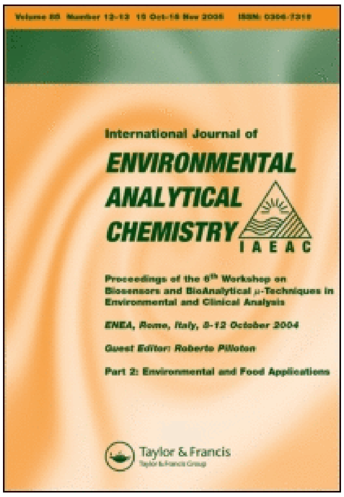

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CHARACTERIZATION OF HUMIC ACIDS ISOLATED FROM ANTARCTIC SOILS

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Humic acids isolated from Antarctic soils have been characterized by FTIR, ^{13}C-NMR and elemental analysis; aminoacid content has been determined after hydrolysis with 6 N HCl. Nitrogen and hydrogen contents of HAs are very high, indicating that more proteinaceous material, covalently bound to the structures, has not undergone remarkable transformation during the humification processes. Moreover, the aliphatic carbon prevails on the aromatic one, owing to the nature of non-ligniferous material as precursor. With the progress of the humification process, obtained in temperate climatic conditions, the proteinaceous material decreases, as shown by the analysis of aminoacids, and the molecular weight of the humic compounds increases whereas aromatic carbon does not.

KEY WORDS: Antarctic soils, humic acids.

INTRODUCTION

The main characteristic of humic substances is the presence of high molecular weight polimeric structures.

Schnitzer *et al.*[1] have recently suggested a carbon skeleton in which alkylbenzene structures play a dominant role in soil humic acids (HA). Because the humification processes strictly depend both on the parent materials and on the physico-chemical and biological parameters, the structures of soil humic acids from Antarctica are probably different from those ones suggested by Schnitzer.

The absence of lignin as precursor may be responsible for a higher proportion of aliphatic structures. Moreover, the lack of humidity may determine a greatest amount of large molecules, because the depolymerization processes are slower. Humic molecules, apparently polymerize progressively during the dry season, while the duration of the humid season appears to be responsible for the decrease in the amount of large molecules because of the depolymerization process[2].

In this paper we have studied some humic acids coming from Antarctic soils, their structures characterized by FTIR, ^{13}C-NMR, elemental analysis have been compared with those ones reported in literature.

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EXPERIMENTAL

Sampling

The soil samples have been collected in the zones listed in Table 1. The Nos. 7 and 8 samples come from damp microclimate zones (near flowing waters and lakes where moss grows profusely).

All samples are superficial (0–15 cm) because under the 0–15 cm layer the permafrost is present; only in the stations Nos. 1 and 9 are collected both superficial (0–15 cm) and lower (15–30 cm) layers.

Procedure

The soil samples were frozen immediately after the collection, then defrozen (six months after), sieved (4 mm) and treated according to the procedure of International Humic Substances Society for extraction of humics, as outlined previously[3].

In the case of sample No. 7, a soil portion was defrozen and then held in temperate climatic condition (30–38°C, regularly damped with water) for three months; humic and fulvic acids were then extracted.

Humic and fulvic acids were characterized by FTIR and ^{13}C-NMR spectrophotometry and elemental analysis; the concentration of carboxyl groups was determined with the calcium acetate method[4].

In some cases both the determination of aminoacids present as component in the humic structures (carried out after hydrolysis with 6N HCl[5]) and the distribution of molecular weights by gel permeation chromatography on Sephadex G resins[6] have been carried out.

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

Table 1 Location of sampling points and extraction yields (%).

Sample No.	*Lat S*	*Long E*	*Yields (%)*	
			HA	*FA*
1	70°20'	165°07'	0.003	0.07
2	70°20'	165°07'	0.08	0.09
3	74°47'	163°38'	0.02	0.07
4	74°43'	164°01'	0	0.06
5	74°43'	164°01'	0.06	0.07
6	74°59'	162°33'	0	0.05
7	74°59'	162°33'	0.13	0.28
8	74°41'	164°02'	0.24	0.17
9	74°41'	164°02'	0.009	0.05

a mathematical formula applied to diffuse reflectance spectra. The samples were prepared by mixing the dried humic acids (1 mg) with anidrous 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 mg) 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 800.000 scans were accumulated.

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

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

RESULTS

Both humic and fulvic acids were extracted from samples collected in damp microclimate zones (samples n. 7 and 8); in the other zones, sometimes only fulvic acids were present. However, in all the samples (except Nos. 7 and 8) the humified matter is poor. Comparing HA extracted from superficial samples (0–15 cm) and those from the corresponding layer underneath (15–30 cm) some differences are evident. The extraction yield for the superficial sample is lower (0.003% and 0.02% for sample n.1 and 0.009% and 0.03% for sample n.9), as observed by several authors in soils with no illuvial horizon^{7,8}. Moreover, FTIR and ^{13}C-NMR spectra show that the superficial HA contain a greater concentration of carboxyl groups (172 ppm) and a higher content of nitrogenous compounds (1660–1540 cm^{-1}), confirmed by N/C ratio (Table 2), probably reflecting aminoacids bound to the humic structures. Aliphatic carbon is present especially as long chains of fatty acids (peak at 32 ppm). On the contrary, HA from the deeper layer have a more heterogeneous composition. In the aliphatic zone (0–50 ppm) peaks corresponding to terminal methyl groups (0–20 ppm) are shown, and in the 20–50 ppm range the signals have equal intensity (Figure 1). Carbon bonded to O or N heteroatoms is evident.

The differences observed may be ascribed to the different stages of the humification processes that in the deeper layer are more advanced. In fact, it is known that the humification processes are accompanied by some chemical modifications such as loss of –COOH functional groups, dehydrogenation, ring closure, increase of aromaticity^{9}. The

Table 2 Elemental analysis of humic acids extracted from different layers of soils: superficial (0–15 cm) (A) and (15–30 cm) (B).

Sample n.	*1*		*9*	
	A	*B*	*A*	*B*
N (%)	8.57 + 0.34	3.96 + 0.16	8.04 + 0.21	3.38 + 0.08
C (%)	53.68 + 2.15	38.70 + 1.55	60.29 + 2.41	38.07 + 1.52
H (%)	8.24 + 0.41	6.93 + 0.41	8.04 + 0.32	4.02 + 0.20
O (%)	29.51 + 1.47	50.41 + 2.21	23.63 + 0.62	54.23 + 2.01
H/C	1.84 + 0.07	2.15 + 0.09	1.60 + 0.05	2.68 + 0.11
N/C	0.14 + 0.01	0.09 + 0.01	0.11 + 0.02	0.08 + 0.11
Ash (%)	12.3 + 2.0	14.0 + 3.0	25.2 + 3.0	23.5 + 2.5

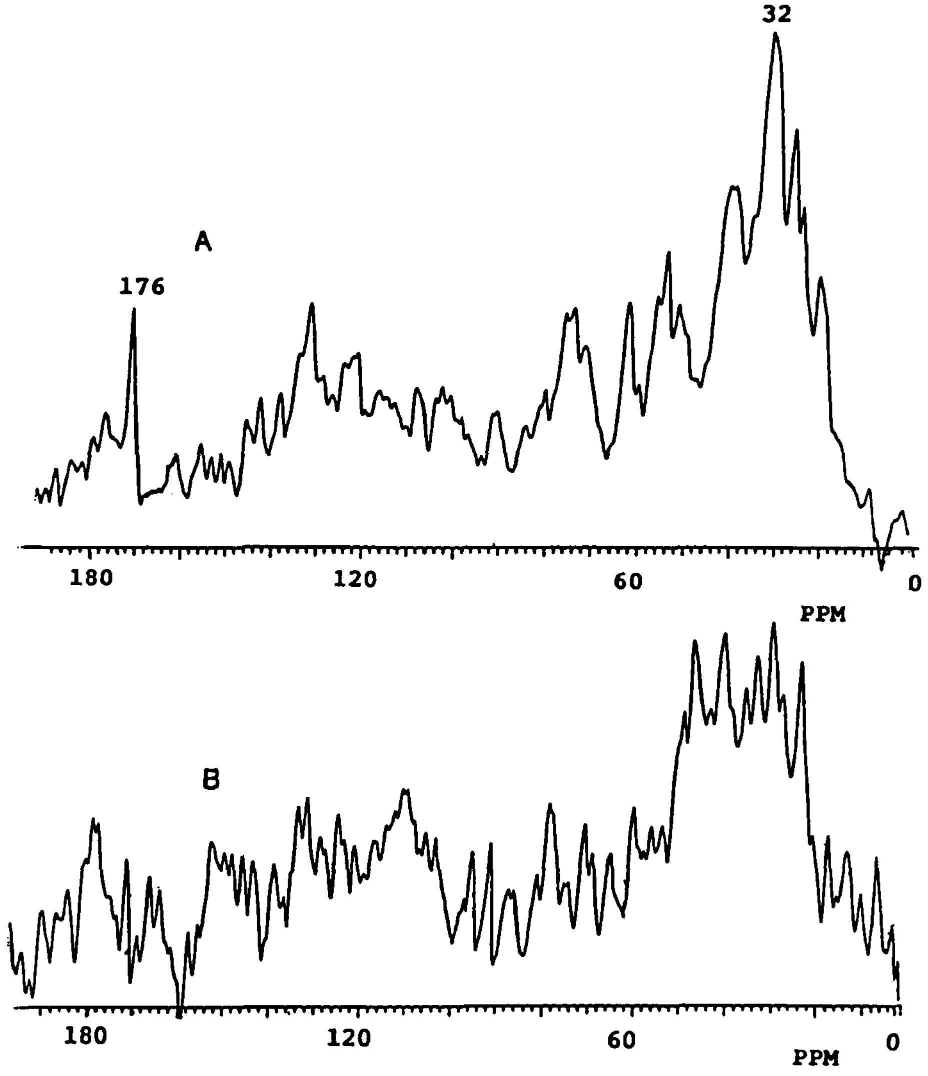

Figure 1 ^{13}C-MNR of HA of superficial (A) and upper (B) layer.

elemental analysis data for humic acids of the lower horizon of soil show that the percentage of C, H, and especially N decrease (Table 2).

Analysing the FTIR and ^{13}C-NMR spectra of humic acids obtained from the superficial samples, some differences are observed between those having mosses (samples Nos. 7 and 8) as precursors and the others. On the contrary, the data of elemental analysis (Table 3) do not differ significantly: only the oxygen content is higher (samples 7 and 8).

The amount of humified matter, both HA and FA, is higher in samples Nos. 7 and 8 (derived by moss), (Table 1) and it is even smaller than that obtained from no Antarctic soils[10].

Table 3 Elemental analyses of humic acids extracted from superficial (0–15 cm) layer of soil samples.

Sample No.	*2*	*3*	*5*	*7*	*8*
N (%)	10.41 + 0.91	8.48 + 0.52	11.30 + 0.67	7.52 + 0.30	8.86 + 0.53
C (%)	66.80 + 2.67	63.20 + 2.14	68.12 + 2.58	59.62 + 1.49	56.02 + 1.68
H (%)	8.93 + 0.27	7.62 + 0.24	9.10 + 0.23	8.17 + 0.16	7.65 + 0.19
O (%)	13.90 + 0.82	20.72 + 0.62	11.53 + 0.57	24.70 + 1.02	27.52 + 1.03
H/C	1.61 + 0.10	1.44 + 0.11	1.60 + 0.09	1.64 + 0.11	1.64 + 0.11
N/C	0.13 + 0.03	0.11 + 0.02	0.14 + 0.02	0.11 + 0.01	0.13 + 0.03
COOH (meq/g)	1.34 + 0.02	1.78 + 0.13	1.78 + 0.14	1.36 + 0.02	1.86 + 0.07
Ash (%)	17.7 + 3.5	22.2 + 3.0	21.4 + 2.5	18.5 + 4.0	10.1 + 2.5

FTIR and ^{13}C-NMR spectra of HA of samples Nos. 7 and 8 show a high adsorption at 1715 cm^{-1} and a signal at 190 ppm, probably due to C = 0 groups of aldehydes and ketones, since the concentration of carboxylic groups is not very high.

However, all Antarctic superficial HA are characterized by the aliphatic carbons prevailing over the aromatic ones. This characteristic can be related with the nature of precursors. In Antarctica the humic substances rise from mosses and algae materials rich in lipids, carbohydrates and proteins, without lignin. The presence of aromatic structures could be prevalently due to residual aromatic aminoacids like tryptophan, phenylalanine and tyrosine of algal origin or to tannins and flavonoids from different origin, or to both of them.

Nitrogen percentage of Antarctic HA is higher (Table 3) than that of the corresponding HA of no Antarctic soils[11-16], probably due to slow transfromations of protein and peptides during the humification processes. The oxigen content and carboxyl groups concentration are generally lower, because of the nature of precursors and the particular climatic conditions. Consequently, in Antarctica the structures of HA of soils are similar to those of marine and lacustrine sediments[3,17-19].

To study the effects of climatic conditions on the Antarctic humic substances we have compared humic acids extracted from sample No. 7 (A) with those of the same sample mantained three months at temperate conditions (30–38°C) (B).

The extraction yield increases (0.13% for A and 0.29% for B), the elemental analysis data do not change significantly and the major oxygen containing groups decrease. The total content of aminoacids, obtained after hydrolysis with 6 N HCl, decreases (2.55 and 2.07 μmol/mg respectively; the signals at 1660 and 1540 cm^{-1} of FTIR spectra (Figure 3) are inverted). Aspartic and glutamic acids decrease significantly (Figure 2). In both samples the percentages of acid, basic and neutral aminoacids are in good agreement with those reported by Stevenson[20] for many soils.

The distribution of molecular weights and the nature of the single fractions are different for the two samples (Table 4). In samples the compounds of the first fraction (1.500–5.000 molecular weight range) show a little content of nitrogen (N/C rate 0.01 instead of 0.08) and a higher percentage of C = C stretching vibrations (H/C rate 0.71 instead of 1.33). ^{13}C-NMR spectrum is not well resolved indicating the complexity and the no repeatibility of the structure of the sample subunits[21,22]. FTIR spectrum shows a signal at 1760 cm^{-1}, probably due to C = 0 stretching of β-ketoesters; in the corresponding fraction of sample A (Figure 3) the prevailing signal appears at 1710 cm^{-1} (carboxyl groups) according to the elemental analysis data. Compounds with a high molecular weight (> 100.000) are present only in sample B (Table 4).

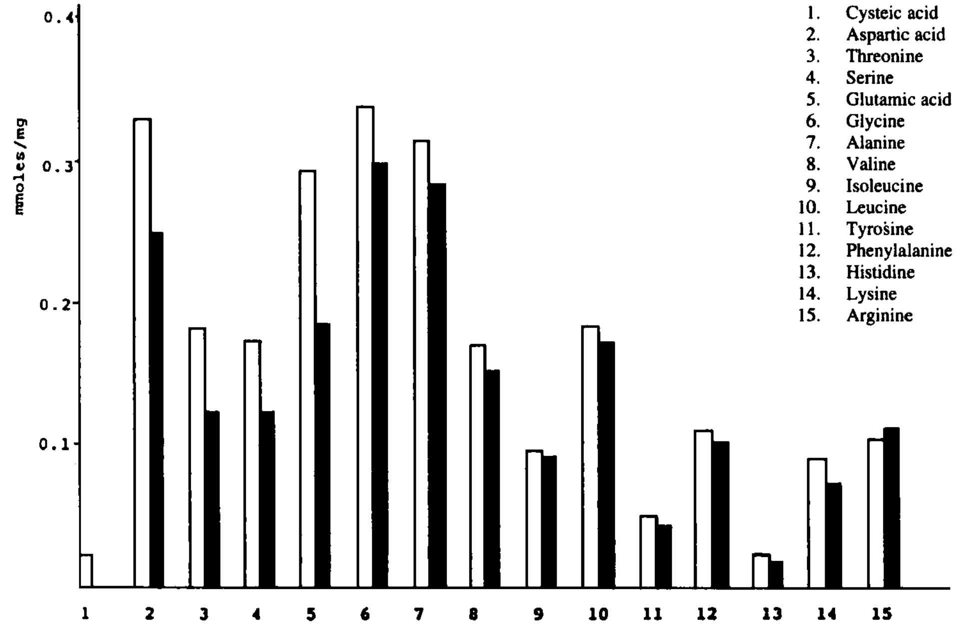

Figure 2 Aminoacid distribution in HA of sample n. 7 before □ and after ■ the progress of humification process.

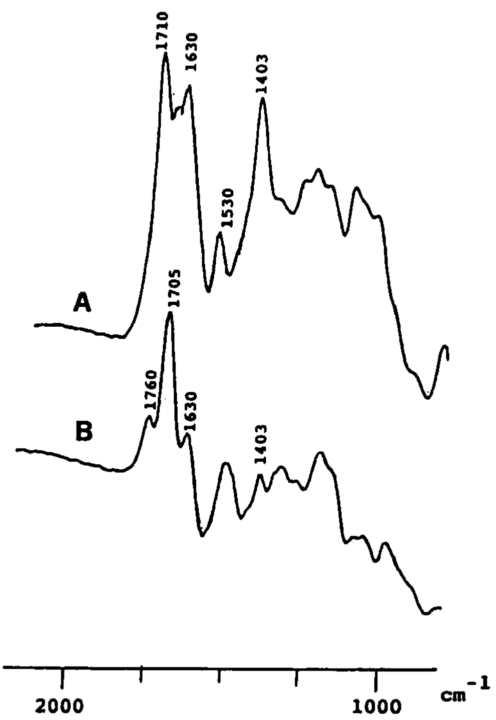

Figure 3 FTIR spectra of HA of the fractions with molecular weight 1500–5000 before (A) and after (B) the progress of the humification process.

Table 4 Elemental analysis, major containing oxygen groups and yields of humic acids extracted from sample No. 7 before (A) and after three months in climatic temperate conditions (B).

Sample No.	*7A*	*7B*
Yield (%)	0.10 + 0.05	0.30 + 0.05
H (%)	8.17 + 0.16	8.40 + 0.24
N (%)	7.52 + 0.30	7.05 + 0.25
C (%)	59.62 + 1.49	61.45 + 2.50
O (%)	24.69 + 1.24	23.10 + 2.05
H/C	1.64 + 0.11	1.64 + 0.11
N/C	0.11 + 0.01	0.10 + 0.01
O/C	0.31 + 0.01	0.28 + 0.01
Ash (%)	18.5 + 2.5	22.0 + 3.5
Aminoacid (nmol/mg)	2552 + 80	2072 + 75
Total acidity (meq/g)	6.06 + 1.03	3.73 + 0.76
–OH groups (meq/g)	4.70 + 1.04	2.71 + 0.77
–COOH groups (meq/g)	1.36 + 0.01	1.02 + 0.01

Table 5 Differences between HA (samples No. 7A and 7B) with different degree of humification (A and B).

		7A			*7B*	
Molecular weight	*Yield*	*N/C*	*H/C*	*Yield*	*N/C*	*H/C*
1500–5000	5.6	0.08	1.33	5.8	0.01	0.71
50000–100000	90.2	0.13	1.50	9.0	0.12	1.47
> 100000	–	–	–	84.0	0.11	1.58

The differences observed point out that the humified process proceeds with the formation of compounds characterized by molecules with high molecular weight. In this process the most abundant fraction (molecular weight 50000–100000) of sample A is probably involved. Moreover, the increase of extraction yield suggests that non-humic materials begin the humified process; the formation of compounds with low molecular weight may be due to the short exposition time.

CONCLUSIONS

Antarctic soils generally contain a low amount of humic compounds, owing to the lack of organic materials.

Humic compounds are present in high amounts only near the lakes and flowing waters there is a high amount of parent materials; in the other sites humic material is present in low quantity because it comes from the snow precipitations[23] or it is derived from the organic matter transported by the wind.

From an environmental point of view, the results obtained point out that the complexing capacity of Antarctic soils towards metals, due to the organic matter, is low. Moreover, the complexing capacity of Antarctic HA could be different owing to the differences observed in the structures.

Acknowledegments

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