Study of Humic and Fulvic Acids in Recent Lagoonal Sediments

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Ácidos húmicos e fúlvicos foram extraídos de sedimentos recentes de uma lagoa costeira no Estado do Rio de Janeiro, Brasil. Estas substâncias foram oxidadas com CuO e os produtos fenólicos foram analisados por cromatografia gasosa (CG). A análise dos sedimentos superficiais demonstrou uma diminuição da influência continental em direção ao cordão litorâneo. No testemunho (460 cm), da base até 380 cm, os ácidos húmicos (AH) estavam ausentes ou em concentrações muito baixas. De 380 a 250 cm as concentrações de AH foram superiores em relação aos ácidos fúlvicos (AF) totais. Acima de 250 cm observou-se um decréscimo nas concentrações de substâncias húmicas (SH).

Humic and fulvic acids were isolated from recent sediments of a coastal lagoon in Rio de Janeiro, Brazil. These substances were oxidized by CuO and the phenolic compounds analysed by GC. The superficial sediments showed a decrease of a continental influence towards the seashore. In the core (460 cm) from the base up to 380 cm, the humic acids (HA) were either absent or found at rather low concentrations. From 380 cm to 250 cm the HA concentrations were higher in relation to the total fulvic acids (FA). Above 250 cm a small decrease was observed in humic substance concentrations.

Key words: *humic acids; fulvic acids; lignins.*

Introduction

The organic matter in recent sediments can be studied as the soluble fraction in organic solvents (hydrocarbons, fatty acids, sterols, carotenoids etc,) and the alkali soluble fraction (humic substances) HS. These comprise the bulk (*ca.*70%) of the organic constituents in soil, water and sediments. Humic and fulvic substances are a group of organic compounds formed in each of the environmental compartments by the decay of organic detritus. These substances originating from aquatic sediments (i.e., those formed in lakes, rivers and oceans) are much more aliphatic than aromatic[1].

The environmental importance of HA and FA is due to the multiplicity of several features, such as: fair resistance to degradation, role played in the food chain, effect on the aesthetic quality of water by imparting color, complexing agents for inorganic ions, and aid in the movement of less soluble organic compounds such as pesticides in the aqueous environment[2].

Derived from the metabolism of detrital organic matter, the HS react among themselves forming complex structures, soluble under alkaline conditions. Several phenomena may influence the humification process, such as bacterial activity, enzymatic action, temperature, pH, quantity in water, and others[3,4].

Chemical analyses of humic substances have revealed biochemical components including proteins, carbohydrates, and lignin structural units[5].

The CuO oxidation products of lignins are used as molecular tracers of vascular plant sources[6,7] and diagenetic histories in sedimentary organic matter. They can be obtained by alkaline oxidation of lignins into simple phenols whose chemical substitution patterns indicate an unambiguous lignin source and whose relative distribution reflects the type of

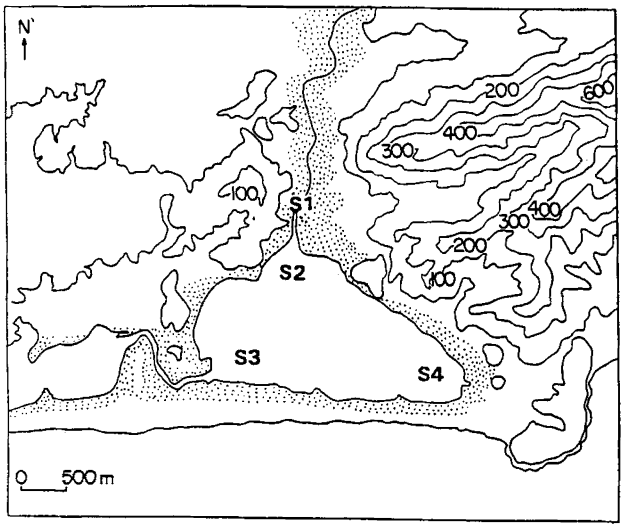

Figure 1. *Sampling stations in Guarapina Lagoon-RJ.*

vascular plant tissue.

The purpose of this paper is to isolate and purify the HA and FA from lagoonal sediments and to apply the same technique of alkaline oxidation of lignins in sediments. The phenolic products obtained from these macromolecules were used as horizontal and vertical tracers of terrestrial organic matter in sediments of Guarapina Lagoon, Rio de Janeiro[8,9]

Experimental

Sampling

The Guarapina Lagoon, located in Rio de Janeiro, has approximately 6380 m^2 of surface area and is protected by a significant sand bar (Figure 1). Its eastern side experiences periodical exchange with the sea station (S4), the west connects with the Padre Lagoon (S3) and the north connects with the estuary of the Rio Caranguejo (S1). These four sites were chosen to evaluate the different contributions and probable horizontal and vertical changes in the lagoon. Sediments were studied from surperficial samples (0 to 10 cm) and from short cores (0 to 50 cm) obtained from the four stations mentioned above, as well as from a core of 460 cm at S2.

Humic substances Isolation and Separation

An aliquot was withdrawn for total organic carbon (TOC) determination (LECO Model WR-12), the dry sediment was weighed, then homogenized and acidified with HCl (6N in proportion 1 g/10 ml). A $20x10^3$ G centrifugation followed in a Kontron H401 cooled centrifuge, rotor A6.14 (6x290 ml), in which the supernatant fulvic acids, "light" crude (FALC) are separated and stored at low temperature for further analysis[10]. The carbonate-free sediment was made alkaline with NaOH under an inert atmosphere (N_2) and centrifuged under the same conditions. The residue with lignins and lipids (LL) was stored while the supernatant fraction was decanted, acidified and centrifuged. The fraction, corresponding to fulvic acids, "heavy" crude (FAHC) was stored in a refrigerator for later analysis. The residue (humic acids + metals + clays) was redissolved in 0.1 N KOH. KCl was added up to a 0.3M concentration in K^+. The mixture was then centrifuged and the resulting precipitate (metals) was analysed by atomic absorption spectroscopy. The resultant solution was centrifuged, after acidification and decantation for 12 hours. The supernatant was discarded and the residue (humic acids and clays) was put into suspension in a 1:1 mixture of 50 ml of 0.1N HCl and 0.3N HF. The mixture was agitated for 12 hours, passed through a GVWP Millipore filter, dried in vacuum and weighed. The operation was repeated until a constant weight was obtained, corresponding to 1% of the weight of the starting sediment. After eliminating the clays, the humic acids were transferred together with distilled water into a Visking cellulose membrane with a 24 Å pore diameter and dialysed during 72 hours, filtered in a GSWP Millipore, dried in vacuum, weighed and ready for analysis.

The residual fractions of FALC and FAHC were purified by successive elutions through a XAD-8 Amberlite resin column, 20-50 mesh, in a 1 ml/g proportion to the initial sediment. The eluents were obtained by gravity at a flow rate of 5 ml/min. After rinsing with distilled water, the eluent from these two steps was rejected. Another leaching with 0.1N NaOH and rinsing with distilled water followed, and the eluent was collected in a polypropylene bottle. It was acidified with 6N HCl to pH 1 and HF was added while agitating to obtain a 0.3N HF concentration in the total volume. After that, a plastic column filled with the same resin was used for another rinsing with distilled water.

The correspondent eluent was discarded. Another leaching with 0.1N NaOH and rinsing with distilled water was carried out. The result of this leaching which represented the FA in the form of sodium salts was then passed through Amberlyst 15 resin saturated with H^+ ions, from which the eluent was discarded. A further leaching with distilled water was reduced by vacuum drying and weighed. With this procedure the proton-saturated FA are obtained ready for analysis.

After the lipid extraction from the LL residue[11], the HA and FA were oxidized to obtain the phenolic products. The following operations are carried out under an inert atmosphere (N_2).

In a stainless steel cylindrical reactor, 20 mg of HA, 1 g of CuO, 50 mg of $Fe(NH_4)_2(SO_4)_2.6H_2O$ and 7 ml of 8% NaOH were reacted at 170°C for 3 hours. The heavy and light FA were put into the reactor according to the quantity obtained from the above procedure and the results were normalized to 20 mg to be compared with the HA. When the reaction was complete, the reactor was water-cooled, its content filtered, triple-rinsed with 8% NaOH and acidified with 6N HCl to pH 1. Then followed three consecutive extractions with ethyl ether, water-rinsing, filtration, drying, weighing and storage in a nitrogen atmosphere. The oxidation products are derivatized with N-trimethylsilylacetamide one hour before the gas chromatographic analyses.

Results and Discussion

The heavy and light designation referring to the FA fractions, corresponds to their different behavior during the centrifugation process and is not directly related to their molecular weights (Table 1).

The HA, light and heavy FA were oxidized in order to obtain the phenolic products derived from lignins. The results were grouped as:

a) HS (HA + heavy FA + light FA);

b) the total ratio FA/HA;

c) the oxidation products of the total FA (FAtox = oxidation

Table 1. *Bulk properties of samples.*

Samples	Depth cm	TOC %	HA mg/g	FA (l) mg/g	FA (h) mg/g	HS mg/g	FA/HA **
01	S2-170	1,95	1,51	0,09	0,28	1,86	0,24
02	180	0,89	0,92	0,10	0,21	1,21	0,33
03	250	6,09	1,92	0,12	0,29	2,33	0,21
04	300	6,64	1,88	0,11	0,31	2,29	0,22
05	340	5,18	2,16	0,22	0,36	2,74	0,27
06	380	4,63	0,11	0,15	0,31	0,55	4,29
07	420	2,61	0,74	0,21	0,32	1,26	0,71
08	460	3,41	nd	0,31	0,24	0,54	nd
09	S1-10	6,17	6,63	0,17	0,24	7,03	0,06
10	S2-10	10,01	1,48	0,11	0,28	1,86	0,26
11	S3-10	8,03	4,87	0,11	0,29	5,27	0,08
12	S4-10	4,11	4,32	0,43	0,67	5,43	0,25
09	S1-10	6,17	6,63	0,17	0,24	7,03	0,06
13	20	4,72	9,97	0,16	0,66	10,78	0,08
17	30	4,16	4,10	0,28	0,11	4,47	0,09
21	40	5,82	8,94	0,02	0,27	9,23	0,03
25	50	5,59	5,61	0,06	0,26	5,92	0,06
10	S2-10	10,01	1,48	0,11	0,28	1,86	0,26
14	20	6,87	13,28	0,16	0,64	14,08	0,06
18	30	7,49	3,32	0,10	0,11	3,53	0,06
22	40	5,22	8,92	0,07	0,29	9,28	0,04
26	50	6,71	5,76	0,07	0,04	5,86	0,02
11	S3-10	8,03	4,87	0,11	0,29	5,27	0,08
15	20	6,78	2,15	0,34	0,27	2,76	0,28
19	30	7,65	0,88	0,23	0,23	1,34	0,52
23	40	10,31	2,58	0,09	0,16	2,83	0,10
27	50	12,28	0,39	0,06	0,30	0,74	0,90
12	S4-10	4,11	4,32	0,43	0,67	5,43	0,26
16	20	7,85	1,44	0,17	0,08	1,69	0,17
20	30	3,52	2,25	0,12	0,14	2,50	0,11
24	40	5,64	3,26	0,06	0,05	3,36	0,03
28	50	4,55	0,45	0,16	0,28	0,88	0,98

TOC - total organic carbon
HA - humic acids
FA (l) - "light" fulvic acids
FA (h) - "heavy" fulvic acids
HS - humic substances
FA/HA - ratio fulvic and humic acids

products of the heavy FA+oxidation products of the light FA)(Table 2).

To facilitate the plant source identification the phenols were grouped into families, based on their chemical substitution patterns (Table 3). The vanillyl phenols occur as oxidation products of all four categories of vascular plants (woods and non-woody tissues of angiosperms and gymnosperms), syringyl phenols are produced only from angiosperm lignin and cinnamyl phenols only from non-woody tissues[5].

As can be seen in Table 1 and Figure 2, the total organic carbon (TOC) is higher in S2, on the other hand, the HS concentrations are the lowest in these surperficial samples. This is probably due to currents entering streams in the lagoon and, which scatter and move the plant detritus, or imput of an important component of the bulk organic matter from other chemical substances. Based on the previous assumptions, the HS in river and lake waters are the same as those in soils, and lignins are the primary source of soil humic substances. Furthermore, the major source of HS in sediments of rivers and lakes is the adjacent soil which is leached or eroded directly into streams during rainfall events[12].

Some methods of HS characterization have shown that stream and soil humic acids are similar in aromatic content, soil HA usually being slightly more aromatic. Stream HA are higher in phenolic content than soil or marine humic acids. Soil FA usually exhibit the highest aromaticity and stream FA are intermediate between marine and soil FA in aromaticity. Marine humic acids are also the lowest in aromaticity, phenolic content and methoxyl content of the three sources of humic acids. These facts suggest that the bulk of the HS in the lagoon is derived from terrestrial sources of these substances.

The HA correspond to 79-95% of the HS in this sample group, considering the lateral sample distribution in the lagoon. The ratio FA/HA provides some information about the environmental conditions of diagenesis and when greater

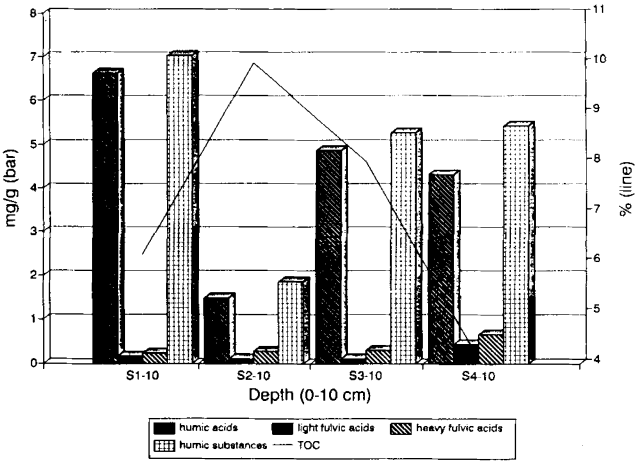

Figure 2. *Humic substances in superficial samples.*

Table 2. *Oxidation products.*

Samples	Depth cm	TOC %	HAox mg/g	FA(l)ox mg/g	FA(h)ox mg/g	FA(t)ox mg/g
01	S2-170	1,95	115,0	230,0	70,0	300,0
02	180	0,89	115,0	105,0	210,0	315,0
03	250	6,09	105,0	140,0	145,0	285,0
04	300	6,64	145,0	75,0	110,0	185,0
05	340	5,18	165,0	35,0	35,0	70,0
06	380	4,63	20,0	40,0	95,0	135,0
07	420	2,61	115,0	30,0	110,0	140,0
08	460	3,41	nd	45,0	135,0	180,0
09	S1-10	6,17	110,0	35,0	100,0	135,0
10	S2-10	10,01	50,0	135,0	85,0	220,0
11	S3-10	8,03	60,0	150,0	70,0	220,0
12	S4-10	4,11	55,0	35,0	25,0	60,0
09	S1-10	6,17	110,0	35,0	100,0	135,0
13	20	4,72	110,0	110,0	35,0	145,0
17	30	4,16	95,0	20,0	195,0	215,0
21	40	5,82	95,0	220,0	120,0	340,0
25	50	5,59	95,0	250,0	110,0	360,0
10	S2-10	10,01	50,0	135,0	85,0	220,0
14	20	6,87	135,0	60,0	40,0	100,0
18	30	7,49	130,0	65,0	180,0	245,0
22	40	5,22	115,0	145,0	90,0	235,0
26	50	6,71	50,0	155,0	545,0	700,0
11	S3-10	8,03	60,0	150,0	70,0	220,0
15	20	6,78	130,0	100,0	200,0	300,0
19	30	7,65	50,0	110,0	325,0	435,0
23	40	10,31	75,0	65,0	125,0	190,0
27	50	12,28	40,0	175,0	95,0	270,0
12	S4-10	4,11	55,0	35,0	25,0	60,0
16	20	7,85	110,0	25,0	390,0	415,0
20	30	3,52	60,0	85,0	175,0	260,0
24	40	5,64	110,0	210,0	565,0	775,0
28	50	4,55	85,0	65,0	105,0	170,0

mg/g - mg of the oxidized product/g from corresponding acid
HAox - humic acid oxidation products
FA(l)ox - light fulvic acid oxidation products
FA(h)ox - heavy fulvic acid oxidation products
FA(t)ox - total fulvic acid oxidation products

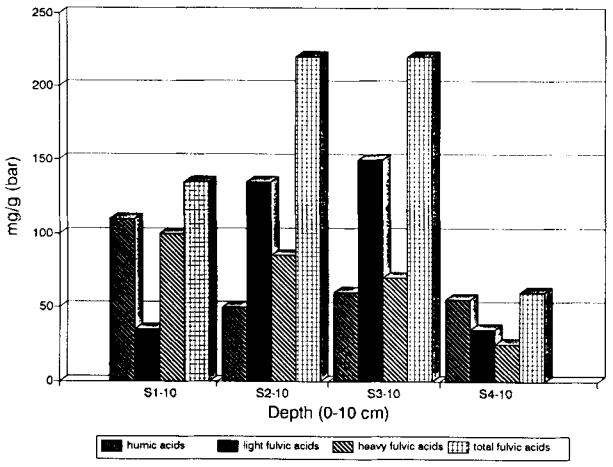

Figure 3. *Oxidation products in superficial samples.*

than 1 corresponds to well preserved organic matter[4]. Generally FA/HA is low, even less 0.5. In the surperficial samples the higher values are observed in S2 and S4. The low average of these values could represent relatively degradated organic matter.

The data of the HAox and the FAtox (0-10 cm), show a decreasing trend in terms of continental influence in the order S1 to S4 (Table 2 and Figure 3). The analyses of the HA and FA oxidation products were carried out by comparison with the corresponding standards in GC (Figure 4) of the 15 compounds the standard mixture, 8 have been chosen. These are characteristic of vascular plants while the others can be

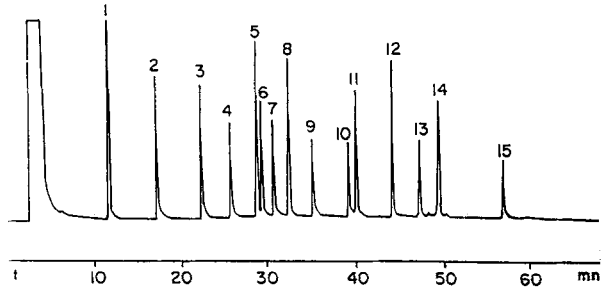

Figure 4. *Gas chromatogram of a standard mixture of Me_3Si derivatives of phenols and carboxylic acids.*

G.C. conditions: capillary column SE-30 (25 m, i.d. 0,2 mm); temperature: 100-270°C, 2°C/min; injector 250°C; detector 300°C; attenuation 1/8; carrier gas N_2; injection 1 l.

Peak identities: 1. benzoic acid; 2. p-hydroxybenzaldehyde; 3. p-hydroxyacetophenone; 4. vanillin; 5. m-hydroxybenzoic acid; 6. ethylvanillin (GC internal standard); 7. acetovanillone; 8. p-hydroxybenzoic acid; 9. syringaldehyde; 10. acetosyringone; 11. vanillic acid; 12. 3,5-dihydroxybenzoic acid; 13. syringic acid; 14. p-coumaric acid; 15. ferulic acid.

Table 3. *Phenolic products from humic acid oxidations.*

Samples	Depth cm	Van ug/mg	Syr ug/mg	Cinn ug/mg	p-hydro ug/mg	Va/Vh	Sa/Sh	Pa/Ph	M
01	S2-170	8,80	8,42	nd	11,59	nd	2,20	2,62	4,50
02	180	8,76	3,58	nd	11,63	nd	nd	2,34	4,10
03	250	nd	nd	11,48	7,05	nd	nd	nd	2,50
04	300	3,02	2,36	nd	11,38	1,08	nd	1,52	2,80
05	340	1,83	nd	nd	6,12	nd	nd	1,71	1,30
06	380	nd	nd	nd	17,60	nd	nd	nd	1,80
07	420	nd	nd	nd	5,60	nd	nd	1,00	0,90
08	460	**	**	**	**	**	**	**	**
09	S1-10	nd	nd	nd	21,90	nd	nd	1,19	3,60
10	S2-10	31,30	36,98	nd	44,96	0,84	1,12	0,91	13,50
11	S3-10	nd	nd	2,75	38,61	nd	nd	1,16	5,60
12	S4-10	nd	26,72	6,06	49,34	nd	nd	0,64	11,00
09	S1-10	nd	nd	nd	21,90	nd	nd	1,19	3,60
13	20	nd	3,48	nd	15,18	nd	nd	1,38	3,70
17	30	nd	nd	nd	16,11	nd	nd	1,77	4,10
21	40	nd	nd	nd	25,75	nd	nd	0,78	3,50
25	50	nd	nd	nd	18,10	nd	nd	2,77	3,50
10	S2-10	31,30	36,98	nd	44,96	0,84	1,12	0,91	13,50
14	20	8,03	6,98	nd	10,74	0,99	nd	1,19	3,40
18	30	10,23	13,54	nd	23,80	1,23	1,11	1,07	5,70
22	40	9,25	18,45	nd	24,10	0,81	0,75	0,80	6,10
26	50	16,54	22,19	nd	72,17	0,65	0,59	0,82	14,40
11	S3-10	nd	nd	2,75	38,61	nd	nd	1,16	5,60
15	20	10,23	17,78	3,25	18,94	1,13	0,82	1,07	5,80
19	30	6,61	nd	nd	48,15	nd	nd	0,68	7,50
23	40	5,53	nd	nd	24,31	nd	nd	0,93	3,60
27	50	nd	nd	nd	34,35	nd	nd	4,22	5,00
12	S4-10	nd	26,72	6,06	49,34	nd	nd	0,64	11,00
16	20	3,96	3,06	nd	21,67	nd	nd	0,83	3,80
20	30	nd	nd	nd	9,30	nd	nd	0,75	1,10
24	40	nd	nd	nd	24,72	nd	nd	0,53	3,50
28	50	nd	nd	nd	7,42	nd	nd	0,62	1,10

Van: vanillyl phenols
Syr: syringyl phenols
Cinn: cinnamyl phenols
p-hydro: p-hydroxy phenols
Va/Vh: vanillic acid/vanillin
Sa/Sh: syringic acid/syringaldehyde
Pa/Ph: p-hydroxybenzoic acid/p-hydroxybenzaldehyde
M: weight percentage of the 14 standards
nd: not determined

found in both vascular or non-vascular plants (i.e., p-hydroxyl phenols, also produced by marine organisms). Benzoic acid, m-hydroxy benzoic acid and 3,5-dihydroxybenzoic acid are among the oxidation products of blue-green and brown algae, but were not produced in significant quantities from vascular plants[13].

The p-hydroxyl phenols are known oxidation products of lignins in gymnosperm woods and non-woody angiosperm tissues. They are common oxidation products of most soils and sediments. However, non-vascular plants and biochemical precursors such as the amino-acid tyrosine, also yield p-hydroxyl phenols making these compounds ambiguous lignin indicators for some samples.

The p-hydroxyl phenols are major oxidation products of all Guarapina Lagoon sediments. Their increase in all samples is probably due to the formation of these compounds from non-lignin biochemicals, such as tyrosine, that were produced by marine organisms. Another source for this rise in all samples is p-coumaric acid which can produce p-hydroxyl phenols under the reaction conditions employed[14]. The cinnamyl phenols (p-coumaric and ferulic acid) are derived from lignins and may have a non-woody angiosperm origin.

In the superficial samples (Table 3) the syringyl phenols in comparison with the vanillyl phenols are more dominant and suggest a non-woody angiosperm contribution.

Previous observations suggest that acid-aldehyde ratios (i.e., Va/Vh; Sa/Sh; Pa/Ph) may reflect the extent to which lignin materials have undergone oxidative degradation in the environment. The ratios are near or slightly higher than 1 in the superficial samples, suggesting a minimal *in situ* oxidative degradation.

The superficial samples 0-50 cm can be placed into 2 subgroups (Tables 1 and 2): S1-S2 with continental charac-

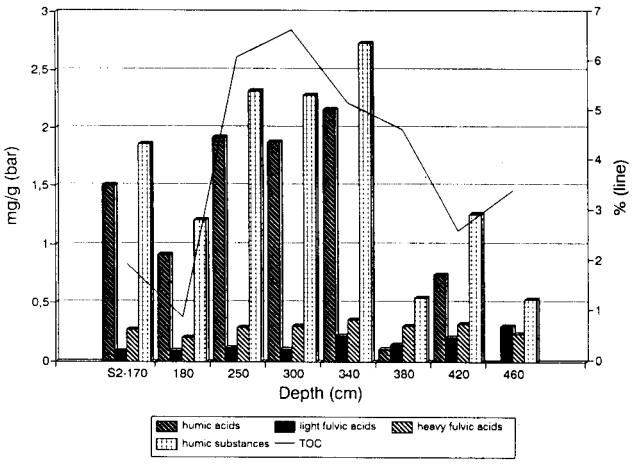

Figure 5. *Humic substances in core S-2.*

teristics and S3-S4 with seashore characteristics. The S1-S2 subgroup has the highest concentration of HS and the HA constitute 94% of the HS. The HA oxidation products show higher yields for S2. In the S3-S4 subgroup, the HS concentrations decrease and the HA percentage relative to HS is 84%; the ratio FA/HA trends to increase with depth and has maximum values of 0.9 in S3 and 0.98 in S4. This suggests that the organic matter is better preserved than in the superficial samples.

Table 3 shows the phenolic oxidation product distribution in the samples from 0-50 cm. The p-hydroxyl phenols are higher than the others in all samples. The subgroup S2 shows a higher phenol average than the others. The syringyl phenols are slightty more dominant than the vanillyl phenols. This may support the assumptions above about the non-woody angiosperm origin in the superficial samples.

In core S2, from 460 to 170 cm, the total organic carbon (TOC) trend parallels the HS concentrations (Table 1, Figure 5). At the bottom core the HA are not detectable. Thus, the concentration of the HS is represented by the sum of the heavy and light FA. At 380 cm the ratio FA/HA reaches 4.29, a maximum level of organic matter preservation. An interpretation for the low HA concentration at 180 cm is leaching of these substances by the lagoon water, over the peat at 250 cm[11].

As can be seen in Table 2 and Figure 6 the HA oxidation products show an irregular distribution from 460 to 340 cm, which is not observed for the total FA oxidation products. In the upper part from 250 to 170 cm the FAtox have high concentrations while at 340 cm is low and consequently the ratio FA/HA shows the lower values. The phenolic products (Table 3) have an important component of p-hydroxyl phenols, but the syringyl phenols are not predominant in comparison with the vanillyl phenols. The acid-aldehyde ratios may be evidence for oxidative degradation of this sedimentary material.

The FA oxidation products are not shown in the tables because they did not contain phenolic products characteristic of lignins in the majority of samples.

Conclusions

The synthesis of the data obtained for the four sampling stations allows the following conclusions:

- a decrease of continental influence towards the seashore is observed in the HA concentrations, and oxidation products of HA and total FA.
- the correlation of the syringyl phenols associated with the vanillyl and p-hydroxyl phenols in the S2 superficial samples may be related with the presence of non-woody angiosperm remains and a minor component from gymnosperm wood.
- the higher ratio of the FA/HA at 380 cm suggests a environment depositional with reducing conditions.
- the production of lignin-derived phenols from CuO oxidation of lagoonal HS is an unambiguous indicator that terrestrially derived carbon is present in these organic sediments.

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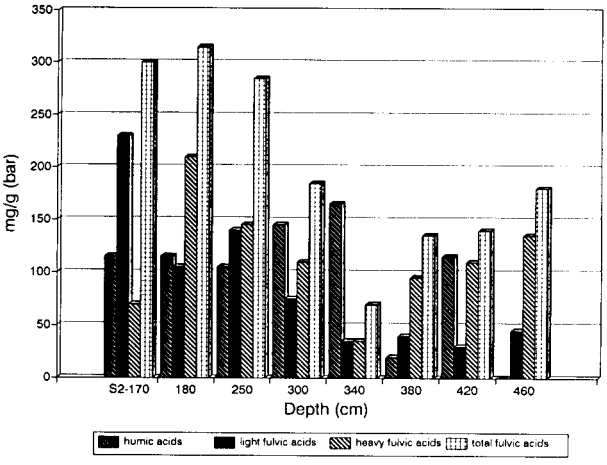

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