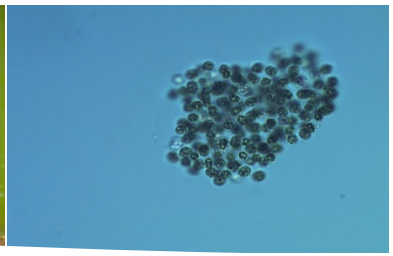
U.S. Environmental Protection Agency, Office of Research and Development

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM




The Impact of Algicide Exposure on Cyanobacterial Responses to Oxidation

Nicholas Dugan
Samantha Smith
Anna Boegehold
Jingrang Lu

Introduction

- Cyanobacteria are a successful phylum of micro-organisms that have flourished for over 1 billion years
- Rising temperatures and nitrogen and phosphorous loadings can cause increased frequency of blooms
- Resource managers work to mitigate these blooms with techniques that include the application of copper-containing algicides

Introduction

- Bloom material can contaminate drinking water treatment facilities
- Potentially toxic cyanobacteria cells are exposed to permanganate (MnO_4^-) oxidation
- Exposure to copper algicides or MnO_4^- imposes pronounced effects on cyanobacterial cells
- What happens when a facility applies MnO_4^- to algicide-treated water pumped from a bloom-impacted reservoir?

Materials & methods

- Microcystin-producing *Microcystis aeruginosa*
- Cyanobacteria at 10^6 cells/L in dechlorinated tap water
- pH = 6, 7, and 8
- $CuSO_4$ dose = 0, 0.1, 0.5, and 1.0 mg Cu/L
- $KMnO_4$ dose = 0 and 1.0 mg/L
- Analyze for:
  - extracellular microcystin and combined (extra + intracellular) microcystin by ELISA
  - extractive chlorophyll-*a*, *in vivo* chlorophyll-*a*, and *in vivo* phycocyanin
  - expression of toxin-producing genes

Materials & methods

Nine trials

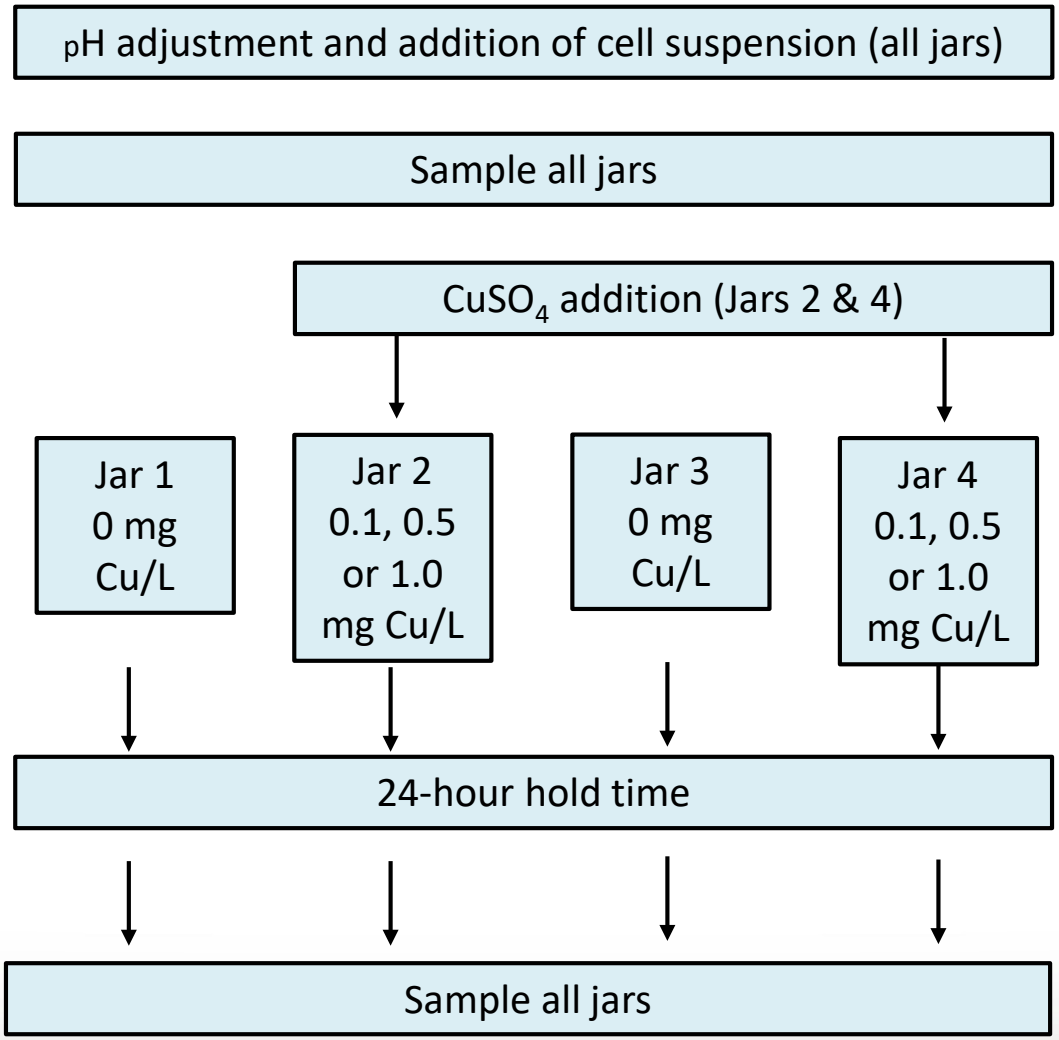
pH	$CuSO_4$	$KMnO_4$
6	0.1	0 & 1
	0.5	
	1.0	
7	0.1	0 & 1
	0.5	
	1.0	
8	0.1	0 & 1
	0.5	
	1.0	

Materials & methods

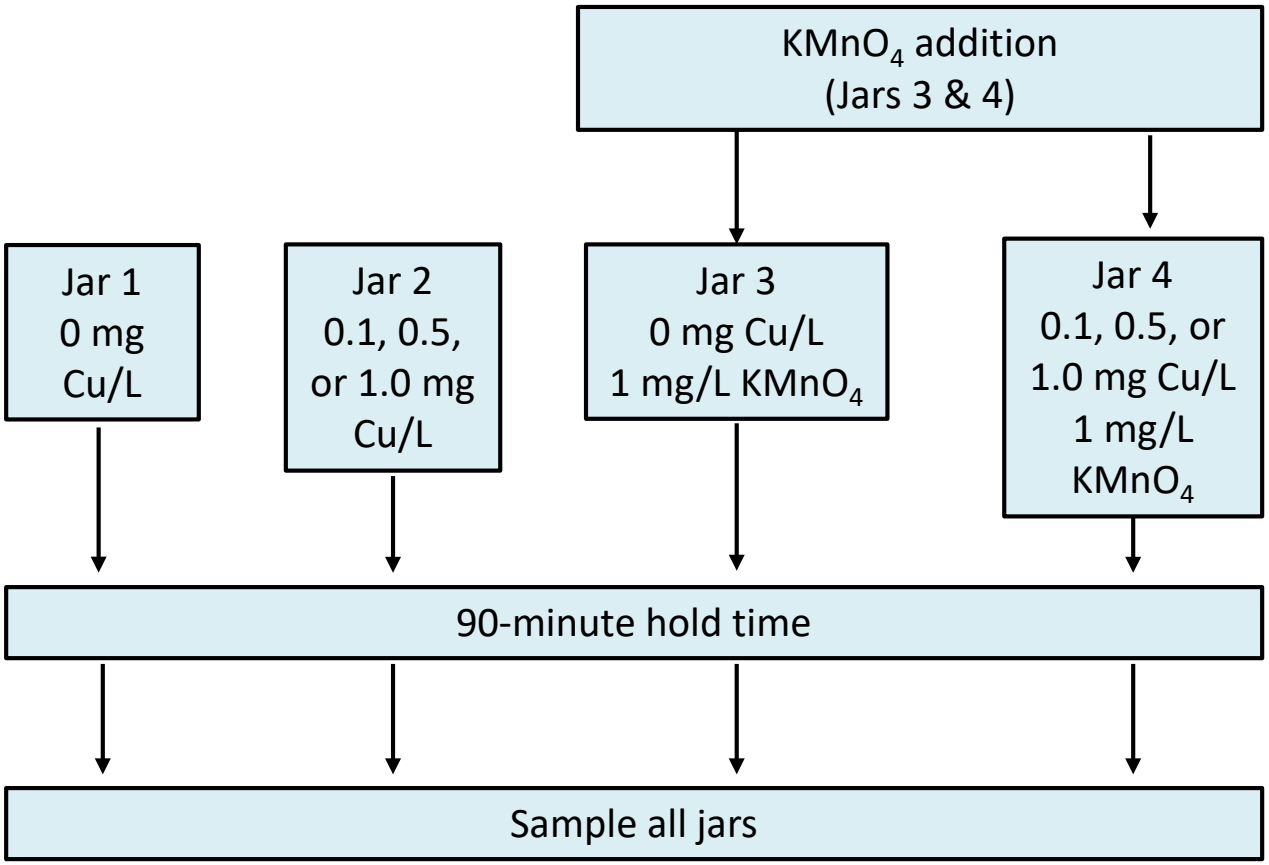
Four jars per trial

Jar	1	2	3	4
$CuSO_4$	-	X	-	X
$KMnO_4$	-	-	X	X

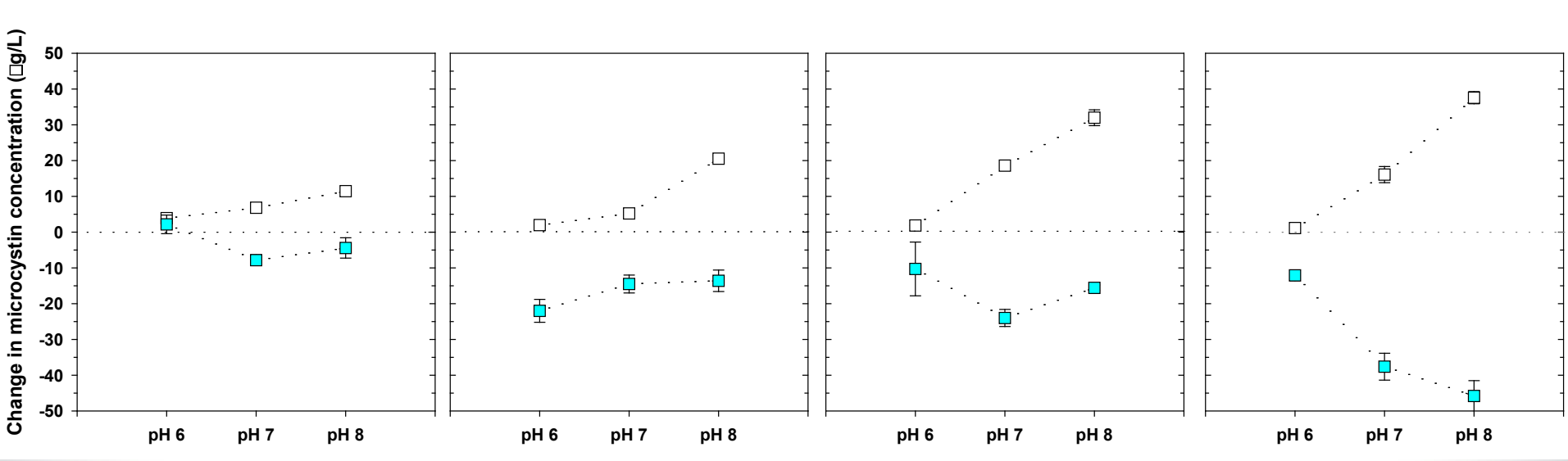
Materials & methods (0 – 24 hours)



Materials & methods (24 – 25.5 hours)

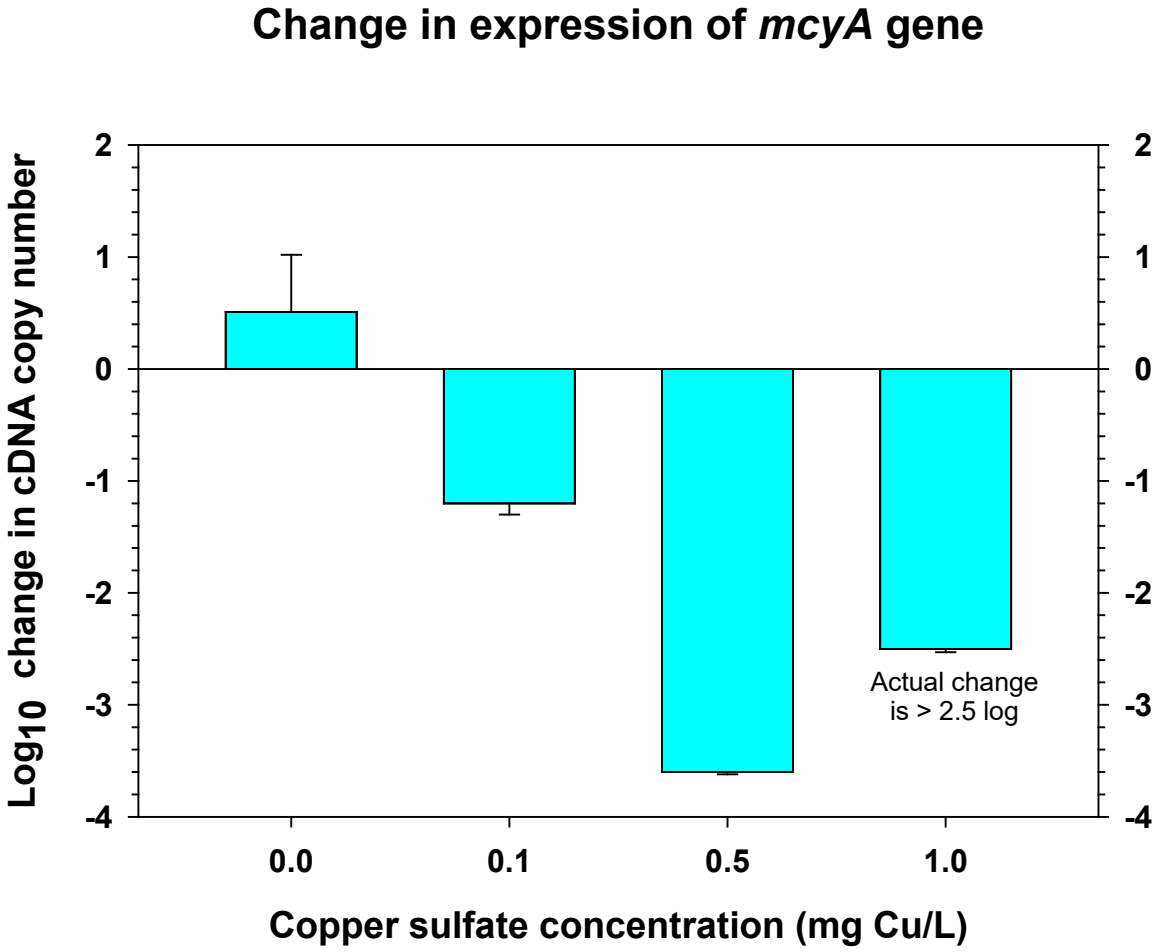


Extracellular & combined microcystin (0 – 24 hours)



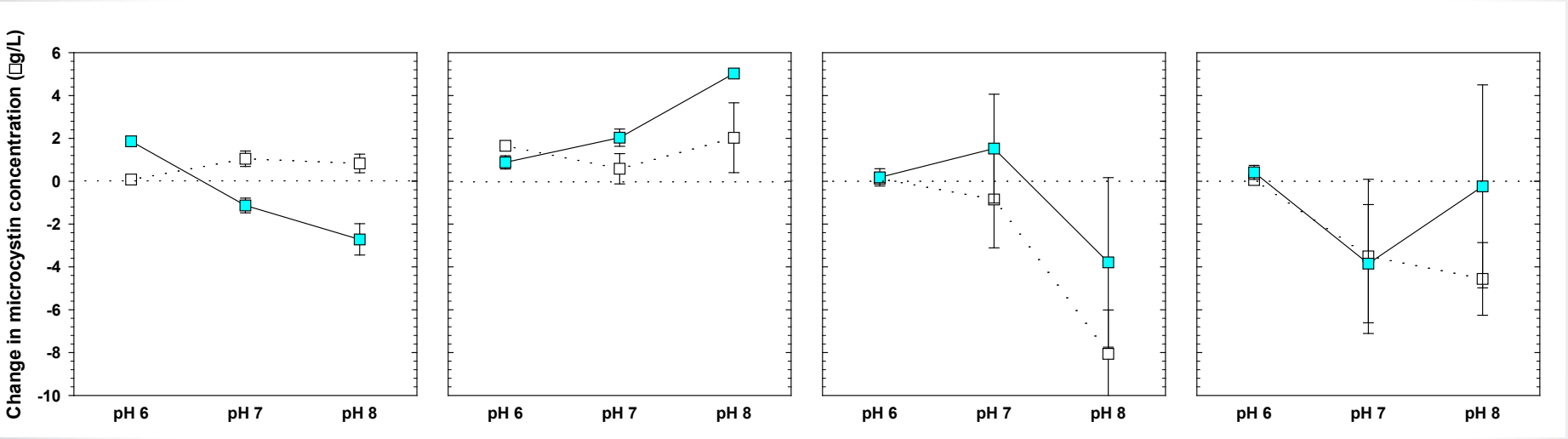
- Extracellular microcystins consistently increased
  - Greatest increases at higher pH
  - At pH 7 & 8, generally greater increases in the presence of copper sulfate
- Combined microcystins consistently decreased
  - Greater decreases in the presence of copper sulfate

Change in expression of gene associated with microcystin production (0 – 24 hours)



Consistent order of magnitude decreases in microcystin gene expression in the presence of copper sulfate

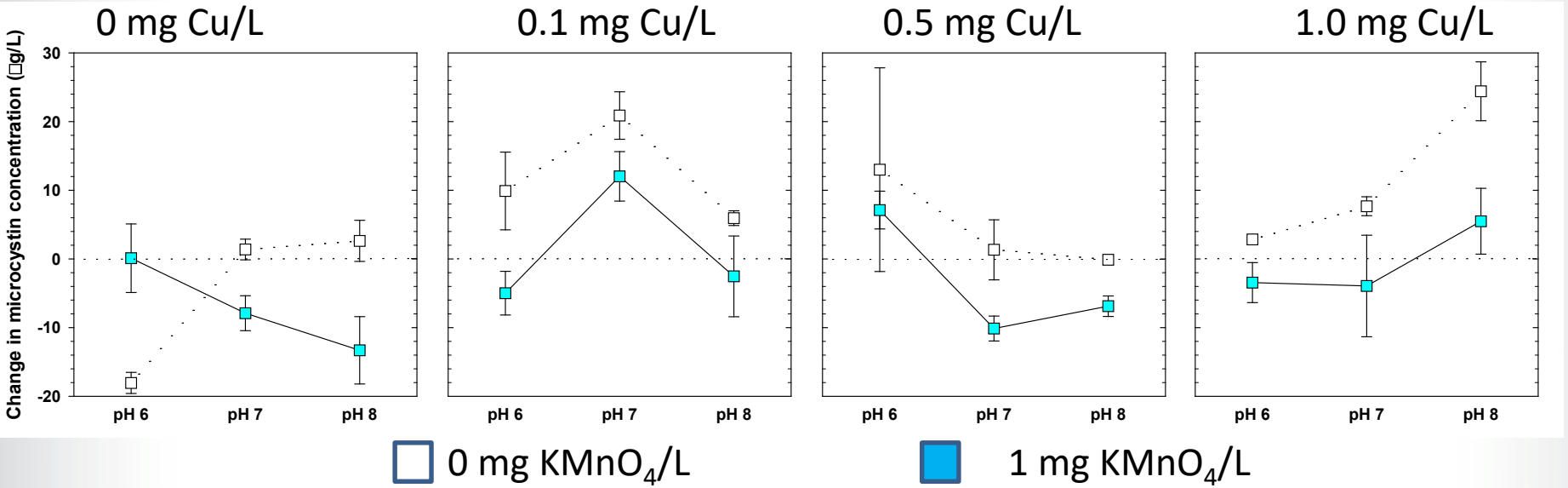
Extracellular microcystin (24 – 25.5 hours)



□ 0 mg $KMnO_4$/L ■ 1 mg $KMnO_4$/L

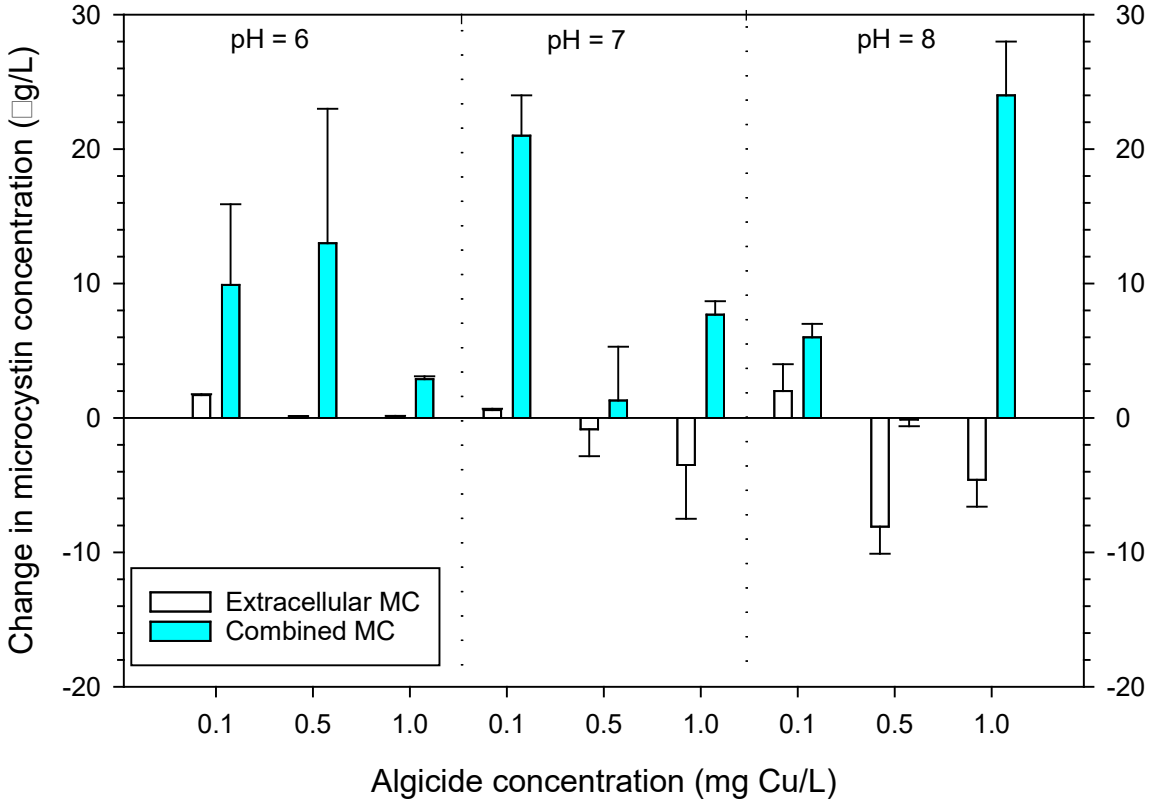
- In general, smallest changes in extracellular microcystins at pH 6
- At pH 7 & 8, in algicide-exposed suspensions, extracellular microcystins generally increased more or decreased less when treated with 1 mg/L $KMnO_4$, compared to 0 mg/l $KMnO_4$

Combined microcystin (24 – 25.5 hours)



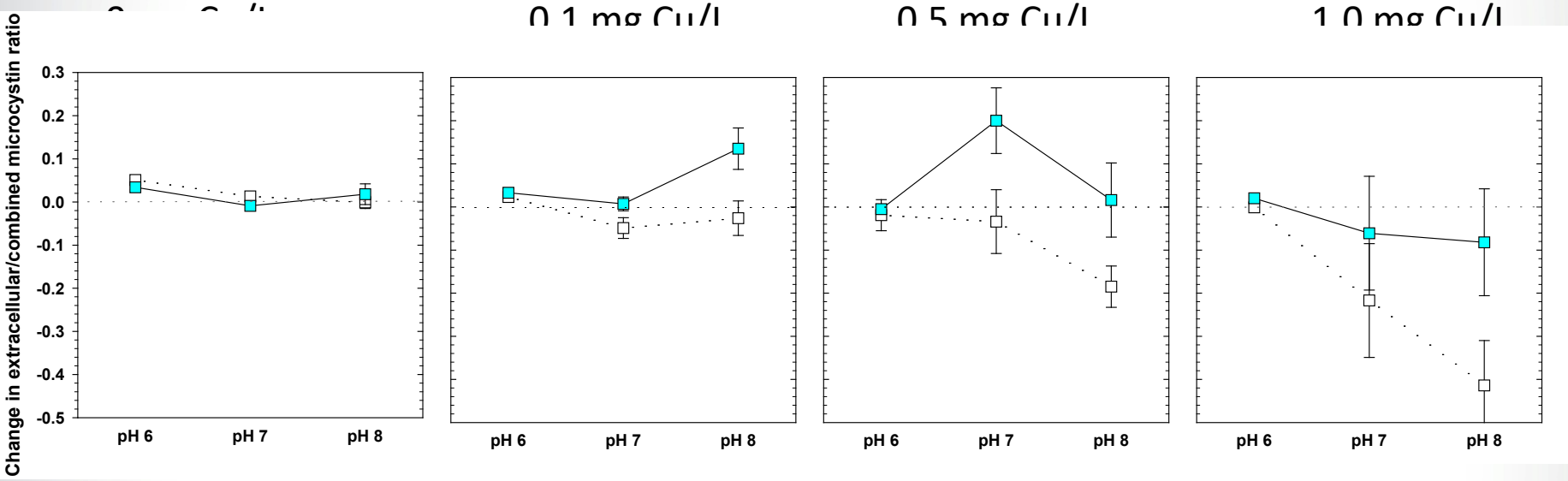
- Except at pH 6 & 0 mg Cu/L, combined microcystins in the absence of $KMnO_4$ remained steady or increased; while combined microcystins in $KMnO_4$ treated suspensions increased less or actually decreased
- Results imply the possibility that intracellular toxin concentrations can recover following algicide exposure and that the recovery can occur over representative time scales

Combined microcystin (24 – 25.5 hours)



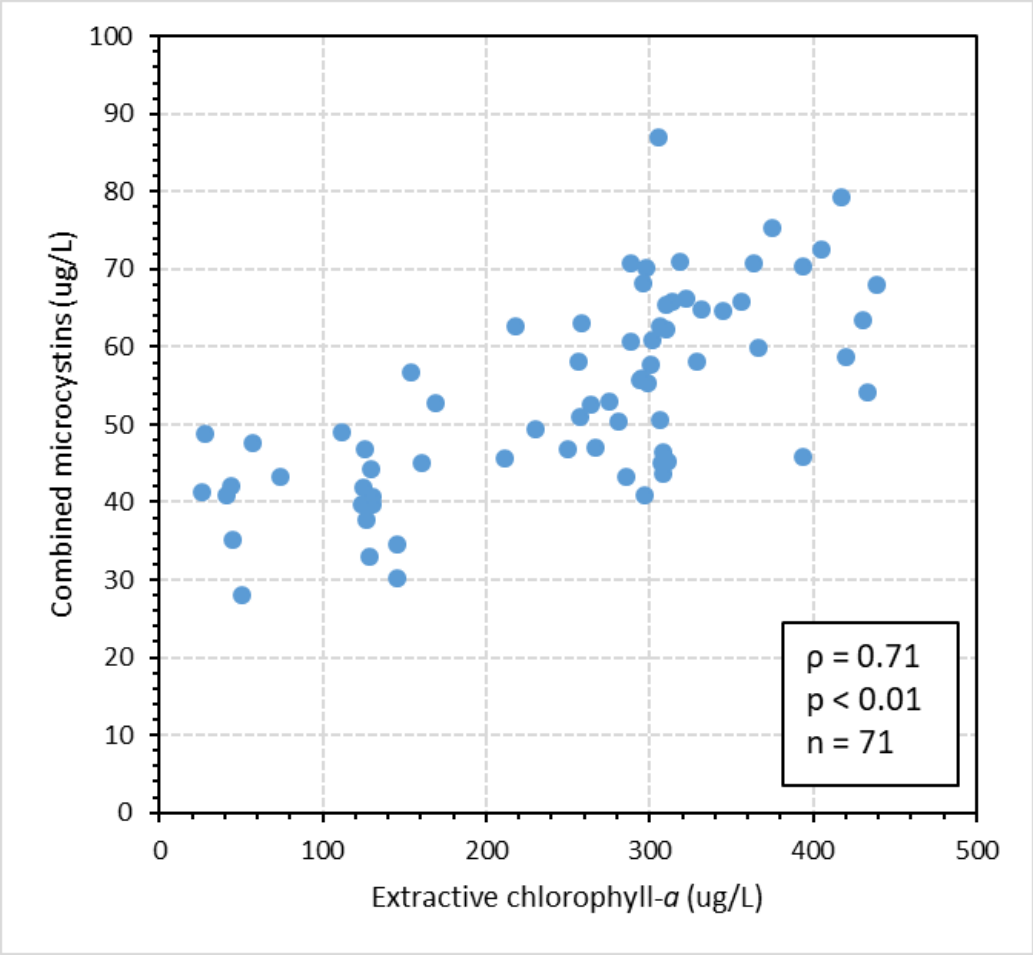
- Results imply the possibility that intracellular toxin concentrations can recover following algicide exposure and that the recovery can occur over time scales that are representative of drinking water treatment

Extracellular/combined microcystin ratio (24 – 25.5 hours)



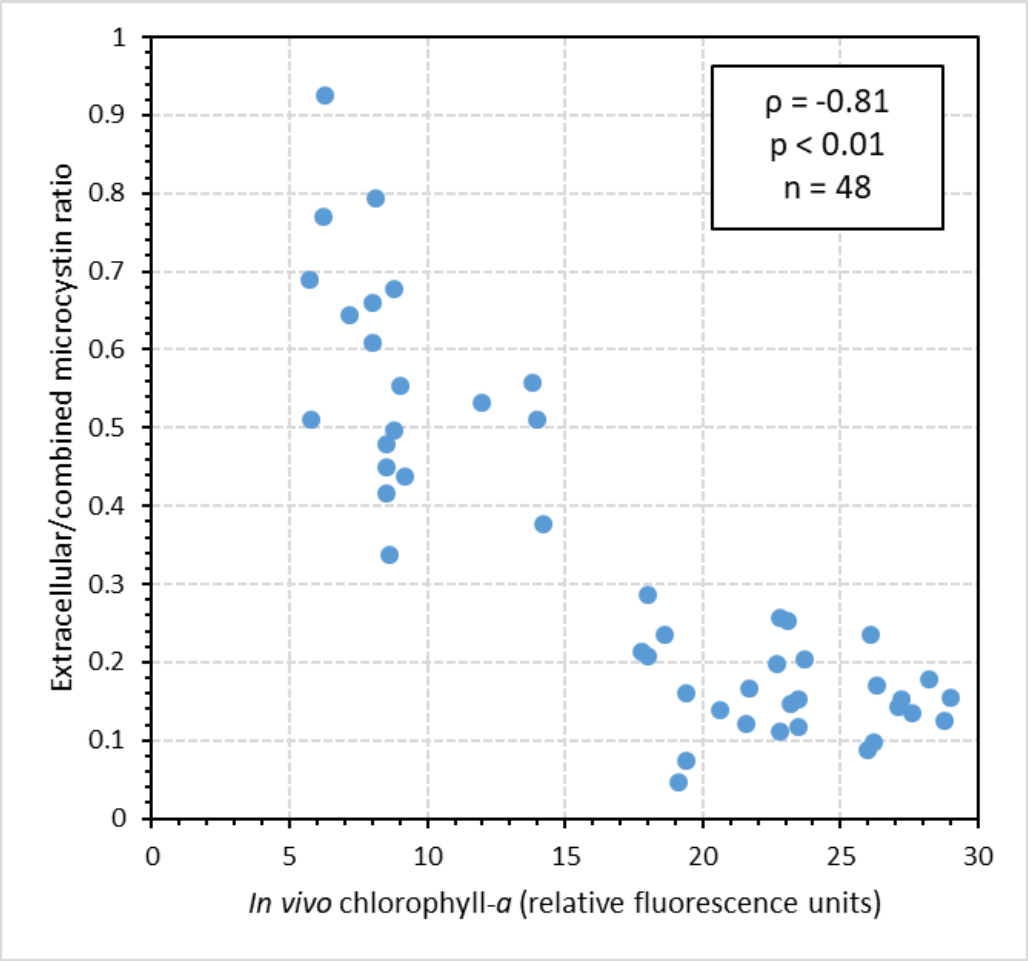
- For suspensions not exposed to $KMnO_4$, the extracellular/combined microcystin ratio remained stable or decreased, and the observed decreases were greater at higher pH levels and algicide exposures
- For suspensions exposed to $KMnO_4$, the extracellular/combined microcystin ratios remained stable, increased, or decreased less than in non-oxidized suspensions

Extractive chlorophyll vs combined microcystins (0 – 24 hours)



The concentration of extractive chlorophyll, an indicator of intracellular pigments, moves in the same direction as the sum of extracellular and intracellular microcystins

In vivo chlorophyll vs extracellular/combined microcystin ratio (24 – 25.5 hours)



In vivo chlorophyll, an indicator of intracellular pigments, moves in the opposite direction of the extracellular/combined microcystin ratio → potentially a rapid indicator of cellular stress

Correlation coefficients, pH 6, 7, & 8 (0 - 24 hours)

	Extracellular microcystin	Combined microcystin	Extracellular/combined microcystin ratio
Extractive chlorophyll-*a*	-0.55 (< 0.01) n = 72	0.71 (< 0.01) n = 71	-0.59 (< 0.01) n = 71
In vivo chlorophyll-*a*	-0.55 (< 0.01) n = 72	0.30 (0.011) n = 71	-0.52 (< 0.01) n = 71
In vivo phycocyanin	-0.14 (0.23) n = 72	0.50 (< 0.01) n = 71	-0.17 (0.16) n = 71

Correlation coefficients, pH 7, & 8 (24 – 25.5 hours)

	Extracellular microcystin concentration	Combined microcystin concentration	Extracellular/combined microcystin ratio
Extractive chlorophyll-*a*	-0.52 (< 0.01) n = 48	0.56 (< 0.01) n = 48	-0.60 (< 0.01) n = 48
In vivo chlorophyll-*a*	-0.78 (< 0.01) n = 48	0.42 (< 0.01) n = 48	-0.81 (< 0.01) n = 48
In vivo phycocyanin	-0.50 (< 0.01) n = 48	0.60 (< 0.01) n = 48	-0.62 (< 0.01) n = 48

Conclusions

- Over the first 24 hours, algicide exposure was associated with increases in extracellular microcystins, decreases in combined microcystins, and decreases in the expression of a gene associated with microcystin production
- Over the final 1.5 hours, non-oxidized suspensions exhibited stable to decreasing extracellular microcystins and stable to increasing combined microcystins → smaller extracellular/combined microcystin ratio → greater relative fraction of toxins inside the cell
- Over the final 1.5 hours, oxidized suspensions tended to exhibit smaller decreases in the extracellular/combined microcystin ratio

Conclusions

- Difference versus non-oxidized suspensions increased at higher algicide doses and pH
- From a drinking water treatment perspective, a greater fraction of toxins inside cells is a favorable situation → higher probability that plant influent toxin burden can be removed through coagulation/flocculation/sedimentation
- Results indicated that extractive and *in vivo* indicators pigment concentrations are strongly correlated with changes in toxin concentrations

Disclaimer

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