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A Comparative Study of Molecular Weight Distribution of Water-Soluble Humic Substances, Humic Acids, and Fulvic Acids Extracted from Sod-Podzolic Soils

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Abstract—The alteration of organic matter from three sod–podzolic soils in the course of the extraction of water-soluble humic substances was studied by means of size-exclusion chromatography using a combined UV–DOC detector. A comparison of water-soluble humic substances with humic and fulvic acids extracted from the same soils was carried out.

Keywords: water-soluble humic substances, molecular weight distribution.

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The dissolved organic substances (DOSs) of soils and, in particular, humic substances (HSs) of soil solutions, are the least studied along the natural humous component; this is related to the low concentration of organic carbon (OC) in the soil solution [4] and also the complexity of the extraction of the water-soluble humic substances (WHSs). On the other hand, there are continuous discussions about the existence of humic acids (HAs) and fulvic acids (FAs) in actual soil systems and whether these are the result of the hydrolysis of soil organic substances during alkali extraction used for their separation [3].

The purpose of this work is to observe the molecular weight changes that occur for the organic substances from aqueous extracts of soils during the extraction of water-soluble humic substances and comparison of their properties with those of humic and fulvic acids extracted from the same soils.

MATERIALS AND METHODS

Preparations of humic substances were extracted from three soils with various states of cultivation: virgin sod–podzolic weakly soddy deep-podzolic medium loamy on the limon under the forest (S^SV; pH_{aq} 5.0, OC 4.3%), cultivated sod–podzolic medium-tillable medium loamy weakly eroded on the limon (S^SC; pH_{aq} 7.8, OC 1.5%), and arable sod–podzolic deep-tillable medium loamy (S^SA; pH_{aq} 7.3, OC 3.8%).

Humic acids were extracted using standard procedures by extraction with 0.1 M NaOH with further precipitation by acidification to pH 1–2 with 0.1 M HCl [3]. Fulvic acids were obtained from the obtained supernatant using the method in [6]. Preparations were purified using electrodialysis. As a result, we obtained three preparations of humic acids (HA–S^SV, HA–S^SC, and HA–S^SA) and fulvic acids (FA–S^SV, FA–S^SC, and FA–S^SA).

In order to separate the preparations of the water-soluble humic substances, a sample (1 kg) of the air-dried soil was passed through a sieve with a mesh size of 1 mm and was placed in distilled water (2 l), thoroughly shaken, and left overnight. The obtained water extract was filtered through a Blue Ribbon paper filter and membrane filter with a pore diameter of 0.45 μm for the separation of the true dissolved organic substances from the colloidal ones. The extraction was carried out using 10 kg of each soil. Filtered extracts were combined, acidified to pH 1–2 with 0.1 M HCl and put through a glass column filled with Amberlite XAD-2 resin to precipitate water-soluble humic substances (Fig. 1). The remaining procedures of their elution from the column and further desalination were analogous to those described for fulvic acids. At each step the samples were taken for analysis by size-exclusion chromatography to control for the changes that were occurring in their solutions. As well, the concentration of organic carbon in the solutions was determined using a Shimadzu 5000 TOC analyzer (Japan); this made it possible to determine the contents of the various fractions of dissolved organic substances in the soils.

The molecular mass of the preparations of humic substances was determined by size-exclusion chromatography using the method from [8]. Their fractionation was performed on a column filled with Toyopearl-HW-50(S) gel (Japan); polydextranes with known molecular mass were used as calibrating substances. The concentration of HAs in the samples analyzed was 1–2 mg OC/l; phosphate buffer served as the mobile phase (0.028 M, pH 6.8), the rate of elution was

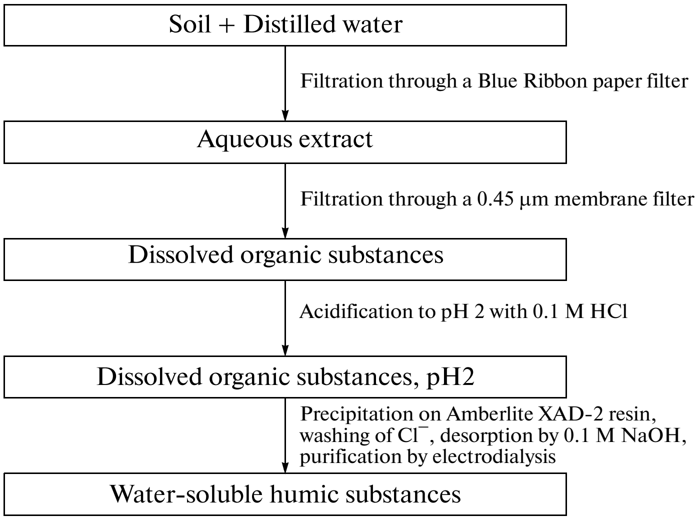

Fig. 1. Scheme of the extraction of water-soluble humic substances in soils.

1 ml/min. Registration of HAs on the column exit was performed by the content of organic carbon in the elute using a flow detector (Graentzel, Germany) and by absorption by the elute at 254 nm with a UV detector. On the basis of the obtained gel chromatograms the molecular mass (MM) of the preparations of humic substances was calculated in the peak (M_p) and the average weight molecular mass (M_w) was calculated using the GelTreat program [5].

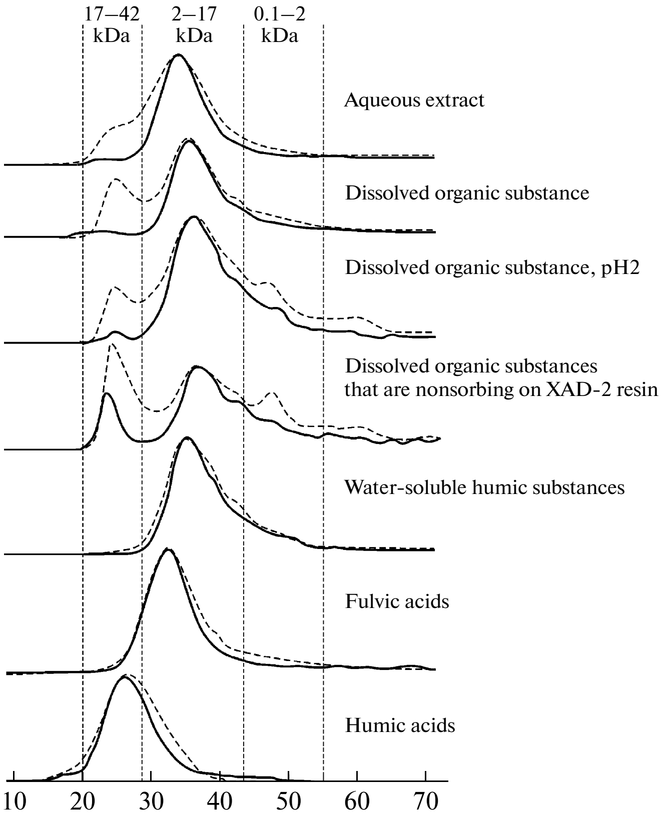

Fig. 2. Gel chromatograms of the preparations of soil organic substances (based on the example of S^SA of a soil): the solid line is UV detection and the dashed line is detection by OC.

RESULTS AND DISCUSSION

Control of the changes that took place in the dissolved soil organic substances at different stages of the extraction of WHSs was performed using size-exclusion chromatography. The simultaneous use of an OC detector and UV absorption made it possible to monitor both the differences in the total pool of the water-soluble organic substances and in its humous component. Corresponding gel chromatograms are given in Fig. 2.

On gel chromatograms of the aqueous extract of the soil (Fig. 2) with detection by OC, two poorly resolved peaks were detected at elution volumes V_e of 26 and 35 ml. The peak that corresponds to the fraction of organic substances with higher molecular mass (17–42 kDa) was characterized by the near absence of absorption at 254 nm. This confirms that substances with the given exit volume were not related to humic substances and were mainly represented by polysaccharides. The presence of a peak on gel chromatograms with UV detection at $V_e = 35$ ml makes it possible to relate the second peak (2–17 kDa) to humic substances.

Filtration of the initial aqueous extract through a 0.45 μm membrane filter, which is usually used for the separation of colloidal organic substances from true dissolved ones, did not give rise to considerable changes in the gel chromatograms. Assuming that the concentration of organic carbon in the DOS solutions was 90–93% of the concentration in aqueous extracts it can be concluded that the content of the colloidal

Table 1. The relative content of fractions with various molecular masses in solutions of organic substances of S^SA for soils at different stages of the extraction of water-soluble humic substances

Preparation of soil organic substances	Relative content of the fractions with various molecular mass, %		
	17–42 kDa	2–17 kDa	0.1–2 kDa
Aqueous extract	17	78	5
Dissolved organic substance	19	77	4
Dissolved organic substance, pH 2	19	65	16
Dissolved organic substance that is nonsorbing on XAD-2 resin	34	38	28
Water-soluble humic substances	5	83	12

organic substances was insubstantial. During acidification of DOSs to pH 2 (Fig. 2) the peak elution volume of the "humic substances" increased from 35 to 38 ml. A second peak formed with an elution volume of 48 ml, for which the absence of absorption at 254 nm is intrinsic. Two hypotheses can be suggested for this phenomenon. The first involves the fact that in the initial dissolved organic substances iron and aluminum complexes with humic substances are present, which are characterized by a minimum of complexation constants in the pH range of 1–3 [2]. Consequently, it can be suggested that with a decrease in pH to 1–2, the breakdown of these complexes takes place, resulting in a decrease in their molecular mass. The second possible explanation is a considerable change of the pH of the solution during acidification of DOSs; this inevitably reflects on the results of chromatographic analysis due to nonexclusion effects, such as sorption on a gel due to the protonation of the charge of humic substances [8]. The mentioned processes led to the shift of the peak on the chromatogram to higher elution volumes.

An additional maximum (V_e = 48 ml) represents the so-called "salt peak," whose exit time coincides with the exit of the phosphate buffer from the column. This maximum is determined by the elution of low-molecular weight acids [7].

As seen from the gel chromatogram of the dissolved organic substances that were nonsorbing on the Amberlite XAD-2 resin, the largest affinity to the sorbent was that of the DOSs of the medium (humous) peak. The relative area of the first and last peaks substantially increased, while the medium peak decreased. The weight parts of the fractions with various molecular masses for these gel chromatograms are given in Table 1.

On the basis of these gel chromatograms, it can be concluded that only the medium peak of the dissolved organic substances with pH 2 (2–17 kDa) (Fig. 2) corresponds to humic substances, since it has its absorption at 254 nm and showed sorption on the XAD-2 resin. In spite of the fact that the area of that peak is 65% of the total content of the organic carbon (Table 1), it can be suggested that the humic substances from the aqueous extract were approximately 65% of the dissolved organic substances. The analogous value for two other soils was 49%. Thus, knowing the concentration of the organic carbon in the solutions of the soil DOSs, the content of the water-soluble humic substances in the soils can be approximately determined. These values were 43.0, 19.0, and 38.0 mg OC/kg soil for S^SV, S^SC, and S^SA, respectively.

The molecular masses in the peak and average-weight molecular masses of the organic substances at different stages of the extraction of the water-soluble humic substances from the soil solution for all the soils under study are given in Table 2. The molecular masses of humic and fulvic acids extracted from those soils are also given.

Table 2. Molecular masses of peak (M_p) and average-weight molecular masses (M_w) of isolated WHS, HA, and FA preparations

Preparation of organic substances in the soil	M_p, kDa			M_w, kDa		
	S^SV	S^SC	S^SA	S^SV	S^SC	S^SA
Aqueous extract	7.6	7.1	9.6	18.5	16.1	15.7
Dissolved organic substance	7.4	7.1	9.5	15.7	15.9	15.4
Dissolved organic substance, pH2	6.0	6.0	6.6	12.8	12.8	11.0
Dissolved organic substance that are nonsorbing on XAD-2 resin	6.1	6.2	6.4	10.5	10.2	17.7
Water-soluble humic substances	6.2	7.2	8.0	6.3	7.2	7.9
Fulvic acids	9.8	8.0	11.2	10.2	8.8	11.7
Humic acids	16.0	17.0	18.3	14.5	16.6	14.2

Note: Confidence interval of M_p and M_w was ±3%.

The data in Table 2 show that with the transfer from the solution of the aqueous extract to the dissolved organic substance, a slight decrease in M_w takes place due to the separation of the colloidal organic substances. The position of the peak shifts slightly to lesser values of molecular mass. During acidification of dissolved organic substances to pH 2, the M_w of the organic substances decreased by 3–4 kDa.

On the basis of the proximity of M_p values of the isolated preparations of humic substances of the aqueous extract and the low-molecular weight (humous) peak of the initial aqueous extract, it can be concluded that method of extraction that was used in this work makes it possible to obtain preparations of humic substances that are similar to those that actually exist in soil solutions.

The M_w values were 8.0–11.7 and 14.2–16.6 kDa for the preparations of humic and fulvic acids, respectively (Table 2). These values correspond well to those given in the literature for analogous preparations of humic acids [1, 3]. For the samples of FA and HA extracted from the same soil, higher values of M_w were observed for the preparations of humic acids; this corresponds to the data of other researchers [3]. On the whole, the preparations of the humic substances can be arranged in the following order according to the increase of their M_w: WHS < FA < HA. As well, the M_w values for fulvic and humic acids exceeded those for water-soluble humic substances by 3 and 8 kDa on average, respectively.

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REFERENCES

1. Anisimova, M.A., Perminova, I.V., and Lebedeva, G.F., Humic Acids Detoxication Ability with Respect to Trifluraline Herbicide, *Pochvoved.*, 1998, no. 9, pp. 1079–1084.
2. Karpukhin, A.I. and Fokin, A.D., Helium Chromatography for Investigating the Fulvic Acids and Ferrum–Fulvic Compounds, in *Osobennosti pochvennykh protsessov dernovo-podzolistykh pochv* (The Features of Soil Processes Taking Place in Soddy-Podzolic Soils), Moscow, 1977, pp. 102–114.
3. Orlov, D.S. and Grishina, L.A., *Praktikum po khimii gumusa* (Practical Works on Humus Chemistry), Moscow: MGU, 1981.
4. Dosskey, M.G. and Bretsch, P.M., Transport of Dissolved Organic Matter through a Sandy Forest Soil, *Soil Sci. Soc. Amer. J.*, 1997, vol. 61, pp. 920–927.
5. Kudryatsev, A.V., Perminova, I.V., and Petrosyan, V.S., Size-Exclusion Chromatographic Descriptions of Humics Substances, *Anal. Chim. Acta*, 2000, vol. 407, nos. 1–2, pp. 193–202.
6. Mantoura, R.F.C. and Riley, J.R., The Use of Gel Filtration in the Study of Metal Binding by Humic Acids and Related Compounds, *Anal. Chim. Acta*, 1975, vol. 78, pp. 193–200.
7. De Nobili, M., Gjessing, E., and Sequi, P., Sizes and Shapes of Humic Substances by Gel Chromatography, in *Humic Substances. II*, Hayes, M.B.H., MacCarthy, P., Malcom, R.L., and Swift, R.S., Eds., 1989, pp. 562–591.
8. Perminova, I.V., Frimmel, F.H., Kovalevskii, D.V., et al., Development of a Predictive Model for Calculation of Molecular Weight of Humic Substances, *Water Res.*, 1998, vol. 32, pp. 872–881.