Comment on bg-2021-312

Anonymous Referee #3

Referee comment on "Investigating the effect of nickel concentration on phytoplankton growth to assess potential side-effects of ocean alkalinity enhancement" by Jiaying Abby Guo et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-312-RC1, 2022

Review of "Investigating the effect of nickel concentration on phytoplankton growth to inform the assessment of ocean alkalinity enhancement" by Guo et al.

Guo and colleagues present an interesting and well written manuscript about the lack of sensitivity of a wide array of phytoplankton species to changes in Ni. This work is timely given the recent intensification of interest in CDR and the threat of Ni toxicity when olivine and other minerals are added to enhance alkalinity.

Overall, I believe that this manuscript is suitable for publication in its current state. I appreciate that the authors clearly articulate the uncertainties and ambiguities involved in extrapolating culture experiments with EDTA to open ocean conditions.

My criticisms at this stage might be filed as suggestions, but I hope the authors consider them, even if they are not required to do so. First, there are several instances, especially in the abstract, discussion, and conclusion, where qualitative terms (e.g. "mildly" "not very pronounced") are used to describe results without any kind of quantitative description or definition afterward. It would be helpful in most of these cases to describe the scale of the (lack of) effect: (e.g. < 10 % change), either as a parenthetical or in a following sentence. The authors explain how small differences in growth rate can quickly result in significant population shifts so clarifying what the authors perceive as mild/small/not very much will be important as future work expands on this research. Similarly, there is a trend in the results section to focus on p-values, without really describing the range of values measured.

Second, the authors should consider removing Section 4.1.3 or integrating it with other sections of the discussion. As is, the information here is not very relevant to the results

and does not really investigate the presence or absence of Ni enzymes in the species investigated. This would seem to require evidence from genomic sequencing or database searches of the organisms used or close relatives. Certainly the word "link" in the subsection title seems misapplied.

Lastly, and least consequentially, the aspect ratio of figure 2 seems to potentially overemphasize the lack of variation found in the data. It occurred to me that more square panels might be a more neutral presentation of the same results.

Below are a few line notes that mostly reinforce the points above. I think the authors have done a very good job here, so there are the final fixes I'd recommend before this is published.

Line 23: "mildly" might be replaced by a quantitative statement based on percent change

Line 27 (and elsewhere): its important to clarify here that EDTA is "synthetic" to avoid confusion that EDTA experiments are direct analogs for natural organic ligands.

Line 50: what does "quality" mean in this context? Would "identity" or "composition" be more clear here?

73: The mention of nitrogenase here seems abrupt. Perhaps another sentence of introduction is warranted here. Note also that Ni is an essential part of hydrogenase enzymes used by some N2-fixers., e.g Tuo et al. 2020 in L&O.

87: please provide more information about the *Synechococcus* sp. strain used. The *Synechococcus* phylogeny can be very confusing/misleading so details regarding strain and/or ecotype membership are essential if others seek to reproduce this work. I was not able to identify the strain based on web searches alone or on the ANACC culture collection webpage.

90: MilliQ does not specify the grade of water. This should be 18.2 mega-ohm cm-1 grade water.

157: it might be reassuring to comment on how the visual minteq calculations differ from the recommended Ni' vs. dNi values of Sunda et al. (2005) in the Algal Culturing Techniques text.

Figure 1: can the species used in panel b and c be named here?

212:an additional explanatory sentence for the k-value here might be needed. It seems like the decision whether a function is over vs. under-fitting remains somewhat arbitrary?

221: can it be state what the concentration of ligands is assumed to be, 0?

222: should be "largely"

313: phrases like "not very pronounced" would be better if replaced by quantitative statements, e.g. <10%.

317: same thing re: "were smaller". Similar issue on 435: "low"

429: Im not aware of specific evidence suggesting that strong Ni ligands will be able to outcompete natural Fe-binding ligands. Perhaps changing "this would" to "this may" would allow for more uncertainty here.

References

Tuo SH, Rodriguez IB, Ho TY. H2 accumulation and N2 fixation variation by Ni limitation in Cyanothece. Limnology and Oceanography. 2020 Feb;65(2):377-86.

Sunda, W.G., Price, N.M. and Morel, F.M., 2005. Trace metal ion buffers and their use in culture studies. Algal culturing techniques, 4, pp.35-63.