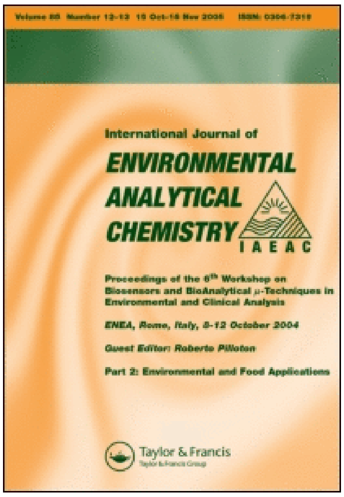

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SEDIMENTARY HUMIC ACIDS IN THE CONTINENTAL MARGIN OF THE ROSS SEA (ANTARCTICA)

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Humic acids (HA) extracted from Antarctic marine sediments were characterized using different techniques (FTIR, NMR, Thermogravimetry, Elemental Analysis and Ionic Exchange Chromatography). Antarctic sedimentary HA show a greater content of nitrogen and a predominant aliphatic nature when compared with data reported in the literature for non Antarctic HA samples. Some differences in the structures, such as the presence of aromatic carbon rings, carbohydrates, fatty acids, etc., have been observed, as a function of sampling zones. The differences are probably due to a different humification process, possibly depending on different trends of temperature and salinity measured down to the water column.

KEY WORDS: Sedimentary humic acids, Antarctica, Ross Sea

INTRODUCTION

The organic part of the sediments may be divided in humic (humic and fulvic acids) and non-humic fractions (carbohydrates, lipids, aminoacids, organic acids, etc.).

The humic fraction is the prevailing part[1] and consists of an heterogeneous mixture of compounds, yellow to black colored, having high molecular weight. Aminoacids and carbohydrates are also part of the humic structures. Oxygen is present within different functional groups (e.g., COOH, OH, C = O, and ethers and esters).

The humic acids extracted from marine sediments may have autochtonous and allochtonous origin, according to some authors[2]. As regards the autochtonous formation a condensation (probably like Maillard's) of carbohydrates, aminoacids and small molecules, has been hypothized[3]. Beside this hypothesis, Harvey *et al.*[4] suggest a mechanism in which fatty acids or polyunsatured glycerides, through an oxidative cross-linking, give rise to fulvic acids first and then humic acids.

The characterization of the structures of humic compounds provides important information about the primary material and the probable formation's mechanism. It represents the starting point to evaluate the capacity of interaction towards metals and different classes of organic compounds (e.g. herbicides and pesticides). In fact, the great

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reactivity of humic substances must be connected with the high number of functional groups: their presence depends both on the humification process and the organic precursors.

The study of humic compounds in Antarctic sediments is important because the starting material is non-ligniferous and the formation's mechanism may be feasibly affected by the typical climatic conditions of Antarctica (very low temperatures, alternation of long periods of light and darkness, lack of humidity, etc...).

EXPERIMENTAL

Sampling

Sediment samples were collected with box-corer in the Antarctic expedition in 1990/91; for each station a superficial (0–15 cm) and the layer below (15–30 cm) of the sediment have been sampled. Immediately after collection the samples were frozen at –30°C.

The sediment samples collected were divided into three groups according to the temperature trend of the water column[5]. Humic acids (HA) from sediments of two groups: n.1 and 2 (zone A) and n.3–6 (zone B) (Table 1) have been analyzed.

Procedure

Fulvic (FA) and humic (HA) acids were extracted from the sediments with the procedure outlined by the International Humic Substances Society[6] with the modifications suggested by Rashid and King[7]. After the NaOH treatment (solubilization of FA_I and HA_I), a series of alternate extractions with HCl 0.1 M and NaOH 0.5 M is carried out in order to solubilize the humic fraction bound to the clay through metal ions (FA_{II} and HA_{II}).

At first time, the I and II fractions were not mixed and were analyzed for elemental composition, thermogravimetry, FTIR and ^{13}C-NMR spectrometry.

Then the two fractions were mixed and again analyzed. Some (HA) samples were subjected to an acid hydrolysis (HCl 6 N) and aminoacids in the hydrolized solutions were identified and quantified by ionic exchange chromatography.

Table 1 Sample location and water depth.

Sample	*Lat.S*	*Long.E*	*Water depth*
1	70°43’4	171°28’3	2285 m
2	70°45’9	172°51’4	2410 m
3	74°25’68	169°35’28	678 m
4	75°01’8	169°56’7	336 m
5	74°38’6	171°32’2	463 m
6	74°46’5	168°10’05	495 m

Apparatus

A FTIR Philips spectrophotometer model P3202 working in diffuse reflectance conditions was used. The results are given in Kubelka Munk units; the Kubelka Munk is a mathematical formula applied to diffuse reflectance spectra. The samples were prepared by mixing the dried humic acids (1 mg) with anhydrous KBr (100 mg).

^{13}C-NMR spectra were determined using a Varian spectrometer model XL–300. The samples were prepared in a NMR tube (5 mm) by dissolving the dried humics (30–10 mg according to the available material) in 1 ml of NaOD 0.5 M. The operating conditions were: 75 MHz, pulse 45°, acquisition time 0.1 sec., delay time 0.5 sec. From 600,000 to 1000,000 scans were accumulated, according to the sample concentration.

Elemental analysis were carried out by a Carlo Erba 240–B model CHN-analyzer in the Microanalysis Laboratory of the Italian Research Council.

Thermogravimetric analysis were carried by a Perkin Elmer TGA thermogravimetric analyzer in N_2 atmosphere, between 50 and 950°C, scanning rate 20°C min^{-1}, 1.5 mg of sample.

A Pharmacia LKB plus 4151 aminoacid analyzer was used, equipped with a cationic exchange resin. Elution was carried out with buffer solutions (citric acid/citrate) at different pH values. Aminoacids were detected with ninhydrin reagent.

RESULTS AND DISCUSSION

Fulvic acids have not been obtained, as observed for Antarctic marine sediment samples[8,9].

Comparison of the ^{13}C-NMR and FTIR spectra (Figure 1) and elemental analysis data of HA_I and HA_{II} (N%, 7.8 and 7.2; C%, 72.2 and 67.8; H%, 8.1 and 7.9, respectively) shows that they are similar; consequently, we have applied the Rashid and King extraction procedure blending HA_I and HA_{II}.

Results reported in Table 2 are referred to the mixture of HA_I and HA_{II}. In this Table are also reported data ranges of the literature[10–13].

The extraction yields for the Antarctic sediments are lower than those of the non-Antarctic ones[2], even though the extraction method we adopted followed the usual scheme, with the addition of a further step. The elemental analyses show that the nitrogen content is higher than the values of the literature, while generally that of oxygen is lower; the H/C ratio is higher.

The FTIR spectra of the humic acids before and after hydrolysis demonstrate that the prevailing part of the nitrogen content is due to the aminoacids bound to the humic structure[14]. In fact, the bands at 1535–1540 cm^{-1} (due to the stretching of peptidic linkage) and at 1690 cm^{-1} (I band of amides) result drastically reduced, whereas the one at 1710 cm^{-1} (stretching $C = O$ of carboxyl groups) is unchanged (Figure 2). Moreover, the N/C ratio decreases after hydrolisis (from 0.1 to 0.05). ^{13}C-NMR spectra of an Antarctic and a non-Antarctic humic acid[15] (Figure 3) show that in the last one the ratio between aliphatic carbon (0–50 ppm) and the aromatic one is lower. Moreover, the 170 and 200 ppm zone, typical of carboxyl, ketonic, amidic and aldehydic groups is higher (if compared with the aliphatic carbon zone), according to the high oxygen content of the non-Antarctic humic acids.

Humic compounds shortage in Antarctic sediments may be attributable both to the poorness of organic substrates and to the rigid climate of Antarctica that makes slower

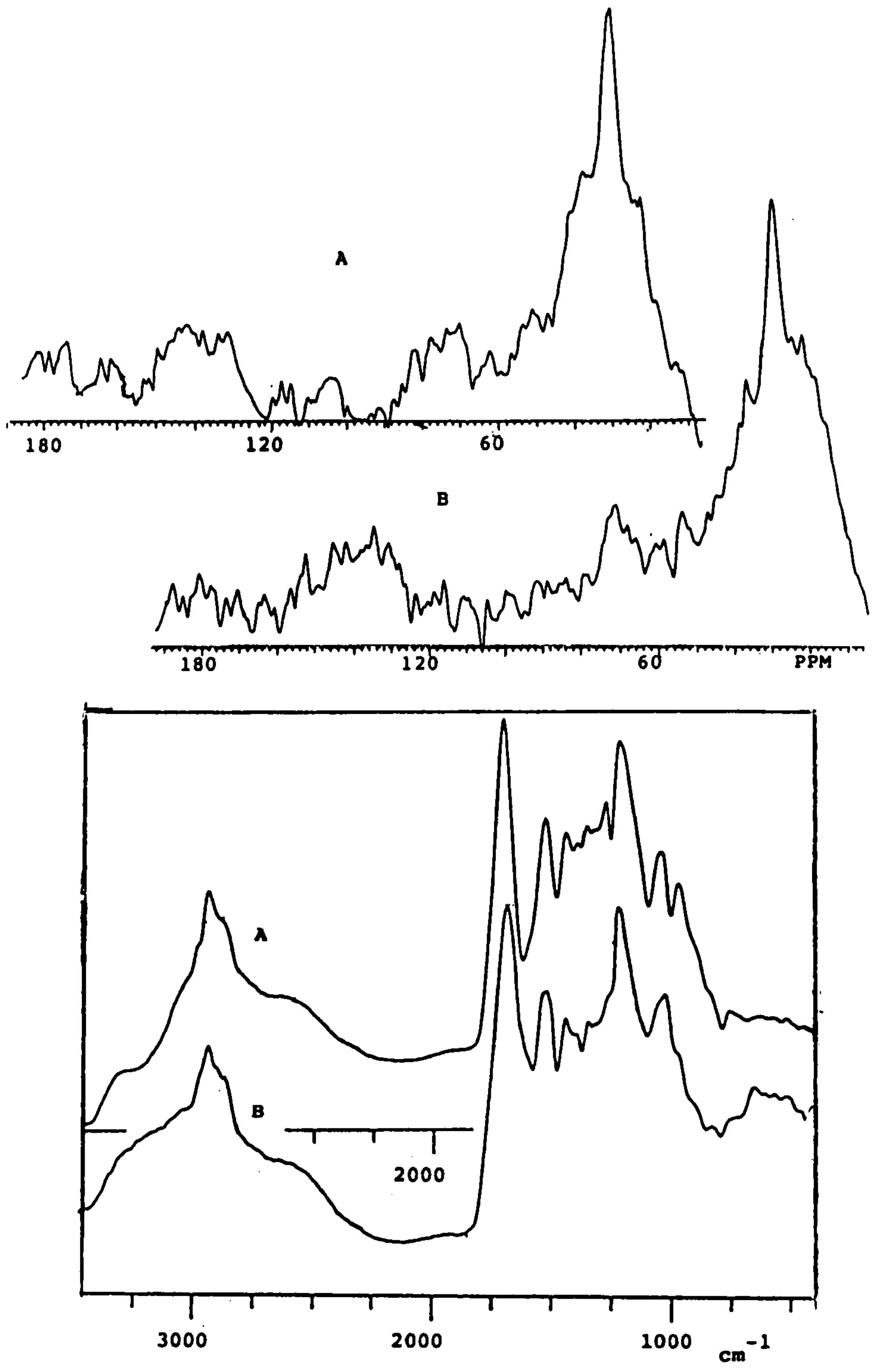

Figure 1 FT-IR and ^{13}C-NMR spectra of HA$_{I}$ (A) and HA$_{II}$ (B).

Table 2 Elemental analysis (%) and yields (%) of HA (ash- free) extracted from superficial and upper layers of sediment samples.

Sample	*Layer*	*N (%)*	*C (%)*	*H (%)*	*O (%)*	*H/C*	*N/C*	*Yield %*
1	superf.	5.98	51.97	6.05	36.0	1.40	0.099	0.028
	upper	5.81	51.16	5.37	37.7	1.26	0.097	0.005
2	superf.	6.78	56.78	7.14	29.3	1.51	0.102	0.043
	upper	6.48	62.23	6.39	24.9	1.23	0.090	0.020
3	superf.	6.15	79.75	6.85	7.25	1.03	0.070	0.082
	upper	5.46	77.46	6.72	10.40	1.04	0.060	0.056
4	superf.	8.23	72.71	9.00	10.1	1.49	0.100	0.187
	upper	6.49	77.99	7.78	7.74	1.20	0.070	0.059
5	superf.	7.10	73.71	8.57	10.6	1.40	0.090	0.095
	upper	7.02	72.56	8.40	12.0	1.39	0.080	0.373
6	superf.	7.53	67.05	8.02	17.4	1.44	0.100	0.231
	upper	5.88	51.81	6.41	35.9	1.48	0.100	0.402

Literature:	*N (%)*	*C (%)*	*H (%)*	*O (%)*	*H/C*	*Yield %*
superf.	3.9–5.5	53–60	4.8–7.1	26–38	1.1–1.5	0.4–0.8
upper	–	–	–	–	–	0.5–0.8

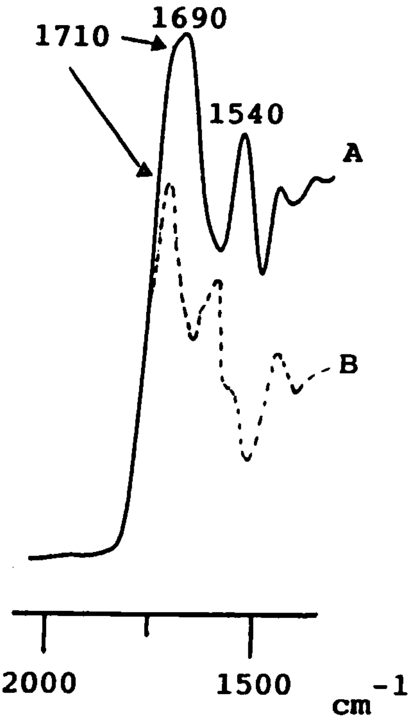

Figure 2 FT-IR spectra of humic acids before (A) and after (B) acid hydrolisis.

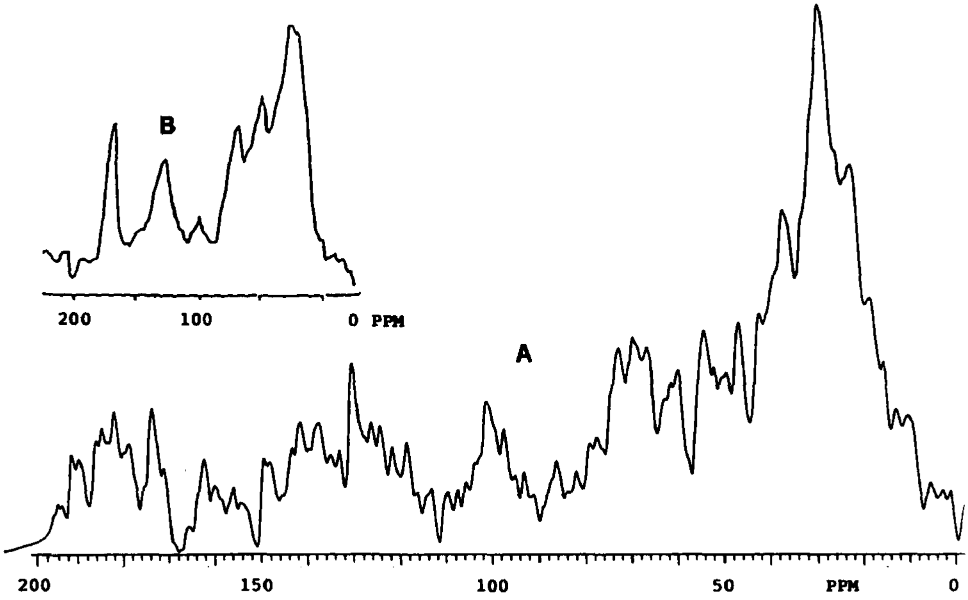

Figure 3 ^{13}C-NMR spectra of an antartic (A) and a non-antartic (B) marine humic acid.

the microbiological activity and the chemical transformations. Also the high nitrogen and the lower oxygen contents may be attributable to an incomplete transformation of proteinaceous materials due both to biological activity considerably reduced[16] and to slow chemical or enzymatic oxidation processes of aliphatic chains.

The low degree of condensation and aromatization, typical of the humic material in marine sediments[17], is particularly marked for the Antarctic marine humic acids (deducible from H/C ratio and ^{13}C-NMR spectra). In fact, the allochthonous contribution from the continent cannot influence the aromaticity of the humic substances, because the terrestrial Antarctic flora is made up to algae and moss devoid of lignin, aromatic precursor of the humic material.

Comparing the values of elemental analysis (Table 2) of humic acids extracted from the superficial and from the upper layer, it may be observed that the humic acids of the superficial layer have higher nitrogen and hydrogen contents. Humic acids of the superficial layer seems to show a lower sedimentary alteration's degree; this process occurs with loss of aliphatic chains and of nitrogen as ammonia, for mineralization of proteins[18,19]. A decrease of functional groups (Figure 4) with the increase of sediment depth is observed. For the nitrogenous groups (amids bands at 1680 and 1540 cm^{-1}) the alteration process is very fast, whereas the oxygen groups (stretching of C–O at 1040 cm^{-1} and stretching of C = O at 1700 cm^{-1}) disappear more slowly[20].

Elemental analysis, FTIR and ^{13}C-NMR of the humic acids extracted from the sediments of A-zone present some differences in respect to that of B-zone, even though they maintain the peculiar characteristics of Antarctic humic acids. The extraction yields for the zone A were significantly lower. Differences are also noted for the elemental

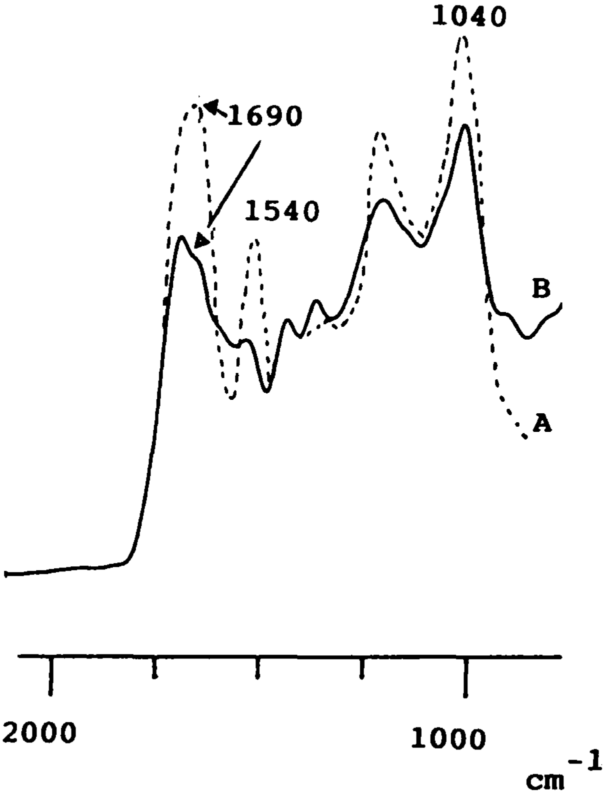

Figure 4 FT-IR spectra of HA extracted from superficial (A) and upper (B) layer of the sediment.

Table 3 Ranges of C, H, N percentages for HA (ash-free) extracted from A- and B-zone.

Zone	*Layer*	*N %*	*C %*	*H %*
A	superf.	6–7	50–60	6–7
	upper	6–7	50–60	5–6
B	superf.	7–8	70–80	7–9
	upper	6–7	50–75	7–8

composition (Table 3). Moreover HA of A-zone show, compared with those of the B-zone (Figure 5), more aromatic structures (^{13}C-NMR: zone 120–130 ppm), and major presence of carbohydrates and alcohols (FTIR: band at 1020–1159 cm^{-1}). HA of B-zone are characterized from long chains of fatty acids (peaks at 32 ppm and 2925, 2865 cm^{-1}).

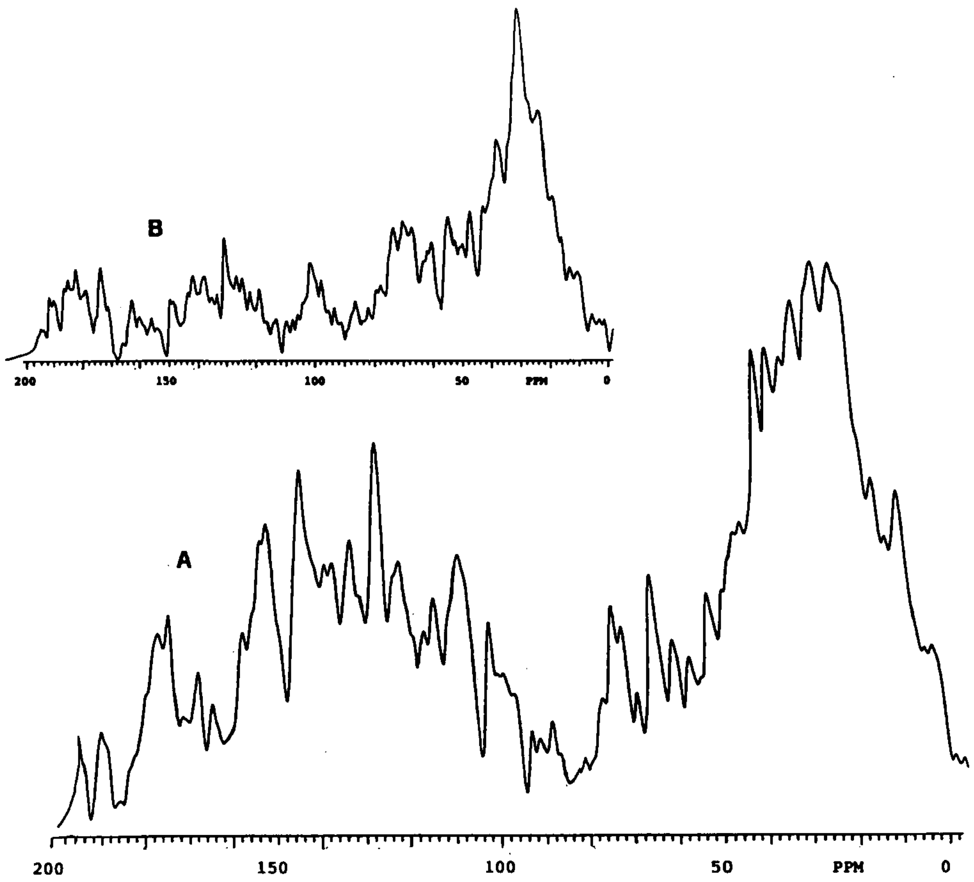

Figure 5 ^{13}C-NMR spectra of HA of the A-zone (A) and the B-zone (B).

The HA's aminoacidic content of both zones is 1–2 micromol/mg of humic acids (on ash-free basis); per cent distribution of aminoacids is reported by the hystogramms in Figure 6.

Glycine, aspartic and glutammic acid, alanine and lysine are the more abundant aminoacids (70% of the total content) in the zone A HA, whereas, in the zone B, they are glycine, aspartic and glutammic acid, alanine and serine (60% of the total content). In the zone A, lysine and glutammic acid have the same weight percentage, whereas in the zone B, the glutammic acid is present in larger amount (10–20% and 4–5%, respectively) according to the literature data[21,22]. In the zone B the basic aminoacids (hystidine, lysine and arginine) are lower than those ones of the zone A.

The differences noted for the two zones cannot derive from precursors (both autochtonous and allochtonous); different humification and sedimentary processes may justify these outcomes (an opposite temperature trend of the water column has been observed).

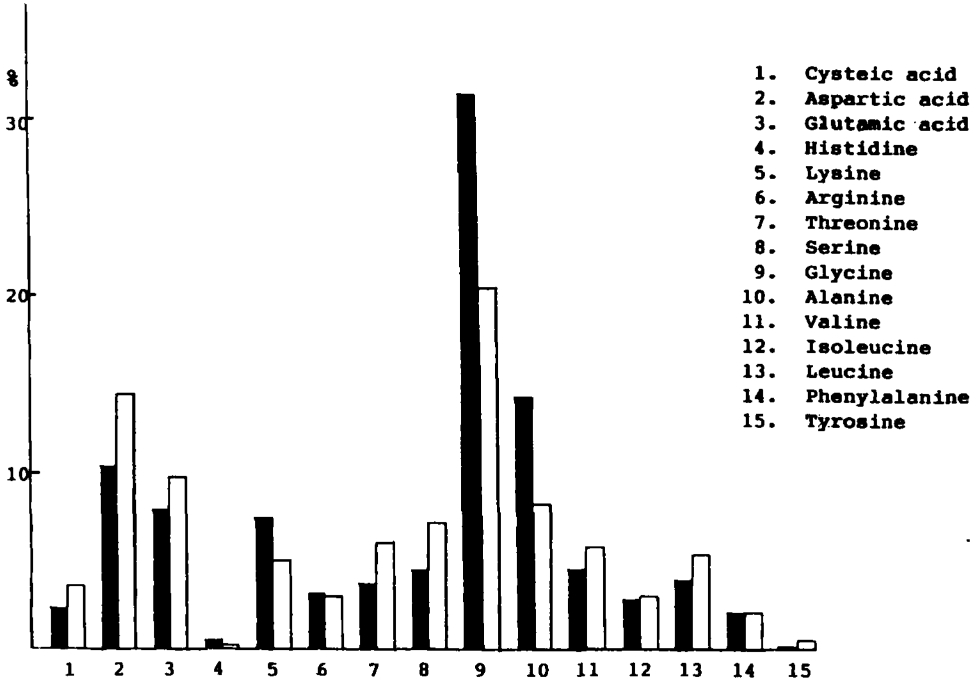

Figure 6 Aminoacid % distribution for HA of the A-zone (black coloured) and of the B-zone (white coloured).

CONCLUSIONS

The HA of marine sediments are characterized by structures that differ from the classic ones because they are affected both by the peculiar nature of precursors and the particular severe climatic conditions of Antarctica. In particular, the structures of Antarctic humic acids are generally less aromatic and their elemental composition presents a higher nitrogen and lower oxygen content.

However, some structural differences for Antarctic sedimentary HA originating from different zones have been noted, probably due to dissimilar environmental conditions (temperature, salinity, sediment depth) in which they have been formed. The observed diversities may be responsable for different complexing (and consequently different metal speciation in the sediments) and sorption capacities.

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