

Evaluation of a Copper Based and Peroxide Based Algaecide for Treatment for Controlling Harmful Algal Blooms in a Recreational Lake

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Abstract

The frequency and intensity of harmful algal blooms is increasing posing a significant risk to surface water used for drinking water and recreation. All algaecide treatments were effective at reducing cyanobacteria within two days of application ($p < 0.05$). Overall, the most significant reductions in cyanobacteria content occurred with full dose of CutrineUltra and remained at less than 600 cells/mL by day 14. Quarter doses of both algaecides exhibited a rebound in cyanobacteria levels between day 7 and 14, indicating that additional treatments would be needed. Extracellular microcystin concentrations were higher on day 2 for PAK-27 treatments, by day 7 for CutrineUltra and full dose PAK-27 + 5 mg natural organic matter.

Keywords

Algae, Cholorophyll-A, Cyanobacteria, Microcystin

1. Introduction

A study of satellite observations from 1982-2019 found that 11.7% of all global lakes in all continents have had harmful algal blooms (HABs) [1]. In 2018, 150 HAB incidences were reported in Canada as well as in all states in the U.S. [2]. HABs occur in water with temperatures above 20°C, ample sunlight and high nutrient levels. With increasing levels of water temperature, the frequency and intensity of HABs are becoming more pronounced [3]. This has led to HABs being considered one of the greatest threats to inland water quality [4]. In addition to increased costs for treating HABs, there have been extreme financial losses in aquaculture and tourism. Japan has reported over \$246 million dollars in aquaculture losses [5]. Inability to use a water body for drinking, agricultural and recrea-

tion have resulted in annual losses of \$2 billion dollars [6].

Human exposure to cyanotoxins produced during HABs occurs via consumption, aerosol inhalation, and dermal contact [3] [7]. With consumption as a key route of exposure, most research has focused on the impact of HABs to drinking water sources. However, HAB incidences in recreational lakes also pose significant harm to human health and the environment. Seventy-five of the waterborne illness reported in the U.S. between 2000 and 2014 were due to algal toxins in recreational waters [8]. Symptoms of exposure to saxitoxins include fever, eye irritation, abdominal pains, rash; microcystin can cause joint pain, gastrointestinal illness fever, liver damage and respiratory distress, while Anatoxin-a can result in seizure, and heart failure and death [8] [9]. One such fatality was reported in Italy after a boater fell into a lake with high anatoxin-a levels [10].

One of the most common methods of in-lake management of HABs is the application of algaecides. Chemical formulations of algaecides can be categorized based on active ingredients as being either copper-based or peroxide-based. Copper algaecides cause cell death by interference with photosynthesis, inhibition of CO₂ fixation and depending on species, inhibition of nitrogen uptake [11] [12]. Although copper-based algaecides have been widely used due to their effectiveness, they have been shown to be toxic to non-target organisms. The impact toward non-target organism in the water column is not the only potential drawback as copper can accumulate in the environment with sediment as the primary sink [13]. The emulsified form of CutrineUltra has been reported to be effective toward specific cyanobacteria and algae at lower concentrations [14] and to be retained in the water column longer (*i.e.*, not sink as fast) as other copper algaecides.

Hydrogen peroxide-based algaecides can hinder photosynthesis, cause oxidative damage to algal cells with little effect toward eukaryotic phytoplankton, and has the advantage that it there is no residual as it breaks down into water and oxygen within days [15] [16]. Researchers have reported that most cyanobacteria are more sensitive to H₂O₂ than green algae [17]. Research associated with the algaecide effectiveness has primarily focused on waterbodies that are used as drinking water sources, with little to no research for lakes used solely for recreational purposes. Often stake holders have different views of the costs for a study and/or algaecide purchase versus the beneficial outcome [18], especially if do not fully believe the harm posed by HABs [19] [20]. This study evaluated the potential of a copper based (CutrineUltra) and a peroxide-based algaecide (PAK-27), each at two dosage levels, for mitigating HABs in a recreational lake. It was hypothesized that i) Both algaecide sources would be effective at managing cyanobacteria levels, ii) PAK-27 would be less harmful to non-target organisms, and iii) Higher dosages would result in higher extracellular toxins.

2. Methods

2.1. Water Source, Sample Collection, and Initial Lake Profile

A Wisconsin Plankton sampler 53 µm mesh (Wildco model 40-A37, U.S.) was

used to physically concentrate cyanobacteria and algae from a recreational lake located in northeast Ohio. The lake covers 1.17 km² and has maximum depth of 8.23 m. The lake is designated solely for recreational use such as boating, swimming, and fishing. However, it has been restricted to non-contact boating only due to microcystin levels >50 µg/L over the last 5 years. The phytoplankton sampler was towed behind the boat at very slow speed. Samples were placed in an 18.9 L bucket until ~1/4 of the bucket was filled. Then raw lake water was added to the mid-point. Buckets of raw lake water were also collected to dilute samples if needed. Samples were transported to the University of Akron lab to initiate the experiments.

A profile of the two sampling locations was conducted. As shown in **Table 1**, cyanobacteria cell counts ranged from 1600 to 14,000 cells/mL depending on the depth. The highest counts of 13,000 cell/mL occurred at a depth of 4 m for site a, and 14,000 cells/mL at 3 m for site b. With this cell count, it was not surprising that dissolved oxygen levels were saturated in water layers above. Similarly, pH readings were also higher in the water layers. Based on the profile readings it was highly probably that the laboratory experiment could be conducted.

Table 1. Profile of sites 1a and 1b Springfield Lake conducted on June 21, 2023.

Depth, m	Temp C	DO mg/L	pH	Cyanobacteria		Chlorophyll		Conductivity µS/cm
				Cells/mL	RFU	µg/L	RFU	
0	23.2	11.39	8.66	3600	1.4	3.1	0.82	706
1	23.0	11.17	8.96	7000	2.2	5.3	1.3	707
2	22.9	11.15	8.68	6300	2.2	4.3	1.09	706
3	21.8	9.81	8.56	6700	2.4	4.3	1.09	705
a 4	18.2	2.18	7.48	13,000	4.9	3.8	1.04	712
5	15.9	0.44	7.31	1600	0.8	1.4	0.39	716
6	11.0	0.10	7.01	2000	0.9	1.6	0.42	720
7	10.5	0.4	6.91	3000	1.4	3.4	0.73	724
0	23.1	11.47	8.75	3400	1.5	3.1	0.77	706
1	23.0	11.51	8.75	7300	2.5	4.6	1.18	706
2	22.8	11.25	8.72	6600	2.3	4.3	1.05	706
b 3	20.7	6.93	8.60	14,000	4.5	5.0	1.27	715
4	17.7	0.56	7.37	7600	2.6	3.5	0.9	714
5	13.6	0.18	7.32	2300	1.0	2.4	0.57	721

2.2. Algaecide Sources and Concentrations

Two algaecides were tested in the bench experiments. The hydrogen peroxide based (H₂O₂) algaecide was PAK27. The second was an emulsified copper based, CutrineUltra. Two dosage levels were tested for each algaecide: the full recommended dose based on the manufacturer's guidelines and one quarter dose of the recommended dose. Previous research with both algaecides has shown that lower than a full dose can be effective at mitigating a bloom [21]-[23]. For each alga-

cide, the doses used were scaled down using the dimensions of the reactors to provide direct correlation to typical field applications in real lakes. For a 1.6 L water volume a quarter dose of PAK-27 was 15 mg (equivalent to 2.6 mg/L H₂O₂) and a full dose was 58 mg (equivalent to 9.99 mg H₂O₂) CutrineUltra's (Arch Chemicals, Inc., U.S.) active ingredient is 27.8% mixed copper ethanolamine complex which corresponds to a 9% metallic copper content. According to manufacturer's guidelines, 0.4 ppm copper concentration needs to be applied per 1.2 gallons per acre-ft. The algaecide should be diluted depending on the application method. Since it is very viscous (396 mPa s at 24°C), a 10:1 dilution was used based on the spray method used and 76 µL after dilution was used for full dose reactor and 19 µL for quarter dose systems. Key characteristics of the algaecides used is given in **Table 2**.

Table 2. Key characteristics of algaecides used in project.

Characteristic	Cutrine Ultra	PAK-27
Active Ingredient	Copper	Hydrogen peroxide
% active ingredient	9	85
Application Rate (mg/L)		0.08 - 44.9
Formulation	Copper ethanolamine emulsified complex	Sodium carbonate peroxyhydrate
Chemical Class	Chelated elemental copper	Hydrogen peroxide oxidant
Mode of Action	Cell toxicant	
Appearance	Viscous blue liquid	White powder
Water Solubility (g/L)	Miscible	150 at 20°C
Boiling Point (°C)	n/a	n/a
Specific Gravity (g/cm ³)	1.20	0.9 - 1.2
pH	10 - 10.5	10.4 - 10.6

2.3. Experimental Set-Up

Each condition was conducted in triplicate. A control set (no amendments) was used to track "normal" cyanobacteria activity. Two sets of jars were used to track effectiveness of PAK-27 dose and two sets to track effectiveness of CutrineUltra dose. In the last set of triplicates, 5 mg Suwannee River natural organic matter (NOM) (International Humic Substances Society, St. Paul MN) was added to determine the impact of NOM on PAK-27 effectiveness.

Each reactor was quantified for phycocyanin, chlorophyll-*a*, NOM, and conductivity at baseline (*i.e.*, time zero prior to algaecide application), and 2 days after algaecide application to track algaecide effectiveness. Data was collected on day 7 and day 14 to evaluate potential rebound of cyanobacteria. Total saxitoxin and total microcystin was quantified at baseline and day 2 with extracellular content of each toxin quantified at baseline, day 2 and day 7.

Each, 2 L wide mouth glass jar was filled with 1.6 L of source water with at least 10,000 cells/mL of cyanobacteria. All jars were placed in a growth chamber (Thermo Scientific Precision Incubator Model 818, U.S.) equipped with soft light

illumination (22 $\mu\text{m}/\text{m}^2\text{s}$, 12 hr. cycle for day 25°C, night 20°C). The cyanobacteria and chlorophyll-a levels were measured after 24 hours. If numbers had not decreased 10% or more, the experiment was started. Measurements were taken from the same location within the jar for each time step. After taking baseline readings and samples, the experiment was initiated by adding the required algaecide dose. All jars were returned to the growth chamber until the next measurement.

2.4. Analytical Methods

A ProDSS sonde (YSI, U.S.) was used to measure phycocyanin (cells/mL) for cyanobacteria counts and chlorophyll-*a* ($\mu\text{g}/\text{L}$) for other photosynthetic organisms. The sonde also tracked the physicochemical characteristics of the water temperature ($^{\circ}\text{C}$) and conductivity ($\mu\text{S}/\text{cm}$).

For NOM, sample pH was adjusted to between 4.0 and 10.0. Next the sample was filtered and absorbance of the supernatant analysed on a portable UV GO! spectrophotometer (Photonic Measurements, United Kingdom) at a wavelength of 254 nm.

The presence of extracellular microcystin and saxitoxin were determined by Enzyme-Linked Immunosorbent Assays (ELISA) Analysis. After each Sonde reading, 10 mL sample was collected from the center of the jar at 5.08 cm below the waterline. Time 0 and day 2 collected samples for each toxin without a filter. Day 7 samples were filtered through a sterile, disposable filter of 0.45 μm pores size. For saxitoxin, 9 mL of sample was collected and amended with 1 mL of preservative. All sample bottles were transported on ice to Enviroscience for analysis.

For Microcystin, Enviroscience used the ABRAXIS microcystins/nodularins (ADDA) ELISA kit (Eurofins Abraxis, United States) with a 0.24 - 5 $\mu\text{g}/\text{L}$ detection range. The ABRAXIS Saxitoxin (PSP) ELISA kit with a range of 0.02 - 0.4 $\mu\text{g}/\text{L}$ detection range was used for STX.

A one-way Analysis of Variance (ANOVA) was used to compare the conditions (Quarter PAK-27, Full PAK-27 etc.) with each other on each day. Tukey's comparison was used to compare all conditions. Minitab® 21.3.1 software was used to conduct the analyses with a $p < 0.05$ considered a significant result.

3. Results and Discussion

3.1. Cyanobacteria, Chlorophyll-a and Toxin Level Without Additional 5 mg NOM

The average baseline cyanobacteria levels prior to algaecide treatments were ~15,000 cells/mL for control and PAK27 jars and ~17,000 cells for Cutrine Ultra jars. As expected, after two days all jars treated with an algaecide decreased by significantly ($p < 0.05$, **Figure 1**). Jars treated with a full dose of CutrineUltra exhibited the most significant decrease to 1701 ± 592 cells/mL ($p < 0.05$). By day 7, the quarter dose of CutrineUltra was also below 1000 cells/mL ($p < 0.05$). Between day 2 and day 7 the cyanobacteria content in jars treated with quarter dose of PAK-27 depicted a significant rebound ($p < 0.05$) to ~26,000 cells/mL which was

higher than that exhibited at baseline conditions (before algaecide). Full dose of CutrineUltra resulted in a continued decreases in cyanobacteria levels until day seven with a slight rebound ($p > 0.05$) between day 7 and 14.

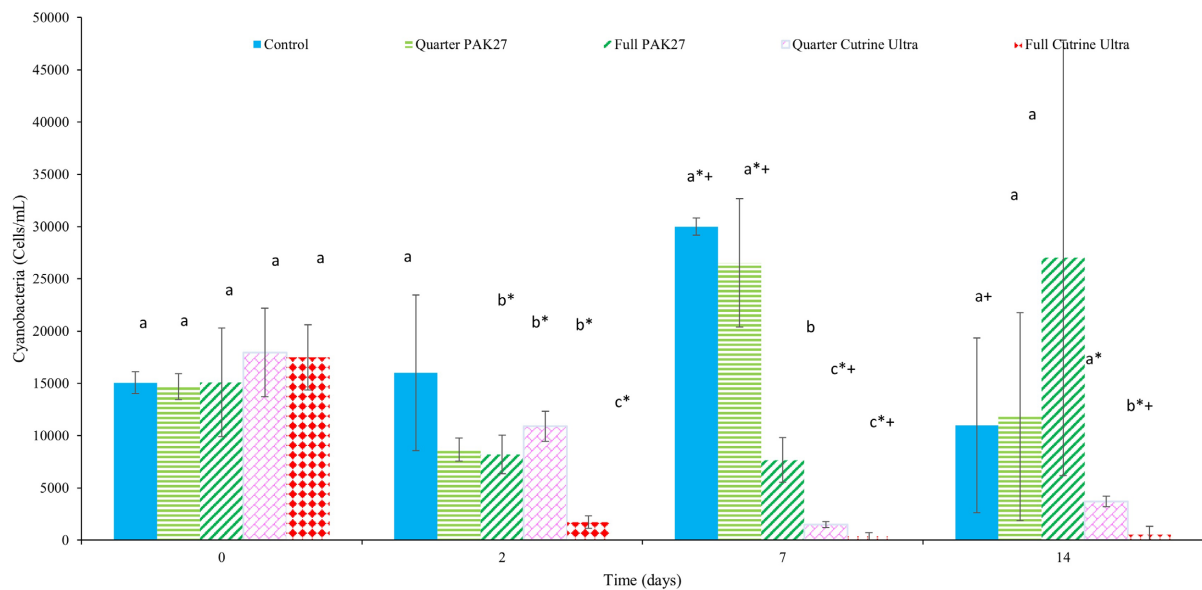


Figure 1. Changes in cyanobacteria versus time. Data shown is the average of triplicate values. Significant difference corresponds to $p < 0.05$. Conditions on the same day that do not share the same letters (a, b, etc.) are significantly different. The asterisk (*) on timesteps indicates a significant different for the same treatment compared to day 0. Plus (+) indicates a significant difference for the same treatment for consecutive days (e.g., days 0 - 2, 2 - 7 and 7 - 14).

Unlike CutrineUltra, PAK-27 treatments continued to exhibit a rebound in cyanobacteria to day 14. Cyanobacteria cell number also rebounded with quarter dose of Cutrine Ultra compared to previous timestep ($p < 0.05$), but to a lesser degree than PAK-27 ($p < 0.05$). Calomeni *et al.* [14] reported that the algal mass rebounded between day two and seven after CutrineUltra treatment. Gao [23] also observed a rebound between day 7 and 14 after the application quarter dose of Cutrine Ultra. The reason for the rebound could be attributed to an increased resistance of some of the cyanobacteria to copper toxicity [24]. Even with the rebound in cyanobacteria content, the day 14 levels in CutrineUltra jars were significantly lower than the baseline levels ($p < 0.05$, Figure 1). This was also evident in the color hue exhibited in the jars where the full CutrineUltra jars had lost almost all color by day 14.

While phycocyanin is unique to cyanobacteria, chlorophyll-a is characteristic of all photosynthetic organisms. As such it was used to assess the impact of the algaecide treatment on cyanobacteria and non-target organisms (*i.e.*, green algae and diatoms). The chlorophyll-a baseline concentration was higher than 30 $\mu\text{g/L}$ the controls and all treatments (Figure 2). Although CutrineUltra doses resulted in a higher reduction in cyanobacteria levels than PAK-27, it also resulted in a higher reduction in chlorophyll-a content. In jars treated with a Full dose of CutrineUltra chlorophyll-a content decreased by 93% lower chlorophyll-a content (p

< 0.05). During the first two days chlorophyll-a in the quarter and full CutrineUltra jars decreased by ~28 $\mu\text{g/L}$ and 32.48 $\mu\text{g/L}$, respectively whereas quarter and full PAK-27 jars decreased by 14.64 $\mu\text{g/L}$ and 24.5 $\mu\text{g/L}$, respectively. The higher reduction in chlorophyll-a indicates that levels of both cyanobacteria and non-target organisms were reduced. In this study, in Quarter PAK-27 and Full PAK-27 the chlorophyll-a increased by 10% ($p < 0.05$) and 382% ($p > 0.05$), respectively over the first week. Based on the cyanobacteria levels shown in **Figure 1** the rebound in chlorophyll-a was a combination of cyanobacteria and green algae. The increase was expected as peroxide-based algaecides at low induce oxidative stress but not death [25]. Buley *et al.* [26] reported a rebound with granulated PAK-27 but took two weeks to occur. For this study, the chlorophyll-a content in jars treated with PAK-27 were slightly lower than the baseline by day 14. Sinha *et al.* [27] reported that 4.0 mg/L PAK-27 application decreased the chlorophyll-a concentration after two weeks. According to his study, >4.0 mg/L H_2O_2 would not be feasible since it can kill other non-target eukaryotic phytoplankton communities.

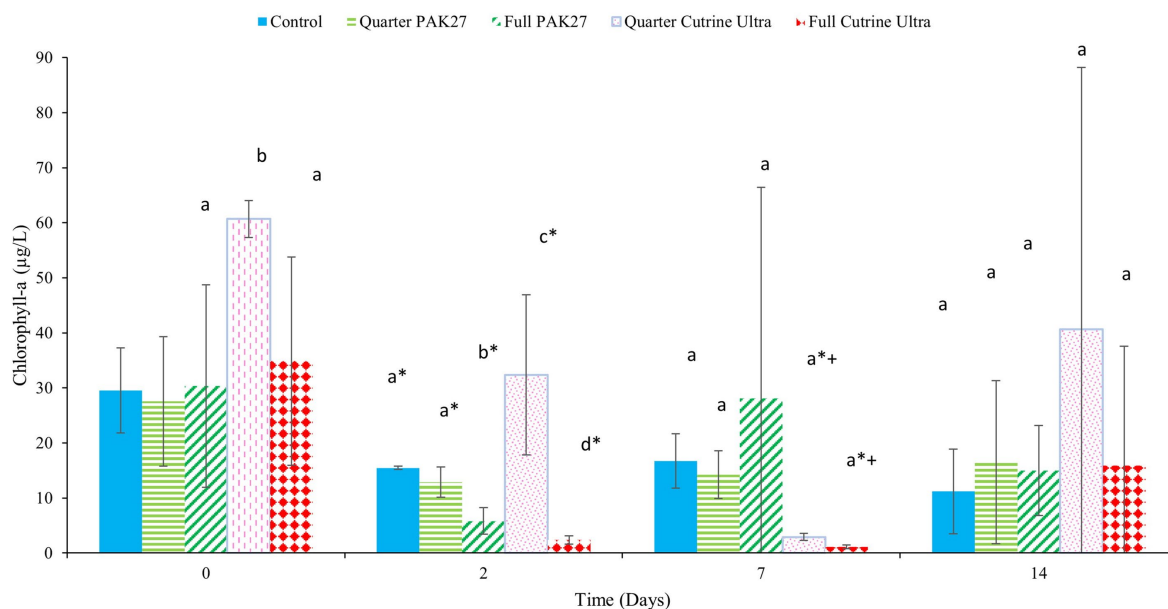


Figure 2. Changes in chlorophyll-a versus time. Data shown is the average of triplicate values. Significant difference corresponds to $p < 0.05$. Conditions on the same day that do not share the same letters (a, b, etc.) are significantly different. The asterisk (*) on timesteps indicates a significant different for the same treatment compared to day 0. Plus (+) indicates a significant difference for the same treatment for consecutive days (e.g., days 0 - 2, 2 - 7 and 7 - 14).

Jars treated with CutrineUltra exhibited less of a rebound than those that received PAK-27. The continued decrease was not surprising as copper is one of the most toxic metals toward algae [28]. By day 14 the jars treated with a full dose of CutrineUltra or Pak-27 had only ~15 $\mu\text{g/L}$ of chlorophyll-a., which was lower than the level present at the start of the experiment.

Another approach to evaluating the effectiveness of algaecide treatments is the amount of cyanotoxins released during cell lysis. For instance, early research indicated that *Microcystis aeruginosa* had a strong correlation copper dose and the

amount of microcystin-LR released [29]. Lefler *et al.* [30] also reported significant reduction in phycocyanin, chlorophyll-a that corresponded to initial release of microcystin. As expected, microcystin was the dominant cyanotoxin present in the samples. For some samples, the baseline extracellular microcystin levels are listed as >5 µg/L. Even with two sets of dilutions the amount was above the detection limit of 5 µg/L, which made a statistical analysis impossible. In general, extracellular microcystin levels increased two days after algaecide treatments followed by a slight decline (Table 3). Shi *et al.* [31] also found that extracellular microcystin increased with higher doses of copper sulfate and peaked two days after treatment followed by a gradual decline. The low levels of saxitoxin were not surprising as microcystin has been the dominant toxin detected in the lake.

Table 3. Extracellular microcystin (MC) and saxitoxin (STX) levels (bdl = below detection limit).

	Day 0		Day 2		Day 7	
	MC (µg/L)	STX (µg/L)	MC (µg/L)	STX (µg/L)	MC (µg/L)	STX (µg/L)
Control	>5	0.0037 ± 0.004	0.98 ± 0.37	0.008 ± 0.001	2.92 ± 1.68	0.009 ± 0.002
Quarter PAK-27	1.31 ± 0.44	0.006 ± 0.00	2.38 ± 0.28	0.006 ± 0.008	3.89 ± 1.59	0.006 ± 0.002
Full PAK-27	0.81 ± 0.00	bdl	3.45 ± 1.04	0.009 ± 0.001	5.14 ± 2.26	0.006 ± 0.002
Quarter CutrineUltra	1.052 ± 0.00	bdl	0.90 ± 1.24	0.016 ± 0.001	8.52 ± 1.50	0.007 ± 0.002
Full CutrineUltra	0.58 ± 0.01	0.001 ± 0.001	0.20 ± 0.09	0.016 ± 0.001	4.60 ± 4.49	0.006 ± 0.002
Quarter PAK-27+5 mg NOM	>5	0.002 ± 0.003	3.68 ± 0.74	0.007 ± 0.002	3.91 ± 3.55	0.008 ± 0.002
Full PAK-27+5 mg NOM	>5	bdl	4.3 ± 0.64	0.009 ± 0.01	9.87 ± 4.05	0.008 ± 0.004

3.2. Effectiveness of PAK-27 with 5 mg NOM

As PAK-27 is a hydrogen peroxide-based algaecide, it's the presence of NOM and other organic constituents can impact effectiveness. At high enough levels, peroxide -based algaecides can degrade cyanotoxins while copper-based cannot [32]. The NOM at baseline conditions was ~10.6 mg/L. For the quarter dose PAK27+ 5 mg NOM and full dose PAK-27+ 5 mg NOM the baseline NOM was ~13 mg/L (Figure 3). NOM levels in all jars amended with algaecide were lower than baseline conditions by day 14. This was attributed NOM consumption during the rebound in cyanobacteria and/or non-target organisms.

All jars treated with PAK-27 depicted a substantial decrease in cyanobacteria levels two days after treatment, with the quarter PAK-27, full PAK-27 and full PAK-27+5 mg NOM being significantly lower ($p < 0.05$, Figure 4(a)). The most significant rebound in cyanobacteria levels between day 2 and day 7 occurred in the quarter PAK-27+ 5 mg NOM (11,793 cells/mL to 41,000 cells/mL). The hydrogen peroxide released by PAK-27 may have been consumed degrading the NOM first, leaving less for control of cyanobacteria cells [33]. By day 14, cyanobacteria levels for quarter PAK-27 and PAK-27+5 mg jars were less than that exhibited at baseline conditions. This was attributed to the higher cell counts during the rebound between day 2 and day 7 using the available nutrients followed by

starvation. Soluble reactive phosphorus levels in reactors that received 5 mg NOM decreased between day 0 and day 2. This was followed by an increase after cell lysis and a decrease between day 7 and 14 while control reactors exhibited a continued increase (Table 4). Zhang *et al.* [34] also found that cyanobacteria blooms released phosphorus during cell death, which was then used by surviving cells. Batch cultures can lead to cell death or stopped production of key intermediates once initial nutrient levels are depleted [35]. This was corroborated by the chlorophyll-a content not exhibiting a significant increase after cyanobacteria levels had decreased (Figure 4(b)).

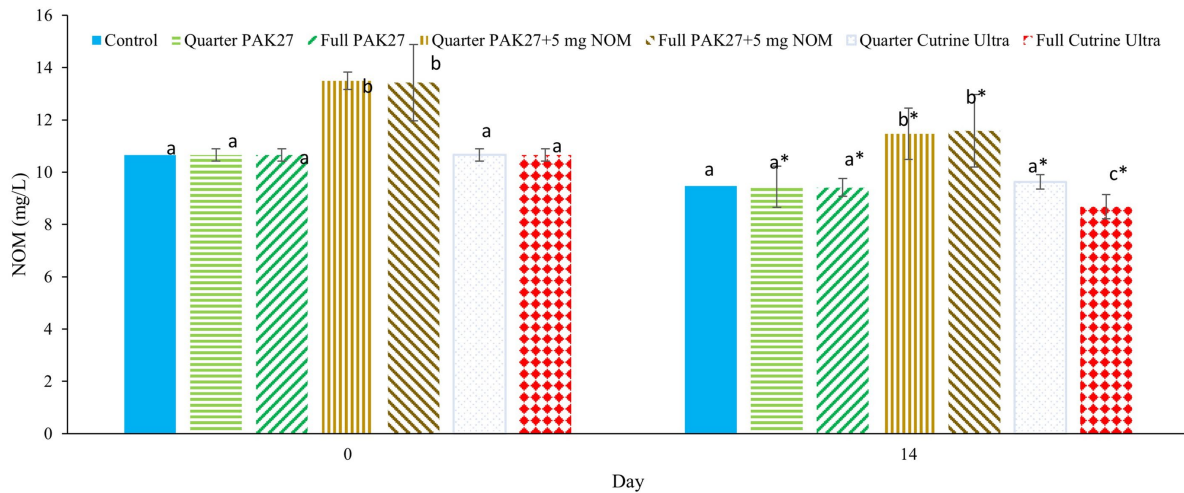
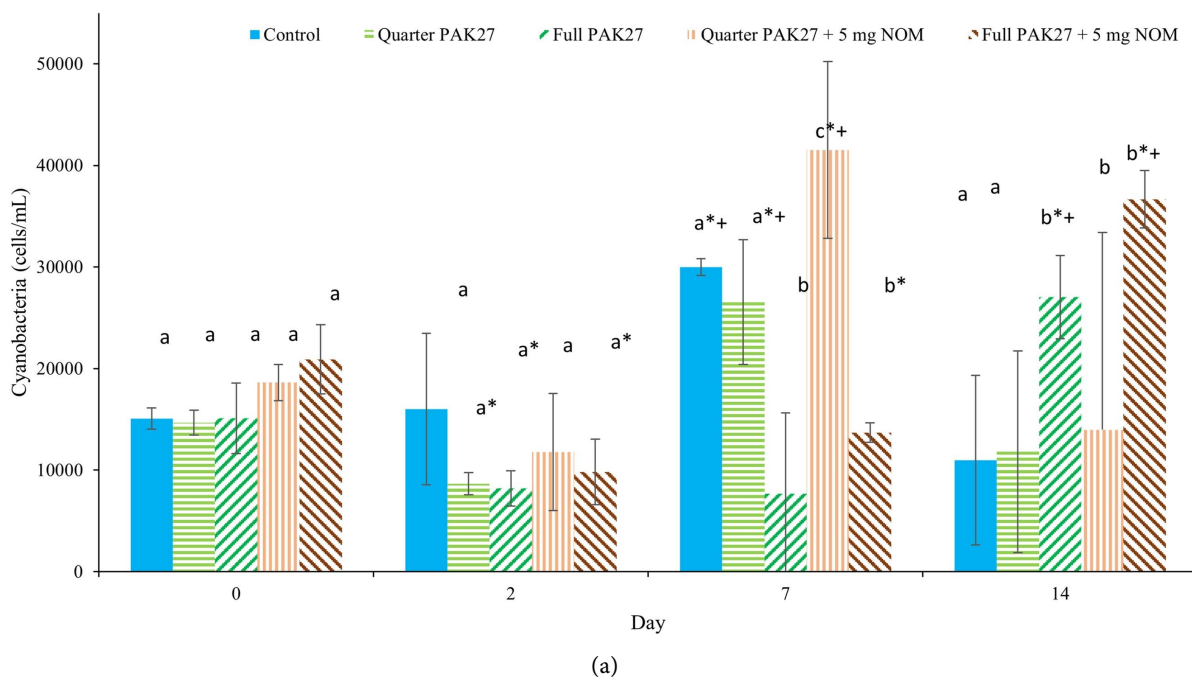


Figure 3. NOM levels across all treatments at the start and end of the experiment. Data shown is the average of triplicate values. Significant difference corresponds to $p < 0.05$. Conditions on the same day that do not share the same letters (a, b, etc.) are significantly different. The asterisk (*) on timesteps indicates a significant different for the same treatment compared to day 0.



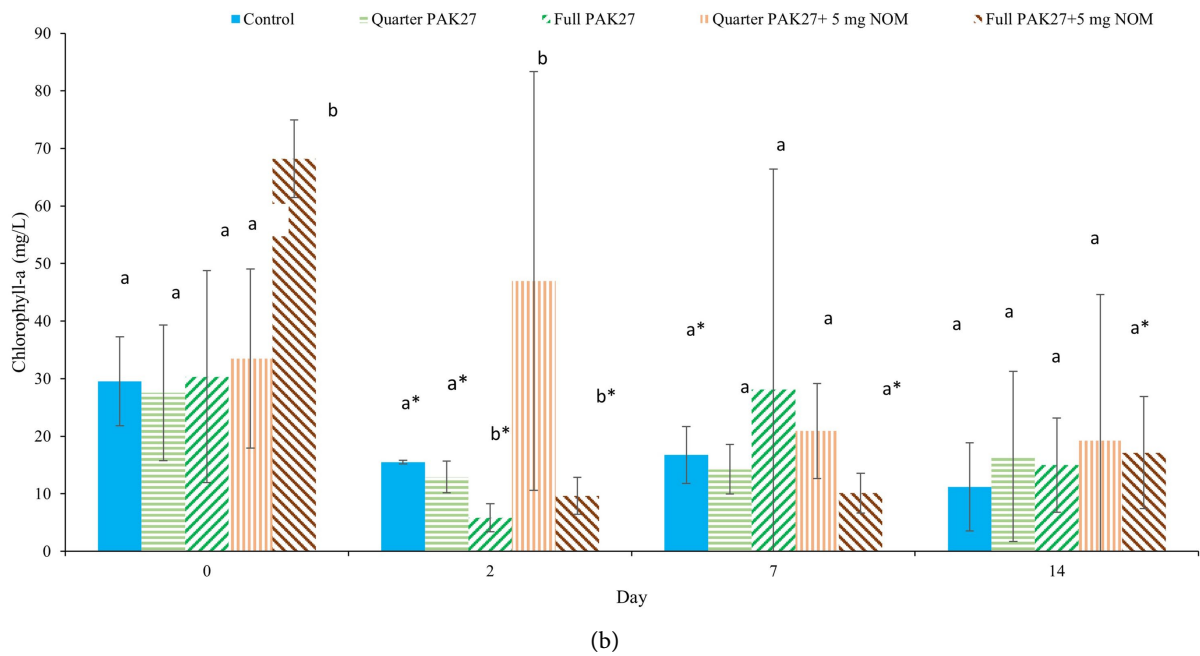


Figure 4. Changes in (a) cyanobacteria and (b) chlorophyll-a versus time for controls, Quarter dose PAK-27, Full dose PAK-27, Quarter dose PAK-27+5 mg NOM and Full dose PAK-27+5 mg NOM. Data shown is the average of triplicate values. Significant difference corresponds to $p < 0.05$. Conditions on the same day that do not share the same letters (a, b, etc.) are significantly different. The asterisk (*) on timesteps indicates a significant different for the same treatment compared to day 0. Plus (+) indicates a significant difference for the same treatment for consecutive days (e.g., days 0 - 2, 2 - 7 and 7 - 14).

Jars that were initial amended with 5 mg NOM had higher microcystin levels after PAK-27 treatments than those that did not (Table 3). For example, by day 14 the extracellular microcystin was 5.14 ± 2.26 in full PAK-27 jars while it was 9.87 ± 4.05 in full Pak-27+ 5 mg NOM.

Table 4. Soluble reactive phosphorous levels measured during each time step.

	Day 0	Day 2	Day 7	Day 14
Control	0.83 ± 0.15	6.79 ± 1.08	10.72 ± 1.67	11.27 ± 1.62
Quarter PAK-27	0.42 ± 0.15	0.54 ± 0.20	0.96 ± 0.03	0.95 ± 0.24
Full PAK-27	0.41 ± 0.15	0.37 ± 0.02	1.01 ± 0.19	1.05 ± 0.10
Quarter CutrineUltra	0.28 ± 0.08	0.23 ± 0.05	0.57 ± 0.05	0.92 ± 0.38
Full CutrineUltra	0.28 ± 0.04	0.52 ± 0.06	0.83 ± 0.08	0.82 ± 0.30
Quarter PAK-27+5 mg NOM	0.42 ± 0.04	0.34 ± 0.05	0.90 ± 1.24	0.54 ± 0.34
Full PAK-27+5 mg NOM	0.39 ± 0.09	0.35 ± 0.05	0.59 ± 0.11	0.47 ± 0.19

4. Conclusion

Both algaecide sources would be effective at managing cyanobacteria levels, with copper-based CutrineUltra resulting in significantly lower levels. As expected, the full dose of algaecide resulted in higher amount of extracellular microcystin. Although PAK-27 is less harmful to non-target organisms, CutrineUltra is recom-

mended as an initial emergency, short term response to get the lake used in the study to manageable conditions. However, the authors do not require copper-based algaecide as long-term solution for residential lake. Additional costs of weekly testing to verify residual copper at safe levels. Once lake has better HAB management can possibly apply copper once early in season and switch to PAK-27 as needed the rest of the season. At this time, lake managers are seeking funds needed to purchase the algaecides to implement the proposed multi-season approach.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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