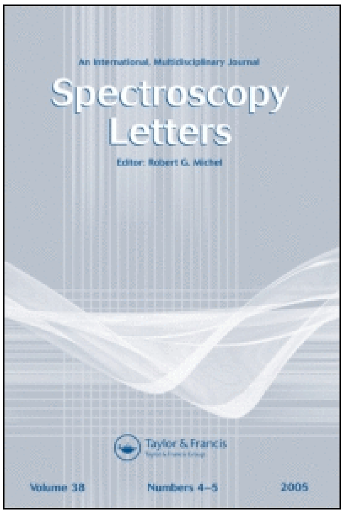

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FTIR TENTATIVE CHARACTERIZATION OF HUMIC ACIDS EXTRACTED FROM ORGANIC MATERIALS

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ABSTRACT

Turkish humic acids, extracted from different materials, were characterized by their infrared spectra and by means of acidity measurements. The information obtained is of great interest to ascertain the nature and origin of humic acid samples.

Key Words: Humic acids; Fourier transform infrared.

INTRODUCTION

The organic matter in soils consists of a mixture of plant and animal residues in various stages of decomposition. Organic substances in soil were synthesized

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chemically and biologically from the breakdown products, by the action of microorganism and small animals.

Humic substances are the main components of soil organic matter, and they occur in soil, peat, coal, natural water, sediments, lignins, and other natural deposits (1), and have a widespread distribution in all terrestrial and aquatic environments (2). Humic substances are composted mainly of humic acids (HA), fulvic acids (FA) and humin. These substances show different solubilities in alkalis and acids HA are soluble in diluted alkali, and precipitates by acidification of the alkaline extract. On the contrary, FA are soluble in diluted alkalis and acids. Humin is the fraction that cannot be extracted from soils and sediments by diluted alkalis and acids (3). HA and FA are very similar in composition and structure, the main difference corresponding to their distribution of molecular weight.

Humic acids are polycondensed polyphenol compounds with heterocyclic rings and functional groups containing N, S, O heteroatoms and methylene bridges (4). All these groups provide a great capability of HA for interacting in the biosphere and thus influencing the transport, decomposition, bioavailability, bioaccumulation, and fate of organic pesticides, consequently affecting their degradation and phytotoxicity (2).

In recent years great interest has grown in the possible recycling of organic residues or wastes in agriculture as soil amendments in order to maintain and increase soil organic matter content.

After application of organic wastes, the soil has the great capability of transform those materials through biodegradation processes leading to humic substance synthesis (5). Before adding organic materials to soil, the chemical characterization of humic acids already present in these materials is necessary to comprehend their transformations in the soil environment.

Humic acids extracted from different origins, like soils, coal, vermicompost, peat, stream water, leonardite and commercial humic acids have been studied with different objectives using infrared analysis. Table 1 summarizes the infrared band assignment of humic acids found in the literature. As can be seen humic acids present common bands at the following frequencies: 3500–3000 cm^{-1} (hydrogen bonded hydroxyl groups and N–H), 3000–2800 cm^{-1} (aliphatic C–H groups), 1725–1600 cm^{-1} (C=O group), 1620–1600 cm^{-1} (C=C), 1460–1450 cm^{-1} (aliphatic C–H groups), 1400–1390 cm^{-1} (O–H, COO^- and C–O groups), 1320–1200 cm^{-1} (C–O and O–H of COOH), 1180–1000 cm^{-1} (C–O of alcohol, ether, esther, polysaccharide groups and O–H of alcohol) noting there are differences as a function of their origin.

In this work, a systematic study of the characterization of humic acids extracted from different soil origins has been conducted, and data found were analyzed as a function of total acidity; taking also into consideration the ash content and origin of the samples. A library, containing the IR spectral data of the samples and reference HA has been created in order to evaluate the comparability of HA of different nature.

Table 1. Band Assignment of the IR Bands of Humic Acids Reported in the Literature

Sample	Band			Reference
	cm^{-1}	Intensity	Groups	
Na-HS (ALDRICH)	3000	Broad	O–H and NH	6
	3000–2800		Aliphatic CH_2 and CH_3	
	1720		C=O of carbon acids, esters, aldehydes or ketones	
	1600	Slight	C=O of ketones, chinones or amides; C=C	
	1384		COO^{-1}; amida	
	1250–1100		Carbonic acids or phenolic or aliphatic alcohols or arylethers	
Commercial Salts (EGA, Fluka, GMS)	1580	Strong	COO^{-1}	7
	1400–1310	Broad	COO^{-1}	
Soil (HA-A and FA-3) Commercial (K&K and Shanghai—SH), Soil(Linyi-1, Linyi-2, Mudanjiang, Beijing, Nanjing, Hangzhou, Changsha, Changchun	3400–3300		O–H; N–H	8
	2940–2900		Aliphatic C–H	
	1725–1720		C=O of COOH and ketones	
	1660–1630		C=O of amide; quinone and/or C=O of H-bonded conjugated ketones	
	1620–1600		Aromatic C=C	
	1590–1517		COO^{-1}; N–H and C=N	
	1460–1450		Aliphatic C–H	
	1400–1390		O–H and C–O of phenolic; C–N; COO^{-1}; C–H of CH_2 and CH_3	
	1280–1200		C–O and O–H of COOH	
	1170–950		C–O of polysaccharide; Si–O of silicate	
Soil (HA-“193 MAR”—Coal of Northern Bohemia)	2900		Aliphatic C–H; H–bonded of O–H or N–H	1
	1706		C=O of COOH and/or ketone	
	1615		Aromatic C=C; H-bonded C=O	
	1463		C–H of methylene or methyl group	
	1377		O–H; C–O; methyl bonding	
	1250		C–O; O–H of COOH	
Peat (PHA)	3500–3000	Strong and broad	O–H	2
	3000–2800		C–H of aliphatic or cyclic groups	
	1720		C=O from carboxylic groups	
	1650–1550	Broad	C=O of COO^-; aromatic C=C	2
	1400		C–H bending of methyl, isopropyl, and tertiary butyl groups, aromatic C–C and COO^-	
	1320–1210		O–H of phenol	
	1180–1000		C–O of alcohols, ethers and esters	

(*continued*)

Table 1. *Continued*

Sample	Band			Reference
	cm^{-1}	Intensity	Groups	
Vermicompost (VHA)	3500–3000	Strong and broad	O−H	2
	3140		N−H	
	3000–2800		C−H of aliphatic or cyclic groups	
	1700–1500	Broad	C=O from carboxylic groups; N−H; aromatic C=C	
	1400		C−H bending of methyl, isopropyl, and tertiary butyl groups, aromatic C−C and COO^{-}	
	1385	Strong	C−N of amine groups	
	1320–1210		O−H of phenol	
	1120–1050	Broad	C−O and COH from alcohols	
HA from peatbog water	3400		O−H	9
	2900		Aliphatic C−H	
	1720		Carboxyl group	
	1610		Carboxylate group	
	1280–1030		C−O of ethers; esters; and others	
HA (aquatic and terrestrial Sample)	3390–3370	Strong	Hydrogen-bonded O−H	10
	2920		Aliphatic C−H	
	1715–1710		C=O of carboxyl and carbonyl groups	
	1650	Broad	Amide	
	1615–1610		Aromatic C=C and COO^{-} groups	
	1520		C=O of amide	
	1385	Medium-weak	O−H; C−O of phenol; COO^{-}; C−H of −CH$_3$	
	1220–1200	Broad	C−O and O−H of COOH	
	1040		Alcohol; O−H; C−O of polysaccharide; Si−O of silicate impurities	
	below 900	Weak	Out-of-plane of aromatic C−H; Aliphatic −CH$_2$−	

EXPERIMENTAL

Samples and Reagents

Commercial humic acid sodium salt (Na-HA) was supplied by Aldrich (Germany). The extraction process and purification of humic acids were performed according to the suggestion of International Humic Substances Society (IHSS), following the procedure described bellow. The origins of the soil samples reported in this paper are summarized in Table 2.

Table 2. Origin of Turkish Humic Acid Samples Characterized on This Study

Origin	Sample	Ash Content (%)
Peat	P1 (Peat 01)	21.51
	P2 (Peat 02)	5.10
Sludge	BFS (Beer Factory Sludge)	3.94
	SS (Sewage Sludge)	53.13
Vegetable	GP (Grape Marc)	1.50
	TEAW (Tea Waste)	0.21
Compost	SMC (Spend Mushroom Compost)	2.69
	CB (Composted Bark)	21.91
Commercial	Aldrich	29.59
Inorganic Soil	SHA (Soil HA)	6.52

Extraction of organic materials and separation into humic acids and fulvic acids.

Ten to 20 g of organic material was weighed into centrifuge tubes and 200 ml of 0.5 mol l^{-1} NaOH was added, the air in the tube was displaced by nitrogen and the system was shaken for 24 hours at room temperature. The dark-colored supernatant solution, after separation from residual material by centrifugation, was acidified to pH 2 with 6 mol l^{-1} HCl and allowed to stand for 24 hours at room temperature to allow for the coagulation of the HA fraction. The soluble material (FA) was separated from the HA by centrifugation.

Purification

HA was shaken for 24 hours at room temperature with 0.5% (v/v) HCl/HF solution. The insoluble residue (HA) was separated from the supernatant by centrifugation, washed with small aliquots of distilled water until be free of Cl^-, using dialysis membranes for to remove low molecular weight constituents, such as aliphatic, aromatic and phenolic acids from high molecular weight HA. The HA were subsequently purified. This procedure was to carried out for all samples, one of then was a commercial sample for Aldrich. All the samples present a dark color. These materials, excluding soil HA, were originated from organic material, and soil HA was extracted from inorganic soil.

Total Acidity and Ash Content Analysis

The total acidity was determined by $BaCl_2$ method: 50 mg of each HA sample was placed in a centrifuge tube and it was added 10 ml of distilled water, 50 ml 0.1 mol l^{-1} $BaCl_2$ and 50 ml 0.1 mol l^{-1} NaOH solution. After shaking for 16 hours, the suspension was filtered and titrated with 0.1 mol l^{-1} HCl.

The ash content of humic acids was obtained by combustion to constant weight at 700°C in an oxygen atmosphere.

Infrared Spectra

The IR spectra of samples were recorded within the range 4000–500 cm^{-1} using a Nicolet 750 Spectrometer. All the samples and KBr were desiccated for 24 hours. Pellets were prepared with 100 mg KBr and 1 mg humic acid by pressing. The spectra were recorded at room temperature. All the pellets were heat for 2 hours at 120°C, after that new spectra were recorded.

RESULTS AND DISCUSSION

Effect of the Temperature on the IR Spectra of Humic Acids

The HA spectra of each sample was obtained from KBr pellets, at 25°C and after drying the pellets at 120°C for one hour. After heating it was observed a reduction of 3410–3080 cm^{-1} band intensity, concerning the O–H absorption of water, as it can be seen in Figures 1A and 1B. Thus, spectra of samples were obtained after drying the pellets in order to avoid differences in their hydration degree.

FTIR Spectra of Humic Acids

Figures 2a through 2f show the IR spectra of the humic acids samples studied. In general, all spectra are almost similar in the position of the main bands, but some differences can be observed in their relative intensity. All the spectra showed bands that could be assigned to the main groups, as: 3410–3083 cm^{-1}(O–H stretching), 2957–2849 cm^{-1} (aliphatic C–H groups), 1420–1332 cm^{-1} (O–H of phenol and alcohol groups). However some bands appeared for specific samples of HA (see Table 3).

The spectrum of the humic acid SS showed a band in the 3623 cm^{-1} which can be assigned to the N–H stretching of N-containing groups. This band is not present in other spectra. On the other hand, it is common to observe bands of N–H stretching of amine at 3490–3180 cm^{-1} and N–H of amide at 3500–3100 cm^{-1}, but these bands can not be observed in the spectra of Turkish humic acids because of the presence of a broad and strong band, assigned to O–H stretching, in the 3410–3083 cm^{-1} range.

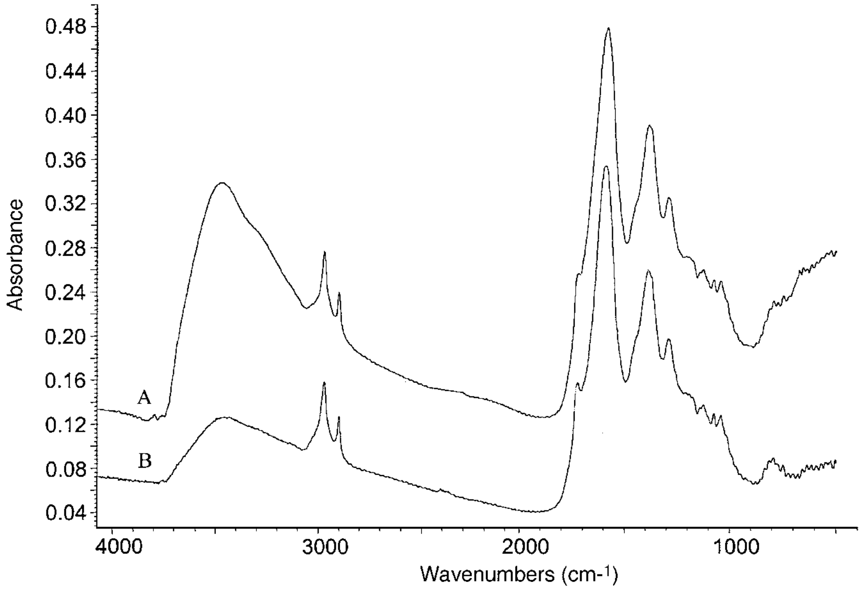

Figure 1. IR spectra of humic acid from Aldrich. (A) without heating; (B) after pellet drying at 120°C for 1 hour.

The bands in the 1700–1500 cm^{-1} range are very strong in the spectra of P1, P2, BFS, GP, TEAW, SMC, SHA and Aldrich, as compared with the spectra of the SS and CB. This fact could be due to the presence of many contribution of C═C and C═O stretching. Furthermore the humic acids SS and CB have a strong band in the 1200–1036 cm^{-1} assigned to aromatic C–H, alcohol C–O and C–O–C of arylethers, thus suggesting the presence of these groups in these samples.

From the aforementioned data it can be seen that HA samples of different origin can be discriminated from their FTIR spectra. However, Figure 2b shows that SS (Sewage Sludge) and BFS (Beer Factory Sludge) can not considered as comparable samples. Sewage sludge humic acid present a totally different IR spectrum than other samples of sludge origin and beer factory sludge spectrum reveals a close structure to that of sample from grape marc and tea waste than the structure of humic acid coming from sewage sludge.

Comparison Between Turkish Humic Acids of Different Origin

To establish some correlation between the HA origin and their IR spectra, it was created a library containing data in the range of 4000–500 cm^{-1}

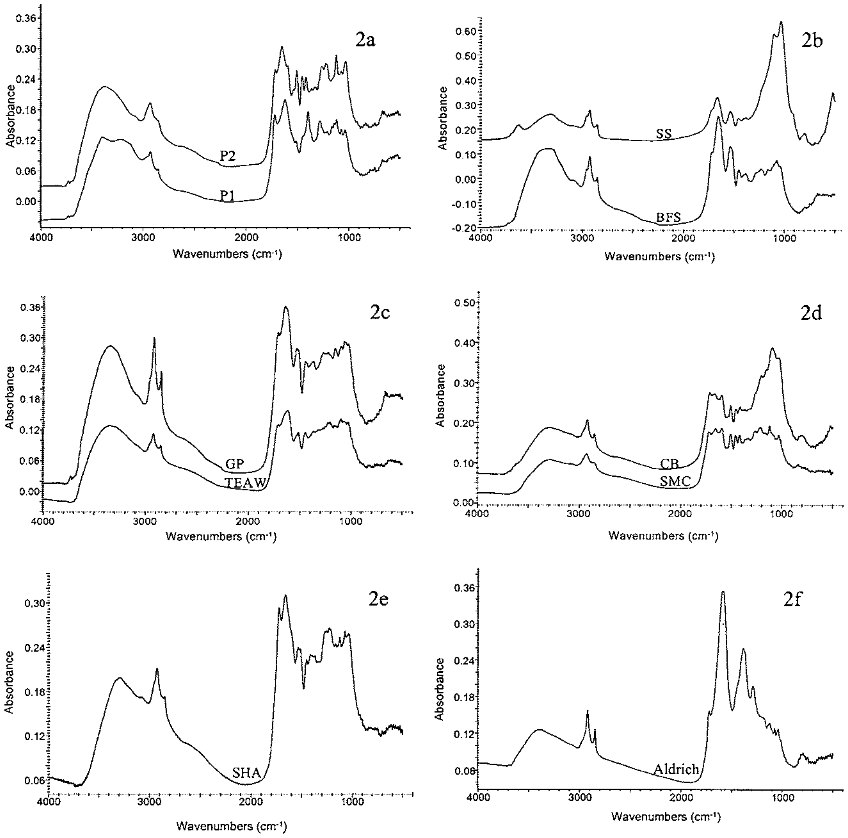

Figure 2. IR spectra of Turkish humic acids. (a) Peat samples, (b) sludge samples, (c) vegetable origin samples, (d) compost samples, (e) inorganic soil, (f) commercial HA.

which were compared with a reference HA sample (Aldrich). Correlation coefficients (r) obtained, making comparisons of the Turkish HA samples with that of Aldrich, varied from 1 to 0.5 (see Table 4), being possible to establish different groups of spectra. However, as it can be seen in the table, sometimes spectra corresponding to the same compound, but registered before and after drying the pellets, were classified in different groups, thus evidencing some limitations of the use of this data base. HA from compost and vegetable origin were localized between 0.66–0.80 correlation coefficient range. HA from peat origin were localized between 0.71–0.85 correlation coefficient range and Peat 01 humic acid presented the more similar spectra with the reference sample. HA from sludge origin were localized between 0.50–0.70 correlation coefficient range and

Table 3. Band Assignment in the IR Spectra of Turkish Humic Acid Samples

Bands		
cm^{-1}	Groups	Samples
3626	N–H	SS
3410–3083	Hydrogen-bonded O–H	P1, P2, BFS, GP, SS, TEAW, CB, SMC, SHA, ALDRICH
2957–2849	Aliphatic C–H	P1, P2, BFS, GP, SS, TEAW, CB, SMC, SHA, ALDRICH
1739	C=O of esther	GP
1720–1705	C=O of ketone and/or carboxylic acid	P2, BFS, GP, TEAW, CB, SMC, SHA
1700	C=O of amine	P1
1665	C=C, C=N of aliphatic imine	SS
1661	C=C, aromatic C–C	BFS, SHA
1659–1537	C=C	P1, P2, BFS, GP, TEAW, CB, SMC, ALDRICH
1537–1514	N–H of amide	P1, SS
1511	COO^-	P2
1506	Aromatic C=C	SMC
1455	N–H of amine and imine	SS
1462–1450	Aliphatic C–H	P2, BFS, GP, TEAW, CB, SMC, SHA
1420–1332	O–H of alcohol and phenol	P1, P2, BFS, GP, SS, TEAW, CB, SMC, SHA, ALDRICH
1330–1315	C–H of alkene, O–H of alcohol and phenol	P1, P2, GP, TEAW, CB
1270–1263	O–H of alcohol, C–O–C of arylether	P2, SMC
1228	O–H of alcohol and phenol, C–O–C of arylethers	BFS
1224–1199	O–H of phenol, C–O–C of arylether	P1, P2, GP, CB, SMC, SHA, ALDRICH
1161–1146	C–O of alcohol, C–O–C of ethers	P1, P2, BFS, GP, TEAW
1126–1124	Aromatic C–H, C–O–C of ethers	P2, SMC, SHA
1116	C–O–C of ethers, C–N of amide	P1
1107–1083	C–O of alcohol, C–O–C of ethers	P1, P2, GP, SS, TEAW, CB, ALDRICH
1060-1033	Aromatic C–H, C–O of alcohol, C–O–C of arylethers	P2, GP, SS, TEAW, CB, SMC, SHA, ALDRICH
834-795	C–H of alkyne	P2, SS, TEAW, SMC, ALDRICH
659	Out-of-plane of C–H	P1

Table 4. Correlation Coefficient Groups of HA Samples Obtained with Comparison of the IR Spectral Data with Reference Sample

Nr. of Group	Correlation Coefficient	Names
01	1.00–0.96	Aldrich Commercial (r = 1.00) Aldrich Commercial - 120° (r = 0.99)
02	0.95–0.91	–
03	0.90–0.86	–
04	0.85–0.81	P1 (peat 01) (r = 0.83) P1 (Peat 01) - 120° (r = 0.81)
05	0.80–0.76	SMC (Spend Mushroom Compost) (r = 0.79) TEAW (Tea Waste) (r = 0.79) SHA (Soil HA) (r = 0.77) GP (Grape Marc) (r = 0.76) P2 (peat 02) (r = 0.76) SMC (Spend Mushroom Compost) - 120° (r = 0.76)
06	0.75–0.71	TEAW (Tea Waste) - 120° (r = 0.75) SHA (Soil HA) - 120° (r = 0.74) CB (Composted Bark) (r = 0.72) P2 (Peat 02) - 120° (r = 0.72)
07	0.70–0.66	GP (Grape Marc) - 120° (r = 0.70) CB (Composted Bark) - 120° (r = 0.68) BFS (Beer Factory Sludge) (r = 0.67)
08	0.65–0.61	-
09	0.60–0.56	BFS (Beer Factory Sludge) - 120° (r = 0.59) SS (Sewage Sludge) (r = 0.55)
10	0.55–0.50	SS (Sewage Sludge) - 120° (r = 0.48)

were the samples for which it was observed the highest differences when compared with the commercial one. HA from inorganic soil origin were localized between 0.71 and 0.80 correlation coefficient values. These data are summarized in Figure 3a.

Using the total acidity as criterion to group the HA samples (see Fig. 3b), it was observed that all HA samples from vegetable origin presented similar total acidity (12 to 13 meq g^{-1}). However, for samples from other origins it was not observed a similar behavior and thus, 10 to 17 meq g^{-1} acidity was found for HA from peat origin, 12 to 17 meq g^{-1} for HA from compost and 14 to higher than 22 meq g^{-1} for HA from sludge.

Data on the ash content, could be used as a criterion of purity of the HA. Vegetable origin samples presented very low ash content (0 to 2%). However, for peat, compost and sludge origin samples, the ash content of some of the studied products was excessively high. So, evidencing, once again, the variability of data

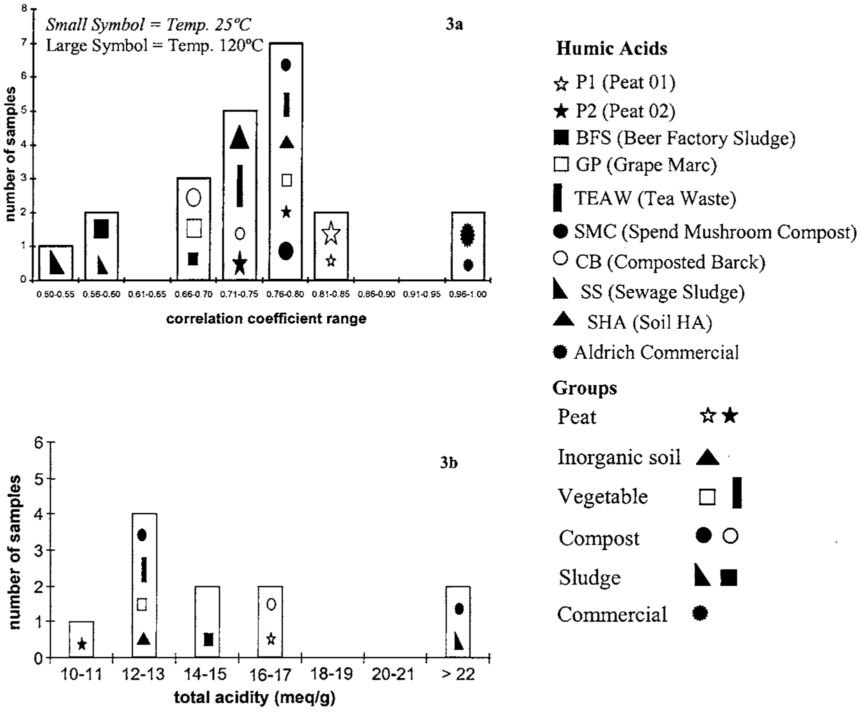

Figure 3. Comparison between Turkish humic acids of different origin using: (a) correlation coefficient values found by comparison of FTIR spectra with a commercial HA sample and (b) total acidity.

concerning the HA and problems found for a simple characterization of these substances using a single criterion.

On the other hand, the regression between the library correlation coefficient which the acidity and ash percentage provided regressions values of 0.29 and 0.24 respectively, thus indicating the absence of a close relation ship between these independent characteristics.

CONCLUSION

From the studies carried out, it can be identified some differences between humic acids of different origin, but being impossible to establish a clear characterization from a single criterion. However, the evidence of big differences in the fingerprinting region of FTIR spectra of HA samples, as a function of their origin, open interesting possibilities for using this information in HA identification.

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