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Influence of alkaline extraction on the characteristics of humic substances in Brazilian soils

André Henrique Rosa [a,*], Luciana Camargo de Oliveira [b], Iramaia Corrêa Bellin [b], Julio Cesar Rocha [b], Luciane Pimenta Cruz Romão [c], Newton Luiz Dias Filho [d]

[a] *Department of Environmental Engineering, São Paulo State University (UNESP), 18087-180 Sorocaba, SP, Brazil*
[b] *Department of Analytical Chemistry, Institute of Chemistry (UNESP), 14800-900 Araraquara, SP, Brazil*
[c] *Department of Chemistry, Federal University of Sergipe (UFS), 49100-000 Aracajú, SE, Brazil*
[d] *Department of Physical and Chemistry, University of the São Paulo State (UNESP), 15385-000 Ilha Solteira, SP, Brazil*

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Abstract

An investigation was made of the influence of alkaline extraction on the characteristics of humic substances extracted from Brazilian soil samples. Humic substances (HS) from seven different soils samples collected in Brazil were extracted using the procedure recommended by the International Humic Substances Society (IHSS). Soils, HS and humins were characterized by thermogravimetry and differential thermal analysis. About 8 mg of each material (soil, HS and humin) were placed in a platinum crucible and heated continuously from 20 to 750 °C at a heating rate of 10 °C min^{-1} in an atmosphere of synthetic air (100 ml min^{-1}). A thermal analysis revealed a difference between the content and structural characteristics of organic matter present in HS and humin fractions in relation to their soils. The results indicated that alkaline extraction alters the characteristics of humic substances during the extraction process, underlining the importance of developing methodologies and analytical procedures that allow organic matter in soils to be studied without extracting it.

Keywords: Humic substances; Humin; Brazilian soils; Extraction; Thermogravimetry and differential thermal analysis

1. Introduction

The main fraction of organic matter contained in soils, peat, sediment and natural water consists of humic substances (HS), which are comprised of a complex mixture of physically heterogeneous substances, with different sizes and molecular weights and a large number of functional groups containing oxygen [1,2]. Due to their structural characteristics, HS control several physical and chemical properties of soil, e.g., stability of aggregates, buffering capacity, sorption of hydrophobic organic compounds and transport, bioavailability and complexation of metals present in the environment [3,4]. Investigations of the structure of humic substances (HS) are important because their structure governs the properties and reactions of these materials in the environment. For decades, the chemical structure of humic substances has been investigated using several techniques, particularly infrared and UV-visible spectroscopy, nuclear magnetic resonance, spin electron resonance, fluorescence and PY–CG–MS to gain a better understanding of the structure of humic matter [5–8].

The structural, chemical and functional properties of HS can be studied in detail in the free state, i.e., when free of inorganic components [4,9]. Therefore, several procedures have been proposed in the literature for the extraction of HS using alkaline solvents, chelating agents, organic solvents and aqueous saline solutions [10–12]. Alkaline solvents were the earliest reagents used for extracting HS and remain the most efficient and widely used [8–10]. The International Humic

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[*] Corresponding author. Tel.: +55 15 32383414; fax: +55 15 32282466.
E-mail address: ahrosa@sorocaba.unesp.br (A.H. Rosa).

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Substances Society (IHSS) recommends a standard procedure based on a 4 h treatment with 0.1 mol L^{-1} NaOH at room temperature in an N_2 atmosphere [10,13,14]. After this period of alkaline treatment, the soluble alkali extract is operationally defined as a humic substance (HS) and can be separated from the residue (humin), which is composed of organic matter linked with mineral phase [6]. However, this procedure has been criticized in the literature because the alkaline extraction can promote some structural alterations in the HS, in particular, auto-oxidation and other chemical modifications, including the breakdown of humic macromolecules and condensation of amino-carbonyls [9,10].

In the field of environmental chemistry it is very important that the results obtained in laboratories can be used to shed light on environmental processes. Thus, it is crucial that HS not be altered during their extraction. In this work, differential thermal analysis and thermogravimetry were used to study the influence of alkaline extraction on the characteristics of organic matter present in seven different soil samples collected in Brazil. Differential thermal analysis (DTA) and thermogravimetry were chosen for this study because they are interesting techniques for studies involving the characterization of organic matter in soils, and because they allow for analyses of original samples without chemical treatment, which is an indispensable characteristic for the type of study proposed here.

2. Experimental

2.1. Chemicals and reagents

Diluted acids and bases required to isolate the HS were prepared by diluting 30% hydrochloric acid solution (p.a. Merck AG, Darmstadt/Germany) and sodium hydroxide monohydrate (p.a. Merck AG), respectively, with high purity water (Millipore-Q system, Millipore GmbH, Eschborn/Germany).

2.2. Collection of samples

The soil samples were collected from soil surfaces (0–20 cm) in different regions of Brazil. The collection sites are depicted in Fig. 1. The dried soil samples were ground and passed through a 2 mm sieve. The fertility of the soil samples (Ca, K, Mg, P, S, CEC) was characterized using the methodology described by Raij et al. [15]. The atomic ratios of C/N were calculated after determining the carbon and nitrogen using a Perkin-Elmer 240 – C elemental analyzer. Table 1 lists the physico-chemical characteristics of the soil samples.

2.3. Humic substances and humin extraction

The humic substances were extracted according to the procedure recommended by [7,10–12]. Five grams of the sieved material were extracted with a volume of 0.1 mol L^{-1} HCl solution equal to 10 times the weight of the sample. The pH of the solution was adjusted between 1 and 2 using 1.0 mol L^{-1} HCl solution. The soil/HCl mixture was shaken for 1 h and the suspension was allowed to settle. The mixture was then centrifuged at $1478 \times g$ for 10 min, and the supernatant separated from the sediment. The latter was neutralized with 1.0 mol L^{-1} NaOH solution at pH 7.0 and a volume of 0.1 mol L^{-1} NaOH solution equal to 10 times the weight of the sample. The mixture was shaken for 4 h under a nitrogen atmosphere and then allowed to settle overnight, after which the soluble supernatant (HS) was separated from the insoluble sediment (humin) by centrifugation at $1478 \times g$ for 10 min. HS and humin were dried at 55 °C in an oven equipped with an exhaust system until they reached a constant weight. The percentage of extracted HS was calculated in relation to the mass of soil subjected to extraction (5.0 g), while the percentage of humin was calculated by subtracting the percentage of HS from 100% (Table 2).

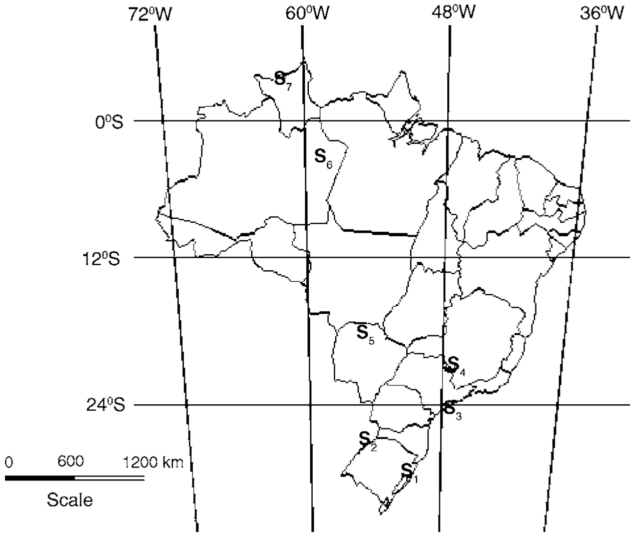

Fig. 1. Soil sample collection sites in Brazil (S_1: Santa Maria (RS), S_2: Bento Gonçalves (RS), S_3: Cananéia (SP), S_4: Ribeirão Preto (SP), S_5: Campo Grande (MS), S_6: Manaus (AM) and S_7: Boa Vista (RO)).

2.4. Differential thermal analysis (DTA) and thermogravimetry (TG)

The intact soil, extracted HS and humin (residue from the alkaline extraction of the HS) samples were ground with a mortar and pestle and sieved through a 70 mesh (0.2 mm) sieve to homogenize them and increase their surface area.

For the DTA and TG analysis, about 8 mg of each material (soil, HS and humin) were placed in a platinum crucible and heated continuously from 20 to 750 °C at a heating rate of 10 °C min^{-1} in a synthetic air atmosphere (100 ml min^{-1}). The DTA and TG curves were recorded simultaneous using a TA Instruments STA-2960 Simultaneous DTA–TGA

Table 1
Physico-chemical characteristics of soil samples

Samples	Location	pH	K (mmol L^{-1})	Ca (mmol L^{-1})	Mg (mmol L^{-1})	H + Al (mmol L^{-1})	CEC (mmol L^{-1})	C (%)	N (%)	Atomic ratio, C/N
S_1	29°68′S, 53°80′W	4.6	7.5	75	20	44	146	35.63 ± 1.50	4.68 ± 0.46	9.0
S_2	29°08′S, 51°32′W	6.5	5.5	354	30	11	401	37.86 ± 0.55	2.80 ± 0.08	15.8
S_3	25°02′S, 47°93′W	4.7	13.1	121	35	80	249	34.48 ± 0.14	1.99 ± 0.02	20.2
S_4	21°10′S, 47°48′W	4.1	1.5	56	14	179	251	23.27 ± 1.53	1.93 ± 0.10	14.1
S_5	20°28′S, 54°37′W	5.1	7.0	143	29	52	231	39.62 ± 0.15	6.65 ± 0.29	6.9
S_6	3°10′S, 60°07′W	4.0	0.7	6	1	47	55	34.29 ± 0.06	7.24 ± 0.02	5.5
S_7	2°49′N, 60°39′W	4.3	1.1	82	11	216	310	55.20 ± 0.37	2.32 ± 0.02	27.8

S_1: soil from Santa Maria; S_2: soil from Bento Gonçalves; S_3: soil from Ilha de Cananéia; S_4: soil from Ribeirão Preto; S_5: soil from Campo Grande; S_6: soil from Manaus; S_7: soil from Boa Vista; H + Al: potential acid; CEC: cationic exchange capability.

thermoanalyzer. Weight losses were calculated from the TG curves (all the TG curves were normalized to the samples' mass).

3. Results and discussion

3.1. Thermogravimetry

The literature contains various reports on the thermal behavior of soils, HS and humins [16–20]. Thermoanalytical techniques (TG/DTA, DSC and PY–CG–MS) are extremely useful in studies of these materials, allowing for analyses of their thermal behavior without requiring their extraction.

Using TG/DTA and PY–MS, Leinweber and Schulten [18] associated the thermal decomposition of compounds present in soils with temperature, observing that, as a rule, phenolic compounds and lignin monomers undergo thermal degradation between 300 and 450 °C, while alkylaromatics and lignin dimers degrade at temperatures ranging from 500 to 650 °C. Sheppard and Forgeron [16] found that the thermal degradation of carbohydrates occurs at temperatures of up to 350 °C.

During the alkaline extraction of soils, part of the organic matter is extracted in the form of HS, while another part is obtained as humin fraction complexed with mineral colloids. Thus, the sum of the organic matter in the extracted fractions (HS and humin) should be approximately equal to that in the original soil. Based on this assumption, we used

Table 2
Mass and percentage of humic substances and humin from different soil samples collected in Brazil

Samples	Mass extracted (g)	Percentage in relation to mass of soil subjected to extraction (%)
HS_1	0.6750	13.50
HS_2	0.5595	11.19
HS_3	0.7880	15.76
HS_4	2.2115	44.23
HS_5	0.6435	12.87
HS_6	1.1220	22.44
HS_7	0.9325	18.65
H_1	4.3250	86.50
H_2	4.4405	88.81
H_3	4.2120	84.24
H_4	2.7885	55.77
H_5	4.3565	87.13
H_6	3.8780	77.56
H_7	4.0675	81.35

$HS_{1–7}$ = humic substances from S_1–S_7; $H_{1–7}$ = humin from S_1–S_7.

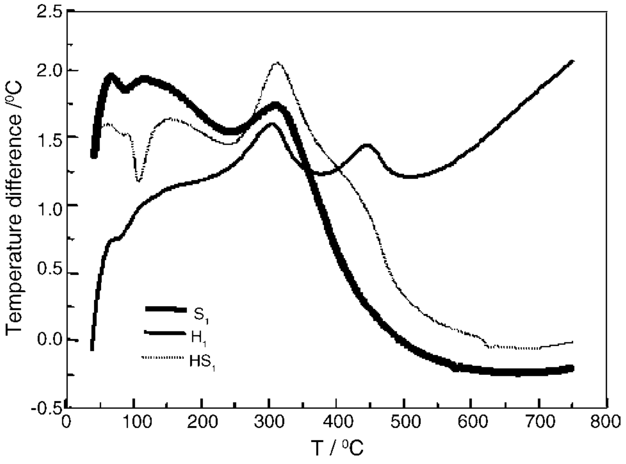

Fig. 2. DTA curves of soil, humin and humic substances from Santa Maria. Conditions: sample mass 8.7329 mg, platinum crucible, synthetic air atmosphere 100 mL min^{-1}, heating temperature 40–750 °C at 10 °C min^{-1}.

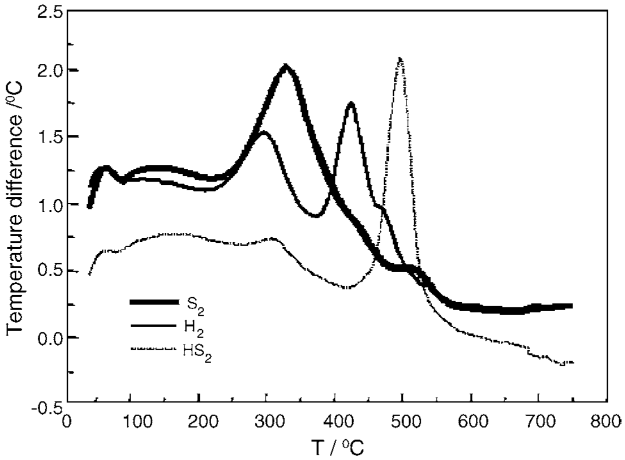

Fig. 3. DTA curves of soil, humin and humic substances from Bento Gonçalves. Conditions: sample mass 8.7329 mg, platinum crucible, synthetic air atmosphere 100 mL min^{-1}, heating temperature 40–750 °C at 10 °C min^{-1}.

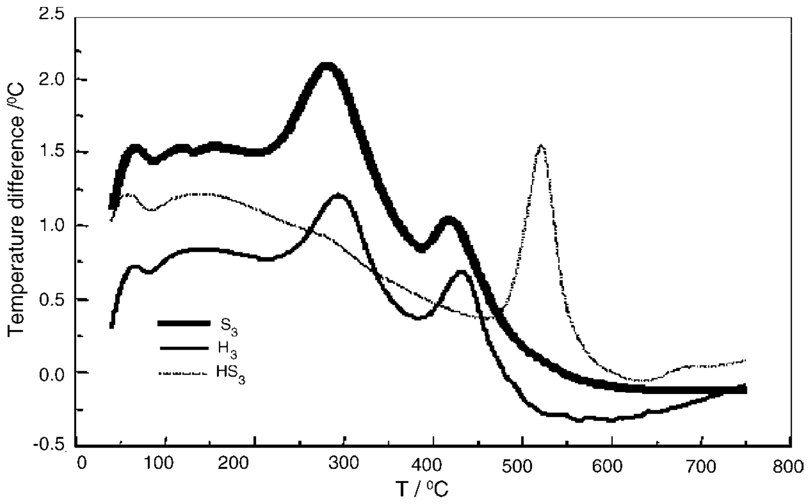

Fig. 4. DTA curves of soil, humin and humic substances from Cananéia. Conditions: sample mass 8.7329 mg, platinum crucible, synthetic air atmosphere 100 mL min^{-1}, heating temperature 40–750 °C at 10 °C min^{-1}.

thermogravimetry to determine the organic mass of the fractions (HS and humin) and to compare the results with soils. To this end, the data were treated as follows:

(1) The TG curves of soils, humin and HS were normalized to the same initial mass (8.73293 mg).
(2) The loss of organic matter from the soil, humin and HS (Table 3) was determined based on a temperature interval of 150–650 °C. This interval of temperature was chosen based on our DTA curves (Figs. 2–8) and the literature [16–18]. In addition, this interval of temperature minimize the influence of the water content and the presence of metal ions that could volatilize during heating, interfering in the results obtained.
(3) The loss of organic matter from the samples of humins and humic substances was corrected based on the percentage of extracted mass (Table 2). For this purpose, the humin and HS mass loss was multiplied by the percentage of extracted mass. This mathematical treatment

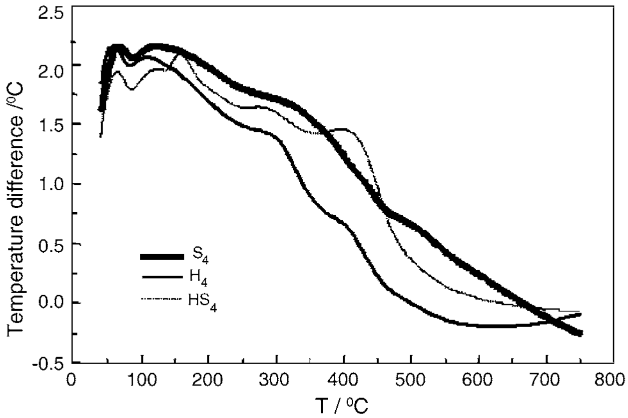

Fig. 5. DTA curves of soil, humin and humic substances from Ribeirão Preto. Conditions: sample mass 8.7329 mg, platinum crucible, synthetic air atmosphere 100 mL min^{-1}, heating temperature 40–750 °C at 10 °C min^{-1}.

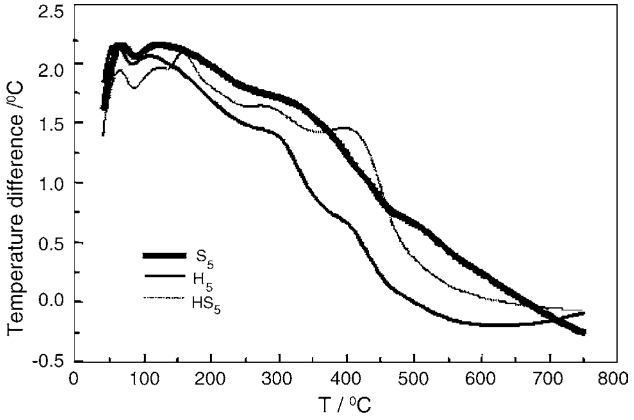

Fig. 6. DTA curves of soil, humin and humic substances from Campo Grande. Conditions: sample mass 8.7329 mg, platinum crucible, synthetic air atmosphere 100 mL min^{-1}, heating temperature 40–750 °C at 10 °C min^{-1}.

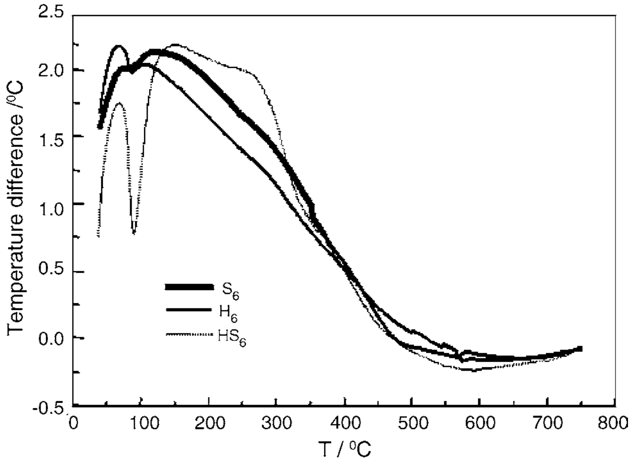

Fig. 7. DTA curves of soil, humin and humic substances from Manaus. Conditions: sample mass 8.7329 mg, platinum crucible, synthetic air atmosphere 100 mL min^{-1}, heating temperature 40–750 °C at 10 °C min^{-1}.

Table 3
Extraction influence and organic matter loss from soils, humin and humic substances as a function of temperature

Samples	150–350 mg	350–500 mg	500–650 mg	Total mass (mg)	EI [%H_x + %HS_x]/S_x
%H_1	0.4106	0.2602	0.0037	0.6745	
%HS_1	0.1318	0.1168	0.0367	0.2853	
%H_1 + %HS_1	0.5424	0.3770	0.0404	0.9598	93.78
S_1	0.6131	0.3411	0.0693	1.0235	
%H_2	0.8868	1.0805	0.1733	2.1406	
%HS_2	0.1075	0.1470	0.0553	0.3098	
%H_2 + %HS_2	0.9943	1.2275	0.2286	2.4504	85.19
S_2	1.5098	1.0806	0.286	2.8764	
%H_3	0.5690	0.5756	0.0938	1.2384	
%HS_3	0.1085	0.1252	0.0935	0.3272	
%H_3 + %HS_3	0.6775	0.7008	0.1873	1.5656	75.01
S_3	0.9959	0.9323	0.1590	2.0872	
%H_4	0.2773	0.3214	0.0441	0.6428	
%HS_4	0.3444	0.5904	0.1039	1.0387	
%H_4 + %HS_4	0.6217	0.9118	0.1480	1.6815	98.94
S_4	0.5562	0.9976	0.1457	1.6995	
%H_5	0.2732	0.3212	0.0518	0.6462	
%HS_5	0.0960	0.1403	0.0370	0.2733	
%H_5 + %HS_5	0.3692	0.4615	0.0888	0.9195	82.34
S_5	0.5163	0.4923	0.1081	1.1167	
%H_6	0.0498	0.0695	0.0444	0.1637	
%HS_6	0.1783	0.1807	0.0238	0.3828	
%H_6 + %HS_6	0.2281	0.2502	0.0682	0.5465	76.87
S_6	0.2020	0.3729	0.1360	0.7109	
%H_7	0.7440	1.0859	0.2916	2.1215	
%HS_7	0.1304	0.3833	0.2055	0.7192	
%H_7 + %HS_7	0.8744	1.4692	0.4971	2.8407	129.58
S_7	0.8838	1.1118	0.1966	2.1922	

%H_x, %HS_x = mass loss considering the percentage of humin and humic substances extracted; %S_x = soil mass loss; %H_x + %HS_x = sum of organic matter loss from humin and humic substances.

was required because, during the alkaline extraction, the organic matter concentrates in the HS fraction, and to study the distribution of organic matter from soil to HS and humin fractions, the mass balance must be considered.

(4) The results of extracted organic matter present in humin and HS were compared with the original soils (Table 3) from which they had been taken, based on the following equation:

$$\mathrm{EI} = \frac{[\%\mathrm{H}_x + \%\mathrm{HS}_x]}{\mathrm{S}_x}$$

where EI is the extraction influence; %H_x the loss of organic matter from the humin fraction; %HS_x the loss of organic matter from the humic substances fraction; %S_x the loss of organic matter from the soils.

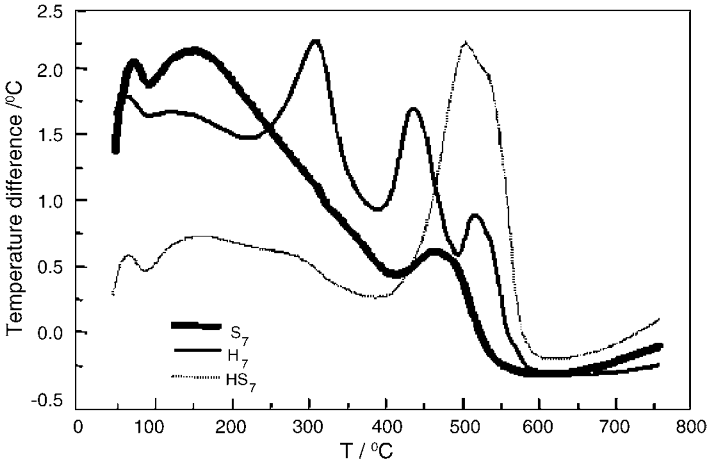

Fig. 8. DTA curves of soil, humin and humic substances from Boa Vista. Conditions: sample mass 8.7329 mg, platinum crucible, synthetic air atmosphere 100 mL min^{-1}, heating temperature 40–750 °C at 10 °C min^{-1}.

The EI (extraction influence) values indicate to what extent the organic matter present in the soils is altered during alkaline extraction. The EI values close to 100 indicate little alteration, while values far from 100 indicate greater alterations.

As can be seen from Table 3, the organic matter extracted from sample S_4 was only slightly modified as a result of alkaline extraction (EI = 98.94). However, the other samples displayed significant differences between the content of organic matter in HS/humin and that in soils. With the exception of sample S_7, all the other samples showed a loss of organic matter during the extraction process, probably due to the oxidation of oxygenated groups present in the organic matter and loss of carbon dioxide.

Mathematically, the results indicated that, with the exception of sample S_4, the organic matter present in the soil was substantially altered through alkaline extraction.

3.2. *Differential thermal analysis*

Figs. 2–8 compare the DTA curves of humin and HS versus the whole soils from which they were extracted.

Figs. 2–8 illustrate the decomposition and thermal stability of the organic matter present in humin and HS and in their respective soils. The DTA curves of humin and HS extracted from soil samples collected in Ribeirão Preto, Manaus and Campo Grande (Figs. 5–7) indicate that these fractions display a thermal behavior similar to that of the soils from which they were extracted. Samples S_1–S_3 and S_7, on the other hand, reveal a considerable difference in the thermal stability of the humin, HS and soils. The exothermic peak at temperatures of 400–500 °C in the DTA curves of HS indicates the presence of compounds with greater thermal stability than that found in humin and soil. The DTA curves of humins display two exothermic peaks associated with a loss of carbohydrates and phenolic compounds [16], which appear less well defined in the soils samples.

The DTA results confirmed that the alkaline treatment of soils causes alterations in the organic matter present in soils, producing two new classes of compounds (humin and HS) whose characteristics differ from those of the original soil.

4. Conclusions

Thermal analysis (TG/DTA) proved to be an interesting technique for characterizing soils and their fractions because it offers the possibility of studying organic matter originally present in soil. The TG/DTA curves showed that alkaline extraction causes modifications in the organic matter during the extraction process. Therefore, our results indicate the need for and importance of developing methods and analytical procedures that allow the behavior and properties of organic matter in soils to be studied without extracting it. This aspect is crucial for the optimal interpretation of results obtained from studies of humic substances, leading to a more realistic understanding of environmental processes.

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