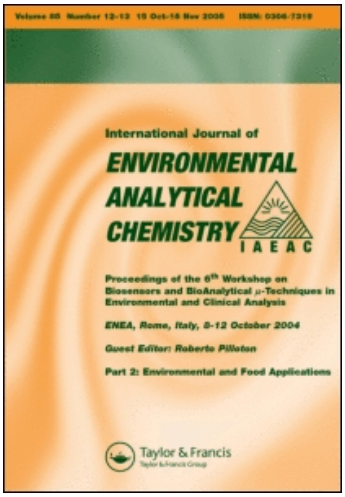

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STUDY OF HUMIC FRACTIONS FROM WATER OF AN ANTARCTIC LAKE

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Soluble humic compounds isolated from water of an Antarctic lake have been characterized by IR, NMR spectrophotometry and fluorimetric measurements. Gel chromatography on Sephadex G resins of fulvic acids, present in the water in high concentration, has evidenced that a great fraction of them consists of compounds having molecular weight greater than 50,000 dalton. The high level of humics and the presence in soluble form of compounds having high molecular weight and similar structure to the humic acids of lake sediments may be due to the high pH value of the water examined.

KEY WORDS: Humic substances, lake water, Antarctica.

INTRODUCTION

Humic substances are natural polyelectrolytic organic compounds of complex structure involving a proportion of more or less condensed aromatic rings, with a large number of -OH and -COOH groups fixed on them[1]. Humics are subdivided into three groups (humic acids, fulvic acids and humins) on the basis of their solubility in basic and acidic solutions, even if this division is artificial. More recently, humic compounds are considered as a mixture of cross-linked polymers of different molecular weights and charge densities[2]; their solubility probably being a function of molecular weight and charge density[3].

Humics are the principal organic components of waters as 60–80% of dissolved organic carbon consists of humic compounds[4]. It is known that pH value of water influences their solubility. Because the water's pH of Antarctic lakes ranges from 8.5 to 10.5, the water

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conditions should favour the solubilization of these polyelectrolytes. Consequently, it is interesting to verify this hypothesis and to study the structures and the molecular weight distribution of soluble humic compounds. In fact, under these conditions humics having high molecular weight may be present. As a result, the complexing ability and the interaction capacity with organic hydrophobic micropollutants may be different from that in natural water.

In this paper we study aquatic humics from an Antarctic lake using Amberlite XAD-8 resins as adsorbent material[5] and Sephadex G resins for molecular weight distributions[6].

EXPERIMENTAL

Material

A water sample collected in the Antarctic Carezza lake (74°43 S, 164°01 E) (100 l) was frozen at −30°C immediately after the sampling. Water temperature and pH at the sampling moment were 10.1°C and 9.8, respectively.

Preparation of fulvic (FA) and humic (HA) acids

After two months the water sample was defrozen, filtered on a 0.45 μm cellulose acetate disc and the filtrate, acidified to a pH 2 by addition of concentrated hydrochloric acid, was passed through two Amberlite XAD-8 columns (500 and 36 ml respectively) as outlined by Thurman[5]. Recovery of humics from the second resin column was carried out with 50 ml of 0.1 M NaOH.

The basic extract, when acidified to pH 2 with hydrochloric acid, yields a solution (FA) and a precipitate (HA). The acid solution was evaporated to obtain a solid sample; FA and HA were dialyzed against water with molecular-porous membranes (MWCO:100 and 1000, respectively) until chloride free and lyophilised.

Sample treatments, apparatus and analysis techniques

The two fractions were analyzed by fluorimetric measurements and by IR and ^{13}C-NMR spectrophotometry. Thermogravimetric and elemental analyses were also performed.

To obtain more information, FA were solubilized in 0.1 N NaOH (25 ml) and fractionated by gel chromatography on Sephadex G resins (Sephadex G 25, G 50, G 75, G 100) according to the Wershaw *et al.* procedure[6]. Two fractions were obtained. These were concentrated, dialyzed (MWCO: 500), dried and characterized by IR and ^{13}C-NMR spectrophotometry.

A fluorescence spectrophotometer Perkin-Elmer LS50-P, computer controlled, was used. The slits were adjusted at 8 mm width, the scan speed was 240 nm/min. No correction procedure was performed.

Three types of fluorescence measurements were carried out: emission spectra, synchro-

nous scan spectra (especially suitable for the analysis of heterogeneous mixtures[7,8]) and multiple emission spectra (already used from Wade *et al.*[9] in the analysis of mixtures of organic compounds dissolved in freshwater).

The emission spectra were recorded in the range 350–630 nm, using 308 nm as exciting wavelength. The spectra were made at different pHs and concentrations (100–5 mg/l for aquatic FA, 20–1.25 mg/l for aquatic and sedimentary HA according to the available material). NaOH was added (final pH was 10.5–11) in order to assure the complete solubilization of the sample. To perform measurements at different pHs, the solutions (40 mg/l for aquatic FA and HA and 9 mg/l for sedimentary HA, respectively), were adjusted to the desired value (± 0.1 pH units) with NaOH or HCl 0.1 M, directly in the quartz cuvette. The pH changes were measured with an Ingold 402M3 microelectrode. Before each measurements session, we checked the cleanness of the cuvettes scanning a "blank" spectrum of triply distilled water whose intensity should not exceed 0.2 arbitrary units.

In synchronous scan spectra both excitation and emission wavelength change, but the wavelength interval is constant (20 nm, value chosen experimentally in order to enhance the peak resolution). Spectra were recorded in the range 350–550 nm (emission wavelength).

Multiple scan spectra were obtained from a graphic elaboration of a series of emission spectra recorded using increasing excitation wavelengths (in our case first excitation wavelength 225 nm, increment 5 nm, 18 spectra recorded for each plot). This set of spectra was elaborated in a three dimensional graph (x and y axes represent excitation and emission wavelength, ranges 225–310 and 350–550 nm respectively, z axis fluorescence intensity). To avoid interferences due to the Raman peak with higher excitation wavelength, we put before the emission monochromator a filter which cut out the wavelength shorter than 350 nm.

A FTIR Philips spectrophotometer model P3202 working in diffuse reflectance conditions was used. The results are given in Kubelka Munk units. The samples were prepared by mixing the dried humics (1 mg) with anydrous KBr (100 mg).

NMR spectra were determined in 0.5 NaOD using a Varian spectrometer model XL-300. The samples were prepared by dissolving the dried humics (30–10 mg according to the available material) in 1 ml of NaOD. 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 in the Microanalysis Laboratory of the Italian Research Council (Carlo Erba 240-B model).

Thermogravimetric analysis were performed with a Perkin Elmer TGA thermogravimetric analyzer in N_2 atmosphere (70 ml/min). The temperature was between 50 and 950°C, at a scanning rate 20°C/min, using 1.5 mg of sample.

RESULTS

The water of Carezza lake contains both fulvic and humic acids in dissolved form; the first are more abundant than the second ones (150 mg and 30 mg, respectively).

TG behaviour of HA (Figure 1A) shows a weight loss in the range 200–450°C due to

elimination of functional groups, carbohydrate degradation, decarboxylation, dehydrogenation (range 200–350°C) (10)(11), and dissociation and breaking down of aromatic structures and polynuclear systems (range 380–450°C) (12)(13). The low ash value points out a small presence of silica bound to humics.

^{13}C-NMR spectrum shows a greater number of signals by comparison with the sedimentary ones (14), probably owing to the poor repeatability of the structure of sample subunits (15). This fact may be related with the high pH value of water: in this condition a wide proportion of humic compounds with different chemical structures may be present in soluble form.

However, aliphatic carbons (0–50 ppm) are evidenced; the phenolic region (usually 140–165 ppm) seem to be greater or, at least, of the same significance of the carboxyl one (usually 165–190 ppm), especially if we consider that in basic solution acidic phenolic carbons can shift to below 170 ppm[16].

IR spectrum (Figure 1A) confirms the presence of carboxyl groups (peak at 1720 cm^{-1}) and points out carbons in CH_2OH and CHOH groups (1350–1260 and 1100–1050 cm^{-1} zones); proteinaceous material is also evidenced (CO adsorption at 1640 cm^{-1} and NH_2 deformation at 1570–1500 cm^{-1}), in good agreement with elemental analysis data (C = 52.3%, H = 5.75%, N = 7.99%, N/C = 0.13, H/C = 1.32). The described IR spectrum is similar to those of Type III (spectral classification of Stevenson[17]) obtained by a copropellic ooze of Mud lake (Florida), largely composed of algal remains not decayed, as outlined by Bradley[18].

Elemental analysis of soluble FA (C = 43.46%, H = 4.14%, N = 3.02%, N/C = 0.059, H/C = 1.14) shows that nitrogen contained compounds are present in low concentration; on the contrary the oxygen content is higher than in HA (O = 49.38% and O = 33.96% respectively). Also in this case, as for all Antarctic humic compounds, the H/C ratio and nitrogen content are high[19].

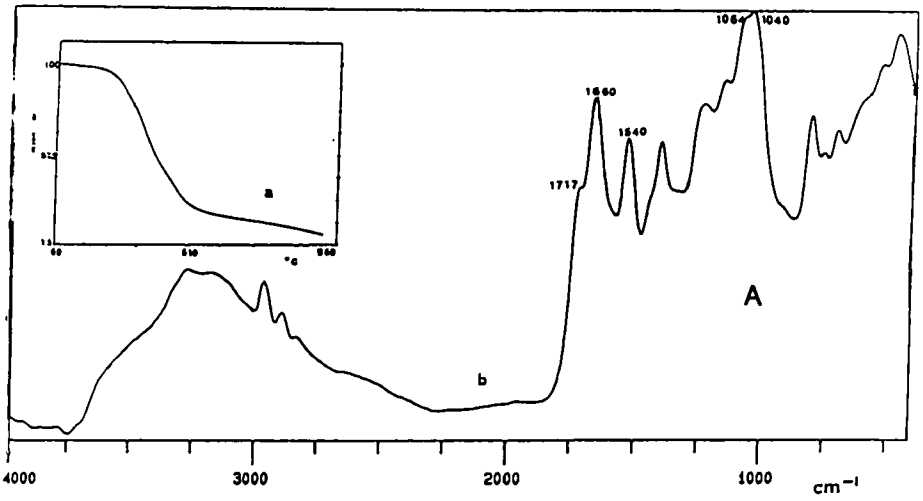

Figure 1 Thermogravimetric behaviour (a) and IR spectrum (b) of aquatic humic acids (A) and aquatic fulvic acids (B).

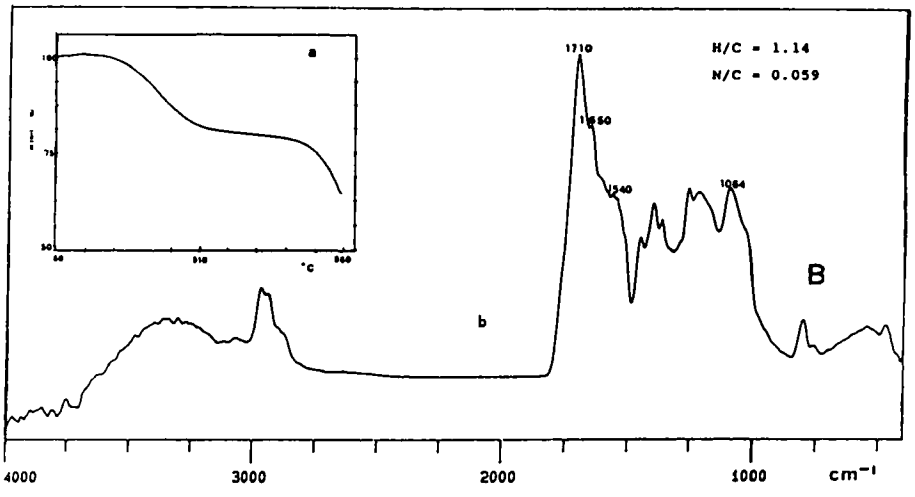

Figure 1 Thermogravimetric behaviour (a) and IR spectrum (b) of aquatic humic acids (A) and aquatic fulvic acids (B).

Thermogravimetric behaviour (Figure 1B) shows two weight losses (in the range 200–450°C and after 800°C). The second may be ascribed to the presence of carbonates which have been formed during the thermogravimetric analysis owing to a very remarkable process of decarboxylation of the samples.

IR spectrum (Figure 1B) of FA can be classified of Type II (17); it is characterized by very strong adsorption near 1710 cm^{-1}, due to carboxyl groups. The -COOH groups may be bonded both to aromatic structures (peak at 1650 cm^{-1}, only partially due to the peptide linkage of proteins, and signals at 160–170 and 110–160 ppm in ^{13}C-NMR) and to aliphatic moieties (signals at 174–186 ppm). Since intense carbohydrate bands occur in the ^{13}C-NMR spectrum, probably a portion of the carboxyl groups is present as uronic acids. Aliphatic carbon percentage is higher than in HA.

Additional informations regarding FA can be obtained from IR and NMR spectra of fractions separated on Sephadex gels. We have obtained two fractions: the first (FA_1, molecular weight ranges from 1500 to 5000) in low concentration and the second (FA_2, greater than 50000) more abundant.

In the FA_1 fraction (Figure 2) the quantity of aliphatic carbon is not higher than the aromatic one; in the aliphatic region the prevailing zone (40–60 ppm) is due to carbon bonded to O or N heteroatoms. The very pronounced peak at 167 ppm can be attributed to carboxyl groups bonded to aromatic rings. The strong adsorption band at 3300–3400 cm^{-1}, present in IR spectrum, may be due to intramolecular bonds.

In the FA_2 fraction (Figure 3) aliphatic carbon prevails (zone 0–48 ppm and peaks at 2980 and 2850 cm^{-1}); protonated aromatic carbons (zone 110–120 ppm and peak at 1660 cm^{-1}) and phenolic region (145–165 ppm) are also present. Carboxyl groups are shown (zone 170–185 ppm and peaks at 1740, 1710, 1550 cm^{-1}): these signals, together with the peak at

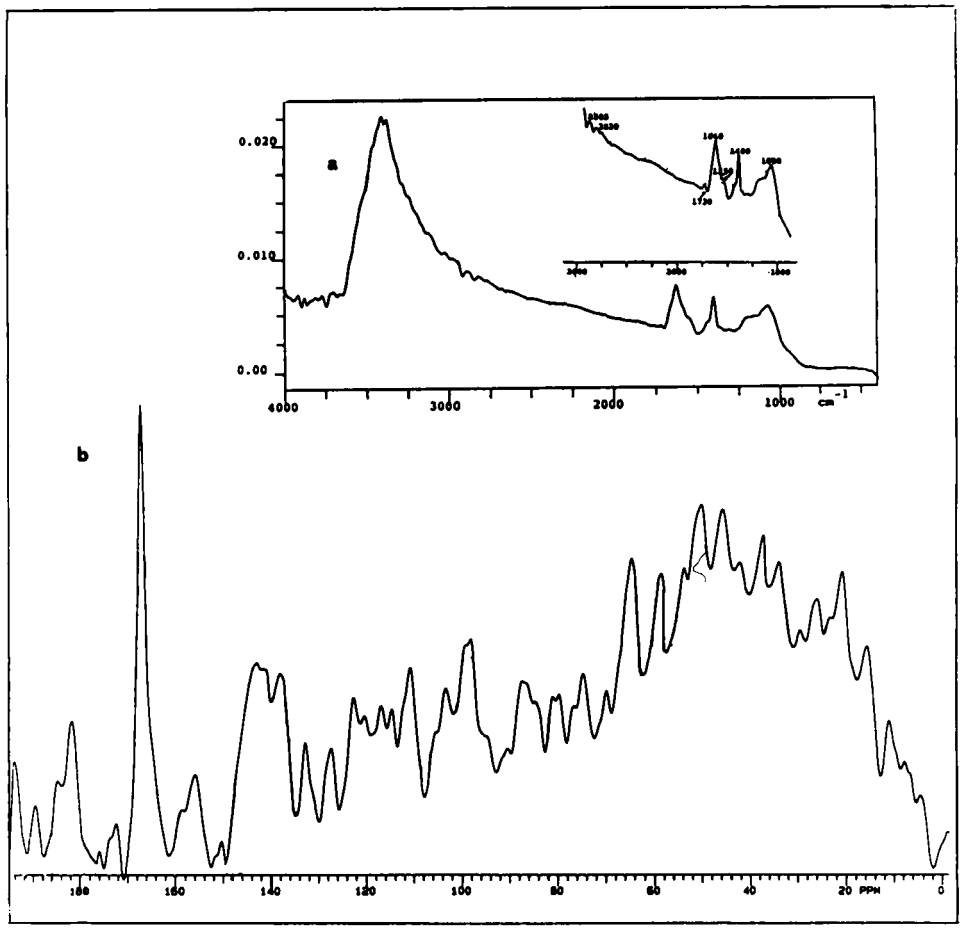

Figure 2 IR spectra (a) and ^{13}C-NMR spectrum (b) of fulvic acid fraction having molecular weight ranges from 1500 to 5000.

32 ppm, generally ascribed to methylene carbon atoms in long aliphatic chains such as those in lipids[20] point out the presence of fatty acids. The structure of this fraction is similar to that of sedimentary HA (14), but exhibits a higher intensity in the acid carbon zone, both phenolic and carboxyl. This fact confirms the hypothesis that the portion of humics with high molecular weight but rich in acid groups may be solubilized owing to the high pH value of water.

Comparing the three soluble fractions, FA_1, FA_2 and HA, it is noted that the aliphatic content increases with molecular weight; the contrary occurs for the acidic region, in particular for the aromatic acid carbon. The fluorescence measurements support the above statements.

The emission spectra of aquatic HA (Figure 4A) show single broad bands (similar to shoulders). The maximum, not well defined, should be situated at 400 nm. FA are much

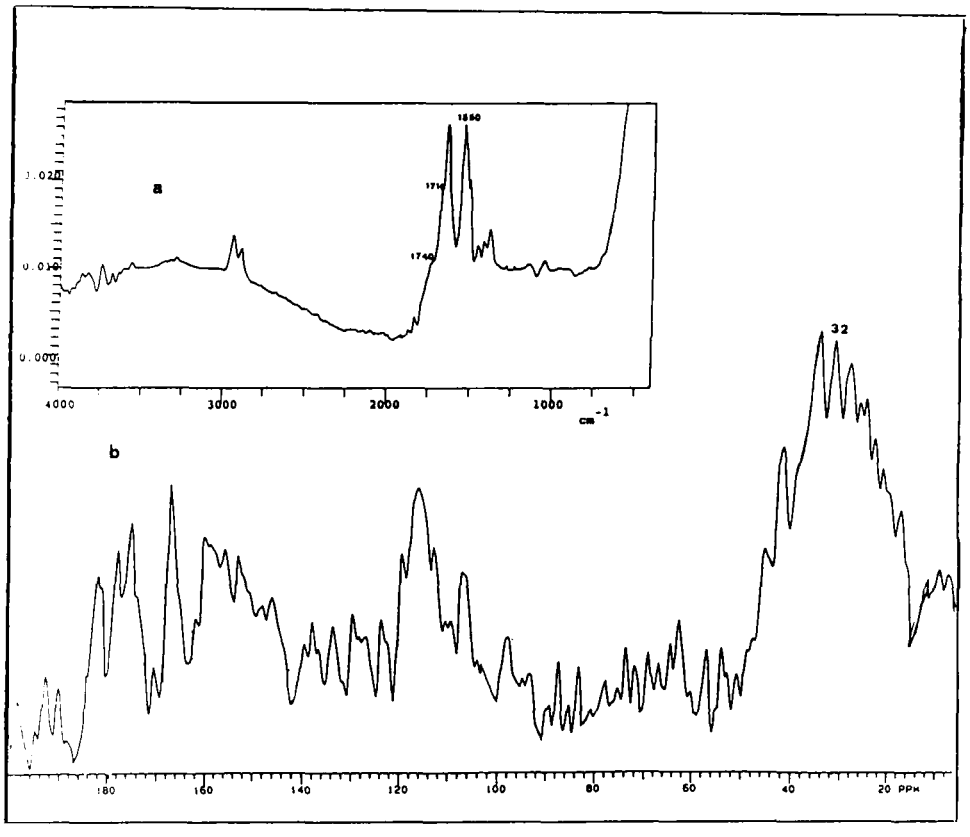

Figure 3 IR spectra (a) and ^{13}C-NMR spectrum (b) of fulvic acid fraction having molecular weight greater to 50000.

more fluorescent and give a spectrum less broad than the humic ones. As the humic material is highly heterogeneous, it is difficult to correlate the emission band to specific fluorophores[21]. However, since the wavelengths of the maximum of soluble FA and HA are not significantly different, we can hypothesize that the average molecular weight of fluorophores[21], the number of aromatic rings and/or conjugated double bonds[22] is more or less the same in aquatic humics. Moreover, the spectrum of the sedimentary HA collected in the same lake is very similar to that of aquatic fulvics. This further confirms the hypothesis that the chemical nature of FA present in water and that of HA of sediments is similar.

On the contrary, the plot of fluorescence intensity vs. concentration of aquatic FA is different from those of HA, both aquatic and sedimentary. In fact, while the plot of aquatic fulvics shows a good correlation between fluorescence intensity and concentration ($r = 0.997$, $n = 4$) in the range 0–40 mg/l, those of HA show a poor correlation ($r = 0.401$, $n = 5$ for aquatic and $r = 0.618$, $n = 5$ for sedimentary HA) even if the range of concentrations considered is narrower (0–20 mg/l). We can hypothesize that this is due to conformation variations of the molecules causing variations of the number of exposed chromophores. On

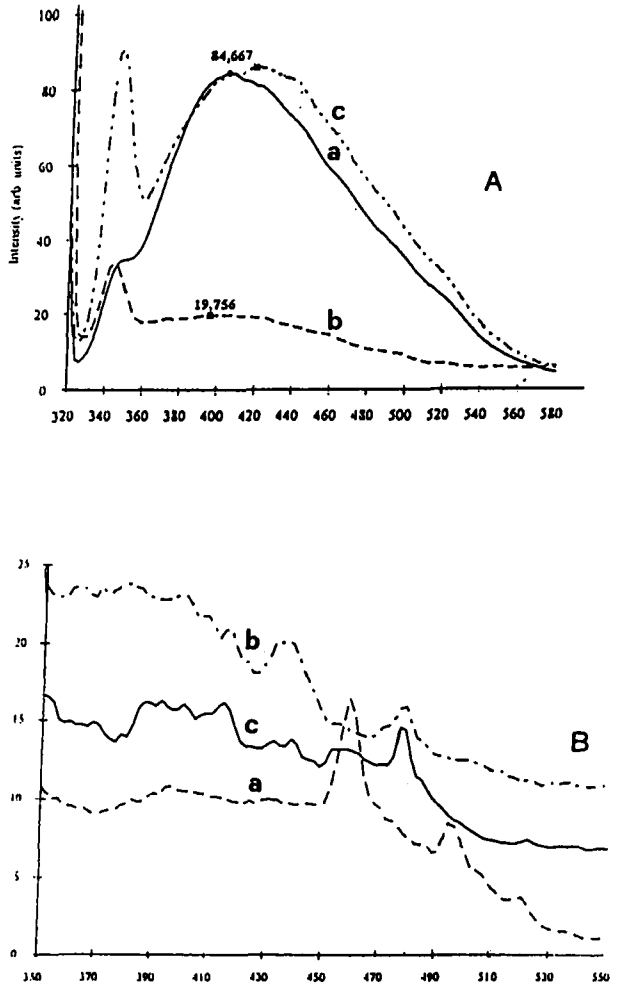

Figure 4 Emission spectra (A) and synchronous scan spectra (B) of aquatic fulvic acids (a), aquatic (b) and sedimentary (c) humic acids.

the other hand, in FA the number of fluorescent groups is more strictly dependent on the solution concentration. The plots of fluorescence intensity vs. pH respectively of HA, both aquatic and sedimentary and of FA (Figure 5) show a general pattern similar in the three solutions. The pH dependence of FA is more pronounced. This seems to point to the presence of a higher quantity of ionizable groups in FA (21) and confirms the results obtained by IR and NMR. We find an increase of intensity with pH, more or less pronounced, up to pH values 6–7, with good correlation ($r = 0.949$, $n = 4$ for aquatic HA, $r = 0.947$, $n = 5$ for sedimentary HA, $r = 0.980$, $n = 4$ for aquatic FA). Moreover, from pH 7 to 10 the linear

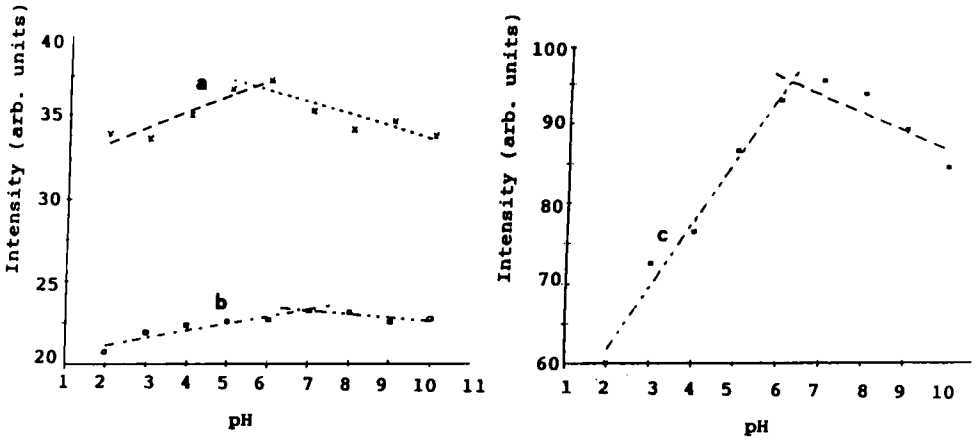

Figure 5 Fluorescence intensity vs. pH of aquatic (a) and sedimentary (b) humic acids and aquatic fulvic acids (c).

regression lines may indicate a slight decrease in intensity. A similar pattern was found by us for HA extracted from marine sediments in Ross sea, but in this case the fluorescence increased going from pH 2 to 5 with more or less scattered data[23]. According to many authors[21] the intensity increases with pH in association with the ionization of phenolic hydroxyl groups, while the presence of carboxyl groups reduces the fluorescence intensity; the presence of an intensity maximum in the emission spectra vs. pH can be related with the ionization of substituted acidic functional groups present on the molecule[24].

Synchronous scan spectra (Figure 4B) of HA, both lacustrine and sedimentary, feature a secondary peak at 478 nm, a sharp peak at 436 nm and a shoulder at 380–420 nm. Similar features can be observed, although in different proportions (main peak at 490 nm, secondary peaks at 460 and 340–430 nm) in synchronous scan spectra of model humic acid-type polymers[25]. Moreover a typical soil humic acid, whose precursors are heterogeneous aromatics, shows the main peak at 475 nm and a shoulder at 390 nm. This fact confirms a certain proportion of aromatics to be present also in HA of Carezza Lake, both aquatic and sedimentary. Aquatic fulvics have only two major peaks in their spectrum.

In Figure 6 we show the multiple scan spectrum of a solution of (a) sedimentary HA (from (14)), (b) aquatic HA and (c) aquatic FA whose concentration was 40 mg/l.

The most structured spectra, also in this case, are those of HA showing in addition to the main peak (exc. 225, em. 388 nm), which is more sharp for sedimentary, another peak at 300 nm exc. and 400 nm em. for sedimentary and a shoulder in the region 260 nm exc. 440 nm em. for aquatic. The spectrum of aquatic FA is even less structured (shows a broad maximum in the region 225 nm exc. and 424 nm em.) and is rather featureless (shows a shoulder between the excitation wavelengths 280–305 nm, in correspondence of the emission wavelength 424 nm). Following the literature[26], from those data we can hipothesize a higher quantity of phenolic groups or conjugated double bonds in the sedimentary HA.

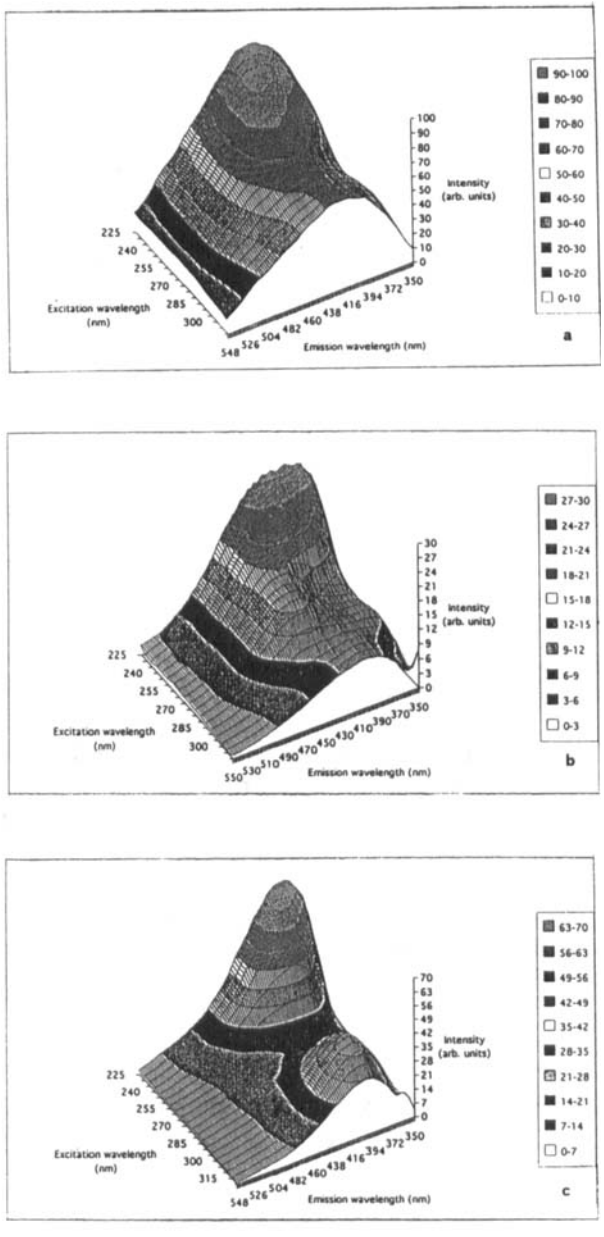

Figure 6 Multiple scan spectra of aquatic (a) fulvic acids, aquatic (b) and sedimentary humic acids (c).

CONCLUSIONS

The humic fractions of humic compounds present in water of the Carezza Lake is remarkable.

The molecular weight of the soluble compounds is generally high, as was obtained by gel chromatography and confirmed by fluorescence measurements. This result is in good agreement with the study of Aho[27] who has found that in polyhumic lakes high molecular weight compounds are dissolved, whereas in oligo humic lakes a substantial quantity of small molecular weight substances are present.

In the structures of soluble humic compounds the phenolic region is remarkable (generally the phenolic character of soluble humics appears to be very little, less than one-third of the total carboxyl content is phenolic[16]). Moreover in HA, both aquatic and sedimentary, fluorophores are more heterogeneous than in FA.

These peculiarities—high molecular weight, significant amount of phenolic groups, high hydroxyl functionality due to carboxyl, phenolic and hydroxyl groups—allow us to conclude that in Carezza lake, and probably in all the Antarctic lakes, the soluble humics have a major structural complexity that may be related to the pH value of water that has a great influence on the equilibrium of not soluble to soluble humic compounds.

Because copper, is preferentially bound to humics having low molecular weight[28,29], whereas lead and mercury are preferentially complexed by humic compounds with high molecular weight (consequently are in greater amount present in sediments), it should be very interesting to study the complexing capacities of Antarctic lacustrine humics and the influence of the structure on the complexing capacity. Moreover, taking into account the characteristics of the coastal Antarctic environment and the air-sea transport phenomena taking place also during the "salt-storms", a further comparison with humic compounds typical of Antarctic sea-surface water should be advisable.

Acknowledgments

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