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CHARACTERISTICS OF HUMIC ACIDS ISOLATED FROM SOILS UNDER VARIOUS FARMING SYSTEMS

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Abstract. Farming systems strongly affect soil humus. In this study some humus characteristics were used to compare the humus quantity and quality under two different farming systems. The field experiment was established in 1990 on an Orthic Luvisols in Slovakia. A higher content of organic carbon (C_T) in topsoil (13.1 $g \cdot kg^{-1}$) was recorded in soil under ecological farming where only organic manures were applied rather than in topsoil from integrated farming (12.5 $g \cdot kg^{-1}$) where both organic and inorganic fertilisers were applied. The quality of humus (HA : FA ratio) was similar in soils under both farming systems. Humic acids (HA) were isolated and analyzed for elemental composition, UV–VIS spectra and thermal properties. HA extracted from soil under ecological farming, as compared with HA from soil under integrated system, were characterised by a lower value of the H : C ratio and by a higher degree of internal oxidation. It shows that HA from ecological farming have higher aromaticity, humification degree and therefore are of a higher quality than HA from integrated farming. A higher aromaticity of humic acids from soil under ecological farming was confirmed by lower values of absorbance ratios ($A_{2/6}$, $A_{2/4}$ and $A_{4/6}$) compared with the other HAs analyzed. The degree of aliphaticity determined thermogravimetrically was lower in HA from ecological farming, as compared with the integrated one, and it confirmed the results of spectral and elemental analyses referring to a higher humic acids quality in ecological farming.

Key words: ecological farming, integrated farming, humus, humic acids

INTRODUCTION

It is well known that different farming systems influence not only the crop yield but also many soil characteristics as well as humus content [Gonet 1989]. For that reason

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farmers can regulate, through a reasonably chosen farming system, some soil properties and organic matter dynamics. Lacko-Bartošová et al. [1999a, b], Zaujec and Tobiašová [1998] stated that the soil under ecological farming system was characterised by higher humus and water-stable aggregates contents and higher values of bulk density and base saturation in comparison with the soil under integrated farming system. The same experimental plots were used for studies of the energy and mineral inputs and outputs [Ciglar et al. 1995]. The results obtained show that the integrated system is more energy-consuming, by an average of 23%, as compared with the ecological system. When the biomass production was analysed, ecological system showed higher biomass productivity (5.5%) and a better energy performance in comparison with the integrated system. Szombathová [1999] investigated organic carbon susceptibility to oxidation by $KMnO_4$ on the same experimental plots and stated that in topsoil from the ecological area there was recorded a higher content of easily oxidised organic carbon (1.35 $g \cdot kg^{-1}$) than in topsoil from the integrated one (0.92 $g \cdot kg^{-1}$). Kubát and Lipavský [1999] compared the organic carbon content (C_T) in soil under different variants of fertilisation after 40 years of farming. In this experiment, the C_T content in soils: without fertilisation, with mineral fertilisation, with organic fertilisers and with combined organic-mineral fertilisation was 12.1, 12.6, 14.3 and 14.7 $g \cdot kg^{-1}$, respectively.

The influence of different farming practices can be properly investigated in cropping systems under continuous long-term management conditions. In such systems, the carbon content, microbial biomass and its activity, mineralization and immobilisation processes, and humification rate are likely to be approaching steady-state needed to sustain productivity. The principal aim of this study was to compare the influence of two different farming systems on the quantity and selected qualitative parameters of soil humus after nine years of farming.

MATERIAL AND METHODS

Soil samples were collected from an area of the Research Experimental Station of the Slovak Agricultural University in Nitra, located at Dolná Malanta, where integrated and ecological farming systems were established in the fall of 1990. Parent material is proluvial sediment mixed with loess and the main soil type is Orthic Luvisols [Hanes et al. 1993]. The A_h horizon (0.0-0.32 m) has a fine to medium crumb structure, firm consistence, loamy texture, without carbonates, with a clear boundary to B_t (0.33-0.65 m), which has a subangular blocky structure, firm consistence, clay-loamy texture and gradual boundary to B_t/C (0.66-0.85 m).

In ecological system IOFAM regulations [Basic standards 1998] and standards for ecological agriculture [Principles of ecological agriculture 1995] valid in the Slovak Republic were accepted. In both farming systems natural processes are supported by crop rotation with intercrops, integrated crop nutrition and fertilisation, regulated and non-chemical plant protection. Farmyard manure (FYM) was incorporated with medium depth ploughing twice during rotation in the amount of 40 $t \cdot ha^{-1}$. The dose of FYM in ecological system is calculated using the balance method based on the macronutrients and plant needs to obtain the yield of 7 $t \cdot ha^{-1}$ of cereals, as well as in integrated system, where manure and also inorganic fertilisers were used. The application rate of inorganic fertilisers was calculated both based on the macroelement contents in soil and plant needs.

The crop rotations:

A) Integrated system: alfalfa, maize (+FYM 40 t·ha^{-1}), maize for silage, winter wheat, sugar beet (+FYM 40 t·ha^{-1}), spring barley (intercrop), common pea, winter wheat.

B) Ecological system: bean + alfalfa, alfalfa, winter wheat (intercrop), maize for silage (+FYM 40 t·ha^{-1}), winter rape (intercrop), common pea (intercrop), maize for silage (+FYM 40 t·ha^{-1}), winter wheat. Together with 37.5% of leguminous crops in rotation the N nutrition was secured.

Detailed information regarding this experiment was presented in earlier papers Lacko-Bartošová et al. [1999a, b]. Soil samples were collected from topsoil (0-0.3 m) under each farming system. To avoid natural variability of soil, for humus extraction and its analysis the average samples obtained by mixing 1000 g of soil material from each of the four replication plots (each plot was 50 m^2) were used:

– Sample No 1 – ecological farming system, only farmyard manure (FYM) was used,
– Sample No 2 – integrated farming system, where both FYM and mineral fertilisers were used,
– Sample No 3 – control soil, sampled before start of the experiment in 1990 [Hanes et al. 1993].

Soil samples were analysed for:

– organic carbon content (C_T) - by Tyurin method [Orlov and Grishina 1981],
– fractional composition of humus – by Tyurin method in Ponomareva-Plotnikova modification [Orlov and Grishina 1981]. Carbon of humic acids (HA) and fulvic acids (FA) fractions were determined.

Humic acids (HA) were isolated using the Orlov method [Orlov and Grishina 1981] and analysed for:

1) elemental composition (CHN 2400 Perkin Elmer Analyser). The oxygen content was estimated from the difference (100% – %C – %H – %N) in relation to the ash-less sample weight. Based on the elemental composition, the values of atomic ratios (H : C, O : C, N : C, O : H) were calculated as well as the internal oxidation degree referring to the formula: $\omega = (2O + 3N - H):C$ where C, H, O, N – represent the contents of carbon, hydrogen, oxygen and nitrogen in atomic percentage [Zdanov 1965];
2) absorption spectra in UV-VIS range (Perkin Elmer UV-VIS Lambda 20 Spectrometer). VIS spectra were performed for 0.02% humic acids solutions in 0.1M NaOH and UV-spectra were determined after fivefold dilution. Based on the determined absorbance values at wavelength: 280 nm (A_{280}), 465nm (A_{465}), and 665 nm (A_{665}), the following factors were calculated: $A_{2/4}$ – absorbances ratio at 280 and 465 nm, $A_{2/6}$ – absorbances ratio at 280 and 665 nm, $A_{4/6}$ – absorbances ratio at 465 and 665 nm [Chen et al. 1977, Kumada 1987, Gonet and Wegner 1994];
3) thermal analysis (Derivatograph C, MOM Hungary), when 40 mg of HA samples were mixed with Al_2O_3 (1:9) and heated in the air atmosphere at the speed of 3.3°C·min^{-1}. From the results of thermal analyses the following indices were calculated:

– weight loss of samples related to the effects registered on DTA curve: endothermic (endo), 1st exothermic (exo1), 2nd exothermic (exo2) and 3rd exothermic (exo3),
– maximum temperatures of the effects registered on DTA curve,
– area under DTA curve,
– parameter 'Z' expressing the ratio of weight loss in low temperature range (endo + exo1) to weight loss in high temperature range (exo2 and exo3). The value Z is proportional to 'aliphaticity' of humic acids [Gonet 1989].

The analysis of C_T content, C in humic and fulvic acids fractions, elemental composition and absorbance parameters were made in three analytical replications.

RESULTS AND DISCUSSION

The results of organic carbon content (C_T) in soil are shown in Table 1. The highest C_T contents were found in topsoils. Under ecological farming system C_T content was 13.1 $g \cdot kg^{-1}$, under integrated C_T = 12.5 $g \cdot kg^{-1}$. During 9 years of different farming the organic carbon content increased in soils under both farming systems (under ecological by 1.2 $g \cdot kg^{-1}$, under integrated by 0.6 $g \cdot kg^{-1}$).

Table 1. Organic carbon content (C_T) in soil and values of HA : FA ratio in humus
Tabela 1. Zawartość węgla organicznego (C_T) w glebie i wartości stosunku HA : FA w próchnicy

Sample – Próbka	Horizon Poziom	C_T $g \cdot kg^{-1}$	HA : FA
No 1 – ecological system Nr 1 – system ekologiczny	A	13.1	0.85
	Bt	7.0	nd – no
	C	1.9	nd – no
No 2 – integrated system Nr 2 – system zintegrowany	A	12.5	0.83
	Bt	6.7	nd – no
	C	1.8	nd – no
No 3 – control sample Nr 3 – próbka kontrolna	A	11.9	0.89
	Bt	3.5	nd – no
	C	1.5	nd – no
$LSD_{0.05}$ – $NIR_{0,05}$ for – dla:			
A horizon – poziomu A		0.56	ns – ni
Bt horizon – poziomu Bt		0.62	nd – no
C horizon – poziomu C		ns – ni	nd – no

nd – no – not determined – nie oznaczono
ns – ni – non-significant difference – różnica nieistotna

There were no significant differences between the values of humus quality parameter HA : FA (the ratio of humic acids carbon to fulvic acids carbon) determined in soils under the two farming systems examined (Table 1).

Elemental composition (in atomic percentage) and values of element ratios for extracted humic acids are shown in Table 2. Humic acids from fertilised soils (samples No 1 and No 2) as compared with humic acids from control soil (sample No 3) were characterised by a higher content of hydrogen. In consequence, HA from control soil had the lowest value of the H : C ratio and the highest degree of internal oxidation degree (ω parameter). The data show that humic acids in soil under both farming systems have a lower aromaticity and humification degree than HA in control soil [Gonet and Wegner 1994].

Table 2. Elemental composition of humic acids isolated from topsoils under ecological (No 1), integrated (No 2) farming systems and control sample (No 3)
Tabela 2. Skład pierwiastkowy kwasów huminowych wyizolowanych z warstwy powierzchniowej gleby o ekologicznym (Nr 1) i zintegrowanym (Nr 2) systemie gospodarowania oraz obiektu kontrolnego (Nr 3)

Sample – Próbka	C	H	N	O	O : C	H : C	N : C	O : H	ω
	in atomic percentage w % atomowych								
No 1	35.0	42.4	2.9	19.7	0.56	1.21	0.083	0.46	0.16
No 2	34.7	44.1	2.9	18.3	0.53	1.27	0.083	0.41	0.03
No 3	35.5	41.8	2.8	19.9	0.56	1.18	0.079	0.48	0.18
$LSD_{0.05}$ – $NIR_{0,05}$	ns – ni	1.22	ns – ni	0.53	ns – ni	0.024	ns – ni	ns – ni	0.018

ns – ni – non-significant difference – różnica nieistotna

Humic acids extracted from soil under ecological farming compared with HA from the integrated one had lower values of the H : C ratio. The decrease in H : C is probably due to a disappearance of aliphatic chains by biological and/or chemical oxidation and relative increase in aromatic groups. An increased content of aromatic parts of macromolecules is connected with a higher stability, maturity and quality of humic acids. A higher oxygen content, which indicates evolution towards oxidation, was supported by an increase in O : C ratio in HA from ecological farming. The ratios obtained were supported by calculated degree of internal oxidation (ω parameter), which was 5 times higher in HA isolated from ecological farming (Table 2).

Absorbance ratios ($A_{2/6}$, $A_{2/4}$ and $A_{4/6}$) confirmed a higher degree of nucleus condensation and aromaticity of humic acids isolated from soil under ecological farming (Table 3).

Table 3. Absorbance ratios of humic acids isolated from topsoils under ecological (No 1), integrated (No 2) farming systems and control sample (No 3)
Tabela 3. Wartości stosunków absorbancji kwasów huminowych wyizolowanych z warstwy powierzchniowej gleby o ekologicznym (Nr 1) i zintegrowanym (Nr 2) systemie gospodarowania oraz obiektu kontrolnego (Nr 3)

Sample – Próbka	$A_{2/6}$	$A_{2/4}$	$A_{4/6}$
No 1 – Nr 1	25.0	5.34	4.68
No 2 – Nr 2	26.9	5.53	4.86
No 3 – Nr 3	26.9	5.54	4.86
$LSD_{0.05}$ – $NIR_{0,05}$	1.04	0.162	0.124

Generally, for humic acids extracted from soils the absorbance values are interpreted according to the following principles: A_{280} – express participation of humic structures characteristic for precursors of humic substances, A_{465} – express participation of ‘young’ humic acids in the initial stage of humification, A_{665} – express participation of 'mature' humic acids characteristic for a well-humified and stable soil organic matter. The lower values of absorbance ratios of HA from ecological farming indicate that they contain much 'matured' substances of a higher humification degree (related to A_{665}) than the substances which are in the initial stage of decomposition (related to A_{280} and A_{465}). The value of $A_{4/6}$ is connected with molecular weigh of humic substances [Chen et al. 1977, Kumada 1987, Gonet and Wegner 1994]. Generally, the values of $A_{4/6}$ are inversely proportional to the average molecular weight.

Differential thermal analysis is also useful for assessing the 'degree of humification' of organic matter in soil. The shapes of DTA and DTG curves of humic acids samples isolated from ecological and integrated farming were substantially different (Fig. 1).

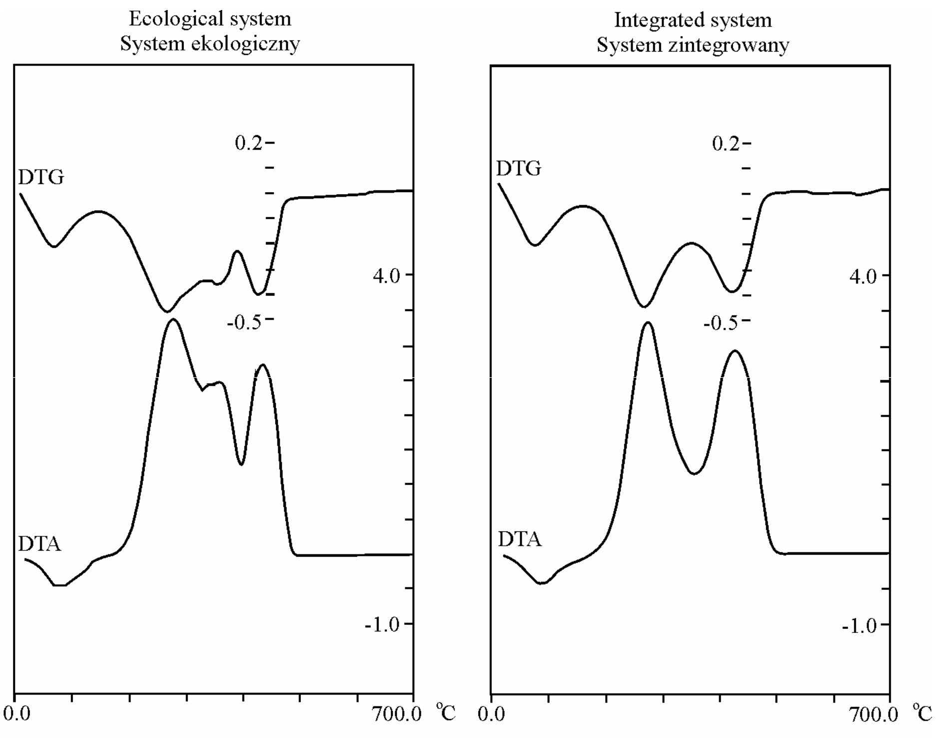

Fig. 1. Thermograms of humic acids isolated from topsoils of ecological (No 1) and integrated farming systems (No 2)

Rys. 1. Termogramy kwasów huminowych wyizolowanych z warstwy powierzchniowej gleby o ekologicznym (Nr 1) i zintegrowanym (Nr 2) systemie gospodarowania

Endothermic effects, which refer to dehydration, were similar to each other in both HA samples. Distinctive differences were found for exothermic effects. The thermogram of HA from integrated farming was more typical for humic acids and contained one endothermic and two exothermic peaks. The shape of DTA and DTG curves of HA isolated from ecological farming is not typical for humic acids as three exothermic peaks were recorded. The 1st exothermic effect, with maximum at low temperature 286°C, was caused by oxidation of simple functional groups and by cleavage of aliphatic structures. Less distinct exothermic peak 2 (taking 23.1% of the total area) can be attributed to the boundary of low and high temperature effect (the maximum temperature effect was 370°C). This peak can be due to the presence of substances having characteristic both – aromatic and aliphatic structures. Flaig et al. [1975] report that at the temperature range of 245-375°C there occurs a cleavage of phenolic hydroxyls and aromatic ethers. The temperature range, where decomposition of condense aromatic rings predominates, is above 350°C. Stevenson [1982] claims that the peak with maximum temperature of 370°C can be caused by a break-up of aromatic structures. The third weak exothermic peak, whose area was 25.5% of the total, had maximum temperature at 445°C (Table 4). This peak clearly corresponds to the cleavage of aromatic structures of humic acids nuclei.

Table 4. Parameters of thermal decomposition of humic acids samples isolated from topsoils of ecological (No 1) and integrated (No 2) farming systems and control sample (No 3)
Tabela 4. Parametry termicznego rozkładu kwasów huminowych wyizolowanych z warstwy powierzchniowej gleby o ekologicznym (Nr 1) i zintegrowanym (Nr 2) systemie gospodarowania oraz obiektu kontrolnego (Nr 3)

Sample Próbka	Maximum temperature of effects recorded on DTA-curves, °C Maksymalna temperatura efektów rejestrowanych na krzywej DTA				Area under DTA effects, % of total area Powierzchnie efektów rejestrowanych pod krzywą DTA, % powierzchni całkowitej				Z
	endo	exo1	exo2	exo3	endo	exo1	exo2	exo3	
No 1 – Nr 1	81	286	370	445	4.0	47.4	23.1	25.5	1.36
No 2 – Nr 2	83	281	–	432	4.5	52.1	–	43.4	1.77
No 3 – Nr 3	87	299	–	480-530	3.3	43.2	–	53.5	0.93

The degree of aliphaticity ‘Z” calculated for HA No 2 extracted from soil under integrated system was 1.77. If the second exothermic peak of HA No 1 isolated from soil under ecological farming was classified as low-temperature, the ‘Z’ parameter would be too high (3.52) and HA No 1 would be classified as highly aliphatic. According to Flaig [1975] and Stevenson [1982], the peak with maximal temperature effect of 370° C was considered corresponding to the cleavage of aromatic functional groups, therefore for the purpose of calculation, the following equation was used: Z = (endo + exo1) : (exo2 + exo3) and the obtained parameter Z = 1.36. A lower degree of aliphaticity of HA from ecological farming obtained thermogravimetrically was in harmony with the results of spectral and elemental analyses (Tables 2 and 3) and refers to a higher ‘maturity’, nucleous condensation and, therefore, a higher quality of humic acids isolated from ecological farming. The lowest value of parameter ‘Z’ (and simultaneously the lowest value of the H : C ratio from elemental composition) was determined for HA from the control soil.

CONCLUSIONS

Both ecological and integrated farming systems result in increased organic carbon content in soil, but the quality of humus (expressed as humic to fulvic acids ratio) shows no significant differences between the two systems.

The elemental composition suggests that humic acids in soil under ecological farming have higher aromaticity and humification degree than humic acids in soil under integrated farming, which was confirmed by lower absorbance ratios of humic acids from ecological system, as compared with the other humic acids.

The degree of aliphaticity estimated thermogravimetrically was lower in humic acids from ecological farming than in humic acids from the integrated one, which confirmed the results of spectral and elemental analyses and which shows a higher stability and quality of humic acids isolated from ecological farming system.

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REFERENCES

Basic standards, 1998. International Federation of Organic Agriculture Movements (IOFAM). Tholey-Theley, 53.

Ciglar J., Streďanská A., Pospíšil R., 1995. Energy analysis of crop rotations in various systems of management. Rostl. Výr. 41, 59-63.

Chen Y., Senesi N., Schnitzer M., 1977. Information provided on humic substances by $E_{4/6}$ ratios. Soil Sci. Soc. Am. J. 41, 352-358.

Flaig W., Beutelspacher H, Rietz E., 1975. Soil components. J.E. Gieseking, Springer-Verlag New York.

Gonet S.S., 1989. Investigations of humic acids by differential thermal analysis. [Badania kwasów huminowych metodą termicznej analizy różnicowej]. Rocz. Glebozn. XL (1), 27-38 [in Polish].

Gonet S.S., Wegner K., 1994. The effect of mineral and organic fertilization on properties of soil humic acids. [In:] Humic Substances in the Global Environment and Implications on Human Health, Elsevier Amsterdam, 607-611.

Hanes J., Mucha V., Sisák P., Slovík R., 1993. Characterization of Orthic Luvisols on experimental base AF SPU Nitra, Dolná Malanta. SPU Nitra, 29 [in Slovak].

Kubát J., Lipavský J., 1999. The dynamics of organic matter and optimal humus content in arable soils. [In:] Proc. Conf. The Balance of Organic Substances and Optimal Stock of Organic Matter in Soil. Praha, VÚRV v Praze-Ruzyni, 60-74 [in Czech].

Kumada K., 1987. Chemistry of soil organic matter. Elsevier Amsterdam.

Lacko-Bartošová M., Zaujec A., Števlíková T., 1999a. Effect of ecological and integrated arable farming systems on crop productivity and soil fertility. [In:] J.E. Olesen, R. Eltun, M.J. Gooding (eds.), Proc. Int. Workshop on Designing and Testing Crop Rotations for Organic Farming, Tjele DARCOF, 297-304.

Lacko-Bartošová M., Zaujec A., Týr Š., 1999b. Productivity and soil fertility of ecological and integrated systems. [In:] B. Badalíková (ed.), Proc. Int. Conf. on Contemporary state and perspectives of the agronomical practices after year 2000. International Soil Tillage Research Organization Brno, 41-44.

Orlov D.S., Grishina, L.A., 1981. Praktikum po chimiji gumusa. MGU Moskva [in Russian].

Principles of ecological agriculture, 1995. VUVR Bratislava, 62.

Stevenson F.J., 1982. Humus chemistry – Genesis, composition, reactions. 3rd ed., Wiley & Sons New York.

Szombathová N., 1999. Comparison of soil carbon susceptibility to oxidation by $KMnO_4$ in different farming systems in Slovakia. Humic Subst. in the Environ. 1, 35-39.
Zaujec A., Tobiašová E., 1998. The effect of different farming systems on contents of non-humic substances in Ortic Luvisols. Humic Subst. in Ecosys. 2, 107-110.
Zdanov J.A., 1965. Average carbon oxidation degree and aminoacids. Biochimija 30, 1257-1259.

CHARAKTERYSTYKA KWASÓW HUMINOWYCH WYIZOLOWANYCH Z GLEBY O ROŻNYCH SYSTEMACH GOSPODAROWANIA

Streszczenie. Sposób zagospodarowania wpływa istotnie na właściwości próchnicy gleb. W pracy określono właściwości substancji humusowych pochodzące z gleby o dwu systemach gospodarowania. Doświadczenie polowe założono na Słowacji w 1990 r. na glebie płowej (Orthic Luvisols). Wyższą zawartością węgla organicznego (C_T) w warstwie powierzchniowej charakteryzowała się gleba, na której stosowano ekologiczny system gospodarowania (13,1 $g \cdot kg^{-1}$) z wykorzystaniem tylko nawozów organicznych w porównaniu z nawożeniem zintegrowanym – organiczno-mineralnym (12,5 $g \cdot kg^{-1}$). Jakość próchnicy (stosunek kwasów huminowych do fulwowych – HA : FA) była zbliżona w obu systemach gospodarowania. Dla wyseparowanych kwasów huminowych przeprowadzono analizy: składu pierwiastkowego, właściwości termicznego rozkładu oraz wykonano widma w zakresie UV-VIS. Kwasy huminowe ekstrahowane z gleby, na której stosowano ekologiczny system gospodarowania, w porównaniu z kwasami huminowymi gleby o zintegrowanym systemie zagospodarowania, charakteryzowały się niższą wartością stosunku H : C i wyższym stopniem utlenienia wewnętrznego. Kwasy huminowe pochodzące z gleby systemu ekologicznego charakteryzują się wyższą aromatycznością, większym stopniem humifikacji oraz lepszą jakością w porównaniu z kwasami huminowymi gleby o zintegrowanym systemie gospodarowania. O wyższej aromatyczności kwasów huminowych gleby użytkowanej w systemie ekologicznym świadczą również otrzymane dla tych kwasów niskie wartości stosunków absorbancji ($A_{2/4}$ $A_{2/6}$ i $A_{4/6}$) w porównaniu z pozostałymi analizowanymi kwasami huminowymi. Otrzymany na podstawie analizy termograwimetrycznej niski stopień alifatyczności kwasów huminowych gleby o ekologicznym gospodarowaniu w porównaniu z systemem zintegrowanym koreluje z otrzymanymi wynikami spektrometrycznymi i analizą składu pierwiastkowego, a tym samym wskazuje na wysoką jakość kwasów huminowych.

Słowa kluczowe: rolnictwo ekologiczne, rolnictwo zintegrowane, próchnica, kwasy huminowe

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