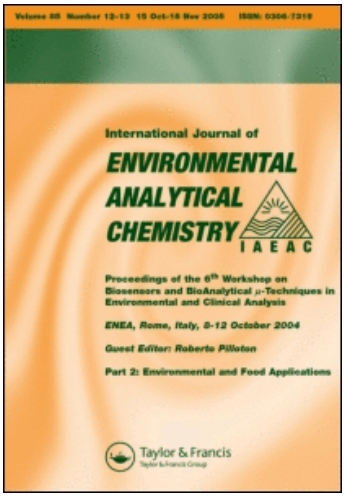

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AQUATIC HUMIC SUBSTANCES IN PACK ICE-SEAWATER-SEDIMENT SYSTEM

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Humic substances were isolated from samples of pack ice, sea water and sediments collected in the Gerlache Inlet and Wood Bay (Antarctica).

The content and structures of the in-ice dissolved humic substances (DHS) and particulate humic substances (PHS) were determined in samples of cores, in the water column sampled under the pack ice, and in the water column and in sediments when sea ice was absent. The organic matter content of ice cores shows that the pack ice behaves as an organic matter "tank". The humification process appears to proceed in the pack ice in which the phytoplanktonic material is trapped during its formation. The concentrations of humic substances isolated from surface seawater sampled under the pack ice layer and when the ice is absent are constant in time. The absence of any increase in humic substance content in surface water and along the water column, when the ice has melted, may be explained by the different salinity values of the ice (from 6 to 20 ‰) and of the seawater (34 ‰), which determine an increase in the sedimentation process rate. This hypothesis is confirmed by fulvic acid/humic acid ratios in sediments that are higher than those found for sediments collected in Antarctic areas where the ice is always absent.

Keywords: Antarctica; pack ice; sea water; sediment; dissolved humic substances; particulate humic substances; sedimentation process; salinity

INTRODUCTION

Dissolved humic substances (DHS) are assumed to be recalcitrant biopolymers that account for at least 10% of the total marine dissolved organic carbon (DOC) pool. In Antarctica the percentage of DHS is smaller and, although some authors [1] hypothesise various sources of Antarctic humic substances (HS) their formation is more than 90% due to phytoplankton decomposition [2–4]. Accord-

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ing to Ishiwatary's model [5] HS are released from decaying cells into seawater. During bacterial attack on decaying phytoplankton, Maillard-type reactions occur among refractory biopolymers, partially degraded biopolymers and regenerated molecules, leading to the formation of HS. In particular, the early stage of HS formation is generally attributed [5–7] to degradation of suspended particulate organic material (POM) degradation. Smith et al. [8] proposed a biochemical mechanism to account for the large scale transfer of organic matter from sinking particles and aggregates to the dissolved phase. The "uncoupled" enzymatic solubilization has been proposed [9] as possible mechanism for the formation of HS in seawater, which confirms the Ishiwatary's model. However, HS may also adsorb on suspended particulate material (PHS) [10].

In seawater 90% of DHS are fulvic acids (FA) [14]. In general, aquatic HS consist of an ensemble of compounds characterised by rather linear structures with a lower molecular weight and a larger number of functional groups, such as carboxyl, hydroxyl, phenolic hydroxyl and nitrogen containing groups, than sedimentary HS. FA are characterized by greater amounts of low-molecular-weight fractions and a higher content of acidic functional groups than humic acids (HA) [11,12]. In particular, Antarctic marine FA are characterised by high nitrogen contents and low aromaticity [9,10,13,14]. The study and characterisation of HS structures provide important information about the source material and its likely formation mechanism.

During the winter, sea ice is formed in the coastal area of the Antarctic continent. Thus, the principal phytoplankta such as diatoms, silico- and dinoflagellates, structural biopolymers such as proteins, polysaccharides, cell wall polymers, and HS aggregates in brine are trapped in the sea ice. Fischer et al. [15] found that in the Weddell Sea phytoplankton production can be related to diatoms released from melting sea ice and that the phytoplankton production under the winter pack ice appears to be minimal. For the Ross Sea [16] the ice melting results in the release of in-ice particulate organic carbon (POC) into the water column [17], thus the content and structures of the in-ice DHS and PHS cores were studied in this work, together with the variations in content and structures of HS in water column sampled under the pack ice and in the water column and sediments when sea ice was absent.

EXPERIMENTAL

Materials

Samples of pack ice, sea water and sediments were collected in the Gerlache Inlet (stations from 1 to 7) and in Wood Bay (station 8) during different Italian

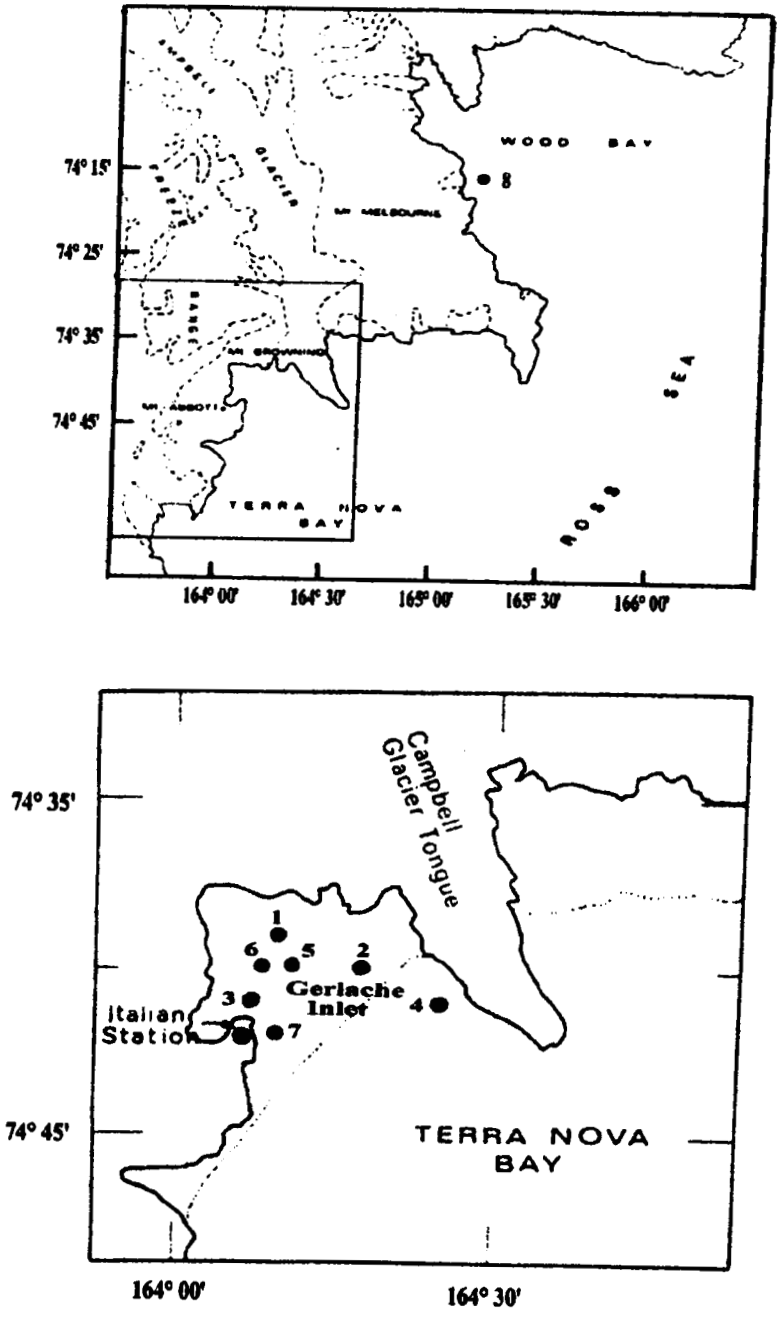

FIGURE 1 Sampling sites

Antarctic Expedition (Figure 1). The sampling stations are described in Table I. Samples at stations 1 to 4 (1996/97) were collected only once under different ice melting conditions; samples at stations 5 to 7 (1997/98) and at station 8 (1998/99) were collected more time from November to February. All the samples were frozen at −30°C immediately after sampling and analysed three months later.

TABLE I Sampling stations

Station	*Site*	*Lat S*	*Long E*	*Features*
1	Gerlache Inlet	74° 39.0′	164° 09.5′	pack ice portion A
				pack ice portion B
				surface seawater
2	Gerlache Inlet	74° 40.1′	164° 17.5′	pack ice portion A
				pack ice portion B
				surface seawater
				sediment
3	Gerlache Inlet	74° 41.2′	164° 06.0′	pack ice portion A
				pack ice portion B
				surface seawater
4	Gerlache Inlet	74° 41.3′	164° 25.0′	surface seawater
5	Gerlache Inlet	74° 40.1′	164° 11.2′	pack ice portion A
				pack ice portion B
				pack ice portion C
				surface seawater
				water (-100 cm)
				water (-380 cm)
				sediment
6	Gerlache Inlet	74° 40.1′	164° 13.9′	pack ice portion A
				pack ice portion B
				pack ice portion C
				surface seawater
				sediment
7	Gerlache Inlet	74° 42.1′	164° 09.1′	pack ice portion A
				pack ice portion B
				pack ice portion C
				surface seawater
8	Wood Bay	74° 15.9′	165° 07.4′	pack ice portion A
				pack ice portion B
				pack ice portion C
				surface seawater
				sediment

Millipore polycarbonate filters with a diameter of 90 mm and pore size of 0.45 μm were used to filter the defrosted samples.

Amberlite XAD-8 resin (Polysciences, INC) was used to extract HS from water and ice samples. In order to eliminate organic compounds (e.g. hydrolyzed acrylic acid) bleeding from XAD 8 columns (500 mL and 36 mL) resin cleaning by Soxhlet sequential extractions for 24 h were performed 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, respectively, and then washed with deionised water until dissolved organic carbon (DOC) concentration was less than 0.3 mg L^{-1} (deionised water).

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

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

All reagents used were analytical grade.

Humic substance recovery from pack ice

Pack ice cores were subdivided into two (superficial portion of ice: **A**, portion in contact with sea water: **B**) or three (superficial portion of ice: **A**, intermediate portion: **B**, portion in contact with sea water: **C**) subsamples. After defrosting the subsample, HS were recovered according to the procedure proposed by Thurman and Malcolm [18]. Briefly, ice subsamples (4–10 L) were filtered on 0.45 μm polycarbonate filter in order to separate dissolved HS from colloidal clay and suspended organic carbon [19]. After acidification to pH 2, the filtered samples were passed through the Amberlite XAD-8 resin column (36 ml). HS was 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 HA (precipitate) from FA (soluble in acid solution). HAs, when present, were purified by dialysis, and FAs by diafiltration [10].

Recovery of PHS was achieved by treating polycarbonate filters with 0.1 M NaOH for 24 hrs in batch conditions and then applying Thurman and Malcolm's procedure [18].

Humic substance isolation from water

The procedure proposed by Thurman and Malcolm [18] used for HS recovery from the ice samples was also applied to HS recovery from the water. The water

samples (100L) were subdivided into two subsamples: one was filtered and after acidification to pH 2 was initially passed through an Amberlite XAD-8 resin column of 500 ml capacity. After recovery from the column, the acidified eluate was passed through an Amberlite XAD-8 resin column of 36 ml capacity in order to obtain the most efficient preconcentration treatment. HS was 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 HA (precipitate) from FA (soluble in acid solution). HAs, when present, were purified by dialysis, and FAs by diafiltration [10].

The other subsample was acidified to pH 2 and directly preconcentrated using the same procedure. The difference between the HS content extracted from the two subsamples represents the HS adsorbed on the particulate material (PHS). Tests showed that the amount recovered using this method is close to that recovered by membrane treatment.

Humic substance extraction from sediments

The FAs and HAs were extracted from sediments using King's procedure (1967) [20]. Briefly, the sediment (200 g) was batched with HCl 0.1 N for 24 h and then twice with NaOH 0.1 N for 24 h. The residue was treated twice with HCl 0.5 N (1 L) and NaOH 0.5 N (1 L), respectively, for 24 h. All basic solutions were acidified to pH 2 with concentrated HCl and the HA precipitate separated by centrifugation from the FA solution. HAs were treated twice with the HF-HCl (0.3 – 0.1 M, respectively) solution for 24 h in batch conditions and then dialysed (1000 D pore size tubular membranes) against bidistilled water until chloride free. Finally, HAs were lyophilised and FAs were purified according to the procedure proposed by Thurman and Malcolm (1981) [18].

Apparatus

An Amicon stirred ultrafiltration cell, model 8400, capacity 50 ml, equipped at the bottom with 500 Dalton membrane disc was used for the FA purification [10]. The cell is connected to a reservoir (5 L) containing nitrogen pressurised (4 atm) diafiltrate solution (deionised water). By varying the operating conditions, the cell can also be used under nitrogen pressure (3.5 atm) without interfacing with the reservoir. In this way it can work in preconcentration conditions.

An FTIR Philips spectrometer model PU9800 operating in diffuse reflectance mode was used for HS characterization. Spectra are given in Kubelka Munk units which represent a mathematical formula applied to diffuse reflectance spectra. In this way, signal intensity varies linearly with variations in sample concentration.

The samples were prepared by mixing the dried HS sample with anhydrous KBr (ratio 1:100).

A Carlo Erba AE11110 CHNS-O model was used for elemental analyses of HS samples.

RESULTS AND DISCUSSION

The dissolved fulvic acid (DFA), particulate fulvic acid (PFA) and particulate humic acid (PHA) concentrations found in the pack ice core portions are reported in Table II. At stations 1, 2, and 3, characterized by different stages of pack ice melting, samples were collected only in January; in stations 5, 6, 7 and 8 (Wood Bay) sampling was repeated more time from November to February.

TABLE II Dissolved fulvic acids, particulate fulvic acids and particulate humic acids concentrations in pack ice and sea water samples A) Samples of 1996/97 Italian Expedition; B) Samples of 1997/98 Italian Expedition

A)

		23–28 january			*february*		
		mg/L			*mg/L*		
Station	*Sample*	*DFA*	*PFA*	*PHA*	*DFA*	*PFA*	*PHA*
1	**PackA**	0,06	n.d.	n.d.			
	PackB	0,26	0,43	n.d.			
	surface	0,11	0,40	n.d.			
2	**PackA**	0,04	0,29	n.d.			
	PackB	0,21	0,25	n.d.			
	surface	0,1	0,13	n.d.			
3	**PackA**	n.d.	0,14	n.d.			
	PackB	0,07	0,11	n.d.			
	surface	0,11	0,20	n.d.			
4	**Pack**					Ice absent	
	surface				0,09	0,02	n.d.

*Standard deviations are lesser that 5%

B)

		7–20 november			*1 december*			*18–28 december*			*1–15 january*			*february*		
		mg/L			*mg/L*			*mg/L*			*mg/L*			*mg/L*		
Station	***Sample***	***DFA***	***PFA***	***PHA***	***DFA***	***PFA***	***PHA***	***DFA***	***PFA***	***PHA***	***DFA***	***PFA***	***PHA***	***DFA***	***PFA***	***PHA***
	PackA	0,16	0,09	0,34	0,17	0,15	0,40	0,11	0,09	0,32						
	PackB	0,32	0,14	0,41	0,26	0,26	0,58	0,22	0,16	0,50					Ice absent	
5	**PackC**	1,44	0,95	1,95	1,80	0,88	0,90	2,42	1,90	1,05						
	surface	0,11	0,03	n.d	0,10	0,06	n.d	0,12	0,02	n.d				0,18	0,70	n.d
	-100m	0,06	0,01	n.d	0,08	0,02	n.d	0,07	0,00	n.d				0,11	0,01	n.d
	-380m	0,09	0,01	n.d										0,12	0,00	n.d
	PackA	0,12	0,07	0,24												
6	**PackB**	0,14	0,08	0,39							0,09	0,07	0,24		Ice absent	
	PackC	0,67	0,59	0,53							0,57	0,32	0,53			
	surface	0,14	0,01	n.d							0,16	0,06	n.d	0,15	0,58	n.d
	PackA	0,18	0,16	0,18							0,01	0,10	0,48			
7	**PackB**	0,17	0,16	0,32							0,15	0,04	0,55		Ice absent	
	PackC	2,99	1,72	0,58							0,93	0,90	0,78			
	surface	0,10	0,02	n.d							0,12	0,02	n.d	0,17	0,82	n.d
	PackA										0,16	0,38	n.d.			
8	**PackB**							0,47	0,36	n.d.	0,34	0,37	n.d.			
	PackC							0,38	0,35	n.d.	0,48	0,18	n.d.			
	surface							0,24	0,41	n.d.	0,12	0,20	n.d.			

*Standard deviations are lesser than 5%

At Gerlache Inlet stations, DFA and PFA concentrations increase from superficial portion (A) to portion in contact with sea water (C or B in the case of stations 1, 2 and 3 where the ice cores sampled were subdivided into only two portions). In stations 5, 6 and 7, PHA are also present and their concentrations increase along the ice cores. In the Wood Bay station HS concentration trend is different probably because the sampling condition is different. In the first sampling the ice cores were 160 cm long while in the second sampling they were 260 cm long. The increase in ice core length was due to the intense snowfalls occurring during the sampling period. The DFA concentration is constant along the ice core in the first sampling but increases from portions A to C in the second one. The PFA content is constant along the length of the ice cores sampled on 18–28 December and decreases in the C portion of the ice cores sampled in January. No PHA are ever found. In a previous paper [22] it has been shown that the Antarctic snow contains HS, in agreement with the presence of HS found in the second sampling. On the other hand, the structural features of HS in portion A are similar to those found in snow [22].

TABLE III Elemental analysis data of dissolved and particulate fulvic acids extracted by pack ice portions

Sample		*C %*	*N %*	*H %*	*S %*	*O %*	*N/C*	*H/C*	*S/C*
Pack A	DFA	44÷51	2.0÷3.3	5.9÷6.9	0.0÷2.8	40÷44	0.04÷0.06	1.56÷1.84	0.00÷0.02
	PFA	46÷48	5.2÷7.4	6.3÷6.8	0.6÷0.8	35÷40	0.09÷0.13	1.60÷1.70	0.00÷0.01
Pack B	DFA	46÷50	4.0÷4.4	6.7÷7.0	1.9÷2.4	37÷41	0.07÷0.08	1.65÷1.76	0.01÷0.02
	PFA	45÷48	3.3÷7.0	5.8÷6.7	0.4÷1.1	38÷45	0.06÷0.12	1.54÷1.70	0.00÷0.01
Pack C	DFA	46÷51	5.2÷7.0	6.3÷6.8	1.1÷1.4	34÷41	0.10÷0.12	1.60÷1.74	0.01÷0.02
	PFA	45÷50	5.3÷8.2	6.2÷7.2	0.6÷0.8	36÷43	0.10÷0.15	1.65÷1.72	0.00÷0.01

*Standard deviations are lesser than 3%

The elemental analysis data (Table III) of DFA and PFA extracted by pack ice are in agreement with literature data of aquatic HS, which are characterised by values of 45–55 % for C, 1.0–3.0 % for N, 2.5–6.5 % for H and 0.5–2.0 % for S [4,10,13]. Only the N content of HS extracted from the C pack ice core portions is higher. Considering that the phytoplanktonic material is trapped in portion C during ice formation, the high N content highlight the presence of HS at an early stage in the humification process. Previous studies [9,14,21] have shown that the pathway for the incorporation of N into the HS macromolecules could be explained by Ishiwatary's model. During bacterial attack on decaying phytoplankton, Maillard-type reactions would occur among refractory biopolymers,

partially degraded biopolymers and regenerated molecules, leading to the formation of HS. Since proteins and carbohydrates are major components of phytoplankton and bacteria, the main characteristics of HS formed in these early stages should primarily reflect the composition of the source material.

IR analysis of DHS and PHS in C pack ice core portions (Figure 2) confirms the presence of high content of proteinaceous and carbohydrate material. The signals at 1650 and 1580 cm^{-1} are due to I and II bands of amides and that at 1050 cm^{-1} can be ascribed to the hydroxyl groups of carbohydrates. The structural features are similar for HS in the C portion but they change from A to C in all stations. In particular, the FTIR spectra of HS isolated from portion A (Figure 3) show an intense peak at 1710 cm^{-1}due to carboxyl groups, which prevails over all other signals.

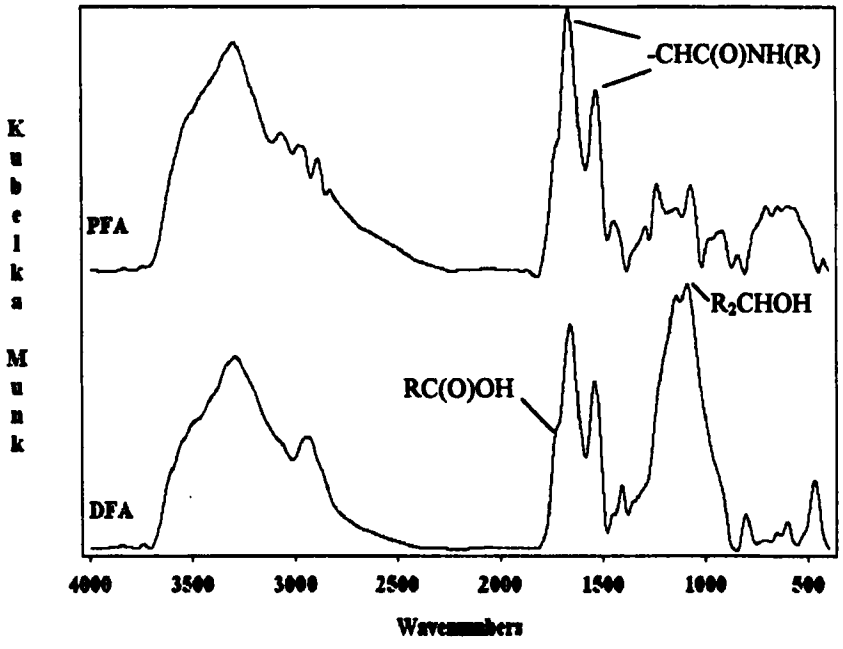

FIGURE 2 FTIR spectra of dissolved (DFA) and particulate (PFA) fulvic acids in portion C ice cores

The structural differences and the different HS concentrations found in the three ice core portions are probably due to different humification stages. HS extracted from portion C are probably formed earlier, while HS present in portion A may be partly due to the transport "via marine aerosol" of HS present in surface waters [22].

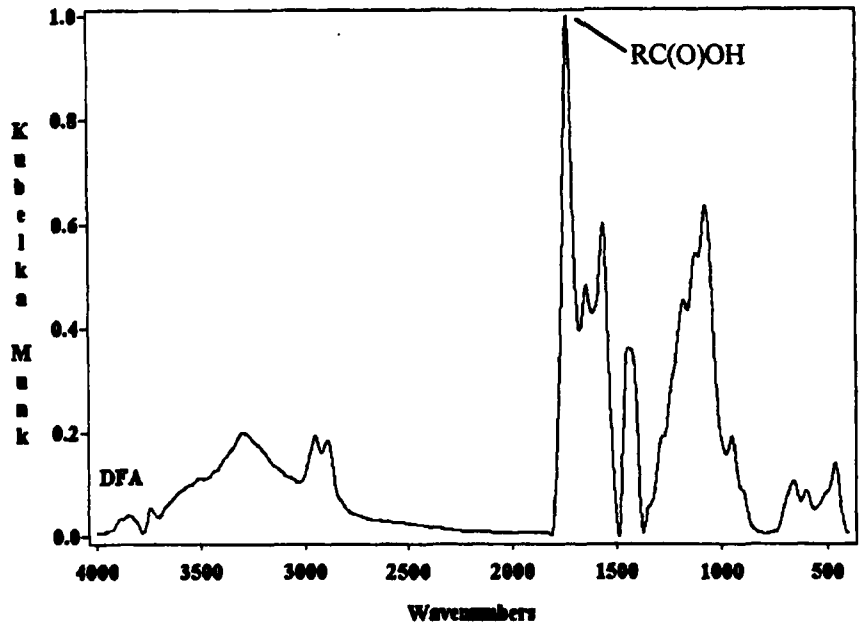

FIGURE 3 FTIR spectrum of dissolved fulvic acids in portion A ice cores

Some differences among the stations can be observed by analysing the data of HS concentrations over time.

In stations 1, 2, and 3 the pack ice cores had a different content of phytoplanktonic material trapped in the B portion, as observed during the sampling on the basis of the coloured layer thickness. In station 1 the brown coloured layer was thicker than that in station 2 and was completely absent in station 3. The amounts of HS and phytoplanktonic material content appear to be closely related. DFA and PFA concentrations actually decrease from station 1 to station 3 in agreement with the release of organic and inorganic matter trapped in the ice into the water column, during melting of ice. Also in stations 6 and 7, where the samples were collected more time from November to February, the amount of DFA and PFA and the phytoplanktonic material content seem to be closely related. The PHA concentrations are equal or increasing but a decrease in the amount of total HS (DFA+PFA+PHA) is observed over time.

In station 5 an increase in DFA concentration is observed in C pack ice core portions over time and no change in PFA and PHA concentrations. These results might be explained by HS formation occurring without release of HS into the water column.

Analysis of HS isolated from surface seawater sampled under the pack ice layer (Table II) and when the ice is absent show that DFA concentrations are constant over time. In station 5, 6, and 7 an increase of PFA concentration is observed sampling surface seawater when the ice is absent; nevertheless no PHA are ever found. The increase in the amount of PFA does not account for the release of HS into the water column during ice melting. The absence of any increase in HS concentration in surface waters and also along the water column, sampled in station 5 may be explained by the different salinity values of the ice (from 6 to 20 ‰) and of the seawater (34 ‰) [23]. When melting pack ice releases HS trapped in the ice, the higher salinity value of the seawater makes the HS less soluble and increases the sedimentation process rate. This phenomenon can be compared with the precipitation process occurring in estuaries [24–27]. The mixing of river water with seawater causes several chemical changes to occur in HS structures, including a stronger contraction of the molecules and the aggregation of high-molecular-weight materials [24]. Further, this hypothesis is confirmed by FA/HA ratios (Table IV) extracted by the sediment sampled in each station when the ice is absent. The FA/HA ratios are higher than those found for sedimentary HS extracted from samples collected in Antarctic areas where the ice is always absent [28,29].

TABLE IV Fulvic and humic acid content in sediments

Station	*FA (mg g^{-1})*	*HA (mg g^{-1})*	*FA/HA*
2	0,36	1,43	0,25
5	0,52	1,94	0,27
6	0,78	2,18	0,36
8	0,40	0,90	0,44

*Standard deviations are lesser than 4%

The structural features of HS isolated from surface seawater are similar to those found in the ice. Comparing FTIR spectra (Figure 4) of seawater DFA with those of DFA in C pack ice, a correlation coefficient higher than 0.95 is obtained. The seawater DFA spectrum (Figure 4) shows signals due to lipid content (2900–2890 cm^{-1}, symmetric and antisymmetric stretching of CH_2 and CH_3 in linear and branched chains) and 1710 cm^{-1} (C=O), and signals due to protein content (1640 and 1580 cm^{-1}, bands of amide I and II), while the OH group content is shown by the large band at 1100 cm^{-1}.

The elemental analysis data of seawater DFA sampled at different depth along the water column are similar (Table V).

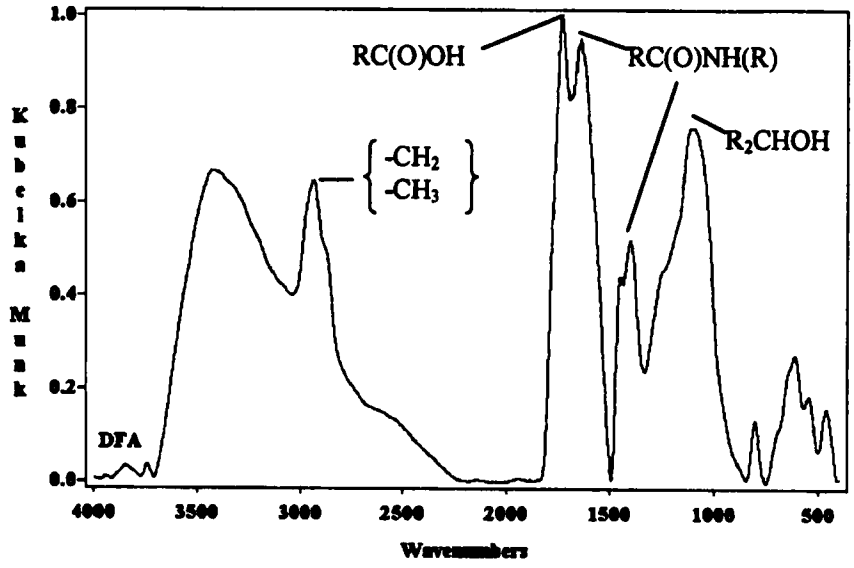

FIGURE 4 FTIR spectrum of dissolved fulvic acids in surface water

TABLE V Elemental analysis data of dissolved fulvic acids extracted by seawaters samples

Sample		*C %*	*N %*	*H %*	*S %*	*O %*	*N/C*	*H/C*	*S/C*
Surface	DFA	47÷50	1.3÷3.5	5.4÷6.9	0.0÷0.5	40÷45	0.02÷0.06	1.33÷1.48	0.00÷0.01
- 100 m	DFA	47÷50	3.0÷3.5	5.5÷6.1	0.6 ÷1.5	39÷44	0.03÷0.06	1.40÷1.48	0.00÷0.01
- 380 m	DFA	49÷50	2.2÷3.6	5.8÷6.4	absent	41÷42	0.04÷0.06	1.41÷1.57	absent

*Standard deviations are lesser than 3%

CONCLUSIONS

Analysis of the organic matter content of pack ice cores indicates that the pack ice is an organic matter "tank". The humification process possibly occurs in pack ice where the phytoplanktonic material is trapped in ice during its formation. The structures and the HS concentration differences in the three ice core portions are probably due to several humification stages. The HS extracted from the C portion are probably formed earlier than HS in portion A, which are probably due to transport "via marine aerosol " of HS present in surface waters.

HS isolated from surface seawater sampled under the pack ice layer and when the ice is absent show concentrations that are constant in time. An increase of PFA concentrations is observed only in samples collected in three stations where the ice was absent. No PHA has ever been found and the increased amount of PFA does not explain the possible release of HS into the water column during ice melting. The absence of HS concentration increase in surface waters and along the water column may be explained by the different salinity values of ice (from 6 to 20 ‰) and seawater (34 ‰), which can determine an increase in the sedimentation process rate (Figure 5). This hypothesis is confirmed by the FA/HA ratios in sediments sampled in each station when ice is absent. The value of FA/HA ratios are higher than those found for sedimentary HS extracted from samples collected in Antarctic areas where ice is always absent.

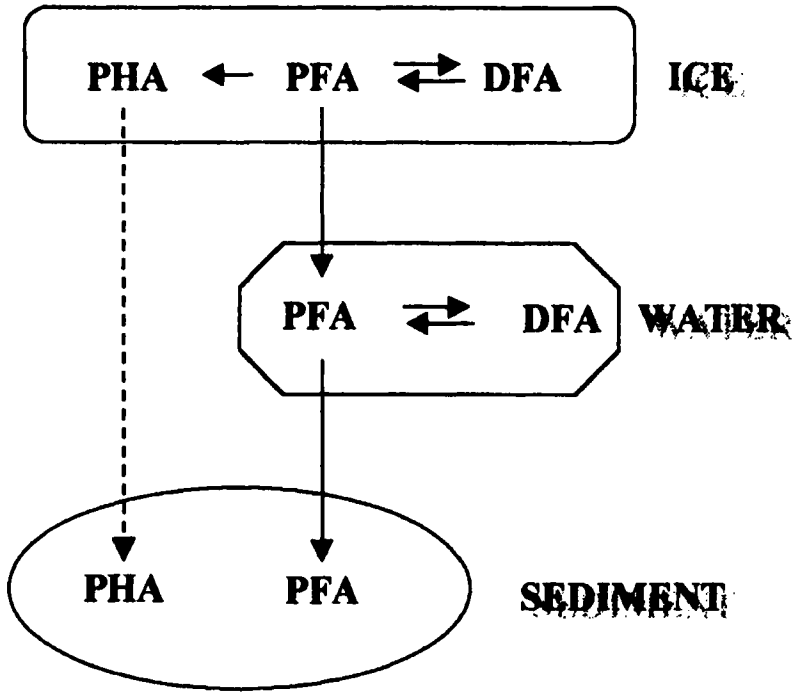

FIGURE 5 Scheme of sedimentation process

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