

PROPERTIES OF HUMUS OF NATURAL FOREST SOIL AND ARABLE SOIL

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Abstract

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The aim of the present paper was to determine the properties of organic matter of forest soil (especially humic acids) representing the natural ecosystem and to compare its properties with agriculturally used soil – located in the vicinity of the forest. To carry out the investigations forest soil (chernozem) was sampled from the nature reserve “Veľký Báb” (Slovakia) and from the agricultural field adjacent to it.

It was shown that in forests soils a constant supply of fresh organic matter significantly modifies the properties of humic acids. Humic acids (HAs) of the humic horizon of the forest soil as compared with HAs of arable soil showed a lower degree of aromaticity, polydispersity, a lower share of carboxyl groups and hydrophilic fractions. It was found that down the profile the HAs molecules contain more carbon and less hydrogen, higher is the share of carboxyl groups and hydrophilic fractions, the lower is the share of aromatic lignin structures and hydrophobic fractions and the higher is the degree of polydispersity. Quality parameters which have been obtained for humic acids of the layer of 40–50 cm can suggest that the process of migration of organic carbon compounds introduced into soil takes places every year together with plant drop very inconsiderably. The highest effect of the plant drop on HAs properties is observed at the soil layer directly adjacent to the organic horizon.

Key words: forest soils, arable soil, humus, humic acids

Introduction

Forests are the most natural formation and at the same time an indispensable factor of the natural equilibrium. Forests serve different functions – ecological, production and social functions. Ecological functions of forests ensure e.g. the stability of water circulation in nature, prevent floods, avalanches and land slides, protect soils from erosion and protect the

landscape from becoming steppe-like, develop the global and local climate, create conditions for maintaining the biological potential of a great number of species and, at the same time, enhance the biodiversity and complexity of the landscape.

In forest ecosystems, a relatively small part of plant production serves the herbivore as food as a living matter, whereas its main matter (88–99%), sooner or later gets into soil (Dziadowiec, 1979). As known, vegetation is the leading soil-forming factor and plays a great role in the development of soil properties. Humus quantity and quality accumulated in soil are essential for soil fertility and an effective operation of ecosystems. A considerable share of humic substances (Dziadowiec, 1993; Gonet, 1997) in soil humus as well as their specific chemical structure make these compounds play an important role in the natural environment. In fact they participate in all the processes which take part in soil and affect their physical, chemical and biological properties. The amount of humus accumulated in soils and the properties of humic substances obtained depend on the course of processes of mineralization and humification of plant drop. The intensity of these processes depends considerably on the chemical composition and the morphology of plant residue entering soil (Aleksandrova, 1980; Almendros et al., 2000; Berg, McLaugherty, 1989; Gonet, Debska, 1998; Lorenz et al., 2004; Osano, Takeda, 2004; Son et al., 2004; Zaujec, 1980). Many of the above authors pay attention to the effect of the ratio of carbon to nitrogen and carbon to some ash elements on the initial stages of the mineralization process. At later stages the intensity of the decomposition of organic matter depends considerably on the content of compounds resistant to the decomposition of lignin mostly (Cortez et al., 1996; Osano, Takeda, 2004). Osano and Takeda (2004) pay attention to the role of the value of the ratio of lignin to nitrogen and lignin to phosphorus in plant litter transformation processes. In forest soils one of the main factors affecting the properties of humic substances will be thus the tree stand species composition. It was demonstrated that e.g. in leaf litter in the humification process the share of humic acids is higher than that of fulvic acids and in coniferous litter there is an equilibrium of both groups of these substances (Dziadowiec, 1990). Kaczmarek (2000) reports on the humic horizon of soil under pine showing a higher share of humic acids than that of fulvic acids and under a mixed forest of pine-and-oak and pine-and-spruce – an advantage of fulvic acids over humic acids. The same author pays attention to a diversity of the properties of humic acids depending on the tree stand species.

Investigating forest soils the tree stand age is very important. In young forests the organic and humic horizons are very poorly developed and have been undergoing constant changes. With time not only do the resources increase but also there is a proportional increase in the amount of matter which undergoes decomposition every year. When the ecosystem reaches the equilibrium between the supply and decomposition, a further increase in resources will not take place (Dziadowiec, 2003). The ecological balance is found in natural forests, which can protect soils representing components of the soil cover typical for a given region from artificial changes in the morphology and physical, chemical and biological changes.

The aim of the present paper was to determine the properties of organic matter of forest soil (especially humic acids) representing the natural ecosystem and to compare its properties with agriculturally used soil – located in the vicinity of the forest.

Material and methods

To carry out the investigations forest soil (chernozem) was sampled from the nature reserve “Veľký Báb” (Slovakia) and from the agricultural field adjacent to it.

“Veľký Báb” nature reserve is the best-preserved natural relict tree stand in the proglacial valley of the Danube. It covers the area of 28.39 ha and has been protected by law since 1966.

The research area fell within the south-western part of the reserve whose main tree stand is made up of oaks (60% of the cover): *Quercus robur* L., *Q. petrae* and *Q. cerris* L. 10% – of common maple (*Acer campestre* L.) and hornbeam (*Carpinus betulus* L.). The shrub layer is dominated by cornel-tree (*Cornus mas* L.). The herbaceous layer is made up of plants typical for dry-ground forest with a considerable amount of the spring aspect species (*Firaca verna*, *Corydalis solida*, *Polygonatum multiflora*). Besides there were found the following species: *Malica uniiflora*, *Doactylis glomerata* ssp., *Polygama*, *Lithrosperum purpureo-coeruleum*, *Poa nemoralis*. Based on the species composition of the area researched, the plant community of *Corneto-Quercetum carpinetum* can be found, which falls into the habitat type of oak-and-hornbeam forests.

Forest soil was sampled from the organic horizon (5–3.5 cm – sub-horizon O1 and 3.5–0 cm – sub-horizon Ofh) and A horizon every 10 cm (0–50 cm). Agricultural soil was sampled from Ap horizon (0–30 cm).

The following were assayed in the soil samples:

- total organic carbon (TOC) and total nitrogen (Nt) – with the VarioMax CN analyzer by Elementar (Germany),
- dissolved organic matter from dry samples was done for 1h with 0.004 M CaCl₂. Carbon (DOC) and nitrogen (DNt) contents in extracts after centrifugation were analyzed with the TOCN Formacs analyzer by Skalar (Breda, the Netherlands) (Gonet et al., 2002).

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 mL H₂O, 5 mL HCl and 5 mL 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:

- elemental composition (Perkin Elmer 2400 CHN analyzer). 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;

- 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

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

- susceptibility to oxidation with hydrogen peroxide by the measurement of decreased HAs solutions absorbance (0.02 % HAs and 1.5 % H₂O₂ in 0.1 M NaOH), at the wavelength of 465 and 600 nm. The susceptibility to oxidation was calculated from the following formula:

$$\%A_x = ((A_0 - A_u)/A_0) * 100\%$$

where: x – wavelength (465 and 600), A_0 – initial absorbance (prior to adding H_2O_2), A_u – absorbance after oxidation (Gonet, 1989);

– 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 $l = 80\%$ (Debska, 2004; Coccozza, Miano, 2002);

– ^{13}C NMR spectra – with the spectrometer MSL-300 by Bruker. Based on the areas defined under peaks (Hayes, 1998; Wilson, 1987; Preston et al., 1998), there was calculated the share of carbon in the following bonds:

Calkil – carbon in alkyl bonds (0–45 ppm),

C-O-alkyl – carbon in C–O bonds (45–108 ppm),

Car – carbon of aromatic bonds (108–140 ppm),

Clig – carbon of aromatic lignin bonds (140–160 ppm),

C-COOH – carbon of carboxyl groups (160–180 ppm) and parameters:

Cal/Car – ratio of carbon of aliphatic bonds (Cal) to carbon of aromatic bonds (Car),

$P = (Clig/(Car+Clig)) \cdot 100\%$ – percentage share of carbon of aromatic lignin bonds (Clig) in the total pool of carbon of aromatic bonds (Car+Clig);

– thermal properties with the use of the Derivatograph C (MOM, Hungary). Samples of 40 mg humic acids were mixed with Al_2O_3 (1:9) and heated in the air at the rate of $3.3\ ^\circ C \cdot min^{-1}$. After that the following indices were calculated:

➤ weight loss of samples related to the effects registered on the DTA curve,

➤ ‘Z’ parameter expressing the ratio of weight loss in the low temperature range (endo + exo1) to weight loss in the high temperature range (exo2). Value ‘Z’ is proportional to ‘aliphaticity’ of humic acids (Gonet, 1989; Debska, Gonet, 1997),

➤ the ratio of the area under the DTA curve to the area under DTG curve corresponding to exothermic effects (DTAexo1/DTGexo1, DTAexo2/DTGexo2). It is possible to accept with certain approximation that the value of this ratio is proportional to calorific value of the sample (Gonet, 1989; Debska, Gonet, 1997);

– 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 μm , 250x4.6 mm. Solutions of humic acids were applied in 0.01 mol/L NaOH of the concentration of 2 mg/mL; injection of the sample – 0.1 mL; solvent – acetonitril–water; solvents flow in the gradient (ratio H_2O : ACN (v/v) over 0–6min. – 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, Fig. 4) fractions in humic acids molecules and the parameter: HIL/HOB (Woelki et al., 1997; Preuße et al., 2000; Debska, 2004; Banach-Szott, 2006);

– high-performance size-exclusion chromatography (HPSEC) (chromatograph HPLC Perkin Elmer Series 200, Diode Array UV detector operating at 254 nm). Humic acids were separated with the TSK G3000SW column (7.5x600 mm). Sodium acetate 0.01 M at pH 7 was used as the eluent. The guard column was TSK G3000SW (7.5x75 mm). Solutions of humic acids were applied in 0.01M CH_3COONa of the concentration of 0.05mg/mL. A sample of 500 μl was injected into the column. The peak area was determined: S0 – total area (cm^2), S1 – the first peak area (%), S2 – the second peak area (%) as well as the S1/S2 ratio (Conte, Piccolo, 1998; Debska et al., 2005).

Tables show the mean values from five replicates. The significance of differences of the parameters determined, depending on the soil sampling depth, was evaluated with Duncan’s test (in Tables only those relations were marked for which the Duncan’s test did not show significant difference).

Results and discussion

The content of carbon (TOC) and nitrogen (Nt) in forest soil samples decreased with depth (Table 1). The highest content of TOC, similarly as Nt, was found in sub-horizon O1 which included mostly oak leaves and dead undergrowth plants. The value of the ratio TOC/Nt for

Table 1. Content of TOC and Nt and the content and share of soluble organic matter (DOC, DNt).

Depth (cm)	TOC	Nt	TOC/Nt	EWO		DNt		DOC/DNt
	g/kg			mg/kg	%	mg/kg	%	
Forest soil								
5–3.5	513	17.0	30.2	9165	1.79	371	2.18 <i>a</i>	24.7
3.5–0	170	8.84	19.2	2475	1.45	200	2.26	12.4
0–10	56.5	4.08	13.9	1200	2.12	89	2.18 <i>a</i>	13.5 <i>b</i>
10–20	33.6	2.75	12.2 <i>b</i> *	526	1.57	39	1.42	13.5 <i>b</i>
20–30	25.0	2.01	12.5 <i>b</i>	466	1.86	33	1.65	14.1
30–40	17.4	1.80	9.7 <i>a</i>	193	1.11	15	0.83	13.0a
40–50	10.9	1.14	9.6 <i>a</i>	159	1.46	12	1.05	13.3 <i>ab</i>
Arable soil								
0–30	12.0	1.29	9.3	150	1.26	15	1.15	10.0

Note: * Values followed by common letter are not significantly different at the 5% level

this sub-horizon was 30.2. In the Ofh layer the content of TOC was about 67% and nitrogen – by about 48% lower, as compared with the content of these elements in O1 sub-horizon, which demonstrates a greater intensity of mineralization of the compounds of carbon than nitrogen (Osano, Takeda, 2004; Berg, McClaugherty, 1989). A high content of TOC and Nt in the layer directly adjacent to the organic horizon (0–10 cm layer) and deeper layers points to the mobility of organic compounds of carbon and nitrogen compounds from the organic horizon to deeper soil layers.

The content of organic carbon in agriculturally used soil was 68.8% lower, as compared with the average content of TOC in the layers from 0 to 30 cm of the forest soil. Arable soil also demonstrated a definitely lower content of nitrogen than the forest soil. A greater accumulation of carbon and nitrogen in forest soil, as compared with the arable soil, is a result of the humus-forming and protective effect of the organic horizon (Dziadowiec et al., 1998; Kaczmarek, 2000). The content of organic matter in arable soils depends on the intensity of organic fertilization (manure, slurry) and the kind of crop rotation used, which is connected with the amount and quality of post-harvest residue in the field (Zaujec, 1980; Gonet, 1989, 1997; Gonet, Debska, 1998; Debska, 2004). Additionally in arable soils the process of mineralization of organic matter is significantly intensified by agrotechnical practices, plough mostly.

Organic matter of soils includes initial materials at different stages of decomposition and large-molecule compounds of specific properties. From the ecological point of view, the fraction of organic matter soluble in water or in salt solutions of pH = 7 (DOM) is of great importance. The highest content of dissolved organic carbon (DOC) and dissolved nitrogen (DNt) were found in O1 sub-horizon of forest soil and the deeper, the lower the

Table 2. Elemental composition (atomic %) of humic acids.

Depth (cm)	C	H	N	O	H/C	O/C	O/H	N/C	ω
Forest soil									
5–3.5	32.3	50.6	2.3 <i>a</i>	14.8	1.57	0.459	0.293	0.071 <i>cb</i>	-0.439
3.5–0	33.7 <i>a</i>	46.1	2.1	18.1	1.37	0.536	0.392	0.062 <i>a</i>	-0.109
0–10	33.4 <i>a</i>	43.0	2.3 <i>a</i>	21.3 <i>a</i>	1.29	0.636	0.495	0.069 <i>b</i>	0.195
10–20	34.9 <i>b</i>	40.9 <i>ab</i>	2.6 <i>c</i>	21.7 <i>a</i>	1.17	0.622 <i>a</i>	0.530 <i>a</i>	0.074 <i>c</i>	0.291
20–30	34.6 <i>b</i>	41.4 <i>b</i>	2.5 <i>bc</i>	21.5 <i>a</i>	1.20	0.622 <i>a</i>	0.520	0.072 <i>bc</i>	0.263
30–40	35.4	40.4 <i>a</i>	2.5 <i>bc</i>	21.7 <i>a</i>	1.14	0.614 <i>a</i>	0.538 <i>a</i>	0.069 <i>b</i>	0.296
40–50	38.3	38.0	2.4 <i>ab</i>	21.3 <i>a</i>	0.99	0.555	0.559	0.063 <i>a</i>	0.308
Arable soil									
0–30	37.2	38.4	2.3	22.1	1.03	0.60	0.58	0.060	0.348

content of this organic matter fraction (Table 1). Arable soil showed a lower content of DOM as compared with forest soil. Similarly the share of DOC and DNt (expressed as % of respectively: TOC and Nt) was lower in arable soil than in forest soil. Forest soil also demonstrated higher values of the ratio DOC/DNt as compared with agriculturally used soil. The results obtained coincide with earlier literature reports on the soil use method (agricultural or forest) significantly determining the share of soluble fraction of organic matter of soils and a greater share of DOM in forest soil resulting from the amount of plant drop supplied (Guggenberger, Zech, 1993).

The main humus components are humic acids and they are the ones which determine the properties and finally the role of humus in the natural environment. The properties of humic substances show some variation and are determined by habitat and anthropogenic conditions (Chen et al., 1978; Gonet, 1989; Drozd, 1994; Gonet, 1997; Howard et al., 1998; Kaczmarek, Dziadowiec, 1999). The elemental composition of the humic acids analyzed is given in Table 2. The lowest content of carbon and oxygen and the highest of hydrogen were found in HAs of the organic horizon of forest soil. In humic acid molecules of forest soil the content of carbon increased and hydrogen decreased with depth and the highest content of carbon and the lowest content of hydrogen were found in HAs isolated from soil sampled from 40–50 cm layer. Humic acids of mineral horizons also demonstrated a higher content of oxygen and nitrogen, as compared with HAs of the organic horizon. The highest content of oxygen was found in HAs molecules of arable soil. HAs of arable soil also demonstrated a higher content of carbon and a lower content of hydrogen as compared with HAs of forest soil of 0 to 40 cm layer.

As a result of the changes in the elemental composition, there occurred changes in the value of atomic ratios of respective elements and the degree of internal oxidation (Table 2).

Table 3. Spectrometric properties and susceptibility to oxidation of humic acids.

Depth (cm)	A ₂₈₀	A ₆₆₅	A _{2/4}	A _{2/6}	A _{4/6}	ΔlogK	%A _{465u}	%A _{600u}
Forest soil								
5–3.5	2.81	0.050	7.58	56.2	7.41	0.996	86.7	86.5
3.5–0	3.96	0.096	6.74	41.1	6.09	0.800	72.6	76.3 <i>a</i>
0–10	4.19	0.137	5.51	30.6	5.56	0.690	66.0	75.9 <i>a</i>
10–20	5.12	0.203	5.10	25.2	4.95	0.628 <i>a</i>	55.9	67.4
20–30	5.55	0.244	4.80 <i>a</i>	22.8 <i>a</i>	4.74	0.624 <i>a</i>	60.3	69.9
30–40	5.33	0.224	4.66 <i>a</i>	23.9 <i>a</i>	5.12	0.650	47.8	62.9
40–50	6.96	0.355	4.35	19.6	4.51	0.592	43.7	58.9
Arable soil								
0–30	6.42	0.340	4.36	18.9	4.34	0.592	44.0	58.9

Values of atomic ratios allow a rough estimation of the structure of humic acid molecules by evaluating the degree of aromaticity (H/C ratio – the higher the value of this ratio, the lower the degree of aromaticity) and the degree of their maturity (O/C, O/H, ω) (Aleksandrova, 1980; Gonet, 1989; Kaczmarek, 2000). The highest value of H/C ratio and the lowest O/C, O/H and the lowest value of the degree of internal oxidation (ω) were found in HAs of O1 sub-horizon. The deeper, it was found the decrease in the value of H/C ratio and an increase in the value of the other parameters. Based on that, one can state that the greater the depth, the greater the degree of maturity of humic acid molecules. It is also worth noting that HAs of agriculturally used soil demonstrated higher values of O/C, O/H and ω parameters and a lower value of H/C as compared with HAs of forest soil of 0–40 cm layers.

As reported in literature, “young” humic acids, mostly of a lower molecular weight and a lower degree of condensation of aromatic structures, reveal higher values of the following coefficients: A_{2/4}, A_{2/6}, A_{4/6} and ΔlogK, as compared with HAs of a high degree of humification (Aleksandrova, 1980; Howard et al., 1998; Debska, 2004; Gonet, Debska, 1998). The absorbance values and coefficients obtained (Table 3) show a low degree of maturity of HAs of the organic horizon. The lowest values of absorbance coefficients were found for HAs of forest soil of 40–50 cm layer and arable soil (Ap horizon – 0–30 cm layer).

Very precious information is generated from the values of susceptibility to oxidization (Table 3). The highest susceptibility to oxidization was found in HAs of organic horizon. In mineral horizons the susceptibility to oxidization of humic acids decreased with depth.

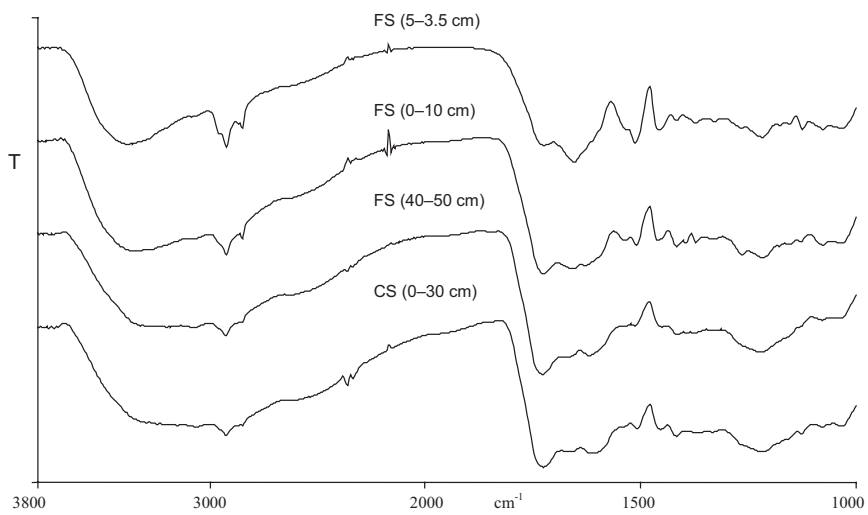


Fig 1. FTIR spectra of the humic acids of forest soil (FS) and arable soil (CS).

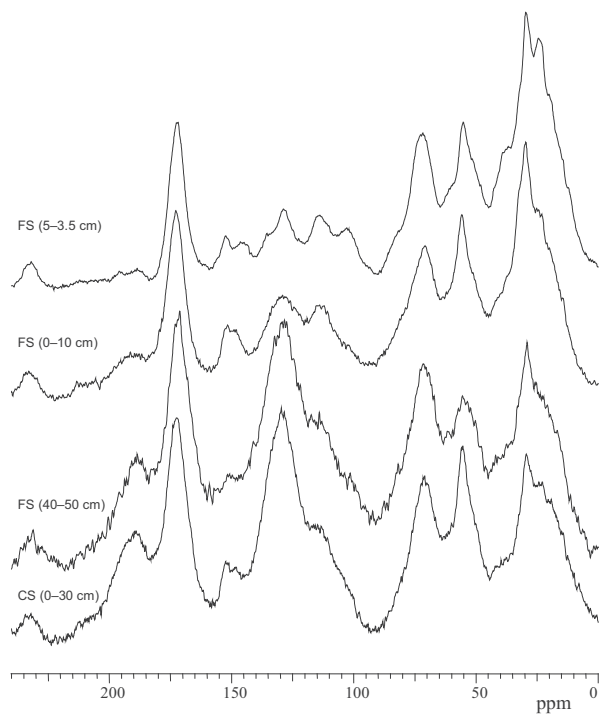


Fig. 2. ^{13}C NMR spectra of the humic acids of forest soil (FS) and arable soil (CS).

The highest resistance to the effect of hydrogen peroxide was found for HAs of forest soil of 40–50 cm layer and HAs of the topsoil used agriculturally. If we assume that a higher susceptibility to oxidation is found for the aliphatic part of molecules of humic acids, while the aromatic one is more resistant (Gonet, 1989), then the results obtained show that humic acids of arable soil demonstrate a higher share of aromatic structures resistant to the effect of hydrogen peroxide, as compared with HAs of forest soil and that with depth the share of aliphatic structures or simple aromatic structures decreases.

Those relationships coincide with the FTIR spectra obtained for the humic acids analyzed (Fig. 1). HAs spectra of forest soil showed that the greater the depth, e.g.:

- the lower the intensity of bands in the ranges pointing to the presence of aliphatic structures (2920–2960 cm^{-1} bands and 2860 cm^{-1}),
- the lower the band in the range pointing to the presence of simple aromatic structures (band 1610–1590 and 1510–1490 cm^{-1}),
- the greater the increase in the intensity of the band at about 1720 cm^{-1} connected with the presence of C=O bond of carboxyl groups.

The lowest share of aliphatic structures and the highest of carboxyl groups were found for HAs of arable soil and HAs of forest soil of 40–50 cm layer.

A low share of carbon found in aliphatic bonds demonstrated in HAs of arable soil and forest soil of 40–50 cm layer is revealed by the spectra ^{13}C NMR obtained (Fig. 2, Table 4). ^{13}C NMR spectra of humic acids generally showed the presence of four peaks of a high intensity in the following ranges: 0–45 ppm, 45–65 ppm, 65–95 ppm and 160–180 ppm, peaks of a lower intensity (mainly in spectra of HAs of a low degree of humification) in the following ranges: 95–108 ppm, 108–125 ppm, 125–140 ppm and 140–160 ppm and the peak of a very low intensity in the range 180–210 ppm. In the spectra of humic acids of mineral horizons, as compared with

Table 4. Share of carbon bonds in humic acids molecules calculated based on the pattern of ^{13}C NMR spectra.

Depth (cm)	Calkil	C-O-alkil	Car	Clig	C-COOH	Cal/Car	P
Forest soil							
5–3.5	39.9	37.1 <i>a</i>	7.1	4.4 <i>b</i>	9.9	6.73	38.3
3.5–0	31.8 <i>a</i>	39.4	8.4	4.7 <i>b</i>	11.4	5.43	35.7
0–10	29.6	37.7 <i>a</i>	10.2	5.1	12.5	4.40 <i>b</i>	33.6
10–20	31.0 <i>a</i>	33.9	11.9	2.9 <i>a</i>	13.2	4.39 <i>b</i>	19.6
20–30	31.1 <i>a</i>	30.1	13.8	2.7 <i>a</i>	14.1 <i>a</i>	3.72 <i>a</i>	16.2
30–40	33.2	32.4	15.6	1.1	13.9 <i>a</i>	3.92 <i>a</i>	6.7
40–50	20.4	23.5	33.9	0.0	14.5 <i>a</i>	1.29	0.0
Arable soil							
0–30	21.1	31.6	18.8	3.9	14.1	2.31	17.3

the spectra of HAs of OI sub-horizon, in the range typical for carbon of aliphatic bonds, there was generally observed a lower intensity of 45–65 ppm band (mostly C-O bonds of methoxyl groups), in the range of 65–95 ppm and in the range of 95–108 ppm, which could suggest the presence of carbon of cellulose and hemicellulose derivatives. However the greatest decrease in the intensity with depth was found for the band connected with the presence of carbon of aromatic lignin structures (140–160 ppm). In HAs spectra isolated from the forest soil sampled from the 40–50 cm layer there was found no peak in the range of 140–160 ppm. Based on the pattern of spectra ¹³CNMR, it was also demonstrated that with depth, in humic acid molecules there increases the share of carbon of carboxyl groups -COOH (the range of 160–180 ppm).

One of the parameters used to evaluate the degree of maturity of HAs molecules is the ratio of Cal/Car (Table 4). The value of this parameter decreases with an increase in the degree of humification of humic acid molecules (Debska, 2004). The highest value of this parameter was found for HAs of OI sub-horizon, while the lowest – HAs of the deepest layer. The values of the Cal/Car ratio obtained for HAs of arable layer were lower as compared with its values obtained for HAs of forest soil isolated from samples taken up to 40 cm deep but higher, as compared with HAs of forest soil of 40–50 cm layer. Besides HAs molecules of arable soil showed a higher share of aromatic lignin bonds (Clig) in the total pool of carbon of aromatic bonds (Clig+Car) as compared with HAs of forest soil of the layers from 20–50 cm.

The presence of non-decomposed lignin and cellulose structures in humic acid molecules points to their chemical young age and, as reported by e.g. Aleksandrova, 1980; Gonet, Debska, 1998; Osano, Takeda, 2004; Lorenz et al., 2004, the greater the degree of humification, the lower the share of these structures in the matter undergoing decomposition. Based on that one can state that humic acids of the organic horizon and the forest topsoil demonstrated a lower degree of maturity (humification), as compared with humic acids of deeper layers and arable soil.

A considerable share of non-decomposed lignin, cellulose structures and hemicelluloses can be seen from the thermograms of humic acids obtained. DTA curves of humic acids generally reveal the presence of a single endothermic effect (endo) and two clearly separated exothermic effects – one in the low-temperature range – up to 350 °C (exo1) and the second one in the high-temperature range (exo2) – over 350 °C. The area of peak exo1 corresponds to burning of this part of humic acids structure which is less resistant to decomposition (mainly aliphatic part), whereas the area of the peak registered for the second exothermic effect corresponds to the destruction of a part of HAs structure of a higher resistance to oxidation, mainly the aromatic part (Dziadowiec, 1979; Gonet, 1989; Debska, Gonet, 1997). Thermograms of HAs of the organic horizon show no clear separation between the first and the second exothermic effect, and the break which occurred between these effects falls within the temperature range corresponding to exo1 (below 350 °C, Fig. 3). The shape of the peak and the range of temperatures in which exo2 occurred shows that HAs were made up of numerous compounds which in the range researched produced a number of difficult to separate, overlapping exothermal effects. Flaig et al. (1975) reports that in the temperature range from 290 to 315 °C there occurs the decomposition of cellulose and the process of degradation of lignin can occur in a wider range of temperatures (250–360°C). Therefore it seems justifiable to claim that a considerably greater share of the area of peak exo2 (Table 5) in HAs molecules of the organic horizon, as compared with HAs of the soil

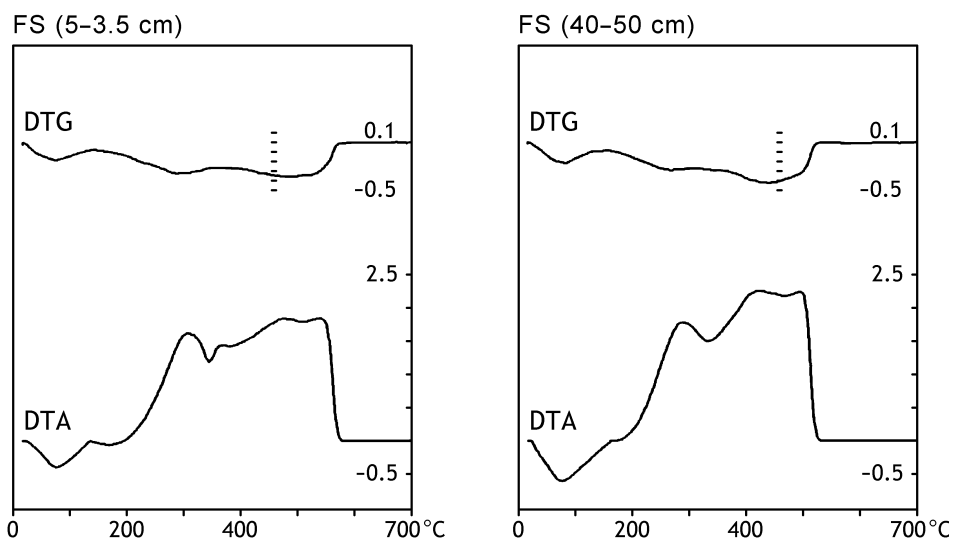


Fig. 3. Selected thermograms of humic acids of forest soil (FS).

Table 5. Parameters of thermal decomposition of humic acids.

Depth (cm)	Area of peaks recorded under the DTG curve (%)			DTAexo1/DTGexo1	DTAexo2/DTGexo2	Z
	endo	exo1	exo2			
Forest soil						
5-3.5	7.9	33.8	58.3	3.72	5.50 <i>a</i>	0.72
3.5-0	10.6	38.8	50.6	4.63 <i>a</i>	5.61 <i>ab</i>	0.97
0-10	11.3	41.4	47.2	5.40 <i>b</i>	6.44 <i>d</i>	1.12
10-20	10.8	33.9	55.3	5.23 <i>b</i>	6.41 <i>d</i>	0.81 <i>a</i>
20-30	11.2	32.6	56.1	5.43 <i>b</i>	6.49 <i>d</i>	0.78 <i>a</i>
30-40	10.7	31.5	57.8	4.88 <i>a</i>	5.90 <i>bc</i>	0.73
40-50	9.7	28.8	61.5	5.93	6.25 <i>cd</i>	0.63
Arable soil						
0-30	12.5	25.6	61.9	4.92	5.64	0.62

layer directly adjacent to the organic horizon was determined by the presence of cellulose and non-decomposed lignin structures. The pattern of thermograms of HAs of forest soil also demonstrates a decreased share of these groups of compounds with depth (the peak becoming narrower and a shift towards higher temperatures).

Based on the parameters defining the first and the second exothermic effect, that is the amount of substance showing a reaction in a given temperature range and the areas under DTA curve corresponding to these effects, values of the DTA/DTG ratio were determined. The value of the DTA/DTG ratio is a parameter characteristic for the burning heat or the energy value of the organic matter (Dziadowiec, 1979; Debska, Gonet, 1997). As it is seen from Table 5, the first exothermal reaction shows higher losses of weight than energy, which makes the substance oxidized in that reaction show a lower heat of burning (lower values of the DTAexo1/DTGexo1 ratio) than in the second exothermal reaction. Besides one shall stress that humic acids of arable soil demonstrated lower values of DTAexo1/DTGexo1 and DTAexo2/DTGexo2 ratios, as compared with HAs of the mineral horizons of forest soil.

The values of parameter Z obtained (the ratio of weight loss in low temperature range (endo + exo1) to weight loss in high temperature range (exo2)) point to the fact that HAs of arable soil show a higher degree of aromaticity as compared with HAs of forest soil. Besides they demonstrate that the greater the depth, the greater the degree of aromaticity of molecules of humic acids (a decrease in parameter Z value). The highest degree of aromaticity was found for HAs of the deepest layer.

In humic acid molecules hydrophilic and hydrophobic fractions can be found. Mutual proportions of both fractions determine the solubility of humic acids and as a result their migration deep the soil profile. The separation of humic acids into hydrophilic and hydrophobic fractions is made with high-performance liquid chromatography (HPLC). As reported by Woelki et al. (1997), Preuße et al. (2000), Debska (2004) and Banach-Szott (2006), peaks falling within the range 3.47–6.00 min. correspond to hydrophilic fractions (HIL), whereas peaks falling within the range 13.42–16.50 min. correspond to hydrophobic fractions (HOB, Fig. 4).

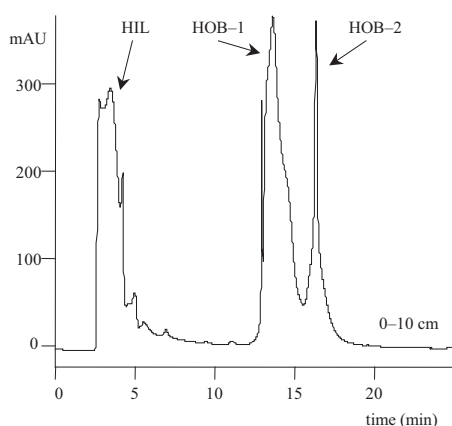


Fig. 4. Selected chromatogram RP-HPLC of the humic acids of forest soil.

Table 6. Share of hydrophilic and hydrophobic fractions in molecules of humic acids and the degree of their polydispersity.

Depth (cm)	HIL	HOB	HIL/HOB	S0 (cm ²)	S1 (%)	S2 (%)	S1/S2
Forest soil							
5–3.5	22.9	77.1	0.297	31.0	52.3	47.7	1.10 <i>a</i>
3.5–0	30.0	70.0	0.429	22.4	55.9	44.1	1.27
0–10	40.9	59.1	0.692	26.8	53.0	47.0	1.13 <i>a</i>
10–20	44.9	55.1	0.815	24.8	53.6	46.4	1.16 <i>a</i>
20–30	47.8	52.2	0.917	24.4	45.1	54.9	0.821
30–40	48.3	51.7	0.934	25.5	38.4	61.6	0.623
40–50	49.9	50.1	0.996	28.2	25.9	74.1	0.350
Arable soil							
0–30	47.3	52.7	0.898	31.5	20.3	79.7	0.255

The share of hydrophilic fractions in HAs molecules of forest soil depended on depth (Table 6). Humic acids isolated from soil sampled from the organic horizon demonstrated a considerably lower share of HIL fraction, as compared with the molecules of humic acids of mineral horizons. Besides the share of HAs of the hydrophilic fraction increased with depth (decrease in the share of hydrophobic fraction).

Changes in the share of hydrophilic and hydrophobic fraction are best seen from the values of the ratio of HIL/HOB (Table 6). In forest soil the values of this parameter increased with depth. HAs of arable soil revealed a higher value of the HIL/HOB ratio, as compared with HAs of forest soil of 0 to 20 cm layers.

Debska (2004), based on the correlations, pointed to the fact that the greater the degree of humification (increase in the value of coefficients: O/H, O/C, ω , share of carboxyl groups and decrease in the value of H/C, $A_{2/4}$, $A_{2/6}$, $A_{4/6}$, $\Delta \log K$ and the share of lignin structures), the greater the share of hydrophilic structure in humic acid molecules, and the lower the hydrophobic fractions. As a result HAs, molecules of a higher degree of “maturity” demonstrated a higher value of the HIL/HOB ratio.

One of the parameters changing in the process of humification is the degree of polydispersity of the molecules of humic substances. The application of HPSEC (High-Performance Size-Exclusion Chromatography) allowed a separation of the isolated humic acids depending on the size of molecules (Conte, Piccolo, 1998; Debska et al., 2005). The analysis of the pattern of chromatograms shows that newly-produced humic acids, at a very early stage of organic matter transformations, record a lower polydispersity as compared with HAs of a greater degree of maturity (HAs of mineral horizons). Chromatograms of HAs of OI sub-horizon show a single wide peak in which, at the retention time of 16 minutes,

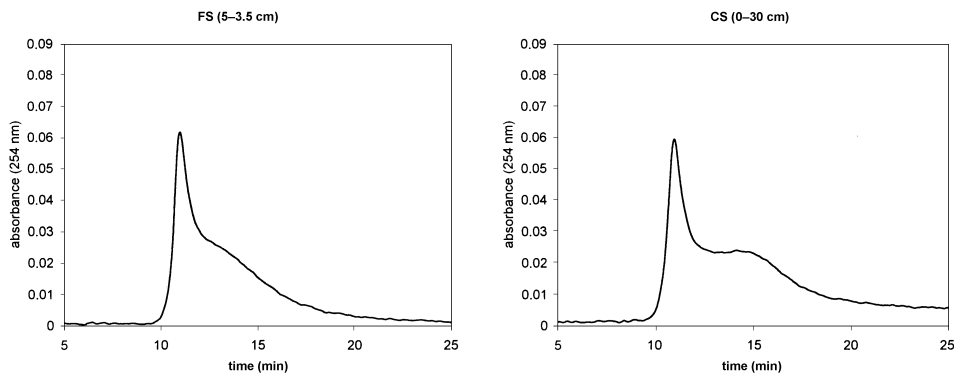


Fig. 5. Selected HPSEC chromatograms spectra of the humic acids of forest soil (FS) – sub-horizon O1 and arable soil (CS).

there was shown a characteristic break, pointing to the tendency of the peak to separate (Fig. 5). However a clear division into two peaks can be observed only for humic acids of the layers below 10 cm. And thus along the progress of the humification process the polydispersity of humic acids molecules increased, the increase in the share of S2 peak area (Table 6). Also Aleksandrova (1980) stressed that humic acids at a very early stage of the humification process of plant material record a higher molecular weight and during the humification process the polydispersity of HAs increases and there is a decrease in the molecular weight of humic acids. As a result of changes in the share of areas of respective peaks, there occurred changes in the value of the ratio of S1/S2. The highest values of this ratio were found for HAs of the organic horizon. With depth the value of this ratio was decreasing and the lowest value of S1/S2 was found in HAs of arable soil.

Conclusion

The present research of properties of humic acids of the natural forest soil whose research area was dominated by oaks and arable soil demonstrated a significant effect of the soil management method on the amount and quality of humus. In forest soils a constant supply of fresh organic matter (quantitatively higher than in arable soils) significantly modifies properties of humic acids. Humic acids of the humic horizon of the forest soil analyzed as compared with HAs of arable soil showed a lower degree of aromaticity, polydispersity, a lower share of carboxyl groups and hydrophilic fractions. Investigations into the properties of humic acids at different depths suggests a statement that the greater the maturity of HAs, in humic acid molecules, the greater the content of carbon and the lower the content

of hydrogen, the greater the share of carboxyl groups and hydrophilic fractions, the lower the share of aromatic lignin structures and hydrophobic fractions and the higher the degree of polydispersity. Quality parameters which have been obtained for humic acids of the layer of 40–50 cm can suggest that the process of migration of organic carbon compounds introduced into soil takes place every year together with plant drop very inconsiderably. The highest effect of the plant drop on HAs properties is observed at the soil layer directly adjacent to the organic horizon.

Properties of humic acids of arable soil similar to the properties of HAs of forest soil of the layer 40–50 cm demonstrate a slight effect of agrotechnical practices (mainly crop rotation and fertilization) on their properties, which can be due to the amount of the plant material supplied to soil and/or chemical composition of the post-harvest residue in soil. Plant material of a narrow C/N ratio and a low content of lignin easily undergoes mineralization processes and as a result their effect on the properties of humic substances is slight. It is worth noting that HAs of arable soil, as compared with HAs of forest soil of the layer of 40–50 cm demonstrated the highest content of oxygen and a higher degree of internal oxidation, which suggests that in arable soils oxidation processes are more intensive than in forest soils.

Translated by the authors

References

- Aleksandrova, L.N., 1980: Soil organic compounds and their transformation processes (in Russian). Nauka, Leningrad.
- Almendros, G., Dorado, J., Gonzalez-Vila, F.J., Blanco, M.J., Lankes, U., 2000: ¹³C NMR assessment of decomposition patterns during composting of forest shrub biomass. *Soil Biol. Biochem.*, 33: 793–804.
- Banach-Szott, M., 2006: Differentiation properties of humic substances ecto and endohumus of soils under the different trees species (in Polish). PhD thesis, UTA, Bydgoszcz.
- Berg, B., McLaugherty, C., 1989: Nitrogen and phosphorus release from decomposing litter in relation to the disappearance of lignin. *Can. J. Bot.*, 67: 1148–1156.
- Chen, Y., Senesi, N., Schnitzer, M., 1978: Chemical and physical characteristics of humic and fulvic acids extracted from soils of the mediterranean region. *Geoderma*, 20: 87–104.
- Cocozza, C., Miano, T., 2002: Structural resolution of metal-humic acids interactions through deconvolution FT-IR spectroscopy. In: Proc. 11th IHSS Meeting, Boston, USA, p. 264–266.
- Conte, P., Piccolo, A., 1998: High pressure size exclusion chromatography (HPSEC) of humic substances: molecular sizes, analytical parameters, and column performance. *Chemosphere*, 38: 517–528.
- Cortez, J., Demard, J.M., Bottner, P., Monrozier, L.J., 1996: Decomposition of mediterranean leaf litters: a microcosm experiment investigating relationship between decomposition rates and litter quality. *Soil Biol. Biochem.*, 28, 4/5: 443–452.
- Debska, B., Gonet, S.S., 1997: Thermal properties of humic acids developed during decomposition of post-harvest residues. *Humic Subst. Environ.*, 1: 73–82.
- Debska, B., 2004: Properties of humic substances of soil fertilized with slurry (in Polish). PhD thesis, UTA, Bydgoszcz.
- Debska, B., Drag, M., Dag, A., 2005: Properties of organic matter and molecular size distribution of humic acids isolated from forest soil. *Latvijas Universitātes Raksti*, 696: 19–28.
- Drozd, J., 1994: Physicochemical properties of humic acids isolated from different soils. In: Senesi, N., Miano, T. (eds), *Humic substances in the global environment and implications on human health*. Elsevier, Amsterdam, p. 273–278.

- Dziadowiec, H., 1979: Changes in energy associated with the humification of forest litter (in Polish). *Studia Soc. Sci. Toruniensis, Sect. D, Botanica, 11*, 1.
- Dziadowiec, H., 1990: Decomposition of litter in selected forest ecosystems (in Polish). PhD thesis, Nicolaus Copernicus University, Torun.
- Dziadowiec, H., 1993: Ecological role of soil organic matter (in Polish). *Zesz. Probl. Post. Nauk Roln., 411*: 269–281.
- Dziadowiec, H., Kaczmarek, J., Kruszynska, M., 1998: Dynamics of leaching dissolved organic carbon and nitrogen from the forest floor of rusty soil in three stand of mixed forest. *Humic Subst. Ecosys., 1*: 43–48.
- Dziadowiec, H., 2003: Selected problems of investigation of humus forest soil. In: Debska, B., Gonet, S.S. (eds), *Humic Substances in Soils and Fertilizers* (in Polish). PTSH Wroclaw, p. 141–166.
- Flaig, W., Beutelspacher, H., Rietz, E., 1975: Chemical composition and physical properties on humic substances. In: *Soil components*. J.E. Gieseking. Springer-Verlag, New York.
- Gonet, S.S., 1989: Properties of humic acids from soil under various fertilizing treatments (in Polish). PhD thesis, UTA, Bydgoszcz.
- Gonet, S.S., 1997: Habitat and anthropogenic factors determining status of soil organic matter (in Polish). *Humic Subst. Environ., 1*: 17–24.
- Gonet, S.S., Debska, B., 1998: Properties of humic acids developed during humification process of post-harvest plant residues. *Environ. Int., 24*: 603–608.
- Gonet, S.S., Debska, B., Pakuła, J., 2002: Content of dissolved organic matter in soil and organic fertilizers. PTSH, Wroclaw.
- Guggenberger, G., Zech, W., 1993: Zur Dynamik geloster organischer Substanzen (DOM) in Fichtenökosystemen-Ergebnisse analytischer DOM-Fraktionierung Z. Pflanzenernäh. Bodenk., *156*: 341–347.
- Hayes, M.H.B., 1998: Humic substances: progress towards more realistic concepts of structures. In: Davies, G., Ghabbour, E.A. (eds), *Humic substances: structures, properties and uses*. Royal Society of Chemistry, Cambridge, p. 1–29.
- Howard, P.J.A., Howard, D.M., Lowe, L.E., 1998: Effects of tree species and soil physico-chemical conditions on the nature of soil organic matter. *Soil Biol. Biochem., 30*, 3: 285–297.
- Kaczmarek, J., Dziadowiec, H., 1999: Humification process of soil organic matter in various forest stand. *Humic Subst. Environ., 1*, 3/4: 27–33.
- Kaczmarek, J., 2000: Influence of various forest stand on properties organic matter of sandy soil (in Polish). PhD thesis, Nicolaus Copernicus University, Torun.
- Lorenz, K., Preston, C.M., Krumrei, S., Feger, K.H., 2004: Decomposition of needle/leaf litter from Scots pine, black cherry, common oak and European beech at conurbation forest site. *Eur. J. Forest Res., 123*: 177–188.
- Osano, T., Takeda, H., 2004: Accumulation and release of nitrogen and phosphorus in relation to lignin decomposition in leaf litter of 14 tree species. *Ecol. Res., 19*: 593–602.
- Preston, C.M., Trofymow, J.A., Niu, J., Fyfe, C.A., 1998: ¹³C CPMAS NMR spectroscopy and chemical analysis of coarse woody debris in coastal forests of Vancouver Island. *For. Ecol. Manage., 111*: 51–68.
- Preuße, G., Friedrich, S., Salzer, R., 2000: Retention behavior of humic substances in reversed phase HPLC. *Fresenius J. Anal. Chem., 368*: 268–273.
- Son, Y., Park, I.H., Yi, M.J., Ojin, H., Kim, D.Y., Kim, R.H., Hwang, J.O., 2004: Biomass, production and nutrient distribution of a natural oak forest in central Korea. *Ecol. Res., 19*: 21–28.
- Wilson, M.A., 1987: *NMR techniques and applications in geochemistry and soil chemistry*. Pergamon Press Oxford.
- Woelki, G., Friedrich, S., Hanschmann, G., Salzer, R., 1997: HPLC fractionation and structural dynamics of humic acids. *Fresenius J. Anal. Chem., 357*: 548–552.
- Zaujec, A., 1980: Chemical and physical characteristics of partially decomposed plant residues of corn, lucerne and wheat (in Slovak). *Poľnohospodárska vysoká škola, Nitra*.