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SPECTRAL CHARACTERISTICS OF HUMIC ACIDS ISOLATED FROM SOUTH MORAVIAN LIGNITE AND SOILS

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Abstract

Humic acids (HA) isolated from lignite and five typical soil types in the south Moravia region were characterized by elemental analysis, FTIR, SFS and ^{13}C NMR spectral methods. Obtained results showed the highest aromaticity degree and mature of lignite HA and Modal Chernozem HA. On the basis mainly on ^{13}C NMR spectra, Cambisol HA were substantial younger and contained significant more aliphatic compounds in comparison to Modal Chernozem and Lignite HA. FTIR spectra divided isolated HA into two groups. The first group included Lignite HA, Modal Chernozem, Haplic Luvisols and Gleyic Stagnosol HA. The second group included Fluvi-Eutric Gleysol and Eutric Cambisol HA. All samples had the same main fluorophore peak at $\lambda_{exc.}/\lambda_{em.}$=468/488 nm. Correlation between RFI indexes and fractional composition of HS was found.

Key words: humic substances, lignite; FTIR;SFS ; ^{13}C NMR spectra

1. Introduction

Unlike most naturally occurring compound, humic substances (HS) are not defined in terms of their chemical composition or functional groups [1]. Instead they are classified into three major groups according to their solubility. That means: humic acids (HA), fulvic acids (FA) and humins. The fractions should not be considered distinct or discrete compounds since each can be further purified to reduce heterogeneity. HS differ in molecular weight, elemental compositoon, acidity, and cation exchange capacity. Fulvic acids are typically composed of a variety of phenolic and benzene carboxylic acids that are held together by hydrogen bonds to form stable polymeric structures [2]. These are associated with polysaccharides that are easily separated by adsorption on charcoal or gel chromatography. The low molecular weight FA has higher oxygen but lower carbon content than HA. There are also more acidic functional groups, particularly COOH in FA molecule. The HA fraction consist of hydroxyphenols, hydroxybenzoic acids, and others aromatic structures with linked peptides, amino compounds, and fatty acids. Soil humins are considered to be no extractable humic type polymers that form strong associations with minerals [2]. Assessment of the best analytical method for complete HS characterization is still being discussed. A difficulty with HS chemical extraction is that they are tedious and labor intensive and not suitable for large numbers of samples. New approaches of spectrometry that include a wide variety of techniques (FTIR, SFS and ^{13}C-NMR) have been successfully applied to the study of HS chemical composition and structure.

FTIR spectroscopy offered insight into HA structural components and identified a variety of infrared bands characterized different molecular structures and functional groups in their molecule.

According to Celi [3], Stuart [4], and Klouda [5] many different transmission methods for obtaining infrared spectra were proposed for example Diffuse Reflectance (DRIFT), Single Reflection Attenuated Total Reflectance (SRATR), Horizontal Attenuated Total Reflectance (HATR) and others [4, 5].

HA performed aromatic mixture with luminescent properties of different fluorophore groups. Synchronous fluorescence spectra (SFS) performed the high resolution of spectral peaks. Fluorescence emission spectra feature a unique broad band with the maximum positioned at the long wavelengths. SFS spectra are capable to characterized content of condensed aromatic ring systems and bear electron-withdrawing substituents, such as carboxyl and carbonyl groups [6,7]. Miano and Senesi [7] also reported that the most efficient fluorophores are indicated to be variously substituted, condensed aromatic rings, and /or highly unsaturated aliphatic chains. Fluorescence of HA is dependent on their origin, molecular weight, concentration, pH, ionic strength, temperature and redox potential. There are following fluorescent methods: excitation scan spectra, emission scan spectra, excitation emission matrix (EEM) = three dimensional excitation and emission scan, synchronous fluorescence spectroscopy (SFS) or synchronous-scan excitation fluorescence spectra or synchronous-scan excitation fluorescence spectra. So in recent years, fluorescence spectroscopy has become widely recognize as a relatively simple, sensitive, and useful technique for HA characterization [6,8,9]. Peuravuori et al. divided fluorescence spectrum into several regions according to certain wavelengths and assumed that certain polycyclic contributors are responsible for humic fluorescence properties [10].

^{13}C - Nuclear Magnetic Resonance (NMR) is a powerful and diverse tool for the elucidation of organic compounds and mixtures structure. ^{13}C-NMR spectrum provides specific information on the chemical structures involving ^{13}C atoms within a molecule. The carbon skeleton of HS is observed rather than the adjacent protons, allowing the functional groups to be detected. Carbon nuclei are spread over a wide range of chemical shifts that effectively separate signals even when carbons have only small differences in diverse structural environments [10]. Carbon structures are determined in relative terms from the chemical shifts that occur when energy is absorbed by a molecular spinning in a magnetic field. Individual carbon types in molecule indicate structure, sorption capacity, binding properties and solution interactions of HS [8,10,11]. The most important step for obtaining good quality ^{13}C-NMR spectra is perhaps sample preparation because paramagnetic species induce fast relaxation of nuclei in close proximity [12]. However, NMR analysis is not always accessible because it is very complex and expensive technique.

It is known that HA isolated from lignite showed typical bands known from other HA soil samples due to aromatic and various C-O structures [13,14]. Therefore lignite represents a valuable organic substrate with mineral inclusion situated on the transformation route from phytomass to a dehydrated, dehydrogenated and deoxidised carbon type complex and water. The one of most attractive way of non - energetic exploitation of lignite is their use as a HS source. Therefore our work was focused on comparison of chemical and spectral properties of lignite and soils HA. Knowing their chemical composition and structure could help us to assess their impact on the environment.

2. Experimental

Samples were taken from south Moravian lignite (locality Mikulčice) and from the topsoil of five typical soil types - Modal Chernozem (locality Bratčice), Haplic Luvisol (locality V. Knínice), Eutric Cambisol (locality Vatín), Gleyic Stagnosol (locality Kameničky), Fluvi - Eutric Gleysol (locality Žabčice).

Humic acids from lignite were isolated from the South Moravian lignite (mine Mikulčice, Czech republic) and purified. HA were isolated following procedures motivated by the Czech standard on determination of HS in coal [14,15] and also the well - known procedures [13,16]. Original material was shaken for 24 hours under nitrogen atmosphere in 0.5 M-NaOH and 0.1 M-$Na_4P_2O_7$ (60 g lignite: 2000 ml of extraction agents) in plastic flasks overnight. Humic acids were precipitated from alkaline extract by adding 6M HCl until pH 2 and treated with a 0.5% (v/v) HCl-HF solution for 24 hours, dialyzed (Spectrapore 3, 3500 Mw cutoff) against distilled water until chloride free and freeze-dried. [17]

Isolation of soil HA was made according to the standard international IHSS method [18,19]. 100g of air-dried soil sample, sieved at mesh size of 1mm, washed by 10 % HCl and stirred for 1-2 hours (decalcination process). After negative reaction for CO_2 (detected by seeing no CO_2), the soil rest washed by 0.05 M HCl. After negative reaction for Ca^{2+} (detected by ammonium oxalate), the soil rest washed by distilled water. After negative reaction for Cl^- (detected by $AgNO_3$), the soil rest was shaken in a 0.1 M NaOH for 7 – 8 hours. We allowed it to precipitate over night and than centrifuge 15 minutes at 5000rpm. Elution with 0.1M NaOH and centrifugation we followed two times and mixed supernatant solutions. Dark-brown solution of HS is precipitated by concentrated HCl to pH=1. The

coagulated HA were decanted, washed several times, extensively purified by 0.5% mixture HCl+HF and dialyzed against distilled water until chloride-free, and freeze-dried.

TOC in selected soils was determined by wet digestion according to [20]. Fractional composition of HS was determined according [18,19]. 5g of air dried soil sample, sieved at mesh size of 1mm and extracted by a mixture (1:1, 0.1M NaOH + 0.1M $Na_4P_2O_7$) for 24h. The sediment was separated by centrifugation at 2800g for 10min, washed with mixture and centrifuge again. Two individual washings were unified with original supernatant, acidified with concentrated H_2SO_4 to pH 1.5. We allowed to precipitate HA overnight. Sum of HS, HA and FA were determined by titrimetric method in aliquot volumes.

Elementary analysis of HA preparations was kindly made in Institute of Rock Structure and Mechanics of the ASCR in Prague. Standard methods of Carlo Erba and elementary CHNS/O analyser - Thermo Finnigan were used.

FTIR spectra were kindly measured in the Zoll Laboratory, Bratislava, Slovak Republic using spectrometer Shimadzu FTIR – 8700, within the range 4000 – 600 cm^{-1}. Standard methods using KBr + HA pellets, HATR and SRATR methods according to [3] were applied.

SFS scan spectra were measured (after filtration and appropriate dilution) within the range 300–600 nm using spectrofluorimeter Aminco Bowman Series 2 (Thermospectronics, Xe-lamp, scan sensitivity 60%, autorange 845 V, bandpass of both monochromators 4nm, relative fluorescence intensity 0-9.99, 2D scan mode, temperature 20°C and the constant difference was ($\Delta\lambda_{em.} - \lambda_{ex.}$) = 20 nm between both excitation and emission monochromators). SFS spectral lines were measured in mixture 0.1M pyrophosphate sodium solution and 0.1M NaOH.

^{13}C Nuclear Magnetic Resonanace (NMR) was carried out on spectrometer Varian INOVA 600 (frequency 150,830 MHz). For NMR experiments 100 mg of isolated HA samples were dissolved in 2.5 ml of 0.5 M NaOH in deuterated water. After 24 hour of intensive stirring 0.5 ml of HA sample was put in 5 mm NMR cell. All ^{13}C - NMR experiments were run at 23°C on a Varian Unity-Inova 600 MHz spectrometer using basic one-pulse experiment with the following set of the acquisition parameters: spectrometer frequency 242.803 MHz; relaxation delay 1s, acquisition time 1.6s; excitation pulse flip angle 45°, spectral width 50000 Hz and a continuous broadband decoupling of the protons. Prior Fourier transformation accumulated data were fitted with exponential function (line broadening 10 Hz). Subdivision of the spectrum was made by the commonly used scheme on Malcolm [21].). Aromatic carbon (C_{ar} %) is represented in the δ 106-157 ppm spectral region. Aliphatic carbon (C_{aliph} %) is represented in the δ 15-106 ppm spectral region. The degree of aromaticity of HA (α) was calculated by the procedure of Hatcher et al. [22].

3. Results and discussions

In this paper comparison of comprehensive changes of soil and lignite HA in south Moravian region were evaluated. By application of HS fractionation in selected soil types we obtained HS sum, HA and FA content. HS contant was decreasing in order: Gleyic Stagnosol > Fluvi-Eutric Gleysol > Modal Chernozem> Haplic Luvisol >Eutric–Cambisol. HS quality given by HA/FA ratio was decreasing in order: Modal Chernozem > Haplic Luvisol > Gleyic Stagnosol > Fluvi-Eutric Gleysol >Eutric–Cambisol (Table 1).

HA isolated from lignite and soils were characterized by elemental composition. Obtained results in atomic % are listed in Table 2. The carbon content of HA ranges from 44.12 % to 34.5 %, hydrogen and nitrogen contents range from 42.4 % to 33.73 % and from 7.6 % to 2.45 % respectively. The HA carbon content was decreasing in order: Elliot 1S102H standard HA > Fluvi-Eutric Gleysol > lignite HA > Modal Chernozem > Eutric-Cambisol > Gleyic Stagnosol > Haplic Luvisol. Hydrogen content was decreasing in order: Haplic Luvisol > Gleyic Stagnosol > Eutric–Cambisol > lignite HA > Modal Chernozem > Fluvi-Eutric Gleysol > Elliot 1S102H standard. Nitrogen content was the highest in lignite HA (7.6 %) and the lowest in Eutric Cambisol HA (2.45 %).

FTIR spectroscopy showed that isolated HA could be divided into two groups. The first group included Lignite HA, Modal Chernozem, Haplic Luvisol and Gleyic Stagnosol humic acids (Fig.1, 2, 3). Their absorbance was due to: (a) aliphatic C-H stretching at 2924 - 2922 and 2855 cm^{-1}; (b) aromatic C=C groups at 1624 - 19 cm^{-1}; (c) phenols at 1404 - 1419 cm^{-1}; (d) carbonyl and carboxyl groups at 1719 - 1718 and 1225 - 23 cm^{-1} (Fig. 2). HA prepared of lignite had mainly higher intensity of composed bands in 1000 - 1200 cm^{-1} (C-O stretch of aliphatic OH, -C-O stretch and OH deformation of -COOH, C-O stretch of polysaccharides). The former is attributable to new C-O stretch vibration of aliphatic alcoholic groups, polysaccharides and various ether groups. We can conclude that lignite HA displayed the highest values of COOH groups.

Table 1. Fractional composition of humic substances in selected soil types (TOC-total organic carbon, HS–humic substances, HA–humic acids, FA-fulvic acids, in numerator: mg/kg , in determinator: % from TOC, RFI-relative fluorescence index)

Soil Types	TOC	HS	HA	FA	HA/FA	RFI
	(%)	(mg/kg)	(mg/kg)	(mg/kg)		
Modal Chernozem	1,8	5,0	4,0	1,0	4	1,20
		27,8	22,2	5,5		
Gleyic Stagnosol	3,65	16,5	10	6,5	1,5	1,21
		45,2	27,4	17,8		
Haplic Luvisol	2,1	5,0	3,0	2,0	1,5	1,43
		23,8	14,3	9,5		
Eutric -Cambisol	1,6	1,0	0,4	0,6	0,7	1,17
		62,5	25	37,5		
Fluvi-Eutric Gleysol	1,4	0,9	0,5	0,4	1,25	1,22
		64,3	35,7	28,6		

Table 2. Elementary composition (atomic %) of HA isolated from lignite and soils

Samples	%C[a]	%H[a]	%N[a]	%O[a]
HA - Modal Chernozem	37,9	39	2,9	20
HA - Gleyic Stagnosol	36	42,4	3	18,4
HA - Haplic Luvisol	34,5	43	2,7	19,7
HA - Eutric Cambisol	36,1	42,4	2,45	19,42
HA -Fluvi-Eutric Gleysol	40,6	34	3	22,34
HA Elliot standard 1S02H	44,12	33,73	2,7	19,42
HA - Lignite	38,8	40,05	7,6	14,55

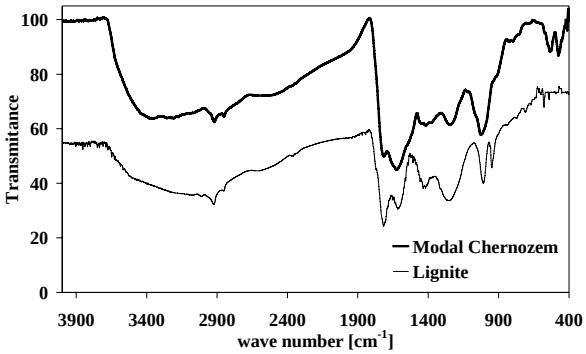

Figure 1. FTIR spectra of HA isolated from Modal Chernozem and Lignite HA

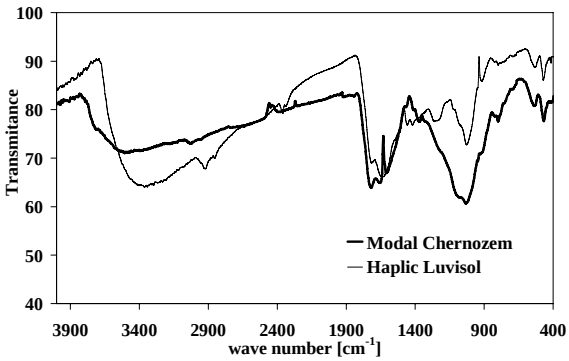

Fig.2 FTIR spectra of HA isolated from Modal Chernozem and Haplic Luvisol

The second group included the Eutric-Cambisol and Fluvi-Eutric Gleysol HA (Fig. 3, 4) with absorbance due to: (a) C-H bands at 2925-2850 cm^{-1} in CH_3 and CH_2 groups of aliphatics; (b) C=O band would be very limited, as suggested by the faint shoulder at 1718-16 cm^{-1}; (c) carboxyl and amide-related ate bands at 1655-54 cm^{-1}; (d) polysaccharide chains at 1045-34 cm^{-1}; (e) O-H and C-O band of various ether and alcoholic groups at 1127-23 cm^{-1} and (f) SO_3 H band at 1100 cm^{-1} (Fig. 3, 4). HA coming from the second group showed less aromatic C=C groups. Composed band in 1000- 1200 cm^{-1} of C-O stretch of aliphatic OH, -C-O stretch and OH deformation of –COOH.

When we compared these results with fractional composition (Table 1) and literature data [23,24] we came to the conclusion that HA isolated from Eutric-Cambisol and Fluvi-Eutric Gleysol were younger and contained more aliphatic and less aromatic compounds to compare with Modal Chernozem, Haplic Luvisol and Gleyic Stagnosol humic acids.

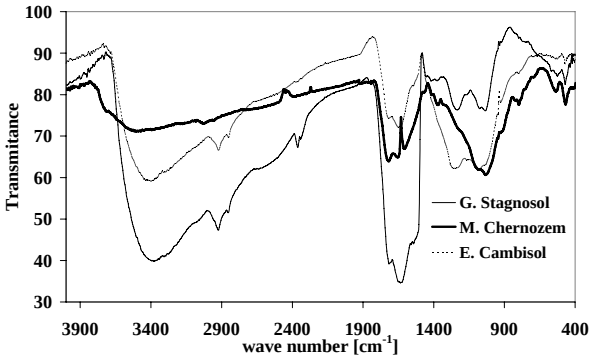

Fig.3 FTIR spectra of HA isolated from Gleyic Stagnosol, Modal Chernozem and Eutric Cambisol

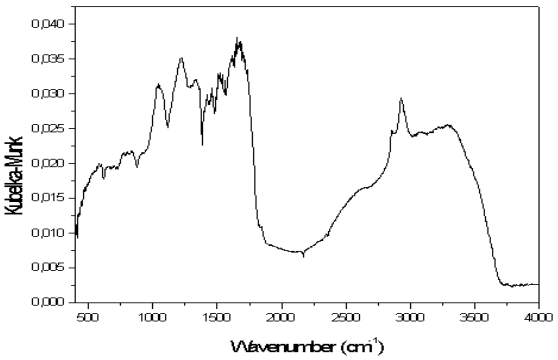

Figure 4. FTIR spectra of HA isolated from Fluvi-Eutric Gleysol

SFS scan spectra of soil samples are given in the Fig. 5. Maximum relative fluorescence intensity gave Gleyic Stagnosol. The lowest fluorescence intensity gave Eutric Cambisol and Fluvi-Eutric Gleysol. All samples exhibited the presence of five main spectral peaks at $\lambda_{ex.}/\ \lambda_{em.}$: 467/487, 481/501, 492/512, 450/470, 339/359 at constant difference of $\Delta\lambda$=20 nm except Lignite HA at 492/512 nm. Spectral behavior (shape of curve) depends on fractional composition of humus (FA and HA content). Gleyic Stagnosol was very specific because contained the highest amount of HA and FA (Table 1). Higher FA content influenced emission peaks at 359 nm and 419 nm that indicated simply phenolic compounds. In generally the high FA content corresponded with higher relative fluorescence intensity at 359 nm (Fig. 6). On the other hand Modal Chernozem and Haplic Luvisol lower FA content corresponded with lower relative fluorescence intensity at 359 nm. Secondary peak at 501 nm for Gleyic Stagnosol was higher than the peak at 487 nm due to polyaromatic moieties presence. Relative fluorescence indexes (RFI) at wavelengths 465/487 were calculated and correlation between RFI and fractional composition of HS was found (Fig. 6)

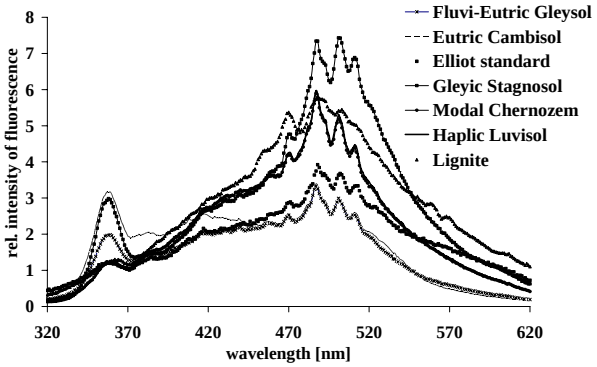

Fig.5 Synchronous fluorescence spectra of HS originated from different sources

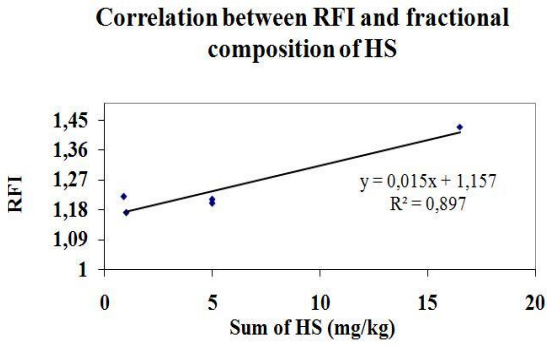

Fig.6 Correlation between RFI indexes (I501/ I478) and fractional composition of HS

^{13}C - NMR spectra showed us structural composition of studied HA. Different groups binding in HA molecule and integral areas are listed in Table 3. The types of carbon in HA molecule are presented in Table 3, 4. The chemical shift is expressed as parts per million (ppm). The intensity of the signal detected and the spectral quality of that signal (signal: noise ratio) are dependent upon the amount of ^{13}C present in the sample and the concentrations. The highest amount of aromatic carbon in Modal Chernozem HA was found (Table 5). Similar concentration of C_{ar} is characterized also for Haplic Luvisol and Lignite HA. The smallest C_{ar} in Eutric Cambisol HA was found. Also the highest degree of aromaticity α in Modal Chernozem and the smallest in Eutric Cambisol HA was determined. Opposite situation was determined for aliphatic moieties. As it can be seen in Table 5, the highest concentration of C_{aliph} in Eutric Cambisol HA was determined. Substantial lower amount of this parameter in Modal Chernozem, Lignite HA and Haplic Luvisol HA was detected. Higher differences in concentration of sp^3 C among HA samples were detected (Table 5). Eutric Cambisol HA showed the highest and Modal Chernozem HA has the lowest concentration of this carbon type. These findings were in agreement with FTIR spectroscopy and fractional composition results. The last confirmed that

HA isolated from Eutric Cambisol were younger and contained more aliphatic and less aromatic compounds. Higher concentration of aromatic carbon moieties was characteristic for HA isolated from Lignite, Modal Chernozem and Haplic Luvisol. Obtained results corresponded with our previous work [25].

Table 3. Integral areas and carbon types for the 13C-NMR spectra

No. of area	Spectral	Types of carbon
1	230 - 184	Carbonyl in keto- and aldehyde
2	184 - 157	Carboxyl in acids or esthers
3	157 - 143	Aromatic C-O
4	143 - 106	Aromatic and olephinic, C-C, C-H
5	106 - 87	Anomers
6	87 - 43	sp3 carbon, C-O, C-N
7	43 - 15	sp3 carbon, C-C

Table 4. Values of relative integral intensities (% of total area) for the ^{13}C-NMR spectra in selected HA samples

Samples / area (ppm)	1	2	3	4	5	6	7
HA - Modal Chernozem	4,45	11,71	6,90	31,74	10,77	21,28	13,20
HA – Lignite	7,06	11,60	8,34	27,55	7,58	14,18	23,70
HA – Haplic Luvisol	3,41	14,99	5,43	31,05	4,18	14,16	26,78
HA Eutric Cambisol	2,47	13,91	4,8	26,1	4,68	21,54	26,5

Table 5. Selected parameters calculated from ^{13}C NMR spectra (%)

Sample/parameters	C_{ar} (157-106ppm)	C_{aliph} (106-15ppm)	sp^3 C (87-15ppm)	α
HA - Modal Chernozem	38,64	45,25	34,48	46
HA - Lignite	35,89	45,46	37,88	47
HA - Haplic Luvisol	36,48	45,12	40,34	44,7
HA - Eutric Cambisol	30,9	52,72	48,04	36,6

4. Conclusions

Significant differences were observed in the spectral properties of different origin HA. FTIR, ^{13}C - NMR and SFS spectra divided isolated HA into two groups according to condensed aromatic ring systems. The first group included Lignite HA, Modal Chernozem, Haplic Luvisol and Gleyic Stagnosol HA. The second group included Fluvi - Eutric Gleysol, and Eutric Cambisol HA. We have found out that HA in the first group had the more ancient origin and consisted of more aromatic groups. Eutric Cambisol and Fluvi-Eutric Gleysol HA reflected less aromatic compounds and more aliphatic structures in their molecule. Presence of FA was also determined by SFS method.

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