SOIL CHEMISTRY

Structure of Humic Acids in Zonal Soils from ^{13}C NMR Data

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Abstract—The structure of humic acids (HAs) in zonal soil types—soddy-podzolic soils (two samples), gray forest soil (one sample), and chernozems (two samples)—was quantitatively studied by ^{13}C NMR spectroscopy. In the series considered, the content of unsubstituted carbon in the aromatic fragments of HAs increased, and the fraction of unsubstituted aliphatic structures decreased. HAs of soddy-podzolic soils were found to be enriched with carbohydrate fragments compared to HAs of chernozems and gray forest soil. The carbon skeleton of HAs from typical rich chernozem contained significantly more aliphatic and carbohydrate fragments compared to typical chernozem, which probably reflected the lower degree of HA transformation in rich chernozem.

INTRODUCTION

The quantity and quality of soil organic matter are important characteristic features of pedogenesis in a specific soil type. A clear relationship exists between the composition and content of organic matter, on one hand, and the bioclimatic and zonal factors, on the other hand [8]. In particular, in the tundra zone with the low intensity of the biological carbon cycle and favorable conditions for the preservation of plant residues, a relatively high (tens of percent) [8] accumulation of carbon in the form of weakly decomposed plant residues is observed; however, humification is of low intensity under these conditions, and the fulvate type of humus is formed. In going to the south, from the taiga to the steppe zone, the intensity of the carbon cycle increases, the accumulation of undecomposed plant residues decreases, and the reserves of humic substances increase [8]. The humus character changes: the fraction of fulvic acids (FAs) decreases, and the relative content of humic acids (HAs) increases.

Humic substances make up 60 to 90% of soil organic matter [6]. A thorough study of the zonal features of humic substances is necessary to better understand their role in the global turnover of carbon and pedogenesis.

Of special interest are HAs, the most reactive and widely distributed components of humic substances. From the practical point of view, attention should primarily be given to HAs from soddy-podzolic, gray forest, and chernozemic soils. This is related to the wide use of these soils in agriculture.

In the last decades, much attention has been paid to studying the structure of HAs from zonal soils.

Trends in the changes in the HA elemental composition when going from north to south were revealed, which indicated that the content of carbon in HAs increases and those of hydrogen and oxygen decrease in the soil series from podzolic to chestnut soils, the content of nitrogen varying insignificantly [10, 16]. Correspondingly, the atom ratios reflecting the molecular structure of HAs are also subjected to zonal changes. The decrease in the H : C ratio in the above series indirectly indicates an increase in the aromaticity of HAs, and the increase in the O : C ratio points to an increase in the degree of oxidation. The C : N ratio increases insignificantly [10, 16]. Analysis of van Krevelen diagrams reveals the dehydration of HAs and the loss of CH_3 groups in going from gray forest soils to chernozems [10].

It should be noted that, in some works, the above trends were not always observed for oxygen. For example, according to the data of Tikhonov et al. [28], the content of carbon increased and that of hydrogen decreased in the series of HAs from surface-gleyed loamy soil, southern chernozem (Altai), and chestnut soil; however, the highest content of oxygen was observed in the HAs from chernozem, chestnut soil was second, and surface gleyed soil was last.

The main trends in the molecular-mass distribution for zonal soil HAs were revealed. For example, for native soils, the weighted average and number average molecular masses decreased and the polydispersity of the molecular-mass distribution decreased in the following series: soddy-podzolic soils, gray forest soils, chernozems [10, 11]. The decrease in the molecular masses reflected a regular increase in the content of aromatic structures in HAs and a decrease in the content of peripheral aliphatic fragments in going from

north to south, which corresponded to Orlov's kinetic theory of humification [10]. The noted decrease in polydispersity, in turn, reflected the relative accumulation of the most thermodynamically stable structures under conditions of steppe pedogenesis [10].

It should be noted that the macromolecular nature of HAs was put into question recently. On the basis of the theoretical assumptions advanced by Wershaw in the 1980s [29], Piccolo et al. performed a number of gel-chromatography experiments [22, 23]. In these experiments, HAs were passed through a gel-chromatographic column in a borate buffer solution (0.02 or 0.1 M, pH 9.2) at different pH values. HAs were first acidified to pH 2 by adding HCl or an organic (acetic or formic) acid. Then, the pH was successively increased by adding KOH, and a gel chromatographic experiment was performed. At the column outlet, in the region of low and neutral pH values, a high-molecular-mass peak was observed in the case of the mineral acid and a low-molecular-mass peak in the case of an organic acid. The authors explained the observed phenomenon by the fact that low-molecular-mass organic acids, in contrast to mineral acids, break hydrogen and hydrophobic (supramolecular) bonds and release separate HA molecules of low molecular masses. On the basis of the data obtained, a conclusion was drawn that humic substances, including HAs, are supramolecular systems: ensembles of molecules with relatively low molecular masses (about several hundred Da) unified into a micelle-like structure by hydrogen and hydrophobic bonds [22, 23]. However, the examination of the chromatographic experiment procedure gives reasons for doubting the conclusions drawn. As was shown in the work of Perminova [21], the addition of a strong mineral acid to establish pH 2 in a solution of humic substances changes its ionic strength by only 0.026 M, while the addition of a weak organic (acetic) acid increases the content of substances in the system by 4.3 M. Thus, these are two incomparable systems.

Moreover, a large negative charge analogous to the gel charge is created on the HAs whose pH was established with HCl in the borate buffer; therefore, these HAs are eluted from the column in the dead volume because of electrostatic repulsion [2]. On the other hand, when pH = 2 was established with an organic acid, humic substances are humified under high ionic strength (4.3 M) and are directly sorbed in the column (the salting-out effect). During the subsequent washing with an alkaline borate buffer, the sorbed HAs are dissolved and gradually eluted from the column; thus, their escape peak corresponds to the low-molecular-mass region [21]. Thus, the works of Piccolo [22, 23] do not provide sufficient reasons to classify humic substances among supramolecular compounds. Nonetheless, this concept is supported by some scientists. In 2005, a critical review was issued in which preference was given to the supramolecular theory of the structure of humic substances [25]. The authors adduce the above approaches of Piccolo et al. as the main argu-

ment for this position. Data are also given on the presence of macromolecules being transformed biomolecules (derivatives of lipids, lignin, nonlignin aromatic compounds, carbohydrates, and proteins) in humic substances, which does not contradict the macromolecular model. The authors consider the ^{15}N NMR spectroscopy data indicating the predominance of amine and amide nitrogen forms over heterocyclic ones as additional proof of the supramolecular theory. However, there is no reason to consider impossible the presence of these compounds in the humic macromolecule. For example, Kononova presented schemes of possible pathways for humus formation, which included the addition of amino acids to pyrocatechols with the retention of the amino group [6].

Thus, the present-day data on the structure of humic substances, including HAs, indicate that this is more likely a molecular ensemble of macromolecules. However, it should always be kept in mind that molecules with relatively low molecular masses are also present in humic substances.

The consideration of humic substances as natural hydrophobic compounds [7] allows the following trends to be revealed: an increase in the proportion of hydrophobic components in the humification horizons for the soil series of humid landscapes: Al-Fe-humus soil, peat-gley soil, gray forest soil; a high content of hydrophobic components was also noted in dark chestnut soil compared to chernozems under semiarid climatic conditions.

Available data on the zonal distribution features of the oxygen-containing (carboxylic and phenolic) functional groups of HAs are contradictory and reveal no clear genetic trend [10]. This is related to the similar acid constants of these groups and, hence, problems of determining the point of equivalence in the titration [10]. Artifacts of different analytical methods upon their application for such complex objects as HAs also complicate the task.

Along with aromatic fragments and functional groups, HAs contain many substituted and unsubstituted aliphatic structures. However, the features of their distribution in zonal HAs are still almost unstudied. This is primarily related to the difficult quantification of these fragments. However, new methods are appearing presently for the analysis of organic substances and the acquisition of new data on the structure of matter.

^{13}C NMR spectroscopy is one of these methods recently adapted for studying the structure of humic substances, including HAs. The recording of the ^{13}C NMR spectra of humic substances in an alkaline water solution with the elimination of the Overhauser effect and with sufficient relaxation delay times provides quantitative information on the distribution of carbon among the structural groups and molecules of HAs [5, 20]. For example, the data obtained by this method for some HAs from surface-gleyed loamy soil, Altai southern chernozem, and chestnut soil indicated a

decrease in the content of aliphatic and polysaccharide fragments and an increase in the content of aromatic carbon and carbon bound to carboxyl and ester groups [28].

It should be noted that the ^{13}C NMR spectra bear information only about a carbon atom and its surroundings, but they give no direct indications regarding such structural parameters as molecular mass and the mutual arrangement of fragments.

The widely used method of solid-phase ^{13}C NMR spectroscopy, along with obvious advantages (higher concentrations of samples, compared to solutions, and lower relaxation times), has significant shortcomings, the main one of which is the significant underestimation of the content of carbon atoms in the aromatic fragments of HAs, which makes the quantification of HA spectra in the solid phase very difficult [11, 24, 27]. For this reason, liquid-phase ^{13}C NMR spectroscopy is used for the acquisition of quantitative structural information [5, 18, 20, 24]. ^{13}C NMR spectroscopy in solutions is capable of describing the distribution of carbon atoms among the structural fragments of HA molecules differing in the chemical shifts of their carbon nuclei. Thus, the distribution patterns of structural fragments can be revealed for the HAs of zonal soils, and new quantitative data on changes in the structure of HAs depending on humification conditions can be acquired. On one hand, this will contribute to the refinement of data on previously studied structural units of HAs (functional groups and aromatic structures); on the other hand, new data on zonal trends in the composition of aliphatic HA fragments will be acquired.

The aim of the work was to study the carbon skeleton structure of HAs in a continuous series from soddy-podzolic soils to chernozems by liquid-phase ^{13}C NMR spectroscopy.

MATERIALS AND METHODS

Selection and characterization of soil samples. HA preparations were isolated from soil samples of the continuous zonal series: soddy-podzolic soils (two samples), gray forest soil (one sample), and chernozems (two samples).

Each soil sample was composed of five individual samples. Individual samples (about 2 kg each) were taken from the upper humus-accumulative horizon (A1) at a depth of 5—15 cm over an area of about 5 m^2.

From the sample obtained (about 10 kg), an average sample (1.5 kg) was formed by quartering; large roots were removed, and the sample was triturated and passed through a sieve with 1-mm mesh.

Samples of virgin soddy-podzolic soils (the 1977 Soil Classification [4]) were taken two times: in 2003 and 2004 in a coniferous forest in the territory of Chashnikovo Educational and Experimental Station, Moscow oblast.

Samples of virgin gray forest soil soils (the 1977 Soil Classification [4]) were taken in the Tul'skie Zaseki broad-leaved forest of the Krapivinskii Reserve (no. 45), Tula oblast, along the route of the summer zonal survey of the Faculty of Soil Science, Moscow State University.

Samples of typical chernozem (the 1977 Soil Classification [4]) were taken from an annually mown steppe plot in the Alekhin Central Chernozemic State Biosphere Reserve, Kursk oblast.

Rich typical chernozem (the 1977 Soil Classification [4]) was sampled from a fallow plot near the Khrenovoe stud farm, Voronezh oblast, along the route of the zonal survey of the Faculty of Soil Science, Moscow State University.

Soil samples were characterized by the content of organic carbon, the $C_{HA} : C_{FA}$ ratio (determined by the Ponomareva−Plotnikova procedure), and pH [1, 9] (Table 1). The content of physical clay (particles <0.01 mm) was also determined, and the soils were classified according to their texture [13] (Table 1).

In the series of soils studied, the content of organic carbon varied from 1.59 to 8.33%, which largely corresponded to published data [15]. A relatively high content of organic content in soddy-podzolic soils should be noted, although these values fell within the range reported for virgin soils of this subtype [12, 15].

The values of the $C_{HA} : C_{FA}$ ratio and pH regularly increase when going from soddy-podzolic soil to gray forest soil and chernozems, which is typical of the zonal series considered [15]. In general, the physicochemical parameters of the samples taken are typical of the zonal soil types under study.

Isolation and characterization of HA preparations. HAs were isolated by the IHSS method [26]. The only difference was that the alkaline extractions were performed in atmospheric air rather than under an argon atmosphere.

Elemental composition was determined on a Carlo Erba Strumentazione model 1106 elemental analyzer (Italy). The content of hygroscopic water in all preparations was taken to be 8% [11]. The content of ash in the isolated HAs was determined by incineration in a quartz tube under an oxygen atmosphere at 750°C for 40 min. The content of oxygen was calculated as the difference between the mass of a dry ash-free sample and the total content of C, H, and N.

The distribution of HA carbon among the structural fragments was determined by ^{13}C NMR spectroscopy [5]. HAs were prepared for NMR study by the dissolution of a 50-g sample in 60 ml of 0.3 M NaOD/D$_2$O. The mixture was put in an ultrasonic bath for 30 min and then centrifuged at 10000 rpm for 5 min; the solution was separated from the precipitate and transferred into a tube 5 mm in diameter for NMR spectroscopic study. The ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer (Germany) at 100 MHz using the Bruker INVGATE pro-

Table 1. Some physicochemical parameters of soils from which HAs were isolated

Soil	C_{org}, %	C_{HA}/C_{FA}	pH_{H_2O}
Virgin loamy sandy soddy-podzolic soil, Moscow oblast (2003)	2.77	0.4	4.7
Virgin loamy sandy soddy-podzolic soil, Moscow oblast (2004)	2.04	1.0	4.7
Virgin loamy sandy gray forest soil, Tula oblast	1.61	1.1	6.6
Clay loamy typical chernozem, annually mown steppe, Kursk oblast	5.52	1.7	6.8
Clay loamy rich typical chernozem, fallow, Voronezh oblast	8.33	2.1	6.8

cedure and a CPMG pulse sequence (the initial pulse was 90°; the registration time of the free induction decay signal was 0.2 s; the relaxation delay was 7.8 s). The duration of an NMR experiment was about 12 h.

To improve the signal-to-noise ratio, the recorded free induction decay signals were subjected to multiplication by a decaying exponential with a spectral line broadening parameter (lb) of 100 Hz followed by the Fourier transform of time-sweep signals into a frequency-sweep spectrum; the frequency was measured in ppm of the spectrometer carrier frequency (100 MHz for the ^{13}C nuclei). The above treatment and transformation of spectra were performed using the MestReC software package. The interval integration of spectra was performed using the GelTreat software package developed by A.V. Kudryavtsev.

The distribution of carbon atoms among different structural fragments was determined by the integration of the corresponding spectral regions. The following assignments of spectral lines were used in accordance with Kovalevskii [5] (ppm):

220−185, carbon of ketone and chinone groups (C=O); 187−165, carbon of carboxyl, ester, and amide groups ((>CO)−O, N); 165−145, carbon of O, N-substituted aromatic fragments (C_{Ar}−O,N); 145−108, carbon of unsaturated and C-substituted aromatic fragments (C_{Ar}); 108−90, carbon bound by simple bonds to two heteroatoms (oxygen or nitrogen) and in humic substances, mainly acetal carbon in cyclic polysaccharides (OC−O,N); 90−48, carbon bound by a simple bond to a heteroatom and mainly entering into the composition of carbohydrate units (this spectral range was additionally separated into subranges: 90−64, secondary carbon atoms (CH−O,N), 64−58, primary carbon atoms (CH_2−O,N), and 58−48, metoxyl carbon (CH_3O)); 48−5, carbon of aliphatic (alkyl) fragments unbound to heteroatoms (CH_n).

The data obtained for the structure of preparations were used to reveal the zonal features of HA structures.

RESULTS AND DISCUSSION

Parameters of the elemental composition of the HAs studied are given in Table 2. The content of HA carbon increased in the series from soddy-podzolic soils to chernozems. This parameter varied from 51.3 (HAs of soddy-podzolic soil sampled in 2003) to 61.2% (HAs of rich chernozem). It should be noted that the content of carbon in the HAs of gray forest soil was slightly higher (by 0.4%) than that in typical chernozem.

The content of hydrogen regularly decreased in the series considered (from 4.9 to 2.7%). The maximum content of oxygen was observed in the HAs of soddy-podzolic soil sampled in 2003; its minimum content was found in the HAs of gray forest soil. No clear decrease in the content of HA oxygen was observed in the series from soddy-podzolic soils to chernozems. This parameter is apparently less characteristic of zonal HAs than the content of carbon or oxygen. It should be noted that similar trends could be observed in the data reported by other authors [3, 17, 28]. This could be related to the fact that more oxygen is present in peripheral functional groups, which are more subjected to changes than the HA nucleus, and, hence, the content of oxygen in HAs will depend on the local factors rather than the zonal factors.

The content of nitrogen was 5.2−6.0, 5.8, and 3.9−4.5% in the HAs of soddy-podzolic soils, gray forest soil, and chernozems, respectively.

The H : C atomic ratio in the considered zonal series of HAs decreased from 1.15 to 0.59, which corresponded to the natural and geographical changes in conditions of humus formation, which were reflected in an increase in the proportion of aromatic structures

Table 2. Elemental composition of HAs from the soils studied

HAs from	Elemental composition, %*				Atom ratios			Ash, %
	C	H	N	O	H/C	O/C	C/N	
Soddy-podzolic soils								
2003	51.3	4.9	5.2	38.6	1.15	0.56	11.6	14.9
2004	55.8	4.9	6.0	33.2	1.06	0.48	10.9	2.0
Gray forest soil	56.7	4.3	5.8	30.7	0.91	0.41	11.4	10.0
Chernozems:								
typical	56.3	3.3	3.9	36.6	0.70	0.49	16.9	2.1
rich typical	61.2	2.7	4.5	31.6	0.59	0.4	15.7	7.0

* Per dry ash-free sample. The content of hygroscopic water was taken to be 8% in all preparations [11].

in HAs and a decrease in the H : C ratio [10]. It was difficult to detect any zonal regularity for the O : C ratio. Its value was 0.48–0.56 for the HAs of soddy-podzolic soils, 0.43 for gray forest soil, and 0.40–0.49 for chernozems. This could be related to the fact that, on one hand, the degree of oxidation of HAs in zonal soils increased from north to south [10] and, on the other hand, the HAs of soddy-podzolic soils had a more developed periphery, which contained more functional groups enriched with oxygen, and the content of oxygen in HAs significantly depended on local factors.

The C : N ratio in HAs increased insignificantly in going from soddy-podzolic soils (10.6–11.6) to gray forest soils (12.0) and increased significantly in chernozems (15.7–16.9).

A fairly good reproducibility of the data should also be noted: for the samples of soddy-podzolic soil taken from the same plot in different years, the H : C ratios differed by 0.09, the O : C ratios differed by 0.08, and the C : N ratios differed by 0.71. The larger difference observed for the last parameter is related to the fact that, first, nitrogen is more mobile in the entire soil and particularly in HAs, and, second, the determination of nitrogen in HAs is usually characterized by a larger error than the determination of carbon and hydrogen, which is related to its lower content in HAs.

On the whole, from the elemental analysis data, the isolated preparations were typical HAs of the corresponding zonal soils [10, 16].

Thus, it was shown that the HA preparations used in the work were typical of the zonal soils from which they were isolated and, hence, their structures could be studied by ^{13}C NMR spectroscopy as the structures of typical zonal soils of Russia.

The obtained ^{13}C NMR spectra are shown in the figure.

The quantitative estimation of spectral intensity distribution in the ^{13}C NMR spectra of the HAs studied is given in Table 3.

As one can see, the spectra of HAs from both soddy-podzolic soils studied are very similar. An increase in the area of peaks in the aromatic region (165–108 ppm) and a decrease in the content of unsubstituted aliphatic carbon (48–5 ppm) are clearly visible in the soil series from soddy-podzolic soils to chernozems. Signals in the region of carbohydrate carbon (90–48 ppm) are more pronounced for the HAs of soddy-podzolic soils than for those of gray forest soil or chernozems. Resonance lines of carboxyl carbon nuclei are distinguished in the region of 187–165 ppm of all spectra, but there is no correlation with the zonal origin of HAs.

The content of aliphatic carbon (CH_n) clearly decreases in going from the HAs of soddy-podzolic soils to those of gray forest soil and chernozems; the maximum difference is 15%. A steady trend toward an increase in the content of carbon in unsubstituted aromatic fragments from 20% in the HAs of soddy-podzolic soils to 44% in typical chernozem is observed in the soil series under study. This fact confirms the supposition about the decrease in the proportion of peripheral fragments and the increase in the content of aromatic structures in HA molecules in going from the taiga conditions of humus formation to the forest-steppe and steppe ones [10]. This could be related to
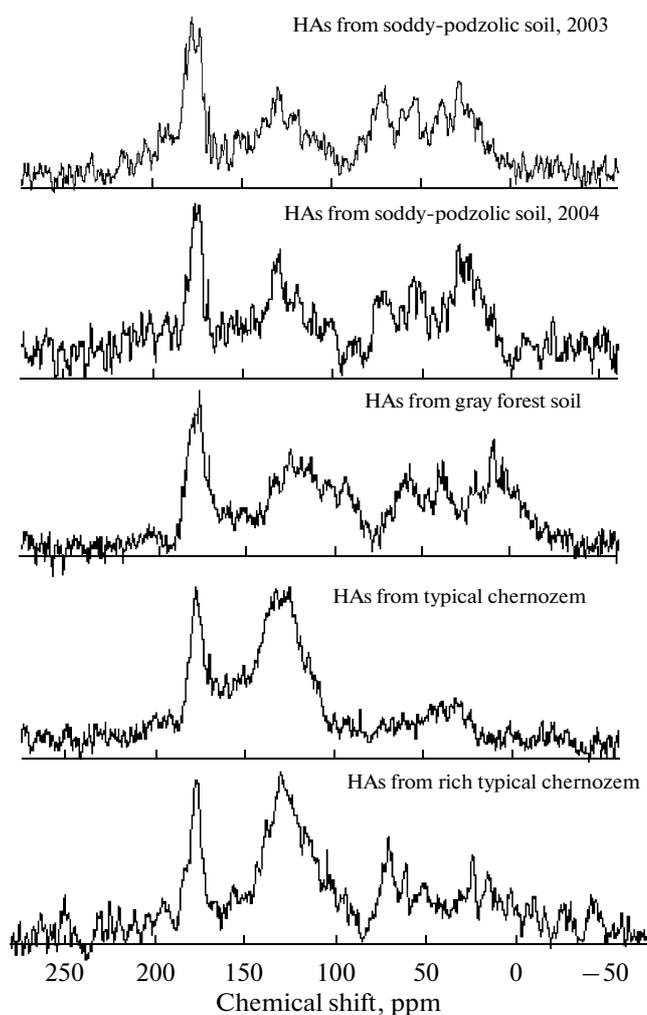
the character of plant residues forming the humus horizon and types of microbial communities. In the soddy-podzolic soil developed under taiga conditions, coniferous litter enriched with lignin is the main source of organic matter. Hence, microbial communities primarily specializing in the biodegradation of lignin are predominant. Therefore, the relative accumulation of aliphatic and carbohydrate structures is observed. Moreover, the prevalence of surface litter in the taiga zone can result in a different mechanism of formation of organic−mineral bonds.

In gray forest soils and chernozems, the main litter is formed in situ with plant roots and interacts with the mineral soil component more rapidly than the surface litter. In addition, it contains more cellulose than coniferous litter [14]. Therefore, microbial communities in these soils can primarily specialize in the decomposition of cellulose, which results in the relative accumulation of lignin derivatives (aromatic components).

No clear zonal trend is observed for substituted aromatic carbon mainly occurring in phenolic fragments. It can be noted that this parameter is lower for the HAs of soddy-podzolic soils (7%) compared to the other soils studied: 13% for the HAs of gray forest soil and typical chernozem and 11% for the HAs of rich chernozem. It can be seen that the HAs of gray forest soil and those of chernozems are very similar in the content of phenolic fragments. This can be related to the fact that the bioclimatic conditions of humus formation in gray forest soils are more similar to those in chernozems than to those in soddy-podzolic soils.

This supposition can also explain the absence of a clear difference between the HAs of chernozems and those of gray forest soil in the content of total carbohydrate fragments (ΣC_{carb}). In general, the value of this parameter was 6% for typical chernozem, 15% for rich chernozem, and 14% for gray forest soil. At the same time, the content of carbohydrate carbon in the HAs of soddy-podzolic soils was significantly higher (19−22%). In most cases, a higher content of separate fractions of carbohydrate fragments is observed in the HAs of soddy-podzolic soils. The less intense biological turnover in soddy-podzolic soils can result in the incomplete microbiological decomposition of hydrocarbon residues, and a fraction of them get into HAs. In addition, more carbon of ketone and chinone groups is observed in the HAs of soddy-podzolic soils.

Special emphasis should be placed on the fact that the content of carbon in carboxyl, ester, and amide groups (range 165−187 ppm), which mainly consists of carboxyl carbon in HAs, was similar in all preparations studied and varied from 16 to 19%. In the considered soil series, the content of carboxylic fragments was probably relatively stable, which explained the absence of a pronounced zonal trend in their distribution, as was also noted in earlier works [10]. On the other hand, further studies, including the comparison of ^{13}C NMR spectroscopy data with the results of

HAs from soddy-podzolic soil, 2003

HAs from soddy-podzolic soil, 2004

HAs from gray forest soil

HAs from typical chernozem

HAs from rich typical chernozem

^{13}C NMR spectra of HAs from the soils studied (signal intensity was normalized to the maximum signal of each spectrum).

potentiometric titration of HA functional groups, are necessary to clarify this issue.

The difference in the carbon skeleton structure between two studied chernozems (typical and rich typical ones) should be noted. The HAs of the rich chernozem contain more aliphatic carbon, as well as carbohydrate fragments by more than 2 times, methoxyl carbon (CH_3O) by more than 2 times, primary carbon (CH_2O) by 3 times, and secondary carbon (CHO) by more than 2.5 times. With respect to these parameters, the HAs of rich chernozem were more similar to the HAs of gray forest soil.

The higher content of carbohydrates in the HAs of rich typical chernozem compared to those of typical chernozem can be explained by the lower transformation of plant residues during their formation probably related to the microclimatic conditions favorable for the preservation of humic substances. There can be, for example, the freezing through of the humus-accumulative horizon or the optimum balance of spring

Table 3. Carbon distribution among the structural fragments of HAs (% of total C)

HAs from	Spectral regions, ppm									Structural descriptors	
	5–48	48–58	58–64	64–90	90–108	108–145	145–165	165–187	187–220	ΣC_{carb}*	$\Sigma C_{Ar}/\Sigma C_{Al}$**
	CHn	CH$_3$O	CH$_2$O	CHO	OCO	C$_{Ar}$	C$_{ArO}$	COO	C=O		
Soddy-podzolic soils											
2003	21	6	3	12	5	20	7	18	6	22	0.6
2004	24	6	3	10	4	20	7	16	10	19	0.6
Gray forest soil	16	4	2	8	2	35	13	19	1	14	1.5
Chernozems:											
typical	9	2	1	3	4	44	13	19	4	6	2.9
rich typical	13	4	3	8	2	41	11	16	3	14	1.8

Notes: * Total carbohydrates (58–108 ppm).; 10.

** Degree of aromaticity.

wetting and summer drying [19], which results in a lower decomposition rate of organic matter and, hence, a higher accumulation of humus in rich chernozem; however, the parameters of its HAs differ from those of the most transformed chernozem HAs of the annually mown steppe, which contain less carbohydrates and aliphatic fragments.

Thus, it can be assumed that the conditions favorable for the preservation of organic matter and the formation of rich chernozems are reflected in the relatively higher content of HA carbohydrate fragments.

The degree of aromaticity of HAs ($\Sigma C_{Ar}/\Sigma C_{Al)}$, which is the ratio between total aromatic carbon and total alkyl fragments reflecting the hydrophobic–hydrophilic balance in HAs, gradually increases in going from the HAs of soddy-podzolic soils (0.6) to those of chernozems (2.9 for typical chernozem). The value of this parameter for rich chernozem is lower (1.8), which reflects its higher content of less decomposed hydrophilic structures (primarily cellulose residues). Hydrophobicity increasing from north to south agrees well with the hydrophobic chromatography data [7].

CONCLUSIONS

The ^{13}C NMR spectroscopic study of the structure of HAs from virgin or fallow soils in the zonal series from soddy-podzolic soils to chernozems showed that the contents of unsubstituted aliphatic carbon and unsubstituted aromatic carbon are the most characteristic zonal parameters of the HA carbon skeleton structure. The former parameter decreases and the latter increases in the series considered, as well as the degree of aromaticity of HAs, reflecting their hydrophilic–hydrophobic balance.

The structure of the HAs of gray forest soil is much more similar to that of chernozems than to that of soddy-podzolic soils, which indicates the similarity of biothermodynamic conditions of humus formation in these soils.

The HAs of soddy-podzolic soils, in contrast to those of gray forest soils and chernozems, contain more carbon related to carbohydrate fragments, which indicates the higher role of polysaccharides in the formation of HAs in these soils.

The HAs of rich chernozem contain more aliphatic and carbohydrate fragments than the HAs of typical chernozem. This can be related to the fact that the decomposition rate of organic matter in rich cher-

nozem is lower, and, hence, a larger amount of humus is accumulated; however, the parameters of its HAs differ from those of the most transformed HAs from the chernozem of the annually mown steppe. The lower decomposition rate of organic matter can be related to the microclimatic features of rich chernozem formation.

REFERENCES

[1] 1. E. V. Arinushkina, *Guide on the Chemical Analysis of Soils* (Mosk. Gos. Univ., Moscow, 1970) [in Russian].

[2] 2. B. G. Belen'kii and L. Z. Vilenchik, *Chromatography of Polymers* (Khimiya, Moscow, 1978) [in Russian].

3. N. P. Bel'chikova, "Some Regularities in the Content and Composition of Humus and the Properties of Humic Acids in the Main Groups of Soils of the Soviet Union," Tr. Pochv. Inst. Im. V.V. Dokuchaeva **38**, 33–58 (1951).

4. *Classification and Diagnostics of Soils of the USSR* (Kolos, Moscow, 1977) [in Russian].

5. D. V. Kovalevskii, A. B. Permin, I. V. Perminova, and V. S. Petrosyan, "Selection of Conditions for the Recording of Quantitative ^{13}C NMR Spectra of Humus Acids," Vest. Mosk. Univ., Ser. 2: Khim. **41** (1), 39–42 (2000).

6. M. M. Kononova, *Soil Organic Matter* (Akad. Nauk SSSR, Moscow, 1963) [in Russian].

7. E. Yu. Milanovskii, *Soil Humus Substances as Natural Hydrophobic–Hydrophilic Compounds* (Geos, Moscow, 2009) [in Russian].

[3] 8. D. S. Orlov, O. N. Biryukova, and N. I. Sukhanova, *Organic Matter in Soils of the Russian Federation* (Nauka, Moscow, 1996) [in Russian].

9. D. S. Orlov and L. A. Grishina, *Laboratory Manual on Humus Chemistry* (Mosk. Gos. Univ., Moscow, 1981) [in Russian].

10. D. S. Orlov, *Soil Humus Acids and the General Theory of Humification* (Mosk. Gos. Univ., Moscow, 1990) [in Russian].

11. I. V. Perminova, Doctoral Dissertation in Chemistry (Moscow, 2000) [in Russian].

12. *Podzolic Soils in the Central and Eastern Regions of European Soviet Union*, Ed. by A. A. Rode, N. A. Nogina, and I. V. Zaboeva (Nauka, Moscow, 1980) [in Russian].

13. *Textbook on the Analysis of Soils and Compilation of Agrochemical Cartograms* (Rossel'khozizdat, Moscow, 1969) [in Russian].

14. *Soil Science: Soils and Pedogenesis*, Ed. by V. A. Kovda and B. G. Rozanov (Vysshaya Shkola, Moscow, 1988) [in Russian].

15. *Soil Science: Soil Types, Their Geography, and Use*, Ed. by V. A. Kovda and B. G. Rozanov (Vysshaya Shkola, Moscow, 1988) [in Russian].

16. I. V. Tyurin, *Soil Organic Matter and Its Role in Fertility* (Nauka, Moscow, 1965) [in Russian].

17. N. V. Khavkina and A. A. Bessarabova, "Nature, Composition, and Properties of Humic Acids from Arable Soils of the Southern Regions of the Far East," Izv. Sib. Otd. Akad. Nauk SSSR, Ser. Biol. Nauk, **3** (15), 16–20 (1983).

18. V. A. Kholodov, A. I. Konstantinov, E. Yu. Belyaeva, et al., "Structure of Humic Acids Isolated by Sequential Alkaline Extraction from a Typical Chernozem," Pochvovedenie, No. 10, 1177–1183 (2009) [Eur. Soil Sci. **42** (10), 1095–1100 (2009)].

19. *Chernozems of the USSR*, Ed. by V. M. Fridland and I. I. Lebedeva (Kolos, Moscow, 1974), Vol. 1 [in Russian].

20. N. Hertkorn, A. B. Permin, I. V. Perminova, et al., "Comparative Analysis of Partial Structures of a Peat Humic and Fulvic Acid Using One and Two Dimensional Nuclear Magnetic Resonance Spectroscopy," J. Environ. Qual. **31**, 375–387 (2002).

21. I. V. Perminova, "Size Exclusion Chromatography of Humic Substances: Complexities of Data Interpretation Attributable to Non-Size Exclusion Effects," Soil Sci. **164**, 834–840 (1999).

22. A. Piccolo, "New Insights on the Conformational Structure of Humic Substances as Revealed by Size Exclusion Chromatography," in *The Role of Humic Substances in the Ecosystems and in Environmental Processes*, Ed. by J. Drozd, S. S. Gonet, N. Senesi, and J. Weber (IHSS-Polish Society of Humic Substances, Wroclaw, Poland, 1997), pp. 19–35.

23. A. Piccolo, S. Nardi, and G. Concher, "Macromolecular Changes of Humic Substances Induced by Interaction with Organic Acids," Eur. J. Soil Sci. **47**, 319–328 (1996).

24. C. M. Preston, "Applications of NMR to Soil Organic Matter Analysis: History and Prospects," Soil Sci. **161**, 144–166 (1996).

25. R. Sutton and G. Sposito, "Molecular Structure in Soil Humic Substances: The New View," Environ. Sci. Technol. **39** (23), 9009–9015 (2005).

26. R. S. Swift, "Organic Matter Characterization," in *Methods of Soil Analysis* (Soil Science Society of America, Madison, WI, 1996), Part 3, pp. 1018–1020.

27. K. A. Thorn, "Nuclear-Magnetic-Resonance Spectrometry Investigations of Fulvic and Humic Acids from the Suwannee River," in *Humic Substances in the Suwannee River, Georgia: Interactions, Properties, and Proposed Structures*, Ed. by R. C. Averett, J. A. Leenheer, D. M. McKnight, and K. A. Thorn (US Geological Survey, Denver, (1994), pp. 141–182.

28. V. D. Tikhova, V. P. Fadeeva, M. I. Dergacheva, and M. M. Shakirov, "Analysis of Humic Acids from Various Soils Using Acid Hydrolysis," Russ. J. Appl. Chem. **81** (11), 1957–1962 (2008).

29. R. L. Wershaw, "A New Model for Humic Materials and Their Interactions with Hydrophobic Organic Chemicals in Soil–Water and Sediment–Water Systems," J. Contam. Hydrol., No. 1, 29–45 (1986).

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