Journal of
Environmental
Monitoring

Cite this: DOI: 10.1039/c1em10150a

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PAPER

Tracking sewage derived contamination in riverine settings by analysis of synthetic surfactants

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Received 15th February 2011, Accepted 3rd May 2011
DOI: 10.1039/c1em10150a

A study has been made of the presence and reactivity of the most commonly used surfactants, both anionic (linear alkylbenzene sulfonates, LAS, and alkyl ethoxysulfates, AES) and non-ionic (alcohol polyethoxylates, AEOs, and nonylphenol polyethoxylates, NPEOs), in water and surface sediments from the middle stretch of the Guadalete River in SW Spain (12 stations). Average values were between 0.1 and 3.7 mg kg^{-1} in sediment, and between 0.2 and 37 μg L^{-1} in water. The sorption of surfactants was dominated by hydrophobic mechanisms, so those homologues having longer alkyl chains (*e.g.* C_{18}AEO) showed higher relative percentages and concentrations in sediments compared with water. Local and sharply higher concentrations of these compounds were observed at three sampling stations (7, 9 and 12), indicating the occurrence of wastewater discharges into the river. By analysing the distributions of different surfactant homologues and their metabolites we were able to distinguish between sewage contamination from sources discharging treated and untreated wastewaters. Upstream (stations 1–2), LAS concentrations were below 30 μg L^{-1} and the composition of their degradation intermediates (sulfophenyl carboxylic acids, SPCs) (160 μg L^{-1}) was dominated by short-chain homologues (C_6–C_9SPCs), indicating that the degradation of this surfactant is at an advanced stage. The highest concentration (487 μg L^{-1}) of SPCs was detected near the effluent outlet of a sewage treatment plant (STP) (station 12). Sampling stations (7 and 9) affected by untreated wastewater discharges were the only ones showing the presence of the most reactive and biodegradable SPC isomers and homologues (*e.g.* C_{11}SPC). Here, LAS reached the highest concentration values measured (>2 mg L^{-1}), and showed a homologue distribution closer to that of commercial mixtures than LAS found at the other stations.

1. Introduction

Surface Active Agents (SurfActAnts) are used in large quantities as active ingredients of detergents and cleaners, as well as in a wide variety of applications such as paints, pesticide formulations, pharmaceuticals, wetting agents, and personal care products. These compounds can be classified into four main groups according to their charge: (1) anionics, (2) non-ionics, (3) cationics and (4) amphoterics;[1] the first and second groups account for the highest production volumes. According to the data reported by CESIO (European Committee of Organic Surfactants and their Intermediates), 1200 ktons of anionic and 1400 ktons of non-ionic surfactants were produced in Europe in 2006; these tonnages represent 87% of total European production of synthetic surfactants. Linear alkylbenzene sulfonates (LAS), alkyl ethoxysulfates (AES) and alkyl sulfates (AS)

Environmental impact

The present research is focused on the determination of the concentrations of widely used surfactants in rivers. These compounds are the most abundant organic contaminants in treated and untreated wastewater, so they can be used as very good markers of sewage-derived pollution. Analysis of the distribution of surfactant components and the presence and levels of their degradation intermediates is performed in this work and allow us to identify the location of sewage sources and to distinguish among different types of wastewaters. The methodology and approaches shown here can be useful to other scientists as they can be easily extrapolated to other aquatic systems impacted by sewage inputs.

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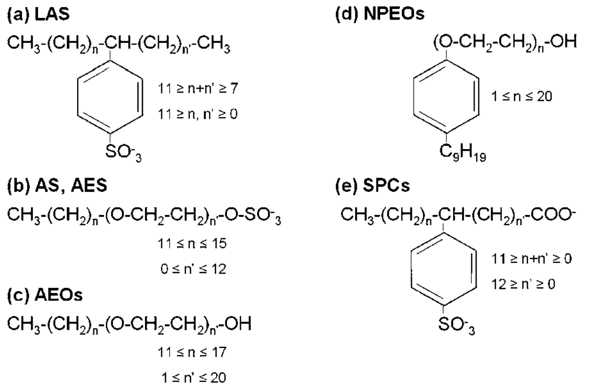

Fig. 1 Chemical structures of (a) linear alkylbenzene sulfonates (LAS), (b) alkyl ethoxysulfates (AES), (c) alcohol polyethoxylates (AEOs), (d) nonylphenol polyethoxylates (NPEOs), and (e) sulfophenyl carboxylic acids (SPCs).

comprise the largest volume of anionic surfactants. LAS are commercially available as a mixture of homologues having from 10 to 13 carbon atoms in their alkyl chain, and isomers resulting from the different attachment positions of the phenyl group along that chain (Fig. 1a). The chemical structure of AES comprises a C_{12-16} alkyl chain bonded to a variable number of ethylene oxide (EO) groups, and a terminal sulfate group (Fig. 1b). Alkyl sulfates (AS) show a structure similar to that of AES but with no EO units. Most of the applications of anionic surfactants[2–4] relate to household and laundry detergents, hand dishwashing liquids, shampoos, and other personal care products. With respect to the non-ionic surfactants, alcohol polyethoxylates (AEOs) and nonylphenol polyethoxylates (NPEOs) are the two major compounds used in Europe. Commercial AEOs are a mixture of homologues having from 12 to 18 carbon atoms in their alkyl chain, which is connected *via* an ether bond to an ethylene oxide chain (Fig. 1c). NPEOs are mixtures of a wide range of ethoxymers (from 1 to 20 EO units), and isomers, depending on the degree of branching of the C_9 alkyl chain (Fig. 1d). Both AEOs and NPEOs are widely used in domestic and industrial applications[5] (*e.g.*, detergents, emulsifiers, wetting and dispersing agents, industrial cleaners, textile, pulp and paper processing), although the potential estrogenicity[6] of NPEO degradation products has resulted in restrictions on their use in recent years.

After their use, surfactant residues are discharged into aquatic ecosystems in treated or untreated wastewaters, and enter various environmental compartments such as surface waters, sediments and biota.[1] Available studies on the presence, environmental behaviour and distribution of these compounds are mainly focused on LAS[7–11] and NPEOs.[11–15] A wide range of LAS concentrations in marine and fresh water[7,10,16–19] has been reported: *e.g.*, from a few μg L^{-1} to several hundred μg L^{-1} in the Tamagawa estuary[7] and the Tega lake[20] (both sites in Japan), the Venice lagoon[21] (Italy), and the Bays of Almeria[18] and Cadiz[8,10,16,19] (both in Spain). NPEO levels in the aqueous phase tend to be slightly lower than those for LAS as a consequence of the higher hydrophobicity and lower production volume of NPEOs. Concentrations of this surfactant in surface waters have been reported all around the world: <0.1 to 100 μg L^{-1} in Mexico,[22] from 0.1 to 14.9 μg L^{-1} (ref. 12,13 and 23) in rivers in Holland, Switzerland and the United States, and from 1 to 25 μg L^{-1} in coastal waters of Israel,[24] Italy,[17,25] Spain[18] and Denmark.[26] In spite of their relatively high solubility, these compounds show a moderate to high sorption capacity, so significant percentages are attached to suspended solids and finally become part of sediments. As a consequence their bioavailability may be reduced, so concentrations of surfactants in surface sediments can be higher than those measured in water, by several orders of magnitude, especially in polluted areas subjected to untreated wastewater discharges: *e.g.*, from 24 to 410 mg kg^{-1} of LAS in the Tamagawa estuary,[7] the Tega lake[20] (Japan) and the Sancti Petri channel[16] (Spain), and from 0.1 to 50 mg kg^{-1} of NPEOs in Barcelona Harbour[18] (Spain) and Jamaica Bay[27] (NY). Available data on the presence and distribution of aliphatic surfactants (AES and AEOs) are more limited compared to data on LAS and NPEOs, in spite of their production volumes being similar, although recent studies have reported AES[16,28,29] and AEO[18,30–33] levels at various sampling sites in Europe, Canada and the United States.

Surfactants, as well as some of their by-products and other detergent related chemicals, have the potential of being source-specific markers for detecting contamination by discharge of industrial and urban wastewaters. This is based on their widespread use, their resistance to chemical and biochemical alteration in sediments (especially under anaerobic conditions), and the physicochemical properties that control their transport.[34,35] NPEOs and linear alkylbenzenes (raw material for the synthesis of LAS) have been used to determine the extent of sewage pollution in Jamaica Bay[36] and Narragansett Bay[37] (United States) by analyzing the distribution of these compounds in surface sediments. They have also been used as geochronological tools[35] due to their relative persistence in the absence of oxygen. In this sense, the historical release of LAS was studied in Swiss lakes by analyzing dated sediment cores.[34] Here, we have determined the presence and longitudinal distribution of LAS and NPEOs, as well as other less studied aliphatic surfactants (AES and AEOs), in surface waters and sediments from the middle stretch of the Guadalete River (Southwest Spain). The main goal of this work is to identify and characterize wastewater sources along the river by measuring surfactant concentrations and analyzing changes that may occur in the composition (in terms of homologue and isomer distributions) of these compounds and their degradation products.

2. Material and methods

2.1. Study area and sample collection

Twelve sampling stations (1–12) located along the middle stretch of the Guadalete River (SW, Spain) were selected for this study (Fig. 2). Sampling was done in spring 2008. Sampling station 1 (36°38′51.40″N, 5°55′49.27″O) is situated twenty kilometres upstream from sampling point 2, so it does not appear on the map. The river is 157 km long and its basin covers an area of 3677 km^2; it flows across the province of Cadiz from the Sierra of Grazalema Natural Park and enters the sea in the northern part of the Bay of Cadiz. Most of the terrain adjacent to the river is used for irrigated crops, mainly sugar beet, cotton, sunflower, wheat and tomatoes and there are also direct discharges from

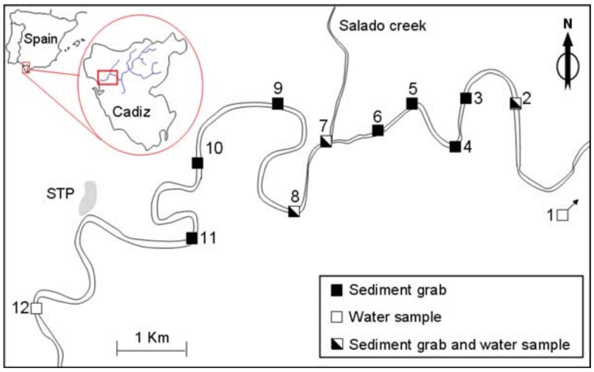

Fig. 2 Map of the Guadalete River showing the location of sampling stations.

farms and individual households. The river also receives the effluent from a sewage treatment plant (STP) located between stations 11 and 12 (see Fig. 2) that collects wastewaters from Jerez de la Frontera (a city of 207 500 inhabitants, year 2009). There is a small dam at station 12 (36°37′54.94″N, 6°7′41.76″O), located at a distance of one kilometre downstream from the STP discharge outlet. Finally, a small creek known as the Salado Stream flows directly into the Guadalete River at station 7 after collecting sewage from small urban areas associated with Jerez de la Frontera, such as Lomopardo, Estella del Marques and Torremelgarejo.

Five water samples (sampling stations 1, 2, 7, 8 and 12) were collected using 2.5 L amber glass bottles and adding 4% of formaldehyde. Ten sediment samples (stations 2 to 11) were obtained from an inflated boat using a Van Veen grab, taking the topmost 10 cm layer of the sediments. Water and sediment samples were maintained at 4 °C during their transport to the laboratory, where they were frozen and stored until their analysis. Sediment samples were then dried at 30 °C in a heater until constant weight, milled and passed through a 0.063 mm sieve. The organic carbon content in sediment grabs was determined by dichromate oxidation, using the method proposed by Gaudette *et al.*[38] with the El Rayis[39] modification and pH measurements were carried out according to Hanlon.[40] The values were between 1.8% and 2.7% for the organic carbon content, and between 7.2 and 7.9 for pH.

2.2. Chemicals

Methanol, triethylamine and acetic acid were of chromatography quality, purchased from Scharlau (Barcelona, Spain); sodium acetate and sodium sulfate were purchased from Panreac (Barcelona, Spain); and water was Milli-Q quality. Solid-phase extraction (SPE) mini-columns (500 mg) were supplied by Varian (Bond Elut C_{18}). Commercial mixtures of AES, AEOs and NPEOs having the following homologue compositions were supplied by the KAO Corporation (KAO, Barcelona, Spain): C_{12} (68.5%), C_{14} (29.8%) and C_{16} (1.7%) for AES; and C_{12} (53.4%), C_{14} (32.6%) and C_{16} (14.0%) for AEOs. Their ethoxylated chains had an average number of 2.94, 9.91 and 10.78 units respectively. A 99% pure $2\Phi C_{16}$ LAS internal standard and an LAS commercial mixture having the following homologue distribution were supplied by Petroquimica Española S.A. (PETRESA, Cadiz, Spain): C_{10} (10.9%), C_{11} (35.3%), C_{12} (30.4%), C_{13} (21.2%) and C_{14} (1.1%). The 99% pure $5\Phi C_5$ to $13\Phi C_{13}$ SPC standards were supplied by F. Ventura (AGBAR, Spain) and J.A. Field (Oregon State University, United States). Some of them were synthesized in our laboratory.

2.3. Analytical methodology

Duplicates of each of the water and sediment samples were extracted and analyzed using an analytical protocol previously developed by Lara Martín *et al.*[41] Briefly, surfactants were extracted from sediments by pressurized liquid extraction (PLE) using methanol at 120 °C and 1500 psi. Subsequently, the methanolic extracts were evaporated until 1 mL and re-dissolved in 100 mL of milli-Q water in an ultrasonic bath. These extracts, as well as the aqueous samples, were purified and pre-concentrated by C_{18} solid-phase extraction (SPE) mini-columns using methanol/acetone 1 : 1 and dichloromethane/ethyl acetate 1 : 1 as elution solvents. Finally, the elution was evaporated until dryness and re-dissolved in 1 mL of methanol/water 8 : 2 solution containing 1 mg L^{-1} of C_{16}LAS (internal standard) and 50 μM of sodium acetate. Recoveries were in the range from 70 to 107% for most homologues.

High performance liquid chromatography-mass spectrometry (HPLC-MS) was used to identify and quantify target compounds. The chromatographic separation was performed using a reversed-phase C-18 analytical column (LiChroCart 100 RP-18, Merck) of 125 mm × 2 mm and 5 μm particle diameter. An LCQ ion-trap mass spectrometer (Thermo) equipped with an electrospray interface (ESI) was used for detection, running from 0 to 38 min in full-scan negative ion mode (ESI^-) for the determination of SPCs, LAS, AS and AES; it was then switched to positive mode (ESI^+) for NPEOs and AEOs. Identification of LAS and SPC homologues was carried out by monitoring their quasi-molecular ions $[M - H]^-$ and their specific fragment ion at *m*/*z* 183 (*m*/*z* 197 for AS and AES). Sodium adducts $[M + Na]^+$ were analyzed for NPEOs and AEOs instead. Concentrations of surfactants and their metabolites were determined by measuring the peak areas of the quasi-molecular ions for anionic compounds and sodium adducts for non-ionics. Calibration curves were prepared using external standard solutions (0.1–20 mg kg^{-1}) and concentrations were corrected according to the intensity of the internal standard. Limits of detection were calculated using a signal to noise ratio of 3 : 1, and were found to be 0.05 μg L^{-1} in water and from 1 to 10 μg kg^{-1} in sediment.

3. Results and discussion

3.1. Surfactant concentrations and location of sewage contamination sources

Fig. 3 shows the longitudinal distribution of anionic (LAS and AES) and non-ionic surfactants (AEOs and NPEOs) in surface sediments along the middle stretch of the Guadalete River. The target compounds were found at a wide range of concentrations; values for AEOs and LAS were of the same order of magnitude (between 0.9 and 3.0 mg kg^{-1} for AEOs, and from 0.1 to 3.7 mg kg^{-1} for LAS), while in the case of AES and NPEOs, the concentrations found were one order of magnitude lower (from 125 to 590 μg kg^{-1}). Similar values have been detected previously

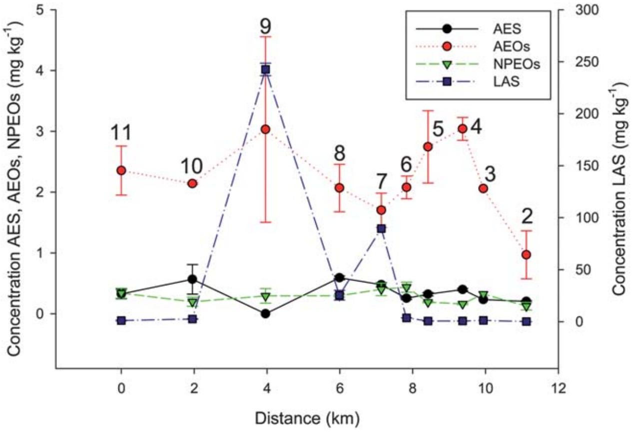

Fig. 3 Surfactant concentrations (mg kg^{-1}) in surface sediments from the Guadalete River.

in other aquatic ecosystems in Spain,[8,10,16,18,28] the United States[29,42] and Germany.[43] For all the synthetic surfactants lower concentrations were found upstream (sampling station 1), in a part of the river that is free of urban and industrial activities. Concentrations of surfactants found at the sampling stations were within the same range as those previously reported in sediment samples from the final stretch of the Guadalete River,[16,19,28] where its estuary joins the Atlantic Ocean. In that study, concentrations of AEOs and NPEOs at some sample stations at the mouth of the estuary[16] were significantly higher (up to 14.4 and 3.7 mg kg^{-1}, respectively) than those reported in the current study, probably as a consequence of the various industrial activities (*e.g.* seafood processing, ports, *etc.*) located in that area. The largest differences along the course of the river studied were found for LAS; although average concentrations were below 3.7 mg kg^{-1}, we detected two 'hot spots' corresponding to sampling stations 7 and 9, where exceptionally high values (89.4 and 242.6 mg kg^{-1}, respectively) were measured. Such high concentrations, however, are unusual and have been only reported in sediments from specific points that are directly affected by untreated urban wastewater discharges, because LAS is widely used in household detergents and cleaners. For example, the Tega lake[20] is a very polluted body of water in Japan where up to 410 mg kg^{-1} of LAS was detected at a sediment sampling station at precisely the place where wastewater is discharged from a population of 400 000 inhabitants. Similar cases have been also reported in estuaries (the Tamagawa estuary,[7] Japan, up to 24 mg kg^{-1}) and coastal waters (the Sancti Petri channel,[16] Spain, up to 67.6 mg kg^{-1}) but, as also is found in the Guadalete River, a few metres away from the sewage discharge outlet concentrations are again at background values (1 mg kg^{-1} or less).

Thus, because concentrations of surfactants are easily measurable and their source can be identified with high specificity, they serve as excellent markers of sewage pollution; they allow us to locate the two main urban sources of contamination in the middle stretch of the Guadalete River. The first (station 7) corresponds to the mouth of the Salado Stream (Fig. 2), which collects untreated wastewaters from urban areas, farms and cottages surrounding the city of Jerez de la Frontera. The second "hot spot" (station 9) turned out to be adjacent to the place where untreated wastewater discharges from Jerez de la Frontera had taken place until the year 1994, when the current STP (located between stations 11 and 12) became operational. Nowadays, however, there are still occasional discharges of untreated sewage into the river when spring and fall rainfalls exceed the capacity of the treatment plant, a fact that would explain the high concentrations detected at station 9. Another "hot spot" was detected at sampling station 12, where concentrations of 12.8 and 12.2 mg kg^{-1} of LAS and AEOs, respectively, were measured in surface sediments[16] obtained from close to the STP discharge outlet (Fig. 2).

Table 1 shows surfactant concentrations in water samples taken at selected sampling stations (Fig. 2). In general terms, these values were below 37 and 5 μg L^{-1} for anionics (LAS and AES, respectively) and below 12 and 2 μg L^{-1} for non-ionics (AEOs and NPEOs). Concentrations reported here are comparable to those previously measured for these compounds in the estuary of the same river[16] and in other aquatic ecosystems in Europe,[12,13,18,32,33] the United States[23,29–31,33] and Canada.[32] At sampling station 7, however, where the Salado Stream flows into the river, notably higher concentrations of AES (72 μg L^{-1}), AEOs (49 μg L^{-1}) and LAS (2766 μg L^{-1}) were measured in the water (Table 1), and 89.4 mg kg^{-1} of LAS were detected in surface sediments here (Fig. 3). AES concentration at station 7 (72 μg L^{-1}) was significantly higher than values previously reported elsewhere[28,29] for this surfactant, and may be related to a combination of the high solubility of this chemical and the urban nature of the wastewater discharged (AES is one of the main active ingredients in shampoos and many other personal care products). AEO values are also certainly among the highest ever reported in the literature,[18,30–33] usually between 0.1 and 37 μg L^{-1}, which is especially significant taking into account their relatively low aqueous solubility (log K_{ow} up to 6.69 depending on the homologue considered). The occurrence of this chemical in the sampling area may be related to its heavy use in household detergents. Conversely, the use of NPEOs was banned for household applications a few years ago in the EU; this would explain the low values (1 μg L^{-1} or less) found compared to the other surfactants, and the absence of a maximum at sampling station 7. On the other hand, as stated above in respect of LAS in surface sediments, LAS levels of the same order of magnitude in water have only been detected before in heavily polluted areas subjected to untreated wastewater discharges. For example, LAS concentrations occasionally exceeded 1500 μg L^{-1} in the central part of the Sancti Petri channel (SW Spain) due to the existence of an urban effluent discharge outlet from a city of 100 000 inhabitants.[10] The steep drops measured in the concentration of target compounds downstream of this station can be explained

Table 1 Concentrations (μg L^{-1}) of anionic (LAS and AES) and nonionic (AEOs and NPEOs) surfactants, and SPCs, in water samples

Station	1	2	7	8	12
AES	5 ± 1	5 ± 1	72 ± 10	4 ± 0	5 ± 1
AEOs	8 ± 1	12 ± 0	49 ± 1	9 ± 0	5 ± 1
NPEOs	0.4 ± 0.1	0.2 ± 0.1	1.2 ± 0.3	0.7 ± 0.0	1.8 ± 0.6
LAS	27 ± 1	15 ± 0	2766 ± 168	37 ± 2	31 ± 1
SPCs	166 ± 24	164 ± 9	225 ± 32	234 ± 12	487 ± 40

not only by dilution but also by the contribution of biological and physicochemical processes, such as biodegradation. In this context, LAS degradation intermediates (SPCs) were identified in the water column reaching above 100 µg L^{-1}. In the case of NPEOs, their polar metabolites (NPECs) were not detected. There is more discussion of this topic in the following section.

3.2. Differential reactivity of synthetic surfactants: sorption and degradation processes

Previous studies[1,44] have identified sorption and biodegradation as two of the main processes responsible for removing surfactants and other organic pollutants from surface waters. In general terms, sorption to the particulate phase (*i.e.* suspended solids, sediments) is enhanced due to the hydrophobic moiety of surfactant molecules, which usually consists of an alkyl chain having a variable number of C atoms. Average sediment-water distribution coefficients (K_{sw}) have been calculated for each of the surfactants and samples, and then plotted (Fig. 4) against average octanol–water partition coefficients[2,3,5] (K_{ow}) for LAS, AES, NPEOs and AEOs. Several differences can be observed, depending on the surfactant considered. First, the anionics (LAS and AES) have lower sorption capacities than the non-ionics (NPEOs and AEOs) due to their lower hydrophobicity ($\log K_{ow} < 3$), since they are charged compounds. There is a wider range of K_{ow} values (Fig. 4) for those surfactants such as AES and AEOs that contain not only one (an alkyl chain) but two (an ethoxylated chain) moieties that are of variable compositions. These chemicals are often sold as complex mixtures of homologues and ethoxymers (sometimes accounting for more than 100 individual compounds). In most cases, however, sorption is determined by hydrophobic mechanisms influenced only by the length of the alkyl chain. As can be seen in Fig. 4, this is confirmed in our case as the increase of average K_{sw} values is directly proportional to the increase in average K_{ow} values.

Differential sorption can be also observed by examining the patterns of surfactant homologues. Fig. 5 shows the average homologue composition of LAS, AES and AEOs in sediment and water samples, as well as the composition of standards used for quantification purposes. In general terms, there is preferential sorption on sediments of those homologues having longer alkyl chains (*e.g.*, C_{13}LAS, C_{16}AES, C_{18}AEO), whereas the aqueous phase is enriched in the more polar homologues of short alkyl chain. This trend is clearly shown in Fig. 5 for LAS, and has been previously reported in other freshwater and also marine environments.[10] Further differences could be observed between AES and AEOs and their respective homologue distributions. In the case of AES, only those homologues with an even number of carbon atoms (C_{12}, C_{14} and C_{16}) in their alkyl chains were detected, whereas the presence of both odd- and even-carbon numbered AEO homologues was observed in every sample. Previous studies[16,19,28] have described similar homologue patterns; the reason for this is that AES, AEOs and many other aliphatic surfactants and organic compounds can be derived from both vegetable/animal fatty acids (a mixture of even-carbon numbered linear homologues) and petrochemical feedstocks (containing odd- and even-carbon numbered homologues). Thus, it should be noted that the AES detected in the sampling area mainly came from the first type of source, although some traces of C_{13} and C_{15}AES (petrochemical homologues) have been found occasionally.[28] On the other hand, AEOs detected probably came from both sources.

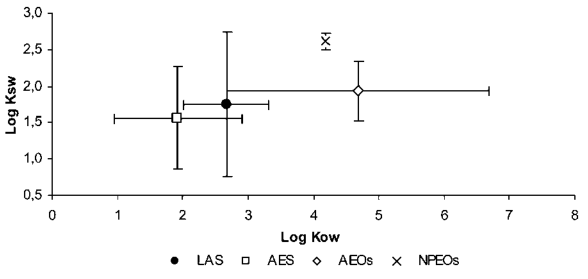

Fig. 4 Average log K_{sw} (sediment-water partition coefficient) *vs.* average log K_{ow} (octanol–water partition coefficient) for each surfactant.

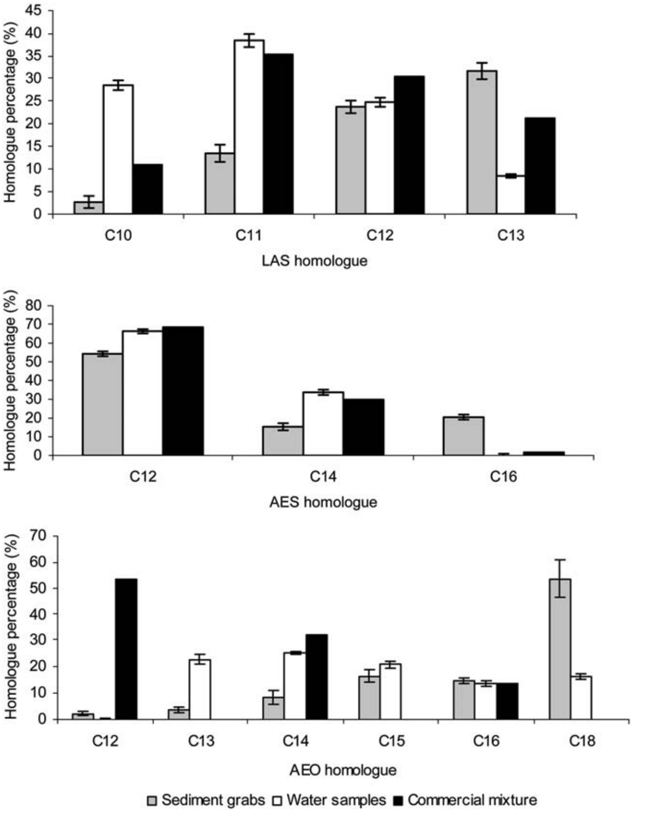

Fig. 5 Average surfactant homologue distributions (%) in sediments, water samples and commercial mixtures.

Degradation, another of the main processes involved in the behaviour and fate of surfactants in the aquatic environment, has been considered here through the study of sulfophenyl carboxylic acids (SPCs, Fig. 1e), biodegradation products resulting from LAS oxidation. These intermediates were mostly found in the aqueous phase, as can be expected considering their high polarity and poor sorption capacity[45] (sorption coefficients $K_d < 8$). Table 1 shows SPC concentrations in water samples taken at selected stations. Their values range between 164 and 487 µg L^{-1}, close to the levels reported in previous studies[8,9,11,17,46] for these compounds, and are notably higher than the rest of the concentrations determined for surfactants (Table 1). The lowest SPC and LAS concentrations (164 and 15 µg L^{-1}, respectively) were observed at sampling point 2 and upstream, where LAS levels are less than one-tenth those of SPCs. Such a relatively large difference in terms of concentration implies that most of the LAS in the water course is residual, and has been or is being actively biodegraded during its transport down the river. The highest SPC values (>200 μg L^{-1}) were found downstream of sampling station 7, which is the place where the Salado Stream joins the river and has been recognized as one of the two "hot spots" commented on in the previous section resulting from the occurrence of untreated sewage discharges (the LAS concentration found in water here is more than 2000 μg L^{-1}). A maximum in SPC concentrations (487 μg L^{-1}) was detected at station 12, within 1 km from the current STP discharge outlet. In contrast to what was observed at station 7, previously treated sewage is discharged here, which is reflected in the relatively low LAS value (31 μg L^{-1}; this is one order of magnitude lower than the SPC concentration at the same point) because most of the surfactant has been already removed in the treatment plant.

The biodegradation pathway of C_{10-13}LAS homologues in surface waters is *via* the generation of SPCs from the initial ω-oxidation of the alkyl chain (C_{10-13}SPCs are formed as primary degradation products) and its progressive shortening[47] by successive α- and β-oxidations (C_{6-9}SPCs appear as secondary products) until the mineralization of LAS is completed. Fig. 6a shows the concentration of SPC homologues measured at selected sampling stations. Upstream, C_{6-9}SPC levels are higher than those for long chain SPCs and LAS homologues, indicating that the surfactant degradation is entering its final phase before mineralization. The distribution and relatively higher abundance of these short-chain SPC homologues have been previously reported in different aquatic ecosystems affected by LAS contamination.[8,47,48] Downstream (from station 7 to 12), however, the SPC pattern changes and there is an enrichment in longer SPC homologues (C_9SPC and higher) that is rarely observed in aquatic systems because these compounds are very easily and quickly biodegraded. As discussed above, sewage sources are located close to sampling stations 7 and 12. We can confirm that wastewaters that are being discharged at both stations are, respectively, untreated and treated by comparing LAS and SPC homologue distributions and concentrations. It can be stated that untreated wastewater discharges take place at station 7 because: (a) LAS levels are unusually high (>2 ppm) (Table 1); (b) LAS homologue distribution (17% C_{10}LAS, 34% C_{11}LAS, 29% C_{12}LAS and 20% C_{13}LAS) here is closer to that commonly found in commercial mixtures (there is only a 1–6% deviation) rather than in the rest of sampling stations (Fig. 5); and (c) LAS degradation is at a very early stage since only very reactive C_9 to C_{13}SPCs can be detected. On the other hand, it can be stated that sewage is treated at the STP and discharged into the river near station 12 because: (a) the highest concentration of SPCs is reached at this point, whereas LAS values are ten-times lower; (b) LAS homologue distribution (34% C_{10}LAS, 36% C_{11}LAS, 21% C_{12}LAS and 9% C_{13}LAS) in water samples is severely biased towards C_{10} and C_{11}LAS as the relatively more hydrophobic and biodegradable longer chain LAS homologues are preferentially removed at the STP by sorption and degradation processes; and (c) the SPC pattern at this station (high concentrations of most SPC homologues) can be explained as a combination of *in situ* biodegradation in surface waters and the effect of the STP effluent.

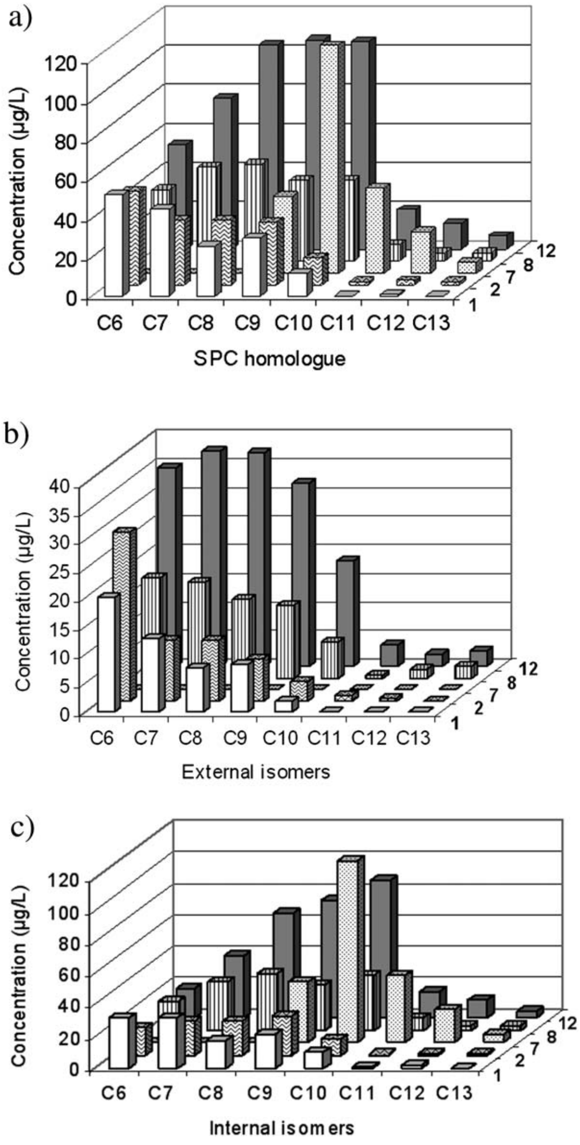

Fig. 6 SPC concentrations in water samples sorted by: (a) homologues, (b) external isomers and (c) internal isomers.

Finally, we have made a deeper study of the degradation of LAS and SPCs by considering the different isomers that are part of every homologue. Isomers were classified into two groups according to their separation by the HPLC column and depending on whether the benzene sulfonic group is attached to the alkyl chain. Commonly the isomers of LAS are expressed as $m\Phi C_n$LAS, where m and n denote the site of the sulfophenyl group link with the alkyl chain ($m = 1$ indicates the C atom at the end of the alkyl chain which is the nearest to the sulfophenyl group) and the length of the alkyl chain respectively (*e.g.*, $2\Phi C_{10}$LAS, $3\Phi C_{10}$LAS and the SPCs derived from their degradation should be considered as external isomers whereas $4\Phi C_{10}$LAS, $5\Phi C_{10}$LAS and SPCs associated to these isomers correspond to internal isomers). Fig. 6b and 6c show the concentrations of both types of SPC isomer, sorted by homologue and sampling station: different patterns can be observed. For external isomers the highest values are reached by short chain C_{6-9}SPC homologues, showing an average length of the alkyl chain of 7.6. The reason for this is that LAS external isomers (and, therefore, the SPCs derived from them) are preferentially biodegraded over internal isomers, due to steric impediments caused by the benzene sulfonic group the closer it is to the terminal carboxylic group in the alkyl chain.[47–49] The same impediment also explains why shorter SPC homologues are degraded more slowly than longer ones, so the concentrations of the first are usually higher in environmental samples. These differences in biodegradability are further confirmed by looking at Fig. 6c and confirming that the average length of the alkyl chain for internal SPC isomers is higher than that for externals (8.3 *vs.* 7.6, respectively); this difference is even clearer at sampling stations directly subjected to wastewater discharges (7 and 12), where the chain length values for internal isomers exceed 10.

4. Conclusions

This study has discussed the distribution and reactivity of the world's most widely used anionic (LAS and AES) and non-ionic (NPEOs and AEOs) surfactants detected in the middle stretch of the Guadalete River (SW Spain). The occurrence and changes in the concentration of surfactants are clearly correlated with the presence of wastewater discharges in such a way that they can be used as effective tracers for monitoring sources of river contamination by sewage. Not only can the location of these sources be identified by observing the presence of maxima in longitudinal concentration profiles along the course of the river, but a detailed analysis of surfactant homologue distributions and determination of degradation products (*e.g.* SPCs) also allows an evaluation of the degree of treatment (if any) applied to that wastewater prior to its release into the aquatic environment. In this context, LAS is especially suitable for distinguishing among different types of urban wastewaters. Partitioning between water and sediment depends mainly on the hydrophobic character of surfactant molecules and their sorption capacity, both properties being higher in line with increasing length of the alkyl chains of LAS, AES and AEO homologues. Biodegradation has been studied by analysis of SPCs (LAS metabolites) in the aqueous phase. Sampling stations located upstream showed very high concentrations of short chain SPC homologues, indicating that the degradation of LAS by oxidation and progressive shortening of the alkyl chain has entered its final stage before mineralization. Downstream, however, the presence of sewage sources has increased the values of very reactive long chain SPC homologues and LAS concentrations, because the degradation process is just starting. We have also reported here, for the first time in a river, how steric impediments can affect the progress of LAS degradation. For this we have analyzed changes in concentrations of SPC internal and external isomers along the course of the river: those isomers defined as "internals" show higher concentrations in the environment as their biodegradation is retarded, while more biodegradable "external" isomers can only be found in short-chain SPC homologues, due to their higher reactivity.

Acknowledgements

We express our gratitude to Erkuden Pérez-Carrera for her assistance during the sampling and to Emiliano M. Gómez (SCCYT UCA) for his technical support with the LC/MS system. This study has been supported by the CICYT (CGL2008-05598) and Marie Curie FP7 BADEPAS (ref. 221686) R&D Projects, and with the help of a FPI fellowship from the Spanish Ministry of Education and Science.

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