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THERMAL ANALYSIS (TG-DTA) AND DRIFT SPECTROSCOPY APPLIED TO INVESTIGATE THE EVOLUTION OF HUMIC ACIDS IN FOREST SOIL AT DIFFERENT VEGETATION STAGES

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Humic acids (HAs) extracted from soils developed under two Norwegian spruce (Picea abies, (L.) Karst) subalpine forests of northern Italy were characterized using chemical, thermal (TG-DTA) and spectroscopic (DRIFT) analyses. The samples were taken from five sites which differed in orientation (northern and southern exposure) and vegetal cover at different old age: grassland, regeneration, immature and mature stands. In general, the thermal patterns of HAs were similar (three exothermic reactions appeared around at 300, 400 and 500°C) in both sites in grasslands and regeneration while a considerable modification appeared in HA from stands of different age at northern and southern exposure site. DRIFT spectroscopy confirmed the differences observed through TG-DTA analysis. In particular the main structural changes were ascribed to modification of carbonyl group and of CH stretching in aliphatic components in each HAs from different sites.

Keywords: *DRIFT, forest soil, humic acids, TG-DTA*

Introduction

Recent ecological investigations have focused particularly on the effects forest vegetation has on humic substances (HS) 'quality' [1, 2]. The 'quality' of HS is usually defined in terms of its potential persistence and sustainability in soil and therefore is of prime interest to know and to evaluate how structural characteristics of humic C vary with location, climatic changes or each succession stage of plants and animals in any given ecosystem. How the vegetation affects the humification process it is not yet well defined because several factors and mechanisms such pseudo-climatic condition, soil pH, redox potential and types of mineral present in the soil itself influence this process [3].

A step towards improving our understanding of humic substances is to develop rapid methods which can provide a complete characterization of humic substances structure and which require little or no sample preparation. The traditional analytical approach entails long purification procedures to remove the impurities using strong mineral acids which can produce artifacts in HS molecular structure. A direct analysis of HS without chemical pre-treatment such as the use of physical rather than chemical procedures is attractive.

Thermal analysis techniques are powerful analytical tools in the investigation the thermal properties of soil organic matter. In particular, thermogravimetry (TG) and derivative thermogravimetry (DTG) were used to examine the thermal nature of soil organic matter in podzols [4, 5]. Additional information was obtained combining thermal analysis with other techniques such as pyrolysis mass spectrometry [6, 7]. Ahmed *et al.* (2002) [8] using DTA analysis found that clay–humus complexes had lower thermal decomposition temperatures than extracted humic acids. They attributed the differences to aromatic rings adsorbed onto the clay's surface. Moreover, this technique is widely used to investigate the genesis of coals [9–12], the humification process of soil organic matter [13], humic substances structure [14–16] and finally composts [17, 18].

To obtain a dynamic picture of the HS thermal pattern however, information regarding their molecular structure are needed. Spectroscopic techniques are powerful tools in distinguishing individual molecular structural differences in a heterogeneous organic mixture. In particular, diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy is considered one of the most sensitive IR techniques for analysis of humic

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substances from different origins [19–21]. The principle of these techniques is based on scattering of incident light in all directions when it is absorbed by powders and/or rough surface. In practice, diffuse reflection spectra strongly dependent upon the conditions under which they are recorded. These spectra can exhibit both absorbance and reflectance features due to contributions from transmission, internal and specular reflectance components as well as scattering phenomena in the collected radiation [22]. Unfortunately, the quality, the amount, the size of the used particle, and how they are packed in the sample holder significantly affect the scattering characteristics of the overlayer material and therefore the quality of the spectra. This problem can be overcome by the use of a silicon carbide (SiC) sampling kit. The SiC substrate is grayish-black and absorbs strongly throughout the whole mid-IR region. These properties violate one of the basic assumptions of the Kubelka-Munk theory, i.e., the need of the presence of a nonabsorbing or weakly absorbing substrate [23].

In the present paper humic acids extracted from soils developed under two Norwegian spruce (*Picea abies*, (L.) Karst) subalpine forests of northern Italy were characterized. The samples were taken from sites differing in orientation exposure and phase of the vegetal cover. The humic acid characteristics were determined using chemical, thermal analysis (TG-DTA) and diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy with the aim to estimate the variation of humic acid in relation to the different environmental conditions.

Experimental

Materials and methods

Study area

The study area is located in the Paneveggio Forest (Trentino) (latitude 48°15', longitude 11°45'), N. Italy, at ca. 1900 m elevation. The average annual rainfall varies between 1207 and 1316 mm and the average annual temperature is 2.4°C. After a preliminary field survey using 1:50,000 vegetation maps, two similar sites were chosen for their similar geomorphology and forest management and their different at northern and southern exposure. Each site comprised different age of stands: high grassland and grassland (0-years), regeneration (<25 years), immature (25–50 years), and mature (<100 years).

Soil samples were air-dried and sieved at 2 mm before analyses. Layers with a thickness ranging from 1 to 10 cm were chosen to study the evolution of organic and humic matter. The samples were called: IS1, IS2, IS3, IS4, IS5, for southern, and IN1, IN2, IN3, IN4, IN5, for northern exposure (Table 1).

Humic acid extraction

About 10 g of air-dried and finely ground samples were extracted under N_2 with 100 mL of 0.5 M NaOH and stirred for 24 h. The suspension was centrifuged at 5.000 g for 30 min and then filtered through a 0.45 μm filter using a Minitan S System (Millipore, Bedford MA–USA). The solution was acidified with 5 M HCl to pH<2 to precipitate the HA and was subsequently centrifuged at 5.000 g for 20 min in order to eliminate the supernatant. The HA were dissolved with NaOH 0.5 M to produce a Na-humate which was dialyzed against Millipore water, using tubing (Cellu Sep H1-USA) with a cut-off of 8000 Da, until a neutral pH was achieved, and was then freeze–dried.

Elemental analysis

Elemental analysis (C, N) was carried out using an Elemental Analyzer–Model EA 1108 (Carlo Erba Milan, Italy).

Organic carbon

The procedure for quantifying the organic matter (OM) has been described by Ciavatta *et al.*, (1989) [24].

Potentiometric titration

Samples were prepared by dissolving 12 mg of the freeze-dried HA in 20 mL of Milli-Q Millipore water containing 0.05 M NaCl to keep the ionic strength constant. The pH was adjusted to about 3 by addition of about 1 mL of 0.05 M HCl. The solutions were titrated to pH 10.5 with 0.05 M NaOH using a VIT90 Radiometer Auto titrator (Radiometer Analytical, France). The titrations were carried out in triplicate at 25°C, under N_2 flow and the delivery range was 10 μL min^{-1} (±0.01). The acidity was calculated from titration curves using the first derivative method (Titramaster 85 Version 5.1 Software Radiometer Analytical, France).

TG-DTA analysis

Thermogravimetric analysis (TG) and differential thermal analysis (DTA) were carried out simultaneously using a TG-DTA92 instrument (SETARAM, France). About 5 mg of lyophilized HA were weighed in allumina crucible and first isothermally heated to 30°C for 2 min, and subsequently headed from 30 to 700°C in a dynamic air atmosphere (air flow 5 L h^{-1}). The heating rate was 10°C min^{-1}. The furnace was calibrated using

Table 1 Some chemical characteristics of two forest soils samples

Samples	Exposure	Cover vegetation	Stand age/years	pH (in H_2O)	pH/in KCl	Organic matter/%	C/N
IS 1	South	High grassland	0	4.3	3.7	12.6	**12.4b**
IS 2	South	Grassland	0	4.3	3.7	7.4	10.5
IS 3	South	Regeneration	<25	4.0	3.7	19.3	22.2
IS 4	South	Immature stand	25–50	3.4	2.8	**21.4a**	13.5
IS 5	South	Mature stand	<100	3.8	3.3	8.62	19.8
IN 1	North	High grassland	0	6.7	6.5	**22.1a**	15.3
IN 2	North	Grassland	0	4.4	3.6	31.9	14.1
IN 3	North	Regeneration	<25	4.0	3.6	17.1	16.6
IN 4	North	Immature stand	25–50	5.3	4.4	3.5	18.8
IN 5	North	Mature stand	<100	3.6	3.1	5.9	**12.8b**

The value are the means of 3 replicates. The value within each column indicated with the same letter (a, b) does not differ at level P=0.05 based

transition temperature of indium and aluminum. Calcined caolinite was used as reference material.

Diffuse reflectance infrared Fourier transform

All the samples were recorded by a Nicolet Impact 400 FT-IR Spectrophotometer (Madison, WI) and fitted with an apparatus for diffuse reflectance (Spectra-Tech. Inc., Stamford, CT). The SiC disks (320-grid-Carb paper obtained from Spectra-Tech) are used to collect a small amount of the samples. Spectra were obtained from the accumulation of at least 200 scans at a resolution of 4 cm^{-1}. The background was performed on the abrasive SiC disk. Analyses of spectral data were performed with Grams/386 spectral software (Galactic Industries, Salem, NH). Peak area integration from 3000 to 2800 cm^{-1} was used to compare the CH groups in aliphatic substances between samples (Table 2).

Statistical analyses

The Statgraphics version 5 plus (Statistical graphics system by statistical graphics corporation) was used for our calculations.

Results and discussion

In Table 1 are shown the chemical characteristics of forest soils at two exposure sites. The Duncan's multiple comparison procedure was performed to determine which means are significantly different from which others. In general, the organic matter content and C/N ratio showed statistically significant differ-

Table 2 Chemical characteristics of humic acids extracted from two studied sites

Samples	C/%	N/%	COOH mmol/100g	CH stretch integration area
IS 1	41	**3.0 ce**	**562 f**	12.00
IS 2	**38 a**	2.7	**521 g**	8.64
IS 3	34	**3.0 ce**	516	15.40
IS 4	25	2.3	590	9.50
IS 5	42	3.3	**562 f**	18.37
IN 1	**38 a**	**2.8 d**	497	21.00
IN 2	47	**3.1 e**	**562 f**	15.56
IN 3	**40 b**	**2.8 d**	**521 g**	16.00
IN 4	**40 b**	3.6	**562 f**	16.35
IN 5	46	**2.9 d**	583	14.33

The values are the means of 3 replicates. The value within each column indicated with the same letter (a, b, c, d, e, f, g) does not differ at level P=0.05t

ences between samples. Similarly the C, N and COOH groups concentrations of HAs, extracted from the two sites, showed statistically significant differences (Table 2).

TG-DTA analysis

The DTA curves of all samples are shown in Fig. 1. All curves presented a first endothermic peak between 90–120°C with a mass loss between 7.5–9.8% corresponding to the dehydration of the samples. In general three main exothermic reactions were observed in both site exposures: the first peak at 300°C was produced by thermal combustion of polysaccharides, decarboxylation of acidic groups and dehydration of hydroxylate aliphatic structures [14, 25]; the second peak, about 400°C, was due to the combustion of aliphatic structures (C16, C18 ω-hydroxy alkanoic acid and ≥C20) derived from plants [7, 26] and animals decomposition, as well as from different pedogenic sources [27]. Their persistence might be also directly affected by the soil's acidic pH and moisture which favor their accumulation in soil. Finally, the third peak at around 500°C was originated by the combustion of aromatic structures and cleavage of C–C bonds [28]. In particular, the TG-DTA curves of high grassland at southern exposure site (Fig. 1 IS1) showed a broad exothermic peak at 327°C with a mass loss of 36.8%, a second exothermic peak at 434°C with a mass loss of 22% and a third exothermic peak at 524°C with a mass loss of 16.8%. In grassland (Fig.1 IS2) only one strong exothermic reactions at 430°C with a shoulder at 324°C with mass losses of 42% and a second peak at 502°C with mass loss of 8% appeared in the curves. The strong exothermic reactions observed in this sample might be due to a higher content in polysaccharides structure than in the HA from high grassland. The thermal pattern of regeneration (Fig. 1 IS3) showed an exothermic peak at 320°C with a mass loss of 26.1%, a second exothermic peak at 430°C with a mass loss between 23.5% and a third peak at 519°C with a mass loss of 12.8%. This sample appeared similar to IS1 but the relative intensity between peaks changed. Specifically, the second peak increased slightly with respect to the previous samples while the other peak decreased considerably. The thermal behavior of HA observed in these vegetation phases seems to be related to interactions between soil and vegetal cover. In IS1 and IS2 samples correspond to phases in which 'pioneer' plants including mosses, lichens and small herbaceous plants are dominant. The organic matter derived from plant residues is characterised by very high polysaccharide content, low content in phenols and no presence in aromatic substances [29]. This might justify the high exothermic reaction of the first peak. However the presence of the third peak is unequivocally related to an enrichment in aromatic components determined by occurrence humification reactions. The thermal pattern of immature stand (IS4) is characterized by two broad, poorly resolved peaks (Fig. 1). Specifically, a peak at 318°C, with a mass loss of about 30%, and a second peak at 478°C with a modest mass loss of 8.5% were observed. The reaction of the first peak seems to be related to decarboxylation reaction of acid groups rather than decomposition of carbohydrates as supported by potentiometric titration and DRIFT spectrum (see below). Finally, thermal reaction of mature stand (IS5) showed an exothermic peak at 319°C with a mass loss of 31.3%, and a second peak at 434°C with a mass loss of 16.8%, and a third peak at 519°C, with a mass loss of 21.1%.

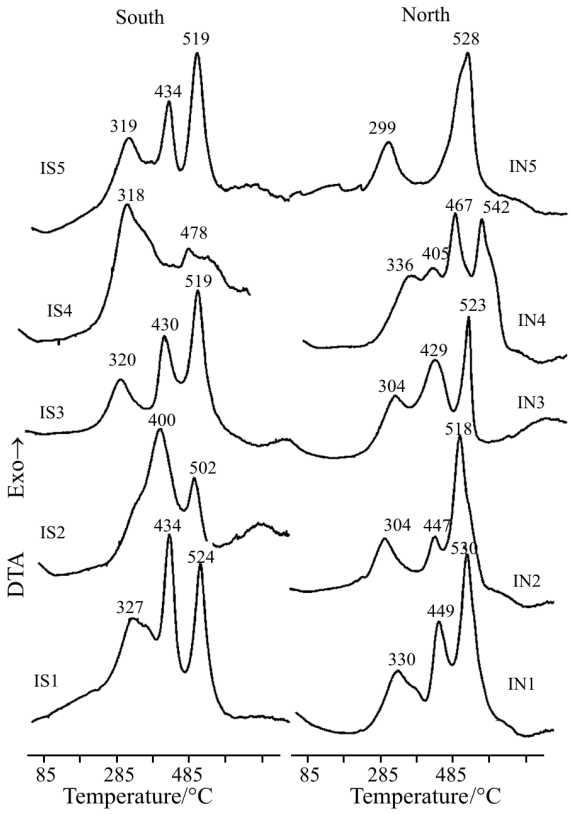

Fig. 1 DTA curves in air atmosphere of HAs extracted from a forest soil differing in southern (IS) and northern (IN) exposure and vegetal cover (Table 1)

The HA grassland samples do not show any significant differences neither between themselves nor between northern and southern site exposure. In detail high grassland (IN1) confirmed the presence of three exothermic peaks at 330, 449 and 530°C, with mass losses of 34.6, 16.7 and 24%, respectively. Similarly grassland (IN2) showed the following peaks at 330, 447 and 518°C, with mass losses of 26, 10 and 25.6%,

respectively (Fig. 1), while IS2 showed a different quantitative mass loss in each peaks as compared to IN2. The thermal pattern of regeneration (IN3) showed the following exothermic peak at 304, 429 and 523°C which are slightly different from the correspondent temperatures of IS3. The mass loss in IN3 of 27.3, 17.3 and 22% at each peak, respectively differs from IS3 slightly in the second and considerably in the third peak.

Thermal pattern of immature stand (IN4) appeared completely different with respect to grassland samples and less different with respect to mature stands (IN5). In detail IN4 showed four peaks at 336, 405, 467 and 542°C, with mass losses of 29.2, 12.9, 23.0, 14.5%, respectively. In the southern site exposure IS4 also showed a peculiar thermal pattern which is similar to others in the first peak while lacking the second peak being substantially smaller in the third peak (478°C). In IN4 the first exothermic reactions might be due to the combustion of mixture of simple sugar and residual hemicelluloses. A further structural modification can be seen in the exothermic reaction of the last peak which showed a shift toward higher temperature with a notable increase in mass loss with respect to the IS4 (Fig. 1). A possible explanation of the different thermal behaviors can be ascribed to effect of a northern site exposure that might directly affect the decomposition rate of needle residues. Finally, the thermal behavior of mature stands (IN5) was characterized by only two exothermic peak at 299 and 528°C with a mass loss of 27.1 and 38.8%, respectively. The temperature displacement of the first peak toward lower temperature might be due to decarboxylation reaction of acid groups rather than sugar decomposition in according to the amount of COOH groups determined (Table 2). A high value in mass loss of the third peak supports the existence of a highly stable structural 'nucleus' with strong bond energies. On the basis of these results we can affirmed that a high content in COOH groups and a simultaneous increase in aromatic components are related to an increase of humification rank. In addition, the disappearance of the peak around 450°C demonstrated a strong modification of humic acid structure. This confirmed how the molecular structures, such as long-chain aliphatic fractions to derived from plants, were no longer recognizable. The thermal differences between IN5 and IS5 might be ascribed to different site exposure because carbon input to the top soil is only due to same contribute of needle decomposition. In summary, at southern site exposure, the HA structure retains more components originated from plant residues while at the northern site, the thermal pattern was similar to that of leonardite HA from the international humic substances society-IHSS (http://www.ihss.gatech.edu) [16].

The structural changes observed using TG-DTA analysis were supported by DRIFT spectroscopy. The vibrational spectra are shown in Figs 2 and 3, respectively. On the whole, the spectra for two analyzed sites were similar and showed similar spectroscopic features. The broad intense band at around 3300 cm^{-1} is attributed to O–H stretching of carboxylic and alcoholic groups [30]. The bands at around 2920 and 2850 cm^{-1} represent aliphatic C–H vibration of aliphatic methyl and methylene groups. The broad band at 2626 cm^{-1} was attributed to the formation of intermolecular hydrogen bonding between OH groups in oxygenated compounds [30]. The carboxylic bands at around 1717 and 1230 cm^{-1} indicated the presence of COOH groups in acid dimers [30]. The bands at around 1650 cm^{-1} likely correspond to carbonyl C=O stretching vibrations in amide I and ketons. The shoulder at 1600 cm^{-1} was assigned to asymmetric stretching of carboxylate (COO^-) and aromatic (C=C) vibration bands [31, 32]. The occurrence of a band at 1507 cm^{-1} can be assigned both to amide II vibrations and to aromatic C=C vibrations from lignin [33]. A broad band appeared around 1400 cm^{-1} due to a methylene bending vibration, methyl symmetrical bending vibration, and COO^- stretching symmetrical vibration, respectively [27, 33, 34]. Due to the complexity of these molecular systems, the bands in this region originate from a mix-

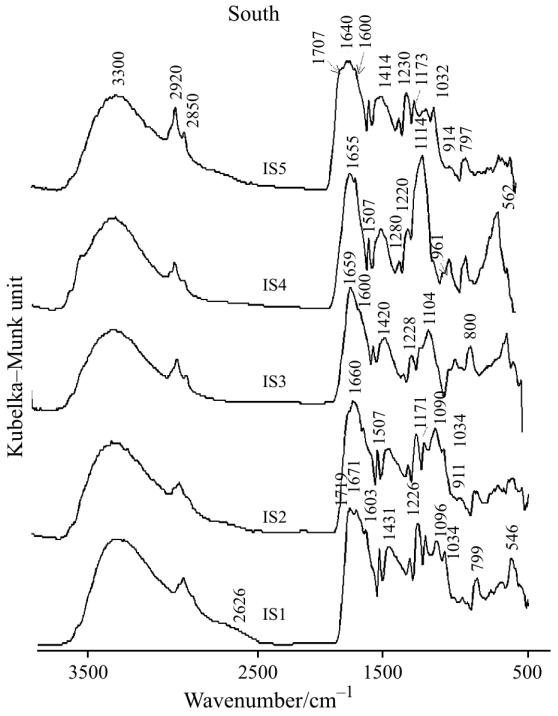

Fig. 2 DRIFT spectra of HAs extracted from southern (IS) site exposure

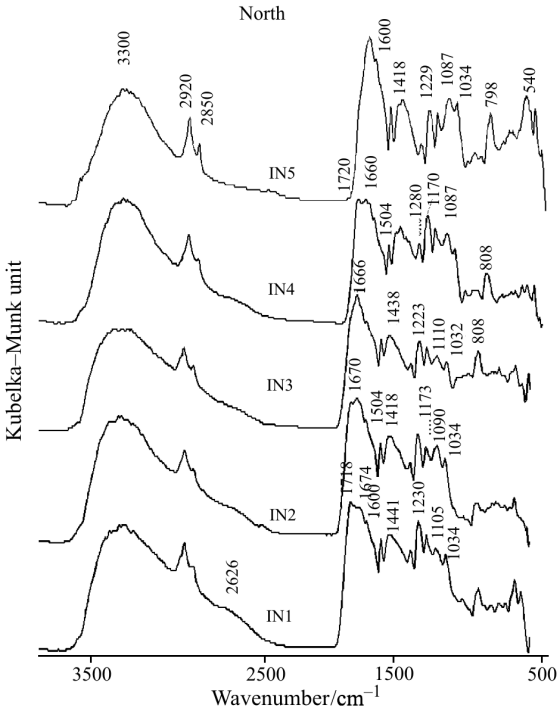

Fig. 3 DRIFT spectra of HAs extracted from northern (IN) site exposure

ture of coupled vibrations. Thus, it is not possible to assign them a specific functional group. A broad band occurs at around 1280 cm^{-1} is assigned to single carbon oxygen bond (C–O) stretching vibration. In the 'fingerprint' region, the absorption bands around 1110–1034 cm^{-1} may represent C–O, C–C or (C–O–C) stretching vibrations in polysaccharides [27]. The strong peak at around 1110 and the region between 790–540 cm^{-1} are indicative of the presence of inorganic materials. In detail, the structural modification observed at southern site exposure between grassland and high grassland (Fig. 2) showed an increase of the intensity of the bands assigned to carboxylic groups and the polysaccharide region. Moreover, comparing grasslands samples to immature stands (IS4), C=O stretching frequency decreased by the order of 10–31 cm^{-1} while the relative intensity increased. It is possible ascribe this decrease of carbonyl frequency to conjugation effect of C=C bounds [30]. The lack of bands at around 2660 and 1717 cm^{-1} might suggest a modification of carboxylic acids composition from dicarboxylic in high grassland to monocarboxylic acids in immature stands (IS4). Finally, the HA of the older stands (IS5) was very similar to high grassland (ISI) but more enriched in aliphatic components. This similar trend was observed in HAs from the northern side exposure (Fig. 3). The main differences regarded the changes of carbonyl stretching frequency by the order of 4–17 cm^{-1} from grassland (IN1) through to mature stands (IN5) and were due to the effect of aryl or polyene conjugation. Furthermore, the polysaccharide region of these samples, especially the band at 1034 cm^{-1}, decreased from grassland to mature stands confirming the result obtained by means of the TG-DTA analysis. Finally, the integration area of CH stretching, calculated for each HA, showed statistically significant differences in relation the vegetal cover and exposition site (Table 2). In particular, aliphatic component appeared higher in the HAs from northern as compared to southern exposure with exception of mature stand at the northern site. Moreover, the lack of a shift of CH band frequency suggested that the aliphatic component in both sites was similar and mainly characterized by long-chain saturate hydrocarbons [31].

Conclusions

In the present paper the combination of thermal analysis (TG-DTA) and DRIFT spectroscopy was applied to the structural characterization of the humic acids extracted from soils sampled under two Norwegian spruce (*Picea abies*, (L.) Karst) sub-alpine forests. The thermal analysis showed the humic acid from high grassland and grassland resulted remarkably similar while the differences between grassland and immature and mature stands appeared to be significant. Similarly, the two stands of different age and at northern and southern exposures showed a substantial variation. From the thermal analysis it may be inferred that the structure of HA is qualitatively different between the two stands and between these and the grasslands (see the differences in thermal profile). The main differences observed using thermal analysis regard aliphatic (second exothermic peak) and aromatic substances (third exothermic peak). Polysaccharides and carboxylic groups (first exothermic peak) did not seem highlight relevant structural differences. DRIFT has, of course, a greater resolution power than thermal analysis when the molecular nature of the differences observed is concerned. The application of TG-DTA and DRIFT techniques has demonstrated to be powerful tools to study and compare humic substances formed in different environmental sites.

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