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Topic

Natural Hazards and Environmental Challenges in the Anthropocene Age

Edited by

Dr. Johnbosco C. Egbueri, Dr. Chaitanya B. Pande and Dr. Quoc Bao Pham

MDPI

https://doi.org/10.3390/w16111624

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Demonstration of Proactive Algaecide Treatments Targeting Overwintering Cyanobacteria in Sediments of an Urban Pond

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Abstract: Most cyanobacteria that form harmful algal blooms (HABs) in inland waterbodies can overwinter in sediments. This field demonstration within an urban pond was conducted to bolster a database on the novel use of algaecide treatments to proactively target overwintering cyanobacteria located in sediments prior to HAB formation. In March 2023, a peroxide-based algaecide was applied to sediments of a water feature located in urban Kansas City, Kansas, and cyanobacteria responses were measured over subsequent weeks and months. Multiple lines of evidence were used to discern the impacts of proactive treatments on overwintering cells in sediments and HAB severity throughout the growing season. Although results of the measured cyanobacterial responses were mixed, three of five lines of evidence indicated proactive algaecide treatments were effective at decreasing the transfer of cyanobacteria to the water column and HAB severity during months when HABs tended to occur. Microcystin concentrations immediately post-treatment (hours) remained at the analytical detection limit (0.10 µg/L) and were below USEPA risk-based thresholds, highlighting the benefits of application prior to the exponential growth phase of toxin-producing cyanobacteria. These results expand the dataset and methodology for field-scale proactive algaecide applications targeting overwintering cyanobacterial cells in sediment to mitigate and delay HAB development.

Keywords: akinetes; harmful algal blooms; HAB; management; mitigation; microcystin; prevention; peroxide; treatment

Citation: Calomeni-Eck, A.; McQueen, A.; Kinley-Baird, C.; Smith, E.; Growcock, B.; Decker, K.; Hampton, S.; Stahl, A.; Boyer, M.; Clyde, G., Jr. Demonstration of Proactive Algaecide Treatments Targeting Overwintering Cyanobacteria in Sediments of an Urban Pond. *Water* **2024**, *16*, 1624. https://doi.org/10.3390/w16111624

Academic Editors: Quoc Bao Pham, Johnbosco C. Egbueri and Chaitanya B. Pande

Received: 6 May 2024
Revised: 20 May 2024
Accepted: 24 May 2024
Published: 6 June 2024

1. Introduction

Harmful algal blooms (HABs), or visible growths of toxin-producing cyanobacteria in freshwater resources, have caused detrimental effects to humans and animals [1]. Ponds in urban environments may be particularly susceptible to HABs and have greater potential for human exposure due to high population densities [2,3]. Management of HABs using U.S. Environmental Protection Agency (USEPA)-registered algaecides is an approach that is used to decrease health risks [4]. A relatively unexplored proactive management approach is the use of algaecides to target overwintering cyanobacteria cells present in sediments during cooler months, prior to the formation of a planktonic HAB [5]. Multiple studies have reported that overwintering cells located in sediments can transfer to the water column and contribute to HABs during the growing season [6–9]. There is initial evidence that proactive

treatment of overwintering cells in sediment may decrease the initial inoculum of cyanobacteria entering the water column, delaying the onset and decreasing the overall impact of HABs [10,11]. However, this approach is novel, with few published examples evaluating the performance of proactive treatments of overwintering cells in sediment [10,12] and few field-scale demonstrations of this approach [11,12] reported.

At the end of a growing season, HABs senesce and settle to the lakebed, where they remain during cooler temperatures [13]. Some of these overwintering cells remain viable during the winter, contributing to the formation of blooms during the following growing season. Most common HAB-producing cyanobacteria can form cells that overwinter in sediments [5]. Collectively, the term overwintering cells is used to describe both specialized akinetes of cyanobacteria from the order Nostocales and non-specialized quiescent and vegetative cells of cyanobacteria such as Microcystis and Planktothrix. The main environmental condition that triggers the germination of akinetes is light, while growth of quiescent vegetative cells is triggered by temperature [5,13]. For example, light at relatively low intensities ($\geq$0.5 µmol $m^{-2}s^{-1}$) has been demonstrated to promote the germination of akinetes, and temperatures ranging from 15 °C to 30 °C are likely environmental triggers stimulating the growth of quiescent vegetative cells, particularly of Microcystis. Environmental conditions triggering HAB formation vary both within an individual lake and among waterbodies, as do densities of overwintering cells [5], indicating the need to identify and prioritize candidate sites for proactive treatment. Candidate sites for proactive treatments can be prioritized using a weight-of-evidence approach [14]. One line of evidence is planktonic transfer potential, which is the potential for cells to transfer from the sediment phase to the water column. Incubation experiments, in which field-collected sediments containing known populations of overwintering cells are placed in suitable environmental conditions for germination and growth, have been used to evaluate planktonic transfer potential and evaluate the performance of algaecide treatments [10,11]. Recent publications have demonstrated that proactive algaecide applications may be effective at decreasing the transfer of overwintering cells to the aqueous phase. In the laboratory, efficacy varied depending on the products used, with some products resulting in a log decrease in planktonic cyanobacterial cell density [10]. A recent field study implementing a proactive algaecide application found no measurable difference in sediment cell densities 3 d post-treatment. Yet, during 9 of 11 sampling events from May to October, planktonic cell densities were lower in treated areas relative to control areas [11]. Due to the novel nature of this proactive approach, additional research is needed to bolster the database and address additional risk-based questions.

Multiple lines of evidence are needed to measure responses of overwintering cells to proactive algaecide treatments, and these responses likely capture different temporal scales. Proactive algaecide treatments aim to achieve a maximum algaecide concentration at the sediment–water interface where overwintering cells remain throughout the cooler months, thereby targeting the benthic phase of the cyanobacterial life cycle [11]. As such, short-term (i.e., days to weeks) responses of overwintering cells are anticipated to manifest in terms of declines in cell densities and viability of cells associated with sediments. However, declines in sediment cell densities may not be immediately apparent as slowed degradation rates anticipated during cooler months would delay the degradation of cellular materials needed to visually distinguish fragmented cells from intact cells during microscopic observation [15]. An additional line of evidence would include a decrease in viability measured as declines in planktonic transfer potential. Changes in planktonic transfer potential would also be apparent in the short term following treatment and can be discerned using incubation experiments [10,11]. Cell densities and planktonic transfer potential are likely critical lines of evidence to evaluate proactive algaecide performance.

Measurement of HAB duration and intensity during months when HABs are anticipated to occur provides another line of evidence for evaluating performance. In theory, lower-than-expected HAB duration and intensity would manifest in the weeks to months post-treatment [11]. However, HABs in freshwater systems are inherently challenging to characterize over extended durations, and measurement techniques (e.g., the timing

and location of samples) can influence outcomes [16]. The spatial distribution of a HAB within a water body is influenced by a variety of factors, including prevailing wind and cyanobacteria buoyancy. Cyanobacteria assemblages and densities can shift horizontally across the water surface as well as vertically within the water column, and differences in spatial distribution can occur in the order of hours [16,17]. Therefore, traditional HAB sampling and analysis methods (e.g., discrete grab samples followed by cyanobacterial identification and enumeration) were combined with the use of high temporal resolution in situ water-quality sondes (e.g., phycocyanin and chlorophyll-*a*) to increase the resolution of HAB measurements [18,19]. Additionally, historical data (2019 to 2023) of HAB advisories were evaluated to compare HAB intensity following this proactive treatment in 2023 with previous HABs at Big Eleven Lake.

Lastly, measurement of HAB toxins is critical for the characterization of relative risks of this proactive approach. Concentrations of microcystins (i.e., a group of hepatotoxins produced by many cyanobacterial species) have been reported in sediments containing overwintering cells [20]. Genetic analyses indicate that messenger RNA for microcystin production can also be detected in sediment [21], indicating that at least some overwintering cells have the potential to produce microcystins. There are often concerns that algaecide treatments may lyse the cellular membrane, consequently releasing cell-bound microcystins into the aqueous phase where they may present a greater potential for human exposure under some exposure pathways (i.e., drinking water treatment) [4,22]. Following release into the aqueous phase, the primary degradation pathway for microcystins in natural systems is biological degradation [22–24]. Therefore, if proactive algaecide treatments are applied during cooler water temperatures, when biological degradation rates are diminished by lower temperatures [25], any microcystins released may persist longer than they might during summer. Released microcystin concentrations measured immediately post-treatment can be compared to USEPA guidance values for recreation and drinking water as a metric of risk. Relatively low densities of overwintering cells in sediments and presumably low microcystin concentrations may render this question irrelevant; however, proactive applications of an algaecide in March provided the opportunity to explore these initial hypotheses.

Our overall goals with this field demonstration were twofold: first, to help a community find an affordable, low-risk, environmentally sustainable means to mitigate HABs in an important urban water feature; and second, to bolster our understanding of the use of proactive algaecide treatments targeting overwintering cyanobacteria cells in sediments. Specifically, our objectives were to (1) measure and compare short-term (i.e., days to weeks) responses of overwintering cells in sediment in terms of densities and planktonic growth potential in treated and control areas, (2) measure and compare long-term (i.e., weeks to months) planktonic HABs in the treated and control areas throughout the growing season, and (3) compare microcystin concentrations immediately post-treatment to USEPA guidance values for recreation and drinking water to inform initial risk-based questions related to microcystin release and delayed degradation following proactive algaecide applications.

2. Materials and Methods

2.1. Selection and Description of the Study Site

Big Eleven Lake was selected based on three primary criteria: (1) a known history of HAB development, (2) limited confounding sources of cyanobacteria, and (3) a potential for overwintering cells to contribute to HAB formation. Big Eleven Lake is a 12,705 m^2 water feature located in urban Kansas City, Kansas (Figure 1). The water feature is used for fishing, and the surrounding park is used for musical events and picnicking. Big Eleven Lake was constructed in 1935 with a gravel bottom and tiered concrete retaining walls [26]; it is the only one that remains of the eleven originally built, so it is historically and socially important to the community. Since construction, concrete has been used to harden the bottom and bank to prevent erosion. Most of the lake bottom is now covered with sediment to a depth of approximately 0.6 m. There are limited shallow areas as banks rapidly deepen from 0.3 m to 2.4 m; the greatest depth is approximately 5 m; cattails grow on some margins

(Figure S1). Big Eleven Lake has high nutrient concentrations within the water column and sediment (Tables S1 and S2). The lake is groundwater fed and has an overflow drain that empties into a combined sewer system; thus, there are limited potential inputs of cyanobacterial cells to the system. This site has experienced reoccurring HABs consisting of *Aphanizomenon*, *Planktothrix*, *Raphidiopsis* (formerly *Cylindrospermopsis*), *Microcystis*, and *Woronichinia.* An extensive survey of sediments was conducted on 7 March 2023, prior to treatment at the site, to confirm the presence of overwintering cells and to identify sediment sampling locations with elevated overwintering cells (Figure 2). Heterogeneity (i.e., orders of magnitude) among sediment sampling locations was anticipated [11] and confirmed with densities of overwintering resting cells ranging from $<2.35 \times 10^3$ to 5.17×10^4 akinetes/g wet sediment. *Planktothrix* trichomes were also overwintering in sediments at the time of sampling and ranged from $<2.35 \times 10^3$ to 2.64×10^4 trichomes/g wet sediment. Overwintering cells of other quiescent cyanobacteria were not identified. Based on this initial survey, sediment sampling locations were refined to target areas with comparable high densities of overwintering cells.

One cove of Big Eleven Lake was identified as a control area (Figure 3). One day prior to treatment initiation, the cove was partitioned using a polyvinyl chloride-coated sediment curtain (Type I Curtain from ACME Environmental, Inc.; Tulsa, OK, USA). The curtain was 3.7 m in height and 30 m long, with an integrated floating line on the top edge and weighted line on the bottom. On each end, it was cinched vertically to fit the shoreline contour and prevent billowing, and then secured on both the shoreline and in the water using Duckbill 88-DB1 Earth Anchors [11]. In situ water-quality sondes were installed at the same time, one each within the treatment and control areas (Figure 3). These two sondes [EXO2 Multiparameter Sonde, YSI Incorporated, Yellow Springs, OH, USA] were programmed to measure phycocyanin and chlorophyll-*a* concentrations once every 15 min. The benefits and limitations of using in situ water-quality sondes for monitoring HABs in the field have been reported [27].

2.2. Proactive Algaecide Treatment

Algaecide treatments consisted of two consecutive treatments of a solid sodium carbonate peroxyhydrate algaecide (GreenClean® PRO [BioSafe Systems, East Hartford, CT, USA]), applied 48 h apart on 21 March 2023 and 23 March 2023. Treatments were applied to yield the maximum label rate to the bottom 0.6 m of the water column. Selection of this algaecide and the application rate were based on anticipated efficacies of sodium carbonate peroxyhydrate algaecides for overwintering cyanobacteria in sediments [8,9]. Algaecide granules were broadcast across the water surface using two custom-built granular spreaders located at the bow of a 4.3 m Carolina Skiff fiberglass boat with a 9.9 horsepower outboard motor. The spreaders were calibrated prior to treatment to dispense product at targeted rates in tandem with a defined boat speed and measured swath width, such that 224 kg/ha was applied to the treatment area (112 kg per 0.3 m of depth for the bottom 0.6 m of the water column). Treatment transects were created 3 m apart (i.e., 3 m swath width) using ExpertGPS Pro (v 8.23) and uploaded onto onboard Lowrance® units for the driver to follow. For each treatment, 313.6 kg of algaecide was applied to the 1.4 ha treated area. Water samples were collected from the shoreline, and peroxide concentrations were measured post-treatment using thiosulfate titration (Hach Company, Loveland, CO, USA).

Figure 1. Map identifying Big Eleven Lake within the urban landscape in Kansas City, Kansas, USA.

7 March 2023
- Confirmation of overwintering cells in Big Eleven Lake

20 March 2023
- Curtain installation to separate treated and control areas
- Collection of pre-treatment samples

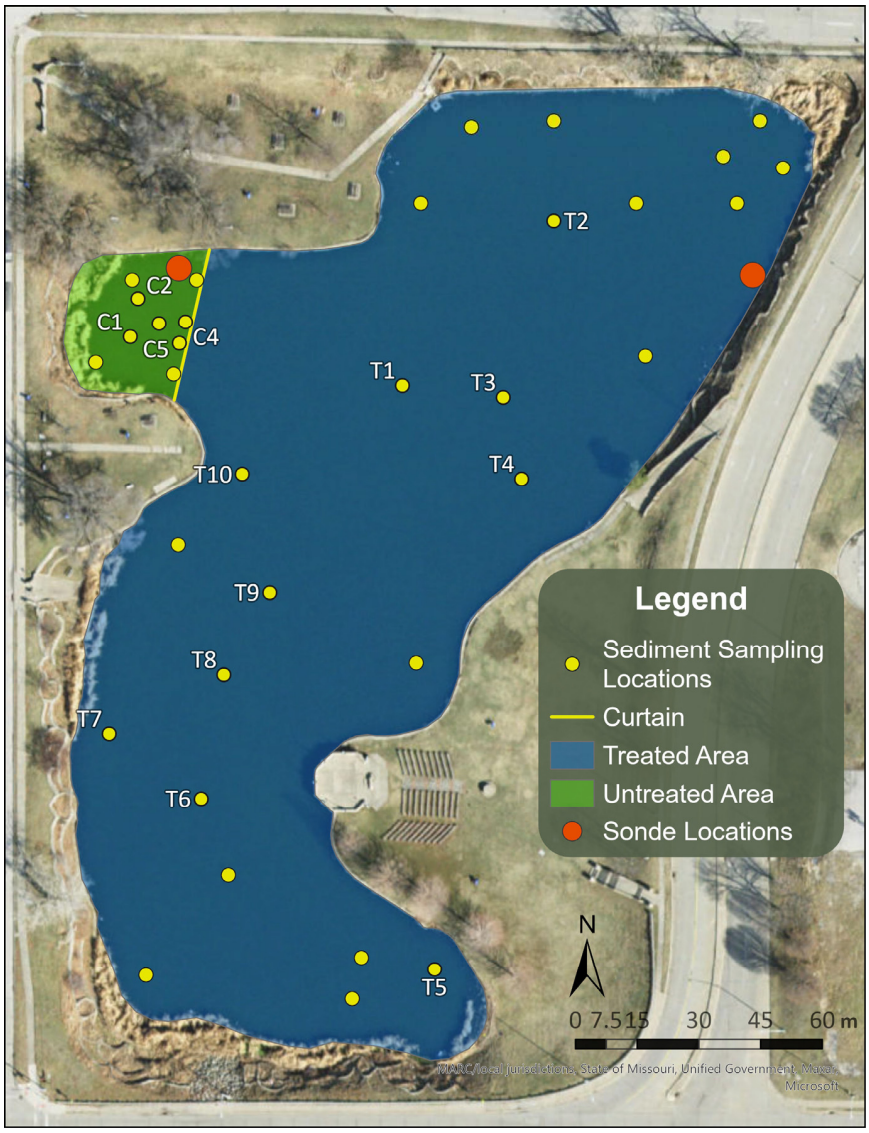
21 March and 23 March 2023
- Two consecutive treatments of algaecide applied
- Microcystin measurements to assess toxin release

30 March 2023
- First set of post-treatment water and sediment samples collected to enumerate and assess viability of overwintering cells

18 April 2023
- Second set of post-treatment water and sediment samples collected to enumerate and assess viability of overwintering cells

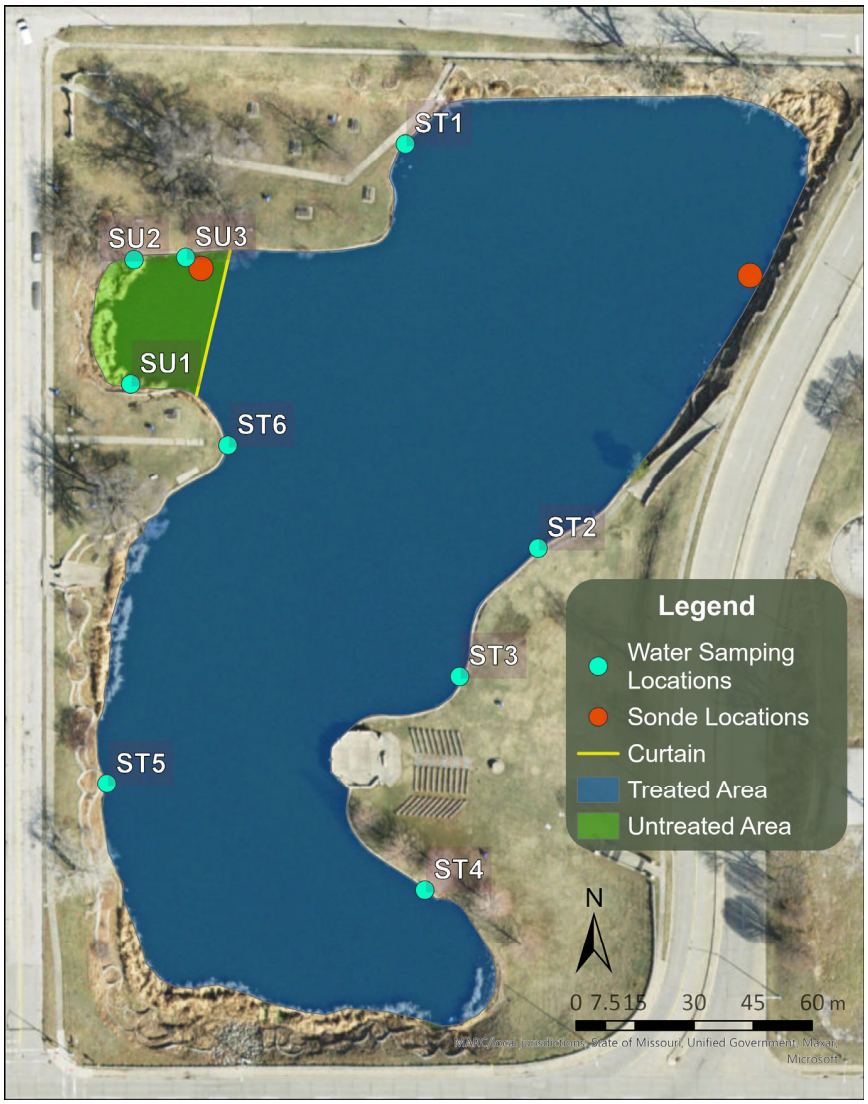
11 April to 6 November 2023
- Long-term monitoring of algaecide performance in planktonic phase

Figure 2. Project timeline.



(**A**)

Figure 3. *Cont.*



(**B**)

Figure 3. Maps showing treated and control areas, sediment (**A**) and water (**B**) sampling locations, and locations of water-quality sondes.

2.3. Short-Term Responses of Overwintering Cells in Sediments

Overwintering cyanobacteria in sediment and water samples were collected 1 d prior to treatment (20 March 2023), as well as 1-week (30 March 2023) and 4-weeks (18 April 2023) post-treatment to discern the responses of overwintering cells to the algaecide. In-lake water samples and sediment samples were collected from an inflatable boat (Sea Eagle 12S; Sea Eagle Boats, Inc., Port Jefferson, NY, USA) equipped with a trolling motor and depth finder (Garmin STRIKER Plus 4 Fishfinder; Garmin, Olathe, KS, USA). Water samples were collected using a Van Dorn sampler from 0.2 m below the surface. Sediment samples were collected with a Petite Ponar Dredge (Wildco, Saginaw, MI, USA), drained, and placed into a stainless steel tray, where sediments (approximately 200 mL) from the top 2 cm were carefully collected using a clean silicone spatula [9]. Equipment was rinsed between uses. All samples were shipped overnight to the U.S. Army Engineer Research and Development Center (ERDC) where they were stored at 4 °C in darkness until analysis.

To quantify overwintering cell densities in sediments, 1 g of wet sediment from each sample was homogenized and placed in a 50 mL conical centrifuge tube with 20 mL to 40 mL of site water (40 mL was used for fine-grained sediments) [10]. Site water added to the tubes was initially filtered through a 0.45 µm pore size nitrocellulose filter paper

to remove cells that would confound analyses. Sediment and site water were rapidly inverted to homogenize cells, and subsamples were immediately transferred to a Palmer–Maloney counting chamber. Overwintering cells were quantified by observing 100 fields of view at 400× magnification with a Motic Panthera C2 (San Antonio, TX, USA) compound microscope. *Microcystis* and *Planktothrix* were present as cells in colonies and trichomes, respectively. For these cyanobacteria, cell numbers were estimated by measuring colony surface areas or trichome lengths using a Gryphax Arktur microscope camera (Jenoptik, Huntsville, AL, USA) and associated firmware, and then were converted to a number of cells using representative examples (i.e., cells/μm^2 for *Microcystis* or cells/μm for *Planktothrix*) from each genus.

Incubation studies were conducted to evaluate the planktonic transfer potential of overwintering cells within treated and control areas. A mass of 10 g of wet sediment and 150 mL of filtered (0.45 μm pore size) site water were placed in 250 mL borosilicate beakers. To simulate suitable growth conditions [14], beakers were placed in a reach-in Darwin Chamber® (St. Louis, MO, USA, KB034 Environmental Chamber) at 25 °C (±0.1 °C) for 14 d. Beakers were covered with a clear polyethylene film during this time to decrease evaporative loss. Targeted nominal light intensities at the mouth of the beaker were 2500 LUX (±600 LUX; approximately 36 μmol $m^{-2}s^{-1}$). After 14 d, cell densities were enumerated in the overlying water. A Palmer–Maloney counting chamber was similarly used to quantify planktonic cell densities of cyanobacteria only. Cyanobacteria cells were enumerated and identified at 100× and 400× magnification, respectively. For low-density samples (<10^5 cells/mL), a minimum of 1/3 of the counting chambers was observed. For high-density samples (≥10^5 cells/mL), a minimum of 21 trichomes or 3607 cells were enumerated.

2.4. Long-Term Performance Monitoring of Planktonic HAB Intensity and Duration

Starting 19 d after treatment (11 April 2023), shoreline grab samples were collected approximately weekly to quantify HAB intensity and duration. Sample events were timed for 11:00 to 16:00 when cyanobacterial cells would be near the surface. At each event, a pole sampler was used to collect water about 1.5 m from the shoreline at nine fixed sites (Figure 3): six within the larger treated area and three within the control area. One aliquot was immediately poured into a 50 mL conical tube and placed in a cooler on ice for overnight shipment to the U.S. Army Engineer Research and Development Center for identification and enumeration of cyanobacteria, while two aliquots were decanted into sample containers to be sent to Kansas Department of Health and Environment laboratories for analysis of microcystins and chlorophyll-*a* concentrations. Samples were maintained at 4 °C until analysis within 3 d post-sampling. Cyanobacteria cell densities were enumerated using a Palmer–Maloney counting chamber as previously described. Data from in situ sondes were also used to augment and interpret data from water samples.

2.5. Microcystin Release and Delayed Degradation

Water samples were collected to inform the potential release of microcystins post-treatment. To assess microcystin release immediately post-treatment [24,28], water samples were collected from the shoreline with a pole sampler. Samples were collected immediately pre-treatment on 21 March 2023 and 0.25 h, 4 h, and 24 h after the first treatment. Additional samples were collected following the second treatment on 23 March 2023, at 1 h, 3 h, and 6 h post-treatment. Two subsamples were taken from each sampling container: one for aqueous microcystins and one for total microcystins. For aqueous microcystins, approximately 15 mL was filtered through a 0.45 μm pore size nitrocellulose filter membrane, and the filtrate was used for analysis. An additional 15 mL of whole water was used for total microcystins. For analysis of total microcystins, microcystins were extracted from cells by adding 1 mL of sample to a Lysing Matrix B tube (MP Biomedicals, CAT#6911-500, Santa Ana, CA, USA). Samples were then homogenized on a Fast Prep homogenizer (MP Biomedicals, Santa Ana, CA, USA) at 4.0 m/s for 1 min, and this was repeated two additional times with samples placed on ice for 1 min between homogenizations. After homogenization, samples were

centrifuged at maximum speed and the resulting supernatant was retained. Total and aqueous microcystins were determined using an ADDA enzyme-linked immunosorbent assay (ELISA) (CAT#520011, Gold Standard Diagnostics, Budapest, Hungary, USEPA Method 546 [29]). The ELISA detection limit was 0.10 µg/L.

To assess the relative risks of proactive treatments in terms of the potential for microcystin release, concentrations of microcystins determined in the aqueous phase immediately post-treatment were compared to USEPA recreational and drinking water guidance values. Contact recreation (e.g., swimming, wading, and boating) is not a primary use of Big Eleven Lake, and this urban water feature is not a potable water source; however, these guidance values provide important risk-based thresholds.

2.6. Statistical Analyses

Based on preliminary observations, sampling, and analysis at Big Eleven Lake, we anticipated a sediment sample location effect. For example, some sediment locations tended to have higher overwintering cell densities and resulted in greater planktonic cell densities after incubation (unpublished data). To control for variability among sediment locations, short-term responses of overwintering cells in sediments were assessed using a repeated-measures one-way ANOVA. However, sample location effects were not significant ($p > 0.05$), and data were subsequently assessed as independent samples. A Shapiro–Wilk test was performed to test for normal distribution of the data, a Brown–Forsythe test was performed to determine homogeneity of variance, and normal distribution of residuals was assessed with Q–Q plots. Only one dataset met the assumption of normal distribution of residuals. Excluding the one exception, Kruskal–Wallis tests were performed with Dunn's post-hoc tests to determine if significant differences existed pre-treatment relative to post-treatment at 1 and 4 weeks. The dataset with normally distributed residuals was that of chlorophyll-*a* determined after a 14 d incubation in sediments within the control area; however, the assumption of homogeneity of variance was not met ($p = 0.022$). Therefore, a Welch test was used with Dunnett T3 post-hoc tests as previously described. All statistical analyses were performed using GraphPad Prism Software version 10.2.0 (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

3.1. Algaecide Treatments

The sediment curtain was effective at excluding algaecide from the control area, as peroxide concentrations were below the detection limit (0.2 mg/L as H_2O_2) in this zone at all sampling intervals from 0.25 h to 24 h after both treatments. Maximum peroxide concentrations of 1.9 mg/L as H_2O_2 and 1.4 mg/L as H_2O_2 for the first and second treatments, respectively, were measured within the water column of the treatment area 15 min after each treatment. Peroxide concentrations within the water column then declined to below the detection limit within 24 h. These data indicate that there was a partial loss of algaecide activity to the water column before the solid product settled to the sediments where overwintering cyanobacteria were located. Field treatments at other locations that used solid sodium carbonate peroxyhydrate algaecides to target algae and cyanobacteria in the benthic phase have also detected peroxide concentrations less than target concentrations in the water column likely due to solid product dissolution and settling rates as well as rapid peroxide reaction rates [30]. Within the US, no algaecide product exists specifically for benthic treatments. To target benthic cyanobacteria, algaecide applicators use techniques such as injecting slurries via weighted drop hoses or utilizing granular spreaders to create a homogenous broadcast of the algaecide granules. However, algaecide products could be altered to decrease activity loss to the water column and increase settling rates while maintaining homogenous coverage of exposure to benthic cyanobacteria.

3.2. Short-term Overwintering Cell Responses in Sediment

Overwintering cell densities before treatment were comparable between control and treated areas. The mean overwintering cell density, consisting of both akinetes and quies-

cent vegetative cells, was 3.43×10^5 (SD = 3.2×10^5) cells/g wet sediment in the control area (n = 5) and was 4.11×10^5 (SD = 8.06×10^5) cells/g wet sediment in the treated area (n = 10). Despite initial efforts to identify sediment sampling locations with similar overwintering cell densities, heterogeneity among samples remained high in both the control area and particularly the treated area. For example, within the treated area, the minimum overwintering cell density was less than the detection limit (2.00×10^3 cells/g wet sediment), and the maximum overwintering cell density was 2.66×10^6 cells/g wet sediment. The heterogeneity of overwintering cell densities in sediments highlights the innate difficulties in assessing statistical differences due to treatments and the need for additional lines of evidence. As such, significant differences were not detected in overwintering cell densities in control and treated areas at 1- and 4-weeks post-treatment when compared to pre-treatment densities (Figure 4).

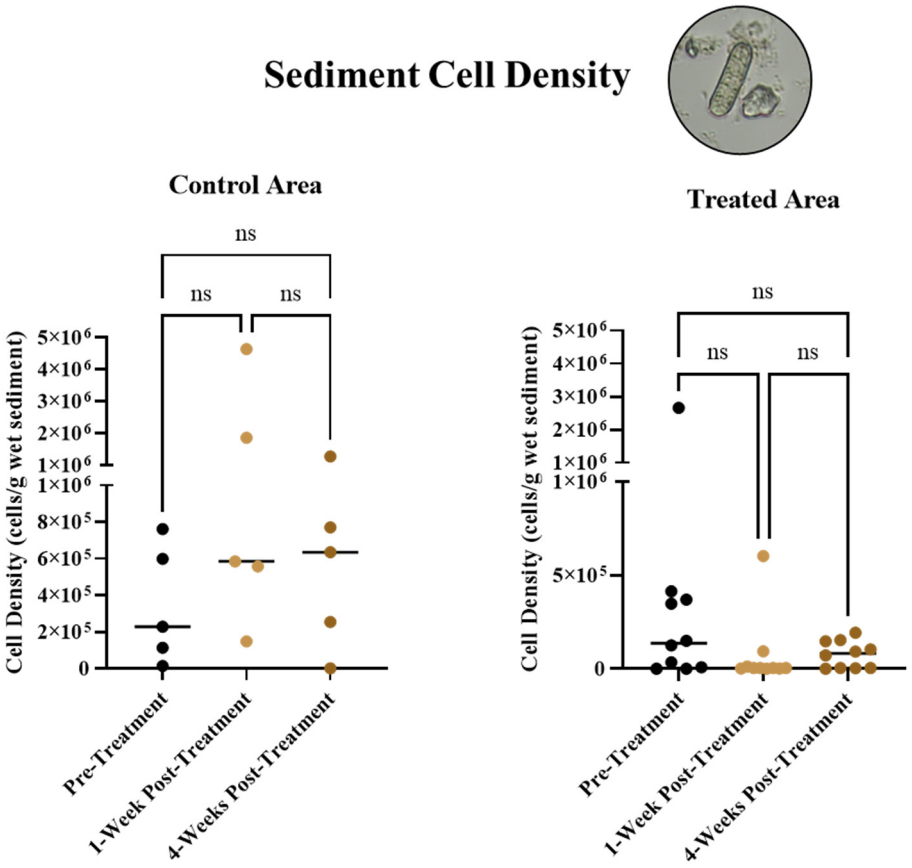


Figure 4. Overwintering cell densities in sediments measured pre-treatment as well as 1- and 4-weeks post-treatment.

Sediments collected pre- and post-treatment were incubated for 14 d with environmental conditions suitable for the germination and growth of overwintering cyanobacteria [5,10,11]. After a 14 d incubation, cyanobacteria cell densities and chlorophyll-*a* concentrations were measured in the overlying water (e.g., planktonic transfer potential). From untreated control sediments, planktonic cyanobacteria cell densities and chlorophyll-*a* concentrations significantly increased between the pre-treatment and 1-week post-treatment sampling periods after incubation in the laboratory (Figure 5). Planktonic cell densities of cyanobacteria did not increase significantly from incubated sediment collected pre-treatment and 1-week post-treatment from the treated area. These data suggest that the number of cyanobacteria capable of transferring to the water column were lower 1-week post-treatment within the treated area as compared to the control area. Significant differences in terms of planktonic cell densities of cyanobacteria or chlorophyll-*a* concentrations were not detected

between pre-treatment and 4-week post-treatment sampling periods in either control or treated areas. Impacts from treatment were not detectable in sediment samples collected 4-weeks post-treatment. Chlorophyll-*a* is a pigment contained in cyanobacteria, green algae, and diatoms, and is therefore not specific to cyanobacteria alone. Chlorophyll-*a* concentrations significantly increased in the overlying water following a 14 d incubation of sediments collected pre-treatment and 1-week post-treatment within both control and treated areas. Significant differences were not detected in terms of chlorophyll-*a* concentrations in overlying water between pre-treatment and 4-week post-treatment sampling intervals within both control and treated areas.

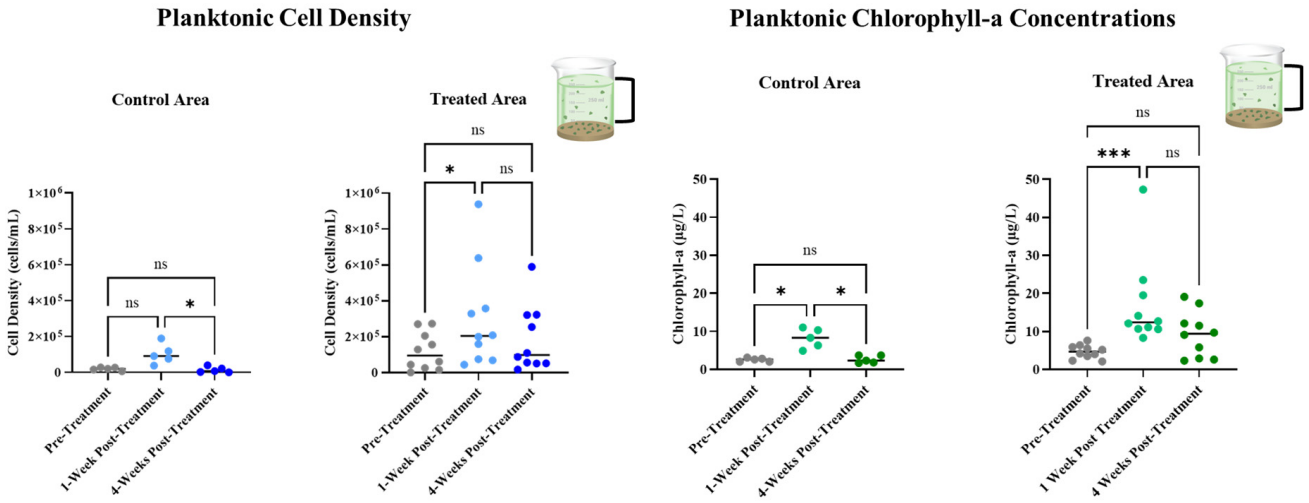


Figure 5. Cell densities and chlorophyll-*a* concentrations measured in overlying water after a 14 d incubation of overwintering cells in sediments collected pre-treatment as well as 1- and 4-weeks post-treatment. * indicates *p*-values $\leq$ 0.05, but >0.01 and *** indicates *p*-values $\leq$ 0.001.

3.3. Long-Term Monitoring of Planktonic HAB Duration and Intensity

HAB duration and intensity were monitored following proactive treatments and during months when HABs were anticipated to occur in Big Eleven Lake (Figures 6 and 7, and Figures S2–S6; Tables S3 and S4). Cyanobacteria densities remained relatively low (<2 $\times$ 10^5 cells/mL) within both control and treated areas from 11 April 2023 to 12 June 2023 without statistical differences between treated and control areas (Figure 6). The cyanobacterial assemblage was dominated by the following genera in series from spring to summer: *Planktothrix*, a mixed assemblage of *Aphanizomenon* and *Planktothrix*, *Microcystis*, and a mixed assemblage of *Planktothrix* and *Raphidiopsis*. Brief ($\leq$1 week) accumulations of cyanobacterial colonies were observed visually in May and June in the treated and control areas and were below public health advisory thresholds during the following sampling event, 1 week later. Seven days post-treatment, chlorophyll-*a* concentrations declined from 74 µg/L to 17 µg/L and did not increase until June in both treated and control areas (Figure S6). Concentrations of total microcystins were below the analytical detection limit of 0.15 µg/L from March to May 2023 (Figure S5). Microcystins peaked at 10.5 µg/L in the control area (n = 2) and 11.5 µg/L in the treated area (n = 2) on 29 June 2023, which corresponded to an increase in cell densities of a mixed assemblage of *Planktothrix* and *Raphidiopsis*. Concentrations of microcystins remained low (<1 µg/L) from 19 July to 23 August 2023. In situ sonde data provided high temporal resolution of algal pigment concentrations (chlorophyll-*a* and phycocyanin) during the post-treatment period (Figure 7 and Figures S2–S4; Tables S3 and S4). Concentrations of phycocyanin trended lower at the sonde located within the treated area relative to the control area.

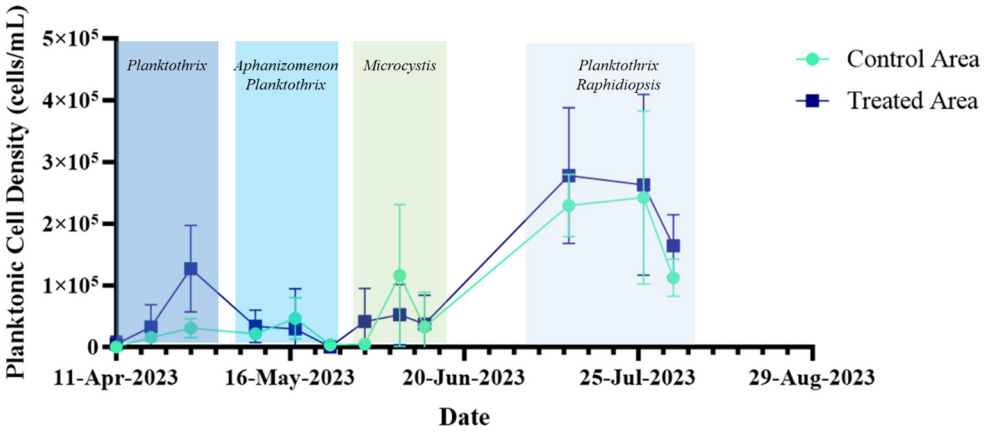


Figure 6. Planktonic cyanobacteria densities post-treatment from 11 April 2023 to 1 August 2023 within control and treated areas.

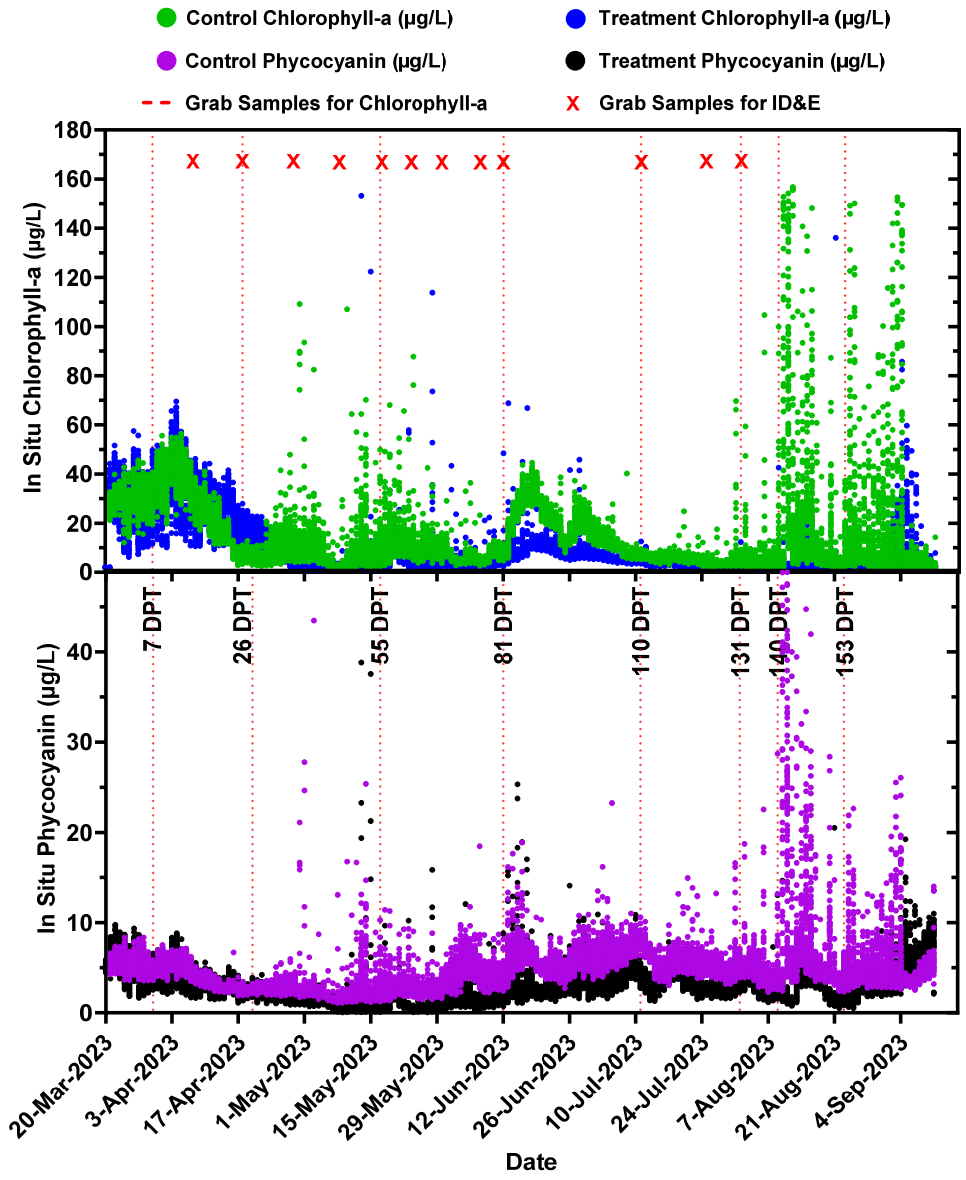


Figure 7. Chlorophyll-*a* and phycocyanin concentrations post-treatment from March to September 2023 from two in situ sondes (n = 1 treated area; n = 1 control area). Identification and enumeration (ID&E) corresponding to grab samples analyzed and presented in Figure 7. Days post-treatment (DPT).

Historical data from KDHE's HAB Response Program, which investigates and informs the public of HAB events in state waters, is an important metric of HAB onset and duration and provides the capacity to make comparisons between years. KDHE's HAB monitoring season is concurrent with the state's recognized water recreation season, 1 April to 31 October. Normally, KDHE investigated only when complaints were received by park staff or the public (interval between site visits > weekly), and advisories are based on cyanobacterial cell density, microcystin concentration, or both. However, Big Eleven Lake was monitored weekly in 2023, which represents higher vigilance and, therefore, higher visibility of possible bloom events. Advisories issued in 2023 were compared with those of prior years, 2019, 2021, and 2022 (2020 sampling was limited due to COVID-19 emergency operations). Following proactive algaecide treatments in 2023, active advisories were delayed relative to prior years, occurring in September rather than in May (as in 2022) or June (as in 2019 and 2021). Public health advisories were active for 6 of 31 weeks of the water recreation season in 2023, compared to 4 weeks in 2022, 20 weeks in 2021, and 18 weeks in 2019 (Figure 8). Notably, an active advisory did not occur during the peak water recreation season, Memorial Day (29 May) to Labor Day (4 September) 2023. Proactive algaecide treatments may have delayed cyanobacterial proliferation until later in the water recreation season. Further, 2023 was the fourth driest year in Kansas since 1901 [31]. Droughts are often named as environmental conditions that make harmful algal blooms more severe, likely due to higher water temperatures [32,33]. This suggests that without proactive treatment, more severe harmful algal blooms might have been anticipated.

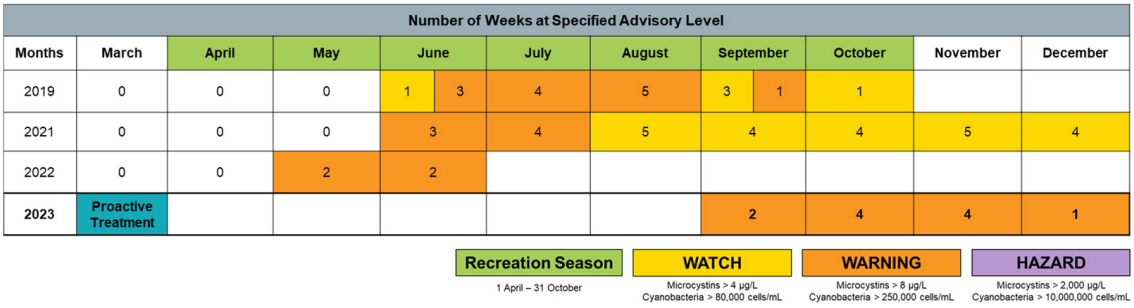


Figure 8. Kansas Department of Health and Environment Harmful Algal Bloom Response Program advisory levels from 2019 to 2023. Limited sampling was conducted during 2020 due to COVID-19 emergency operations. Table reflects advisories based on quantitative results for cyanobacteria cell densities or microcystin concentrations. Advisories based on visual determination are omitted.

3.4. Weight-of-Evidence—Performance of Proactive Algaecide Application

Metrics used to evaluate proactive algaecide treatment performance resulted in mixed outcomes. Weight-of-evidence approaches can be used to organize separate lines of evidence in an uncomplicated and coherent manner for the interpretation and presentation of data [14,34].

The five separate lines of evidence presented in this assessment were as follows:

1. Planktonic cyanobacteria densities, chlorophyll-*a*, and microcystin concentrations from shoreline grab samples collected throughout the active HAB season;
2. Historical active advisory data;
3. Planktonic algal pigment (phycocyanin and chlorophyll-*a*) concentrations from in situ sondes;
4. Planktonic transfer potential results of the 14 d incubation of site-collected sediments containing overwintering cells post-treatment;
5. Overwintering cell densities in sediments.

Three of five lines of evidence support effective proactive treatment performance at Big Eleven Lake. These were historical active advisory data, planktonic algal pigment concentrations from in situ sondes, and planktonic transfer potential. Two of five lines of

evidence suggest that proactive treatment performance had no impact on HAB severity within Big Eleven Lake (Table 1).

Table 1. Weight-of-evidence approach used to evaluate proactive algaecide treatment performance in Big Eleven Lake. Open circles indicate no impact and pluses indicate positive performance of proactive algaecide treatments.

Lines of Evidence	Outcome
Planktonic cyanobacteria densities, chlorophyll-*a* concentrations, and microcystin concentrations from grab samples collected throughout the active HAB season	○
Historical active advisory data	⊕
Planktonic algal pigment concentrations from sondes collected throughout the active HAB season	⊕
Planktonic cell densities following 14 d incubation of sediments collected immediately post-treatment	⊕
Overwintering cell densities in sediments collected immediately post-treatment	○

3.5. Microcystin Release and Delayed Degradation

Following proactive treatments, microcystin concentrations remained at the analytical detection limit (0.10 µg/L) of the ELISA. Aqueous microcystin concentrations ranged from below the detection limit to 0.11 µg/L, and total microcystin concentrations ranged from below the detection limit to 0.15 µg/L (Figure 9). Microcystin concentrations were approximately 2 to 3 times lower than the USEPA drinking water health advisory level of 0.3 µg/L for bottle-fed infants and pre-school-aged children [35], and approximately 11 to 15 times lower than the USEPA drinking water health advisory level of 1.6 µg/L for individuals over the age of 6 years old. Further, microcystin concentrations were approximately 50 to 70 times lower than the USEPA recreational value of 8 µg/L [36]. For this site and treatment conditions, there was no evidence to suggest that microcystins and their release would be an increased risk from early season treatments due to low microcystin concentrations within the system at the time of proactive treatments. This highlights the importance of implementing proactive management action before microcystin-producing cyanobacteria have the time to increase density and production.

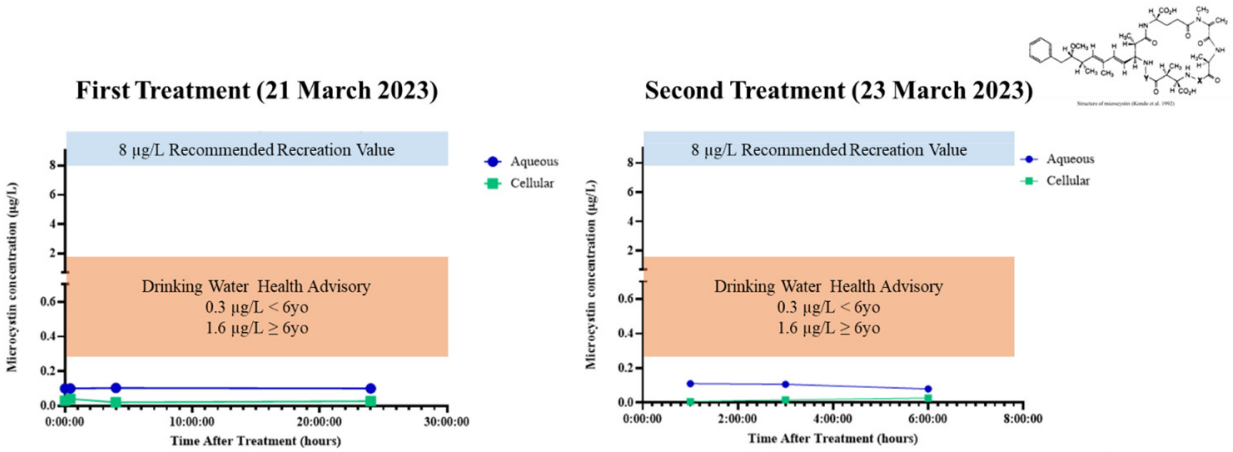


Figure 9. Aqueous and cell-bound concentrations of microcystins measured immediately post-treatment on 21 and 23 March 2023 compared to USEPA human health risk thresholds.

4. Conclusions

A demonstration of the novel use of a peroxide algaecide to target overwintering cyanobacteria in sediments was performed in an urban water feature located in Kansas City, Kansas. Three of five lines of evidence support a decrease in HAB severity during months that HABs were anticipated to occur. Notably, following proactive algaecide treatments in 2023, active advisories were delayed relative to prior years (2019, 2021, and 2022), occurring in the third week of September after the peak recreation period, Memorial Day (29 May 2023) to Labor Day (4 September 2023). Immediately following proactive treatments, microcystin concentrations remained close to the analytical detection limit of 0.10 µg/L and were lower than USEPA human health risk thresholds. Microcystin data collected within hours of proactive treatments applied in March highlight the importance of managing HABs prior to exponential cyanobacterial growth to decrease potential microcystin risks. Ultimately, evidence of effective proactive algaecide treatment performance was mixed. However, these results expand the dataset and methodology for future field-scale proactive algaecide applications targeting overwintering cyanobacteria in sediments to mitigate and delay HAB development.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/w16111624/s1: Figure S1: Bathymetric map of Big Eleven Lake, Kansas City, Kansas, USA; Table S1: Surface water nutrient concentrations in Big Eleven Lake; Table S2: Average, minimum, and maximum sediment nutrient concentrations in Big Eleven Lake; Figure S2: Mean in situ algal pigment and turbidity in control and treatment zones of Big Eleven Lake averaged in 2-week intervals; Figure S3: Violin plots of in situ chlorophyll-*a* in control and treatment zones of Big Eleven Lake as totals and monthly; Figure S4: Violin plots of in situ phycocyanin in control and treatment zones of Big Eleven Lake as totals and monthly; Figure S5: Microcystin concentrations measured in the pre-treatment, control, and treatment zones of Big Eleven from March to August 2023; Figure S6: Chlorophyll-*a* concentrations measured in the pre-treatment, control, and treatment zones of Big Eleven from March to October 2023; Figure S7: Mean in situ temperature in control and treatment zones of Big Eleven Lake; Table S3: In situ chlorophyll-*a* concentrations in Big Eleven Lake; Table S4: In situ phycocyanin concentrations in Big Eleven Lake; Table S5: In situ conductivity in Big Eleven Lake. Table S6: In situ dissolved oxygen concentrations in Big Eleven Lake; Table S7: In situ turbidity in Big Eleven Lake; Table S8: In situ temperature in Big Eleven Lake.

Author Contributions: Conceptualization, A.C.-E., A.M., C.K.-B., E.S., M.B. and G.C.J.; methodology, all authors; formal analysis, A.C.-E. and A.M.; resources, E.S., B.G., K.D., S.H., A.S. and M.B.; Writing—original draft preparation, A.C.-E., A.M., C.K.-B. and E.S.; writing—review and editing, all authors; funding acquisition, A.M. and A.S.; All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the United States Army Corps of Engineers (USACE) Aquatic Nuisance Species Research Program (ANSRP), funding account code U4384107 and AMSCO code 008284. This research was also supported by the Kansas State Water Plan Fund for HAB Mitigation.

Data Availability Statement: Data are available from the authors by request.

Acknowledgments: The Unified Government of Wyandotte County and Kansas City, Kansas for permission to work at Big Eleven Lake. The Unified Government Parks Department for assistance with curtain installation and the Unified Government Public Works for background data and support. The Kansas Department of Health and Environment's Bureau of Water Director, Tom Stiles, for project coordination and technical assistance, and the agency staff who assisted with field work: Shawn Weber, Jack Lapin, Britini Jacobs, Caitlin Schoeck, and Sasha Bornstein. Dorcas Thiam and Haley Martin from Kansas Health and Environment Laboratories who performed toxin analyses. Nate Long and Adam Charlton from Aquatic Control, Inc. for applying the algaecide during field treatments of Big Eleven Lake. Maps were created by Aleksandra Ostojic from the U.S. Army Engineer Research and Development Center. Analysis of microcystins immediately following algaecide treatments were performed by Brianna Fernando from the U.S. Army Engineer Research and Development Center.

Conflicts of Interest: The authors declare no conflicts of interest.

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