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Hydrology and Earth System Sciences Discussions

Interactive comment on "Cyanobacterial and microcystins dynamics following the application of hydrogen peroxide to waste stabilisation ponds" *by* D. J. Barrington et al.

Anonymous Referee #1

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In the manuscript "Cyanobacterial and microcystins dynamics following the application of hydrogen peroxide to waste stabilization ponds", the authors present the results from two laboratory studies and one field study where cyanobacteria and microcystin were reduced using differing doses of hydrogen peroxide. The primary weakness of this study was the lack of replication in the 2nd and 3rd experiments. Additionally, it appears that the authors used some kind of pseudo replication in statistical differences among treatments, which is invalid. The lack of true replication should be explicitly noted early in the ms to put the experiments in context for the reader and statistical tests should be used for experiment one, where n=2. Although the field experiment did not have an actual control and the 2nd lab experiment was not replicated, I still feel

the results are worthy of publication given the "before and after" presentation. Overall, the data show that in field trials cyanobacteria and microcystin were reduced after a hydrogen peroxide treatment – which is a somewhat new and novel treatment for cyanobacteria. Thus, this work cannot be recommended for publication until after the pseudo replication issue is taken care of and some other revisions have been made.

Specific major comments

1. On lines 20-25 on page 2074, the authors should include more information on the enumeration methods of Hotzel and Croome (i.e., how many natural units were counted or how many cells were counted before counts were completed?)

2. A small discussion of the difference between maturation and facultative WSPs would help the reader understand the project better. Additionally, in the same section the authors should define the rational for choosing a maturation WSP for some of the work and a facultative WSP for other parts of the work. The authors could probably fit this in to the study site area section.

3. 20 L aquariums are not mesocosms. These are laboratory nanocosms/microcosms at best. Suggest changing mesocosms to nancosms/microcosms or laboratory nanocosms/microcosms.

4. How did spatial/temporal scale affect the differences between lab and field experiments? Where differences really only due to the lack of microcystin-degrading bacteria? The difference in scale will need to be noted in the reasons that results differed unless the authors are certain, and can show they are certain in the text of the ms, that scale did not play a role.

5. On each of the 3 figures, it is unclear how standard error bars were generated. For example, the manuscript explicitly states that the 1st lab trial used 2 controls and 2 treated mesocosms; yet the text description states that standard errors are for n = 10 for cyanobacteria measurements and n = 3 for microcystin concentrations. Obviously the

authors have calculated standard error from some kind of pseudo replicate sampling plan- but details on how this was done are missing from the manuscript. The authors need to have error bars from actual replicates, not pseudo-replicate samples. When the switch to actual replicate error bars is made, figure 2 and 3 should not have error bars and statistical hypothesis testing cannot be conducted for experiments 2 and 3. All of this information needs to be explicitly stated in the "data analyses" section before this manuscript can proceed.

6. Are the units for chlorophyll on figures 1 and 2 correct? If so, why are there such large increases in chl-a in the control mesocosm in figure 1- was this due to a malfunction by the probe or an actual increase in cyanobacteria? – Although this is explained in the text as an interference with the probe, the question of whether cyanobacteria were actually reduced is not answered. Please include the results, even if only qualitative, in the next version of the manuscript.

7. Figures 1 and 2 are somewhat confusing at first glance, and the readers need to read through half the ms before the figures can be completely understood. Figures should allow the reader to look at only the figure to understand the information presented without reading the ms. One way to fix this problem, and reduce the number of graphs, is to compare everything to the control by using a ratio between the treatment and control data (i.e., dividing the treatment data by the control data). This approach would clearly show if the treatments were higher or lower compared to the control, and by how much, while simplifying the graphics. Additionally, it would remove the rather large units on the y-axis of the cyano graphs and replace it with a unit less metric that would explicitly show if day X was Y-fold higher or lower than the control.

Minor comments

8. Lines 8-10 on page 2068: Would reword this sentence to ....total phytoplankton chlorophyll a led to the lysis of cyanobacteria cells, in turn causing the release of dissolved microcystin into the environment. Additionally, I suggest the authors changed

"death" and "killed" to lyse or lysis throughout the paper – see lines 29 of page 2070, 16 of page 2079, and 23 of page 2079 as just a few.

9. Line 13 and 15 of page 2068- capitalize Chlorophyta here and elsewhere

10. Line 15 of page 2068- does earlier refer to upstream? Or from the facilitative WSP? A discussion of how these ponds work and the order of the treatment train would clear this up in the ms (see major comment 2 above).

11. On line 23 of page 2068- suggest a period after animals, start a new sentence with "Both. . ."

12. Line 19 of page 2074- is there a reference for "the resulting absence of fluorescence indicates a reduction in viable phytoplankton biomass"?

13. Line 1 of page 2075- was the dissolved fraction stored in the same manner as the intracellular MC?

14. On page 2075- please state how total MC was calculated. It seems as though you added intracellular and dissolved, but it is not explicitly stated.

15. On page 2075- Are there any references for your extraction technique? Was it the same as the previous study (Barrington, Ghadouani, Ivey 2011)?

16. On page 2075- why were samples "pooled"?

17. Line 19 of page 2078- quantitatively define how much MC concentrations increased.

18. Line 18 of page 2080- ". . .were present at very low concentrations" -quantitatively define low.

19. Line 3 of page 2081- figure 3a does not show abundance, yet this indicates that it does. Do the authors mean figure 3c?

20. Figure 3 appears to be presented out of order (with 3a and then 3c being presented

in the text first and second).

Interactive comment on Hydrol. Earth Syst. Sci. Discuss., 10, 2067, 2013.