

## Molecular Size Distribution and Hydrophilic and Hydrophobic Properties of Humic Acids Isolated from Forest Soil

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**Abstract:** The aim of the present paper was to determine hydrophilic and hydrophobic properties and the degree of polydispersity of humic acids depending on their degree of maturity and the properties of the plant material participating in the process of humification. The study involved humic acids isolated from samples taken from organic and mineral horizons of forest soils. The samples were taken under the tree stands of: pine with an admixture of hardbeam, European beech, elm, fir, spruce, and thuja. It was demonstrated that the properties of humic acids of the organic horizon and mineral horizons are determined by the kind of plant litter, mainly by the tree species. The humification process is connected with an increase of the proportion of humic acids of lower molecular size as compared to the proportion of molecules greater in size, and with an increase of hydrophilic fractions and a decrease of hydrophobic fractions. Based on the correlation relationships, it was shown that the degree of polydispersity of molecules of humic acids is related to their hydrophilic and hydrophobic properties. Humic acids with a larger proportion of high-molecular fractions demonstrate also a higher proportion of hydrophobic fractions.

**Keywords:** forest soil; humic acids; HPSEC; hydrophobic fractions; hydrophilic fractions

It is currently assumed that humic substances constitute a three-dimensional flexible structure built of benzene, naphthalene, and phenanthrene rings connected with chain structures (SCHULTEN *et al.* 1991). Humic substances can form bonds with e.g. carbohydrates, proteins, fats, biologically active compounds, and silty minerals. The properties of humus depend both on the habitat factors such as: the soil type, plant cover, climatic conditions, and on anthropogenic factors (GONET 1997; LIU *et al.* 2006). Therefore, the qualitative and quantitative composition of humic substances can change depending on the origin and the degree of maturity. As reported in literature (ALEKSANDROVA 1980; ZAUJEC *et al.* 1996; HOWARD *et al.* 1998; GONET

& DEBSKA 1999), the chemical composition of the plant material is a major factor shaping the properties of the humic substances produced and the course of humification process. One of the parameters changing in the humification process is the degree of polydispersity of humic substances molecules (ALEKSANDROVA 1980; DEBSKA *et al.* 2005) and the proportions of hydrophobic and hydrophilic fractions (DEBSKA 2004). The above parameters are important from the ecological point of view as the proportions of these fractions determine the solubility of humic acids and, as a result, their migration down the soil profile.

High performance size exclusion chromatography (HPSEC) can be used to analyse the molecular size

distribution (MSD) of organic macromolecules. In HPSEC, molecules larger than the gel pores move rapidly through the column with the eluent but smaller molecules penetrate into the pores of gel in relation to both their size and shape as well as the pore size distribution of the gel (VARTIAINEN *et al.* 1987; CONTE & PICCOLO 1998; NISSINEN *et al.* 2001; BELYAEVA *et al.* 2006; CONTE *et al.* 2006).

High performance liquid chromatography can be applied in a normal phase pattern: polar adsorbent (hydrophilic) and less polar solvent, as well as in the reverse pattern (RP), when the stationary phase is somewhat polar or non-polar (e.g. C8, C18), and the solvent polar. The application of HPLC in RP allows for an analysis of samples in water solutions, which is of considerable importance in the case of humic substances, offering them a possibility of separation into fractions of a varied degree of hydrophobic character. According to WOELKI *et al.* (1997), PREUSSE *et al.* (2000), DEBSKA (2004), BANACH-SZOTT and DEBSKA (2006), the fractions of the retention times of 3.0–6.0 min demonstrate greater hydrophilic properties, while those of the retention times between 12.0 and 17.0 min are ever more hydrophobic. The fractions of lower retention times are commonly referred to as hydrophilic, while those of higher retention times – hydrophobic. It should be stressed that the course of the chromatograms of humic substances depends considerably on e.g. the method of the sample preparation for analysis (concentration, solution pH, solutions filtering method) as well as on the parameters of chromatographic separation (inject volume, eluent elution power, column parameters, eluent flow rate) (WOELKI *et al.* 1997; PREUSSE *et al.* 2000).

The aim of the present paper was to determine hydrophilic and hydrophobic properties and the degree of polydispersity of humic acids depending on their degree of maturity and the properties of the plant material participating in the process of humification. The study involved humic acids isolated from samples taken from organic and mineral horizons of forest soils.

## MATERIAL AND METHODS

Haplic Luvisols were samples from the forest experimental area of the Arboretum of the Forest Experiment Department (FED), Warsaw Agricultural University (WAU), at Rogow in the vicinity

of Skierniewice (Poland). FED WAU is located between 51°45' and 51°55' of northern altitude and between 19°50' and 20°10' of eastern longitude. The height above the sea level ranges from 146 to 223 m. The thermal vegetation period at Rogow is about 210 days long; for the beginning of April to the beginning of November. The average annual air temperature is +7°C, the average July temperature is +17°C, the average January temperature is –3°C, while the average annual total precipitation is 590 mm.

In the research carried out in FED Rogow, forest soils were sampled from under the following tree stands:

- pine with the admixture of hardbeam (*Pinus silvestris* L. and *Carpinus betulus* L.) – trees about 70 years old, tree layer cover 75% (pine 55%, hardbeam 20%), shrubs 20%, herbaceous layer 10%, mossy layer 30% (RO);
- European beech (*Fagus sylvatica* L.) – trees about 90 years old, tree layer cover 85%, no shrubbery layer, no undergrowth (RB);
- elm (*Ulmus Laevis* Pall.) – trees about 40 years old, tree layer cover 80%, no shrubbery layer, no undergrowth (RW);
- fir (*Abies alba* Miller) – trees about 60 years old, tree layer cover 85%, no shrubbery layer, no undergrowth (RJ);
- spruce (*Picea abies* L. Karsten) – trees about 90 years old, tree layer cover 75%, shrubs 20%, herbaceous layer 30%, mossy layer 40–60% (RS);
- thuja (*Thuja plicata* D. Don. ex Lamb.) – trees about 90 years old, tree layer cover 85%, no shrubbery layer, sporadic undergrowth (RT).

Humic acids were extracted from the soil samples by means of the commonly applied method recommended by IHSS. The research involved humic acids from O1 subhorizon – the samples were marked with symbol 1, and mineral horizons directly adjacent to the organic horizon (horizon A) – the samples marked with symbol 2, and the eluvial horizon (horizon E) – the samples marked with symbol 3.

The separated humic acids were subjected to the following determination:

polydispersity with the use of 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 (7.5 mm × 600 mm). Sodium acetate 0.01 mol/dm<sup>3</sup> 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 0.01M CH<sub>3</sub>COONa of the concentration of 0.05 mg/cm<sup>3</sup>. A sample of 500 µl was injected into the column. The following peak areas were determined:  $S_0$  – total area,  $S_1$  – the first peak area,  $S_2$  – the second peak area as well as the  $S_1/S_2$  ratio (VARTIAINEN *et al.* 1987; NISSINEN *et al.* 2001).

Hydrophilic and hydrophobic properties were determined using liquid chromatograph HPLC Series 200 with DAD detector by Perkin-Elmer. The separation involved the use of the column X-Terra C18, 5 µm, 250 × 4.6 mm. The solutions of humic acids were applied in 0.01 mol/dm<sup>3</sup> NaOH of the concentration of 2 mg/cm<sup>3</sup>; injection of the sample – 0.1 cm<sup>3</sup>; solvent – acetonitril–water; solvents flow in the gradient (ratio H<sub>2</sub>O:ACN (v/v) over 0–6 min – 99.5:0.5, 7–13 min – 70:30, 13–20 min – 10:90); detection – at the wavelength of 254 nm. The proportion of hydrophilic (HIL) and hydrophobic (HOB = HOB-1 + HOB-2) fraction in humic acids molecules and the parameter HIL/HOB were determined based on the areas under peaks (Figure 2) (WOELKI *et al.* 1997; PREUSSE *et al.* 2000; DEBSKA 2004; BANACH-SZOTT & DEBSKA 2006).

The tables show the mean values from four replicates. The significance of differences of the parameters determined, depending on the soil sampling depth, was evaluated with Duncan's test. To define the similarities (differences) across the objects,

the cluster analysis was made (Figure 3). The relationships between the characters researched were defined by means of the correlation coefficients. The above relationships were determined using statistical software STATISTICA MS.

## RESULTS AND DISCUSSION

In forest soils, humus accumulates in different horizons, yet mostly in organic and humic horizons. The organic horizon is formed from the accumulation of organic residues from the annual litter of trees and shrubs and undergrowth from the accumulation on the surface of mineral part of soils. The humic horizon (A) is created from dead underground parts of plants and dead residues of overground plant parts transported by soil animals, while the accumulation of humus in iluvial horizons (E) is mostly due to the migration of soluble humic compounds from the surface horizons: organic and humic (DZIADOWIEC 2003).

### Degree of polydispersity of humic acids molecules

While separating the humic acids analysed with the HPSEC method, with the humic acids isolated from the samples taken from horizon O1 under all the tree stands a single wide peak was obtained demonstrating a tendency to separate at the reten-

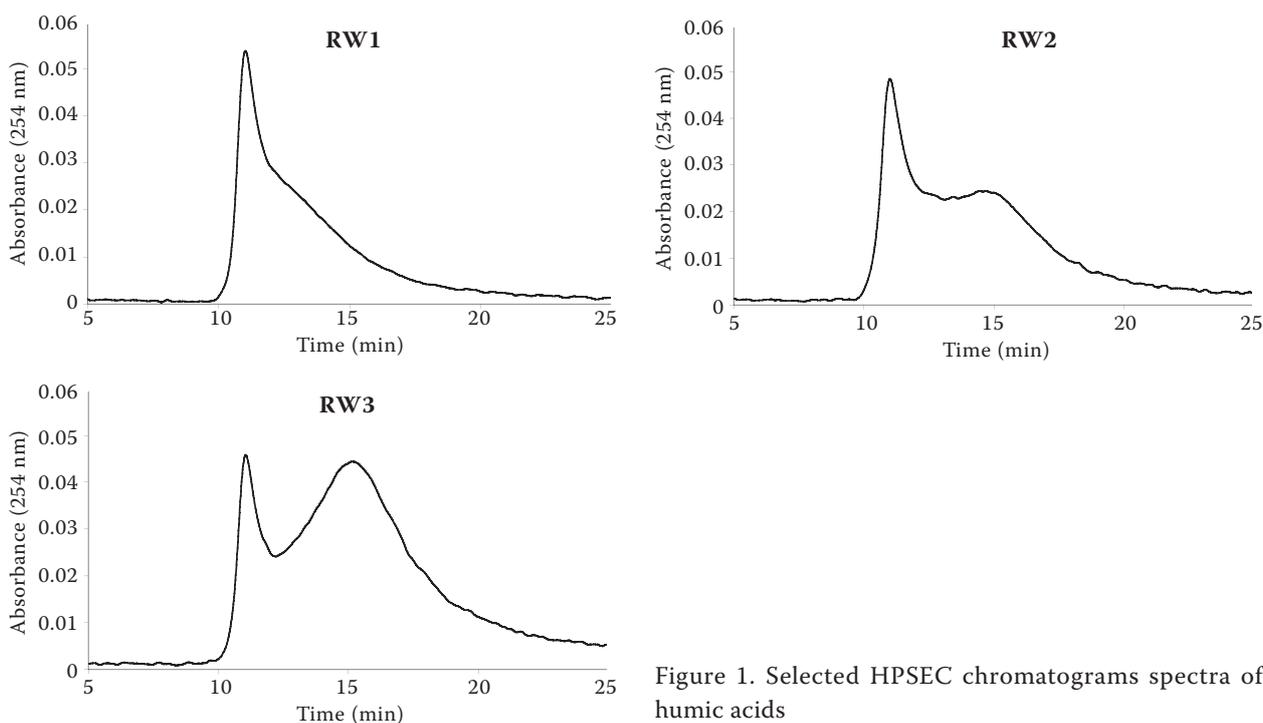


Figure 1. Selected HPSEC chromatograms spectra of humic acids

Table 1. Parameters of chromatographic resolution of humic acids obtained by HPSEC methods

Sample	$S_0$ (cm <sup>2</sup> )	$S_1$ (%)	$S_2$ (%)	$S_1/S_2$
<b>Stand of pine with an admixture of hardbeam</b>				
RO1	11.8 <i>a</i> *	39.8 <i>c</i>	60.2 <i>a</i>	0.661 <i>c</i>
RO2	23.5 <i>b</i>	28.5 <i>b</i>	71.5 <i>b</i>	0.399 <i>b</i>
RO3	23.5 <i>b</i>	20.9 <i>a</i>	79.1 <i>c</i>	0.264 <i>a</i>
<b>Beech stand</b>				
RB1	14.7 <i>a</i>	42.9 <i>c</i>	57.1 <i>a</i>	0.751 <i>c</i>
RB2	21.4 <i>b</i>	34.6 <i>b</i>	65.4 <i>b</i>	0.529 <i>b</i>
RB3	22.8 <i>b</i>	29.4 <i>a</i>	70.6 <i>b</i>	0.416 <i>a</i>
<b>Elm stand</b>				
RW1	17.0 <i>a</i>	40.0 <i>b</i>	60.0 <i>a</i>	0.667 <i>b</i>
RW2	21.4 <i>b</i>	43.9 <i>b</i>	56.1 <i>b</i>	0.783 <i>c</i>
RW3	32.4 <i>c</i>	18.5 <i>a</i>	81.5 <i>c</i>	0.226 <i>a</i>
<b>Fir stand</b>				
RJ1	16.5 <i>a</i>	45.5 <i>c</i>	54.5 <i>a</i>	0.835 <i>c</i>
RJ2	19.7 <i>b</i>	35.5 <i>b</i>	64.5 <i>b</i>	0.550 <i>b</i>
RJ3	21.2 <i>b</i>	25.9 <i>a</i>	74.1 <i>c</i>	0.350 <i>a</i>
<b>Spruce stand</b>				
RS1	19.4 <i>a</i>	46.9 <i>b</i>	53.1 <i>a</i>	0.883 <i>c</i>
RS2	22.7 <i>b</i>	41.0 <i>a</i>	59.0 <i>b</i>	0.695 <i>b</i>
RS3	38.0 <i>c</i>	30.3 <i>c</i>	69.7 <i>c</i>	0.435 <i>a</i>
<b>Thuja stand</b>				
RT1	6.4 <i>a</i>	29.7 <i>a</i>	70.3 <i>b</i>	0.422 <i>b</i>
RT2	21.6 <i>b</i>	33.3 <i>b</i>	66.7 <i>a</i>	0.500 <i>c</i>
RT3	23.5 <i>b</i>	25.5 <i>a</i>	74.5 <i>b</i>	0.342 <i>a</i>

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

tion time of about 16 minutes (Figure 1). However, a clear separation into two peaks is observable only with humic acids separated from mineral horizons, adjacent to the organic horizon (horizon A), and with humic acids extracted from eluvial horizon (horizon E). Therefore, the total area of the peaks ( $S_0$ ) of humic acids from horizon O1 is lower as compared with the total areas obtained for HAs of horizons A and E (Table 1).

The measurement conditions applied as well as the column selection (TSK GEL 3000SW) facilitated the separation of the humic acids isolated in the dependence on the molecule size. For that reason, the first peak which is the one of a shorter reten-

tion time, covers under its surface, the molecules greater in size, while the peak of a longer retention time is characteristic for molecules smaller in size (VARTIAINEN *et al.* 1987; NISSINEN *et al.* 2001).

CONTE *et al.* (2006) indicated that the fraction with the largest molecular size was richest in aliphatic structures and contained a substantial amount of carbohydrate-like carbon. This results in a strong mutual association due to weak hydrophobic interactions and hydrogen-bonding, and the formation of large supramolecular structures which eluted at short elution times during HPSEC separation.

The proportion of the peak area ( $S_1$ ) in HPSEC spectra of humic acids ranged from 18.5 to 46.9,

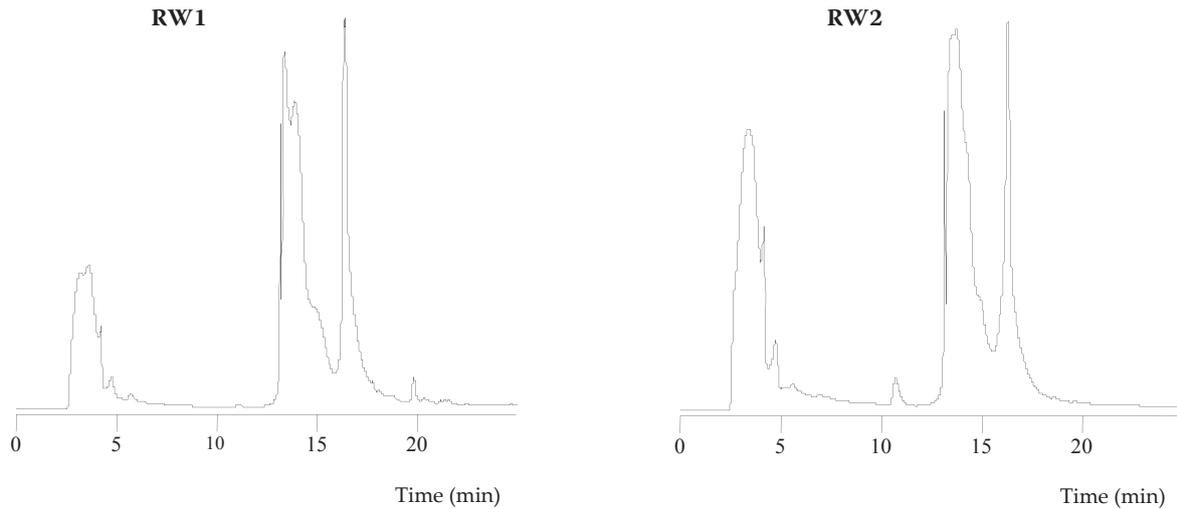


Figure 2. Selected chromatogram RP-HPLC of humic acids

and so peak II appeared to be dominant, which is also demonstrated by the values of the ratio of the peak areas  $S_1/S_2$  (Table 1). In most cases, it was observed that the greater the depth, the lower the proportion of peak I – of a shorter retention time, and the greater the proportion of peak II – of a longer retention time. Changes in the areas of the respective peaks affected the values of the ratio of  $S_1/S_2$  defining the degree of polydispersity of humic acids molecules. The higher the value of this ratio, the larger the proportion of molecules greater in size in humic acids molecules. The highest values of the ratio  $S_1/S_2$  were found with humic acids of subhorizon O1, while the lowest – humic acids of

horizon E. Humic acids isolated from the samples taken from the elm and thuja stands are an exception – the ratio of the area of peaks  $S_1/S_2$  is higher with humic acids of horizon A than with those of subhorizon O1. One can therefore conclude that, in general, the greater the sampling depth, the greater the number of molecules small in size, and the greater the sampling depth, the lower the number of molecules large in size.

The degree of polydispersity of humic acids molecules depended not only on the sampling depth but it was also determined by the tree species. Of the humic acids of subhorizon O1, the highest values of  $S_1/S_2$  parameter were found for humic acids of

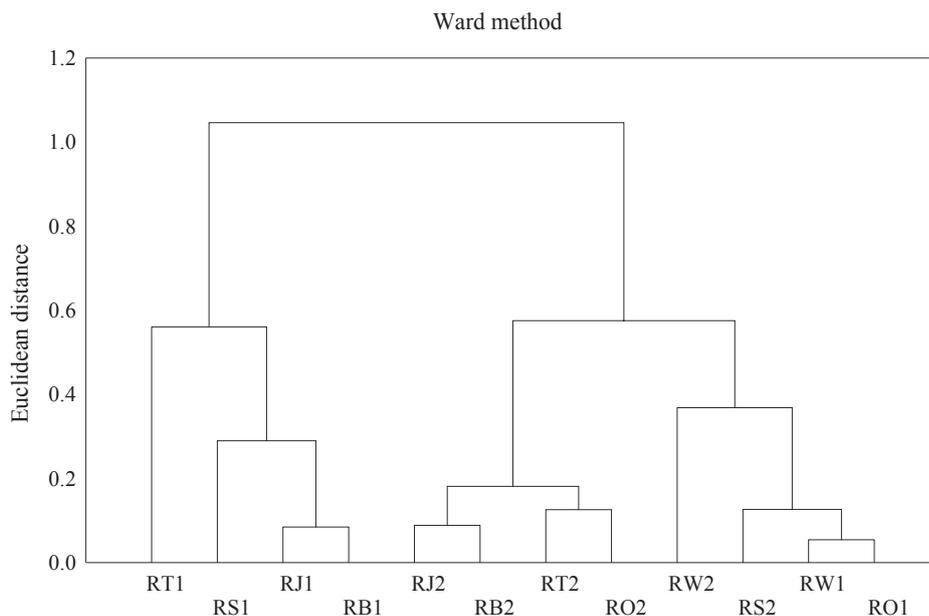


Figure 3. Cluster analysis of humic acids

the spruce and fir stands, while the lowest – the thuja stand. The values of the ratio  $S_1/S_2$  obtained for humic acids of horizon E ranged from 0.226 (elm stand) to 0.435 (spruce stand).

The analysis of the chromatogram patterns demonstrates that newly-created humic acids (humic acids of subhorizon OI) show, at a very early stage of organic matter transformation, a lower polydispersity and a relatively high average molecular weight as compared with humic acids of a higher degree of maturity (humic horizon humic acids). The greater the progress of the humification process, the greater the polydispersity of humic acids molecules and, as a result, a decreased average molecular weight. Similarly, ALEKSANDROVA (1980) stressed that humic acids at a very early stage of

the humification process of the plant material demonstrate a higher average molecular weight, and that during the humification process a decrease takes place in the average molecular weight of humic acids. According to the author, HAs are created as a result of oxidation of high-molecular products of plant residues decomposition.

#### Hydrophobic and hydrophilic properties of humic acids molecules

In the molecules of the humic acids analysed, hydrophilic (HIL) and hydrophobic fractions (HOB-1 and HOB-2) were isolated. The peaks found on the chromatograms (Figure 2) ranging from 3.4 to 5.5 min correspond to hydrophilic fractions, while

Table 2. Proportions of hydrophilic and hydrophobic fractions in molecules of humic acids

Sample	HIL	HOB-1	HOB-2	$\Sigma$ HOB	HIL/HOB
<b>Stand of pine with an admixture of hardbeam</b>					
RO1	28.1 <i>a</i>	40.7 <i>a</i>	31.2 <i>b</i>	71.9 <i>b</i>	0.391 <i>a</i>
RO2	37.0 <i>b</i>	42.0 <i>b</i>	21.0 <i>a</i>	63.0 <i>a</i>	0.587 <i>b</i>
RO3	37.9 <i>b</i>	43.8 <i>b</i>	18.3 <i>a</i>	62.1 <i>a</i>	0.610 <i>c</i>
<b>Beech stand</b>					
RB1	24.8 <i>a</i>	49.7 <i>b</i>	25.5 <i>b</i>	75.2 <i>b</i>	0.330 <i>a</i>
RB2	37.4 <i>b</i>	43.8 <i>a</i>	18.8 <i>a</i>	62.6 <i>a</i>	0.597 <i>b</i>
RB3	37.2 <i>b</i>	41.4 <i>a</i>	21.4 <i>a</i>	62.8 <i>a</i>	0.592 <i>b</i>
<b>Elm stand</b>					
RW1	30.8 <i>a</i>	54.7 <i>b</i>	14.5 <i>b</i>	69.2 <i>b</i>	0.445 <i>a</i>
RW2	41.4 <i>b</i>	44.9 <i>a</i>	13.7 <i>a</i>	58.6 <i>a</i>	0.706 <i>b</i>
RW3	42.6 <i>b</i>	45.5 <i>a</i>	11.9 <i>a</i>	57.4 <i>a</i>	0.742 <i>c</i>
<b>Fir stand</b>					
RJ1	25.5 <i>a</i>	57.0 <i>b</i>	17.5 <i>b</i>	74.5 <i>b</i>	0.342 <i>a</i>
RJ2	40.6 <i>b</i>	45.0 <i>a</i>	14.4 <i>a</i>	59.4 <i>a</i>	0.683 <i>b</i>
RJ3	41.9 <i>b</i>	45.1 <i>a</i>	13.0 <i>a</i>	58.1 <i>a</i>	0.721 <i>c</i>
<b>Spruce stand</b>					
RS1	10.6 <i>a</i>	63.6 <i>b</i>	25.8 <i>b</i>	89.4 <i>b</i>	0.119 <i>a</i>
RS2	34.3 <i>b</i>	48.0 <i>a</i>	17.7 <i>a</i>	65.7 <i>a</i>	0.522 <i>b</i>
RS3	34.0 <i>b</i>	47.8 <i>a</i>	18.2 <i>a</i>	66.0 <i>a</i>	0.515 <i>b</i>
<b>Thuja stand</b>					
RT1	11.7 <i>a</i>	67.6 <i>c</i>	20.7 <i>b</i>	88.3 <i>b</i>	0.133 <i>a</i>
RT2	33.9 <i>b</i>	48.1 <i>a</i>	18.1 <i>a</i>	66.2 <i>a</i>	0.512 <i>b</i>
RT3	36.9 <i>b</i>	41.1 <i>b</i>	22.0 <i>ab</i>	63.1 <i>a</i>	0.585 <i>c</i>

the peaks ranging from 13.4 to 16.5 min correspond to hydrophobic fractions (HOB) (WOELKI *et al.* 1997; PREUSSE *et al.* 2000; DEBSKA 2004). Due to a poor separation of the peaks corresponding to hydrophilic fractions, these were considered to be a single hydrophilic fraction (HIL). As to the 13.4–16.5 min range, 2 fractions were separated: one from 13.4 to 14.8 min referred to as HOB-1, and another one from 16.3 to 16.5 min referred to as HOB-2.

Humic acids isolated from the samples taken from subhorizon O1 demonstrated a considerably lower peak intensity of a shorter retention time (HIL) as compared with HAs of horizons A and E. The peak intensity corresponding to hydrophobic fractions was considerably higher as compared with the intensity of these peaks corresponding to the hydrophilic fraction.

Table 2 presents the proportion of the respective fractions in humic acids molecules and the values of the ratio HIL/HOB. The proportion of the fraction hydrophilic in character (HIL) in humic acids molecules depended on the sampling depth and location. It was demonstrated that humic acids extracted from the organic horizon revealed a significantly lower proportion of fraction HIL as compared with the humic acids extracted from deeper soil layers. The proportion of hydrophilic fraction in humic acids of subhorizon O1 ranged from 10.6 (humic acids of spruce stand) to 30.8% – the elm stand. The proportion of the fraction hydrophilic in character in humic acids molecules increased with depth. The highest amount of hydrophilic fraction was found in humic acids molecules isolated from the samples taken from horizons E of soils of elm and fir stands. The lowest proportion of hydrophilic fraction in humic acids molecules of mineral horizons of soils was recorded for the spruce stand, while the humic acids of mineral horizons of soils of the pine and European beech stands demonstrated similar proportions of hydrophilic fraction.

The proportion of hydrophobic fraction of a shorter retention time (HOB-1) in humic acids molecules of organic horizons ranged from 40.7 (the pine stand) to 67.6% (thuja stand), while in humic acids of mineral horizons it ranged from 41.1 to 48.1%. Thus, the changes in the proportion of fraction HOB-1 showed opposite tendencies as compared with the changes in the proportion of fraction HIL.

The proportion of hydrophobic fraction of a longer retention time (HOB-2) in humic acids

molecules extracted from the samples taken from organic soil horizons, just like in HOB-1, was higher as compared with its proportion in humic acids of the humic horizon and deeper soil layers.

The changes in the proportions of hydrophilic and hydrophobic fractions are best defined by the values of the ratio of HIL/HOB (Table 2). With the results obtained, it was demonstrated that the values of the ratio of HIL/HOB obtained with humic acids isolated from soil sampled from subhorizon O1 depended on the tree species. The lowest values of this ratio were found with humic acids of organic horizons on the spruce and thuja stands, while the highest values – humic acids of the elm stand. An increase was observed in the value of the ratio of HIL/HOB with depth, due to which the humic acids of mineral horizons demonstrated higher values of this ratio as compared with the humic acids of subhorizon O1. Of HAs of mineral horizons, just like HAs of subhorizon O1, the highest values of HIL/HOB were shown for humic acids on the elm stand, while the lowest values – humic acids of the spruce and thuja stands. The relationships obtained show a strong effect of the plant litter properties on the ‘hydrophobic character’ of the humic acids produced.

To determine the similarities (differences) of the objects researched, a cluster analysis was carried out (STATISTICA MS). Objects of similar properties are located on dendrograms in homogenous groups. The cluster analysis, whose results are given in Figure 3, used the parameters which characterise both the hydrophobic and hydrophilic properties (HIL/HOB) and the polydispersity of humic acids molecules ( $S_1/S_2$ ). The dendrograms obtained show clearly that the properties of humic acids of the organic horizon and mineral horizons are determined by the kind of plant litter (chemical composition, morphology).

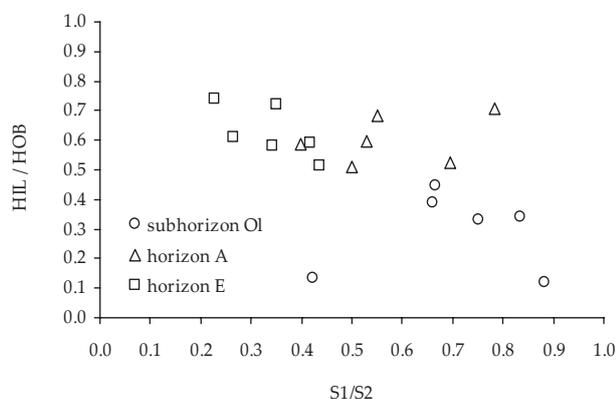
DEBSKA (2004) showed that, as based on the correlation relationships, the greater the degree of humification (an increase in the values of coefficients O/H, O/C,  $\omega$ , and in the proportion of carboxyl groups, and a decrease in the values H/C,  $A_{2/4}$ ,  $A_{2/6}$ ,  $A_{4/6}$ ,  $\Delta \log K$  and in the proportion of lignin structures) the greater the proportion of hydrophilic fraction in humic acids molecules, and the lower the proportion of hydrophobic fraction. As a result, the molecules of humic acids of a higher degree of ‘maturity’ demonstrate a higher value of the ratio HIL/HOB (HAs of mineral horizons).

Table 3. Significant values of correlation coefficients between the parameters determined

Parameter	HIL	HOB	HIL/HOB
$S_1$	-0.483	0.483	-0.517
$S_2$	0.483	-0.483	0.517
$S_1/S_2$	-0.496	0.496	-0.524

As seen from the correlation relationships given in Table 3, the proportions of hydrophilic and hydrophobic fractions (HOB) in humic acids molecules are connected with their degree of polydispersity. The lower the proportion of the molecules larger in size in humic acids molecules, the greater the proportion of fraction HIL and the lower the proportion of fraction HOB. Similarly, the value of the ratio HIL/HOB shows a negative correlation with the value of the ratio  $S_1/S_2$ .

The relationships given in Figure 4 show clearly that the process of humification is connected with a decrease in the value of the ratio  $S_1/S_2$  and an increase in the value of the ratio HIL/HOB. The highest values of the ratio  $S_1/S_2$  and the lowest of HIL/HOB were obtained with humic acids of subhorizon Ol, composed of hardly-changed plant residues at an early stage of transformation.

Figure 4. Relationship between parameters  $S_1/S_2$  and HIL/HOB

## CONCLUSIONS

- The research results obtained demonstrated that the properties of humic acids of the organic horizon and mineral horizons are determined by the kind of plant litter, mostly tree species.
- Molecules of humic acids show a higher proportion of the fraction possessing hydrophobic

properties as compared with the proportion of hydrophilic fraction.

- The humification process is connected with an increase in the proportion of the fraction smaller in size in humic acids molecules, as compared with the proportion of molecules greater in size, and with an increase of the proportion of hydrophilic fractions and a decrease of that of hydrophobic fractions.
- The degree of polydispersity of humic acids molecules is connected with their hydrophilic and hydrophobic properties. Humic acids molecules with a larger proportion of high-molecular fraction demonstrate a more hydrophobic character.

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