Controlling *Microcystis aeruginosa* blooms in a freshwater system: a comparative assessment across three chemical algaecides

S. Dia, I. Alameddine, D. Salam & M. El-Fadel
*Department of Civil and Environmental Engineering,
Faculty of Engineering and Architecture,
American University of Beirut, Lebanon*

Abstract

Freshwater systems are facing mounting challenges as a result of excessive nutrient loading with 20 to 40% of global lakes and reservoirs considered eutrophic and unable to support their historical designated uses because of water quality impairments. While long-term basin-wide management measures that aim at reducing point and non-point pollution sources are invariably well known, they are often hard to implement in many watersheds due to socio-economic and political constraints. In this context, the use of algaecides can provide a temporary solution towards controlling excessive algal blooms, especially in the event of a toxic algal bloom. This study examined under laboratory conditions the efficacy of three algaecides (Copper Sulphate, Potassium Permanganate, and Diquat) on the hepatotoxin producing freshwater cyanobacteria *Microcystis aeruginosa*. Algaecide efficacy was measured over 96 hrs. The results showed that Diquat and Copper Sulphate were significantly more effective than Potassium Permanganate for controlling *Microcystis aeruginosa*. Reduction rates were temporarily variable, with the largest rates reported between 48 and 72 hrs of treatment. Optimal dosages for the three algaecides were developed, while ensuring that application rates were environmentally safe.

Keywords: algaecides, Copper Sulfate, Potassium Permanganate, Diquat, Microcystis aeruginosa.

1 Introduction

Harmful algal blooms (HABs) are responsible for the impairment of a large portion of the world's reservoirs and lakes [1, 2]. Generally, HABs are stimulated by excessive anthropogenic loading of nutrients reaching waterbodies, particularly phosphorus and nitrogen [3–7]. Cyanobacteria are the dominant algae type responsible for HABs and the impairment of freshwater systems [8]. Some cyanobacteria are known for the release of cyanotoxins, which can cause fish kills, affect human health, and lead to the loss of key ecological functions and services [6, 9–11]. Of particular concern is *Microcystis*, a cyanobacterium with strains capable of releasing hepatotoxic toxins, given its dominance in eutrophic freshwater systems, including some of the world's largest lakes and reservoirs [6]. *Microcystis* affected systems are often heavily impacted by human activities, especially non-point pollution sources [12–14]. Moreover, weather is another important factor that appears to play a critical role in its proliferation, with warm and static conditions promoting its blooms [5, 7].

Various control measures have been proposed to counteract these blooms including the application of chemical algacide, enhancing mechanical mixing, manipulating the food web, and decreasing nutrient loads. While the latter is often the most effective solution on the long run, specifically when targeting point and non-point nutrient sources, its implementation is often hindered by socio-economic challenges and the need to implement a comprehensive watershed-level management plan (e.g. Total Maximum Daily Load programs). The use of algacide is invariably considered as the most practical short-term solution to deal with cyanobacteria blooms.

The most commonly used algacide is Copper Sulfate (CuSO_4); it has been in use since the early 1900s. It is inexpensive, accessible, and highly effective on almost all species of cyanobacteria [8, 15]. It controls algae by competing with magnesium in the chlorophyll molecule or by inhibiting the electron transport in photosystem II [16]. Another algacide that has been in use since the early 1960s is Potassium Permanganate (KMnO_4). Its use has been promoted by its low environmental hazard as compared to copper [17]. Hydrated MnO_2 released from the KMnO_4 treatment has been shown to promote the coagulation of surface algae cells [18]. Additionally, KMnO_4 preoxidation was shown to be highly effective in promoting the coagulation of *Microcystis aeruginosa* [19]. Another promising algacide is Diquat, a widely used herbicide that has been available commercially since the 1950s. It acts specifically on photosynthetic tissues and interferes with electron transport, replacing Nicotinamide Adenine Dinucleotide Phosphate (NADP). Philips, *et al.* [20] examined the effect of diquat on the growth of several species of phytoplankton and cyanobacteria; they found that 7 out of 10 tested species showed sensitivity. *Microcystis aeruginosa* was one of those affected. Diquat has been recently used across eight New Zealand lakes to control submerged aquatic weeds [21].

This study examines the effectiveness of Copper Sulphate, Potassium Permanganate, and diquat in controlling *Microcystis aeruginosa* blooms. A laboratory experiment was conducted with the use of cultured blooms propagated

from raw water collected from a hyper-eutrophic reservoir. Different dosages were tested with regards to their achieved reductions over 5 days. Dosages were statistically compared in an effort to define the optimal dosage for each algaecide. Finally, the three algaecides were compared with regards to their optimal dose and ability to minimize environmental risks.

2 Methodology

2.1 Sampling location and culturing

Surface water samples were collected from the Qaraoun Lake (Figure 1), a hypereutrophic reservoir draining a heavily agricultural basin that suffers from point and non-point source pollution [22, 23]. The recent proliferation of *Microcystis aeruginosa* in the lake is impairing its use for irrigation, as the algae clog sprinklers and pumps. Samples from the reservoir were collected in the summer season (August-September 2015), following a *Microcystis aeruginosa* bloom. Surface water samples were collected at approximately 10 cm below the water surface. Concurrently, water quality samples were collected and analysed for pH, temperature, conductivity, and dissolved oxygen. These were used to establish the lab conditions set for algae culturing.

Figure 1: Qaraoun reservoir with the map on the right showing a *Microcystis aeruginosa* bloom captured by the Landsat 8 satellite on July 4, 2013.

2.2 Experimental procedure

Freshly collected *Microcystis aeruginosa* samples were grown in 20 L glass beakers that were provided with a 12:12 light:dark cycle, enriched with nutrients, 25°C water temperature, and bubbled with air to ensure no carbon limitation and enhance mixing. The batch was routinely sub-cultured to maintain logarithmic growth phase. After achieving healthy cell density $> 1.0 \times 10^6$ cells/ml of

Microcystis aeruginosa, samples were diluted using filtered lake water to achieve a final concentration of approximately 10,000 cells/ml. Note that most *Microcystis aeruginosa* cells were colonial. Cell density and composition were determined by the use of a hemocytometer under a Zeiss fluorescence microscope (Axiovert 200). For chlorophyll-a analysis, a known volume of sample with a magnesium carbonate buffer was filtered through a membrane filter paper, which was stored overnight at -20°C to facilitate bursting of algal cells. Chlorophyll-a was then extracted using a 90% acetone solution and 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)) measured on a HACH DR3900 spectrophotometer [24].

Algaecides were prepared from stock concentrates, which were diluted to obtain the desired dosages. CuSO₄ was supplied from Sigma Aldrich as Copper (II) sulfate pentahydrate (209198 ACS reagent), Diquat as Diquat dibromide monohydrate (Supleco N11816 analytical standard), and Potassium Permanganate (Merck and Co. M5080). The stock solutions of CuSO₄ and KMnO₄ were tested using graphite furnace atomic absorption spectrophotometry (USEPA method 200.9), and High Performance Liquid Chromatography was used for diquat (USEPA method 549.2). For CuSO₄, dosages of 0.2, 0.5, 0.8, and 1 mg/L were prepared. KMnO₄ efficacy was tested under 1, 2, and 3 mg/L concentrations; diquat was prepared under 1 and 2 mg/L concentrations.

Inhibition tests were conducted in 250 ml Erlenmeyer flasks that were prefilled with the diluted (10,000 cells/ml) *Microcystis aeruginosa* culture media. According to the developed EPA inhibition procedures [25], the flasks were placed on shelves and irradiated with 3 florescent lamps that provide 4306 lux. Three replicates were used as controls. Similarly, inhibition tests for each algaecide-dose combination were conducted in triplicate samples.

Temperature, pH, and dissolved oxygen (DO) were monitored daily throughout the experiment across all flasks. The pH varied between 8.7 and 9.4, temperature was between 23.6 and 24.4°C, and DO levels were between 11.40 and 14.26 mg/L. 50 ml samples were collected from each flask at 24h, 48h, 72h, and 96h contact time. The collected samples were used to count *Microcystis aeruginosa* cells and to determine chlorophyll-a concentrations that act as a surrogate of algae biomass [24]. During cell counts, the state of the cells was monitored, with emphasis placed on whether the *Microcystis aeruginosa* were colonial or unicellular. At the end of the experiment (96h), nutrients content, hardness, turbidity, and the chemical residuals of each algaecide were tested. The latter was used to ensure that the applied dosages were within safe environmental standards.

2.3 Statistical analysis

The effect of dosage for the three algaecides was assessed, while accounting for potential changes in efficacy over time. This was conducted through the use of a 2-way ANOVA. The potential for an interaction between dose and time was assessed through the inclusion of an interaction term. The analysis was conducted in the software R [26]. Tukey's method for multiple comparisons was used to identify differences of significance between dosages and time. Note that

chlorophyll-a concentrations were square root-transformed to achieve normality. Additionally, multiple linear regression models were developed for each dose to predict changes in chlorophyll-a concentrations as a function of time. The developed models were used to determine the change in the rate of chlorophyll drop over time and the time at which the minimum concentration was achieved. These two metrics were used to compare efficacy across dosages and algaecides. The results were used to define the optimal dose for the three algaecides.

3 Results and discussion

All three algaecides proved effective in controlling *Microcystis aeruginosa*, albeit with marked differences in efficacy. The observed drop in chlorophyll-a concentrations exhibited a similar pattern across algaecides, with the lowest chlorophyll-a concentrations observed after 48 hr contact time (Figure 2). CuSO₄ proved to be highly effective in controlling *Microcystis aeruginosa* across all doses. Colonies broke down into individual cells within the first 24 hr. Reductions in chlorophyll-a concentrations were affected by the applied dose; moreover, the effect of the dose was found to be a function of exposure time. Statistically, average chlorophyll-a concentrations decreased as the CuSO₄ dose was increased (p -value < 0.05). Reductions of 55 to 60% were observed after 48 hr of treatment, even with the lowest dosage (0.2 mg/L). Inhibition increased with dosage, reaching 90% at 48 hr with a dose of 0.5 mg/L (Figure 2). For both the 0.8 and 1 mg/L dose, 90% reduction was achieved after 24 hr, with an inhibition of 99% following 48 hr of exposure. No statistical difference was discerned between 0.8 and 1 mg/L CuSO₄; both were equally effective (p -value 0.94), indicating that the former is the optimal dose to use. Observed reductions are consistent with results reported by Fan *et al.* [15], who reported reductions of 98% at a CuSO₄ dose of 1 mg/l.

The temporal changes in average chlorophyll-a concentrations were statistically different over the four days of the experiment (p -value < 0.05), except for levels observed on 48 and 72 hr (p -value = 0.20) and levels observed on 24 and 96 hr (p -value = 0.997). The former coincides with the period where the lowest chlorophyll-a concentration was measures, while the latter is an indication of *Microcystis aeruginosa* recovery, whereby chlorophyll-a concentrations started to increase and reached levels similar to those observed after 1 day of treatment (Figure 2). Yet, microscopic inspection on the fifth day showed that the recovery was still not able to produce colonies, with most cells remaining in an unattached form (Figure 3). The recovery could be due to the development of resistant mutants that are able to survive under high Cu⁺² concentrations [27]. Lowest chlorophyll-a concentrations were observed following 48 hrs of treatment.

KMnO₄ exhibited the least effectiveness in reducing *Microcystis aeruginosa*. Colonial cells which were disrupted under the highest applied dose only (3 mg/L), following 48 hr of treatment (Figure 3). With the 2 mg/L KMnO₄ dose, colonies persisted even though the average colony size decreased after 72 hr. At the lowest dosages (1 mg/L), colonies were largely unaffected throughout the entire period of the experiment (Figure 2). Average chlorophyll-a concentrations were not

statistically different between the 1 and 2 mg/L algacide dose (p -value = 0.055). However at the 3 mg/L dosage, the concentrations were statistically lower than those observed under the two lower dosages. Under the 3 mg/L KMnO_4 dosage, 75 to 80% inhibition was achieved after 48h. Knappe [19] reported an 80% efficiency for *Microcystis aeruginosa* removal when samples were treated with 3 mg/L of KMnO_4 . Lowest concentrations were observed on the second day following treatment (Figure 2). Note that after 96 hr following treatment with KMnO_4 , chlorophyll-a concentrations across all dosages were not statistically different than those observed under the control (p -value = 0.3, 0.53, and 0.35 for 3, 2, and 1 mg/l, respectively), indicating that the *Microcystis aeruginosa* was able to make a full recovery even under the highest KMnO_4 dose applied (Figure 2).

Figure 2: Changes in chlorophyll-a concentrations over time across the three tested algacides: (a) reduction across the 4 CuSO_4 dosages as compared to the control; (b) reductions under the 3 KMnO_4 dosages as compared to the control; (c) reductions under the 2 diquat dosages as compared to the control.

Diquat was found to be highly effective under the two tested dosages (0.5 and 1 mg/L) when compared with the control (p -value < 0.05). No statistically significant difference in inhibition was observed between the two dosages. Microscopic observations showed that the colonies disappeared under both dosages during the first 24h; unicellular *Microcystis aeruginosa* were also found to disappear after 48 hrs (Figure 3). After 24h chlorophyll-a levels were found to have dropped by around 50%. Diquat's effect was most pronounced after 48 hr of treatment, where reductions approached 100% (Figure 2). Recovery of *Microcystis aeruginosa* was largely negligible after 96 hr; concentrations were 17 and 11% of levels measured under the control for 0.5 mg/L and 1 mg/L

respectively. This highlights the persistent algaecidal effect that diquat has on *Microcystis aeruginosa* colonies. The efficacy of diquat on cyanobacteria control has been reported by Phlips *et al.* [20], where strong inhibition (>90%) was observed even for dosage as low as 0.3 mg/L.

Figure 3: Microscope observations of *Microcystis aeruginosa* colonies after 48 hr: (a) control; (b) 0.8 mg/L CuSO₄; (c) 3 mg/L KMnO₄; (d) 0.5 mg/L diquat.

Chlorophyll-a temporal dynamics following the application of algaecides was best described by a quadratic regression model. The functional form of the model permits accounting for the rapid initial decrease associated with algaecide inhibition followed by a potential recovery phase, during which *Microcystis aeruginosa* starts to recover. Table 1 shows the developed models for the optimal dose for each of the three algaecides (CuSO₄ = 0.8 mg/L; KMnO₄ = 3 mg/L; diquat = 0.5 mg/L). Figure 4 compares the models and their prediction intervals with actual data observed. The quadratic regressions appear to capture the dynamics reasonably well, especially for CuSO₄ and diquat ($R^2 > 0.9$). The low R^2 associated with KMnO₄ is a direct result of the large variability among the replicates and the low inhibition action of the algaecide. The minimum chlorophyll-a concentration occurred at 62, 60, and 71 hr for CuSO₄, KMnO₄, and diquat respectively (Figure 4). These results highlight that the diquat’s algaecidal effect on *Microcystis aeruginosa* and the initiation of algae recovery tend to lag by ~10 hr when compared to the two other algaecides. Nevertheless, diquat’s rate of inhibition is higher than that for CuSO₄ and KMnO₄ (Figure 4), underscoring the superior efficacy of diquat as an algaecide.

Table 1: Multiple linear regression models representing chlorophyll-a dynamics with time (T) following the application of the algaecides.

Algaecide (optimal dose)	Regression model	R ²
CuSO ₄ (0.8 mg/L)	Chl-a = 112.4 – 3.71×T + 0.03×T ²	0.95
KMnO ₄ (3 mg/L)	Chl-a = 141.1 – 3.58×T + 0.03×T ²	0.49
Diquat (0.5 mg/L)	Chl-a = 191.4 – 5.68×T + 0.04×T ²	0.92

Figure 4: (a) Drop in chlorophyll-a concentration under a CuSO_4 dose of 0.8 mg/L; (b) drop in chlorophyll-a concentration under a KMnO_4 dose of 3 mg/L; (c) drop in chlorophyll-a concentration under a diquat dose of 0.5 mg/L. The dashed horizontal line represents initial conditions; black solid circles represent average measured concentrations; dashed vertical lines show the range of observed concentrations (minimum and maximum); solid black lines represent modelled chlorophyll-a reductions over time; (*) represent the 95% confidence intervals around model predictions.

Residual concentrations of algacides after 96 hr of exposure were below threshold standards for drinking water. Under the optimal algacide dose of 0.8 mg/L of CuSO_4 , the residual concentration of copper was below 0.6 mg/L (WHO standards for $\text{Cu} < 2$ mg/L [28]). For KMnO_4 , residual Mn concentrations did not exceed 0.15 mg/L, even when the algacide dose was 3 mg/L. The WHO guideline value for Mn in potable water is 0.4 mg/L [28]. Given that diquat is strongly adsorbed to soil and is rarely reported in drinking water sources, the WHO proposed an acute health-based value of 20 mg/L [28]. The residual concentration when 0.5 mg/L diquat was used was finally below 0.1 mg/L.

Figure 5: The change in the rate of algaecide inhibition over time for the three algaecides (CuSO_4 dose = 0.8 mg/L; KMnO_4 dose = 3 mg/L; diquat dose = 0.5 mg/L).

4 Conclusion

The following conclusions can be drawn based on the results of this study:

- The three tested algaecides (CuSO_4 , KMnO_4 , and Diquat) were effective in controlling *Microcystis aeruginosa* blooms when compared to the control, albeit with different efficiencies.
- Diquat proved to be the most effective algaecide in terms of reducing algae concentrations and inhibiting their recovery. In the same context, KMnO_4 exhibited the least effectiveness. While CuSO_4 was found to be an effective algaecide, its effectiveness diminished with time. This may point towards the promotion of Cu^{+2} tolerant *Microcystis aeruginosa* cells.
- All three tested algaecides showed statistically significant differences in efficiency as a function of dose and exposure time. Lowest concentrations were observed after an exposure time between 48 and 72 hrs.

Acknowledgements

The authors thank the United States Agency for International Development for providing support for this work through the USAID-NSF PEER initiative (Grant Number –AID-OAA-A_I1_00012) in conjunction with additional support from the University Research Board at the American University of Beirut.

References

- [1] M. Leira, G. Chen, C. Dalton, K. Irvine, and D. Taylor, "Patterns in freshwater diatom taxonomic distinctness along an eutrophication gradient," *Freshwater Biology*, vol. 54, pp. 1–14, 2009.
- [2] X. Wang, Y. Wang, L. Liu, J. Shu, Y. Zhu, and J. Zhou, "Phytoplankton and eutrophication degree assessment of Baiyangdian Lake Wetland, China," *The Scientific World Journal*, vol. 2013, 2013.
- [3] 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*, vol. 36, pp. 45–56, 1996.
- [4] 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*, vol. 6, pp. 519–530, 2007.
- [5] A. M. Michalak, E. J. Anderson, D. Beletsky, S. Boland, N. S. Bosch, T. B. Bridgeman, *et al.*, "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*, vol. 110, pp. 6448–6452, 2013.
- [6] H. W. Paerl and T. G. Otten, "Harmful cyanobacterial blooms: causes, consequences, and controls," *Microbial ecology*, vol. 65, pp. 995–1010, 2013.
- [7] 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*, vol. 44, pp. 7756–7758, 2010.
- [8] I. Chorus and J. Bartram, *Toxic cyanobacteria in water: A guide to their public health consequences, monitoring and management*: Spon Press, 1999.
- [9] D. M. Anderson, P. M. Glibert, and J. M. Burkholder, "Harmful Algal Blooms and Eutrophication: Nutrient Sources, Composition, and Consequences," *Estuaries*, vol. 25, pp. 704–726, 2002.
- [10] H. W. Paerl, H. Xu, M. J. McCarthy, G. Zhu, B. Qin, Y. Li, *et al.*, "Controlling harmful cyanobacterial blooms in a hyper-eutrophic lake (Lake Taihu, China): The need for a dual nutrient (N & P) management strategy," *Water Research*, vol. 45, pp. 1973–1983, 2011.
- [11] I. R. Falconer, "Is there a human health hazard from microcystins in the drinking water supply?," *Acta hydrochimica et hydrobiologica*, vol. 33, pp. 64–71, 2005.
- [12] J. N. Galloway, A. R. Townsend, J. W. Erisman, M. Bekunda, Z. Cai, J. R. Freney, *et al.*, "Transformation of the nitrogen cycle: recent trends, questions, and potential solutions," *Science*, vol. 320, pp. 889–892, 2008.
- [13] C. Gobler, T. W. Davis, K. Coyne, and G. Boyer, "Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake," *Harmful Algae*, vol. 6, pp. 119–133, 2007.

- [14] R. A. Smith, R. B. Alexander, and G. E. Schwarz, "Natural background concentrations of nutrients in streams and rivers of the conterminous United States," *Environmental Science & Technology*, vol. 37, pp. 3039–3047, 2003.
- [15] 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*, vol. 47, pp. 5153–5164, 2013.
- [16] D. Jančula and B. Maršálek, "Critical review of actually available chemical compounds for prevention and management of cyanobacterial blooms," *Chemosphere*, vol. 85, pp. 1415–1422, 2011.
- [17] H. Kemp, R. Fuller, and R. Davidson, "Potassium permanganate as an algicide," *Journal (American Water Works Association)*, vol. 58, pp. 255–263, 1966.
- [18] J.-J. Chen and H.-H. Yeh, "The mechanisms of potassium permanganate on algae removal," *Water Research*, vol. 39, pp. 4420–4428, 2005.
- [19] D. R. Knappe, *Algae detection and removal strategies for drinking water treatment plants*: American Water Works Association, 2004.
- [20] E. J. Philips, P. Hansen, and T. Velardi, "Effect of the herbicide diquat on the growth of microalgae and cyanobacteria," *Bulletin of environmental contamination and toxicology*, vol. 49, pp. 750–756, 1992.
- [21] J. Clayton and F. Matheson, "Optimising diquat use for submerged aquatic weed management," *Hydrobiologia*, vol. 656, pp. 159–165, 2010.
- [22] ELARD, "Business Plan for Combating Pollution in Qaraoun Lake," United Nations Development Programme (UNDP). Beirut, Lebanon, 2011.
- [23] BAMAS, "Litani Water Quality Management Project: Technical Survey Report," Beirut, Lebanon, 2005.
- [24] American Public Health Association (APHA), American Water Works Association (AWWA), and Water Environment Federation (WEF), *Standard Methods of Water and Wastewater*, 22 ed.: American Public Health Association, 2012.
- [25] 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.
- [26] R Development Core Team, "R: A language and environment for statistical computing," R Foundation for Statistical Computing, Vienna, Austria 2015.
- [27] L. García-Villada, M. Rico, M. a. Altamirano, L. Sánchez-Martín, 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 algicide," *Water Research*, vol. 38, pp. 2207–2213, 4, 2004.
- [28] World Health Organization, *Guidelines for drinking-water quality: recommendations*, 3 ed. vol. 1. Geneva, Switzerland: World Health Organization, 2004.