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*Soil Chemistry*

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# USE OF HPLC, Py-GCMS, FTIR METHODS IN THE STUDIES OF THE COMPOSITION OF SOIL DISSOLVED ORGANIC MATTER

*Abstract.* The study has determined the composition of dissolved organic matter in Luvisols, Fluvisols and Histosols using spectroscopic (FTIR) and chromatographic (HPLC and Py-GCMS) methods. It has been found that aliphatic hydrocarbons (linear) containing from 4 to 12 atoms of carbon constitute the dominant group of compounds included in the dissolved organic matter (DOC). The preparations isolated from Histosols and Luvisols demonstrated a higher proportion of hydrophobic fraction with a longer retention time probably containing more compounds with long-chain aliphatic and simple aromatic structure than the DOC of Fluvisols. The differences in infrared spectra are evident particularly in the wave number between 1650–1030 $cm^{-1}$. The DOC of Histosols is richer in aromatic compounds (range 1620 $cm^{-1}$) but the DOC of Luvisols and Fluvisols is richer in alkene chains and hydroxyl (OH) and methoxy ($OCH_3$) groups. The results showed differences in the composition of the DOM across the soils, caused their genesis.

The presence of organic matter is one of the most essential soil characteristics, which is connected with the key role played by humus in most processes which occur in the pedosphere [13]. In terms of ecology, a very important role in the soil environment is played by the most mobile and quickly decomposing humus fraction; the dissolved organic matter (DOM) [10]. The DOM is a reactive fraction of organic matter which controls a number of chemical, physical and biological processes which occur in the soil and other components of environment [2].

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The dissolved organic matter may inhibit or release minerals from soil [23], nutrients [9] and hazardous compounds [17]. The DOM is thus one of the components of the environment which, indirectly or directly, affects the state of pollution of soils and waters as well as, as reported by Kalbitz and Kaiser [14], global warming.

The effect of the dissolved organic matter on the processes which occur in the soil ecosystem and the physical and physicochemical degradation of soil depends not only on the concentration, but also on its chemical composition [3]. Huang et al. [12] report on the DOM not being a simple mixture of monomeric or dimeric compounds. Kalbitz and Kaiser [15] claim that aromatic compounds are probably the most stable part of the DOM. Sleighter et al. [21] claim that the DOM which occurs in soil is more aromatic than the DOM which occurs in water and contains more carboxyl and hydroxyl functional groups. According to Gonet et al. [10], the DOM composition may include carbohydrates, proteins, fats, hydrocarbons and their derivatives, low-molecule fractions of humus acids as well as many other simple organic compounds. Aiken [1] reports on the DOM composition containing simple organic compounds demonstrating non-specific humus substances (fatty acids, organic compounds, amino acids, sugars) as well as water-soluble substances which are humus in nature.

The aim of the paper has been to determine dominate organic carbon structures in the DOM originated from soils using the infrared spectroscopy (FTIR) as well as HPLC and Py-GCMS chromatographic methods.

## MATERIAL AND METHODS

The research used dry soil samples which differed significantly in the content of organic carbon (TOC, Table 1). Soil was sampled from the layer Ap (A) of soil under agricultural use (Luvisols) and meadow (Fluvisols, Histosols). The content of organic carbon (TOC) and total nitrogen (Nt) were assayed by means of the analyser Vario Max CNS supplied by Elementar (Germany).

The dissolved organic matter (DOM) was extracted with 0.004 M $CaCl_2$ at the ratio: soil sample: extractant as 1:10 (for Histosols 1:50); extraction time 1 h [10]. Having completed the extraction, the solutions were centrifuged, the contents of the DOC and DNt were sampled to be assayed and from the residue water was evaporated and the sediment was pulverised.

The content of the dissolved organic carbon (DOC) and nitrogen (DNt) in the DOM solutions were determined by means of the analyser Multi N/C 3100 (Analityk Jena). The content of the DOC and DNt was expressed in mg $kg^{-1}$ d.m. of soil as well as % share in the pool, the TOC and Nt, respectively.

For the isolated DOM preparations, the following analyses were performed:

– infrared spectrometry. Infrared spectra were made in the range of 400-4000 $cm^{-1}$, for tablets 3 mg DOM in 800 mg KBr (by means of the spectrometer FT-IR Spectrum BX supplied by Perkin-Elmer);

– the chromatographic separation into hydrophilic and hydrophobic fractions. The DOM chromatographic separation was made by means of the liquid chromatograph HPLC Series 200 provided by Perkin–Elmer equipped with DAD (diode array detector). The analytic column X-Terra C18 with the molecules 5 μm in size and the dimensions of 250 × 4.6 mm I.D was used. The samples of the dissolved organic matter were solved in acetonitrile. The following conditions of the analyses were applied: for the separation – the eluent with the following composition: acetonitrile-water and gradient program of elution (Fig. 1). The injection was 100 μl, detection was performed at the wavelength of 254 nm. Based on the areas determined under peaks, the percentage share of hydrophilic (HIL) and hydrophobic fractions (HOB 1, HOB 2, HOB 3) was calculated [7, 20, 25];

– pyrolysis gas chromatography with mass spectrometry. The analysis of the spectra of the studied samples of dissolved organic matter was performed using the gas chromatograph Turbo Mass Gold Instrument provided by Perkin Elmer and pyrolysis Pyrojector II provided by SGE. Each sample was exposed to pyrolysis at the temperature of 770°C and then the products of pyrolysis were directly transferred to silicone chromatographic column Elite – 5MS provided by Perkin Elmer, 30 m long, 0.25 mm in inner diameter and the film thickness of 0.25 μm. Helium was used as the carrier gas at the flow rate of 1.5 ml $min^{-1}$. For the MS analysis purposes, the following temperature program was applied: initial furnace temperature 40°C, maintained for 5 min, increase in temperature 10°C $min^{-1}$ to 300°C, end temperature maintained for 15 min. The conditions for the mass spectrometer were as follows: the temperature of the source of ions: 150°C, electron energy: 70 eV. The products of the pyrolysis were identified using the NIST mass spectra library and the comparison with data reported in literature.

## RESULTS AND DISCUSSION

The content of organic carbon in the samples ranged from 13.77 g $kg^{-1}$ (Luvisols) to 384.2 g $kg^{-1}$ (Histosols). The highest total nitrogen was found in the Histosols sample (25.90 g $kg^{-1}$), and the smallest – Luvisols (1.26 g $kg^{-1}$). The highest content of extractable organic carbon was recorded in Histosols, while the lowest – Luvisols; a similar relationship occurred for extractable nitrogen (DNt). The highest percentage share of the DNt was demonstrated for Luvisols, while the lowest – the Histosols sample. The share of the DOC in the organic carbon pool was inconsiderable and highest for the Luvisols sample, and the lowest – for Fluvisols (Table 1). As reported by literature [10], the DOC content

generally correlates with the contents TOC. Participation DOC often shows an inverse relationship, i.e. a negative correlation with the TOC or lack thereof.

TABLE 1. BASIC PROPERTIES OF THE INVESTIGATED SOILS

| Sample | TOC | Nt | DOC | DNt | DOC (%) | DNt (%) | TOC/ Nt | DOC/ DNt |
|---|---|---|---|---|---|---|---|---|
| | g kg$^{-1}$ | | mg kg$^{-1}$ | | | | | |
| Luvisols | 13.77 | 1.26 | 126.20 | 41.90 | 0.92 | 3.33 | 10.93 | 3.01 |
| Fluvisols | 34.02 | 2.69 | 191.00 | 50.30 | 0.56 | 1.87 | 12.65 | 3.80 |
| Histosols | 384.2 | 25.90 | 2577.00 | 114.90 | 0.67 | 0.44 | 14.83 | 22.43 |

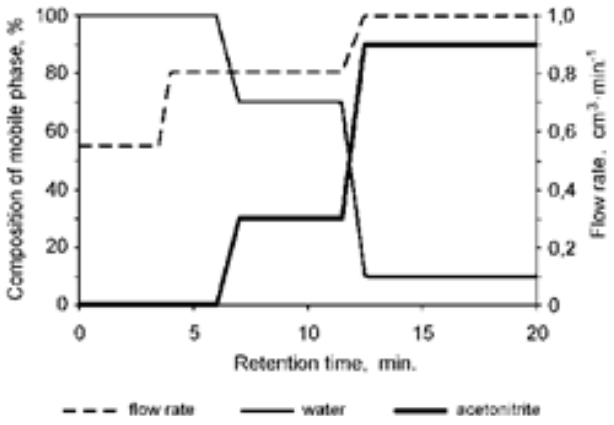


Fig. 1. Gradient program used to separate DOM.

The quality features of the DOM fractions isolated were determined with the use of spectroscopic and chromatographic methods. The methods of spectroscopy are an important tool used in the environmental analyses, mostly to study the structure of complex organic compounds. The FTIR is a very important quality method used to provide characteristics of chemical groups of organic substances; the method is commonly used in the analyses of soils and organic matter [4]. The application of the FTIR facilitates collecting information on the aromaticity of the DOM molecules and the content of functional groups, carbohydrates and simple organic compounds [16, 19]. In the FTIR spectra of the preparations of dissolved organic matter (Fig. 2), the presence of the band in the range of wave numbers 3100-3600 cm$^{-1}$ was found. The band corresponds to stretching vibrations of groups OH in alcohols, phenols and acids as well as stretching vibrations NH. The band (inflexion) in the range of 2920-2960 cm$^{-1}$ is conditioned by the presence of groups $-CH_3$ and $=CH_2$, the bands in the range of wave numbers 1600-1660 cm$^{-1}$ are connected with the occurrence of stretching vibrations C=O (1630-

1680 cm$^{-1}$), deformation vibrations NH (1620-1660 cm$^{-1}$) in primary amides, and stretching amides C=C (1610 cm$^{-1}$) in the aromatic ring. The band 1600-1625 cm$^{-1}$ also points to the presence of groups $COO^-$. In the range 1600-1660 cm$^{-1}$, the bands derived from the vibrations of groups C=N may occur, as well as C=C coupled with the carbonyl group. The band in the range of wave numbers 1440-1460 cm$^{-1}$ corresponds to the deformation vibrations of groups $–CH_3$ and $=CH_2$, the band with the maximum at about 1384 cm$^{-1}$ connected with the presence of groups $COO^-$ and bonds C–H in the aliphatic chain, bands 1030-1080 cm$^{-1}$ may be related to the presence of groups $OCH_3$ as well as OH in alcohols. The bands which occur in the range of 900-400 cm$^{-1}$ are connected with the presence of aromatic bonds and the presence of inorganic compounds [5, 11, 18, 22].

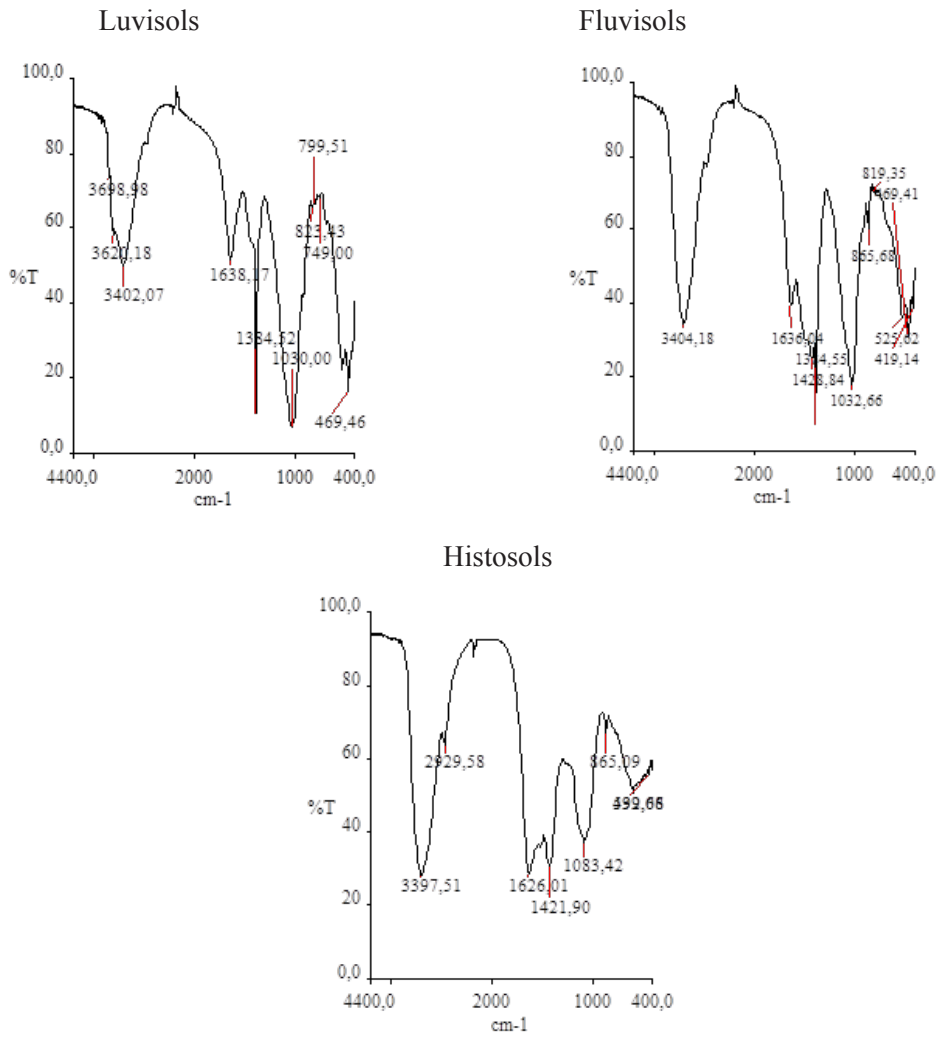


Fig. 2. Infrared spectra of the analyzed fraction of DOM from the different type of soils.

The pattern of infrared spectra clearly points to the difference in the structure of the DOM preparations. The least mineral compounds was contained in the preparations derived from Histosols, showing the highest intensity of the bands in the range of wave numbers 1600-1660 $cm^{-1}$ as well as 1440-1460 $cm^{-1}$. In the DOM spectra from Histosols , inflexion at 1500 $cm^{-1}$was identified, which points to the possibility of the occurrence of aromatic bonds.

However, the pattern of infrared spectra of the DOM preparations received from Luvisols and Fluvisols points to the presence of aliphatic chains in their composition, containing multiple bonds and carboxyl and amine functional groups. The DOM fraction isolated from Luvisols and Fluvisols is richer in alkene chains and hydroxyl groups (OH), methoxy ($OCH_3$) groups, as compared with the DOM isolated from Histosols.

The HPLC method application facilitates the separation of the molecules found in the DOM into hydrophilic and hydrophobic fractions. As reported by the organic matter fractions, the research performed [6-8], the peaks which occur in the range from 0 to 6 min correspond to the fractions with hydrophilic properties whereas the peaks with longer retention times – to the fractions with hydrophobic properties.

In the preparations studied, hydrophobic fractions with the following retention times: HOB 1 from 12.3 to 13.3; HOB 2 from 13.3 to 16.2; HOB 3 from 16.2 to 18.9 min were separated (Fig. 3).

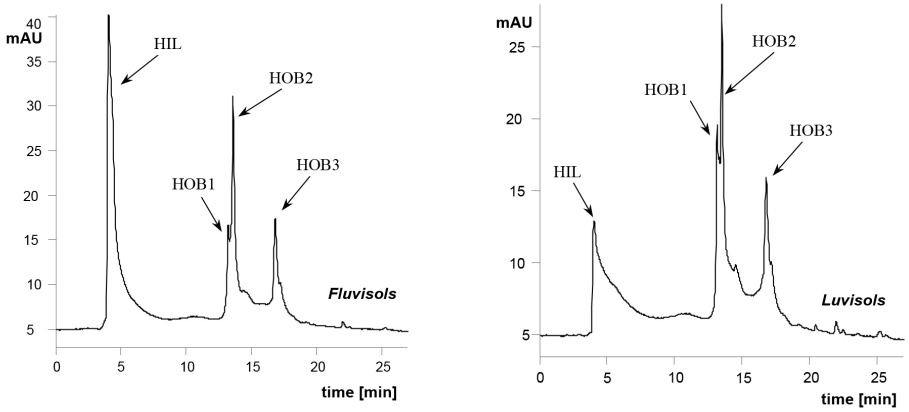


Fig.3. HPLC chromatograms of fractions of soil dissolved organic matter.

The percentage share of hydrophilic fractions ranged from 31.86% to 66.54%, and hydrophobic – from 33.46 % to 68.14 % (Table 2). A two-fold higher percentage share of hydrophobic fractions was noted for the DOM preparations isolated from Luvisols and Histosols, as compared with the DOM of Fluvisols.

The DOM isolated from Histosols and Luvisols showed a much higher share of hydrophobic fractions with a longer retention time, as compared with

the DOM of Fluvisols. It is also worth stressing that the fractions dominating among hydrophobic fractions were HOB 1 and HOB 2 fractions. The percentage share of those fractions in the DOM preparations accounted for: 23.36% – Fluvisols; 44.87% – Histosols; 45.66% – Luvisols, respectively. Drawing on the literature reports [25], one may assume that the DOM preparations isolated from Luvisols and Histosols showed a higher percentage share of the HOB 3 fraction that most probably contains more compounds with long aliphatic chains or simple aromatic structures.

TABLE 2. SHARE (%) OF HYDROPHILIC (HIL) AND HYDROPHOBIC (HOB) FRACTIONS

| Sample | HIL | HOB 1 | HOB 2 | HOB 3 | ΣHOB |
|---|---|---|---|---|---|
| Luvisols | 32.63 | 9.64 | 36.02 | 21.71 | 67.37 |
| Fluvisols | 66.54 | 5.06 | 18.30 | 10.10 | 33.46 |
| Histosols | 31.86 | 10.84 | 34.03 | 23.27 | 68.14 |

Pyrolysis gas chromatography with mass spectrometry is an instrumental tool facilitating a quantitative identification of respective DOM compounds [24]. The compounds identified being a part of dissolved organic matter are presented in the Table 3. As seen from the data given in the Table 3, the differences in the DOM qualitative composition isolated from Luvisols and Fluvisols and the DOM isolated from Histosols occurred, e.g. the DOM Histosols showed the presence of sulphur-containing compounds.

The identified products of pyrolysis of the studied DOM were divided into groups: aliphatic hydrocarbons, aromatic hydrocarbons and hydrocarbons aliphatic-aromatic. Aliphatic hydrocarbons (chain-like) containing from 4 to 12 carbon atoms constitute the dominant group of the compounds contained in the DOM (Table 4). The percentage share of aliphatic hydrocarbons ranged from 53.64% to 57.28%. The highest percentage share of that group of compounds was demonstrated for Luvisols and the lowest – Fluvisols. The percentage share of hydrocarbons aliphatic-aromatic was, in general, similar to the percentage share of aromatic compounds. The hydrocarbons aliphatic-aromatic and aromatic were dominated by compounds with a single benzene ring. The highest percentage share of aromatic hydrocarbons was found for the sample isolated from Histosols (25.6%), while the highest percentage share was found for hydrocarbons aliphatic-aromatic – the sample isolated from Fluvisols (24.25%).

TABLE 3. IDENTIFIED PRODUCTS IN THE PYROLYSIS OF SOIL DOM

| Aliphatic hydrocarbons | Aromatic hydrocarbons | Aliphatic-aromatic hydrocarbons |
|---|---|---|
| 1,3-butadien | benzen | etylobenzen |
| 1,3-butadien,2-metyl | ksylen | 2-fenylonitroetan |
| methylenecyclopentene** | bifenyl | 2-ethyl-6-(2,4,6-trimethylphelnyl) benzothiazole* |
| spiro(2,4)hepta-2,6-diene | naphthalene,1-methyl | indene |
| 5,6-dimethylenebicyclo(2,2,1)-2-heptene | 1-naftol | 1H-indene,2,3-dihydro-1-methyl |
| | acetonaphthalene* | azulene |
| | | 1H-indole-5-carbonitrile* |
| | | 1H-indene,1-ethylidene ** |
| | | 5,8-epoxy-5,8-dihydroisoquinoline** |
| | | 3-benzylpyridine** |
| | | fluorene |
| | | 1-naphthalenol,3-methyl** |
| | | 9-metylofluorene |
| | | indeno-inden |

* compounds found only in Histosols;
** compounds found only in Luvisols and Fluvisols.

TABLE 4. THE SHARE OF INDIVIDUAL GROUPS OF HYDROCARBONS (%) IN DOM

| Sample | Aliphatic hydrocarbons | Aromatic hydrocarbons | | Aliphatic-aromatic hydrocarbons | |
|---|---|---|---|---|---|
| | | Numbers of rings | | | |
| | | 1 | 2 | 1 | 2 |
| Luvisols | 57.28 | 19.22 | 3.02 | 16.65 | 3.82 |
| Fluvisols | 53.64 | 19.01 | 3.10 | 18.30 | 5.95 |
| Histosols | 54.73 | 23.02 | 2.58 | 19.07 | 0.61 |

## CONCLUSIONS

1. The results point clearly that the methods: infrared spectroscopy, HPLC (High Performance Liquid Chromatography) and Py-GCMS – the analysis of the DOM pyrolysis products with the method of mass spectroscopy may be applied to determine the structure (composition) and/or differences in the DOM composition of various origin.

2. The application of the said methods showed differences in the quantitative and qualitative composition of compounds being a part of the dissolved organic matter. The differences may affect the role played by the DOM fraction in the soil environment as well as in the freshwaters.

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## WYKORZYSTANIE HPLC, Py-GCMS I FTIR W BADANIACH SKŁADU ROZPUSZCZALNEJ MATERII ORGANICZNEJ GLEB

W pracy badano skład rozpuszczalnej materii organicznej (RMO) gleb (Luvisols, Fluvisols and Histosols) przy zastosowaniu metod spektroskopowych (FTIR) oraz chromatograficznych (HPLC i Py-GCMS). Stwierdzono, że dominującą grupą związków wchodzących w skład RMO są węglowodory alifatyczne (łańcuchowe) zawierające od 4 do 12 atomów węgla. Preparaty RMO wyizolowane z torfu i gleby płowej charakteryzujące się wyższym udziałem frakcji hydrofobowych o najdłuższym czasie retencji, zawierały najprawdopodobniej więcej związków o długich łańcuchach alifatycznych oraz proste struktury aromatyczne w porównaniu z RMO mady. Przebieg widm w podczerwieni wyraźnie wskazał różnicę w składzie badanych preparatów RMO, szczególnie w zakresie liczb falowych między 1650-1030 $cm^{-1}$. Preparaty RMO wyizolowane z torfu były bogatsze w związki aromatyczne (pasmo 1620 $cm^{-1}$) a frakcja RMO wyizolowana z gleby płowej i mady ciężkiej była bogatsza w łańcuchy alkenowe i grupy hydroksylowe (OH) i metoksylowe ($OCH_3$). Otrzymane wyniki badań wykazały różnice w składzie RMO pomiędzy glebami, wynikające z ich genezy.