

## ***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 #2**

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In this paper the authors Barrington et al describes the dynamics of cyanobacterial species, microcystis and the associated toxin, microcystin in a waste stabilization pond (WSP), after treatment with H<sub>2</sub>O<sub>2</sub>. Two lab trials (in 20 L scale) were conducted to establish conditions prior to the H<sub>2</sub>O<sub>2</sub> treatment of WSP, which was about 8000 m<sup>3</sup> in volume. The authors have conducted similar field trials before, such field studies are rare, complicated and expensive to perform, hence the information gathered from such field trials are useful to water managers, therefore should be published. However, there are some flaws in the manuscript, these should be addressed.

The authors describe two lab trials, the first trial (here after described as trial1) using a

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high cell count ( $5 \times 10^7$  cells/mL) together with a high concentration (about 80  $\mu\text{g/L}$ ) of toxin with a control and a treatment, both in duplicate (altogether 4 experiments). The second lab trial involves a lower cell count ( $3 \times 10^6$  cells/mL), approximately 1/10th that of trial1, done as a single experiment, using a control and 3 different concentrations of  $\text{H}_2\text{O}_2$  as treatment. The  $\text{H}_2\text{O}_2$  concentrations in the second trial, in relative terms, varied from 1, 10 and 100. The toxin count in this trial was low, under 1  $\mu\text{g/L}$ .

#### Specific comments

(1) Statistics shown in the lab trials 1 and 2 are somewhat ambiguous. There are no replications, except in trial1, which was done in duplicate. The error bars shown for trial 2 are a measure of how well the microcystins and chlorophyll-a were estimated within a single experiment and are not true statistics of a replicated experiment. The authors could argue, the trails were done only to establish the  $\text{H}_2\text{O}_2$  concentration necessary to use in the field experiment, hence no replications were attempted. This concept, in my opinion, is valid, however, it should be explicitly expressed in the manuscript and in the statistical discussion and corrected accordingly.

(2) The units used to express the concentration of  $\text{H}_2\text{O}_2$  is difficult to visualise at a first glance, rather 0.02 mg  $\text{H}_2\text{O}_2$  / $\mu\text{g}$  Chl a, 0.2 mg  $\text{H}_2\text{O}_2$  / $\mu\text{g}$  Chl a and 2 mg  $\text{H}_2\text{O}_2$  / $\mu\text{g}$  Chl a is recommended. This is however, a recommendation only since non-technical people reading the manuscript may have an easy grasp of the concentrations used.

(3) Were the pH and temperature of the experiments measured? The oxidative power of  $\text{H}_2\text{O}_2$  varies under different conditions such as the temperature, pH, the presence of catalytic ions such as  $\text{Fe}^{2+}$  and also influenced by UV. The mechanism of breakdown of living cells and microcystins present in water with  $\text{H}_2\text{O}_2$  is different. Perhaps it would be useful to report the pH and the temperature of the experiments, if these have been measured. There may be temperature differences between lab trails and the field trial as well.

(4) If the molecular masses of the microcystins were identified (page 2076), what were

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they? Different microcystins may behave differently to H<sub>2</sub>O<sub>2</sub> treatment (oxidation kinetics may be different between different microcystin analogues).

(5) If the samples were pooled (page 2075) and analysed for microcystins, how did the error bars in the figures were derived? The pooling is not clear, please clarify.

(6) Sample sonication (page 2075 and elsewhere) seems to have done using a sonic bath, rather than using a sonic probe, which is the standard practice for cell lysis. In general, about 5 minutes of sonication time is sufficient to break open microcystis cells with a probe. Please clarify why the times are so long.

(7) Is Figure 1 necessary? The conditions used in experiment 1 is so different (cell count and microcystin concentration) to actual field study, is it necessary to present this figure?

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