13C NMR and Mass Spectrometry of Soil Organic Matter

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Abstract: Liquid state, high resolution 13C NMR spectroscopy and mass spectrometry were used to study the composition and structure of soil organic matter (SOM) using soil extracts from two long-term experiments at the Rothamsted Experimental Station. Both one- and two-dimensional NMR techniques were applied. 13C NMR sub-spectra of the CH\textsubscript{n} (n = 0…3) groups, obtained by the Distortionless Enhancement by Polarisation Transfer (DEPT) technique, were used for the elucidation of the qualitative and quantitative composition of humic and fulvic acids in the soils. The chemical structure of SOM was further analysed at the molecular level through Fast Atom Bombardment Mass Spectrometry (FABMS) and Gas Chromatography-Mass Spectrometry (GC/MS). Humic and fulvic extract results were not only compared to each other, but also to the solid state 13C NMR results for the complete soil sample.

Keywords: soil organic matter, 13C NMR, DEPT, FABMS, GC/MS, Rothamsted soils

1 Introduction

High resolution Fourier transform NMR spectroscopy is a relatively new technique in the field of soil science. The complex nature of soil organic matter (SOM) makes the investigation of soils and soil extracts by NMR spectroscopy difficult [1]. The application of the NMR spectroscopy in the studies of soils and soil organic matter for both solid...
samples and liquid extracts has been discussed by selected authors including Randall et al. [1] and Preston [2]. $^{13}$C NMR spectroscopy is useful among the molecular spectroscopic methods available for SOM characterisation. It identifies partial structures of the SOM chemical components. Additional structural information for liquid extracts is obtained by the application of the NMR techniques of Distortionless Enhancement by Polarisation Transfer (DEPT), SEMUT-90 (sub-spectral editing using a multiple quantum trap) [3] and two-dimensional (2D) NMR experiments [1]. DEPT, SEMUT-90 and 2D J-resolved spectroscopy have been used previously to study aquatic humic substances [4].

Unfortunately, polarisation transfer and 2D NMR techniques have had limited application in the characterisation of SOM. The spectra obtained to date have been at relatively low magnetic fields ranging up to 9 T [4, 5, 6, 7], allowing low dispersion.

Molecular structure for SOM can be elucidated by mass spectrometric studies using analytic techniques developed for samples at low vapour pressure and thermal stability, or high molecular weight, such as biological macromolecules, biopolymers, oligosaccharides, drugs, carbohydrates, etc. Separation techniques and mass spectrometric techniques, such as gas chromatography or liquid chromatography may be used to analyse complex mixtures.

Pyrolysis-mass spectrometry, a method pioneered by Schulten [8], was developed and widely applied [9, 10, 11, 12] for soil characterisation. This method pyrolyses samples directly under a vacuum in the ion source of the mass spectrometer at temperatures ranging from $50^\circ$ C to $750^\circ$ C. Thermal decomposition products are then identified by soft ionisation mass spectrometry [11]. Another example of how Pyrolysis-gas chromatography/mass spectrometry is used is the chemical characterisation of the organic matter in soils [13, 14, 15].

The following report results from a long-term study conducted at the Rothamsted Experimental Station, UK, on the composition and structure of SOM by a combination of high-field $^{13}$C NMR spectroscopy (at magnetic fields of 14.1 T and 9.1 T), Fast Atom Bombardment Mass Spectrometry (FABMS), and Gas Chromatography-Mass Spectrometry (GC/MS).

The qualitative and quantitative compositions of humic and fulvic acids in the above-mentioned soils were measured using $^{13}$C NMR, DEPT and 2D $^1$H-$^{13}$C heteronuclear correlation experiments (HMQC, Heteronuclear Multiple Quantum Correlation spectroscopy).

FABMS and GC/MS experiments yielded further information on the chemical structures of SOM subunits at the molecular level. These techniques are advantageous in that they avoid the preliminary thermal decomposition of the samples. FABMS is a particularly suitable technique for polar or labile samples, yielding ionised molecular species for molecular weights up to $\sim 20$ kDa. Since FABMS has been less successful with non-polar samples, gas chromatography-mass spectrometry was employed to analyse the humic acids of the soils without a preliminary thermal decomposition.
2 Materials and Methods

2.1 Soils and Sample Preparation

Two soils, High-field Grass (H) and Geescroft Field (G) were sampled from a long-term field experiment (more than 135 years old) conducted at the Rothamsted Experimental Station, (51° 48’ 03” N, 0° 21’ 10” W). Sample H was taken in 1981 from a mature grassland area which had received nitrogen fertiliser over a period of 135 years. It was taken at a depth of 10 cm showing a pH of 6.12 and a carbon content of 4.7%.

Sample G was taken in 1991 from a plot of agricultural land which had not received manure or carbonate treatment since 1881. Sample G was taken at a depth of 15 cm indicating a pH of 5.7 and a carbon content of 0.9%.

The humic substances of H and G were extracted with 0.5 M NaOH according to the procedure described in the literature [16]. The humic (HH, HG) and fulvic (FH, FG) acids were separated by acidification (6 M HCl, pH < 1) of the alkaline soil extracts. They were isolated and purified by the method described above [17].

2.2 NMR Spectroscopy

The liquid state NMR spectra were recorded on Bruker AMX-600 and Bruker 400 spectrometers, operating at 150.9 and 100.6 MHz for $^{13}$C respectively. The solutions were obtained by dissolving 50 - 100 mg (according to solubility) of the sample in 0.5 ml 0.5 M NaOD/ D$_2$O under nitrogen. The solutions were then centrifuged prior to the NMR experiments on a Europe 24M centrifuge at 18 000 c/s for 20 minutes. A sample temperature of 37° C was used, and chemical shifts were referenced to internal TSP (sodiumtrimethylsilylpropionate).

The $^{13}$C NMR spectra were recorded using the inverse-gated decoupling technique, with the decoupler off except during the acquisition time, in order to obtain $^{13}$C spectra decoupled from the protons but without Nuclear Overhauser Enhancement (NOE). The spectroscopic parameters were as follows: a pulse width of 6 μsec; an acquisition time of 0.8 s; and a relaxation delay of 2.0 s. More than 30,000 scans were normally accumulated and a 50 Hz line-broadening applied.

The DEPT ($\theta_{45^0}$, $\theta_{90^0}$ and $\theta_{135^0}$) spectra of the humic (HH and HG) and fulvic (FH and FG) acids were obtained under the same conditions as the $^{13}$C NMR spectra, with the use of $\tau = (2^{1/2}J_{CH})^{-1} = 3.45$ μs, (whereby $t$ is the delay between the pulses). A line broadening of 50 Hz and more than 10,000 scans were accumulated. The sub-spectra of CH, CH$_2$, CH$_3$ and C-quaternary carbons were obtained by addition and subtraction of the DEPT spectra, according to the expressions 1 - 4 below.

\[
\text{C-quaternary} = ^{13}\text{C NMR-DEPT}\Theta_{45^0} \\
CH = \text{DEPT}\Theta_{90^0} \\
CH_2 = \text{DEPT}\Theta_{45^0} - \text{DEPT}\Theta_{135^0}
\]
\[ CH_3 = (\text{DEPT}\Theta_{45^\circ} + \text{DEPT}\Theta_{135^\circ}) - \text{DEPT}\Theta_{90^\circ} \] (4)

The 2D $^1$H-$^{13}$C heteronuclear correlation spectra (HMQC) were obtained using the standard Bruker software, with a spectral width of ca 30,000 Hz for $^{13}$C and ca 7,000 Hz for $^1$H, a relaxation delay of 2 s, 128 increments, and an FT size of 1K 512.

2.3 Mass Spectrometry

For the FABMS experiments, a CRATOS MS890 mass spectrometer was used. The samples were run with xenon (Xe) FAB from a thioglycerol matrix. Gas chromatography/mass spectrometry was used for the humic acids. For the gas chromatographic separation, a GC Hewlett Packard 5890 gas chromatograph equipped with a SGE 25QC2/ BPI - 0.25 column (25m 0.22mm 0.25m. Bonded 100% Me Silicone) was employed. The starting temperature for the GC separation was 60$^\circ$C and the final temperature was 300$^\circ$C, with heating rates of 8$^\circ$C per min. The mass spectra were then produced by a Jeol JMS-AX 505W spectrometer, using the Electron Impact (EI) mode.

The analysis of FABMS and GC/MS spectra was done using the library-search program of the mass-spectral service at King’s College, University of London. The data from "Eight peak index of mass spectra"-MSDS, was compared with mass spectral data from the literature [8, 9, 10, 11] and with structural information obtained from the NMR spectroscopy investigations from The Institute of Organic Chemistry, Bulgarian Academy of Sciences.

3 Results and Discussion

The extraction procedures applied to soils are may change slightly the nature of the soil organic matter. Nonetheless, they offer possibilities for more detailed structural elucidation of these complex mixtures compared with solid state NMR on untreated soils (‘complete’ soils). Solid state NMR spectroscopy has been applied to a wide range of soil samples; however, there are associated problems of quantification and loss of structural details [1, 2, 18, 19, 20, 21]. Although extraction procedures are invasive, they are advantageous in that some of the inorganic matter is removed reducing the paramagnetism of the samples. The percentage of SOM in the samples is increased. The soil-extracts may be investigated by solution-state NMR spectroscopy, which gives better resolution of the spectra, especially with the use of high field NMR spectrometers and also one- and two-dimensional techniques [3, 4, 17]. Both humic (HH, HG) and fulvic (FH, FG) acids were isolated and investigated separately to obtain more information about the composition and chemical structure of the SOM in sample soils H and G. The $^{13}$C NMR spectra of humic and fulvic acids demonstrated many overlapping resonances which were difficult to resolve even at high field (100.6 MHz for 13C). The use of DEPT pulse techniques produced a completely edited set of DEPT sub-spectra which were used for a quantitative evaluation [3, 4, 22].
3.1 $^{13}$C Solution State NMR Spectra

The spectral assignments were based on the 1D $^{13}$C NMR, DEPT, the sub-spectra for CH$_3$, CH$_2$, CH and C-quaternary (Fig. 1, 2) carbons, and the 2D $^1$H-$^{13}$C heteronuclear correlation spectra (Fig. 3).

![Fig. 1 150.9 MHz $^{13}$C NMR and DEPT spectra showing CH$_3$, CH$_2$, CH and C-quat. carbons in humic acids of soil High-field Grass](image)

The results are presented in Table 1. The quantitative distribution (in %) of CH$_3$, CH$_2$, CH and C-quaternary carbons was calculated on the basis of the integral intensity of the spectral regions in the DEPT spectra and sub-spectra [3, 4, 22]. The sum total of the quantitative distribution (in %) of CH$_n$ (n = 0, 1, 2 and 3) for every one spectral area was compared to those obtained from the $^{13}$C NMR spectra recorded using the inverse-gated decoupling technique. The data are presented in Tables 2 and 3.

From the data presented in Table 1, the difference in the composition and chemical structure of the humic and fulvic acids is obvious (Fig. 1, 2). Compared to the fulvic acids FH and FG, the $^{13}$C NMR spectra of humic acids are more complicated. The difference is the domination of aromatic and aliphatic structures in the humic acids, whereas carbohydrate structures prevail in the fulvic acids. The spectral regions at 105 - 145 ppm and 145 - 160 ppm in the $^{13}$C NMR spectra of humic acids were assigned...
to alkyl and O-substituted aromatic carbons, respectively. The strong peaks at 118.1 and 128.6 ppm are characteristic for the ortho- and meta-aromatic carbons in phenols, and those at 50.0 and 55 - 60 ppm for the carbons of methoxy and ethoxy groups, and can be easy identified in the 2D 1H/ 13C correlated spectrum (cross peaks at 118.1/ 6.5 ppm, 128.6/ 7.4 ppm, 50.0/ 3.4 ppm and 55 - 60/ 3.6-4.0 ppm) (Fig. 3). The resonance signals at 45 - 65, 65 - 80 and 80 - 90 ppm are assigned to the carbons of the aliphatic chains in lignin-like structures (18). The peaks at 47-60 ppm in the CH-sub-spectra of HH (Fig. 1), probably originate from the methine carbons of the propane side chains in lignins. The presence of these resonances shows the lignin-like nature of the humic acids.

In the 13C NMR spectra of the fulvic acids FH and FG, the resonance peaks of 95 - 108 ppm were assigned to anomeric carbons, those of 65 - 80 ppm to C-2 - C-5 carbons, and of 63 - 70 ppm to C-6 in sugar units from oligo- and polysaccharides. The assignment of DEPT spectra and sub-spectra of CH, CH2 carbons confirm the presence of carbohydrate structures in the fulvic acids. The resonance peaks of 170 - 190 ppm, as well as those in the aliphatic region, show the presence of carboxylic acids or esters. The very weak peaks of 40 - 45 ppm and 55 - 60 ppm were assigned to carbons in CH2NH2 and CHNH2 functional groups in amino acids. From the sub-spectra of the methyl carbons...
the amount of the CH$_3$O- groups (59-64 ppm) was determined.

3.2 MASS Spectrometry

The proposed chemical structures involved in the SOM of humic and fulvic acids, identified on a basis of FABMS, are shown in Tables 4 and 5, respectively. Signals with the same m/z ratio have been found in the FABMS spectra of the fulvic acids FG and FH, but with a difference in the relative intensities. The intensities were normalised with respect to the signal at m/z 80 for fulvic acids (FH and FG) and that at m/z 130 for humic acids. It was observed in the FABMS experiment that the signal intensity at m/z 133 (M+1) increased with time by one at m/z 195 (M+1). This could be the result of decomposition of glucoronic acid to glutaric acid or of the decomposition of carbohydrate structures. A substantial difference in the FABMS spectra of the humic acids HG and HH was observed, resulting from the presence of high molecular hydrocarbons or alcohols in HG (Table 5).

HG and HH were also investigated by gas-chromatography/mass spectrometry (GC/MS). The gas-chromatographic separation for some of the mixture components was poor, especially for those at lower retention times (RT = 3.5 - 28 min); so their identifica-
tion was difficult. The gas-chromatograms of the humic acids HH and HG are presented in Figure 4.

![Gas-chromatograms of the humic acids: a. HH; b. HG](image)

The fraction compositions were estimated by Electron Impact Mass spectrometry (EIMS). The main EI-signals (m/z) and proposed associated structures are presented in Table 6. The signals due to monomeric units of lignin-like structures (m/z 124, 126, 140, 154, 168, 182, 196, 210) were found at RT 8.4, 10.6, 11.7, 17.2 min in the gas-chromatograms of HG and HH. Additional fractions in HG (at RT = 20.6, 23.3, 25.9, 28.4, 30.9, 32.9, 39.9, 44.0, 46.0 min) were observed. The analysis of EIMS spectra has shown the presence of high-molecular weight aliphatic fractions. The compositions of the humic acids HH and HG in the gas chromatograms are presented in Table 7.

### 3.3 Structural Elucidation of the Samples

The structural elucidation of the humic acids HH and HG was achieved not only on the basis of $^{13}$C NMR and FABMS but also from the data obtained from GC/MS by the use of Electron Impact Mass spectrometry (EIMS). The GC/EIMS experiments have been used because of the inadequate information obtained by the application of FABMS to the humic acids, which could be explained by the presence of non-polar structures in the humic acids. FABMS is particularly suitable for polar molecules but less successful with non-polar samples [23]. On the basis of the $^{13}$C NMR data of HH and HG, a high percentage for the aromatic and aliphatic carbons was found (Table. 2). Most of the aromatic residues in the humic acids HG were substituted, as shown by the high (16.7 %) amount of quaternary carbons in the aromatic spectral area (110 - 145 ppm). On the other hand a doubled amount for the methine carbons in the aromatic spectral area of HH was
found. The predominant presence of CH₂ groups and especially of methylene carbons in long-chain hydrocarbons (22.3 ppm) for HH was established. The low content of methyl, methine and quaternary carbons in the spectral region 5 - 40 ppm indicated the lower content of branched aliphatic chains in HH, in comparison to HG (Table 2). The higher percentage of carboxylic carbons in HG indicated the increased content of carboxylic acids. The analysis of GC/EIMS spectra has shown the supplementary presence of high-molecular weight aliphatic fractions and carboxylic acids (mass range m/z 324 - 450) in the HG sample which has not been found in HH (Table 6 and 7).

The ¹³C NMR data show that the percentage of O-substituted methine and methylene carbons is highest in the fulvic acids. An increased content of CH-OH(R), CH₃O and COOH(R) carbons in FG was observed (Table 3). This could be explained by the greater abundance of alcohols, glycols, amino and carboxylic acids and their derivatives. The high percentage of carboxylic carbons and the low percentage of methylene and methyl carbons, shows the presence of dicarboxylic acids, substituted in the chain. The carboxylic acids in the fulvic acid FG are more substituted in the chain, as shown by the higher percentage of methine and quaternary carbons in the aliphatic spectral region (Table. 3). In Table 4, the FABMS results are in general agreement with the NMR data although a higher relative intensity of the signals characteristic for alcohols (m/z 61, 62), proteins (m/z 131), carboxylic acids or their derivatives (m/z 286) was found.

Both ¹³C NMR and mass spectral data show that the main difference in the chemical composition of SOM of the soils High-field Grass and Geescroft Field is the presence of high-molecular aliphatic compounds (hydrocarbons, alcohols or carboxylic acids with long aliphatic chains) in the latter.

The results of this investigation may be compared with those obtained on the whole soils by solid state ¹³C NMR spectroscopy [24] by combining the results for the humic and fulvic components for each of the two soils. Agreement after normalisation is good except for two spectral areas: the 160 - 190 ppm (carbonyl) and 10 - 50 ppm (alkyl) regions. For these, the solid state NMR results are approximately two thirds lower. This may be attributed to complications in the CP MAS NMR experiments which were the lack of an enhancement for carbon atoms, which have no approximate protons.

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| Assignment as functional groups | Chem. shifts (ppm) of HH | Chem. shifts (ppm) of FH | Chem. shifts (ppm) of HG | Chem. shifts (ppm) of FG |
|--------------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| CH₃ in n-alkyl chain           | 13.4                    | -                       | 15.5                    | -                       |
| CH₃ in branched chain          | 17.0, 17.9              | 18.7, 19.6              | 17 - 26                 | 18 - 20                 |
| CH₃CO-, CH₃N-                  | 21.8                    | -                       | 28.0                    | 25 - 30                 |
| CH₃-CH₂                         | 22.0                    | -                       | 21.1                    | -                       |
| CH₂ in n-alkyl chain           | 29.1                    | -                       | 25.4 - 32.6             | 24.9                    |
| C-quat.                        | 29.3                    | 33.7, 39.6              | 33.1                    | 40.0                    |
| CH in alkyl chain              | 25.0, 29.4              | -                       | 24.1 - 45.5             | 30 - 40                 |
| CH₂ in branched chain          | 35 - 40                 | -                       | 30 - 40                 | 30 - 40                 |
| CH₂-N                           | 46.5, 47.7              | 40 - 45                 | 40.5                    | 45 - 50                 |
| CH-N                            | 55 - 60                 | 55 - 60                 | 50 - 65                 | 50 - 60                 |
| CH(CHOH)₂                      | 45 - 65                 | 50 - 60                 | 50 - 65                 | 50 - 60                 |
| CH₃O-                           | 55.5                    | 59 - 65                 | 59.1                    | 64.2                    |
| CH₂OH                           | 60.0, 60.5              | 63.9, 65.5              | 59.1                    | 63.3                    |
| CH₂OR                           | 68.5                    | 68.6, 71.4              | 64.1                    | 65.2                    |
| CHOH, CHOR                     | 65 - 90                 | 70 - 90                 | 65 - 90                 | 70 - 90                 |
| -O-CH-O-, -O-C-O-              | 95 - 105                | 90 - 115                | 95 - 110                | 95 - 105                |
| CH-aromatic, olefinic          | 105 - 140               | -                       | 105 - 140               | -                       |
| CH - o- to COH(R)              | 118.1                   | 18.1                    | 105 - 117               | -                       |
| CH - m- to COH(R)              | 128.6                   | -                       | 131.7                   | -                       |
| CH in olefinic chain           | 139.8                   | -                       | 138.0                   | -                       |
| C-quat. Ar, alkyl subst.       | 120 - 145               | -                       | 100 - 145               | -                       |
| C-quat. Ar with -OH(R)         | 145 - 160               | -                       | 150 - 165               | -                       |
| COOH(R)                        | 170 - 190               | 170 - 190               | 170 - 190               | 170 - 190               |

Table 1 Chemical shift assignment of the resonance peaks in $^{13}$C NMR spectra of humic and fulvic acids of High-field Grass (H) and Geescroft Field (G) soils.
| Spectral area (ppm) | $^{13}$C NMR (%) | C-quat. (%) | CH (%) | CH$_2$ (%) | CH$_3$ (%) | $\sum$ CH$_n$
|-------------------|--|--|--|--|--|--|
| 10 - 50           | 25.0 | 25.7 | 0.5 | --- | 0.8 | 9.7 | 22.3 | 13.4 | 1.7 | 2.5 | 25.3 | 25.6 |
| 50 - 70           | 10.1 | 8.6 | --- | --- | 1.6 | 3.4 | 6.2 | 3.5 | 2.4 | 2.2 | 10.2 | 9.1 |
| 70 - 95           | 12.6 | 11.5 | --- | --- | 12.1 | 11.8 | --- | --- | --- | --- | 12.1 | 11.8 |
| 95 - 145          | 29.3 | 29.4 | 9.5 | 16.7 | 20.3 | 12.6 | --- | --- | --- | --- | 29.8 | 29.3 |
| 145 - 165         | 6.7  | 5.3 | 6.7 | 5.3 | --- | --- | --- | --- | --- | --- | 6.7  | 5.3 |
| 165 - 190         | 16.3 | 19.5 | 16.3 | 19.5 | --- | --- | --- | --- | --- | --- | 16.3 | 19.5 |

**Table 2** Quantitative distribution (%) of CH$_3$, CH$_2$, CH and C-quaternary carbons in the humic acids of the soils High-Field Grass (HH) and Geescroft Field (HG).
| Spectral area (ppm) | $^{13}$C NMR (%) | C-quat. (%) | CH (%) | CH$_2$ (%) | CH$_3$ (%) | $\sum CH_n$ |
|---------------------|-----------------|-------------|--------|-----------|-----------|------------|
| 10 - 50             | FH 10.2, FG 14.5, FH 1.9, FG 3.2 | FH 4.9, FG 7.2, FH 6.3, FG 1.3 | 10.4 | 14.4 |
| 50 - 90             | FH 64.9, FG 56.6 | FH 38.7, FG 41.9, FH 26.0, FG 11.3, FH 1.9, FG 2.1 | 66.6 | 55.3 |
| 90 - 110            | FH 11.1, FG 9.5, FH 5.2, FG 0.5 | FH 5.8, FG 9.0 | 11.0 | 9.5 |
| 165 - 190           | FH 13.8, FG 19.4, FH 13.8, FG 19.4 | ---- | ---- | ---- | 13.8 | 19.4 |

Table 3: Quantitative distribution (%) of CH$_3$, CH$_2$, CH and C-quaternary carbons in the fulvic acids of the soils High-Field Grass (FH) and Geescroft Field (FG).
| Mol. weight m/z | Rel. signal intensity in FG (%) | Rel. signal intensity in FH (%) | Proposed elemental composition | Proposed chem. structure |
|-----------------|--------------------------------|--------------------------------|--------------------------------|--------------------------|
| 58              | 14.2                           | 6.2                            | C₃H₆O                         | Acetone                  |
| 61              | 19.7                           | 15.5                           | C₂H₇ON                        | Aminoethanol             |
| 62              | 24.8                           | 13.8                           | C₂H₆O₂                        | Ethyleneglycol           |
| 80              | 100                            | 100                            | C₄H₄N₂                        | Pyrimidine               |
|                 |                                 |                                | C₄H₄N₂                        | Pyridazine               |
|                 |                                 |                                | C₆H₈                          | Hexatriene               |
| 130             | 50.8                           | 23.1                           | C₁₀H₁₀                         | Methylindene             |
| 131             | 99.2                           | 71.8                           | C₅H₉O₃N                       | Alanine, N-acetyl        |
|                 |                                 |                                | C₆H₁₃O₂                       | Leucine                  |
|                 |                                 |                                | C₅H₉O₃N                       | Glycine, N-acetyl, methyl ester |
| 132             | 61.8                           | 66.7                           | C₅H₈O₄                        | Glutaric acid            |
|                 |                                 |                                | C₄H₈O₃N₂                      | Glycine, N-glycyl        |
|                 |                                 |                                | C₅H₈O₄                        | Pentoses                 |
| 133             | 19.7                           | 25.1                           | C₄H₇NO₄                       | Aspartic acid            |
| 139             | 24.0                           | 20.8                           | C₄H₇N₆                        | Methylmelamine           |
| 154             | 52.7                           | 70.5                           | C₅H₁₀N₆                       | Dimethylmelamine         |
| 194             | 42.5                           | 67.2                           | C₆H₁₀O₇                       | Glucuronic acid          |
|                 |                                 |                                | C₇H₁₄O₆                       | Inositol, 4-C-methyl      |
|                 |                                 |                                | C₇H₁₄O₆                       | Fructose, 3-O-methyl      |
| 252             | 18.5                           | 18.3                           | C₉H₁₅O₆B₃                     | Inositol, tris(methylboronate) |
| 286             | 393.7                          | 11.7                           | C₁₄H₂₆O₄N₂                    | Leucine, N-acetylglycyl−, butyl ester |
|                 |                                 |                                | C₁₄H₂₆O₄N₂                    | Alanine, N-acetylvalyl−, butyl ester |
|                 |                                 |                                | C₁₀H₂₂O₅S₂                    | Glucose, diethyl mercaptal |
| 311             | 13.4                           | 12.7                           | C₁₂H₁₇O₅N₅                    | Guanosine, N, N−dimethyl  |

*Table 4* Proposed chemical compounds, in the fulvic acids FG and FH, identified by FABMS spectrometry.
| m/z | Rel. signal intensity in HG (%) | Rel. signal intensity in HH (%) | Proposed elemental composition | Proposed chem. structure |
|-----|-------------------------------|--------------------------------|-------------------------------|--------------------------|
| 130 | 100                           | 100                            | C_{10}H_{10}                  | Methylindene             |
|     |                               |                                | C_{10}H_{10}                  | Divinylbenzene           |
|     |                               |                                | C_7H_{14}O_2                  | Propanoic acid, butyl ester |
| 152 | 54.8                          | 68.0                           | C_{10}H_{16}O                 | 2,4-Decadienal           |
|     |                               |                                | C_9H_{12}O_2                  | Phenol, 4-propoxy-       |
|     |                               |                                | C_8H_{8}O_3                   | Hydroxymethoxybenzaldehyde |
| 174 | 10.7                          | —                              | C_{10}H_{22}O_2               | Decadiol                 |
|     |                               |                                | C_{13}H_{20}                  | Benzene, heptene         |
| 194 | 20.3                          | 10.4                           | C_{11}H_{14}O_3               | 2-Allyloxy-1, 3-dimethoxybenzene |
|     |                               |                                | C_{11}H_{14}O_3               | Phenol, 2, 6-dimethoxy-4-propenyl |
| 220 | 14.7                          | 2.3                            | C_9H_{12}                     | Ethylmethylbenzene       |
| 236 | 47.9                          | —                              | C_{17}H_{32}                  | Heptadecadiene           |
| 278 | 12.9                          | —                              | C_{20}H_{42}O_2               | 1,4-Eicosanediol         |
| 324 | 7.8                           | —                              | C_{23}H_{48}                  | Tricosane                |
| 410 | 7.1                           | —                              | C_{30}H_{50}                  | Squalene                 |

Table 5 Proposed chemical compounds, in the humic acids HG and HH, identified by FABMS spectrometry.
| EI-signal m/z | Proposed elemental composition | Proposed structure                           |
|---------------|--------------------------------|---------------------------------------------|
| 124           | C\(_7\)H\(_8\)O\(_2\)         | Guaiacol                                    |
| 126           | C\(_6\)H\(_6\)O\(_3\)         | Hydroxymethylfuraldehyde                    |
| 140           | C\(_7\)H\(_8\)O\(_3\)         | Hydroxyguaiacol                             |
| 154           | C\(_8\)H\(_{10}\)O\(_3\)      | Syringol                                    |
| 168           | C\(_9\)H\(_{12}\)O\(_3\)      | Methylsyringol                              |
| 182           | C\(_9\)H\(_{10}\)O\(_4\)      | Syringaldehyde                              |
| 196           | C\(_{10}\)H\(_{12}\)O\(_4\)   | Syringylethanone                            |
| 210           | C\(_{11}\)H\(_{14}\)O\(_4\)   | Synapyl alcohol                             |
| 214           | C\(_{14}\)H\(_{30}\)O          | 1-Tetradecanol                              |
| 242           | C\(_{16}\)H\(_{34}\)O          | 1-Hexadecanol                               |
| 282           | C\(_{20}\)H\(_{42}\)          | Eicosane                                    |
| 310           | C\(_{22}\)H\(_{46}\)          | Octadecane, –tetramethyl                    |
| 324           | C\(_{23}\)H\(_{48}\)          | Tricosane; Docosane, 6–methyl               |
| 336           | C\(_{24}\)H\(_{48}\)          | Cyclotetracosane                            |
| 338           | C\(_{24}\)H\(_{50}\)          | Nonadecane, –pentamethyl; Tetracosane       |
| 342           | C\(_{22}\)H\(_{46}\)O\(_2\)   | Ethanol, 2-eicosyloxy                       |
| 352           | C\(_{25}\)H\(_{52}\)          | Eicosane, -pentamethyl                      |
| 355           | C\(_{23}\)H\(_{46}\)O\(_2\)   | High carboxylic acid                        |
| 364           | C\(_{26}\)H\(_{52}\)          | 1-Hexacosene                                |
| 366           | C\(_{26}\)H\(_{54}\)          | Hexacosane                                  |
| 394           | C\(_{28}\)H\(_{58}\)          | Docosane, 7-hexyl                           |
| 408           | C\(_{27}\)H\(_{52}\)O\(_2\)   | Docosenoic asid, tetramethyl–, methyl ester |
| 410           | C\(_{30}\)H\(_{50}\)          | Squalene                                    |
| 422           | C\(_{30}\)H\(_{62}\)          | Triacontane; Hexamethyltetrasosane          |
| 436           | C\(_{31}\)H\(_{64}\)          | 11-Decylleneicosane                         |
| 450           | C\(_{32}\)H\(_{66}\)          | Dotriacontane                               |

Table 6 Assignment of the EI-signals (m/z) in the masspectra of chromatographic fractions of humic acids HH and HG to proposed structures.
| GC-fractions RT (min) | Found in   | Main m/z signals in EI/MS (see Table 6) |
|-----------------------|------------|----------------------------------------|
| 8.4                   | HG, HH     | 126, 154, 182, 210                      |
| 10.6                  | HG, HH     | 124, 168, 182, 210                      |
| 11.7                  | HG, HH     | 126, 196, 214, 242                      |
| 17.2                  | HG, HH     | Mixture                                |
| 20.6                  | HG         | 310, 342                               |
| 22.7                  | HG, HH     | Mixture                                |
| 23.3                  | HG         | 324                                    |
| 25.9                  | HG         | 338                                    |
| 27.9                  | HG, HH     | 341, 430, 503                          |
| 28.4                  | HG         | 352                                    |
| 30.9                  | HG         | 366                                    |
| 32.9                  | HG         | 336                                    |
| 33.3                  | HG, HH     | 380                                    |
| 35.5                  | HG         | 394                                    |
| 35.8                  | HG, HH     | 410                                    |
| 37.5                  | HG, HH     | 364                                    |
| 37.8                  | HG, HH     | 408                                    |
| 39.9                  | HG         | 422                                    |
| 42.0                  | HG, HH     | 436                                    |
| 44.0                  | HG         | 355, 450                               |
| 46.0                  | HG         | 355, 394                               |

**Table 7** Composition of the fractions in the gas-chromatograms of HH and HG.