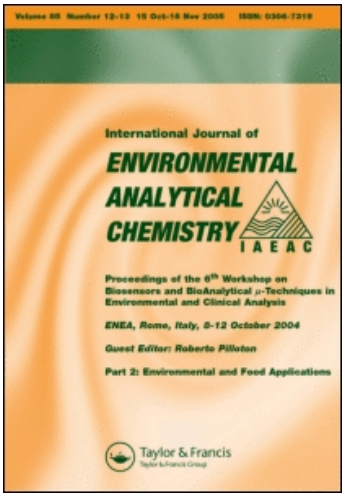

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Fulvic acid enrichment in the microlayer of the Gerlache Inlet Sea (Antarctica): preliminary results

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FULVIC ACID ENRICHMENT IN THE MICROLAYER OF THE GERLACHE INLET SEA (ANTARCTICA): PRELIMINARY RESULTS

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The aim of this work was to study the presence of fulvic acids in Antarctic waters, as they appear to be principal film-forming components in the surface microlayer. We obtained a series of samples during two Antarctic Italian Expeditions in 1998/99 and 2000/01 and studied the distribution and the structural differences between fulvic acids extracted from both microlayer and subsurface waters.

Fulvic acids are concentrated in the microlayer and the enrichment factor between microlayer and subsurface water differs between samples. The enrichment factor values for fulvic acids lie between those found for dissolved organic carbon (DOC) and particulate organic carbon (POC) in the literature. Fulvic acids extracted from the microlayer were found to be different in structure from those present in the subsurface layer and enriched in sulphur content. We hypothesised that sulphur-containing compounds are slightly bound and/or occluded in fulvic acid structures. The sulphur-containing compounds analysed in the microlayer could be dimethylsulfide (DMS) and/or its products stemming from photochemical and biological oxidation reactions.

Keywords: Microlayer; Aquatic fulvic acids; Enrichment factor; Refractory organic matter; Antarctica

INTRODUCTION

The thin layer of water at the air–sea interface represents a unique physico-chemical habitat for marine biota. The microlayer is subject to constant alteration by a number of dynamic and non-equilibrium processes that take place at the interface. A wide variety of organic compounds was found to be enriched in microlayer samples relative to subsurface waters. The microlayer enrichment is influenced by different mechanisms such as high surface tension of the air–water interface, atmospheric deposition, wind stress, heat and mass transfer across the interface, molecular diffusion and thermodiffusion, turbulent mixing of elements between the microlayer and bulk water, settling of particles within the water column, solar radiation and biochemical transformation within the layer. Nevertheless, research on the microlayer is important

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for an understanding of the effects on sea-to-air transfer of chemical substances and organisms through bubble bursting and aerosol formation. These studies are also important to an understanding of the role of organic matter in photochemical and biological transformations at the sea surface and of the impact of anthropogenic pollutants on the marine food chain, which can influence the reproductive cycles of marine species.

Both dissolved and particulate materials accumulate in the microlayer with enrichment factors up to 10^3 [1]. The interesting feature is that different constituents concentrate in the microlayer to different extents. Much work on the organic composition of the microlayer [2–5] has focussed on the measurement of lipid materials such as fatty acids, fatty alcohols and hydrocarbons, which are well known for their surface activity and/or high insolubility in seawater. Nevertheless, lipids were not the major component of the total organic carbon (TOC) in microlayer and sub-surface waters. Mean enrichment factors were 1.1–2.4 for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), urea, carbohydrate, lipids and the soluble inorganic nutrients NH_4^+, NO_2^-, NO_3^-, PO_4^{3-} and SiO_3^{2-}; 1.3–2.0 for ATP, chlorophyll-a, microplankton and bacteria and 1.1–3.7 for particulate organic carbon (POC), particulate organic nitrogen (PON) and dissolved and particulate protein [6–8]. Yang [9] highlighted the fact that dimethylsulphide which is the dominant volatile reduced sulphur compound found in surface seawater, showed an apparent enrichment in the surface microlayer compared to the underlying water. The sea surface microlayer plays an important role in the global biogeochemical sulphur cycle.

As regards organic and inorganic pollutants, a number of early papers report enrichment of chlorinated insecticides, polychlorinated biphenyls and aromatic hydrocarbons in microlayer samples [10–12] and of trace elements such as heavy metals [13]. Hunter and Liss [13] explained the presence of heavy metals by metal–ion complexation with organic ligands enriched in the microlayer.

In this article we report preliminary inquiries about the presence of fulvic acids in the surface microlayer. These compounds seem to be principal components in superficial film forming; moreover, they play a fundamental role in binding the organic and inorganic pollutants, influencing the sea-to-air transfer of pollutants. We also investigated the enrichment factor and the structural characterisation of fulvic acid extracted from microlayer to explore possible differences in both composition and structure.

EXPERIMENTAL

Materials

Samples of microlayer and subsurface water were collected in January and February in the Gerlache Inlet (Ross Sea, Antartica) during two Italian Antarctic Expeditions (1998/99 and 2000/01). The sampling station, sampled at different times, was located at 74° 41.6′ S and 164° 11.2′ E. During all the sampling the sea was quiet.

The microlayer, consisting of a superficial water film (thickness 40–80 μm), was collected using a Multi-Use Microlayer Sampler (MUMS). The collector consists of a rotating glass cylinder inserted in a rotating structure. The whole collector is located in a floating structure. Adsorbed film is removed from the drum by a Mylar scraper. The liquid is collected by a Teflon system, composed of a three-way valve and a

membrane pump. This set-up guarantees the interruption of the collection sampling as well as the washing of the pipeline. Contemporaneously, a second parallel channel is used to collect subsurface water, here considered around −0.50 m. The floating structure is powered by an electric motor and a system of batteries, and all the principal functions are radio controlled [14].

Solid adsorbers and collectors base their capacity for film recovery on an adsorption mechanism at the air–water interface. This method emphasises the adsorbable surface-active species and may not give 100% recovery of the non-polar or highly water-soluble components in the surface microlayer, which are less competitive for adsorption sites [15].

All the samples were frozen at −30°C immediately after sampling and were analysed three months later.

Amberlite XAD-8 resin (Polysciences, Inc) was used to extract humic substances (HS) from the microlayer and water samples. In order to eliminate organic compounds (e.g. hydrolysed acrylic acid) bleeding from XAD 8 columns (500 mL and 36 mL), resin cleaning was performed for 24 h by Soxhlet sequential extractions with methanol, diethyl ether, acetonitrile and methanol. The resin was then packed in glass columns and rinsed five times with 0.1 N NaOH and 0.1 N HCl, successively, and then washed with deionised water until the DOC concentration was less than 0.3 mg L^{-1} (deionised water).

Spectra/Por Cellulose Ester tubular membranes (diameter 10 mm, volume 0.79 mL per 1 cm length) with molecular weight cut-off of 500 Da, were used for dialysis. Deionised water was used as the washing solution.

Millipore Cellulose Acetate ultrafiltration membranes (diameter 76 mm) with molecular weight cut-off of 500 Da were used for ultrafiltration.

All reagents used were analytical grade.

Humic Substance Isolation from Microlayers and Seawaters

The procedure proposed by Thurman and Malcolm [16] was used for recovery of humic substances (HSs) from microlayer and sea waters. Briefly, after acidification to pH 2, the microlayer samples (4–10 L) were passed through the Amberlite XAD-8 resin column (36 mL). HSs were recovered from the columns with 0.1 M NaOH, and the alkaline extract was then acidified to pH 2 with HCl in order to separate humic acids (HAs, precipitate) from fulvic acids (FAs, soluble in acid solution). HAs, when present, were purified by dialysis and FAs by diafiltration [17].

After acidification to pH 2, the water samples (50 L) were also passed through an Amberlite XAD-8 resin column (500 mL). After recovery from the column, the acidified eluate was passed through an Amberlite XAD-8 resin column (36 mL) to obtain the most efficient preconcentration. HSs were recovered from the columns with 0.1 M NaOH, and the alkaline extract was then acidified to pH 2 with HCl in order to separate HAs from FAs as above. HAs, when present, were purified by dialysis and FAs by diafiltration [17].

Chemical and Spectroscopic Analyses

The carbon, hydrogen, nitrogen and sulphur elemental content of HSs was determined with a Carlo Erba model EA11110 CHNS-O Element Analyser. Samples for Fourier

transform infra-red (FTIR) spectroscopy were prepared by mixing 1 mg of the HA with 100 mg of oven-dried KBr (110°C). The spectra were recorded using a Philips model PU9800 FTIR spectrophotometer working in diffuse reflectance conditions. Reflectance spectra were converted to Kubelka Munk units, which are directly proportional to the concentration of the scattering medium. 100 scans were recorded with a resolution of 4 cm^{-1} and normal apodization.

^{13}C NMR spectra were determined in NaOD (0.5 M) by a Varian spectrometer, model XL-300. Samples were prepared by dissolving the dried residue (30 mg) in 1 mL NaOD in an NMR tube (5 mm). The operating conditions were: 75 MHz, pulse 45°, acquisition time 0.1 s, delay time 0.5 s. About 800 000 scans were accumulated. Spectra were obtained with broadband decoupling. Under these conditions, a clear spectrum should be obtained with a distinct single peak for each carbon atom; because of the molecular complexity of samples, the spectra show many poorly resolved signals.

Molecular weight fractionation using Centriplus Amicon concentrators, supported low-binding membranes with a molecular weight cut-off of 3000 Da, was carried out with a VISMARA centrifuge model 3225R (max speed 8000 *g*).

The spin time was 190 min (centrifugal force 3000 *g*) to obtain a final retentate volume of 0.5 mL. Initial solution was 500 mg L^{-1} of FAs solubilised in 10 mL in 0.5 M NaOD. Since concentrator membranes contain trace amounts of glycerine, the membranes were washed before use by centrifuging them with 0.1 M NaOH (three times for 1 h) and removing the residual NaOH by washing with deionised water until a neutral pH value was obtained.

RESULTS AND DISCUSSION

Fulvic acids are present both in subsurface and in microlayer waters. In subsurface water their concentration in the different samples does not vary much. However, the concentration range in microlayer water is variable from 0.6 to 2.4 mg L^{-1} and the differences observed are random (Table I). Consequently, FAs are more concentrated in the microlayer and the enrichment factor between microlayer and subsurface waters varies among the samples in the range 2.5–8.

TABLE I Total fulvic acid amounts

Date of samples	*Total Fulvic acid* (mg L^{-1})	
	Microlayer	*Subsurface*
23/1/99	1.6	0.3
28/1/99	0.7	0.2
2/2/99	1.0	0.4
4/2/99	0.9	0.2
3/2/01	1.1	0.2
5/2/01	1.6	–
7/2/01	0.8	–
12/2/01	0.6	–
14/2/01	2.4	0.3

Relative standard deviations are lesser than 5%.

The enrichment factor values we found lie between those previously reported for DOC and POC: microlayer DOC values are for the most part similar to those of the corresponding subsurface, with enrichment factors >1.5 being exceptional. Enrichment factor values for POC are generally in the range 6–10 [4].

As regards FA characterisation, FTIR spectrum of microlayer FAs (Fig. 1a) display an absorption band in the region of 1150–1040 cm^{-1} which is split into two only partially resolved peaks, one of which is centred at 1147 cm^{-1}. This adsorption band, characteristic of humic compounds [18], is assigned to the C–O stretching of alcohols and/or carbohydrates, but this region can be ascribed also to sorptions of alkyl and aryl sulphoxides, sulphates, etc. Usually the sulphur content in humic compounds is low and then the principal assignment is given to oxygen groups. Conversely, the spectrum of subsurface fulvic compounds (Fig. 1b) also shows the splitting of band adsorption in the region 1150–1050 cm^{-1} but the peak centred at 1147 cm^{-1} has a lower intensity than that shown by microlayer samples. Moreover, the spectra of both subsurface and microlayer water show absorption bands at 1650, 1540 and 1230 cm^{-1}, indicating the presence of peptides by the C=O stretching and N–H and C–N deformation of amidic linkages, and a strong signal at 1710 cm^{-1} assigned to C=O asymmetric stretching of carboxyl groups. The aliphatic character of fulvic macromolecules is highlighted by the presence of absorptions at around 2950 and 2800 cm^{-1} due to asymmetric and symmetric stretchings of methyl and methylene groups.

^{13}C NMR spectra of microlayer and subsurface layer FAs (Fig. 2a,b) show some differences in the composition of structural units. Aromatic signal intensities are stronger in the subsurface spectrum; in particular the main contributions are the phenolic signal around 150 ppm and the signal at 140 ppm associated with aromatic rings. Microlayer FAs show a greater aliphatic nature with well separated signals at 32 ppm (long-chain fatty acids) and at 38 ppm (branched forms). In both spectra the maximum intensity in the *O*-alkyl region is at 74 ppm.

The difference observed in chemical structures could be attributable to a selective enrichment. Indeed, the molecular weight distribution shows that FAs in the microlayer are predominantly compounds of large molecular size. In the microlayer the >3000 Da fraction constitutes 100% of the FAs, while in the subsurface samples only 70% of the FAs are composed of the >3000 Da fraction, the remaining 30% falling into the <3000 Da fraction. According to literature data [19] molecules of larger size are more aliphatic in nature while molecules of smaller size are more aromatic.

Finally, the elemental analysis data (Table II) show that the fulvic macromolecules extracted from microlayer and subsurface waters differ mainly in their sulphur content. Fulvic compounds from the microlayer show a greater sulphur content than those from subsurface water (the S/C ratio ranges from 0.007 to 0.068 for microlayer samples and from 0.004 to 0.013 for samples from subsurface water). The enrichment of sulphur content is more clearly shown by comparing the S/C value for microlayers FSs with the S/C value for subsurface FSs. The ratio is always greater than unity (Table III). This result supports the hypothesis of selective enrichment. The ratio between the N/C value for FSs extracted from the microlayer and that for FSs extracted from subsurface water is much smaller than the ratio between the S/C values, indicating that amino acids are not the source of the sulphur enrichment. We suggest that the presence of sulphur could derive from dimethyl sulphide

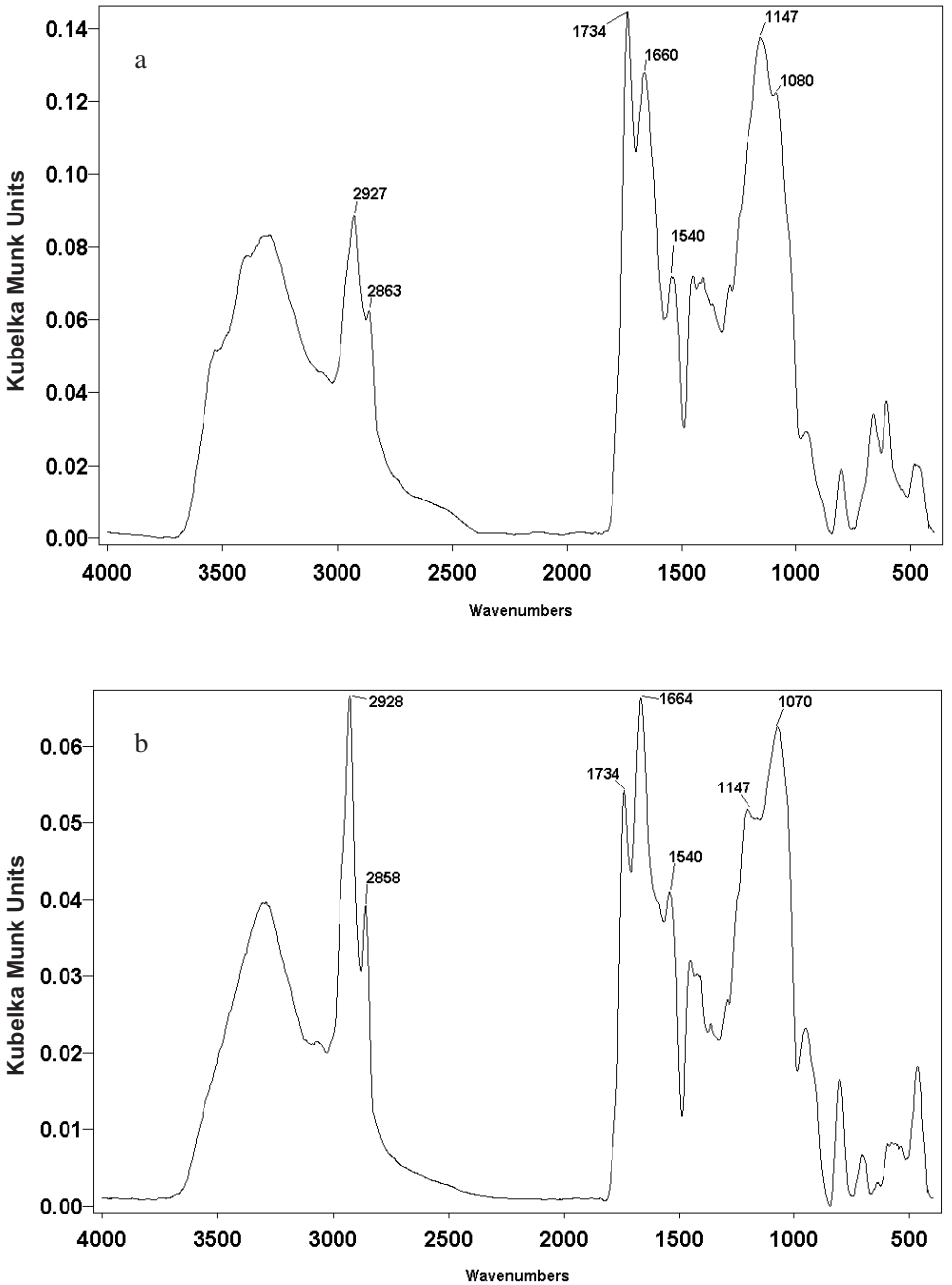

FIGURE 1 FTIR spectra of fulvic acids extracted from (a) microlayer and (b) subsurface waters.

(DMS) photochemical oxidation reactions and/or their products stemming from photochemical and biological oxidation reactions which are very fast at the air–water interface. The sulphur-containing products are then weakly bound to and/or occluded in fulvic acid structures.

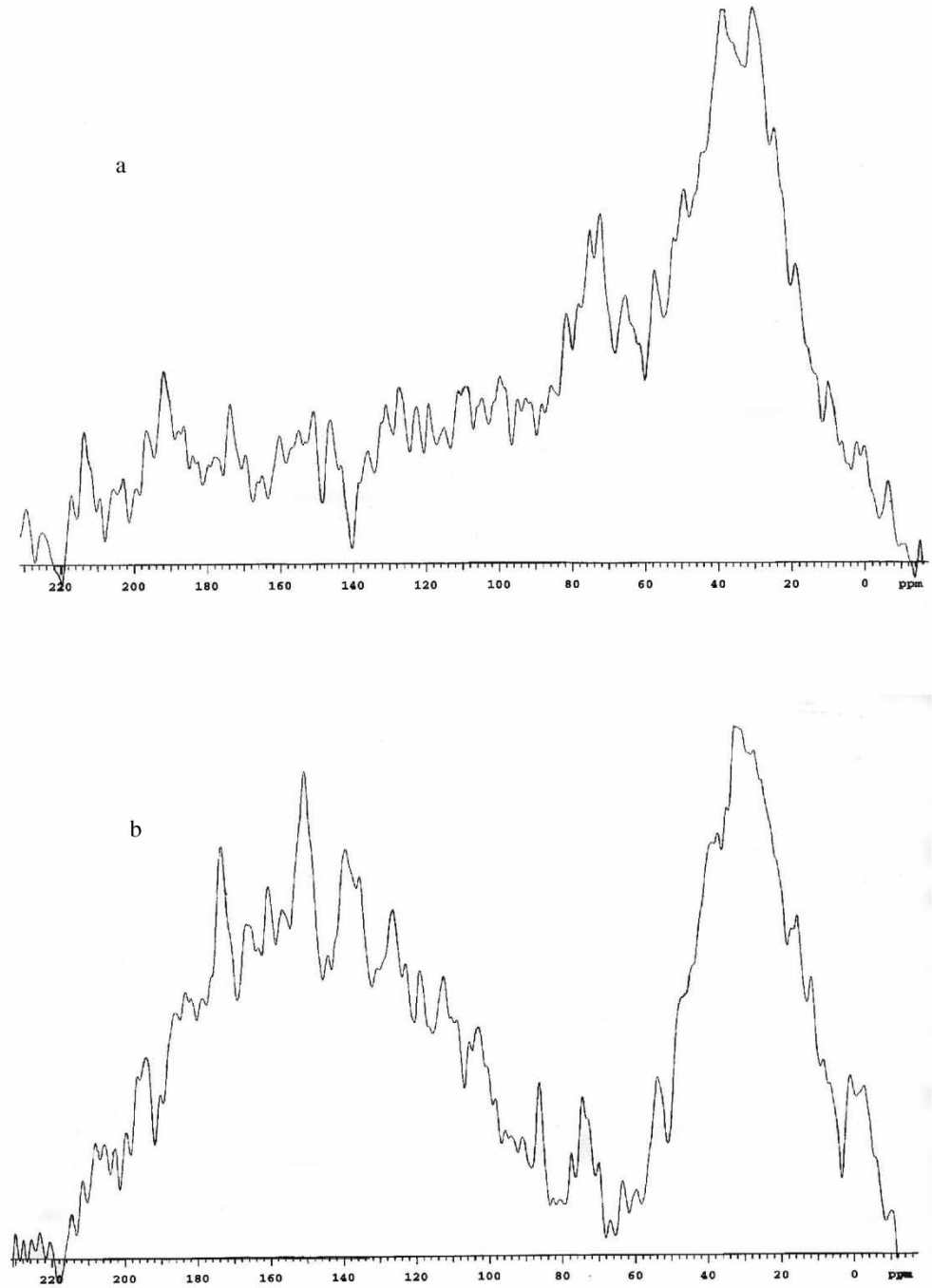

FIGURE 2 ^{13}C NMR spectra of fulvic acids extracted from (a) microlayer and (b) subsurface waters.

TABLE II Elemental analysis (no ash contents correction) and atomic ratios of the total fulvic acids

Date of sample	*Element content* (%)				*Element ratio*	
	N	*C*	*H*	*S*	*N*/*C*	*S*/*C*
Microlayer FA						
23/1/99	3.08 ± 0.04	34.42 ± 1.02	4.63 ± 0.09	4.26 ± 0.03	0.077	0.046
28/1/99	3.78 ± 0.06	33.70 ± 0.62	4.73 ± 0.06	1.10 ± 0.01	0.096	0.012
2/2/99	2.57 ± 0.03	32.41 ± 0.94	4.52 ± 0.08	3.16 ± 0.03	0.068	0.036
4/2/99	2.98 ± 0.04	28.03 ± 0.98	4.58 ± 0.09	5.07 ± 0.05	0.091	0.068
3/2/01	3.80 ± 0.05	24.51 ± 0.55	4.48 ± 0.11	0.88 ± 0.06	0.133	0.013
5/2/01	4.23 ± 0.02	34.74 ± 0.68	5.63 ± 0.12	1.07 ± 0.07	0.104	0.011
7/2/01	2.52 ± 0.06	27.70 ± 0.92	4.75 ± 0.09	0.55 ± 0.06	0.078	0.007
12/2/01	1.82 ± 0.04	25.86 ± 0.86	4.78 ± 0.10	2.37 ± 0.03	0.060	0.034
14/2/01	3.45 ± 0.04	28.67 ± 0.88	4.91 ± 0.15	1.01 ± 0.04	0.103	0.013
Subsurface FA						
23/1/99	5.06 ± 0.08	36.91 ± 1.12	4.98 ± 0.09	0.45 ± 0.02	0.117	0.004
28/1/99	4.21 ± 0.06	39.38 ± 1.33	5.84 ± 0.11	0.73 ± 0.00	0.092	0.007
2/2/99	5.90 ± 0.04	38.56 ± 1.26	4.92 ± 0.08	0.66 ± 0.02	0.131	0.006
4/2/99	2.51 ± 0.04	33.85 ± 1.28	5.05 ± 0.06	1.15 ± 0.02	0.063	0.013
3/2/01	2.75 ± 0.06	21.25 ± 1.33	3.90 ± 0.21	0.40 ± 0.01	0.111	0.007
5/2/01	–	–	–	–	–	–
7/2/01	–	–	–	–	–	–
12/2/01	–	–	–	–	–	–
14/2/01	3.62 ± 0.08	33.70 ± 1.51	5.30 ± 0.32	1.19 ± 0.04	0.092	0.013

TABLE III Ratios between N/C and S/C values for fulvic acids from microlayer and from subsurface samples

Date of sample	$(N/C)_M/(N/C)_S$	$(S/C)_M/(S/C)_S$
23/1/99	0.66	11.50
28/1/99	1.04	1.70
2/2/99	0.52	6.00
4/2/99	1.44	5.20
3/2/01	1.20	1.86
14/2/01	1.12	1.00

CONCLUSION

The sea surface microlayer was found to be enriched in FAs. Characterisation of fulvic compounds extracted from microlayer samples demonstrated that the molecular weight distribution and the structural nature of the units is different between the microlayer and subsurface water. Moreover, a greater sulphur content is present in microlayer FAs, probably due to the products of DMS photochemical oxidation reactions, such as alkyl sulphoxides. A selective enrichment is hypothesized. The data discussed in this work on the enrichment of surface-active fulvic acids in the microlayer are preliminary and further studies of large numbers of samples are necessary in order to generalise this finding.

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