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Evaluation of the Destruction of the Harmful Cyanobacteria, *Microcystis aeruginosa*, with a Cavitation and Superoxide Generating Water Treatment Reactor

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Abstract Cyanobacterial/Harmful Algal Blooms are a major issue for lakes and reservoirs throughout the U.S.A. An effective destructive technology could be useful to protect sensitive areas, such as areas near water intakes. The study presented in this article explored the use of a reactor called the KRIA Water Treatment System. The reactor focuses on the injection of superoxide (O_2^-), which is generated electrochemically from the atmosphere, into the water body. In addition, the injection process generates a significant amount of cavitation. The treatment process was tested in 190-L reactors spiked with water from cyanobacterial contaminated lakes. The treatment was very effective at destroying the predominant species of cyanobacteria, *Microcystis aeruginosa*, organic matter, and decreasing chlorophyll concentration. Microcystin toxin concentrations were also reduced. Data suggest that cavitation alone was an effective treatment, but the addition of superoxide improved performance, particularly regarding removal of cyanobacteria and reduction of microcystin concentration.

Keywords Cyanobacterial/harmful algal blooms (CHAB) · Cyanobacteria · Microcystin · Superoxide · Cavitation

Numerous studies have documented the impacts of Cyanobacterial/Harmful Algal Blooms (CHABs) in the United States and throughout the world (Beaver et al. 2014; Heiskary et al. 2014; Oberholser et al. 2006; Makhera et al. 2011; Mou et al. 2013; Persaud et al. 2015; Roelke et al. 2013). These organisms can adversely affect aquatic life through rapidly decreased dissolved oxygen, prevention of light penetration, and limit gas exchange. Furthermore, certain algae and cyanobacteria species can release toxins in water, such as microcystin, that can harm people, kill livestock and affect other aquatic life (Zimba et al. 2001; Otten and Pearl 2015). For example, Lake Erie suffered record setting CHABs in 2013 (Michalak et al. 2013), and in 2014, cyanobacterial blooms in Lake Erie, U.S.A., necessitated the closure of the water treatment intake for the City of Toledo OH and bottled drinking water had to be provided to the city's residents (Egan 2014).

There are numerous means of controlling algal blooms. Ultimately, preventing these blooms by controlling land use nutrient loading from surface water runoff, is considered the most effective means (Oberholser et al. 2006; Beaver et al. 2014). However, implementing such controls is challenging due to spatial and temporal constraints. Therefore effective, localized source treatments are needed that can destroy cyanobacteria and algae in hot spot areas, such as water intakes, sensitive environmental areas, and in areas used for livestock watering. However, in some cases, physical destruction of the algal biomass can have the unintended result of releasing biotoxins into the water (Li et al. 2014).

The KRIA Water Treatment System (tradename, Premier Materials, Minneapolis, MN) is a unique reactor that combines several potential mechanisms for controlling algal blooms including; cavitation, microbubbles, and the generation of superoxide radicals. The literature has indicated that physical cavitation can be an effective means of cyanobacterial deactivation (Li et al. 2014) and superoxide has been speculated to be effective at treating microcystin

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toxin (Shephard et al. 1998). A previous study confirmed the production of superoxide, and also showed that the KRIA can supersaturate dissolved oxygen and increase electrical conductivity (due to the charged oxygen species) in a 50 gal (190 L) reactor (Medina et al. 2015). This study explored the effectiveness of the KRIA reactor for algal blooms in a controlled setting.

Materials and Methods

The KRIA ionizing water treatment system is designed to be deployed into rivers, streams, lakes and ponds, although it could be effectively applied into tanks and engineered reactors. It has an intake where water is drawn into the reactor, and a discharge, where water is returned (Fig. 1). Superoxide is produced from atmospheric air, which air is drawn into the system, and electrochemically treated, resulting in the conversion of molecular oxygen into the superoxide anion (Fig. 1). The method of ionization, described as "ionization by collision", involves reaction in a magnetic field as the air is drawn through a bed of ceramic balls with reactive minerals, which are not specified (Kunio et al. 1999). The negatively charged air is then pressurized and stored in a reservoir tank. This superoxide is charged into the discharge of the system. The discharge is also a key part of the treatment, as it is designed to promote reactions by cavitation in addition to superoxide reactions (Kunio et al. 1999). Additional information on the KRIA reactor can be found in Medina et al. (2015).

Two identical test reactors were prepared, these being lined 55 gallon (209 L) drums. A top was engineered by cutting holes for the reactor inlet/discharge to limit volatilization while allowing for the KRIA nozzles to be inserted in the water. The two test reactors were filled with 170 L of dechlorinated tap water, which is used for fish cultivation studies. Reactors were then spiked with 40 L of water collected from lakes suffering from severe algal blooms. Two sets of samples were collected from lakes in California, and a third from a bay in Lake Erie, from an Ohio location near the city of Toledo. Samples were collected by onsite personnel using surface scoop samplers and placed in 5 gallon buckets, sealed and shipped by overnight mail to the laboratory. At the laboratory, the buckets were opened and placed under plant growth lights and used in studies within 24 h of their receipt.

Algal/cyanobacterial counts were conducted by Phycotech (St. Joseph, MI). Volatile matter was determined using gravimetric loss on ignition, using ASTM method D7348 (ASTM International 2013). A 50 mL portion of solution was taken and placed in a preweighed crucible, then dried at 105°C. The crucible was then weighed again to measure the total solids. The crucible was then combusted at 500°C to combust the organic compounds, and was weighed after cooling to room temperature to measure the organic content. Total suspended chlorophyll was extracted and measured spectrophotometrically (ESS 1991). Turbidity was measured using a Hach 2100P Turbidometer, which was calibrated prior to use. Microcystin toxin concentrations were measured by GreenWater Laboratories (Palatka, FL). The cells were lysed using ultrasonication and the microcystin was then measured using an Enzyme Linked Immunosorbent Assay method with a detection limit of 0.15 ug/L (as described in Rivasseau et al. 1999).

Individual studies were conducted on samples from three water bodies, respectively. Study 1 used samples from Clear Lake in California (provided by Ms. Carolyn Ruttan, Invasive Species Coordinator, Lake County, CA) to spike the reactors and involved 40 min exposures to the

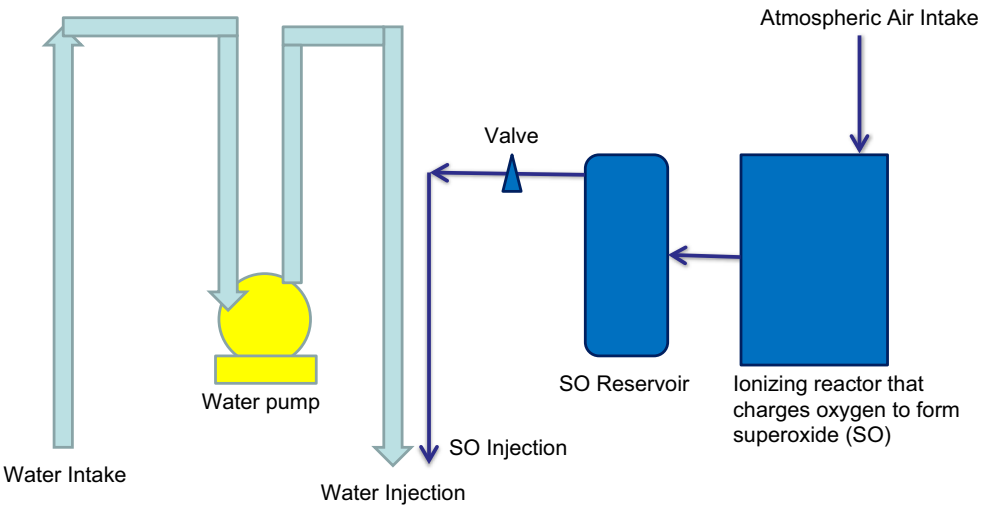

Fig. 1 Schematic of the KRIA Water Treatment System

KRIA reactor with and without superoxide charging. An untreated control was maintained for comparison. Study 1 did not include the algal/cyanobacterial counts. The 40 min exposures had been based on treatment studies focused on chemical contaminants like diesel and gasoline (Medina et al. 2015). However, it became apparent that the reactions were much faster. Consequently, Study 2 also used samples from Clear Lake, but with 5 min exposures to the KRIA. It also included a control—spiked with algae but not treated in any way with the KRIA. Study 3 focused on the 5 min treatment of a sample collected from Lake Erie using only the KRIA with superoxide. Due to the large scale of the reactors, the limited amount of spiking solutions, and the time requirements to do each experiment, this experiment did not employ replication.

Results and Discussion

Treatment using the KRIA showed almost immediate results in removing visible cyanobacterial cells. Figure 2 shows a photograph comparing a sample collected at 5 min from an untreated control reactor to one sample treated by the KRIA reactor, in this case with the superoxide valve off. The difference in color and turbidity was striking, showing virtually no color or turbidity after treatment.

Analysis of the intreated samples by Phycotech indicated that over 90 % of the algal/cyanobacterial species were *M. aeruginosa*. Table 1 shows algal cell counts and biovolume data from two studies (Study 1 did not have count data). In Study 2, a reduction observed in the control and in the two KRIA treatments, with and without superoxide. The reduction in the KRIA without superoxide was nearly twice that of the control and more than four times that of the control when exposed to superoxide. The % biovolumes decreased in all cases, but the decreases were larger with the KRIA treatments. The effect was most pronounced with the KRIA with superoxide, which had an 80 % reduction compared to a 32 % reduction for the control. Study 3, which examined the treatment of a sample from Lake Erie with the KRIA with superoxide charging, had a 23 % reduction in *M. aeruginosa* and 27 % reduction in the % biomass.

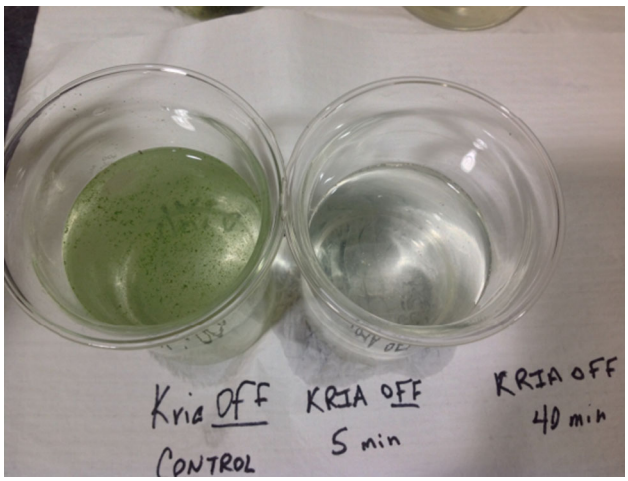

Fig. 2 Comparison of samples collected from control and KRIA treated (with superoxide valve off)

Figure 3 shows normalized volatile matter concentrations in samples collected from Study 1 at 1, 5 and 40 min of KRIA treatment with superoxide, compared to an untreated control (Study 1). The treatment resulted in a sharp, 70 %, drop in volatile matter within 1 min, followed by a more gradual reduction to reach an 82 % reduction at 40 min. The control also had a sharp initial drop, but not as much as the KRIA treated reactor. The reduction in volatile matter in the control at 40 min was 37 %. Similarly, KRIA without superoxide had a 75 % reduction after 5 min of treatment (Study 2), and the Lake Erie study (KRIA with superoxide) had a 65 % reduction (Study 3).

Treatment with the KRIA system showed a powerful effect on chlorophyll concentrations (Fig. 4, Study 1). Chlorophyll concentrations dropped 80 % in the Kria treated (with SO) reactor while actually increased in the control. Decreases were also found in Kria without SO (90 % in 5 min, Study 2) and in the Lake Erie treated samples (75 % in 5 min, Study 3).

Turbidity is the measure of light scattering in water and is a function of particles in the water. Algal cells can act as light scattering particles and typically account for some portion of turbidity in water. In these experiments, with no other turbidity sources entering or exiting the system, it is reasonable to assume that the bulk of any changes in turbidity are related to algal concentrations. Figure 5 summarizes turbidity measurements in Study 1. The KRIA-treated with superoxide sparging sample had >90 % reduction of turbidity after 40 min treatment. There was no reduction of turbidity in the control. Similarly, KRIA-treated without superoxide (Study 2) and Lake Erie treatment (Study 3) both had 84 % reductions in turbidity after 5 min of treatment.

KRIA treatments with and without superoxide charging resulted in substantial reduction of microcystin toxins compared to controls. Study 1 and 2 were treatments of Clear Lake samples, but the starting levels in Study 1 were lower than that in Study 2 (146 vs. 555 ug/L). In Study 1, which was a 40 min treatment, the control had only a 5 % reduction in microcystin concentration compared to 68 % and 77 % reduction in KRIA treatments without and with superoxide, respectively. In Study 2, which focused on a 5 min treatment, the control had a 61 % reduction in microcystin levels, and this was substantially less than the

Table 1 Counts of the cyanobacteria *Microcystis aeruginosa*

Study	Treatment	Initial count (NU/mL)	Final count (NU/mL)	% Count change	Initial biovolume (um^3/mL)	Final biovolume (um^3/mL)	% Biomass change
Study 2—California Lake with high cell density	Control	3.70E+07	3.08E+07	17	1.72E+09	1.18E+09	32
	KRIA with superoxide	3.40E+07	6.32E+06	81	1.10E+09	2.16E+08	80
	KRIA without superoxide	1.72E+07	1.17E+07	32	5.34E+08	3.37E+08	37
Study 3—Lake Erie	KRIA with superoxide	7.56E+05	5.81E+05	23	3.83E+07	2.80E+07	27

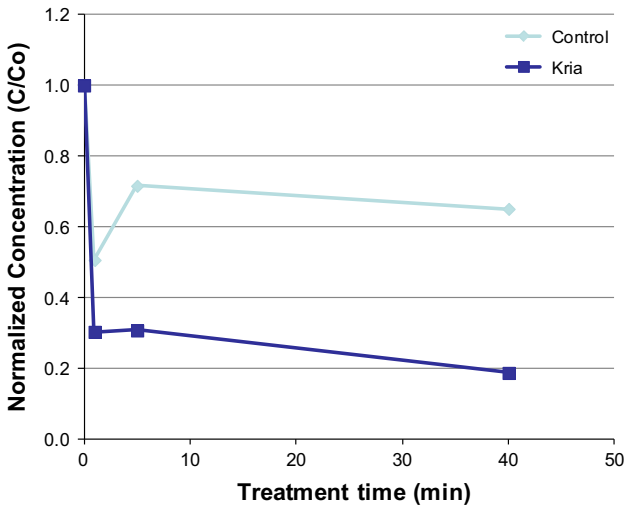

Fig. 3 Changes in volatile matter (as a surrogate for organic matter) for the untreated control versus the KRIA treated reactor over a 40 min experiment (Study 1)

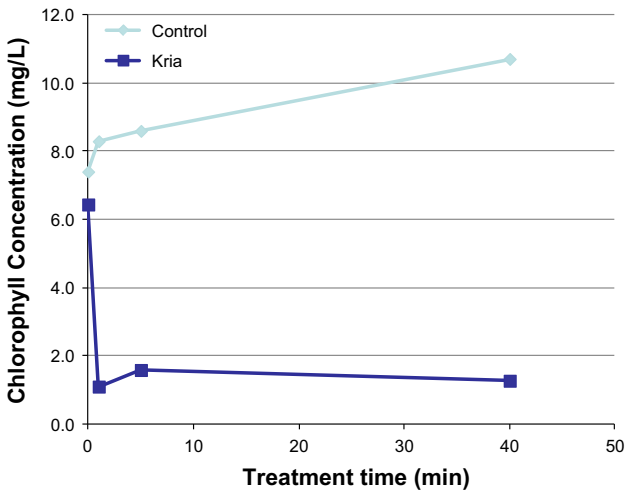

Fig. 4 Changes in chlorophyll concentration for the untreated control versus the KRIA treated reactor over a 40 min experiment (Study 1)

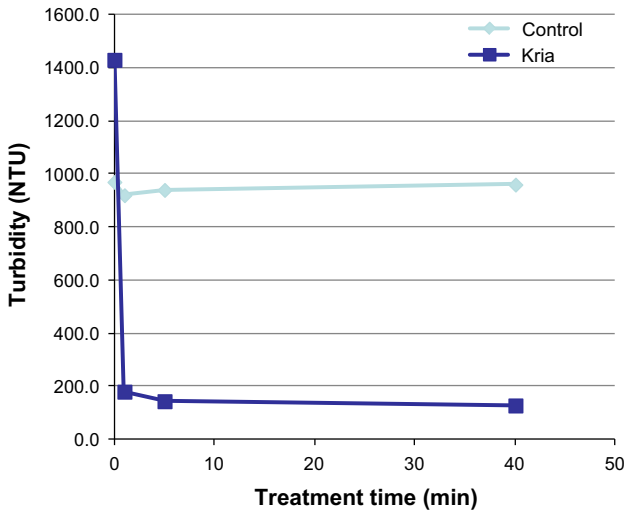

Fig. 5 Turbidity comparison of untreated control versus KRIA with superoxide (Study 1)

KRIA treatments of 87 % and 92 % reductions found in KRIA treatments without and with superoxide, respectively. Superoxide charging improved performance on the order of 5 %–9 %. The microcystin levels in Study 3 (Lake Erie) were much lower. We found a 67 % reduction after a 5 min KRIA with superoxide treatment. These destruction rates are much faster than those found for biodegradation of microcystin. For example, Ramani et al. (2012) found 74 % reduction in a 20-day incubation (Table 2).

Because of the nature of the study, replication was not applied. So, it is not possible to indicate statistical significance. However, the magnitude of changes by a range of data presented (counts, volatile matter, chlorophyll and turbidity) strongly support the destruction of *M. aeruginosa* by the KRIA system. The results consistently indicate that substantial reductions were obtained by using the KRIA without the superoxide system. This further suggests that cavitation is a primary mechanism in the effectiveness of

Table 2 Microcystin toxin concentrations

Study	Treatment	Treatment time (min)	Time zero microcystin concentration (ug/L)	Post treatment microcystin concentration (ug/L)	% Reduction
Study 1—California Lake with medium cell density	Control	40	146	138	5
	KRIA with Superoxide	40	146	34	77
	KRIA without Superoxide	40	138	44	68
Study 2—California Lake with high cell density	Control	5	555	215	61
	KRIA with superoxide	5	555	47	92
	KRIA without superoxide	5	215	28	87
Study 3—Lake Erie	KRIA with superoxide	5	5.5	1.8	67

the system. However, the addition of superoxide charging as a secondary mechanism improved performance. This tandem effect of cavitation coupled with superoxide provided by the KRIA Water Treatment System was most pronounced in the cell count data, but also occurred in the microcystin toxin treatment, demonstrating an effective strategy to destroy *M. aeruginosa* cell and its biotoxin.

The changes in volatile matter and suspended chlorophyll suggest that the treatment resulted in the oxidation of the organic material associated with the cyanobacteria, indicating cellular disruption. Li et al. (2014) found that the physical action of cavitation resulted in lysing of *M. aeruginosa* cells which led to a spike of microcystin levels due to release of intracellular toxins. Similarly, Wu et al. (2012) used cavitation to lyse cyanobacteria, but then used ozone to destroy the toxins. This is contrary to our results, as we found reductions in microcystin toxins in KRIA treated samples even without superoxide sparging. Shephard et al. (1998) reported that radicals (superoxide and hydroxyl) generated from titanium dioxide reactions were effective at degrading microcystin toxin. Moreover, studies have shown that cavitation alone can generate radicals, to the point of degrading polychlorinated biphenyls (Zhang and Hua 2000). We hypothesize that these radicals, whether generated by the superoxide generator or by cavitation, result in microcystin toxin degradation. In some cases, cavitation may only be strong enough to lyse the cyanobacteria, as in the studies of Li and Wu. But a strong enough cavitation field could generate enough radicals to initiate toxin degradation.

Effective treatment is probably not feasible for most lakes and ponds due to their large size. However, most CHAB occurrences are found near shores or bays. For example Davis et al. (2014) found most growth in near shore areas. By applying treatment in problematic areas, it may be possible to minimize the effects of CHAB events.

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References

ASTM International (2013) ASTM D7348-13. Standard test methods for loss on ignition. In: LOI of solid combustion residuals, West Conshohocken

Beaver J, Manis E, Loftin K, Graham J, Pollard A, Mitchell R (2014) Land use patterns, ecoregion, and microcystin relationships in US lakes and reservoirs: a preliminary evaluation. Harmful Algae 36:57–62

Davis TW, Watson SB, Rozmarynowycz MJ, Ciborowski JJH, McKay RM, Bullerjohn GS (2014) Phylogenies of microcystin-producing cyanobacteria in Lower Laurentian Great Lakes suggest extensive genetic connectivity. PLoS One 9(9):e106093

Egan D (2014) Toxic algal blooms shuts off water, but green slime limited so far to Toledo. The Plain Dealer. http://www.cleveland.com/outdoors/index.ssf/2014/08/toxic_algal_bloom_shuts_off_wa.html

Environmental Sciences Section (ESS) (1991) ESS Method 150.1. Chlorophyll-spectrophotometric. Inorganic Chemistry Unit. Wisconsin State Lab of Hygiene, Madison. http://www.epa.gov/grtlakes/lmmb/methods/methd150.pdf

Heiskary S, Lindon M, Anderson J (2014) Summary of microcystin concentrations in Minnesota lakes. Lake Reserv Manag 30:268–272

Kunio F, Goro S, Takeshi N (1999) Method for water quality improvement of rivers, lakes, and ponds, and its device. Certificate of Patent. Japan Patent Office. Patent Number 3280643. Translated to English

Li P, Song Y, Yu S (2014) Removal of *Microsystis aeruginosa* using hydrodynamic cavitation: performance and mechanisms. Water Res 62:241–248

Makhera M, Gumbo JR, Chigayo K (2011) Monitoring of microcystin-LR in the Luvuvhu River catchment: implications for human health. Afr J Biotechnol 10:405–412

Medina VF, Morrow AB, Wade R (2016) Testing of the KRIA ionizing water treatment system for waters contaminated with diesel, PCBs, and nutrients (nitrogen forms). U.S. Army Engineer Research & Development Center Technical Note. ERDC/EL TR-16-03

Michalak AM, Anderson EJ et al (2013) Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. PNAS 110(16): 6448–6452

Mou X, Lu X, Jacob J, Sun S, Heath R (2013) Metagenomic identification of bacterioplankton taxa and pathways involved in microcystin degradation in Lake Erie. PLOS One 8(4):e61890. http://www.theguardian.com/commentisfree/2015/jul/27/alaska-global-warming-relocation?CMP=share_btn_tw

Oberholser PJ, Botha A-M, Cloete TE (2006) Toxic cyanobacteria blooms in a shallow, artificially mixed urban lake in Colorado, USA. Lakes Reserv Res Manag 11:111–123

Otten TG, Pearl HW (2015) Health effects of toxic cyanobacteria in the U.S. drinking and recreational waters: our current understanding and proposed direction. Curr Environ Health Rep 2:75–84

Persaud A, Paterson A, Dillon P, Winter J, Palmer M, Somers K (2015) Forecasting cyanobacteria dominance in Canadian temperate lakes. J Environ Manag 151:343–352

Ramani A, Rein K, Shetty KG, Jayachandran K (2012) Microbial degradation of microcystin in Florida's waters. Biodegradation 23:35–45

Rivasseau C, Racaud P, Deguin A, Hennion M-C (1999) Evaluation of an ELISA kit for the monitoring of microcystins (cyanobacteria toxins) in water and algae water samples. Environ Sci Technol 33:1520–1527

Roelke DL, Brooks BW, Grover JP, and Lundgren VM (2013) Golden algae control: efficacy of hydraulic manipulations in covers of Lake Granbury. U.S. Army Engineer Research & Development Center Technical Report. ERDC/EL CR-13-1

Shephard GS, Stockenstrom S, DeVilliers D, Engelbrecht WH, Sydenham EW, Wessels GFS (1998) Photocatalytic degradation of cyanobacterial microcystin toxins in water. Toxicon 36: 1895–1901

Wu Z, Shen H, Ondruschka B, Zhang Y, Wang W, Bremmer D (2012) Removal of blue–green algae using the hybrid method of hydrodynamic cavitation and ozonation. J Hazard Mater 235:152–158

Zhang GM, Hua I (2000) Cavitation chemistry of polychlorinated biphenyls: decomposition mechanisms and rates. Environ Sci Technol 34:1529–1534

Zimba PV, Khoo L, Gaunt PS, Brittain S, Carmichael WW (2001) Confirmation of catfish, *Ictalurus punctatus* (Rafinesque), mortality from Microcystis toxins. J Fish Dis 24:41–47