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

SAFE AND SUSTAINABLE WATER RESOURCES RESEARCH PROGRAM

Impacts of Early-Stage Drinking Water Treatment on Cyanobacterial Toxin Release and Degradation

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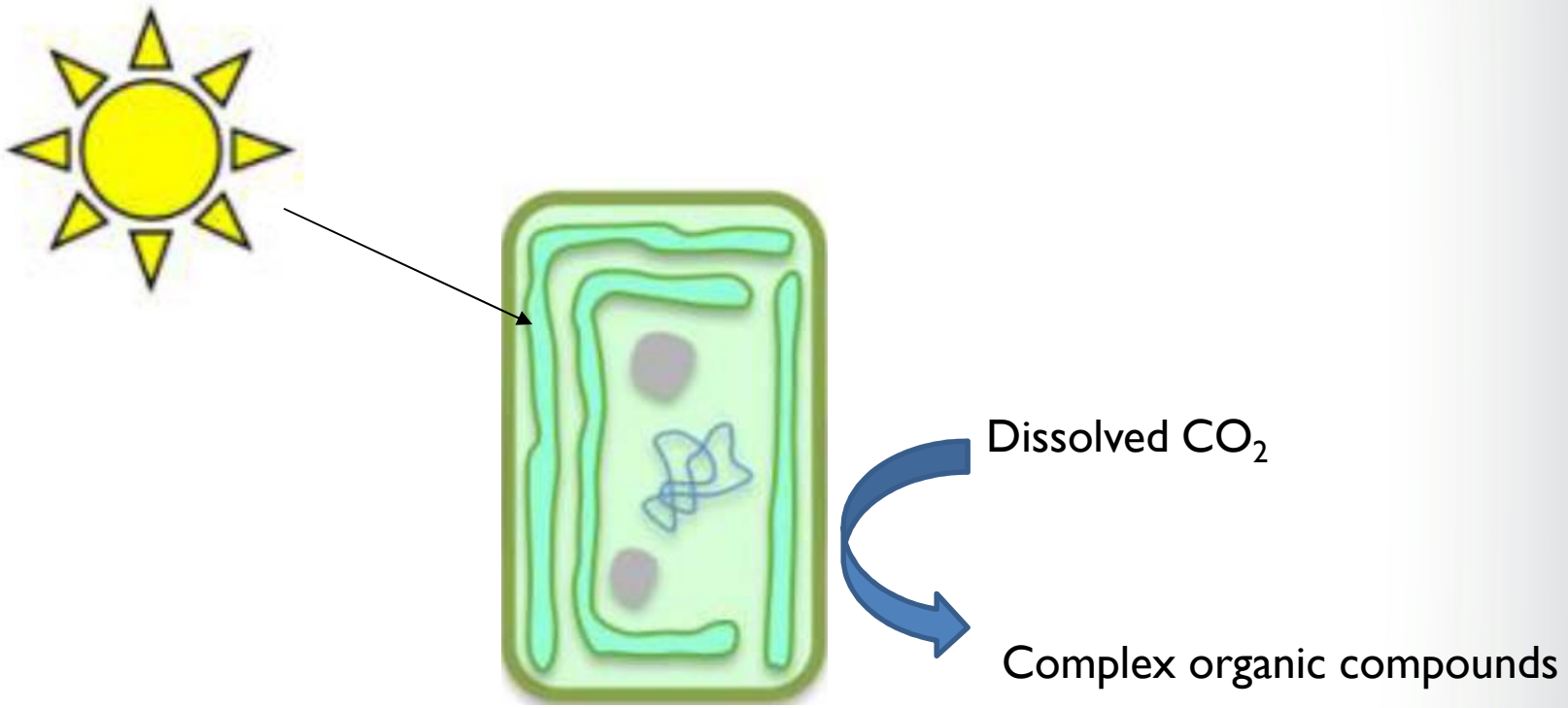
Presentation outline

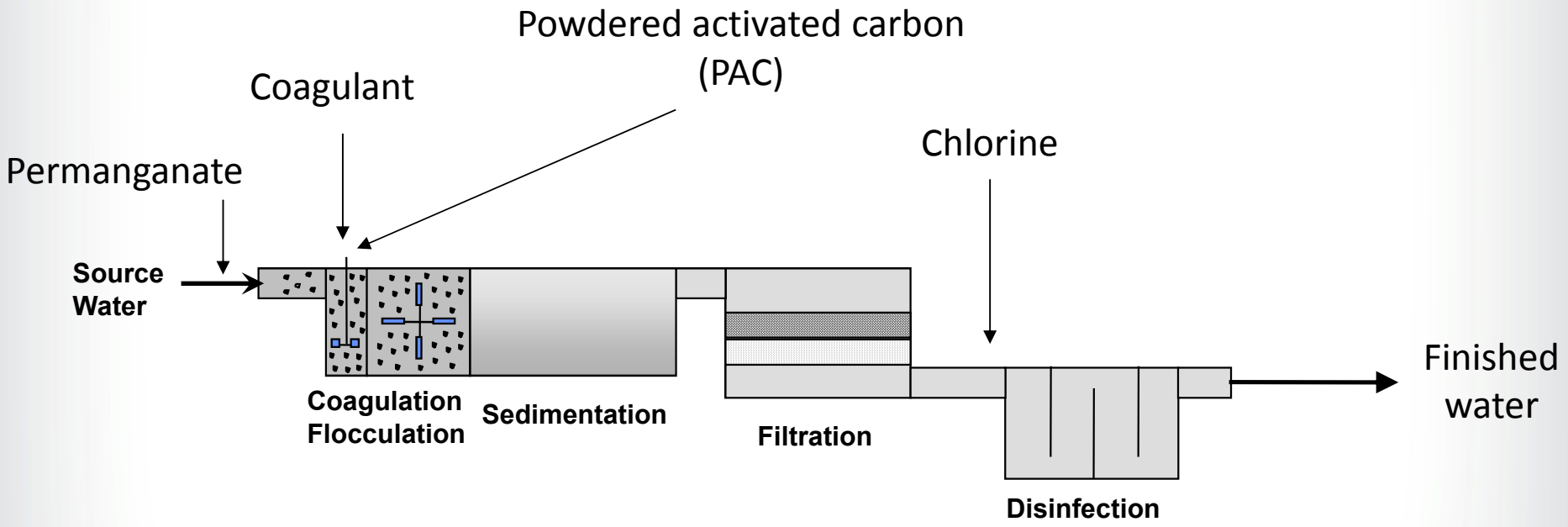
- Background information
- Research goals
- Methods
- Results
- How do our results compare to work performed by other groups?
- How do we interpret and apply the information?

Background: cyanobacteria



Adapted from: Kamennaya, N.A., et al; *Minerals* 2012:2:338-364

Background: surface water treatment process



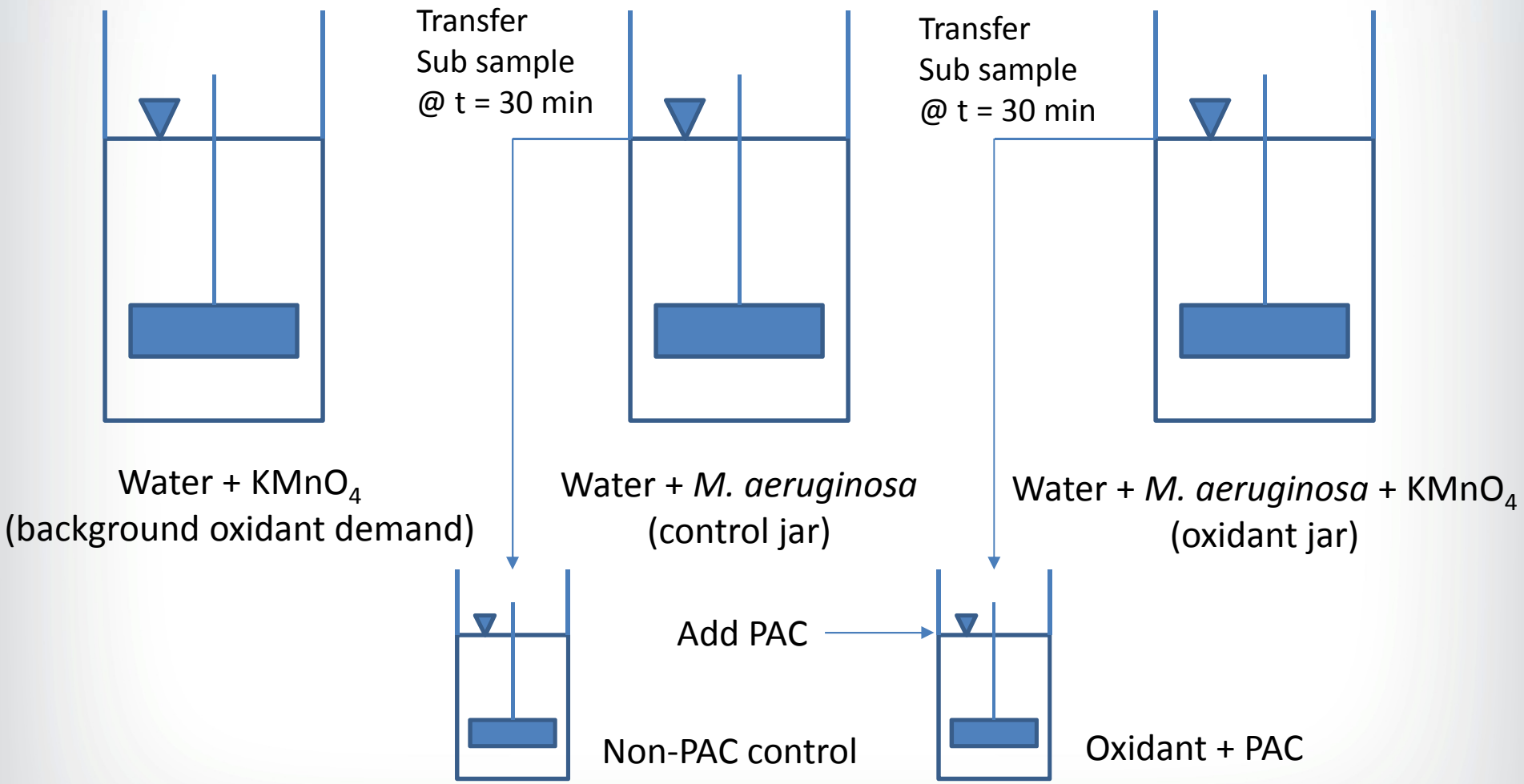
Background: potassium permanganate and powdered activated carbon

- Potassium permanganate ($KMnO_4$):
  - Taste and odor control
  - Control of zebra mussels in intake structures
  - Potentially induces oxidative stress in cyanobacterial cells → may lead to the release of toxins into solution
  - Oxidizes toxins released into solution
- Powdered activated carbon (PAC):
  - Taste and odor control
  - Control of trace organics (herbicides, insecticides, etc.)
  - Adsorbs cyanobacterial toxins released into solution
  - Neutralizes $KMnO_4$

Research goals

- Investigate the interaction of $KMnO_4$, pH, PAC and turbidity on the release and subsequent degradation of cyanobacterial toxins from intact *M. aeruginosa* cells
- $KMnO_4$: 1, 2.5 and 5 mg/L
- pH: 7 and 9
- PAC: 0 and 10 mg/L
- Turbidity: < 0.1, 5 and 20 NTU

Experimental set-up



Three primary jars sampled @ t = 0, 15, 30, 90 minutes. PAC flasks sampled @ t = 90 minutes

Methods

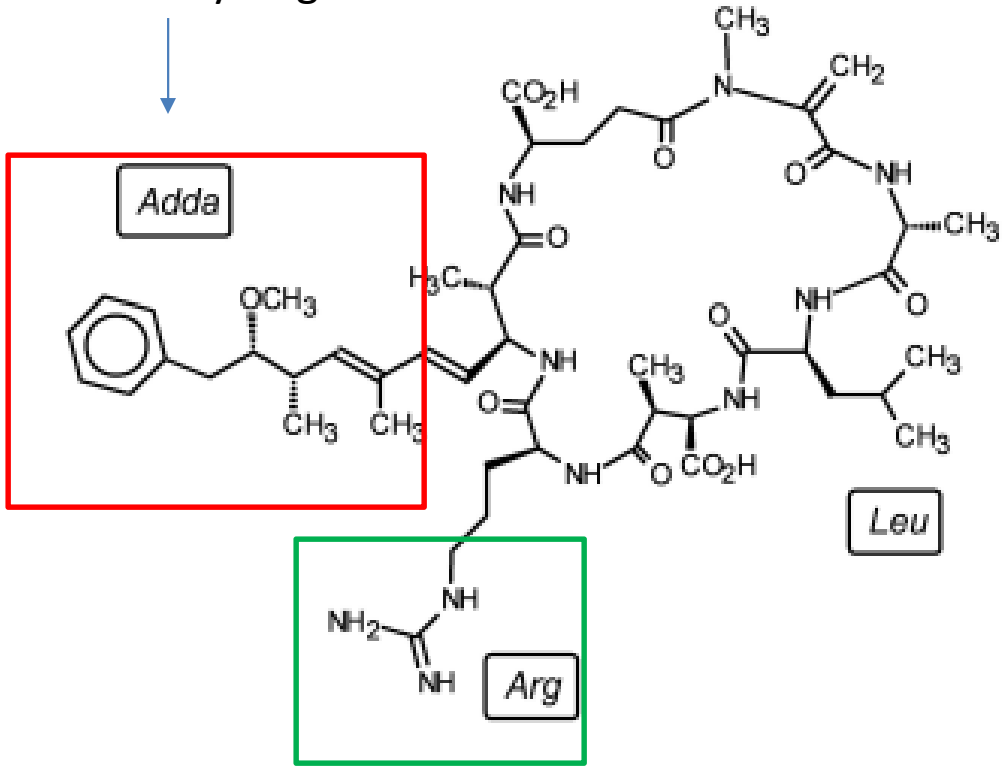
- Microcystins analyzed by enzyme linked immunosorbent assay (ELISA):
  - Total
    - 3 x freeze thaw cycles at -20 °C
    - Centrifuge → analyze supernatant
  - Extracellular
    - Filter through glass fiber filter → analyze filtrate
- Cell membrane status:
  - SYTOX Green stain
  - Permeates compromised cell membranes and binds with DNA
- Chlorophyll-*a*:
  - Filter through glass fiber filter (same filter used for extracellular toxin determination
  - Disrupt filter and cells, extract into organic solvent, measure fluorescence

Methods

- Total cells:
  - Manual count under microscope
  - Hemacytometer
  - 400X magnification
- Phycocyanin:
  - In vivo, non-extractive analysis

Microcystin molecule

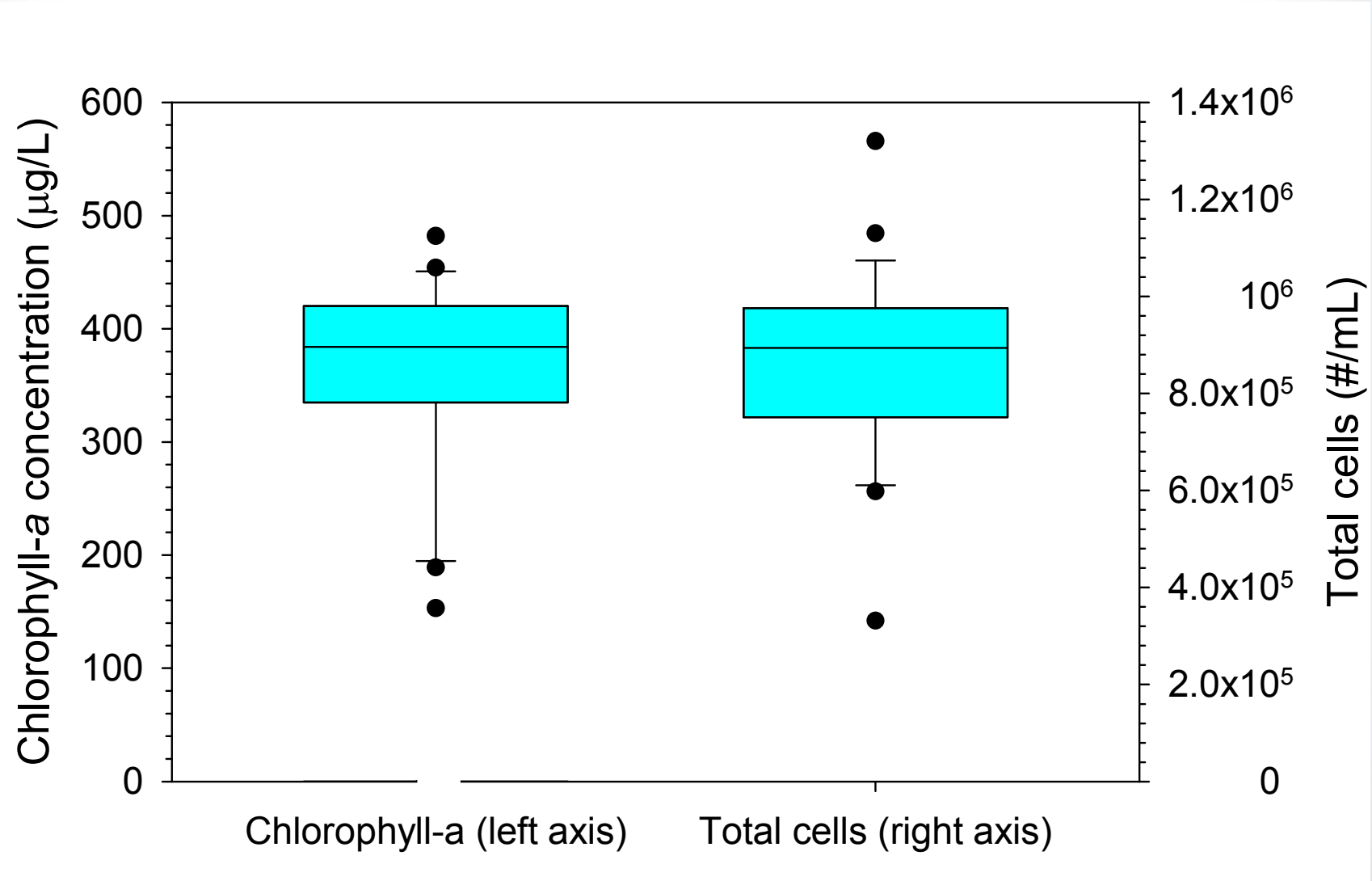
ELISA assay target



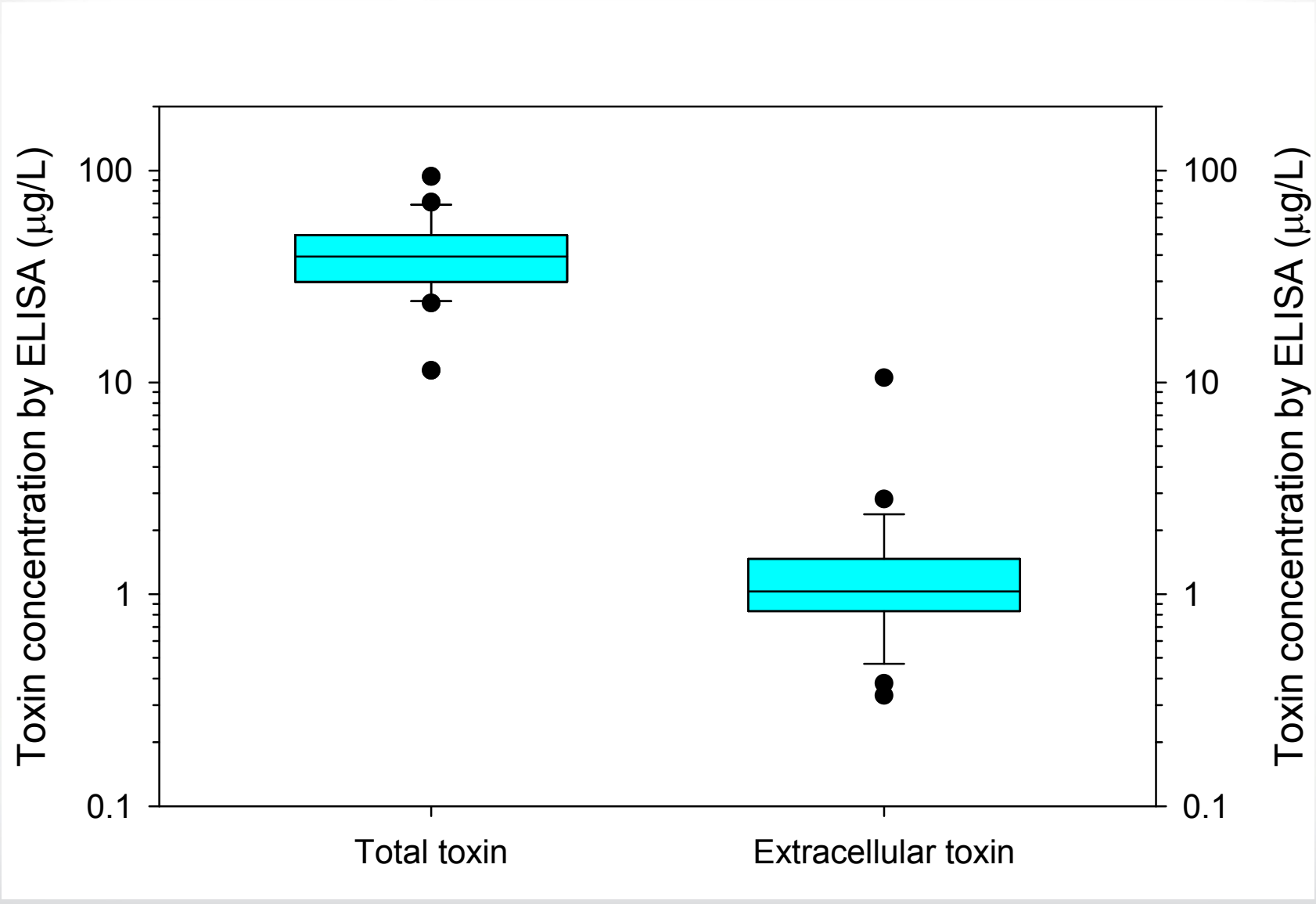
Microcystin-LR

Results

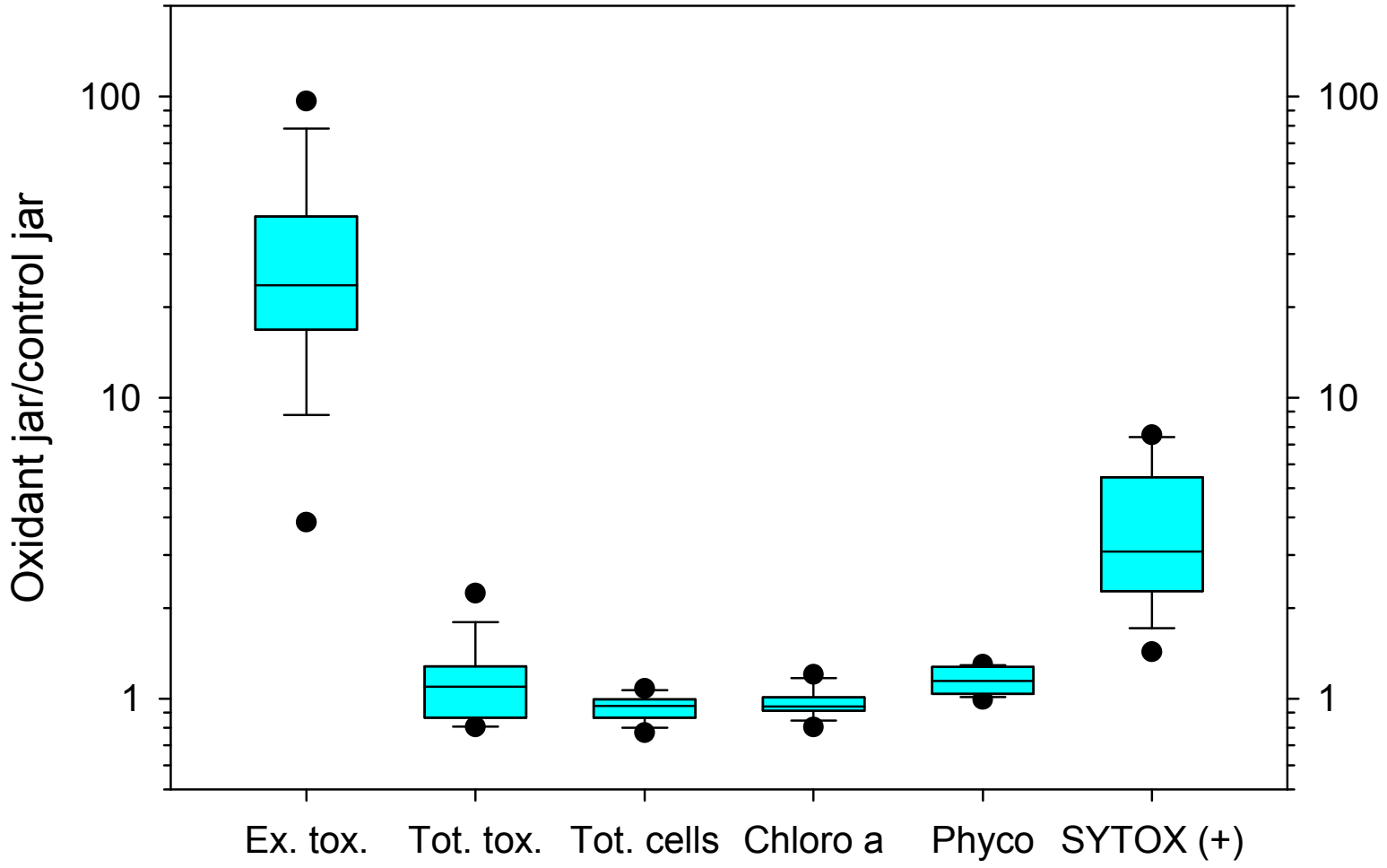
Initial chlorophyll-*a* concentrations and cell titers



Initial total and extracellular toxin concentrations

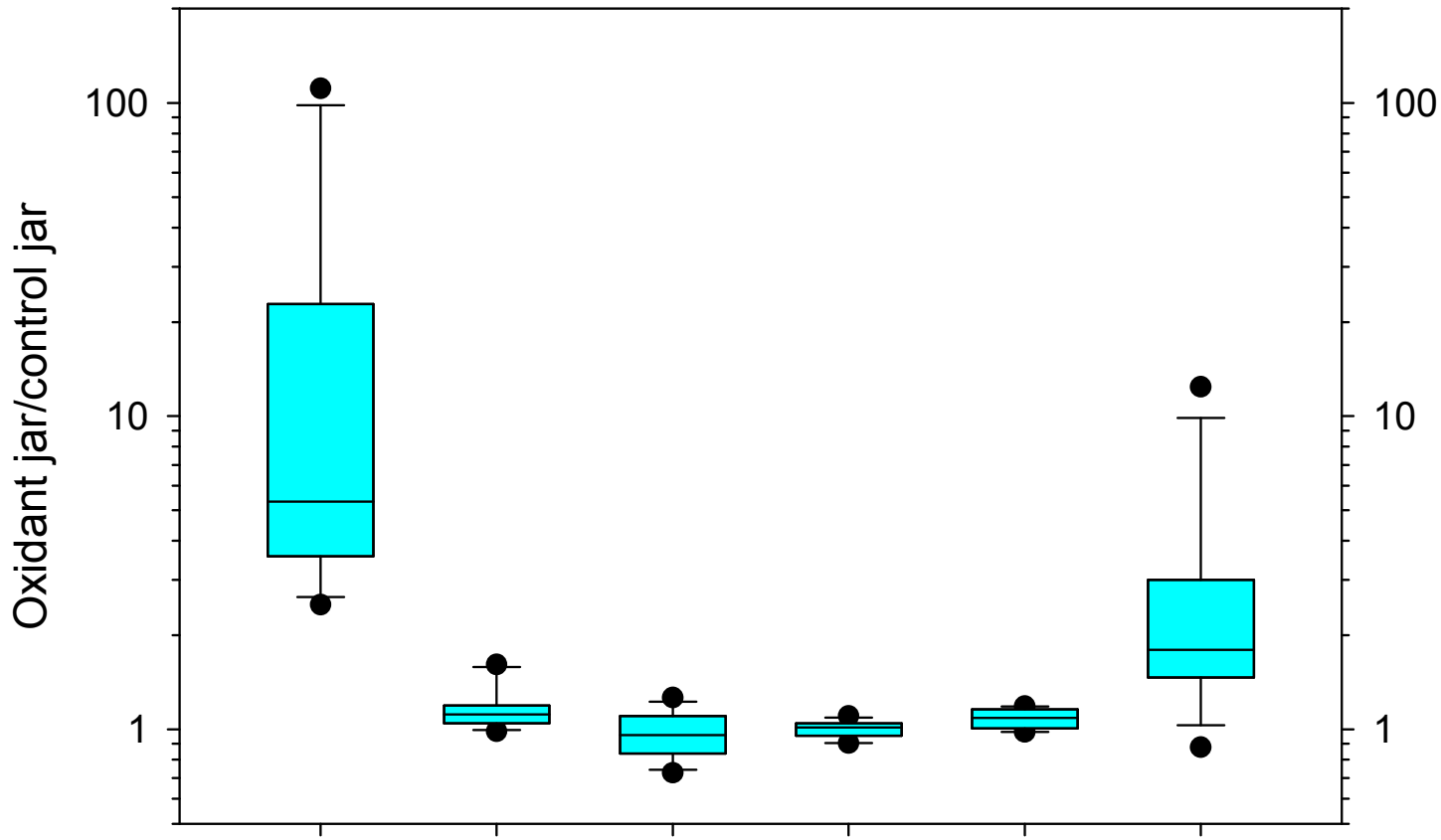


Response for six parameters at pH 7, t = 15 minutes



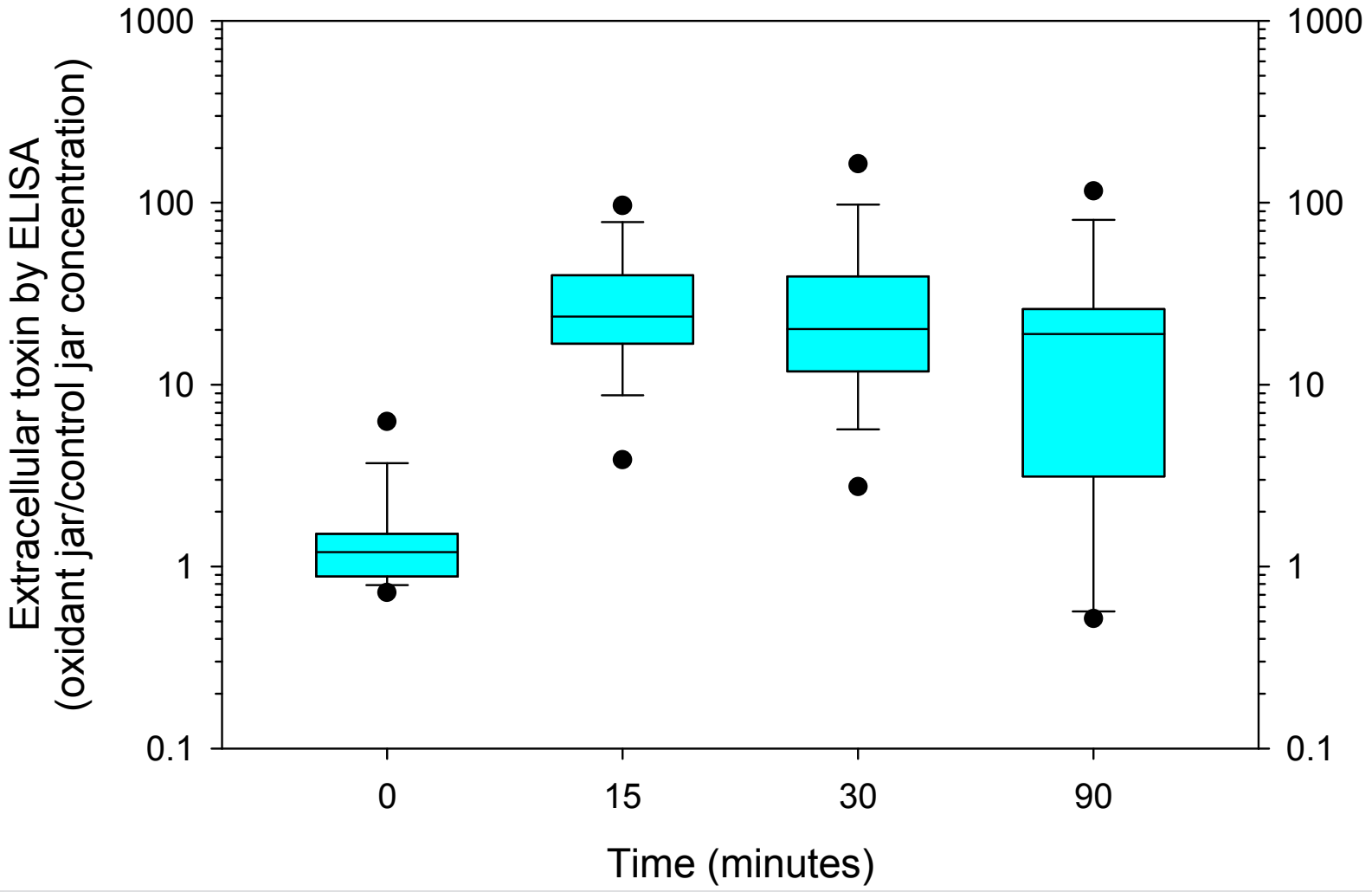
	Ex. tox.	Tot. tox.	Tot. cells	Chloro a	Phyco	SYTOX (+)
Median ratio @ t = 0:	(1.2)	(1.0)	(0.98)	(1.0)	(1.0)	(1.2)

Response for six parameters at pH 9, t = 15 minutes

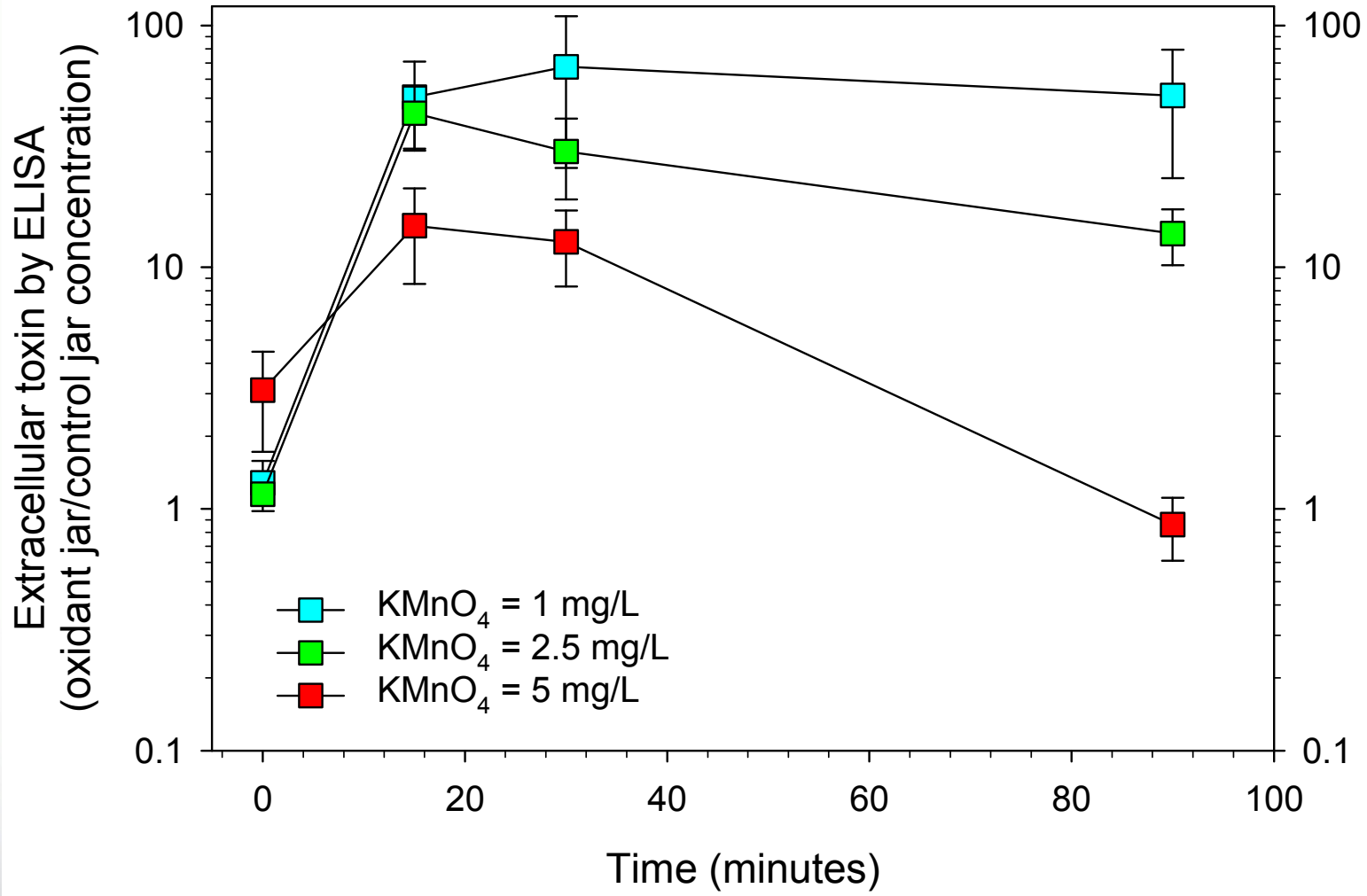


	Ex. tox.	Tot. tox.	Tot. cells	Chloro a	Phyco	SYTOX (+)
Median ratio @ t = 0:	(1.3)	(1.1)	(0.92)	(1.0)	(0.98)	(1.1)

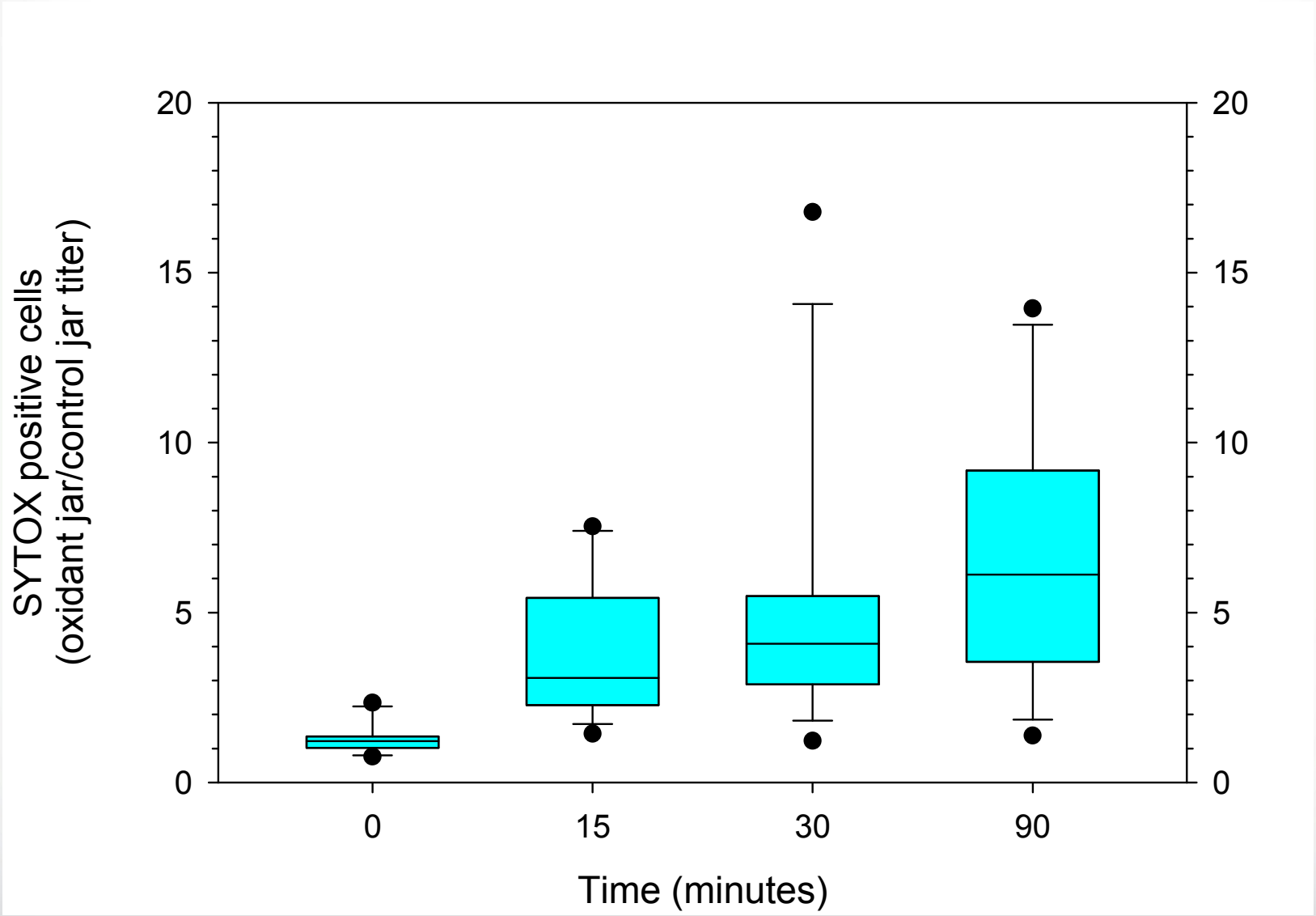
Extracellular toxin concentrations: all pH 7 trials



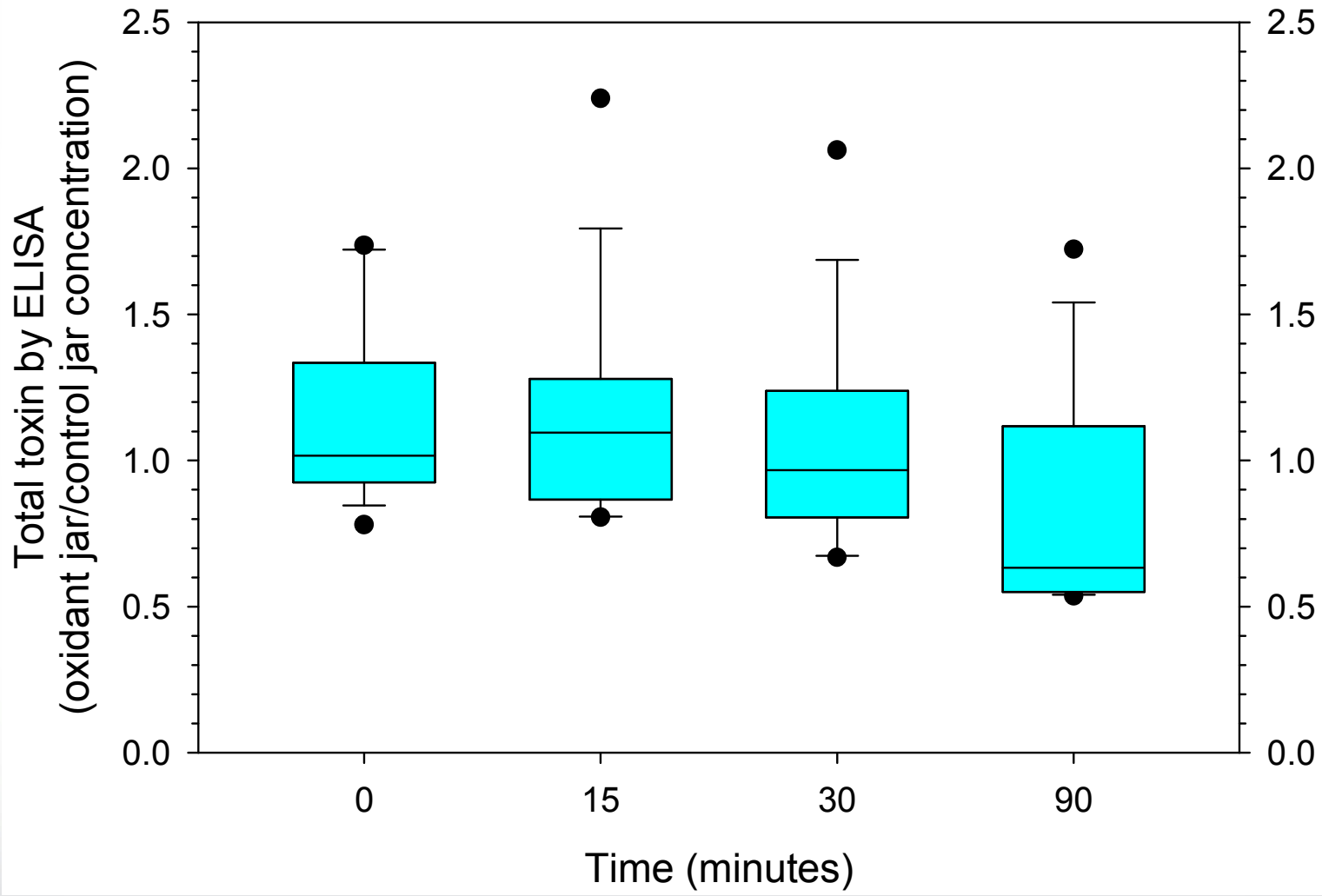
Extracellular toxin concentrations (pH = 7, turbidity < 0.1 NTU)



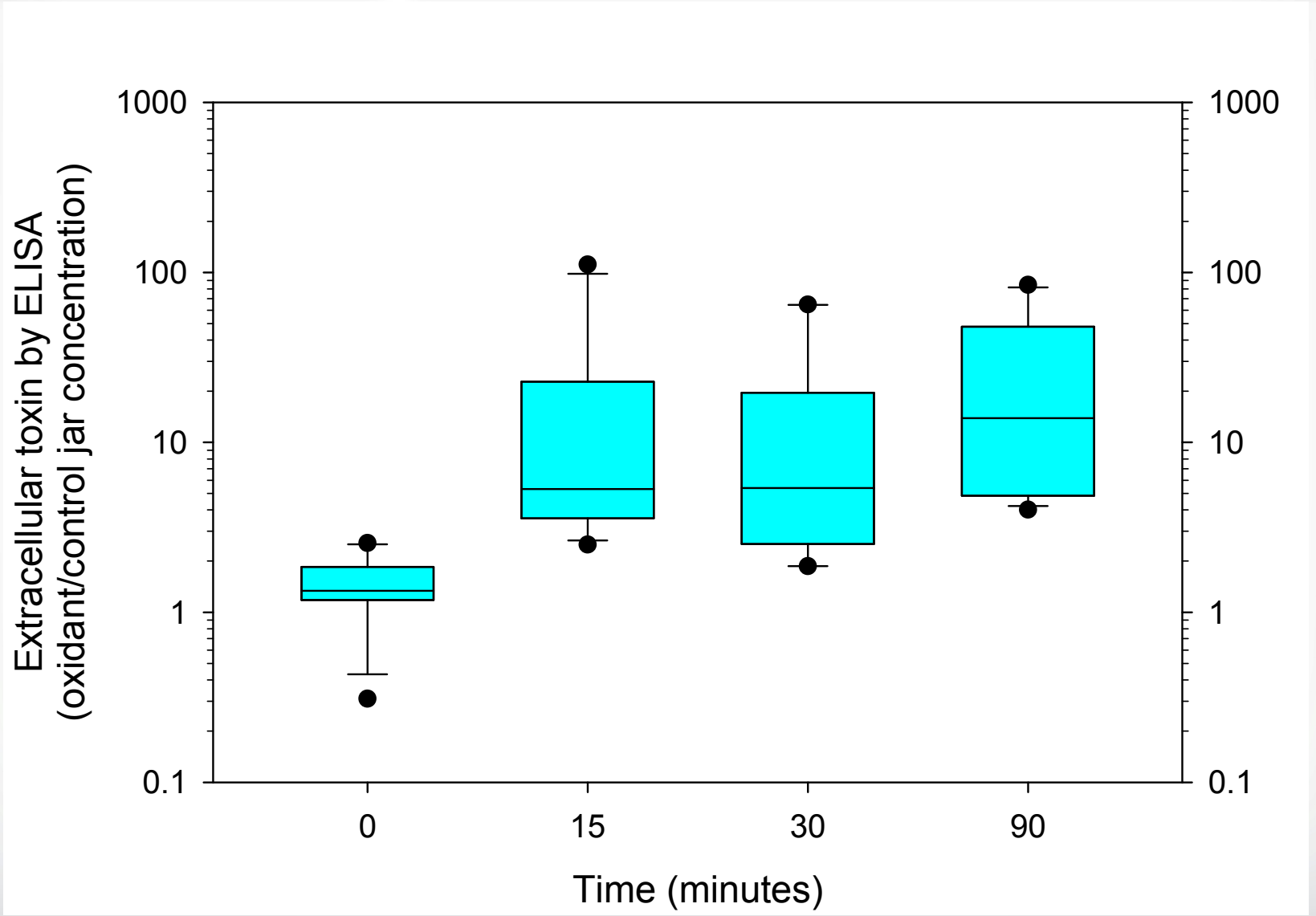
SYTOX positive cells: all pH 7 trials



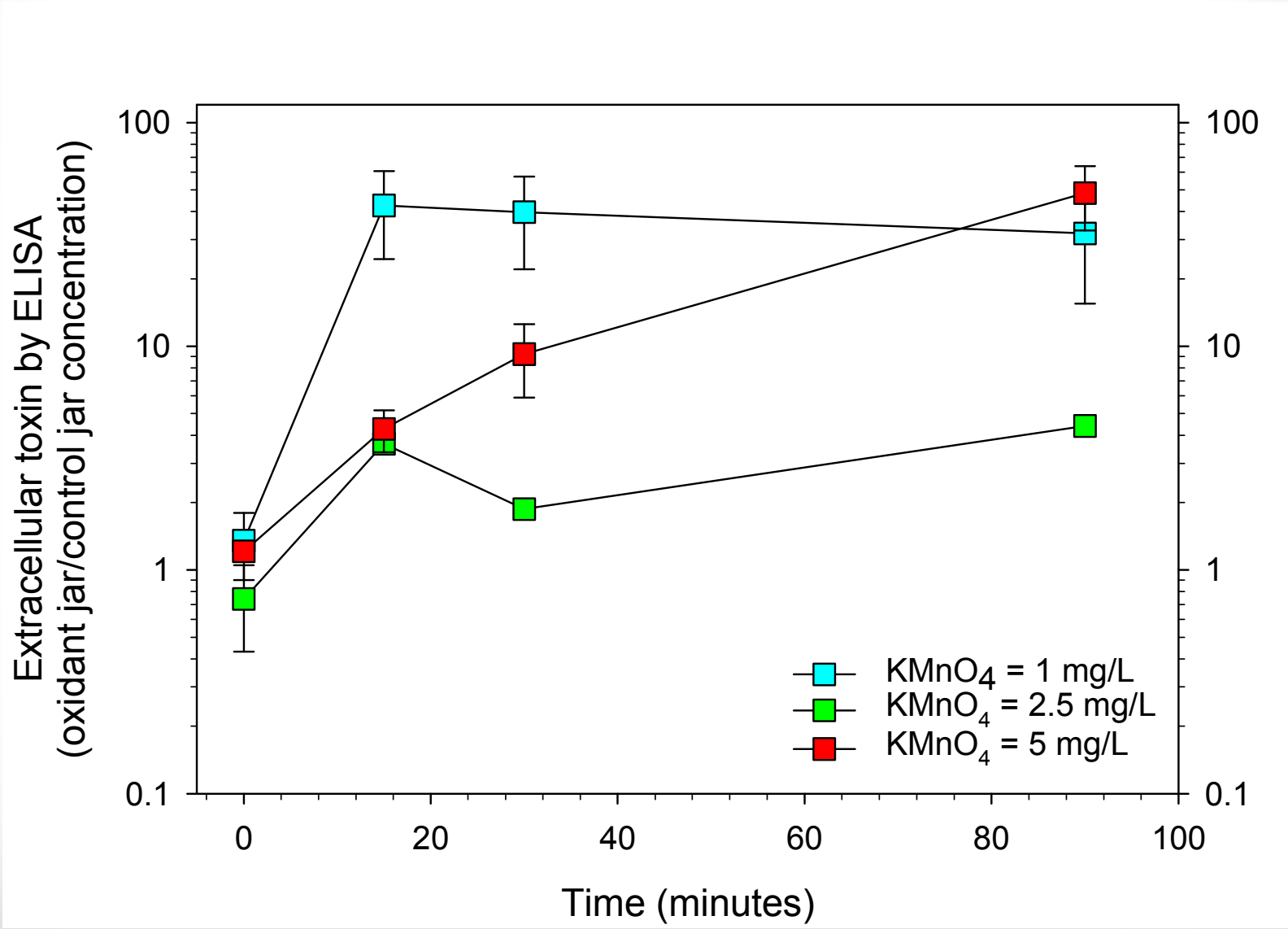
Total toxin concentrations: all pH 7 trials



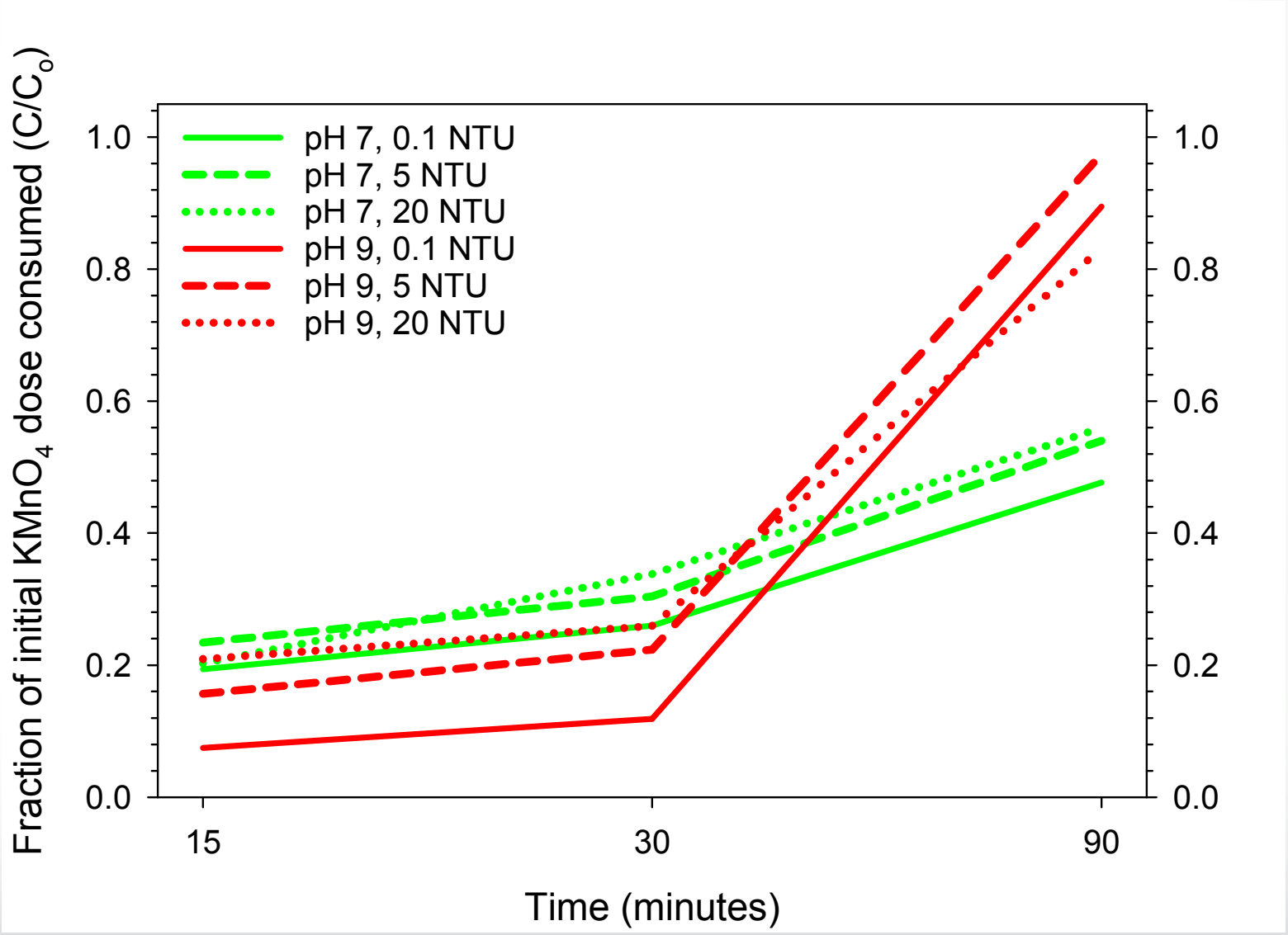
Extracellular toxin concentrations: all pH 9 trials



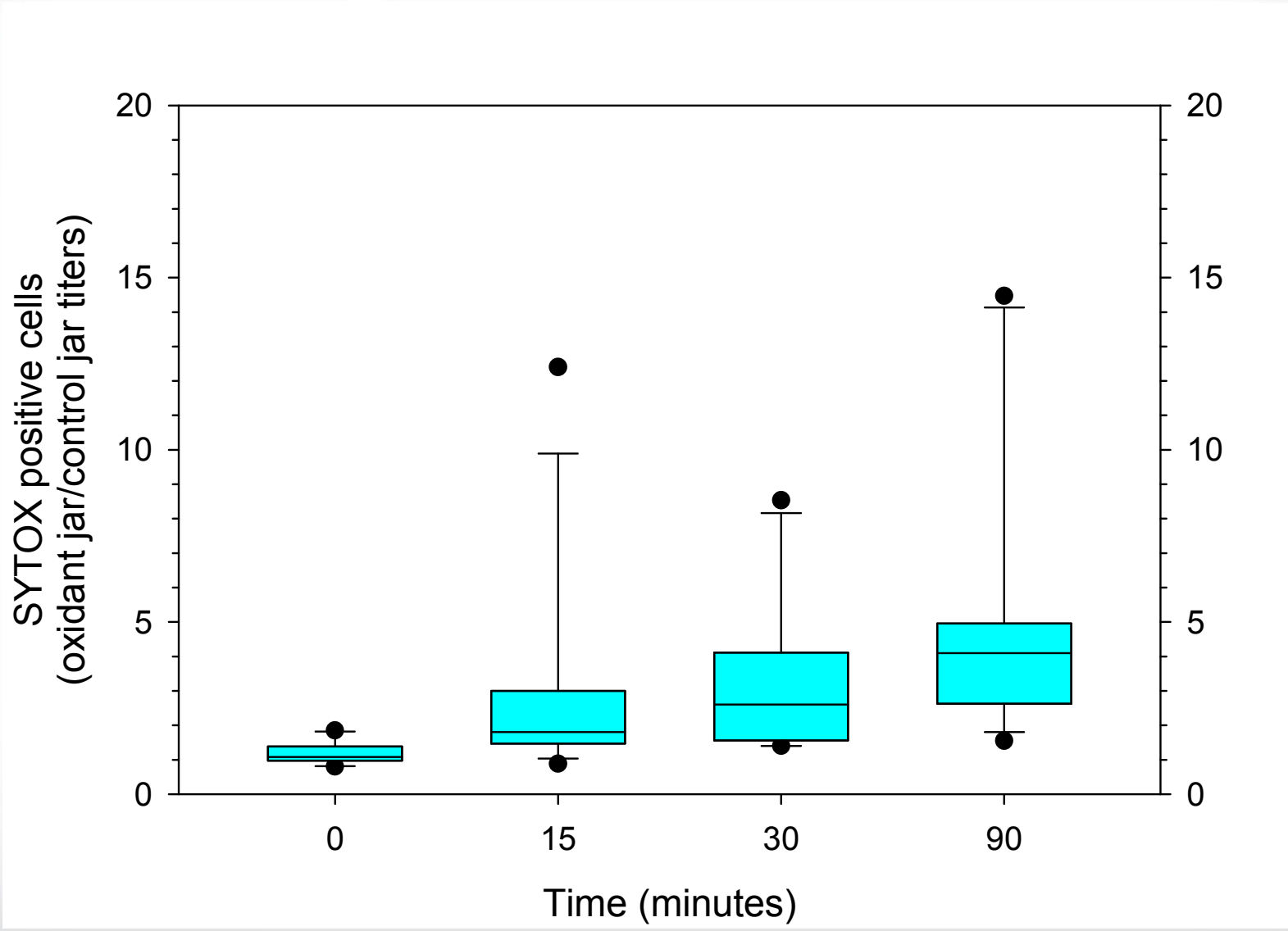
Extracellular toxin concentrations (pH = 9, turbidity < 0.1 NTU)



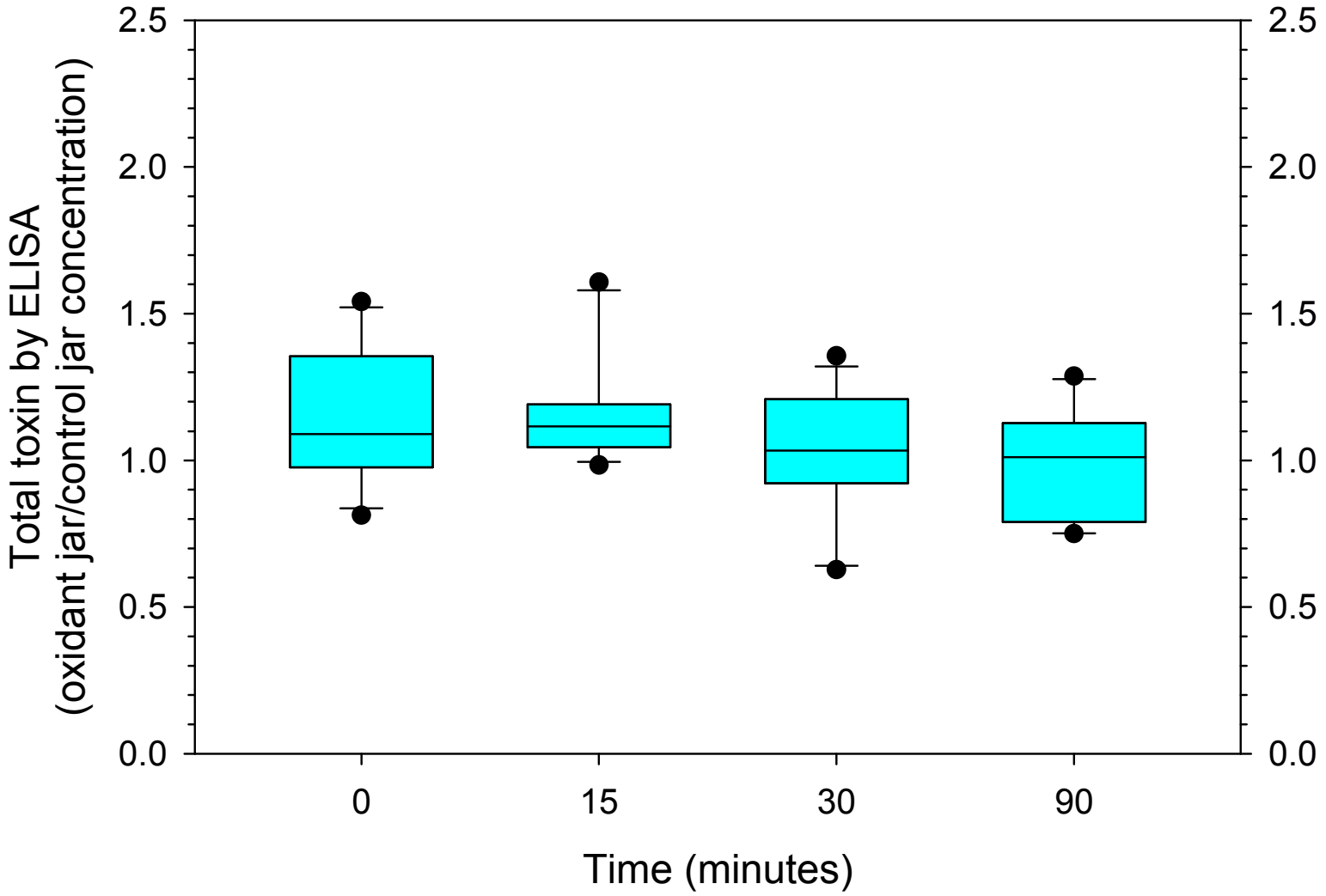
$KMnO_4$ consumption at 5 mg/L dose



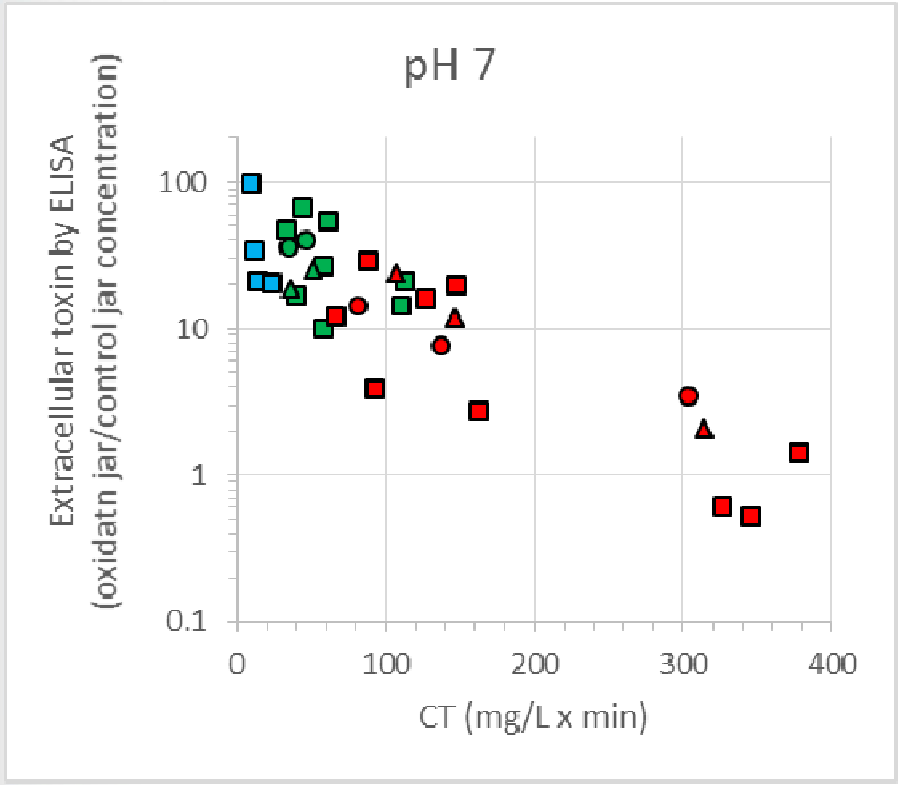
SYTOX positive cells: all pH 9 trials

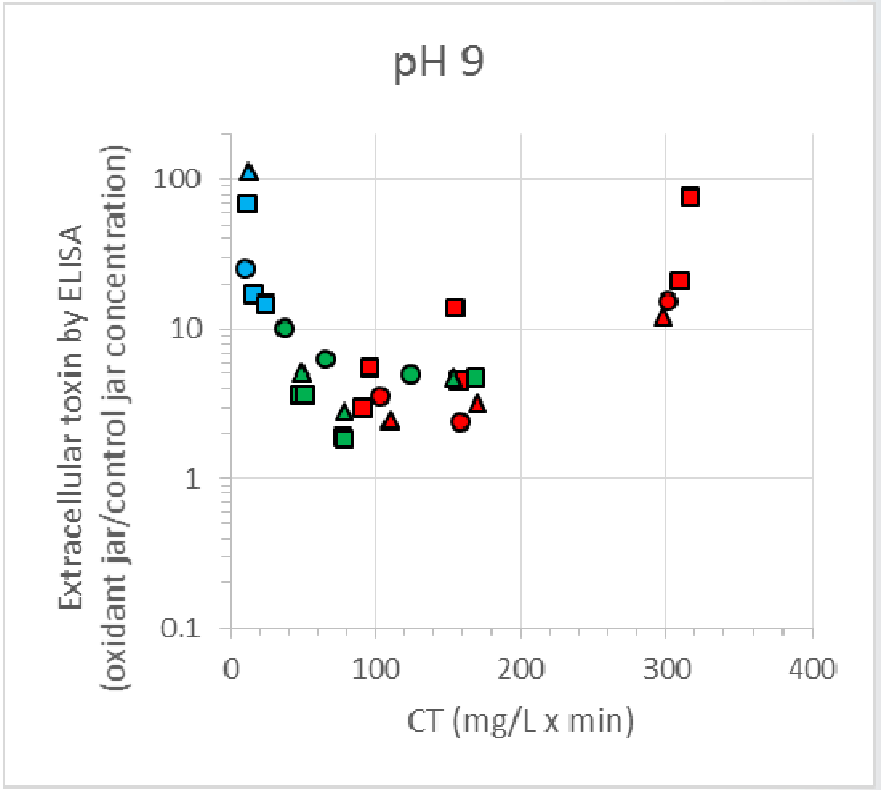


Total toxin concentrations: all pH 9 trials



Extracellular toxin vs. CT

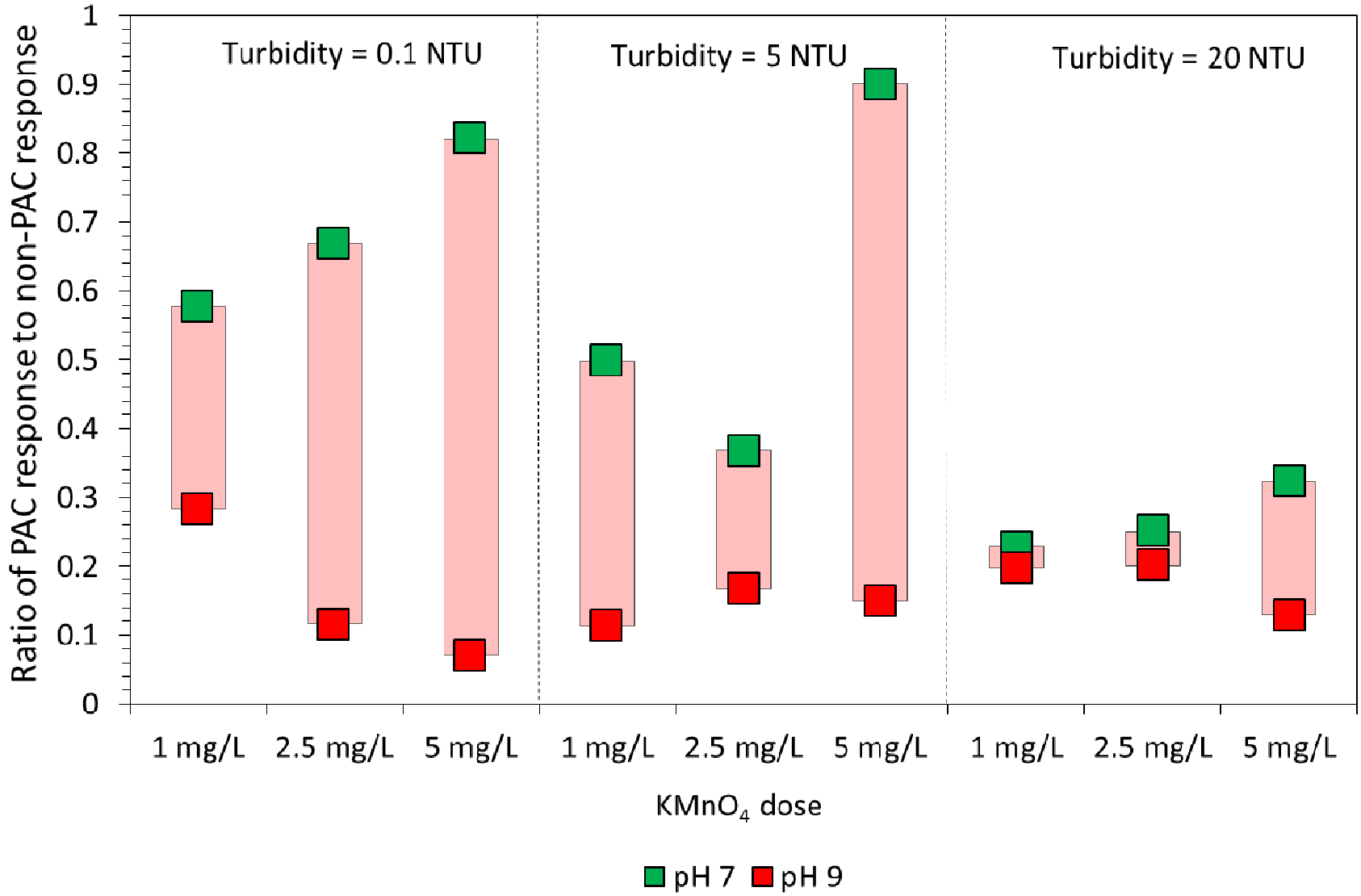




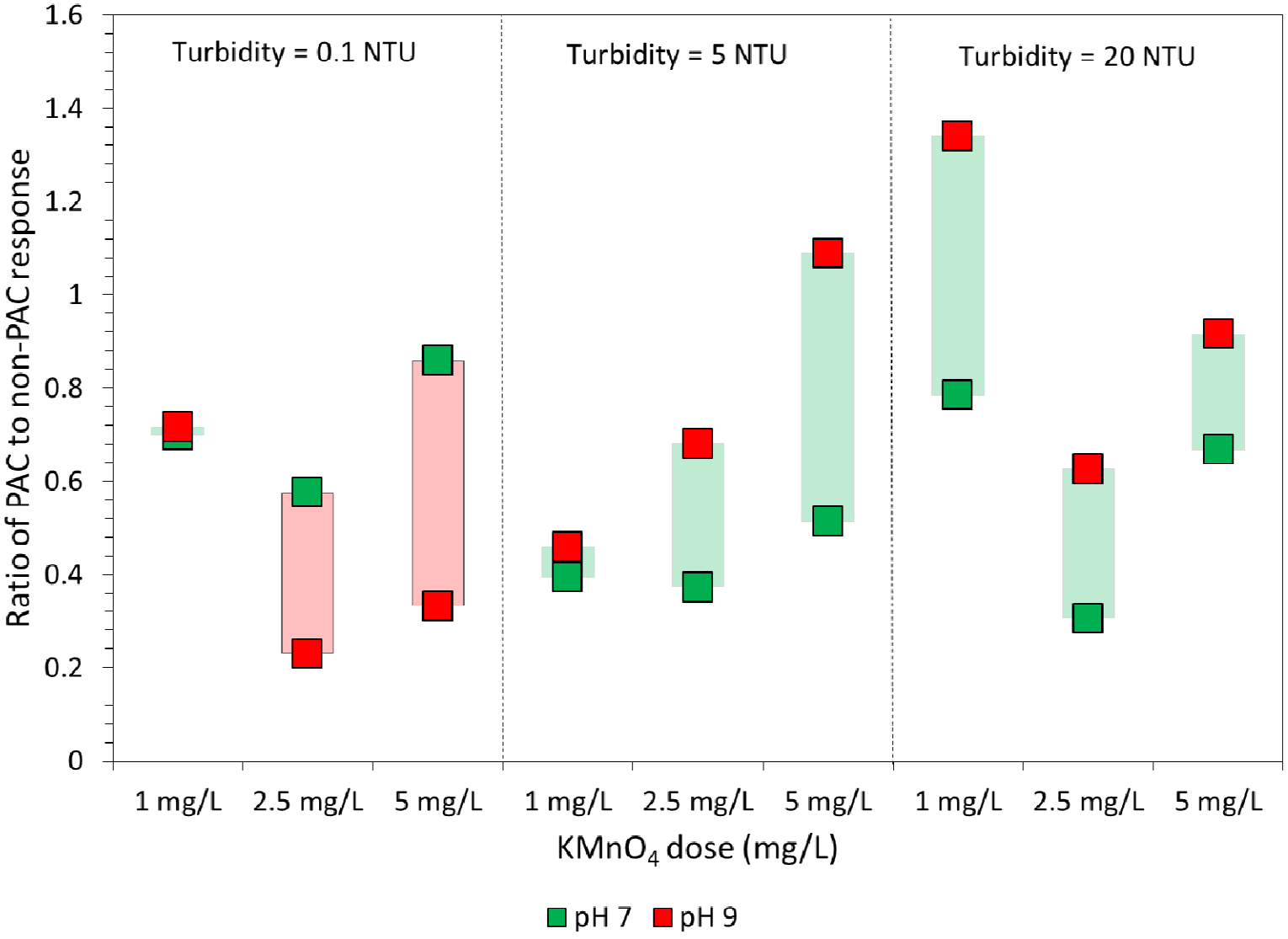
□ Turbidity = 0.1 NTU
△ Turbidity = 5 NTU
○ Turbidity = 20 NTU

$KMnO_4$: Cyan = 1 mg/L, green = 2.5 mg/L, red = 5 mg/L

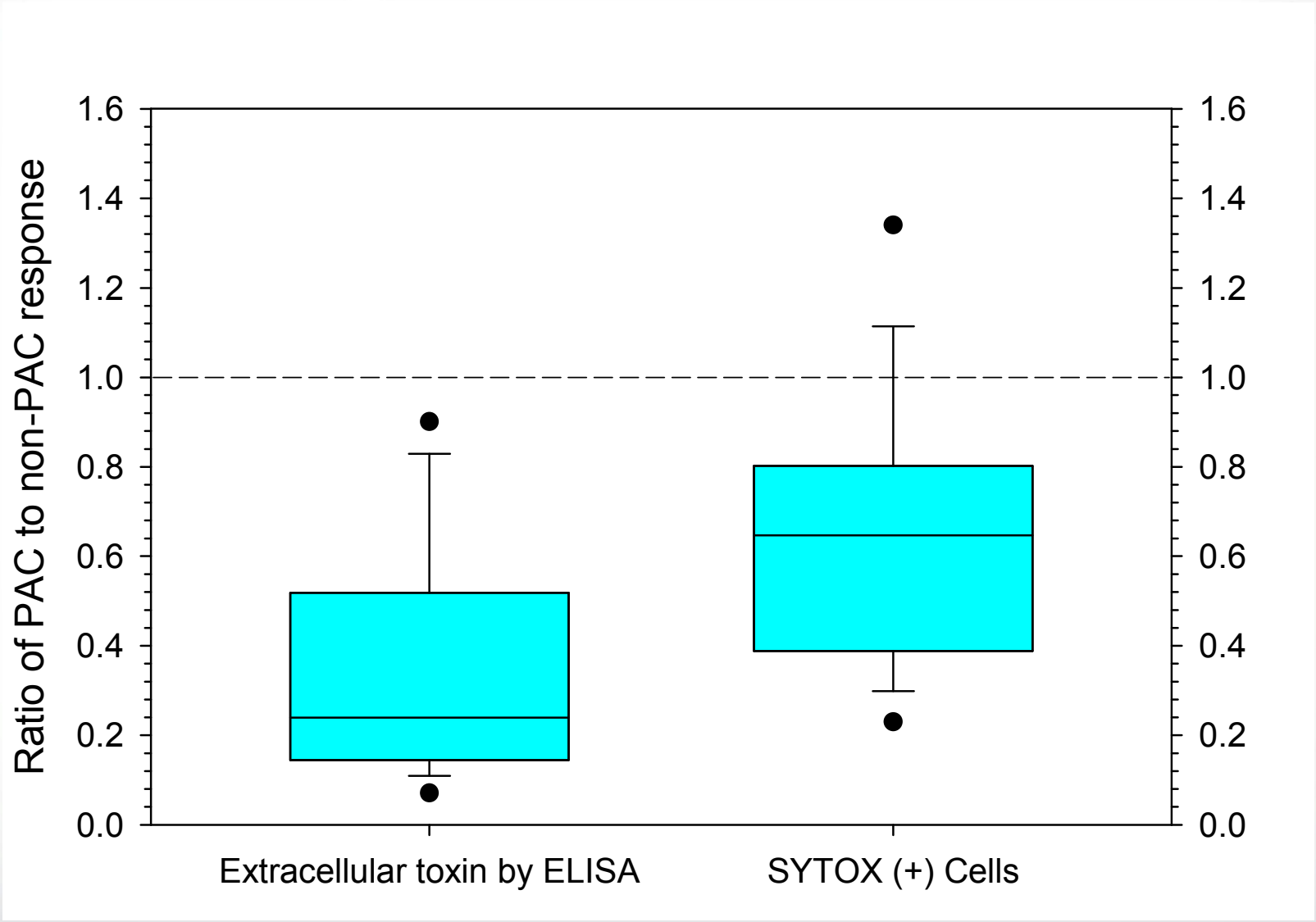
PAC impact on extracellular toxin response as a function of pH, turbidity and $KMnO_4$ dose



PAC impact on SYTOX (+) response as a function of pH, turbidity and $KMnO_4$ dose



PAC addition – summary of impacts for all pH, turbidity and oxidant levels



How do our results compare?

Fan et al, Chemosphere 2013:92:5:529-534. Impact of Potassium Permanganate on Cyanobacterial Cell Integrity and Toxin Release and Degradation

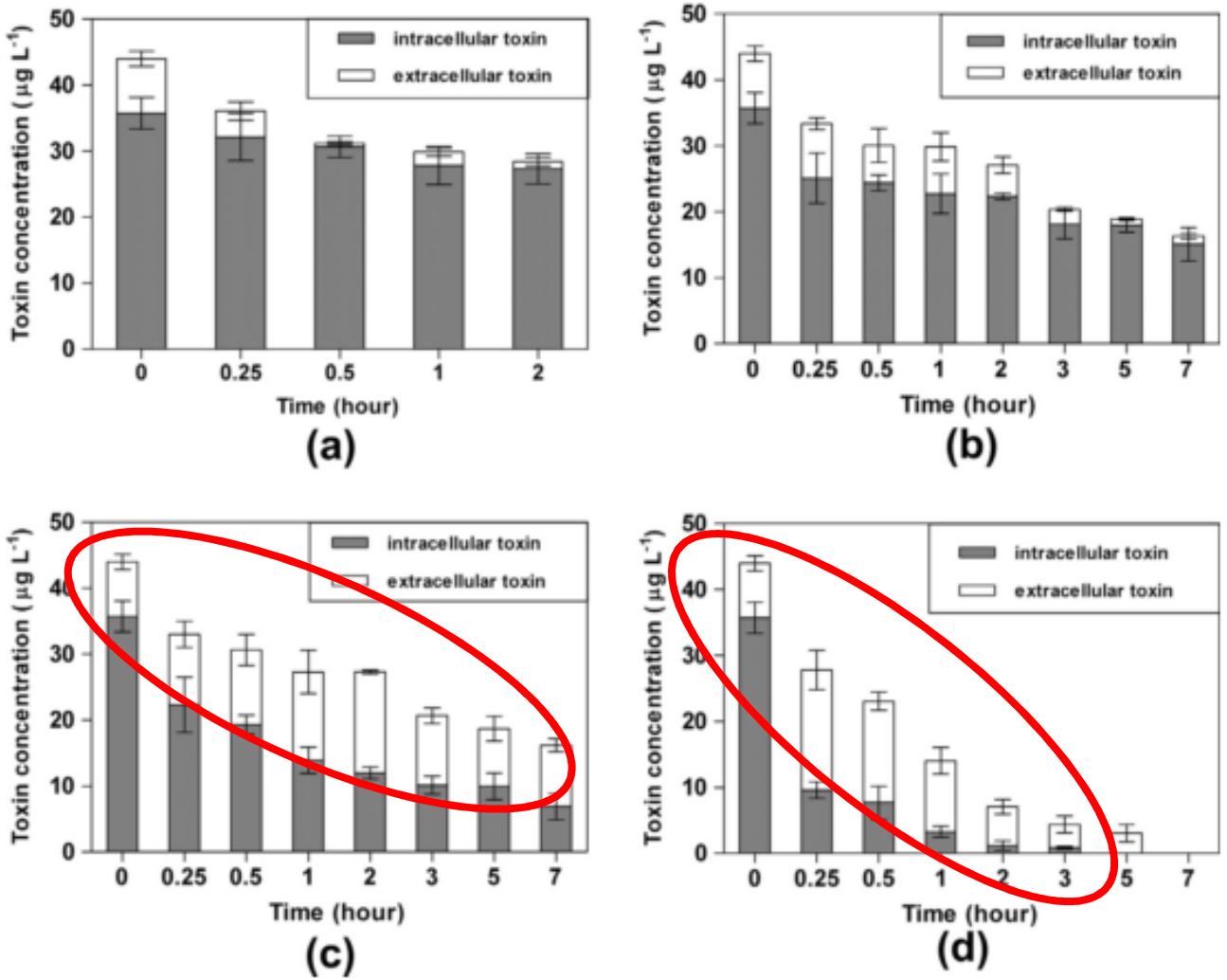
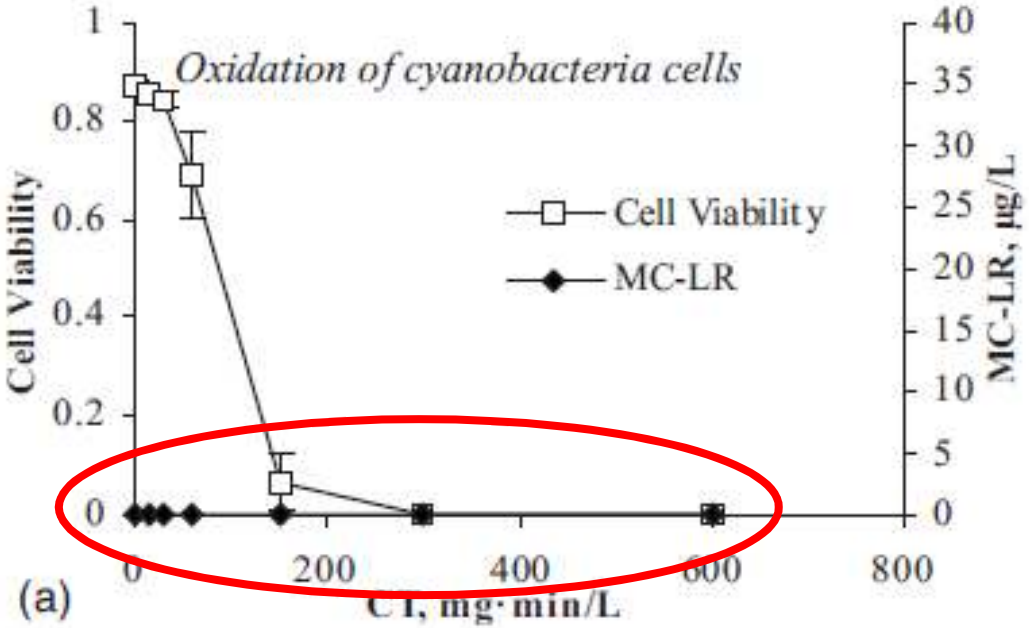


Fig. 3. Intracellular and extracellular microcystin concentrations after treatment with $KMnO_4$ (up to 7 h contact time) with $KMnO_4$ at concentrations of (a) 1 mg L^{-1}; (b) 3 mg L^{-1}; (c) 5 mg L^{-1}; and (d) 10 mg L^{-1}. Error bars equal ± standard deviation.

Ding et al, ASCE Journal of Environmental Engineering 2010:136:1:2-11. Release and Removal of Microcystins from *Microcystis* During Oxidative-, Physical-, and UV-Based Disinfection



How do our results compare?

Group	$KMnO_4$ dose (mg/L)	pH	Target cell titer (x 10^6/mL)	Aqueous medium	Toxin analysis	Result
USEPA, 2016	1, 2.5, 5	7 and 9	1.0	De-Cl_2 tap water	ELISA	Extracellular toxin release and accumulation at all $KMnO_4$ doses
Fan et al, 2013	1, 3, 5, 10	7.5	0.7	ASM-1 growth medium	LC-UV	Extracellular toxin release and accumulation at 5 and 10 mg/L $KMnO_4$ doses
Ding et al, 2010	1 - 2	7.6	2.0	Buffered saline	LC-MS	No extracellular toxin release or accumulation observed

How do we interpret and apply the information?

Interpret and apply

- Evidence has been generated that cyanobacterial cells can release toxins when exposed to $KMnO_4$
- Observed magnitudes of releases and conditions under which releases occur vary between research groups
- Released toxins react with residual $KMnO_4$
- Released toxins adsorb onto PAC
- Drinking water treatment personnel should be cognizant of these mechanisms

Interpret and apply

- Maintain vigilance:
  - Monitor for cyanobacterial biomass and toxins at plant intake
  - Monitor for toxins (total and extracellular, if possible) through treatment process once toxins detected at intake
  - Think through contingency issues:
    - What do I do if I detect an extracellular toxin release?
    - Can I shut off my $KMnO_4$ for a day? One week? Two weeks?
    - How much can I boost my PAC feed before I have problems with my feeders, problems with my sludge and carry-over to my filters?
    - Where am I with respect to DBP compliance? How much could I boost my chlorine dose before I hit non-compliance?

Applying Optimization Concepts to HABs

- EPA's Drinking Water Optimization Team (OGWDW, Technical Support Center, Cincinnati, OH) recently initiated a project with Ohio EPA to:
  - Develop evaluation tool to assess how prepared a system is for a HAB event
  - Identify "factors" which may limit a system's ability to effectively treat a HAB
- Process will be based on turbidity optimization concepts and approach – with a strong emphasis on the importance of optimizing plant processes (clarification, filtration) for turbidity, while including considerations for treating HABs.

Disclaimer

U.S. Environmental Protection Agency, through its Office of Research and Development, funded and managed, or partially funded and collaborated in, the research described herein. It has been subjected to the Agency's peer and administrative review and has been approved for external publication. Any opinions expressed in this paper are those of the author(s) and do not necessarily reflect the views of the Agency, therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Thank You!

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Supplementary material

Methods

- Cells cultured in BG-11 growth medium
- Centrifuged, washed 3X, and resuspended in de-Cl_2 tap water
- De-Cl_2 tap water buffered to pH 7 and 9 with phosphate and borate buffers, respectively
- T = 20 – 22 °C
- 2L glass beakers on a Phipps & Bird jar test apparatus equipped with steel paddles
- 30 rpm stirring speed

Methods: Initial de-Cl_2 water quality (prior to adding *M. aeruginosa* suspension)

Parameter	Mean (mg/L)
NO_3^-	0.73
NO_2^-	< 0.012
NH_4^+	< 0.031
PO_4^{3-}	0.17
Hardness (as $CaCO_3$)	110
Total organic carbon	0.80