

Preparative Yield and Properties of Humic Acids Obtained by Sequential Alkaline Extractions

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Abstract—The preparative yield, composition, and structure of humic acids obtained by sequential alkaline extractions from two soils (a soddy-podzolic soil under forest and a typical chernozem in treatment with permanent black fallow of a long-term experiment since 1964) have been studied. The preparative yield of humic acids from the first extraction is 0.40 and 0.94% for the soddy-podzolic soil (Retisols) and the chernozem, respectively. The preparative yield from the second extraction is lower by several times, and the yield from the third extraction is lower by an order of magnitude. The study of the obtained preparations by elemental analysis, gel-permeation chromatography, and ¹³C NMR spectroscopy has shown insignificant changes in the elemental, molecular-weight, and structural-group composition of humic acids among the extractions. It has been supposed that this is related to the soil features: typical climatic factors for the formation of soil subtype in the case of soddy-podzolic soil and the land use in the long-term experiment in the case of typical chernozem. It has been concluded that a single extraction is sufficient for the separation of humic acids and the preparation of a representative sample.

Keywords: humic preparations, separation of humic substances, soil organic matter, long-term field experiments, soddy-podzolic soils, chernozems, Chernozems, Retisols

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INTRODUCTION

Preparations of humic acids (HAs) are obtained by two main methods: the method adopted in the Russian school of soil science [8] and that recommended by the International Humic Substances Society (IHSS) [24]. The both methods are based on the following general principles: alkaline extraction of humic substances from soils, separation of HAs and fulvic acids (FAs), purification, and drying. However, there are some distinctions in the separation procedures:

—The IHSS recommends a single alkaline extraction of humic substances [24]; the Russian procedure involves a multiple extraction [7].

—According to the IHSS procedure [24], the alkaline extraction is performed in a nitrogen atmosphere [24]; in the Russian version, it is operated in the air [7].

—In the IHSS method, the coagulation of mineral suspensions is performed with HAs separated from FAs, and KCl is used as a coagulant [24]; in the Russian method, the operation is performed with NaCl in an alkaline extract of humic substances before their separation into HAs and FAs [8].

—In the IHSS method, the final step is the treatment of the HA precipitate with a mixture of 0.1 M HCl and 0.3 M HF for the complete removal of fine

mineral suspensions [24]; the Russian method does not include this operation.

The most significant difference, which can affect the quality of the obtained preparations and the labor intensity of the procedure, is the number of alkaline extractions used for the isolation of the preparation. As was noted above, the IHSS method recommends a single alkaline extraction [24]. In the Russian procedure, humic substances are extracted with an alkali at least three times, and the extracts are combined for the preparation of an integral sample, from which the HA preparation is then isolated. The multiple extractions are explained by potential differences in the properties and composition of humic substances among the extract portions [7, p. 122].

The number of alkaline extractions is the key difference between the procedures. First, the single extraction utilizes 5 L of the solution for 500 g of soil, while the triple extraction requires 15 L of the solution, which significantly increases the labor inputs. Second, the single extraction takes no more than 24 h, compared to three days for the triple extraction, during which soil is in the alkaline solution, which can affect the structure of humic substances because of alkaline or microbial destruction. Third, the triple

increase in the solution volume inevitably reduces the yield of the preparation because of the increased labor content. However, the Russian procedure presumably gives larger amounts of more representative HA preparations.

Thus, the comparison of the HA preparations obtained by the Russian and the IHSS procedures requires the comparison of the structures and properties of preparations obtained by the single and triple extractions from soils.

Some tendencies were revealed earlier in the studies of the elemental composition, molecular-weight distribution, and carbon distribution among the functional groups for HAs of three sequential alkaline extractions from a typical chernozem of annually mown steppe (fallow for more than 100 year) [11, 13] and a native gray forest soil [3]. It was shown that the preparation obtained by the first extraction significantly differs from the following two preparations, which are very similar to each other. The HAs in the first extract from the typical chernozem contained more carbon and less nitrogen than the HAs in the next extracts; the content of oxygen remained almost unchanged. Therefore, the H/C atomic ratio in the following extracts increased and the C/N ratio decreased compared to the first extract. The average weighted molecular weight of the first-extraction HAs was lower, and those for the next extractions were similar. According to the ^{13}C NMR data, the carbon skeleton of the first-extraction HAs contained less aliphatic fragments and more aromatic and carboxyl fragments [11]. Analogous tendencies in the distribution of carbon among the functional groups were noted for the HAs obtained by the next alkaline extractions from gray forest soils [13]. It should be noted that a similar approach (use of humic substances from separate sequential extractions) was applied earlier in a series of works performed at the St. Petersburg State University [14–16], which showed that only the first and second extractions provide significant information on the structure of humic substances.

It was shown that the HAs obtained by the first extraction from the typical chernozem of annually mown steppe and the native gray forest soil differ from those isolated from the next extracts. However, their differences remain within the ranges typical for the HAs of these soils.

Nonetheless, all the noted differences among the preparations obtained by the sequential alkaline extractions are leveled in the integral preparation. This is related to the fact that the preparative yield of HAs from the first extraction exceeds their total yield from all next extractions by several times. The weight yield of HA preparation from the typical chernozem decreased with each following extraction by 3–4 times [11]; for the gray forest soil, the amount of preparation obtained in the first extraction exceeded that in the second extraction by almost 10 times [13]. Thus, it was shown for native (perennially fallow) soils (typical

chernozem and gray forest soil) that the integral preparation obtained by mixing all alkaline extracts inherits the properties of the HAs from the first extraction because of their significantly higher yield.

It follows from the aforesaid that the isolation of HA preparations from chernozems and gray forest soils by multiple extractions with the combination of extracts gives neither appreciable increase in the preparation yield nor changes in its composition compared to the preparation obtained by the single extraction. The working volume of extraction solution increases by several times. This increases the labor content and the probability of losses, which reduces the relative yield of preparation and the efficiency of the entire isolation procedure. Therefore, the single extraction is preferred for the preparative isolation of HAs from these soils.

For the wider use of the above recommendation and its universalization, we studied the composition and structure of HAs obtained by sequential alkaline extractions from a soddy-podzolic soil under forest and a typical chernozem used in the experimental treatment with permanent black fallow since 1964. The former soil was selected as a representative of the 'soddy-podzolic soils-gray forest soils-chernozems' zonal series for the completion of the series of works on this problem. The organic matter of the latter soil (typical chernozem in the treatment with permanent black fallow) is characterized by the highest degree of humification for this soil type in the background of the minimum content of organic matter (plant residues have not entered into the soil since 1964 [5]). The use of this soil can contribute to the assessment of HA yield from sequential alkaline extractions at the extreme exhaustion of soil. The two soils also remained close to similar contents of organic matter.

The aim of this work was to study the preparative yield, composition, and structure of HAs obtained by sequential alkaline extractions from a soddy-podzolic soil under forest and a typical chernozem from the experimental treatment with permanent black fallow since 1964.

OBJECTS AND METHODS

Soils. Soddy-podzolic soil was taken under a pine forest in the Chashnikovo Research and Experimental Station of the Moscow State University in Moscow oblast. Typical chernozem was sampled in the treatment with permanent black fallow of the long-term experiment of the Dokuchaev Soil Science Institute established in the area of the Kursk Research Institute of Agroindustrial Production in 1964.

A mixed soil sample was composed of five individual samples. Individual samples (about 2 kg each) were taken from the humic (A1) or plow (Ap) horizon (at a depth of 5–15 cm) on a plot of about 5 m in radius. From the obtained sample (of about 10 kg), an average sample of 1.5 kg was formed by quartering.

In the samples, the content of organic matter (C_{org}) was determined by digestion in potassium dichromate according to the Tyurin method with spectrophotometric detection [8], and the pH value was determined at a soil : water ratio of 1 : 2.5 [1]. For the soddy-podzolic soil, the content of C_{org} was 2.77% and pH was 4.7; for the typical chernozem under permanent black fallow, $C_{\text{org}} = 2.79\%$ and $\text{pH} = 6.5$. The obtained values correspond to the parameter ranges reported in the literature [10].

From the prepared and characterized soil samples, HA preparations were obtained using a procedure combining the Russian method [8] and the IHSS method [24] of HA isolation. Several alkaline extractions of humic substances were performed according to the Russian method, but the obtained extracts were not combined, and HAs were isolated from each extract separately. The composition and structure of the obtained preparations were assessed from the elemental composition, molecular weight distribution (MWD), and carbon distribution among the functional groups.

Isolation of HA preparations. For the isolation of HA preparations, five portions of 100 g were taken from the average sample; thus, the total weight of soil taken was 500 g. Each portion was decalcified by the addition of 1 M HCl to reach a pH of 1–2; 0.1 M HCl was then added to a final soil : water weight ratio of 1 : 10. The obtained suspension was periodically mixed for 6 h and left to stand overnight; then, the supernatant was separated from the solid soil phase by decantation. The decalcified soil was neutralized by adding 1 M NaOH to pH 7, and 0.1 M NaOH was then added to a final soil : water weight ratio of 1 : 10. The suspension was periodically mixed for 6 h and left to stand overnight. Twenty four hours after the beginning of the extraction, the alkaline extraction was separated from soil by decantation and centrifugation (3000 rpm, 5 min); at this stage, all five extracts obtained from five soil portions were combined into one extract (extract 1), which was stored. Each of the same five soil portions was then treated with 0.1 M NaOH at a ratio of 1 : 10, periodically mixed for 6 h, and left overnight; the five alkaline extracts obtained 24 h later were combined and stored (extract 2). This procedure was repeated once more to obtain extract 3. Each of the three extracts was used for the isolation of HA preparations by separating FAs and purifying HAs from inorganic impurities. HAs were separated from FAs by precipitation; the solution was acidified with 6 M HCl to pH 1–2, and the HA precipitate was separated by centrifugation. Then, HAs were dissolved again in a minimum volume of 0.1 M KOH, and KCl was added to a K^+ concentration of 0.3 M for the coagulation of fine mineral particles. The coagulated solid impurities were separated by centrifugation. Next, HAs were reprecipitated and treated with a mixture of 0.1 M HCl and 0.3 M HF to remove finely dispersed silicon-containing impurities in accordance with the IHSS procedure [24]. The treated HA suspen-

sion was purified by dialysis, dried on a rotary evaporator, and held over P_2O_5 in a desiccator for at least 21 days. Thus, preparations of HAs from soddy-podzolic soil were obtained from extracts 1, 2, and 3. The yield of HAs in each extraction was estimated by weighing the obtained preparations.

Characterization of HAs preparations.¹ The composition and structure of the obtained preparations were assessed from elemental composition; their MWD was determined by gel-permeation chromatography, and the carbon distribution among the functional groups was determined by ^{13}C NMR spectroscopy.

The elemental composition of HA preparations was determined on a Carlo Erba Strumentazione 1106 CHN analyzer (Italy). The content of hygroscopic water in all the preparations taken was equal to 8% [9]. The content of ash in the isolated HAs was determined by gravimetry in an individual soil portion by combustion in quartz tubes at 750°C in an oxygen atmosphere for 40 min. The content of oxygen was calculated from the difference between the weight of dry ash-free soil portion and the total content of C, H, and N.

The molecular-weight distribution of HAs was determined by gel-permeation chromatography according to the described procedure [19–22]. An Abimed gel permeation chromatograph (Gilson, France) consisted of a column, a pump, an automated sampler, and a flow-through ultraviolet detector. The column was 25 mm \times 20 cm in size. Gel Toyopearl TSK HW-55S (Toso Haas, Japan) was used as a stationary phase; a 0.028 M phosphate buffer with pH 6.8 was used as a mobile phase. The void volume of the column was determined with blue dextran (20000 kDa); its total volume was determined with acetone (48 Da). They were equal to 12 and 34 mL, respectively. Sodium salts of polystyrene sulfonic acids with peak molecular weights of 14.00, 20.70, 45.10, and 80.84 kDa, respectively (Polymer Standard Service, Germany), were used for calibration. From the obtained chromatograms and calibration curves, the average weight molecular weight (M_w) and average numeric molecular weight (M_n) were determined, as well as polydispersity (M_w/M_n) [2, 18]. The yield from the column was determined for assessing the potential sorption of substance on the gel. Blanc experiments showed that 85% of the applied HAs were recovered on average.

^{13}C NMR spectroscopy in the liquid phase is one of the most informative methods for the structural study of organic substances with stochastic structures, including HAs. This technique allows for description of the distribution of carbon in HA molecules among their structural fragments with different chemical shifts. Thus, ^{13}C NMR spectroscopy is capable of estimating the content of structural units in the carbon

¹ The preparations were characterized at the Chemical Faculty of the Moscow State University.

Table 1. Yields of HA preparation in sequential alkaline extractions

Alkaline extraction no.	HA yield	
	% of soil weight	% of C _{org}
Soddy-podzolic soil under forest		
1	0.40	14.4
2	0.12	4.3
3	0.01	0.4
Typical chernozem, permanent black fallow		
1	0.94	33.6
2	0.25	8.9
3	0.05	1.9

skeleton of HAs [4, 17, 19, 23]. Therefore, the structural distinctions among the HAs isolated by sequential alkaline extractions can be detected by ¹³C NMR spectroscopy.

HA samples for the NMR study were prepared by dissolving an 80-mg portion in 0.6 mL of 0.3 M NaOD/D₂O. The mixture was put in an ultrasonic bath for 30 min and centrifuged at 18 g for 5 min; the solution was separated from the precipitate and transferred into a 5-mL NMR tube. The ¹³C NMR spectra were recorded on a Bruker Avance 400 spectrometer (100 MHz ¹³C) using a CPMG pulse sequence with the first 90° pulse, a free-induction-decay recording time of 0.2 s, and a relaxation delay time of 7.8 s. The duration of an NMR experiment was about 12 h.

To improve the signal-to-noise ratio, the recorded signals of free induction decay were multiplied by the descending exponential with a spectral line (1b) broadening of 100 Hz and Fourier transform to convert the time-domain signal to a frequency spectrum measured in ppm of the spectrometer carrier frequency (100 MHz for ¹³C nuclei). This processing and transformation of spectra are performed using MestReC software. The integration of spectra over intervals was performed using GelTreat software (A.V. Kudryavtsev).

The distribution of carbon atoms among the different structural fragments was determined by the integration of the corresponding spectral regions. The following assignments according to [4, 19] were made in the following spectrums (ppm): 220–187, carbon atoms of ketone and quinone groups (C_{C=O}); 187–165, carbon atoms of carboxyl, ester, and amide groups (C_{COO-H, R}); 165–145, carbon atoms of O,N-substituted aromatic fragments (C_{Ar-O, N}); 145–108, carbon atoms of unsubstituted and C-substituted aromatic fragments (C_{Ar-H, R}); 108–48, carbon atoms of O,N-substituted aliphatic fragments (C_{Alk-O, N}); 48–5, carbon atoms of aliphatic fragments unbound to heteroatoms (C_{Alk-H, R}).

RESULTS AND DISCUSSION

The preparations obtained in each extraction were weighed before analysis to estimate the preparative yield. It follows from the data in Table 1 that the preparative yield of HAs from the soddy-podzolic soil decreased by more than 3 times after the second extraction and by 40 times after the third extraction compared to the first extraction. For the chernozem under permanent black fallow, a decrease in the HA yield by almost 4 times was observed after the second extraction and by almost 30 times after the third extraction. Similar ratios were obtained when the preparation yield was calculated per organic carbon in the soil sample rather than per total sample.

It is interesting that the preparative yield from the soddy-podzolic soil was half that from the chernozem, although the contents of organic carbons were similar in both soils. The higher yield of HAs from the chernozem can be related to their higher content in this soil [6, 8]. The decrease in the preparative yield of HAs in each following extraction was of the same order of magnitude for the both examined soils.

Similar results were obtained earlier for the typical chernozem under annually mown steppe [11]; the yield of HAs in the first extraction (1.18%) was similar to that for the chernozem under permanent fallow, and a decrease in the yield by 4 and almost 13 times after the second and third extractions, respectively, compared to the first extraction was noted. For the virgin gray forest soil, the preparative yield after the first extraction (0.45%) was similar to that observed for the soddy-podzolic soil [13]. However, the second extraction from the gray forest soil fell out of the observed series: the yield decreased by 10 times compared to the first extraction; this result could be an outlier caused by an error or related to the specific features of this soil type. The former supposition is most probably true, because the third extraction from the gray forest soil also showed a decrease in HA yield by 11 times compared to the first extraction, and this value is of the same order of magnitude that those obtained for chernozems and soddy-podzolic soil.

The elemental composition parameters (atomic ratios) of the HA preparations obtained by sequential extractions are given in Table 2. In the soddy-podzolic soil, no significant changes in the atomic ratios were observed after the consecutive extractions; the obtained parameters were close to the mean values reported for these soils [7].

In the typical chernozem under permanent black fallow, a significant difference was noted between the H/C ratios in the HAs from the first and the second extractions (0.54 and 0.75, respectively). This fact can be indicative of an increase in the proportion of aliphatic fragments in the HAs isolated by the second extraction. In addition, the O/C ratio in the HAs from the second extraction was lower, which could indicate a higher content of oxidized HAs in the first extract.

This could be related to the general destructive direction of organic matter transformation in the experimental treatment with permanent black fallow since 1964. It may be supposed that the more oxidized HAs are more easily extracted. It should be noted that the atomic ratios in the HAs of the third extraction from the chernozem occupy an intermediate position between those in the HAs of the first and second extractions.

To estimate the contribution of the HAs from each alkaline extraction to the mixed preparation obtained by the combined extractions, the average weighted values (M) were calculated for all parameters from the equation

$$M = \frac{\sum p_i M_i}{\sum p_i},$$

where p_i is the mathematical weight of the parameter for the i th extraction (estimated from the proportion of the HA yield for each extraction, Table 1), and M_i is the parameter value for the i th extraction.

The average weighted values of atomic ratios for the HAs from the soddy-podzolic soil completely corresponded to the values obtained for the first extraction. For the chernozem HAs, the weighted average values also little differed from those of the first extraction; the differences for the H/C, O/C, and C/N ratios were 0.4, 0.1, and 0.5, respectively, which was close to the experimental error.

The MWDs of the obtained HAs were studied by gel-permeation chromatography. The gel chromatograms of HA preparations are given in Fig. 1. The chromatograms of all the obtained preparations had monomodal distributions, which indicated a significant compensation of nonexclusion effects [20]. In the chromatograms of the soddy-podzolic soil HAs, a small peak was observed in the region of void volume, which most probably indicated the appearance of a high-molecular-weight fraction. In general, it may be concluded that in both soddy-podzolic soil and chernozem, the HA chromatograms for all three consecutive extractions had similar shapes, which pointed to analogous MWDs for the HAs obtained from the same soil by consecutive extractions.

From the obtained chromatograms, the main MWD parameters (Table 3) were calculated [20]: the average weight (M_w) and average numeric (M_n) molecular weights, as well as the preparation polydispersity (M_w/M_n). In general, the average weighted molecular weights of HAs from the soddy-podzolic soil exceeded the M_w values for HAs from the typical chernozem under fallow, which agreed with the literature data [7, 22].

No clear tendencies were revealed for the HAs obtained by sequential extractions from the soddy-podzolic soil, and most of the parameters little differed from one another. It should be noted that the value of M_w for the HAs of the second extraction from this soil

Table 2. Atomic ratios in HAs from sequential alkaline extractions (calculation for ash-free sample, moisture 8% [9])

Alkaline extraction no.	H/C	O/C	C/N
Soddy-podzolic soil under forest			
1	1.06	0.45	10.9
2	1.08	0.43	11.0
3	1.07	0.44	10.8
M^*	1.06	0.45	10.9
Typical chernozem, permanent black fallow			
1	0.54	0.42	19.6
2	0.75	0.36	17.9
3	0.59	0.38	16.0
M	0.58	0.41	19.1

* Here and below, M is the average weighted parameter value for all three extractions.

Table 3. Molecular weight distribution parameters in HAs from sequential alkaline extractions

Alkaline extraction no.	M_w	M_n	M_w/M_n
	kDa		
Soddy-podzolic soil under forest			
1	31.6	2.9	11.1
2	34.9	3.2	10.9
3	30.0	3.8	8.0
M	32.3	3.0	11.0
Typical chernozem, permanent black fallow			
1	22.1	3.0	7.4
2	27.4	2.4	11.6
3	25.2	4.1	6.2
M	23.3	2.9	8.2

exceeded that for the HAs of the first extraction by 3.3 kDa; however, the M_w for the HAs of the third extraction was lower than that for the first extraction by 1.6 kDa. The HAs isolated by consecutive extractions from the soddy-podzolic soil under forest probably had similar MWD values.

The HAs obtained by consecutive extractions from the chernozem under permanent black fallow also had similar characteristics. A small increase in the values of M_w for the second and third extractions compared to the first extraction should be noted.

The weighted average values of MWD parameters were almost identical to the values obtained for the first extraction.

The distribution of carbon atoms among the functional groups of HAs (carbon skeleton) was studied by

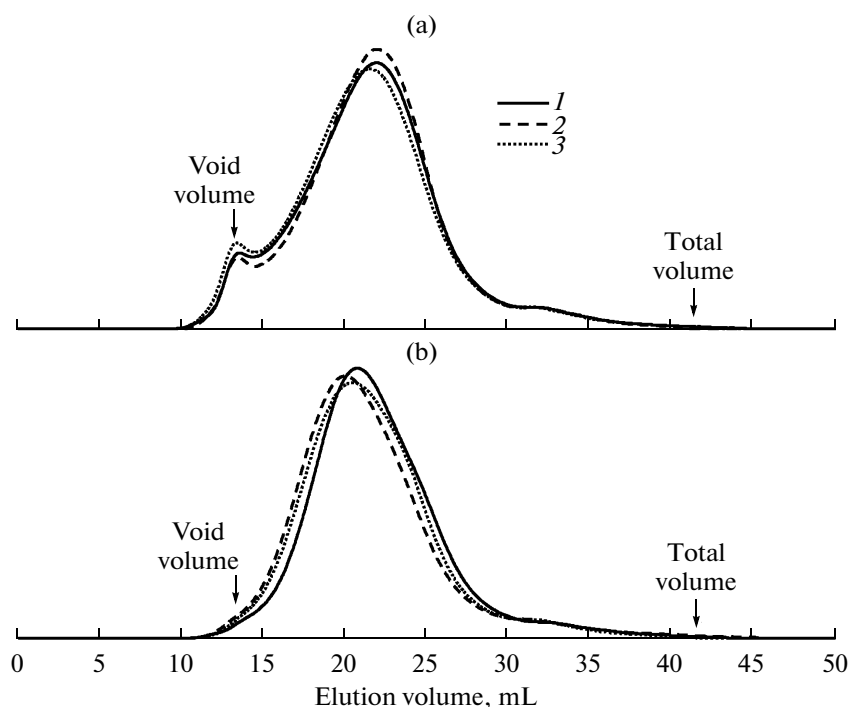


Fig. 1. Gel chromatograms of HA preparations obtained by sequential alkaline extractions from (a) soddy-podzolic soil under forest and (b) typical chernozem of the experimental treatment with permanent black fallow since 1964 (here and below: (1)–(3) alkaline extraction numbers); all chromatograms are normalized to the unit area.

^{13}C NMR spectroscopy. The spectra of the HAs obtained by the consecutive extractions are given in Fig. 2a for the soddy-podzolic soil and in Fig. 2b for chernozemic soils.

The spectra of HAs obtained by three consecutive extractions from the soddy-podzolic soil are characterized by the presence of intensive signals in the region of unsaturated aliphatic carbon (48–5 ppm); many small peaks in the region of 108–48 ppm, where the signals of heteroatom (O, N) substituents at carbon atoms (including carbohydrate and amine fragments) are located; a peak in the region of aromatic carbon (165–108 ppm); and a well-defined peak assigned to the carbon of carboxylic groups (187–165 ppm).

The spectra of all HAs from the typical chernozem under fallow is characterized by the presence of intensive signals in the region of aromatic carbon (165–108 ppm), carboxylic groups (187–165 ppm), and unsubstituted aliphatic carbon (48–5 ppm). The spectral intensity is low in the region of 108–48 ppm, where the signals of heteroatom (O, N) substituents at carbon atoms are located.

The comparison of the spectra of HAs from the soddy-podzolic soil and the chernozem show some characteristic differences. The soddy-podzolic soil shows many relatively small peaks of substituted and unsubstituted aliphatic carbon and aromatic carbon; only the peak of carboxyls is well defined. The chernozem shows a dominating peak of aromatic carbon and a well-defined peak of carboxylic carbon. The spec-

tra of HAs obtained by the consecutive alkaline extractions from each of the analyzed soils differ little.

Data on the quantitative estimation of spectral intensity distributions in the ^{13}C NMR spectra of HA preparations obtained by three sequential alkaline extractions from the studied soils are given in Table 4.

The comparison of the ^{13}C NMR data for the forest soddy-podzolic soil and the typical chernozem under permanent black fallow reveals some distinctions between the HAs from these soils. The HAs from the soddy-podzolic soil have more substituted and unsubstituted aliphatic carbon. Aromatic fragments prevail in the chernozem HAs. The contents of carboxyl and carbonyl groups are similar in both soils. The revealed tendencies largely agree with the earlier ^{13}C NMR data for HAs from zonal soils of Russia [12].

The distributions of carbon among the functional groups in the HAs of all three sequential alkaline extracts from the forest soddy-podzolic soil are similar. The second extract contains slightly less carbon of unsubstituted aliphatic groups and slightly more C-substituted alkanes than the first and third extracts. The calculated weighted average parameters of carbon distribution are almost completely similar to those obtained for the first extract.

The HAs from the chernozem under permanent black fallow showed almost identical carbon skeleton structures after all the sequential extractions. The calculated weighted average parameters of carbon distribution are identical to those of the first extraction.

Thus, the ^{13}C NMR data indicate similar structures of HAs obtained by sequential alkaline extractions from both the forest soddy-podzolic soil and the typical chernozem under permanent black fallow. However, it was shown earlier for a long-term fallow typical chernozem and a gray forest soil that the structure of HAs of the first alkaline extraction differs from those of the two next extractions: an increase in the proportion of aliphatic fragments and a decrease in that of aromatic fragments were observed in both cases [11, 13].

CONCLUSIONS

Sequential alkaline extractions had almost no effect on the composition and structure of HAs from the studied soils. However, differences between the HAs of sequential alkaline extractions from undisturbed (native or fallow) gray forest soil and chernozem under annually mown steppe were noted earlier [11, 13]. An increase in the content of aliphatic fragments, a decrease in the content of aromatic fragments, and an increase in the average weighted molecular weights of HAs from the second and third extracts compared to those from the first extract were observed for these soils.

It was shown that the HAs in sequential alkaline extracts from the soddy-podzolic soil under forest had almost identical compositions and structures. This can be related to the formation features of this soil type. On the one hand, the percolative water conditions and the relatively short vegetation period do not favor the accumulation and humification of organic matter [7]. On the other hand, the unfavorable conditions of humus formation apparently favor the accumulation of HAs with sufficiently uniform extractabilities, in contrast to the HAs from the steppe and forest-steppe zones, where a more diverse molecular ensemble of HAs is formed under nonpercolative water conditions.

The absence of differences among the HAs of sequential alkaline extracts from the chernozem of permanent black fallow can be related to the features of the long-term field experiment on this soil. No plant residues have entered the soil since 1964. During this time, all plant residues present in the soil were humified, and low-stable humic substances were degraded or converted to more stable compounds. All these changes resulted in the relatively uniform structure and composition of HAs in this soil. Therefore, there are no significant differences among the HAs in the sequential alkaline extracts from this soil.

The observed tendencies could be explained by the diversity of organic matter in the soil. The higher the diversity, the more significant the difference in the composition and structure of HAs from different extracts, and vice versa: the more homogeneous the organic matter, the lower the differences among the HAs from sequential soil extracts.

The diversity of organic matter in soils is determined by the features of zonal formation and the land

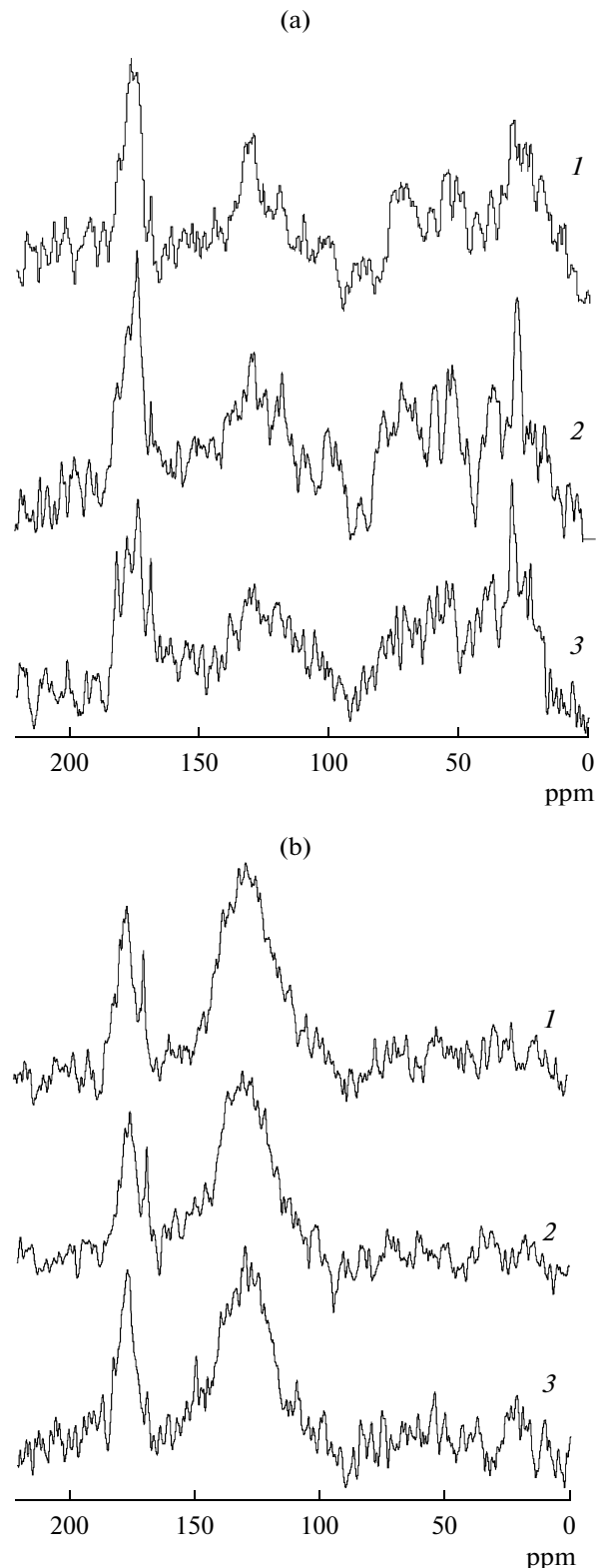


Fig. 2. ^{13}C NMR spectra of HAs obtained by sequential alkaline extractions from (a) soddy-podzolic soil under forest and (b) typical chernozem of the experimental treatment with permanent black fallow.

Table 4. Carbon distribution among the functional groups in HAs from sequential alkaline extractions

Alkaline extraction no.	Spectral regions, ppm					
	5–48 $C_{\text{Alk-H, R}}$	48–108 $C_{\text{Alk-O, N}}$	108–145 $C_{\text{Ar-H, R}}$	145–165 $C_{\text{Ar-O, N}}$	165–187 $C_{\text{COO-H, R}}$	187–220 $C_{\text{C=O}}$
Soddy-podzolic soil under forest						
1	24	23	20	7	16	10
2	19	30	19	9	16	6
3	25	25	20	8	15	7
<i>M</i>	23	25	20	7	16	9
Typical chernozem, permanent black fallow						
1	9	12	46	7	18	8
2	9	12	45	10	17	8
3	13	12	41	6	16	6
<i>M</i>	9	12	46	8	17	8

use. According to the general theory of humification advanced by Orlov [8], selection of quasi-stable compounds occurs in the soil under specific humification conditions. Consequently, more humus accumulates under favorable humification conditions (e.g., in chernozems). The favorable conditions of humification probably favor a higher physicochemical diversity of the accumulated substances. On the other hand, the relatively unfavorable zonal conditions of humus accumulation, e.g., in soddy-podzolic soils, result in not only smaller accumulation of humic substances, but also lower physicochemical diversity of the formed humus. The land use should have a similar effect on the diversity of the molecular ensemble of humic substances. The land use favorable for humus accumulation, which is aimed at the preservation and accumulation of organic matter, will increase the physicochemical diversity of humic substances. The unfavorable land use causing a decrease of humus reserve and limiting the input of fresh residue can result in a relative decrease in the diversity of humic substances.

These suppositions explain the absence of strong differences in the composition and structure of HAs from the sequential alkaline extracts in our work, while a difference between the HAs of the first and the next extracts was noted earlier for native (fallow) gray forest soil and chernozem.

The current work, as well as the early works on this subject [11, 13], demonstrates a significant decrease in the yield of HAs for each following extraction. The yields of HAs in the second and third extractions are lower than in the first extraction by at least 3 and 10 times, respectively. Thus, the first alkaline extraction makes the major contribution to the properties of the integral HA preparation obtained by several extractions.

In summary, based on the performed study, one may recommend to use a single alkaline extraction for the separation of HA preparations. In the case of an

especially valuable and small soil sample, two extractions are more appropriate. In addition, the sequential alkaline extractions (optimally, the first and the second ones) could be separately studied for the investigation of minor HA fractions and the assessment of their physicochemical diversity.

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