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Properties of humic acids in meadow soils irrigated with the slope-and flooding system

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Abstract

The aim of the research has been to determine the effect of many-year irrigation of unique grasslands on the properties of humic acids defining the quality of organic matter. The research was performed based on the soil (*Albic Brunic Arenosol*, the A, AE and Bsv horizons) sampled from Europe's unique complex of permanent grasslands irrigated continuously for 150 years, applying the slope-and-flooding system; the Czerskie Meadows. The soil samples were assayed for the content of total organic carbon (TOC) and the particle size distribution. HAs were extracted with the Schnitzer method and analysed for the elemental composition, spectrometric parameters in the UV-VIS range, hydrophilic and hydrophobic properties and the infrared spectra were produced. The research results have shown that the HAs properties depended on the depth and the distance from the irrigation ditch. The HAs of the A horizon of the soils were identified with a lower "degree of maturity", as reflected by the values of atomic ratios (H/C, O/C, O/H), absorbance coefficients, and the FT-IR spectra, as compared with the HAs of the Bsv horizon. The HAs molecules of the soils sampled furthest from the irrigation ditch were identified with a higher degree of humification, as compared with the HAs of the soils sampled within the closest distance. The results have demonstrated that many-year grassland irrigation affected the structure and the properties of humic acids.

Keywords: Meadow soil, Humic acids, Elemental composition, UV-VIS, HPLC

1. Introduction

Meadow soils provide good conditions for the accumulation and immobilisation of organic substance ¹. For that reason, those ecosystems stand for the land use preventing from organic carbon (OC) losses in soils. The OC losses, as a result of the soil processes, are CO₂ and CH₄ gas emissions as well as soluble organic carbon leaching to groundwaters. It was demonstrated that meadow ecosystems help limiting OC losses from soil and

they are important for a balanced management of that element in the environment^{2, 3, 4, 5, 6}. Besides, permanent grassland soils are an important reservoir of organic carbon (OC), and its resources are similar or higher than the carbon resources in forest soils.

The main factor of organic matter are humus substances being most common organic compounds found in nature^{7, 8, 9}. Humus substances are produced as a result of the processes of transformation and decomposition together referred to as “humification”. The term “humus substances” is used for a scientific description of specific nutrients of a complex structure which can be isolated and fractioned in many ways¹⁰. Those compounds take part in all the processes in soil and they affect its physical, chemical and biological properties, for example, improving soil buffering capacity, supplying plants with available micronutrients, immobilizing organic contaminants and metals^{11, 12, 13, 14}. Humus substances also determine the production potential of soil by taking part in the global carbon cycling, they perform environmental functions related to carbon sequestration and a release of CO₂ to the atmosphere^{15, 16}. With the research of the properties of humic acids, one can determine the advancement of transformation and decomposition processes.

To evaluate the properties of humic acids, the elemental composition and the values of H/C, O/C, O/H and N/C atomic ratios determined from it are used. The parameters facilitate an approximate determination of the structure of the humic acid molecules by evaluating the degree of condensation of aromatic rings (H/C ratio) and the degree of maturity (O/C, O/H, N/C ratios)^{17, 18, 19, 20}.

The structural characteristics of humic acids, especially identifying the functional groups, are assayed from the infrared spectra (FTIR)^{21, 22, 23, 24}. The parameters which, e.g., reflect the character and the origin of humic acids are the values of absorbance of their solutions in the UV-VIS spectrum: A₂₈₀, A₄₆₅, A₆₆₅ and the coefficients of absorbance A_{2/4}, A_{2/6}, A_{4/6}, ΔlogK. They are important indicators of the organic matter humification advancement^{21, 24, 25, 26, 27, 28, 29, 30}.

Interesting information on humic acid transformation is provided by the results of the high-performance liquid chromatography. Banach-Szott and Dębska³¹, Debska et al.³², Preuße et al.³³, Sierra et al.³⁴, Woelki et al.³⁵, with the chromatographic analysis, separated the hydrophilic (HIL) and hydrophobic (HOB-1 and HOB-2) fractions. The ratios of those fractions affect the solubility of humic acids and, as a result, their migration deep down the soil profile. According to Debska et al.³⁶ and Debska and Gonet³⁷, the higher the degree of humification, the higher the share of hydrophilic fraction in the molecules of humic acids, and the lower the share of hydrophobic fractions. As a result, the HAs molecules with a higher degree of “maturity” showed a higher HIL/ΣHOB value.

Thanks to the studies of humic acids properties, one can determine the advancement of the processes of transformation and decomposition of organic matter which is the source of organic carbon. Due to increasing soil degradation and total organic carbon (TOC) losses due to, e.g., changes in soil use and an intensified agricultural production, the local, regional and global soil protection has become one of the key goals of the European Community policies³⁸. Importantly, due to a specific biodiversity, meadow ecosystems, including the ones the research was performed in, are one of the key methods of the agricultural land use preventing the losses of organic carbon in soils. With that in mind, the aim of the present paper has been to determine the impact of many-year irrigation of unique grasslands on the properties of humic acids determining the quality of organic matter.

2. Materials and Methods

2.1. Materials

Soil was sampled from perennial grasslands of the unique “Czersk Meadow Complex”, continuously irrigated for about 150 years with the slope-and-flooding system. The name “Czersk Meadow Complex” covers a historic term for perennial grasslands with the total area of almost 2000 hectares which used to be considered meadow and pastureland, and arranged in various parts of the Tuchola Forest³⁹. For meadow irrigation, mostly the rivers of the Tuchola Forest, the Brda and the Wda River, were used. A sensation is the fact that the waters from the irrigation ditches were distributed across the meadows with a system of channels, ditches and overflow furrows creating a unique irrigation system referred to as the slope-and-flooding system.

The soils (*Albic Brunic Arenosol*, the A, AE and Bsv horizons) were sampled in three replications 5, 15 and 25 m away from the irrigation ditch in the area of Kamionna (53°50' N; 18°09' E), Cegielnia (53°53' N; 18°07' E) and Podlesie (53°51' N; 18°08' E) quarters. The soil samples were dried in room temperature and sieved (2 mm). In total the research covered 9 soil profiles (a total of 27 soil samples).

2.2 Methods

2.2.1. Soil analysis

For air-dry soil samples, the following analyses were made:

- the content of total organic carbon (TOC) and total nitrogen (Nt). The content of organic carbon and total nitrogen were assayed with the Vario Max CN analyser provided by Elementar (Germany). The content of TOC and the content of Nt were expressed in g kg⁻¹ of d.w. of soil⁴⁰;
- pH – in the suspension of distilled water and soil with the pH-meter MultiCal pH 540 GLP WTW⁴¹;
- grain size composition was determined applying the aerometric method⁴²;

2.2.2. *Extraction of humic acids*

Humic acids (HAs) were extracted and purified with standard methods using the following procedure:

- decalcification (24 h) with 0.05 M HCl (1:10 w/v). After centrifugation, the residue was washed with distilled water till neutral;
- extraction (24 h) of the remaining solid with 0.5 M NaOH (1:10 w/v), with occasional mixing, followed by centrifugation;
- precipitation (24 h) of humic acids from the resulting alkaline extract with 2 M HCl to pH=2 and centrifugation;
- purification of the resulting humic acids as follows: the humic acid residue was treated with a mixture of HCl/HF (950 mL H₂O, 5 mL HCl, 5 mL HF) over a 24 h period, followed by centrifugation. The humic acid residue was treated with distilled water until a zero reaction to chloride was achieved.

The preparations were lyophilised and powdered in agate mortar. Ash content in the humic acids preparations was lower than 2%.

2.2.3. *Characteristics of humic acids*

In the separated humic acids the following were assayed:

- elemental composition (Perkin Elmer 2400 CHN analyser). The H/C, O/C, O/H, N/C atomic ratios and ω (internal oxidation degree) were calculated according to the formula:

$$\omega = (2O + 3N - H) : C$$

where: O, N, H, C – content in atomic %;

- hydrophilic and hydrophobic properties were determined with liquid chromatograph HPLC Series 200 with DAD detector by Perkin-Elmer. The separation involved the use of column X-Terra C18, 5 μ m, 250 x 4.6 mm. The solutions of humic acids were applied in 0.01 mol/L NaOH of the concentration of 2 mg/mL; injection of the sample – 100 μ L; solvent: acetonitrile–water; solvents flow in the gradient (ratio H₂O:ACN (v/v) over 0-6 min. – 99.5 : 0.5, 7-13 min. – 70 : 30, 13-20 min. – 10 : 90); detection – at the wavelength of 254 nm. Based on the areas determined under the peaks, the share of hydrophilic (HIL) and hydrophobic (Σ HOB = HOB1 + HOB2) fractions in humic acid molecules and the parameter HIL/ Σ HOB were determined^{33, 35, 36}.
- UV-VIS absorption spectra (Perkin Elmer UV-VIS Spectrometer, Lambda 20). VIS spectra were obtained from 0.02% humic acid solutions in 0.1 M NaOH and UV-spectra after fivefold dilution.

Absorbance was measured at 280 nm (A_{280}), 400 nm (A_{400}), 465 nm (A_{465}), 600 nm (A_{600}) and 665 nm (A_{665}) was used to calculate the coefficient values:

$A_{2/4}$ – 280 nm and 465 nm absorbance ratio

$A_{2/6}$ – 280 nm and 665 nm absorbance ratio

$A_{4/6}$ – 465 nm and 665 nm absorbance ratio

$$\Delta \log K = \log A_{400} - \log A_{600}^{25};$$

- infrared spectra (Perkin-Elmer FT-IR Spectrometer, Spectrum BX) over 400 – 4400 cm^{-1} were produced for HAs (3 mg) in KBr (800 mg). Deconvolution was applied, with a filter making the bands of $\gamma = 4$ narrower, and using the process of smoothing, for which the length parameter was $l = 80\%$ ²².

2.2.3. Statistical analyses

The soil properties were treated with descriptive statistics: arithmetic mean, minimum value, maximum value, standard deviation, coefficient of variation. The statistical analyses of humic acids involved the analysis of variance of the data derived from a single two-factor experiment in a randomised split-plot. The tables present the mean values from three replications. The significance of differences was evaluated using the Fisher test (LSD) at a significance level of $\alpha = 0.05$. The similarities across the objects were evaluated using cluster analysis. The method involves dividing the data set into groups to produce clusters in which the elements are similar to one another and, at the same time, different from the elements from the other groups. The groups of similar treatments are presented in a form of dendrogram. In a given group the smaller the Euclidean distance, the more similar the objects. Data clustering was performed with the Ward method ⁴³. The analysis was made after data standardization. The cluster analysis was performed based on the elemental composition (H/C, O/H, O/C, ω), spectrometric parameters ($A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta \log K$) as well as the HIL/ Σ HOB parameter. The method involves dividing the data set into groups to produce clusters where the elements are similar to one another and, at the same time, different from the elements of the other groups. The relationships were determined using the Statistica MS 12.0 statistics software.

3. Results and Discussion

3.1. Basic parameters of soils

A long-term, surface irrigation of soils with the slope-and-flooding system affected the contents of TOC and Nt in the soil samples (Table 1). The results confirm that the expanded root system of graminaceous plants increases the content of organic matter in the AE and Bsv horizons of soils. The importance of the processes enhancing the enrichment in organic matter in the upper layers of the meadow soil profile is also stressed ^{44, 45, 46}.

Under constant meadow irrigation, in general, the highest values of the TOC/Nt ratio were reported for the soil sampled closer to the irrigation ditches, which is the key consequence below the nitrogen content in those samples. It was found that the highest TOC/Nt value was noted for the AE horizon of the soils of the highest carbon content. The soil pH values ranged from 5.3 to 7.0 and they were changing inconsiderably, which is confirmed by a very low value of the coefficient of variation (CV) (Table 2). The soils showed a high content of the sand fraction (2.0-0.05 mm), which ranged from 86.57%-93.97% and a very low content of clay fraction (<0.002 mm) accounting for 0.32% (Table 2).

3.2. Elemental composition of humic acids

Basic indicators used in an assessment of humic acid properties include elemental composition and the values of atomic ratios calculated from the composition (Table 3). The H/C values (1.10-1.35) were similar to the values reported by Zdenek and Tesarova⁴⁷ and they show that the structure of the humic acids corresponds to the aromatic systems coupled with the aliphatic chain, containing up to 10 atoms of C¹⁷. The H/C values above 1 can point to the advantage of aliphatic structures in the molecules of humic acids⁴⁸. Humic acids showed a significant decrease in the value of the H/C ratio with depth, whereas the distance from the irrigation ditch affected the value of the ratio only in the HAs molecules of the soils in Cegielnia; an increase in the parameter value with an increase in the distance was recorded. It was shown that a decrease in the value of the H/C ratio indicates an increase in the degree of the aromaticity of humic acids, which, in turn, suggests an increase in their degree of humification (degree of maturity)^{47, 49, 50}. Dergacheva et al.⁵¹ stress that the value of the H/C ratio depends on the conditions the humic acids originated in. The parameters used to describe the advancement of the process of humification also provide the degree of internal oxidation of the HAs molecules and the ratios O/C, O/H, N/C and ω . Higher ω , O/C, O/H values and lower H/C values correspond to the humic acids with a higher “degree of maturity”^{52, 53}. For the HAs molecules, the N/C values ranged from 0.054 to 0.081, O/C from 0.529 to 0.635, and the O/H values ranged from 0.435 to 0.515. The value of parameter ω fell within the range from 0.041 to 0.208. The HAs molecules of soils, in general, showed an increase in the value of O/H and parameter ω and a decrease in the value of the N/C ratio with depth, which points to a higher degree of maturity of the HAs molecules of the Bsv horizon, as compared with the humic acids of the A horizon. There were demonstrated no definite dependencies between the distance of the soil sampling from the watercourse. Only in the case of humic acids of the soils sampled in Podlesie, the values of the ratio O/C, O/H and parameter ω were decreasing together with the distance from the watercourse.

3.3. Spectrometric parameters of humic acids in the UV–Vis range

Based on absorbance values of humic acids in the UV-VIS range and the coefficients of absorbance $A_{2/4}$, $A_{2/6}$, $A_{4/6}$, $\Delta\log K$, the following properties can be determined: the degree of advancement of organic material humification as well as changes in the properties of the humic acids which occur due to various anthropogenic factors^{24, 26, 27, 28, 29, 30}. The HAs molecules were identified with a decrease in the values of $A_{2/4}$, $A_{2/6}$, $A_{4/6}$, $\Delta\log K$ with depth (Table 4). Drawing on literature reports^{54, 55}, it was found that the humic acids of the A horizons revealed a lower degree of humification, as compared with the humic acids of deeper horizons. The distance factor had a significant effect on the spectrophotometric parameters of the HAs molecules of the soils sampled in Cegielnia and in Kamionna. In Kamionna the coefficients of absorbance of the HAs sampled 5 m away from the irrigation ditch were higher, as compared with the sites further away from the watercourse. Whereas, in Cegielnia, with an increasing depth, only a significant decrease in the value $\Delta\log K$ was recorded. The values of the coefficients of absorbance $A_{2/6}$ and $A_{4/6}$ in this location were also decreasing, however, they were insignificant. The results suggested that the further from the watercourse, the higher the degree of humification in the molecules of HAs in the soil sampled from those locations. As for the humic acids of the soils sampled in Podlesie, there were observed decreased, although insignificant, values of the coefficients of absorbance $A_{4/6}$ and $\Delta\log K$ with an increase in the distance, which can suggest an increase in the degree of humification of the molecules of humic acids. Importantly, the values of the coefficients of absorbance $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta\log K$ were lower than the values for the HAs molecules of meadow soil reported by Drąg et al.⁵⁶. Similarly, Jończak⁵⁷, investigating, e.g., the humic acids of meadow soils sampled in the Slawno Plain, Poland, Kobierski et al.⁵⁸ for the molecules of HAs of the soils sampled from the floodplain between the Vistula riverbed (Poland) and the flood embankment recorded higher values of the coefficient of absorbance $A_{4/6}$. It confirms a high degree of transformation of organic matter in the soils, and thus a high humus quality.

3.4. Analysis of the Fourier transform infrared (FTIR) spectra of humic acids

The FTIR spectra of the HAs (Fig. 1) revealed the presence of the absorption bands the ranges of which and their corresponding functional groups are given in Table 5^{59, 60, 61}. The intensity of the absorption bands in the molecules of HAs depended on the depth and, to a little extent, on the distance from the irrigation ditch. The FT-IR spectra of humic acids of the soils in Kamionna, Podlesie and in Cegielnia showed a lowered intensity of the bands in the range of 3300 cm^{-1} , $2920\text{-}2960\text{ cm}^{-1}$ and the bands with a low intensity at 2850 cm^{-1} deep down the soil profile. With depth, there was observed an increase in the intensity of the bands within the range of $1730\text{-}1710\text{ cm}^{-1}$ pointing to an increase in the share of carbonyl groups in the acids and ketones. Interestingly, the deeper down the soil profile the greater the gradual disappearance of the bands of $1660\text{-}1620\text{ cm}^{-1}$ related to,

e.g., the presence of amide nitrogen structures in the humic molecules. The intensity of the bands characteristic for the bonds C-C in the aromatic compounds ($1500-1520\text{ cm}^{-1}$) and the bands in the range of $1460-1440\text{ cm}^{-1}$ and in $1420-1400\text{ cm}^{-1}$ was decreasing with the sampling depth. Interpreting the FTIR spectra in terms of the effect on the properties of humic acids, a considerable variation was found. Generally, the FT-IR spectra of the HAs molecules of soils showed an increase in the intensity of the band in the range of $1730-1710\text{ cm}^{-1}$ together with the distance from the watercourse.

3.5. Hydrophilic and hydrophobic nature of humic acids

The degree of organic matter humification is also related with the hydrophilic-hydrophobic properties of humic acids determined with the chromatographic analysis. Similarly Debska et al. ^{32, 36, 50}, Preuße et al. ³³ Woelki et al. ³⁵ a separation into fractions was recorded. Fractions of a retention time of 3.0–8.0 min show greater hydrophilic properties, whereas the fractions of a retention time between 12.0 and 25.0 min become more and more hydrophobic (Fig. 2). The share of hydrophilic fraction ranged from 37.71% to 40.76%. The share of hydrophobic fraction was higher and it ranged from 59.24% to 62.29% (Table 6). In Kamionna and in Cegielnia the share of the fractions in the HAs molecules depended significantly on the soil sampling depth. It was found that the share of hydrophilic fraction was increasing and the share of hydrophobic fraction was decreasing deep down the soil profile, a similar tendency was observed in Podlesie. The distance from the watercourse, on the other hand, did not have a significant effect on the share of those fractions. The changes in the share of respective fractions resulted in changes in the value of the HIL/ Σ HOB ratio. Parameter HIL/ Σ HOB, as shown by, e.g. Debska et al. ^{32, 36, 50}, is connected with the degree of organic matter humification; the value of the parameter increases with an increase in the degree of the maturity of the molecules of humic acids. The values of parameter HIL/ Σ HOB ranged from 0.606 to 0.690 (Table 6). With the statistical analysis it was found that the values of the parameters in humic acids in Kamionna and in Cegielnia depended on the depth; the HAs molecules of the AE and Bsv horizons of soils demonstrated higher values of the ratio of HIL/ Σ HOB than the HAs molecules of the A horizon of soils. Similar, insignificant, tendencies were noted also in the humic acids of the soils sampled in Podlesie. The results point to a lower degree of maturity of the HAs of the A horizon of the soils, as compared with the humic acids of the other soil horizons. The effect of the distance on the hydrophilic-hydrophobic properties was significant for HAs in Cegielnia. However, in general, the humic acids of the soils sampled 25 m away from the watercourse identified a higher degree of humification (the highest values of the ratio HIL/ Σ HOB), as compared with the humic acids of the soils sampled 5 m away from the irrigation ditch. Similar dependencies were reported by Banach-Szott et al. ⁶² for the humic acids of the Fluvisols sampled in the

floodplains of the Vistula River; the values of the ratio $HIL/\Sigma HOB$ were increasing with the distance from the riverbed. Interestingly, the values of that parameter recorded by Banach-Szott et al.⁶² were slightly higher, ranging from 0.72 to 0.81. Thus, the HAs molecules were identified with a slightly lower degree of maturity than the humic acids of Fluvisols.

To acquire complete information on the differences (similarities) in the chemical composition of HAs, depending on the depth and the distance from the irrigation ditch, the cluster analysis was applied based on the elemental composition, spectrometric parameters as well as on the HIL-HOB properties dividing HAs into two groups (Fig. 3a). In the first group, similar properties were found for the HAs molecules of the Bsv horizon of the soil sampled in Podlesie and in Cegielnia, while in the second group there were identified two subgroups. The first subgroup covered the humic acids of the A horizon of the soils sampled in Kamionna and in Podlesie. In the second subgroup, similar properties were found for the HAs molecules of the AE horizon of the soil sampled in Podlesie and in Cegielnia as well as for the HAs of the Bsv horizon of the soils from Kamionna and the humic acids of the A horizon of the soils sampled in Cegielnia and the AE horizon of the soils sampled in Kamionna. As seen from the diagram, the soil sampling depth was the factor determining the properties of HAs.

A cluster analysis was performed based on the properties of humic acids of soils for each of the sampling sites (Fig 3b – Fig 3d). The dendrograms definitely point to the HAs isolated from the soil sampled 5 m away from the irrigation ditch in Kamionna and Cegielnia as significantly different from the soil sampled 15 and 25 m away. As for the Podlesie sampling site, the distance was not the factor which definitely determined the humic acid properties.

4. Conclusions

The structure of the HAs molecules corresponded to the aromatic systems coupled with the aliphatic chain containing up to 10 atoms of C. The atomic ratios, the spectrometric properties as well as the hydrophilic-hydrophobic properties point to a high maturity of the molecules of the humic acids of meadow soils continuously irrigated for more than 150 years. The molecules of humic acids demonstrated an increase in the degree of humification with depth. The distance from the watercourse determined the time of the water retention and, as a consequence, the humic acids of the soils sampled furthest from the irrigation ditch, in general, showed a higher degree of humification, as compared with the humic acids of the soils sampled closest.

Irrespective of the changes in the humic acid parameters, depending on the soil sampling distance or depth, the results identified that many-year grassland irrigation results in humic acids with a high degree of humification, which confirms the importance of meadow soils for carbon sequestration process.

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Ethics declarations

Competing interests

The authors declare that they have no conflict of interest.

Contributions

A. Dziamski conceived the project, provided funding of the project.

M. Banach-Szott conceived the project, performed lab work and analysis and developed the manuscript.

All authors reviewed the manuscript.

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Table 1. Results of the analysis of variance for the basic chemical parameters of soils

Factor		TOC (g kg ⁻¹)	Nt (g kg ⁻¹)	TOC/Nt	TOC (g kg ⁻¹)	Nt (g kg ⁻¹)	TOC/Nt	TOC (g kg ⁻¹)	Nt (g kg ⁻¹)	TOC/Nt
Kamionna				Cegielnia			Podlesie			
Distance (m)	5	22.7	1.83	14.55	39.2	2.35	17.35	35.0	2.78	13.19
	15	29.4	2.02	16.87	27.3	2.46	12.76	34.2	2.97	11.71
	25	40.2	3.04	14.20	32.1	3.16	10.93	37.4	3.07	12.85
LSD		1.58	0.431	Ns	0.56	0.136	1.293	2.01	0.168	1.262
Horizon	A	14.2	0.99	17.00	12.3	0.77	16.06	17.8	1.51	12.44
	AE	47.5	4.17	11.43	44.6	3.81	11.81	56.5	4.91	11.52
	Bsv	30.6	1.73	17.19	41.8	3.39	13.17	32.4	2.40	13.78
LSD		1.05	0.045	2.138	0.32	0.163	2.871	1.59	0.132	1.356

Table 2. Grain size composition of the soil samples

Parameter (Unit of measure)	Mean	Range	SD	CV (%)
pH in H ₂ O	-	5.3-7.0	-	-
Sand (%)	90.67	86.57-93.97	2.02	2.2
Silt (%)	9.12	6.03-13.03	1.75	19.3
Clay (%)	0.321	0.1-1.1	0.264	82.2

SD standard deviation, CV coefficient of variation

Table 3. Results of the analysis of variance for the values of atomic ratio of humic acids

Factor		H/C	N/C	O/C	O/H	ω
Kamionna						
Distance (m)	5	1.29	0.068	0.622	0.484	0.160
	15	1.27	0.067	0.635	0.501	0.198
	25	1.28	0.067	0.614	0.481	0.162
LSD		0.005	ns	0.010	0.010	ns
Horizon	A	1.34	0.077	0.620	0.462	0.131
	AE	1.28	0.071	0.626	0.488	0.181
	Bsv	1.21	0.054	0.625	0.515	0.208
LSD		0.025	0.006	ns	0.026	0.062
Cegielnia						
Distance (m)	5	1.10	0.061	0.529	0.478	0.132
	15	1.24	0.071	0.582	0.464	0.121
	25	1.35	0.081	0.620	0.450	0.108
LSD		0.053	0.015	0.014	ns	ns
Horizon	A	1.29	0.072	0.580	0.449	0.082
	AE	1.24	0.071	0.570	0.462	0.115
	Bsv	1.16	0.070	0.582	0.480	0.165
LSD		0.051	ns	ns	0.015	0.033
Podlesie						
Distance (m)	5	1.273	0.068	0.609	0.473	0.145
	15	1.259	0.069	0.591	0.467	0.124
	25	1.253	0.063	0.542	0.435	0.041
LSD		ns	ns	0.022	0.020	0.063
Horizon	A	1.317	0.073	0.586	0.444	0.080
	AE	1.284	0.070	0.595	0.459	0.111
	Bsv	1.184	0.058	0.562	0.472	0.120
LSD		0.060	0.006	0.021	0.012	0.024

LSD (Least significant difference)

Table 4. Results of the analysis of variance for the coefficients of absorbance of humic acids

Factor		$A_{2/4}$	$A_{2/6}$	$A_{4/6}$	$\Delta \log K$
Kamionna					
Distance (m)	5	6.45	33.81	5.23	0.675
	15	5.25	24.49	4.66	0.628
	25	5.28	23.51	4.46	0.632
LSD		0.417	3.60	0.63	0.025
Horizon	A	6.05	29.59	4.86	0.688
	AE	5.55	26.50	4.73	0.654
	Bsv	5.38	25.72	4.75	0.593
LSD		0.409	Ns	ns	0.051
Cegielnia					
Distance (m)	5	5.21	24.72	4.74	0.697
	15	4.94	23.37	4.72	0.614
	25	5.33	24.19	4.53	0.535
LSD		0.288	Ns	ns	0.042
Horizon	A	5.54	27.06	4.88	0.645
	AE	5.14	24.11	4.69	0.614
	Bsv	4.79	21.11	4.42	0.588
LSD		0.230	0.994	0.420	0.028
Podlesie					
Distance (m)	5	5.05	24.47	4.90	0.671
	15	5.19	24.90	4.71	0.629
	25	5.18	25.12	4.77	0.610
LSD		0.128	Ns	ns	ns
Horizon	A	5.87	30.94	5.28	0.700
	AE	5.12	24.16	4.71	0.647
	Bsv	4.41	19.39	4.39	0.564
LSD		0.195	2.44	0.32	0.031

LSD (Least significant difference)

Table 5. List of peaks (bands) present in FT-IR spectra

Wavenumber (cm ⁻¹)	Assignment ^a
3400-3100	O-H stretching of alcohols, phenols and acids, N-H stretching
3100-3000	C-H groups of aromatic and alicyclic compounds
2960-2920; 2850	asymmetric and symmetric C-H stretching of CH ₃ and CH ₂ group
1730-1710	C = O stretching of carboxyl, aldehyde, ketone group
1660-1620	C = O of stretching of amide groups; N-H deformation
1610-1600	C – C stretching of aromatic rings
1550-1530	N-H deformation, C = N stretching (amide II bands)
1520-1500	C-C stretching of aromatic rings
1460-1440	C-H asymmetric of CH ₃ and CH ₂
1420-1400	C-O stretching and OH deformation of phenols
1380-1320	C-N aromatic amine, COO-, C-H stretching
1280-1200	C-O stretching of aryl ethers, esters and phenols
1160-1030	C-O stretching alcohols, ethers and polysaccharides

^a Enev et al.⁵⁹, Zhang et al.⁶⁰, Hayes & Swift⁶¹

Table 6. Results of the analysis of variance for the share (%) of hydrophilic (HIL) and hydrophobic (HOB) fractions in the molecules of humic acids

Factor		HIL	ΣHOB	HIL/ΣHOB	HIL	ΣHOB	HIL/ΣHOB	HIL	ΣHOB	HIL/ΣHOB
Kamionna				Cegielnia			Podlesie			
Distance (m)	5	38.88	61.12	0.637	39.62	60.38	0.657	39.85	60.15	0.663
	15	40.04	59.96	0.668	38.18	61.82	0.620	40.16	59.84	0.671
	25	39.81	60.19	0.662	40.42	59.58	0.680	40.48	59.52	0.682
LSD		ns	ns	ns	ns	ns	0.057	ns	ns	ns
Horizon	A	38.57	61.43	0.628	37.71	62.29	0.606	39.90	60.10	0.664
	AE	39.83	60.17	0.662	39.92	60.08	0.664	39.82	60.18	0.690
	Bsv	40.34	59.66	0.676	40.59	59.41	0.686	40.76	59.24	0.663
LSD		1.374	1.374	0.037	2.264	2.264	0.061	ns	ns	ns

LSD (Least significant difference)

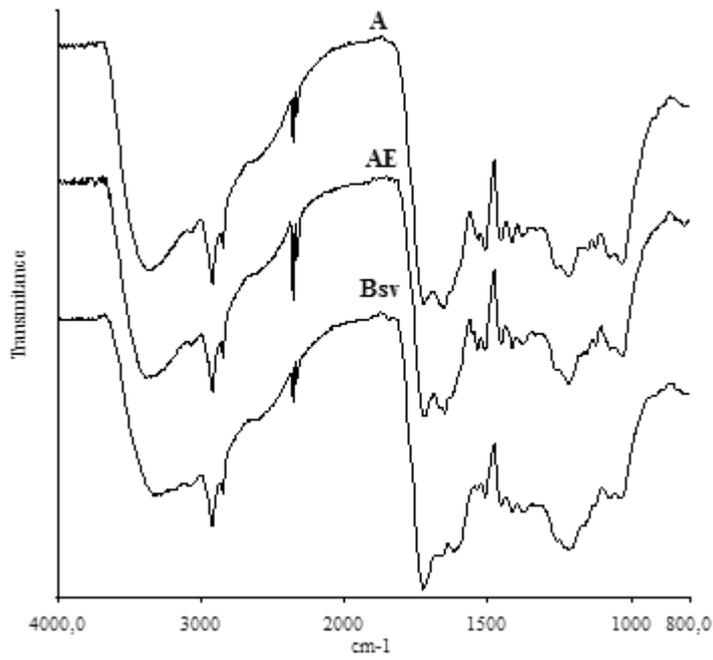


Fig. 1 FT-IR spectra of humic acids of the soils sampled in Podlesie 25 m away from the watercourse

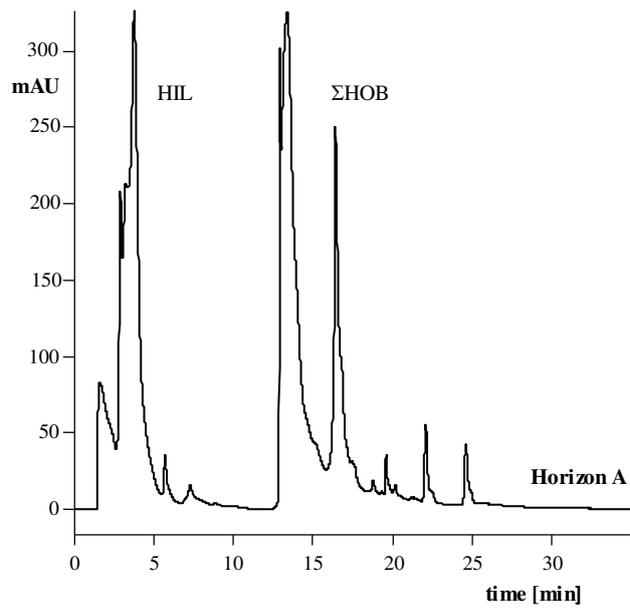


Fig. 2 RP-HPLC chromatogram of humic acids of the soils sampled in Podlesie 25 m away from the watercourse

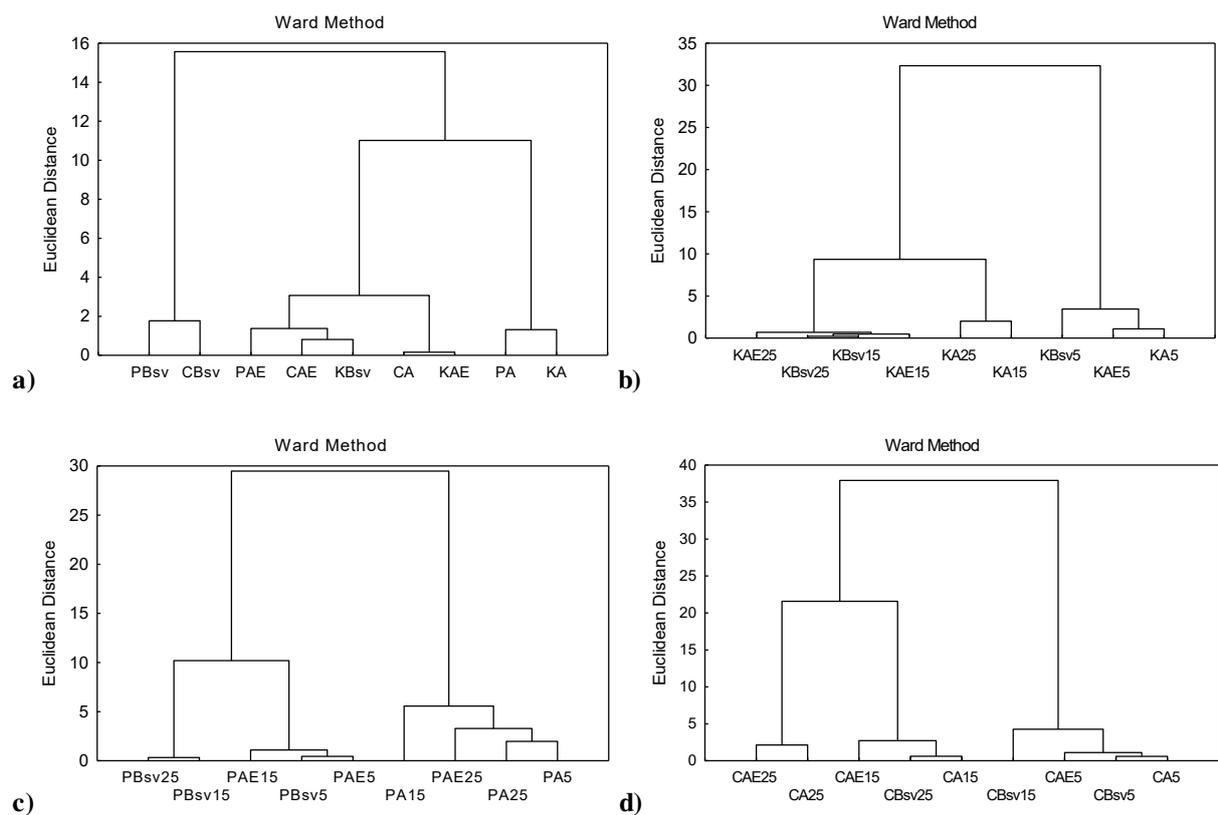


Fig. 3 Cluster analysis determined based on humic acids parameters: H/C, O/H, O/C, ω , $A_{2/4}$, $A_{2/6}$, $\Delta\log K$, HIL/ Σ HOB **a)** for HAs of all the sampling locations **b)** for HAs of the soils in Kamionna **c)** for HAs of the soils in Cegielnia **d)** for HAs of the soils of Podlesie (K – Kamionna, C – Cegielnia, P – Podlesie)

Figures

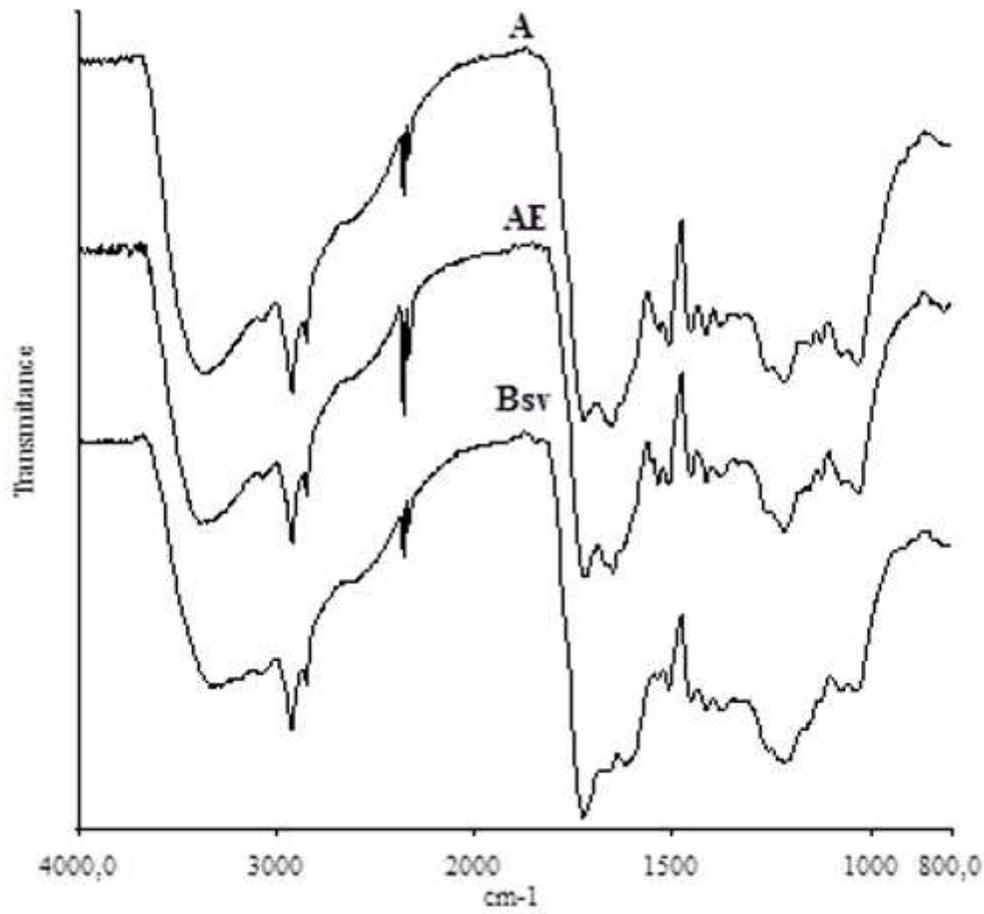


Figure 1

FT-IR spectra of humic acids of the soils sampled in Podlesie 25 m away from the watercourse

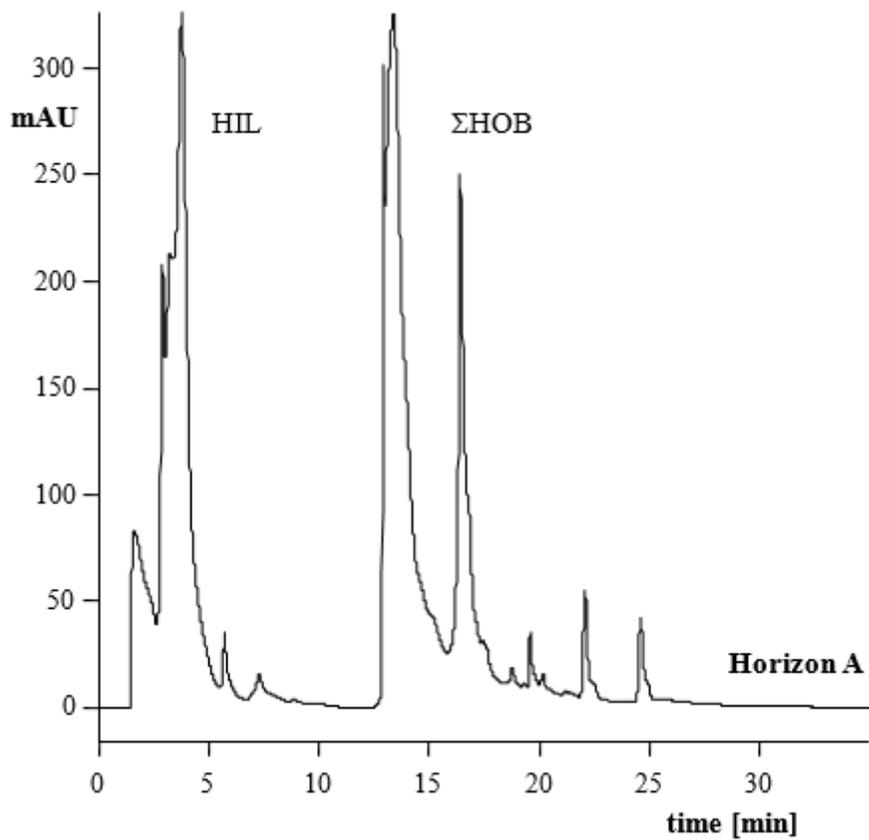


Figure 2

RP-HPLC chromatogram of humic acids of the soils sampled in Podlesie 25 m away from the watercourse

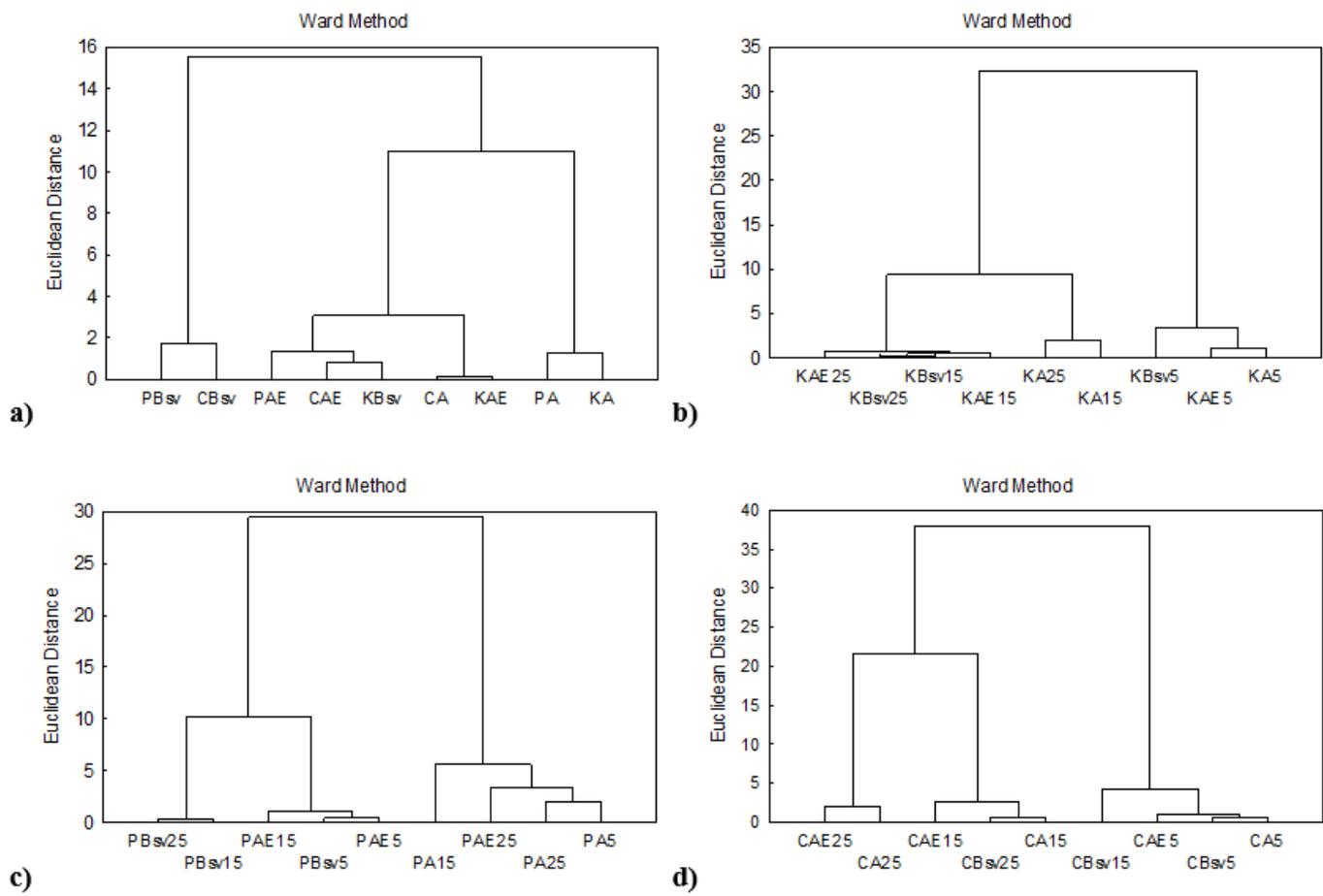


Figure 3

Cluster analysis determined based on humic acids parameters: H/C, O/H, O/C, ω , A2/4, A2/6, $\Delta\log K$, HIL/ Σ HOB a) for HAs of all the sampling locations b) for HAs of the soils in Kamionna c) for HAs of the soils in Cegielnia d) for HAs of the soils of Podlesie (K – Kamionna, C – Cegielnia, P – Podlesie)