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Sorption–desorption kinetics and toxic cell concentration in marine phytoplankton microalgae exposed to Linear Alkylbenzene Sulfonate

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ABSTRACT

Linear Alkylbenzene Sulfonates (LAS) are ubiquitous surfactants. Traces can be found in coastal environments. Sorption and toxicity of C_{12}-LAS congeners were studied in controlled conditions (2–3500 μg C_{12}LAS/L) in five marine phytoplanktonic species, using standardized methods. IC_{50} values ranged from 0.5 to 2 mg LAS/L. Sorption of $^{14}C_{12}$-6 LAS isomer was measured at environmentally relevant trace levels (4 μg/L) using liquid scintillation counting. Steady-state sorption on algae was reached within 5 h in the order dinoflagellate > diatoms > green algae. The sorption data, fitted a L-type Freundlich isotherm, indicating saturation. Desorption was rapid but a low LAS fraction was still sorbed after 24 h. Toxic cell concentration was 0.38 ± 0.09 mg/g for the studied species. LAS toxicity results from sorption on biological membranes leading to non-specific disturbance of algal growth. Results indicate that LAS concentrations in coastal environments do not represent a risk for these organisms.

1. Introduction

Linear Alkylbenzene Sulfonates (LAS) are anionic surfactants commonly used in household and industrial detergents with an annual global production of 4×10^6 tons (Zhu et al., 1998). They have replaced soap in detergents since the 1960s (Augier, 1992), with a per capita consumption of 1–2 kg/year in Western Europe and United States, and up to 3.5 kg in Japan (González-Mazo and Forja, 1998; Mackay et al., 1996). After use, most of LAS is disposed off with wastewater and is efficiently eliminated from the aqueous phase by biological treatment (typically between 95% and 99%) through biodegradation and adsorption on sludge (Mungray and Kumar, 2008). The remaining LAS in the treated effluents is further removed in receiving waters via biodegradation, sorption on suspended particles and deposition, limiting geographic dispersion to a few kilometers around urban effluent discharges (González-Mazo and Forja, 1998; Takada and Ogura, 1992). However, constant untreated effluent discharge along urbanized coasts can lead to chronic contamination of marine waters, sediments and organisms at the μg/L and μg/g levels (Lara-Martín et al., 2005; León et al., 2000; Sáez et al., 2000).

Marine unicellular algae are part of the suspended particulate organic matter found in the water column. They represent a key food source for the growth stages of bivalve molluscs, larval stages of some crustacean species and very early growth stages of some fish species (IPCS, 1996; Walsh, 1988). The hydrophobic nature of algal cell walls makes microalgae good candidates for LAS sorption through van der Waals interactions due to their alkyl chain (Sáez et al., 2001; Wang et al., 1997). Sorbed LAS can exert toxicity on cells through the denaturation and the binding of proteins in the cell wall and consequently the alteration of membrane permeability to nutrients and chemicals (Blasco et al., 1997; Hampel et al., 2001). Although LAS have a non-specific mode of action, the sensitivity of different algal species, as measured by toxicity tests in accordance with OECD guidelines (OECD, 1984, 2002), is highly variable. The measured toxicity values vary by three orders of magnitude depending on the homologues and isomers (Lewis, 1990).

In this study, five algal species *Tetraselmis levis* (green alga), *Dunaliella tertiolecta* (green alga), *Thalassiosira pseudonana* (diatom), *Skeletonema costatum* (diatom), and *Prorocentrum minimum* (dinoflagellate) present in the N-W Mediterranean coastal waters were chosen to assess LAS sorption and desorption kinetics and LAS toxicity. The aim of the study is to contribute to evaluate the potential risk of LAS to coastal phytoplankton.

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2. Materials and methods

2.1. Chemicals and organisms

A mixture of C_{12}-LAS isomers (five different congeners from C_{12}-2-LAS to C_{12}-6-LAS) was used in the toxicity experiments whereas a particular radiolabelled isomer: $^{14}C_{12}$-6-LAS was used in kinetics studies. The latter had a specific activity of 12.9 mCi/mmol. Stable and radiolabelled C_{12}-LAS were obtained from the Procter & Gamble Company. All other chemicals were HPLC grade purchased from Merck.

Five marine phytoplanktonic species were selected: the green algae *T. levis* (diameter: 5–7 μm) and *D. tertiolecta* (diameter: 5–7 μm), the diatoms *T. pseudonana* (diameter: 3.5–5 μm) and *S. costatum* (diameter: 4–5.5 μm) and the dinoflagellate *P. minimum* (diameter: 10–13 μm). Test algal species were selected from IAEA-MEL axenic culture collections, using standard methods from the APHA, AWWA and WPCF (Blasco et al., 2003), according to their ability to grow under similar conditions (Fisher et al., 1984; Lu et al., 2005). Their different cell wall structure (carbohydrate or silica) and shape but also the total surface area were taken into account to tentatively explain the differences in affinity of the algae to C_{12}-LAS. Algal cell dry weight was determined by filtering and drying algae from aliquots of culture of known concentration on glass-fiber filters (Zhu and Lee, 1997). Algal cell diameter was estimated by microscopic observation using a stage micrometer (S12-stage micrometer, Pyser-SGI with 2 μm divisions). Algal surface and volume were estimated from measured cell diameter, assuming a spherical shape.

2.2. Toxicity tests

C_{12}-LAS toxicity was assessed by measuring the growth inhibition of the five algal species using the methodology described in the OECD updated guideline 201 (2002) and according to Moreno-Garrido et al. (2000). Briefly, experimental double-glass bottles were filled with 150 ml of 0.2 μm-filtered seawater, enriched with F/2 medium lacking EDTA (Guillard and Ryther, 1962), inoculated with a specific cell density in order to reach an initial algal concentration close to 10^5 cell/ml and placed in a culture chamber at 20 ± 0.2 °C with a 12 h/12 h light cycle. Salinity was 38 p.s.u. and pH showed low variation (8.7 ± 0.4). Cells were exposed in triplicate to 0, 0.2, 0.4, 0.8, 1.5 and 3.5 mg/L for 72 h. Every 24 h, cell density was measured on a Fuchs Rosenthal haemocytometer (Paul Marienfeld GmbH & Co., Lauda-Königshofen, Germany).

Percentage of growth inhibition (f_x, %) was fitted to a sigmoid curve (Eq. (1)) as a function of the LAS concentration in water (C_w, mg/L). The model was fitted to the raw data using the non-linear estimation module of Statistica® 6.1 (StatSoft Inc., 1998) with the quasi-Newton method for calculating least squares. Parameters a, b and growth inhibition (IC_{50}, mg/L) were determined by iterative adjustments:

$$f_x = \frac{a}{1 + e^{\frac{-(C_w - IC_{50})}{b}}} \quad (1)$$

2.3. Sorption and desorption kinetics

Algal cells were filtered and suspended in 1 L double-glass bottles in order to reach similar initial density as previously described. The bottles were filled with 0.2 μm-filtered seawater enriched with F/2 medium lacking EDTA and spiked with 10 Bq/ml of $^{14}C_{12}$-6-LAS (equivalent to 4 μg/L). During the exposure period, cells were sampled in triplicate (3 × 50 ml) and filtered on 1 μm-polycarbonate filters (Osmonics), after 0, 1, 2, 3, 4, 6, 8 and 24 h. The ^{14}C activity of the samples was analysed using liquid scintillation counting. Briefly, the filters were placed in 20-ml glass vials, with 10 ml of scintillation cocktail (UltimaGold XR, Perkin Elmer) and counted using a liquid scintillation counting (LSC) analyzer (Tri-Carb 2900 TR, Perkin Elmer). LAS degradation was not expected during the 24 h period of experiment performed with filtered seawater (Terzic et al., 1992) and 100% of the measured ^{14}C activity was assumed to represent the parent compound, C_{12}-6-LAS. Basic parameters (temperature, salinity, pH, cell density) and ^{14}C radioactivity in aqueous phase were controlled at each sampling time. No variation in $^{14}C_{12}$-6-LAS concentration was observed in the water and sorption to the bottle wall was negligible. It is therefore assumed that sorption on phytoplankton cells was not limited by LAS concentrations.

A first-order one-compartment kinetic sorption model was used to describe the LAS concentration on cells, C_t (mg LAS/kg algae dry weight) over time t (h) (Eq. (2)), as a function of the exposure water concentration (Hamelink et al., 1971; Neely et al., 1974). The sorption coefficient at steady-state (K_{ss}, L/kg) and the desorption rate constant (k, h^{-1}) were calculated by iterative adjustments using Statistica® as previously described:

$$C_t = K_{ss}(1 - e^{-kt}) \times C_w \quad (2)$$

At the end of the exposure period, the cells were filtered and resuspended in 0.2 μm-filtered seawater enriched with F/2 medium lacking EDTA. The same protocol was used to measure desorption kinetics after 0, 1, 2, 3, 4, 6 and 24 h.

C_{12}-LAS desorption was expressed in terms of percentage of remaining activity, *i.e.* sorbed LAS concentration at time t divided by initial sorbed LAS concentration measured on the cells at the beginning of the decontamination period. The percentage of remaining activity was plotted against time. The kinetics were described by a two-component exponential model (Eq. (3)), where A_t and A_0 are the remaining activities (%) at time t (h) and 0, respectively, and k is the biological desorption rate constant (h^{-1}) that allows the calculation of the biological half-life ($T_{b1/2} = \ln(2)/k$). The 's' subscript refers to a short-lived component (s component) while the 'l' subscript refers to a long-lived component (l component) (Whicker and Schultz, 1982). The short lived-component is a model for the loss of the proportion of LAS pool that is weakly sorbed on cells, while the long-lived component is a model of the loss of the fraction of the LAS pool that is tightly bound on the cells:

$$A_t = A_{0s}e^{-k_s t} + A_{0l}e^{-k_l t} \quad (3)$$

2.4. Sorption isotherms

Experimental 250 mL double-glass bottles were used for cell incubation. The bottles were filled with 0.2 μm-filtered seawater, enriched with F/2 medium and inoculated with algae in order to reach similar initial algal density as previously described. After an adaptation period of 24 h, cells were counted on Fuchs Rosenthal haemocytometer and seawater was spiked with $^{14}C_{12}$-6-LAS (5 Bq/ml) and completed with non-radiolabelled C_{12}-LAS in order to reach 2.1, 2.7, 3.7, 7.5 or 13.5 μg/L. For three species (the green algae and *T. pseudonana*), the experiment was conducted with two additional exposure concentrations: 100 and 1000 μg C_{12}-LAS/L. Each exposure concentration was tested in triplicate during 10 h in order to reach steady-state adsorption on cells for all algal species. LAS concentration sorbed on cells was determined as previously described.Data was fitted to a linear Freundlich isotherm (Rico-Rico et al., 2009b; Sáez et al., 2001) using the following equation:

$$\log C_a = \log K + n \log C_w \quad (4)$$

where C_a (mg/kg) and C_w (mg/L) are the surfactant concentrations sorbed on the cells and in the aqueous phase at equilibrium, respectively, K_f (mg/kg)(L/mg)^n is the Freundlich constant, and n provides information about the linearity of the isotherm.

2.5. Toxic cell concentration (TCC)

Toxic cell concentration (TCC) was calculated as the sorbed LAS concentration associated with growth inhibition, as suggested by Mayer et al. (1998). The K_{ss} values used to calculate the TCC values (Eq. (5)) were estimated from the sorption isotherm data (Fig. 4). The TCC_{50} value was obtained similarly to IC_{50} using the sigmoid curve described in Eq. (1):

$$TCC = K_{ss} \times IC \quad (5)$$

3. Results and discussion

3.1. Growth inhibition of algae exposed to LAS

Algal species exposed to dissolved LAS for 72 h presented a growth inhibition pattern following classical dose–response curves (Fig. 1). Growth inhibition was observed for all the tested exposure concentrations; from 0.2 to 3.5 mg C_{12}-LAS/L. Data were fitted to sigmoid curves and the IC_{50} (72 h) values are presented in Table 1. The values ranged from 0.55 to 1.99 mg/L, which is in the range of IC_{50} values obtained with marine algae exposed to a commercial LAS mixture in similar experimental conditions (*viz.* 0.24–1.9 mg/L) (Debelius et al., 2008). This confirms that the tested C_{12}-LAS congeners are good model compounds to approximate the fate and effects of LAS mixtures traditionally used in household products; *i.e.* $C_{11.6}$-LAS (Van de Plassche et al., 1999), and can be used in toxicity tests for experimental simplification and standardisation.

In the literature, LAS toxic concentration has been reported for different freshwater and marine microalgae to vary by three orders of magnitude, from 0.1 to 100 mg/L (Lewis, 1991; Pavlic et al., 2005). The wide range of toxic concentrations may be due to the use of non-standardized LAS mixtures with varying alkyl chain length, and/or phenyl isomers distribution (Blasco et al., 2003) in toxicity tests. Indeed, some authors reported homologue-specific 72 h IC_{50} values in the range 1.4–13 and 0.18–1.2 mg/L for C_{11} and C_{13} homologues, respectively (Hampel et al., 2001; Moreno-Garrido et al., 2001). The values obtained by these authors overlap the values found in this work (0.55–1.99 mg/L), (Hand and Williams, 1987; Westall et al., 1999). From the studies cited above and our results, toxicity ratio between C_{11}-LAS and C_{12}-LAS or between C_{12}-LAS and C_{13}-LAS are 3.5 (1.6–6.5). This ratio is in the same order of magnitude with data from Rico-Rico et al. (2010) who compared sorption coefficient values between different LAS homologues (in the range 1.6–5.0). Garcia et al. (2002) proposed a simple structure–activity relationship based on LAS hydrophobicity, where sorption coefficient is increased by a factor 2.6–2.8 for each additional methylene group. As a first approximation, the relationship between the LAS alkyl chain length, the hydrophobicity of the homologue and its toxicity to algae allows extrapolating other LAS homologues specific toxicity to the five algal species from our results.

Table 1
Parameters (±SE) from sigmoid curve model (Eq. (1)) fitted to a 72 h – toxicity test (inhibition growth) with five marine algal species exposed to the concentration range 0.2–3.5 mg C_{12}-LAS/L.

Algal group	Algal species	*a*	*b*	IC_{50} (mg/L)
Diatoms	*T. pseudonana*	91 ± 11	0.19 ± 0.08	0.55 ± 0.10
	S. costatum	100 ± 2	0.23 ± 0.02	0.77 ± 0.03
Green algae	*T. levis*	102 ± 26	0.85 ± 0.35	1.99 ± 0.73
	D. tertiolecta	89 ± 8	0.07 ± 0.01	1.66 ± 0.46
Dinoflagellate	*P. minimum*	103 ± 7	0.20 ± 0.07	0.99 ± 0.10

The toxicity of LAS generally presents a high interspecies variability (Blasco et al., 2003; Debelius et al., 2008; Lewis, 1991). The sensitivity of microalgae to a particular homologue can vary by up to a factor 10 among phyla (Moreno-Garrido et al., 2001). In our study, IC_{50} values for tested algae were significantly different (Freidman statistical test, $p < 0.05$) with 0.55 ± 0.10 and 0.77 ± 0.03 mg/L for the two diatoms (*T. pseudonana* and *S. costatum*, respectively), 0.99 ± 0.10 mg/L for the dinoflagellate (*P. minimum*) and 1.66 ± 0.46 and 1.99 ± 0.73 mg/L for the green algae (*D. tertiolecta* and *T. levis*, respectively) which represented the least sensitive group (Table 1). Green algae have been identified as the least sensitive taxonomic group and authors agreed with the fact that *Tetraselmis* and *Dunaliella* are part of the most resistant genera (Blasco et al., 2003; Moreno-Garrido et al., 2001; Pavlic et al., 2005).

The difference in sensitivity between species could be due to the microalgae density used in toxicity tests of different studies. From the literature it has been observed that increasing cell density drastically reduces sensitivity to many contaminants (Moreno-Garrido et al., 2000) including LAS (Debelius et al., 2008). In this work, we used similar microalgae densities (10^5 cells/ml) in order to reach the same toxic cellular quote, estimated around 4×10^{-8} µg C_{12}-LAS/cell (Moreno-Garrido et al., 2000). Fig. 2 presents the IC_{50} values for the different microalgae species according to their surface–volume ratio. An inverse relationship (dashed line) was observed for the diatoms and the dinoflagellate, suggesting that the smaller size microalgae are relatively more sensitive to LAS than the bigger cells. However, the IC_{50} values obtained for the green algae departed from this trend by a factor 2 compared to the other microalgae species. This relative low sensitivity to

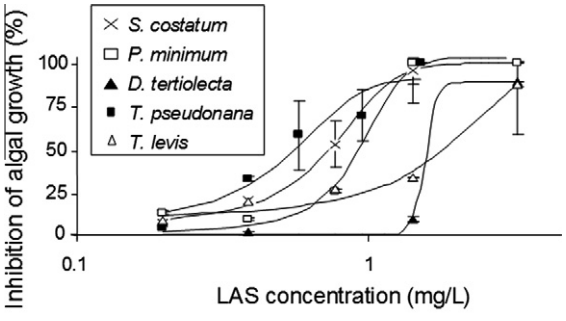

Fig. 1. Concentration–response plots for the phytotoxicity (growth inhibition) of C_{12}-LAS, related to measured aqueous concentration (mg/L) at the end of the 72 h exposure period, fitted by a sigmoid curve.

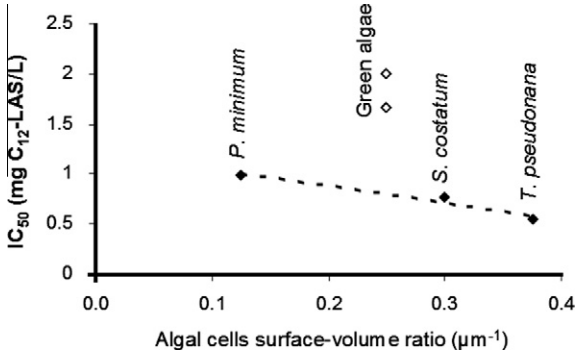

Fig. 2. IC_{50} values (mg C_{12}-LAS/L72 h) plotted vs. algae surface-volume ratio (µm^{-1}) for the five marine algal species.

LAS suggests that the components of the green algae cell wall composed of a characteristic membrane-like trilaminar sheath (TLS) are less sorptive to LAS than the components of the cell walls of diatoms and the dinoflagellate. Such structure has been reported to influence the resistance of green algae to LAS (Corre et al., 1996).

3.2. Sorption and desorption kinetics

The use of radiotracer and LSC technique allowed assessing C_{12}-6-LAS kinetics for the five algal species exposed to 4 μg/L. This exposure concentration is representative of coastal areas receiving treated wastewater discharges (Temara et al., 2001). Classic technique used in previous studies (HPLC/UV) is much more complex to implement and is much less specific (Sáez et al., 2001). LAS kinetics could not be assessed below 50 μg/L. Sorption and desorption kinetics of LAS on the five algae are presented in Fig. 3. During the accumulation experiment, LAS sorption could be detected after one hour of exposure and steady-state was reached within 5 h of exposure for all the tested microalgae. Sorption kinetics data were fitted to a first-order one-compartment model with a determination coefficient (R^2) ranging from 82% to 92%. Steady-state sorption coefficient values (K_{ss}) varied among algae. The lowest K_{ss} values were obtained for the green algae between 1800 ± 400 and 2700 ± 150 L/kg for *T. levis* and *D. tertiolecta*, respectively, whereas the highest values were obtained for the dinoflagellate *P. minimum* with 9200 ± 900 L/kg (Table 2). Diatoms presented intermediary K_{ss} values, 5200 ± 800 and 5700 ± 300 L/kg for *T. pseudonana* and *S. costatum*, respectively. The affinity of the algal species for C_{12}-LAS is therefore in the following increasing order: green algae < diatoms < dinoflagellate.

When the exposed cells were suspended in clean seawater, LAS were rapidly desorbed from the cells with a $T_{b1/2}$ value ranging between 2 and 6 h (Fig. 3). After 6 h of desorption, more than 50% of the initially adsorbed LAS were desorbed from the cells and after 24 h, up to 95% were desorbed. A similar reversible process has been reported for LAS sorption on sediment, with rapid and efficient desorption observed in a few hours (Hand and Williams, 1987). The loss kinetics data were fitted to a first-order two-compartment exponential model (Eq. (3)) and the determination coefficients (R^2) were ⩾90%. The long-lived component desorption rate could not be estimated from our data after 24 h (rate not significantly different from infinite) except for the dinoflagellate *P. minimum* with a $T_{b1/2}$ value of approximately 38 h. However the present data indicate that a minor fraction of LAS (<20%) sorbed on cells of *P. minimum* during the exposure period could remain on the cell wall for longer periods. In the literature, desorption rate constant is generally derived from exposure experiments, by using a one-compartment exponential model (Hwang et al., 2003). Such model mathematically supposes that LAS does not remain in the test organisms, contrary to the two-compartment model used in this work, leading to the generally accepted conclusion of a rapid and complete desorption of LAS. However it is our recommendation to experimentally assess desorption for longer periods of time if a significant LAS fraction is still sorbed on cells after 24 h. This would help estimating the potential presence of LAS in marine food chains.

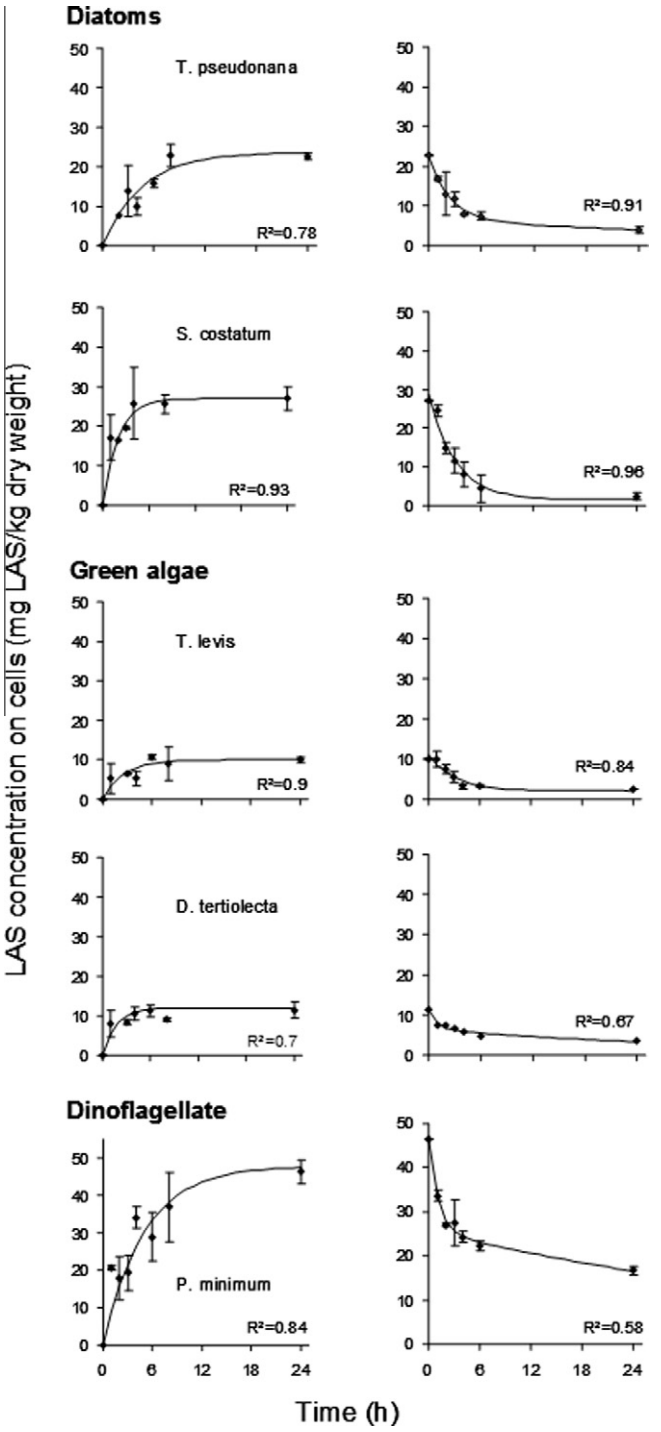

Fig. 3. LAS cell concentration on five marine algal species exposed to 4 μg/L dissolved C_{12}-LAS during 24 h followed by 24 h desorption. Data were fitted to a one-compartment first-order kinetic model for the exposure period, and to a two-compartment first-order kinetic model for the desorption period.

3.3. Sorption isotherms

According to the literature, sorption coefficient of the 5 C_{12}-LAS isomers present less than 0.2 log unit difference (Rico-Rico et al., 2009a). We therefore assume that sorption coefficient results obtained from one isomer (C_{12}-6-LAS) give a good representation of the C_{12}-LAS congeners sorption coefficient. The method used in this work K_{ss} values (Fig. 4) were inversely related to the LAS exposure concentration and decreased by one order of magnitude, in the test concentration range 2.1–1000 μg/L, for every species (Fig. 4a for green algae; Fig. 4b for diatoms and dinoflagellate). This relation illustrates the saturation processes occurring on the cell wall when increasing the LAS exposure concentration in the surrounding water. The data presented in Fig. 4 were fitted to a Freundlich isotherm. The K_f and n parameters are presented in Table 3 and describe a typical L-type Freundlich isotherm ($n < 1$) characteristic of sorption saturation for the highest exposure concentrations. As a general pattern, the K_f values obtained for algae

Table 2
Parameters (±SE) from the one-compartment (Eq. (2)) and two-compartment (Eq. (3)) first order kinetic models fitting LAS cell concentration in five marine algal species during sorption (4 μg/L C_{12}-LAS) and desorption periods, respectively.

Algal group	Algal species	K_{ss} (L/kg)	Sorbed LAS (mg/kg)	A_{0s} (%)	k_s (h^{-1})	A_{0l} (%)	k_l (h^{-1})
Diatoms	*T. pseudonana*	5200 ± 800	26 ± 2	71 ± 13	0.4 ± 0.1	29 ± 13	–[a]
	S. costatum	5700 ± 300	24 ± 3	95 ± 9	0.33 ± 0.06	5 ± 6	–[a]
Green algae	*T. levis*	1800 ± 400	9 ± 1	89 ± 14	0.31 ± 0.09	21 ± 10	–[a]
	D. tertiolecta	2700 ± 150	10 ± 1	41 ± 13	1.1 ± 0.4	59 ± 13	–[a]
Dinoflagellate	*P. minimum*	9200 ± 900	44 ± 6	45 ± 4	0.9 ± 0.2	55 ± 4	0.02 ± 0.005

[a] Values not significantly different from 0.

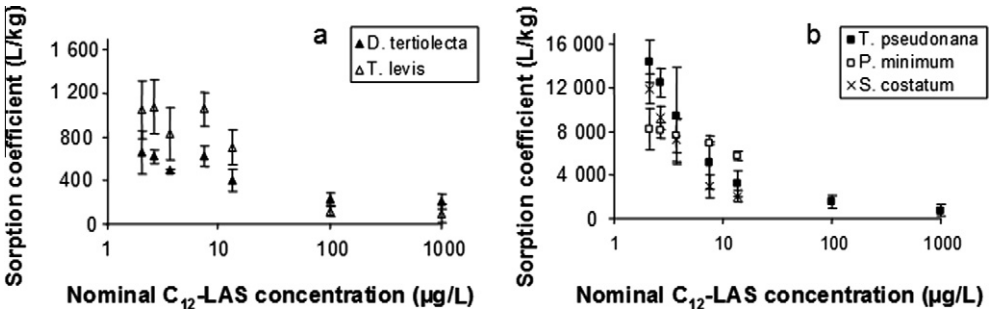

Fig. 4. Steady-state sorption coefficients (K_{ss} L/kg), for a – the green algae and b – marine algal species exposed to a range of C_{12}-LAS concentrations from 2.5 to 1000 μg/L.

Table 3
Freundlich isotherm constants (K_f and n) and standard error (SE) relating the sorbed C_{12}-LAS concentration on the different algal species with the initial dissolved LAS exposure concentration in the surrounding water varying from 2.1 and 13.5 μg/L.

Algal group	Algal species	K_f[a] ± SE	n ± SE	R^2 (%)
Diatoms	*T. pseudonana*	95 ± 11	0.18 ± 0.02	97
	S. costatum	33 ± 6	0.04 ± 0.03	43
Green algae	*T. levis*	363 ± 244	0.8 ± 0.1	95
	D. tertiolecta	185 ± 127	0.8 ± 0.1	95
Dinoflagellate	*P. minimum*	2680 ± 41	0.81 ± 0.02	99

[a] K_f unit is $(mg/kg)(L/mg)^n$.

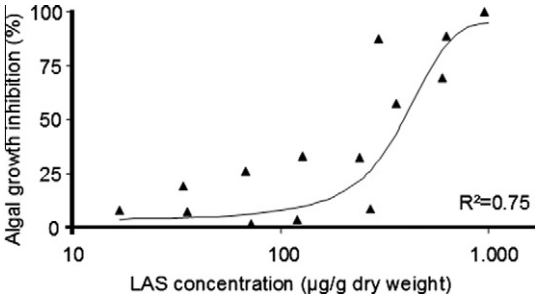

Fig. 5. Concentration–response plots for the phytotoxicity (growth inhibition) of C_{12}-LAS, related to derived internal cell concentration (CC, mg/g dry weight) at the end of the 72 h experiment for *T. pseudonana*, *D. tertiolecta* and *T. levis*.

were in the same range as the ones reported for C_{12}-LAS sorbed on sediment: 22–3550 $(mg/kg)(L/mg)^n$ (Fytianos et al., 1998; Rico-Rico et al., 2009b). The K_f values were the lowest for the diatoms (33 ± 6 and 95 ± 11 $(mg/kg)(L/mg)^n$), intermediate for the green algae (185 ± 127 and 363 ± 244 $(mg/kg)(L/mg)^n$) and the highest for the dinoflagellate (2680 ± 41 $(mg/kg)(L/mg)^n$).

3.4. Toxic cell concentration (TCC)

TCC refers to the phytotoxicity of the sorbed concentration of a compound (Mayer et al., 1998). For three (green algae and dinoflagellate) out of the five algal species, the K_{ss} values could be estimated for C_{12}-LAS exposure concentrations up to 1000 μg/L. For these species only, these values were used to compute specific LAS concentrations sorbed on cells. Growth inhibition plotted against internal LAS concentrations presented a classical dose–response curve, that allowed calculating TCC_{50} values (Mayer et al., 1998). For all the three algal species, the concentration–response relationships were similar (Fig. 5) and a common TCC_{50} value of 0.38 ± 0.09 mg C_{12}-LAS/g algae dry weight (representing 1 mmol C_{12}-LAS/kg of algae dry weight) was estimated (Table 4). This value is in the range of the lethal body burden (LBB) values obtained for aquatic organisms exposed to C_{12}-LAS over similar periods (0.08–2.8 mmol/kg) (Hwang et al., 2003; Versteeg and Rawlings, 2003). This concentration range has been considered as a "minimum toxicity" caused by the accumulation of the compounds on the cell. These findings are in agreement with the concept of baseline toxicity, meaning that LAS exert an acute toxicity by a relatively non-specific mode of action, also called polar narcotic toxicity (Hwang et al., 2003). Toxicity of surfactants is caused by their lipophilic alkyl chain that is responsible for the denaturation of cell walls and the alteration of membrane permeability to nutrients and chemicals (Lewis, 1990; Rieβ and Grimme, 1993). The results indicate that LAS toxicity to marine algae is mainly driven by the affinity of the cell wall to sorb LAS and therefore depends on the size of the cells and on the specific structure of the cell wall.

Table 4
Variables ± SE and critical toxic cell concentration from the sigmoidal curve fitted to a 72 h – toxicity test (inhibition growth) with three marine algal species according to a range of internal C_{12}-LAS concentrations (R^2 = 78%).

Parameters	a	b	TCC (mg/g)
Fitting curve	89 ± 12	0.11 ± 0.06	0.38 ± 0.09

In the present study, algae exposed to 4 μg/L dissolved LAS, sorbed between 0.006 and 0.029 mg LAS/g, whereas TCC_{50} value

is estimated at 0.38 mg/g. Sorbed LAS is thus between 10 and 60 times lower than TCC_{50}. In a coastal environment with a dissolved LAS concentration of 4 μg/L, the sorbed LAS concentration on algae would thus be 10–60 times lower than the TCC_{50} value. This safety margin is sufficient to conclude that short term exposure to environmentally realistic concentrations of LAS would represent low risk of acute toxicity to marine algal species.

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