1.2 $CuSO_4$

$CuSO_4$ proved to be effective in controlling *Microcystis* across all dosages. Microscopic observations showed that *Microcystis* colonies broke down into individual cells within the first 24 hrs. Chlorophyll-a concentrations decreased on average by 20 % at the lowest tested dose of 0.2 mg/L (Figure 2.c). Yet, reductions exceeded 75% after 24 hrs of treatment at higher dosages (0.5, 0.8, and 1 mg/L). These results show that $CuSO_4$ has a faster inhibition action as compared to the Diquat concentrations used. After 48 hrs of being subjected to the highest dose (1 mg/L), chlorophyll-a concentrations dropped to 5 % of their original values. Similar removal efficiencies were reported by Fan et al. [91]. The net regrowth of *Microcystis* following $CuSO_4$ application occurred earlier than Diquat. Recovery following $CuSO_4$ treatment started at 72 hrs versus 96 hr for Diquat. Moreover, the recovery rate of *Microcystis* following $CuSO_4$ treatment

was higher than that observed for Diquat, with measured concentrations at 96 hrs statistically similar to those observed at 24 hrs (p-value = 0.99).

$CuSO_4$ was also found to be potent in controlling *Aphanizomenon flos-aquae*; yet its efficacy was lower than that of Diquat (Figure 2). Under microscopic observations, the decolonization of *Aphanizomenon* was apparent following the first day of treatment. Reductions after 48 hrs of contact time did not exceed 75% of the original chlorophyll-a concentration at the lowest tested dose (0.2 mg/L). At higher dosages, reductions reached 87%, 90% and 95% after 48 hrs of contact to 0.5, 0.8, and 1 mg/L respectively. No regrowth was evident across all tested dosages (Figure 2.d).

3.1.3 KMnO4

$KMnO_4$ was less effective than Diquate and $CuSO_4$ in controlling *Microcystis*. Overall, measured chlorophyll-a concentrations following the application at the two lower dosages (1 and 2 mg/L) were found to be statistically similar to the control (p-values = 0.99 and 0.11 respectively). At the highest dosage of 3 mg/L, the removal rate after 48 hrs did not exceed 80% on average (Figure 2.e). Chlorophyll-a concentrations appeared to rebound after 72 hrs and on day 4 (96 hrs) were statistically similar to levels in the control across all dosages (Figure 2.e). While $KMnO_4$ proved to be ineffective in controlling *Microcystis*, it proved to be a good inhibitor of *Aphanizomenon*. Colonies of *Aphanizomenon* declined significantly even after 24 hrs of treatment across all dosages. Dosages of 2 mg/L and higher achieved removals in excess of 98% at 72hrs. Previous studies suggested the need for a higher (5 mg/L) dosage for controlling *Aphanizomenon* [57]. 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. Both dosages achieved more

than 98% reductions at 96 hrs (Figure 2.f). No net regrowth was apparent, with measured chlorophyll-a levels largely stable at 72 and 96 hrs (p-value = 0.17).

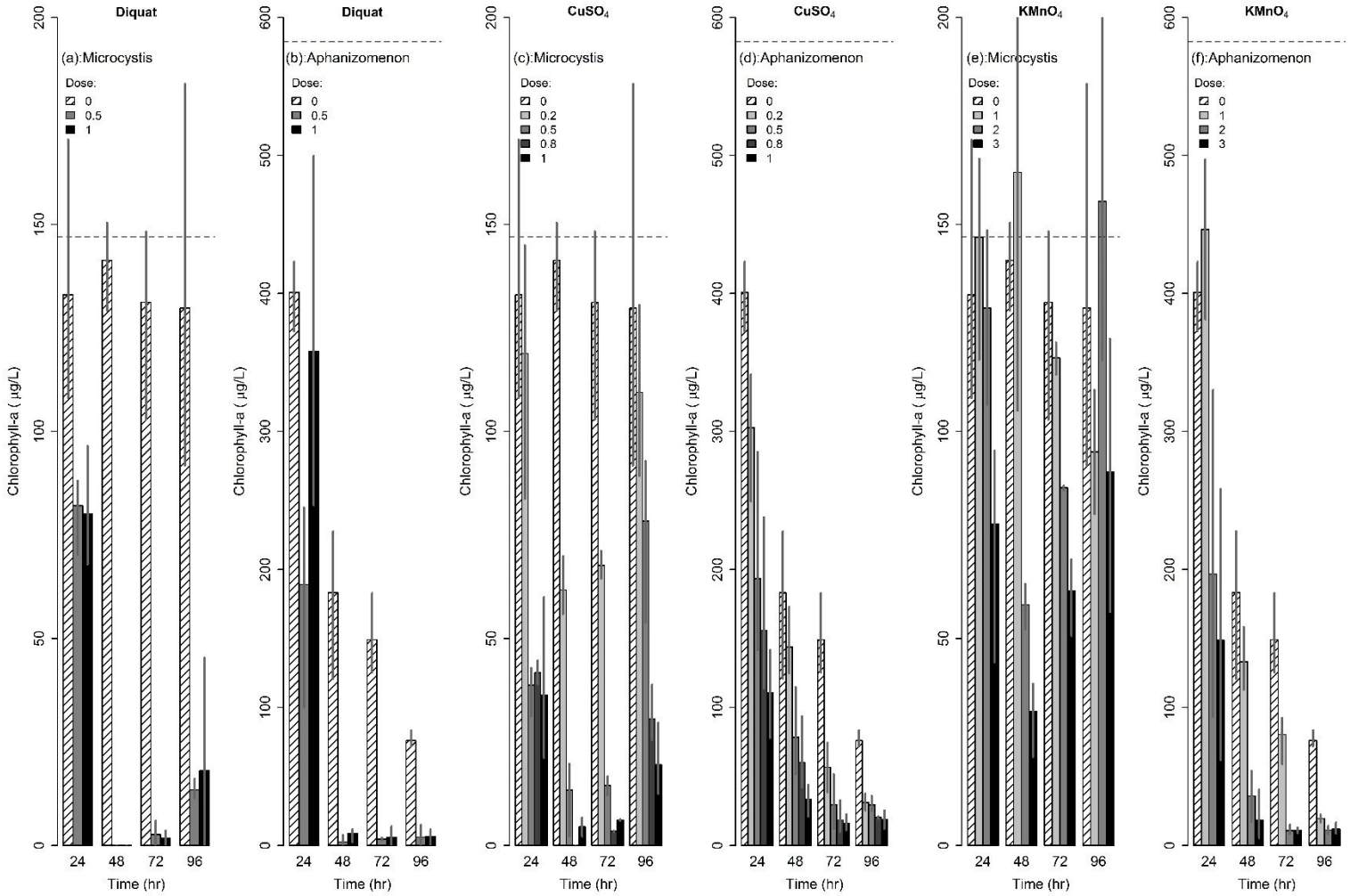


Figure 2. Changes in chlorophyll-a concentrations following algaecide treatment for *Microcystis* and *Aphanizomenon*

3.2 Modeling algaecidal potency

3.2.1 Microcystis

One the main features observed in this study was 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 was reported in previous studies [40, 92]. Yet, the dynamics of regrowth have not been discussed nor modeled. The proposed quadratic model proved to capture the dynamics of *Microcystis auerginosa*, especially under high algaecide dosages (Figure 3). Yet, 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 Diquat and $CuSO_4$ (Figure 3).

The rates at which *Microcystis* concentrations dropped across the three algaecides under the different dosages are shown in Figure 4. Diquat had the lowest *Microcystis* recovery rate among all tested algaecides, thus potentially requiring the least frequent application (Figure 4.a). Net regrowth tended to occur at least after 70 hrs of treatment. As for $CuSO_4$, the model predicted that regrowth will exceed inhibition between 55 and 64 hrs following treatment across the four tested dosages (Figure 4.b). Given the fact that *Microcystis aeruginosa* exhibited resilience against $KMnO_4$ treatment at the lower dosages, it was difficult to separate the inhibition phase from regrowth. This manifested in the absence of a second order regression term in the algae dynamics model for the 1 mg/L dose (Figure 4.c). At the 2 and 3 mg/L dosages, the $KMnO_4$ treatment did show evidence of an initial net inhibition phase that was followed by a new recovery phase (Figure 3 and Figure 4.c). Clearly, the use of $KMnO_4$ is not suitable for the control of *Microcystis aeruginosa* blooms.

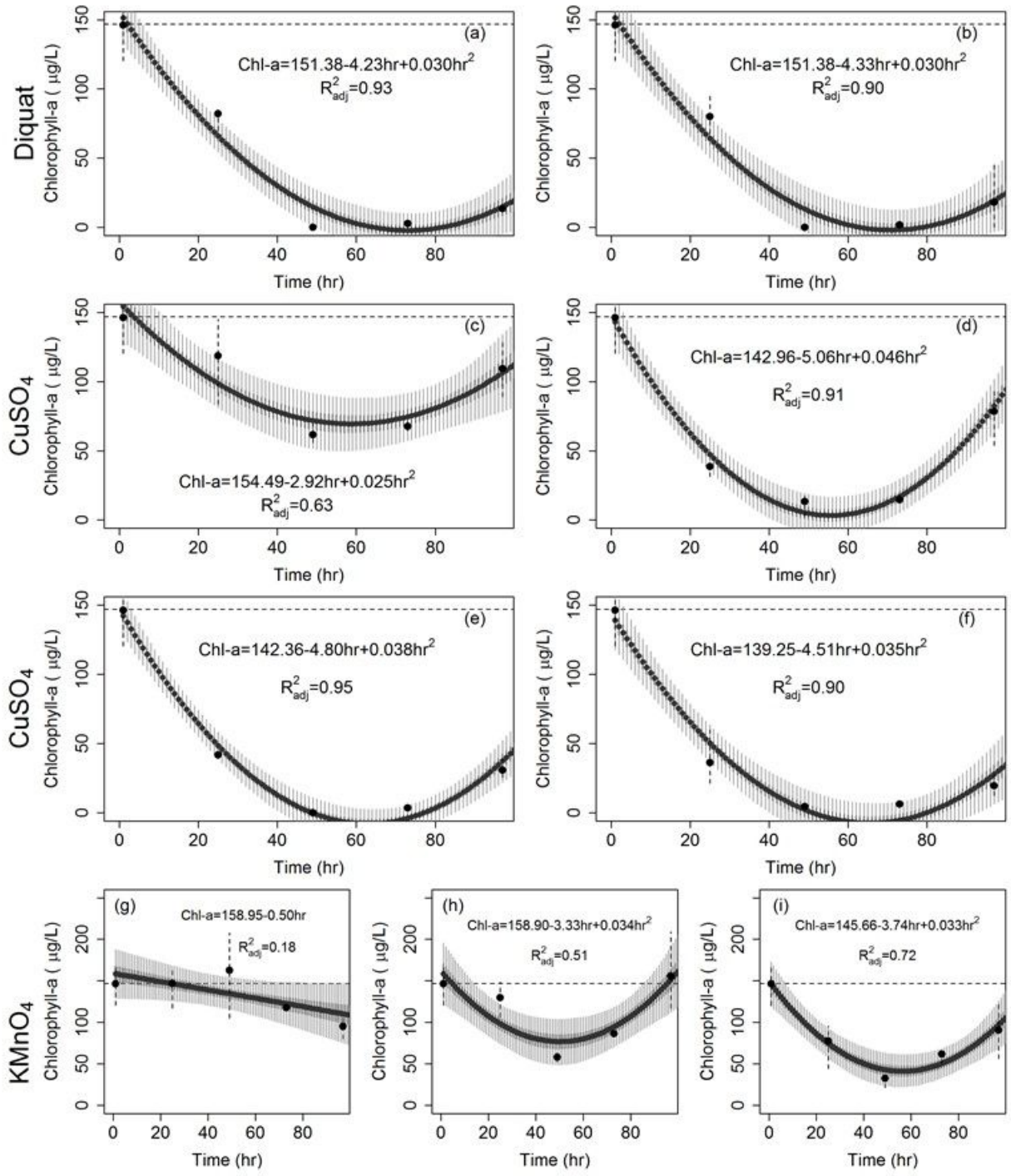


Figure 3 Drop in Chlorophyll-a concentration of *Microcystis* after the application of (a) Diquat = 0.5 mg/L, (b) Diquat = 1 mg/L, (c) $CuSO_4$ =0.2 mg/L, (d) $CuSO_4$ = 0.5 mg/L, (e) $CuSO_4$ = 0.8 mg/L, (f) $CuSO_4$ =1 mg/L, (g) $KMnO_4$ = 1 mg/L, (h) $KMnO_4$ = 2 mg/L, and (i) $KMnO_4$ = 3 mg/L. The dashed horizontal line shows initial conditions; solid circles represent median concentrations; dashed vertical lines show the range of observed concentrations. Solid thick vertical grey segments and solid light grey vertical segments represent the 50% and 95% model predicted chlorophyll-a concentrations respectively.

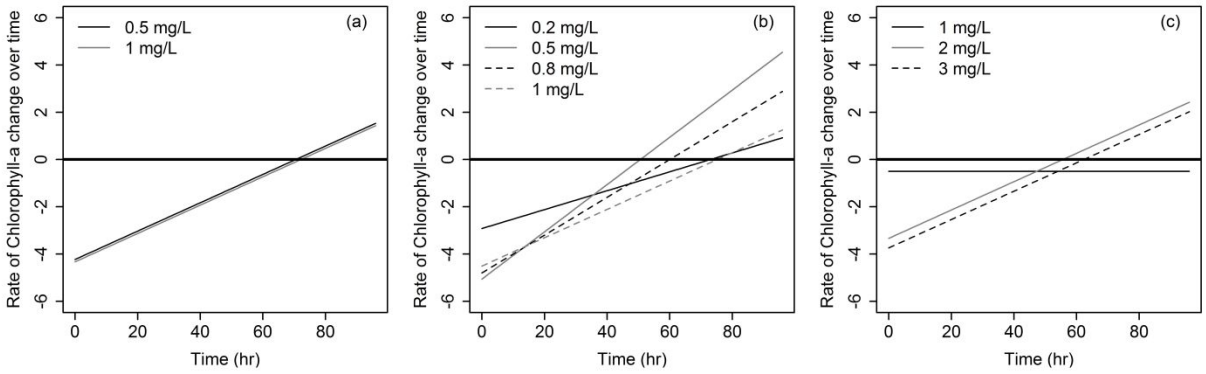


Figure 4 Rates of inhibition and recovery of *Microcystis* over time for (a) Diquat; (b) $CuSO_4$; and (c) $KMnO_4$. Rates below the solid black horizontal line (y= 0) indicates dominance of inhibition with a net decrease in concentrations, while rates above (y= 0) represent dominant regrowth dynamics with a net increase in concentrations

3.2.2 *Aphanizomenon*

Given the absence of net regrowth following treatment across the three tested algaecides, the first order decay models were suitable to explain the change in *Aphanizomenon* over time (Figure 5). Diquat exhibited the highest chlorophyll-a decay rate of all three algaecides, with a rate of 1.44/day for both dosages. The decay rate for $KMnO_4$ was 0.96/day across the three tested dosages, while the rate for $CuSO_4$ ranged between 0.72 and 0.96/day depending on the dosage used. Thus while the applied dosages of KMnO4 and CuSO4 proved to be effective in suppressing the *Aphanizomenon* levels, the use of Diquat was able to achieve a similar suppression in a shorter time interval. As can be seen in Figure 5, the adopted first order decay models had a tendency to underestimate the initial drop in chlorophyll-a concentrations across the applied Diquat dosages. This was also true under the high dosages of $CuSO_4$ and $KMnO_4$. The mismatch between the model and the data indicates that the initial (first 48 hours) removal rates appear to be faster than what can be predicted by the first order decay model, which assumes that the rate is temporally invariant.

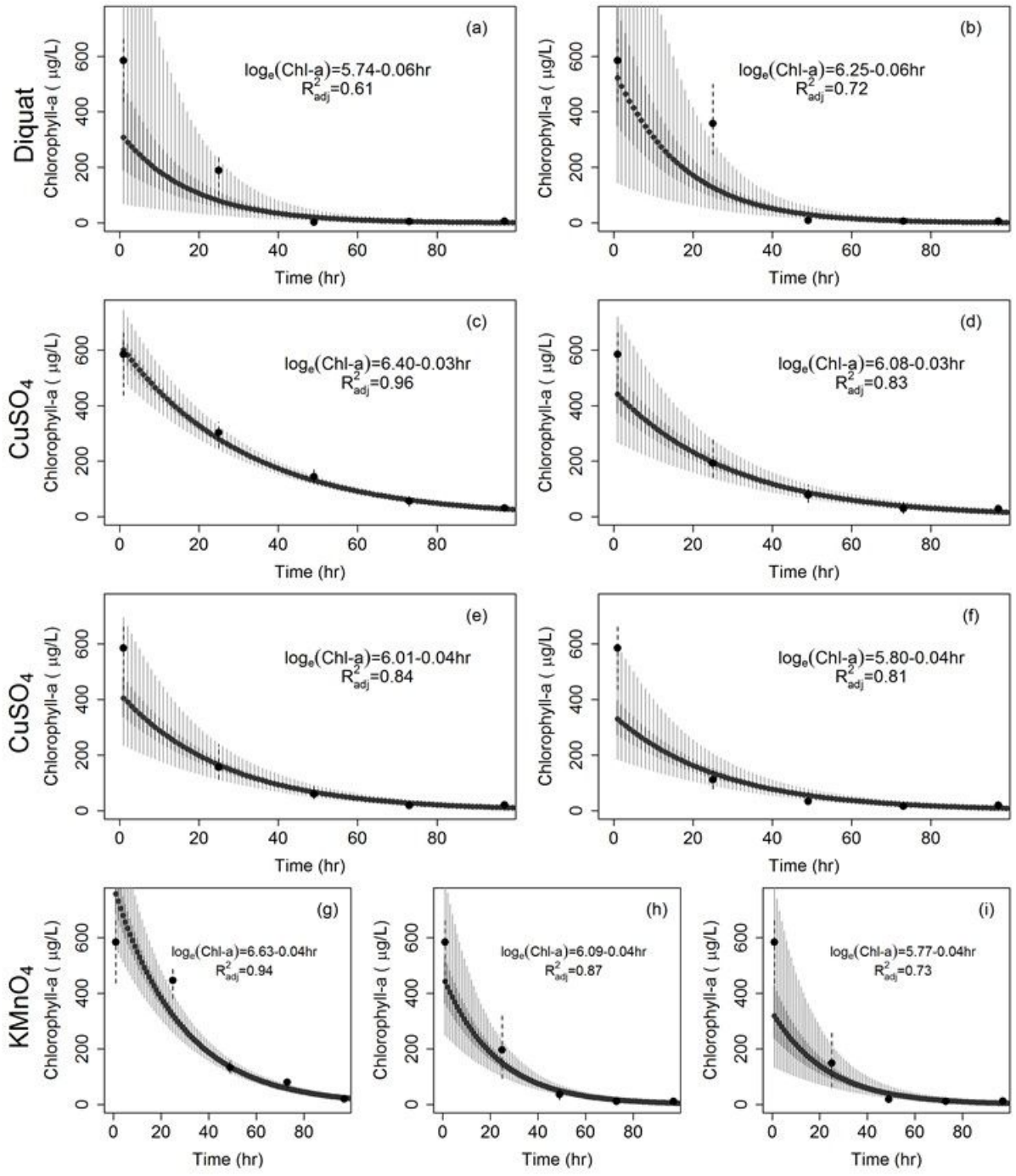


Figure 5 Drop in Chlorophyll-a concentration of *Aphanizomenon* after the application of (a) Diquat =0.5 mg/L, (b) Diquat =1 mg/L, (c) $CuSO_4$ =0.2 mg/L, (d) $CuSO_4$ =0.5 mg/L, (e) $CuSO_4$ =0.8 mg/L, (f) $CuSO_4$ =1 mg/L, (g) $KMnO_4$ =1 mg/L, (h) $KMnO_4$ =2 mg/L, and (i) $KMnO_4$ =3 mg/L. The dashed horizontal line represents initial conditions; black solid circles represent average measured 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 CONCLUSIONS

This study established the efficacy of Diquat as an efficient chemical algaecides that can be used to control two important nuisance cyanobacteria that are known to form HABs and produce toxins. The results showed that Diquat and $CuSO_4$ outperformed $KMnO_4$ in controlling *Microcystis aeruginosa* and both were able to inhibit and retard its regrowth. *Microcystis aeruginosa* levels after 72 hrs of algaecidal treatment with the highest tested dosage of Diquat (1 mg/L) were found to be statistically lower that the levels achieved with the highest tested dose of $CuSO_4$ (1 mg/L). *Microcystis* levels after 96 hr of treatment rebounded across all algaecides. Yet, the lowest average chlorophyll-a concentrations achieved after 96 hrs of contact time with the highest dosage across the three algaecides was found to be associated with Diquat (8.9 µg/L of chlorophyll-a as compared to 16.4 and 92.1 µg/L for $CuSO_4$ and $KMnO_4$ respectively); yet these levels were not found to be statistically different from the levels achieved with the 1 mg/L $CuSO_4$ treatment at the 95% confidence level due to variability observed between the triplicates. $KMnO_4$ proved to be ineffective in controlling *Microcystis*; yet it performed satisfactory with *Aphanizomenon*. While the applied dosages of $KMnO_4$ and $CuSO_4$ proved to be effective in suppressing *Aphanizomenon*, the use of Diquat was able to achieve similar suppressions in a shorter time interval. As such, the results underscore the need for proper algae characterization during a bloom event prior to algaecide selection and application. While the efficacy of Diquat has been shown to vary by species (e.g. [64, 68]), previous studies have shown that $CuSO_4$ tends to have a broad spectrum of action [40, 93, 94]. Changes in diversity can have a cascading effect on the freshwater food web [35]. The study also showed that the algaecidal impact on *Microcystis aeruginosa* was best described by a concave quadratic decay model that is capable of capturing

the effect of decreasing proportional pressures. This is in contrast to the first order decay model structure that to a large extent captured the *Aphanizomenon flos-aquae* dynamics over time.

Note that this study did not explore the confounding effects that ambient environmental conditions may have on the effectiveness of the three tested algaecides. In the case of Diquat, the presence of high levels of suspended particulates in the water column can cause Diquat to bind to these sediments, reducing its bioavailability [63, 95]. For $CuSO_4$, the degree of chelation in the water column can affect its residence time and thus may change its efficacy [42, 43, 96, 97]. As for $KMnO_4$, increased hardness of the water has been linked to better algal removal rates [59]. Future work should also examine the scalability and transferability of the results to the lake level, where algal densities can be more than two orders of magnitude higher (~ 1-2 million cells/ml) than those set by the EPA algal inhibition test guidelines [83].

While algaecide applications were shown to be 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 reduced efficacy over time due to the development of algaecide-resistant mutant strains. Several studies have reported the development of resistant strains of nuisance algal species following exposure to water contaminants [46, 98, 99]. As such, 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, while there is no reported health concerns regarding the residual levels of the algaecides in the treated water, the possibility of toxin release upon cell lysis following the use of algaecides is a serious concern that needs attention [92, 100, 101]. Tests conducted on the residuals levels of Copper, Permanganate, and Diquat after 96 hrs of treatment (summarized in the online Supplemental Material), showed that all concentrations, irrespective of dose, were within the standards set for

drinking water [8]. Yet, the long-term ecotoxicty associated with these residual levels of algaecides may be of concern. Ecological stresses have been documented even at low dosages of copper [102, 103] and Diquat [104].

CONFLICTS OF INTEREST

There are no conflicts to declare.

ACKNOWLEDGMENTS

This study was supported through the United States Agency for International Development through the USAID-NSF PEER initiative (grant # AID-OAA-A-I1-00012) and the American University of Beirut University Research Board. The manuscript was extracted from the thesis of Sara Dia titled "Efficacy of three algaecides on algal blooms in hypereutrophic lakes" that was submitted to the American University of Beirut in fulfillment of the requirements of her MS Degree on June 2016 (https://scholarworks.aub.edu.lb/handle/10938/11127). A presentation of the work was conducted at the Water Pollution 2016 conference, Venice, Italy.

REFERENCES

1. H. W. Paerl and J. T. Scott, Throwing fuel on the fire: synergistic effects of excessive nitrogen inputs and global warming on harmful algal blooms, *Environmental Science & Technology*, 2010, **44**, 7756-7758.

2. M. Leira, G. Chen, C. Dalton, K. Irvine and D. Taylor, Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient, *Freshwater Biology*, 2009, **54**, 1-14.

3. D. M. Anderson, P. M. Glibert and J. M. Burkholder, Harmful algal blooms and eutrophication: nutrient sources, composition, and consequences, *Estuaries*, 2002, **25**, 704-726.

4. G. M. Hallegraeff, A review of harmful algal blooms and their apparent global increase, *Phycologia*, 1993, **32**, 79-99.

5. I. Chorus and J. Bartram, *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*, Spon Press, 1999.

6. J. Heisler, P. M. Glibert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, Q. Dortch, C. J. Gobler, C. A. Heil and E. Humphries, Eutrophication and harmful algal blooms: a scientific consensus, *Harmful algae*, 2008, **8**, 3-13.

7. H. K. Hudnell, *Cyanobacterial harmful algal blooms: state of the science and research needs*, Springer Science & Business Media, 2008.

8. WHO, *Guidelines for drinking-water quality: recommendations*, World Health Organization, 2004.

9. I. R. Falconer, Is there a human health hazard from microcystins in the drinking water supply?, *Acta hydrochimica et hydrobiologica*, 2005, **33**, 64-71.

10. J. Beaver and K. Havens, Seasonal and spatial variation in zooplankton community structure and their relation to possible controlling variables in Lake Okeechobee, *Freshwater Biology*, 1996, **36**, 45-56.

11. M. A. Doblin, K. J. Coyne, J. M. Rinta-Kanto, S. W. Wilhelm and F. C. Dobbs, Dynamics and short-term survival of toxic cyanobacteria species in ballast water from NOBOB vessels transiting the Great Lakes—implications for HAB invasions, *Harmful Algae*, 2007, **6**, 519-530.

12. A. M. Michalak, E. J. Anderson, D. Beletsky, S. Boland, N. S. Bosch, T. B. Bridgeman, J. D. Chaffin, K. Cho, R. Confesor and I. Daloğlu, Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions, *Proceedings of the National Academy of Sciences*, 2013, **110**, 6448-6452.

13. H. W. Paerl and T. G. Otten, Harmful cyanobacterial blooms: causes, consequences, and controls, *Microbial Ecology*, 2013, **65**, 995-1010.

14. E. Vardaka, M. Moustaka-Gouni, C. M. Cook and T. Lanaras, Cyanobacterial blooms and water quality in Greek waterbodies, *Journal of Applied Phycology*, 2005, **17**, 391-401.

15. I. Ohtani, R. E. Moore and M. T. Runnegar, Cylindrospermopsin: a potent hepatotoxin from the blue-green alga Cylindrospermopsis raciborskii, *Journal of the American Chemical Society*, 1992, **114**, 7941-7942.

16. WHO, Guidelines for drinking-water quality, *World Health Organization*, 2011, **216**, 303-304.

17. H. W. Paerl, R. S. Fulton, P. H. Moisander and J. Dyble, Harmful freshwater algal blooms, with an emphasis on cyanobacteria, *The Scientific World Journal*, 2001, **1**, 76-113.

18. Y. Liu, W. Chen, D. Li, Y. Shen, G. Li and Y. Liu, First report of aphantoxins in China—waterblooms of toxigenic Aphanizomenon flos-aquae in Lake Dianchi, *Ecotoxicology and environmental safety*, 2006, **65**, 84-92.

19. A. E. Strong, Remote sensing of algal blooms by aircraft and satellite in Lake Erie and Utah Lake, *Remote Sensing of Environment*, 1974, **3**, 99-107.

20. W. W. Carmichael, C. Drapeau and D. M. Anderson, Harvesting of Aphanizomenon flos-aquae Ralfs ex Born. & Flah. Var. flos-aquae (Cyanobacteria) from Klamath Lake for human dietary use, *Journal of Applied Phycology*, 2000, **12**, 585-595.

21. J. Huisman, P. van Oostveen and F. J. Weissing, Species dynamics in phytoplankton blooms: incomplete mixing and competition for light, *The American Naturalist*, 1999, **154**, 46-68.

22. J. Huisman, J. Sharples, J. M. Stroom, P. M. Visser, W. E. A. Kardinaal, J. M. Verspagen and B. Sommeijer, Changes in turbulent mixing shift competition for light between phytoplankton species, *Ecology*, 2004, **85**, 2960-2970.

23. P. Visser, B. Ibelings, B. Van Der Veer, J. Koedood and R. Mur, Artificial mixing prevents nuisance blooms of the cyanobacterium Microcystis in Lake Nieuwe Meer, the Netherlands, *Freshwater Biology*, 1996, **36**, 435-450.

24. B. W. Ibelings, B. Kroon and L. R. Mur, Acclimation of photosystem II in a cyanobacterium and a eukaryotic green alga to high and fluctuating photosynthetic photon flux densities, simulating light regimes induced by mixing in lakes, *New Phytologist*, 1994, **128**, 407-424.

25. V. H. Smith and D. W. Schindler, Eutrophication science: where do we go from here?, *Trends in Ecology & Evolution*, 2009, **24**, 201-207.

26. E. Jeppesen, J. Peder Jensen, M. Søndergaard, T. Lauridsen, L. Junge Pedersen and L. Jensen, in *Shallow Lakes '95: Trophic Cascades in Shallow Freshwater and Brackish Lakes*, eds. L. Kufel, A. Prejs and J. I. Rybak, Springer Netherlands, Dordrecht, 1997, DOI: 10.1007/978-94-011-5648-6_17, pp. 151-164.

27. M. T. Dokulil and K. Teubner, Cyanobacterial dominance in lakes, *Hydrobiologia*, 2000, **438**, 1-12.

28. J. J. Elser, The pathway to noxious cyanobacteria blooms in lakes: the food web as the final turn, *Freshwater Biology*, 1999, **42**, 537-543.

29. A. Herrero, A. M. Muro-Pastor and E. Flores, Nitrogen control in cyanobacteria, *Journal of bacteriology*, 2001, **183**, 411-425.

30. T. D. Geer, A. J. Calomeni, C. M. Kinley, K. J. Iwinski and J. H. Rodgers, Predicting In Situ Responses of Taste-and Odor-Producing Algae in a Southeastern US Reservoir to a Sodium Carbonate Peroxyhydrate Algaecide Using a Laboratory Exposure-Response Model, *Water, Air, & Soil Pollution*, 2017, **228**, 53.

31. W. Edmondson, Phosphorus, nitrogen, and algae in Lake Washington after diversion of sewage, *Science*, 1970, **169**, 690-691.

32. N. Jaworski, RETROSPECTIVE STUDY OF THE WATER-QUALITY ISSUES OF THE UPPER POTOMAC ESTUARY, *Reviews in Aquatic Sciences*, 1990, **3**, 11-40.

33. W. Ashworth, The Late, Great Lakes-An Environmental History.*Journal*, 1986.

34. J. Heisler, P. M. Glibert, J. M. Burkholder, D. M. Anderson, W. Cochlan, W. C. Dennison, Q. Dortch, C. J. Gobler, C. A. Heil, E. Humphries, A. Lewitus, R. Magnien, H. G. Marshall, K.

Sellner, D. A. Stockwell, D. K. Stoecker and M. Suddleson, Eutrophication and harmful algal blooms: A scientific consensus, *Harmful Algae*, 2008, **8**, 3-13.

35. D. Jančula and B. Maršálek, Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms, *Chemosphere*, 2011, **85**, 1415-1422.

36. L. E. Brand, W. G. Sunda and R. R. Guillard, Reduction of marine phytoplankton reproduction rates by copper and cadmium, *Journal of Experimental Marine Biology and Ecology*, 1986, **96**, 225-250.

37. A.-H. Le Jeune, M. Charpin, V. Deluchat, J.-F. Briand, J.-F. Lenain, M. Baudu and C. Amblard, Effect of copper sulphate treatment on natural phytoplanktonic communities, *Aquatic Toxicology*, 2006, **80**, 267-280.

38. M. J. Hanson and H. G. Stefan, Side effects of 58 years of copper sulfate treatment of the fairmont lakes, minnesota1.*Journal*, 1984.

39. G. T. Moore and K. F. Kellerman, *Copper as an algicide and disinfectant in water supplies*, US Government Printing Office, 1905.

40. J. Fan, L. Ho, P. Hobson and J. Brookes, Evaluating the effectiveness of copper sulphate, chlorine, potassium permanganate, hydrogen peroxide and ozone on cyanobacterial cell integrity, *Water Research*, 2013, **47**, 5153-5164.

41. J. F. Elder and A. J. Horne, Copper cycles and CuSO4 algicidal capacity in two California lakes, *Environmental management*, 1978, **2**, 17-30.

42. A. Calomeni, J. H. Rodgers and C. M. Kinley, Responses of Planktothrix agardhii and Pseudokirchneriella subcapitata to Copper Sulfate (CuSO4· 5H2O) and a Chelated Copper Compound (Cutrine®-Ultra), *Water, Air, & Soil Pollution*, 2014, **225**, 2231.

43. K.-P. Tsai, Management of Target Algae by Using Copper-Based Algaecides: Effects of Algal Cell Density and Sensitivity to Copper, *Water, Air, & Soil Pollution*, 2016, **227**, 238.

44. E. A. Crafton, J. Glowczewski, D. W. Ott and T. J. Cutright, In situ field trial to evaluate the efficacy of Cutrine Ultra to manage a cyanobacteria population in a drinking water source, *Environmental Science: Water Research & Technology*, 2018, **4**, 863-871.

45. P. Pandey, C. Singh and S. Singh, Cu uptake in a cyanobacterium: fate of selected photochemical reactions, *Current microbiology*, 1992, **24**, 35-39.

46. L. Garcia-Villada, M. Rico, M. Altamirano, L. Sánchez-Martin, V. López-Rodas and E. Costas, Occurrence of copper resistant mutants in the toxic cyanobacteria Microcystis aeruginosa: characterisation and future implications in the use of copper sulphate as algaecide, *Water Research*, 2004, **38**, 2207-2213.

47. M. Gledhill, M. Nimmo, S. J. Hill and M. T. Brown, The toxicity of copper (II) species to marine algae, with particular reference to macroalgae, *Journal of Phycology*, 1997, **33**, 2-11.

48. T. Hall, J. Hart, B. Croll and R. Gregory, Laboratory‐scale investigations of algal toxin removal by water treatment, *Water and Environment Journal*, 2000, **14**, 143-149.

49. J.-J. Chen, H.-H. Yeh and I.-C. Tseng, Effect of ozone and permanganate on algae coagulation removal–Pilot and bench scale tests, *Chemosphere*, 2009, **74**, 840-846.

50. P. Roccaro, C. Barone, G. Mancini and F. Vagliasindi, Removal of manganese from water supplies intended for human consumption: a case study, *Desalination*, 2007, **210**, 205-214.

51. H. Kemp, R. Fuller and R. Davidson, Potassium permanganate as an algicide, *Journal (American Water Works Association)*, 1966, **58**, 255-263.

52. D. Ellis, C. Bouchard and G. Lantagne, Removal of iron and manganese from groundwater by oxidation and microfiltration, *Desalination*, 2000, **130**, 255-264.

53. E. Rodríguez, G. D. Onstad, T. P. Kull, J. S. Metcalf, J. L. Acero and U. von Gunten, Oxidative elimination of cyanotoxins: comparison of ozone, chlorine, chlorine dioxide and permanganate, *Water Research*, 2007, **41**, 3381-3393.

54. J. Ma and W. Liu, Effectiveness and mechanism of potassium ferrate (VI) preoxidation for algae removal by coagulation, *Water Research*, 2002, **36**, 871-878.

55. H. Zhao, L. Wang, H. Zhang, X. Wu, B. Zhao and F. Han, Effect of potassium permanganate dosing position on the performance of coagulation/ultrafiltration combined process, *Chinese Journal of Chemical Engineering*, 2018, **26**, 89-95.

56. M. Yagi, M. Kajino, U. Matsuo, K. Ashitani, T. Kita and T. Nakamura, Odor problems in lake Biwa, *Water Science and Technology*, 1983, **15**, 311.

57. H. G. Peterson, S. E. Hrudey, I. A. Cantin, T. R. Perley and S. L. Kenefick, Physiological toxicity, cell membrane damage and the release of dissolved organic carbon and geosmin by Aphanizomenon flos-aquae after exposure to water treatment chemicals, *Water Research*, 1995, **29**, 1515-1523.

58. A. M. Lazur and R. P. E. Yanong, *The Use of Potassium Permanganate in Fish Ponds*, University of Florida, Gainesville, FL, 2013.

59. J.-J. Chen and H.-H. Yeh, The mechanisms of potassium permanganate on algae removal, *Water Research*, 2005, **39**, 4420-4428.

60. S. Dash, S. Patel and B. K. Mishra, Oxidation by permanganate: synthetic and mechanistic aspects, *Tetrahedron*, 2009, **65**, 707-739.

61. H.-Q. Wang, T.-G. Mao, B.-D. Xi, L.-Y. Zhang and Q.-H. Zhou, KMnO4 pre-oxidation for Microcystis aeruginosa removal by a low dosage of flocculant, *Ecological Engineering*, 2015, **81**, 298-300.

62. B. Liu, F. Qu, W. Chen, H. Liang, T. Wang, X. Cheng, H. Yu, G. Li and B. Van der Bruggen, Microcystis aeruginosa-laden water treatment using enhanced coagulation by persulfate/Fe(II), ozone and permanganate: Comparison of the simultaneous and successive oxidant dosing strategy, *Water Research*, 2017, **125**, 72-80.

63. J. Clayton and F. Matheson, Optimising diquat use for submerged aquatic weed management, *Hydrobiologia*, 2010, **656**, 159-165.

64. E. J. Phlips, P. Hansen and T. Velardi, Effect of the herbicide diquat on the growth of microalgae and cyanobacteria, *Bulletin of environmental contamination and toxicology*, 1992, **49**, 750-756.

65. Wisconsin Department of Natural Resources, *Diquat Chemical Fact Sheet*, Wisconsin Department of Natural Resources, Madison, WI, 2012.

66. H. G. Peterson, C. Boutin, P. A. Martin, K. E. Freemark, N. J. Ruecker and M. J. Moody, Aquatic phyto-toxicity of 23 pesticides applied at expected environmental concentrations, *Aquatic Toxicology*, 1994, **28**, 275-292.

67. S. M. Bartell, K. R. Campbell, C. M. Lovelock, S. K. Nair and J. L. Shaw, Characterizing aquatic ecological risks from pesticides using a diquat dibromide case study III. Ecological process models, *Environmental Toxicology and Chemistry*, 2000, **19**, 1441-1453.

68. K. K. Schrader and C. S. Tucker, Evaluation of diquat as a potential algicide for controlling the musty-odor-producing cyanobacterium, Oscillatoria perornata, in catfish aquaculture ponds, *Journal of Applied Aquaculture*, 2003, **14**, 149-154.

69. A. Melendez, R. Kepner Jr, J. Balczon and J. Pratt, Effects of diquat on freshwater microbial communities, *Archives of Environmental Contamination and Toxicology*, 1993, **25**, 95-101.

70. M. E. DeLorenzo, G. I. Scott and P. E. Ross, Toxicity of pesticides to aquatic microorganisms: a review, *Environmental Toxicology and Chemistry*, 2001, **20**, 84-98.

71. G. Jager, Herbicides, *Chemistry of Pesticides. New York: Wiley*, 1983, 322-392.

72. G. Zweig, N. Shavit and M. Avron, Diquat (1, 1′ -ethylene-2, 2′ -dipyridylium dibromide) in photoreactions of isolated chloroplasts, *Biochimica et Biophysica Acta (BBA)-Biophysics including Photosynthesis*, 1965, **109**, 332-346.

73. USAID, *Litani water quality management project: rapid review report.*, Development Alternatives, Inc. (DAI), 2005.

74. ELARD, *Business Plan For Combating Pollution of The Qaraoun Lake*, Report LB-EQM-UND-CPQ-10, 2011.

75. A. Atoui, H. Hafez and K. Slim, Occurrence of toxic cyanobacterial blooms for the first time in Lake Karaoun, Lebanon, *Water and Environment Journal*, 2013, **27**, 42-49.

76. M. Bouvy, N. Ba, S. Ka, S. Sane, M. Pagano and R. Arfi, Phytoplankton community structure and species assemblage succession in a shallow tropical lake (Lake Guiers, Senegal), *Aquatic Microbial Ecology*, 2006, **45**, 147-161.

77. H. W. Paerl and C. S. Tucker, Ecology of blue‐green algae in aquaculture ponds, *Journal of the World Aquaculture Society*, 1995, **26**, 109-131.

78. H. Tsukada, S. Tsujimura and H. Nakahara, Seasonal succession of phytoplankton in Lake Yogo over 2 years: effect of artificial manipulation, *Limnology*, 2006, **7**, 3-14.

79. USEPA, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving waters to Freshwater Organisms.*, United States Environmental Protection Agency, U.S. Environmental Protection Agency Office of Water (4303T),1200 Pennsylvania Avenue, NW Washington, DC 20460, 2002.

80. R. R. Guillard, in *Culture of marine invertebrate animals*, Springer, 1975, pp. 29-60.

81. J. Hodgeson, W. Bashe and J. W. Eichelberger, *Method 549.2: Determination of Diquat and Paraquat in Drinking Water by Liquid-solid Extraction and High Performance Liquid Chromatography with Ultraviolet Detection*, United States Environmental Protection Agency, Office of Research and Development, National Exposure Research Laboratory, 1997.

82. J. Creed, T. Martin and J. O'Dell, *Method 200.9 determination of trace elements by stabilized temperature graphite furnace atomic absorption, revision 2.2*, 1994.

83. P. A. Lewis, D. J. Klemm, J. M. Lazorchak, T. J. Norberg-King, W. H. Peltier and M. A. Heber, *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms*, US Environmental Protection Agency, Environmental Monitoring Systems Laboratory, 1994.

84. E. W. Rice, L. Bridgewater, F. Water Environment, A. American Water Works and A. American Public Health, *Standard methods for the examination of water and wastewater*, American Public Health Association, Washington, D.C, 2012.

85. A. Eaton and M. Franson, American Water Works Association & Water Environment Federation 2005 Standard Methods for the Examination of Water and Wastewater, *Washington, DC*, 2005.

86. A. J. Calomeni and J. H. Rodgers, Evaluation of the utility of six measures for algal (Microcystis aeruginosa, Planktothrix agardhii and Pseudokirchneriella subcapitata) viability, *Ecotoxicology and environmental safety*, 2015, **111**, 192-198.

87. R Core Team, *R: A Language and Environment for Statistical Computing.*, R Foundation for Statistical Computing, Vienna, Austria, 2015.

88. M. Di Fonzo, B. Collen and G. M. Mace, A new method for identifying rapid decline dynamics in wild vertebrate populations, *Ecology and evolution*, 2013, **3**, 2378-2391.

89. A. Gelman, Y.-S. Su, M. Yajima, J. Hill, M. G. Pittau and J. Kerman, arm: Data analysis using regression and multilevel/hierarchical models. 2015, *URL https://CRAN. R-project. org/package= arm. R package version*, 2016, 1.9-3.

90. S. S. Qian, *Environmental and ecological statistics with R*, CRC Press, 2016.

91. J. Fan, R. Daly, P. Hobson, L. Ho and J. Brookes, Impact of potassium permanganate on cyanobacterial cell integrity and toxin release and degradation, *Chemosphere*, 2013, **92**, 529-534.

92. D. I. Greenfield, A. Duquette, A. Goodson, C. J. Keppler, S. H. Williams, L. M. Brock, K. D. Stackley, D. White and S. B. Wilde, The effects of three chemical algaecides on cell numbers

and toxin content of the cyanobacteria Microcystis aeruginosa and Anabaenopsis sp, *Environmental management*, 2014, **54**, 1110-1120.

93. E. C. de Oliveira-Filho, R. M. Lopes and F. J. R. Paumgartten, Comparative study on the susceptibility of freshwater species to copper-based pesticides, *Chemosphere*, 2004, **56**, 369-374.

94. M. J. Hanson and H. G. Stefan, SIDE EFFECTS OF 58 YEARS OF COPPER SULFATE TREATMENT OF THE FAIRMONT LAKES, MINNESOTA 1, *JAWRA Journal of the American Water Resources Association*, 1984, **20**, 889-900.

95. J. H. J. Rodgers, B. M. Johnson and W. M. Bishop, Comparison of Three Algaecides for Controlling the Density of Prymnesium parvum, *Journal of the American Water Resources Association (JAWRA)*, 2010, **46**, 153-160.

96. C. Murray-Gulde, J. Heatley, A. Schwartzman and J. Rodgers Jr, Algicidal effectiveness of clearigate, cutrine-plus, and copper sulfate and margins of safety associated with their use, *Archives of Environmental Contamination and Toxicology*, 2002, **43**, 19-27.

97. D. A. Salam, E. American University of Beirut. Faculty of and P. Architecture. Interfaculty Graduate Environmental Sciences, Dissertation/Thesis, 2006.

98. E. Costas, E. Carrillo, L. M. Ferrero, M. Agrelo, L. Garcia-Villada, J. Juste and V. López-Rodas, Mutation of algae from sensitivity to resistance against environmental selective agents: the ecological genetics of Dictyosphaerium chlorelloides (Chlorophyceae) under lethal doses of 3-(3, 4-dichlorophenyl)-1, 1-dimethylurea herbicide, *Phycologia*, 2001, **40**, 391-398.

99. V. Lopez-Rodas, M. A. R. Agrelo, E. Carrillo, L. M. Ferrero, A. Larrauri, L. MartÍN-Otero and E. Costas, Resistance of microalgae to modern water contaminants as the result of rare spontaneous mutations, *European Journal of Phycology*, 2001, **36**, 179-190.

100. S. Zhou, Y. Shao, N. Gao, Y. Deng, J. Qiao, H. Ou and J. Deng, Effects of different algaecides on the photosynthetic capacity, cell integrity and microcystin-LR release of Microcystis aeruginosa, *Science of The Total Environment*, 2013, **463**, 111-119.

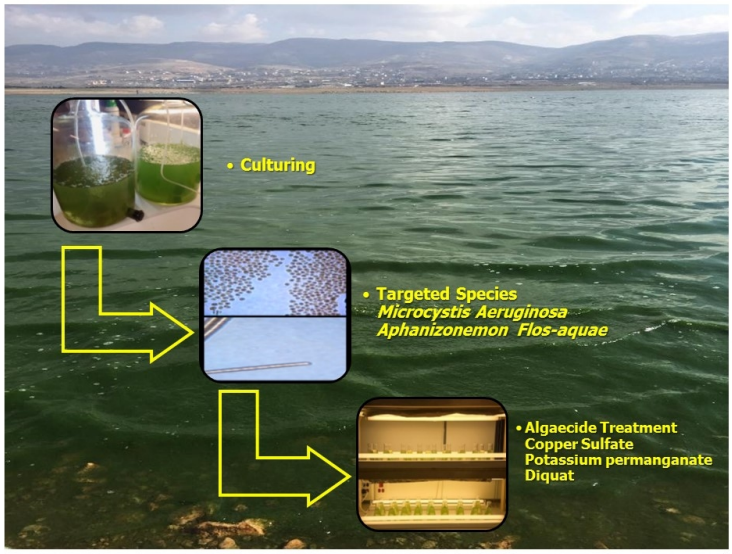
101. G. J. Jones and P. T. Orr, Release and degradation of microcystin following algicide treatment of a Microcystis aeruginosa bloom in a recreational lake, as determined by HPLC and protein phosphatase inhibition assay, *Water Research*, 1994, **28**, 871-876.

102. Y. E. Roman, K. A. C. De Schamphelaere, L. T. H. Nguyen and C. R. Janssen, Chronic toxicity of copper to five benthic invertebrates in laboratory-formulated sediment: Sensitivity comparison and preliminary risk assessment, *Science of The Total Environment*, 2007, **387**, 128-140.

103. W. Sanchez, O. Palluel, L. Meunier, M. Coquery, J.-M. Porcher and S. Aït-Aïssa, Copper-induced oxidative stress in three-spined stickleback: relationship with hepatic metal levels, *Environmental Toxicology and Pharmacology*, 2005, **19**, 177-183.

104. M.-A. Coutellec, G. Delous, J.-P. Cravedi and L. Lagadic, Effects of the mixture of diquat and a nonylphenol polyethoxylate adjuvant on fecundity and progeny early performances of the

pond snail Lymnaea stagnalis in laboratory bioassays and microcosms, *Chemosphere*, 2008, **73**, 326-336.



338x190mm (96 x 96 DPI)