Abstract. The aim of the work was to determine the elemental composition, hydrophilic and hydrophobic properties and polydispersity of humic acids of soils under different management regimes. The research involved forest and agricultural soil samples. Humic acids were exposed to the following analyses: elemental composition, division into hydrophilic and hydrophobic as well as low- and high-molecular fractions with chromatographic methods. The present results, confirmed by the cluster analysis, showed that the basic factor conditioning the properties of humic acids is the soil type, but also one can disregard neither the soil management method nor the agricultural practices applied.

The content and quality of humus are directly and indirectly determined by physical, chemical, biological and environmental soil properties. The content and properties of organic matter of soil are conditioned not only by soil-and-climatic factors but also by the soil management (use) methods. In forest soils one of the basic factors determining the properties of the organic horizon is the tree stand species composition [6, 7, 10]. The tree stand species composition is also affected by the properties of deeper horizons as a result of mobility of nutrients from surface layers. In the soils under agricultural use the soil humus properties are mostly determined by post-harvest residue left after the harvest of crops [1, 8, 9].

The parameters which are changing in the humification process, besides elemental composition, are the degree of polydispersity of the molecules of humus substances [1, 4] and the share of hydrophobic and hydrophilic fractions [2, 3]. The above parameters are important from the ecological perspective since the proportions of these fractions determine the solubility of humic acids and, as a result, their migration down the soil profile.

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Properties of Humic Acids of Soil Under Different Management Regimes

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The aim of the present paper was to determine the elemental composition, hydrophilic and hydrophobic properties and polydispersity of humic acids of soils which differ in the management method and the farming regimes applied.

MATERIAL AND METHODS

For investigation, soil samples were used from depth 0.02 – 0.10 m: four samples from Bab locality and three samples from Dolna Malanta locality (Slovakia) [15, 16]. Soil samples from intensively cultivated soils were compared with the control samples from adjoining forest soil.

Soil samples from Bab were compared in pairs (1, 2 and 3, 4):
- sample No. 1 – forest soil, classified as Luvic Phaeozems;
- sample No. 2 – cultivated soil, classified as Haplic Chernozem;
- sample No. 3 – forest soil, classified as Orthic Luvisols;
- sample No. 4 – cultivated soil, classified as Orthic Luvisols.

The second chosen locality was Dolna Malanta of the area of the Experiment Station of the Slovak Agricultural University of Nitra. Soil samples were collected from ecological and integrated farming systems and from the near castle park (control sample). Parent material in this locality is proluvial sediment mixed with loess and the main soil type is Orthic Luvisols [16]. Three soil samples were characterised in this locality:
- sample No. 5 – ecological farming regime, where only farmyard manure (FYM) was used;
- sample No. 6 – integrated farming regime, where both FYM and calculated doses of inorganic fertilizers were used. (The application rate of inorganic fertilizer is calculated on the basis of the macroelement concentration in the soil and plant requirements);
- sample No. 7 – the nearby park, approximately 250 m from the above sites.

From all the soils there were extracted humic acids for which the following analyses were made:
- the elemental composition (Perkin Elmer 2400 CHN analyzer). On the basis of the elemental composition the values of atomic ratios: H/C and ω – internal oxidation degree were calculated according to the formula:
  \[ \omega = \frac{2O + 3N - H}{C}, \]
  where: O, N, H, C – content in atomic percentage;
- hydrophilic and hydrophobic properties were determined with the liquid chromatograph HPLC Series 200 with DAD detector by Perkin-Elmer. The separation involved the use of column X-Terra C18, 5 µm, 250 x 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 – 0.1 mL; solvent – acetonitril–water; solvents flow in the gradient (ratio H₂O : ACN (v/v) over 0-6 min – 99.5 : 0.5, 7-13 min – 70 : 30, 13-20 min – 10 : 90); detection – at the wavelength of 254 nm.
Based on the areas determined under peaks (Fig. 1), the share of hydrophilic (HIL) and hydrophobic (HOB=HOB-1 + HOB-2) fractions in humic acids molecules and the parameter: HIL/HOB \[2, 3, 12, 13\] were determined; – 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 x 600 mm). Sodium acetate 0.01 mol/L at pH=7 was used as eluent. The guard column was TSK G3000SW (7.5 x 75 mm). Solutions of humic acids were applied in 0.01 M CH$_3$COONa of the concentration of 0.05 mg/mL. A sample of 100 $\mu$L 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 $S_1/S_2$ ratio \[4, 11, 14\].

RESULTS AND DISCUSSION

The highest content of carbon was determined in the molecules of HAs of Haplic Chernozem (sample No. 2) The content of carbon in HAs No. 2 was 4.52 pp higher as compared with HAs of Luvic Phaeozems (1) and on average 3.63 pp higher than in the molecules of humic acids of Orthic Luvisols (HAs marked with symbols 3,4,5,6, Table 1). The content of nitrogen in HAs of the soils investigated ranged from 2.37 to 2.86. The highest content of this element was reported for HAs of soil of ecological and integrated farming regimes. A higher content of oxygen was recorded, in general, for HAs of forest soils as compared with HAs of arable soils. The HAs molecules of a lower content of hydrogen showed, in general, higher values of the degree of internal oxidation (parameter $\omega$, Table 1). For HAs isolated from the soils sampled in Bab there were noted higher values of parameter $\omega$ in HAs molecules of arable than forest soils. The lowest values of the degree of internal oxidation were reported for HAs of soil of an integrated farming regime.

\[
\begin{array}{ccccccc}
\text{Sample No.} & \text{C} & \text{H} & \text{N} & \text{O} & \text{H/C} & \omega \\
1 & 33.75 & 43.18 & 2.79 & 20.29 & 1.28 & 0.171 \\
2 & 38.27 & 39.42 & 2.68 & 19.64 & 1.03 & 0.206 \\
3 & 34.47 & 42.37 & 2.46 & 20.70 & 1.23 & 0.186 \\
4 & 34.40 & 41.96 & 2.37 & 21.27 & 1.22 & 0.223 \\
5 & 34.95 & 42.44 & 2.86 & 19.74 & 1.21 & 0.161 \\
6 & 34.73 & 44.09 & 2.84 & 18.34 & 1.27 & 0.033 \\
7 & 36.24 & 40.02 & 2.40 & 21.33 & 1.10 & 0.272 \\
\end{array}
\]

TABLE 1. ELEMENTAL COMPOSITION OF HUMIC ACIDS (IN ATOMIC PERCENTAGE)
A comparison of two farming methods: integrated and ecological showed that HAs isolated from the soil samples of the integrated farming regime demonstrated a higher content of hydrogen and, as a result, a higher value of the H/C ratio and a lower content of oxygen and a lower value of the degree of internal oxidation, as compared with HAs of soil where the ecological farming regime was applied. As reported by Szombathova et al. [16], lower values of the H/C ratio recorded for HAs of soil of ecological farming regime are probably due to a disappearance of aliphatic chains by biological and/or chemical oxidation and relative increase in aromatic groups. An increased content of aromatic parts of macromolecules is connected with higher stability, maturity and quality of humic acids.

One of the parameters affecting e.g. the sorption capacity of humic acids are their hydrophilic and hydrophobic properties. The division into hydrophilic and hydrophobic fractions of humic acids is made using the RP-HPLC method (Fig. 1). Fractions of hydrophilic (HIL) character correspond to peaks within the range from 2.50 to 6.10 min, while fractions within the range from 11.20 to 17.60 min are hydrophobic fractions (HOB). Fraction HOB-1 is a hydrophobic fraction of a shorter retention time from 11.20 to 15.60 and fraction HOB-2 ranges from 15.60 to 17.60 min. The share of hydrophobic fractions considerably exceeded the share of hydrophilic fractions (Table 2). The share of hydrophilic fractions in the molecules of HAs ranged from 32.1 to 42.2 %, and thus the fraction which is dominant in the molecules of HAs is the fraction of hydrophobic properties. The lowest number of hydrophilic fractions was contained in HAs of soils of ecological and integrated farming regimes (Table 2). For the other soils, irrespective of their management method, the share of hydrophilic fraction ranged from 40.6 to 42.2 %.

Fig. 1. Selected RP-HPLC chromatogram of humic acids.
The differences in the share of hydrophilic and hydrophobic fractions resulted in the differences in the calculated values of the HIL/ΣHOB ratio. The highest values of this parameter were recorded for HAs of Orthic Luvisols – forest soil. The lowest values of the HIL/ΣHOB ratio were reported for HAs of soils of ecological and integrated farming regimes. As reported earlier [2, 3, 5], the value of parameter HIL/ΣHOB is connected with the degree of maturity of HAs molecules (the greater the degree of maturity, the higher the values of HIL/ΣHOB) and it is also a character which is specific to the soil type formed in specific soil and climatic conditions. The parameters reported defining the hydrophilic and hydrophobic properties of HAs point clearly that they are determined by the soil type as well as, to some extent, by the soil use method and the farming regimes applied.

Sample HPSEC chromatograms are given in Fig. 2. The pattern of chromatograms suggests that the molecules of humic acids include two fractions. The first peak, of a shorter retention time, falling from about 13.00 to 16.00 min, corresponds to the fraction of humic acids the molecules of which are bigger in size, while the other one the maximum of which occurred most frequently at the retention time of about 19.00 min is found for the molecules smaller in size. The share of high-molecular fraction in the molecules of humic acids of the soils researched ranged from 13.7 to 21.5%, the fraction of smaller molecules was then dominant. The greatest share of the high-molecular fraction was recorded for humic acids of Orthic Luvisols under forest management (locality Dolna Malanta). Of the HAs of arable soils, most high-molecular fraction was contained in HAs of soil of ecological farming regime. The share of S1 fraction in the molecules of HAs of soil of integrated farming regime was lower than in HAs of soil where the ecological farming regime was applied but higher than in the molecules of HAs of the other arable soils.

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>HIL (%)</th>
<th>HOB-1 (%)</th>
<th>ΣHOB (%)</th>
<th>HIL/ΣHOB</th>
<th>S1 (%)</th>
<th>S1/S2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40.6</td>
<td>51.1</td>
<td>59.4</td>
<td>0.683</td>
<td>20.6</td>
<td>0.259</td>
</tr>
<tr>
<td>2</td>
<td>41.4</td>
<td>49.5</td>
<td>58.6</td>
<td>0.706</td>
<td>14.1</td>
<td>0.164</td>
</tr>
<tr>
<td>3</td>
<td>42.2</td>
<td>50.7</td>
<td>57.8</td>
<td>0.730</td>
<td>13.7</td>
<td>0.159</td>
</tr>
<tr>
<td>4</td>
<td>41.8</td>
<td>50.8</td>
<td>58.2</td>
<td>0.717</td>
<td>14.9</td>
<td>0.157</td>
</tr>
<tr>
<td>5</td>
<td>34.4</td>
<td>55.4</td>
<td>65.6</td>
<td>0.525</td>
<td>19.8</td>
<td>0.248</td>
</tr>
<tr>
<td>6</td>
<td>32.1</td>
<td>56.5</td>
<td>67.9</td>
<td>0.473</td>
<td>17.7</td>
<td>0.215</td>
</tr>
<tr>
<td>7</td>
<td>41.2</td>
<td>52.7</td>
<td>58.9</td>
<td>0.700</td>
<td>21.5</td>
<td>0.274</td>
</tr>
</tbody>
</table>
Parameter S1/S2 (the ratio of the share of high-molecular fraction to the share of low-molecular fraction) is assumed as the index of polydispersity of the molecules of humic acids. The higher the value of this parameter, the higher the polydispersity of HAs [1, 3, 5]. The highest values of parameter S1/S2 were reported for humic acids of forest soils: Luvic Phaeozems and Orthic Luvisols. Of the arable soils, higher values of ratio S1/S2 were reported for HAs of soil located in Dolna Malanta than the HAs of soils sampled in Bab.

In order to determine the similarities (differences) of the objects investigated, a cluster analysis was carried out. The objects of similar properties are located on dendrograms in homogenous groups. The analysis was based on all the parameters defined and the results are presented in Fig. 3. The dendrogram differentiates

![Fig. 2. Selected HPSEC chromatogram of humic acids](image)

![Fig. 3. Cluster analysis based on parameters determining humic acids properties.](image)
between the two main groups: one was made up of humic acids isolated from the soil sampled from the experimental plots in Dolna Malanta where integrated and ecological farming regimes were applied. The other group defines two subgroups: one with HAs of forest soils (Luvic Phaeozems – locality: Bab, Orthic Luvisols – locality: Dolna Malanta), the other one: HAs of Orthic Luvisols – locality Bab (forest and arable soil); that group included also HAs of Haplic Chernozem. The results, verified with the cluster analysis, demonstrated that the basic factor conditioning the properties of humic acids is the soil type, however, one can disregard neither their management method nor the agricultural practices.

CONCLUSIONS

1. The factors determining the properties of humic acids included the soil type, the management method, the agricultural practices applied as well as the conditions the soils were formed in.

2. Humic acids of soils under agricultural management located in Bab showed a lower content of hydrogen and higher values of the degree of internal oxidation as compared with HAs of forest soils.

3. Humic acids of soils of an integrated and ecological farming regimes demonstrated lower values of the degree of internal oxidation and ratio HIL/ΣHOB as compared with the other HAs.

4. Humic acids of an integrated farming regime, as compared with HAs of soil where the ecological farming regime was applied, revealed higher values of ratio H/C and lower values of HIL/ΣHOB, S1/S2 and the degree of internal oxidation.

REFERENCES

CAŁOŚĆ I PRÓCHNICY GLEB O ZRÓŻNICOWANYM ZAGOSPODAROWANIU

Celem niniejszej pracy było określenie składu pierwiastkowego, właściwości hydrofilowo-hydrofobowych oraz polidispersyjności kwasów huminowych gleb różniących się sposobem zagospodarowania i stosowanymi systemami uprawy. Do badań wykorzystano próbki gleb leśnych i użytkowanych rolniczo. Kwasy huminowe wyizolowane ogólnie przyjętą metodą Schnitzera poddano analizom: składu pierwiastkowego, rozdziału na frakcje hydrofilowe i hydrofobowe oraz nisko- i wysokocząsteczkowe metodami chromatograficznymi.

Otrzymane wyniki badań, potwierdzone analizą skupień wykazały, że podstawowym czynnikiem warunkującym właściwości kwasów huminowych jest typ gleby, ale również nie można pominać sposobu zagospodarowania gleb, czy też stosowanych zabiegów uprawowych.