

Original Research

Content of Phenolic Compounds in Fulvic and Humic Acid Fractions of Forest Soils

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Abstract

The aim of the present paper was to determine the content of phenolic compounds in fulvic acid extracts and the hydrolyzates of humic acids depending on their degree of maturity and plant litter properties. The research involved samples from organic and mineral horizons of forest soils. Phenolic compounds were identified with the chromatographic method (HPLC). The present research demonstrated that fractions of fulvic acids showed a higher content of aldehydes and phenolic acids as compared with the hydrolyzates of humic acid fractions. The parameter which differentiates humus acid properties of subhorizon O1 produced under different tree species is the ratio of the share of vanillyl, syringyl and cinnamyl compounds (V:S:C), while humification is connected with decreased content of aldehydes and phenolic acids which occur in hydrolyzates of humic acid fractions and fulvic acid extracts. In hydrolyzates of humic acid and fulvic acid fractions together with sampling depth, the values of parameter V+S+C decrease, which confirms that the parameter can be used to research the humification process of the plant material.

Keywords: humic acids, fulvic acids, phenolic compounds, forest soils

Introduction

Humification and mineralization in soils are connected with the origin of the organic material and so with the plant cover. In forest soils one of the basic factors defining the properties of the organic horizon and deeper soil horizons is the species composition of the tree stand which, through the plant litter and nutrient leaching from tree crowns, affects the soils [1-3]. The chemical composition of the plant litter, its decomposition rate and humus resources have been well known already [1, 4]. However, besides humus in soil the problem of the properties of humic substances playing an important role in soil functioning is of similar importance.

Literature offers a lot coverage on the structure and properties of humic substances and the course of the humification process [5-7]. However, a complex structure of

humic substances and its variation call for new research methods to give maximum information on the structure and directions of the transformation.

Research of the structure has demonstrated that in humic acid molecules are found non-decomposed lignin fragments [5, 8, 9]. For that reason these compounds are considered one of the main precursors of aromatic carbon in humic substance molecules [5]. Changes in the content of lignin and its degree of transformation in plant material undergoing decomposition can be evaluated by plant material oxidation or soil samples with CuO or with acidic hydrolysis [2, 8, 10-12]. These methods facilitate a release of aldehydes and phenolic acids from lignins, among which the following can be distinguished:

- vanillyl compounds (V) – total content of vanillin and vanillic acid, obtained from coniferyl alcohol,
- syringyl compounds (S) – total content of syringaldehyde and syringic acid, obtained from sinapyl alcohol,

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- cinnamyl compounds (C) – total content of ferulic acid, caffeic acid and coumaric acid, obtained from coumaryl alcohol [8, 13].

As demonstrated by Higuchi et al. [14], Crawford [13] and Fengel and Wegener [15], mutual proportions of coniferyl, sinapyl and coumaryl alcohol and, as a result, the content of vanillyl, syringyl and cinnamyl compounds in lignin depend on the plant species.

As reported in literature [8, 10, 16], the structure of lignin fractions from plant material undergoes a considerable transformation during humification and so research into lignin decomposition products facilitates the collection of data on the degree of decomposition of plant residue in soil.

The acidic hydrolysis method was also applied to investigate humic substances of different origin [17-20]. Serve et al. [18] researched phenolic extracts of humic acids isolated from soils and plants. Based on the chromatogram patterns the authors demonstrated differences in the contents of phenolic compounds in humic acids of soils and humic acids of plant material – humic acids of soils demonstrated much lower contents of phenolic compounds than humic acids of the plants researched.

Banach and Debska [20] showed differences in the composition of phenolic compounds among fractions of humic and fulvic acids depending on the soil type. The authors making a comparison of hydrolyzates of humic acid fractions of chernozem, brown soil and luvisol, demonstrated that the highest contents of phenolic compounds were found in hydrolyzates of the fraction of humic acids isolated from chernozem. The fraction of fulvic acids of *Haplic Luvisols* and brown soil revealed a higher amount of phenolic compounds as compared with the fraction of humic acids, while the opposite relationship was shown in the case of the fraction of fulvic acids and the fraction of humic acids of chernozem.

One of the latest analytical methods used to investigate humic substances is high performance liquid chromatography (HPLC). The application of HPLC to identify phenolic compounds released e.g. in the process of acidic hydrolysis of humic acids can also provide new information on the organic matter humification process. Some authors [2, 10, 12, 18, 19] suggest that the share of some structural compounds of humic acids can be an indicator of the degree of lignin decomposition and so it can be an indirect indicator of the degree of humification.

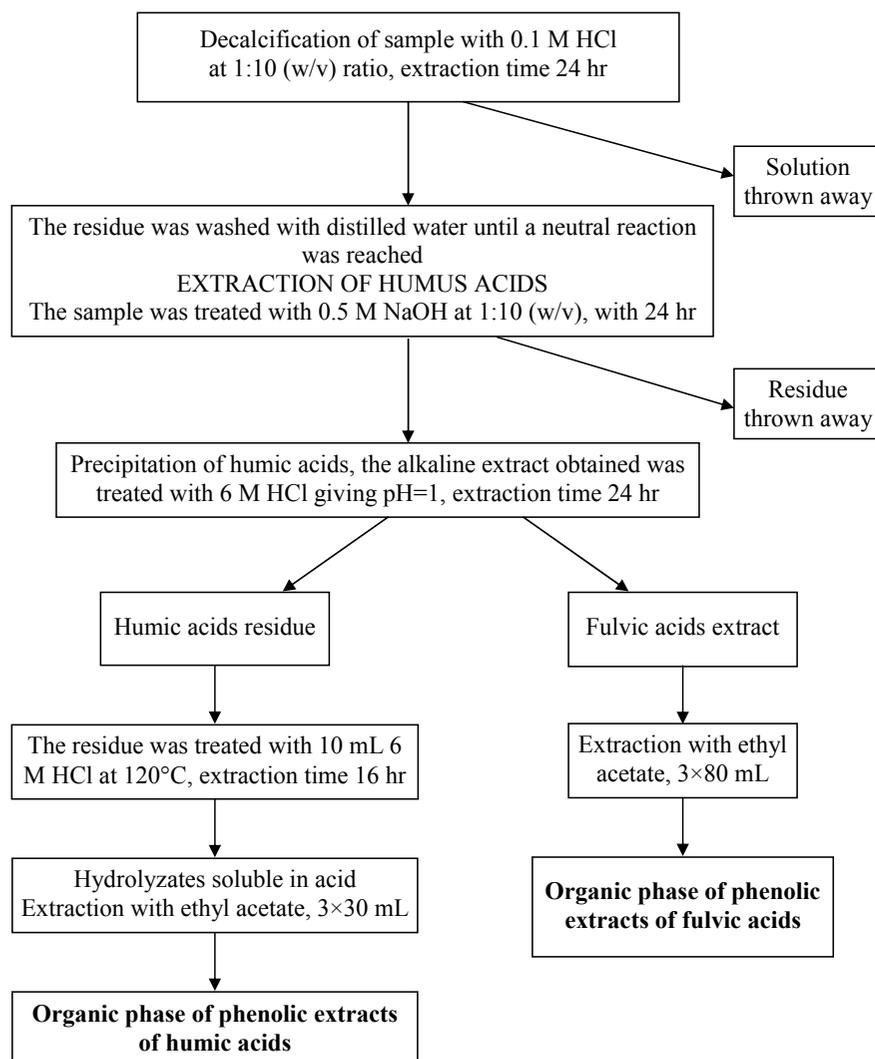


Fig. 1. Scheme of extraction procedure of phenolic compounds.

The aim of the present paper was to determine the content of phenolic compounds in fulvic acid extracts and hydrolyzates of humic acid depending on the degree of their maturity and the plant litter properties. The objective was realized with samples of humic and fulvic acids taken from organic and mineral horizons of forest soils (*Haplic Luvisols*) on the stands of oak, spruce and thuja.

It was also assumed that qualitative and quantitative analysis of phenolic compounds contained in the humic and fulvic acid fractions (similarly to determination of phenolic compounds in hydrolysates obtained directly from soils used by the majority of authors) will allow us to assess the degree of transformation of lignin as the main index connected with the degree of humification (maturation) of soil organic matter.

Materials and Methods

The research used forest soil sampled from the area of the Forest Experiment Department, Warsaw Agricultural University (WAU), at Rogow. FED WAU is located in the northeastern part of the Łódź Uplands, between 51°45' and 51°55' of northern latitude and between 19°50' and 20°10' of eastern longitude. The thermal vegetation period at Rogow is about 210 days long; the beginning of April through the beginning of November. The most essential data on the climatic conditions of this area:

Mean annual air temperature – +7°C,
Mean July temperature – +17°C,
Mean January temperature – -3°C,
Mean annual precipitation total – 590 mm.

FED Rogow is a unit of Warsaw Agricultural University established for forest experimentation and educating students of different faculties and the University. It offers education and scientific service as well as managing implementation and serving economic functions. FED Rogow consists of the Arboretum, Forestry Management, Natural and Forest Education Centre, Training Centre Nursery and the Centre of Animal Breeding.

Forest soil (*Haplic Luvisols*) was sampled in September 2004 in 5 replications according to the pattern given in Table 1 under the following tree stands:

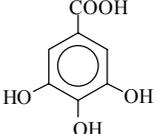
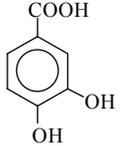
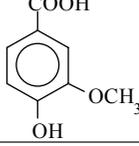
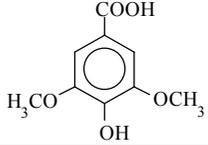
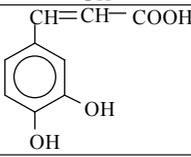
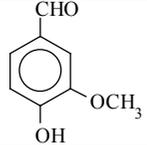
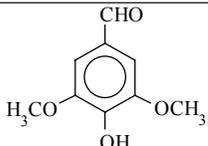
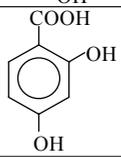
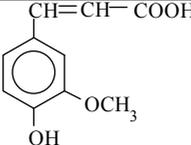
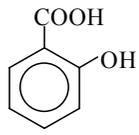
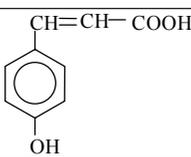
- oak (*Quercus robur* L.), trees about 90 years old, tree layer cover of 75%, shrubby layer cover of 15%, green plants layer cover of 30% and mossy layer cover of 5%,
- spruce (*Picea abies* L. Karsten), trees about 90 years old, tree layer cover of 75%, shrubby layer cover of 30%, green plants layer cover of 25% and mossy layer cover of 50%,
- thuja (*Thuja plicata* D.Don.ex. Lamb.) trees about 90 years old, tree layer cover of 85%, green plants layer cover of 10% and mossy layer cover of 5%.

Table 1. Description of soil samples and content of total organic carbon (TOC) and total nitrogen (Nt).

| Sample | Depth (cm) | Horizon | TOC (g kg ⁻¹) | Nt (g kg ⁻¹) | TOC/Nt |
|--------------|------------|---------|---------------------------|--------------------------|----------|
| Oak stand | | | | | |
| RD01 | 5-4 | Ol | 474±11* | 11.0±0.5 | 43.1±1.1 |
| RD02 | 4-0 | Ofh | 204±7 | 9.0±0.4 | 22.7±0.7 |
| RD1 | 0-8 | A1 | 37.4±2.2 | 2.63±0.13 | 14.2±0.3 |
| RD2 | 8-20 | A2 | 11.7±0.9 | 1.09±0.09 | 10.7±0.6 |
| RD3 | 20-30 | E | 6.6±0.4 | 0.90±0.06 | 7.3±0.1 |
| Spruce stand | | | | | |
| RS01 | 7-5 | Ol | 545±11 | 15.9±0.6 | 34.3±0.6 |
| RS02 | 5-1 | Of | 484±10 | 16.9±0.6 | 28.6±0.6 |
| RS03 | 1-0 | Oh | 261±7 | 8.7±0.4 | 30.0±0.8 |
| RS1 | 0-3 | AOh | 55.3±3.5 | 2.22±0.19 | 24.9±1.3 |
| RS2 | 3-20 | AE | 9.4±0.8 | 0.81±0.07 | 11.6±0.5 |
| Thuja stand | | | | | |
| RT01 | 3-2 | Ol | 508±12 | 7.9±0.3 | 64.1±1.6 |
| RT02 | 2-0 | Ofh | 387±11 | 12.6±0.4 | 30.7±0.5 |
| RT1 | 0-5 | A1 | 45.2±3.7 | 2.47±0.16 | 18.3±0.8 |
| RT2 | 5-18 | A2 | 20.2±2.6 | 1.25±0.08 | 16.2±1.6 |
| RT3 | 18-40 | E | 9.0±0.6 | 0.76±0.05 | 11.8±0.2 |

* - standard deviation for 5 replications

Table 2. List of models applied in qualitative and quantitative analysis of phenolic extracts.

| No. | Compound | Chemical formula | Symbol |
|-----|---|--|--------|
| 1. | Gallic acid (3,4,5-trihydroxybenzoic acid) |  | GA |
| 2. | Protocatechuic acid (3,4-dihydroxybenzoic acid) |  | PA |
| 3. | Vanillic acid (4-hydroxy-3-methoxybenzoic acid) |  | VA |
| 4. | Syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid) |  | SYA |
| 5. | p-Hydroxybenzoic acid (4-hydroxybenzoic acid) |  | p-HBA |
| 6. | Caffeic acid (3,4-dihydroxy-trans-cinnamic acid) |  | CA |
| 7. | Vanillin (4-hydroxy-3-methoxybenzaldehyde) |  | VAN |
| 8. | Syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde) |  | SYAL |
| 9. | 2,4-Dihydroxybenzoic acid |  | DHBA |
| 10. | Ferulic acid (4-hydroxy-3-methoxy-trans-cinnamic acid) |  | FERA |
| 11. | Salicylic acid (2-hydroxybenzoic acid) |  | SA |
| 12. | p-Coumaric acid (4-hydroxy-trans-cinnamic acid) |  | p-CA |

The extraction of phenolic compounds was made following the pattern given in Fig. 1. 10g of sample was weighed for extraction.

The obtained organic phase of phenolic extracts was vaporized completely and then dissolved in 5 mL of CH₃OH.

The chromatographic separation of solutions containing phenolic compounds was made with HPLC Series 200 by Perkin-Elmer equipped with the DAD detector. The analytical column by Waters X-Terra C18 was applied: molecule size of 5 µm, 250 × 4.6 mm in size I.D. The pre-column contained the same material as the analytical one.

The mobile phase consisted of:

eluent A: H₂O:CH₃CN:CH₃COOH (84:14:2)

and eluent B: CH₃CN.

Samples for HPLC analysis were prepared by combining 1 mL of extract of phenolic compounds and 1 mL of eluent A. The injection was 20 µL. The detection was made at the wavelength of 254 nm. The gradient separation program was used for the flow rate of 1 mL·min⁻¹. The initial composition of the mobile phase was 100% of eluent A, concentration of eluent B was increasing linearly during analysis. The gradient was completed after 49 min., when the content of eluent B was 10%.

Phenolic compounds were identified and their quantity was determined based on model solutions of phenolic compounds. The list of models applied is given in Table 2.

The HPLC analysis of model solutions was made just like for phenolic extracts. Based on the areas determined under peaks of the model solutions chromatograms obtained at different concentrations, there were plotted standard curves of the relationship between the peak area and the concentration. Based on the model curves the following were determined: content (µg·mL⁻¹) of phenolic compounds in extracts of fulvic acids and hydrolyzates of humic acids [2, 17-19]. The content of vanillyl (V), syringyl (S) and cinnamyl compounds (C) was calculated:

V – total content of vanillin (VAN) and vanillic acid (VA), (VAN+VA),

S – total content of syringaldehyde (SYAL) and syringic acid (SYA), (SYAL+SYA),

C – content of ferulic acid (FERA), caffeic and coumaric acids were not determined

and the following parameters were calculated:

V+S+C – sum of phenols of vanillyl, syringyl and cinnamyl type,

V:S:C – ratio of the share of respective compounds [8, 9].

Table 3. Content of phenolic compounds in the extracts of fulvic acids. For symbols, see Table 2.

| Sample | Horizon (Depth) (cm) | GA | PA | VA | SYA | p-HBA | VAN | SYAL | DHBA | FERA | SA |
|--------------|----------------------|---------------------|------------------|--------------------|-------|-------|-------|-------|-------|-------|-------------------|
| | | µg/mL | | | | | | | | | |
| Oak stand | | | | | | | | | | | |
| RD01 | Ol | 10.9 _c * | 11.8 | 17.4 | 3.34 | 7.45 | 0.728 | 10.7 | 2.29 | 13.2 | 7.03 |
| RD02 | Ofh | 0.960 _b | 2.13 | 10.0 | 2.67 | 2.83 | 0.248 | 2.56 | 0.232 | 2.11 | 1.71 |
| RD1 | A1 (0-8) | 0.901 _a | 0.989 | 1.27 | 0.445 | 0.604 | 0.087 | nd | 0.186 | 0.192 | 0.442 |
| RD2 | A2 (8-20) | 0.913 _a | 0.179 | 0.501 | 0.322 | 0.427 | 0.112 | nd | 0.067 | 0.085 | nd |
| RD3 | E (20-30) | nd | nd | nd | nd | nd | nd | nd | nd | nd | nd |
| Spruce stand | | | | | | | | | | | |
| RS01 | Ol | 64.4 | 112 _a | 20.6 _d | 16.4 | 119 | 7.87 | nd | 3.74 | 1.11 | 8.24 _b |
| RS02 | Of | 117 | 123 _b | 20.0 _d | 10.2 | 80.8 | 6.83 | nd | 3.40 | nd | 8.87 _b |
| RS03 | Oh | 74.3 | 119 _b | 10.8 _c | 5.17 | 35.8 | 3.61 | nd | 0.607 | nd | 9.91 _c |
| RS1 | AOh (0-3) | 20.4 | 134 _d | 1.64 _b | 1.28 | 0.991 | 0.759 | nd | 0.292 | nd | 3.00 _a |
| RS2 | AE (3-20) | 9.86 | 124 _c | 0.223 _a | 0.060 | 0.239 | 0.243 | nd | nd | nd | nd |
| Thuja stand | | | | | | | | | | | |
| RT01 | Ol | 11.4 | 13.0 | 3.42 | 1.03 | 4.75 | 1.71 | 1.41 | nd | 3.98 | 118 _c |
| RT02 | Ofh | 6.14 | 10.3 | 2.74 | 2.57 | 19.3 | 1.17 | 2.28 | nd | 1.97 | 123 _c |
| RT1 | A1 (0-5) | 1.48 | 2.70 | 0.587 | 0.076 | 4.07 | 0.857 | 0.406 | nd | 0.192 | 16.8 _b |
| RT2 | A2 (5-18) | nd | 1.70 | 0.155 | nd | 1.55 | 0.190 | 0.053 | nd | nd | 3.25 _a |
| RT3 | E (18-40) | nd | 0.307 | nd | nd | 0.452 | 0.042 | nd | nd | nd | nd |

nd – not detected

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

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 Duncan's test did not show significant difference).

Results

The concentrations of the aldehydes and phenolic acids in hydrolyzates of the fraction of humic acids and extracts of fulvic acids determined with the model curve method are given in Tables 3 and 5. Fig. 2 presents sample chromatograms of solutions containing phenolic compounds.

Content of Phenolic Compounds in the Fraction of Fulvic Acids

Extracts of fulvic acids of organic horizons of soils demonstrated higher contents of phenolic compounds as compared with the extracts of fulvic acids of mineral horizons (Table 3). In the extracts of fulvic acids obtained from soil sampled from the thuja stand, no 2,4-dihydroxybenzoic

acid was recorded (DHBA), whereas on the spruce stand no syringaldehyde (SYAL) was found, while ferulic acid (FERA) was recorded only in the extracts of subhorizon OI.

Extracts of fulvic acids showed a decrease in the content of phenolic compounds deep in the soil profile (Table 3). One shall note, however, that extracts of fulvic acids isolated from soil sampled from the thuja stand demonstrated significantly higher contents of syringic acid (SYA), syringaldehyde (SYAL) and 4-hydroxybenzoic acid (p-HBA) in the Ofh subhorizon as compared with the subhorizon OI. Extracts of fulvic acids of subhorizon OI on the spruce stand showed lower contents of gallic acid (GA) and protocatechuic acid (PA) as compared with their contents in the subhorizon Of and Oh.

The highest content of gallic acid (GA), protocatechuic acid (PA), 4-hydroxybenzoic acid (p-HBA) and vanillin (VAN) was found in extracts of fulvic acids from the spruce stand, lower extracts of fulvic acids isolated from soil sampled from the thuja stand, and the lowest – extracts of fulvic acids from the oak stand. Just the opposite relationships were demonstrated for the content of ferulic acid. However, one shall note that on the spruce stand it occurred only in subhorizon OI.

Table 4. Content of vanillyl (V), syringyl (S) and cinnamyl (C) compounds and the values of parameter V+S+C in the extracts of fulvic acids and hydrolyzates of the fraction of humic acids.

| Sample | Horizon (Depth) (cm) | FAs | | | | HAs | | | |
|--------------|----------------------|-------|-------|-------|-------|-------|-------|-------|-------|
| | | V | S | C | V+S+C | V | S | C | V+S+C |
| µg/mL | | | | | | | | | |
| Oak stand | | | | | | | | | |
| RD01 | OI | 9.13 | 1.26 | nd | 10.4 | 18.1 | 14.0 | 13.2 | 45.3 |
| RD02 | Ofh | 17.8 | 2.31 | nd | 20.1 | 10.3 | 5.23 | 2.11 | 17.6 |
| RD1 | A1 (0-8) | 2.92 | 0.534 | nd | 3.45 | 1.36 | 0.445 | 0.192 | 2.00 |
| RD2 | A2 (8-20) | 0.525 | 0.163 | nd | 0.688 | 0.613 | 0.32 | 0.085 | 1.02 |
| RD3 | E (20-30) | 0.199 | nd | nd | 0.199 | nd | nd | nd | nd |
| Spruce stand | | | | | | | | | |
| RS01 | OI | 38.9 | 3.32 | 8.51 | 50.7 | 28.5 | 16.4 | 1.11 | 46.0 |
| RS02 | Of | 42.4 | 3.57 | 10.6 | 56.6 | 26.8 | 10.2 | nd | 37.0 |
| RS03 | Oh | 44.7 | 2.75 | 3.08 | 50.5 | 14.4 | 5.17 | nd | 19.6 |
| RS1 | AOh (0-3) | 4.79 | 0.323 | nd | 5.11 | 2.40 | 1.28 | nd | 3.68 |
| RS2 | AE (3-20) | 0.273 | nd | nd | 0.273 | 0.466 | 0.060 | nd | 0.526 |
| Thuja stand | | | | | | | | | |
| RT01 | OI | 7.08 | 0.234 | 0.112 | 7.43 | 5.13 | 2.44 | 3.98 | 11.5 |
| RT02 | Ofh | 18.3 | 0.655 | 0.373 | 19.3 | 3.91 | 4.85 | 1.97 | 10.7 |
| RT1 | A1 (0-5) | 9.95 | 0.230 | 0.070 | 10.3 | 1.44 | 0.482 | 0.192 | 2.11 |
| RT2 | A2 (5-18) | 2.54 | 0.077 | nd | 2.62 | 0.345 | 0.053 | nd | 0.398 |
| RT3 | E (18-40) | 0.526 | 0.107 | nd | 0.633 | 0.042 | nd | nd | 0.042 |

nd – not detected

The change in the content of phenolic compounds in extracts of fulvic acids resulted in the calculated contents of vanillyl (V), syringyl (S) and cinnamyl type compounds (C) (Table 4). Cinnamyl compounds (C) account for the content of ferulic acid. However, in the extracts no caffeic and coumaric acids were determined, and thus values C are equivalent to the content of ferulic acid.

Extracts of fulvic acids of the organic horizon of soils demonstrated higher contents of phenolic compounds V, S and C type, as compared with extracts of fulvic acids of mineral horizons of soils. Besides, the greater the soil sampling depth, the lower the contents.

The highest contents of phenolic compounds of vanillyl and syringyl type in the entire soil profile were found in extracts of fulvic acids obtained from soil sampled from the spruce stand and the lowest – in the samples obtained from under the thuja stand. The highest contents of phenolic compounds type C (ferulic acid) were found in extracts of fulvic acids isolated from soil sampled from the oak stand.

The concentrations of phenolic compounds in the solutions of fulvic acids resulted in the values of parameter V+S+C (Table 4). The value of parameter V+S+C, similar to the values of parameters V, S and C, decreased with soil

sampling depth. Values of parameter V+S+C obtained for extracts of fulvic acids of subhorizon OI ranged from 11.5 µg/mL for the RT01 sample (the thuja stand, subhorizon OI) to 46.0 µg/mL for the RS01 sample (the spruce stand, subhorizon OI) and 45.3 µg/mL – oak stand.

Content of Phenolic Compounds in Hydrolyzates of the Fraction of Humic Acids

In general, it was observed that the highest contents of phenolic acids were found in hydrolyzates of the fraction of humic acids of subhorizon Of (Ofh) (Table 5). In mineral horizons of soils the content of phenolic compounds was lower than in organic horizons and decreased with soil sampling depth, irrespective of the tree species. In hydrolyzates of the fraction of humic acids on all the stands there was no 2,4-dihydroxybenzoic acid (DHBA). Besides, on the oak stand there was no syringic acid and ferulic acid, on the spruce stand – syringic acid, and on the thuja stand – syringaldehyde. In the hydrolyzates of humic acids, similar to the extracts of fulvic acids, no caffeic and coumaric acids were determined.

Table 5. Content of phenolic compounds in hydrolyzates of the fraction of humic acids. (For symbols, see Table 2).

| Sample | Horizon (Depth) (cm) | GA | PA | VA | SYA | p-HBA | VAN | SYAL | DHBA | FERA | SA |
|--------------|----------------------------|-------|--------|-------|-------|-------|-------|-------|------|-------|-------|
| | | µg/mL | | | | | | | | | |
| Oak stand | | | | | | | | | | | |
| RD01 | OI | 1.57 | 2.36 | 8.60 | nd | 0.392 | 0.528 | 1.26 | nd | nd | 9.92 |
| RD02 | Ofh | 2.43 | 3.24 | 17.1 | nd | 0.808 | 0.695 | 2.31 | nd | nd | 18.0 |
| RD1 | A1 (0-8) | 1.22 | nd | 2.60 | nd | 0.523 | 0.317 | 0.534 | nd | nd | 1.90 |
| RD2 | A2 (8-20) | 0.963 | nd | 0.400 | nd | 0.148 | 0.125 | 0.163 | nd | nd | 0.675 |
| RD3 | E (20-30) | 0.335 | nd | 0.199 | nd | nd | nd | nd | nd | nd | nd |
| Spruce stand | | | | | | | | | | | |
| RS01 | OI | 34.8 | 8.78b* | 35.1 | nd | 6.31 | 3.81 | 3.32 | nd | 8.51 | 13.0 |
| RS02 | Of | 53.8 | 17.9c | 39.0 | nd | 20.2 | 3.38 | 3.57 | nd | 10.6 | 39.0 |
| RS03 | Oh | 11.4 | 17.6c | 43.5 | nd | 1.13 | 1.23 | 2.75 | nd | 3.08 | 3.99 |
| RS1 | AOh (0-3) | 0.782 | 0.178a | 4.64 | nd | 0.072 | 0.145 | 0.323 | nd | nd | 0.494 |
| RS2 | AE (3-20) | 0.411 | nd | 0.273 | nd | nd | nd | nd | nd | nd | nd |
| Thuja stand | | | | | | | | | | | |
| RT01 | OI | 5.12 | 4.08 | 5.81 | 0.234 | 0.695 | 1.27 | nd | nd | 0.112 | 1.90 |
| RT02 | Ofh | 6.78 | 11.1 | 15.3 | 0.655 | 2.44 | 2.98 | nd | nd | 0.373 | 20.2 |
| RT1 | A1 (0-5) | 1.61 | 0.218 | 9.19 | 0.230 | 0.946 | 0.761 | nd | nd | 0.070 | 4.64 |
| RT2 | A2 (5-18) | 0.893 | 0.087 | 2.54 | 0.077 | 0.213 | nd | nd | nd | nd | 0.308 |
| RT3 | E (18-40) | 0.330 | nd | 0.526 | 0.107 | nd | nd | nd | nd | nd | nd |

nd – not detected

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

In mineral horizons of soil, similar to organic horizon, vanillyl compounds were dominant, while cinnamyl compounds were identified only in hydrolyzates of HAs the organic horizon on the thuja stand (Table 4). Besides, in the deepest soil layers there were no syringyl compounds (exception – the RT3 sample, thuja stand, horizon E).

The values of parameter V+S+C obtained for hydrolyzates of humic acids of organic horizons of soils were definitely higher than mineral horizons (Table 4). The highest value of parameter V+S+C was found for the fraction of humic acids of subhorizon O1 on the spruce stand. In the deepest soil layers in which there were found vanillyl, syringyl or cinnamyl compounds the value of this parameter ranged from 0.199 (RD3, oak stand, horizon E) to 0.633 $\mu\text{g/mL}$ (RT3, thuja stand, horizon E).

One shall stress that the content of phenolic compounds in hydrolyzates of the fraction of humic acids was in general lower, except for vanillic acid, as compared with their content in extracts of fulvic acids. Vanillic acid, irrespective of the tree species and the soil sampling depth, occurred always in greater amounts in hydrolyzates of humic acids.

Discussion

Phenolic compounds released from humic acids provide much more precious information on the organic matter humification process. As reported in literature [2, 12, 17-23], both the qualitative and the quantitative composition of the phenolic compounds obtained depend on the research material.

Based on the results obtained, it was demonstrated that the content of identified aldehydes and phenolic acids in extracts of fulvic acids and humic acids isolated from forest soil samples depended not only on the tree species but also on the soil sampling depth (Tables 3 and 5). A decrease with depth in the concentration of phenolic compounds in fractions of humic and fulvic acids was a consequence of a higher degree of humification. A constant inflow of fresh organic matter in the organic horizons causes the existence of material of low humification degree.

Furthermore, it was demonstrated that the content of phenolic compounds in hydrolyzates of the fraction of humic acids was lower in most cases, except for vanillic

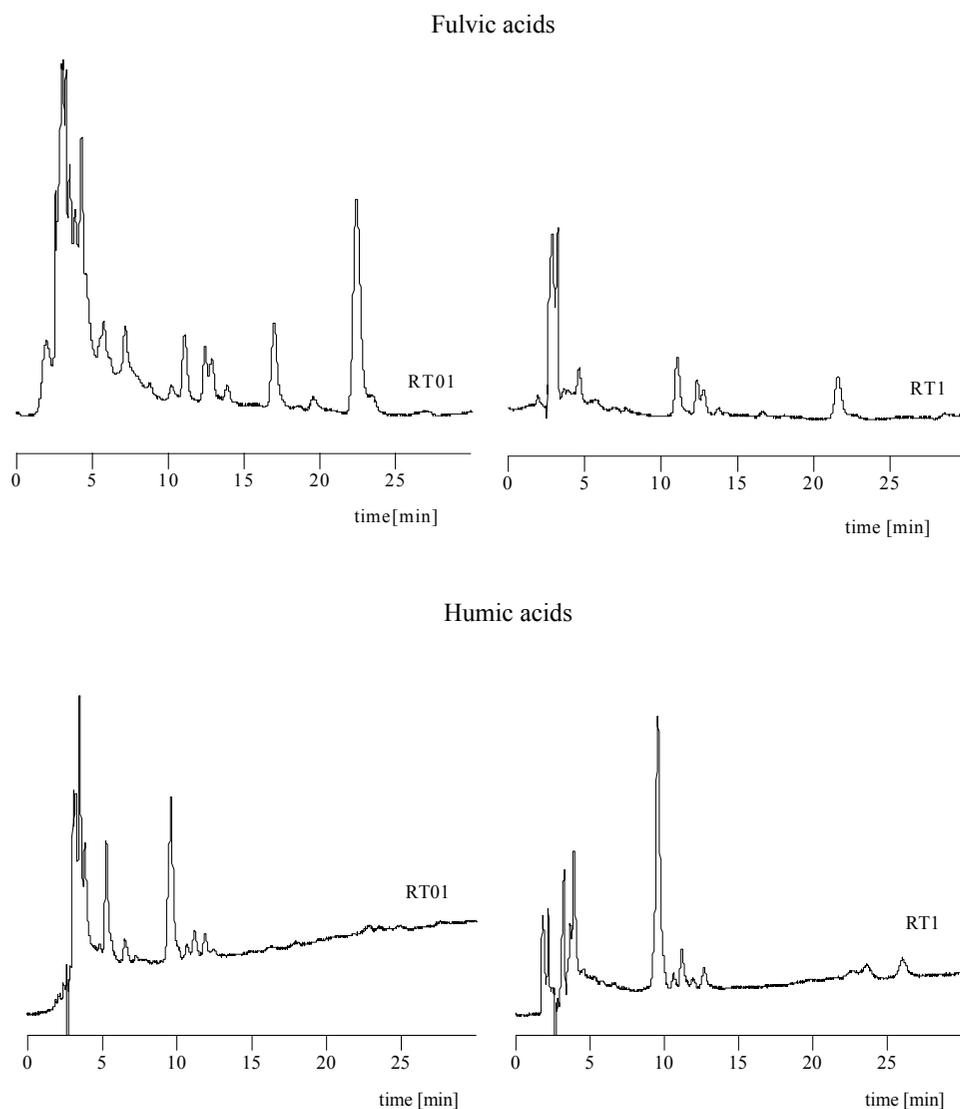


Fig. 2. Sample chromatograms of phenolic compounds which occurred in the extracts of fractions of fulvic acids and humic acids (RT01 – thuja stand, subhorizon O1; RT1 – thuja stand – horizon A1).

acid, as compared with their content in extracts of fulvic acids. It is commonly known that in fulvic acids, due to genetic conditions, the qualitative composition is the same as in humic acids. The differences between these groups of compounds occur in the quantitative composition. Fulvic acids as compared with humic acids demonstrate a lower degree of condensation of the aromatic nucleus and so they contain more simple aromatic structures, which can be confirmed by the present results.

The progress of the humification process can be seen from the changes in the value of parameter V+S+C defined as a measure of undisturbed, uncondensed lignin structures. The results given in the present paper (Table 4) coincide with literature reports [11, 12, 24], according to which the value of parameter V+S+C decreases with the soil profile depth and so decreases with an increase in the degree of humification of the material researched. The values of parameter V+S+C obtained for the fraction of fulvic acids and the fraction of humic acids of subhorizon OI were also determined by the plant litter properties. The values of parameter V+S+C obtained for extracts of fulvic acids and hydrolyzates of humic acids of subhorizon OI of soils increased in the following order:

spruce stand \geq oak stand $>$ thuja stand

Aldehydes and phenolic acids released from lignin in the form of vanillyl compounds obtained from coniferyl alcohol, syringyl compounds from sinapyl alcohol and cinnamyl compounds from coumaryl alcohol occur in lignin in specific proportions. Reports by Crawford [13] and Higuchi et al. [14] demonstrated that mutual proportions of coniferyl, sinapyl and coumaryl alcohol in lignin depended on the plant species. Angiospermous plants lignin contains approximately the same amounts of vanillyl and syringyl compounds and a few cinnamyl compounds (49:46:5), while gymnospermous plants lignin shows a clear advantage of vanillyl compounds over the others (80:6:14). Some authors [15] note that the proportions of these compounds in lignin of spruce can also be 94:1:5.

With the above in mind we determined mutual proportions of the shares of respective vanillyl (V), syringyl (S) and cinnamyl (C) compounds – V:S:C, for the fraction of fulvic acids and hydrolyzates of humic acids and the total fraction HAs + FAs, which to some extent allowed for referring the values calculated to the values obtained for lignin.

Extracts of humus acids (HAs + FAs) of subhorizon OI on the oak stand demonstrated a similar share of syringyl and cinnamyl compounds and an advantage of vanillyl compounds (ratio V:S:C – 49:28:23, Table 6). The share of vanillyl compounds was similar to the total share of syringyl and cinnamyl compounds. Extracts of humus acids of subhorizon OI on the spruce and thuja stands showed a considerable advantage of vanillyl compounds (Table 6). For the extracts being the sum of HAs + FAs, isolated from soil sampled from subhorizon OI, there were then obtained similar values of the ratio of V, S and C compounds to the value of the ratio of these compounds in lignin, which demonstrates a high share of lignin in the processes of humus acid formation in forest soils.

Table 6. Ratios of the share of vanillyl (V), syringyl (S) and cinnamyl (C) compounds in humus, humic and fulvic acids.

| Sample | V:S:C | | |
|--------------|-----------|-----------|-----------|
| | HAs+FAs | HAs | FAs |
| Oak stand | | | |
| RD01 | 49:28:23 | 88:12:00 | 40:31:29 |
| RD02 | 74:20:06 | 89:11:00 | 58:30:12 |
| RD1 | 78:18:04 | 85:15:00 | 68:22:10 |
| RD2 | 67:28:05 | 84:16:00 | 60:32:08 |
| RD3 | 100:00:00 | 100:00:00 | nd |
| Spruce stand | | | |
| RS01 | 70:20:10 | 77:06:17 | 62:36:02 |
| RS02 | 74:15:11 | 75:06:19 | 72:28:00 |
| RS03 | 84:11:05 | 89:05:06 | 74:26:00 |
| RS1 | 82:18:00 | 94:06:00 | 65:35:00 |
| RS2 | 92:08:00 | 100:00:00 | 89:11:00 |
| Thuja stand | | | |
| RT01 | 64:14:22 | 95:03:02 | 44:21:34 |
| RT02 | 74:18:08 | 95:03:02 | 37:45:18 |
| RT1 | 92:06:02 | 97:02:01 | 68:23:09 |
| RT2 | 96:04:00 | 97:03:00 | 87:13:00 |
| RT3 | 84:16:00 | 83:17:00 | 100:00:00 |

nd – not detected

Proportions of V, S and C type compounds in extracts of fulvic acids isolated from soil sampled from subhorizon OI on the oak and thuja stands were as follows: 40:31:29 and 44:21:34, whereas extracts of fulvic acids of subhorizon OI on the spruce stand, just like the extracts of humic acids, revealed a considerable advantage of vanillyl compounds over the others: 62:36:2. It was also observed that the share of vanillyl compounds in the extracts of humic acids and in the extracts of fulvic acids of mineral horizons was higher than in extracts of these acids isolated from soil sampled from subhorizon OI, whereas hydrolyzates of the fractions of humic acids of subhorizon OI in general showed a considerably greater share of vanillyl compounds, as compared with the extracts of fulvic acids (Table 6). Changes in the share of phenolic compounds (increase in the share of vanillyl compounds in the humic horizon) in the extracts of humic and fulvic acids researched coincide with the results reported by Johansson et al. [10]. The authors demonstrated that in the decomposition process of spruce needles there is an increase in the share of vanillyl compounds and a slight decrease in the share of cinnamyl compounds.

Conclusions

1. The fraction of fulvic acids demonstrated a higher content of aldehydes and phenolic acids as compared with the hydrolyzates of fractions of humic acids. Hydrolyzates of the fractions of humic acids of sub-horizon O₁ showed a higher share of vanillyl compounds in the pool of vanillyl, syringyl compounds and ferulic acid (component of cinnamyl compounds), as compared with the extracts of fulvic acids.
2. The parameter which differentiates the properties of humus acids of subhorizon O₁ produced under different tree species is the ratio of the share of vanillyl, syringyl and cinnamyl compounds (V:S:C). Humus acids of subhorizon O₁ on the oak stand revealed a similar or slightly higher share of the total syringyl and cinnamyl compounds to the share of vanillyl compounds, while humus acids from coniferous tree stands (spruce, thuja) – a considerable advantage of vanillyl compounds.
3. The degree of maturity of organic matter is connected with decreased content of aldehydes and phenolic acids which occur in hydrolyzates of fractions of humic acids and extracts of fulvic acids. In hydrolyzates of humic acids and fractions of fulvic acids, along with soil sampling depth, the values of parameter V+S+C decrease.

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