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Application of liquid chromatography–electrospray-tandem mass spectrometry for the identification and characterisation of linear alkylbenzene sulfonates and sulfophenyl carboxylates in sludge-amended soils

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Abstract

A novel procedure was developed for the simultaneous determination of linear alkylbenzene sulfonates (LAS) and their major metabolites, sulfophenyl carboxylates (SPC), in sludge-amended soil. After pressurised liquid extraction with methanol/water (90:10) and a clean-up on C_{18} solid-phase extraction cartridges, final analysis was done by ion-pair liquid chromatography–electrospray–tandem mass spectrometry (LC–ESI–MS/MS). With this method, SPC with 5–13 carbon atoms in the aliphatic side chain were identified for the first time in agricultural soils treated with sewage sludge. Quantification of LAS and SPC in soil from 10 field sites, which differed in the history of sludge application, gave total concentrations of 120–2840 $\mu g\,kg^{-1}$ for LAS and of 4–220 $\mu g\,kg^{-1}$ for SPC. The data provided evidence for rapid biodegradation of LAS in the initial phase after sludge amendment with a transitory build-up of high concentrations of, mainly, short-chain SPC. Trace amounts of residual LAS and SPC were detected in soils having received the last sludge treatment 10 days to 4 years prior to sampling.

Keywords: Surfactants; Linear alkylbenzene sulfonates; Sludge-amended soil; Sulfophenyl carboxylates; Electrospray-tandem mass spectrometry

1. Introduction

In the Member States of the European Union the agricultural use of sewage sludge is, along with disposal to landfills, the most popular disposal route. Of the ca. 8 million tonnes (dry matter; dm) of sludge produced in European wastewater treatment plants every year, the percentage of reused sludge varies largely between the nations; for the year 2005 the reuse rates of sewage sludge for the most populated countries are predicted to reach levels as high as 50% in Germany, 54% in Spain, 65% in France and 71% in the United Kingdom [1]. Despite the valuable properties of sewage sludge, such as relatively high levels of organic matter and essential plant nutrients, the widespread application of sewage sludge in agriculture needs to be critically evaluated in view of the concomitant presence of a variety of inorganic and organic contaminants that may adversely affect crops and/or soil organisms [2]. Furthermore, the potential leaching of these contaminants into the subsoil may pose a threat to ground water supplies [3], which represent an important source of drinking water in Europe.

Against this background, the European Commission elaborated a draft of a "Working Document on Sludge" [4] to promote the use of sewage sludge in agriculture whilst improving the safety and harmonizing quality standards. This draft proposes cut-off limits for a series of organic micropollutants among which figure the anionic linear alkylbenzene sulfonate surfactants (LAS; Fig. 1). These surface-active substances are listed as a restricted compound in the draft document (cut-off value for agricultural use was set to 2600 $mg\,kg^{-1}$) due to their presence at high levels in sludge. In aerobic sludge concentrations have been reported to be in the range 100–500 $mg\,kg^{-1}$ [5–7], but in anaerobically digested sludge reported levels were between 1000 and 20,000 $mg\,kg^{-1}$ [6,8,9] with some exceptionally high values

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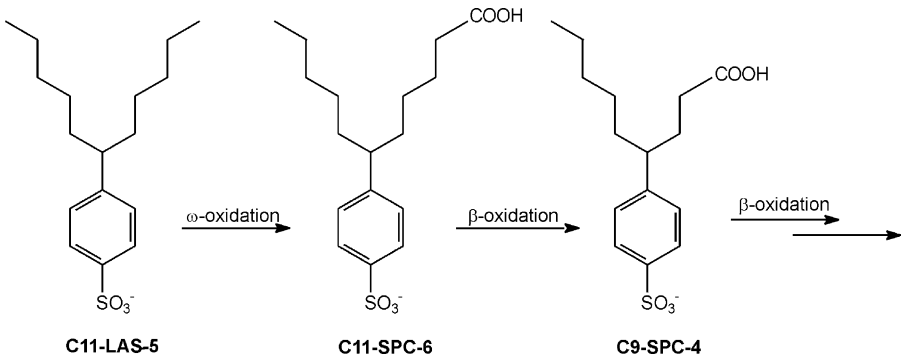

Fig. 1. Scheme illustrating microbial degradation of C11-LAS into C11-SPC and further to C9-SPC. Number after compound acronym indicates position of the sulfophenyl moiety on the carbon chain.

of up to 52,000 mg kg^{-1} [10]. In the latter sludge type, LAS concentrations are substantially higher compared with the aerobic sludge as the aromatic sulfonates do not biodegrade to a substantial extent under anaerobic conditions.

The first methods for the determination of LAS components—comprising the four alkyl homologues C10–C13, each of which with its positional phenyl isomers—in samples of sludge-amended soils date back to the late 1980s [5,11,12]. In these studies, Soxhlet or reflux extraction with methanol, clean-up on solid-phase extraction cartridges (reversed-phase (RP) or strong anion-exchange material) and analysis by high-performance liquid chromatography (HPLC) equipped with ultraviolet (UV) or fluorescence (FL) detectors were employed. By the mid-1990s, a series of methods had been published dealing with the occurrence, distribution and biodegradability of LAS in soils. These studies reported LAS levels in soil samples of about 50–150 mg kg^{-1} [5,13,14] directly after sludge application, which thereafter dropped rapidly within a few weeks to concentrations in the low mg kg^{-1}/high μg kg^{-1} range (limits of detection (LOD) achieved with UV or FL detectors typically between 0.2 and 1.0 mg kg^{-1}). Under field conditions, half-lives of LAS in soils were calculated to be 7–33 days [5,15]. This ready biodegradability and also the potential for ultimate mineralisation were corroborated by laboratory data gathered from studies on ^{14}C-LAS [16–18]. In the recent past, and in particular since the release of the EU "Working Document on Sludge" [4], the question on the whereabouts [2,19–21] and on possible risks [23–28] of LAS in soils has received further attention with studies performed by both industrial and academic researchers. Despite the large body of literature dealing with the fate and behaviour of LAS in sludge-amended soils—a comprehensive review on this subject is given in [29]—a gap of knowledge still exists with respect to the identity and occurrence of their degradation products in the terrestrial environment. It has been known for almost 40 years [30] that aquatic microbial communities biotransform LAS into sulfophenyl carboxyates (SPC; Fig. 1), via a mechanism initiated with an ω-oxidation of the alkyl side chain followed by a series of β-oxidations which result in the formation of a complex mixture of SPC homologues each of which with its positional phenyl isomers (Fig. 1) [30–32].

With the advent of sophisticated and robust mass spectrometric instruments equipped with atmospheric pressure ionisation interfaces the analysis of polar organic compounds became possible, and the identification of LAS degradation intermediates at trace amounts was accomplished in wastewaters [33], river waters [34,35], coastal waters [36] and even in drinking waters [37,38]. But as yet the presence of SPC in sludge-amended soils has not been confirmed, nor could other metabolites be determined in natural soils (though specific LAS-degrading soil bacteria cultured under laboratory conditions were identified [39,40]). This can be traced to the lack of sensitive and selective methods for identifying SPC in such complex matrices, while the lack of authentic standards has posed a further problem for a reliable quantitative analysis.

Thus, the objectives of the present work were: (i) to develop a sensitive analytical protocol for the simultaneous determination of LAS and SPC in sludge-amended soils employing pressurised liquid extraction (PLE); (ii) to unequivocally identify and characterise SPC by means of electrospray–tandem mass spectrometry (LC–ESI–MS/MS); (iii) to compare environmental concentrations of both sulfonated compound classes in soil samples from agricultural fields differing in the history of sewage sludge application.

2. Experimental

2.1. Standards and reagents

HPLC-grade 'Suprasolv' solvents water, acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Acetic acid (96%, puriss.) and hydrochloric acid (p.a.) were from Merck, triethylamine (>99.5%, puriss.) from Sigma–Aldrich (Madrid, Spain). Ethylenedinitrilo tetraacetic acid, di-sodium salt (EDTA–Na_2; p.a.) was obtained from Boehringer Mannheim (Mannheim, Germany). Commercial LAS with low dialkyl tetralinsulfonate content (<0.5%)

were supplied by Petroquímica Española S.A. in a single standard mixture with the proportional composition of the different homologues as follows: 3.9% C10, 37.4% C11, 35.4% C12 and 23.1% C13. The 5-(4-sulfophenyl)-valerate, C5-SPC-1, (>98%) was kindly supplied by Dr. Ventura (AGBAR, Barcelona, Spain), the 10-(4-sulfophenyl)-undecanoate, C11-SPC-2, (>98%) was a gift from Prof. González-Mazo (University of Cádiz, Spain). Stock solutions of LAS and SPC standards were prepared at a concentration of 1 μg μL^{-1} by dissolving adequate amounts of the solids in methanol. Aliquots of stock solutions were used to prepare dilution series in water/acetonitrile (95:5) containing 5 mM of the ion-pairing agent (IPA) acetic acid/triethylamine.

2.2. Site description and sampling

Soil samples were collected on August 19 and 20, 2002 at 10 selected sites in the province of Barcelona (Catalonia) in the Northeast of Spain (Table 1). At all sites, used for cultivation of winter crops, sludge had been applied at least once since 1997 when its application began to be controlled and managed by a company adjusting the origin and quantity of sewage sludge for application to the nutrient requirements of the crop to be cultivated in the next growing period. The sludge application was generally carried out in September/October at a rate of between 8 and 20 tonnes (dm) per hectare. After spreading of the sludge, the soil was ploughed to a depth of between 15 and 20 cm. The soil types were typically calcareous with a low to moderate content of organic carbon (Table 1). Representative sampling of the topsoil (0–20 cm) was performed with an Auger sampler (Eijkelkamp, Giesbeek, The Netherlands) at a frequency of approximately 10 subsamples per hectare. Individual subsamples were wrapped in aluminium foil and placed in plastic bags for transport to the laboratory. Subsamples from the same field were pooled and freeze-dried. Moisture content of the fresh field samples was calculated by backweighing the dried samples. Average water content was 8.0% with a range from 5.0 to 12.9%. In order to reduce contamination of the samples by LAS during sample handling to a minimum, the use of cleaning agents in the laboratory was strictly avoided. All glassware was backed at 400 °C overnight.

2.3. Sample extraction and clean-up

Prior to extraction the samples were sieved (<2 mm) and homogenised in a mortar. A 10 g-sample of sludge-amended soil, mixed with pre-washed diatomaceous earth, was loaded into 11 mL cells and extracted with methanol/water (90:10) using a PLE device (Dionex ASE® 200; Sunnyvalley, CA, USA). The extraction cell was maintained at 100 bar and 120 °C during the extraction (three cycles of 5 min each). The collected extract of about 22 mL was supplemented with 9 mL EDTA–Na_2 (3%) to prevent precipitation of insoluble calcium salts of LAS, and the organic solvent was completely removed using rotary-evaporation apparatus. To the remaining aqueous residue, 100 mL of HPLC grade water was added and the pH adjusted to 3.0 with 10% HCl. This solution was then sucked at a rate of ∼3 mL min^{-1} through a solid-phase extraction cartridge containing 500 mg LiChrolut RP-C_{18} material (Merck; Lot. L649631) previously conditioned with 2 × 3 mL methanol and 2 × 3 mL water (pH 3.0). After drying the cartridge, it was eluted with 3 × 2 mL methanol and the eluate reduced to dryness under a gentle stream of nitrogen. The residue was reconstituted in 1 mL water/acetonitrile (95:5) containing 5 mM IPA, and filtered through a disposable filter holder (Schleicher & Schuell, Dassel, Germany) prior to injection into the HPLC. Procedural blanks (N = 3)

Table 1
History of sludge application and soil characteristics at investigated sites in the province of Barcelona, Spain

	Fonollosa[a]	Sabadell[b]	Castellcir[c]	Calonge[d]	Pujalt-N[d]	Pujalt-C[d]	Pujalt-SD[d]	Castellvell[c]	Montseny[c]	St. Joan[b]
Year of application										
1997	MND	MND	MND	MND	MND	–	–	–	–	–
1998	MND	MND	MD	MND	MND	MND	MND	–	–	–
1999	–	MND + P	MND	MND	MND	MND	MND	MD + P	–	–
2000	–	MD + P	MD	MD	MD	MD	MD	MD + P	MD + MND	–
2001	–	MD + P	MND	MD	MD	MD	MD	MD + P	MD	–
2002	–	–	–	–	–	–	–	–	–	MD[e] + P[e]
Soil parameter										
Sand (%)	37.3	30.9	30.2	27.1	28.1	18.8	18.8	51.2	63.4	34.1
Lime (%)	42.1	44.4	50.2	49.7	47.9	56.8	55.1	36	25.7	49.1
Loam (%)	20.6	24.7	19.6	23.2	24.0	24.4	26.1	12.8	10.9	16.8
C_{org} (%)	1.2	2.0	3.4	3.5	3.5	1.0	2.6	0.9	1.8	1.1
pH	8.2	8.1	8.4	8.0	8.3	8.5	8.2	8.6	7.1	8.0

MD: municipal sewage sludge, digested; MND: municipal sewage sludge, not digested; P: paper sludge (all data provided by sludge-administrating company).
[a] Bages.
[b] Administrative area ('comarca') of Vallés Occidental.
[c] Vallés Oriental.
[d] Anoia.
[e] Sludge spreading on August 10, 2002.

consisting of diatomaceous earth were extracted along with each batch of real samples.

2.4. Liquid chromatography

Analyses were performed on a Waters 2690 series Alliance HPLC (Waters, Milford, MA, USA) with a quaternary pump. Separation was performed at ambient temperature (22 °C) on a Merck Superspher 60 RP-select B column (125 mm × 2 mm, 5 μm particles) under a gradient elution of mobile phase A (water + 5 mM IPA) and mobile phase B (acetonitrile + 5 mM IPA) at a flow rate of 200 μL min^{-1} (t = 0 min, 95% A; t = 2 min, 95% A; t = 17 min, 20% A; t = 21 min, 20% A; t = 22 min, 95% A for 16 min). The sample injection volume was set at 50 μL. During the first 1.5 min of each run the LC stream was directed to the waste and then re-directed without post-column splitting into the ionsource by switching an instrument-integrated six-port valve. Once the MS data acquisition completed (22 min) the valve was switched back to the initial position (see next section).

2.5. Mass spectrometry

A bench-top triple quadrupole mass spectrometer Quattro LC from Micromass (Manchester, UK) equipped with a pneumatically assisted electrospray probe and a Z-spray interface was used for the MS/MS analyses. Nitrogen gas (99.999% purity) was used as the desolvation and cone gas, and argon as the collision gas (3.6×10^{-5} mbar). For MS/MS optimisation of C6-SPC through C10-SPC as well as of C12- and C13-SPC, extracts of biologically treated wastewater, prepared according to [34], were analysed chromatographically applying distinct collision energies at intervals of 5 units for virtually identical multiple reaction monitoring (MRM) transitions in the same run. All data were acquired and processed using Masslynx V4.0 software. Quantitative LC–MS/MS analyses were carried out in multiple-reaction monitoring (MRM) mode. The ESI–MS/MS was operated in negative ion mode with the following instrument settings: capillary voltage: 2.8 kV; source temperature: 120 °C; desolvation temperature: 350 °C; desolvation gas flow rate: 600 L h^{-1}; cone gas flow rate: 50 L h^{-1}; cone voltage: 30 V; extractor: 7 V; RF lens: 0.6 kV; resolution: 12.0; ion energy: 1.0 V; entrance: −2; exit energy: 1; multiplier: 650; inter-channel delay 0.02 s; interscan delay: 0.02 s. The retention time windows are given in Table 2 along with the transitions monitored. Primary transitions were used for quantification and the secondary transitions for confirmation.

2.6. Method validation

Soil samples from Calonge and Castellvell (Table 1) were spiked in triplicate with an LAS mixture (500 μg kg^{-1}) and the two SPC standards (50 μg kg^{-1} each) for recovery experiments. After 24 h of equilibration, the fortified samples were extracted with the above described extraction protocol and subsequently subjected to the clean-up procedure. Analysis by LC–ESI–MS/MS gave recoveries of 71–85% for the LAS homologues (standard deviation: 7.9–14%) and between 52 and 68% for the SPC homologues (7.3–18%). Calibrations curves were constructed by injecting standard solutions in the range from 10 to 2500 pg μL^{-1} (10, 20, 50, 100, 200, 500, 1000, 2500 pg μL^{-1}). Linearity for all of the six individual reference compounds (C10 to C13-LAS, C5- and C11-SPC) was achieved in this concentration range with correlation coefficients of $R \geq 0.996$. The two early eluting C5- and C6-SPC were quantified based on the C5-homologue, while C11-SPC was used as reference compound for the higher homologues recorded in the second time window. The limits of quantification (LOQ) for the entire method were calculated

Table 2
Retention time windows and MS/MS transitions monitored (collision energy)

1.5–8.0 min		8.0–16.0 min		16.0–22.0 min	
C5-SPC	m/z 257 > 170 (20) m/z 257 > 183 (30) m/z 257 > 80 (55)	C7-SPC	m/z 285 > 183 (30) m/z 285 > 119 (50)	C10-LAS	m/z 297 > 183 (35) m/z 297 > 119 (50)
C6-SPC	m/z 271 > 183 (30) m/z 271 > 80 (55)	C8-SPC	m/z 299 > 183 (30) m/z 299 > 119 (50)	C11-LAS	m/z 311 > 183 (35) m/z 311 > 119 (50)
		C9-SPC	m/z 313 > 183 (30) m/z 313 > 119 (50)	C12-LAS	m/z 325 > 183 (35) m/z 325 > 119 (50)
		C10-SPC	m/z 327 > 183 (30) m/z 327 > 119 (50)	C13-LAS	m/z 339 > 183 (35) m/z 339 > 119 (50)
		C11-SPC	m/z 341 > 183 (35) m/z 341 > 119 (50)		
		C12-SPC	m/z 355 > 183 (35) m/z 355 > 119 (50)		
		C13-SPC	m/z 369 > 183 (35) m/z 369 > 119 (50)		

by using a signal-to-noise ratio (S/N) of 10. For the studied SPC, the LOQ varied from 0.3 to 0.8 $\mu g\,kg^{-1}$ with highest values for the short-chain C5- and C6-SPC. (The response factor of C5-SPC was about three times lower than that of C11-SPC.) The method quantification limit for the anionic surfactant could not be calculated owing to LAS contamination in procedural blanks corresponding typically to a level of about 25 $\mu g\,kg^{-1}$. Concentrations of LAS determined in real samples were corrected by the blank value. Sensitivity of LAS detection, however, was not an issue as the environmental concentrations measured in the soils were so high that five-fold dilution of the samples (St. Joan: 25-fold) was necessary prior to injection so as to be in the linear range of the calibration curve. The robustness of the LC–MS method was verified by injecting the dilution series at the beginning and the end of each sequence. The decrease in signal intensity was always less than 10% for all reference compounds.

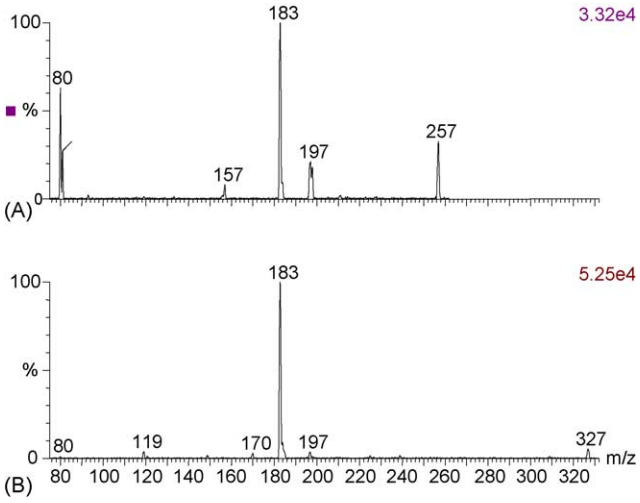

Fig. 2. (−)-ESI–MS/MS daughter ion spectra of (A) C5-SPC (m/z 257) and (B) C10-SPC (m/z 327) in extract of soil sample from St. Joan. Spectra were recorded using collision energy of 20 and 35, respectively (arbitrary units).

3. Results and discussion

3.1. Analytical method

In present work we describe the first analytical method based on LC–ESI–MS/MS for the quantitative determination of LAS, including their major degradation products SPC, in samples of sludge-amended soils. Extraction of the soil samples was carried out employing PLE. This technique offers a series of advantages over conventional extraction techniques (ultrasonication or Soxhlet extraction) such as high degree of automation, rapid extraction, low solvent consumption and good extraction efficiencies. The PLE has already been successfully used for recovering LAS from solid environmental samples like soil [41] and riverine sediment [42–44]. In the present study satisfactory recoveries were achieved for all target analytes ranging between 52 and 85% with relative standard deviations of $\leq$18% ($n = 3$).

The use of a sophisticated detection technique providing both high sensitivity and selectivity was of the utmost importance in view of the requirement to detect and identify SPC in environmental samples of high complexity, at concentrations expected to be markedly lower than those of the parent compounds. A tandem MS equipped with an ESI interface was the instrument of choice. Owing to the very polar nature of SPC, a sufficient chromatographic retention on an RP column entailed the use of an IPA fulfilling the requirement of being compatible with the ESI interface. Equimolar amounts (5 mM) of acetic acid and triethylamine had proved to enable retention of even the short-chain (sc)-SPC, C5 and C6 [34]. The chromatographic separation achieved occurred at the expense of sensitivity, since the IPA adversely affected the ionisation process [45], reducing, e.g. the signal intensity of LAS by a factor of eight. In order to reduce this undesirable effect of the IPA, lower concentrations in the mobile phase were tested, but it was observed (at 2 mM IPA) that the retention times of the SPC shifted irreproducibly. Moreover, peak broadening was observed for the carboxylated compounds. For these reasons an IPA concentration of 5 mM was maintained despite its detrimental effect on the sensitivity.

3.2. Mass spectrometric identification of SPC

Through the acquisition and interpretation of (−)-ESI–MS/MS daughter ion spectra of the selected SPC parent ions, the homologues C5–C13 (parent ions: m/z 257, 271, 285, 299, 313, 327, 341, 355 and 369) could be unambiguously identified. The spectra of C5-SPC and C10-SPC are shown in Fig. 2. The fragmentation pattern are in agreement with those of previously gathered spectra that were recorded on a distinct mass spectrometer (with ESI source) analysing aqueous samples of a degradation experiment with C12-LAS under controlled laboratory conditions [31]. In Fig. 2 the most abundant signal in both spectra, detected at m/z 183, corresponds to 4-styrene sulfonate. Other characteristic fragments are assigned to 4-styrene phenolate at m/z 119 and $[SO_3]^-$ at m/z 80. As all of the LAS and SPC homologues produced the ion m/z 183 with high intensity, the ion transition $[M - H]^- > m/z$ 183 was monitored in quantitative analysis, operating the MS in the very sensitive and highly selective MRM mode. Under these conditions the analysis of a soil extract yielded the LC chromatogram given in Fig. 3. Undisturbed baselines enabled the unequivocal identification of the different SPC homologues numbered consecutively from 5 through 13 according to the carbon atom number in the alkyl chain. As each homologue is composed of a series of phenyl isomers, a set of signals rather than a single peak is obtained for a homologue group. These are best resolved in the case of C6-SPC, with four identifiable components (arrows pointing at minor components). With increasing chain length the number of possible isomers increases notably, thus the closely eluting isomers form a ‘bunch’ of signals.

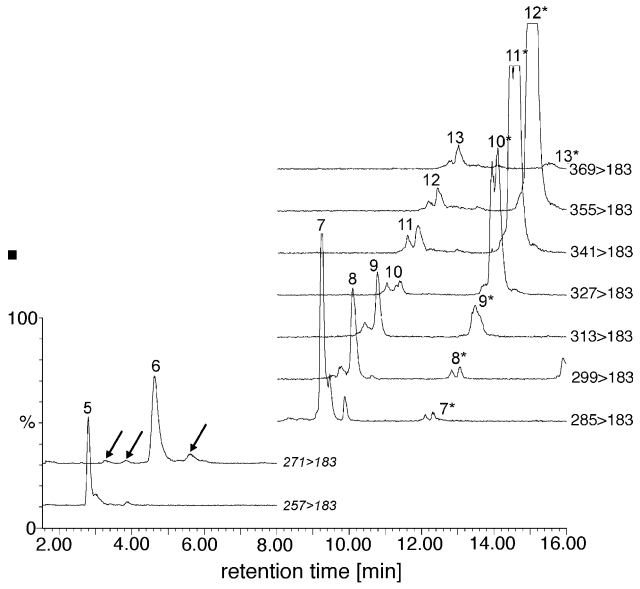

Fig. 3. (−)-LC–ESI–MS/MS chromatogram showing elution of SPC in the sample taken at St. Joan site (extract five-fold diluted). Peak assignment according to number of carbon atoms in oxidised alkyl chain. Signals marked with asterisks (n^*) are unknown compounds (see text). All mass traces are at the same scale (*y*-axes are linked).

In the mass traces corresponding to the homologues C7- to C13-SPC a second series of peaks with $\Delta m/z = 14$ appears (masses of parent ions confirmed by acquiring full scan spectra), marked in the Fig. 3 with asterisks, at retention times of about 3 min after the identified SPC. In this series the peaks 11^* and 12^* represent the most intensive signals. Attempts were made to identify these compounds through their (−)-ESI daughter ion mass spectra, but these were qualitatively identical to those of SPC. From the mass spectrometric information gained and the chromatographic behaviour observed, it can be speculated that: (a) they contain alkyl homologues ($\Delta m/z = 14$) apparently structurally related to LAS and SPC as they yield the fragment *m*/*z* 183 characteristic of both compound classes; (b) they were of less polar nature than the isobaric SPC, which exhibited substantially weaker retention on the RP column; (c) the heavy homologues—assuming that they corresponded to degradative products—accumulated to quite high levels indicative of slower biodegradation; (d) should these unidentified compounds derive from LAS, then the structure of the anionic surfactant had been modified in such a manner that species of higher molecular weight were formed, possibly through the addition of one or several oxygen atoms.

Besides SPC, a further class of metabolites in the pathway of LAS biodegradation are dicarboxylated SPC (SPdC), formed by oxidative attack on both sides of the alkyl chain and subsequent further breakdown by β-oxidations. These intermediates were identified by mass spectrometric techniques in sewage treatment plants and surface waters [33] and in liquors from laboratory test devices simulating the biodegradation of LAS [31,46]. Therefore, extracts of soil samples were examined for the occurrence of these very polar compounds using full scan mode (scan range: *m*/*z* 100–500; cone voltage: 30 V) as well as the more sensitive MRM mode scanning the characteristic ion transitions $[M-H]^- > [M-H-CH_3COOH]^-$ and $[M-H]^- > [M-H-CO_2-H_2O]^-$ with a collision energy of 30 (for a detailed description of the fragmentation behaviour of SPdC refer to [29,31]). No evidence for the presence of SPdC was obtained. The apparent lack of these species in the soil samples might be due to their low levels in sludge-amended soils, since concentrations of SPdC in aquatic media were reported to be lower as compared with the SPC [33] (see next section).

Table 3
Concentrations (μg kg^{-1} soil, dm) of LAS and SPC in samples of soil treated with sewage sludge

	Fonollosa	Sabadell	Castellcir	Calonge	Pujalt-N	Pujalt-C	Pujalt-SD	Castellvell	Montseny	St. Joan
C10-LAS	13	41	15	28	57	37	22	31	19	152
C11-LAS	37	164	48	129	122	101	74	125	64	767
C12-LAS	38	174	46	168	109	90	75	141	69	1049
C13-LAS	34	177	43	192	109	110	87	146	98	874
Σ LAS	**122**	**556**	**152**	**517**	**396**	**338**	**258**	**443**	**249**	**2839**
Ø Cn-LAS[a]	11.8	12.0	11.9	11.8	11.7	11.9	11.9	11.8	12.0	11.9
C5-SPC	<LOQ	<LOQ	<LOQ	<LOQ	n.d.[b]	n.d.	n.d.	<LOQ	<LOQ	41
C6-SPC	<LOQ	n.d.	<LOQ	<LOQ	n.d.	n.d.	n.d.	<LOQ	<LOQ	83
C7-SPC	0.5	0.8	0.5	1.1	<LOQ	<LOQ	<LOQ	0.5	1.9	34
C8-SPC	0.4	0.6	0.5	1.2	<LOQ	<LOQ	<LOQ	0.5	1.9	18
C9-SPC	0.3	0.6	0.8	1.4	<LOQ	<LOQ	<LOQ	0.6	3.3	13
C10-SPC	<LOQ	0.9	1.0	1.6	<LOQ	<LOQ	<LOQ	<LOQ	3.1	5.6
C11-SPC	0.9	3.0	1.2	3.2	1.4	1.4	<LOQ	2.0	3.9	10
C12-SPC	1.2	3.7	1.6	4.3	2.0	1.7	<LOQ	2.4	2.6	8.7
C13-SPC	1.2	2.8	1.7	4.5	1.7	1.4	<LOQ	1.9	3.0	8.1
Σ SPC	**4.4**	**13**	**7.4**	**17**	**5.1**	**4.5**	**–**	**7.9**	**20**	**221**

Significance were not determined for values appearing in bold.
[a] Mean length of alkyl chain.
[b] Not detected.

3.3. Results of field monitoring

The concentrations of the LAS homologues analysed in the soil samples are compiled in Table 3. With the exception of the St. Joan sample, the LAS surfactant content was quite similar at all locations, ranging from 122 to 556 $\mu g\,kg^{-1}$. These values are comparatively low compared to those reported in previous studies reporting data on the occurrence of LAS in samples taken after a period of several weeks to months after sludge application (in the present work samples were taken at least 10 months after the last sludge application; exception: St. Joan). In all studies of which the authors are aware, optical detection systems were employed with LOD in the sub-mg kg^{-1} range (0.2 mg kg^{-1} (UV/FL [47]); 0.1–5 mg kg^{-1} (UV [48]), 0.3–1.4 mg kg^{-1} (FL [49])). For example, Berna et al. [5] detected ∼1 mg kg^{-1} LAS in soil 90 days after sludge application, while Holt et al. [15] reported 0.7 mg kg^{-1} after 60 days and <0.2 mg kg^{-1} after 21 days, respectively, in experiments carried out at different field sites. In contrast, de Ferrer et al. [13] reported on significantly higher residual LAS concentrations of 16.7 mg kg^{-1} after a period of 62 days. Giger et al. [14] also reported a relatively high LAS concentration of 5.0 mg kg^{-1} on a permanent pasture site 104 days after sludge application. However, it has to be taken into consideration that the initial concentrations directly after sludge amendment varied by more than order of magnitude among the studies cited above.

By far the highest LAS concentration listed in Table 3 corresponds to the sample from St. Joan (2840 $\mu g\,kg^{-1}$), the field that had received sludge 10 days prior to sampling. The kinetics of LAS breakdown in sludge-amended soils has been demonstrated to approximate a first-order kinetics, thus an exponential decrease in concentration has been frequently observed. In the studies of Holt and Bernstein [47] the LAS level dropped from 145 to 20 mg kg^{-1} within 10 days and then further to 16 mg kg^{-1} after another 10 days. A similar trend was reported by de Ferrer et al. [13] with a starting concentration of 155 mg kg^{-1} and a decrease to 56 mg kg^{-1} after 6 days; an LAS content of 28 mg kg^{-1} soil was measured after a total elapsed time of 15 days. In the work performed by Giger et al. [14] LAS biodegradation was reported to amount to 62% after only 10 days (reduction from 45 to 17 mg kg^{-1}), and after 23 days a further 8.4% of the initially present LAS had disappeared. Spreading of sewage sludge on arable land resulted in an LAS concentration of 2.6 mg kg^{-1} [50], which had declined to 1.5 mg kg^{-1} after 8 days and further to 0.6 mg kg^{-1} after another 10 days. Regarding the frequency of sludge application and the LAS levels measured in the soil samples (ignoring the field at St. Joan), our data confirm an extensive removal of LAS through biodegradation (confirmed by the presence of metabolites; see below). No evidence was obtained that the anionic surfactant was accumulated as a result of repetitive sludge application (up to five applications in consecutive years).

As reported, the kinetics of LAS microbial degradation is a function of the alkyl chain length; degradation rates increase with length of the aliphatic carbon chain, and external phenyl isomers (i.e. those in which the sulfophenyl ring is attached close to the chain end) are more rapidly biotransformed than internal phenyl isomers [30]. Supposing the alkyl chain length to be the conclusive factor in the degradation rate, enrichment over time of the shorter homologues in sludge-amended soils should be observed. Such a trend was not observed in the present study where the mean chain length for the soil samples varies between 11.7 and 12.0 (Table 3). No significant differences in the concentration of each homologue during the degradation process were likewise stated in [15] suggesting that no preferential biodegradation of specific chain length took place. Contrasting findings were formulated in [51] reporting on an increase in the average carbon atom number from 12.1 to 12.4 after more than 10 months (C10-LAS concentration not considered). Faster degradation of the lighter homologues was attributed to the preferential sorption of the heavier ones to the soil according to their higher hydrophobicity. Hence, soil composition may ultimately play the deciding role in the fate of each homologue.

In the studies carried out on the fate of LAS in sludge-amended soils, aerobic biodegradation of the synthetic surfactant has been demonstrated to be the chief removal mechanism preventing LAS from accumulating in regularly amended soil. Nonetheless, this study provides the first evidence on the identity, occurrence and fate of their breakdown intermediates in natural soil environments. Employing (−)-ESI–MS/MS, a set of polar SPC was identified for the first time in real samples from arable land treated with municipal sewage sludge. The concentrations of individual SPC homologues ranged between <LOQ and 4.5 $\mu g\,kg^{-1}$ (St. Joan: 83 $\mu g\,kg^{-1}$) and the sum of all homologues measured was between 4.4 and 20 $\mu g\,kg^{-1}$ (St. Joan: 221 $\mu g\,kg^{-1}$), one to two orders of magnitude lower than total LAS concentrations. Hence, it is not surprising that these low amounts of SPC have escaped detection in previous analyses utilising optical detection systems with inferior sensitivity and selectivity compared with LC–ESI–MS/MS.

The long-chain (lc)-SPC with 10–13 aliphatic carbon atoms dominated the profile in all of the samples with the exception of St. Joan (see below). These pattern are distinct from those typically found in water samples from sewage treatment plants or rivers, in which the mid-chain (mc)-SPC with seven or eight carbon atoms in the side chain are the most abundant species [34,37]. This difference can be attributed to the nature of the bacterial communities, involved in the LAS/SPC degradation, as well as the physico-chemical properties of the surrounding medium. As far as the sample from St. Joan is concerned, the peak pattern is largely shifted towards the sc-SPC making up more than half the total SPC concentration in this soil. This observation can be attributed to the fact that the biodegradation of LAS had been recently initiated and was still progressing, i.e. lc-SPC were still being

liberated and subsequently broken down. Quite high amounts of the sc-SPC built up and transiently accumulated in the soil since the biodegradation rate—assuming breakdown of the carboxylated species through β-oxidation—decreases with chain shortening [31]. This process can be supposed to continue as long as LAS are converted to yield lc-SPC. Finally, a quasi-steady state in LAS biodegradation is reached and time allows the sc-SPC to disappear almost completely.

4. Conclusions

LC–ESI–MS/MS has proved to be a powerful tool in unambiguously identifying LAS and SPC at trace levels in sludge-amended soils. We concluded that the anionic surfactant LAS was rapidly biodegraded in agricultural soils with high conversion rates immediately after the sludge amendment. Residues of LAS persisted over longer periods of time in soil, presumably due to very low bioavailability. In future investigations it will be of pronounced interest to elucidate the impact of soil composition as well as of climatic conditions on the fate of SPE in sludge-amended soils. Hence, it will be necessary to collect data from various sites of distinct specifications. With regards to potential hazardous effects of residues of LAS surfactant in soils in the upper $\mu g\,kg^{-1}$ range—effects of the less abundant SPC are beyond discussion due to their comparatively low toxicity values [52,53]—a probabilistic risk assessment of LAS in agricultural soils was recently presented deriving a predicted no-effect concentration (PNEC) from a series of measured effect concentrations for soil invertebrates, terrestrial plants and microbial soil parameters [27]. Applying the estimated PNEC of $4.6\,mg\,kg^{-1}$ [27] to the present study, the conclusion can be inferred that the LAS residues do not pose a significant risk to fauna, plants and essential functions of agricultural soils.

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