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Bioconcentration of linear alkylbenzene sulfonates and their degradation intermediates in marine algae

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Abstract Sorption experiments using different homologues of linear alkylbenzene sulfonate (LAS) and sulfophenylcarboxylic acid (SPC) on several marine microalgae have been carried out. The steady state seems to be reached in the first 4 hours. Longer exposure times lead to biodegradation of the compound and, therefore, to an overestimation of the bioconcentration factor. Sorption coefficients are higher for *Nannochloropsis gaditana*, for example, 1,293 Lkg^{-1} for C_{11}-LAS and 525 Lkg^{-1} for C_{11}-SPC versus 727 Lkg^{-1} for C_{11}-LAS and 28 Lkg^{-1} for C_{11}-SPC for *Dunaliella salina*. For both algae an increase in the sorption coefficient is observed when the polarity of the compound decreases (C_5-SPC<C_{11}-SPC<C_{11}-LAS≈C_{12}-LAS). The sorption of C_{11}-LAS on *D. salina* is fitted to a linear Freundlich isotherm (K=176±0.02, n=0.964±0.02)

Introduction

Linear alkylbenzene sulfonate (LAS) is the most widely used anionic surfactant in detergent products nowadays [1]. Although a wastewater treatment plant removes a high percentage of LAS, it and its degradation intermediates (sulfophenylcarboxylic acids, SPCs) exist in marine coastal areas [2].

The toxicity of these surfactants on marine organisms depends on their bioconcentration factor (BCF). This factor is defined as the concentration of the compound for the organisms or specified tissue thereof divided by its concentration in the surrounding medium at the steady state. Some authors [3, 4, 5] assert that this factor can be estimated using linear regression models based on the chemical hydrophobicity, more specifically the octanol–water partitioning coefficient (K_{OW}). According to its logK_{OW} (2.0152) LAS should have some affinity for biological tissue lipids. For other surfactants, such as alcohol ethoxylates [6], the K_{LIPW} (liposome–water partitioning coefficient) calculated by equilibrium dialysis or ultracentrifugation is employed to estimate the BCF. However, other authors [7, 8, 9] disagree about the validity of these relationships considering the BCF dependence with the species, size and environmental factors.

In earlier bioconcentration experiments reported by several authors [10], the concentration of radiolabelled LAS was determined by liquid scintillation counting. The data obtained are not specific for an individual compound and, therefore, it is not possible to distinguish among the original compounds and their biotransformation intermediates. An accurate determination of the BCF requires a specific quantification of the chemical and its metabolites. Because of this, few LAS bioconcentration experiments have been carried out [9, 11, 12], and practically there is none for SPC, in contrast with the extensive data available about the aquatic toxicity of LAS [13, 14].

Phytoplankton plays a key role in aquatic ecology [15, 16]. Most of the information about algae refers to toxicity [17, 18, 19, 20, 21, 22], mainly in freshwater environments, and recently in marine ones [23, 24]. To the best of our knowledge there is no information about the bioconcentration of LAS and SPC in marine algae. Although few works briefly refer to LAS sorption on algae [25, 26], the literature sorption data on algae mainly refer to other compounds, such as metals [27, 28, 29, 30, 31]. Green algae are more resistant to toxic than the other taxonomic classes, showing EC50 values (growth inhibition test) about 2 or 3 orders of magnitude higher for LAS than for metals [23, 31]. For *Nannochloropsis gaditana* Lubián, one of the two alga species tested, EC50 values of 1.38±0.10 and 0.18±0.01 mg L^{-1} have been reported for C_{11}-LAS and C_{13}-LAS, respectively [23]. Algae are at the bottom of the food chain, and if LAS is accumulated by these organisms a biomagnification process could occur; therefore, every level of the food chain will accumulate these chemicals as far as the food chain does not purify them. In this work we realised static (C_{12}- and C_{11}-) LAS

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and (C_5- and C_{11}-) SPC homologue bioconcentration assays using two different marine microalgae: *N. gaditana* Lubián and *Dunaliella salina* Teodoresco in different states, alive and dead. The aim of this work was to characterise the LAS and SPC bioconcentration on marine algae depending on the different hydrophobicity of the chemicals and the different organisms. More specifically, the objectives were the following:

1. To characterise the process kinetically in order to determine the time to reach the steady state.
2. To determine the different BCFs depending on the compound and the algae tested.
3. To fit the process to a known theoretical sorption model.

Material and methods

Water formaldehyde and methanol (MeOH) of high-performance liquid chromatography grade and extra pure NaCl were purchased from Scharlab, Barcelona, Spain. KH_2PO_4 was purchased from Panreac, Barcelona, Spain. $NaClO_4$ and tetraethylammonium hydrogen sulfate (TEAHS) were purchased from Merck, Darmstadt, Germany. The C_{18} and SAX minicolumns were purchased from Supelco, Bellefonte, Pa. Seawater was collected from the Cádiz Bay area, southern Spain (see Table 1 for the seawater characteristics) and filtered using GF/C (Whatman) filters and sterilised using a Sterivac-GP10 vacuum-driven filtration unit (Millipore). The chromatographic column was a Lichrosorb RP-8 column of 250-mm length and 4.6-mm internal diameter, with a particle size of 10 m, purchased from Teknokroma.

The LAS homologue standards (undecyl benzene and dodecyl benzene sulfonate), with purities of 99.0 and 99.5%, respectively, were supplied by Petroquímica Española, Cádiz. The sulfophenylundecanoic acid (C_{11}-SPC) was synthesised at the University of Cádiz, with more than 99% purity, by sulfonation of the corresponding phenylundecanoic acid following the procedure described in Ref. [32]. C_5-SPC was donated by F. Ventura.

Two different green algae species were used: *N. gaditana* obtained from the Marine Microalgae Culture Collection of the Instituto de Ciencias Marinas of the CSIC and *D. salina* cultured in the Wet Laboratories of the Marine Sciences Faculty of Cádiz University. Some characteristics of the two algae [33, 34] are displayed in Table 2. *N. gaditana* has a smaller size and a cellular wall which *D. salina* lacks. This one has two flagella. The algae were collected in the exponential growth phase with a density of around 100×10^6 cellmL^{-1} for *N. gaditana* and $2–3\times10^6$ cellmL^{-1} for *D. salina*. Centrifugation for 15 min at 1,380*g* and 25 °C concentrated them in order to obtain about 0.2 g biomass. The cell number was counted using Neubauer counting chambers.

Table 1 Characteristics of the sterilised sea water (*nd*=not detected)

Sea water	
Salinity	36.25
pH	8.13
Alkalinity (mmolL^{-1})	2.43
HCO_3^- (mmolL^{-1})	2.10
LAS (μgL^{-1})	nd
PO_4^{3-} (μmolL^{-1})	0.79
SiO_2 (μmolL^{-1})	27.72
NO_3^- (μmolL^{-1})	3.33
NO_2^- (μmolL^{-1})	1.14
NH_4^+ (μmolL^{-1})	0.27

Table 2 Characteristics of the marine algae

Class	Dunaliellaceae	Chlorophyceae
Family	Monodopsidaceae	Eustigmatophyceae
Species	*Nannochloropsis gaditana* Lubián	*Dunaliella salina* Teodoresco
Cellular volume (μm^3)	8	230
Protein percentage	36.87	35.76
Lipid percentage	41.33	41.75

The different experiments carried out to reach the different objectives can be grouped in three batches. The first batch of experiments was carried out with 200 μgL^{-1} C_{11}-LAS and C_{11}-SPC on both algae and measurements were made at several exposure times. The sampling frequency was high at the beginning of the experiment and decreased with time, in order to determine the kinetic sorption that allows us to establish the time to reach the steady state. The second batch of experiments was carried out with 200 μgL^{-1} C_5-SPC, C_{11}-SPC, C_{11}-LAS and C_{12}-LAS on live and dead *N. gaditana* and *D. salina* for 12 h in order to establish the influence of the compound, the algal type and the state on the BCF. The sorption of C_{11}-LAS on dead *D. salina* was studied in the last batch of experiments at different concentrations (50, 100, 200, 400 and 600 μgL^{-1}) in order to establish a sorption isotherm. All the experiments were carried out in duplicate. Blanks, under same conditions but without algae, were also carried out for each experiment in order to determine the sorption to the test-tube walls.

Around 0.2 g algae was resuspended in 50 mL sterilised seawater in plastic centrifuge tubes. Subsequently LAS or SPC homologues were added to obtain the test concentration. All tubes, samples (containing algae) and blanks (only with sterilised water) were placed in a culture chamber at 25 °C, lighted and shaken continuously. Afterwards they were centrifuged (25 °C, 15 min and 1,380*g*) and the pellet was discarded. The surfactant concentration was measured in the supernatant of the samples and in the blanks. Sorption to the walls was determined in the blank supernatant and the amount of LAS in the algae was calculated by subtracting the amount measured in the water phase and the amount sorbed on the walls of the tube from the total amount added.

For LAS determination, a 50-mL sample, acidified to pH 3 with HCl, was passed through a C18 minicolumn and an SAX minicolumn, following the procedure described in Ref. [35]. For the SPC determination, KH_2PO_4 (0.05 molL^{-1}) and NaCl, up to an ionic strength of 5 molL^{-1}, were added to 50 mL sample. The sample was passed through a Bond Elut C_{18} minicolumn and an SAX minicolumn according to the procedure described in Ref. [36]. The final eluate obtained was evaporated in a heating block under a gentle stream of N_2, and resuspended in 1 mL of the same mobile phase used in the chromatographic step.

The LAS and SPC were analysed using a HP 1050 HPLC chromatograph equipped with a fluorescence detector ($\lambda_{excitation}$=225 nm, $\lambda_{emission}$=295 nm). LAS homologues and C_{11}-SPC were analysed under isocratic conditions, where the mobile phase used was MeOH/H_2O (80:20, v/v) with 10 gL^{-1} $NaClO_4$, meanwhile C_5-SPC was analysed under gradient conditions mixing solvent A (100% water) and solvent B (80% methanol/20% water and 1.25 mmolL^{-1} TEAHS). The LC eluant conditions varied from 90% of solvent A and 10% of solvent B to 100% of solvent B in 51 min. Isocratic conditions were maintained until complete elution of all the compounds. One hundred microlitres of sample was injected. The LAS and SPC concentrations were determined by measuring the peak areas using external standards. These standards were treated in the same way as the samples.

Results and discussion

The curve of the ratio of the concentration of C_{11}-LAS and C_{11}-SPC on algae and water (C_a/C_w) versus time is shown in Fig. 1. C_a is the concentration of the chemical on the algae expressed in micrograms per kilogram and C_w is the concentration on the water expressed in micrograms per

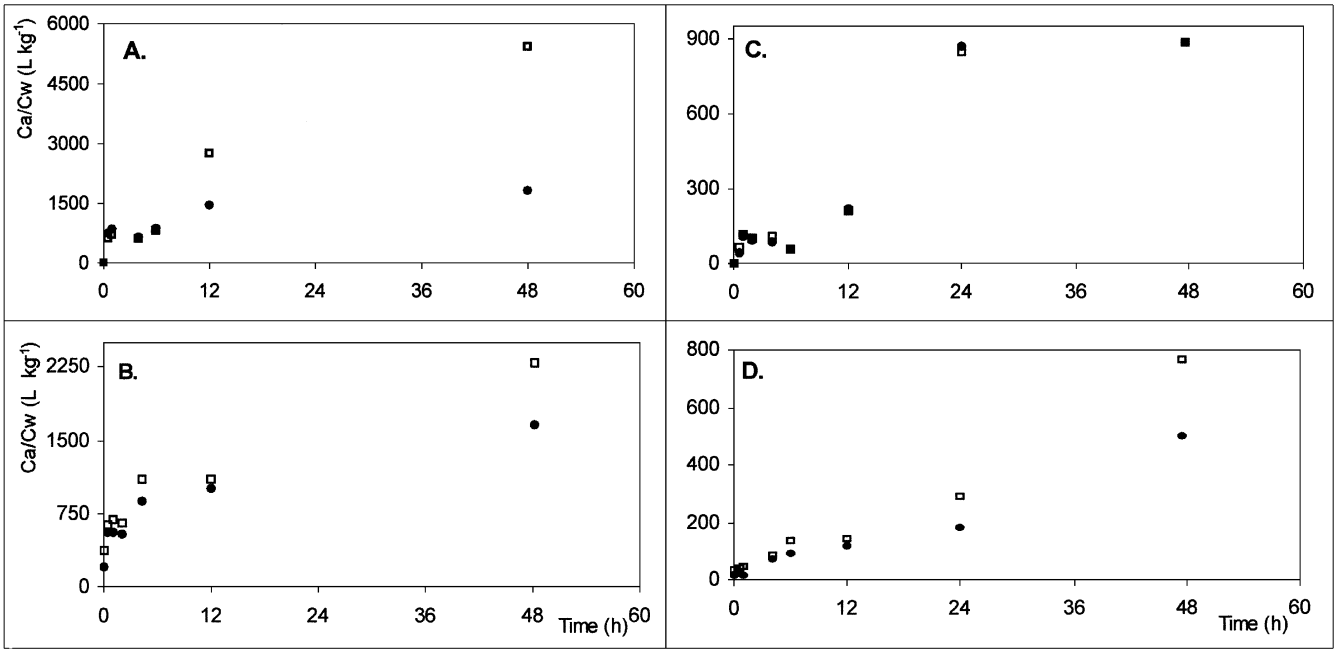

Fig. 1 Sorption curves to determine the steady state for C_{11}-linear alkylbenzene sulfonate (*LAS*) on **a** *Nannochloropsis gaditana*, **b** C_{11}-LAS on *Dunaliella salina*, **c** C_{11}-sulfophenylcarboxylic acid (*SPC*) on *N. gaditana*, and **d** C11-SPC on *D. salina*. The experiments were carried out in duplicate

litre; consequently, the ratio units are litres per kilogram. An increase in the C_a/C_w ratio during the first few hours was observed for C_{11}-LAS on *N. gaditana* (Fig. 1a) and on *D. salina* (Fig. 1b). An apparently stable ratio of C_a/C_w is reached after 4 h. The values obtained agree with the values for the marine sediment sorption experiment described in the literature [35]. However, the C_a/C_w ratio increased sharply until 48 h (end of the experiment), where the standard deviations were also high. As far as C_{11}-SPC is concerned, its sorption on *N. gaditana* and on *D. salina* is shown in Fig. 1c and d, respectively. It has the same tendency as was observed for C_{11}-LAS but with smaller values of the C_a/C_w ratios, which were very low in the first hours of exposure. This fact is due to the more hydrophilic character of the degradation intermediates, or in other words, their lower affinity for the biological lipids.

After 6-h exposure, and for both algae, in all the chromatograms of C_{11}-LAS in water, the presence of degradation intermediates was observed. As an example, the broken line in Fig. 2 shows the chromatogram of C_{11}-LAS in water corresponding to the experiment realised with *N. gaditana* at an exposure time of48 h. Therefore, the decrease in the concentration of C_{11}-LAS in water was due to biodegradation; in this way the BCF values were overestimated. Considering the sterilisation of the seawater prior to its use in the experiments, the bacteria responsible for the LAS degradation should come from the medium surrounding the algae. In the chromatograms corresponding to the same experiment but realised with dead algae (Fig. 2, continuous line) no degradation was observed. The C_a/C_w ratio for C_{11}-LAS on live *D. salina* at 48 h was 1,981 Lkg^{-1}, meanwhile for C_{11}-LAS on dead *D. salina* at 48 h it was 720 Lkg^{-1}. Nevertheless, dead and live algae

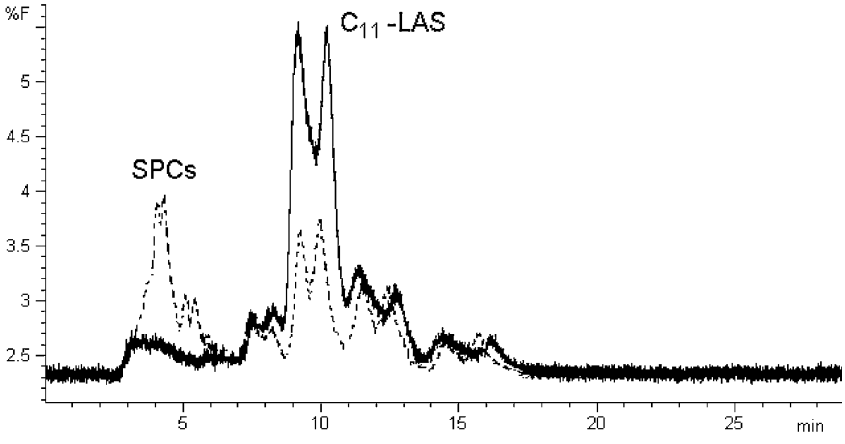

Fig. 2 High-performance liquid chromatography–fluorescence overlaid chromatograms of C_{11}-LAS in water after 48 h exposure on different states of *N. gaditana*. The *continuous line* corresponds to dead alga and *broken line* to live alga

Table 3 Sorption coefficients with their standard deviation obtained for 200 μgL^{-1} linear alkylbenzene sulfonate (*LAS*) and sulfophenylcarboxylic acid (*SPC*) homologues on *N. gaditana* and *D. salina* exposed for 12 h. The values in *parentheses* correspond to the total concentration ratios: sum of chemical concentration on algae and chemical concentration on tube divided by the chemical concentration in water. The sorption coefficients are expressed in litres per kilogram

	N. gaditana	*D. salina*
C_5-SPC	180± 40	0
C_{11}-SPC	525±138	28± 2
C_{11}-LAS	1293±197 (2502±332)	727±195 (1742±405)
C_{12}-LAS	274± 11 (2321± 41)	710± 0 (2526± 0)

exposed for a short period of time (fewer than 6 h), before degradation started, showed similar C_a/C_w ratios (810±26 and 700±42 Lkg^{-1} for C_{11}-LAS on live and dead *D. salina*, respectively), which allows us to think that absorption by the algae could have little significance compared with adsorption, as has been suggested before [37].

The BCFs must be estimated either at shorter exposure times (with the steady state reached) in order to avoid biodegradation or by calculating the sorption coefficient on dead algae. The first alternative is experimentally difficult to achieve, mainly considering the rapid degradation of SPCs. Because of this, the following experiments were designed by employing dead algae. In order to determine the sorption coefficients in the proper way, the batch of dead algae experiments were exposed to 200 μgL^{-1} C_5-SPC, C_{11}-SPC, C_{11}-LAS and C_{12}-LAS for 12 h. The sorption coefficients employing dead algae (no biodegradation) for 12 h of exposure time are shown in Table 3. Considering the similar lipid percentage of both algae (Table 2) *N. gaditana* showed higher sorption coefficient values than *D. salina*. The smaller size of *Nannochloropsis*, which has a higher surface/volume ratio, and its cellular wall, which *Dunaliella* lacks, would explain the bigger sorption coefficient values of *N. gaditana*.

Both algae had small values of the SPC sorption coefficient as was expected owing to the lower affinity (higher polarity than the LAS homologues) for the biota. If the SPC homologues are compared, C_{11}-SPC shows higher sorption values than C_5-SPC, which shows no sorption for *D. salina*. When C_{11}-LAS and C_{12}-LAS are compared, C_{11}-LAS shows higher sorption coefficient values than C_{12}-LAS. It looks like the C_{11} homologue (with less hydrophobic character) has more affinity for the biota than C_{12}, but this fact cannot be confirmed because in the C_{12}-LAS blanks (no alga) there was a high sorption on the tube walls. The total sorption ratio values (the concentration of LAS on the algae plus the concentration of LAS on the tubes walls divided by the concentration of LAS in water) are shown in parentheses in Table 3. If we compare these data, the results are similar for both LAS homologues. For the same alkyl chain length, C_{11}-LAS and C_{11}-SPC, differences in the sorption coefficients are observed owing to the more polar character of the SPC. In general, it can be stated that an increase in the sorption coefficient is observed when the polarity of the compound decreases (C_5-SPC<C_{11}-SPC<C_{11}-LAS≈C_{12}-LAS).

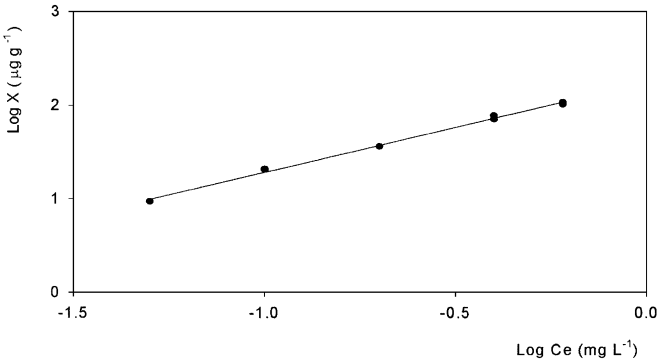

Fig. 3 Linearisation of Freundlich's isotherm for the sorption of C_{11}-LAS on the dead alga *D. salina* after 12 h exposure. *X* represents the amount of C_{11}-LAS sorbed per unit of sorbent and C_e is the equilibrium concentration of C_{11}-LAS in solution

The sorption of C_{11}-LAS at 25 °C with the initial range of C_{11}-LAS concentrations on dead *Dunaliella* is shown in Fig. 3. The data are fitted to a linear Freundlich isotherm of the type

$$\log X = \log K + n \log C_e,$$

where X is the amount of LAS adsorbed per unit of adsorbent (micrograms per gram), C_e is the equilibrium concentration of LAS in solution (milligrams per litre), K is a constant related to the binding energy, which can be defined as the distribution or sorption coefficient and represents the amount of LAS adsorbed in the biota per unit equilibrium concentration (micrograms per gram), and n is a measure of the sorption intensity. We established K=176±0.02 and n=0.964± 0.02. Comparable results using marine sediment have been reported [29] at similar LAS concentrations to those used in the present work and the following parameters were obtained: K=142±59 and n=1.38±0.29.

Conclusions

The sorption equilibria for LAS and SPC in the algae *N. gaditana* and *D. salina* were reached at 4 h, in accordance with the sediment data in the literature. *N. gaditana* showed higher sorption coefficients than *D. salina* for all the compounds tested. Since biodegradation was observed after 6 h, the sorption coefficients were determined at 12 h by employing dead algae to avoid overestimation. A sorption coefficient increase was detected with the decrease in the polarity of the compounds (C_5-SPC<C_{11}-SPC<C_{11}-LAS≈C_{12}-LAS). The C_{11}-LAS sorption on *D. salina* was fitted to a linear Freundlich isotherm (K=176±0.02, n=0.964±0.02).

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References

1. Britton LN (1998) J Surf Deterg 1:109
2. González-Mazo E, Quiroga JM, Sales D, Gómez-Parra A (1997) Toxicol Environ Chem 59:77
3. Hawker DW, Connell DW (1986) Ecotox Environ Safe 11:184
4. Tolls J, Sijm DTMH (1995) Environ Toxicol Chem 14:1675
5. Tolls J, Haller M, Labee E, Verweij M, Sijm DTMH (2000) Environ Toxicol Chem 19:646
6. Müller MT, Zehnder JB, Escher BI (1999) Environ Toxicol Chem 18:2191
7. Barron MG (1990) Environ Sci Technol 24:1612
8. Haitzer M, Höss S, Traunspurger W, Steinberg C (1998) Chemosphere 37:1335
9. Tolls J, Haller M, Seinen W, Sijm DTHM (2000) Environ Sci Technol 34:304
10. Tolls J, Kloepper-Sams P, Sijm DTHM (1994) Chemosphere 29:693
11. Tolls J, Haller M, De Graaf I, Thijssen MATC, Sijm DTMH (1997) Environ Sci Technol 31:3426
12. Tolls J, Lehmann MP, Sijm DTHM (2000) Environ Toxicol Chem 19:2394
13. Kimerle RA (1989) Tenside Surfactants Deter 26: 169
14. Lewis MA (1992) Water Res 26:1013
15. Christensen ER, Nyholm N (1984) Environ Sci Technol 18:713
16. Skoglund RS, Swackhamer DL (1994) In: Baker LA (ed) Environmental chemistry of lakes and reservoirs. American Chemical Society, Washington, DC, pp 559, 570
17. Ernst R, Gonzales CJ, Arditti J (1983) Environ Pollut 31:159
18. Yamane AN, Okada M, Sudo R (1984) Water Res 18:1101
19. Chawla G, Viswanathan PN, Devi S (1988) Ecotox Environ Safe 15:119
20. Lewis MA (1990) Ecotox Environ Safe 20:123
21. Lewis MA (1995) Environ Poll 87:319
22. Verge C, Moreno A (1996) Tenside Surfactants Deterg 33:166
23. Hampel M, Moreno-Garrido I, Sobrino C, Lubián LM, Blasco J (2001) Ecotox Environ Safe 48:287
24. Moreno-Garrido I, Hampel M, Lubián LM, Blasco J (2001) Fresenius J Anal Chem (in press)
25. Davis E, Gloyna E (1969) J Am Oil Chem Soc 46:604
26. Chawla G, Viswanathan PN, Devi S (1987) Environ Exp Bot 3:311
27. Crist RH, Martin JR, Carr D, Watson JR, Clarke HJ (1994) Environ Sci Technol 28:1859
28. Gonzalez-Davila M, Santana-Casiano JM, Perez-Peña J (1995) Environ Sci Technol 29:289
29. Santana-Casiano JM, González-Dávila M, Perez-Peña J, Millero FJ (1995) Mar Chem 48:115
30. Santana-Casiano JM, González-Dávila M, Laglera LM, Perez-Peña J, Brand L, Millero FJ (1997) Mar Chem 59:95
31. Moreno-Garrido I, Lubián LM, Soares AMVM (2000) Ecotox Environ Safe 47:112
32. Marcomini A, Di Corcia A, Samperi R, Capri S (1993) J Chromatogr 647:219
33. Chrétiennot-Dinet MJ (1990) Atlas du phytoplancton marin, vol 3. Centre National de la Recherche Scientifique, Paris, pp 20, 64
34. Lubián LM, Yúfera M (1989) In: Yúfera M (ed) Acuicultura intermareal. Instituto de Ciencias Marinas de Andalucía, Cádiz, p 69
35. González Mazo E, Forja JM, Gómez-Parra A (1998) Environ Sci Technol 32:1636
36. León VM, González-Mazo E, Gómez-Parra A (2000) J Chromatogr A 889:211
37. Rubio JA, González-Mazo E, Gómez-Parra A (1996) Mar Chem 54:171