

# Properties of Water-Soluble Organic Substances Isolated from Soils Using Centrifugation and Vacuum Filtration

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**Abstract**—This paper examines the composition and properties of water-soluble organic substances in relation to their localization in different parts of soil pore space, which determines the organic matter's susceptibility to biodegradation. Water-soluble organic substances contained in the sod and humus horizons of a Mollic Gleysol were extracted from pores more and less than 30  $\mu\text{m}$  in diameter using sequential centrifugation of soil samples saturated to the full moisture capacity. The obtained solutions do not differ statistically significantly in the total concentration of carbon and phenolic compounds or in their absorption and fluorescence spectra; however, a strong variation in parameter values (the maximum and minimum values differ by 4–15 times) indicates that the composition of extracted substances is heterogeneous. Concentrations of simple phenolcarboxylic acids (PCAs) in solutions extracted from large pores in the humus horizon are 4–8 times higher in comparison with those extracted from smaller pores. Salicylic, benzoic, and cinnamic acids predominate in large pores (75% of the total amount); while *p*-hydroxybenzoic and benzoic acids predominate in small pores (67% of the total amount). Dissolved organic matter present in the ELBTg horizon of a podzolic soil was isolated from pores more and less than 14.7  $\mu\text{m}$  in diameter at a soil moisture level of 22% using tension lysimeters. Dissolved organic matter extracted from large pores contains more C, N, and phenolic compounds; based on the absorption spectra, it has a larger molecular weight and a higher aromaticity degree. Organic matter contained in small pores is more hydrophobic, and its C : N ratio varies in a wider range (i.e., it is more resistant to decomposition). Phenolic compounds are predominantly confined to large pores; this ensures their preservation in soil due to the transportation to lower horizons with water flows and subsequent chemical stabilization in the form of organomineral compounds.

**Keywords:** water-soluble organic matter, pore diameter, tension lysimeters, soil solution, gleysols, retisols

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## INTRODUCTION

Water-soluble organic substances present in soils are essential components of the global carbon and nitrogen cycles. The chemical and biochemical activity of water-soluble organic matter (WSOM) in soils is diverse; it varies in time and space and depends on such factors as sources of its inflow into the soil, vegetation type, land use type, soil and climatic conditions, etc. Many studies [7, 10, 14] examine these interrelationships, but effects exercised by some factors still remain unclear. For instance, data on the correlation between the WSOM composition and properties on the one hand and its localization in different parts of soil pore space on the other hand are insufficient and often contradictory [29], even though such information is required to forecast such processes as organic matter mineralization, sorption, and stabilization in soil or its removal with runoff.

Moisture contained in soil represents a system of fractions with different mobility degrees and different composition; the latter is extremely important. It is

established that solutions contained in different categories of pores, gravitational moisture, and capillary moisture differ significantly in concentrations of cations, anions, organic carbon, and nitrogen [3, 17]. Qualities and composition of organic substances are understood to a lesser extent, but even the available data confirm differences between them [4, 8, 13, 25]. The different availability of large and small soil pores for solutions, plant roots, and decomposing organisms determines the possibility for and direction of transformation processes affecting organic substances present in these pores and their qualitative characteristics. Concurrently, a variety of physicochemical conditions (humidity, wetting/drying dynamics, diffusion rates of gases and substances, redox regime, water affinity (i.e., hydrophilic–hydrophobic properties) of surfaces, etc.) leads to an uneven distribution of microorganisms in soil pore space. It is established that more intensive microbiological activities and greater bacterial populations are confined to microaggregates [19], while fungi predominantly colonize macroaggregates

[6]. Also, different numbers and types of bacteria populate the microaggregate surface and internal space that features favorable conditions for anaerobic microorganisms [6]. Different taxa of organisms, including aerobes and anaerobes, transform organic matter in different ways, which is reflected in the specificity of WSOM qualitative properties. In smaller pores, the WSOM composition is significantly affected by microbial enzymes and conditions determined by the isolated existence [26]. In some soils, pores less than 2  $\mu\text{m}$  in diameter occupy up to 50% of the volume; in such pores, organic matter can be transformed only due to the limited diffusion of enzymes and virtually does not undergo mineralization [18]. By contrast, conditions in larger macropores associated with predominant moisture flows are favorable from the hydrological perspective for rapid WSOM mineralization and renewal, since these zones feature a more intensive moisture dynamics and a high microbiota density and composition diversity, while sorption in such pores is limited [13].

Overall, the WSOM amount and qualitative composition in different pores are affected by physicochemical, biological, and hydrological factors operative in the soil. The purpose of this study was to examine the content and properties of water-soluble organic matter in different fractions of the soil solution.

## MATERIALS AND METHODS

The subject of this study was water-soluble organic matter contained in two modal soils present in Tsentral'nolesnoy State Nature Biosphere Reserve: muck-humus gley soil (the sod (AY) and humus (AU) horizons) and texturally differentiated podzolic soil (the ELBTg horizon). Descriptions of both soils are provided in earlier studies [2, 3]. According to the World Reference base for Soil Resources 2014 (WRB 2014), the studied soils belong to gleysols and retisols, respectively. WSOM contained in the muck-humus gley soil was isolated by centrifugation in a laboratory environment. The analyzed samples represented unground specimens with an undisturbed structure consisting of aggregates of different sizes. The soil samples were placed in perforated centrifuge tubes, saturated to the full moisture capacity with distilled water, left for some time to drain free moisture, and then centrifuged sequentially at 600 and 6000 rpm (45 and 3720 g, a Rotofix 32A centrifuge) to extract solutions from pores of different sizes. The extraction technique is described in more detail in [3]. The extraction conditions correspond to the following pressure values:  $pF = 2$  and  $pF = 3.9$  ( $pF = -\log|P|$ , where  $P$  is the soil moisture pressure (cm water column)). According to Jurin's law, the application of such conditions results in the isolation of two soil solution fractions: from pores  $\geq 30 \mu\text{m}$  and from pores  $0.4\text{--}30 \mu\text{m}$  in diameter. Ten solution samples were extracted for each

pore category from the sod horizon and six samples from the humus horizon.

WSOM contained in the podzolic soil was isolated in the field in summer (first half of June). The soil moisture content at the time of sampling was 22%. Monoliths of the ELBTg horizon 15 cm in diameter and 22 cm high were cut off from profile section walls using a pipe segment; solutions were immediately isolated from the monoliths by the tension lysimetry method using ceramic filters with pores  $0.8\text{--}0.9 \mu\text{m}$  in diameter. A modern mechanical automobile pump with a back-pressure valve (Russia) was used to create vacuum. Two soil solution fractions were isolated by applying pressure values of 20 and 80 kPa resulting in the drainage of pores  $\geq 14.7 \mu\text{m}$  and less than  $14.7 \mu\text{m}$  ( $3.7\text{--}14.7 \mu\text{m}$ ) in diameter. In total, eight samples were obtained (four samples for each pore category).

All isolated solutions were filtered through membrane filters with a pore diameter of  $0.45 \mu\text{m}$  prior to the analysis. In all solutions, the content of organic carbon and nitrogen was determined using a liquid TOC Elementar analyzer (Germany); the samples were diluted to concentrations of  $10\text{--}30 \text{ mg/L}$  (with an error of 2%). The content of soluble phenolic compounds (SFCs) was determined photometrically [1]. 5 mL of 20%  $\text{Na}_2\text{CO}_3$  solution and 0.5 mL of the standard Folin-Ciocalteu reagent (PanReac AppliChem) were added to a solution aliquot of 20 mL; then distilled water was added to dilute the sample to a volume of 50 mL. The samples were subsequently left for 40 min for color development. The optical density of the solutions was measured at a wavelength  $\lambda = 725 \text{ nm}$  using a Hach Lange DR 2800 spectrophotometer. A calibration curve was constructed using gallic acid, a typical component of plant residues. UV absorption spectra were recorded for all solutions (an SF-2000 spectrophotometer); a number of coefficients used to describe WSOM were computed [11, 22, 28]: extinction coefficient  $E_{250}$  (an aromaticity degree indicator),  $E_{250} : E_{365}$  ratio (a molecular weight indicator [22]), and the integral extinction coefficient  $E_{\Sigma(254\text{--}465 \text{ nm})}$  (the total absorption intensity index [28] computed as the sum of extinction coefficients at wavelengths of 254, 260, 272, 280, 285, 300, 340, 350, 365, 400, and 465 nm). In addition, fluorescence spectra were recorded for the muck-humus gley soil (an LS-45 spectrofluorimeter). The organic matter humification index (HIX) was computed as the ratio between the long-wavelength (435–480 nm) fluorescence intensity and the total (300–480 nm) fluorescence intensity at an excitation wavelength of 254 nm [21]. The qualitative composition and content of phenolcarboxylic acids (PCAs) were determined for WSOM isolated from the humus horizon of the muck-humus gley soil. PCAs were preconcentrated on Diapak-P cartridges (BioKhimMak). The cartridges were preconditioned by flushing with 5 mL of acetone (extra purity grade) using a syringe and then, with 20 mL of bidistilled

**Table 1.** Properties of WSOM isolated from the muck–humus gley soil (mean values; max and min values are provided in parentheses)

Horizon	No. of samples	Pore diameter, $\mu\text{m}$	C, mg/L	SFC, mg/L	SFC, % of C	HIX	$E_{\Sigma(254-465 \text{ nm})}$ , L/mg C <sup>-1</sup> cm <sup>-1</sup>
Sod	10	>30	218.7 (95.8–408.5)	17.0 (8.2–32.3)	9.1 (3.8–16.6)	0.980 (0.966–0.994)	0.157 (0.033–0.384)
	10	0.4–30	262.6 (33.2–468.5)	19.2 (9.5–34.1)	16.7 (3.0–21.8)	0.946 (0.838–0.992)	0.283 (0.069–1.023)
Humus	3–6	>30	721.4 (603.3–813.2)	34.5 (31.4–36.3)	4.7 (4.4–5.3)	0.844 (0.723–0.965)	0.304 (0.262–0.345)
	3–6	0.4–30	710.9 (437.3–905.5)	31.9 (29.2–34.3)	4.1 (3.7–4.4)	0.986 (0.960–0.999)	0.250 (0.202–0.300)

water. Using a peristaltic pump, filtered solution samples (50–100 mL) were flowed through the prepared cartridges at a rate of 3 mL/min; after that, 20 mL of distilled water were flowed through the cartridges (the rinse was discarded). Concentrated phenolic compounds were washed off the cartridge with 5 mL of acetone using a syringe. The solvent was evaporated at room temperature; the concentrated samples were dissolved in acetonitrile; and the PCA content (4-*p*-hydrobenzoic, ferulic, *p*-coumaric, salicylic, benzoic, and cinnamic acids) was determined using high-performance liquid chromatography (an Agilent 1100 chromatograph) with a photometric detector at a wavelength of 280 nm. The separation was performed using a Diasfer 110–118 column 4 × 250 mm in size with a particle diameter of 5  $\mu\text{m}$ . Sample dosing volume: 1  $\mu\text{L}$ ; eluent: acetonitrile–water–1% trifluoroacetic acid (ratios from 20 : 75 : 5 to 70 : 25 : 5 for 20 min, exposure: 5 min, rate: 1 mL/min). The ratio between hydrophilic and hydrophobic fractions was determined for WSOM isolated from the podzolic soil using reversed-phase chromatography (a Biologic LP chromatograph, BioRad). The separation was performed on hydrophobized agarose gel (Octyl Sepharosa CL-4B, Pharmacia); column size: 1 × 10 cm; flow rate: 1 mL/min. Two fractions were obtained; the hydrophilic fraction was eluted with Tris-HCl buffer (pH = 7.0); the hydrophobic fraction, with 0.3% SDS-Na solution. The share of the hydrophobic fraction was computed as the share of the area under the respective peak on the curves (22200 recording integrator LKB Bromma).

Statistical data processing was performed in Statistica 10. The Mann–Whitney U test was used to assess the statistical significance of differences at a significance level of 0.05.

## RESULTS

Properties of WSOM isolated from the muck–humus gley soil are presented in Table 1.

The concentration of water-soluble carbon in solutions extracted from large and small pores in the sod horizon varies significantly: the range of fluctuations reaches 1.5 orders of magnitude; this is especially true for solutions extracted from the 0.4–30- $\mu\text{m}$  pore category. The mean values amount to some 218–260 mg/L. The content of soluble phenolic compounds (SFCs) varies somewhat less: on average, their concentrations in solutions extracted from pores of different sizes are close and amount to some 18 mg/L; however, the minimum and maximum values differ by 4–5 times. The relative share of SFCs in the total organic carbon content in small pores is generally higher than in large ones. The integral extinction values in the range of 254–465 nm vary from 0.157 to 0.283 L/mg C cm (i.e., they are also slightly higher in WSOM isolated from smaller pores, but the variation of this parameter is very strong: the spread of values also reaches 1.5 orders of magnitude, which indicates that the qualitative composition of isolated organic substances is heterogeneous). The humification index (HIX) values [21, 29] computed on the basis of the fluorescence spectra for organic matter extracted from large and small pores are similar: 0.946 and 0.980. None of the examined properties of WSOM isolated from different pores are statistically significantly different (values of the  $U$  coefficient range from 41 to 78, which is greater than  $U_{cr} = 23$ ;  $n_1 = n_2 = 10$ ).

The average carbon content in solutions isolated from the humus horizon is almost three times higher in comparison with the sod horizon: 721.4 and 710.9 mg/L (in large and small pores, respectively), while the SFC content is 1.5–2 times higher. The extinction coefficients of substances isolated from the humus horizon and from the sod horizon are of the same order of magnitude: 0.250–0.304 L/(mg C cm) on average; the same applies to the humification indices.

In the humus horizon, organic substances extracted from different pores do not differ statistically significantly: neither in the content of phenolic com-

**Table 2.** Concentrations of phenolcarboxylic acids ( $\mu\text{mol/L}$ ) in solutions isolated from the humus horizon of the muck–humus gley soil

Acid	Solution from pores $>30 \mu\text{m}$	Solution from pores $0.4\text{--}30 \mu\text{m}$
4- <i>p</i> -hydrobenzoic	1.44	1.78
Ferulic	1.24	0.32
<i>p</i> -coumaric	1.06	0.24
Salicylic	4.07	0.64
Benzoic	5.28	1.22
Cinnamic	2.32	0.29

pounds ( $U = 2 > U_{cr} = 1$ ,  $n_1 = n_2 = 4$ ,  $p = 0.6$ ) nor in spectral characteristics ( $U = 1 > U_{cr} = 0$ ,  $n_1 = n_2 = 3$ ,  $p = 0.28$ ); variation ranges of all parameters, especially extinction coefficients, are narrower in comparison with the sod horizon, which indicates a greater WSOM homogeneity. Differences were registered in the qualitative composition of individual phenolcarboxylic acids. Concentrations of all identified PCAs (Table 2), except for *p*-hydroxybenzoic acid, were 4–8 times higher in solutions extracted from larger pores where they reached several micromoles per liter; benzoic, cinnamic, and salicylic acids predominated: their content amounted to 75% of the total content of all acids. In small pores, the scatter of PCA concentrations was greater: from tenths of micromoles to micromoles per liter with the predomination of *p*-hydroxybenzoic and benzoic acids (67% of the total amount).

Solutions isolated from the humus and sod horizons differ statistically significantly in the total concentration of organic carbon ( $p = 0.006$ ) and SFCs ( $p = 0.01$ ) (pores of the same diameter are compared). Both parameters reach higher values in the humus horizon.

Properties of organic substances present in situ in the liquid phase of the podzolic soil are provided in Table 3. In this particular case, water-soluble organic matter extracted from pores larger and smaller than  $14.7 \mu\text{m}$  differs statistically significantly ( $U = 0 < U_{cr} = 1$ ,  $n_1 = n_2 = 4$ ,  $p = 0.02$  and  $0.03$ ) in all studied param-

eters. WSOM in solutions isolated from large pores contains more C, N, and SFCs and features a lower light absorption capacity and a smaller share of the hydrophobic fraction in comparison with substances extracted from small pores.

## DISCUSSION

The studied properties of WSOM contained in different pores of the sod horizon of the *muck–humus gley soil* do not differ statistically significantly. All properties vary significantly, which is reflected in their variation ranges (Table 1). The parameters of WSOM isolated from pores  $30$  to  $0.4 \mu\text{m}$  in size vary especially significantly. Perhaps, this is because the extraction of respective solutions drains pores belonging to different categories. According to Zsolnay [29], organic matter in the soil solution is represented by three fractions contained in different pores:  $d > 6 \mu\text{m}$  (fraction 3),  $d = 0.2\text{--}6 \mu\text{m}$  (fraction 2), and  $d < 0.2 \mu\text{m}$  (fraction 1). Different properties of these fractions are determined by different mechanisms transporting substances into the respective pores, different intensity of this transportation, different susceptibility of pores to dehydration, involvement of biota in the metabolism, etc. This is why different WSOM fractions can pass into solutions extracted from small pores in different amounts depending on the ratio between pore categories in each analyzed soil sample.

The properties of WSOM isolated from the humus horizon are more homogenous; the variability of parameters within each category is smaller. A possible reason is the difference in WSOM sources. In natural environments, organic substances enter the soil solution from humus, fresh and decomposing plant residues, with undercrown runoff, and with root and microbial exudates. In the experiment, the main WSOM source in the humus horizon is soil humus; while in the sod horizon, root residues make a significant contribution. With regards to PCAs, their concentration in large pores is an order of magnitude higher, even though their share relative to the total organic matter content (in carbon equivalent) remains small, amounting to tenths of a percent. PCA amounts

**Table 3.** Properties of WSOM isolated from different pores of the ELBTg horizon of the podzolic soil (mean values; max and min values are provided in parentheses)

WSOM solution from pores with a diameter of	No. of samples	C (mg/L)	N (mg/L)	C : N	SFC (mg/L)	$E_{250}$ (L/mg C cm)	$E_{250} : E_{365}$	HPB (%)
$\geq 14.7 \mu\text{m}$	3 (4*)	16.6 (13.2–19.7)	1.5 (0.9–2.1)	15.3 (11.0–20.8)	2.6 (2.3–2.9)	0.027 (0.024–0.032)	5.1 (4.8–5.2)	20 (13–29)
$14.7\text{--}3.7 \mu\text{m}$	4	10.2 (9.4–10.9)	0.5 (0.4–0.6)	24.3 (19.6–31.1)	1.3 (1.1–1.7)	0.022 (0.020–0.024)	6.2 (5.6–6.7)	35 (31–38)

\*For C, N, and C : N.

in solutions isolated from small pores are consistent with concentrations measured in podzol centrifugates [8].

The content of phenolic monomers in natural soil solutions is usually small, since they are rapidly oxidized and easily sorbed by the soil [20]. Phenolic compounds are sorbed more intensely in small pores and in the presence of minerals belonging to the iron hydroxide group; a number of studies [5, 9, 12] show that these factors reduce concentrations of phenolic compounds in soil solutions. For instance, it was shown for a podzol [8] that the PCA concentration in natural moisture of small pores (i.e., capillary moisture) decreases down the profile starting with the Bh horizon already (by half); while, in moisture contained in large pores, a decrease in PCA concentration is registered lower in the profile (only in the Bf horizon), due to their sorption by iron hydroxides. According to Kaiser and Guggenberger [13], during storm rainfalls, the content of dissolved phenolic compounds at a depth of 90 cm in gravitational moisture rapidly moving through the rendzina macropores does not differ from their concentrations in solutions flowing down from under the litter, but is significantly higher in comparison with their concentration in capillary moisture isolated using tension lysimeters (at a pressure of 10–20 kPa). Extinction coefficients and the share of the hydrophobic fraction in the composition of dissolved organic matter demonstrate similar patterns: they decrease from the soil surface to a depth of 90 cm only in capillary moisture. The authors believe that lower SFC concentrations in small pores, whose conditions are close to equilibrium, are determined by sorption that virtually does not occur in large pores.

The ratio between soluble PCAs identified in the course of the laboratory experiment reflects their distribution in soil pores at the time of sampling, and their smaller amounts in smaller pores can be explained by their better sorption in accordance with the patterns described above.

The absence of more significant differences in the composition of WSOM isolated from different horizons may be partly explained by the fact that the soil was completely saturated with moisture prior to the liquid phase extraction: this procedure could facilitate the transfer of substances between pores and contribute to the mixing of solutions. Concurrently, differences in concentrations of substances in different pores are manifested even in aquiferous horizons [29], and the identified differences in concentrations and ratios of phenolcarboxylic acids also confirm that solutions do not homogenize completely, and dissolved organic substances from small pores retain their individual features. The specificity of dissolved organic substances present in such pores should be manifested more clearly in soils with lower moisture content levels and greater shares of small pores; the data obtained for the natural soil solution isolated from a podzolic soil confirm this (see below).

Based on the extinction coefficients, organic matter present in solutions extracted from the ELBTg horizon of the *podzolic soil* features higher aromaticity degrees in large pores: the specific extinction values in the entire ultraviolet range are 1.5–1.8 times higher in comparison with small pores. The same results were obtained for Cambisols [25]. Solutions isolated from gravity lysimeters featured higher organic carbon concentrations ( $20.8 \pm 1.2$  and  $11.2 \pm 1.2$  mg C/L) and higher extinction coefficients  $E_{254}$  ( $0.043 \pm 0.001$  and  $0.034 \pm 0.001$  L/mg C cm) in comparison with tension lysimeters.

The SFC content in solutions extracted from large pores is also two times higher, and it can be assumed that aromatic organic components are largely represented in the podzolic soil by phenolic compounds. The same patterns were identified for rendzina soils: the SFC content in gravitational and capillary moisture was 8.5 and 5.8 mg/g C, while the  $E_{260}$  values were 0.032 and 0.024 L/mg C cm, respectively (13). Overall, WSOM of large pores contains more phenolic compounds, both in the podzolic soil (the total PCA content) and in the muck–humus gley soil (individual PCAs). The elevated concentrations of phenols in better aerated large pores are consistent with the fact that simple PCAs are products of plant residue decomposition by fungi and aerobic bacteria. On the other hand, it was shown [27] that the application of simple phenolic acids to the soil, including *p*-coumaric acid, increases the water resistance of soil aggregates and is accompanied by an increase in the fungal population in soils representing the respective variants of the experiment. Taking that fungal hyphae also act as a stabilization factor for microaggregates, it can be concluded that an elevated concentration of phenolic compounds in large moisture-conducting pores increases in various ways the hydrophobization of surfaces that are in contact with the solution, thus, contributing to a better preservation of organic substances in small intra-aggregate pores. In addition, in podzols and podzolic soils, dissolved phenolic compounds are mainly localized in macropores, which contributes to their transfer with moisture flows to lower horizons where they sorb on iron hydroxides, thus, undergoing chemical stabilization. Sorption occurs most efficiently on nanoparticles of metastable minerals (allophanes, imogolite, and ferrihydrite), and the authors of [15] believe that this mechanism plays the main role in the formation of stable organic carbon reserves in lower soil horizons. Organic matter from larger pores of the studied podzolic soil also contains more N and C (Table 3). Their concentrations there are 2–3 times higher. Similarly, a number of studies [4, 8, 25] note higher carbon concentrations in gravitational moisture compared to capillary moisture. One of the reasons may be frequent changes in wetting/drying conditions in large pores that contribute to the formation of water-soluble organic substances.

Concurrently, the C : N ratio in larger pores is 1.6 times lower than in small ones (Table 3); this may indicate a greater OM lability since these properties are inversely correlated [24]. The authors of [23] classify organic substrates (plant residues) whose C : N ratio is <18 as rapidly degradable and organic substrates with C : N = 18–27 as degradable at a moderate rate. According to this classification, dissolved organic substances from different pores of the podzolic soil (Table 3) fall into different stability classes: more stable substances are contained in smaller pores. Toosi et al. [25] compare properties of organic matter in solutions isolated from forest Cambisols using tension and gravity lysimeters and conclude that rapidly flowing gravitational moisture removes components that are more susceptible to biodegradation. It is necessary to keep in mind thought that, aside from the C : N ratio, the stability of organic matter is determined by a number of other factors, including substrate type, time, etc.

The share of the hydrophobic (HPB) fraction in the composition of organic matter in solutions isolated from large pores is 20%; in solutions isolated from small pores, it is 35%. Based on the spectral index  $E_{250}/E_{365}$  that inversely correlates with molecular weight [22], organic substances in small pores have a lower molecular weight in comparison with those contained in large pores.

Overall, the studied properties of organic substances dissolved in liquid phases of the middle ELBTg horizon of the podzolic soil extracted from pores more and less than 14.7  $\mu\text{m}$  in diameter differ significantly. OM contained in solutions from large pores has a higher molecular weight, features higher hydrophilicity and aromaticity degrees, and contains more C, N, and phenolic compounds. Substances contained in small pores generally have a lower molecular weight (based on the  $E_{250}/E_{365}$  value) and are more hydrophobic and probably more resistant to degradation (based on a broader C : N variation range). As is known, such factors as isolation in small pores and hydrophobicity contribute to the organic matter stabilization in soils; one of the reasons is its lower availability to microorganisms [16]. The identified differences in the distribution of soluble phenolic compounds also potentially contribute to the preservation of this WSOM fraction in the soil: phenolic compounds are more easily transported to lower horizons, where their interaction with iron compounds ensures the most effective stabilization.

## CONCLUSIONS

1. Water-soluble organic substances contained in the sod and humus horizons of the muck–humus gley soil and isolated from pores 0.4–30  $\mu\text{m}$  and >30  $\mu\text{m}$  in diameter do not differ statistically significantly in the total concentration of carbon and phenolic compounds; a possible reason for this is partial mixing of

solutions from different pore categories in the course of their extraction.

2. A strong variation in properties of organic matter isolated from the muck–humus gley soil (the maximum and minimum values differ by 4–15 times), especially in extinction coefficients, indicates that the composition of extracted substances is heterogeneous.

3. Concentrations of phenolcarboxylic acids in solutions extracted from large pores in the humus horizon of the muck–humus gley soil are 4–8 times higher in comparison with solutions extracted from smaller pores. Salicylic, benzoic, and cinnamic acids predominate in large pores; while *p*-hydroxybenzoic and benzoic acids, in small ones (75 and 67% of the total amount, respectively).

4. Dissolved organic substances present in situ in pores of different diameters in the ELBTg horizon of the podzolic soil differ statistically significantly in all studied parameters. Organic matter from pores more than 14.7  $\mu\text{m}$  in diameter contains more C, N, and phenolic compounds; based on absorption coefficients in the ultraviolet range, it has a larger molecular weight and a higher aromaticity degree. Organic matter of solutions contained in small pores is more hydrophobic, and its C : N ratio varies in a wider range (i.e., it is potentially more resistant to decomposition).

5. Phenolic compounds are predominantly confined to large pores; in natural environments, this contributes to their transportation with water flows to lower horizons featuring most favorable conditions for sorption and biochemical stabilization.

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## COMPLIANCE WITH ETHICAL STANDARDS

*Conflict of interests.* The authors declare that they have no conflicts of interest.

*Statement on the welfare of humans or animals.* This article does not contain any studies involving humans or animals performed by any of the authors.

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