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

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Abstract

Increasing the marine CO₂ 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 CO₂ 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
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 CO₂ 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 (CaCO₃), olivine (Mg,Fe)₂SiO₄, 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 (Butenschoen 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.
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 of a moving ship was compared to Cu(OH)$_2$ and NaOH on marine microalgae. 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 *Skeletonema 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 *Skeletonema 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.
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 $2.6 \times 10^5$ - $1.4 \times 10^6$ 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.
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 1L 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 CO₂ (< 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.
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 display) 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 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 10⁶ 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).
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 (r²) 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 60 m 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 time point and final time point (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 (EC₅₀) 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.
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 concentration 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; $\chi^2=6.3$, $P<0.05$), 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.

<table>
<thead>
<tr>
<th>Day</th>
<th>Mg(OH)$_2$</th>
<th>NaOH</th>
<th>Ca(OH)$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>43%</td>
<td>43%</td>
<td>43%</td>
</tr>
<tr>
<td>2</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
</tr>
<tr>
<td>3</td>
<td>1%</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

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.
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).
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).

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC$_{50}$</td>
<td>111</td>
<td>82</td>
</tr>
<tr>
<td>LOEC</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>NOEC</td>
<td>25</td>
<td>25</td>
</tr>
</tbody>
</table>
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\(_{(z=-5.8, P<0.01;\; Table\; 3)}\)). 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 Skeletonema 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.

<table>
<thead>
<tr>
<th></th>
<th>Low Mg(OH)(_2): concentrations</th>
<th>High Mg(OH)(_2): concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 mg L(^{-1})</td>
<td>10 mg L(^{-1})</td>
</tr>
<tr>
<td>Day 0</td>
<td>Cells mL(^{-1})</td>
<td>% Contr.</td>
</tr>
<tr>
<td>0</td>
<td>412 96</td>
<td>446 104</td>
</tr>
<tr>
<td>1</td>
<td>907 101</td>
<td>858 96</td>
</tr>
<tr>
<td>2</td>
<td>1107 91</td>
<td>1110 92</td>
</tr>
<tr>
<td>3</td>
<td>1180 91</td>
<td>1210 94</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Group</th>
<th>Organism</th>
<th>% of total biodiversity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>Diatoms</td>
<td>Chaeotoceros spp.</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Skeletonema spp.</td>
<td>29</td>
</tr>
<tr>
<td></td>
<td>Other diatoms</td>
<td>16</td>
</tr>
<tr>
<td>Dinoflagellate</td>
<td>Dinoflagellate</td>
<td>6</td>
</tr>
<tr>
<td>Unspecified</td>
<td>Monad</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Flagellate</td>
<td>4</td>
</tr>
</tbody>
</table>

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
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 into 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 underlying 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.
(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

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