Toxicity of Synthetic Surfactants to the Marine Macroalga, *Ulva lactuca*

Kanaji Masakorala · Andrew Turner · Murray T. Brown

Abstract The toxicities of three synthetic surfactants to the marine macroalga, *Ulva lactuca*, have been examined by monitoring chlorophyll *a* fluorescence quenching. The anionic surfactant, sodium dodecyl sulphate (SDS), exerted no measurable toxicity over the concentration range 0–10 mg L^{-1}, while presence of the non-ionic surfactant, Triton X-100 (TX), elicited a small reduction in photochemical efficiency that was independent of concentration. The cationic surfactant, hexadecyltrimethylammonium bromide (HDTMA), incurred a dose-dependent response to ~3 mg L^{-1} (EC$_{50}$=2.4 mg L^{-1}), but a reduction in toxicity thereafter. Presence of TX had little effect on the toxicity of HDTMA but an equimolar concentration of SDS directly offset the impact of HDTMA on photochemical efficiency. Relative toxicities of the surfactants are attributed to differences in affinity for the algal surface and tendencies to disrupt cell membranes and interact with intracellular macromolecules. Non-linear dose responses and antagonistic effects are attributed to non-specific interactions between molecules of the same surfactant and electrostatic interactions between molecules of different amphiphilic character.

Keywords Synthetic surfactants · Macroalgae · Toxicity · Chlorophyll fluorescence

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1 Introduction

Synthetic surfactants have a wide variety of domestic, industrial, agricultural and remedial applications and are, therefore, important contaminants of many aquatic environments. Consequently, much attention has been directed towards their environmental distributions and behaviour and their toxic effects on a variety of fresh water and marine biota (Utsunomia et al. 1997; Venhuis and Mehrvar 2004; Jonkers et al. 2005; Mungraya and Kumar 2008; Turner and Xu 2008; García-Luque et al. 2009). The fate and persistence of surfactants is largely dictated by their rate of degradation, principally through microbial activity, and tendency to sorb to natural particles. With respect to the latter, and because of the net negative charge of most geosolids and biosolids in the aquatic environment, interactions are greater for cationic and non-ionic surfactants than for anionic

amphiphiles (Atia and Radwan 1997; Jones-Hughes and Turner 2005). The precise responses of organisms to surfactants are dependent on biotic factors (e.g. species, life stage, nutritional status), environmental conditions (e.g. temperature, dissolved oxygen concentration, presence of other contaminants and sorbents) and the physico-chemical properties of the surfactant (e.g. solubility, charge of the hydrophile, alkyl chain length of the hydrophobe) (Pantani et al. 1995; Verge and Moreno 1996; Oros et al. 1997; Jørgensen and Christoffersen 2000; Pavlić et al. 2005). In general, however, surfactant toxicity appears to be determined by the affinity of the amphiphile for the external surface and its consequent tendency to penetrate the cell membrane (Rosen et al. 2001), and cationic surfactants are more toxic than anionic ones of similar molecular weight or equivalent hydrophobe (Lewis 1990; Dirilgen and Ince 1995; Sandbacka et al. 2000; García et al. 2001).

In coastal marine environments, macroalgae are potentially exposed to relatively high concentrations of synthetic surfactants discharged in waste effluents or employed as oil-spill dispersants or sorbents (Banerjee et al. 2006; Zoller 2006; Lara-Martín et al. 2008). Compared with other organisms, however, the toxicity of surfactants to marine macroalgae is poorly defined (Singer et al. 1995; Han et al. 2007). In this study, we examine the effects of three chemically contrasting, commercially important surfactants on the green, benthic macroalga, Ulva lactuca. The surfactants we employ, characterised in Table 1, are hexadecyltrimethylammonium bromide (HDTMA), a cationic quaternary ammonium compound used as a disinfectant, fabric softener, germicide and hair conditioner, Triton X-100 (TX), a non-ionic octylphenol polyethoxylate used as a wetting agent, emulsifying agent and polymer stabiliser, and sodium dodecyl sulphate (SDS), an anionic amphiphile used in laundry, shampoos and pharmaceuticals (Lewis 1990; Jones-Hughes and Turner 2005). The genus Ulva has a cosmopolitan distribution in shallow coastal areas and U. lactuca is a common member of seaweed communities in the littoral and sublittoral shores of NW Europe. Because of their ability to withstand moderately polluted conditions and accumulate many contaminants, Ulva species are considered to be useful biomonitors and important test organisms in ecotoxicological studies (Haritonidis and Malea 1999, Lee and Wang 2001; Han et al. 2009; Masakorala et al. 2008; Turner et al. 2009). We used chlorophyll a fluorescence quenching as a non-invasive measure of toxicity in our experiments because of its ease of measurement and sensitivity with respect to algal–surfactant interactions (Pérez et al. 2009; van Wijk et al. 2009).

2 Materials and Methods

2.1 Sampling and Reagent Preparation

Before being used in the experiments, all plasticware and glassware were soaked in 0.5 M HCl for at least 24 h and rinsed twice with distilled, deionised Milli-Q water (MQW). Filtered (<5 μm) sea water ($S=33.5$; pH=8.1; dissolved organic carbon=250 μm) was

Table 1 Physico-chemical properties of the surfactants studied (Masakorala et al. 2008 and references therein)

Surfactant	Molecular formula	Monomer mass (g mol^{-1})	Solubility[a] (M)	CMC[b] (mM)	K_{ow}[c]	$t_{1/2}$[d] (h)
Sodium dodecyl sulphate (SDS)	$CH_3(CH_2)_{11}OSO_3Na$	288.4	0.35	12	50	<50[e]
Hexadecyltrimethylammonium bromide (HDTMA)	$CH_3(CH_2)_{15}N(CH_3)_3Br$	363.9	0.10	0.93	1,500	40
Triton X-100 (TX)	$C_8H_{17}-C_6H_4-(OCH_2-CH_2)_{9.5}OH$	~625	Miscible[f]	0.26	72,000	150

[a] Aqueous solubility at 25°C

[b] Critical micelle concentration in pure water at 25°C

[c] Calculated octanol-water partition coefficient

[d] Indicative degradation half-life in natural waters at 10–20°C

[e] SDS typically undergoes a period of acclimation before degradation

[f] Miscible in all proportions

available on tap in the laboratory after having been collected in bulk from Plymouth Sound, SW England, at high water. Chemical reagents were purchased from Sigma, Fisher or Merck/VWR and were of analytical grade or equivalent. Individual stock solutions of the surfactants (1,000 mg L^{-1}) were prepared in MQW in 100 mL plastic volumetric flasks and stored in the dark at 4°C (SDS and TX) or at room temperature (HDTMA) until use (within 48 h).

Samples of *U. lactuca* were collected from tidal rock pools at Wembury, a protected beach 7 km to the SE of Plymouth, England. Samples were transported in zip-locked polyethylene bags to the laboratory where all epiphytic flora and fauna and adherent material were carefully removed. Clean, healthy samples were placed in aerated 10 L plastic tanks containing sea water and acclimatised for about 3 days at 15°C under 250 µmol m^{-2} s^{-1} photosynthetic active radiation generated by 'daylight' fluorescence lamps on a 12 h light/dark cycle. Discs of 14 mm in diameter were then cut from the central area of thalli with the sharpened end of a polyethylene cylinder and maintained for a further 24 h prior to commencement of the exposure experiments.

2.2 Experimental and Analysis

Experiments were carried out in loosely covered 150 mL polyethylene terephthalate beakers under the conditions described above and under continuous, orbital agitation at about 100 rpm. Each of six replicates per treatment was conducted in 50 mL of sea water containing two algal discs to which surfactants (either individually or in combination) were added to attain a concentration ranging from 0 to 10 mg L^{-1}. Experiments proceeded for 24 h (in order to minimise amphiphile degradation; Table 1) before discs were carefully removed using plastic tweezers and excess sea water gently shaken off. The chlorophyll-*a* fluorescence of algal discs was determined at the termination of the experiments using a Hansatech Instruments pulse-modulated fluorescence monitoring system which had been calibrated using the accompanying Modfluor *v*.2.02 software. Discs were initially dark-adapted for 15 min in order to allow relaxation of non-photochemical quenching before readings of F_m, the maximum fluorescence yield, and F_o, the initial fluorescence yield, were recorded. The maximum quantum efficiency of

photosystem II (PS II) in the dark-adapted state is expressed as the ratio of variable to maximal chlorophyll fluorescence, $F_v/F_m = (F_m - F_o)/F_m$. In selected treatments, the recovery of *U. lactuca* was examined by measuring photosynthetic response after a subsequent 24-h incubation period in clean sea water.

In order to evaluate the extent of cell membrane damage incurred by the surfactants, leakage of dissolved free amino acids (DFAA) was determined fluorometrically, according to the method outlined by Parsons et al. (1984). Thus, 3 mL of borate-buffered solution, containing o-phthaldialdehyde and 2-mercaptoethanol, were added to thawed 3 mL sea water aliquots that had been sampled at the end of the treatments and stored at −20°C in 20 mL screw-capped polyethylene test tubes. The contents were allowed to stand for 2 min before fluorescence was measured in a 1 cm cell using a Perkin Elmer LS50B spectrophotometer at an optimal excitation wavelength of 342 nm and an optimal emission wavelength of 452 nm. The standard curve was obtained using glycine solutions up to a concentration of 1.0 µM and the results were calculated as glycine equivalents with a detection limit of 4 nM.

2.3 Statistical Methods

Statistical analysis was performed using MINITAB *v*.14. Chlorophyll *a* fluorescence data obtained from the exposure experiments were analysed using the general linear model two-way analysis of variance (ANOVA) and DFAA data by one-way ANOVA. Significant differences between individual mean values ($p < 0.05$) were ascertained by Tukey's post hoc test.

3 Results

3.1 Influence of Individual Surfactants on PS II

The effects of different concentrations of the individual surfactants on the ratio F_v/F_m, the potential photochemical efficiency of PS II, of *U. lactuca* suspended in natural sea water are shown in Fig. 1. The extent and nature of the response varied among the surfactants studied. Thus, relative to the surfactant-free controls, no measurable effect was elicited by the anionic surfactant, SDS, over the

Fig. 1 Efficiency of photochemical energy conversion (F_v/F_m) ▶ of *U. lactuca* after 24 h as a function of added concentration of **a** HDTMA, **b** SDS and **c** TX. *Error bars* represent 1 SD about the mean of six measurements

concentration range studied. With respect to the non-ionic surfactant, TX, a small but significant reduction in the efficiency of photochemical energy conversion relative to the controls was observed at a concentration of 0.5 mg L^{-1} (~0.8 μM), but further increases in surfactant concentration were not accompanied by further reductions in F_v/F_m.

The presence of the cationic surfactant, HDTMA, caused a significant reduction in photochemical response relative to the surfactant-free controls throughout the concentration range studied. However, a linear, dose-dependent response culminated in a minimum in F_v/F_m at about 3 mg L^{-1} (~8 μM), an effect that was replicated precisely when the experiment was repeated. Linear regression analysis of the first six data points resulted in 24 h EC_{50} and EC_{10} values (based on 50% and 10% reductions in F_v/F_m) of 2.4 and 0.45 mg L^{-1}, respectively.

Release of intracellular dissolved free amino acids (DFAA), indicative of cell membrane damage, was also detected in the presence of HDTMA (Fig. 2) but not SDS or TX. Moreover, the concentration of DFAA released by the effects of the cationic surfactant correlated with the reciprocal of F_v/F_m throughout the concentration range of HDTMA employed in the
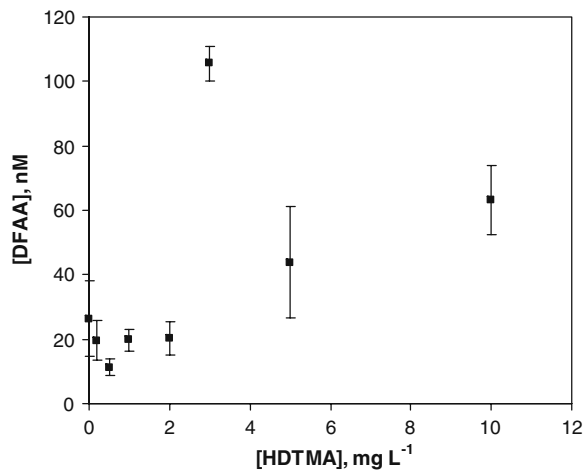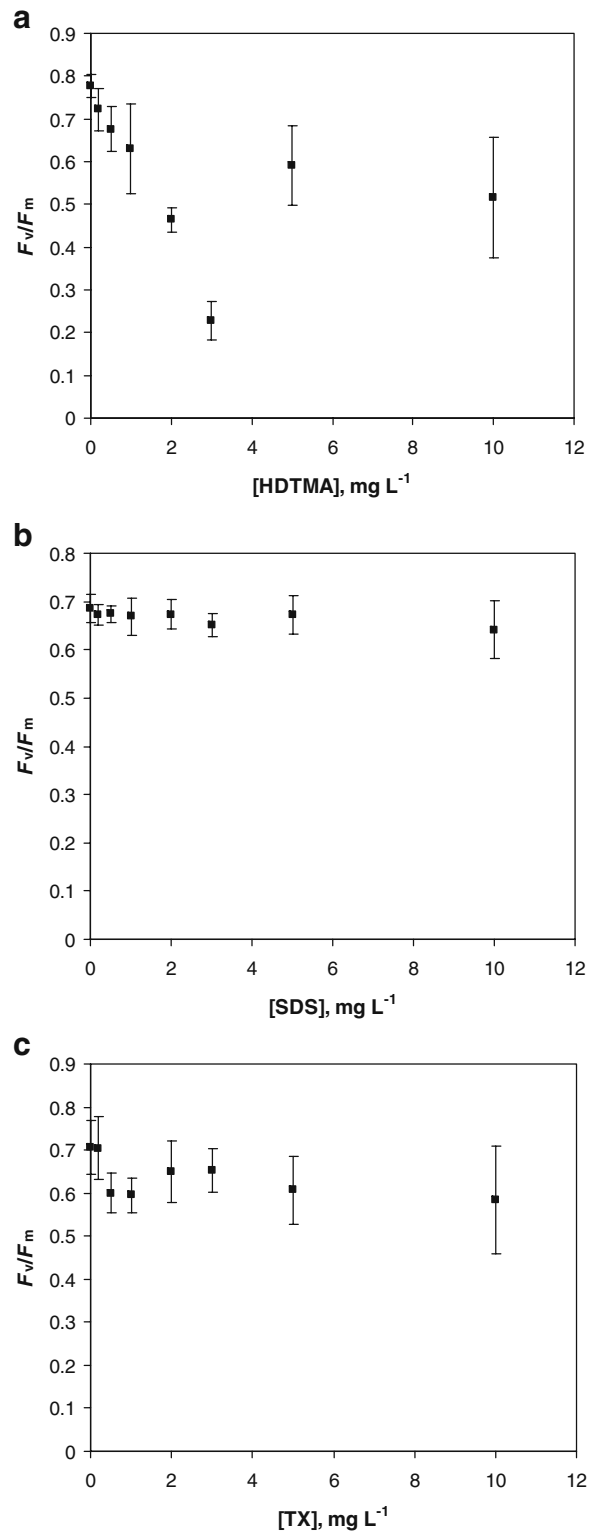

Fig. 2 Concentration of dissolved free amino acids (DFAA) released by *U. lactuca* after 24-h incubation as a function of added concentration of HDTMA. *Error bars* represent 1 SD about the mean of six measurements

experiment ($r=0.92$; $p<0.01$). Further experiments revealed that the suppression of photochemical efficiency was greatest between HDTMA concentrations of about 2–3 mg L^{-1} (Fig. 3), and that recovery of *U. lactuca* was not attained by immersion of the alga in clean sea water for 24 h.

The effects of different quantities of SDS and TX on the toxicity of a fixed concentration of HDTMA (2.4 mg L^{-1}, or its 24 h EC_{50}) are shown in Fig. 4. While the presence of TX did not appear to affect the toxicity of the cationic surfactant, increasing concentrations of SDS reduced the effect such that it was directly offset (and F_v/F_m was similar to that in the surfactant-free controls) at about 2–3 mg L^{-1}.

4 Discussion

4.1 Relative Toxicities of the Surfactants

Over the concentration range studied, and according to fluorescence quenching, the order of surfactant toxicity to *U. lactuca* is: HDTMA >>TX>SDS. This observation can, largely, be attributed to the extent of interaction between the algal surface and the amphiphile and the ease of penetration of the latter through
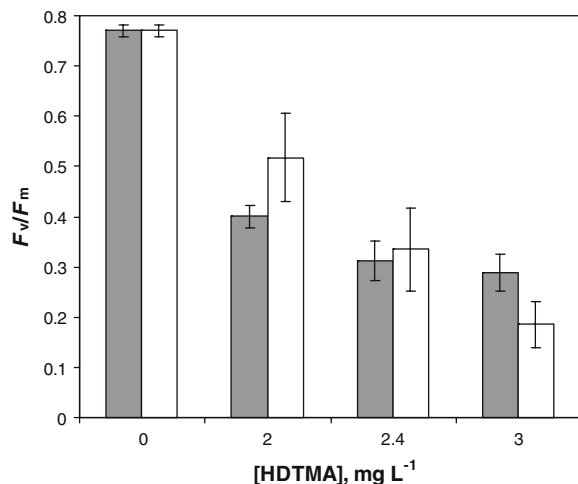
Fig. 4 Efficiency of photochemical energy conversion (F_v/F_m) of *U. lactuca* after 24-h incubation in the presence of 2.4 mg L^{-1} of HDTMA and variable concentrations of SDS (*filled squares*) or TX (*empty triangles*). *Error bars* represent 1 SD about the mean of six measurements

the cell membrane (Rosen et al. 2001). Although the sorption of the surfactants was not determined, it most likely involves non-specific (e.g. hydrophobic) interactions between the hydrophobe and apolar sites of the algal cell wall, and electrostatic attraction between the hydrophile and polar moieties of surface functional groups (van Wijk et al. 2009). Thus, the ionic head of SDS is repelled by the net negative charge of the algal surface and sorption is limited to non-specific interactions between the alkyl tail of the surfactant and neutral regions of the algal surface. Non-specific interactions between the alga and TX are stronger than those involving SDS because of the greater hydrophobicity of the non-ionic molecule (Table 1). In contrast, binding of HDTMA involves both ion exchange and non-specific interactions and is, therefore, more heterogeneous but highly favourable (Jones-Hughes and Turner 2005; van Wijk et al. 2009).

The subsequent molecular mechanisms of surfactant toxicity are not well understood. Moreover, a single surfactant may induce multiple effects on an individual organism or, at a given concentration, may exhibit very different toxicities to different organisms (Venhuis and Mehrvar 2004; Li 2008). With regard to algae, it appears that amphiphiles interfere with the phospholipid bilayer and bind with or denature membrane proteins, thereby altering the organisation, stability and permeability of the membrane (Cserháti et al. 2002). Once inside the cell, surfactants may affect

Fig. 3 Efficiency of photochemical energy conversion (F_v/F_m) of *U. lactuca* as a function of added concentration of HDTMA. *Grey bars* denote samples that were incubated for 24 h in the presence of HDTMA and *white bars* samples that were subsequently incubated for 24 h in clean sea water. *Error bars* represent one standard deviation about the mean of six measurements
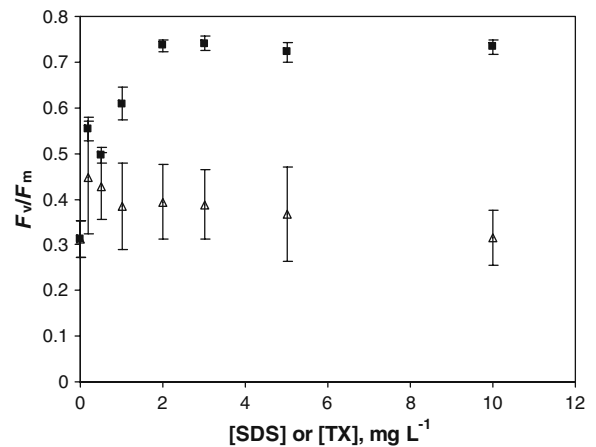
thylakoid organisation and chlorophyll synthesis, and are able to interact electrostatically or hydrophobically with enzymes and other bioactive molecules, resulting in their malfunction or a reduction in their activity (Walker and Evans 1978; Lürling 2006; Pérez et al. 2009).

Lack of measurable toxicity displayed by SDS over the concentration range studied implies limited interactions with cell membranes and/or intracellular, bioactive macromolecules of *U. lactuca*. SDS is not particularly toxic towards algae and invertebrates at environmentally realistic concentrations (Tozumcalgan and Atayguneyman 1994; Guilhermino et al. 2000; Sandbacka et al. 2000). Moreover in some laboratory studies, this and other anionic amphiphiles have been shown to promote algal growth by serving as a direct source of carbon or, through moderate permeation effects, allowing a greater influx of nutrients or an efflux of carbon-rich, nutritionally viable lysis products (Lewis 1990; Dirilgen and Ince 1995). Evidence suggests that growth stimulation also occurs in the environment since the presence of a variety of synthetic anionic surfactants in coastal sea water has been accompanied by an increase in phytoplankton productivity (Tkalin et al. 1993). Measurable, but non-dose-dependent toxicity of TX is, qualitatively, consistent with its greater hydrophobicity and consequent propensity to interact with biotic surfaces and intracellular molecules (Oros et al. 1997). The toxicity of HDTMA to *U. lactuca* and other organisms (e.g. Walker and Evans 1978; Sandbacka et al. 2000) is greater than its hydrophobicity (e.g. octanol–water partition coefficient; Table 1) would imply as a consequence of its ability to interact electrostatically with biological surfaces and macromolecules, and its propensity to denature cellular proteins (Lewis 1990).

4.2 Effects of Amphiphilic Interactions on Toxicity

The minimum in F_v/F_m resulting from exposure to increasing concentrations of HDTMA cannot be attributed to interactions of the surfactant with the algal surface or an ambient solute since any non-linear effects (e.g. relating to the availability or saturation of binding sites) would result in an asymptotic dose–response. Most likely, the effect results from physico-chemical interactions between HDTMA molecules above a specific concentration. The critical micelle concentration (CMC; the concen-

tration above which micelles are formed) of HDTMA in pure water is considerably greater than the concentrations employed in the present experiments. However, CMCs of surfactants are reduced in saline water because ions partly neutralise the electrostatic antagonism between charged head groups (Kalmanzon et al. 1992; Xu and Boyd 1995). We are not aware of measurements of the CMC of HDTMA in sea water but surmise that it occurs at a concentration coincident with the minimum in F_v/F_m observed in our experiments. Thus, below this value toxicity towards *U. lactuca* is proportional to the concentration of molecularly disperse HDTMA, while above it the availability of molecular HDTMA, hence its toxicity, is dramatically reduced.

Because of its affinity for negatively charged binding sites, HDTMA also reacts with non-biological surfaces and macromolecules in the environment. In sea water, reactions with suspended sediment reduce its aqueous concentration (Jones-Hughes and Turner 2005) while electrostatic and hydrophobic interactions with polar and apolar moieties of amphiphilic, heterogeneous polyelectrolytes (e.g. humic substances) are predicted to reduce its chemical reactivity and availability to marine biota (Otto et al. 2003). The concentration of dissolved organic carbon in the coastal sea water used in our experiments was about 250 μM, or at least an order of magnitude greater than the molar concentration of added HDTMA, suggesting that a high proportion of the synthetic amphiphile is bound in the aqueous phase as, for example, stable ion pairs (Otto et al. 2003). This requires that molecular HDTMA is intrinsically more toxic to *U. lactuca* than our observations imply, or that available binding sites on the algal surface are stronger or more abundant than those on natural polyelectrolytes.

The anionic surfactant, SDS, interacts only weakly with heterogeneous polyelectrolytes like humic acids (Yee et al. 2006), but its presence reduces the toxicity of HDTMA to *U. lactuca*. Moreover, at roughly equimolar concentrations, the toxicity of HDTMA is directly offset. It appears, therefore, that hydrophobic and electrostatic interactions between these synthetic surfactants are stronger (or at least more favourable) than those between either of the surfactants and the binding sites on ambient macromolecules or on the surface of the seaweed. In contrast, presence of the non-ionic surfactant, TX, does not significantly affect

the toxicity of HDTMA, suggesting limited interaction between the surfactants in the aqueous phase and little competition for apolar sorption sites on the algal surface, or at least those that elicit a toxic response.

4.3 Environmental Implications

Total surfactant concentrations in surface waters heavily impacted by industrial or municipal effluents may be as high as 10 mg L^{-1} (Guilhermino et al. 2000). In contaminated coastal environments, however, aqueous concentrations of individual surfactants or surfactant homologues below a few tens of $\mu g\ L^{-1}$ are more typical. For instance, in sea water in the vicinity of waste water treatment plants, concentrations of (anionic) linear alkylbenzenesulphonates (LAS) of up to about 25 $\mu g\ L^{-1}$ are reported (González et al. 2004; Zoller 2006). Higher concentrations of LAS have been measured in estuaries, but concentrations of (non-ionic) nonylphenol ethoxylates and alcohol ethoxylates are generally below 10 $\mu g\ L^{-1}$ (Jonkers et al. 2005; Lara-Martín et al. 2008). As far as we are aware, concentrations of cationic surfactants in sea water have not been determined. However, a recent study indicates levels of quaternary ammonium compounds in estuarine sediment of up to 74 $\mu g\ g^{-1}$ (Li and Brownawell 2009). Based on an equilibrium sediment–sea water partitioning of HDTMA of between about 10^4 and 10^5 mL g^{-1} (Jones-Hughes and Turner 2005), an upper estimate of the aqueous concentration of such compounds is a few $\mu g\ L^{-1}$.

Clearly, of the surfactants considered in the present study and at environmentally realistic concentrations, HDTMA has the greatest potential to exert an impact on marine macroalgae. Its precise effects will depend on its absolute concentration, the nature of the organism exposed (e.g. thickness and chemical composition of the cell wall) and the presence of other amphiphiles and contaminants. With regard to the latter, the toxicity of HDTMA is predicted to be reduced in the presence of co-disposed anionic surfactants of similar persistence, and by natural polyelectrolytes, such as humics and organic exudates. Electrostatic interactions between HDTMA and the algal cell wall may confer a more hydrophobic surface, attracting co-existent non-polar xenobiotics, while damage to the cell membrane may accelerate the internalisation or efflux of a range of contaminants (Masakorala et al. 2008).

5 Conclusions

Of the three contrasting synthetic surfactants studied, the cationic, quaternary ammonium compound, HDTMA, elicited the greatest reduction in photosynthetic response by *U. lactuca*. The response was dose-dependent to about 3 mg L^{-1}, with an EC_{50} of 2.4 mg L^{-1}, but a reduction in toxicity was observed at higher concentrations. The anionic surfactant, SDS, was not measurably toxic in the current experiments, but its presence reduced the toxicity of HDTMA, possibly because of electrostatic and hydrophobic interactions between aqueous molecules of the different amphiphiles. At environmentally realistic concentrations, persistent, cationic surfactants are predicted to incur the greatest impacts on coastal primary producers.

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