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4 **microalgae in alkaline mineral concentrations simulating the**
5 **initial concentrations after ship-based dispersions**

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14 **Ocean Alkalinity Enhancement impacts: Regrowth of marine**
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22

23 **Abstract**

24 Increasing the marine CO₂ absorption capacity by adding alkaline minerals into the world's oceans is a promising
25 marine carbon dioxide removal (mCDR) approach to increase the ocean's CO₂ storage potential and mitigate
26 ocean acidification. Still, the biological impacts of dispersion of alkaline minerals needs to be evaluated prior to
27 its field deployment, especially the impacts of the initial discharge causing local and temporary extreme
28 alkalinity/pH changes. In this study, the toxicity effect on marine microalgae of two commonly used alkaline
29 minerals, calcium hydroxide (Ca(OH)₂) and magnesium hydroxide (Mg(OH)₂), by adding the same equivalent
30 molar concentration of hydroxyl ions. Cultures of marine green microalgae *Tetraselmis suecica* were exposed
31 to Ca(OH)₂ or Mg(OH)₂, in concentrations mimicking the initial high concentrations following a dispersion
32 scenario from a ship. A short-term exposure with high alkaline mineral concentration called "dispersion phase"
33 was followed by a dilution step and a "regrowth" phase over six days. There was no detectable effect of Mg(OH)₂
34 treatment on algae growth either after the dispersion phase or during the regrowth phase, compared to control
35 treatments. The Ca(OH)₂ treatment resulted in very few living algal cells after the dispersion phase, but a similar
36 growth rate was observed during the regrowth phase as was for the Mg(OH)₂ and control treatments. Standardized
37 whole effluent toxicity (WET) tests were carried out with a range of Mg(OH)₂ concentrations using a sensitive
38 marine diatom, *Skeletonema costatum*, which confirmed the relative low toxicity effect of Mg(OH)₂. Similar
39 biological effects were observed on natural microalgae assemblages from a local seawater source when applying
40 the same Mg(OH)₂ concentration range and exposure time used in the WET tests. The results suggest that
41 Mg(OH)₂ is relatively safe compared to Ca(OH)₂ with respect to marine microalgae.

42

43 1 Introduction

44 It is widely recognized that reducing carbon dioxide emissions is not sufficient to accomplish the goals of the
45 Paris agreement of 2015, limiting global warming and ocean acidification (Pathak et al., 2022). Accordingly, there
46 is an urgent need for additional carbon dioxide removing approaches. Many different marine dioxide carbon
47 removal (mCDR) approaches are currently under evaluation (GESAMP, 2019), including artificial
48 upwelling/downwelling, nutrient fertilization, deep sea storage, electrochemical ocean carbon dioxide removal,
49 macroalgal/microalgal cultivation, marine ecosystem restoration, and ocean alkalinity enhancement (OAE). In
50 general, the principle of some of these approaches is based on acceleration of the natural process of absorption
51 and long-term storage of the excess atmospheric carbon dioxide by the ocean (Siegel et al., 2021, NASEM, 2021).
52 Among them, OAE has been put forward as one of the most promising approaches, because the acidification
53 remediation process itself triggers the reduction of the atmospheric carbon dioxide level (Renforth and Henderson,
54 2017). Hence, when the aqueous carbon dioxide deficit, generated by the addition of alkaline mineral, returns to
55 the initial equilibrium with atmospheric carbon dioxide, the final pH still remains slightly higher than the initial
56 pH, while calcite (most stable polymorph of calcium carbonate CaCO_3) level and aragonite (crystal structure of
57 calcium carbonate) saturation state are elevated. The aragonite saturation state is commonly used to track ocean
58 acidification (Qing-Jiang et al., 2015). The most studied alkaline minerals for OAE approaches are limestone
59 (CaCO_3), olivine ($\text{Mg,Fe}_2\text{SiO}_4$), sodium hydroxide (NaOH) and calcium hydroxide (Ca(OH)_2) (DOSI, 2022).
60 While the latter mineral has been evaluated for large scale application on the Mediterranean Sea (Butenschön et
61 al., 2021), a large-scale study involving field deployment of olivine in coastal waters off New York, USA is
62 currently being performed (Tollefson, 2023). Magnesium hydroxide has also recently been studied (Yang et al.,
63 2023; Hartmann et al., 2022). Its relatively low water solubility allows it to be added in a larger amount without
64 reaching harmful pH levels (Tollefson, 2023) and will potentially increase the durability of the alkalization
65 effect. Following this, in addition to raw material source scalability (Caserini et al., 2022), alkalization efficiency
66 and solubility are important criteria of OAEs (Hartmann et al., 2022; Ilyina et al., 2013). Moreover, the effects on
67 the aquatic environment need to be considered, including the biological impact of the initial discharge of high
68 alkaline mineral concentrations upon dispersion causing local and temporary extreme alkalinity/pH changes.
69 Accordingly, Bach et al., (2019) and Burns and Corbett (2020) pointed out that before approval of the alkaline
70 mineral dispersion at global scale, a risk assessment of the toxicity effect of the alkaline minerals on marine
71 organisms must be performed. Thus, it is crucial to consider not only the toxicity effect, if any, of the final low
72 alkaline mineral concentration after expected final dilution into ocean, but also the potential initial toxicity effect
73 of the initial hot spot discharge of the alkaline mineral on local organisms. These discharges upon dispersion
74 might be local and temporary, but it is important to consider that they would be applied at a global scale. These
75 local and temporary effects will potentially include increased cation levels (Mg^{2+} and Ca^{2+}), increased bicarbonate
76 and carbonate ions, pH increase or decrease of dissolved carbon dioxide. Perturbations that potentially form
77 impact hotspots, affecting phytoplankton species composition and growth, resulting in impacts higher up in the
78 food chain (Bach et al., 2019). Biological impacts will strongly depend on the spatial and temporal scale of
79 alkaline mineral dispersion, and studies must therefore use realistic alkaline mineral dispersion scenarios.

80 In this study, the biological impact of initial and temporary discharge of Mg(OH)_2 concentrations expected from
81 dispersion from a moving ship was compared to Ca(OH)_2 on marine microalga. This was done by exposing
82 cultured *Tetraselmis suecica* to the above alkaline minerals. The toxicity of Mg(OH)_2 was then further investigated

83 by using a sensitive microalgal species, in a recognized and standardized whole effluent toxicity (WET) test with
84 cultured diatom *Skeletonoma costatum*. Additional experiments were performed for further toxicity assessment
85 of Mg(OH)₂ on a natural microalgal assemblage from local seawater.

86

87 **2 Methods**

88 The study was performed in three steps. In the first step, the toxicity effect was studied by exposing marine alga
89 to alkaline minerals in successive concentrations mimicking dispersion from a moving ship. These experiments
90 were carried out with *Tetraselmis suecica*, a standard test organism in toxicity studies (Ebenezer et al., 2017; Li
91 et al., 2017; Seoane et al., 2014; Vagi et al., 2005). In the second step, toxicity effects of the alkaline minerals
92 were verified by a standardized WET ecotoxicology assay with *Skeletonoma costatum*, a more sensitive marine
93 algal species (Petersen et al., 2014, Wee et al., 2016), by using the recognized 72 hours growth inhibition test
94 (ISO 10253:2016). In the third step, the toxicity effect was studied by exposing a natural assemblage of marine
95 algal species from the Oslofjord, Drøbak, Norway to similar Mg(OH)₂ concentrations used in the WET tests. All
96 experiments were carried out in non-airtight containers to allow ambient CO₂ to re-equilibrate with seawater used
97 for the experiments.

98

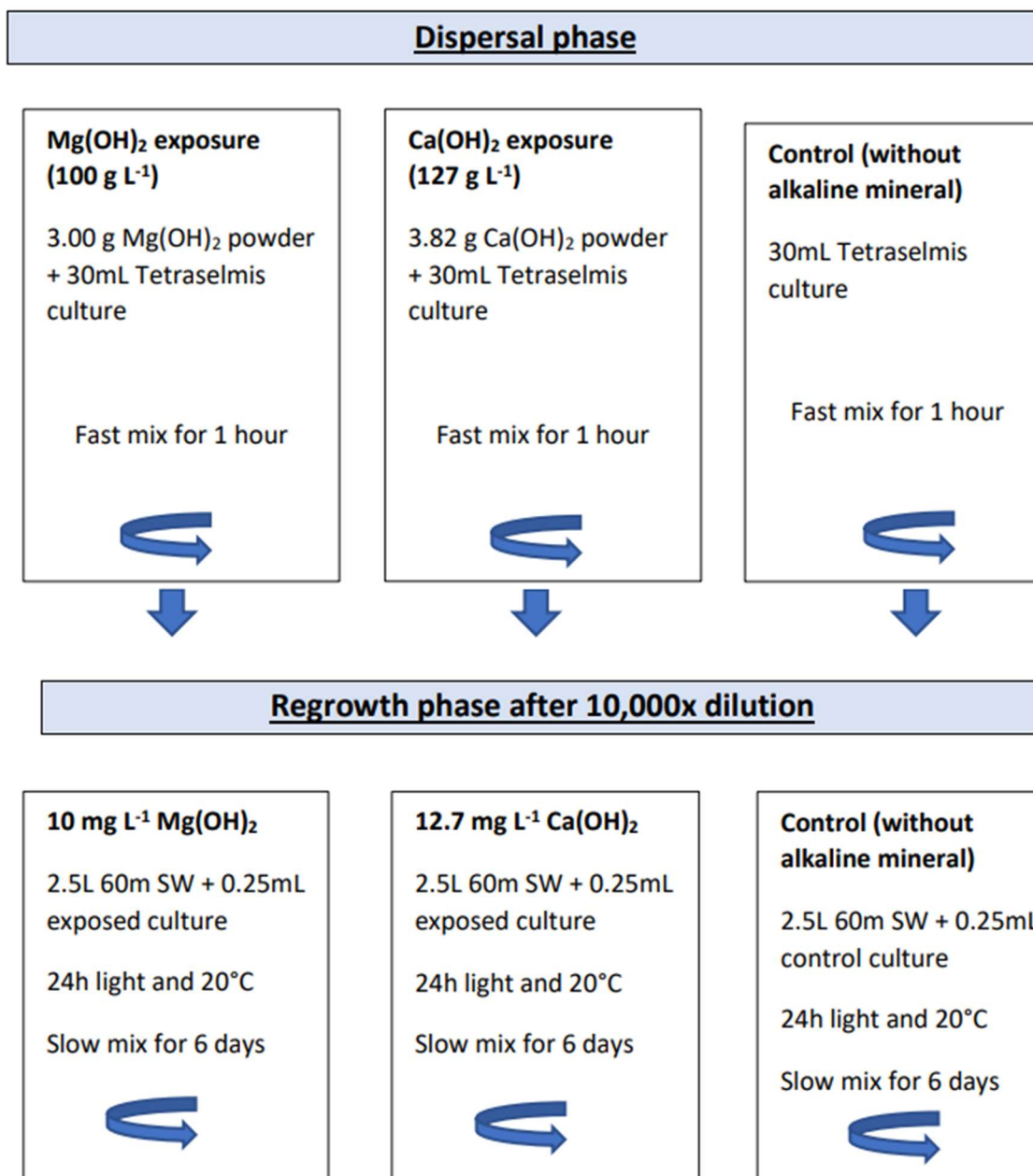
99 **2.1 Exposure of *Tetraselmis suecica* to simulated dispersion of alkaline minerals from a moving ship**

100 The expected distribution of a slurry of Mg(OH)₂ during its dispersion from the ship's discharge point on the
101 surface of the oceans was determined utilizing computational fluid dynamic (CFD) models (FORCE Technology
102 Inc., Denmark) and the Bottom RedOx Model (BROM) (Yakushev et al., 2017). In those models, both the forced
103 and natural mixing effects of the Mg(OH)₂ by the ship's propeller and physical oceanic processes (as waves,
104 convection, currents, etc.), respectively, in the ship's wake were simulated with different scenarios, including
105 propeller motion, velocity of tangential ocean currents, Mg(OH)₂ slurry discharge rate/dissolution rate/settling
106 rate, ship size and ship speed. Dilution was observed with an immediate minimum dilution rate of 1/1000 within
107 2 minutes after injection, followed by an additional minimum dilution rate of 1/7000 during the next 5 hours and
108 a final minimum dilution rate of 1/154000 during the following next 5 hours. Moreover, the tonnage capacity and
109 operating costs of a ship were also considered together with a final Mg(OH)₂ concentration target of < 1 mg L⁻¹.
110 Taken together, this suggested that the dispersion rate of 500 kg s⁻¹ would be the most realistic applicable scenario.
111 From this dispersion rate, it was concluded that marine organisms would be exposed to < 100 g L⁻¹ approximately
112 for less than one hour followed by a dilution to <10 mg L⁻¹ over a period of 10 hours.

113 To investigate biological impact of Mg(OH)₂ and compare it with Ca(OH)₂, cultures of *Tetraselmis suecica* were
114 exposed to these three alkaline minerals during a simulated dispersion phase (as described above) followed by a
115 regrowth phase (Fig 1). In the dispersion phase, 30 mL of *Tetraselmis suecica* cultures (see further down), in
116 exponential growth with a cell density range within 2.6 x 10⁵ - 1.4 x 10⁶ cells mL⁻¹, were exposed to the alkaline
117 minerals in 50 mL glass beakers with continuous mixing at approximately 300 rpm with a magnetic stirrer (VELP
118 Scientifica) for 1 hour. To achieve similar concentrations of hydroxide ions in the different alkaline mineral
119 treatments, algae were exposed to either 100 g L⁻¹ (or 1.7 M) of Mg(OH)₂ or 127 g L⁻¹ (or 1.7 M) of Ca(OH)₂
120 (Fig.1).

121 In the regrowth phase, a subsample from each exposure media was diluted by 10,000 in local seawater and algal
122 cell density was monitored for 6 days. The dilution was performed by mixing 0.25 mL subsample to 2.5 L ambient
123 60 m deep seawater from the Oslofjord (Fig.1). The diluted subsamples were incubated in 3 L glass beakers in a
124 20°C temperature-controlled climate room with 24h light (2x 21W Philips Pentura Mini) and continuous mixing
125 with a magnetic stirrer (VELP Scientifica; 100 rpm approximately). The measured light intensity was within 20-
126 60 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. As the beakers were left uncovered, evaporated water volume was replaced every 24h
127 (except for week-end period) by an equivalent volume of ultrapure water. Effects of each alkaline mineral were
128 investigated in triplicates, including both the exposure and regrowth phases; resulting in total of nine bioassays
129 which were conducted in NIVA's laboratory in Oslo between November 2021 and January 2022. Each bioassay
130 study was conducted with one or two alkaline minerals in parallel and were repeated three times for each alkaline
131 mineral with new cultures of *Tetraselmis suecica*, except for two of the NaOH studies which were started on the
132 same day from the same algal culture. In addition, control bioassays excluding the addition of alkaline minerals
133 were performed in parallel to each alkaline mineral exposure including a dispersal phase followed by a regrowth
134 phase.

135 The ambient Oslofjord seawater was unfiltered and unsterilized water collected from 60 m depth just outside of
136 NIVA's marine research station located at Drøbak, 40 km south of Oslo. The water quality of this seawater is
137 stable year-round with a temperature of approximately 7°C. This water is representative of ocean regions; i.e. rich
138 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
139 of dissolved organic carbon (DOC), 6 mg L⁻¹ of total suspended solids (TSS) and very low biological load with
140 < 1 cell mL⁻¹ of algae and less than 500 CFU mL⁻¹ of heterotrophic bacteria.
141



142

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

145

146 Before exposure, the algae were collected from 1 L laboratory cultures of *Tetraelmis suecica* (NIVA-3/10;
 147 Norwegian Institute for Water Research, Oslo, Norway). At first, a 50 mL algal culture was prepared by semi-
 148 static cultivation in a 100 mL glass flask with 50 mL of autoclaved 20% Z8 culture medium with addition of
 149 vitamins (Kotai, 1972). The medium culture was inoculated with 5-10 mL of the *T. suecica* culture from NIVA's
 150 algal culture collection. The culture was incubated for ~1 week with fluorescent light tubes giving 20-60 μmol
 151 photons m⁻² s⁻¹, provided by cool-white fluorescence lamps (TLD 36W/950, Philips, London, UK), on an Infors
 152 Multicrom 2 incubator shaker (Infors AG, Bottmingen, Switzerland) at 20 ± 2°C, with orbital shaking at 90 rpm.
 153 After incubation, the culture was used for the inoculation of the 1L culture, except for ~10 mL which was held

154 back to start a new 50 mL culture by adding 40 mL of freshly prepared Z8 medium in same culture conditions as
155 described above. The 1 L culture was prepared by static cultivation with 1 L autoclaved 20 % Z8 medium with
156 addition of 1 mL L⁻¹ vitamins in 2-liter glass culture bottles. Approximately 40 mL of the 50 mL stock culture
157 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
158 in a 20°C temperature-controlled room for approximately one week.

159 The culture medium was prepared at least 24 h before usage to allow the equilibrium of media components. The
160 20% Z8 culture medium was made by mixing 0.2 L of Z8 medium into 0.8 L seawater, and shortly aired with CO₂
161 (< 1 min) to avoid precipitation of salts during autoclaving. The seawater was pasteurized seawater collected from
162 60 m depth in the Oslofjord. The medium was autoclaved for 15 minutes at 121°C. 1 mL L⁻¹ of vitamins stock
163 solution was added to the 20% Z8 medium (Kotai, 1972).

164 The studied alkaline minerals were magnesium hydroxide (CAS number: 1309-42-8), calcium hydroxide (CAS
165 number: 1305-62-0) and sodium hydroxide (CAS number: 1310-73-2); all with ≥97.0% purity. Magnesium
166 hydroxide (Batch No. 18417-01A) was provided by Negative Emission Materials, Inc. via a factory in Canada
167 producing the mineral by hydrometallurgy process and purification from natural magnesium silicate. The two
168 other alkaline minerals were purchased from Sigma-Aldrich (United Kingdom).

169 The density of living *Tetraselmis suecica* was determined using the double staining method with fluorescein
170 diacetate (FDA) and 5-chloromethylfluorescein diacetate (CMFDA) (NSF, 2010). This double staining method,
171 FDA/CMFDA, is based on the validation work of the US Navy Research Laboratory to distinguish between living
172 and dead cells after disinfection by a ballast water treatment (Steinberg et al., 2011). This viability method is the
173 only one recognized by both the International Maritime Organization (IMO) and the United States Coast Guard
174 (USCG) for approval of ballast water discharge from 70,000 commercial ships at a global scale (USCG, 2012,
175 IMO, 2018).

176 The following staining protocol was used: A 2.5 mM CMFDA stock solution was prepared by dissolving 1 mg of
177 CMFDA in 0.86 ml DMSO (Dimethylsulphoxide). It was then divided into 50 μl batches and stored at -20 °C.
178 The 5 mM FDA stock solution was prepared by dissolving 10 mg FDA in 4.8 ml DMSO. The FDA stock solution
179 was divided into 100 μl batches and stored at -20 °C. For each analysis, a 4 ml subsample was collected and 4 μl
180 of 10% HCl was added, bringing the pH back to approximately 8 prior to staining. 4 μl of each stock solution was
181 added to each subsample, resulting in final concentrations of 2.5 μM CMFDA and 5μM FDA. The subsamples
182 were then incubated in darkness for 10 minutes, after which they were loaded into 1 ml Sedgewick-Rafter counting
183 chambers etched with 1-mm 2 grids. Chambers were examined at 100x magnification using compound
184 epifluorescent microscopes with standard blue light excitation (480 nm) and green bandpass emission (530 nm)
185 filter cubes. Samples were counted within a 45-minute period after incubation. The stained *Tetraselmis suecica*
186 cells were counted in triplicate (3x 1 mL) The untreated algal samples without alkaline mineral were used as
187 positive controls. Both *T. suecica* and local diatoms are nearly 100% stainable with these stains according to our
188 15 years of experience with this method in our local seawater. Samples treated with sodium hydroxide (NaOH)
189 to increase the pH to approximately 14 were used as negative controls. No fluorescence could be observed in the
190 negative controls, indicating an instant kill effect of the algal cells.

191 Temperature, salinity and pH in the bioassays were measured in-situ by using a calibrated handheld WTW
192 Multimeter (WTW Multi 3620 IDS/3420 IDS displayer) with a conductivity probe (TetraCon 925 Xylem) and a
193 pH-electrode (SenTix 945P). The three-point calibration method with Hamilton pH-buffer solutions (4, 7 and 10)
194 was used for the calibration of the pH electrode, according to WTW instructions. The temperature in the test
195 waters varied within a range of 18-23°C for all experiments during the 6 days of regrowth phase as all experiments
196 were conducted at room temperature. The same temperature was registered in the alkaline test waters compared
197 to the corresponding control waters. The salinity of the test waters, with or without alkaline mineral, was around
198 32-33 PSU at the start of the 6 days regrowth phase for all experiments. The salinity stayed relatively stable for
199 most of the regrowth phase, except for the last day with an increase up to 35-36 PSU on average. This increase
200 was due to the evaporation of the test water at room temperature during the week-end period included at the end
201 of the 6 days of experimentation.

202

203 **2.2 Whole Effluent Toxicity (WET) test**

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

208 A 100 mg L⁻¹ Mg(OH)₂ sample was diluted by using a modified ISO 10253 media, except that no Fe-EDTA stock
209 solution was added, as the tested compound Mg(OH)₂ showed to be affected by the presence of EDTA causing
210 precipitation of Mg(OH)₂. A preliminary study was made to verify the microalgal growth in this modified media.
211 Although less growth was observed when compared to normal ISO 10253 media, the specific daily growth rate
212 was still greater than 0.9 d⁻¹, which was considered as valid. A total of six concentrations of Mg(OH)₂ was tested
213 (1, 10, 25, 50, 75 and 100 mg L⁻¹). The test was performed with 15 mL samples in 30 mL glass vials. Each
214 concentration was tested in triplicate with 6 replicates for each control (one control set with normal ISO 10253
215 and another control set with modified ISO 10253); same number of replicates for analysis of blank samples but
216 without microalgae added.

217 All samples were inoculated with 5 x 10⁶ cells L⁻¹ of *S. costatum* from an exponentially growing laboratory culture
218 and incubated on a shaking table at 20±1°C under continuous illumination of 63 µM m⁻² s⁻¹ of photosynthetic
219 active radiation (PAR).

220 The cell density was determined by FDA and CMFDA double staining and fluorescence at 645 nM in SpectraMax
221 iD3 microplates after approximately 24, 48 and 72 hours (±2h). The fluorescence measurements were directly
222 correlated to the algal density as a correlation factor (r²) of 1 between the measured fluorescence and the cell
223 density was calculated. The fluorescence values of the exposed samples without algae (blanks) were measured to
224 investigate potential biases caused by effect of the tested substance on the fluorescence readings. As no such
225 effects were detected, no further transformation of data was necessary.

226 The temperature, pH and salinity were measured in-situ at the beginning and at the end of each WET test. The
227 temperature varied from 19.9 to 20.3°C for both WET tests. The pH at the start of the experiment varied from
228 8.089 to 9.376 in all vials for both tests, with increasing pH for increasing Mg(OH)₂ concentrations as expected.

229 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
230 with 32-35 PSU in all vials during the entire experiment for both tests.

231 **2.3 Natural assemblage of ambient marine algal test**

232 For the preparation of the ambient algal culture, either a 25 L grab-sample from the surface water of Oslofjord
233 was directly used for the test or a 2 L subsample was mixed to 2 L of 60 m deep seawater from Oslofjord for
234 further algal growth. For growth, the culture was incubated in a 5 L glass beaker in a climate-room at 20°C and
235 with constant light from fluorescent light tubes of 20-60 $\mu\text{M m}^{-2} \text{s}^{-1}$ for four days. The total density of algal cells
236 in the culture after incubation was approximately 1000 cell mL^{-1} . 500 mL of the culture was then mixed, in a 2 L
237 glass beaker with a magnetic stirrer at approximately 90 rpm, added to 1500 mL of a prepared $\text{Mg}(\text{OH})_2$
238 suspension resulting in $\text{Mg}(\text{OH})_2$ concentrations of 1, 10, 25, 50, 75 and 100 mg L^{-1} and initial algal density of
239 approximately 125-250 cell mL^{-1} . The $\text{Mg}(\text{OH})_2$ suspensions were prepared by mixing 2.7 mg, 27 mg, 66 mg,
240 133 mg, 200 mg or 270 mg of $\text{Mg}(\text{OH})_2$ in 1.5 L of unfiltered 60 m seawater from Oslofjord, with a magnetic
241 stirrer (300 rpm) over the night prior test start. The final solutions were slowly mixed continuously with a magnetic
242 stirrer at approximately 90 rpm, in a climate room at 20°C and with constant light from fluorescent light tubes of
243 20-60 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ for 72 hours. The water quality and algal density was monitored daily in each beaker,
244 the same methods described in 2.1. Moreover, cell count and viability were quantified using the same protocol as
245 in 2.1., with fluorescence measured at 645 nm. For the control treatments, 500 mL of the ambient algal culture was
246 mixed with 1.5 L of unfiltered 60m deep seawater from Oslofjord, without $\text{Mg}(\text{OH})_2$, and incubated as described
247 above. Those tests were carried out on different weeks. Therefore, different control treatments applied for 1-10
248 $\text{mg/L Mg}(\text{OH})_2$ treatments, 50-75 $\text{mg/L Mg}(\text{OH})_2$ treatments and 100 $\text{mg/L Mg}(\text{OH})_2$ treatment (see Appendix
249 C). Aliquots from the 100 mg L^{-1} treatment were collected from the initial timepoint and final timepoint ($t=3$ d)
250 for microscopy-based assessment of community composition by taxa.

251 252 **2.4 Data analysis**

253 Effects on *T. suecica* cell survival with $\text{Ca}(\text{OH})_2$, and $\text{Mg}(\text{OH})_2$ in simulated dispersions from a moving ship were
254 analyzed with a Student's t-test with type of alkaline mineral as independent grouping variable and % survival
255 compared to control treatments after the regrowth phase as the dependent variable. Data were log transformed to
256 obtain similar variation between groups.

257 In the WET test, the growth rate of *S. costatum* in each $\text{Mg}(\text{OH})_2$ sample was calculated from the logarithmic
258 increase of cell density from start to 72 hours, and expressed as percentage of the growth rate of control samples.
259 The concentrations causing 50% growth inhibition (EC_{50}) were calculated using a non-linear regression analysis
260 of the growth rate versus log cell concentration of control water (Hill, 1910; Vindimian et al. 1983). The non-
261 observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were calculated
262 using Dunnett's test/ t-test for non-homogenous variance and Williams Multiple Sequential t-test for homogenous
263 variance.

264 Effects of $\text{Mg}(\text{OH})_2$ on the natural marine algal assemblage was investigated by dividing the different exposure
265 concentrations (1, 10, 25, 50, 75 and 100 mg L^{-1}) within two groups based on the LOEC (25 mg L^{-1}) from the
266 WET test. This resulted in one low concentration group (1, 10 and 25 mg L^{-1}) and one high concentration group
267 (50, 75 and 100 mg L^{-1}). The difference in % survival compared to control treatment between the high and low

268 concertation groups was investigated by a Student's t-test. This approach, with three replicates in each group,
 269 allowed us to investigate effects of increased Mg(OH)₂ concentrations.

270 **3 Results**

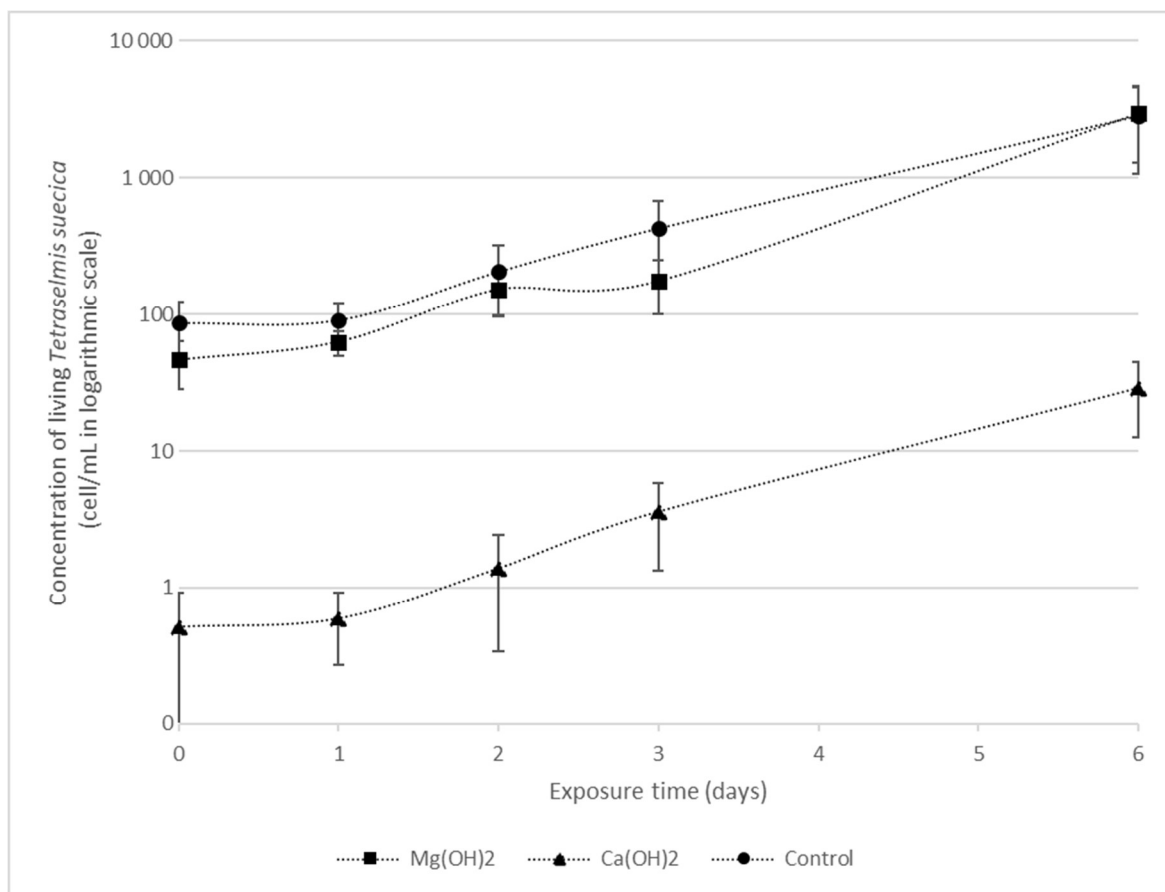
271 **3.1 Exposure of *Tetraselmis suecica* to simulated dispersion of alkaline minerals from a moving ship**

272 There were significant differences in living cells of *Tetraselmis suecica* (% survival compared to control
 273 treatments; Table 1) between the alkaline minerals in the end of the regrowth phase (Student's t-test; t=9.4,
 274 P<0.0001), which were reflected in both the dispersion and the regrowth phases. At the start of the regrowth phase,
 275 the surviving cell densities in the Mg(OH)₂ treatments were similar to the ones observed in control treatment,
 276 while only one living cell was observed in one of the Ca(OH)₂ treatments (Day 0; Table 1). In the Mg(OH)₂ and
 277 Ca(OH)₂ treatments, algal cell densities increased during the regrowth phase (Day 1-6; Table 1). At the end of the
 278 regrowth phase, the algal cell densities in the Mg(OH)₂ treatments were similar as in control treatments, while the
 279 algal cell densities in Ca(OH)₂ treatments showed lower values than in control treatments (Fig.2).

280 **Table 1. Densities of living *Tetraselmis suecica* (cell mL⁻¹) and their relation to control treatment (% Contr.) during the**
 281 **regrowth phase of a bioassay mimicking dispersion of the alkaline minerals Mg(OH)₂ or Ca(OH)₂ from a ship. Before**
 282 **the regrowth phase, algae were exposed to either 100 g L⁻¹ Mg(OH)₂ or 127 g L⁻¹ Ca(OH)₂ (achieving similar amount**
 283 **of hydroxide in the different alkaline mineral suspensions) for 1h. After this, subsamples from each treatment were**
 284 **diluted 10 000 times and algae growth were studied during a 6-day regrowth phase. Each alkaline mineral was assayed**
 285 **in triplicates. Values at day zero corresponds to 1h after dilution and effects of each alkaline mineral was investigated**
 286 **in triplicates.**

Day	Mg(OH) ₂						Ca(OH) ₂					
	Replicate 1		Replicate 2		Replicate 3		Replicate 1		Replicate 2		Replicate 3	
	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
1	40	62	64	145	84	53	1	0.66	0	0	1	2.5
2	72	63	129	168	256	64	-		0	0	3	3.5
3	101	72	249	199	-	-	6	0.60	0	0	4	3.6
6	1040	84	1533	263	6217	128	56	0.68	1	0.11	29	2.3

287

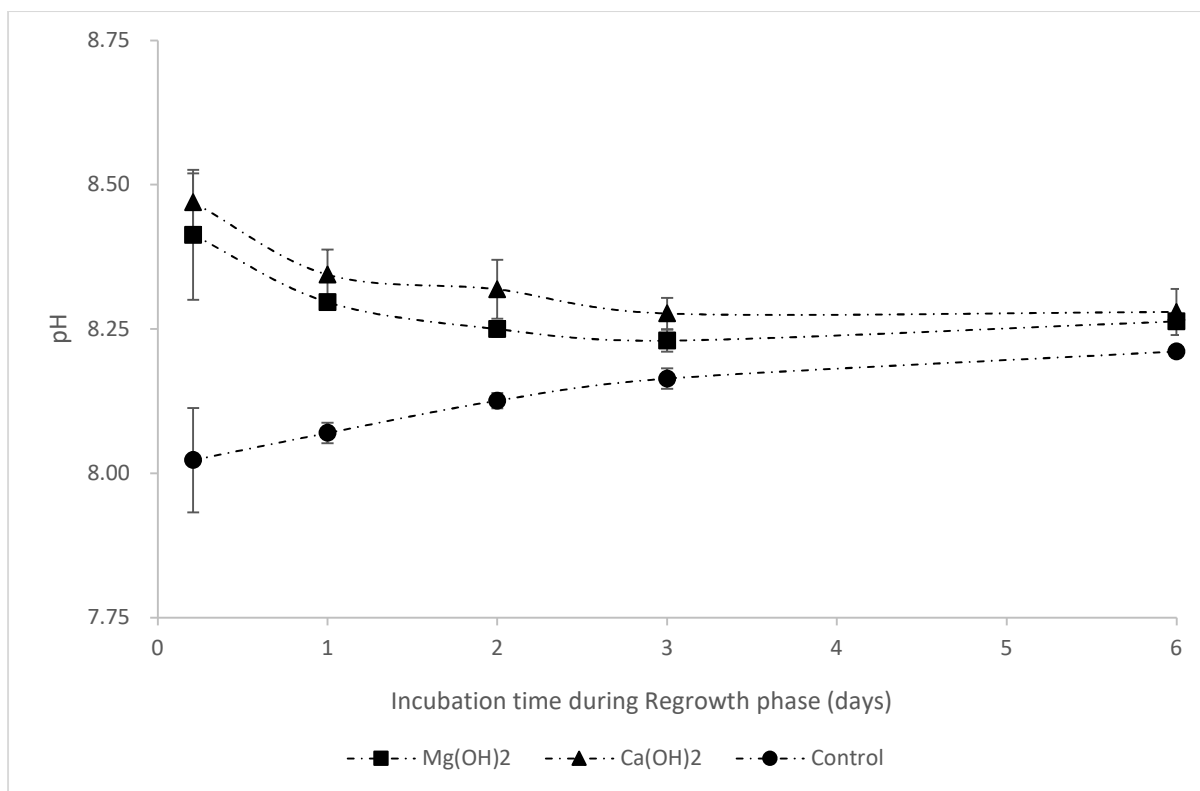


288

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

294

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



298

299 **Figure 3. pH during the regrowth phase in a bioassay mimicking dispersion of the alkaline minerals Mg(OH)₂ or**
 300 **Ca(OH)₂ from a ship. Before the regrowth phase, algae were exposed to either 100 g L⁻¹ Mg(OH)₂ or 127 g L⁻¹ Ca(OH)₂**
 301 **(achieving similar concentrations of hydroxide ions in the different alkaline mineral solutions) for 1h. After this,**
 302 **subsamples from each treatment were diluted 10 000 times to achieve the following concentrations during the regrowth**
 303 **phase; 10 mg L⁻¹ Mg(OH)₂ or 12.7 mg L⁻¹ Ca(OH)₂.**

304

305 3.3 WET tests

306 The results of the lowest observed effect concentration (LOEC) and the non-observed effect concentration
 307 (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
 308 Mg(OH)₂ concentration causing 50% algal growth inhibition was close to 100 mg L⁻¹ in both tests; within a range
 309 of 82-111 mg L⁻¹ (Table 2).

310 **Table 2. Results of the duplicate Whole Effluent Toxicity (WET) tests (WET tests 1 and 2) for three endpoints (EC₅₀,**
 311 **LOEC and NOEC) after 72 hours exposure of the marine microalgae *Skeletonema costatum* with a total of six different**
 312 **concentrations of magnesium hydroxide (1, 10, 25, 50, 75 and 100 mg L⁻¹). Those concentrations were prepared by**
 313 **diluting an initial Mg(OH)₂ solution in the algal culture medium, prior to algal inoculation. The initial solution was a**
 314 **freshly prepared 1 L suspension of 100 g L⁻¹ Mg(OH)₂ in ambient 60m deep seawater from Oslo fjord. EC₅₀:**
 315 **concentration causing 50% algal growth inhibition. LOEC: lowest observed effect concentration. NOEC: non-observed**
 316 **effect concentration (NOEC).**

Endpoint	WET tests, Mg(OH) ₂ (mg L ⁻¹)	
	1	2
EC ₅₀	111	82
LOEC	50	50
NOEC	25	25

317

318

319 3.4 Natural assemblage of ambient marine algal species

320 There was a significant difference in algal survival between the low concentrations group (1, 10 and 25 mg L⁻¹
 321 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₎=-
 322 5.8, P<0.01; Table 3). The analysis of the algal biodiversity composition in the 100 mg L⁻¹ Mg(OH)₂ suspension
 323 showed that the dominant surviving species were diatoms, including *Skeletonoma spp.*, with 80% and 94% of the
 324 total on Day 0 and Day 3, respectively. The biodiversity composition of the natural algal assemblage in beginning
 325 and at the end of the experiment for the 100 mg L⁻¹ Mg(OH)₂ treatment is given in Table 4.

326 **Table 3. Densities of living ambient algal cells (cell mL⁻¹), and their survival in percentage compared to corresponding**
 327 **control water without Mg(OH)₂ (% Contr.), during 3 days of exposure to six different concentrations of Mg(OH)₂ (1,**
 328 **10, 25, 50, 75 and 100 mg L⁻¹) when incubated in 20°C temperature-controlled room with constant light. Low and high**
 329 **concentration groups refer to the groups used in the Student’s t-test, see 2.4 statistics for more information.**

Day	Low concentration						High concentrations					
	1 mgL ⁻¹		10 mgL ⁻¹		25 mgL ⁻¹		50 mgL ⁻¹		75 mg L ⁻¹		100 mgL ⁻¹	
	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	1167	92	1197	94	2117	106	551	28	563	28	396	7

330

331

332 4 Discussion

333 4.1 Dispersal model and experimental design

334 The current ship dispersal model suggests a dilution rate of 1/1000 over a 2-minute period in the near field of the
 335 wake, given a dispersal rate of 500 kg s⁻¹. This is consistent with a recent study where the dispersal of Ca(OH)₂
 336 from a ship was modeled. The study showed that dilution rates could vary between 710-7100, depending on the
 337 diffusion potential of the Ca(OH)₂, at a dispersal rate of 100 kg s⁻¹, 810 m in the wake behind the ship (Caserini
 338 et al., 2021). This distance corresponds to 2 minutes at the modeled ship speed of 25 km h⁻¹. Another study from
 339 the Cefas Burnham Laboratory, in which maximum (but safe levels of) discharge of industrial waste from ships
 340 was sought after, calculated ship discharge dilutions rates of 1/10,000 within 5 minutes was possible (C.Vivian,
 341 pers.comm.). Thus, the model in the current paper predicts dilution rates that are within what other model suggests.
 342 Still, regarding to models of safe laves of discharge of industry waste, it is important to note that maximum
 343 dispersal (discharge) is not the sole criteria for ocean alkalinity enhancement, but rather an intermediate between
 344 a high dispersal rate for maximum input and a low dispersal rate to promote maximum dissolution for the alkaline
 345 material of choice. For example, in the dispersal model scenario used for designing the experiments in the current
 346 study, a 1/10,000 dilution after 1 hour resulted in a final concentration of Mg(OH)₂ and Ca(OH)₂ of 10 and 12.7
 347 mg/L, respectively. At these concentrations, both alkaline materials are expected to fully dissolve for optimal CO₂
 348 uptake while also not resulting in elevated calcium carbonate saturation states leading to “runaway” secondary
 349 precipitation of calcium carbonate (e.g., secondary precipitation was observed at Ω_{Ar} > 7 for Ca(OH)₂ on the
 350 timescale of 4-5 h; Moras et al., 2022). Still it cannot be excluded that some uncontrolled CaCO₃ precipitation
 351 could have occurred at 100 mgL⁻¹ of Mg(OH)₂ and 127 mgL⁻¹ Ca(OH)₂ during the initial 1 h of exposure in the
 352 present study.

353

354 **4.2 Regrowth of *Tetraselmis suecica***

355 Similar algal densities were observed in both control and Mg(OH)₂ treatments at the beginning of the regrowth
356 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
357 g L⁻¹ in pure water and around 0.008 g L⁻¹ in seawater (Yang et al., 2023). For comparison, the solubility of
358 Ca(OH)₂ is 1.73 g L⁻¹ at 20-25°C. Accordingly, pH increased during the dispersion phase from approximately 8.0
359 to 9.5 in the Mg(OH)₂ treatment which was lower compared to the expected pH of 12 in Ca(OH)₂ (Hartmann et
360 al., 2022). However, pH was similar at the beginning of the regrowth period for both alkaline mineral treatments
361 at ~8.3-8.6 (Fig. 3), giving similar potential regrowth conditions. The similar growth rates observed in controls,
362 Mg(OH)₂-added and Ca(OH)₂-added treatments (Fig. 2) suggests that the algae previously exposed to 100 g L⁻¹
363 Ca(OH)₂ were able to recover during this phase, at least when the algae were incubated in optimal culture
364 conditions which might not be the case in natural oceanic conditions. Taken together, our data indicated high algal
365 mortality in Ca(OH)₂ at the high concentrations of 127 g L⁻¹ during the first hour after the alkaline mineral
366 discharge from a moving ship, while no such toxic effect was observed when algae were exposed to Mg(OH)₂.
367 This emphasizes that the local and temporary biological impact of alkaline mineral discharge in the initial phase
368 of the dispersion, in addition to alkalinity increase capability, needs to be considered when evaluating mCDR
369 strategies. Following this, it is important to keep in mind that in this study the toxicity comparison was based on
370 the criteria that each alkaline mineral should have the same hydroxide content, not taking in account difference in
371 alkalinity enhancement between the alkaline minerals.

372 **4.3 Growth inhibition test with *Skeletonoma costatum***

373 The results from the WET tests indicate that no growth inhibition of *S. costatum* was observed for Mg(OH)₂
374 concentrations equal or below to 25 mg L⁻¹ (NOEC). This is somewhat in accordance with the simulated dispersion
375 test, showing no growth inhibition of *T. suecica* during the 6 days of regrowth phase in 10 mg L⁻¹ magnesium
376 hydroxide. The results from dispersion phase indicate no or low effect of 1 h of exposure with 100 g L⁻¹
377 magnesium hydroxide on *T. suecica*. The WET tests indicated a 50% growth inhibition effect of Mg(OH)₂
378 concentrations (EC₅₀) between 82 and 111 mg L⁻¹ after 72 h of exposure. This toxicity effect might be explained
379 by the temporary local CO₂ limitation impact, limiting the algal growth, due to increasing pH at these high alkaline
380 mineral concentrations. These EC₅₀ values were much higher than Mg(OH)₂ solubility of ~ 12.2 mg L⁻¹ in pure
381 water (Yang et al., 2023). This raises questions regarding the cause of growth inhibition in the current study. It
382 has been suggested that trace metals, such as Cr, Mo, Ni, Pb in industrial and natural mineral products used as
383 alkaline minerals may impair organism growth (Bach et al., 2019; Hartmann et al., 2022). However, this might
384 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.
385 Further studies are needed to verify and investigate the underlying mechanism for the growth inhibition of *S.*
386 *costatum* observed in the current WET tests.

387 **4.4 Regrowth test with assemblage of ambient algal species**

388 The same toxicity effect of Mg(OH)₂ was observed in the tests performed with local marine algal species; i.e. no
389 significant toxicity effect of Mg(OH)₂ concentrations below 25 mg L⁻¹ but significant toxicity effect for
390 concentrations above 50 mg L⁻¹. *Skeletonoma spp.* was represented in the natural assemblage, as one of the
391 dominant species, while *Skeletonoma costatum* was used in the WET tests. This suggests that the results from the

392 WET tests using laboratory monoculture are still representative and applicable to similar species growing in
393 natural marine environment. Thus, the results from the natural seawater test demonstrated that toxicity effects
394 observed with $Mg(OH)_2$ on laboratory cultures might be applicable to a wider range of marine algal species.

395 Thus, both the simulated dispersion scenario, the WET tests and ambient algal tests results suggest that $Mg(OH)_2$
396 is a suitable alkaline enhancement mineral with respect to minimizing biological impacts on marine microalgae
397 during temporary and local extreme alkaline mineral discharge upon initial phase of the dispersion. While our
398 studies focused on marine microalgae, most other studies focused on the impact of OAE on organisms with
399 calcium carbonate containing parts and therefore sensitive to seawater acidification (Cripps et al., 2013, Fakhraee
400 et al., 2023, Gomes et al., 2016, Renforth and Henderson, 2017). Microalgae play an important role as primary
401 producers and impacts may be reflected in the entire marine ecosystem by affecting higher trophic-level
402 organisms, such as zooplankton and fish (Pauly and Christensen, 1995; Chassot et al., 2010). Accordingly,
403 microalgae are considered a useful and crucial indicator to evaluate the deterioration of environmental quality
404 (Lee et al., 2023). Thus, the current study applying microalgae assays to investigate the effects of $Mg(OH)_2$
405 suggests a low negative biological impact of $Mg(OH)_2$. However, it is important to keep in mind that these
406 laboratory assays, in addition to approximate the biological impact, are employed because they are relatively fast
407 and cost-effective. Thus, further studies on other functional groups and species are required for ensuring a low
408 impact of the OAE.

409

410 **5 Conclusion**

411 The bioassays based on initial local and temporary discharge simulation from scenario of alkaline mineral
412 dispersion from ship demonstrated that $Mg(OH)_2$ resulted in lower biological impacts on marine microalgae when
413 compared to $Ca(OH)_2$. Further laboratory studies must be completed to include a wider range of biological
414 biodiversity from different trophic levels and on a larger scale, such as in mesocosm studies, prior to field
415 deployment. The observed low negative biological impact of $Mg(OH)_2$ was confirmed by the standardized toxicity
416 test using a more sensitive marine algae species, but also by the tests with a wider range of local ambient marine
417 algal species. Additionally, there are potentially positive biological impacts of OAE, including remediation of
418 ocean acidification conditions by reducing pH and increasing saturation state of calcium carbonate, which were
419 not addressed in this study. Overall, these results indicate that $Mg(OH)_2$ is a suitable mineral for OAE application.
420 Still, it is important to consider that $Mg(OH)_2$ needs to maintain in suspension right below the ocean's surface to
421 be an effective OAE. Thus, in addition to further toxicity assessment of $Mg(OH)_2$ on aquatic environment,
422 techniques for optimization of its dissolution, including injection and distribution methods, in seawater needs to
423 be performed.

424

425 **6 Data availability**

426 The raw data are presented in Appendix A for the Tetraselmis test, in Appendix B for the WET tests and in
427 Appendix C for the natural algal assemblage test.

428

429 **7 Author contribution**

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

434 **8 Competing interests**

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438 to be more connected to their peers' work, and to partner with experts in conservation nonprofit management to
439 execute bold initiatives. More info here: <https://www.windwardfund.org/about-the-fund/>. The authors declare that
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448

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645 Appendix A – Raw data for the *Tetraselmis* bioassay studies

646

647 The Table 1 of the manuscript was generated from the raw data presented in Table A1.

648 **Table A1. Daily averages (n=3) of density of living *Tetraselmis suecica* (cell mL⁻¹) during the regrowth phase (Day 0 –**
 649 **Day 6) of the triplicate tests mimicking dispersion of the alkaline minerals Mg(OH)₂ or Ca(OH)₂ from a ship. Before**
 650 **the regrowth phase, algae were exposed to either 100 g L⁻¹ Mg(OH)₂ or 127 g L⁻¹ Ca(OH)₂ (resulting in similar molar**
 651 **concentration of hydroxide in the two alkaline mineral suspensions) for 1 hour. After this, subsamples from each**
 652 **treatment were diluted 10 000 times and algae growth were studied during a 6-day regrowth phase. Each alkaline**
 653 **mineral treatment and corresponding control treatment was assayed in triplicates. Values at day zero corresponds to**
 654 **1h after dilution and effects of each alkaline mineral was investigated in triplicates.**

655

Day/Replicate #	Density averages (n=3) of living <i>Tetraselmis suecica</i> (cell.mL ⁻¹)											
	Mg(OH) ₂			Control			Ca(OH) ₂			Control		
	Treated			Control			Treated			Control		
	1	2	3	1	2	3	1	2	3	1	2	3
Day 0	27	30	82	32	31	156	0	0	1	116	152	34
Day 1	40	64	84	65	44	159	1	0	1	152	89	39
Day 2	72	129	256	115	77	399	-	0	3	-	361	86
Day 3	101	249	-	141	125	-	6	0	4	1012	766	110
Day 6	1040	1533	6217	1245	583	4844	56	1	29	8275	930	1230

656

657 **Table A2. Daily water quality measurements (pH, temperature and salinity) in the treated and control test waters**
 658 **during the 6-day regrowth phase of the triplicate tests (Test 1, Test 2, Test 3) when mimicking dispersion of the alkaline**
 659 **minerals Mg(OH)₂ or Ca(OH)₂ from a ship.**

660

days	Mg(OH) ₂ - Treated water									Mg(OH) ₂ - Control water									
	pH			Temp.(°C)			Salinity (PSU)			pH			Temp.(°C)			Salinity (PSU)			
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	
0	8.23	8.70	8.31	19.9	20.6	19.6	31.7	31.8	33.6	0	7.93	8.38	7.92	18.7	19.8	18.8	31.8	31.8	-
1	8.29	8.33	8.27	21.6	21.7	21.9	32.8	32.7	33.7	1	8.05	8.07	8.00	21.1	21.6	22.4	32.3	32.8	33.7
2	8.25	8.28	8.22	21.9	21.2	22.2	33.7	32.4	34.1	2	8.12	8.13	8.08	21.3	21.3	21.8	33.3	32.7	34.0
3	8.20	8.26	-	21.4	21.1	-	35.4	32.1	-	3	8.13	8.15	-	21.2	21.1	-	34.9	32.4	-
6	8.26	8.25	8.28	21.2	21.0	22.5	41.6	32.8	34.5	6	8.24	8.21	8.21	21.0	21.2	22.5	40.4	33.4	34.0

days	Ca(OH) ₂ - Treated water									Ca(OH) ₂ - Control water									
	pH			Temp.(°C)			Salinity (PSU)			pH			Temp.(°C)			Salinity (PSU)			
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	
0	8.57	8.42	8.42	18.2	19.1	18.7	33.7	33.9	33.3	0	7.90	-	7.99	19.1	-	18.6	33.9	-	33.5
1	8.43	8.29	8.31	21.3	23.7	22.1	33.6	35.4	33.4	1	8.08	8.13	8.09	23.3	21.0	21.4	35.5	33.6	33.7
2	8.37	-	8.27	21.3	-	22.1	33.5	-	33.4	2	-	8.16	8.14	-	21.0	21.4	-	33.5	33.7
3	8.33	8.26	8.24	21.5	25.2	22	33.5	33.9	32.8	3	8.23	8.17	8.14	25.1	21.0	21.4	33.7	33.5	32.7
6	8.24	8.36	8.24	21.4	25.4	22.1	34.5	37.3	33.9	6	8.22	8.19	8.20	25.2	21.2	21.7	37.6	34.5	34.0

661

662

663 Appendix B – Raw data for the WET tests

664

665 The Table 3 of the manuscript was generated from the raw data presented in Table B1 and Table B2. The
 666 complete laboratory analysis reports can be provided upon request.

667

668 **Table B1. Calibration data for WET Test 1 and for WET Test 2 to correlate the fluorescens measurements to the cell**
 669 **density of *Skeletonoma costatum*. The cell density was determined by fluorescence with SpectraMax iD3 microplate**
 670 **after approximately 72 hours (± 2 h). The fluorescence measurements were directly correlated to the algal density as a**
 671 **correlation factor (r^2) of 1 between the measured fluorescence and the cell density was calculated.**

WET test 1- calibration data		WET test 2- calibration data	
Cell counts	Fluorescence	Cell counts	Fluorescence
9767	21129	7722	20909
34407	91377	28320	60447
105747	194737	169517	267903
581800	1533120	543317	623790

672

673 **Table B2. Fluorescens measurements of the control and Mg(OH)₂ treatments for WET Test 1 and WET Test 2 after**
 674 **72 hours exposure according to ISO 10253:2016. A total of six concentrations of Mg(OH)₂ was tested (1, 10, 25, 50, 75**
 675 **and 100 mg L⁻¹). Each concentration was tested in triplicate, with 6 replicates for each control (one control set with**
 676 **normal ISO 10253 and another control set with modified ISO 10253).**

Fluorescence results for WET Test 1-72h								
Replicate #	Controls		Mg(OH) ₂ concentration in mg.L ⁻¹					
	Normal control	Modified control	1	10	25	50	75	100
1	1741942	492151	581669	854536	752064	316455	227769	114436
2	1629608	582180	593910	775861	780683	334224	198120	111869
3	1720051	332864	542791	816187	705611	329265	234354	113917
4	1885773	514530						
5	2048400	398823						
6	1973322	481943						

Fluorescence results for WET Test 2-72h								
Replicate #	Controls		Mg(OH) ₂ concentration in mg.L ⁻¹					
	Normal control	Modified control	1	10	25	50	75	100
1	2124534	640947	775797	1044538	1184687	514139	168631	59714
2	2188199	671593	713625	920976	1196415	441565	212443	50273
3	2203985	679313	713790	988564	1274252	453043	170141	53626
4	2344184	634189						
5	2194617	445427						
6	2209858	671270						

677

678

679

680 Appendix C – Raw data for the natural algal assemblage tests

681

682 Table 3 of the manuscript was generated from the raw data presented in Table C1 below.

683

684 **Table C1. Daily triplicate enumeration of density of living ambient algal cells (cell mL⁻¹) with FDA/CMFDA method in**
 685 **Mg(OH)₂ treated and control treatments during 3 days of exposure to six different concentrations of Mg(OH)₂ (1, 10,**
 686 **25, 50, 75 and 100 mg L⁻¹) when incubated in 20°C temperature-controlled room with constant light. Some of those**
 687 **tests were conducted separately with therefore different control waters. Those tests were carried out on different weeks.**
 688 **Therefore, different control treatments were applied with one control for 1-10 mg/L Mg(OH)₂ treatments, one control**
 689 **for 50-75 mg/L Mg(OH)₂ treatments and one control for 100 mg/L Mg(OH)₂ treatment.**

		Densities of living ambient algae (cell.mL ⁻¹)								
		Mg(OH) ₂ Treated (cell.mL ⁻¹)						Control (cell.mL ⁻¹)		
		Low concentrations			High concentrations			for the corresponding treatments with		
Replicate #		1 mg.L ⁻¹	10 mg.L ⁻¹	25 mg.L ⁻¹	50 mg.L ⁻¹	75 mg.L ⁻¹	100 mg.L ⁻¹	1-10 mg.L ⁻¹	25-75 mg.L ⁻¹	100 mg.L ⁻¹
Day 0	1	420	443	220	278	192	212	407	264	240
	2	447	470	254	210	252	250	480	238	276
	3	370	423	264	268	266	230	403	258	222
Day 1	1	955	860	745	400	303	250	875	785	550
	2	895	825	700	450	275	280	910	715	666
	3	870	890	690	463	338	282	910	655	662
Day 2	1	1040	1110	1630	550	338	300	1340	1380	2733
	2	1120	1190	1570	450	330	308	1000	1130	3183
	3	1160	1030	1390	485	315	333	1290	1240	2950
Day 3	1	1200	1240	2000	580	560	377	1220	1860	5925
	2	1160	1180	2280	483	600	400	1360	2050	5425
	3	1140	1170	2070	590	530	410	1240	2080	4750

690

691

692