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Phytoplankton Response to Increased Nickel in the Context of Ocean Alkalinity Enhancement

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Abstract

Ocean alkalinity enhancement (OAE) is considered one of the most promising approaches to actively remove carbon dioxide (CO_2) from the atmosphere by accelerating the natural process of rock weathering. This approach involves introducing alkaline substances sourced from natural mineral deposits such as olivine, basalt, and carbonates or obtained from industrial waste products such as steel slags, into seawater and dispersing them over coastal areas. Some of these natural and industrial substances contain trace metals, which would be released into the oceans along with the alkalinity enhancement. The trace metals could serve as micronutrients for marine organisms at low concentrations, but could potentially become toxic at high concentrations, adversely affecting marine biota. To comprehensively assess the feasibility of OAE, it is crucial to understand how the phytoplankton, which forms the base of marine food webs, responds to ocean alkalinization and associated trace metal perturbations. In this study, we investigated the toxicity of nickel on three representative phytoplankton species across a range of Ni concentrations (from 0 to 100 µmol L^{-1} with 12 µmol L^{-1} synthetic organic ligand). The results showed that the growth of the tested species was impacted differently. The low growth inhibition and high IC50 (concentration to inhibit growth rate by 50 %) revealed that both the coccolithophore *Emiliania huxleyi* and the dinoflagellate *Amphidinium carterae* were mildly impacted by the increase in Ni concentrations while the rapid response to exposure of Ni, high growth rate inhibition, and low IC50 of *Thalassiosira weissflogii* indicate low tolerance to Ni in this species. In conclusion, the variability in phytoplankton sensitivity to Ni suggests that for OAE applications with Ni-rich materials caution is required and critical toxic thresholds for Ni must be avoided.

1 Introduction

The progressive release of anthropogenic carbon dioxide (CO_2) into the atmosphere since the industrial revolution resulted in a multitude of environmental challenges, including global warming, ocean acidification, ecosystem alteration, increasing frequency of extreme climatic events, and food insecurity (Pörtner et al., 2022). To keep the effects of climate change within acceptable limits, about 1 to 15

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$GtCO_2$ yr^{-1} must be captured by 2100 (Rogelj et al., 2018). Carbon capture activities, known as negative emission technologies (NETs), have moved to the limelight of discussion as they will need to be implemented over the next two decades to meet the climate targets and limit global warming to < 2°C (Shepherd, 2009; Allan et al., 2021). One of the promising NETs is to accelerate natural rock weathering by introducing finely ground alkaline products on land (Enhanced Weathering, EW) or into the surface ocean (Ocean Alkalinity Enhancement, OAE) to remove CO_2 from the atmosphere (Minx et al., 2018; Bach et al., 2019). OAE, in addition to enhancing the buffering capacity of seawater, has the co-benefit of mitigating ocean acidification (Köhler et al., 2010). Natural alkaline minerals, as well as by-products from industrial activity, are potential candidates for EW and OAE (Taylor et al., 2016; Renforth, 2019). Among the most recognized alkaline minerals, olivine rocks have gained considerable attention (Schuiling and Krijgsman, 2006; Hartmann et al., 2013). These rocks contain high amounts of trace metals, e.g., nickel (Ni) and chromium (Cr) (Montserrat et al., 2017; Amann et al., 2020) that through OAE could affect coastal and off-shore systems, possibly influencing marine communities (Gaillardet et al., 2014).

In seawater, Ni is present at low concentrations (Donat et al., 1994; Mackey et al., 2002; Saito et al., 2004) and acts as a micronutrient when urea serves as the nitrogen source (Muyssen et al., 2004; Egleston and Morel, 2008). However, this metal at elevated concentrations may emerge as a concern to marine ecosystems, due to its toxicity, bioaccumulation, and biogeochemical cycling (Sclater et al., 1976; Hall and Anderson, 1995; Horvatić and Peršić, 2007; Debelius et al., 2011; DeForest and Schlekat, 2013; Martínez-Ruiz and Martínez-Jerónimo, 2015; Karthikeyan et al., 2018). In olivine-based OAE scenarios, Ni concentrations could rapidly rise above critical levels that become harmful to marine organisms (Montserrat et al., 2017; Hartmann et al., 2022). To date, studies on high Ni concentrations are scarce. In this study, we examined the impacts of Ni on three representative marine phytoplankton species: the marine diatom, *Thalassiosira weissflogii*, the dinoflagellate, *Amphidinium carterae*, and the coccolithophore, *Emiliania huxleyi*. This research aimed to (1) investigate how different phytoplankton species respond to a gradient of Ni concentrations and (2) compare the inter-species differences in metal sensitivity.

2 Materials and methods

2.1 Strain and culture conditions

Experiments were conducted with cultures of *Emiliania huxleyi* B92/11 (Plymouth Marine Laboratory), *Amphidinium carterae* CCAP1102 (University of Oldenburg), and *Thalassiosira weissflogii* CCMP1336 (Bigelow Laboratory for Ocean Sciences). Algae were cultivated in sterile-filtered (0.2 µm) f/2 media prepared with artificial seawater (Guillard and Ryther, 1962; Kester et al., 1967). Media were enriched with essential trace metals buffered by EDTA (12 µmol L^{-1}). Cells grew at 18°C with a 12:12h light and dark cycle under 200 µmol photons m^{-2} s^{-1} of photosynthetically active radiation (PAR). Media

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were acclimated to the incubation temperature prior to inoculation from the precultures to avoid a potential thermal shock.

2.2 Species response to Ni exposure

To determine the toxicity of nickel, a stock solution (as $NiCl_2 \times 6H_2O$) was prepared, with a nominal value of 50 mmol L^{-1}. All bottles were soaked with 10 % HCl (Fisher) for 24 h and rinsed with Milli-Q water before the experiment. Stock solutions of Ni were added to the algal media for different Ni concentration treatments (0.01, 0.1, 1, 5, 10, 20, 50, and 100 µmol L^{-1}) and the control with exclusive f/2 medium. Experiments were performed in triplicate 75 mL falcon flasks. All cultures were gently turned by hand twice a day to avoid cells from settling. Samples were always collected at the same time of day between 9:00 a.m. and 10:00 a.m. One-milliliter samples of culture were collected in sterile 2 mL microtubes. The cell density was determined daily with a flow cytometer (BD Accuri™ C6). To minimize the cell metabolic impact on the carbonate chemistry of the medium, the maximum cell densities of *A. carterae, T. weissflogii,* and *E. huxleyi* in the main experiment never exceeded 12000 cells mL^{-1}, 15000 cells mL^{-1}, and 130000 cells mL^{-1}, respectively.

2.3 Growth rate and IC50 value determination

Growth rates (μ; d^{-1}) were calculated from cell density following:

$$\mu = \frac{ln(c_f) - ln\,(c_0)}{d}, \quad (1)$$

where c_0 and c_f are the cell densities at the beginning and end of the experiment, respectively; *d* is the duration of the experiment.

The toxic response was expressed as:

$$I = (1 - \frac{\mu_{inhibited}}{\mu_{control}}) \times 100, \quad (2)$$

where *I* is the growth inhibition and μ is the growth rate.

Dose-response curves were constructed for growth rate following Stephenson et al. (2000). Nonlinear regression models were determined by the least square method. Model equations were chosen based on scatter plots of the growth rates of the different species. The sigmoidal model was applied as:

$$Y = \frac{t}{1+(\frac{C}{u})^B}, \quad (3)$$

where *Y* is the growth rate and *C* is the Ni concentration. The parameter *t* is the control response, and *u* and *B* define the location and shape of the equation, respectively. The concentrations to inhibit growth rate by 50 % (IC50) were located from the dose-response curves.

2.4 Nickel measurement

At the end of the growth experiment, 30 mL media of each sample were filtered with 0.2 µm sterile disc filters to remove the algae. The filtered media were collected for Ni measurements. The total nickel

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concentrations were measured with ThermoFisher Scientific ElementXR while the free Ni^{2+} concentrations were calculated with Visual MINTEQ 3.1 to ensure that the targeted concentrations were reached.

2.5 Statistical analysis

Data represent means ± standard deviations (N = 3). ANOVA was performed on the cell density and growth rates to assess the effect of Ni concentrations. Differences among treatments were tested with Tukey's HSD ANOVA test. Significant differences were reported at the 95 % confidence level. All statistics were conducted in the Rstudio environment (R packages "tidyverse" and "ggplot2"; Posit team, 2023; R Core Team, 2023).

3 Result

3.1 Ni concentrations

Table 1. Targeted, measured, and free concentrations of Ni for the different phytoplankton cultures at the end of the experiment.

	Targeted Ni concentration (μmol/L)	Measured Ni concentration (μmol/L)	Free Ni^{2+} concentration (μmol/L)
Stock	5×10^4	4.16×10^4	
A. carterae	0	0.01	0
	0.01	0.01	8.0×10^{-6}
	0.1	0.06	7.9×10^{-5}
	1	0.72	1.6×10^{-3}
	5	3.60	0.03
	10	7.05	0.17
	20	16.20	4.47
	50	40.60	22.26
	100	80.58	51.64
E. huxleyi	0	0.00	0
	0.01	0.00	0
	0.1	0.12	9.2×10^{-5}
	1	0.88	2.2×10^{-3}
	5	3.89	0.03
	10	7.32	0.19
	20	15.34	3.90
	50	40.17	21.95
	100	78.60	50.19
T. weissflogii	0	0.01	0
	0.01	0.07	3.0×10^{-6}
	0.1	0.07	9.3×10^{-5}
	1	0.77	1.8×10^{-3}
	5	3.99	0.03
	10	8.01	0.27
	20	16.50	4.68
	50	40.91	22.48
	100	77.80	49.60

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The stock solution was not acidified to avoid pH changes in the culture media. For this reason, nickel carbonate precipitation occurred in the stock solution and approximately 80 % of the targeted Ni concentrations were achieved (Table 1, Fig. S1). Free Ni^{2+} was chelated by ligand at low concentrations but concentrations of free Ni^{2+} increased with elevated total Ni (Table 1, Fig. 1). Ligand chelated more Ni^{2+} with elevated total Ni concentration, while the binding ability decreased. More than 60 % free Ni^{2+} were beyond ligand binding capacity at the highest Ni concentration.

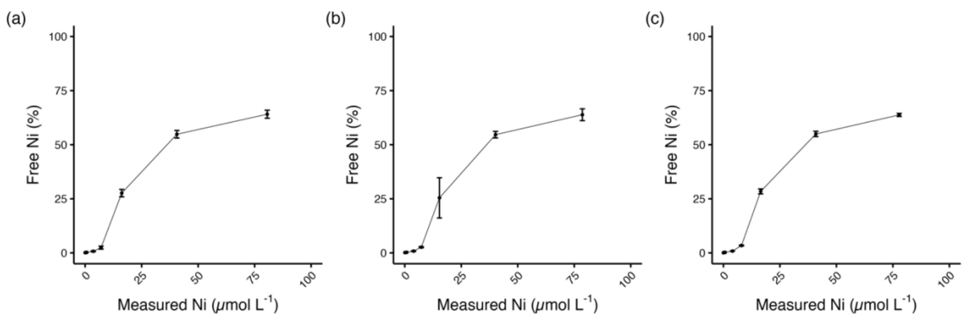

Fig. 1. Percentage of free Ni^{2+} in (**a**) *A. carterae*, (**b**) *E. huxleyi*, and (**c**) *T. weissflogii* cultures at the end of the experiment. Error bars denote standard deviations (N = 3).

3.2 Growth response and cell density accumulation

All three species survived the highest tested concentrations. With increasing Ni, the cell densities and growth rates of the three species decreased, albeit differently.

3.2.1 *Amphidinium carterae*

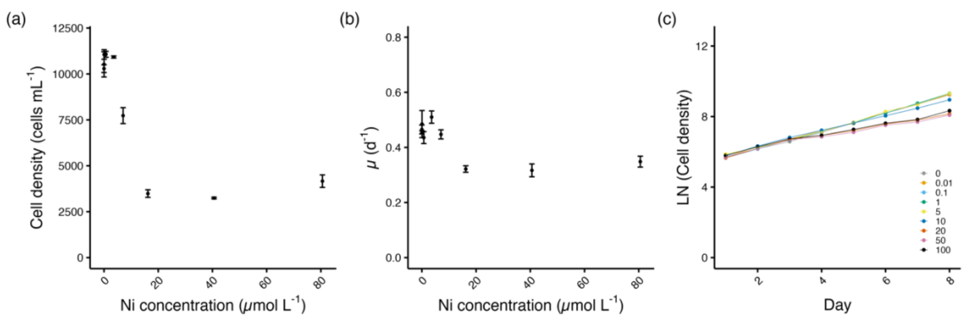

Fig. 2. Growth performance of *A. carterae*. (**a**) Cell densities (cells mL^{-1}), and (**b**) Growth rates (d^{-1}) plotted against measured Ni concentrations on the final experimental day. (**c**) Log-transformed cell densities plotted against time (day) to indicate the time of response; targeted Ni concentrations (μmol L^{-1}) used for clarity. Error bars denote standard deviations (N = 3).

The cell densities of *A. carterae* decreased significantly from 7.1 μmol L^{-1} and the growth rates from 16.2 μmol L^{-1} Ni concentrations ($p < 0.05$; Fig. 2a, Fig. 2b). Growth was not inhibited until day 4 after the exposure to Ni (Fig. 2c). From 16.2 to 80.6 μmol L^{-1} Ni concentrations, we observed the maximum

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decrease in cell density of about 59–66 %, with the growth rate decreasing up to 30 % at the highest Ni concentration compared to the control (Fig. 2a, Fig. 2b).

3.2.2 *Emiliania huxleyi*

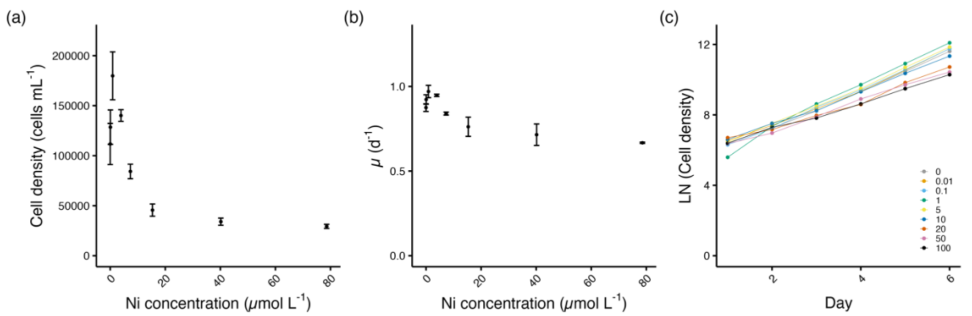

Fig. 3. Growth performance of *E. huxleyi*. (**a**) Cell densities (cells mL^{-1}), and (**b**) Growth rates (d^{-1}) plotted against measured Ni concentrations on the final experimental day. (**c**) Log-transformed cell densities plotted against time (day) to indicate the time of response; targeted Ni concentrations (µmol L^{-1}) used for clarity. Error bars denote standard deviations (N = 3).

The cell densities and growth rates of *E. huxleyi* increased with Ni until 4 µmol L^{-1}. At 0.9 µmol L^{-1} Ni, a 63 % increase in the cell density was observed ($p < 0.01$). A similar trend was observed for the growth rate that increased by about 11 %, however, statistically not significant ($p = 0.07$).
From 15.3 to 78.6 µmol L^{-1} Ni, the cell densities decreased significantly between 57-72 % compared to the control ($p < 0.05$; Fig. 3a). The decrease in growth rate reached up to 24 % at the highest Ni concentration compared to the control (Fig. 3b). The growth of *E. huxleyi* was inhibited from day 3 after being exposed to Ni (Fig. 3c).

3.2.3 *Thalassiosira weissflogii*

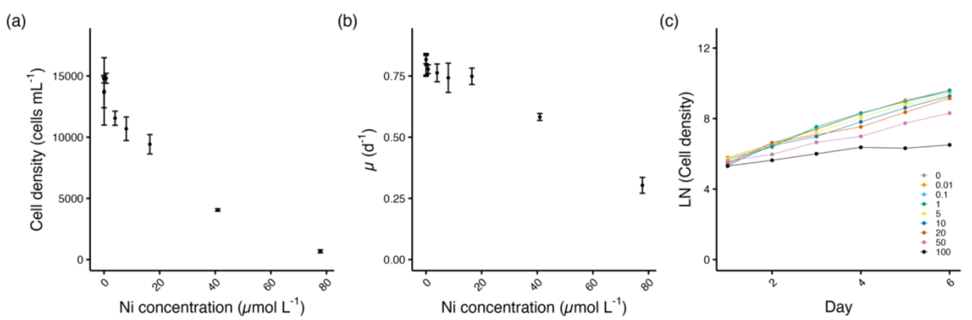

Fig. 4. Growth performance of *T. weissflogii*. (**a**) Cell densities (cells mL^{-1}), and (**b**) Growth rates (d^{-1}) plotted against measured Ni concentrations on the final experimental day. (**c**) Log-transformed cell densities plotted against time (day) to indicate the time of response; targeted Ni concentrations (µmol L^{-1}) used for clarity. Error bars denote standard deviations (N = 3).

The cell densities of *T. weissflogii* decreased significantly from 8.0 µmol L^{-1} and the growth rates from 40.9 µmol L^{-1} Ni concentrations ($p < 0.05$; Fig. 4a, Fig. 4b). The cell density was reduced by 95 % at the highest Ni concentration (Fig. 4a). The growth rate of *T. weissflogii* decreased by 60 % at the highest

Ni concentration (Fig. 4b) compared to the control. After being exposed to Ni, *T. weissflogii* reacted immediately from day 2 (Fig. 4c).

3.3 Determination of IC50

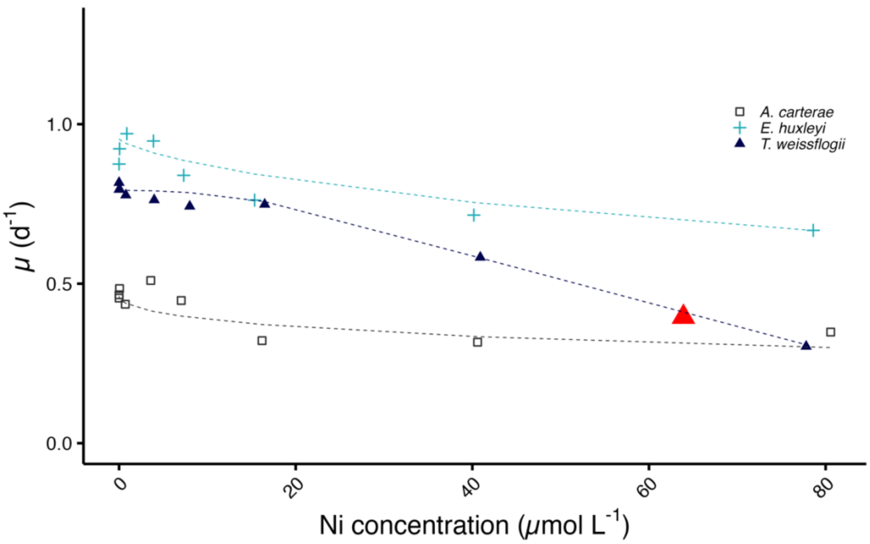

Fig. 5. Predicted growth curves plotted against measured Ni concentrations. The red triangle denotes the IC50 value of *T. weissflogii*. Note that the predicted IC50 values of *A. carterae* and *E. huxleyi* are not shown as they exceed the highest tested Ni concentration.

The diatom *T. weissflogii* has the lowest IC50 value with a concentration of 63.9 µmol L^{-1} while the IC50 values for *A. carterae*, and *E. huxleyi* exceed the highest tested Ni concentration (Fig. 5).

4 Discussion

Trace metals are required by phytoplankton for numerous physiological processes and biochemical reactions. Up to today, it is still complex to disentangle the distinct roles of each element. Ni, for example, is widely recognized to be “bio-required” in several species when urea is utilized as a nitrogen source (Bartha and Ordal, 1965; Pederson et al., 1986; Price and Morel, 1991). However, to our knowledge, no studies have reported that phytoplankton could benefit from supplemented Ni when cultivated in nitrate-enriched media while only a few studies have documented the tolerances of different taxa to progressively increased (possibly toxic) Ni concentrations (Horvatić and Peršić, 2007; DeForest and Schlekat, 2013; Martínez-Ruiz and Martínez-Jerónimo, 2015; Panneerselvam et al., 2018). Nickel in natural seawater has a concentration lower than 10 nmol L^{-1} (Gerringa et al., 2021; John et al., 2022), and exists mainly in the form of free Ni^{2+} (Donat et al., 1994; Achterberg and Van Den Berg, 1997; Saito et al., 2004). Basic and ultrabasic rocks, which are widely recognized source minerals for OAE, would introduce high amounts of Ni into seawater during mineral dissolution. In a previous batch reaction experiment using forsterite olivine sand, an increase of 100 µmol L^{-1} alkalinity was associated with a parallel increase of approximately 3 μmol L^{-1} dissolved Ni during the non-stoichiometric dissolution process (Montserrat et al., 2017). In recent studies on the stability of alkalinity, it has been suggested that using alkaline solutions, rather than reactive alkaline particles, is a safe way to avoid the secondary precipitation of carbonates (Moras et al., 2021; Hartmann et al., 2022). In real-world applications, the release of alkaline solutions at discrete locations could potentially lead to "hotspots" of alkalinity and associated increases of Ni in seawater (Bach et al., 2019; Caserini et al., 2021). Alkalinity enhancement modelling studies suggest that the phytoplankton may be impacted by the cumulative effects of alkalinity and released trace metals from recurring local addition (Ilyina et al., 2013, Feng et al., 2017).

In this study, we focused on the impacts of a gradient of Ni concentrations on three key species that belong to different phytoplankton groups. The results showed that while the three tested species were able to survive in all treatments, they displayed adverse responses to high Ni concentrations. The diatom species *T. weissflogii* was the most sensitive species, with an instant reaction to the exposure of Ni and a decrease in cell density when Ni increased to 4.0 μmol L^{-1}. At the highest Ni concentration, its growth rate was reduced by 60 %. The dinoflagellate *A. carterae* and coccolithophore *E. huxleyi* are more tolerant to Ni enrichment, with less inhibition in growth rate. The growth rates of *A. carterae* and *E. huxleyi* remained relatively constant beyond a certain threshold and the IC50 values of these two species exceeded the highest tested Ni concentration. The cell densities of *E. huxleyi* were even enhanced when Ni was supplied at low concentrations. 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 Ca^{2+} and Mg^{2+} transport systems; the uptake of Ca^{2+} and Mg^{2+} may compete with Ni for the transport pathways and reduce the uptake of Ni in *E. huxleyi* (Deleebeeck et al., 2009). Similar strategies to counteract metal stress were observed in the other species. The dinoflagellate *A. carterae* produces strong ligands to reduce free metal levels (Croot et al., 2000). Production of metal chelators was also reported in diatoms and green algae under metal stress (Gerringa et al., 1995; Gonzalez-Davila et al., 1995). The release of phytochelatin-metal complex probably is a detoxification mechanism of the diatom *T. weissflogii* (Lee et al., 1996). Sequestering metals into a vacuole or storage complexes or binding metals with small chaperones are also adaptive strategies to buffer the uptake of metals (Blaby-Haas and Merchant, 2012).

Due to the increasing interest in olivine-based alkalinization applications, recent studies investigated the effects of Ni on marine phytoplankton in the context of OAE. In the study by Guo et al. (2022), most of the tested phytoplankton species did not exhibit growth inhibition in response to high Ni

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concentrations. The inconsistency between our results and those of Guo et al. (2022) could be attributed to the different amounts of bio-available Ni. Guo et al. (2022) utilized a chelator at a high concentration (100 µmol L^{-1} EDTA), while in our experiment 12 µmol L^{-1} EDTA was added. EDTA can chelate free trace metal ions, forming metal-EDTA complexes. The ligand serves as a buffer by increasing trace metal availability when trace metal concentrations are low and decreasing trace metal reactivity at excess levels (Van den Berg and Nimmo, 1987). Several studies documented that phytoplankton is sensitive to free Ni^{2+} rather than total dissolved Ni (Canterford and Canterford, 1980; Morel et al., 1991; Dupont et al., 2010). Indeed, the lower amount of EDTA used in our study led to orders of magnitude higher free Ni^{2+} compared to that of Guo et al. (2022). We presume that the variance in the growth inhibition between this study and that of Guo et al. (2022) arises from the discrepancy of free Ni^{2+} determined by the different amounts of EDTA used in the two studies. Contrarily, Hutchins et al. (2023) showed that most phytoplankton taxa were irresponsive to Ni independent of the concentrations of Ni species and EDTA. 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. Thus, the synthetic olivine dissolution yielded lower Ni concentrations, possibly without reaching threshold values of toxicity compared to those tested in our experiment.

Several studies investigated the impact of Ni at high concentrations on various marine organisms, albeit outside the specific context of OAE. These studies showed a range of sensitivities to Ni among different groups. For example, some diatom and copepod species that exhibited low IC50 or LC50 values (Horvatić and Peršić, 2007; Huang et al., 2016), could be susceptible to released nickel in the context of OAE (see Table 2). On the contrary, the lethal concentration of Ni for the dinoflagellate *Prorocentrum donghaiense* and the diatom *Skeletonema costatum* was found to be 1.7 mmol L^{-1}, which is unlikely to be encountered during the process of OAE (Huang et al., 2016). Taken together, the introduction of Ni through olivine-based OAE has the potential to shift the taxonomic composition of natural phytoplankton communities. 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 if Ni-rich materials are to be deployed.

Table 2. IC50 and LC50 values (µmol L^{-1}) of different marine organisms. LC50 is the concentration of a material expected to be lethal to 50 % of the tested organisms.

Taxa	Species	Time (h)	Test water	Ni	IC50 (µmol L^{-1})	LC50 (µmol L^{-1})	EDTA (µmol L^{-1})	Reference
Diatom	*Odontella mobiliensis*	96	Natural	-	5.28		/	(Karthikeyan et al., 2018)
Diatom	*Coscinodiscus centralis*	96	Natural	-	10.56		/	(Karthikeyan et al., 2018)
Diatom	*Skeletonema costatum*	72	Natural	-	154		/	(Huang et al., 2016)
Diatom	*Phaeodactylum tricornutum*	72	Natural	-	124.04		/	(Horvatić and Peršić, 2007)
Diatom	*Thalassiosira weissflogii*	120	Synthetic	$NiCl_2 \cdot 6H_2O$	63.9		12	this study

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Dinophyta	*Prorocentrum donghaiense*	96	Natural	-	185		/	(Huang et al., 2016)
Dinophyta	*Amphidinium carterae*	120	Synthetic	$NiCl_2·6H_2O$	>100		12	this study
Cocco-lithophore	*Emiliania huxleyi*	96	Synthetic	$NiCl_2·6H_2O$	>100		12	this study
Copepod	*Oithona similis*	96	Natural	-	47.37		/	(Karthikeyan et al., 2018)
Copepod	*Acartia danae*	96	Natural	-	39.87		/	(Karthikeyan et al., 2018)
Copepod	*Amphiascus tenuiremis*	96	Natural	-		11.76	/	(Hagopian-Schlekat et al., 2001)
Copepod	*Tigriopus brevicornis*	96	Natural	$NiSO_4·6H2O$		3.41	/	(Barka et al., 2001)
Copepod	*Tisbe holothuriae*	48	Synthetic	$Ni(CH_3CO_2)_2 · 4H_2O$		44.30	/	(Verriopoulos and Dimas, 1988)

To prevent the potential ecological impacts of Ni in the process of OAE, Ni could be removed during the preparation of alkaline solutions. Numerous techniques that have been employed to remove Ni from wastewater could provide insights into the removal of Ni in olivine (Kadirvelu et al., 2001; Kim et al., 2002; Kalyani et al., 2004; Papadopoulos et al., 2004; Fu et al., 2007; Decostere et al., 2009). For example, chemical precipitation is an effective and the most widely used method in the industry. Though varying success has been achieved, these methods are associated with high costs, operational drawbacks, and the potential for secondary pollution (Fu and Wang, 2011). Nowadays Ni is a highly demanded metal resource for battery manufacture. A novel approach has been proposed to recover Ni for enhancing the supply of critical battery metals (Wang et al., 2023; Wang and Dreisinger, 2023). This technique could be useful in the context of OAE, contributing to the mitigation of ecological impacts on the one hand and reducing the costs of OAE application on the other. For OAE applications, metal-free resource minerals, such as limestone, could also be considered. These minerals could provide a viable option for the required application without introducing harmful elements into the ocean (Bach et al., 2019; Caserini et al., 2022). The limestone is abundantly available and could meet the requirement for large-scale deployment of OAE. In addition, its economic costs for extraction and transportation are relatively low compared to olivine (Caserini et al., 2022). However, it is essential to acknowledge that the calcination of limestone demands a substantial amount of energy and necessitates proper capture and storage of the released CO_2. To comprehensively assess the applicability and scalability of various material deployments, further investigation and research are warranted.

5 Conclusions

The goal of this study was to examine the response of three phytoplankton species representative for different taxonomic groups to the exposure of elevated Ni, which may occur in the process of OAE. The results demonstrated that the tested phytoplankton species exhibited varying responses to excess Ni. The diatom *T. weissflogii* displayed a high sensitivity to elevated Ni, evident from its rapid growth inhibition response, high growth inhibition, and low IC50 value. In contrast, the low growth inhibition and high IC50 values of *A. carterae* and *E. huxleyi* indicate that these two species are more tolerant to excess Ni. The variability in sensitivity to Ni among different species highlights the importance of avoiding critical toxic thresholds of Ni concentrations. The recovery of Ni from Ni-rich materials and the usage of alternative clean minerals would avoid adverse impacts on the phytoplankton community, enhancing the feasibility and scalability of ocean alkalization. In summary, the varying responses to Ni among different species make it clear that the impacts of Ni cannot be neglected, and that caution is needed in setting the threshold for Ni in OAE applications with Ni-rich materials.

Data availability. The raw data will be made available by the authors, without undue reservation. The data will be submitted to Pangaea, https://www.pangaea.de/.

Author contributions. XX and UR designed the experiment and XX carried them out. XX conducted statistical analyses and prepared the manuscript with contributions from all authors.

Competing interests. The contact author has declared that none of the authors has any competing interests.

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