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Environmental behaviour and ecotoxicity of cationic surfactants towards marine organisms

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Environmental behaviour and ecotoxicity of cationic surfactants towards marine organisms

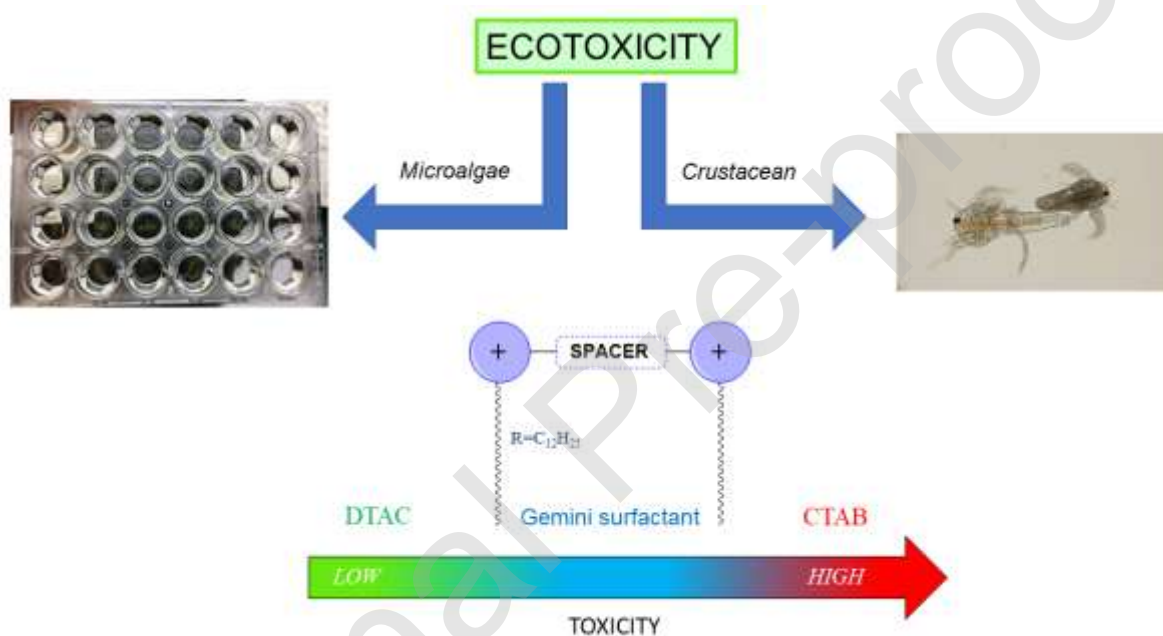
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Graphical abstract



Highlights

- Cationic surfactants have shown differentiated toxicity towards marine species;
- CTAB is the most toxic to marine species among tested cationic surfactants;
- Cationic surfactants are not readily biodegradable in artificial seawater.

Abstract

Cationic surfactants are surface-active compounds that can be found in many products, including household and cleaning agents. As a consequence, they tend to be discarded into water streams, ultimately ending up in the aquatic environment. In spite of this environmental issue, studies describing their effects towards marine species are lacking. The aim of this study was therefore to evaluate the short-term exposure effects of two commercial cationic surfactants and three novel gemini surfactants on four marine species, the green microalgae *Nannochloropsis gaditana* and *Tetraselmis chuii*, the diatom *Phaeodactylum tricorutum*, and the crustacean *Artemia salina*. Furthermore, biodegradation and size distribution of the cationic surfactants in artificial seawater were also studied by UV-Visible spectrophotometry and dynamic light scattering, respectively.

Ecotoxicity tests revealed that the commercial cationic surfactant *N*-cetyl-*N,N,N*-trimethylammonium bromide is toxic to all tested marine species while *N*-dodecyl-*N,N,N*-trimethylammonium chloride and 1,4-bis-*[N*-(1-dodecyl)-*N,N*-dimethylammoniummethyl]benzene dibromide showed the lowest toxicity among the tested cationic surfactants. Besides the novel insights regarding the effects caused by these five cationic surfactants, this work opens prospects for the replacement of commercially available surfactants by more environmentally friendly alternatives.

Keywords

Cationic surfactants, gemini, ecotoxicity, seawater, biodegradation

1. Introduction

The global use of surfactants increases every year and is expected to reach US\$ 28.8 billion until 2023 (Brycki et al., 2017), which attests to its importance in daily life. Cationic surfactants, which include quaternary ammonium salts, comprise one of the four main groups of surfactants (Brycki et al., 2017). Due to their unique properties, such as emulsification, dispersion, surface or biological activity, cationic surfactants have found numerous industrial applications including those pertaining to chemistry, pharmacy, cosmetic, biotechnology, metallurgy or petrochemistry (Garcia et al., 2017, 2019; Lei et al., 2019; Nałęcz-Jawecki et al., 2003; Olkowska et al., 2014). As a result of accidental leakages, lack of appropriate sewage treatment and specific industrial applications such as antifouling coatings or remediating materials, cationic surfactants have been detected in both freshwater and marine environments and can persist in the environment, becoming a source of ecotoxicity (Biswas et al., 2019; Figueiredo et al., 2019; Olkowska et al., 2014; Piola and Grandison, 2017; Wang et al., 2018). Most of the reported ecotoxicity studies have concerned fate and hazard of cationic surfactants in freshwater environments (Garcia et al., 2016, 2019; Kaczerewska et al., 2018), while there is still a lack of information for seawater. For instance, in the recent review by Jackson *et al.* (Jackson et al., 2016) a summary of marine data values reported for surfactants was presented. Most of the data concerned alkyl sulphates (anionic surfactants) with less than 5% of data reported being associated with ditallow dimethyl ammonium chloride (DTDMAC), a cationic surfactant and a representative fabric softener (Jackson et al., 2016; Roghair et al., 1992). For other commercially available compounds such as *N*-dodecyl-*N,N,N*-trimethylammonium chloride (DTAC) or *N*-cetyl-*N,N,N*-trimethylammonium bromide (CTAB; also known as hexadecyltrimethylammonium bromide HDTMA) (source: European Chemicals Agency www.echa.europa.eu) data are still limited, as well. Both DTAC and CTAB are widely used as emulsifiers and softeners. CTAB, in particular, is used during the synthesis of silica nanomaterials utilized in maritime coatings applications (Chen et al., 2008; Mirmohseni et al., 2019; Singh et al., 2014). The environmental behaviour and ecotoxicity assessment in the marine compartment of such compounds is therefore crucial, in order to understand and predict

eventual impacts of existing or novel surfactants in the marine organisms. Moreover, due to the lack of marine ecotoxicity data, risk assessment for new and existing substances rely on extrapolations based on freshwater information assuming that their sensitivities are identical. Nevertheless, some studies have already suggested that certain marine organisms are more sensitive than freshwater species (Jackson et al., 2016). Hence, insufficient understanding of the relation between the sensitivity to chemicals of marine, estuarine and freshwater organism may lead to differences in toxicity estimates. Data provision regarding the hazard to marine species will therefore decrease this uncertainty (Leung et al., 2001; Wheeler et al., 2002).

Gemini surfactants constitute a new subgroup of cationic surfactants (Karaborni et al., 1994), which are composed of two hydrophilic and two hydrophobic groups linked by a spacer (Liu et al., 2018). Due to their more complex structure, they have exceptional properties, superior in comparison to single chain surfactants analogues: lower critical micelle concentration (CMC) (In and Zana, 2007), better wetting ability, higher surface activity and corrosion inhibition efficiency, meaning that lower amount of gemini surfactants is needed to reach the desired effect (Brycki et al., 2017; Garcia et al., 2017; Liu et al., 2018). Altogether, these features make gemini surfactants cost-effective and more sustainable. Moreover, by changing the molecular structure, namely spacer, length of hydrophobic chain, hydrophilic part or counterion, their properties can be controlled and new molecules with designed features may be synthesized (Liu et al., 2018). Previous reports showed that gemini surfactants also exhibit lower toxicity in freshwater compared to monomeric salts (Garcia et al., 2016, 2019; Kaczerewska et al., 2018), making them environmentally-friendly replacements for conventional cationic surfactants.

Considering the abovementioned information, the aim of this study was to assess the behaviour and effects of five cationic surfactants, two monomeric (commercially available) and three gemini, to the marine microalgae *Nannochloropsis gaditana* and *Tetraselmis chuii*, the diatom *Phaeodactylum tricorutum*, and the crustacean *Artemia salina*, following standard protocols. Complementary studies including biodegradation and size distribution of surfactants in artificial seawater were also carried out for the first time for gemini surfactants in order to interpret their action from chemical, compositional and colloidal perspectives.

2. Materials and methods

2.1. Materials

N-dodecyl-*N,N,N*-trimethylammonium chloride (**DTAC**) ($\geq 98\%$), *N*-cetyl-*N,N,N*-trimethylammonium bromide (**CTAB**) ($\geq 98\%$), were purchased from Sigma-Aldrich. Gemini surfactants were synthesised according to the methods described in literature, i.e. 3-oxa-1,5-pentamethylene-bis(*N*-dodecyl-*N,N*-dimethylammonium) dichloride (**12-O-12**) (Garcia et al., 2016), 3-oxa-1,5-tetramethylene-bis(*N*-dodecyl-*N*-hydroxyethyl-*N*-methylammonium) dichloride (**MOH-12**) (Kaczerewska et al., 2018) and 1,4-bis-[*N*-(1-dodecyl)-*N,N*-dimethylammoniummethyl]benzene dibromide (**QSB2-12**) (Garcia et al., 2016). The structures of these compounds are presented in Figure 1.

The chemical structure of gemini surfactants was confirmed by comparing proton nuclear magnetic resonance (^1H NMR) spectra, and Fourier-transform infrared spectroscopy (FTIR) data, and melting points with previous studies (Kaczerewska, 2017). In all ^1H NMR spectrum triplet and multiple signals at 0.88 ppm and 1.25-1.37 ppm, which show the presence of protons from methyl and methylene groups of long alkyl chains, as well as signals at 3.60-4.02 ppm from protons of carbon atoms next to quaternary nitrogen atoms were detected. For the gemini surfactants with spacer functionalized with oxygen atoms (12-O-12 and MOH-12), signals from protons of methylene groups in the immediate vicinity of the oxygen were observed at 4.06-4.30 ppm. In the case of an aromatic ring as a spacer, signals from methylene groups were found

at 5.28 ppm, due to a stronger deshielding effect of the aromatic ring than the oxygen atom. For QSB2-12 a singlet at 7.82 ppm is characteristic of the disubstituted benzene ring. The FTIR spectrum showed signals at wavenumbers characteristic of the bonds: C-H stretching absorption typical for alkanes (from long alkyl chains), in the range 2840 to 3000 cm^{-1} ; and rocking vibrations of $(\text{CH}_2)_n$, for $n \geq 4$, around 720 cm^{-1} (present only in case of long alkyl chains). The stretching vibration of $(=\text{C}-\text{H})$ from a benzene ring was observed around 3010 cm^{-1} for QSB2-12, and the presence of characteristic O-H stretching absorption at 3288 cm^{-1} was observed for MOH-12. Moreover, in all three cases, disappearance of stretching vibration of C-halogen (Cl or Br) around 675 cm^{-1} was observed, which confirmed the successful synthesis of dimeric quaternary ammonium salts. Finally, the melting points of the gemini surfactants were: 223-225, 154-156 and 218-219°C for 12-O-12, MOH-12 and QSB2-12, respectively (Kaczerewska, 2017).

The dye methyl orange (Orange II) (85%) and chloroform (anhydrous, $\geq 99\%$) were purchased from Sigma-Aldrich.

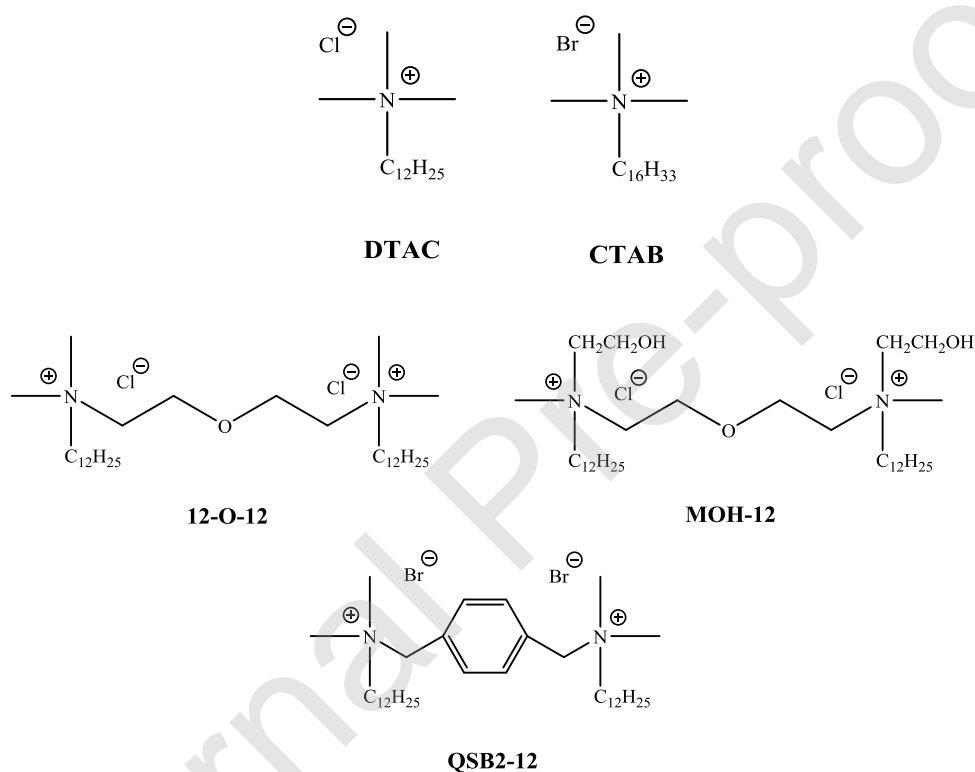


Figure 1 Structure of the tested compounds.

2.2. Test organisms

Cultures of green microalgae *Nannochloropsis gaditana* (Ochrophyta: Eustigmataceae) and *Tetraselmis chuii* (Chlorophyta: Chlorodendrales) and diatoms *Phaeodactylum tricornutum* (Heterokontophyta: Naviculales) were prepared using sterile 0.45 μm -filtered artificial seawater (ASW; prepared with Tropic Marin® Pro Reef pharmaceutical-grade salt; salinity 35), enriched with culture medium “Optimedium” purchased from Aqualgae SL. They were maintained in vials with approximately 150 mL of culture medium, to allow gas exchange, at a temperature of $19 \pm 1^\circ\text{C}$ and photoperiod conditions of 16:8h (light: dark) and daily agitated. In order to create the growth curve of each culture, a dilution series with factor of two was performed (with eight

points), and the cell density was monitored by fluorescence (excitation: 475 ± 10 nm; emission: 645 ± 20 nm) with a Biotek® microplate spectrophotometric reader, and correlated with cell density calculated using an optical microscope and a Neubauer hemocytometer. The initial cell density was approximately 10^4 cells/mL, and the regression equation between cell density (Y) and optical density (OD) (X) was calculated as:

$$Y = 36.84X - 41765 \quad (r^2 = 0.996), \text{ for } N. \textit{ gaditana};$$

$$Y = 34.89X - 19681 \quad (r^2 = 0.980), \text{ for } T. \textit{ chuii};$$

$$Y = 291.89X - 315415 \quad (r^2 = 0.999), \text{ for } P. \textit{ tricorutum}.$$

Dry cysts of the brine shrimp *Artemia salina* (Crustacea, Anostraca) were hydrated during 30 min in 300 mL of reverse osmosis water with strong aeration. A sample was then observed in a stereo microscope and, after confirming their full hydration, 700 mL of ASW was added to correct for salinity 25. After 16-24 h at $26\pm 1^\circ\text{C}$ (24 h light), hatched organisms were washed with new ASW before use. In this study, *A. salina* was used in the stage second-third instar, reached after > 24 h.

2.3. Methods

2.3.1. Biodegradation in artificial seawater

Biodegradation under exposure to light of the cationic surfactants DTAC, 12-O-12, MOH-12 and QSB2-12 was followed by UV-Visible spectrophotometry, based on complexation of cationic surfactant with methyl orange (Orange II), chloroform extraction, ASW:chloroform phase separation and subsequent spectrophotometric measurement. Orange II was added in excess to provide total complexation. The methods available in the literature (Scott, 1968; Wang and Langley, 1975) were adjusted for dimeric cationic surfactants. Monomeric salts form the complex with Orange II in 1:1 stoichiometry, while dimeric salts were 1:2 (Figure 2).

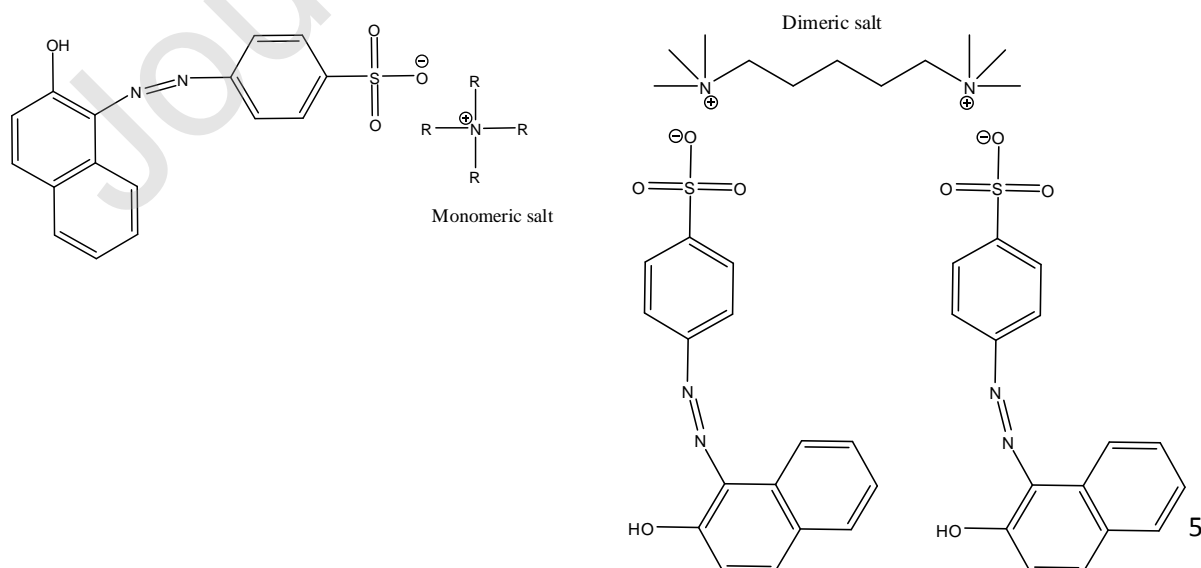


Figure 2 Structure of the complex Orange II: cationic surfactants.

The complex was dissolved in chloroform phase by rapidly shaking. The chloroform phase is heavier than the ASW phase and it was separated by gravity. The intensity of the orange colour in the chloroform phase is directly proportional to the concentration of the complex and the intensity of the colour can be measured spectrophotometrically. The absorbance curves cover a wavelength range of 300-500 nm. The absorbance curves for all tested cationic surfactants showed an absorbance maximum at 486 nm. The wavelength of maximum absorbance for Orange II salts in chloroform remains invariant over a wide range of pH values (Scott, 1968).

The methyl Orange (Orange II) stock solution was prepared as follows: 0.05 g of Orange II was dissolved in 100 mL of ASW and then stored, protected from light. For the preparation of the surfactant stock solutions 10 mg of the cationic surfactant was dissolved in 100 mL of ASW.

Samples for calibration curves were obtained in the following manner: 15 mL of surfactant solution (100 mg/L) were mixed with 1 mL of ethanol (added to aid cationic transfer), 3 mL of Orange II solution (added in excess-256 %) and 10 mL of chloroform. The sample was shaken rapidly to dissolve the Orange II complex in chloroform. The chloroform phase (with orange colour) was separated gravitationally and collected in 50 mL volumetric flasks. The extraction was repeated until the chloroform phase was visually colour-free. The final volume of the surfactant solution in chloroform was 50 mL and the concentration of the surfactant was 30 mg/L. Other standard solutions for calibration curves were prepared by dilution of 30 mg/L solution (A) (Table S1). Calibration curves were plotted as a function of mg/L of cationic surfactant versus absorbance.

For the biodegradation studies the cationic surfactants were prepared in ASW. Concentrations varied between 10-30 mg/L. Subsequently, each sample was treated as described in the preparation of samples for calibration curves (extraction with chloroform). Absorbance was measured at 486 nm and the concentration of the surfactant was determined from the appropriate calibration curve. The quantification was carried out at 0, 24, 48 and 72 hours after preparing the solution in ASW. For each sample, three replicates were prepared.

2.3.2. Dynamic Light Scattering (DLS)

A Zetasizer Nano-ZS (Malvern Instruments, UK) was used to perform dynamic light scattering (DLS) measurements, at a scattering angle of 173°. Filtered ASW (0.45 µm; salinity 35) and distilled water were used to prepare solutions of DTAB, CTAB, 12-O-12, MOH-12 and QSB2-12. Two sets of DLS measurements were done: at low concentrations (used for ecotoxicity tests) and at concentrations higher than critical micelle concentrations (CMC). For the first set, two concentrations of the tested cationic surfactants (1 and 10 mg/L) in ASW were prepared and then the average hydrodynamic size was measured at 25°C, up to 72 h. For each sample, measurements were carried out at least in triplicate. The aim of the second set of measurements was to compare the aggregation behaviour of the cationic surfactants in distilled water and ASW. For this reason, DLS measurements were performed at concentrations higher than their CMC values for both media (distilled water and ASW) (Table S2) (Garcia et al., 2017; Kaczerewska et al., 2018; Vatta and Kaur, 2017): 5000 mg/L for 12-O-12, MH-12,

QSB2-12 and CTAB, and 10000 mg/L for DTAC. Measurement in ASW were not possible for QSB-12 due to problems with solubility at 25°C. The average hydrodynamic size was only measured at 25°C after the solutions were prepared. For each sample, measurements were performed at least three times.

2.3.3. Ecotoxicity testing

All ecotoxicity tests were carried out in ASW, filtered at 0.45 µm, at room temperature (19±1 °C) and photoperiod of 16:8 h (light: dark) for *N. gaditana*, *T. chuii* and *P. tricornerutum* and, at a temperature of 25±1°C and photoperiod of 24 h dark for *A. salina*. There was no replacement of medium during exposure.

Exposure concentration ranges were chosen based on preliminary tests (0.01, 0.1, 1, 10, 100 mg/L) and differ depending on the species and compound (Table S3).

Short-term effects on the three photosynthetic species were evaluated through 72 h growth inhibition tests, according to the guidelines OECD 201 (OECD, 2011) and ISO 10253 (ISO 10253, 2016) with some adaptations for 24-well microplate (Figueiredo et al., 2019). For each compound five concentrations plus one negative control (ASW only), with four replicates per treatment, were tested. An exception was made for CTAB (*N. gaditana*) where six concentrations were included. Each replicate contained 1 mL of exposure solution (ASW for the control) and 1 mL of microalgae sample. The test cell density was monitored daily by fluorescence (excitation: 475 nm; emission: 645 nm) at 0, 24, 48 and 72 h. In order to provide consistent results, each sample was re-suspended immediately before reading. The 72 h growth inhibition was calculated following the equation:

$$\% Ir = \frac{\mu_c - \mu_T}{\mu_c} \times 100$$

where % *Ir* is the percent inhibition in average specific growth rate; μ_c is the mean value for average specific growth rate (μ) in the control group and μ_T corresponds to the average specific growth rate for the treatment replicate.

The evaluation of the acute toxicity of the tested compounds to *A. salina* followed the standard protocol ASTM E-1440-91 (ASTM E1440-91, 2012) with adaptations for 24-well microplate (Figueiredo et al., 2019). For each compound five concentrations plus a negative control (ASW only), with three replicates per treatment, were tested. Each replicate contained 1 mL of exposure solution (ASW for the control) and ten specimens. Mortality/immobilization was checked after 24 and 48 h.

2.3.4. Statistical analysis

The percentage of photosynthetic species growth inhibition and percentage of crustaceans' survival were plotted against concentration, in a logarithmic scale. The IC/LC₅₀ (i.e., the concentration that caused 50% mortality or inhibition) was determined by a non-linear regression method with the software *Graphpad Prism v.6.0*. For each compound and species, the non-linear regression equation that best fits the data was chosen, considering the R² value, the absolute Sum of Squares and the 95% Confidence Intervals. Based on the results, the toxicity of each compound was classified according to the EC Directive 93/67/EEC repealed by Regulation (EC) No 1907/2006 (EC, 2006).

3. Results and discussion

3.1. Biodegradation in artificial seawater

The absorbance of the cationic surfactant-Orange II complex was measured at 486 nm ($A_{486 \text{ nm}}$) and values increased with the increasing concentration of the resulting complex. An example of data for the complex QSB2-12:Orange II is presented in Figure 3.

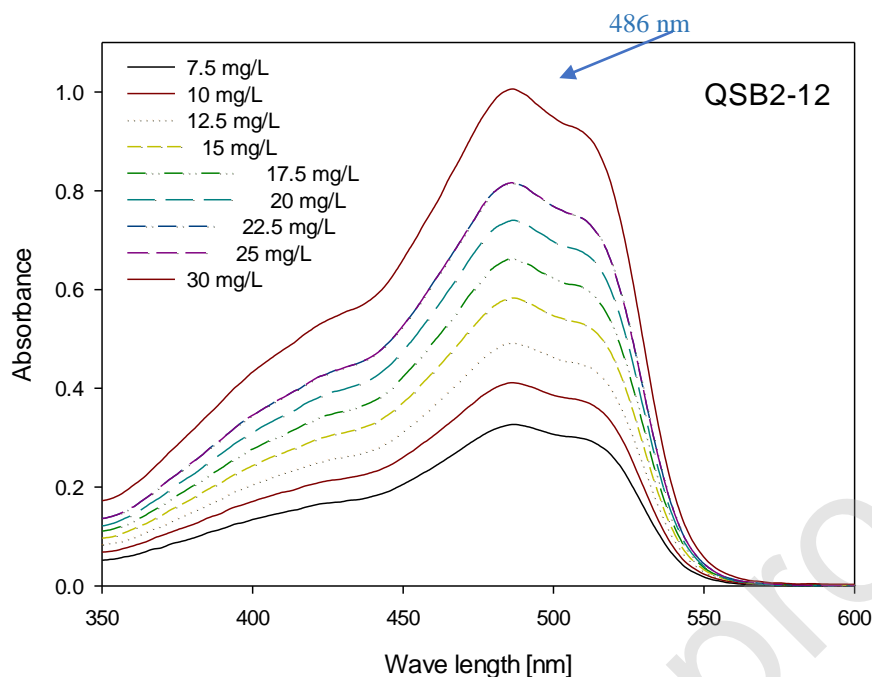


Figure 3 Absorbance measured at wavelength 486 nm of the complex QSB2-12:Orange II, at different concentrations of QSB2-12.

As mentioned in the experimental section, the wavelength of maximum absorbance for Orange II salts in chloroform is fixed over a wide range of pH values. However, in order to confirm that no changes occurred in the tested systems during the biodegradation tests, pH values of surfactants' solutions were monitored up to 72 h (Table 1) (pH meter: HACH, PHB101-01 Probe). The indicated time slot was chosen as it corresponds to the timeframe of conducted ecotoxicity tests.

Table 1 pH values daily measured in the surfactant solutions after preparation (0 h) and up to 72 h, at different concentrations.

Compound		Time [hours]			
		0	24	48	72
12-O-12	1 mg/L	8.06±0.05	8.07±0.06	8.07±0.04	8.04±0.03
	10 mg/L	8.05±0.01	8.05±0.02	8.07±0.02	8.03±0.02
MOH-12	1 mg/L	7.66±0.02	7.66±0.02	7.68±0.01	7.75±0.03
	10 mg/L	7.74±0.02	7.76±0.01	7.75±0.04	7.76±0.01

QSB2-12	1 mg/L	7.72±0.01	7.72±0.02	7.71±0.01	7.84±0.02
	10 mg/L	7.82±0.01	7.76±0.05	7.83±0.02	7.87±0.02
DTAC	1 mg/L	7.97±0.01	7.97±0.03	8.02±0.02	8.03±0.04
	10 mg/L	8.05±0.01	8.04±0.01	8.06±0.04	8.05±0.01
CTAB	1 mg/L	7.73±0.02	7.73±0.02	7.73±0.02	7.86±0.03
	10 mg/L	7.73±0.03	7.84±0.02	7.85±0.03	7.86±0.05

Calibration curves were plotted as Absorbance as a function of Concentration of cationic surfactant (Figure S1), to infer on the biodegradation of the cationic surfactants in time, using linear regression parameters. Concentrations of the cationic surfactants after 0, 24, 48 and 72 h are presented in Table 2.

Table 2 Average values of calculated concentrations of cationic surfactants after 0, 24, 48 and 72 h.

Compound	Time [hours]	Concentration [mg/L]
DTAC	0	22.2±0.2
	24	21.9±0.8
	48	21.8±0.6
	72	22.0±0.5
12-O-12	0	16.7±0.4
	24	17.2±0.5
	48	17.1±0.5
	72	17.2±0.6
MOH-12	0	9.89±0.59
	24	10.5±0.7
	48	10.5±0.9
	72	10.7±0.7
QSB2-12	0	12.1±0.7
	24	12.4±0.5
	48	12.2±0.2
	72	12.4±0.2
CTAB	0	25.7±0.3
	24	25.1±2.8
	48	25.4±3.0

72

26.8±1.2

Based on the results presented in Table 2, the tested cationic surfactants are shown to not be biodegradable in artificial seawater up to 72 h, the time duration of the ecotoxicological tests. Thus, the obtained results suggest that surfactants' concentration was stable during the ecotoxicity testing.

Biodegradability is a desired property as it helps to reduce some negative effects of substances to the environment by mineralization to carbon dioxide, water, mineral salts and biomass, or by alteration in their chemical structure leading to loss of some specific properties (Brycki et al., 2014). After the molecule played its functional role and is released into the water, the biodegradation process would have led to the disappearance of the parental molecule. Nonetheless, if considering some applications of cationic surfactants such as antifouling agents, corrosion inhibitors or preservatives, short-term biodegradation may not always be a favourable feature. When the surfactant needs to have efficacy in the aquatic environment under light or oxygen exposure, biodegradation could lead to a decrease of surfactant's concentration, affecting its performance. Therefore, a compromise between surfactant degradation and the application for which it was initially designed must be considered. One of the possible ways of promoting degradation of these compounds can be through a process similar to what is commonly done in wastewater treatment plants, where the effective degradation of organic compounds is accomplished by bacteria consortia (Brycki et al., 2014).

3.2. DLS measurements

Size distribution of tested cationic surfactants was determined by dynamic light scattering in two sets. The first set was performed on two low concentrations, 1 and 10 mg/L, in ASW from 0 to 72 h. All DLS measurements showed a high polydispersity index ($Pdl > 0.5$) indicating heterogeneity and possible presence of large aggregates (Figueiredo et al., 2019). Due to this, the Z-average diameter measurements could not be used. The hydrodynamic size of the molecules was based on the average (at least three repetitions) value of peaks (intensity distribution) of each measurement (Table 3). Signals for pure ASW were found between 400-500 nm and no significant changes were observed over time (up to 72 h) and they may result from salt particles smaller than the pore size of the filters (0.45 μm). Solutions with cationic surfactants showed a wider size distribution, with peaks ranging from small diameters (around 100 nm) to slightly bigger ones (around 500 nm). For 12-O-12 and MOH-12, at 10 mg/L concentration, larger diameters were observed with peaks centred between 700-1000 nm. Within the range of tested concentrations, the measured effects on aggregation are not significant as concentrations are much lower than critical micelle concentration (CMC) values (980 mg/L 12-O-12, 920 mg/L MOH-12, 840 mg/L QSB2-12, 394 mg/L CTAB and 4220 mg/L DTAC (Garcia et al., 2017; Kaczerewska et al., 2018; Vatta and Kaur, 2017)) and changes in colloidal properties were not observed.

Table 3 Hydrodynamic size (average \pm standard deviation) [nm] of tested cationic surfactants at 1 and 10 mg/L in ASW, from 0 h to 72 h.

Compound

Time [hours]	Concentration [mg/L]					
		12-O-12	MOH-12	QSB2-12	CTAB	DTAB
0	1	469±47	239±17	310±22	200±14	307±30
	10	420±35	261±24	393±42	293±30	299±49
24	1	411±39	196±12	236±17	91±10	341±35
			360±26			
	10	344±38	306±27	360±26	255±27	349±36
48	1	365±27	129±12	199±19	185±10	244±16
	10	522±55	391±42	227±13	190±20	307±23
			1059±184			
72	1	425±31	335±17	183±12	304±18	171±12
				745±116		286±17
	10	397±31	326±26	279±38	190±19	273±20
		522±63	839±98			

Hydrodynamic size [nm] of ASW: 367 nm (0 h), 441 nm (24 h), 498 nm (48 h), 531 nm (72 h).

The second set of DLS measurements was carried out to investigate whether ASW affected the surfactants' aggregation behaviour above CMC. DLS measurements were performed at surfactants' concentrations much higher than CMC values: 5000 mg/L for 12-O-12, MOH-12, QSB2-12 and CTAB, and at 10000 mg/L for DTAC, in both ASW and distilled water (Table 4). For this concentration range, changes in hydrodynamic size were detected. Typically, micelles' morphology differs from that of monomers and simple aggregates as they are compact structures (Brycki et al., 2017). Therefore, the obtained results in this second set of measurements show that cationic surfactants formed slightly bigger micelles in ASW than in distilled water, although in both cases hydrodynamic diameters were lower than 10 nm. These findings are consistent with the formation of spherical micelles (Kaczerewska et al., 2018). It has been already reported that addition of salt to the aqueous solution of cationic surfactant results in decreased CMC values. Screening of the repulsion between cationic head groups of the surfactant in the presence of counter ions favours early micelles formation (Kumar, 2012). For zwitterionic surfactants, which also belong to a group of ionic surfactants, changes in micelles size have been observed. Depending on aggregates morphology, increasing the ionic strength of the medium leads to a decrease (asymmetric monomers) or an increase (symmetric monomers) in their hydrodynamic size (Borisova et al., 2012). The increase in diameter of micelles made of symmetric cationic surfactants may be associated with the phenomena observed for zwitterionic surfactants.

Table 4 Hydrodynamic size (distribution by intensity) of cationic surfactants at concentrations higher than CMC.

Compound	Size [nm]	
	Distilled water	ASW
12-O-12	1.0±0.4	5.1±0.1
MOH-12	2.2±0.6	5.3±1.1
QSB2-12	1.5±0.4	-
CTAB	0.8±0.2	6.3±0.9
DTAC	2.9±0.5	4.2±0.8

3.3. Ecotoxicity testing

Dose-response curves for microalgae (*N. gaditana* and *T. chuii*), diatoms (*P. tricornutum*) and crustaceans (*A. salina*) are presented in Figure 4a-d for cationic surfactants. IC₅₀ and LC₅₀ values for photosynthetic organisms and crustaceans are summarized in Table 5 and 6, respectively. Among the tested cationic surfactants, CTAB was the most deleterious for all photosynthetic species being classified as toxic to *T. chuii* and very toxic to *N. gaditana* and *P. tricornutum*. The surfactants 12-O-12 and CTAB were the most toxic compounds to *A. salina*. The least toxic surfactants were QSB2-12 to both *N. gaditana* and *P. tricornutum*, and DTAC to *T. chuii*, both classified as harmful, being 17-times less toxic than CTAB. Both QSB2-12 and DTAC were not toxic to *A. salina*. Although for the freshwater species *D. magna* cationic surfactants' toxicity decreases with decreasing surfactant hydrophobicity (Garcia et al., 2016), a different pattern was noticed for the tested marine species. It was observed for all tested species that the gemini surfactant with a benzene ring as a spacer (QSB2-12) exhibited the highest IC₅₀ values and therefore the lowest toxicity. Comparing the structure of the gemini surfactants, it can be stated that QSB2-12 is the most hydrophobic due to the presence of an aromatic ring. In terms of monomeric salts, the opposite trend was observed. DTAC presents lower hydrophobicity and toxicity than CTAB and has a shorter alkyl chain in comparison to CTAB (Cave and Fatehi, 2015; Miraglia et al., 2011). The pattern observed for marine species was unexpected. Trying to explain the pattern without additional tests could be inappropriate as the degree of correlation between freshwater and seawater species may be influenced by physiological differences between species, physicochemical differences among media and methodological differences among tests (Wheeler et al., 2002). In order to explain the pattern and to compare the results more studies are recommended, including organisms of different ecological traits and tests with other surfactants and similar chemicals. Moreover, additional tests are needed to allow imaging the effect of the surfactants on a cellular level, that may explain a way of damaging. One example is the cryo-TEM that may provide new insights at the cellular level.

b)

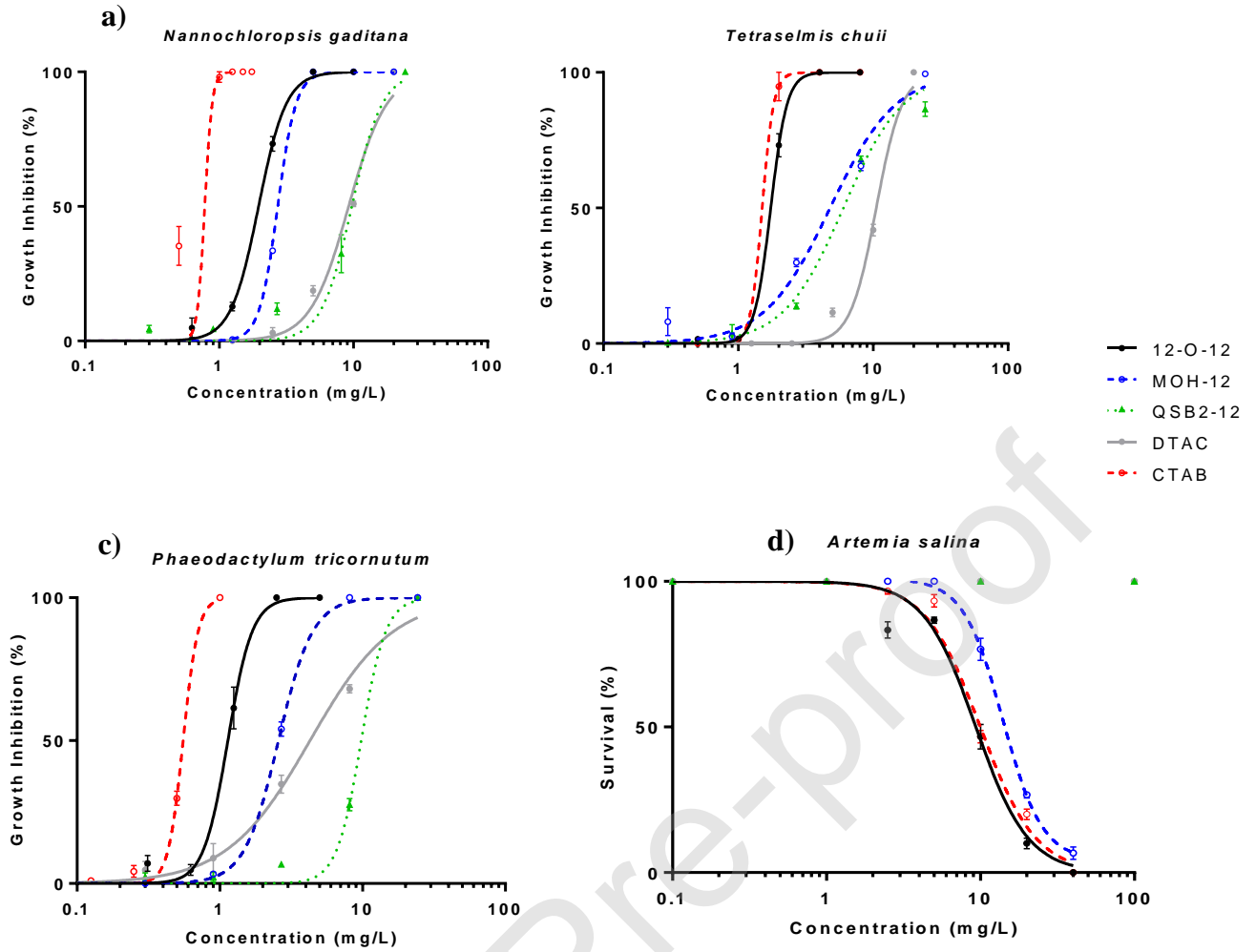


Figure 4 Dose-response curves of the microalgae *Nannochloropsis gaditana* and *Tetraselmis chuii* (a,b), the diatom *Phaeodactylum tricornerutum* (c) and the crustacean *Artemia salina* (d) exposed to tested cationic surfactants. Microalgae and diatom data are expressed as growth inhibition, while for crustaceans the endpoint is survival (%).

Table 5 Median growth inhibition concentration (72 h-IC₅₀) for the microalgae *Nannochloropsis gaditana* and *Tetraselmis chuii* and the diatom *Phaeodactylum tricornerutum* calculated for the tested cationic surfactants.

Marine species	Contaminant	IC ₅₀ [mg/L]	CI 95% [mg/L]
<i>N. gaditana</i>	12-O-12	1.96	1.88-2.05
	MOH-12	2.73	2.54-2.93
	QSB2-12	9.73	8.39-9.85
	DTAC	9.20	8.62-9.83
	CTAB	0.780	0.751-0.809
<i>T. chuii</i>	12-O-12	1.74	1.64-1.85
	MOH-12	4.80	4.02-5.72
	QSB2-12	6.02	5.46-6.63
	DTAC	10.6	10.1-11.0
	CTAB	1.50	1.29-1.75
<i>P. tricornutum</i>	12-O-12	1.14	1.07-1.22
	MOH-12	2.58	2.51-2.65
	QSB2-12	9.79	8.48-11.3
	DTAC	4.29	3.74-4.91
	CTAB	0.554	0.513-0.598

Table 6 Median lethal concentration values (48 h-LC₅₀) for the crustacean *Artemia salina* during an acute exposure to the tested cationic surfactants.

Contaminant	LC ₅₀ [mg/L]	CI 95% [mg/L]
12-O-12	9.34	7.72-11.3
MOH-12	14.0	11.3-17.2
QSB2-12	>100	-
DTAC	>100	-
CTAB	9.98	8.82-11.3

Ecotoxicity data for cationic surfactants in the marine environment is scarce, although cationic surfactants are an important subgroup of the surfactants. As mentioned above, marine ecotoxicity data for cationic surfactants available are mainly from DTDMAC exposure to bacteria, algae, invertebrates and fish. However, different species are used within the same taxonomic group which makes difficult to compare toxicity endpoints. For the other groups of surfactants, comparison of toxicity is easier as the same marine species are used. The anionic surfactant sodium lauryl ether sulphate (SLES) exhibits LC₅₀=3.15 mg/L (*Artemia salina*) (Zillioux et al., 1973) and is classified as a toxic compound, being more than 30-times more toxic than DTAC and QSB2-12 (non-toxic to *Artemia salina*). Another popular detergent and emulsifier, alkylpolyoxyethylene alkyl ether (AES) is categorized as harmful (LC₅₀=11.97 mg/L) (Liwarska-Bizukojc et al., 2005). Another study (Sibila et al., 2008) revealed that AES is very toxic to the marine diatom *P. tricornutum* (EC₅₀=0.50 mg/L), being more toxic than all

cationic surfactants tested in the present study. Polysorbates are well-known representatives of non-ionic surfactants that find application in the food industry (Komaiko and McClements, 2016). Polyoxyethylene sorbitans Tween 80 and 85 have been tested for both marine species and are regarded as non-toxic (ECHA). However, not all non-ionic surfactants are environmentally-friendly. Polyoxyethylene alkyl ethers (AE), which are a mixture of moieties with a different number of methylene groups in alkyl chains used in analytical chemistry (Berthod, 2001), exhibit a $LC_{50}=0.62$ mg/L (*A. salina*) for structures with $n=11-30$ (Liwerska-Bizukojc et al., 2005), three orders of magnitude more toxic than DTAC or QSB2-12. Overall, the presented comparison with other surfactants shows that, whenever their properties allow, cationic surfactants DTAC and QSB2-12 could be considered as more environmental-friendly replacements for some of the anionic and non-ionic surfactants currently in use.

Among the tested cationic surfactants, only CTAB was assessed in terms of ecotoxicity effects in marine organisms (Liang et al., 2013; Masakorala et al., 2011). CTAB inhibits the growth of the green algae *Chlorella vulgaris* by 86% at concentration 0.6 mg/L (Liang et al., 2013), whereas it causes a 50% reduction in photosynthetic response of the macroalga *Ulva lactuca* at 2.4 mg/L (EC_{50}), therefore being classified as toxic. Moreover, this action is attributed to interactions between the algal surface and the amphiphile which ease the perforation of the algae cell: electrostatic interaction of negatively charged surface of the algae and the hydrophilic part of the cationic surfactant, as well as non-specific interactions of the hydrophobic part of CTAB and apolar sites of the algae cells.

It is worth mentioning that, taking into consideration present data from DLS measurements, due to low exposure concentrations, well below CMC values of tested cationic surfactants, no relation between molecular size and ecotoxicity data can be established.

4. Conclusions

The present work provides new data on ecotoxicity and biodegradation of cationic surfactants in seawater. Among the tested cationic surfactants, CTAB turned out to be the most toxic to marine species and QSB2-12 and DTAC the least. QSB2-12 was shown to be 12-times less toxic to *N. gaditana*, 4-times less to *T. chuii*, and 17-times less to *P. tricornutum* than CTAB and non-toxic to *Artemia salina*, while CTAB is classified as a toxic surfactant. The observed pattern differs from that in freshwater. In order to explain the differences, further studies which would include more organisms of different ecological traits, other surfactants and similar chemicals, need to be carried out. Moreover, further studies should also include additional tests such as cryo-TEM, in order to explain the effect of the surfactants on a cellular level.

None of the tested surfactants are biodegradable in ASW, suggesting that their concentration was fixed over the time of ecotoxicity testing. Moreover, DLS measurements revealed the heterogeneous nature of the tested samples. No relationship between the hydrodynamic size of the samples and surfactant concentration and structure, as well as ecotoxicity data, was found due to the low concentrations tested, well below CMC values. By comparing size distribution in distilled water and ASW, at concentrations higher than CMC, it was confirmed that ASW did not disturb micelles formation, in spite of slightly bigger micelles of spherical shape being formed in ASW. Anyway, some caution has to be taken considering that in natural environment bacteria may have a role on chemical degradation. Therefore, organic chemical concentration may not be stable in time, decreasing the expected effect.

Overall, the obtained results can be used as data for selection of chemicals in safe-by-design approaches, particularly relevant in early stages of materials development.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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6. Supplementary data

Supplementary data associated with this article can be found in an additional file.

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