Image caption: Algal bloom in a fisheries farm pond.

Mitigating Cyanobacterial Blooms and Cyanotoxins in Hypereutrophic Ponds Following the Application of a Granular Hydrogen Peroxide-Based Algaecide

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Abstract: To control cyanobacterial blooms and their toxins, the efficacy of a newly developed granular compound (sodium carbonate peroxyhydrate 'SCP', trade name 'PAK® 27') containing hydrogen peroxide (H_2O_2) as the active ingredient was investigated. First, the dose efficacy of the SCP that corresponded to 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 8.0 mg/L H_2O_2 was tested for 10 days in small-scale tanks installed in 0.1-acre experimental hypereutrophic ponds dominated by blooms of the toxic cyanobacterium *Planktothrix sp.* SCP ranging from 2.5- 4.0 mg/L H_2O_2 selectively killed *Planktothrix sp.* without major impacts on either eukaryotic phytoplankton (e.g., diatom *Synedra sp.*, green algae *Spirogyra sp.* and *Cladophora sp.*) or zooplankton (e.g., rotifers *Brachionus sp.* and cladocerans *Daphnia sp.*). Based on these results, SCP at 2.5 mg/L and 4.0 mg/L H_2O_2 were homogeneously introduced into entire water volume of the experimental ponds in parallel with untreated control ponds. Temporal analysis indicated that *Planktothrix sp.* blooms collapsed remarkably in both 2.5 mg/L and 4.0 mg/L H_2O_2 treatments. Both treatments also were accompanied by an overall reduction in the total microcystin concentration. At 2.5 mg/L H_2O_2, the growth of eukaryotic phytoplankton (*Synedra* and *Cladophora sp.*) increased, but these populations along with zooplankton (*Brachionus* and *Daphnia sp.*) were suppressed at 4.0 mg/L H_2O_2. The longevity of 2.5 and 4.0 mg/L H_2O_2 treatment effects were up to 5 weeks. In addition, the added granular algaecide degraded within a few days, thereby leaving no long-term traces of H_2O_2 in the environment.

Key Points:

- Cyanobacterial blooms and their toxins are potential threat to aquatic animals.
- Granular H_2O_2 based sodium carbonate peroxyhydrate (SCP) compound was investigated.
- SCP at 2.5 and 4.0 mg/L H_2O_2 effectively suppressed cyanobacterial bloom and toxin.
- SCP left no footprint of H_2O_2 in water; hence, SCP is an eco-friendly compound.

Introduction

Cyanobacterial blooms have been increasingly reported and are progressively becoming a major water quality issue in pond, lakes, and river ecosystems throughout Arkansas, thus impacting their fisheries resources. There are several strategies suggested to remove cyanobacterial blooms. Reducing nutrient loads (typically phosphorus) to prevent eutrophication is probably the best strategy (Conley et al., 2009; Matthijs et al., 2012; Smith and Schindler, 2009), though it often requires several years for the effect to be realized. Dredging of nutrient-rich sediments from pond bottoms followed by a phosphorus-binding clay treatment is the simplest remedial approach to eliminate phosphorus loads. However, these practices are associated with high operating costs, slow action, and the outcomes are not always predictable or effective (Robb et al., 2003; Van Oosterhout and Lurling, 2011). Additional strategies such as artificial pond mixing also may restrain cyanobacterial populations (Huisman et al., 2004; Visser et al., 1996), but is economically infeasible in most cases. Chemical alternatives including herbicides (e.g., diuron), copper-based compounds (e.g., copper sulfate), and alum have been used for many decades. However, there are concerns with lengthy environmental persistence and risks of ecotoxicity to other non-target aquatic biota, including green algae, zooplankton, and fishes (Jancula and Marsalek, 2011). High-frequency sonication is a newer method of selectively bursting gas vesicles and vacuoles in cyanobacteria, which disrupts cell membranes and retards photosynthetic activity (Rajasekhar et al., 2012). Although this technique kills the cyanobacterial blooms by lysing their cells, it has no effect on the toxins. Consequently, following mass cell ruptures, large amounts of cyanotoxins are released into surrounding waters, which often deteriorates rather than resolves the water-quality issues.

In light of the well-documented problems associated with cyanobacterial blooms and their toxins, there is a corresponding need for an environmentally-benign treatment that rapidly restrains the cyanobacterial populations while also destroying their toxins. Recently, hydrogen peroxide (H_2O_2) has been proven useful in selectively reducing cyanobacteria in mixed phytoplankton communities (Barrington et al., 2013; Bauza et al., 2014; Drabkova et al., 2007; Matthijs et al., 2012; Wang et al., 2012). The algaecidal action of H_2O_2 occurs via the formation of free hydroxyl radicals (OH^-) in the solution, which in turn, inhibit electron transport and photosynthetic activity by rendering photosystem II inactive, and thus causing cellular death. Nevertheless, adding large volumes of pure H_2O_2 solution directly into water bodies poses safety concerns, and also is likely to spill during broadcasting, transportation, and storage. An attractive alternative to traditional H_2O_2 solution is sodium carbonate peroxyhydrate (SCP), which is a relatively new, dry granulated H_2O_2-based algaecide (USEPA, 2004). When added to water, SCP decomposes rapidly and liberates H_2O_2 and sodium carbonate.

In the present study, our primary goal was to examine the use of this granulated H_2O_2-based algaecide (SCP) for treating cyanobacterial blooms in ponds. We hypothesized that adding SCP to hypereutrophic experimental ponds would selectively suppress cyanobacterial overgrowth and destroy the associated toxins. We also proposed that SCP added to ponds would degrade within a few days, and that no long-term traces of H_2O_2 would remain. Findings of this study will provide insights into the current knowledge base of effective, rapid, and safe technologies to successfully control cyanobacterial blooms in Arkansas water resources and beyond.

Methods

Experimental Site and Algal Bloom Culture

Experimental trials using the granular SCP-based algaecide were performed in a series of ponds located at the Aquaculture Research Station on the campus of the University of Arkansas at Pine Bluff (UAPB). The experiments were performed at two different scales: small-scale trials done in outdoor tanks and full-scale trials conducted in experimental ponds. A total of six experimental ponds (0.1-acre each with average depth of 1.2 m) were filled with shallow well water, and fertilized with an inorganic fertilizer and commercially available de-oiled rice bran to stimulate phytoplankton growth. In early July 2017, water from a nearby hypereutrophic pond (i.e., 'seed stock') was used to inoculate each of the six experimental ponds. Nutrients (inorganic fertilizer and de-oiled rice bran) were added, as needed, throughout the culture phase until hypereutrophic, cyanobacteria-dominated conditions were obtained. Average values and range of the various physico-chemical parameters measured in experimental ponds prior to the SCP treatments are provided in Table 1.

Preparation of SCP Dilutions

The SCP-based algaecide used in this study is marketed as SePRO 'PAK® 27' (active ingredient ~ 27% H_2O_2; USEPA Registration number, 67690-76, SePRO Corporation, Carmel, IN, U.S.A.). The physical properties and characteristics of PAK® 27 are outlined in Table 2.

Small-Scale Outdoor Tank Experiment

Small-scale tank experiments were performed first to screen for the most appropriate dose of SCP (quantified as H_2O_2 concentrations) for the full-scale pond application. Three circular 75-L tanks were installed in each of the six hypereutrophic algal bloom ponds in early August 2017. Each tank was filled with water (up to 65 L) from the respective algal bloom ponds. SCP (as PAK® 27) at 5.56,

Table 1. Mean values ± S.E of the physico-chemical and biological parameters of control and the treatment ponds prior to the SCP (PAK® 27) application.

	Control	SCP (2.5 mg/L H_2O_2)	SCP (4.0 mg/L H_2O_2)
Water temperature (°C)	24.4 ± 0.6	25.8 ± 0.5	24.2 ± 0.4
Transparency (cm)	19.92 ± 1.12	20.94 ± 0.94	18.86 ± 1.24
pH	8.62 ± 0.20	8.48 ± 0.11	8.82 ± 0.14
Dissolved oxygen (mg/L)	2.84 ± 0.34	2.76 ± 0.29	3.04 ± 0.26
Total hardness (mg/L as $CaCO_3$)	187 ± 12	182 ± 13	196 ± 17
Total alkalinity (mg/L as $CaCO_3$)	119 ± 9	102 ± 12	121 ± 10
Conductivity (µS/cm)	385 ± 18	371 ± 10	405 ± 21
Ammonia – N (mg/L)	0.92 ± 0.08	0.96 ± 0.12	0.89 ± 0.14
Nitrite – N (µg/L)	35.0 ± 4.2	41.0 ± 3.8	39.0 ± 4.2
Nitrate – N (mg/L)	0.37 ± 0.03	0.43 ± 0.03	0.39 ± 0.03
Total Nitrogen (TN, mg/L)	8.06 ± 0.34	7.96 ± 0.29	7.79 ± 0.31
Total Phosphorus (TP, mg/L)	1.71 ± 0.09	1.76 ± 0.10	1.72 ± 0.14
TN:TP	4.71 ± 0.17	4.52 ± 0.19	4.53 ± 0.14
Chlorophyll a (µg/L)	1002 ± 84	989 ± 72	1112 ± 81
Planktothrix sp. (10^6 cells per mL)	1.09 ± 0.10	1.11 ± 0.12	1.08 ± 0.09

Table 2. Physical and chemical properties of PAK® 27 (Source: Pak® 27 Technical Data Sheet).

Ingredient	Property
Sodium Carbonate Peroxyhydrate (active ingredient)	> = 85.0 %
Carbonic acid sodium salt	< =13.0 %
Sodium silicate SiO_2/ Na_2O	< =1.5 %
EPA Registration no.	68660-9-67690
CAS No.	15630-89-4
Physical state	Free flowing white granules
Mean Particle Size	350 – 650 (µm)
Alkalinity (%Na_2CO_3)	67
Solubility	150 g/L
pH	10.4-10.6 (10.1 g/L)
Bulk density	900-1200 kg/m^3

7.41, 9.26, 11.11, 12.96, 14.81, 18.52 and 29.63 mg/L was mixed into each tank to achieve final concentrations of 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 8.0 mg/L H_2O_2 respectively. This design also included one control to which no SCP was added. Each of the eight treatments and the control were conducted in duplicate.

Full-Scale Pond Experiment and Sampling

Based on the results of the small-scale tank experiments, which are reported in the Results and Discussion section, concentrations of 2.5 mg/L (low dose) and 4.0 mg/L (high dose) H_2O_2 as SCP were chosen for further study in full-scale ponds. Two ponds were treated with 2.5 mg/L H_2O_2, two ponds were treated with 4.0 mg/L H_2O_2, and the remaining two ponds received no treatments and served as control ponds. The experimental design consisted of first sampling the water on day 1 following the initiation of SCP treatments followed by daily sampling for the next 10 days. This was followed by weekly sampling from week 2 through week 6.

Sampling Protocols and Analytical Techniques

All phytoplankton were identified to the lowest practical taxonomic level via 200X, 400X, 600X (oil), or 1000X (oil) magnifications by using a 0.1-mm hemocytometer under an optical microscope (Axiostar plus, Zeiss, USA). Zooplankton composition and numbers was determined using Sedgewick Rafter counting cell and viewed at either 100X or 150X. Total microcystin concentrations were determined using Abraxis microcystins assay kit (product No. 520011). Standard water quality parameters were determined through a portable multi-probe field meter (HQ40D portable multi meter, HACH) and HACH assay kits (method details are provided in the Table 3 legends).

Statistical Analysis

All data are presented as mean ± standard error (S.E.). For comparisons among treatment and control groups, one-way completely randomized analyses of variance (ANOVA) were performed; if significant differences were detected, among-treatment differences were assessed using Dunnett's test. Student's two-tailed t-test was used for single comparisons. A probability level of 0.05 was used for rejection of all null hypotheses.

Results and Discussion

Selective Toxicity and Dose Optimization of Granular H_2O_2 Algaecide (SCP) Towards Cyanobacterial Blooms

The present study tested the feasibility of a commercially available SCP granular algaecide (PAK® 27) that would release H_2O_2 when added to the water as a means of selectively eliminating cyanobacteria from mixed phytoplankton communities. In this study, determination of the correct dosage through a small-scale tank experiment was a critical step for the effective application at the full-scale pond level. The tank experiments suggested that the addition of the SCP corresponding to 2.5 mg/L H_2O_2 and greater sig-

nificantly reduced the dominating cyanobacterium *Planktothrix sp.* population (Figure 1). However, concentrations of 5 mg/L H_2O_2 and greater would not be feasible, as non-targeted eukaryotic phytoplankton communities (e.g., green algae *Spirogyra sp.*, *Cladophora sp.* and the diatom *Synedra sp.*) and herbivorous zooplankton (e.g., the rotifer *Brachionus sp.* and cladoceran *Daphnia sp.*) appeared sensitive to these elevated levels (Figures 2 and 3). On the basis of these findings, SCP corresponding to 2.5 mg/L and 4.0 mg/L H_2O_2 were selected for application in experimental ponds to investigate optimal suppression of cyanobacteria without affecting the remaining, non-target plankton community.

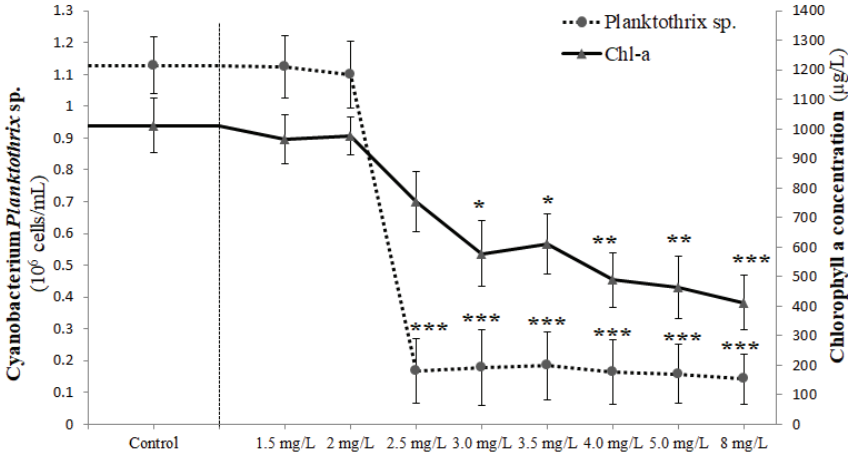

Figure 1. Changes in the cyanobacterium *Planktothrix sp.* abundance (dotted line) and chlorophyll a concentrations (solid line) in tanks after 10 days with different concentrations of H_2O_2 as SCP (PAK® 27). Values are means ± S.E. Asterisks (*) indicates a significant difference between the exposure groups (n=6) and the respective control (n=6) (*P < 0.05; **P < 0.01; ***P < 0.001).

Plankton Dynamics in the SCP Treated Ponds

The application of 2.5 mg/L H_2O_2, in the form of SCP in the full-scale experimental ponds reduced the abundance of cyanobacterium *Planktothrix sp.* (Figure 4), whereby other phytoplankton classes (e.g., green algae *Cladophora sp.* and the diatom *Synedra sp.*) exhibited a conspicuous increase in abundance (Figures 5A,5B). This finding suggested that eukaryotic phytoplankton species in the 2.5 mg/L H_2O_2 -SCP treated ponds exploited the cyanobacterial collapse and mobilized the available nutrients, which would otherwise have been rapidly exhausted by the cyanobacteria bloom. This was supported by an initial significant increase in ammonia (Table 3). Another possibility could include the presence of nitrifying bacteria (i.e., oxidizing ammonia to nitrite and to nitrate), based on a gradual increase in nitrite and nitrate in all treated ponds after 3 weeks (Table 3). Furthermore, comparatively greater total phosphorus content in the treated ponds relative to controls was consistent with the reduction in cyanobacterial blooms in treatment ponds, which rendered phosphorus more bioavailable in the water column (Table 3). We also observed that the abundance of herbivorous zooplankton (*Brachionus* and *Daphnia sp.*) strongly declined in the 4.0 mg/L H_2O_2 -SCP applied ponds in contrast to those that received 2.5 mg/L H_2O_2 (Figures 6A,6B). It is very likely that the oxidative damage induced by a higher dose of 4.0 mg/L H_2O_2 is beyond the tolerance range

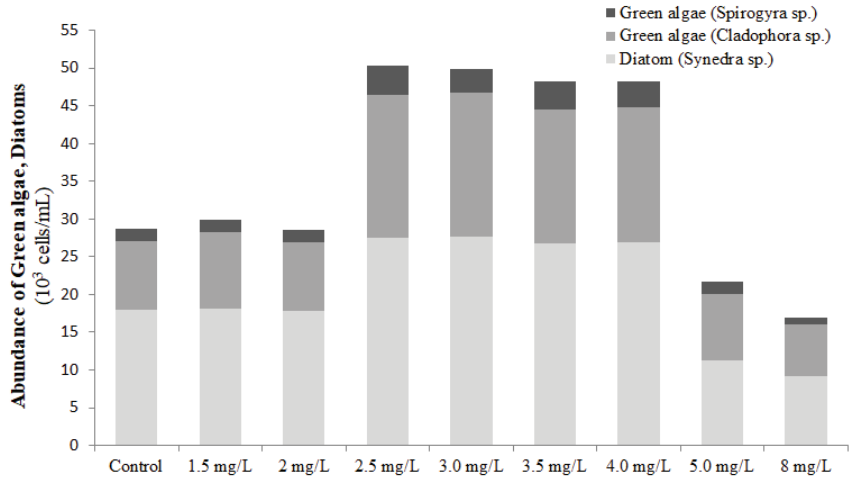

Figure 2. Abundance of green algae (*Spirogyra sp.* and *Cladophora sp.*) and diatom (Synedra sp.) in the tanks after 10 days with different concentrations of H_2O_2 as SCP (PAK® 27). Data show the means (n=6) of two duplicate tanks per treatment.

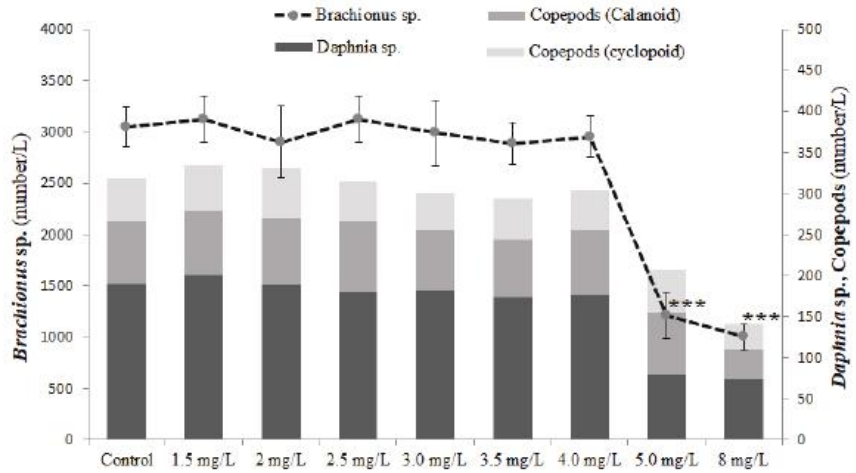

Figure 3. Abundance of zooplankton in the tanks after 10 days with different concentrations of H_2O_2 as SCP (PAK® 27). Line graph represents the population dynamics of rotifers (*Brachionus sp.*) while cladocerans (*Daphnia sp.*) and copepods (calanoid, cyclopoid) are illustrated as bar graphs. Data show the means (n=6) of two duplicate tanks per treatment.

Table 3. Temporal dynamics of water quality parameters of experimental ponds over the duration of 6 weeks following application with 2.5 mg/L and 4.0 mg/L H_2O_2 as SCP (PAK® 27).

Parameter	Treatment	Days						
		1	2	3	4	5	6	7
Chlorophyll a (µg/L)	Control	1048 ± 89	1086 ± 89	1025 ± 105	1000 ± 86	938 ± 114	942 ± 88	929 ± 84
	2.5 mg/L	1070 ± 89	1030 ± 78.4	1023 ± 85	966 ± 157	740 ± 112	790 ± 85	725 ± 82
	4.0 mg/L	1115 ± 86	1060 ± 87	1078 ± 86	944 ± 157	680 ± 132	713 ± 81	621 ± 115*
Water Temperature (°C)	Control	25.8 ± 0.8	25.2 ± 0.4	23.1 ± 1.1	23.1 ± 1.1	19.5± 1.2	19.1 ± 0.7	22.8 ± 0.9
	2.5 mg/L	26.2 ± 1.0	25.2 ± 0.7	25.5 ± 0.7	25.5 ± 0.7	20.5 ± 0.8	19.0 ± 1.1	21.3 ± 0.7
	4.0 mg/L	26.1 ± 0.8	25.8 ± 0.9	23.9 ± 1.1	23.9 ± 1.1	20.9 ± 1.4	18.6 ± 0.6	22.6 ± 0.9
pH	Control	8.62 ± 0.33	8.61 ± 0.11	8.62± 0.24	8.68± 0.27	8.67± 0.23	8.64± 0.21	8.62± 0.23
	2.5 mg/L	8.51± 0.41	8.53± 0.32	8.62± 0.16	8.64± 0.16	8.62± 0.09	8.66± 0.31	8.52± 0.16
	4.0 mg/L	8.81± 0.21	8.80± 0.25	9.16 ± 0.27	9.18± 0.21	9.4± 0.20*	9.41± 0.22 *	9.39± 0.21 *
Transparency (cm)	Control	19.87 ± 1.23	18.83 ± 1.33	19.01 ± 1.12	21.11 ± 1.12	22.22 ± 1.89	23.34 ± 1.67	22.22 ± 1.21
	2.5 mg/L	20.88 ± 1.11	17.99 ± 2.00	20.02 ± 1.11	20.12 ± 1.32	21.01 ± 1.09	22.09 ± 1.75	22.0 ± 1.89
	4.0 mg/L	18.86 ± 1.09	19.09 ± 2.01	21.11 ± 1.06	22.00 ± 1.44	20.09 ± 1.90	19.98 ± 1.82	21.00 ± 1.92
Total alkalinity (mg/L as $CaCO_3$)	Control	119 ± 9	112 ± 9	119 ± 8	110 ± 8	120 ± 13	121 ± 12	111 ± 12
	2.5 mg/L	102 ± 8	117 ± 12	109 ± 8	116 ± 9	111 ± 8	118 ± 12	122 ± 13
	4.0 mg/L	121 ± 9	127 ± 14	131 ± 7	128 ± 10	127 ± 13	134 ± 13	139 ± 13
Conductivity (µS/cm)	Control	384 ± 24	376 ± 22	365 ± 24	381 ± 16	389 ± 24	387 ± 26	377 ± 23
	2.5 mg/L	376 ± 22	368 ± 24	389 ± 26	378 ± 18	389 ± 15	375 ± 24	376 ± 26
	4.0 mg/L	401 ± 17	378 ± 25	399 ± 27	376 ± 20	408 ± 27	410 ± 25	424 ± 27
Dissolved oxygen (mg/L)	Control	2.84 ± 0.21	3.01 ± 0.26	2.38 ± 0.19	2.46 ± 0.23	3.04 ± 0.16	3.41 ± 0.17	2.88 ± 0.28
	2.5 mg/L	2.76 ± 0.31	3.02 ± 0.32	2.67 ± 0.33	2.33 ± 0.33	2.90 ± 0.33	3.13 ± 0.35	2.81 ± 0.32
	4.0 mg/L	3.01 ± 0.24	2.89 ± 0.30	2.99 ± 0.23	2.01 ± 0.23	3.19 ± 0.26	3.21 ± 0.29	2.89 ± 0.30
Total hardness (mg/L as $CaCO_3$)	Control	182 ± 7.8	190 ± 7.8	178 ± 13.2	181 ± 11.7	180 ± 12.9	189 ± 11.5	190 ± 12.2
	2.5 mg/L	187 ± 7.7	186 ± 9.2	180 ± 7.6	182 ± 12.3	190 ± 8.2	192 ± 13.2	188 ± 15.8
	4.0 mg/L	196 ± 7.1	192 ± 10.1	189 ± 13.3	190 ± 12.7	183 ± 14.3	190 ± 13.7	185 ± 14.8
Ammonia – N (mg/L)	Control	0.92 ± 0.11	0.91 ± 0.08	0.88 ± 0.11	0.97 ± 0.11	0.91 ± 0.10	0.89 ± 0.14	0.92 ± 0.12
	2.5 mg/L	0.96 ± 0.12	0.90 ± 0.12	0.91 ± 0.12	0.88 ± 0.10	0.82 ± 0.11	0.88 ± 0.10	0.90 ± 0.09
	4.0 mg/L	0.89 ± 0.12	0.88 ± 0.12	0.79 ± 0.09	0.91 ± 0.12	0.94 ± 0.07	1.02 ± 0.11	0.89 ± 0.07
Nitrite – N (µg/L)	Control	39.2 ± 5.80	41.1 ± 5.61	43.7 ± 5.80	39.5 ± 5.67	37.2 ± 5.67	40.2 ± 6.18	45.5 ± 5.61
	2.5 µg/L	38.6 ± 5.73	37.4 ± 5.61	41.3 ± 5.22	33.5 ± 5.61	28.7 ± 5.73	29.5 ± 5.61	30.3 ± 5.80
	4.0 mg/L	40.2 ± 5.03	40.1 ± 5.80	39.6 ± 5.99	30.2 ± 6.50	29.4 ± 5.80	30.1 ± 5.73	28.2 ± 5.86*
Nitrate – N (mg/L)	Control	0.43 ± 0.05	0.44 ± 0.05	0.44 ± 0.05	0.45 ± 0.04	0.49 ± 0.04	0.48 ± 0.04	0.47 ± 0.04
	2.5 mg/L	0.41 ± 0.02	0.41 ± 0.02	0.39 ± 0.01	0.38 ± 0.03	0.46 ± 0.03	0.34 ± 0.04*	0.41 ± 0.03
	4.0 mg/L	0.39 ± 0.02	0.37 ± 0.03	0.38 ± 0.03	0.37 ± 0.02	0.28 ± 0.03	0.32 ± 0.03**	0.40 ± 0.03
Total Nitrogen (mg/L)	Control	8.04 ± 0.39	7.77 ± 0.38	8.11 ± 0.46	7.97 ± 0.34	7.76 ± 0.41	7.87 ± 0.28	8.02 ± 0.31
	2.5 mg/L	7.10 ± 0.41	6.96 ± 0.41	8.78 ± 0.43	8.63 ± 0.44	7.48 ± 0.44	8.27 ± 0.45	8.51 ± 0.49
	4.0 mg/L	7.79 ± 0.39	7.29 ± 0.37	8.44 ± 0.38	8.46 ± 0.37	6.97 ± 0.28	8.09 ± 0.32	8.95 ± 0.40
Total Phosphorus (mg/L)	Control	1.72 ± 0.13	1.75 ± 0.13	1.78 ± 0.13	1.69 ± 0.13	1.70 ± 0.15	1.70 ± 0.14	1.58 ± 0.14
	2.5 mg/L	1.88 ± 0.13	1.84 ± 0.12	1.89 ± 0.12	1.80 ± 0.11	1.82 ± 0.14	1.81 ± 0.11	1.87 ± 0.13
	4.0 mg/L	1.73 ± 0.12	2.03 ± 0.14	2.09 ± 0.14	1.72 ± 0.12	1.92 ± 0.09	1.99 ± 0.10	1.80 ± 0.12

Table 3 continued. Temporal dynamics of water quality parameters of experimental ponds over the duration of 6 weeks following application with 2.5 mg/L and 4.0 mg/L H_2O_2 as SCP (PAK® 27).

					Weeks		
8	**9**	**10**	**2**	**3**	**4**	**5**	**6**
917 ± 84	1148 ± 62	1142 ± 115	1130 ± 127	966 ± 126	889 ± 116	807 ± 149	987 ± 90
698 ± 87	651 ± 110**	649 ± 77**	614 ± 170*	510 ± 142*	311 ± 139**	394 ± 122*	678 ± 69*
622 ± 78*	602 ± 135**	569 ± 175**	544 ± 191*	571 ± 157*	231 ± 153**	389 ± 147*	601 ± 73**
21.1 ± 0.3	22.4 ± 0.5	23.4 ± 0.6	21.4 ± 0.8	20.1 ± 0.9	18.4 ± 0.2	15.4 ± 0.6	14.4 ± 0.5
21.7 ± 0.7	21.3 ± 0.9	22.0 ± 1.1	21.0 ± 0.7	18.9 ± 0.7	19.2 ± 0.6	16.2 ± 0.7	15.1 ± 0.8
21.2 ± 0.7	21.6 ± 0.8	22.1 ± 0.3	20.8 ± 0.7	20.8 ± 0.7	18.8 ± 0.5	15.8 ± 0.6	14.0 ± 0.6
8.59 ± 0.21	8.62 ± 0.23	8.71± 0.25	8.73± 0.22	8.71± 0.27	8.71± 0.27	8.67± 0.31	8.64± 0.21
8.59± 0.22	8.54± 0.42	8.57± 0.22	8.62± 0.22	8.52± 0.21	8.52± 0.21	8.61± 0.20	8.59± 0.24
8.96± 0.25	8.97± 0.24	8.86± 0.21	8.91± 0.29	9.02± 0.22	9.16± 0.22	8.94± 0.21	9.06± 0.18
20.09 ± 2.02	21.0 ± 2.21	20.09 ± 2.26	22.09 ± 1.90	20.09 ± 1.65	21.21 ± 1.56	22.99 ± 1.45	21.90 ± 2.10
21.20 ± 1.89	23.78 ± 1.78	24.02 ± 2.12	23.98 ± 1.90	22.89 ± 1.91	23.33 ± 1.88	22.45 ± 2.12	22.34 ± 2.09
22.32 ± 1.67	20.01 ± 2.12	19.05 ± 1.23	21.39 ± 1.78	21.08 ± 1.78	22.98 ± 1.90	19.01 ± 1.91	20.98 ± 2.14
115 ± 12	131 ± 15	111 ± 16	121 ± 13	112 ± 12	124 ± 11	121 ± 10	119 ± 15
124 ± 13	121 ± 12	112 ± 15	112 ± 13	103 ± 13	111 ± 13	125 ± 15	129 ± 15
148 ± 12*	140 ± 12	138 ± 9	130 ± 12	132 ± 12	139 ± 13	136 ± 13	130 ± 15
392 ± 24	378 ± 31	397 ± 32	378 ± 27	378 ± 23	381 ± 22	390 ± 20	382 ± 24
389 ± 26	391 ± 25	369 ± 30	381 ± 27	375 ± 27	391 ± 26	366 ± 30	362 ± 24
412 ± 23	432 ± 24	429 ± 17	398 ± 25	390 ± 25	401 ± 27	410 ± 27	405 ± 27
2.31 ± 0.28	2.34 ± 0.22	2.64 ± 0.22	1.65 ± 0.26	2.01 ± 0.21	2.38 ± 0.16	2.26 ± 0.27	2.04 ± 0.26
2.32 ± 0.31	2.21 ± 0.31	2.48 ± 0.38	1.75 ± 0.29	2.12 ± 0.28	2.61 ± 0.36	2.78 ± 0.35	2.58 ± 0.35
2.67 ± 0.29	2.52 ± 0.29	2.42 ± 0.35	2.27 ± 0.36	2.32 ± 0.29	2.72 ± 0.36	2.88 ± 0.37	2.70 ± 0.38
191 ± 11.9	185 ± 15.1	191 ± 12.5	190 ± 13.0	196 ± 11.3	182 ± 14.7	190 ± 9.9	201 ± 10.2
184 ± 13.3	188 ± 11.6	201 ± 13.2	200 ± 14.4	190 ± 14.4	186 ± 14.6	192 ± 16.0	189 ± 14.9
189 ± 13.3	186 ± 13.3	188 ± 12.9	201 ± 13.5	204 ± 12.9	190 ± 14.9	201 ± 14.4	205 ± 13.4
0.88 ± 0.12	0.89 ± 0.11	0.9 ± 0.11	0.92 ± 0.08	0.91 ± 0.10	0.91 ± 0.11	0.86 ± 0.12	0.89 ± 0.11
0.90 ± 0.11	1.31 ± 0.11**	1.34 ± 0.14**	1.21 ± 0.11*	1.27 ± 0.12*	0.98 ± 0.13	1.09 ± 0.12	1.04 ± 0.12
1.22 ± 0.11*	1.32 ± 0.11**	1.29 ± 0.13*	1.23 ± 0.12*	1.30 ± 0.12*	1.08 ± 0.12	1.07 ± 0.11	1.01 ± 0.08
46.4 ± 5.73	44.6 ± 8.34	47.1 ± 8.54	52.3 ± 7.71	51.9 ± 5.80	49.4 ± 8.28	47.3 ± 5.67	50.4 ± 6.82
28.4 ± 5.47*	31.1 ± 8.41	28.3 ± 8.22	29.6 ± 7.83*	30.7 ± 6.24*	47.6 ± 5.48	42.4 ± 6.62	48.5 ± 6.11
29.8 ± 5.77*	28.5 ± 5.86	31.1 ± 5.86	33.2 ± 8.09	29.3 ± 6.88*	42.5 ± 6.94	48.3 ± 6.43	46.8 ± 5.67
0.49 ± 0.05	0.46 ± 0.03	0.46 ± 0.04	0.46 ± 0.03	0.47 ± 0.05	0.47 ± 0.05	0.49 ± 0.04	0.48 ± 0.05
0.28 ± 0.03***	0.31 ± 0.03**	0.39 ± 0.03	0.39 ± 0.03	0.41 ± 0.03	0.48 ± 0.03	0.50 ± 0.03	0.47 ± 0.03
0.31 ± 0.05**	0.31 ± 0.04 **	0.35 ± 0.03 *	0.38 ± 0.031	0.39 ± 0.029	0.47 ± 0.035	0.51 ± 0.026	0.48 ± 0.032
8.26 ± 0.39	7.97 ± 0.39	8.13 ± 0.41	7.63 ± 0.48	8.03 ± 0.50	8.28 ± 0.47	8.50 ± 0.47	8.16 ± 0.49
9.22 ± 0.48	9.30 ± 0.47*	9.82 ± 0.50*	9.75 ± 0.46**	9.19 ± 0.46	8.99 ± 0.47	8.67 ± 0.47	8.16 ± 0.48
9.15 ± 0.31	9.87 ± 0.31**	9.76 ± 0.35*	9.96 ± 0.38**	8.97 ± 0.46	8.16 ± 0.39	8.33 ± 0.51	7.99 ± 0.48
1.59 ± 0.15	1.59 ± 0.14	1.33 ± 0.14	1.21 ± 0.14	1.17 ± 0.15	1.22 ± 0.15	1.18 ± 0.15	1.08 ± 0.15
1.71 ± 0.19	1.67 ± 0.09	1.58 ± 0.12	1.53 ± 0.15	1.32 ± 0.13	1.46 ± 0.15	1.35 ± 0.16	1.33 ± 0.15
1.99 ± 0.19	1.84 ± 0.09	1.59 ± 0.14	1.51 ± 0.16	1.45 ± 0.12	1.53 ± 0.15	1.51 ± 0.16	1.42 ± 0.15

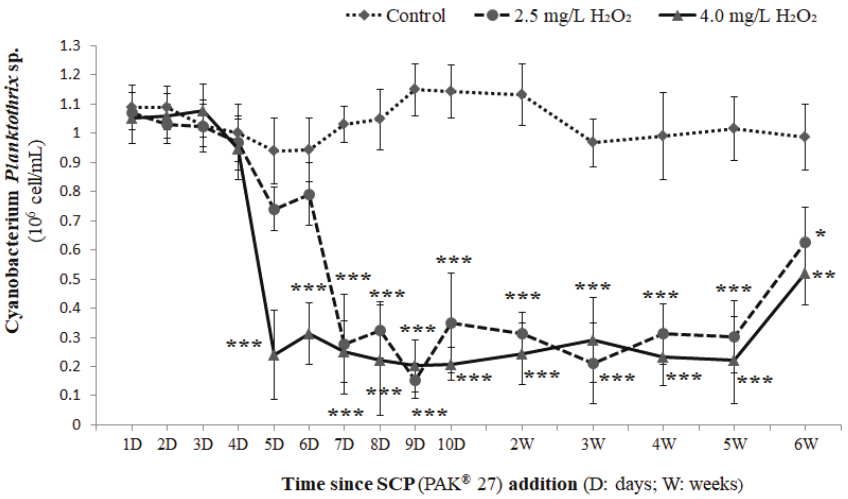

Figure 4. Temporal changes in the cyanobacterial *Planktothrix sp.* abundance in ponds over 6 weeks of treatments with 2.5 mg/L and 4.0 mg/L H_2O_2 as SCP (PAK® 27). Values are means ± S.E. Asterisks (*) indicate a significant difference between the treatment groups (n=8) and control (n=8) at the same sampling period (*P < 0.05; **P < 0.01; ***P < 0.001).

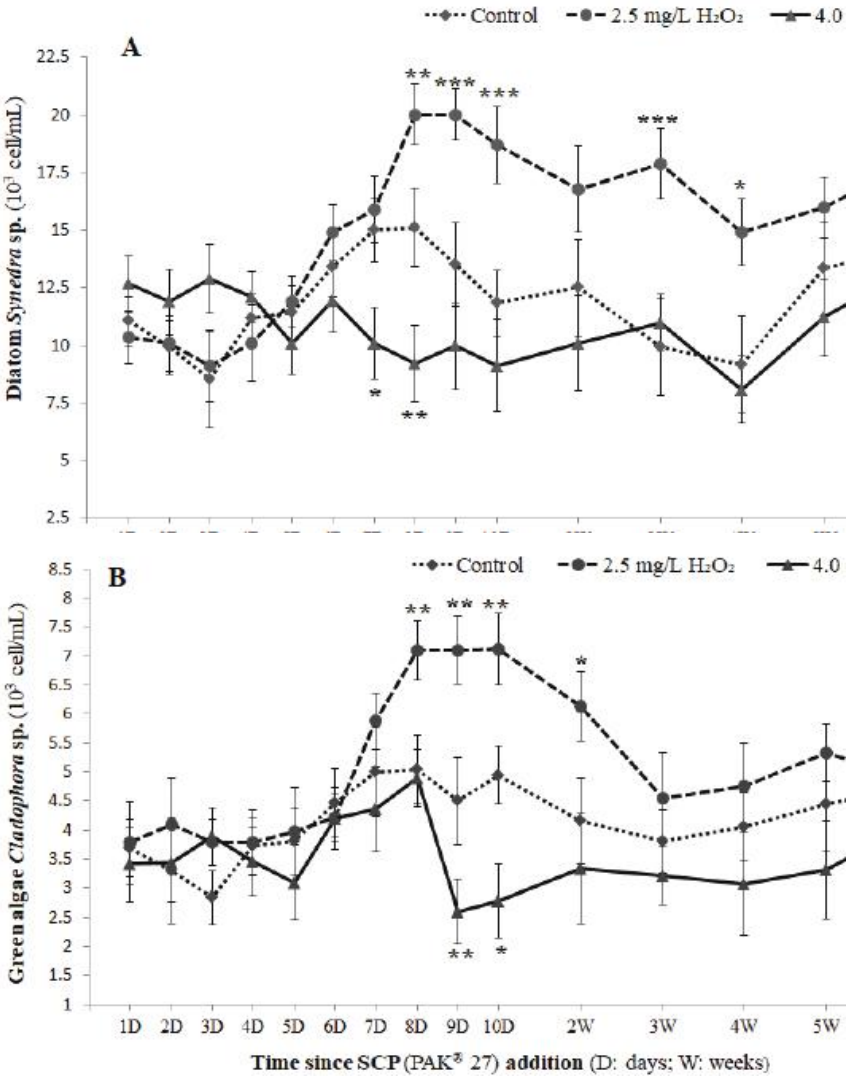

Figure 5. Temporal variations in the dynamics of eukaryotic phytoplankton (A) diatoms *Synedra sp.* and (B) green algae *Cladophora sp.* populations in ponds over 6 weeks of treatments with 2.5 mg/L and 4.0 mg/L H_2O_2 as SCP (PAK® 27). Values are means ± S.E. Asterisks (*) indicate a significant difference between the treatment groups (n=8) and control (n=8) at the same sampling period (*P < 0.05; **P < 0.01; ***P < 0.001).

of these zooplankton groups. This reduction in herbivorous zooplankton might have also been potentially coupled with the reduction of eukaryotic phytoplankton richness that limits the supply of phytoplankton as a food source.

Cyanotoxin Degradation and Environmental Feasibility of SCP-Based Algaecide

A potential risk associated with the massive cyanobacterial lysis is the copious release of internally produced cyanotoxins into the surrounding water (Westrick et al., 2010). For instance, the persistence of cyanotoxins has the potency to kill food fish, cause food safety issues, or adversely affect product quality (Sinden and Sinang, 2016). Hence, the timely control of not merely the cyanobacterial blooms, but also their associated toxins from the culture system is essential. Copper-containing algaecides (e.g., Captain and K-Tea) are effective in controlling cyanobacterial populations; however, evidence suggests that these chemicals cannot mitigate cyanotoxins or microcystin concentrations (Greenfield et al., 2014; Jones and Orr, 1994; Kenefick et al., 1993). This study provides strong evidence that the total microcystin concentrations are dramatically reduced by H_2O_2 applications in the form of SCP-based algaecide (Figure 7). The oxidation of the H_2O_2 fraction of the SCP granules may have catalyzed the production of hydroxyl and hydroperoxyl radicals that induced the oxidative cleavage of microcystins. This process, in effect, degrades microcystins into peptide residues by either modifying the Adda-moiety or breaking the amino-acid ring structure of the microcystins (Antoniou et al., 2008; Liu et al., 2003).

Aquaculturists, water resource managers, and water authorities should consider not only the efficiency, but also the ecological consequences of cyanobacteria bloom prevention and control approaches. In this study, the H_2O_2 added in the form of SCP-'PAK® 27' rapidly degraded in the water column, usually within 3 to 4 days (Figure 8), which suggests that this product is unlikely to leave any significant environmental footprint. Consequently, the SCP-based algaecide seems to exert minimal detrimental consequences on aquatic food webs compared to

other algaecides (e.g., copper-based compounds) that have a more lengthy environmental persistence.

Conclusions

With the current scenario of increased frequencies of cyanobacterial blooms worldwide, largely due to anthropogenic activities, an environmentally compatible management strategy is crucial that not only controls the blooms, but also their toxins. To address this issue, the efficacy of a newly developed granular H_2O_2 based SCP algaecide (PAK® 27) application for full-scale hypereutrophic ponds was assessed following a dose range-finding test in outdoor tanks. The applications of SCP at both 2.5 and 4.0 mg/L H_2O_2 substantially reduced cyanobacteria *Planktothrix sp.* cell numbers. However, given the minimal effects on non-target eukaryotic algae and zooplankton, the 2.5 mg/L H_2O_2 concentration as SCP had practical advantages over the 4.0 mg/L H_2O_2 concentration for reducing cyanobacteria and diminishing the likelihood of recurring cyanobacteria blooms. Furthermore, the present study also revealed that the added H_2O_2 as PAK® 27 degrades within a few days, and thus leaves no long-term traces in the environment. Overall, these results suggest that SCP based PAK® 27 algaecide is effective at both removing cyanobacterium Planktothrix and microcystins, while also being environmentally benign. However, the optimal dosage may also depend on the species composition of the cyanobacteria. In the future, conducting similar experiments with other genera of dominating cyanobacterial blooms (e.g., *Microcystis* or *Anabaena sp.*) will be crucial.

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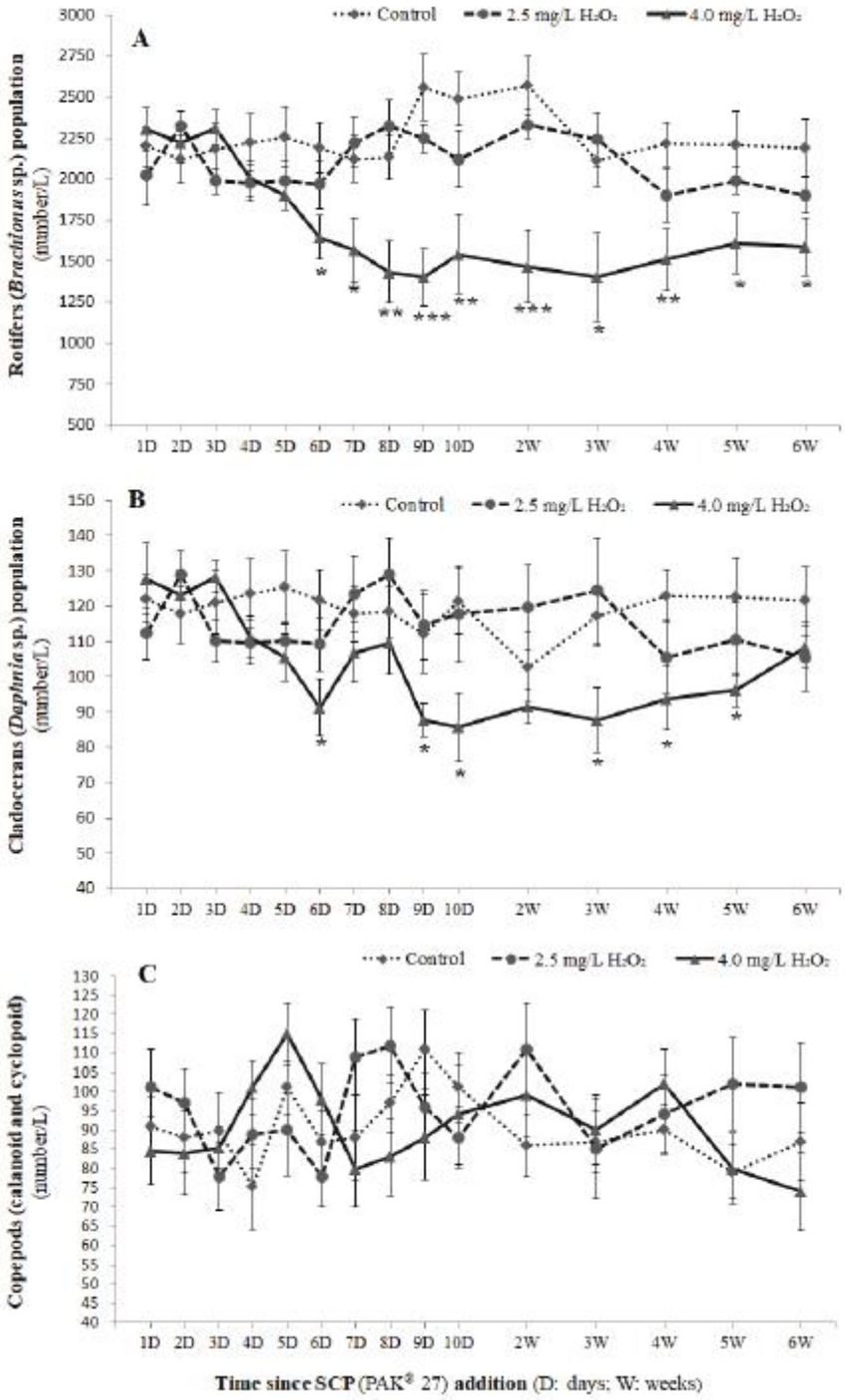

Figure 6. Abundance patterns of zooplankton (A) *Brachionus sp.*, (B) *Daphnia sp.* and (C) copepods in ponds over 6 weeks of treatments with 2.5 mg/L and 4.0 mg/L H_2O_2 as SCP (PAK® 27). Values are means ± S.E. Asterisks (*) indicate a significant difference between the treatment groups (n=8) and control (n=8) at the same sampling period (*P < 0.05; **P < 0.01; ***P < 0.001).

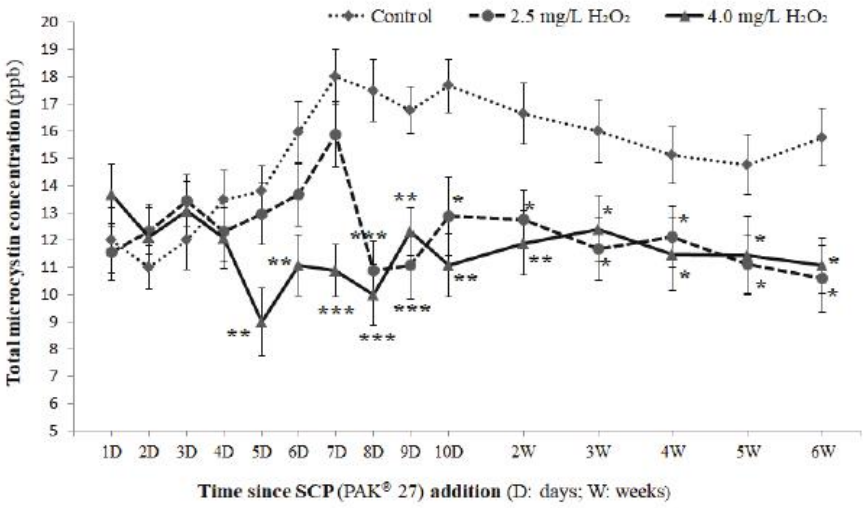

Figure 7. Changes in microcystin concentrations (ppb) in ponds over 6 weeks of treatments with 2.5 mg/L and 4.0 mg/L H_2O_2 as SCP (PAK® 27). Values are means ± S.E. Asterisks (*) indicate a significant difference between the treatment groups (n=8) and control (n=8) at the same sampling period (*P < 0.05; **P < 0.01; ***P < 0.001).

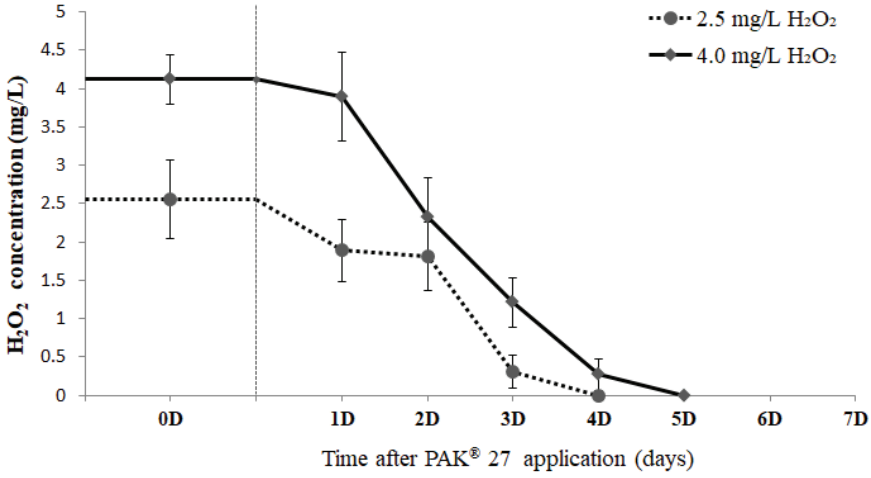

Figure 8. Degradation profile of 2.5 mg/L and 4.0 mg/L H_2O_2 applied as SCP (PAK® 27) in ponds. Values are means ± S.E (n=8).

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