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Are Surfactant-Modified Zeolites Toxic to Non-Target Microorganisms?

Leah A. Constantinou, Robin N. Kaur and Gary S. Caldwell *

School of Natural and Environmental Sciences, Newcastle University, Newcastle upon Tyne NE1 7RU, UK; constantinouleah@gmail.com (L.A.C.); robin_kaur@hotmail.com (R.N.K.)
* Correspondence: gary.caldwell@newcastle.ac.uk; Tel.: +44-191-208-6660

Abstract

Zeolites are naturally abundant, low-cost aluminosilicate minerals commonly found in sedimentary rock. The surface chemistry of zeolite can be modified via cationic surfactant loading, termed a surfactant-modified zeolite (SMZ), that can be used as an antimicrobial technology in water treatment processes. This raises the possibility that SMZs could be utilised to treat blooms of harmful algae and cyanobacteria; however, there is a lack of understanding of the toxicity of SMZs to non-target microorganisms, including non-problematic algae and cyanobacteria. To address this knowledge gap, this research investigates whether hexadecyltrimethylammonium-bromide (HDTMA-Br) SMZs are toxic to the cyanobacterium *Synechococcus elongatus* and the microalgae *Chlorella vulgaris*, *Nannochloropsis oculata* and *Duniallela salina*. The cells were exposed to natural zeolite, HDTMA-Br surfactant and HDTMA-Br SMZ for 24 h and analysed 2- and 26 h post-exposure via flow cytometry and imaging pulse amplitude modulated fluorometry. There was an overall trend of reduced cell density in the SMZ and surfactant treatments. The SMZ treatment reduced the effective PSII quantum yield (Y(II)) but increased the quantum yield of regulated energy dissipation for *C. vulgaris*. When exposed to the surfactant treatment, no Y(II) signals were detected from any species. We conclude that SMZs are toxic to non-target microorganisms, with resilience dependent upon cell wall structure.

Keywords: zeolites; water treatment; algae blooms; toxicity; phytoplankton; cyanobacteria

1. Introduction

Eutrophication may be defined as a rise in the rate of organic matter provided to an ecosystem and is fuelled by enhanced inputs of nitrogen and phosphorous [1]. Excess phosphorous can trigger harmful blooms of microalgae and cyanobacteria (HABs; [2]). HABs can result in the death of flora and fauna [3] by reducing dissolved oxygen concentrations or by producing biotoxins [4–8]. Options to manage blooms and any resulting biotoxins are limited, both in situ and as part of drinking water treatment processing; generally necessitating integrated management [8–10].

Phosphorous is a limiting nutrient for algae and cyanobacteria metabolism, therefore decreasing phosphorous concentrations should prevent HAB formation [11–13]. However, processes such as internal phosphorous loading can proceed following the reduction of phosphorous concentrations in a water body. Internal phosphorous loading is concerned with the continuous phosphorous cycling between the sediment and water of a lake, thereby allowing algae growth to continue [12,14]. Treating the internal release of phosphorous is vital to impede eutrophication [15].

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Natural zeolites (NZs) are a group of low-cost and readily available aluminosilicate minerals, which can be utilised against environmental contamination [16,17] and wastewater treatment, including phosphorous capture [18,19]. NZs are also being considered as deployable agents to control bloom development in situ [20,21]. NZs form the tectosilicate subclass, with clinoptilolite the most commonly occurring form [16,17]. NZs bear crystal-like chemical characteristics which support unique physiochemical properties, such as a sieve-like mechanism and ion exchange, as well as an extensive cation exchange capacity and a large surface area to volume ratio [16,17,22]. These properties allow them to act as efficient catalysts and adsorbents and also allow for efficient modification via chemical and thermal treatment [22]. NZ particles are hydrophilic and bear a negative net surface charge when deposited in water [17,23].

Modifying the surface charge of NZ particles with cationic surfactants (termed surfactant-modified zeolites, SMZ; [24]) has demonstrated potential for the remediation of water contaminants [17,25], potentially enabling cost-effective treatment of nonpolar organic contaminants, and cations and anions [24–27]. The surfactant's sorption to NZ depends on the surfactant's chain length and cation exchange capacity [26,27]. Hexadecyltrimethylammonium (HDTMA; formula $C_{19}H_{42}N^{+}$) is a surfactant utilised for NZ surface alteration. A counterion, such as bromide, stabilises the molecule's charge [17,24]. The sorption maximum of HDTMA is approximately 200% of the NZ's cation exchange capacity [27]. HDTMA forms bilayers on the surface of NZ at the surfactant loading maximum. Electrostatic forces bond the layers beneath. These forces are formed by a reaction between the surfactant's positively charged head group and the negative charge of the NZ surface. Lastly, hydrophobic interactions between the surfactant's tail group hold the upper and lower layers together. The formation of bilayers alters the NZ's surface charge; thus, the NZ forms strong attractions with anionic pollutants such as phosphate ions [26,27].

Despite an abundance of research concerning SMZs as anion exchangers, there is a lack of relevant environmental risk assessments concerning SMZs for water remediation. Theoretically, SMZs should bind to excess phosphate ions to mitigate eutrophication and bloom development. However, there is uncertainty about how SMZs may affect environmental health. External stressors can trigger cell lysis, potentially instigating the release of intracellular cyanotoxins into water bodies [28,29]. This presents an issue due to the difficulty of removing individual cyanotoxins as opposed to cyanobacterial cells. Thus, the feasibility of SMZs for water remediation is dependent on whether cell damage occurs. This technology could potentially promote cell lysis and contaminate resources such as drinking water [29]. This response has been observed in cyanobacteria treated by conventional remediation methodologies, such as filtration and flocculation [30].

Additionally, HAB treatments need to be highly specific to target the bloom-forming species. Hydrogen peroxide treatments have had detrimental impacts on non-target species, such as phytoplankton [31]. Phytoplankton are vital primary producers in trophic networks, responsible for the carbon transfer between trophic levels. There is potential for SMZs to cause the removal of non-target phytoplankton, which could affect ecosystem stability [32]. Therefore, there is the need for relevant environmental risk assessments concerning the utilisation of SMZs for water remediation. This research addresses some of these knowledge gaps by investigating whether SMZs could affect non-target phytoplankton if used to prevent or mitigate HABs. To achieve this, the toxic capacity of natural and SMZs to three microalgae and a cyanobacterium are evaluated.

2. Materials and Methods

2.1. Cell Cultures

Chlorella vulgaris (CCAP 211/12) and *Synechococcus elongatus* (CCAP 1479/1A) cultures were grown in BG-11 medium (Culture Collection of Algae and Protozoa (CCAP); all chemicals from Merck, Gillingham, UK), with initial mean cell densities of 1.1×10^5 and 7.3×10^5 cells mL^{-1}, and *Dunaliella salina* (CCAP19/18) and *Nannochloropsis oculata* (CCAP 849/1) were cultured in F2 medium (Culture Collection of Algae and Protozoa (CCAP); all chemicals from Merck, Gillingham, UK), with initial mean cell densities of 1.2×10^5 and 9.9×10^5 cells mL^{-1} [33]. All cultures were grown in 250 mL Erlenmeyer flasks. Growth media were autoclaved at 121 °C for 15 min before vitamins were added [33,34]. The cultures were grown statically under an 18:6-h L:D photoperiod with a 200 µmol m^{-2} s^{-1} light intensity at 19 ± 1 °C [33,34]. Aseptic technique was followed by ensuring all glassware was cleaned and autoclaved before use [33].

2.2. Zeolite Preparation and HDTMA-Br Loading

NZ samples and HDTMA-Br (99% purity; CAS-Number: 57-09-0) were obtained from Merck Life Science UK Limited (Gillingham, UK). The aqueous HDTMA-Br solution was produced by adding 5.4 g of the HDTMA-Br crystals to 223 mL of Milli-Q® water (Avidity Science, Buckingham, UK). A magnetic stirrer (Stuart CB161, Cole-Parmer, UK) was used for 120 s at 400 RPM, to produce an aqueous HDTMA-Br solution at 67 mM concentration [17].

The HDTMA-Br surfactant-NZ loading was performed at 25 °C by combining 18 g of NZ with 54 mL of the HDTMA-Br solution. The solution was shaken at 60 RPM for 24 h. The surfactant-NZ mixture was filtered using a vacuum pump (KNF Neuberger Laboport, Freiberg, Germany) with 110 mm diameter 180 µm filter paper. The supernatant was discarded and the residual SMZ was washed with 40 mL of Milli-Q® water. This process was repeated three times and then left to air dry at 25 °C [17,26].

2.3. Algal and Cyanobacterial Assays

Algae and cyanobacteria were exposed to the three experimental conditions (NZ, HDTMA-Br surfactant, and HDTMA-Br SMZ) over 24 h. Exposure conditions were the same as those used for sample incubation (19 ± 1 °C, 18:6-h photoperiod). Four grams of each treatment was added to 25 mL of the corresponding growth media (*C. vulgaris* and *S. elongatus*, BG-11; *D. salina* and *N. oculata*, F2) to produce treatment solutions (n = 3; [33,34]) in individual culture flasks (25 mL working volume). Initial cell concentrations were 1.1×10^5, 7.3×10^5, 1.2×10^5 and 9.9×10^5 cells mL^{-1} for *C. vulgaris*, *S. elongatus*, *D. salina* and *N. oculata*, respectively.

2.4. Cell Counts

Cell counts were conducted following vortexing with a BD Accuri™ C5 Flow Cytometer (488 nm blue laser) with BD Accuri™ C6 software (version 1.0.264.21) (Wokingham, UK) at 2- and 26-h post-exposure. Milli-Q® water was passed through the instrument following the analysis of individual replicate sets. Side and forward scatter were utilised as an acquisition threshold for intracellular complexity and cell number to obtain cell counts. Individual cells were gated against a logarithmic scale and cell diameters to obtain representative counts and exclude contaminants (e.g., debris; [33]). Fifty microlitres of cell suspension were analysed per sample [33]. Cell density (cells mL^{-1}) measurements were obtained from the transformation of initial cell counts.

2.5. Photosystem II Analysis

Photosystem II (PSII) performance was determined with a Maxi-Imaging PAM fluorometer (Heinz Walz GmbH, Effeltrich, Germany). A 1 mL sample of exposed cells was collected for all replicates, 24 h post-exposure. The samples were concentrated via centrifugation for 20 min at 760.24 *g* at 20 °C. The remaining supernatant was discarded, and the pellet was reconstituted in 200 µL of fresh growth media (*C. vulgaris* and *S. elongatus*, BG-11; *D. salina* and *N. oculata*, F2). Individual 200 µL replicates were pipetted into each well of a clear 24-well flat bottom microplate (Corning Incorporated, New York, NY, USA) in triplicate for the determination of PSII efficiency. To maximise the oxidation of PSII reaction centres, microplates were dark-acclimated for 30 min [33]. The gain, measuring light frequency, and light intensity were set to produce current fluorescence yields (Ft) at a minimum of 0.1 relative units. For *S. elongatus*, measuring light frequency was set to 8, measuring light intensity to 12, gain to 15 and damping to 3 (this decreases extraneous signal noise). For *C. vulgaris*, *N. oculata* and *D. salina*, measuring light frequency was set to 1, measuring light intensity to 2, gain to 15 and damping to 4. The camera's aperture lens was adjusted to 1 for all analyses [34].

Dark-acclimated microplates were exposed to actinic light at gradually increasing intensity; increases in photosynthetically active radiation (PAR) occurred in 20 s increments until PAR values of 617–708 were attained [33,34]. Measurements of the effective PSII quantum yield (Y(II)) and the quantum yield of regulated energy dissipation (Y(NPQ)) were obtained and displayed as relative units.

2.6. Statistical Analysis

All statistical analyses and graphical representations were carried out using RStudio (Posit PBC, Version 4.3.3, MA, USA). Data were checked for homogeneity of variances and normality assumptions with Levene's test and the Shapiro-Wilk test; all data conformed to these assumptions. Cell density data were normalised against the experimental controls to obtain the percentage change in cell density relative to the treatment concentration, with time. These data were expressed as the mean ± standard deviation (S.D.). Y(II) and Y(NPQ) data were expressed as the mean ± standard error against PAR. Further analysis was conducted using a one-way analysis of variance (ANOVA) to investigate the significance of time and experimental conditions within each species for growth against time. A two-way ANOVA was used to investigate the significance of time, experimental condition and species for Y(II) and Y(NPQ) parameters. Multiple comparisons were conducted with Tukey's HSD Post Hoc test for non-parametric analysis ($p < 0.05$). No formal outlier analysis was performed, and all replicate values were retained in the dataset.

3. Results

3.1. Cell Growth

Cell density was measured at 2- and 26 h following exposure to SMZ, surfactant, or NZ (Figure 1). The NZ treatment produced the largest mean percentage change in cell density relative to the experimental controls for *C. vulgaris*, from 518 ± 458% at hour 2 to 614 ± 642% at hour 26. *D. salina* also increased to 78 ± 69% at hour 2 before decreasing to 0% at hour 26. The SMZ treatment produced increases in the mean percentage change in cell density relative to the controls for *C. vulgaris* and *D. salina*. *C. vulgaris* increased by 431 ± 264% at hour 2, followed by a decrease to 290 ± 184% at hour 26; *D. salina* displayed an initial increase of 134 ± 195% at hour 2, which dropped to 0% at hour 26. The surfactant treatment produced an increase in the mean percentage change in cell density relative to the control for *C. vulgaris*, from 0% at hour 2 to 322 ± 456% at hour 26. *D. salina* displayed a small increase from 17.8 ± 28% at hour 2, which decreased slightly to 17.5 ± 33% at

hour 26. For all treatments, there were no increases in the mean percentage change in cell density relative to the controls for *N. oculata* or *S. elongatus*. Significant differences were found between the experimental condition and time point for *N. oculata* and *S. elongatus* (One-way ANOVA: df = 7, f = 12.36, p = <0.001; df = 7, f = 17.01, $p < 0.001$, respectively). There were no significant differences found between the experimental condition and time point for *C. vulgaris* and *D. salina* (One-way ANOVA: df = 7, f = 2.37, $p = 0.07$; df = 7, f = 1.13, $p = 0.39$, respectively). A Levene's test confirmed homogeneity of variance ($p > 0.005$ for all species), validating the use of parametric analysis. There was an overall trend of the SMZ and surfactant treatments reducing cell density, which indicates toxicity to microorganisms.

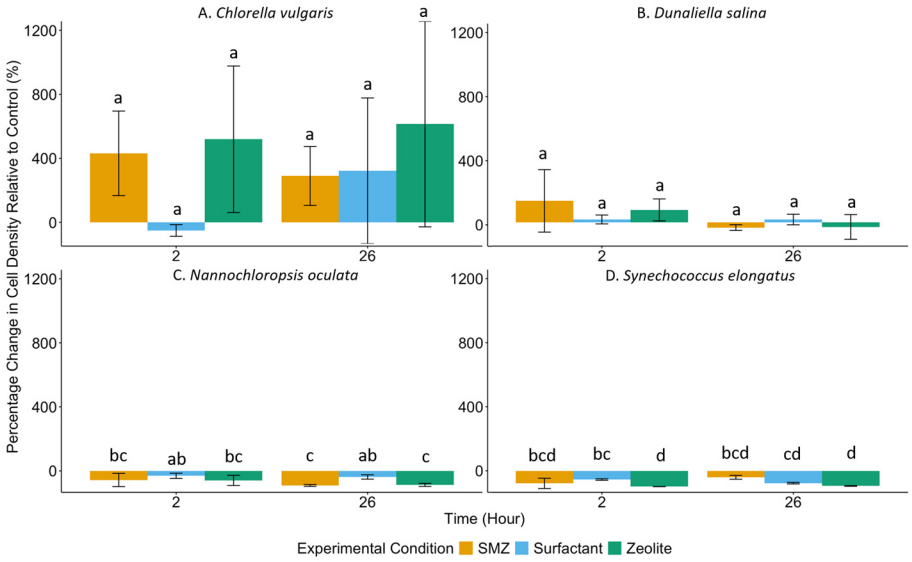

Figure 1. Mean percentage change in cell density (cells mL^{-1}) relative to the controls of each species ± standard deviation (S.D.) in 24 h following exposure to experimental groups (n = 3). Measurements were taken at time points 2 and 26 h in the assay from three replicates per species, experimental group and time point. Microalgae (**A**) *Chlorella vulgaris*, (**B**) *Dunaliella salina*, (**C**) *Nannochloropsis oculata* and cyanobacteria (**D**) *Synechococcus elongatus* were exposed to three experimental groups: surfactant-modified zeolite (SMZ), surfactant, and natural zeolite (NZ). Controls were cell suspensions in clean growth mediaLower case letters represent statistical differences between experimental groups and time points between each species wherein means that do not share a letter are significantly different ($p < 0.05$; Tukey's HSD Post Hoc).

3.2. Effective PSII Quantum Yield

All plots displayed an inversely proportional relationship between Y(II) and PAR (Figure 2). The NZ treatment produced the largest Y(II) measurement for *D. salina*, which was 0.134 ± 0.013 at 7 PAR, before plateauing to 0 at 192 PAR at hour 2; at hour 26 Y(II) was 0.135 ± 0.013 at 7 PAR, before plateauing to 0.010 ± 0.018 at 62 PAR. *C. vulgaris* exhibited Y(II) readings of 0.051 ± 0.078 at 7 PAR, before plateauing to 0.170 ± 0.014 at 400 PAR at hour 2, whereas hour 26 Y(II) was 0.153 ± 0.013 at 5 PAR, before plateauing to 0.016 ± 0.014 at 705 PAR. *N. oculata* displayed Y(II) measurements of 0.076 ± 0.031 at 7 PAR, before plateauing to 0 at 62 PAR at hour 2 and from 0.085 ± 0.012 at 7 PAR to 0.008 ± 0.014 at 707 PAR at hour 26. Lastly, *S. elongatus* had Y(II) readings of 0.027 ± 0.024 at 8 PAR to 0 at 63 PAR at hour 2 and 0.035 ± 0.024 at 8 PAR to 0 at 63 PAR at hour 26. The SMZ treatment produced a drop in Y(II) for *C. vulgaris*, from 0.120 ± 0.024 at 5 PAR to 0 at 60 PAR at hour 2 and from 0.110 ± 0.016 at 5 PAR to 0 at 60 PAR at hour 26. All measurements for the SMZ treatment group for *D. salina*, *N. oculata* and *S. elongatus* were 0. The surfactant treatment group produced Y(II) readings of 0 for all species. Significant differences were found between species and experimental conditions (Two-way ANOVA: df = 9, f = 4.828,

p = <0.001). When exposed to the SMZ treatment, significant differences were found between *N. oculata* and *S. elongatus* compared to the control (Tukey post hoc, $p < 0.05$). Significant differences were also found between the SMZ and NZ treatments for *D. salina* (Tukey post hoc, $p < 0.05$). When exposed to the SMZ treatment, significant differences were found in comparison to the controls (Tukey post hoc, $p < 0.05$). There was an overall trend of the SMZ and surfactant treatments reducing Y(II) efficiency, which indicates toxicity to microorganisms.

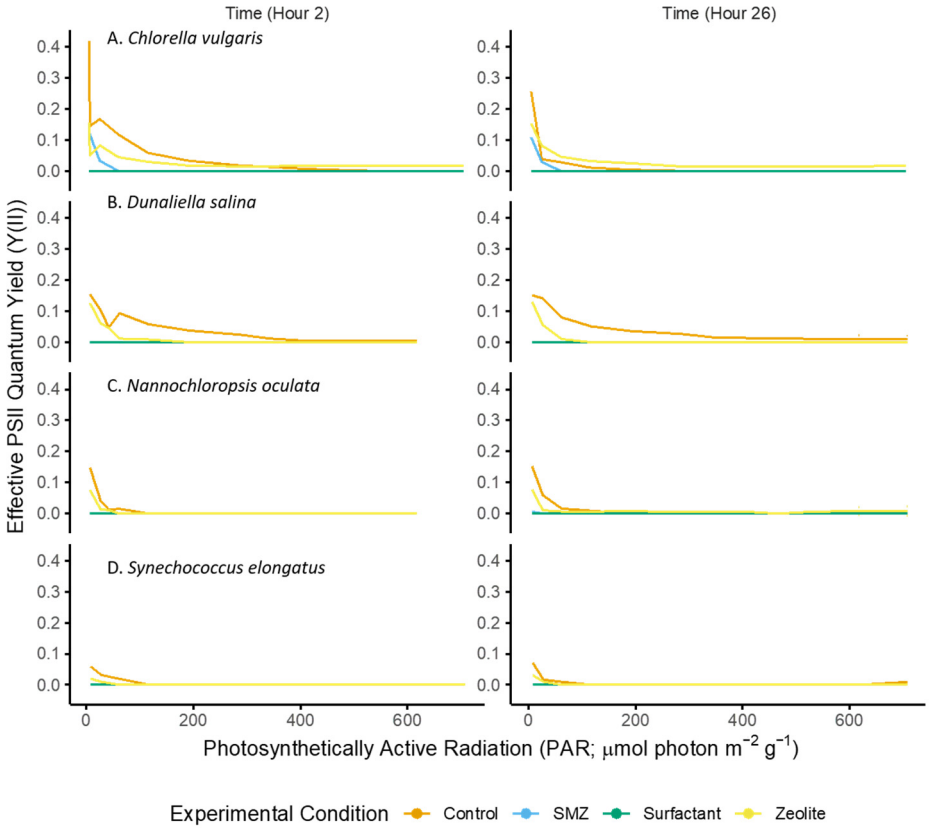

Figure 2. Mean effective photosystem II (PSII) quantum yield (Y(II)) ± standard deviation (S.D.) of microalga (**A**) *Chlorella vulgaris*, (**B**) *Dunaliella salina*, (**C**) *Nannochloropsis oculata* and cyanobacteria (**D**) *Synechococcus elongatus*, exposed to surfactant-modified zeolite (SMZ), surfactant, and natural zeolite (NZ) for 24 h (n = 3). Y(II) is displayed against increasing photosynthetically active radiation (PAR) with the units µmol photon m^{-2} g^{-1}. Measurements were taken at time points 2 and 26 h in the assay from three replicates per species, experimental group and time point. Controls were cell suspensions in clean growth media.

3.3. Quantum Yield of Regulated Energy Dissipation

All plots displayed the trend of an initial exponential increase in Y(NPQ) with PAR before eventually plateauing (Figure 3). There were no signals of Y(NPQ) produced for any species exposed to the surfactant treatment. The NZ treatment however, exceeded the Y(NPQ) performance of the controls for all species but *N. oculata*, which exhibited a spike followed by a rapid decrease, before eventually reaching 0.31 ± 0.048 at 617 PAR at hour 2; hour 26 displayed a gradual increase, but compared to hour 2 there was a decrease to 0.13 ± 0.138 at 617 PAR. *D. salina* also followed this pattern, with an initial spike and drop at hour 2 then plateauing to 0.3 ± 0.117 at 617 PAR; however, the initial Y(NPQ) measurements at hour 26 formed a smooth curve which plateaued relative to the control with 0.35 ± 0.054 at 617 PAR. *S. elongatus* Y(NPQ) readings remained similar at both time points, whereas *C. vulgaris* exceeded the control at hour 26 with 0.38 ± 0.277 Y(NPQ) at 705 PAR. The SMZ treatment produced the highest Y(NPQ) measurement for *C. vulgaris*,

with a smooth curve reaching 0.5 ± 0.013 at 705 PAR at hour 2; however, this dropped to a gradual inversely proportional relationship which reached 0.29 ± 0.020 at 705 PAR at hour 26. Significant differences were found between species and experimental conditions (Two-way ANOVA: df = 9, f = 27.17, p = <0.001). Significant differences were found between all experimental conditions and species groups (Tukey post hoc, $p < 0.05$). There was an overall trend of the SMZ and surfactant treatments reducing Y(NPQ) efficiency, which indicates toxicity to microorganisms.

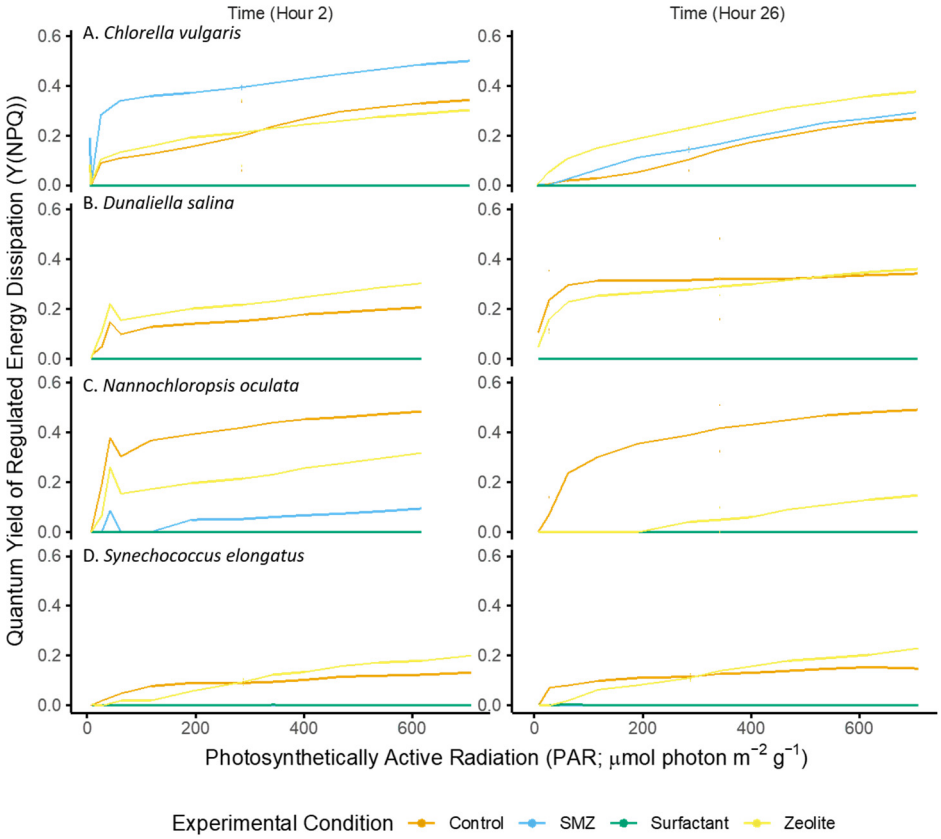

Figure 3. Mean quantum yield of regulated energy dissipation (Y(NPQ)) ± standard deviation (S.D.) of microalgae (**A**) *Chlorella vulgaris*, (**B**) *Dunaliella salina*, (**C**) *Nannochloropsis oculata* and cyanobacteria (**D**) *Synechococcus elongatus*, exposed to surfactant-modified zeolite (SMZ), surfactant, and natural zeolite (NZ) for 24 h (n = 3). Y(NPQ) is displayed against increasing photosynthetically active radiation (PAR) with the units µmol photon m^{-2} g^{-1}. Measurements were taken at time points 2 and 26 h in the assay from three replicates per species, experimental group and time point. Controls were cell suspensions in clean growth media.

4. Discussion

This study investigated the toxicity of a SMZ—a candidate deployable control for the development of harmful algae blooms—to non-target microalgae and cyanobacteria by examining changes in cell density and PSII parameters. The 24 h exposure period was selected to assess the acute toxicity of SMZs under controlled conditions. While it does not capture long-term environmental persistence, it provides an initial indication of whether SMZ exposure may induce cellular stress in water-treatment scenarios.

When treated with SMZ the cell densities of *D. salina* and *C. vulgaris* increased following 2 h but decreased by 26 h, indicating potential toxicity. SMZ exposure decreased Y(II) and Y(NPQ) signals. Y(II) is an important parameter for investigating physiological states and Y(NPQ) reveals the utilisation of energy for non-primary photochemistry [35]. The positive control (unmodified natural zeolite, NZ) did not impact on the photophysiology,

with Y(II) exceeding or equivalent to the algae-only control, except for *D. salina*. Y(NPQ) followed the same trend apart from the *N. oculata* treatment. The negative control (surfactant) produced no Y(II) or Y(NPQ) signals from any species, although minor Y(II) signals were detected for *C. vulgaris*. These data indicate that SMZ exposure negatively affects PSII photochemistry [35,36] which may negatively affect electron transport linked to energy dissipation [33]. This is consistent with the known mechanism of cationic surfactants, which can interact with lipid-rich cellular membranes and increase permeability, thereby reducing photosynthetic efficiency [35–37]. *N. oculata* demonstrated some resilience to the SMZ, however, Y(NPQ) was still lower than the control and no readings were detected for *D. salina* and *S. elongatus*. Decreased Y(NPQ) is associated with the failure of the PSII energy dissipation defence system [33], with the SMZ preventing the dissipation of excess excitation energy through thermal pathways. When *C. vulgaris* was exposed to SMZ, Y(NPQ) performance exceeded the control treatment. Therefore, increased Y(NPQ) demonstrates that *C. vulgaris* was still physiologically equipped to defend itself via the dissipation of excessive light energy into heat. Photoinhibition is likely to have occurred in species which displayed reduced PSII efficacy, which indicates damage to important PSII proteins. This cascades to the inability to activate PSII reaction centers and an overall decreased photosynthetic capacity [37].

The *C. vulgaris* cell wall constitutes a rigid glucosamine structure, encased within a thin polymeric matrix [38,39]. The matrix forms a triple-layered sheath, which provides resilience against exposure to non-oxidative chemical components, thus this mechanism can be extended to defence against cell damage by SMZs; however, toxicity indications were still present [39]. *D. salina* has a thin elastic plasma membrane, as opposed to a rigid polysaccharide cell wall [40,41]. This species thrives in extreme, hypersaline environments and can easily outcompete other species due to its ability to maintain homeostasis when exposed to stressors [42,43]. Cell density decreased when *N. oculata* was exposed to SMZs, despite the complexity of its cell wall. *N. oculata* has a bilayer structure, constituted of a hydrophobic algaenan outer layer and a cellulose inner layer [44] which does not appear well equipped to defend against damage from SMZ exposure [45]. The *S. elongatus* cell wall comprises a lipoprotein outer layer and a peptidoglycan-cellulose inner layer [39,46]. *S. elongatus* was alive but may not have been at the optimum level of health [39]. The differential susceptibility of the studied species to SMZ exposure may be attributed to structural differences in the cell wall. Species-specific resistance to SMZ exposure likely reflects differences in cell envelope composition, which may influence surfactant penetration and downstream photophysiological damage [38,39,44–46]. The large standard deviations observed for some treatments indicate biological heterogeneity among replicates.

While not significant in the current study, zeolites that were not surfactant modified have been shown to be beneficial for microalgae growth within pond-based aquaculture systems, resulting in higher fish yields [47] and in microalgae biofilms [48]. HDTMA-chloride SMZs have been shown to eliminate 100% of *Escherichia coli* [49,50]. Surfactants attack lipid membrane structures, resulting in increased membrane permeability [39]. This is damaging for important cell components, triggering cell lysis [39,51]. If SMZ treatment was implemented in natural environments, the responses from non-target microorganisms, as evaluated here, may have broad ecological impacts that could affect ecosystem functioning, trophic interactions and community dynamics. Organisms which are more tolerant of SMZs, such as *C. vulgaris*, could outcompete less tolerant species, such as *S. elongatus*. This could trigger shifts in community diversity with likely trophic level consequences [52].

The toxicity indicated in this study contrasts with prior research which concluded that HDTMA attached to the NZ surface was nontoxic to microorganisms. The present results

may reflect toxicity from the surface-bound SMZ and/or surfactant release during exposure, rather than from the zeolite carrier alone [24,50]. However, unlike this study, previous research did not quantify the toxicity of desorbed surfactants to microorganisms [26,50]. During SMZ preparation, 3.618 mmol HDTMA-Br was introduced via the loading solution, representing an absolute upper bound for potential release over the 24 h exposure period. Although SMZs are designed to retain surfactants on the zeolite surface, prior studies have reported surfactant desorption, suggesting that surfactant release may contribute to the toxicity observed in this study [24,50]. Nevertheless, surfactant release was not directly quantified in this study, so the extent of desorption during exposure cannot be confirmed. Zeolite with HDTMA is also known to be toxic to grazers of microalgae, including *Daphnia pulex* [53]. Despite the toxicity of SMZs to algae and cyanobacteria, nonionic and anionic surfactants are less toxic than cationic surfactants, as utilised in this study [54–57]. This also links to findings which state that cationic surfactants are biologically degradable in aerobic environments; however, when NZs are functionalised, the surfactant may not function in the same way [55]. For future research, a less toxic surfactant may be more suitable for NZ modification and could be nontoxic to non-target microorganisms. Future work should combine biological endpoints with chemical quantification of surfactant desorption to distinguish carrier effects from leached-surfactant toxicity and assess desorption kinetics. Spectrophotometric or Total Organic Carbon analysis of the leachate is strongly recommended. Further, risks posed by loss of surfactant from the NZ surface need to be assessed [58]. Overall, SMZs are toxic to non-target microorganisms and further research is needed to understand the possible ecological impacts of implementing SMZs in water treatment.

5. Conclusions

This study demonstrates that surfactant-modified zeolites (SMZs), while promising for water treatment applications, exhibit measurable toxicity to non-target microalgae and cyanobacteria. Reductions in PSII efficiency and altered energy dissipation responses indicate disruption to photosynthetic function, with species-specific resilience likely linked to cell wall structure. Notably, the toxicity appears largely driven by the surfactant component, raising concerns about unintended ecological impacts, including shifts in community composition and trophic dynamics. These findings highlight the need for careful risk assessment and optimisation of SMZ formulations, particularly through the exploration of less toxic surfactants, before their safe and effective deployment in aquatic environments.

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