

# CHEMICAL CHARACTERIZATION OF SOIL ORGANIC MATTER FRACTIONS BY ANALYTICAL PYROLYSIS-GAS CHROMATOGRAPHY-MASS SPECTROMETRY

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(Received March 17th, 1985; accepted July 5th, 1985)

## SUMMARY

Curie-point pyrolysis-gas chromatography-mass spectrometry has been used to chemically characterize fulvic acid, humic acid, hyatomelanic acid and polysaccharide fractions of a representative soil. A detailed study of all the pyrolysis products was made. By comparing the results of this study with previous pyrolysis data for lignins, degraded lignins, polysaccharides, proteins, etc., we have been able to obtain more detailed information about the chemical composition of the pyrolysis products from different soil organic matter fractions. It is shown that fulvic acid fractions consist mainly of polysaccharide and/or carbohydrates and polyphenols, that humic acid and humin fractions are complex mixtures of several biopolymers such as polysaccharides, partially degraded lignins, peptides and lipids and that the hyatomelanic acid fraction represents mainly lipid materials.

## INTRODUCTION

Plant residues constitute an important organic component of soils. Living, dying and dead tissues representing wide varieties of chemical substances undergo biochemical and chemical degradation reactions. The compounds produced in this way, the so-called humic substances, are very complex in nature and are thought to be more stable than the starting materials.

The classical method of fractionation of humic substances is based on their solubility in alkalis and acids. The major fractions thus obtained are

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humic acid, fulvic acid and humin. A further fractionation of humic acid into hyatomelanic acid, an ethanol-soluble fraction, is sometimes performed. In addition to humic substances, polysaccharides are quantitatively important compounds present in soil organic matter [1].

Numerous investigations have been undertaken to characterize the chemical structure of soil organic matter fractions by means of chemical degradation techniques [2]. More recently, pyrolysis-mass spectrometric studies of different humic fractions and related materials (e.g. fungal melanins, lignins, polysaccharides) have been reported. This analytical approach is used as a fingerprinting technique and clearly shows similarities and differences between different fractions present in soils [1,3-5].

However, to obtain an insight into the structural composition of the organic matter more firm identifications of the pyrolysis products, which reflect structural moieties present within the polymeric matrix, are required.

In this study we used Curie-point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) to identify the pyrolysis products of the polysaccharide, fulvic acids, humic acid and humin and also the hyatomelanic acid fraction of a representative soil. By comparing the results of this study with those of previous Py-GC-MS studies of lignins, degraded lignins, polysaccharides and fulvic acids [6,7], we have been able to obtain more detailed information about the chemical composition of the pyrolysis products from different soil organic matter fractions.

## EXPERIMENTAL

### *Soil sample*

The soil sample used was obtained from the A<sub>1</sub> horizon of a brown soil (Typic Xerochrept) in the northern part of the province of Huelva, Spain.

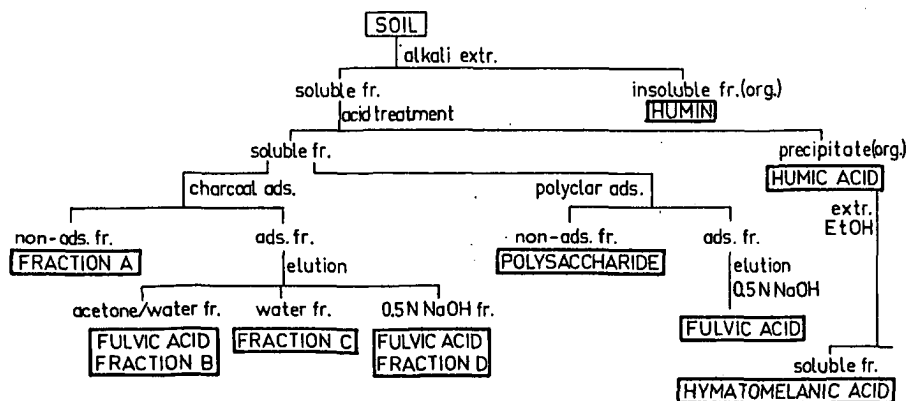


Fig. 1. Fractionation of soil organic matter (for details, see ref. 1).

TABLE 1

Elemental composition of soil organic matter fractions [1]

Fraction	C (%)	H (%)	N (%)	O (%)	Ash (%)
Polysaccharide	37.8	6.8	2.1	53.3	22.0
Humin	55.1	6.6	4.2	34.1	2.2
Humic acid	51.4	5.8	4.1	38.7	5.5
Hymatomelanic acid	57.8	7.5	1.1	33.6	2.5
Fulvic acid (Polyclar)	47.9	5.2	2.6	44.3	11.5
Fulvic acid (fraction B)	49.9	6.3	1.4	42.4	6.5
Fulvic acid (fraction D)	39.5	4.3	2.5	53.7	21.1

The altitude was 480 m and the vegetation consisted of an uncultivated prairie with gramineous plants, *Medicago* and *Trifolium*. The sample taken represented a depth of 0–10 cm and had a pH of 5.6 in water, a carbon content of 3.5% and a nitrogen content of 0.4%. The applied fractionation procedure for soil organic matter is described elsewhere [1]. For the reader's convenience we have included Fig. 1, which indicates the different fractions studies and the procedural pathways by which they were obtained. Table 1 shows the elementary composition of the isolated soil organic matter fractions on a dry and ash-free basis [1].

#### *Pyrolysis–gas chromatography–mass spectrometry*

The samples were suspended in methanol. One droplet of the suspension (10–20  $\mu\text{g}$  of sample) was applied to a ferromagnetic wire with a Curie temperature of 510°C. The temperature rise time was about 0.15 s and the wire was held at the end temperature for 10 s.

The Py–GC–MS analyses were carried out using a pyrolysis unit similar to that described by Meuzelaar et al. [8] modified for use at high temperatures [9]. The pyrolysis products were separated on a capillary glass WCOT column (28 m  $\times$  0.5 mm I.D.) coated with CP-Sil 5 (1.25  $\mu\text{m}$  film thickness) held at 0°C for 5 min and subsequently programmed to 300°C at a rate of 5°C/min. Helium was used as the carrier gas at a rate of 1.6 ml/min. The chromatograph (Varian Model 3200) was coupled to a Varian-MAT 44 quadrupole mass spectrometer operated in the electron impact (EI) mode at 80 eV and with a cycle time of 2 s.

#### RESULTS AND DISCUSSION

Pyrolysis products were identified by comparing their EI mass spectra with mass spectral libraries [10,11] and with mass spectra and GC retention times of standard compounds. Subsequently, Py–GC–MS data obtained

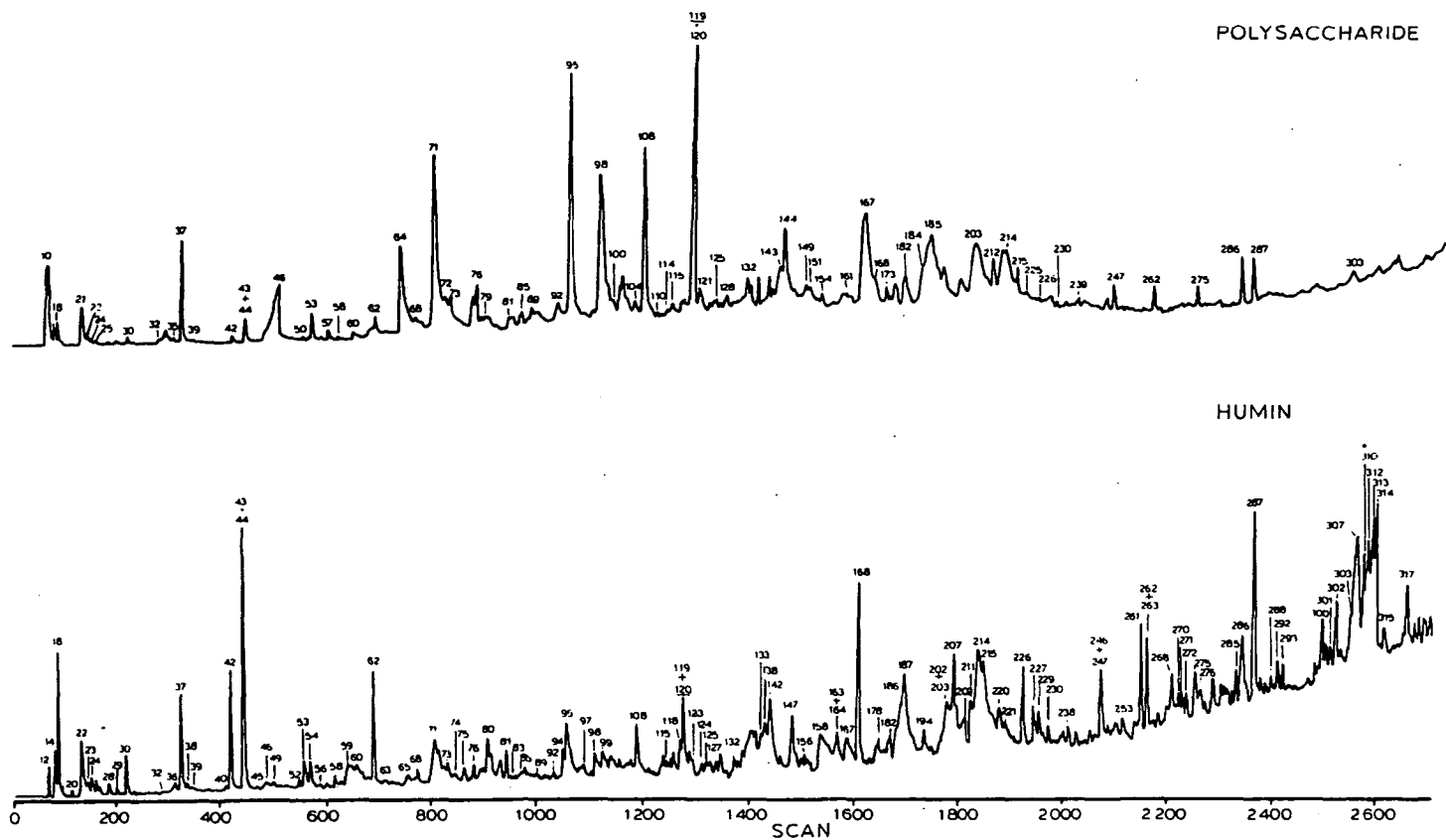


Fig. 2. Pyrolysis-gas chromatography-mass spectrometry of soil polysaccharide and humin. Peak identifications are given in Table 2. Underlined numbers indicate minor contributions to the peak.

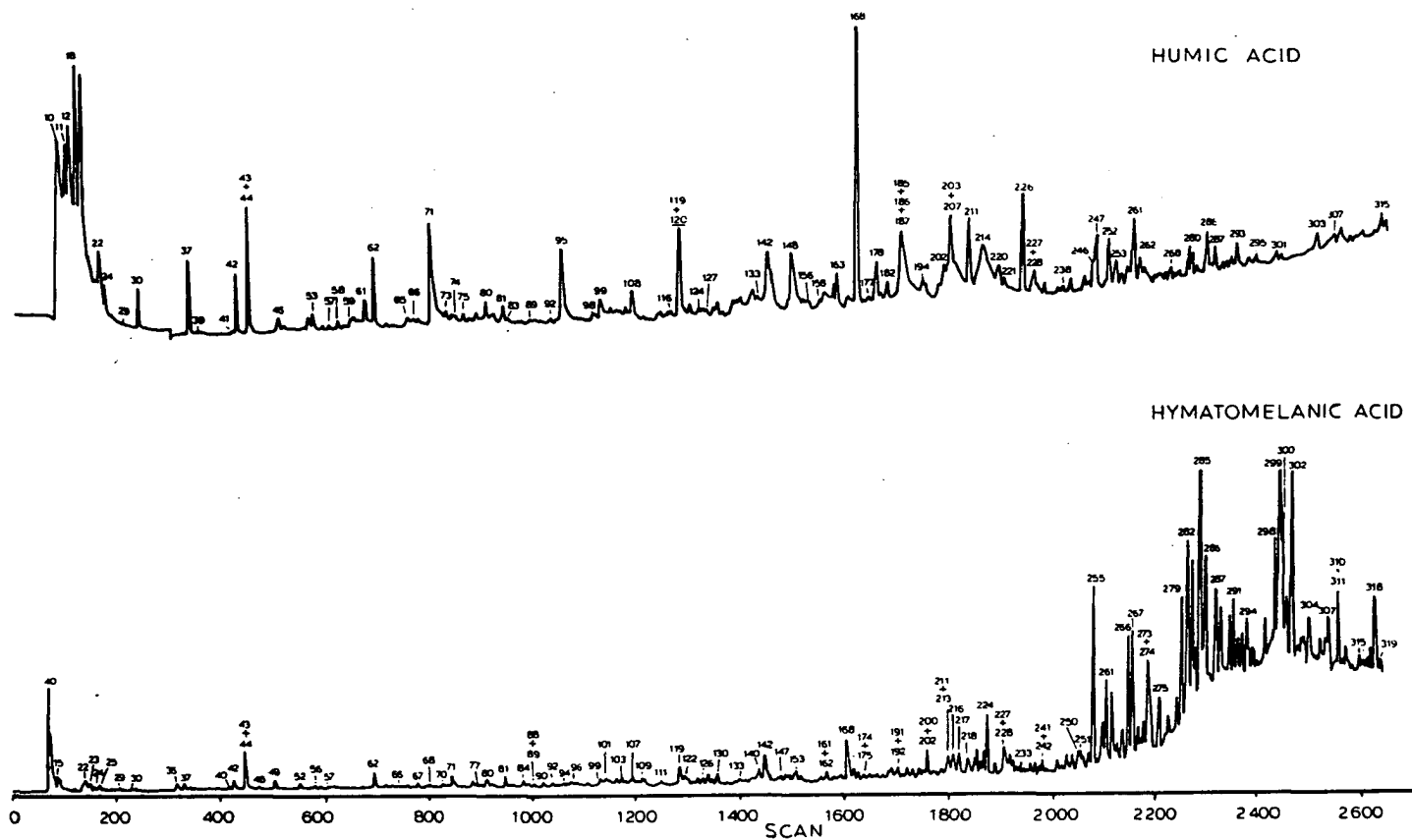
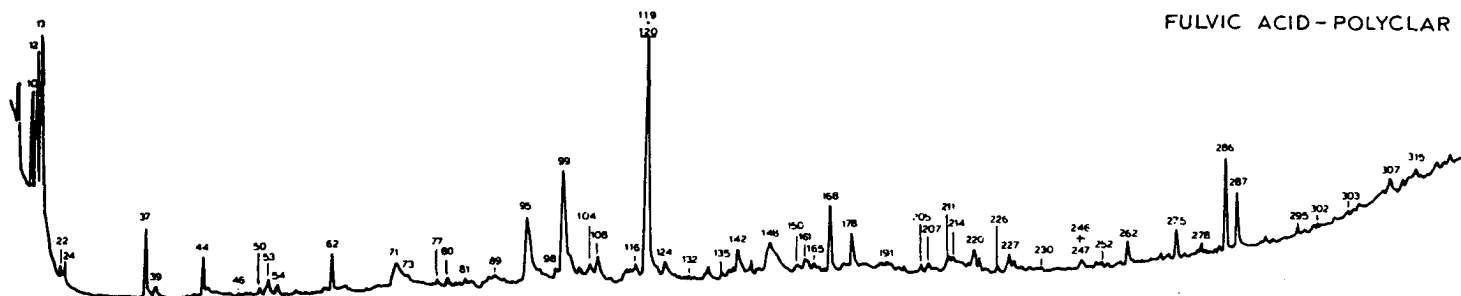
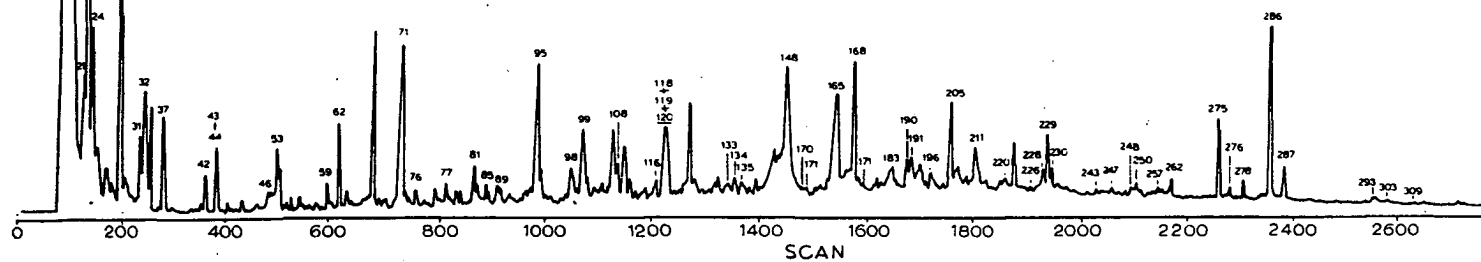


Fig. 3. Pyrolysis-gas chromatography-mass spectrometry of humic acid and hymatomelanic acid. Peak identifications are given in Table 2. Underlined numbers indicate minor contributions to the peak.

FULVIC ACID - POLYCLAR



FULVIC ACID - B



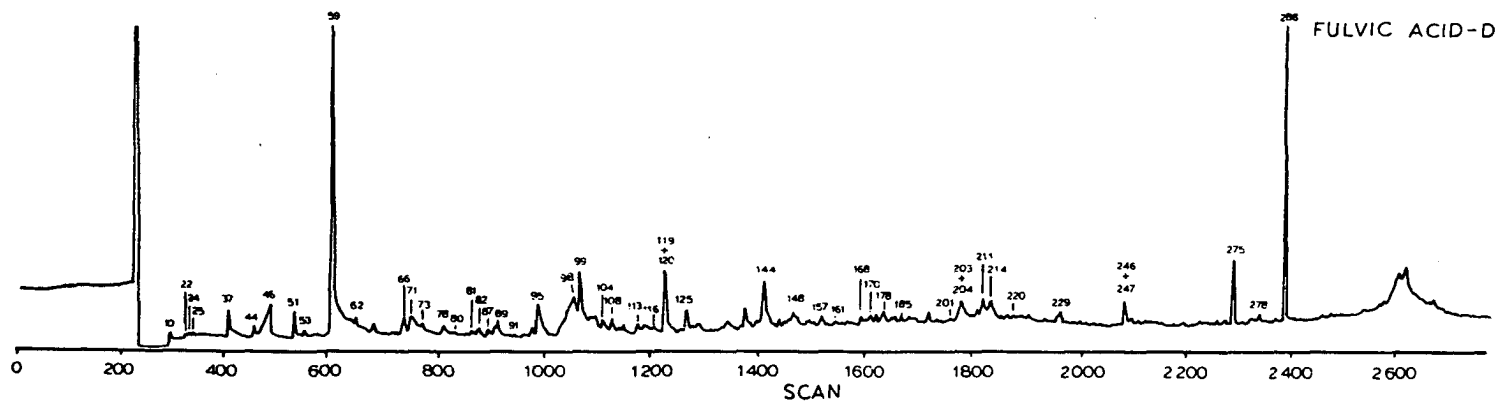


Fig. 4. Pyrolysis-gas chromatography-mass spectrometry of fulvic acid fractions. Peak identifications are given in Table 2. Underlined numbers indicate minor contribution to the peak.

TABLE 2

Pyrolysis products of soil organic matter fractions as identified by pyrolysis-gas chromatography-mass spectrometry

Peak No. *	Compound	Origin **
1	Carbon monoxide	
2	Carbon dioxide	
3	Methane	
4	Ethene	
5	Ethane	
6	Hydrogen sulphide	Pr
7	Propene	
8	Propane	
9	Methanol	Ps, Lg
10	Sulphur dioxide	
11	Hydrochloric acid	
12	Chloromethane	
13	Acetaldehyde	Ps
14	2-Methylprop-1-ene	
15	But-1-ene	
16	Buta-1,3-diene	
17	<i>n</i> -Butane	
18	Methanethiol	Pr
19	<i>trans</i> -But-2-ene	
20	3-Methylpent-1-ene	
21	2-Propenal	Ps
22	Acetone	Ps
23	Pent-1-ene	
24	Furan	Ps
25	Iodomethane	
26	<i>n</i> -Pentane	
27	Ethanethiol	Pr
28	<i>cis</i> -Pent-2-ene	
29	Cyclopentadiene	Ps
30	2-Methylpropenal	Ps
31	3-Buten-2-one	Ps
32	Butane-2,3-dione	Ps
33	2-Methylpentane	
34	Cyclopentane	
35	Methyldihydrofuran	Ps
36	Hex-1-ene	
37	2-Methylfuran	Ps
38	<i>n</i> -Hexane	
39	3-Methylfuran	Ps
40	Hexatriene	
41	Hexadiene	
42	3-Methylbutanal	Pr
43	2-Methylbutanal	Pr
44	Benzene	Ps, Pr, Lg
45	Thiophene	
46	Acetic acid	Ps
47	2-Methylcyclobutanone	Ps



TABLE 2 (continued)

Peak No. *	Compound	Origin **
48	Cyclohexane	
49	Cyclohexene	
50	Ethylfuran	Ps
51	1,4-Dioxan	
52	Hept-1-ene	
53	2,5-Dimethylfuran	Ps
54	2,4-Dimethylfuran	Ps
55	<i>n</i> -Heptane	
56	Dimethylfuran	Ps
57	Vinylfuran	Ps
58	N-Methylpyrrole	Pr
59	Pyridine	Pr
60	5-Methyl-2(5H)-furanone	Ps
61	Pyrrole	Pr
62	Toluene	Pr, Lg
63	Methylthiophene	
64	$\gamma$ -Crotonolactone	Ps
65	Dihydropyran	Ps
66	Oct-1-ene	Lp
67	Oct-2-ene	Lp
68	3-Furaldehyde	Ps
69	<i>n</i> -Octane	Lp
70	<i>n</i> -C <sub>5</sub> Fatty acid methyl ester	Lp
71	2-Furaldehyde	Ps
72	Acetamide	Ps
73	Benzenemethanol	
74	Methylpyrrole	Pr
75	Methylpyrrole	Pr
76	Furfuryl alcohol	Ps
77	Ethylbenzene	Pr, Lg
78	Methylpyridine	Pr
79	3-Methylcyclopent-2-en-1-one	Ps
80	<i>m</i> - and/or <i>p</i> -xylene	Pr, Lg
81	Styrene	
82	2-Methylcyclopent-2-en-1-one	Ps
83	<i>o</i> -Xylene	Lg
84	Non-1-ene	Lp
85	C <sub>3</sub> -Alkylfuran	Ps
86	Furancarboxylic acid	Ps
87	C <sub>3</sub> -Alkylfuran	Ps
88	<i>n</i> -Nonane	Lp
89	$\alpha$ -Angelicalactone	Ps
90	<i>n</i> -C <sub>6</sub> Fatty acid methyl ester	Lp
91	Dimethylpyridine	Pr
92	$\alpha$ -Methylbenzenemethanol	
93	C <sub>4</sub> -Alkylfuran	Ps
94	Benzaldehyde	Lg
95	5-Methyl-2-furaldehyde	Ps
96	C <sub>3</sub> -Alkylbenzene	
97	C <sub>3</sub> -Alkylbenzene	

TABLE 2 (continued)

Peak No. *	Compound	Origin **
98	4-Hydroxy-5,6-dihydro-2H-pyran-2-one	Ps
99	Phenol	Ps, Pr, Lg
100	Trimethylcyclopentenone	Ps
101	$\alpha$ -Methylstyrene	
102	Dec-1-ene	Lp
103	Iso-C <sub>7</sub> fatty acid methyl ester	Lp
104	2-Hydroxy-3-methyl-2-cyclopenten-1-one	Ps
105	C <sub>3</sub> -Alkylpyridine	Pr
106	<i>n</i> -Decane	Lp
107	<i>n</i> -C <sub>7</sub> Fatty acid methyl ester	Lp
108	4-Hydroxy-6-methyl-5,6-dihydro-2H-pyran-2-one	Ps
109	Indene	
110	Furan-2,5-aldehyde	Ps
111	<i>o</i> -Cresol	Lg
112	C <sub>3</sub> -Alkylbenzene	
113	C <sub>3</sub> -Alkylbenzene	
114	5-(2-Hydroxyethylidene)-2H-furanone	Ps
115	Hexane-2,3,4-trione	Ps
116	3-Hydroxy-6-methyl-3,4-dihydro-2H-pyran-2-one	Ps
117	2-Furyl hydroxymethyl ketone	Ps
118	<i>p</i> -Cresol	Pr, Lg
119	Guaiacol	Lg
120	Levogluconone	Ps
121	3-Hydroxy-6-methyl-2H-pyran-2-one	Ps
122	<i>n</i> -C <sub>7</sub> Fatty acid	Lp
123	Ethylstyrene	Lg
124	3-Hydroxy-2-methyl-4H-pyran-4-one	Ps
125	3-Acetoxypyridine	Ps
126	Undec-1-ene	Lp
127	Benzyl cyanide	Pr
128	5-Hydroxy-2-methyl-4H-pyran-4-one	Ps
129	<i>n</i> -Undecane	Lp
130	<i>n</i> -C <sub>8</sub> Fatty acid methyl ester	Lp
131	Methylpentane-1,5-dioate	Lp
132	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Ps
133	Ethylphenol	Lg
134	C <sub>4</sub> -Alkylbenzene	
135	C <sub>4</sub> -Alkylbenzene	
136	C <sub>2</sub> -Alkylphenol	Lg
137	C <sub>4</sub> -Alkylbenzene	
138	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub> (chitin)	Ps
139	Naphthalene	
140	<i>n</i> -C <sub>8</sub> Fatty acid	Lp
141	C <sub>2</sub> -Alkylphenol	Lg
142	Methylguaiacol	Lg
143	3,5-Dihydroxy-2-methyl-4H-pyran-4-one	Ps
144	1,4:3,6-Dianhydro- $\alpha$ -D-glucopyranose	Ps
145	Methylnaphthalene	
146	Dodec-1-ene	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
147	Iso-C <sub>9</sub> fatty acid methyl ester	Lp
148	Vinylphenol	Lg
149	Resorcinol	Ps, Lg
150	2-Methylthiophenol	
151	Anhydrohexose	Ps
152	<i>n</i> -C <sub>9</sub> Fatty acid methyl ester	Lp
153	Methylhexane-1,6-dioate	Lp
154	5-(Hydroxymethyl)-2-furaldehyde	Ps
155	<i>n</i> -Dodecane	Lp
156	Amino acid dimer	Pr
157	1-Indanone	
158	Amino acid dimer	Pr
159	Methylthiophenol	
160	C <sub>6</sub> -Alkylbenzene	
161	Ethylguaiaicol	Lg
162	<i>n</i> -C <sub>9</sub> Fatty acid	Lp
163	Indole	Pr
164	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub> (chitin)	Ps
165	Phthalic anhydride	
166	Iso-C <sub>10</sub> fatty acid methyl ester	Lp
167	1,4-Dideoxy-D-glycero-hex-1-enopyranos-3-ulose	Ps
168	Vinylguaiaicol	Lg
169	Tridec-1-ene	Lp
170	Indan-1,3-dione	
171	1(3H)-Isobenzofuranone	
172	<i>n</i> -Tridecane	Lp
173	C <sub>7</sub> H <sub>7</sub> NO <sub>3</sub> /C <sub>8</sub> H <sub>11</sub> NO <sub>2</sub> (chitin)	Ps
174	<i>n</i> -C <sub>10</sub> Fatty acid methyl ester	Lp
175	Methylheptane-1,7-dioate	Lp
176	Amino acid dimer	Pr
177	Amino acid dimer	Pr
178	2,6-Dimethoxyphenol	Lg
179	Amino acid dimer	Pr
180	Amino acid dimer	Pr
181	Amino acid dimer	Pr
182	Eugenol	Lg
183	Benzenepropionic acid	Lg
184	C <sub>8</sub> H <sub>9</sub> NO <sub>2</sub> (chitin)	Ps
185	Levogalactosan	Ps
186	Amino acid dimer	Pr
187	Methylindole	Pr
188	<i>n</i> -C <sub>10</sub> Fatty acid	Lp
189	Biphenyl	Lg
190	Methylphthalic anhydride	
191	Vanillin	Lg
192	Iso-C <sub>11</sub> fatty acid methyl ester	Lp
193	Amino acid dimer	Pr
194	<i>cis</i> -Isoeugenol	Lg
195	Tetradec-1-ene	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
196	Methylphthalic anhydride	
197	Amino acid dimer	Pr
198	<i>n</i> -Tetradecane	Lp
199	<i>n</i> -C <sub>11</sub> Fatty acid methyl ester	Lp
200	Methyloctane-1,8-dioate	Lp
201	4-Methyl-2,6-dimethoxyphenol	Lg
202	Amino acid dimer	Pr
203	Levoglucofane (mannose?)	Ps
204	Phthalide	
205	Dimethyl phthalate	
206	Amino acid dimer	Ps
207	<i>trans</i> -Isoeugenol	Lg
208	Amino acid dimer	Pr
209	Amino acid dimer	Pr
210	Ethyl- $\alpha$ -ethyl hexanoate	Lp
211	Acetoguaiacone	Lg
212	Trianhydro-2-acetamido-2-deoxyglucose (C <sub>8</sub> H <sub>9</sub> NO <sub>3</sub> )	Ps
213	<i>n</i> -C <sub>11</sub> Fatty acid	Lp
214	Levoglucofane (glucose)	Ps
215	C <sub>8</sub> H <sub>9</sub> NO <sub>3</sub> (chitin)	Ps
216	3,4-Dimethoxyacetophenone	Lg
217	Iso-C <sub>12</sub> fatty acid methyl ester	Lp
218	Methyl vanillate	Lg
219	4-Ethyl-2,6-dimethoxyphenol	Lg
220	(4-Hydroxy-3-methoxy)phenylpropan-2-one	Lg
221	<i>n</i> -Pentadecane	Lp
222	Tributyl phosphate	
223	<i>n</i> -C <sub>12</sub> Fatty acid methyl ester	Lp
224	Methylnonane-1,9-dioate	Lp
225	C <sub>8</sub> H <sub>11</sub> NO <sub>4</sub> (chitin)	Ps
226	4-Vinyl-2,6-dimethoxyphenol	Lg
227	<i>n</i> -C <sub>12</sub> Fatty acid	Lp
228	Propioguaiacone	Lg
229	Diethyl phthalate	
230	Vanillic acid	Lg
231	Dihydroxydimethylbenzaldehyde	Lg
232	4-Allyl-2,6-dimethoxyphenol	Lg
233	Iso-C <sub>13</sub> fatty acid methyl ester	Lp
234	Anteiso-C <sub>13</sub> fatty acid methyl ester	Lp
235	Dianhydro-2-acetamido-2-deoxyglucose	Ps
236	Hexadecadiene	Lp
237	Hexadec-1-ene	Lp
238	<i>n</i> -Hexadecane	Lp
239	C <sub>8</sub> H <sub>9</sub> NO <sub>3</sub> (chitin)	Ps
240	<i>n</i> -C <sub>13</sub> Fatty acid methyl ester	Lp
241	Methyldecane-1,10-dioate	Lp
242	Syringaldehyde	Lg
243	Tributyl phosphate	
244	<i>n</i> -C <sub>13</sub> Fatty acid	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
245	Iso-C <sub>14</sub> fatty acid methyl ester	Lp
246	4- <i>trans</i> -Propenyl-2,6-dimethoxyphenol	Lg
247	Biphenol	Lg
248	C <sub>9</sub> -Alkylphenol	
249	Acetosyringone	Lg
250	C <sub>9</sub> -Alkylphenol	
251	Heptadecadiene	Lp
252	4-Propyl-2,6-dimethoxyphenol	Lg
253	Heptadec-1-ene	Lp
254	<i>n</i> -Heptadecane	Lp
255	<i>n</i> -C <sub>14</sub> Fatty acid methyl ester	Lp
256	Methylundecane-1,11-dioate	Lp
257	C <sub>9</sub> -Alkylphenol	
258	4-Propenol-2,6-dimethoxyphenol	Lg
259	4-Propanol-2,6-dimethoxyphenol	Lg
260	C <sub>9</sub> -Alkylphenol	
261	Prist-1-ene	Lp
262	<i>n</i> -C <sub>14</sub> Fatty acid	Lp
263	Prist-2-ene	Lp
264	Anthracene	
265	10-Methyl-C <sub>14</sub> fatty acid methyl ester	Lp
266	Iso-C <sub>15</sub> fatty acid methyl ester	Lp
267	Anteiso-C <sub>15</sub> fatty acid methyl ester	Lp
268	Octadec-1-ene	Lp
269	Methyl ferulate	Lg
270	<i>n</i> -Octadecane	Lp
271	Iso-C <sub>15</sub> fatty acid	Lp
272	Anteiso-C <sub>15</sub> fatty acid	Lp
273	<i>n</i> -C <sub>15</sub> Fatty acid methyl ester	Lp
274	2,6-Dimethoxyphenyl-4-propionic acid	Lg
275	Diisobutyl phthalate	
276	<i>n</i> -C <sub>15</sub> Fatty acid	Lp
277	10-Methyl-C <sub>15</sub> fatty acid methyl ester	Lp
278	Dialkyl phthalate	
279	Iso-C <sub>16</sub> fatty acid methyl ester	Lp
280	Phytadiene	Lp
281	Nonadec-1-ene	Lp
282	Iso-C <sub>16:1</sub> fatty acid methyl ester	Lp
283	Anteiso-C <sub>16:1</sub> fatty acid methyl ester	Lp
284	<i>n</i> -Nonadecane	Lp
285	<i>n</i> -C <sub>16</sub> Fatty acid methyl ester	Lp
286	Dibutyl phthalate	
287	<i>n</i> -C <sub>16</sub> Fatty acid	Lp
288	<i>n</i> -C <sub>16</sub> Fatty acid ethyl ester	Lp
289	10-Methyl-C <sub>16</sub> fatty acid methyl ester	Lp
290	Iso-C <sub>17</sub> fatty acid methyl ester	Lp
291	Anteiso-C <sub>17</sub> fatty acid methyl ester	Lp
292	Eicos-1-ene	Lp
293	<i>n</i> -Eicosane	Lp

TABLE 2 (continued)

Peak No. *	Compound	Origin **
294	<i>n</i> -C <sub>17</sub> Fatty acid methyl ester	Lp
295	<i>n</i> -C <sub>17</sub> Fatty acid	Lp
296	10-Methyl-C <sub>17</sub> fatty acid methyl ester	Lp
297	Iso-C <sub>18</sub> fatty acid methyl ester	Lp
298	C <sub>18:2</sub> Fatty acid methyl ester	Lp
299	C <sub>18:1</sub> Fatty acid methyl ester	Lp
300	C <sub>18:1</sub> Fatty acid methyl ester	Lp
301	<i>n</i> -Heneicosane	Lp
302	<i>n</i> -C <sub>18</sub> Fatty acid methyl ester	Lp
303	<i>n</i> -C <sub>18</sub> Fatty acid	Lp
304	10-Methyl-C <sub>18</sub> fatty acid methyl ester	Lp
305	Iso-C <sub>19</sub> fatty acid methyl ester	Lp
306	Anteiso-C <sub>19</sub> fatty acid methyl ester	Lp
307	Dialkyl phthalate	
308	Docos-1-ene	Lp
309	<i>n</i> -Docosane	Lp
310	<i>n</i> -C <sub>19</sub> Fatty acid methyl ester	Lp
311	Methylhexadecane-1,16-dioate	Lp
312	Terpenoid	Lp
313	Terpenoid	Lp
314	Terpenoid	Lp
315	<i>n</i> -C <sub>19</sub> Fatty acid	Lp
316	Tricos-1-ene	Lp
317	<i>n</i> -Tricosane	Lp
318	<i>n</i> -C <sub>20</sub> Fatty acid methyl ester	Lp
319	<i>n</i> -C <sub>20</sub> Fatty acid	Lp
320	Dialkyl phthalate	
321	<i>n</i> -C <sub>21</sub> Fatty acid	Lp
322	<i>n</i> -C <sub>22</sub> Fatty acid	Lp

\* Peak numbers as shown in Figs. 2—4.

\*\* Ps = polysaccharide; Pr = protein; Lg = lignin; Lp = lipid.

for well defined polymers such as amylose [12], chitin [13], proteins and peptides [14] and lignins [6] allowed a detailed recognition of typical pyrolysis products.

The reconstructed ion chromatograms of the pyrolysis mixtures obtained from the soil organic matter fractions are shown in Figs. 2–4. The peak numbers in these figures correspond with the numbers mentioned in Table 2. Owing to the vast number of identified compounds in each pyrolysate (e.g., 175 in the hymatomelanic acid fraction), it was not possible to label all peaks in the chromatograms; therefore, only major peaks are indicated in the figures.

Most of the major compounds are well known pyrolysis products of biologically produced substances (polysaccharides, lignins, peptides, lipids, etc.). A number of compounds clearly indicate the presence of pollutants.

## *Polysaccharides*

The compounds listed in Table 2 labelled Ps are pyrolysis products which are thought to be characteristic for polysaccharides. These products have been identified in the pyrolysates of cellulose, amylose and soil polysaccharide [7,12,15]. The abundant presence of anhydrosugars, pyranones and furans in the soil polysaccharide fraction indicates that this fraction consists almost entirely of hardly or non-biodegraded polysaccharides probably originating from residual plant polysaccharides and newly made microbial polysaccharides.

Compounds 185 and 203 have been tentatively identified as "levogalactosan" and "levomannosan". This identification is based on their mass spectral data (identical mass spectra when compared with levoglucosan), on the well known occurrence of galactose and mannose moieties in soil polysaccharides [16] and on the absence of these compounds in the pyrolysates of polyglucoses such as cellulose and amylose.

Compounds 116 (3-hydroxy-6-methyl-3,4-dihydro-2H-pyran-2-one) has been reported previously by Saiz-Jimenez and De Leeuw [7] in the pyrolysate of a soil polysaccharide. This compound is the same as that reported by Van der Kaaden et al. (ref. 12, peak 32) in pyrolysates of amylose.

The distribution pattern of the polysaccharide pyrolysis products encountered in the humin and humic acid fractions is similar to that observed in the soil polysaccharide fraction. In the fulvic acid B fraction the furans and to some extent the pyranones and levoglucosenone are clearly present. However, the anhydrosugars are hardly or not present. This might indicate that in this fraction there are monosaccharide moieties present in structures other than polysaccharides. A phenolic glycoside structure has been proposed for this fraction [17].

In the pyrolysis of carbohydrates, acidic conditions catalyse the formation of furans, in close analogy with the dehydration reactions of carbohydrates under aqueous acidic conditions, while alkaline conditions catalyse the breakdown of the sugar molecule to carbonyl compounds through reverse aldol condensation mechanisms [12]. In the absence of additives (as in this work), both types of reactions take place, but the ratio of furans to carbonyl compounds suggests slightly acidic conditions during pyrolysis. Because all fractions were obtained in the acidic form [1] and suspended in methanol to coat the ferromagnetic wires, it is understandable that the pyrolysis will preferentially produce furans over carbonyl compounds.

Cyclopentenones (compounds 79 and 82) have been reported in pyrolysates of soil organic matter [7,18]. Bracewell et al. [18] considered cyclopentenones as major pyrolysis products of aliphatic polycarboxylic acids and polymaleic acid and suggested that aliphatic polycarboxylic acids are important components in soil organic matter. This suggestion is not supported by previous pyrolysis data [7]. Further, cyclopentenones have been identified

as pyrolysis products from amylose [12] and are well known burned sugar aroma components [19].

Several pyrolysis products present in the polysaccharide and humin fractions (compounds 72, 125, 138, 164, 173, 184, 212, 215, 225, 235 and 239) have been found in pyrolysates of chitin [13]. It can be speculated that the chitin contribution in soil organic matter is mainly derived from fungi.

### *Lipids*

It is very likely that a major part of the lipids encountered in the pyrolysates (peaks labelled Lp) are mainly the result of evaporation and are not generated from polymeric frameworks by pyrolysis. The lipid components are predominantly present in the hymatomelanic acid fraction obtained after ethanol extraction of the humic acid fraction. This also indicates that the lipids are mainly freely occurring components, which are easily extractable as such.

Aliphatic hydrocarbons have been detected in almost every plant, animal and microorganism examined, and therefore obvious sources of soil hydrocarbons are plant and animal residues and the soil microbial populations. However, it is improbable that animal residues contribute much directly to soil hydrocarbons. The series of *n*-alkanes and *n*-alkenes ranging from C<sub>2</sub> to C<sub>23</sub> are encountered in a number of soil organic matter fractions and possibly originate from cuticle materials [20] and microbial populations [21].

Acyclic isoprenoid hydrocarbons such as prist-1-ene, prist-2-ene and phytadiene were identified in the humic acid, hymatomelanic acid and humin fractions. The phytyl side-chain of chlorophyll *a* is believed to be the source of phytadienes [22]. Recently, it has been reported that tocopherols are likely sources of pristenes, as both flash pyrolysis and thermal degradation of  $\alpha$ -tocopherol yield prist-1-ene as a major pyrolysis product [23].

Morrison [24] has reported that many substances of a lipid nature, particularly of plant origin, are likely to be present in soils. Such substances would include tocopherols and porphyrins from higher plants and may accumulate as resistant remnants of plant residues undergoing humification. Further, Wagner and Muzorewa [25] considered that lipids extracted from soil organic matter may also be of microbial origin. Microbially synthesized products of a lipid nature in soil may become incorporated into soil humus without undergoing major degradative modifications.

The methyl esters encountered are thought to be procedural artifacts formed from the free fatty acids during the preparation of the pyrolysis samples using methanol as the suspension liquid.

Although the saturated and unsaturated straight-chain fatty acids are the main components in soil organic matter fractions, they are not very characteristic as almost any organism contains these fatty acids. The relatively abundant presence of iso-, anteiso- and 10-methyl fatty acids with chain



lengths ranging from  $C_{14}$  to  $C_{20}$  are highly characteristic for a microbial input of fungi and bacteria [26,27]. The  $\alpha,\omega$ -diacids might also be the result of bacterial degradation, although an origin from higher plant waxes cannot be ruled out [28].

There can be little doubt that steroids and terpenoids of various types occur in soils, but in spite of the abundance and variety of terpenoids in plants, there are only a few reports of their presence in soils [29]. Three compounds (312, 313 and 314) were tentatively identified as terpenoid hydrocarbons, based on their mass spectral fragmentation pattern ( $m/z$  95, 109, 123, 149, 163, 191, 203, 207) [30]. These compounds (more detailed structures are as yet unknown) occur only in the humin and hymatomelanic acid fractions.

### *Lignins*

The compounds listed in Table 2 labelled Lg are well known and characteristic pyrolysis products of lignins and degraded lignins [6]. The compounds identified show that they are contributions from grasses, higher plants and/or trees, as all three types of lignin building blocks (*p*-coumaryl, coniferyl and syringyl derivatives) are present.

Obviously, lignin is present in all fractions; however, in the soil polysaccharide and the hymatomelanic acid fractions the lignin contribution is minor. Substantial amounts of lignins are present in the humic acid, the humin and the fulvic acid fractions. The distribution of the lignin pyrolysis products indicates that the lignins are partly biodegraded, as the relative amounts of  $C_3$ -alkyl components are low and as carbonyl and carboxyl functional groups are clearly present [6].

### *Proteins and peptides*

A number of pyrolysis products are of a protein origin and are labelled Pr in Table 2. Among them are the so-called "amino acid dimers", originating from valine, leucine and isoleucine pairs. This type of characteristic pyrolysis product, the structures of which are not yet completely known, are also encountered as major components in the pyrolysates of polyamino acids [14]. The peptides are present mainly in the humin and the humic acid fractions, indicating that the relatively high percentage of nitrogen as measured in these fractions (Table 1) originates from peptides.

### *Miscellaneous*

A number of components present cannot be ascribed to well defined biopolymers, because they are not known as pyrolysis products of the studied biopolymers or they are not pyrolysis products at all.

Sulphur dioxide might originate from sulphonated materials (e.g., sulphonated polysaccharides). The sulphur content of different soil polysaccharide fractions ranges between 4 and 10% [31]. Occasionally, sulphur-containing compounds such as thiophenes and thiophenols have been identified in humic acid and fulvic acid fractions, respectively.

Chlorine compounds (hydrochloric acid and chloromethane) probably arise from the hydrochloric acid employed in the fractionation and purification procedures. At present, no explanation can be given for the presence of iodomethane and 1,4-dioxane.

Among the pyrolysis products some compounds considered as pollutants were identified. Dialkyl phthalates were the most prominent, especially in fulvic acid fractions. The origin of the pollutants may be diverse [32] and they interact with the soil organic matter fractions, either in the soil or during the extraction and fractionation procedures.

### *Nature of the soil organic matter fractions*

Having discussed the origin of the pyrolysis product, it is now convenient to survey the main groups of compounds identified in each soil organic fraction.

#### *Soil polysaccharide*

The fraction obtained by the Polyclar filtration, called soil polysaccharide, consists almost entirely of polysaccharides. The major pyrolysis products encountered are well known and specific pyrolysis products of polysaccharides. Trace amounts of lignin pyrolysis products and fatty acids are also present.

#### *Humin*

The pyrolysate of this fraction consisted of a complex mixture. The main series of pyrolysis products encountered originate from polysaccharides and lignins. Lipids are significantly present, including alkanes, alkenes, fatty acids, terpenoids and pristenes. The presence of peptides is also evident. Pollutants are minor products.

#### *Humic acid*

The major pyrolysis compounds encountered are lignin derivatives. Polysaccharide products are also clearly present, whilst peptide pyrolysis products are less prominent. Lipids are minor components.

#### *Hymatomelanic acid*

This fraction consists almost entirely of lipids including alkanes, alkenes, acyclic isoprenoid hydrocarbons, fatty acids and aliphatic dicarboxylic acids. Lignin pyrolysis products are minor compounds.

#### *Fulvic acid (Polyclar)*

The initially retained fraction during the Polyclar filtration, called fulvic acid, contains mainly two series of pyrolysis products. Most abundant are the lignin pyrolysis products. A series of pyrolysis products originating from polysaccharides are also clearly present. Fatty acids and dialkyl phthalates are encountered in minor amounts.

#### *Fulvic acid B (charcoal)*

This fraction shows two main series of pyrolysis products originating from lignins and/or polyphenols and carbohydrates. Dialkyl phthalates are present, in addition to minor amounts of fatty acids.

#### *Fulvic acid D (charcoal)*

Major products in the pyrolysate of this fraction are dialkyl phthalates and pyridine. Lignin and carbohydrate pyrolysis products are less important than in the other fulvic acid fractions.

### CONCLUSIONS

1. In the A<sub>1</sub> horizon of the studied Typic Xerochrept soil about 10% of the total organic matter is present as carbohydrate in the soil polysaccharide fraction [1]. In addition, various amounts of polysaccharide moieties are present in the humic fractions.

2. The structures of the pyrolysis products encountered in the humic acid fraction are also observed in more or less pure lignin preparations and in polysaccharides. Hence, there is no need to assume that the pyrolysable part of the humic acid fraction consists of a condensed lignin-polysaccharide structure; a simple mixture of these two biopolymeric substances can explain the results satisfactorily.

3. As already pointed out for the humic acid fraction, the pyrolysis data for the other humic fractions also indicate that there is no direct need to assume that humic substances are generated by condensation reactions of lipids, carbohydrates, amino acids, etc. On the contrary, mixtures of more or less biodegraded biopolymers and originally present low-molecular-weight compounds explain the pyrolysis data very well.

4. The chemical contents of the studied humic fractions are probably determined by the solubility of the individual components (e.g., polyphenolic substances such as lignins dissolve in base, but precipitate on acidification; most polysaccharides remain in aqueous solution; lipids do not dissolve in water but do so in ethanol; lipoproteins, glycolipids, glycoproteins, etc. [33] will end up in almost any fraction).

5. Our results indicate that hymatomelanic acid, a term introduced by Hoppe-Seyler in 1889, cannot be considered as a humic fraction, as it

consists almost entirely of lipid compounds extractable from the humic acid fraction. Probably extraction of the intact soil with toluene-methanol, for example, prior to fractionation would considerably reduce or completely eliminate the humatmelanic acid fraction.

6. It is clear from the results obtained from the different fulvic acid fractions that the chemical composition of these fractions is a consequence of the fractionation procedure followed. These data are in agreement with previous observations [34].

7. Finally, the Py-GC-MS technique as applied in this study is a powerful method for chemically characterizing considerable amounts of soil organic matter.

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