Image caption: A harmful algal bloom of *Microcystis* at Bryce Davis Park in West Fayetteville, Arkansas in August 2019. Photo from Dr. Brad Austin, Arkansas Water Resources Center.

An In Situ Approach to Harmful Algal Blooms: Simultaneous Treatment of Cyanobacteria and Cyanotoxins in Natural Water Sources Using Catalytic Nanoparticle-Fiber Nets

Dr. Lauren Greenlee[1*] and Dr. Wen Zhang[2]

[1]Ralph E. Martin Department of Chemical Engineering, University of Arkansas, [2]Department of Civil and Environmental Engineering, University of Arkansas
*Corresponding author

Abstract: Harmful algal blooms (HABs) and their associated cyanotoxins that are produced cause negative environmental, water quality, and human health impacts. Few treatment approaches currently exist that can treat both HAB cyanobacteria and cyanotoxins without a pump-and-treat requirement or negative environmental impacts. This research focuses on an innovative in situ approach based on the concept of a catalytic fishing net that is retrievable and reusable. Research results demonstrate that titanium dioxide (TiO_2) and iron oxide nanoparticle catalysts cause HAB cyanobacteria deactivation through a flocculation mechanism. The cyanotoxin microcystin-LR is removed by TiO_2 through UV light activated catalytic degradation.

Key Points:

- Harmful algal blooms (HABs) and the associated cyanotoxins occur in surface waters globally and in Arkansas.
- Current HAB and cyanotoxin treatment options involve significant disadvantages, including a pump-and-treat requirement, only addressing either HABs or the cyanotoxin(s), and possible environmental impacts.
- A fishing net concept is used as an in situ approach to simultaneously deactivate HAB cyanobacteria and degrade cyanotoxins.
- Non-toxic titanium dioxide (TiO_2) and iron oxide nanoparticle (NP) catalysts were tested for HAB cyanobacteria deactivation, with flocculation as the primary deactivation mechanism.
- TiO_2 NPs removed microcystin-LR cyanotoxin through UV-initiated catalytic degradation, where cyanotoxin adsorption plays a minimal role.

Introduction

Harmful algal blooms (HABs) are globally increasing in frequency and distribution due to excessive nutrient runoff from agriculture and worsening eutrophication of water sources (He et al., 2016; Marsalek et al., 2012). HABs are cyanobacteria that accumulate biomass and produce cyanotoxins such as microcystin-LR, and an estimated 25 to 75% of HABs are toxic (Blaha, Babica, & Marsalek, 2009; Meng, Savage, & Deng, 2015). Cyanobacteria and cyanotoxins are becoming more prevalent and are severely damaging ecosystems because of nutrient pollution; cyanobacteria have negative impacts on ecosystem functions, such as organism relationship disturbances, biodiversity changes, light conditions, and oxygen concentrations (Blaha et al., 2009). Several animal studies have shown evidence of microcystins exhibiting tumor promoting properties (Blaha et al., 2009).

Cyanobacteria and cyanotoxins adversely impact human health and promote negative health effects like liver damage, immunotoxicity, and neurotoxicity (Marsalek et al., 2012). Annually, the U.S. alone spends $2.2 to 4.6 billion on methods, including filtration, flocculation, coagulation, or sedimentation, to battle the effects of HABs (Marsalek et al., 2012; Meglic, Pecman, Rozina, Lestan, & Sedmak, 2017; Meng et al., 2015). However, these methods are only temporary solutions, and key disadvantages associated with these techniques include detrimental environmental impacts and inefficiency (Marsalek et al., 2012). To address these disadvantages, we posed the research question: Can an in situ treatment approach based on the concept of a retrievable fishing net be used to deactivate HABs in the source water, without the need to pump and treat the water and with minimal to no environmental impacts?

Our objectives for this project were to test commercially available and experimentally synthesized catalyst materials, immobilize nanoparticle (NP) catalysts on a polymer fiber net, and evaluate the NP-fiber net concept for in situ treatment of HAB cyanobacteria and cyanotoxin. Our approach specifically focused on non-toxic titanium dioxide (TiO_2) and iron oxide (Fe_2O_3) as the catalyst materials, and our preliminary results from this past year suggest that our approach works through a dual flocculation / catalytic degradation mechanism. Our initial studies over the past year focused on understanding the performance of the catalytic materials alone with future work to include and progress to catalyst-immobilized fiber materials.

As mentioned, HABs are a global phenomenon; therefore, Arkansas is also affected by the devastating consequences of HABs. One study showed the mortality event of certain types of catfish located in Mississippi, Alabama, Arkansas, and Louisiana ponds linked to microcystin-LR poisoning (Zimba et al., 2001). Microcystin-LR was detected in water samples and in catfish liver tissue, and fish were killed within 24 hours of being exposed to toxic bloom-infested pond water (Zimba et al., 2001). Given the prevalence and importance of surface waters in the state of Arkansas for human recreation, environmental health, fresh water supply, and municipal and industrial development, the occurrence of HABs has a direct impact on Arkansas state economic vibrancy and environmental health.

Methods

Microcystis aeruginosa *Growth and Preservation*

M. aeruginosa (strain #2386) in suspension was obtained from the UTEX algae center at the University of Texas, Austin, and maintained in autoclaved BG-11 medium as instructed. Flasks of *M. aeruginosa* were set near a window, allowing for adequate sunlight. The growth of *M. aeruginosa* was monitored by measuring the optical density at 680 nm. Fresh BG-11 medium was supplemented into existing culture every 21 days to maintain algal growth (UTEX Culture Collection of Algae, 2009).

Microcystin-LR Stock Preparation

A 500 μg film of microcystin-LR (MC-LR) was purchased from Cayman Chemical, and the film was dissolved in methanol to obtain a 500 mg/L concentration of MC-LR. Five mg/L MC-LR stock solutions were made by diluting with deionized water. From 5 mg/L stock solutions, 1 mg/L solutions were created, which minimize freezing and refreezing of MC-LR samples. All samples were frozen and stored at -20˚C in a freezer.

Experimental Protocol for Microcystis aeruginosa

NP treatment impacts were discerned by adding different concentrations of TiO_2 and Fe_2O_3 to *M. aeruginosa* suspended cell solutions. Both NPs were prepared by Dr. Greenlee's lab at the University of Arkansas and the stock solutions of 1 mg/L concentration were used. Prior to each experiment, cell morphology and concentration were assessed using a Nikon NiE upright light microscope and a Beckman Multisizer 4 Coulter counter, respectively. Ten-mL samples were prepared in 15 mL centrifuge tubes with *M. aeruginosa* diluted in phosphate buffer saline (PBS) at a 1:10 ratio. After samples were prepared, centrifuge tubes were gently vortexed to encourage even cell distribution throughout the PBS. Samples of the supernatant were taken for cell concentration measurement prior to NP addition, measuring initial concentrations of *M. aeruginosa* in cells/mL with diameters ranging from 2.5 to 4 μm. All cell concentration measurements were taken using the Coulter counter, prepared in 20 mL accuvettes with 20 μL added of supernatant to 10 mL of Isoton III Diluent as the electrolyte. Prior to being added to the accuvette, the electrolyte was filtered using a 0.22 μm syringe filter. The 20 μm aperture tube was

used for Coulter counter readings, and the Coulter counter was operated using the volumetric operating mechanism. After initial cell concentrations were recorded in all samples, NPs were vortexed to evenly mix them. Treatment amounts of NPs were then added to each tube, with no NPs added to the two control tubes (Table 1). Cell concentration was again measured three hours following NP addition and once every 24 hours for three days after NP addition using the Coulter counter. Throughout the experiment, samples were left sitting upright in a 15 mL centrifuge holder near the window.

All experiments were conducted in duplicate. Results were analyzed using Z-score (Equation 1), which considers values greater than an absolute value of 2 to suggest significant differences (alpha=0.05); because this analysis involved decreasing concentrations over time, Z-scores less than -2 were considered significant cell removal. In the Z-score calculation, X represents the individual cell concentration of each tube, $\bar{X}$ represents the average cell concentration of all tubes prior to treatment, and S represents the sample standard deviation of all tubes prior to treatment.

Equation 1. Z-Score Calculation, $Z=(X - \bar{X})/S$

Finally, percent of cells removed was calculated by subtracting the final cell concentration of each tube from the initial average cell concentration of all tubes in the experiment. This value was then divided by the initial average cell concentration of all tubes in the experiment and multiplied by 100. Duplicates of percent cell removal were then averaged to gain percent cell removal of each treatment in each experiment.

Experimental Protocol for Microcystis-LR

The impacts of TiO_2 NP on MC-LR were investigated through water, MC-LR, and TiO_2 batch experiments. Commercial AEROXIDE TiO_2 P 90 NP with an average particle size of 14 nm were used due to high surface area and mixed crystal structure, which contribute to good photocatalytic activity. Concentrations of 0.5 or 1 g/L stock solutions of TiO_2 were made prior to each experiment and vortexed to ensure suspension of NPs. Before each experiment, 1 mg/L MC-LR stock solutions were thawed. Five-20 mL batches of water, MC-LR, and TiO_2 were added to quartz crystal Erlenmeyer flasks and capped with stoppers. Starting MC-LR and TiO_2 concentrations were 200 μg/L and 0.25 g/L, respectively. Batches were mixed with an orbital shaker at 100 rpm for a maximum of 60 minutes. Control experiments excluded TiO_2 NPs and/or ultraviolet (UV) light. For UV experiments, a UV lamp was the source of UV-A light with a wavelength of 365 nm and an intensity of 230 $\mu W/cm^2$ at 3 inches. Samples collected during experiments were centrifuged at 7500 rpm for roughly 20 minutes and added to vials for liquid chromatography–mass spectrometry (LC-MS) analysis. Standard concentration points of 200, 150, 100, 50, 10, and 0 μg/L MC-LR were made for each experimental sample set and standard curves were plotted.

Table 1. Experimental design for NP treatment on *M. aeruginosa.*

Treatment	Concentration NPs (mg/L)	Tube Number
Titanium Dioxide – low concentration	25	F1, F2
Iron (III) Oxide – low concentration	25	F3, F4
Titanium Dioxide – high concentration	50	F5, F6
Iron (III) Oxide – high concentration	50	F7, F8
Control	0	F9, F10

Initially, Amicon Ultra-4 regenerated cellulose centrifugal filter units with 3 and 10 KDa pore sizes were utilized to filter out NPs from samples prior to LC-MS analysis. Filtration ensured NP removal as a precaution for LC-MS instrumentation. Control experiments indicated that the concentration of MC-LR decreased significantly once MC-LR was centrifuged with the filter units. Syringe filters with 0.2 μm pore size made of PVDF also showed decreased MC-LR starting concentration after filtration of samples. The method for NP removal was modified due to filter unit complications. Samples were centrifuged in 15 mL centrifuge tubes for 20 minutes, and TiO_2 pellets accumulated on the side of the container. The supernatant was sampled.

Controls tested included no NPs, no UV light, UV light + no NPs, and no UV light + NPs. Adsorption of MC-LR to NPs was investigated by a constant 200 μg/L MC-LR concentration and varied TiO_2 concentrations.

Results and Discussion

Cell Morphology

M. aeruginosa cell morphology was confirmed using 100x oil emersion light microscopy (Figure 1). Cells were moving rapidly throughout the media; only *M. aeruginosa* cells were seen in the sample.

Flocculation

Preliminary experimentation revealed flocculation as the main method of algal removal. This is illustrated in Figure 2, which shows cells prior to treatment (left), after TiO_2 NP treatment (center), and after Fe_2O_3 NP treatment (right). The untreated sample showed cell movement, while the treated samples showed little movement of cells.

Flocculation was further evident in results through Coulter counter measurements, showing a decrease in cell concentration of the supernatant. When *M. aeruginosa* was treated with varying concentrations of NPs, it was revealed that increased concentrations lead to faster flocculation. All four high-concentration NP treatments showed significant reduction in cell concentration by 24 hours, while only

one replicate in the low-concentration iron NP treatment achieved the same (Table 2, Figure 3).

Concerning the effectiveness of the NP treatments, percent cells removed at 72 hours for each treatment is listed in Table 3. Despite these high removal rates, no samples achieved enough algae removal for the concentration to fall below 20,000 cells/mL, which is the threshold the EPA specifies as low probability of human health risk (D'Anglada, n.d.). Cell removal could likely be improved by increasing concentration or amount of time treated.

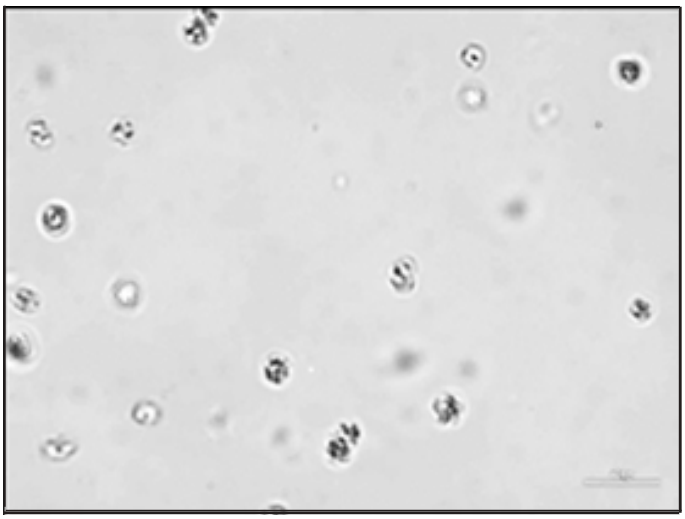

Figure 1. *M. aeruginosa* cell morphology, 10 μm scale bar.

LC-MS Analysis of MC-LR

The standard MC-LR concentration points 200, 150, 100, 50, 10, and 0 μg/L for Standards 1, 2, 3, and 4 from LC-MS analysis were used to calculate an average standard curve for experiments. Table 4 shows the multiple-reaction monitoring (MRM) LC-MS peak area data used to plot an average standard curve seen in Figure 4. Standard points are consistent between experimental runs.

MC-LR + water, MC-LR + water + 0.25 g/L TiO_2, MC-LR + water + UV light, and MC-LR + water + 0.25 g/L TiO_2 + UV light control experiments were tested over a 60 minute time period. Only MC-LR + water and MC-LR + water + UV light controls are shown in Table 5 since NP controls were tested with centrifugal filter units. Filter units affected MC-LR concentrations and produced inconclusive results. The microcystin-LR concentrations were reduced by 5.7% and 7.43% for the MC-LR + water and MC-LR + water + UV light controls, respectively. There is no significant evidence of MC-LR degradation by these control parameters.

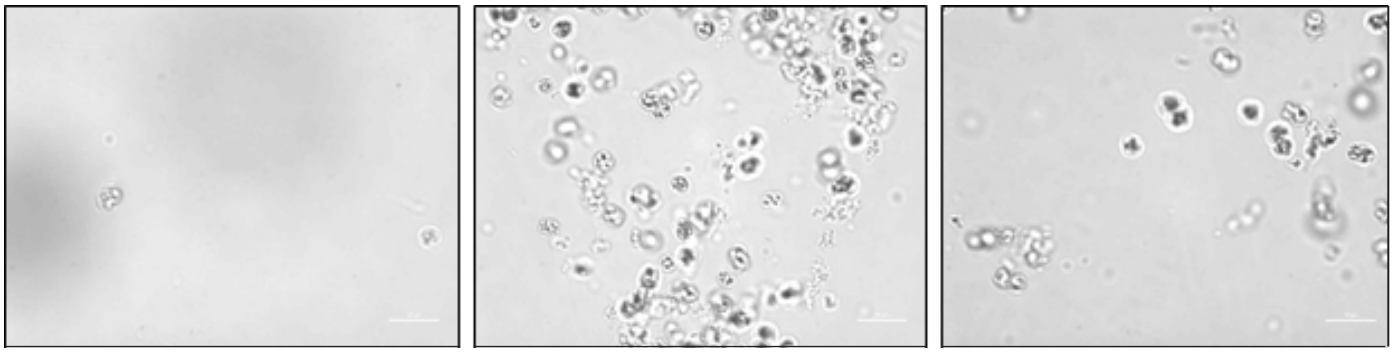

Figure 2. Images of *M. aeruginosa* prior to dilution in PBS and NP treatment (left), after TiO_2 NP treatment (center), and after Fe_2O_3 treatment (right). The scale bar is 10 μm.

Table 2. Z-score over cell concentration change after treatment of varying concentrations; significant reduction in cell concentration is represented in bold text.

Treatment	Hours Elapsed:	3	24	48	72
Titanium Dioxide – low concentration	Tube 1 z-score	0.49	-0.53	**-2.97**	**-3.11**
	Tube 2 z-score	0.69	0.32	0.47	**-3.11**
Iron (III) Oxide – low concentration	Tube 3 z-score	-0.35	**-2.1**	-1.89	**-2.68**
	Tube 4 z-score	**-6.46**	-0.8	-0.03	-1.84
Titanium Dioxide – high concentration	Tube 5 z-score	0.53	**-3.36**	**-2.17**	**-3.04**
	Tube 6 z-score	0.45	**-3.04**	**-2.1**	**-4.3**
Iron (III) Oxide – high concentration	Tube 7 z-score	1.02	**-3.36**	**-3.04**	**-4.11**
	Tube 8 z-score	0.69	**-3.81**	**-3.95**	**-4.3**
Control	Tube 9 z-score	0.45	0.81	0.19	-0.08
	Tube 10 z-score	0.47	6.68	0.26	0.42

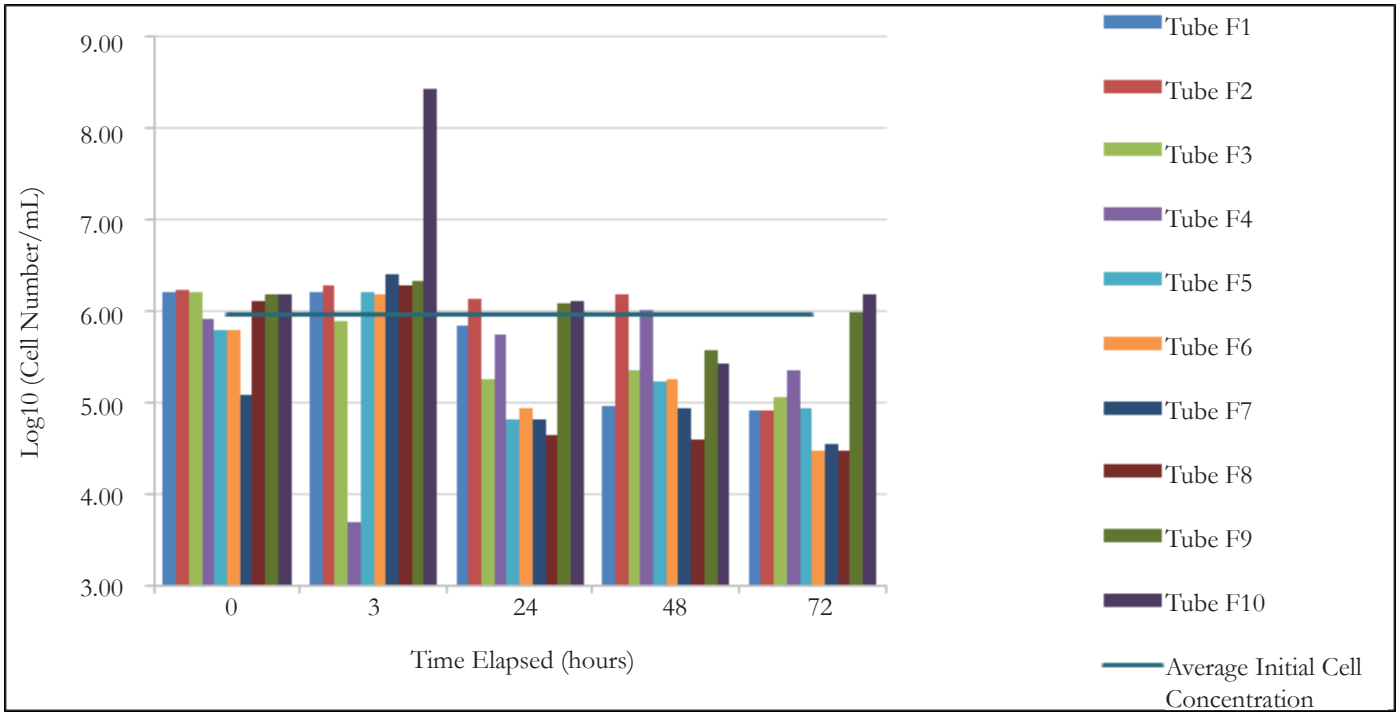

Figure 3. Cell concentration change over the treatment time. Tubes 1 and 2 were treated with the low concentration of TiO_2, tubes 3 and 4 were treated with the low concentration of Fe_2O_3, tubes 5 and 6 were treated with the high concentration of TiO_2, tubes 7 and 8 were treated with the low concentration of Fe_2O_3, and tubes 9 and 10 were the control.

Adsorption testing with varying TiO_2 concentrations and a constant 200 µg/L MC-LR concentration also produced inconclusive results due to centrifuge filtration units. Additional experiments have shown that MC-LR is not removed significantly by TiO_2 without UV light activation, suggesting adsorption is not a primary removal mechanism and that UV activation of catalytic activity is necessary for MC-LR removal.

Table 3. Percent algae removed from each treatment.

Treatment	Percent Algae Removed
Titanium Dioxide – low concentration	93.10%
Iron (III) Oxide – low concentration	85.10%
Titanium Dioxide – high concentration	95.00%
Iron (III) Oxide – high concentration	97.20%

Conclusions

Results this far have allowed us to discover that HAB cyanobacteria are deactivated even in the absence of light (i.e., no UV activation of catalyst activity), which suggests a flocculation mechanism for deactivation. Experiments on MC-LR have demonstrated repeatable quantitative standard curves, no adsorption of MC-LR onto TiO_2 NPs and significant adsorption of MC-LR onto some experimental materials, motivating changes in experimental procedure. Results also suggest that removal of MC-LR requires a catalytic function, in our system initiated by UV light. Based on our results thus far, we recommend further investigation of the catalyst-fiber composite for HAB-cyanotoxin mixed contaminant scenarios to evaluate the dual flocculation-catalytic degradation mechanism. We also recommend a focus on

Table 4. Multiple-Reaction Monitoring (MRM) Liquid Chromatography–Mass Spectrometry Peak Area for Standards 1, 2, 3, 4.

	Standard 1	Standard 2	Standard 3	Standard 4		
Microcystin-LR Concentration (µg/L)	Multiple-Reaction Monitoring (MRM) Chromatogram Peak Area				Average MRM Peak Area	Standard Deviation MRM Peak Area
10	4,734	6,269	5,451	5,990	5,611.03	585
50	28,220	33,134	26,979	30,467	29,700.04	2,344
100	59,339	68,557	52,398	59,936	60,057.45	5,733
150	86,055	101,592	78,510	90,531	89,172.00	8,359
200	110,275	138,010	102,819	124,187	118,822.80	13,473

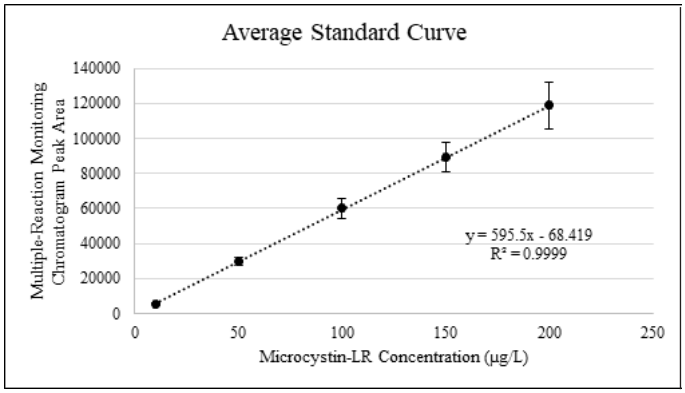

Figure 4. Microcystin-LR Average Standard Curve for Standards 1, 2, 3, and 4 with Standard Deviation Error.

Table 5. Microcystin-LR + water (no UV light + no TiO_2) and microcystin-LR + water + UV light (no TiO_2) control experiments.

	MC-LR Concentration (µg/L)	
Time (minutes)	**MC-LR + water**	**MC-LR + water + UV light**
0	193	202
5	186	191
10	183	190
15	181	193
20	187	185
40	189	182
60	182	187

catalyst-immobilized net configuration testing and optimization, as well as testing with real water samples that have been obtained from HAB-contaminated surface water sources. The State of Arkansas directly benefits from this research, where development of technologies based on our concept could enable HAB remediation in lakes without negative environmental impacts. This research also broadly benefits USGS, in that our focus and research will inform the agency on how HABs and associated cyanotoxins might be remediated throughout the US. The fishing net concept could be applied in a wide range of surface water scenarios, which would enable use across regions and general applicability.

Acknowledgements

This material is based upon work supported by the United States Geological Survey under grant agreement No. G16AP00040 and administered by the Arkansas Water Resources Center. The views and conclusions contained in this document are those of the authors and should not be interpreted as representing the opinions or policies of the U.S. Geological Survey.

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