https://doi.org/10.5194/bg-2023-138
Preprint. Discussion started: 17 August 2023

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Biological impact of ocean alkalinity enhancement of magnesium hydroxide on marine microalgae using bioassays simulating ship-based dispersion

Stephanie Delacroix[1], Tor Jensen Nystuen[1], Erik Höglund[1], Andrew L. King[1]

[1]Norwegian Institute for Water Research, Økernveien 94, 0579 Oslo, Norway

Corresponding author: Stephanie Delacroix (sde@niva.no)

Abstract

Increasing the marine CO_2 absorption capacity by adding alkaline minerals into the world's oceans is a promising marine carbon dioxide removal (mCDR) approach to increase the ocean's CO_2 storage potential and mitigate ocean acidification. Still, the biological impacts of dispersion of alkaline minerals needs to be evaluated prior to its field deployment. In this study, the toxicity effect on marine microalgae of two commonly used alkaline minerals, calcium hydroxide ($Ca(OH)_2$) and sodium hydroxide (NaOH), was compared with magnesium hydroxide ($Mg(OH)_2$), by applying the same concentration of hydroxyl radicals (OH^-) for each component. Cultures of marine green microalgae *Tetraselmis suecica* were exposed to NaOH, $Ca(OH)_2$ or $Mg(OH)_2$ in concentrations mimicking dispersion scenarios from a ship which included short-term exposure with high alkaline mineral concentration called "dispersion phase" followed by a dilution and "regrowth" phase over six days. There was no detectable effect of $Mg(OH)_2$ treatment on algae growth either after the dispersion phase or during the regrowth phase, compared to control treatments. The $Ca(OH)_2$ treatment resulted in very few living algal cells after the dispersion phase, but a similar growth rate was observed during the regrowth phase as was for the $Mg(OH)_2$ and control treatments. The NaOH treatment resulted in no surviving algae after the dispersion phase and during the regrowth phase. Standardized whole effluent toxicity (WET) tests were carried out with a range of $Mg(OH)_2$ concentrations using a sensitive marine diatom, *Skeletonema costatum,* which confirmed the relative low toxicity effect of $Mg(OH)_2$. Similar biological effects were observed on natural microalgae assemblages from a local seawater source when applying the same $Mg(OH)_2$ concentration range and exposure time used in the WET tests. The results suggest that $Mg(OH)_2$ is relatively safe compared to $Ca(OH)_2$ and NaOH with respect to marine microalgae.

1 Introduction

In average, the pH of open ocean surface decreased from 8.15 to 8.00 between 1950 and 2021 (Terhaar et al. 2023); with a rate of change from -0.017 to -0.027 pH units per decade since the late 1980s (Canadell et al., 2021). Models predict that the rate of decrease in pH will continue, or even double, by the end of this century in business-as-usual scenarios (Joos et al., 2011; Orr et al., 2005); reaching an unprecedented rate, never recorded in the entire geological history (Hönisch et al., 2012). The primary cause of the decrease in ocean pH, termed ocean

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acidification (OA), is the increase of the anthropogenic atmospheric emissions of carbon dioxide (Broecker and Takahashi, 1977; Broecker et al., 1979; Feely et al., 2004; Guinotte and Fabry, 2008; Orr et al., 2005). Approximately 25% of anthropogenic CO_2 is absorbed by the ocean, which results in decreased pH and decreased concentrations of carbonate ions available for forming calcium carbonate in the marine biota (Broecker and Takahashi, 1977; Feely et al., 2004; Orr et al., 2005). Marine organisms which are dependent on carbonate ions for the formation of their shell and skeletons, such as corals, mollusks, echinoderms and foraminifera, are especially vulnerable to ocean acidification (Guinotte and Fabry, 2008). Therefore, the three main consequences are negative impacts on; 1) organisms that rely on carbonate-based shells and skeletons, 2) organisms sensitive to lower pH and 3) organisms higher up the food chain that feed on these sensitive organisms. Thus, OA can have devastating socio-economic consequences, by affecting the provision of ecosystem services such as fisheries, tourism and coastal protection (Andersson et al., 2015). For example, shellfish have been shown to be negatively affected by reduced growth and survival of larvae and juveniles under OA scenarios (Dupont et al., 2013; Hettinger et al., 2013; Whiteley, 2011). In addition, the changes in seawater chemical composition due to OA might modify the abundance and toxicity of the harmful algal blooms, which would also negatively impact the shellfish production by accumulation of the algal toxins within the shellfish (Falkenberg et al., 2020). Moreover, such algal blooms can damage the gills of fish in open sea cages, resulting in large losses in the aquaculture industry (Riebesell et al., 2018).

It is widely recognized that reducing the carbon dioxide emissions is not sufficient to accomplish the goals of the Paris agreement of 2015, limiting global warming and OA (Canadell et al., 2021). Accordingly, there is an urgent need for additional carbon dioxide removing approaches. At least seven different marine dioxide carbon removal (mCDR) approaches are currently under evaluation, including; artificial upwelling/downwelling, nutrient fertilization, deep sea storage, electrochemical ocean carbon dioxide removal, macroalgal/microalgal cultivation, marine ecosystem restoration, and ocean alkalinity enhancement (OAE). In general, the principle of these approaches is based on acceleration of the natural process of absorption and long-term storage of the excess atmospheric carbon dioxide by the ocean (Siegel et al., 2021, NASEM, 2021). Among them, OAE has been put forward as one of the most promising approaches, because the acidification remediation process itself triggers the reduction of the atmospheric carbon dioxide level (Renforth and Henderson, 2017). The most studied alkaline minerals for OAE approaches are limestone ($CaCO_3$), olivine $(Mg,Fe)_2SiO_4$, sodium hydroxide (NaOH) and calcium hydroxide ($Ca(OH)_2$) (DOSI, 2022). While the latter mineral has been evaluated for large scale application on the Mediterranean Sea (Butenschön et al., 2021), a large scale study involving field deployment of olivine in coastal waters off New York, USA is currently being performed (Tollefson, 2023). Magnesium hydroxide has also recently been studied (Yang et al., 2023; Hartmann et al., 2022); its relatively low water solubility allows it to be added in a larger amount without reaching harmful pH levels (Tollefson, 2023).

For the selection of the suitable alkaline minerals for OAE, both raw material source scalability (Caserini et al., 2022), alkalinization efficiency and durability are important criteria to evaluate (Hartmann et al., 2022; Ilyina et al., 2013). However, the effects on the aquatic environment need to be considered, including the biological impact of high alkaline mineral concentrations as well as dispersion techniques. Accordingly, Bach et al., (2019) and Burns and Corbett (2020) pointed out that before approval of the alkaline mineral dispersion at global scale, a risk assessment of the toxicity effect of the alkaline minerals on marine organisms must be performed.

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Potentially, there will be considerable local/regional effects of dispersion of alkaline minerals. Increased cation levels (Mg^{2+} and Ca^{2+}), increased bicarbonate and carbonate ions, temporary local pH increase or temporary local decrease of dissolved carbon dioxide might cause perturbation hotspots affecting phytoplankton species composition and growth, resulting in impacts higher up in the food chain (Bach et al., 2019). Biological impacts will strongly depend on the spatial and temporal scale of alkaline mineral dispersion, and studies must therefore use realistic alkaline mineral dispersion scenarios.

In this study, the biological impacts of $Mg(OH)_2$ concentrations expected from dispersion from a moving ship was compared to $Ca(OH)_2$ and NaOH on marine microalga. This was done by exposing cultured *Tetraselmis suecica* to the above alkaline minerals. The toxicity of $Mg(OH)_2$ was then further investigated by using a sensitive microalgal species, in a recognized and standardized whole effluent toxicity (WET) test with cultured diatom *Skeletonoma costatum*. Additional experiments were performed for further toxicity assessment of $Mg(OH)_2$ on a natural microalgal assemblage from local seawater.

2 Methods

The study was performed in three steps. In the first step, the toxicity effect was studied by exposing marine alga to alkaline minerals in successive concentrations mimicking dispersion from a moving ship. These experiments were carried out with *Tetraselmis suecica*, a standard test organism in toxicity studies (Ebenezer et al., 2017; Li et al., 2017; Seoane et al., 2014; Vagi et al., 2005). In the second step, toxicity effects of the alkaline minerals were verified by a standardized WET ecotoxicology assay with *Skeletonoma costatum*, a more sensitive marine algal species, by using the recognized 72 hours growth inhibition test. In the third step, the toxicity effect was studied by exposing a natural assemblage of marine algal species from the Oslofjord, Drøbak, Norway to similar $Mg(OH)_2$ concentrations used in the WET tests.

2.1 Exposure to simulated dispersion of alkaline minerals from a moving ship

The expected distribution of a slurry of $Mg(OH)_2$ during its dispersion from the ship's discharge point on the surface of the oceans was determined utilizing computational fluid dynamic (CFD) models (FORCE Technology Inc., Denmark) and the Bottom RedOx Model (BROM) (Yakushev et al., 2017). In those models, both the forced and natural mixing effects of the $Mg(OH)_2$ by the ship's propeller and physical oceanic processes (as waves, convection, currents, etc.), respectively, in the ship's wake were simulated with different scenarios, including propeller motion, velocity of tangential ocean currents, $Mg(OH)_2$ slurry discharge rate/dissolution rate/settling rate, ship size and ship speed. Dilution was observed with an immediate dilution factor of 1000 within 2 minutes after injection, followed by an additional dilution factor of 7 during the next 5 hours and a final dilution factor of 22 during the following next 5 hours. Moreover, the tonnage capacity and operating costs of a ship were also considered together with a final magnesium hydroxide concentration target of < 1 mg L^{-1}. Taken together, this suggested that the dispersion rate of 500 kg s^{-1} would be the most realistic applicable scenario. From this dispersion rate, it was concluded that marine organisms would be exposed to < 100 g L^{-1} approximately for less than one hour followed by a dilution to <10 mg L^{-1} over a period of 10 hours.

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To investigate biological impact of $Mg(OH)_2$ and compare it with $Ca(OH)_2$ or NaOH, cultures of *Tetraselmis suecica* were exposed to these three alkaline minerals during a simulated dispersion phase (as described above) followed by a regrowth phase (Fig 1). In the dispersion phase, 30 mL of *Tetraselmis suecica* cultures (see further down), in exponential growth with a cell density range within 2.6 x 10^5 - 1.4 x 10^6 cells mL^{-1}, were exposed to the alkaline minerals in 50 mL glass beakers with continuous mixing at approximately 300 rpm with a magnetic stirrer (VELP Scientifica) for 1 hour. To achieve similar concentrations of hydroxide ions in the different alkaline mineral treatments, algae were exposed to either 100 g L^{-1} (or 1.7 M) of $Mg(OH)_2$, 127 g L^{-1} (or 1.7 M) of $Ca(OH)_2$ or 141 g L^{-1} (or 3.4 M) of NaOH (Fig.1).

In the regrowth phase, a subsample from each exposure media was diluted by 10,000 in local seawater and algal cell density was monitored for 6 days. The dilution was performed by mixing 0.25 mL subsample to 2.5 L ambient 60 m deep seawater from the Oslofjord (Fig.1). The diluted subsamples were incubated in 3 L glass beakers in a 20°C temperature-controlled climate room with 24h light (2x 21W Philips Pentura Mini) and continuous mixing with a magnetic stirrer (VELP Scientifica; 100 rpm approximately). The measured light intensity was within 20-60 µmol photons m^{-2} s^{-1}. As the beakers were left uncovered, evaporated water volume was replaced every 24h (except for week-end period) by an equivalent volume of ultrapure water. Effects of each alkaline mineral were investigated in triplicates, including both the exposure and regrowth phases; resulting in total of nine bioassays which were conducted in NIVA's laboratory in Oslo between November 2021 and January 2022. Each bioassay study was conducted with one or two alkaline minerals in parallel and were repeated three times for each alkaline mineral with new cultures of *Tetraselmis suecica*, except for two of the NaOH studies which were started on the same day from the same algal culture. In addition, control bioassays excluding the addition of alkaline minerals were performed in parallel to each alkaline mineral exposure including a dispersal phase followed by a regrowth phase.

The ambient Oslofjord seawater was unfiltered and unsterilized water collected from 60 m depth just outside of NIVA's marine research station located at Drøbak, 40 km south of Oslo. The water quality of this seawater is stable year-round with a temperature of approximately 7°C. This water is representative of ocean regions; i.e. rich in oxygen but poor in inorganic and organic contents, with 0.7 mg C L^{-1} of particulate carbon (POC), 1.1 mg C L^{-1} of dissolved organic carbon (DOC), 6 mg L^{-1} of total suspended solids (TSS) and very low biological load with < 1 cell mL^{-1} of algae and less than 500 CFU mL^{-1} of heterotrophic bacteria.

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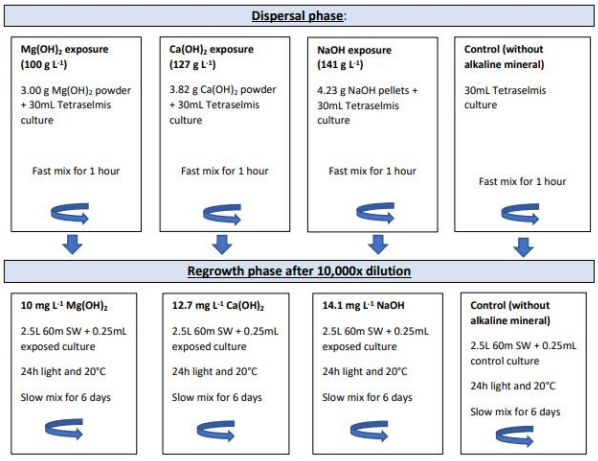

Figure 1. Schematic illustration of the experimental set-up including the dispersion phase in 50 mL glass beakers followed by the dilution step and the regrowth phase of the exposed algal cells in 3 L glass beakers.

Before exposure, the algae were collected from 1 L laboratory cultures of *Tetraelmis suecica* (NIVA-3/10; Norwegian Institute for Water Research, Oslo, Norway). At first, a 50 mL algal culture was prepared by semi-static cultivation in a 100 mL glass flask with 50 mL of autoclaved 20% Z8 culture medium with addition of vitamins (Kotai, 1972). The medium culture was inoculated with 5-10 mL of the *T. suecica* culture from NIVA's algal culture collection. The culture was incubated for ~1 week with fluorescent light tubes giving 20-60 µmol photons m^{-2} s^{-1}, provided by cool-white fluorescence lamps (TLD 36W/950, Philips, London, UK), on an Infors Multicrom 2 incubator shaker (Infors AG, Bottningen, Switzerland) at 20 ± 2°C, with orbital shaking at 90 rpm. After incubation, the culture was used for the inoculation of the 1L culture, except for ~10 mL which was held back to start a new 50 mL culture by adding 40 mL of freshly prepared Z8 medium in same culture conditions as described above. The 1 L culture was prepared by static cultivation with 1 L autoclaved 20 % Z8 medium with addition of 1 mL L^{-1} vitamins in 2-liter glass culture bottles. Approximately 40 mL of the 50 mL stock culture was added to 1 L of medium. The culture was exposed to fluorescent light tubes of 20-60 µM m^{-2} s^{-1} and placed in a 20°C temperature-controlled room for approximately one week.

The culture medium was prepared at least 24 h before usage to allow the equilibrium of media components. The 20% Z8 culture medium was made by mixing 0.2 L of Z8 medium into 0.8 L seawater, and shortly aired with CO_2 (< 1 min) to avoid precipitation of salts during autoclaving. The seawater was pasteurized seawater collected from 60 m depth in the Oslofjord. The medium was autoclaved for 15 minutes at 121°C. 1 mL L^{-1} of vitamins stock solution was added to the 20% Z8 medium (Kotai, 1972).

The studied alkaline minerals were magnesium hydroxide (CAS number: 1309-42-8), calcium hydroxide (CAS number: 1305-62-0) and sodium hydroxide (CAS number: 1310-73-2); all with ≥97.0% purity. Magnesium hydroxide (Batch No. 18417-01A) was provided by Negative Emission Materials, Inc. via a factory in Canada

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producing the mineral by hydrometallurgy process and purification from natural magnesium silicate. The two other alkaline minerals were purchased from Sigma-Aldrich (United Kingdom).

Density of living *Tetraselmis suecica* was determined by using the double staining method with Fluorescein Diacetate (FDA) and 5-chloromethylfluorescein diacetate (CMFDA) (NSF, 2010). For each analysis, a 4 mL subsample was collected and added 4 µl of 10% HCl. The sample was then stained by adding 4 µl of FDA/CMFDA double stain and incubated during 10 minutes in the dark. The stained *Tetraselmis suecica* cells were counted in triplicate (3x 1 mL) in a Sedgwick Rafter counting chamber using fluorescence microscope (Leitz Aristoplan, CoolLED pE-300 lite) with 485-530 nm excitation-emission filter combination and 100x magnification.

Temperature, salinity and pH in the bioassays were measured in-situ by using a calibrated handheld WTW Multimeter (WTW Multi 3620 IDS/3420 IDS displayer) with a conductivity probe (TetraCon 925 Xylem) and a pH-electrode (SenTix 945P). The temperature in the test waters varied within a range of 18-23°C for all experiments during the 6 days of regrowth phase as all experiments were conducted at room temperature. The same temperature was registered in the alkaline test waters compared to the corresponding control waters. The salinity of the test waters, with or without alkaline mineral, was around 32-33 PSU at the start of the 6 days regrowth phase for all experiments. The salinity stayed relatively stable for most of the regrowth phase, except for the last day with an increase up to 35-36 PSU in average. This increase was due to the evaporation of the test water at room temperature during the week-end period included at the end of the 6 days of experimentation.

2.2 Whole Effluent Toxicity (WET) test

The WET test consisted of a marine algal growth inhibition test of 72 hours performed by NIVA's ecotoxicity laboratory according to NIVA's standard procedure which is based on International Standard ISO 10253: Water Quality – Marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. In this study, the diatom *S. costatum* (NIVA-strain BAC 1) was used as test organism.

A 100 mg L^{-1} $Mg(OH)_2$ sample was diluted by using a modified ISO 10253 media, except that no Fe-EDTA stock solution was added, as the tested compound $Mg(OH)_2$ showed to be affected by the presence of EDTA causing precipitation of $Mg(OH)_2$. A preliminary study was made to verify the microalgal growth in this modified media. Although less growth was observed when compared to normal ISO 10253 media, the specific daily growth rate was still greater than 0.9 d^{-1}, which was considered as valid. A total of six concentrations of $Mg(OH)_2$ was tested (1, 10, 25, 50, 75 and 100 mg L^{-1}). The test was performed with 15 mL samples in 30 mL glass vials. Each concentration was tested in triplicate with 6 replicates for each control (one control set with normal ISO 10253 and another control set with modified ISO 10253); same number of replicates for analysis of blank samples but without microalgae added.

All samples were inoculated with 5 x 10^6 cells L^{-1} *of S. costatum* from an exponentially growing laboratory culture and incubated on a shaking table at 20±1°C under continuous illumination of 63 µM m^{-2} s^{-1} of photosynthetic active radiation (PAR).

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The cell density was determined by fluorescence with SpectraMax iD3 microplate after approximately 24, 48 and 72 hours (±2h). The fluorescence measurements were directly correlated to the algal density as a correlation factor (r^2) of 1 between the measured fluorescence and the cell density was calculated. The fluorescence values of the exposed samples without algae (blanks) were measured to investigate potential biases caused by effect of the tested substance on the fluorescence readings. As no such effects were detected, no further transformation of data was necessary.

The temperature, pH and salinity were measured in-situ at the beginning and at the end of each WET test. The temperature varied from 19.9 to 20.3°C for both WET tests. The pH at the start of the experiment varied from 8.089 to 9.376 in all vials for both tests, with increasing pH for increasing $Mg(OH)_2$ concentrations as expected. The pH at the end of the experiment varied from 8.270 to 8.540 in all vials for both tests. The salinity was stable with 32-35 PSU in all vials during the entire experiment for both tests.

2.3 Natural assemblage of ambient marine algal test

For the preparation of the ambient algal culture, either a 25 L grab-sample from the surface water of Oslofjord was directly used for the test or a 2 L subsample was mixed to 2 L of 60 m deep seawater from Oslofjord for further algal growth. For growth, the culture was incubated in a 5 L glass beaker in a climate-room at 20°C and with constant light from fluorescent light tubes of 20-60 µM m^{-2} s^{-1} for four days. The total density of algal cells in the culture after incubation was approximately 1000 cell mL^{-1}. 500 mL of the culture was then mixed, in a 2 L glass beaker with a magnetic stirrer at approximately 90 rpm, added to 1500 mL of a prepared $Mg(OH)_2$ suspension resulting in $Mg(OH)_2$ concentrations of 1, 10, 25, 50, 75 and 100 mg L^{-1} and initial algal density of approximately 125-250 cell mL^{-1}. The $Mg(OH)_2$ suspensions were prepared by mixing 2.7 mg, 27 mg, 66 mg, 133 mg, 200 mg or 270 mg of $Mg(OH)_2$ in 1.5 L of unfiltered 60 m seawater from Oslofjord, with a magnetic stirrer (300 rpm) over the night prior test start. The final solutions were slowly mixed continuously with a magnetic stirrer at approximately 90 rpm, in a climate room at 20°C and with constant light from fluorescent light tubes of 20-60 µmol photons m^{-2} s^{-1} for 72 hours. The water quality and algal density was monitored daily in each beaker. For the control treatment, 500 mL of the ambient algal culture was mixed with 1.5 L of unfiltered 60m deep seawater from Oslofjord, without $Mg(OH)_2$, and incubated as described above. Aliquots from the 100 mg L^{-1} treatment were collected from the initial timepoint and final timepoint (t=3 d) for microscopy-based assessment of community composition by taxa.

2.4 Data analysis

Effects on *T. suecica* cell survival with $Ca(OH)_2$, NaOH, and $Mg(OH)_2$ in simulated dispersions from a moving ship were analyzed with a Kruskal-Wallis analyses of variance (ANOVA) with type of alkaline mineral as independent grouping variable and % survival compared to control treatments after the regrowth phase as the dependent variable.

In the WET test, the growth rate of *S. costatum* in each $Mg(OH)_2$ sample was calculated from the logarithmic increase of cell density from start to 72 hours, and expressed as percentage of the growth rate of control samples. The concentrations causing 50% growth inhibition (EC_{50}) were calculated using a non-linear regression analysis of the growth rate versus log cell concentration of control water (Hill, 1910; Vindimian et al. 1983). The non-observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were calculated

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using Dunnett's test/ t-test for non-homogenous variance and Williams Multiple Sequential t-test for homogenous variance.

Effects of $Mg(OH)_2$ on the natural marine algal assemblage was investigated by dividing the different exposure concentrations (1, 10, 25, 50, 75 and 100 mg L^{-1}) within two groups based on the LOEC (25 mg L^{-1}) from the WET test. This resulted in one low concentration group (1, 10 and 25 mg L^{-1}) and one high concentration group (50, 75 and 100 mg L^{-1}). The difference in % survival compared to control treatment between the high and low concertation groups was investigated by a Student's t-test.

3 Results

3.1 Exposure to simulated dispersion of alkaline minerals from a moving ship

There were significant differences in living cells of *Tetraselmis suecica* (% survival compared to control treatments; Table 1) between the alkaline minerals in the end of the regrowth phase (Kruskal-Wallis ANOVA; $\chi2=6.3$, $P<0.05$), which were reflected in both the dispersion and the regrowth phases. At the start of the regrowth phase, the surviving cell densities in the $Mg(OH)_2$ treatments were similar to the ones observed in control treatment, while only one living cell was observed in one of the $Ca(OH)_2$ treatments and no survival algae could be observed in the NaOH treatments (Day 0; Table 1). In the $Mg(OH)_2$ and $Ca(OH)_2$ treatments, algal cell densities increased during the regrowth phase (Day 1-6; Table 1). No living cells were observed in the NaOH treatments (Fig 2). At the end of the regrowth phase, the algal cell densities in the $Mg(OH)_2$ treatments were similar as in control treatments, while the algal cell densities in $Ca(OH)_2$ treatments showed lower values than in control treatments. Still, no living algal cell could be observed in the NaOH treatments on Day 6 (Day 6; Table 1).

Table 1. Densities of living *Tetraselmis suecica* (cell mL^{-1}) and their relation to control treatment (% Contr.) during the regrowth phase of a bioassay mimicking dispersion of the alkaline minerals $Mg(OH)_2$, $Ca(OH)_2$ or NaOH from a ship. Before the regrowth phase, algae were exposed to either 100 g L^{-1} $Mg(OH)_2$, 127 g L^{-1} $Ca(OH)_2$ or 141 g L^{-1} NaOH (achieving similar amount of hydroxide in the different alkaline mineral suspensions) for 1h. After this, subsamples from each treatment were diluted 10 000 times and algae growth were studied during a 6-day regrowth phase. Each alkaline mineral was assayed in triplicates. Values at day zero corresponds to 1h after dilution and effects of each alkaline mineral was investigated in triplicates.

	$Mg(OH)_2$						$Ca(OH)_2$						$Ca(OH)_2$					
	Replicate 1		Replicate 2		Replicate 3		Replicate 1		Replicate 2		Replicate 3		Replicate 1		Replicate 2		Replicate 3	
Day	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.	Cells ml^{-1}	% Contr.
0	27	84	30	97	82	53	0	0	0	0	1	2.9	0	0	0	0	0	0
1	40	62	64	145	84	53	1	0.66	0	0	1	2.5	0	0	0	0	0	0
2	72	63	129	168	256	64	-		0	0	3	3.5	0	0	0	0	0	0
3	101	72	249	199	-	-	6	0.60	0	0	4	3.6	0	0	0	0	0	0
6	1040	84	1533	263	6217	128	56	0.68	1	0.11	29	2.3	0	0	0	0	0	0

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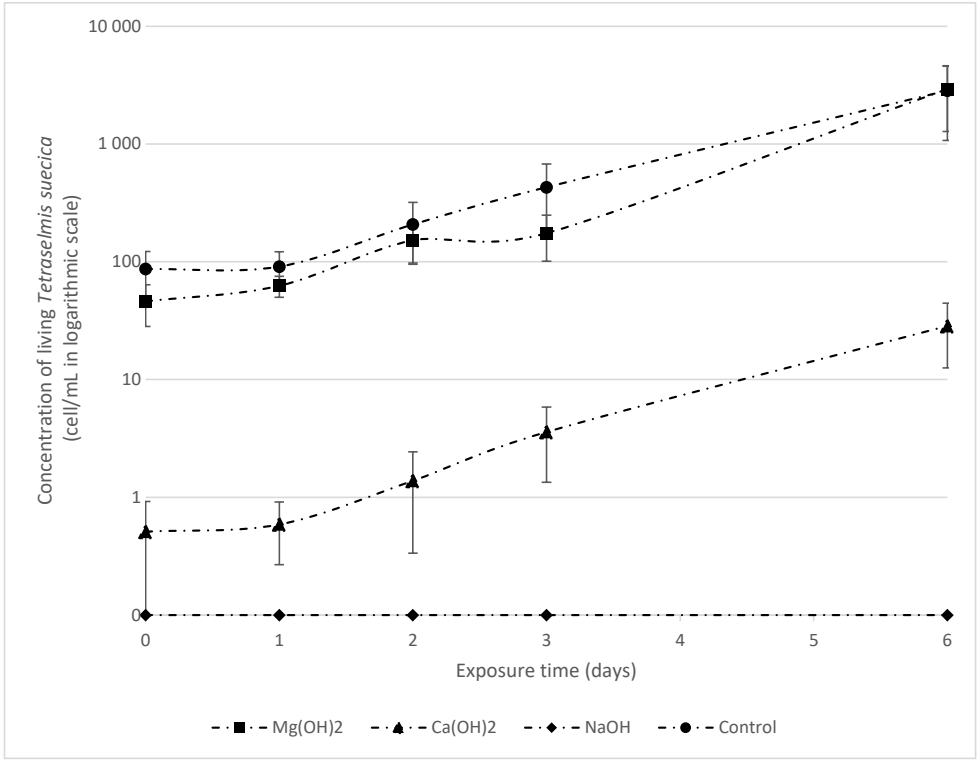

Figure 2. Densities of living Tetraselmis suecica (cell mL^{-1}) during the regrowth phase of a bioassay mimicking dispersion of the alkaline minerals Mg(OH)$_2$, Ca(OH)$_2$ or NaOH from a ship. Before the regrowth phase, algae were exposed to either 100 g L^{-1}, Mg(OH)$_2$, 127 g L^{-1} Ca(OH)$_2$ or 141 g L^{-1} NaOH (achieving similar concentrations of hydroxide ions in the different solutions) for 1h. After this, subsamples from each treatment were diluted 10 000 times and algae growth were studied during the 6 days regrowth phase.

3.2 pH

pH in the control treatments were around 8.0-8.2 during the regrowth phase (Fig. 3). While alkaline mineral treatments resulted in elevated pH (~ 8.5) at day one after dilution step. Where upon, pH decreased and reached similar values as control treatments in day 3 for all alkaline mineral treatments (Fig. 3).

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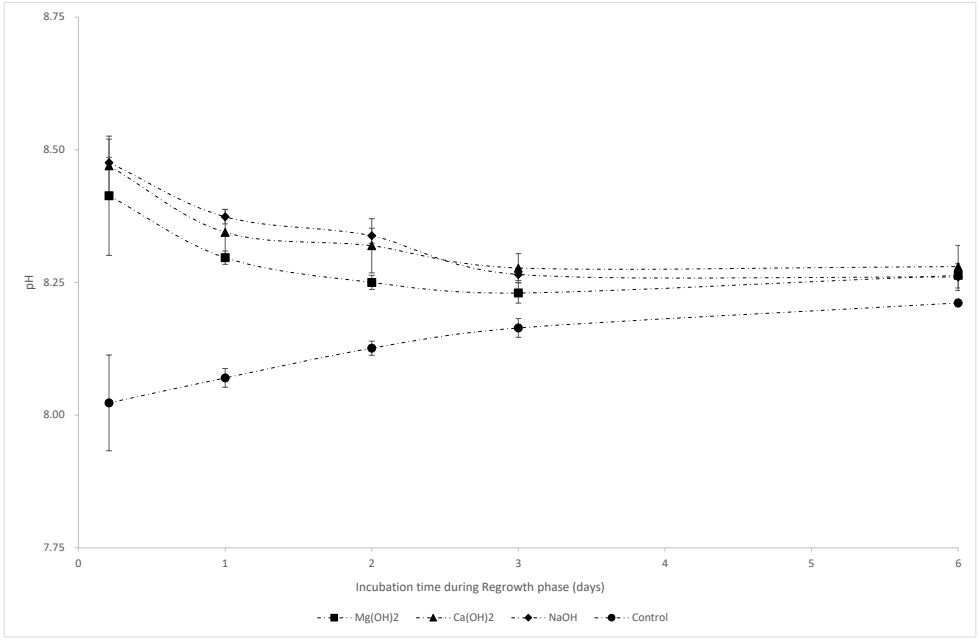

Figure 3. pH during the regrowth phase in a bioassay mimicking dispersion of the alkaline minerals $Mg(OH)_2$, $Ca(OH)_2$ or NaOH from a ship. Before the regrowth phase, algae were exposed to either 100 g L^{-1} $Mg(OH)_2$, 127 g L^{-1} $Ca(OH)_2$ or 141 g L^{-1} NaOH (achieving similar concentrations of hydroxide ions in the different alkaline mineral solutions) for 1h. After this, subsamples from each treatment were diluted 10 000 times to achieve the following concentrations during the regrowth phase; 10 mg L^{-1} $Mg(OH)_2$, 12.7 mg L^{-1} $Ca(OH)_2$ or 14.1 mg L^{-1} NaOH.

3.3 WET tests

The results of the lowest observed effect concentration (LOEC) and the non-observed effect concentration (NOEC) of $Mg(OH)_2$ were similar in both WET tests; with 50 mg L^{-1} and 25 mg L^{-1} $Mg(OH)_2$, respectively. The $Mg(OH)_2$ concentration causing 50% algal growth inhibition was close to 100 mg L^{-1} in both tests; within a range of 82-111 mg L-1 (Table 2).

Table 2. Results of the duplicate Whole Effluent Toxicity (WET) tests on the marine microalgae *Skeletenoma costatum* with freshly prepared 1 L suspension of 100 g L^{-1} $Mg(OH)_2$ in ambient 60m deep seawater from Oslo fjord. A total of six concentrations of magnesium hydroxide (1, 10, 25, 50, 75 and 100 mg L^{-1}) were tested by diluting the suspension in culture medium. EC50: concentration causing 50% algal growth inhibition. LOEC: lowest observed effect concentration. NOEC: non-observed effect concentration (NOEC).

	WET tests, $Mg(OH)_2$ (mg L^{-1})	
Endpoint	1	2
EC_{50}	111	82
LOEC	50	50
NOEC	25	25

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3.4 Natural assemblage of ambient marine algal species

There was a significant difference in algal survival between the low concentrations group (1, 10 and 25 mg L^{-1} $Mg(OH)_2$) and the high concentrations group (50, 75 and 100 mg L^{-1} $Mg(OH)_2$) after three days of exposure ($t_{(4)}$=-5.8, P<0.01; Table 3). The analysis of the algal biodiversity composition in the 100 mg L^{-1} $Mg(OH)_2$ suspension showed that the dominant surviving species were diatoms, including *Skeletonoma spp.*, with 80% and 94% of the total on Day 0 and Day 3, respectively. The biodiversity composition of the natural algal assemblage in beginning and at the end of the experiment for the 100 mg L^{-1} $Mg(OH)_2$ treatment is given in Table 4.

Table 3. Densities of living ambient algal cells (cell mL^{-1}), and their survival in percentage compared to control water without $Mg(OH)_2$ (% Contr.), during 3 days of exposure to six different concentrations of $Mg(OH)_2$ (1, 10, 25, 50, 75 and 100 mg L^{-1}) when incubated in 20°C temperature-controlled room with constant light. Low and high concentration groups refer to the groups used in the Student's t-test, see 2.4 statistics for more information.

	Low $Mg(OH)_2$ concentrations						High $Mg(OH)_2$ concentrations					
	1 mg L^{-1}		10 mg L^{-1}		25 mg L^{-1}		50 mg L^{-1}		75 mg L^{-1}		100 mg L^{-1}	
Day	Cells mL^{-1}	% Contr.	Cells mL^{-1}	% Contr.	Cells mL^{-1}	% Contr.	Cells mL^{-1}	% Contr.	Cells mL^{-1}	% Contr.	Cells mL^{-1}	% Contr.
0	412	96	446	104	246	97	252	99	237	93	231	94
1	907	101	858	96	712	99	438	61	305	42	271	43
2	1107	91	1110	92	1530	122	495	40	328	26	313	11
3	1180	91	1210	94	2140	109	531	27	580	30	388	7

Table 4. Algal biodiversity composition (in % of the total algae) in 100 mg L^{-1} $Mg(OH)_2$ treatment at the initial (Day 0) and last (Day 3) timepoint of the 3 day natural assemblage experiment with local ambient seawater from Oslofjord.

		% of total biodiversity	
Group	Organism	Day 0	Day 3
Diatoms	*Chaetoceros spp.*	39	5
	Skeletonema spp.	29	50
	Other diatoms	16	35
Dinoflagellate	Dinoflagellate	6	1
Unspecified	Monad	6	10
	Flagellate	4	0

4 Discussion

Similar algal densities were observed in both control and $Mg(OH)_2$ treatments at the beginning of the regrowth phase (Day 0, Table 1). This could be related to the short exposure time or to the low solubility of $Mg(OH)_2$; 0.012 g L^{-1} in pure water and around 0.008 g L^{-1} in seawater (Yang et al., 2023). For comparison, the solubility of $Ca(OH)_2$ and NaOH is 1.73 g L^{-1} and 1000 g L^{-1} at 20-25°C, respectively. Accordingly, pH increased during the dispersion phase from approximately 8.0 to 9.5 in the $Mg(OH)_2$ treatment which was lower compared to the expected pH of 12 in $Ca(OH)_2$ and pH of 14 in NaOH treatments (Hartmann et al., 2022). However, pH was similar at the beginning of the regrowth period for all three alkaline mineral treatments at ~8.3-8.6 (Fig. 3), giving similar potential regrowth conditions. The similar growth rates observed in controls, $Mg(OH)_2$-added and $Ca(OH)_2$-added treatments (Fig. 2) suggests that the algae previously exposed to 100 g L^{-1} $Ca(OH)_2$ were able to recover during this phase, at least when the algae were incubated in optimal culture conditions which. might not

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be the case in natural oceanic conditions. Taken together, our data indicated high algal mortality in $Ca(OH)_2$ and NaOH at the high concentrations of 127 and 141 g L^{-1}, respectively, during the first hour after the alkaline mineral discharge from a moving ship, while no such toxic effect was observed when algae were exposed to $Mg(OH)_2$. This emphasizes that the biological impact of alkaline mineral dispersion, in addition to alkalinity increase capability, needs to be considered when evaluating mCDR strategies. Following this, it is important to keep in mind that in this study the toxicity comparison was based on the criteria that each alkaline mineral should have the same hydroxide content, not taking in account difference in alkalinity enhancement between the alkaline minerals. Yang et al. (2023) showed that 12 mg L^{-1} $Mg(OH)_2$ resulted in a stable and efficient alkalinity enhancement in seawater and Hartmann et al. (2022) demonstrated that $Mg(OH)_2$ was 2.4 times more effective in alkalinity enhancement of seawater compared to $Ca(OH)_2$. This supports $Mg(OH)_2$ as a relatively safe and effective alkaline mineral.

The results from the WET tests indicate that no growth inhibition of *S. costatum* was observed for $Mg(OH)_2$ concentrations equal or below to 25 mg L^{-1} (NOEC). This is somewhat in accordance with the simulated dispersion test, showing no growth inhibition of *T. suecica* during the 6 days of regrowth phase in 10 mg L^{-1} magnesium hydroxide. The results from dispersion phase indicate no or low effect of 1 h of exposure with 100 g L^{-1} magnesium hydroxide on *T. suecica*. The WET tests indicated a 50% growth inhibition effect of $Mg(OH)_2$ concentrations (EC_{50}) between 82 and 111 mg L^{-1} after 72 h of exposure. This toxicity effect might be explained by the temporary local CO_2 limitation impact, limiting the algal growth, due to increasing pH at these high alkaline mineral concentrations. These EC50 values were much higher than $Mg(OH)_2$ solubility of ~ 12.2 mg L^{-1} in pure water (Yang et al., 2023). This raises questions regarding the cause of growth inhibition in the current study. It has been suggested that trace metals, such as Cr, Mo, Ni, Pb in industrial and natural mineral products used as alkaline minerals may impair organism growth (Bach et al., 2019; Hartmann et al., 2022). However, this might not be the case here as the $Mg(OH)_2$ powder used in this study was 97-98% ultrapure with <0.01% Ni or Cr. Further studies are needed to verify and investigate the underlaying mechanism for the growth inhibition of *S. costatum* observed in the current WET tests.

The same toxicity effect of $Mg(OH)_2$ was observed in the tests performed with local marine algal species; i.e. no significant toxicity effect of $Mg(OH)_2$ concentrations below 25 mg L^{-1} but significant toxicity effect for concentrations above 50 mg L^{-1}. *Skeletonoma spp.* was represented in the natural assemblage, as one of the dominant species, while *Skeletonoma costatum* was used in the WET tests. This suggests that the results from the WET tests using laboratory monoculture are still representative and applicable to similar species growing in natural marine environment. The biological biodiversity (Table 4) of the local source water included both algal species with hard cell wall made of silicate (diatoms as *Chaetoceros spp.* and *Skeletonoma spp.*), dinoflagellates, monads, and unspecified flagellates. Thus, the results from the natural seawater test demonstrated that toxicity effects observed with $Mg(OH)_2$ on laboratory cultures might be applicable to a wider range of marine algal species.

Thus, both the simulated dispersion scenario, the WET tests and ambient algal tests results suggest that $Mg(OH)_2$ is a suitable alkaline enhancement mineral with respect to minimizing biological impacts on marine microalgae. Our studies focused on marine microalgae, while most studies on biological impacts of alkaline minerals were using species being dependent on carbonate for their development and therefore sensitive to seawater acidification

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(Cripps et al.,2013, Fakhraee et al., 2023, Gomes et al., 2016, Renforth and Henderson, 2017). microalgae play an important role as primary producers and impacts may be reflected in the entire marine ecosystem by affecting higher trophic-level organisms, such as zooplankton and fish (Pauly and Christensen, 1995; Chassot et al., 2010). Accordingly, microalgae are considered a useful and crucial indicator to evaluate the deterioration of environmental quality (Lee et al., 2023). Thus, the current study applying microalgae assays to investigate the effects of $Mg(OH)_2$ suggests a low negative biological impact of $Mg(OH)_2$. However, it is important to keep in mind that these laboratory assays generally are considered as simple, fast and cost-effective and further studies on other functional groups and species are required.

5 Conclusion

The bioassays based on scenario of alkaline mineral dispersion from ship demonstrated that $Mg(OH)_2$ resulted in lower biological impacts on marine microalgae when compared to $Ca(OH)_2$ and Na(OH). Further studies must be completed to include a wider range of biological biodiversity from different trophic levels and on a larger scale, such as in mesocosm studies. The observed low negative biological impact of $Mg(OH)_2$ was confirmed by the standardized toxicity test using a more sensitive marine algae species, but also by the tests with a wider range of local ambient marine algal species.

6 Data availability

Data will be made available upon request.

7 Author contribution

SD established the study plan, collected all data for data analyses and drafted the first version of this manuscript. EH was involved in statistical analyzes and writing up the manuscript in collaboration with all authors. TN performed the laboratory experiments (both dispersion and regrowth phases) and recorded the biological and chemical analyses results. AK was involved in the quality assurance of the final manuscript.

8 Competing interests

NIVA received funding from Negative Emissions Material Inc. (Claymont, USA) to perform the study and from Windward Fund (Washington, USA) for the writing of this publication after results disclosure agreement with Negative Emissions Material Inc. The Windward Fund was founded in response to donors who expressed a desire to be more connected to their peers' work, and to partner with experts in conservation nonprofit management to execute bold initiatives. More info here: https://www.windwardfund.org/about-the-fund/. The authors declare that they have no conflict of interest.

9 Acknowledgments

We would like to thank Dr. Evgeniy Yakushev (NIVA) for the development of the BROM model for magnesium hydroxide specific application, Dr. August Tobiesen (NIVA) and Dr. Ana Catarina Almeida (NIVA) for their

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expertise contribution during test plan and/or experiments execution of bioassays and/or WET tests. This material is based upon work supported by funding from Negative Emissions Material Inc. (Claymont, USA) to perform both the bioassays and the WET tests. NIVA has received funding from Windward Fund (Washington, USA) for the writing of this publication under the Master Services Agreement No. Windward-NOR16-MSA-2023.

10 References

Andersson, A. J., Kline, D. I., Edmunds, P. J., Archer, S. D., Bednaršek, N., Carpenter, R. C., Chadsey, M., Goldstein, P., Grottoli, A. G., and Hurst, T. P.: Understanding ocean acidification impacts on organismal to ecological scales, Oceanography, 28, 16-27, doi: 10.5670/oceanog.2015.27, 2015.

Bach, L. T., Gill, S. J., Rickaby, R. E., Gore, S., and Renforth, P. J. F. i. C.: CO2 removal with enhanced weathering and ocean alkalinity enhancement: potential risks and co-benefits for marine pelagic ecosystems, Frontiers in Climate, 1, 7, doi: 10.3389/fclim.2019.00007, 2019.

Broecker, W., and Takahashi, T. J. N. A. A. M.: Neutralisation of fossil ful CO2 by marine calcium carbonate, in: The fate of fossil fuel CO2 in the oceans, edited by: Andersen, N.R., Malahoff, A. 213, 1977.

Broecker, W. S., Takahashi, T., Simpson, H., and Peng, T.-H.: Fate of fossil fuel carbon dioxide and the global carbon budget, Science, 206, 409-418, doi: 10.1126/science.206.4417.409, 1979.

Burns, W., and Corbett, C. R.: Antacids for the sea? Artificial ocean alkalinization and climate change, One Earth, 3, 154-156, doi: 10.1016/j.oneear.2020.07.016, 2020.

Butenschön, M., Lovato, T., Masina, S., Caserini, S., and Grosso, M.: Alkalinization scenarios in the Mediterranean Sea for efficient removal of atmospheric CO2 and the mitigation of ocean acidification, Frontiers in Climate, 3, 614537, doi: 10.3389/fclim.2021.614537, 2021.

Canadell, J.G., P.M.S. Monteiro, M.H. Costa, L. Cotrim da Cunha, P.M. Cox, A.V. Eliseev, S. Henson, M. Ishii, S. Jaccard, C. Koven, A. Lohila, P.K. Patra, S. Piao, J. Rogelj, S. Syampungani, S. Zaehle, and K. Zickfeld: Global Carbon and other Biogeochemical Cycles and Feedbacks. In Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 673–816, doi:10.1017/9781009157896.007, 2021.

https://doi.org/10.5194/bg-2023-138
Preprint. Discussion started: 17 August 2023

436 Caserini, S., Storni, N., and Grosso, M.: The availability of limestone and other raw materials for ocean
437 alkalinity enhancement, Global Biogeochemical Cycles, 36, e2021GB007246, doi: 10.1029/2021GB007246,
438 2022.
439
440 Chassot, E., Bonhommeau, S., Dulvy, N.K., Mélin, F., Watson, R., Gascuel, D., and Le Pape. O.: Global marine
441 primary production constrains fisheries catches, Ecology Letters, 13, 4, 495-505, doi: 10.1111/j.1461-
442 0248.2010.01443.x, 2010.
443
444 Cripps, G., Widdicombe, S., Spicer, J., and Findlay, H.S.: Biological impacts of enhanced alkalinity in Carcinus
445 maenas, Marine Pollution Bulletin, 71, 1-2, 190-198, doi: 10.1016/j.marpolbul.2013.03.015, 2013.
446
447 DOSI (2022). "Ocean Alkalinity Enhancement." Deep Ocean Stewardship Initiative Policy Brief.
448 https://www.dosi-project.org/wpcontent/uploads/Alkalinity-Enhancement-Policy-Brief.pdf
449
450 Dupont, S., Dorey, N., Stumpp, M., Melzner, F., and Thorndyke, M.: Long-term and trans-life-cycle effects of
451 exposure to ocean acidification in the green sea urchin Strongylocentrotus droebachiensis, Marine biology, 160,
452 1835-1843, 10.1007/s00227-012-1921-x, 2013.
453
454 Ebenezer, V., and Ki, J.S.: Toxic Effects of Aroclor 1016 and Bisphenol A on Marine Green Algae Tetraselmis
455 Suecica, Diatom Ditylum Brightwellii and Dinoflagellate Prorocentrum Minimum, The Korean Journal of
456 Microbiology 52, 3, 306–312. doi:10.7845/KJM.2016.6050, 2016.
457
458 Fakhraee, M., Li, Z., Planavsky, N.J., and Reinhard, C.T.: A biogeochemical model of mineral-based ocean
459 alkalinity enhancement: impacts on the biological pump and ocean carbon uptake, Environmental Research
460 Letters, 18, 044047, doi: 10.1088/1748-9326/acc9d4, 2023.
461
462 Falkenberg, L. J., Bellerby, R. G., Connell, S. D., Fleming, L. E., Maycock, B., Russell, B. D., Sullivan, F. J.,
463 Dupont, S. J. and Health, P.: Ocean acidification and human health, International Journal of Environmental
464 Research and Public Health, 17, 4563, doi: 10.3390/ijerph17124563, 2020.
465
466 Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F.: Impact of
467 anthropogenic CO2 on the CaCO3 system in the oceans, Science, 305, 362-366, doi: 10.1126/science.109732,
468 2004.
469
470 Gomes, H.I., Mayes, W.M., Rogerson, M., and Stewart, D.I.: Alkaline residues and the environment: a review
471 of impacts, management practices and opportunities, Journal of Cleaner Production, 112, 3571-3582, doi:
472 10.1016/j.jclepro.2015.09.111, 2016.
473
474 Guinotte, J. M., and Fabry, V. J.: Ocean acidification and its potential effects on marine ecosystems, Annals of
475 the New York Academy of Sciences, 1134, 320-342, doi: 10.1196/annals.1439.013, 2008.

https://doi.org/10.5194/bg-2023-138
Preprint. Discussion started: 17 August 2023

476
477 Hartmann, J., Suitner, N., Lim, C., Schneider, J., Marín-Samper, L., Arístegui, J., Renforth, P., Taucher, J., and
478 Riebesell, U. Stability of alkalinity in ocean alkalinity enhancement (OAE) approaches–consequences for
479 durability of CO 2 storage, Biogeosciences Discussions, 2022, 1-29, doi: 10.5194/bg-2022-126, 2022.

480 Hönisch, B., Ridgwell, A., Schmidt, D.N., Thomas, E., Gibbs, S.J., Sluijs, A., Zeebe. R., Kump, L., Martindale,
481 R.C., Greene, S.E., Kiessling, W., Ries, J., Zachos, J.C., Royer, D.L., Barker, S., Marchitto Jr., T.M., Moyer, R.,
482 Pelejero, C., Ziveri, P., Foster, G.L., and Williams, B.: The Geological Record of Ocean Acidification, Science,
483 335,1058-1063, doi:10.1126/science.1208277, 2012.

484 Hettinger, A., Sanford, E., Hill, T. M., Lenz, E. A., Russell, A. D., and Gaylord, B.: Larval carry-over effects
485 from ocean acidification persist in the natural environment, Global Change Biology, 19, 3317-3326, doi:
486 doi.org/10.1111/gcb.12307, 2013.
487
488 Hill. A.V.: The possible effects of aggregation of the molecules of haemoglobin on its dissociation curves. J.
489 Physiol, 40, IV-VII, 1910.
490
491 Ilyina, T., Wolf-Gladrow, D., Munhoven, G., and Heinze, C. J. G. R. L.: Assessing the potential of calcium-
492 based artificial ocean alkalinization to mitigate rising atmospheric CO2 and ocean acidification, Geophysical
493 Research Letters, 40, 5909-5914, doi: 10.1002/2013GL057981, 2013.
494
495 Joos, F., Frölicher, T.L., Steinacher, M., and Plattner, G-K.: Impact of climate change mitigation on ocean
496 acidification projections, edited by: gattuso, J-P., hansson, L., 272, 2011.
497
498 Kotai, J.., Oslo: Instructions for preparation of modified nutrient solution Z8 for algae, Norwegian Institute for
499 Water Research, 11, 5, 1972.
500
501 Lee, J., Hong, S., An, S. A., and Khim, J. S.: Methodological advances and future directions of microalgal
502 bioassays for evaluation of potential toxicity in environmental samples: A review, Environment International,
503 107869, doi: 10.1016/j.envint.2023.107869, 2023.
504
505 Li, J., Schiavo, S., Rametta, G., Miglietta, M.L., La ferrara, V., Wu, C., and Manzo, S.: Comparative toxicity of
506 nano ZnO and bulk ZnO towards marine algae Tetraselmis suecica and Phaeodactylum tricornutum, Environ Sci
507 Pollut Res 24, 6543–6553, doi:10.1007/s11356-016-8343-0, 2017.
508
509 National Academies of Sciences, Engineering, and Medicine (NASEM): A Research Strategy for Ocean-based
510 Carbon Dioxide Removal and Sequestration, Washington, DC, The National Academies Press., doi:
511 10.17226/26278, 2021.
512

https://doi.org/10.5194/bg-2023-138
Preprint. Discussion started: 17 August 2023

513 Nestler, H., Groh, K. J., Schönenberger, R., Behra, R., Schirmer, K., Eggen, R. I., and Suter, M. J.-F. J. A. t.:
514 Multiple-endpoint assay provides a detailed mechanistic view of responses to herbicide exposure in
515 Chlamydomonas reinhardtii, Aquatic toxicology, 110, 214-224, doi: 0.1016/j.aquatox.2012.01.014, 2012.
516
517 NSF International. Generic Protocol for the Verification of Ballast Water Treatment Technology. U.S.
518 Environmental Protection Agency, Washington, DC, EPA/600/R-10/146, 2010.
519
520 Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida,
521 A., and Joos, F.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying
522 organisms, Nature, 437, 681-686, doi: 10.1038/nature04095, 2005.
523
524 Pauly, D., and Christensen, V.: Primary production required to sustain global fisheries, Nature, 374, 255-257,
525 doi: 10.1038/374255a0, 1995.
526
527 Renforth, P., and Henderson, G.: Assessing ocean alkalinity for carbon sequestration, Reviews of Geophysics,
528 55, 636-674, 10.1002/2016RG000533, 2017.
529
530 Riebesell, U., Aberle-Malzahn, N., Achterberg, E. P., Algueró-Muñiz, M., Alvarez-Fernandez, S., Arístegui, J.,
531 Bach, L. T., Boersma, M., Boxhammer, T., and Guan, W.: Toxic algal bloom induced by ocean acidification
532 disrupts the pelagic food web, Nature Climate Change 8, 1082-1086, doi: 10.1038/s41558-018-0344-1, 2018.
533
534 Seoane, M., Rioboo, C., Herrero, C., and Cid, A.: Toxicity induced by three antibiotics commonly used in
535 aquaculture on the marine microalga Tetraselmis suecica (Kylin) Butch, Marine Environmental Research, 10, 1-
536 7, doi: 10.1016/j.marenvres.2014.07.011, 2014.
537
538 Siegel, D. A., DeVries, T., Doney, S., and Bell, T.: Assessing the sequestration time scales of some ocean-based
539 carbon dioxide reduction strategies, Environmental Research Letters 16, 104003, doi: 10.1088/1748-
540 9326/ac0be0, 2021.
541
542 Terhaar, J., Frölicher T.L., and Joos F.: Ocean acidification in emission-driven temperature stabilization
543 scenarios: the role of TCRE and non-CO2 greenhouse gases., Environmental Research Letters 18, 024033, doi:
544 10.1088/1748-9326/acaf91, 2023.
545
546 Tollefson, J.: Start-ups are adding antacids to the ocean to slow global warming. Will it work?, Nature, 618,
547 902-904, 10.1038/d41586-023-02032-7, 2023.
548
549 Vagi, M.C., Kostopoulou, M.M., Petsas, A.S., Lalousi, M.E., Rasouli C.H., and Lekkas, T.D.: Toxicity of
550 organophosphorous pesticides to the marine alga *Tetraselmis suecica*, Global NEST Journal, 7, 2, 222-227,
551 researchgate.net/publication/291025132, 2005.
552

https://doi.org/10.5194/bg-2023-138
Preprint. Discussion started: 17 August 2023

553 Vindimian. E., Robaut. R., and Fillion, G.: A method for co-operative and non-cooperative binding studies
554 using non-linear regression analysis on a microcomputer, J. Appl. Biochem, 5, 261-268, PMID: 6689608, 1983.
555
556 Whiteley, N.: Physiological and ecological responses of crustaceans to ocean acidification, Marine Ecology
557 Progress Series, 430, 257-271, doi: 10.3354/meps09185, 2011.
558
559 Yakushev, E. V., Protsenko, E. A., Bruggeman, J., Wallhead, P., Pakhomova, S. V., Yakubov, S. Kh., Bellerby,
560 R. G. J., and Couture, R.-M. 2017: Bottom RedOx Model (BROM v.1.1): a coupled benthic–pelagic model for
561 simulation of water and sediment biogeochemistry, Geosci. Model Dev., 10, 453-482, doi: 10.5194/gmd-10-
562 453-2017, 2017.
563
564 Yang, B., Leonard, J., and Langdon, C.: Seawater alkalinity enhancement with magnesium hydroxide and its
565 implication for carbon dioxide removal, Marine chemistry, 104251, doi: 10.1016/j.marchem.2023.1042512023.
566