Properties of Humic Acids in Meadow Soils Irrigated With the Slope-And Flooding System

Magdalena Banach-Szott (mbanach@utp.edu.pl)  
University of Science and Technology

Andrzej Dziamski  
University of Science and Technology

Research Article

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

DOI: https://doi.org/10.21203/rs.3.rs-535526/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Properties of humic acids in meadow soils irrigated with the slope-and-flooding system

Magdalena Banach-Szott¹, Andrzej Dziamski²

¹University of Science and Technology, Department of Biogeochemistry and Soil Science, 6 Bernardynska St., 85-029 Bydgoszcz, Poland
²University of Science and Technology, Department of Botany and Ecology, 7 Kaliskiego St., 85-796 Bydgoszcz, Poland

*Corresponding author: mbanach@utp.edu.pl; tel: (+48)523749538; fax: (+48)523749506

ORCIDs:
Magdalena BANACH-SZOTT: https://orcid.org/0000-0002-9981-0841

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 substance¹. 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\textsuperscript{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\textsuperscript{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 ways\textsuperscript{10}. 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\textsuperscript{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 CO\textsubscript{2} to the atmosphere\textsuperscript{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)\textsuperscript{17, 18, 19, 20}.

The structural characteristics of humic acids, especially identifying the functional groups, are assayed from the infrared spectra (FTIR)\textsuperscript{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: \(A_{\text{280}}\), \(A_{\text{465}}\), \(A_{\text{665}}\) and the coefficients of absorbance \(A_{\text{24}}\), \(A_{\text{26}}\), \(A_{\text{46}}\), \(\Delta \log K\). They are important indicators of the organic matter humification advancement\textsuperscript{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 Dębska\textsuperscript{31}, Debska et al.\textsuperscript{32}, Preuße et al.\textsuperscript{33}, Sierra et al.\textsuperscript{34}, Woelki et al.\textsuperscript{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.\textsuperscript{36} and Debska and Gonet\textsuperscript{37}, 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/\(\Sigma\)HOB 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 policies. 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 Forest. 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 soil;
- pH – in the suspension of distilled water and soil with the pH-meter MultiCal pH 540 GLP WTW;
- grain size composition was determined applying the aerometric method.
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 H$_2$O, 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 $\omega$ (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 μm, 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 μL; solvent: acetonitrile–water; solvents flow in the gradient (ratio H$_2$O: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 ($\Sigma$HOB = HOB1 + HOB2) fractions in humic acid molecules and the parameter HIL/$\Sigma$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:

- $A_{280}/A_{465}$ absorbance ratio
- $A_{280}/A_{665}$ absorbance ratio
- $A_{465}/A_{665}$ 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, $\omega$), spectrometric parameters ($A_{234}$, $A_{266}$, $A_{466}$ and $\Delta \log K$) as well as the HIL/ΣHOB 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. 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, 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 Drag et al. Similarly, Jończak investigating, e.g., the humic acids of meadow soils sampled in the Slawno Plain, Poland, Kobierski et al. 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. 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$^{-1}$, 2920-2960 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 1730-1710 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 1660-1620 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 cm\(^{-1}\)) and the bands in the range of 1460-1440 cm\(^{-1}\) and in 1420-1400 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 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/ΣHOB ratio. Parameter HIL/ΣHOB, 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/ΣHOB 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/ΣHOB 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/ΣHOB), 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/ΣHOB were increasing with the distance from the riverbed. Interestingly, the values of that parameter recorded by Banach-Szott et al. 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.
Acknowledgements

The research has been made as part of 1047/B/P01/2010/39 research project, financed by the Ministry of Science and Higher Education.

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.

References

1. Wiesmeier, M. et al. Soil organic carbon storage as a key function of soils - A review of drivers and indicators at various scales. *Geoderma* **333**, 149-162. https://doi.org/10.1016/j.geoderma.2018.07.026 (2019).

2. Minami, K., Goudriaan, J.E.A., Lanlinga, E.A. & Kimura T. Significance of grasslands in emission and absorption of greenhouse gases in *Proceedings of the XVIIth International Grassland Congress* **2**, 1231-1238 (1993).

3. Conant, R.T., Paustian, K. & Elliott, E.T. Grassland management and conversion into grassland: effects on soil carbon. *Ecol. Appl.* **11**, 343-355 (2001).

4. Mannetje, L.T. Advances in grassland science. *NIAS-Wagen. J. Life. Sci.* **50(2)**, 195-221 (2003).

5. Lal, R. Sequestering carbon in soils of agro-ecosystems. *Food Policy* **36**, 533-539 (2011).

6. Kämpf, I., Hölzel, N., Störrle, M., Broll, G. & Kiehl, K. Potential of temperate agricultural soils for carbon sequestration: A meta-analysis of land-use effects. *Sci. Total Environ.* **566-567**, 428-435 doi.org/10.1016/j.scitotenv.2016.05.067 (2016).

7. Stevenson, F.J. *Humus chemistry – genesis, composition reactions.* (Wiley, Chichester, 1994).

8. Senesi, N. & Miano T.M. *Humic substances in the global environment and implications on human health* (Elsevier, Amsterdam, 1994).

9. Schlesinger, W.H. *Biogeochemistry: An Analysis of Global Change* (Elsevier, 1997).

10. Hayes, M.H.B. & Swift, R.S. Vindication of humic substances as a key component of organic matter in soil and water. *Adv. Agron.* **163**, 1-37. doi:10.1016/bs.agron.2020.05.001. (2020).
11. Lanyi, K. Assessment of the relations between the spectroscopic characteristics of soils and their ability to adsorb organic pollutants. *Microchem. J.* **79**, 249-256 (2010).

12. McCarthy, P. The principles of humic substances. *Soil Sci.* **166**, 738-751 (2001).

13. Yamashita, Y., Jaffé, R., Maie, N. & Tanoue, E. Assessing the dynamics of dissolved organic matter (DOM) in coastal environments by excitation emission matrix fluorescence and parallel factor analysis (EEM-PARAFAC). *Limnol. Oceanogr.* **53**, 1900-1908 (2008).

14. Canellas, L.P. et al. Chemical composition and bioactivity properties of size-fractions separated from a vermicompost humic acid. *Chemosphere* **78**, 457-466. doi:10.1016/j.chemosphere.2009.10.018 (2010).

15. Lal, R. Soil carbon sequestration in Latin America in *Carbon Sequestration in Soils of Latin America* (eds. Lal, R., Cerri, C.C., Bernoux, M., Etcheves, J. & Cerri, E.) 49-64 (Food Products Press, New York, 2006).

16. Hayes, M.H.B. & Clapp, C.E. Humic substances: considerations of compositions, aspects of structure, and environmental influences. *Soil Sci.* **166**, 723-737 (2001).

17. Visser, S. A. Application of van Krevelen’s graphical statistical method for the study of aquatic humic material. *Environ. Sci. Technol.* **17**(7), 412-417 (1983).

18. Fuentes, M., Baigorri, R., González-Gaitano, G. & García-Mina, J.M. The complementary use of 1H NMR, 13C NMR, FTIR and size exclusion chromatography to investigate the principal structural changes associated with composting of organic materials with diverse origin. *Org. Geochem.* **38**, 2012-2023. doi:10.1016/j.orggeochem.2007.08.007 (2007).

19. Trubetskaya, O. E., Trubetskoy, O.A., Voyard, G. & Richard C. Determination of hydrophobicity and optical properties of soil humic acids isolated by different methods. *J. Geochem. Explor.* **132**, 84-89. org/10.1016/j.gexplo.2013.06.004 (2013).

20. Boguta, P., D’Orazio, V., Sokolowska, Z. & Senesi, N. Effects of selected chemical and physicochemical properties of humic acids from peat soils on their interaction mechanisms with copper ions at various pHs. *J. Geochem. Explor.* **168**, 119-126. doi.org/10.1016/j.gexplo.2016.06.004 (2016).

21. Tan, K.H. *Principles of soil chemistry*. (Dekker, New York, 1998).

22. Cocozza, C. & Miano, T. Structural resolution of metal-humic acids interactions through deconvolution FT-IR spectroscopy in *Proceedings of the 11th IHSS Meeting* (eds. Davis, G. & Ghabbour, E.A.) 264-266 (IHSS, Boston, USA, 2002).

23. Pajączkowska, J., Sułkowska, A., Sułkowski, W.W. & Jędrzejczyk, M. Spectroscopic study of the humification process during sewage sludge treatment. *J. Mol. Struct.* **651-653**, 141-149 (2003).
24. Polak, J., Bartoszek, M., Żądło, M., Kos, A. & Sułkowski, W.W. The spectroscopic studies of humic acid extracted from sediment collected at different seasons. *Chemosphere* **84**, 1548-1555 (2011).

25. Kumada, K. *Chemistry of soil organic matter* (Elsevier, Amsterdam, 1987).

26. Gonet, S.S. & Debska, B. Properties of humic acids produced during decomposition of plant residues in soil. *Rostl. Vyroba* **45**(10), 455-460 (1999).

27. Morán Vieyra, F.E., Palazzi, V.I., Sanchez de Pinto, M.I. & Borsarelli C.D. Combined UV–Vis absorbance and fluorescence properties of extracted humic substances-like for characterization of composting evolution of domestic solid wastes. *Geoderma* **151**(3-4), 61-67 (2009).

28. Weishaar, J.L. et al. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. *Environ. Sci. Technol.* **37**, 4702-4708 (2003).

29. Chen, J., Gu, B., LeBoeuf, E., Pan, H. & Dai, S. Spectroscopic characterization of the structural and functional properties of natural organic matter fractions. *Chemosphere* **48**, 59-68 (2002).

30. Rodríguez, F.J., Schlenz, P. & García-Valverde, M. Monitoring changes in the structure and properties of humic substances following ozonation using UV-Vis, FTIR and H NMR techniques. *Sci. Total Environ.* **541**, 626-637 (2016).

31. Banach-Szott, M. & Debska, B. Chromatographic separation of humic acids of a forest soil in *Humic substances – linking structure to functions* (eds. Frimmel, F.H. & Abbt-Braun, G.) 225-228 (Karlsruhe, Germany, 2006).

32. Debska, B., Banach-Szott, M., Dziański, A. & Gonet, S.S. Chromatographic characteristics (HPLC, HPSEC) of humic acids of soil fertilised with various organic fertilisers. *Chem. Ecol.* **26**(S2), 49-57 (2010).

33. Preuße, G., Friedrich, S. & Salzer, R. Retention behavior of humic substances in reversed phase HPLC. *Fresenius J. Anal. Chem.* **368**, 268-273 (2000).

34. Sierra, M.D., Giovanela, M., Parlati, E. & Soriano-Sierra E.J. 3D-fluorescence spectroscopic analysis of HPLC fractionated estuarine fulvic and humic acids. *J. Brazilian Chem. Soc.*, **17**(1), 113-124 (2006).

35. Woelki, G., Friedrich, S., Hanschmann, G. & Salzer, R. HPLC fractionation and structural dynamics of humic acids. *Fresenius J. Anal. Chem.* **357**(5), 548-552 (1997).

36. Debska, B., Drag, M. & Banach-Szott, M. Molecular size distribution and hydrophilic and hydrophobic properties of humic acids isolated from forest soil. *Soil Water Res.* **2**(2), 45-53 (2007).

37. Debska, B. & Gonet, I. Share of hydrophilic and hydrophobic fractions in humic acids formed as a result of post-harvest residue decomposition. *Pol J. Soil Sci.* **40**(1), 57-65 (2007).
38. COM 231 Final. Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions. Thematic Strategy for Soil Protection, Brussels. 2006. Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:52006DC0231&from=EN (accessed on 26 September 2006).

39. Sabiniarz, A. Łąki Czerskie w aspekcie historycznym (Historic outline of the Czersk Meadows). Ląkarstwo w Polsce (Grassland Science in Poland) 9, 184-194 (2006). In Polish

40. Debska, B., Dlugosz, J., Piotrowska-Dlugosz, A. & Banach-Szott, M. The impact of bio-fertilizer on the soil organic matter status and carbon sequestration – results from a fiel-scale study. J. Soil. Sediment. 16(10), 2335-2343. https://doi.org/10.1007/s11368-016-1430-5 (2016).

41. Van Reeuwijk, L.P. Procedures for Soil Analysis (International Soil Reference and Information Centre, Wageningen, 2002).

42. Różański, Sz., Jaworska, H., Matuszezak, K., Nowak, J. & Hardy, A. Impact of highway traffic and the acoustic on the content and spatial distribution of heavy metals in soils. Environ. Sci. Pollut. Res. 24, 12778-12786. https://doi.org/10.1007/s11356-017-8910-z (2017).

43. Ward, J.H. Hierarchical grouping to optimise an objective function. J. Am. Stat. Assoc. 58, 236-244 (1963).

44. Kuzyakov, Y. Review: factors affecting rhizosphere priming effects. J. Plant Nutr. Soil Sc. 165(4), 382-396 (2002).

45. Van Camp, L. et al. Reports of the Technical Working Groups establish under the thematic strategy for soil protection. Vol. IV: Contamination and Land Management. EUR 21319 EN. Luxembourg: Office for Official Publications of the European Communities, JRC28870, 2004.

46. Khalid, M., Soleman, N. & Jones, D.L. Grassland plants affect dissolved organic carbon and nitrogen dynamics in soil. Soil Biol. Biochem. 39, 378-381. doi:10.1016/j.soilbio.2006.07.007 (2007).

47. Zdenek, F. & Tesarova, M. Microbial degradation and transformation of humic acids from permanent meadow and forest soils. Int. Biodeterior. Biodegr. 54, 225-231. doi:10.1016/j.ibiod.2004.06.006 (2004).

48. Amir, S. et al. Structural study of humic acids during composting of activated sludge-green waste: Elemental analysis, FTIR and $^{13}$C NMR. J. Hazard. Mater. 177(1-3), 524-529 (2010).

49. Orlov, D.S. Humus acids of soils (Balkema, Rotterdam, 1986).

50. Debska, B., Drąg, M. & Tobiasova, E. Effect of post-harvest residue of maize, rapeseed, and sunflower on humic acids properties in various soils. Pol. J. Environ. Stud. 21(3), 603-613 (2012).
51. Dergacheva, M.I. et al. Ratio of elements in humic acids as a source of information on the environment of soil formation. *Contemp. Probl. Ecol.* **5**, 497-504. doi: 10.1134/S1995425512050022 (2012).

52. Sanchez-Monedero, M.A., Cegarra, J., Garcia, D. & Roig, A. Chemical and structural evolution of humic acids during organic waste composting. *Biodegradation* **13**, 361-371 (2002).

53. Gonet, S.S., Debska, B., Zaujec, A., Banach-Szott, M. & Szombathova, N. Effect of the tree species and soil-and-climate conditions on the properties of humus in forest soils in *Role of Organic Matter in the Environment* (eds. Gonet, S.S. & Markiewicz, M.) 61-98 (PTSH, Wroclaw, Poland, 2007). In Polish

54. Tinoco, P., Almendros, G., Gonzalez-Vila, F.J., Sanz, J. & Gonzalez-Perez, J.A. Revisiting molecular characteristics responsive for the aromaticity of soil humic acids. *J. Soil. Sediment.* **15**, 781-791. doi: 10.1007/s11368-014-1033-y (2015).

55. Filcheva, E. et al. Quantitative and qualitative characterisation of humic products with spectral parameters. *J. Soil. Sediment.* **18**(8), 2863-2867. doi: 10.1007/s11368-018-2021-4 (2018).

56. Drąg, M., Debska, B. & Dziamski A. Properties of humic substances of forest and meadow soil in the area of the Wierzchlas Reserve. *Humic Subst. Ecosys.* **7**, 141-151 (2007).

57. Jończak, J. Soil organic matter properties in stagnic luvisols under different land use types. *Acta Agroph.* **20**(4), 565-576 (2013).

58. Kobierski, M., Kondratowicz-Maciejewska, K., Banach-Szott, M., Wojewódzki, P. & Peñas Castejón, J.M. Humic substances and aggregate stability in rhizospheric and non-rhizospheric soil. *J. Soil. Sediment.* **18**(8), 2777-2789. doi: 10.1007/s11368-018-1935-1 (2018).

59. Enev, V., Pospisilova, L., Klucakova, M., Liptaj, T. & Doskocil, L. Spectral characterization of selected humic substances. *Soil Water Res.* **9**(1), 9-17. https://doi.org/10.17221/39/2013-SWR (2014).

60. Zhang, J. et al. Effects of long-term fertilization on soil humic acid composition and structure in Black Soil. *Plos One* **12**(11), 1-14. https://doi.org/10.1371/journal.pone.0186918 (2017).

61. Hayes, M.H.B. & Swift, R.S. An appreciation of the contribution of Frank Stevenson to the advancement of studies of soil organic matter and humic substances. *J. Soil. Sediment.* **18**(4), 1212-1231. https://doi.org/10.1007/s11368-016-1636-6 (2018).

62. Banach-Szott, M., Kobierski, M. & Kondratowicz-Maciejewska, K. Humic substances in Fluvisols of the Lower Vistula floodplain, North Poland. *Environ. Sci. Pollut. Res.* **25**(24), 23999-24002. doi: 10.1007/s11356-018-2454-8 (2018).
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| Kamionna            | Cegielnia           | Podlesie| 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 H₂O                   | -    | 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} | ΔlogK |
|-------------|---------|---------|---------|-------|
|             | 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 & Swift\(^ {61}\)
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 |
|-----------------|------|------|----------|------|------|----------|------|------|----------|
|                 | 5    | 15   | 25       |      |      |          |      |      |          |
| Kamionna        | 38.88| 40.04| 39.81    | 39.62| 38.18| 40.42    | 39.85| 38.18| 40.48    |
| Cegielnia       | 61.12| 59.96| 60.19    | 60.38| 61.82| 59.58    | 60.15| 62.29| 59.52    |
| Podlesie        | 0.637| 0.668| 0.662    | 0.657| 0.620| 0.680    | 0.663| 0.606| 0.664    |
| LSD             | ns   | ns   | ns       | ns   | ns   | 0.057    | ns   | ns   | ns       |
| Horizon         |      |      |          |      |      |          |      |      |          |
| A               | 38.57| 39.83| 40.34    | 37.71| 39.92| 40.59    | 39.90| 39.82| 40.76    |
| AE              | 61.43| 60.17| 59.66    | 62.29| 60.08| 59.41    | 60.10| 60.18| 59.24    |
| Bsv             |      |      |          | 0.606| 0.664| 0.686    | 0.664| 0.690| 0.663    |
| LSD             | 1.374| 1.374| 1.374    | 2.264| 2.264| 2.264    | 2.264| 2.264| 2.264    |

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, $A_{244}$, $A_{266}$, $Δ\log K$, HIL/∑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)
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, $\omega$, A2/4, A2/6, $\Delta$logK, HIL/∑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)