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PARAMETERS OF ORGANIC MATTER OF SOIL IN THE CULTIVATION OF *Miscanthus sacchariflorus*

PARAMETRY MATERII ORGANICZNEJ GLEBY POD UPRAWĄ TRAWY *Miscanthus sacchariflorus*

Abstract: Selected properties of organic matter in the humus horizon of soil fertilised with sludge at 10, 20 and 30 Mg · ha⁻¹ and with mineral fertilisers (NPK), were examined in the third year after harvesting biomass of *Miscanthus sacchariflorus*. The following were analysed: TOC, N_i in the soil; the following were analysed in the humic acids isolated from samples – elemental composition, atomic ratios, degree of internal oxidation, absorption coefficient, hydrophilic and hydrophobic properties, IR and ¹³C NMR spectra (the latter two were used for determination of carbon species). The organic carbon content was found to increase in the pot fertilised with the largest dose of the sludge fertiliser (30 Mg · ha⁻¹), while the nitrogen content increased in all the pots fertilised with the organic waste material, as compared with the control pot. Humic acids, isolated from the pots fertilised with sludge and mineral fertilisers (NPK), were found to have a higher degree of internal oxidation. The variance of the other quality parameters of those acids was not significant. Fertilisation with sludge did not significantly affect the properties of humic acids in the third year of cultivation of the test grass, which is important regarding the balance of the soil.

Keywords: *Miscanthus sacchariflorus*, sewage sludge, organic matter, infrared (IR) spectra, HPLC, ¹³C NMR

Introduction

The growing demand for energy, with its increasingly restricted supply, has been encouraging scholars to seek alternative sources. New energy sources are increasingly often sought among agricultural products. Plant species cultivated as material providing energy should be highly capable of fixing solar energy and transforming it to organic matter. They should also be capable of taking up nutrients from soil and their growth rate should be high. It is necessary to be able to obtain a high yield of biomass at a low

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cost of cultivation. Therefore, it is important to select an appropriate plant, suitable for a local habitat (soil, climate).

Among the plants cultivated as energy sources are grasses of genus *Miscanthus*, whose features – according to Hryniewicz and Grzybek [1] – include low energy consumption. The plants have thick, rigid stems filled with spongy cores and contain large amounts of lignin and cellulose. *Miscanthus* grasses can be used for 20 years, with most of financial outlays being made during the first year. It is an advantage of some of its species, eg *Miscanthus sacchariflorus*, that it can be cultivated on soils fertilised with sludge [2]. Using sludge for fertilisation of energy plants enables one to achieve higher biomass yield and better physicochemical soil properties [3].

The aim of the study was to evaluate the effect of NPK fertilisation of varied rates of fertilisation with sludge of *Miscanthus sacchariflorus* on the content of organic carbon and nitrogen in soil and on the properties of humic acids.

Material and methods

Samples taken from the humus horizon of the soil used in the cultivation of *Miscanthus sacchariflorus*, in the third year after biomass harvest, from plots with varied fertilisation options: no fertilisation – control (sample marked as “a”); mineral fertilisation (NPK) with the element ratio of 1 : 0.8 : 1.2 (“b”); 10^4 kg · ha⁻¹ of dry matter of sludge (“c”); $2 \cdot 10^4$ kg · ha⁻¹ of dry matter of sludge (“d”); $3 \cdot 10^4$ kg · ha⁻¹ of dry matter of sludge (“e”), were used in the study.

The sludge is characterised in Table 1 [2]. The amount of nitrogen introduced to soil with the NPK fertilisers was equivalent to the amount of nitrogen in $2 \cdot 10^4$ kg · ha⁻¹ of dry matter of sludge.

Table 1

Chemical composition of waste activated sludge [2]

Dry matter [%]	pH	TOC	N	TOC : N	P	K
		[g · kg ⁻¹]	[g · kg ⁻¹ d.m.]			
24.5	6.5	351	41.50	8.5 : 1	20.78	2.90

The following were determined in the samples total organic carbon (TOC) and total nitrogen (N_t) – with the VarioMax CN analyzer by Elementar (Germany). *Humic acids* (HAs) were extracted and purified according to standard methods using the following procedure:

- decalcification with 0.05 M HCl at 1 : 10 (w/v) ratio, extraction time 24 h. After centrifugation of the solution, the residue was washed with distilled water until a neutral reaction was reached;

- extraction of humic acids: solid remaining after decalcification was subjected to 0.5 M NaOH at 1 : 10 (w/v), with 24 h extraction time, occasionally mixed, then centrifuged humic acids extract;

- precipitation of humic acids: the alkaline extract obtained was treated with 2 M HCl giving pH = 2, after 24 hours the precipitate of humic acids was separated by centrifugation;

– purification of humic acids, the residue of humic acids obtained was treated with a mixture of HCl-HF (990 cm³ H₂O, 5 cm³ HCl and 5 cm³ HF) over 24 hours and subsequently separated in a centrifuge. This action was repeated three times. After purification the residue of humic acids was treated with distilled water until a zero reaction to chlorides was achieved, then freeze-dried.

The extracted humic acids were analyzed for:

a) elemental composition (Perkin Elmer CHN analyzer, Series II 2400). On the basis of elemental composition the values of atomic ratios: H/C, O/C, O/H, N/C and ω – internal oxidation degree were calculated according to the formula:

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

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

b) absorption spectra in UV-VIS range (Perkin Elmer UV-VIS Spectrometer, Lambda 20). VIS spectra were performed for 0.02 % humic acids solutions in 0.1 M NaOH and UV-spectra were determined after fivefold dilution. Absorbance measured at the wavelengths 280 nm (A_{280}), 400 nm (A_{400}), 465 nm (A_{465}), 600 nm (A_{600}) and 665 nm (A_{665}) were used to calculate 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,
- $\Delta \log K = \log A_{400} - \log A_{600}$;

c) infrared spectra (Perkin-Elmer FT-IR Spectrometer, Spectrum BX). FT-IR spectra for the range 400–4400 cm⁻¹ were done for HA samples of 3 mg in 800 mg KBr. To carry out IR spectra processing, the deconvolution method was applied, with a filter making the bands of $\gamma = 4$ narrower, and the process of smoothing, for which the length parameter was $l = 80\%$ [4];

d) ¹³C NMR spectra – with the spectrometer MSL-300 by Bruker. Based on the areas defined under peaks [5, 6], there was calculated the share of carbon in the following bonds:

- C_{al} – carbon in aliphatic bonds (0–108 ppm),
- C_{ar} – carbon of aromatic bonds (108–145 ppm),
- C_{lig} – carbon of aromatic lignin bonds (145–165 ppm),
- C-CO – carbon of carboxyl groups (165–180 ppm) and carbonyl groups (180–200 ppm)

and parameters:

– C_{al}/C_{ar} – ratio of carbon of aliphatic bonds (C_{al}) to carbon of aromatic bonds and lignin bonds ($C_{ar} + C_{lig}$);

e) hydrophilic and hydrophobic properties with the use of liquid chromatograph HPLC Series 200 with DAD detector (Perkin-Elmer). The separation involved the use of column X-Terra C18, 5 μ m, 250 · 4.6 mm. Solutions of humic acids were applied in 0.01 mol/dm³ NaOH of the concentration of 2 mg/cm³; injection of the sample – 0.1 cm³; solvent–acetonitril–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 peaks, the share of

hydrophilic (HIL) and *hydrophobic* (HOB = HOB-1 + HOB-2) fractions in humic acids molecules and the parameter: HIL/HOB [4, 7, 8].

Results and discussion

The TOC content in the soil samples ranged from 28.47 (plot fertilised with NPK) to 32.25 g · kg⁻¹ (plot with 3 · 10⁴ · ha⁻¹ of sludge) (Table 2). It ranged from 29.61 to 30.64 g · kg⁻¹ in the soil of the other plots, regardless of fertilisation option. The nitrogen content (N_t) in the soil of the plots where no sludge was added, was similar – ca 1.5 g · kg⁻¹. The addition of sludge to soil increased the content of N_t by 19 to 45 %, as compared with soil with no fertilisation; the largest growth was caused by addition of 30 Mg · ha⁻¹ of sludge. An increase in the amount of N_t in soil fertilised with sludge reduced the TOC/N_t ratio, as compared with soil with no fertilisation (control) and that fertilised with NPK.

Table 2

TOC and N_t content and ratio values of TOC/N_t in the analyzed soil samples

Object	TOC	N _t	TOC /N _t
	[g · kg ⁻¹]		
Control	30.64	1.52	20.16
NPK	28.47	1.51	18.85
10 Mg · ha ⁻¹ of the sludge*	30.78	1.95	15.78
20 Mg · ha ⁻¹ of the sludge	29.61	1.81	16.36
30 Mg · ha ⁻¹ of the sludge	32.25	2.21	14.59

* – waste activated sludge.

Humic acids are one of the most important fractions of organic matter; they contribute to physical, biological and chemical properties of soil and immobilise toxic compounds, *etc* [4, 9].

The main elements which comprise *humic acids* (HAs) include carbon, hydrogen, oxygen and nitrogen. The elemental composition of humic acids, expressed as weight and atom percent, is shown in Table 3.

Table 3

Elemental composition of humic acids

Object	C	H	N	O	C	H	N	O
	weight [%]				atomic [%]			
Control	51.49	5.34	4.30	38.87	34.70	43.17	2.48	19.65
NPK	52.10	5.19	4.17	38.54	35.48	42.41	2.43	19.68
10 Mg · ha ⁻¹ of the sludge*	52.35	5.17	4.24	38.24	35.68	42.29	2.48	19.55
20 Mg · ha ⁻¹ of the sludge	51.46	5.22	4.20	39.12	34.98	42.62	2.44	19.95
30 Mg · ha ⁻¹ of the sludge	51.43	5.26	4.39	38.91	34.86	42.82	2.55	19.78

* – waste activated sludge.

Humic acids in soil fertilised with larger doses of sludge (20 and 30 Mg · ha⁻¹) contained smaller amounts of carbon as compared with HAs in soil fertilised with NPK and sludge at 10 Mg · ha⁻¹. The hydrogen content in HA molecules in the pots fertilised with sludge and NPK was lower than or close to (30 Mg · ha⁻¹ of sludge), as compared with humic acids in soil where no fertilisation was applied. The nitrogen and oxygen content in HAs of the soil did not differ much, regardless of the fertilisation option. A slightly higher content of oxygen was found in HAs in soil fertilised with sludge at 20 Mg · ha⁻¹. The H/C ratio lay within the range from 1.19 to 1.24 (Table 4). No significant differences in the N/C, O/C or O/H values were found. Soil fertilisation with NPK and sludge at 10 to 30 Mg · ha⁻¹ increased the degree of internal oxidation in HA molecules. The lowest value of the parameter was determined for HAs in soil with no fertilisation.

Table 4

Value of atomic ratios ω and degree of internal oxidation of humic acids

Object	H/C	N/C	O/C	O/H	ω
Control	1.24	0.072	0.57	0.46	0.103
NPK	1.20	0.069	0.55	0.46	0.120
10 Mg · ha ⁻¹ of the sludge*	1.19	0.069	0.55	0.46	0.119
20 Mg · ha ⁻¹ of the sludge	1.22	0.070	0.57	0.47	0.132
30 Mg · ha ⁻¹ of the sludge	1.23	0.073	0.57	0.46	0.126

* – waste activated sludge.

The value of absorbance at the wavelengths of $\lambda = 280, 400, 465, 600$ and 665 nm was used to determine the absorbance ratios $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta\log K$ (Table 5). The $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta\log K$ coefficients can be used to evaluate the advancement of humification of organic matter and to characterise the resultant humic substances [4, 10, 11]. The absorbance values for humic acids isolated from soil taken from plots where fertilisers were applied were usually higher as compared with the control. The absorbance coefficients $A_{2/4}$, $A_{2/6}$, $A_{4/6}$ and $\Delta\log K$ HAs for soil fertilised with NPK and sludge at 10 and 20 Mg · ha⁻¹ were lower compared with the soil with no fertilisation applied and soil fertilised with sludge at 30 Mg · ha⁻¹.

Table 5

Value of absorbance at the wavelengths of humic acids

Object	A ₂₈₀	A ₄₀₀	A ₄₆₅	A ₆₆₅	A _{2/4}	A _{2/6}	A _{4/6}	$\Delta\log K$
Control	2.93	1.21	0.626	0.104	4.68	28.23	6.03	0.790
NPK	3.00	1.28	0.667	0.113	4.50	26.47	5.89	0.777
10 Mg · ha ⁻¹ of the sludge*	2.94	1.26	0.656	0.111	4.48	26.54	5.93	0.782
20 Mg · ha ⁻¹ of the sludge	2.87	1.22	0.633	0.110	4.53	26.10	5.76	0.780
30 Mg · ha ⁻¹ of the sludge	2.81	1.21	0.621	0.102	4.52	27.54	6.09	0.796

* – waste activated sludge.

In the molecules of the humic acids analyzed there were isolated hydrophilic (HIL) and hydrophobic fractions (HOB-1 and HOB-2). Peaks found on chromatograms (Fig. 1) ranging from 2.4 to 7.5 min. correspond to hydrophilic fractions, while peaks ranging from 17.0 to 25.0 min. correspond to hydrophobic fractions (HOB) [4, 7, 8].

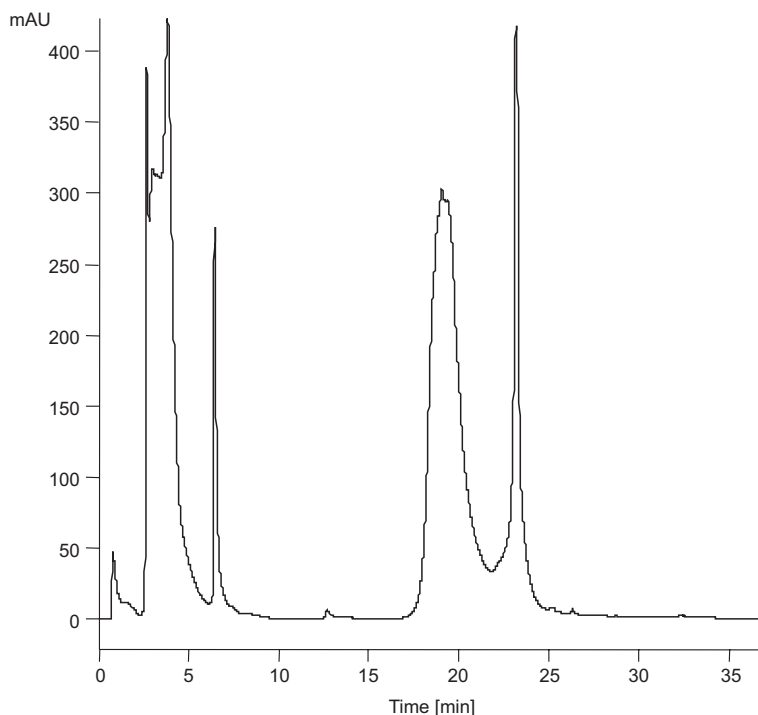


Fig. 1. Example of the chromatogram (HPLC) of humic acids (plot fertilised with 20 Mg · ha⁻¹ of sludge)

Fraction HOB-1 is a hydrophobic fraction of a shorter retention time from 17.2 to 21.0 and fraction HOB-2 ranges from 21.0 to 25.0 min. The share of hydrophobic fractions considerably exceeded the share of hydrophilic fractions (Table 6).

Table 6

Parameters describing the hydrophilic and hydrophobic properties of humic acids

Object	HIL-1	HIL-2	Σ HIL	HOB-1	HOB-2	Σ HOB	HIL/HOB
Control	42.3	4.5	46.8	38.6	14.6	53.2	0.878
NPK	41.5	4.6	46.1	39.5	14.4	53.9	0.854
10 Mg · ha ⁻¹ of the sludge*	40.7	4.6	45.3	40.6	14.1	54.7	0.829
20 Mg · ha ⁻¹ of the sludge	41.5	4.5	46.0	39.3	14.7	54.0	0.852
30 Mg · ha ⁻¹ of the sludge	41.2	4.7	45.9	39.5	14.6	54.1	0.850

* – waste activated sludge.

The application of NPK fertilisers and varied rates of fertilisation with sludge slightly modified hydrophilic and hydrophobic properties of analyzed humic acids. The HAs of objects fertilised with sludge and mineral fertilisers characterized by slightly lower contribution of hydrophilic fractions and higher contribution of hydrophobic ones, compared with the not fertilised soil.

Above relationships indicate slight effect of type of fertilisation on the properties and structure of humic acids (in the third year after application), which is confirmed by ^{13}C NMR and IR spectra.

In the spectra of the HAs (Fig. 2), there was identified the presence of the band in the range of wavenumbers $3100\text{--}3600\text{ cm}^{-1}$ corresponding to the stretching vibration of --OH groups in alcohols, phenols and acids and NH . The inflection noted in the range of wavenumbers $3000\text{--}3100\text{ cm}^{-1}$ is connected with the presence of CH bonds (valence vibration) of aromatic and alicyclic compounds. The bands in the $2920\text{--}2960\text{ cm}^{-1}$ range and around 2850 cm^{-1} are conditioned by the presence of groups --CH_3 and =CH_2 (symmetric and antisymmetric stretching vibration). The $1710\text{--}1730\text{ cm}^{-1}$ band points to the presence of carbonyl groups C=O (stretching vibration) in the acids and ketones and the bands in the range of wavenumbers $1600\text{--}1660\text{ cm}^{-1}$ are connected with the occurrence of stretching vibration C=O ($1630\text{--}1680\text{ cm}^{-1}$), NH deformation vibration ($1620\text{--}1660\text{ cm}^{-1}$) in the primary amides, and C=C stretching vibration (1610 cm^{-1}) in

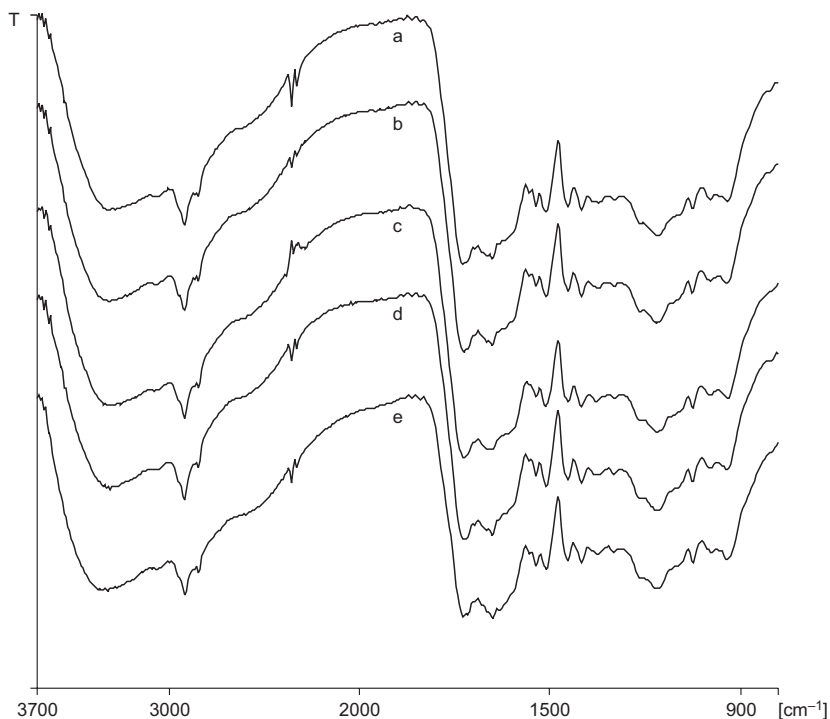


Fig. 2. FT-IR spectra of humic acids: a – no fertilisation; b – fertilisation with NPK; c – $10\text{ Mg} \cdot \text{ha}^{-1}$ of dry matter of sludge; d – $20\text{ Mg} \cdot \text{ha}^{-1}$ of dry matter of sludge; e – $30\text{ Mg} \cdot \text{ha}^{-1}$ of dry matter of sludge

the aromatic ring. In the 1600–1660 cm^{-1} range there can also occur bands derived from the vibration of groups C=N, and C=C coupled with the carbonyl group. The band at around 1540 cm^{-1} is determined by the presence of amine groups (deformation vibration), the 1500–1520 cm^{-1} band is characteristic for aromatic compounds (C=C). The band in the range of wavenumbers 1440–1460 cm^{-1} corresponds to the deformation vibration of groups $-\text{CH}_3$ and $=\text{CH}_2$, the band 1400–1420 cm^{-1} points to the presence of groups O–C–O in esters and C=O in carboxyl acids, and OH groups. The 1320–1380 cm^{-1} band is connected with the presence of groups COO and CH bonds in the aliphatic chain. In the 1200–1280 cm^{-1} range there occur bands of C–O groups of phenols, carboxyl acids, esters and ethers, and the bands in the range 1130 cm^{-1} and 1030–1080 cm^{-1} point to the presence of polysaccharides. They can be also related to the presence of groups OCH_3 which occur in lignins and OH in alcohols [10, 12].

The IR spectra of humic acids in all the variants under study was generally similar (Fig. 2). An intensive 1710–1730 cm^{-1} band is noteworthy, indicating a high proportion of carbonyl groups in humic acid molecules. The proportion of C of carboxyl (165–180 range) and carbonyl (180–200 range) groups, calculated from the ^{13}C NMR results for soil samples from the control plot, from the plot where NPK fertilisers were applied and from one where sludge was applied at 30 t/ha, ranged from 19.4 to 21.5 % (Table 7).

Table 7

Percentage of selected forms of carbon in humic acids molecules calculated on the basis of ^{13}C NMR spectra

Object	C_{al}	C_{ar}	C_{lig}	C–CO	$C_{\text{al}}/C_{\text{ar}} + C_{\text{lig}}$
Control	51.2	21.4	5.9	21.5	0.53
NPK	50.4	22.4	7.8	19.4	0.59
30 $\text{Mg} \cdot \text{ha}^{-1}$ of the sludge*	53.1	21.7	5.4	19.8	0.51

* – waste activated sludge.

The carbon content in aliphatic structures ranged from 50.4 (HAs in the plot fertilised with NPK) to 53.1 (HAs in the plot fertilised with 30 $\text{Mg} \cdot \text{ha}^{-1}$ of sludge). Humic acids isolated from samples of soil fertilised with NPK contained slightly more carbon in aromatic structures as compared with HAs of the control and the pot with the largest dose of sludge. This results in higher values of $C_{\text{ar}}/(C_{\text{al}} + C_{\text{lig}})$ for HAs from the plot fertilised with NPK.

Conclusions

1. After three years of cultivation of *Miscanthus sacchariflorus* in soil fertilised with sludge, the organic carbon content was found to increase in the plot fertilised with the largest dose of the sludge fertiliser (30 $\text{Mg} \cdot \text{ha}^{-1}$), while the nitrogen content increased in all the plots (10, 20, 30 $\text{Mg} \cdot \text{ha}^{-1}$) fertilised with the organic waste material.

2. Humic acids of the organic matter, isolated from the plots fertilised with sludge and mineral fertilisers, were found to have a higher degree of internal oxidation

compared with the control. The variance of the other quality parameters of those acids was not significant.

3. Fertilisation of soil with sludge did not significantly affect the properties of humic acids in the third year of cultivation of the test grass, which is important in regard to the balance of the soil fertilisation in *Miscanthus* cultivation.

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Abstrakt: Badano wybrane właściwości materii organicznej poziomu próchnicznego gleby nawożonej osadem ściekowym w dawce 10, 20 i 30 Mg · ha⁻¹ oraz mineralnie NPK, w trzecim roku po zbiorze biomasy trawy *Miscanthus sacchariflorus*. Analizowano: C_{org}, N_t w glebie, a w wydzielonych kwasach huminowych – skład pierwiastkowy, stosunki atomowe, stopień utlenienia wewnętrznego, wartość współczynnika absorpcji, właściwości hydrofilowo-hydrofobowe, widma w zakresie podczerwieni (IR) oraz ¹³C NMR i na ich podstawie określono formy węgla. Stwierdzono zwiększenie zawartości węgla związków organicznych na obiekcie nawożonym największą dawką osadu ściekowego (30 Mg · ha⁻¹), a azotu na wszystkich obiektach

nawożonych tym odpadowym materiałem organicznym, w stosunku do obiektu kontrolnego. Kwasy huminowe materii organicznej wydzielone z gleby obiektów nawożonych osadem ściekowym i mineralnie NPK cechowały się wyższymi wartościami stopnia utlenienia wewnętrznego. Zróżnicowanie pozostałych parametrów jakościowych tych kwasów nie było jednoznaczne. Nawożenie osadem ściekowym nie wpłynęło istotnie na zmianę właściwości kwasów huminowych w trzecim roku uprawy trawy testowej, co jest ważne z punktu widzenia stanu równowagi nawożonej gleby.

Słowa kluczowe: *Miscanthus sacchariflorus*, osady ściekowe, materia organiczna, widma IR, HPLC, ¹³C NMR