Humic Compounds in Seawater and Marine Sediments from Antarctica

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HUMIC COMPOUNDS IN SEAWATER AND MARINE SEDIMENTS FROM ANTARCTICA

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This paper reports the results of various types of analysis and physico-chemical characterization of several samples of humic compounds extracted from Antarctic seawater and marine sediments. Several features of Antarctic humic compounds are evidenced. In seawater only fulvic acids are present. Fluorescence spectra of filtered seawater are similar to those of aquatic fulvics extracted from the same location. In sediments, only humic acids are present; they are characterized by a high aliphatic fraction and large quantities of aminoacids, as shown by NMR and IR. The particulate is a mixture of inorganic and organic matter, the latter consisting of proteinaceous material, carbohydrates and lipids.

KEY WORDS: Aquatic humics, sedimentary humics, chemico-physical characterizations, metals, Ross sea.

INTRODUCTION

In sea water organic matter is present both in the particulate (not filtered through a 0.45 μm filter) and in the "dissolved" fractions. Humic compounds, according to many authors, comprise up to 70% of the "dissolved" organic matter both in marine and groundwater at relatively low concentration (from 20 ug/l in deep water to over 30 mg/l in surface water[1]).

Moreover, humic substances are also present in soils and in marine and lacustrine sediments. From the environmental point of view humic compounds are excellent complexing agents for trace metals[2-5] and also promote the solubilization of organic compounds, such as pesticides and hydrocarbons[6,7] because of their surface activity.

Many studies concerning the nature of aquatic humic substances have been performed[8-11], but prior to characterization, aquatic humic substances have to be concentrated and

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isolated from other organic compounds and inorganic species, such as metals and dissolved silica.

Several methods have been developed to isolate and concentrate humic substances from water[12], including macroporous resin adsorption[1,11,13-17].
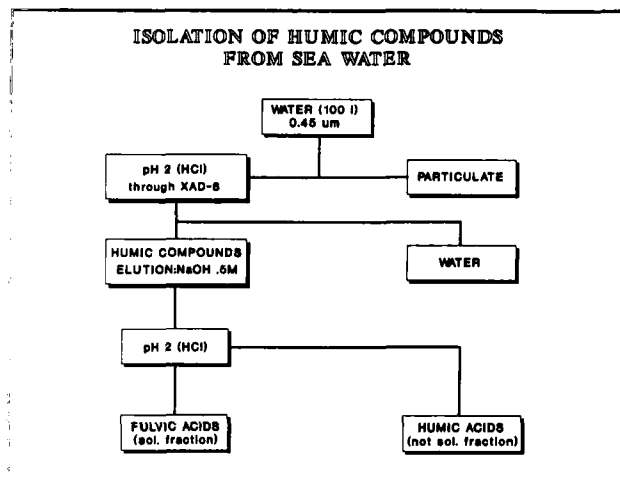
In this research we studied aquatic marine humic compounds using Amberlite XAD-8 resins as the adsorbent material. The first part of our investigation regards marine humic substances in sea water; the second part describes the same type of compounds in the corresponding sediment, in an effort to find the possible correlations between the soluble and insoluble humic compounds. Particular attention was also given to the particulate fraction.

EXPERIMENTAL

Materials and procedures.

The marine water samples examined in this study were collected in the Ross Sea (Antarctica) at 74° 38′ 9 Lat S and 167° 14′ 69 Long E.

Water was collected at the surface (A) and at the bottom (B); the depth was 980 m. At the sampling time the bottom water temperature was −1.898°C, compared to +0.108°C at the surface. The samples (100 1) were immediately passed through a 0.45 μm cellulose acetate filter. We acidified the filtrate to a pH 2 by the addition of concentrated hydrochloric acid, then passed through an Amberlite XAD-8 column prepared as previously outlined[1] and subsequently stored at +4°C. After six months we recovered the humic substances from the resin column, following the procedure described by Thurman[1]. Details of the method used for the recovery and concentration of aquatic humic substances are given in Figure 1.

ISOLATION OF HUMIC COMPOUNDS FROM SEA WATER

WATER (100 l)
0.45 um

pH 2 (HCl)
through XAD-8

PARTICULATE

HUMIC COMPOUNDS
ELUTION:NaOH .5M

WATER

pH 2 (HCl)

FULVIC ACIDS
(sol. fraction)

HUMIC ACIDS
(not sol. fraction)

Figure 1   Method for the recovery and concentration of aquatic humic substances.

We also collected one set of sediment samples by box-corer at the same site. It consisted of two portions, the first, (A), of surface sediment (0–15 cm) and a second, (B), of deeper sediment (15–30 cm). The two samples were immediately stored at −20°C. After six months humic and fulvic acids were extracted using the method of the International Humic Substances Society, outlined in a previous paper[18]. The total content, (T), and the amount of metals present in various phases (exchangeable and bound to the carbonate fraction "A"; reductive phase bound to manganese-iron oxides "B"; bound to sulphide and organic phase "C") were determined by spectrochemical techniques. Both the total solubilization of the sample and the selective solubilization of the metals were carried out by the procedures described by Cosma et al.[19].

Apparatus and methods of analysis

Elemental analysis was carried out in the Microanalysis Laboratory of the Italian Research Council, Monte Libretti, Rome (Italy) using a Carlo Erba 240-B model.

^{13}C-NMR spectra were determined in 0.5 N NaOD on a Varian spectrometer model XL-300. The samples were prepared by dissolving the dried residue (30 mg) in 1 ml of NaOD in a NMR tube (5 mm). The operating conditions were: 75 MHz, pulse 45°, acquisition time 0.1 sec., delay time 0.5. From 350,000 to 600,000 scans were accumulated for sediment and from 1,200,000 to 1,500,000 for water humic compounds, respectively.

IR spectra were recorded by means of a FTIR Philips spectrophotometer model P3202 working in diffuse reflectance conditions. The results are given in kubelka munk units. The samples were prepared by mixing the dried residue with anydrous KBr.

Thermogravimetric analysis was performed by a Thermogravimetric balance model TGA7, working in nitrogen atmosphere between 50 and 950 °C, heating rate 20°C/min, 1.5 mg of sample.

For metal determinations, a Varian Zeeman AA 300 spectrophotometer equipped with AA Graphite Atomizer and with Zeeman background corrector, or a Jobin Yvon JV 24 inductively coupled plasma atomic emission spectrometer, equipped with a Reyton 2300 RF plasma torch fitted with a Scott glass spray chamber and a Meinhard concentric glass nebulizer supplied with a Gilson LLA peristaltic pump were used.

In order to perform the fluorescence measurements, all the humic substances extracted from water and sediments were solubilized in 5 ml of NaOH 0,1 N then, for each sample, the stock solution was brought to its final volume with tridistilled water, sonicated, and finally diluted to the desired concentrations. No solution was filtered. The pH of the stock solutions ranged from 5.7 to 6.5. Fluorescence spectra were scanned with a Perkin-Elmer LS-50 computer-driven fluorescence spectrophotometer. The lamp excitation spectrum was automatically corrected. The results are given in arbitrary units. We performed emission fluorescence spectra (range 320–550 nm and 308 nm excitation wavelength) and synchronous scan excitation spectra (range 320–550 nm, delta 15 nm).

We also did dynamic surface tension (DST) measurements using a TRSV (Time Resolved Surface Viscoelastometer), a computer-driven apparatus especially designed for working with small quantities[20].

TABLE 1   Elemental analysis (%) (on the ash-free basis) of the different samples.

sample	C	H	N	H/C	N/C
surface water	32.95	3.51	0.47	1.28	0.012
deep water	28.77	4.87	4.52	2.03	0.13
superficial sediment	61.23	7.31	6.95	1.43	0.097
deeper sediment	48.60	7.37	5.47	1.81	0.096
particulate	30.61	5.41	6.33	2.11	0.18

RESULTS AND DISCUSSION

Water dissolved humic substances

The humic substances extracted from the deep and the surface water samples behave as fulvic acids according to the definition given in the available literature (acid and base soluble compounds). They differ one from the other both in composition and in structure. Humic acids (base soluble and acid insoluble compounds) are absent. We have obtained similar results with coastal Antarctic surface water samples.
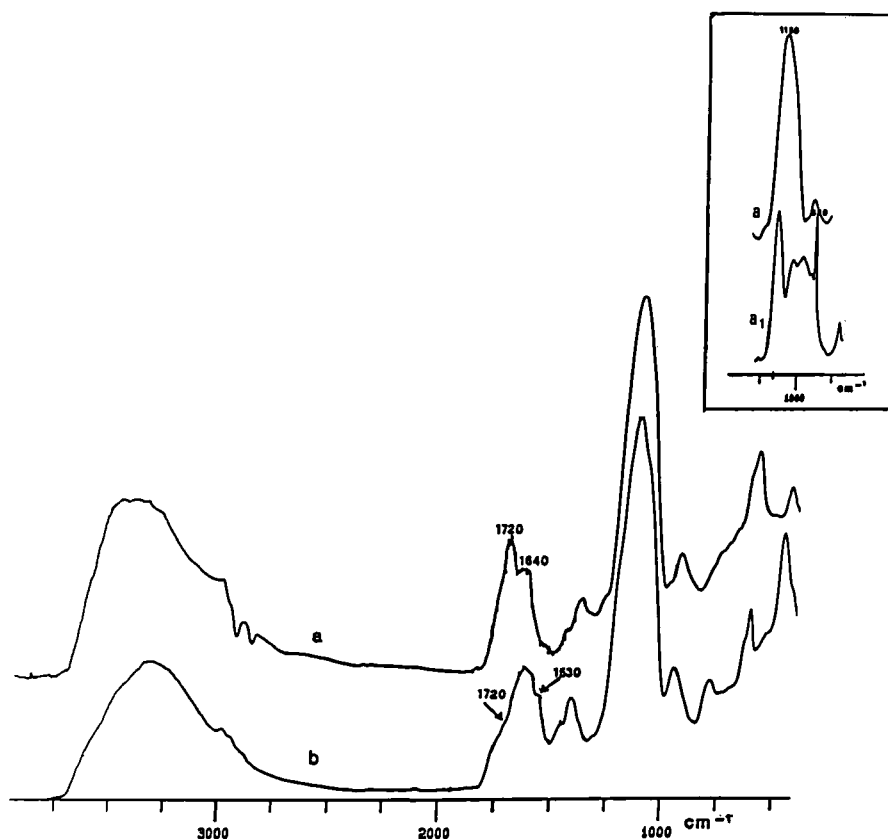
Figure 2   IR spectra of fulvic acids recovered from surface seawater (a) and from deep seawater (b). Detail of IR spectra of surface sample after thermogravimetric analysis carried out in the range 0–540°C (a₁).

The fraction of fulvic acids extracted from surface water contains a small quantity of nitrogen, (see Table 1) indicating a slight presence of proteins and compounds containing nitrogen. On the other hand, in the bottom sample, nitrogen is present in greater quantity and the N/C ratio is high.

The H/C ratio increases with the water depth. This result, according to the theory, should be an indication of lower condensation degree and aromatic structure composition in the humic substances obtained from bottom water, but IR and ^{13}C-NMR spectra (Figures 2 and 3) do not confirm this hypothesis. In fact, although the aromatic zone is not well evident in both the samples, it appears, nevertheless, more pronounced in the second one. So in the present case the smaller value of the H/C ratio observed for surface humic compounds may be related to the content of carboxyl groups, which is much greater in surface humic compounds, as can be drawn from IR and ^{13}C-NMR spectra.

The aliphatic region is the prevailing zone in both spectra and is given prevalently by short and branched chains. In the bottom sample, the aromatic zone (120–140 ppm) is distinguishable, even if the spectrum is not well resolved, probably due to the poor repeatability of the sample subunit structure.

A comparison of IR spectra of the two samples shows interesting differences.

The surface humic substances show a very strong absorption band near 1720 cm^{-1} (due to the CO stretching of carboxyl groups) which is considerably lower in the bottom water sample. On the other hand, the bottom water sample shows a shoulder at 1530 cm^{-1} and a strong band at 1640 cm^{-1} indicating presence of proteinaceous compounds (carbon-hydrogen and
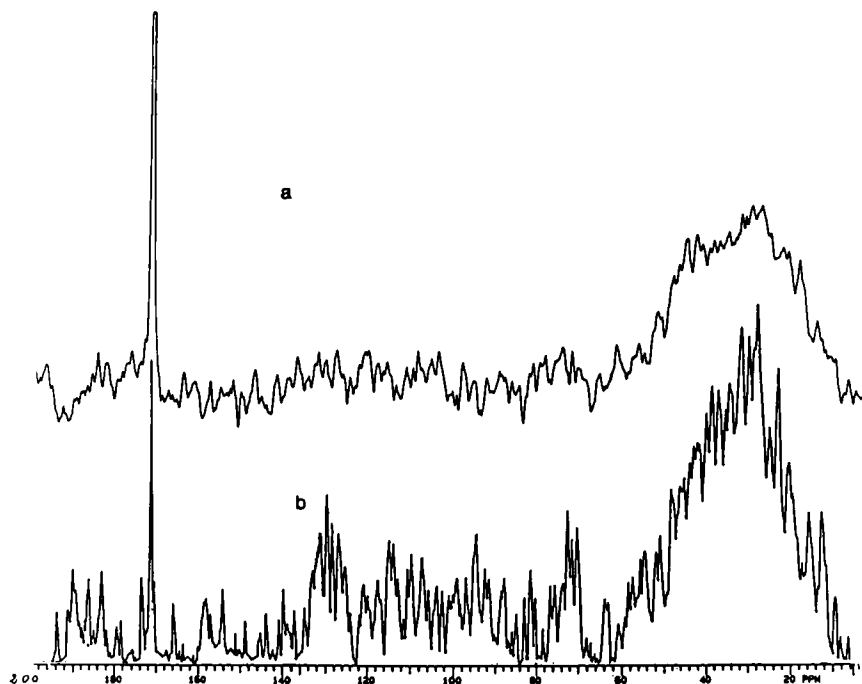

Figure 3 ^{13}C–NMR spectra of fulvic acids recovered from surface seawater (a) and from deep seawater (b).

carbon-nitrogen peptide bond of proteins), this, in agreement with the high N/C value. Moreover, the band in the 1450–1380 cm^{-1} zone (due to the OH deformation and CO stretching of phenolic OH groups and/or to CH deformation of CH_2 and CH_3 groups) is wider.

In the spectrum of bottom water samples, the adsorption band of silica at 1100 and 800 cm^{-1} are well evident, but the strong signal in the 1200–1100 cm^{-1} zone is not due to the silica alone. Its intensity is too great if compared to the one at 800 cm^{-1} and it, probably, can be associated to cyclic ethers and/or carbohydrates. In the surface sample, the band at 800 cm^{-1} is absent, allowing us to attribute the band at 1200–1100 cm^{-1} to the presence of cyclic ethers and/or carbohydrates only. These assignments are confirmed both by the low contents of ash and by the IR spectrum of the residue obtained after a thermogravimetric analysis carried out in the 0–540°C range, in which the adsorption band at 1200–1100 cm^{-1} is almost absent and so a very low silica concentration is evidenced (Figure 2a$_1$, panel).
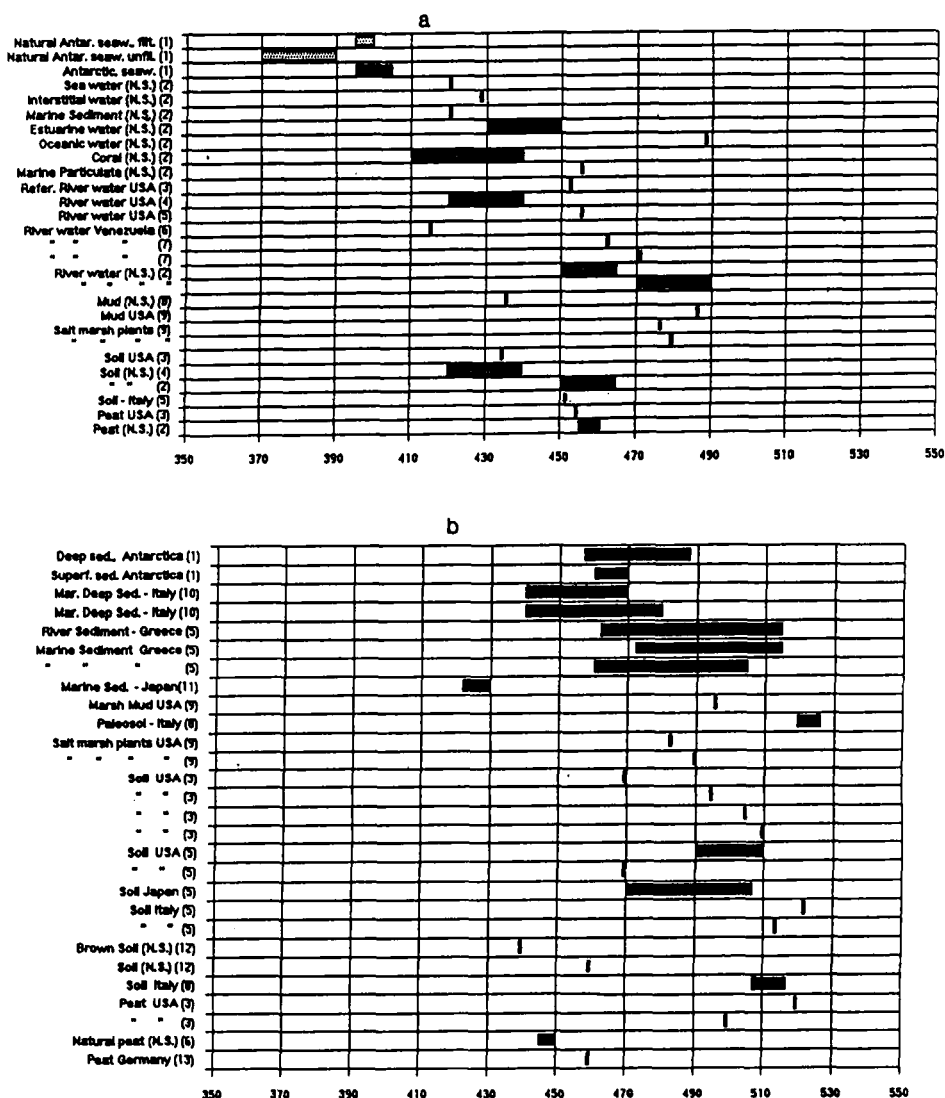
Fluorescence emission spectra have only been performed on fulvics extracted from surface water, due to the low amount available. The spectra offer scarce information, showing only a broad weak band in the 400–450 nm range (Figure 4). As suggested in the literature[21], these characteristics confirm the presence, as fluorophores, of a small number of aromatic groups and/or aliphatic insaturated groups, probably arranged in complicated, heterogeneous structures. The emission spectrum of these fulvic acids is very similar to that of the filtered seawater sampled from the same location, whereas the spectrum of the unfiltered seawater is different (see Figure 4): the emission maximum falls at very short wavelengths and the intensity is lower.

Only Ewald *et al.*[22] have performed fluorescence emission measurements on Antarctic seawater, in particular on unfiltered samples (Weddel Sea, 40 m depth). Their emission maximum occurs at shorter wavelengths (303 nm) than ours, but the spectra are not directly comparable, since the excitation wavelength was of 230 nm (much shorter than ours). They did not perform spectra on filtered seawater, or any extraction procedure. These authors
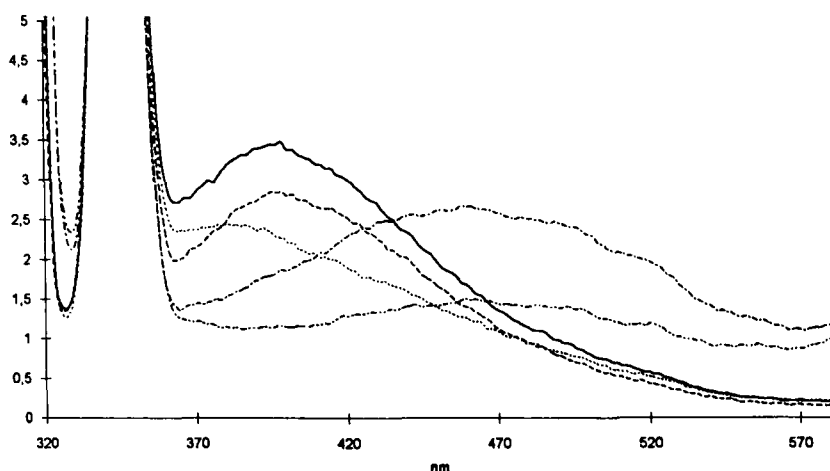
Figure 4   Fluorescence emission spectra of surface filtered water (——), unfiltered water (. . . .), fulvics of surface seawater (- - - -), humics of surface sediment (— —) and of deep sediment (– ·· –).

compare the obtained spectrum with another one obtained from seawater coming from the Gironde estuary, and conclude that humic substances of continental origin are not present in Antarctica samples. This conclusion is confirmed by our results. In fact, Figure 5a. shows a comparison between the maxima of the emission spectra of our samples and those found by various authors[21,23-31] for fulvic acids extracted from various matrixes and locations. The

Figure 5 Comparison of maximum wavelengths of fulvic (a) and humic (b) acid fluorescence emission spectra extracted by various matrixes and locations. (N.S.) Sample location not specified in reference. The number in brackets denotes the reference source: (1) This work; (2) Senesi[21]; (3) Miano[27]; (4) Ewald[23]; (5) Senesi[31]; (6) Saar[29]; (7) Vegas-Villarrùbia[28]; (8) Senesi[30]; (9) Alberts[26]; (10) Unpublished data; (11) Hayase[25]; (12) Visser[24]; (13) Mueller-Wegener[38].

emission maxima of our samples are lower than other samples taken from marine environments, and much lower than the emission maxima of fulvic acids from various terrestrial matrixes. This, as found by many authors[21,27], may possibly indicate that in our samples the fluorophores are less structured and that the degree of polymerization is lower than in other cases. It must be taken into account that the excitation wavelength is often different from the one we used (308 nm), ranging from 310 to 360 nm, but according to Miano[27], up to a certain limit the excitation wavelength influences only the intensity of the emission spectrum and not the wavelength of the maximum.

Emission spectra on fulvic acid solutions at different concentrations show that the intensity is proportional to the solution concentration (in the range 0.5–12 mg/dm^3, r=0.98, n=5). In Fig.6a, the plot of the maximum of the emission fluorescence vs. pH, obtained with a solution of seawater fulvics whose concentration was 10 mg/dm^3, is shown. It can be seen
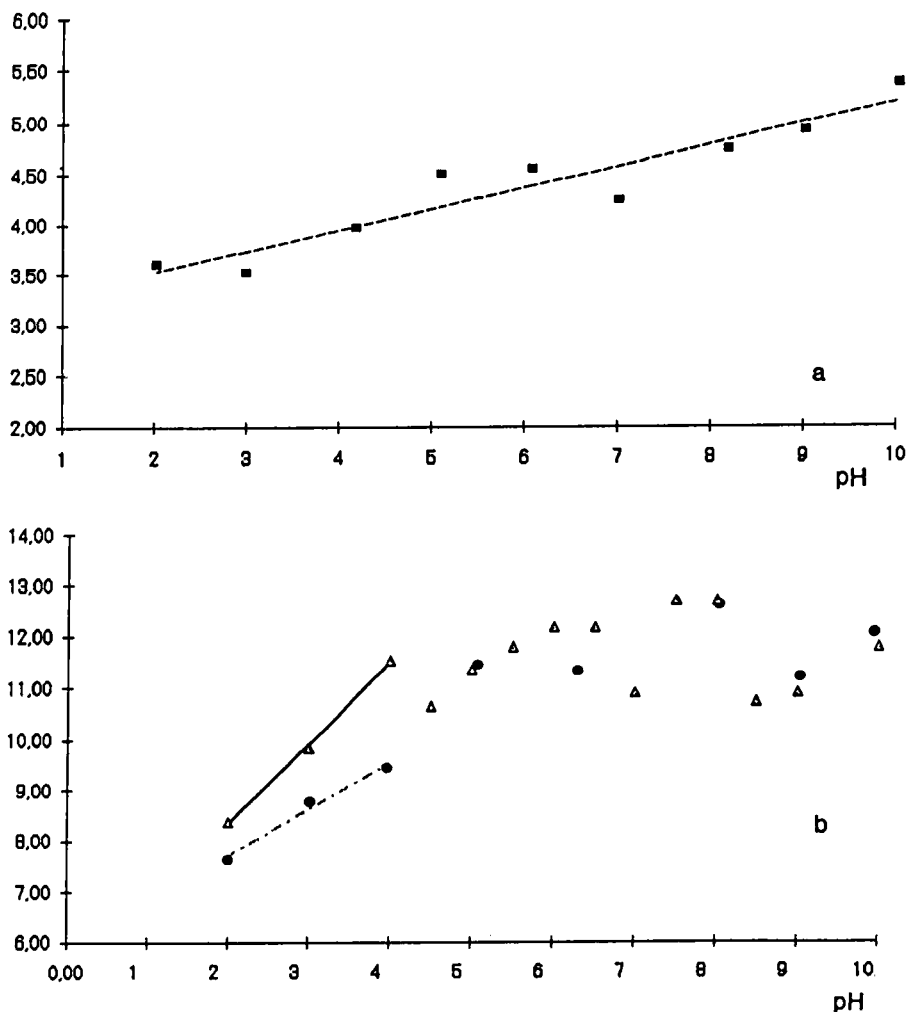
Figure 6 pH vs. intensity seawater fulvic acids (a) and sedimentary humic acids (b): surface sample (———) and deep sample (- - - -).

from the graph that there is a slight increase in intensity with increasing pH ($r=0.94$, $n=9$). This probably points to the presence of acidic groups, especially –COOH, whose ionization, taking place at a higher pH, increases the emission efficiency[27].

The synchronous scan spectra of our fulvic acids are similar to those of fulvics coming from marine aquatic environments as reported in literature[21].

Replicate DST measurements performed on solutions of fulvic acids extracted from surface water demonstrate a good reproducibility. The diffusional model was fitted to experimental data. The standard deviation between the observed and the calculated values showed that the fitting was not good, so we concluded that these solutions do not behave according to the single diffusional model. Since the solution concentration was relatively low, this behaviour can be attributed to concomitant processes on the same temporal scale, such as molecular rearrangements at the interface, typical of macromolecules.

Humic substances in sediment

In all of the portions of sediment examined the humic fraction is formed only by humic acids. Fulvic acids are not present, as described by us in a previous paper[18].

The values obtained show that in the sediment samples that are in contact with water, the contents of carbon, hydrogen and nitrogen (Table 1) are generally greater than those reported in the literature[32–36], and in agreement with the values found by Campanella *et al.*[18] in other Antarctic sediments. In the deeper sample, carbon and nitrogen contents are lower than in the upper layer, whereas the hydrogen concentration exhibits no significant change.

Both the samples are characterized by a high aliphatic fraction in which branched chains do not prevail (Figure 7a).

Large quantities of aminoacids are associated with humic acids, mainly by covalent linkage (peaks at 1640 and 1530 cm^{-1} in IR spectra eliminated in those obtained on the residues after hydrolysis).

Moreover, in the surface sample, carbohydrates are present, as indicated by ^{13}C-NMR and IR spectra. In both the sediment portions there is evidence of carboxyl groups. These groups are more apparent in ^{13}C-NMR spectra performed on the residues obtained after hydrolysis with 6 N HCl, indicating that not all the carboxyl groups are present as aminoacids.

We also performed fluorescence emission spectra on these samples, both on the humics extracted from the sediment in contact with water and on the deeper ones. In Figure 4 we show the spectra of these samples together with those of fulvics extracted from water and two spectra of natural seawater. The emission maxima for the sediment samples are higher, but the intensity is lower (all considered solutions had the same concentration, 2.5 mg/l). The deep sediment is even less fluorescent than the other, and shows a very strong Rayleigh scattering. This indicates to a high degree of micellization, which causes a very strong internal quenching. On the other hand, as discussed above, the higher maximum for both the samples suggests the presence of fluorophores having a higher degree of complexity than those of the fulvics in seawater.

Figure 5b shows a comparison between the maxima of the emission spectra of our samples and those found by various authors[21,24–27,30,31,37,38] for humic acids extracted from various
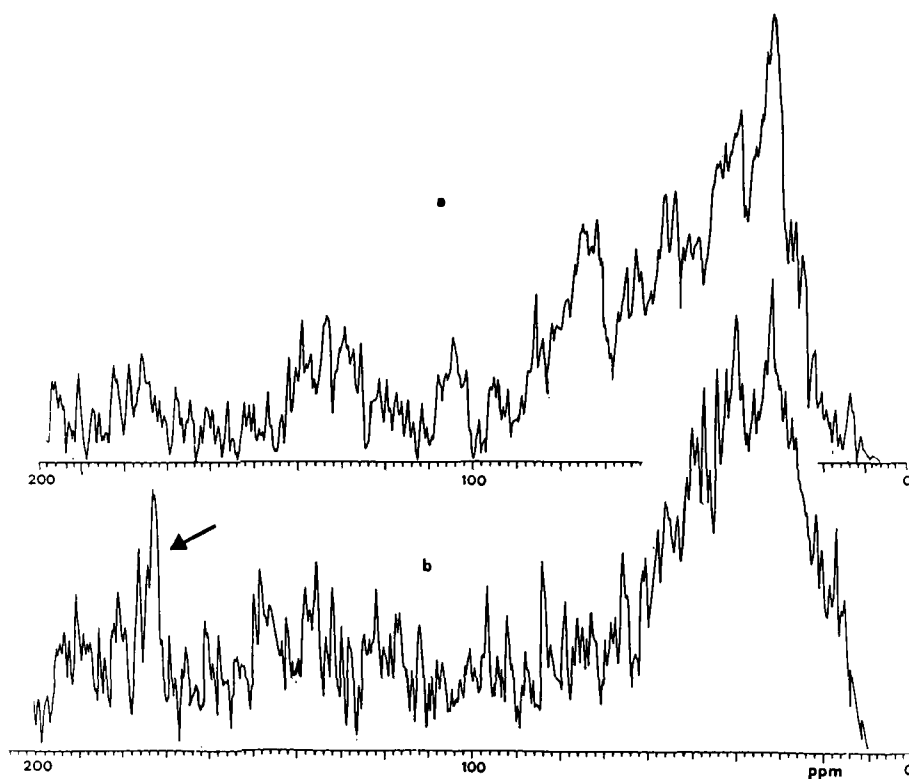
Figure 7 ^{13}C–NMR spectra of sedimentary humic acids: surface sample (a) and surface sample after acid hydrolysis (b).

matrixes and locations. It can be seen that the maximum wavelengths of Antarctic samples do not markedly differ from the other ones.

For these samples, we also carried out emission spectra on solutions at different concentrations and pH's. We found that the intensity is proportional to the solution concentration (in the range 0.5–12 mg/dm^3, r=0.99, n=5) both for the deep and for the surface HA's. Figure 6b, shows the plot of the maximum of the emission fluorescence vs. pH for two humic solutions (20 mg/dm^3) extracted from the first and from the second layer respectively. It can be seen from the graph that there is an increase in intensity with increasing pH in the 2–5 range (r=0.99 both for the deep and surface sediment sample, n=4) in both cases, more pronounced than that of seawater fulvics. It is noteworthy that in this range the surface sample plot is steeper than that of the deeper sample, and that the surface sediment humics are markedly more fluorescent than the other ones, whereas in the 5–10 range, there is no appreciable difference. For higher pH, the data are more or less evenly scattered, indicating a very poor correlation. This seems to point out a more complicated acid-base behaviour in sediment humics than in the water fulvics. We may establish the hypothesis that in sediment humics at higher pH's some other ionization process takes place, due to different functional groups, compensating the increased emission efficiency given by COOH groups.

TABLE 2 Total content (ug/g) and amount of metals present in the selective extraent solutions.

Metal	A	B	C	T
Cu	1.59	1.50	7.94	22.9
Pb	1.30	1.56	3.25	11.9
Zn	5.11	16.3	30.1	117
Cd (ng/g)	20	22	54	230
Fe	n.d.	1.73×10^3	1.50×10^4	2.45×10^4
Cr	1.85	3.65	17.1	106
Co	0.67	0.92	2.43	10.5
Mn	5.55	37.9	132	371
Al	72	3.74×10^3	3.25×10^4	4.19×10^4

A: CH_3COONH_4 1 M pH 5; B: $NH_2OH.HCl$ 1 M + CH_3COOH 25% (V/V); C: HNO_3 8 N; T: Total attack (HF + Acqua regia-Teflon Bomb) n.d. = not significant.

We also performed spectra on the same samples after hydrolysis. Considering the emission scans, it is noteworthy that the hydrolized HA extract from the superficial sediment is much more fluorescent than the corresponding non-hydrolized extract, whereas the contrary happens for deep sediment HA's; in this case the maximum wavelength is lower, pointing out, as previously discussed, a significant decrease in the complexity of the molecules. The marked decrease of turbidity (i.e. scattering) should also be noted for both the superficial and the deep hydrolized sample. This can be gauged by the decrease in the Rayleigh peak shoulder. In synchronous scans, both of the hydroliyzed samples show lower intensities.

The amounts of metals present in the sediment, and the distribution in the selective extracting solutions are reported in Table 2. Table 3 summarizes the concentrations of metals in the NaOH solution (fulvic and humic acids simultaneously present), in the humic and fulvic fraction respectively and in the HCl solution relative to the different steps of the procedure used for the isolation of humic compounds[18].

The average imprecision at the 95% confidence level, calculated from five replicate determinations, is ±5% for total attack (3± for copper), ±8%, ±6%, ±10%, for the A, B and C solutions respectively.

TABLE 3 Metal concentration (ug/g) in HCl solution, in NaOH solution (fulvic and humic acids simultaneously present), in humic and fulvic fraction.

Metal	HCl solution	Humic substances	Humic acids	Fulvic acids
Cu	0.15	0.61	0.44	0.14
Pb	0.48	0.048	0.021	0.025
Zn	1.72	0.49	0.32	0.11
Cd (ng/g)	7.56	0.093	0.042	0.040
Fe	1.18×10^2	3.45	3.10	1.87
Cr	1.84	0.026	0.010	0.012
Co	0.60	0.046	0.023	0.020
Mn	27.6	0.40	0.19	0.18
Al	7.71×10^2	1.29×10^2	1.02×10^2	20.4

Aluminium and iron are the macro elements, and the aluminium content is higher than that of iron, as reported in a previous paper[19]. Cadmium is the metal present at the lowest concentration level.

For all of the metals, an increase in concentration can be observed when passing from the "A" to the "C" solution. In the last one, all of metals are present as 20% to 40% of the total content. Only aluminium and iron concentrations are higher (78% and 61% respectively). However, by comparing the data of the "C" solution (Table 2) with those reported in Table 3 it becomes evident that the metals are present in the "C" solutions mainly as sulphides. Only a small part of the metals is associated with humic compounds (not more than 25% of "C" concentration), also when considering the fraction present in 0.1 M HCl solution. This fraction may be solubilized by an acid-base action if the metal-humic compound bond is not very strong.

Aluminium and iron are present in the "C" solution only in inorganic form.

As regards chromium, the bulk of the metal is present in the residual matrix (80%). For
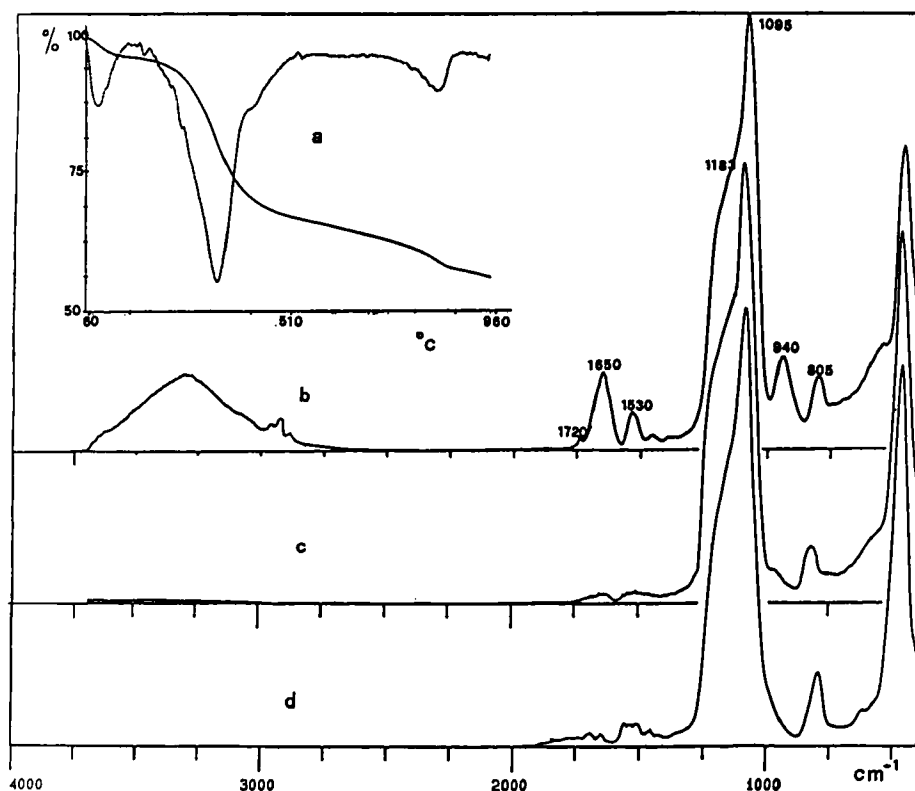
Figure 8 Thermogram (a) and IR spectra of the particulate (b), of the particulate after thermogravimetric analysis in the range 0–540°C (c) and 0–880°C (d).

other metals, except for aluminium and iron, 40%–50% of the total content is present in the residual matrix. Only aluminium is solubilized at a very high concentration (87%).

Particulate matter

The residue recovered from the 0.45 μm filter (superficial water sample, 2.34 mg/l) is a mixture of organic and inorganic compounds present in suspension at the moment of the sampling.

The inorganic fraction (ash 50%) is prevalently given by clay, as can be deduced from IR spectra (Figure 8) performed on the residues of thermogravimetric analysis carried out in the temperature 0–540 and 0–880°C ranges (loss zones in the thermogram of the particulate, Figure 8a).

The predominant adsorption band (1200–1100 cm^{-1}, due to the stretching of carbohydrates and SiO) is the same in the three spectra and is not modified after heating, thus indicating the presence of a high amount of silica, whereas the absence of bands at 1480 and 880 cm^{-1} may be related to a negligible amount of carbonates.

The organic matter, probably a residue of marine organisms, consists mainly of protein-aceous material (very high N/C ratio, adsorption bands at 1654 and 1530 cm^{-1}). IR spectra show no the presence of carbohydrates due to the great amount of silica which doesn't allow us to evidence the decrease of the peak at 1100 cm^{-1} after heating. To the contrary, the presence of lipids is well evident in IR spectra obtained on the residue of the particulate after treatment with diethylether (CH_2 and CH_3 stretching at 2980 cm^{-1} -COOH stretching at 1720 cm^{-1}, and CO vibration at 1400 cm^{-1}, Figure 9).

In the water sample collected near the water-sediment interface, the content of particulate (0.56 mg/l) was too low to allow any characterization. The inorganic fraction is 32%.
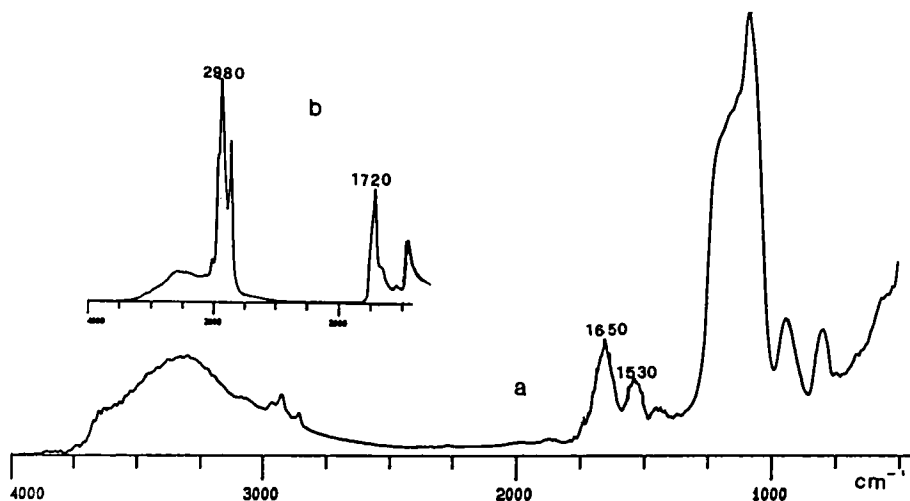
Figure 9 IR spectra of the particulate (a) and of the particulate (b) extracted with diethylether.

CONCLUSIONS

The main conclusions of the present study can be summarized as follows:

In sea water, only humic compounds having the characteristics of fulvic acids (acid and base soluble compounds) are present.

The surface water contains fulvic acids different in structure from the ones present in the bottom sample. Both samples contain fulvic acids. They show a high content of carboxyl groups which makes this material an excellent complexing agent for metal ions.

The fluorescence emission spectra of filtered seawater and fulvics coming from seawater are similar. A slight increase in intensity with increasing pH is observed in the maximum of the fluorescence emission of fulvics.

The structure of fulvic acids of bottom water is more complicated than that of surface water and is similar to that of surface sediment humic acids. Consequently, an equilibrium between non-soluble and soluble humic compounds may be hypothisized. These acids are particularly rich in nitrogen.

Fulvic acids are not present in the sediment.

The humic acids from the two sediment sets are different in structure. Both are characterized by a high prevalence of aliphatic structures.

The maximum of the emission fluorescence of humics increases in intensity with increasing pH, only in the 2–5 range. The increase is more pronounced than that in seawater fulvics.

Only a small part of metals is associated with humic compounds.

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