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Original Research

Effect of Post-Harvest Residue of Maize, Rapeseed, and Sunflower on Humic Acids Properties in Various Soils

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Abstract

The aim of our paper was to determine the properties of humic acids produced as a result of decomposition (under controlled conditions) of post-harvest residue of maize, rapeseed, and sunflower in different soil types (haplic luvisol, chernozems, haplic arenosols, and haplic gleysols). The extraction of humic acids was performed from the samples obtained directly once the post harvest residue was mixed with soil material, and after 360 days of incubation. The properties of humic acids were determined based on elemental analysis HPLC and HPSEC. The qualitative parameters of humic acids demonstrated that introducing sunflower post-harvest residue into the soil causes the formation of humic acids typical for soils without any additives. On the other hand, properties of humic acids after introducing maize post-harvest residue into soil are most modified.

Keywords: plant residues, humic acids, elemental composition, HPLC, HPSEC

Introduction

Many biological, chemical, and physical soil properties depend directly or indirectly on the presence of organic matter in soil, the main components of which are humus substances. Therefore, organic matter is the key factor in forming and maintaining soils of high quality [1-6]. Bearing this in mind, it becomes necessary to limit organic matter mineralization, and thus limit greenhouse gas emissions by aiming at stabilization and even increasing its resources. One of the agro-technical treatments affecting the content as well the quality of humus is adequate crop selection in crop rotation [7-10]. It is common knowledge that post-harvest plant residue introduced into soil undergoes mineralization and humification, thus showing a specific effect on the content and the quality of humus in soils [8-11]. Next to the amount of post-harvest residue introduced into soil, yet another factor conditioning their decomposition process is quality (the chemical composition of post-harvest residue). The main factors determining the rate of plant residue mineralization processes include the content of carbon, nitrogen, and phosphorus; the C/N and C/P ratio values and the share of easily-decomposable compounds (monosaccharides, proteins, hemicellulose, starch) [8, 9, 12-16].

As reported by Cortez et al. [17], and Osano and Takeda [16] at further stages of decomposition, the intensity of the process depends considerably on the resistant compounds content, mostly lignins. Osano and Takeda [16] point to the role played by the values of the ratio of lignins to nitrogen, as well as lignins to phosphorus in plant material transformation processes.

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Considering the applicable literature reports [10, 18], post-harvest residue decomposition is also determined by soil properties. Fast decomposition is observed in soils of high bioactivity. The decomposition slow-down occurs in soils of low bioactivity and poorly-aerated soils, as well as when facing excessive or insufficient moisture, low temperature, and low pH. Factors inhibiting mineralization also include bacteriostatic compounds, e.g. tannins, phenols, and resins [19].

It is common knowledge that the agricultural and ecological importance of humus substances (including humic acids) comes not only from their soil resources but also from their physicochemical properties. In some processes that occur in soils, the quality of humus substances plays a much greater role than its total content.

With that in mind, the aim of the present paper was to determine the properties of humic acids formed as a result of the decomposition process (under controlled conditions) of post-harvest residue of maize, rapeseed, and sunflower in different soil types (haplic luvisol, chernozems, haplic arenosols, and haplic gleysols). The properties of humic acids were determined based on the analysis of their elemental composition and chromatographically (HPLC and HPSEC). The application of chromatographic analysis allows us to define polydispersity and hydrophilic-hydrophobic properties. These are directly influenced by humic acid solubility and susceptibility to biodegradation, as well as affecting their sorption potential.

Material and Methods

The research material was made up by post-harvest residue (mixture of straw and roots) of maize (M), rapeseed (R), and sunflower (S). The samples of the following soils were used as soil substrate for research: haplic luvisol (HL), chernozems (Ch), haplic arenosols (HA), and haplic gleysols (HG), sampled from the arable layer (0-30 cm). The post-harvest residue was mixed with soil samples at a ratio of 1:10. The research involved the use of samples obtained right after mixing post-harvest residue with soil material (samples marked with symbol 0) and after 360 days of incubation (samples marked with symbol 360, e.g. samples marked 'HL' are haplic luvisol without post-harvest residue added, and the sample marked 'HLM-0' stands for haplic luvisol mixed with post-harvest residue of maize prior to incubation). The incubation was performed in plastic pots (3 pots for each variant) at 25ºC, and moisture was maintained at 60% of full water capacity.

Humic acids were extracted from both fractions with 0.5 mol/L NaOH solution after earlier removal of carbonates with 0.01 mol/L HCl. Humic acids were precipitated with HCl solution at pH 2 and then purified with the mixture HCl+HF. The preparations were lyophilized and powdered in agate mortar. Ash content in the humic acid preparations was lower than 2%. The separated humic acids were analyzed for:

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

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

...where: O,N,H,C are content in atomic %.

- polydispersity with the use of high performance size exclusion chromatography (HPSEC) (Perkin Elmer Series 200 chromatograph HPLC, diode array UV detector operating at 254 nm). Humic acids were separated with a TSK G3000SW column (7.5 mm × 600 mm). Sodium acetate 0.01 mol/L at pH=7 was used as eluent. The guard column was TSK G3000SW (7.5 mm × 75 mm). Solutions of humic acids were applied in 0.01 M CH_3COONa of the concentration of 0.05 mg/mL. A sample of 100 μL was injected into the column. The peak area was determined: S_1 – the first peak area, S_2 – the second peak area as well as the $S_{1/2}$ ratio [20, 21].
- 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 × 4.6 mm. 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 – acetonitril–water; solvents flow in the gradient (ratio H_2O: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 (ΣHOB=HOB-1+ HOB-2) fractions in humic acid molecules and the parameter HIL/ΣHOB [22-24] were determined.

The results were verified by analysis of variance with the use of the Tukey test at the significance level of α=0.05, for multifactor experiments without reps (ANALWAR statistics software), for the following factors:

- Factor I – soil type (HL – haplic luvisol, Ch – chernozems, HA – haplic arenosols, HG – haplic gleysols
- Factor II – post-harvest residue type (M – maize residue, R – rapeseed residue, S – sunflower residue)
- Factor III – incubation time of post-harvest residue with soil material (0, 360 days).

The evaluation of similarities of the treatments researched was made using cluster analysis. The relationships between the characters were defined with the coefficients of correlation. The above relationships were determined with STATISTICA MS statistics software.

Results and Discussion

Research Material Characteristics

The post-harvest residue of maize, as compared with the other plant materials applied in the experiment, demonstrated the highest content of organic carbon and easily decomposable compounds (monosaccharides, proteins, and starch – C_1, Table 1) and the lowest content of heavily decomposable compounds (C_3 – lignins). The low content of lignins resulted in the lowest values of the ratio of lignin/N and lignin/P (respectively, 13.2; 100), obtained for

Table 1. Chemical composition of post-harvest residue and the share of easily-decomposable compounds (C_1), slow-decomposable (C_2), and heavily-decomposable compounds (C_3) [10].

Residue type	Ash	C	N	P	C/N	C/P	*C_1	C_2	C_3
	%	g/kg					%		
Maize (M)	6.8	487	13.6	1.8	35.8	270	19.4	62.6	18.0
Rapeseed (R)	14.5	444	12.9	1.6	34.4	277	10.7	56.5	32.9
Sunflower (S)	10.6	433	14.4	1.8	30.1	240	15.3	58.4	26.3

*C_1 – proteins, monosaccharides, and starch; C_2 – hemicelullose and cellulose; C_3 – lignins

Table 2. Basic chemical properties of soils [25].

Soil type	pH in H_2O	TOC	IC	N_t	TOC/N_t	Clay fraction
		g/kg				[%]
Haplic luvisol (HL)	6.33	13.9	–	1.55	8.97	11
Chernozems (Ch)	7.87	21.1	15.4	2.53	8.34	8
Haplic arenosols (HA)	6.31	5.1	–	0.93	5.48	4
Haplic gleysols (HG)	7.45	17.7	0.13	2.33	7.60	34

post-harvest residue of maize. Interestingly, however, the residue demonstrated the highest content of slow-decomposable compounds: cellulose, hemicelullose (C_2), and the highest value of the C:N ratio.

The rapeseed post-harvest residue showed the lowest content of nitrogen and easily-decomposable compounds (C_1), and the highest – of lignins – C_3, Table 1, and thus the widest ratio of lignins-to-nitrogen as well as lignins-to-phosphorus (respectively, 25.5, 205).

The sunflower post-harvest residue contained the most nitrogen and demonstrated the lowest values of C/N and C/P ratios (Table 1). The value of the lignins/N ratio was 18.3 and lignins/P – 146.

The incubation experiment was performed under optimal conditions, namely at the moisture of 60% of full water capacity and temperature of 25ºC, at which the mineralization of the plant matter is most intensive. The pH of the soils selected for the present experiment ranged from 6.31 for haplic arenosols to 7.87 for chernozems (Table 2). The highest content of TOC and N_t was reported in Chernozem samples, while the lowest was in haplic arenosols samples. The TOC/N_t ratio assumed the highest value for haplic luvisol, and chernozems showed a value similar to that reported for haplic luvisol, whereas for haplic gleysols it was 7.60 and for haplic arenosols the value was lowest (5.48) (Table 2).

An essential factor, besides the above named, affecting the intensity of the pattern of mineralization and humification processes, is the grain-size composition of soils [26, 27]. Jurcova et al. [27], investigating the decomposition of various plant residue in soils, of various content of clay fraction, found that the higher the content of that fraction, the lower the plant residue mineralization intensity. The highest content of clay fraction was found for haplic gleysols and the lowest one for haplic arenosols (Table 2).

Humic Acid Characteristics

The basic chemical characteristic of humus substances is their elemental composition. Many authors [7-9, 24], investigating the decomposition of plant materials, demonstrated that ‘young’ humic acids show a higher content of hydrogen and a lower content of carbon, as compared with mature humic acids.

In the present experiment it was shown that the content of C, H, and N depends not only on the selected soil type but also on the type of post-harvest residue introduced into soil and changes during incubation (Tables 3 and 4). Having introduced post-harvest residue of maize, there was recorded a significant decrease in the content of carbon in humic acid molecules, for haplic arenosols only. Adding post-harvest residue of rapeseed resulted in a decrease in the content of carbon in humic acid molecules in haplic arenosols and haplic gleysols and, having introduced post-harvest residue of sunflower, there also was reported a decrease in the content of carbon in humic acids of haplic luvisol. Interestingly, the lowest significant changes in the content of carbon in humic acid molecules were observed in maize variants, which could have been due to the highest carbon content in the residue compared with the other studied samples (Table 1). Adding post-harvest residue, however, did not result in any changes in the carbon content in humic acids in chernozems. In the humic acid molecules in chernozems, irrespective of the variant, the carbon content ranged from 33.4 to 34.6.

After a direct addition of post-harvest residue, we observed in some cases a decreasing content of carbon in humic acid molecules, and during incubation we noted an

Table 3. Elemental composition (atomic %) of humic acids and the values of atomic ratio and the degree of internal oxidation.

Variant	C	H	N	O	H/C	N/C	O/C	O/H	ω
HL*	35.9	42.3	2.7	19.0	1.18	0.076	0.53	0.45	0.109
HLM-0	34.7	45.3	2.3	17.7	1.30	0.066	0.51	0.39	-0.085
HLM-360	34.6	43.4	2.7	19.3	1.25	0.077	0.56	0.44	0.089
HLR-0	34.6	47.3	2.5	15.6	1.37	0.073	0.45	0.33	-0.244
HLR-360	34.5	44.1	2.7	18.7	1.28	0.079	0.54	0.42	0.044
HLS-0	32.0	49.3	2.8	15.9	1.54	0.087	0.50	0.32	-0.288
HLS-360	35.1	42.1	2.9	20.0	1.20	0.082	0.57	0.47	0.185
Ch*	33.4	45.0	3.7	17.9	1.35	0.111	0.54	0.40	0.060
ChM-0	33.5	46.6	2.3	17.6	1.39	0.067	0.53	0.38	-0.138
ChM-360	33.7	44.4	3.1	18.7	1.32	0.091	0.56	0.42	0.067
ChR-0	33.4	47.5	2.8	16.3	1.42	0.084	0.49	0.34	-0.194
ChR-360	34.6	44.2	3.4	17.7	1.28	0.098	0.51	0.40	0.041
ChS-0	33.4	47.3	3.2	16.1	1.42	0.097	0.48	0.34	-0.165
ChS-360	33.8	43.9	3.5	18.7	1.30	0.103	0.55	0.43	0.119
HA*	37.3	42.8	2.8	17.0	1.15	0.075	0.46	0.40	-0.009
HAM-0	35.4	44.7	2.4	17.5	1.26	0.067	0.49	0.39	-0.072
HAM-360	35.2	43.2	2.5	19.1	1.23	0.070	0.54	0.44	0.068
HAR-0	33.5	46.8	2.2	17.5	1.40	0.066	0.52	0.37	-0.157
HAR-360	35.3	43.3	2.8	18.6	1.23	0.078	0.53	0.43	0.060
HAS-0	33.4	48.1	2.5	15.9	1.44	0.076	0.48	0.33	-0.260
HAS-360	35.3	43.6	2.7	18.3	1.24	0.078	0.52	0.42	0.036
HG*	34.4	45.7	3.4	16.5	1.33	0.100	0.48	0.36	-0.066
HGM-0	35.0	46.1	2.6	16.3	1.32	0.074	0.47	0.35	-0.165
HGM-360	34.2	43.9	2.7	19.2	1.28	0.079	0.56	0.44	0.075
HGR-0	33.6	47.9	2.7	15.9	1.43	0.079	0.47	0.33	-0.239
HGR-360	35.0	44.5	3.0	17.5	1.27	0.085	0.50	0.39	-0.015
HGS-0	32.6	47.0	2.3	18.1	1.44	0.072	0.56	0.39	-0.114
HGS-360	34.0	43.9	3.0	19.1	1.29	0.089	0.56	0.44	0.100

*For soils without additives we give mean values for humic acids isolated prior to incubation and after 360 days of incubation.

increase in the content of that element. The processes that occur during incubation gave rise to the formation of humic acids which, showed in the case of the variants involving haplic arenosols, as prior to incubation, still lower carbon content as compared with humic acids of haplic arenosols without additives. The relationships reported can come from differences in the intensity of decomposition of fresh plant material introduced into soil, which is a result of their chemical composition and the properties of soils as such. It is known that adding fresh organic matter to soil can intensify the mineralization of organic matter of a high degree of humification contained in soil.

Humic acids of soils without plant residue added demonstrated a lower content of hydrogen than after they were introduced into soil. Having added post-harvest residue into soils, a higher content of that element was reported for humic acids of soils with sunflower post-harvest residue, as compared with humic acids of the maize variants.

The post-harvest residue decomposition process, irrespective of the residue and soil types, was connected with a decrease in its content. After incubation the humic acids of haplic gleysols and chernozems mixed with post-harvest residue demonstrated similar (or lower) levels, and humic acids of haplic luvisol and haplic arenosols – similar (or higher) content of hydrogen, as compared with the humic acids without any additives.

Literature reports [7-9] show that the content of nitrogen in humic acid molecules depends on its content in plant residues. This was also confirmed by our study. An average higher content of this element was reported for humic acids of soils with rapeseed and sunflower residue than with maize residue (Table 4). In the present experiment the incubation time resulted in an increase in the content of nitrogen in the molecules of humic acids. We have also found out that nitrogen content in the molecules of humic acids was also the soil type. Humic acids of chernozems mixed with

Table 4. Results of the analysis of variance for the elemental composition (atomic %), atomic ratios and the degree of internal oxidation of humic acids.

Variant	C	H	N	O	H/C	N/C	O/C	O/H	ω
Factor I – soil									
HL	34.7	44.5	2.7	18.1	1.29	0.076	0.52	0.41	-0.012
Ch	33.7	45.5	3.2	17.6	1.35	0.095	0.53	0.39	-0.019
HA	35.4	44.4	2.6	17.6	1.26	0.072	0.50	0.40	-0.043
HG	34.1	45.6	2.9	17.4	1.34	0.085	0.51	0.38	-0.060
LSD	0.90	1.10	0.26	n.s.	0.06	0.014	n.s.	n.s.	n.s.
Factor II – post-harvest residue type									
‘0’	35.2	43.9	3.1	17.6	1.25	0.088	0.50	0.40	0.023
M	34.6	44.7	2.5	18.2	1.30	0.074	0.53	0.41	-0.020
R	34.3	45.7	2.8	17.2	1.34	0.080	0.50	0.38	-0.088
S	33.7	45.7	2.9	17.8	1.36	0.086	0.53	0.40	-0.048
LSD	0.89	1.10	0.26	n.s.	0.06	n.s.	0.030	n.s.	0.070
Factor III – time									
0 days	34.1	46.2	2.7	16.9	1.36	0.079	0.50	0.37	-0.126
360 days	34.8	43.8	2.9	18.4	1.26	0.084	0.53	0.42	0.059
LSD	0.46	0.56	0.13	0.49	0.03	n.s.	0.015	0.016	0.036

n.s. – non-significant difference

post-harvest residue, similarly as the humic acids of chernozems without post-harvest residue added, recorded a significantly higher content of nitrogen, as compared with the humic acids of another soils (Table 4). Those relationships confirmed that the elemental composition is characteristic for a given soil type, and only to some extent can it be modified by the properties of organic materials introduced into soil.

Completely different was the behaviour of oxygen in HA molecules. Statistical analysis did not demonstrate the relationship between oxygen content and soil type and the type of post-harvest residue. The content of this element, however, was changing throughout the incubation time. The amount of oxygen in humic acid molecules was higher after a year of incubation, as compared with its content in the humic acids isolated from soil samples before the start of incubation. The humification process is thus connected with organic matter oxidation processes [1].

Changes in the elemental composition are usually accompanied by changes in the value of atomic ratios of respective elements. They allow us to assess the structure of humic acid molecules roughly by evaluating the degree of aromatic ring condensation (the H/C ratio) and the degree of their maturity (O/C, O/H, ω) [1, 8, 9, 24, 28].

The H/C values recorded ranged from 1.15 to 1.54, which demonstrates that the humic acids contain aromatic sets coupled with the aliphatic chain including up to 10 carbon atoms. Rice and MacCarthy [29] report on aromatic/unsaturated carbon as being dominant in humic acid molecules at low values of the H/C ratio, while aliphatic carbon – at high values. The values of the H/C ratio for humic acids of soils without additives were, in general, lower than when they were mixed with the residue (Table 3). Having introduced plant materials into soils, the lowest values of that ratio were demonstrated for humic acids of the variants with maize. The results of the statistical analysis showed that for humic acids of soils with post-harvest residue after incubation there was a clear decrease in the H/C values (Tables 3 and 4), which can suggest an increase in the degree of their aromaticity, which comes from the results reported by e.g. Gonet and Debska [8, 9]. Similarly, Albrecht et al. [30] noted an increase in the share of aromatic and phenolic carbon and a decrease in the share of polysaccharides and other aliphatic structures in the fresh organic matter composting process. As reported by Rosell et al. [31], the lower the value of the H/C ratio in humic acids, the higher their stability, degree of aromatic rings condensation, and degree of organic matter humification.

The organic matter humification process is closely connected with an increase in the content of oxygen and a decrease in the content of hydrogen [8, 9, 32]. Changes in the content of oxygen and hydrogen are, in general, accompanied by changes in the value of the O/H and O/C atomic ratios. The higher the O/H ratio, the higher the humification of humic acid molecules. The value of the O/C ratio is an indicator of their degree of oxidation. The values of the O/H and O/C ratios in humic acids molecules separated after 360 days of incubation from soil samples mixed with plant residue were, in general, significantly higher as compared with the values characteristic for humic acids of soils mixed with plant residues before incubation (Tables 3 and 4). However, there was found no significant effect of the soil type and the post-harvest reside on the values of the parameters discussed.

An important parameter describing the advancement of the plant materials humification process is also the degree of internal oxidation (ω) of humic acid molecules. The parameter can also serve to provide characteristics of the humus substances formed [8, 9, 24]. The degree of internal oxidation of humic acids was calculated based on Zdanow's formula (1965) (cited after [9]), which considers both the bonds of carbon with oxygen as well as with hydrogen and nitrogen. Humic acids separated from soils without any additives demonstrated higher values of the degree of internal oxidation as compared with the humic acids of soils mixed with residue (Tables 3, 4). Therefore, introducing residue into soils clearly decreases the degree of oxidation, which is obvious in Fig. 1 and diagrams presenting changes in parameters O/H and ω, while humic acids isolated from the soil samples with additives after incubation demonstrate higher values of the degree of internal oxidation than prior to incubation, which is connected with progress in the process of plant residue humification (Tables 3 and 4, Fig. 1). The values of the degree of internal oxidation were modified by the type of post-harvest residue, irrespective of soil type. The average highest values of that parameter were recorded for maize variants and the lowest for the humic acids isolated from soil samples containing rapeseed post-harvest residue (Table 4).

The application of HPSEC (high-performance size-exclusion chromatography) allowed a separation of the isolated humic acids depending on molecule size [20, 33-37]. The chromatogram patterns (Fig. 2) can show the presence of two fractions in the molecules of humic acids. The first peak, of a shorter retention time (about 14 min), corresponds to that fraction of humic acids the molecules of which are greater in size (fraction S_1), while the second one the peak of which occurred most frequently at the retention time of about 19 min. is characteristic for molecules smaller in size (fraction S_2). Conte et al. [37], and Piccolo et. al. [38] point to the differences in the chemical properties of respective fractions of humic acids. Canellas et al. [39]

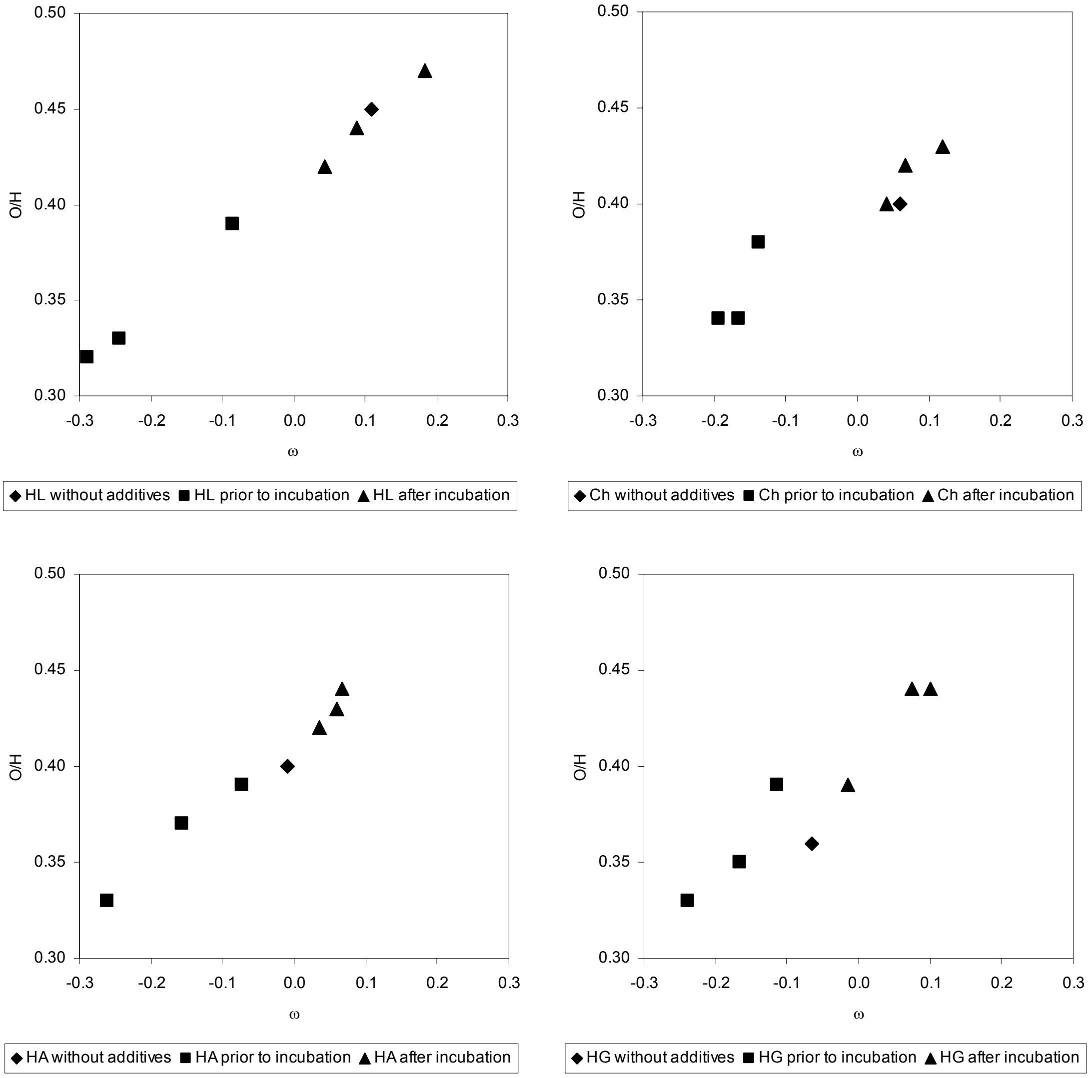

Fig. 1. Diagrams presenting the relationships of the O/H ratios and ω.

report on high-molecular fractions showing a higher content of carbon and nitrogen, as compared with the low-molecular-weight fractions.

Introducing post-harvest residue into soil, in general, resulted in an increase in the share of high-molecular-weight fraction in humic acids (fraction S_1, Tables 5, 6). However, the share of that fraction of humic acids, irrespective of soil type and the the type of post-harvest residue, decreased throughout the incubation time, thus increasing the share of the low-molecular-weight fraction. And so humic acids isolated from the soil samples mixed with post-harvest residue prior to incubation demonstrated a lower share of the fraction defined as the low-molecular one, as compared with the humic acids after incubation. As a consequence of changes in the share of respective fractions there occurred changes in parameter S_1/S_2, referred to as the index of polydispersity that was decreasing throughout incubation. There was found no clear-cut effect of the post-harvest residue type on the polydispersity of humic acid molecules.

Ceritini et al. [40] claim that the direction of changes in the size of humic acid molecules (increase/decrease) depends on the mechanism of organic matter transformation. The results reported by Aleksandrowa [7] showed that humic acids at a very early stage of the plant material humification process demonstrate a greater molecular weight, while during the humification process there occurs a decrease in the molecular weight of humic acids. The present results can thus suggest that newly-produced humic acids at an early stage of organic matter transformation, showing a greater share of large-molecular-weight fraction, show a relatively large molecular weight as compared with the humic acids of a higher degree of maturity (the humic acids isolated from soil samples mixed with post-harvest residue after a year of incubation). With that in mind, it can be observed that the greater the progress in the humification process, the lower the average molecular weight.

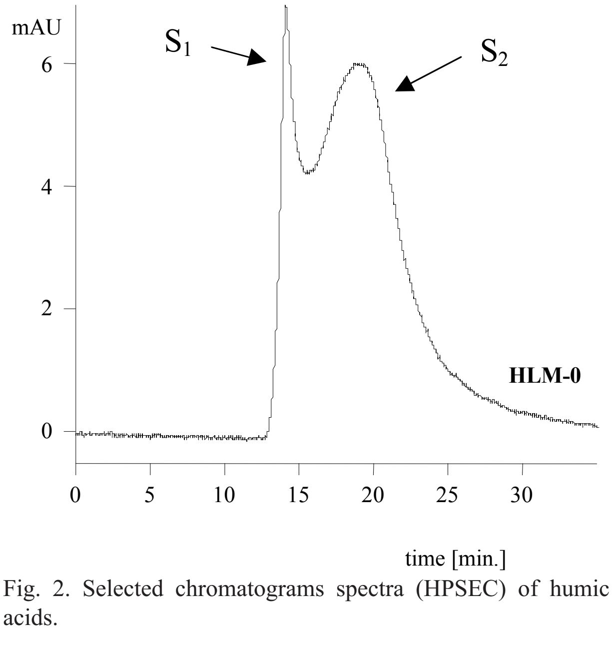

Fig. 2. Selected chromatograms spectra (HPSEC) of humic acids.

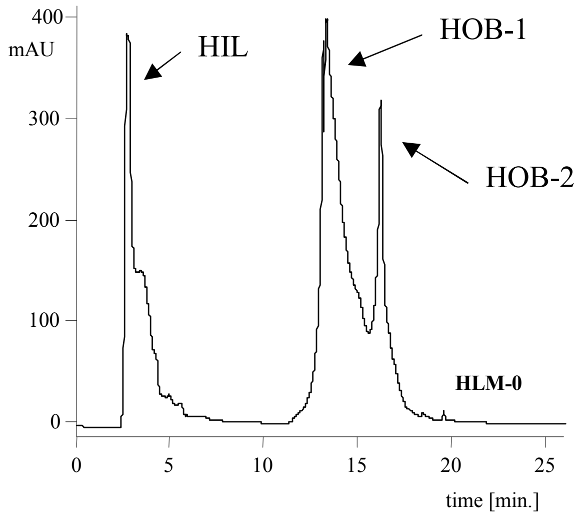

Fig. 3. Selected chromatogram spectra (HPLC) of humic acids.

Interesting information on the humic acids transformation is provided by high performance liquid chromatography (HPLC). As a result of the present chromatographic analysis, similar to Woelki et al. [22], Preuße et al. [23], and Debska [21, 24], there was reported a division into hydrophilic (HIL) and hydrophobic (HOB-1 and HOB-2) fractions (Fig. 3). The division of humic acids into respective fractions is based on differences in hydrophobicity. The peaks, ranging from 2.44 to 5.50 min., correspond to hydrophilic fractions (HIL), while those with a later retention time – to hydrophobic fractions (HOB), whereas hydrophobic fractions, the peaks of which ranged from 12.80 to 16.10 min., were referred to as HOB-1, and the peaks of a longer retention time (16.10-18.30 min.), due to a clear division from the peaks of a shorter retention time, were referred to as HOB-2.

The humic acids of soils without additives showed higher shares of HIL fractions than the humic acids of soils mixed with post-harvest residue, prior to incubation (Tables 5, 6). The share of fractions hydrophilic in character depended on soil type, the type of post-harvest residue, and incubation time. The incubation time of soils with plant residue, in general, increased the share of hydrophilic fractions. Most hydrophilic fraction was contained in the humic acids of the variants with haplic gleysols, and among post-harvest residue it was the sunflower that turned out to be the organic material the properties of which (the chemical composition) enhance the formation of humic acid molecules, of new structures containing sets of hydrophilic properties. Despite the increase in the share of hydrophilic fraction in the humic acids molecules of soils incubated with post-harvest residue, still after the end of incubation, for most variants the share of that fraction was lower than in the humic acids of soils without any additives. Irrespective of soil type, the greatest differences across the shares of hydrophilic fractions in humic acids of soils without additives and the humic acids of the variants containing post-harvest residue were recorded for the humic acids of variants with maize.

Table 5. Share (%) of hydrophilic and hydrophobic fractions and high- and low-molecular-weight fractions in humic acid molecules.

Variant	HIL	HOB-1	HOB-2	∑HOB	HIL/HOB	S_1	$S_{1/2}$
HL*	31.9	53.7	14.4	68.1	0.47	10.0	0.111
HLM-0	19.4	57.3	23.3	80.6	0.24	25.4	0.34
HLM-360	28.9	55.9	15.3	71.2	0.41	9.4	0.10
HLR-0	23.7	53.4	23.0	76.4	0.31	22.3	0.29
HLR-360	31.4	55.0	13.5	68.5	0.46	14.3	0.17
HLS-0	26.4	51.1	22.5	73.6	0.36	21.9	0.28
HLS-360	34.2	52.8	13.0	65.8	0.52	12.6	0.14
Ch*	36.4	50.5	13.5	63.6	0.57	20.4	0.260
ChM-0	15.4	60.4	24.3	84.7	0.18	29.0	0.41
ChM-360	28.6	53.6	17.7	71.3	0.40	8.7	0.10
ChR-0	22.8	53.3	23.9	77.2	0.30	19.6	0.24
ChR-360	31.9	49.8	18.3	68.1	0.47	13.3	0.15
ChS-0	35.0	47.2	17.9	65.1	0.54	28.9	0.41
ChS-360	34.8	50.9	14.3	65.2	0.53	9.9	0.11
HA*	34.1	53.9	12.0	65.9	0.52	22.4	0.289
HAM-0	22.4	56.9	20.6	77.5	0.29	11.3	0.13
HAM-360	26.6	53.8	19.6	73.4	0.36	13.1	0.15
HAR-0	27.0	51.0	22.0	73.1	0.37	27.4	0.38
HAR-360	30.2	54.7	15.1	69.8	0.43	20.7	0.26
HAS-0	30.7	48.6	20.7	68.7	0.44	24.0	0.32
HAS-360	37.0	51.3	11.6	62.9	0.59	19.0	0.23
HG*	35.3	49.3	15.4	64.7	0.55	6.4	0.070
HGM-0	23.8	50.1	24.9	75.0	0.32	16.3	0.20
HGM-360	31.6	54.4	14.0	68.4	0.46	10.3	0.11
HGR-0	29.5	47.5	23.0	70.5	0.42	23.5	0.31
HGR-360	36.1	48.6	15.3	63.9	0.57	16.2	0.19
HGS-0	35.6	46.8	17.5	64.3	0.55	19.3	0.24
HGS-360	36.5	48.9	14.6	63.5	0.58	11.2	0.13

*For soils without additives there are given mean values for humic acids isolated prior to the incuba-tion and after 360 days of incubation

The total share of hydrophobic fractions, similarly to the hydrophilic ones, was determined by soil type, post-harvest residue type, and incubation time (Tables 5, 6). Having introduced post-harvest residue into soils, the share of ΣHOB fraction was higher to compare with it its share in the humic acids of soils without additives (exception: GeSl-0). The incubation of soils with plant materials decreased the total share of the hydrophobic fraction in the humic acid molecules. Significant changes in the hydrophobic fractions during the time of incubation were a result of changes mainly in the HOB-2 fraction, and thus the fraction of more hydrophobic properties. Egeberg and Alberts [41] showed that the fraction of hydrophobic properties has a higher percentage of bigger molecules. In consequence a higher share of hydrophobic fraction also means that high-molecular fraction is also higher.

Changes in the share of hydrophilic and hydrophobic fractions are best presented by the values of the HIL/ΣHOB ratio (Tables 5, 6). It can be observed that introducing plant residue into soils significantly modified the values of the ratio, namely the HIL/ΣHOB values were lower for humic acids with additives to compare with humic acid of soils without plant residue. The decrease is evident especially for maize residue (Table 6). Statistical analysis demonstrated

Table 6. Results of the analysis of variance for the share (%) of hydrophilic and hydrophobic fractions as well as high- and low-molecular-weight fractions in humic acids.

Variant	HIL	HOB-1	HOB-2	∑HOB	HIL/∑HOB	S_1	$S_{1/2}$
Factor I – soil							
HL	28.5	54.1	17.4	71.5	0.405	15.74	0.193
Ch	30.2	51.9	17.9	69.8	0.445	18.78	0.242
HA	30.3	53.0	16.7	69.6	0.441	20.04	0.255
HG	33.0	49.4	17.5	66.9	0.499	13.70	0.164
LSD	3.41	3.01	n.s.	3.35	0.066	5.54	0.087
Factor II – post-harvest residue type							
"0"	34.4	51.7	13.8	65.6	0.528	14.80	0.182
M	24.6	55.3	19.9	75.3	0.333	15.44	0.192
R	29.1	51.7	19.3	70.9	0.416	19.66	0.249
S	33.8	49.7	16.5	66.2	0.514	18.35	0.232
LSD	3.41	3.01	3.19	3.35	0.066	n.s.	n.s.
Factor III – time							
0 days	28.1	51.9	19.9	7108	0.403	20.51	0.267
360days	32.8	52.3	14.8	67.1	0.492	13.62	0.160
LSD	1.75	n.s.	1.63	1.72	0.034	2.84	0.045

n.s. – non-significant difference

that the average highest value of the ratio was recorded for the humic acids of haplic gleysols, while the lowest was for the humic acids of haplic luvisol with additives (Table 6).

The present relationships show at the same time that the hydrophilic-hydrophobic properties depend on the 'degree of maturity' of humic acid molecules. The higher the degree of plant material humification, the higher the values of the HIL/ΣHOB ratio, which is due to an increase in the share of hydrophilic fractions and a decrease in the share of hydrophobic fractions in the humic acid molecules. The relationships also point to the share of hydrophilic and hydrophobic fractions in the molecules of humic acids depending, on the one hand, on the type of post-harvest residue introduced into soil, and being mostly a result of differences in the chemical composition of the post-harvest residue and, on the other hand, being conditioned by the soil genesis.

Based on the selected quality parameters (C, H, N, HIL, HOB-2, HIL/ΣHOB, S_1/S_2) of humic acids, the analysis of clusters was made. The dendrograms show that the treatments of similar properties are located in homogenous groups. The relationships (Fig. 4) demonstrate that, having introduced post-harvest residue, humic acids are produced, the properties of which differ from the properties of the humic acids of soils without additives significantly. The properties of humic acids of haplic gleysols and chernozems mixed with sunflower post-harvest residue were the only ones found in the same group with the humic acids of soils without additives. From the group with the humic acids of the variants with post-harvest residue we identified subgroups, and an effect of post-harvest residue type on HA properties was confirmed.

The sunflower post-harvest residue decomposition process in soils gives rise to humic acids, the properties of which are most similar to the properties of the humic acids of soils without additives (Fig. 5). Introducing maize post-harvest residue into soil modifies the humic acid properties not only right after being introduced but also after a year of decomposition considerably, which points to its slow decomposition, even though the basic chemical properties of post-harvest residue: the C/P ratio, the content of lignins

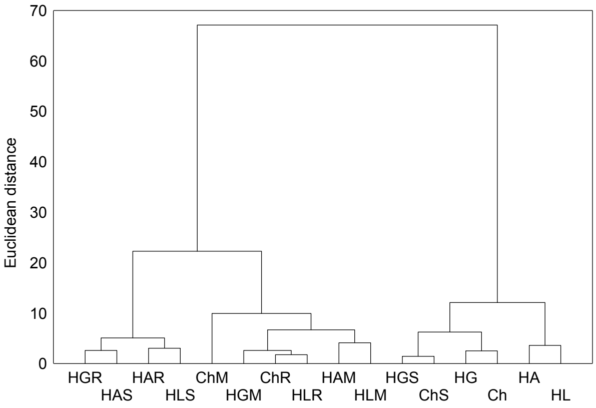

Fig. 4. Cluster analysis of humic acids without additives and prior to incubation.

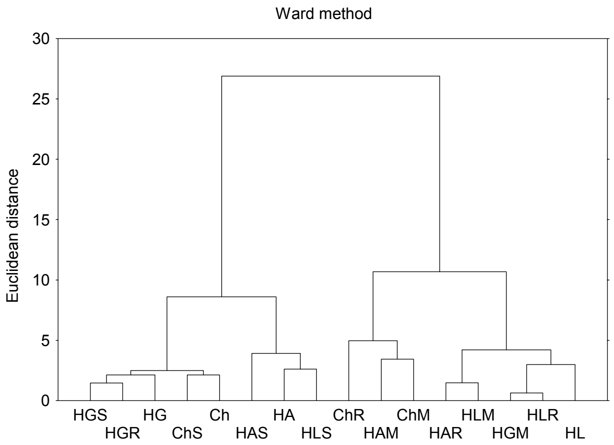

Fig. 5. Cluster analysis of humic acids without additives and after incubation.

and the ratio of lignins to nitrogen and lignins to phosphorus would suggest that, as compared with the maize residue, rapeseed post-harvest residue should undergo decomposition processes more slowly. The low rate of the maize post-harvest residue decomposition must be due to the highest, of all the residues investigated, content of C and C/N ratio as well as the compounds representing a low-decomposition-rate group (cellulose, hemicellulose (C_2)), as well as a result of the tissue structure of the residue investigated.

Conclusions

1. Introducing post-harvest residue into soils resulted in, irrespective of the soil type and the chemical composition of post-harvest residue, an increase in the content of hydrogen, a decrease in the value of the degree of internal oxidation, a decrease in the share of hydrophilic fractions, and in the value of the HIL/HOB ratio in humic acid molecules.
2. An increase in the degree of 'maturity' of humic acid molecules was, in general, connected with a decrease in the content of hydrogen, an increase in the content of oxygen and the value of the degree of internal oxidation, a decrease in the share of high-molecular-weight fraction and the value of $S_{1/2}$ parameter, as well as an increase in the share of hydrophilic fractions and the value of the HIL/ΣHOB ratio. The parameters can be used to evaluate the degree of advancement of the plant material humification process, irrespective of the soil type and the type of post-harvest residue introduced into soil.
3. Introducing sunflower post-harvest residue into soil results in a production of humic acids, the properties of which are most similar to the humic acids of soils without additives. Those that are most modified are the properties of humic acids after introducing maize post-harvest residue into soils.

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