

We greatly appreciate the valuable comments and critical reading of the manuscript made by the anonymous reviewers, which were useful in improving the scientific quality of the manuscript. Please find below our answers to the Reviewers comments.

RC1: ['Comment on bg-2023-130'](#), Anonymous Referee #1, 19 Sep 2023

Xin et al. measured the cell density of three marine phytoplankton species under different Ni concentrations to assess the influence of Ni on phytoplankton in the context of ocean alkalinity enhancement (OAE). They also measured the total dissolved Ni concentrations and calculated the free Ni concentrations in the growth media to examine the availability or toxicity of Ni. Overall, this study contributes to the current understanding of OAE-related Ni influence. The comments provided below are intended to assist the authors in refining their articles for publication.

Line 17: Please add a sentence to explain why you chose to study Ni and how it relates to OAE materials.

Response: Thank you for the above suggestion. We have added information explaining the importance of Ni and its relationship to OAE material. The text has been modified as follows: *“As one of the most abundant metals in OAE source materials, understanding the impacts of nickel (Ni) on the phytoplankton is critical for the OAE assessment.”*

Line 17: As previously mentioned, Ni can act as a micronutrient at low concentrations. Please consider that dissolved Ni can have both positive and negative effects on phytoplankton growth. Change 'toxicity of nickel' to 'influence/effects of nickel'.

Response: We rephrased the sentence considering the reviewer's comment.

Line 18: Change “a range of Ni concentrations” to “9 Ni concentration gradients”.

Response: We changed the text accordingly.

Line 20 -24: Considering the results revealed some growth enhancement from Ni addition, the fertilization effects of Ni should be mentioned as well. Please write the specific inhibition effects and potential fertilization effects on phytoplankton, such as “XX% of growth rate inhibition” and “IC50 value was XX Ni concentrations”.

Line 22: The sentence “rapid response to exposure of Ni” is unclear.

Response: We have broadened the discussion on *E. huxleyi* increase in cell density in chapter 4.1 and have underlined that this growth alteration cannot be dismissed. But in order to keep the flow of the text, we decided not to add this information in the abstract. We have added the detailed concentration of Ni accordingly and modified the text as follows:

*“The impacts of elevated Ni varied among the tested phytoplankton species. The coccolithophore *Emiliana huxleyi* and the dinoflagellate *Amphidinium carterae* exhibited a growth rate inhibition of about 30% and 20%, respectively, at the highest Ni concentrations. The half-maximal inhibitory concentration (IC50, at which the growth rate is inhibited by 50%) of both species exceeded the tested range of Ni. This suggests that both species were only mildly affected by the elevated Ni concentrations. In contrast, the diatom *Thalassiosira weissflogii* displayed a considerably higher sensitivity to Ni, with a 60% growth rate inhibition at the highest Ni concentration and an IC50 value of 63.9 $\mu\text{mol L}^{-1}$.”*

Line 23: Add “diatom” before “Tholassiosira weissflogii”.

Response: Added to the text.

Line 58: The word “metal sensitivity” is a wide concept and considering only Ni was investigated in this study, I suggest changing it to “Ni sensitivity”.

Response: We agree and have changed the text accordingly.

Line 65: “EDTA”, please write out the full name the first time the abbreviation is mentioned.

Response: We added the full name of EDTA.

Table 1. There were standard deviations in Fig.1 so please add the standard deviations in Table 1 for the measured Ni concentrations and potentially free Ni concentrations as well.

Response: Thank you for the above suggestion. The standard deviations are added in the revised version of this manuscript.

Line 111-114: Why did the NiCl₂ stock solution (line 70) precipitate as nickel carbonate when its maximum solubility is 10.73 mol/L, while the stock solution was only 50 mmol/L? What caused this precipitation of NiCl₂?

Response: This could be attributed to the ability of nickel to form complexes with anionic species rather than chloride, resulting in the formation of compounds (here nickel carbonate) during the invasion of air (Gad, 2023). The solubility of nickel carbonate is 1.58 mmol/L, which is comparatively lower compared to that of NiCl₂. This is confirmed by the visual precipitated particles in the stock solution bottle.

Ref: Gad SC.: Nickel chloride, Reference Module in Biomedical Sciences, Elsevier; <https://doi.org/10.1016/B978-0-12-824315-2.00704-1>, 2023

Fig 2,3,4: It's hard to distinguish the cell density and growth rates in low Ni concentrations (0-5 umol/L). Please consider editing the figure to enhance the differentiation of data points in this low Ni concentration range. It appears that one data point may have been lost or obscured, possibly due to the similarity in results between the control (0 umol/L) and 0.01 umol/L conditions?

Response: We are aware that some data points are not well-readable due to similar results. All measurements are available in Figures 2, 3 and 4. For Figure 3, due to a technical problem, the measurement of *E. huxleyi* at 0.01 μmol/L was not successful. Thus, the data point was not plotted in the graph. We have specified the missing point in the caption. All the values of the growth rates are now available in Table S1 in the supplementary.

Discussion: I suggest giving each main discussion paragraph a subtitle. This will help to keep the audience engaged with the clear outlines.

Response: Thanks for this comment. We have separated the discussion into two subsections: “4.1 Effects of Ni on marine plankton; 4.2 Implication for the deployment of ocean alkalinity enhancement.”

Line 174: Please provide a reference for the sentence “Basic and ultrabasic rocks, which are widely recognized source minerals for OAE, would introduce high amounts of Ni into seawater during mineral dissolution”.

Response: We added a reference as requested.

Renforth, P.: The negative emission potential of alkaline materials, Nat. Commun., 10, 1–8, <https://doi.org/10.1038/s41467-019-09475-5>, 2019.

Line 179: (Hartmann et al., 2022) should be (Hartmann et al., 2023).

Response: We have corrected it.

Line 185-209 This paragraph draws comparisons between the responses of three different species based on the results, but it requires some further refinement:

Line 193: The influence of Ni at low concentrations (0-5 umol/L) is just as important at high concentrations since the natural Ni concentrations are around 10 nmol/L. It's likely the added Ni from OAE will fall into the range of 0-5 umol/L, and E. huxleyi had enhanced growth rates in this range. Therefore, please discuss why E. huxleyi benefited from supplied Ni at low concentrations.

Response: Thank you for the above suggestion. We have added the possible explanation for the enhancement in *E. huxleyi* growth as follows:

“Considering the high tolerance of E. huxleyi to several other trace metals such as copper and cadmium (Brand et al., 1986), it is not surprising that this species was found to be mostly unaffected by Ni in our study. For example, to counteract high Cu concentrations, E. huxleyi, regardless of the needs of the cells, can continuously produce organic Cu-ligand (Echeveste et al., 2018). Another study postulates that E. huxleyi survives the Cu stress through an efficient efflux system by exporting intracellular metals (Walsh and Ahner, 2014). We speculate that E. huxleyi may apply analogous strategies to grow at high nickel concentrations. Furthermore, Ni was shown to interact with Ca²⁺ and Mg²⁺ transport systems; the uptake of Ca²⁺ and Mg²⁺ may compete with Ni for the transport pathways and reduce the uptake of Ni in E. huxleyi (Deleebeeck et al., 2009). Interestingly, we observed an enhancement in the cell densities of E. huxleyi at low Ni concentrations. Ni serves as a necessary micronutrient to the Ni-containing enzyme urease in phytoplankton when the primary nitrogen source is urea (Price and Morel, 1991). However, this does not apply to our study. To the best of our knowledge, there are no clear reports indicating the positive effects of nickel as a nutrient when nitrate serves as the nitrogen source. One possible explanation might be that the introduction of low-dose toxins prompted an increased rate of cell division, a phenomenon known as hormesis. Studies on various phytoplankton groups revealed a similar dose-response pattern, where low doses exhibited beneficial effects and high doses led to toxicity. In these investigations, hormesis was attributed to low increased levels of Cd (Brand et al., 1986) and Cu (Brand et al., 1986; Pérez et al., 2006; Yang et al., 2019). This interpretation differs from the notion of metal limitation. Considering Ni, a slight increase in concentrations positively impacted multiple chlorophyll fluorescence parameters associated with photosynthesis in terrestrial plants, which was explained as a hormetic response (Moustakas et al., 2022). Another potential explanation is that Ni may, to some extent, contribute to the functionality of superoxide dismutase enzymes which are vital components in an organism's defense against oxidative stress (Sunda 2012). Nevertheless, this growth alteration should not be dismissed, as it could indirectly impact the competitive dynamics within ecosystems containing multiple phytoplankton species.”

Brand, L. E., Sunda, W. G., and Guillard, R. R.: Reduction of marine phytoplankton reproduction rates by copper and cadmium, *J. Exp. Mar. Biol. Ecol.*, 96, 225-250, [https://doi.org/10.1016/0022-0981\(86\)90205-4](https://doi.org/10.1016/0022-0981(86)90205-4), 1986.

Moustakas, M., Moustaka, J. and Sperdouli, I.: Hormesis in photosystem II: a mechanistic understanding, *Curr. Opin. Toxicol.*, 29, 57-64, <https://doi.org/10.1016/j.cotox.2022.02.003>, 2022.

Price, N. M. and Morel, F. M. M.: Colimitation of phytoplankton growth by nickel and nitrogen, *Limnol. Oceanogr.*, 36, 1071– 1077, <https://doi.org/10.4319/lo.1991.36.6.1071>, 1991.

Pérez, P., Estévez-Blanco, P., Beiras, R. and Fernández, E.: Effect of copper on the photochemical efficiency, growth, and chlorophyll a biomass of natural phytoplankton assemblages, *Environ. Toxicol. Chem.*, 25, 137-143, <https://doi.org/10.1897/04-392R1.1>, 2006.

Sunda, W.G.: Feedback interactions between trace metal nutrients and phytoplankton in the ocean, *Front. Microbiol.*, 3, 204, <https://doi.org/10.3389/fmicb.2012.00204>, 2012.

Yang, T., Chen, Y., Zhou, S. and Li, H.: Impacts of aerosol copper on marine phytoplankton: A review, *Atmosphere*, 10, 414, <https://doi.org/10.3390/atmos10070414>, 2019.

Line 195-204: If *E. huxleyi* and *A. carterae* produce more organic ligands to decrease the free Ni concentrations, we might observe a decline in free Ni levels at the end of the experiment. Conducting a significance test on the free Ni concentrations among the three species could help determine if the presence of additional ligands reduces Ni toxicity. However, variations in metal quotas among different species introduce additional uncertainty when testing this hypothesis. I recommend that the author revise this discussion.

Response: The free Ni concentrations were obtained from the Visual MINTEQ 3.1 software calculation and the values are therefore not coming from direct measurements. Since the ligand concentrations are needed as input for the calculations, we cannot provide a significance test for this study to detect the presence of ligands.

Line 208: The discussion in this section lacks clarity. While the paragraph explains the potential detoxification mechanism in the diatom *T. weissflogii*, it fails to address why *T. weissflogii* exhibited higher sensitivity and lower tolerance to high Ni concentrations.

Response: Thank you for pointing this issue out. Detox mechanisms for *T. weissflogii* are known for this species. However, these studies are based on other trace metals while there isn't any study dedicated to Ni detoxification mechanism. We can therefore only hypothesize that this species applies a similar mechanism for Ni. However, this mechanism does not conflict with *T. weissflogii* sensitivity to Ni, which resulted in being higher compared to the other two tested species. We rephrased the text to clarify. "*The growth rate of T. weissflogii was unperturbed until about 40 μmol/L and dropped rapidly beyond this threshold.*"

Line 212: The cited reference Guo et al., (2022) used different Ni concentrations (0-50 μmol/L) and gradients, so please write the specific Ni concentrations compared here to avoid potentially misleading information. Change "in response to high Ni concentrations" to specific Ni concentrations.

Response: We added the specific Ni concentrations: "*In the study by Guo et al. (2022), most of the tested phytoplankton species did not exhibit growth inhibition in response to the tested Ni concentrations, ranging between 0 and 50 μmol L⁻¹.*"

Line 220: "...in our study led to orders of magnitude..." how many orders of magnitude?

Response: The number of orders is added. "*Indeed, the lower amount of EDTA employed in our study led to five orders of magnitude higher concentration of free Ni²⁺ at the target concentration of 50 μmol L⁻¹ compared to that of Guo et al. (2022).*"

Line 227: “... contained a low amount of Ni...” what’s the concentration of Ni used in Hutchins et al. (2023)?

Response: The specific concentration is added. *“However, the study was conducted in a coastal enhanced weathering scenario where the Ni-release process would be gradual (i.e., years) and the olivine utilized for the experiment contained a low amount of Ni, measuring 0.13 $\mu\text{mol L}^{-1}$ at the highest concentration.”*

Line 231: “These studies showed a range of sensitivities to Ni among different groups.” Please change the word “groups” to a more specific description, like “plankton” etc.

Response: Thank you for the suggestion. The word has been changed to plankton.

Line 231: “LC 50” Please explain or at least write out the full name when the abbreviation first appears.

Response: We rephrased the sentence and added an explanation for LC50 as follow: *“For example, certain diatom species with low IC50 and copepod species with LC50 (concentration expected to be lethal to 50 % of the tested organisms), could potentially be vulnerable to the nickel released in the context of OAE (see Table 2).”*

Line 234: I suggest using the IC50 instead of the “lethal concentration” if you have the information because in this study IC50 was calculated and discussed.

Response: IC50 is not an appropriate indicator for larger organisms with low growth rates. We have rephrased the sentence to remove ambiguity as a former reply.

Line 235: Move “(Huang et al., 2016)” after the “1.7 mmol/L”.

Response: We have moved the reference accordingly.

Line 229-239: Considering the information presented in Table 2 and the potential impact of nickel addition mentioned in line 176, it is advisable for the authors to delve deeper into the discussion of a nickel threshold in the context of OAE projects.

Response: We took into account the reviewer’s comment. The discussion has been expanded accordingly.

“DeForest and Schlekot (2013) suggested a threshold of 20.9 $\mu\text{g L}^{-1}$ Ni (0.35 $\mu\text{mol L}^{-1}$) as the Predicted No Effect Concentration (PNEC) for chronic Ni toxicity in marine organisms. In a coastal OAE scenario, with a short water residence time, the low Ni released from alkaline particles is unlikely to impact the ecosystem due to the slow dissolution rate (Hutchins et al. 2023; Table 2). In the open ocean, olivine must be ground to a very small size (less than 1 μm) before sinking out of the surface mixed layer (Köhler et al. 2013; Meysman and Montserrat, 2017). Thus, olivine has the potential to release a high quantity of Ni above the IC50 and LC50 values reported in Table 2 for most species. The perturbation could be minimal if the mixing with surrounding waters could rapidly dilute the alkaline solution before impacts in plankton species occur. Therefore, the deployment of alkalinity enhancement in zones with high mixing dynamics could meet the PNEC requirement. Taken together, the introduction of Ni through olivine-based OAE has the potential to shift the taxonomic composition of natural phytoplankton communities. Hence, the observed species-specific sensitivities towards the release of Ni underline that caution is needed in terms of magnitude and temporal mode (e.g., weekly, monthly, seasonal, and annual release) of ocean alkalization to alleviate the cumulative effects of Ni.”

DeForest, D. K. and Schlekot, C. E.: *Species sensitivity distribution evaluation for chronic nickel toxicity to marine organisms*, *IEAM*, 9, 580-589, <https://doi.org/10.1002/ieam.1419>, 2013.

Köhler, P., Abrams, J.F., Völker, C., Hauck, J., and Wolf-Gladrow, D.A.: *Geoengineering impact of open ocean dissolution of olivine on atmospheric CO₂, surface ocean pH and marine biology*, *Environ. Res. Lett.*, 8, 014009, <https://doi.org/10.1088/1748-9326/8/1/014009>, 2013.

Meysman, F.J. and Montserrat, F.: *Negative CO₂ emissions via enhanced silicate weathering in coastal environment*, *Biol. Lett.*, 13, 20160905, <https://doi.org/10.1098/rsbl.2016.0905>, 2017

Line244: Please add a reference for the sentence “Nowadays Ni is a highly demanded metal resource for battery manufacture”.

Response: Thank you for the above suggestion. We added the following reference:
Turcheniuk, K., Bondarev, D., Amatucci, G.G. and Yushin, G.: Battery materials for low-cost electric transportation, *Mater. Today*, 42, 57-72, <https://doi.org/10.1016/j.mattod.2020.09.027>, 2021.

Line 253: Limestone is not a metal-free resource mineral (Šiler, 2018). In fact, nearly all minerals used in OAE contain metals, including elements like Ca and Mg. Please change it into a more accurate description.

Response: We have rephrased the sentence following the reviewer’s suggestion as: “*For OAE applications, minerals containing less heavy metals, such as quicklime produced from limestone, could also be considered (Gabe and Rodella, 1999; Šiler et al, 2018).*”

Gabe, U. and Rodella, A. A.: Trace elements in Brazilian agricultural limestones and mineral fertilizers, *Commun. Soil. Sci. Plant. Anal.*, 30, 605–620, <https://doi.org/10.1080/00103629909370231>, 1999.

Šiler, P., Kolářová, I., Bednárek, J., Janča, M., Musil, P. and Opravil, T.: The possibilities of analysis of limestone chemical composition, *IOP Conf. Ser.: Mater. Sci. Eng.*, 379, 012033, <https://doi.org/10.1088/1757-899X/379/1/012033>, 2018.

Line 253-257: The use of limestone differs significantly from that of olivine. Limestone cannot be employed directly as an OAE material, making the example provided somewhat misleading. A revision of this discussion is necessary to provide clarity and context for the subsequent discussion on energy consumption.

Response: Thank you for the above suggestion. We have changed limestone to quicklime so that the application of olivine and quicklime are comparable.

Please review and ensure the accuracy of the reference format, including the inclusion of all necessary information for each citation. Specifically, please make sure to use subscript "2" for "CO₂," superscript "2+" for "Mg²⁺," avoid capitalizing journal titles, provide DOI numbers, and use standard abbreviations for journal names.

Response: Thank you for the above suggestion. We have revised the reference format and ensured the format meets the requirements of Biogeosciences.

References:

Šiler, P.: The possibilities of analysis of limestone chemical composition, Mater. Sci. Eng., 2018