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# ELEMENTAL COMPOSITION AND STRUCTURE OF HUMIC ACIDS IN SOD-PODZOLIC ARABLE SOIL AND ITS VIRGIN ANALOGUES IN LONG-TERM STATIONARY EXPERIMENT

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**ABSTRACT**

The effect of crop rotations with different saturation by legumes (0; 28.6; 42.9 percent of legumes) and continuous barley on the elemental composition and structure of humic acids in sod-podzolic heavy loamy soil *(Eutric Albic Retisols (Abruptic, Loamic, Cutanic)* was studied in long-term (since 1977) stationary experiment in Perm Region. The arable soil was compared with virgin analogues (under mixed forest, poaceous-forb meadow) and neglected field. The carbon content in humic acids of arable and virgin sod-podzolic soil was 28.3-33.5, hydrogen 40.1-47.5, oxygen 19.6-27.9, nitrogen 2.1-2.5 and sulfur 0.1-0.2 percent. The differences in humic acids composition of arable and virgin soils result from the qualitative composition of the organic soil material, its amount and the intensity of anthropogenic impact. The saturation of field crop rotation with legumes up to 42.9 percent caused the raising the aliphatic groups share in the structure of soil humic acids (the maximum H / C ratio 1.67 was noted). Using five-field grain rotation promoted the increasing of stable aromatic structures and nitrogen decline in humic acids (the minimum H / C ratio 1.20 and maximum C / N ratio 16.2 was noted). The highest content of oxygen-containing groups and high degree of humic acid oxidation (W = + 0.53), were found in humic acids of neglected field soil (C / O - 0.95). That may indicate the most favorable conditions for the humification of organic matter. According to the results of Fourier IR spectroscopy, the most branched structure, consisting from large amount of individual monomers of various molecular weights with a large number of aliphatic groups, had humic acids in soil under crop rotation with high legumes saturation and soil under mixed forest. Aromatic structures were more intensely expressed in humic acids spectra of the soil under conventional crop rotation and continuous barley.

**Key words:** *humic acids, elemental analysis, IR spectroscopy, crop rotation, neglected field, continuous barley.*

## INTRODUCTION

In the second half of the 20th century, it was believed that the predominant part of soil organic matter (SOM) was represented by humic substances - stable high molecular polymers with heterocyclic compounds enriched by nitrogen, with aromatic core and extensive aliphatic periphery, which give great variety and specificity of its properties and functions. The ratio between himic (HA) and fulvic acids (FA) reflects the SOM quality (Kholodov. et al., 2011; Semenov et al., 2013; Ivanov et al., 2017) These views contradict with modern studies carried out by Russian and foreign scientists at the beginning of the 21st century. New information about genesis, composition and structure of humic substances was provided by instrumental methods of analyses (Fourier IR spectroscopy, , mass spectroscopy, nuclear magnetic resonance, X-ray structural analysis, electronic microscopy, etc.) (Wershaw, 2004; Kleber, Johnson, 2010; Xu et al., 2017).

Three-layer SOM model has been proposed, with biomolecules of amphiphilic organic matter (OM) self-linkage into supramolecular aggregates ordered by non-valent interactions on chemically active surfaces of mineral particles (Kleber et al., 2007; Baveye, Wander, 2019; Kholodov et al., 2020). The multilayer model explains the stability and biodegradability inherent to SOM, much better than the polymer concept (Piccolo, 2002; Olk et al., 2019).

According to the opinion of International Humic Substances Society (IHSS) and the American Soil Science Society, the main criterion for determining humic substances today is still the solubility in alkalis (Kleber, Lehmann, 2019). The different solubility of humic substances in acid- alkaline media is the basis for their division into humic acids, fulvic acids and non-extractable residue (humin). Among humic substances, humic acids (HA) are of particular importance, since they possess high functional activity, determine the specificity of the water, physical, chemical and thermal properties of the soil. Their content and structure depend on the conditions of soil formation and change under anthropogenic impact on the soil (Orlov, 1990; Kholodov et al., 2011).

The aim of given research is to reveal the influence of various land use methods on the elemental content and structure of humic acids in arable soil and to compare it with virgin analogues.

## MATERIAL AND METHODS

The experimental work was fulfilled in 2019–2021 in long-term stationary field experiment (founded in 1977) on the experimental farm of Perm Agricultural Research Institute. The experimental plots located on sod-podsolic heavy loam soil. Experimental scheme: 1. continuous barley; 2. five-course grain field rotation (barley, winter rye, spring wheat, barley, oat; with 0 percent of legumes); 3. seven-course conventional field rotation (manured bare fallow, winter rye, spring wheat as cover crop for red clover, first year clover, second year clover, barley, oat; with 28.6 percent of legumes) 4. seven-course field rotation (green manure fallow (clover), winter rye, spring wheat as cover crop for red clover, first year clover, second year clover, barley, oat as cover crop for red clover; with 42.9% of

legumes) 5. neglected field (no tillage since 1978). There were no mineral fertilizers application in presented variants. The soil had the following agrochemical characteristics by the time of experiment foundation: $pH_{KCl}$ 5.2 - 5.3; hydrolytic acidity 2.1 - 2.3 cmol (eq)/kg, the the sum of exchange bases (S) was 14.0-15.5 cmol (eq) / kg, the content of organic carbon according to Tyurin was 1.10-1.12%, available phosphorus $P_2O_5$ 225-240 mg/kg, exchange potassium $K_2O$ - 196-204 mg / kg (according to Kirsanov). Soil samples for research were taken in the fall of 2018 from the 0-20 cm layer in two non-contiguous replications.
To identify the processes of organic matter transformation, arable soils were compared with their virgin analogs: under mixed-deciduous forest with rich grass cover and poaceous-forb meadow. The following tree species were presented in the forest: coniferous - spruce (*Pícea ábies*), fir (*Ábies sibírica*), pine-tree ( *Pinus sylvestris)*, deciduous - birch (*Betula pendula*), aspen (*Populus tremula*) and maple (*Acer platanoides*). Mountain ash (*Sorbus aucuparia*), linden (*Tilia cordata*), alder (*Álnus incána*), bird cherry, (*Prunus padus*) etc. formed well developed forest undergrowth. Oxalis, oxalis-ferns and forb-poaceous-ferns plant communities prevailed in the ground cover. The thickness of the forest litter under the forest canopy was about 3 cm. The species composition of the natural poaceous-forb meadow: poaceous grasses – 62.0 percent; legumes – 13.5 percent; forbs – 24.5percent. Soil samples under the mixed forest were taken from the layer 3-20 cm. Preparations of humic acids were separated according to the classical method of the Russian School of Soil Science, which differs from the recommendations of the International Humic Substances Society (IHSS) (Swift, 1996). The soil was extracted with alkali at least three times in ordinary air, then combined extract is analyzed. The elemental composition of humic acids was determined on a CHN - elemental analyzer after Perkin - Elmer company (USA), the amount of oxygen was calculated by difference (all calculations are given for ash free preparations); IR absorption spectra were recorded on a VERTEX-80v Fourier spectrometer (Bruker company, Germany) in the range 4000-400 cm-1 at a spectral resolution of 2 cm-1. The spectra were processed using the OPUS software package.
The research work was carried out in the IV agroclimatic district of Perm Region, located in the geographical subzone of the southern taiga and mixed coniferous-deciduous forests (Agro-climatic resources…,1979). In accordance with the soil-ecological zoning, the territory of Perm Region belongs to the Vyatka-Kama soil province. The climate is temperate continental with cold, long, snowy winters and warm short summers. The sum of the average daily temperatures above 10°C is 1700-1900. The duration of the active growing season with temperatures above 10°C is on average 115 days, with temperatures above 15°C – 60 days. The district belongs to the zone of sufficient moisture: hydrothermal coefficient (HTC) –1.4, annual precipitation sum is 470-500 mm, evaporation from the soil surface is about 340 mm. The number of days with snow cover averages 176 (Eremchenko et al., 2016).

## RESULTS AND DISCUSSION

The main peculiars of the studied soils is the low carbon content in the upper layers: under mixed forest -1.57%, poaceous -forb meadow -1.25, in arable soil - 1.01-1.47% (depending on the experiment treatment), high acidity (pH 4.2; 4.8 and 4.8-5.5 respectively) (Table 1). Nitrogen content was medium and high, total C:N ratio was 8.3-9.4, under mixed forest it was high – 5.9, under the meadow - average - 8.4 (according the scale by Orlov, Grishina, 1981).

Table 1. Agrochemical properties of sod-podzolic soil and ecophysiological indicators of microflora condition under different land use

| Type of land using | $C_{org}$, % | $pH_{KCl}$ | S | Ha | Ca | Mg | $P_2O_5$, | $K_2O$ | $N_{tot}$ | Cmic | SIR |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | cmol (eq) / kg, | | | | mg/kg | | | μg/g | μg/g C-$CO_2$/g hour |
| Continuous barley, | 1.01 | 5.1 | 22.5 | 3.1 | 18.6 | 2.0 | 440 | 212 | 1078 | 413 | 10.3 |
| Grain crop rotation ( 0% of legumes ) | 1.07 | 5.2 | 20.1 | 3.2 | 17.9 | 3.6 | 420 | 188 | 1295 | 189 | 5.2 |
| Conventional crop rotation 28.6 % of legumes ) | 1.47 | 5.5 | 21.5 | 3.0 | 17.2 | 3.2 | 537 | 322 | 1729 | 316 | 8.3 |
| Crop rotation (42.9 % of legumes) | 1.21 | 4.9 | 21.7 | 3.5 | 18.2 | 2.9 | 314 | 219 | 1428 | 257 | 5.1 |
| Neglected field | 1.36 | 4.8 | 19.8 | 4.8 | 17.2 | 2.8 | 473 | 247 | 1554 | 440 | 10.9 |
| Mixed forest | 1.57 | 4.2 | 20.0 | 6.4 | 12.0 | 3.2 | 168 | 177 | 2660 | 1236 | 30.9 |
| Poaceous-forb meadow | 1.25 | 4.8 | 21.2 | 2.2 | 13.9 | 2.5 | 290 | 175 | 1490 | 571 | 14.3 |
| $LSD_{05}$ | 0.19 | 0.2 | $F_ф<F_т$ | 0.4 | 1.2 | $F_ф<F_т$ | 58 | 34 | 111 | 128 | 1.2 |

The noted differences in the carbon content in the experiment variants are characteristic of sod-podzolic soil, poor in humus and COM. For more fertile soils, the difference may not be so noticeable. So in research of Seremešić et al (2021), on chernozem soil the average content of SOM was similar for organic and conventional management practices – 3.09% and 3.08%, respectively. Thus, the additional research is required for each type of soil and region with different natural conditions. The studied soils, in accordance with the approximate scale of soil enrichment with microflora, correspond to the gradations of "very poor" and "poor" (Zvyagintsev et al., 2005) The virgin soil was characterized by the minimum content of all microorganisms types (Zavyalova et al., 2020; Zavyalova et al.,2021). The carbon content of microbial biomass (Cmic) varied from 189 in grain rotation to 1236 μg/g soil under mixed forest. The maximum value of substrate-induced respiration (SIR) was recorded in the study of soil under mixed forest - 30.9 μg C-CO2/g hour. To obtain information about the structure of supramolecular aggregates, the presence of basic constitutional elements in their structural fragments, and the transformation direction of organic matter under the influence of natural and anthropogenic factors, we used the method of elemental analysis in the study of humic acids. Expressing the results of elemental analysis in atomic percent gives the information about the changes that occur with humic substances during soil formation. Ratios H:C, O:C and C:N characterize the direction of transformation processes of humic acids under anthropogenic impact to the soil. The H:C ratio determines the degree of enrichment of the HA structure with aromatic fragments, O:C - the degree of oxidation, C:N reflects the role of nitrogen-containing components in the construction of humic acids (Orlov, 1990; Gasanova et al., 2018). In the arable soil of the long-term stationary experiment, where various methods of land use are studied, a low carbon content was found in HA structure of the soil under the crop rotation with legumes saturation– 42.8 percent (two fields of clover and lupine) - 28.35 at. percent (Table 2) and high hydrogen content –47.43 at. percent. In that variant, the maximum H/C ratio was noted, equal to 1.67, indicating the predominance of aliphatic groups in the structure of supramolecular HA aggregates. The same data were obtained by other researches (Popov, 2004; Milkheev, Tsybenov, 2018). The predominance of organic matter mineralization processes over the accumulation of humic substances was noted in the soil in grain crop rotation and under continuous barley, where the loss of carbon over 41 years of the experiment was 7-9 percent compared with the initial level when the experiment was founded. Sometimes the total loss may be about y 30% of initial soil organic carbon in the topsoil. (Seremešić et al, 2021). Under these conditions, during the destruction of organic material, aliphatic groups disintegrate rapidly and aromatic structural fragments with a higher carbon content remain. For these treatments, a lower H/C ratio was noted: 1.20–1.28 and that indicates the increase in the aromatic structures share in supramolecular associations when the soil is depleted by carbon and nutrients. A high degree of oxidation (W=0.23) of HA in grain crop rotation indicates the depth of humification of organic matter (Semenov et al., 2013; Gasanova et al., 2018).

Table 2. Elemental content of soil humic acids in long-term experiment with various types of land use

| Variant | Content, % | | | | | Atomic ratios | | | Degree of oxidation, (*W*) |
|---|---|---|---|---|---|---|---|---|---|
| | C | H | O | N | S | H/C | O/C | C/N | |
| Continuous barley | 48.56<br>33.36 | 5.17<br>42.65 | 41.36<br>21.33 | 4.18<br>2.47 | 0.76<br>0.20 | 1.28 | 0.64 | 13.5 | -0.001 |
| Grain crop rotation ( 0% of legumes ) | 46.60<br>33.47 | 4.67<br>40.29 | 44.60<br>24.05 | 3.36<br>2.07 | 0.48<br>0.13 | 1.20 | 0.72 | 16.2 | 0.23 |
| Conventional crop rotation 28.6 % of legumes ) | 49.59<br>32.84 | 5.64<br>44.85 | 39.97<br>19.87 | 3.94<br>2.24 | 0.86<br>0.22 | 1.37 | 0.61 | 14.7 | -0.16 |
| Crop rotation (42.9 % of legumes) | 43.93<br>28.35 | 6.12<br>47.43 | 45.15<br>21.87 | 3.79<br>2.10 | 1.03<br>0.25 | 1.67 | 0.77 | 13.5 | 0.13 |
| Neglected field | 40.34<br>29.50 | 4.57<br>40.13 | 50.86<br>27.92 | 3.67<br>2.30 | 0.58<br>0.16 | 1.36 | 0.95 | 12.8 | 0.53 |
| Mixed forest | 47.91<br>30,51 | 6.21<br>47.48 | 41.32<br>19.75 | 3.79<br>2.07 | 0.79<br>0.19 | 1,56 | 0.66 | 14.4 | -0.26 |
| Poaceous-forb meadow | 49.11<br>32.07 | 5.83<br>45.73 | 40.02<br>19.62 | 4.22<br>2.36 | 0.84<br>0.21 | 1.43 | 0.61 | 13.6 | -0.20 |

Footnotes:

* Above the line - mass fraction, below the line - atomic fraction (all calculations are given for ash free preparations)

The widest C/N ratio - 16.2, was found in the soil HA in the grain crop rotation, that showed their depletion with nitrogen, because they are formed from nitrogen-poor plant residues of grain crops (Orlov, 2019). The HA of neglected field soil were characterized by minimum content of hydrogen and maximum content of oxygen. The O/C ratio of humic acids in the considered variants increased with a decrease of the anthropogenic influence on the soil and was maximum in soil HA under neglected field – 0.95.

The oxidation of humic acids (W) varied from –0.26 to +0.53 owing to the type of land use. The maximum content of oxygen-containing groups was determined in HA of the neglected field soil. The high degree of HA oxidation, the positive sign of this indicator may serve as evidence of the most favorable conditions for the humification of organic matter in the soil under neglected field (Abakumov, 2009; Motuzova et al., 2012; Gorbov, Bezuglova, 2013).

Despite the fact that the total biomass of the forest biocenosis is much larger compared to the neglected field biomass, the soil under it can accumulate more carbon. This is owing to the alienation of biomass during timber harvesting. The cultivation of long cycle forest species, whose economic uses are not dedicated exclusively to wood production (fruit trees, rubber trees and so on ) has comparative advantages in relation to the short-cycle tree species because carbon remains stored in the vegetation for longer periods ( Torres et al., 2020).

The data of elemental analysis of HA from virgin sod-podzolic soil under forest and meadow indicate that under the conditions of leaching regime, low content of exchange bases, humic acids with a low content of carbon and nitrogen in supramolecular aggregates composition were formed from plant residues enriched with cellulose-lignin complex. Low microbiological activity led to decrease of organic matter (plant residues) mineralization, what led in turn to increase of aliphatic groups share, the H:C ratio was 1.43-1.56.

The conducted studies have showed that humic acids separated preparatively from the arable soil in long-term stationary experiment and its virgin analogues, correspond to the average values for the class of humic acids of sod-podzolic soils in terms of constitutional elements content (C, H, N, O). The aliphatic fragments dominated in humic acids structure, the C:H ratio was 1.20-1.67. The similarity of humic acids in arable and virgin soils resulted from almost the same set of formation factors: soil, plants, moisture, heat, microorganisms; the differences were in the qualitative composition of organic material in the soil and its quantity, different intensity of anthropogenic impact, and different random factors (Semenov et al., 2013).

Infrared spectroscopy is the obligatory and most important diagnostic method for studying humic substances. The method makes it possible to identify atomic groups and provides information about the type of chemical bonds and structural elements of humic acid molecules (Orlov,1990; Motuzova et al., 2012). The set and intensity of the absorption bands make it possible to estimate the role of aromatic and aliphatic fragments in the structure of supramolecular aggregates. It was found in the comparative study of the spectra, that humic acids from different types of soils have the same type of IR spectra, which allows to speak about the general way of their genesis. IR spectra are used as a characteristic diagnostic sign of humic acids, that makes it possible to reveal some features associated with the conditions of their formation (Shevtsova et al., 2019; Starykh et al., 2019; Zherebtsov et al., 2019).

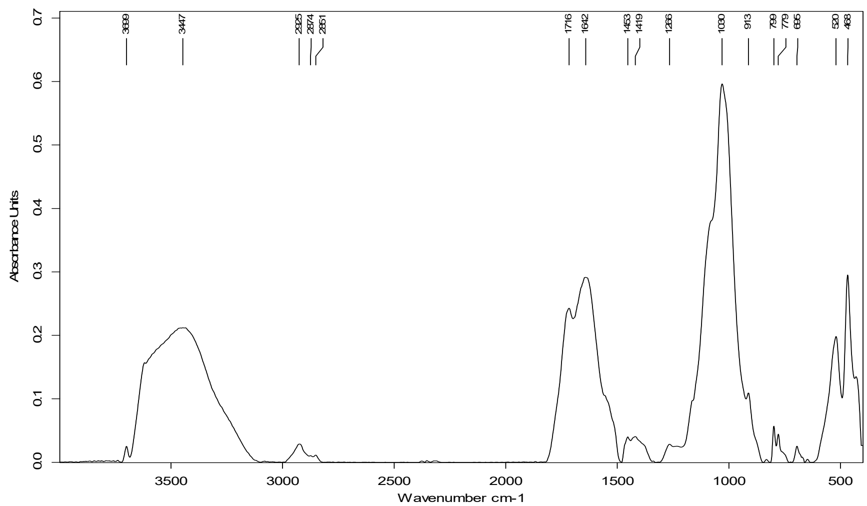


Figure 1. Infrared spectrum of humic acids in sod-podzolic soil under the mixed forest

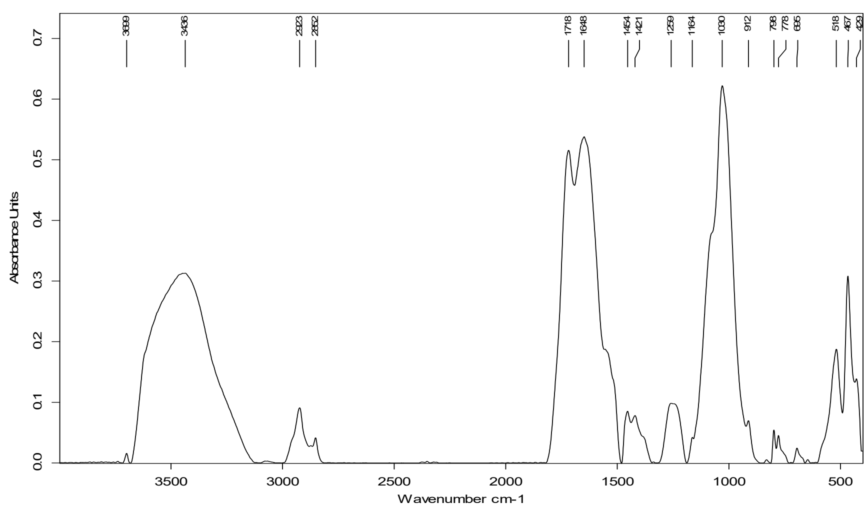


Figure 2. Infrared spectrum of humic acids in sod-podzolic soil under poaceous-forb meadow

The IR-spectra of humic acid preparations from the soil under mixed forest and poaceous-forb meadow are typical for sod-podzolic soils (Fig. 1, 2). They are characterized by a large set of absorption bands. Absorption bands in the region of 2800-3000 $cm^{-1}$ were resulted from valence vibrations C-H of methyl ($CH_3$) and methylene ($CH_2$) groups. A stronger absorption in this region was observed in the spectrum of HAs in the soil under poaceous-forb meadow, what indicates a greater amount of aliphatic components of relatively low molecular weight in the structure of supramolecular aggregates compared with HA under the mixed forest. A wide absorption band in the zone of 3300-3500 $cm^{-1}$ is responsible for hydrogen bonds. Intensive absorption in the region of 1700-1720 $cm^{-1}$ was owing to wavering of the >C=O groups of carboxylic acids.

The presence of aromatic groups in HA aggregates was indicated by the absorption band at 1605-1670 $cm^{-1}$, which was owing to valence vibrations of conjugated double bonds of carbon atoms. The band (shoulder) in the region of 1510 $cm^{-1}$ indicated the presence of aromatic C=C bonds in the macromolecule composition but its intensity was weak. Absorption in the zone of 1400–1470 $cm^{-1}$ can be attributed to deformation vibrations of the C–H bond in $CH_2$ groups. Absorption bands with a maximum at 1200-1280 $cm^{-1}$ were resulted from wavering of the C–O bond of simple ethers and similar compounds. It is possible that absorption in that region was caused by asymmetric valence vibrations in the C-O-C groups, which was confirmed by the characteristic symmetric vibrations of this group in the region of 1030 $cm^{-1}$.

The investigated humic acids of the soils in long-term experiment have absorption bands in a wide wavelength range from 500 to 4000 cm-1. The studied methods of land use had little effect on the presence of the most characteristic atomic groups and the intensity of the absorption bands resulted from the fluctuations of these groups (Fig. 3-7). The intensive absorption band at 3436-3465 cm-1 was owing to valence wavering of OH groups linked by intermolecular hydrogen bonds.

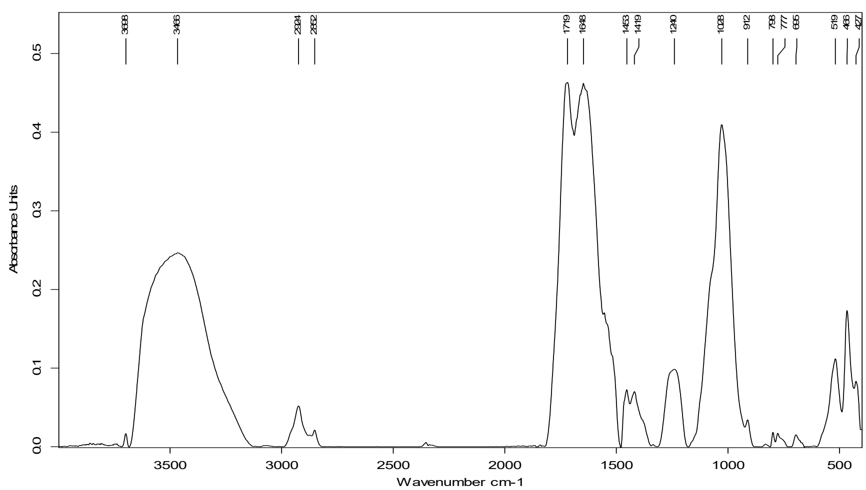


Figure 3. Infrared spectrum of humic acids in sod-podzolic soil under continuous barley

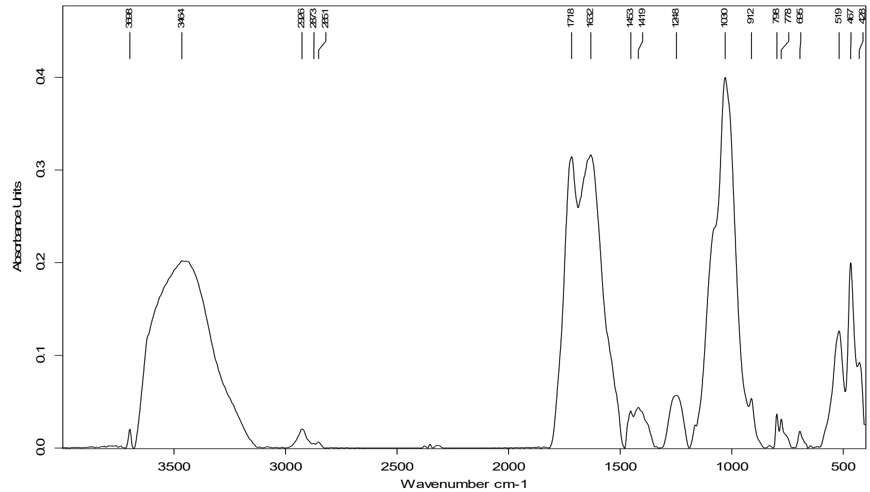


Figure 4. Infrared spectrum of humic acids in sod-podzolic soil under grain crop rotation (no legumes)

Absorption in that area was maximum for humic acids of the soil under typical crop rotation, minimum - for neglected field soil. Absorption bands (2929-2924 and 2849-2875 $cm^{-1}$) were resulted from C-H valence vibrations of methyl ($CH_3$) and methylene ($CH_2$) groups. These absorption bands proved themselves most intensively in the spectra of soil humic acids in typical crop rotation, which indicated the presence of sufficient amount of terminal methyl groups in the structure of these acids. The intensity of that band was about the same for the soil HA in the grain crop rotation and the crop rotation with high content of legumes, what can be explained by the destruction of aliphatic structures and, consequently, by decrease in the content of methyl and methylene groups in them. In the case of humic acids in typical crop rotation, the opposite trend was observed, that is, the absorption intensity of these bands increased due to content raise of aliphatic groups in them. The presence of the above groups was confirmed by absorption bands in the zone of 1454-1418 $cm^{-1}$.

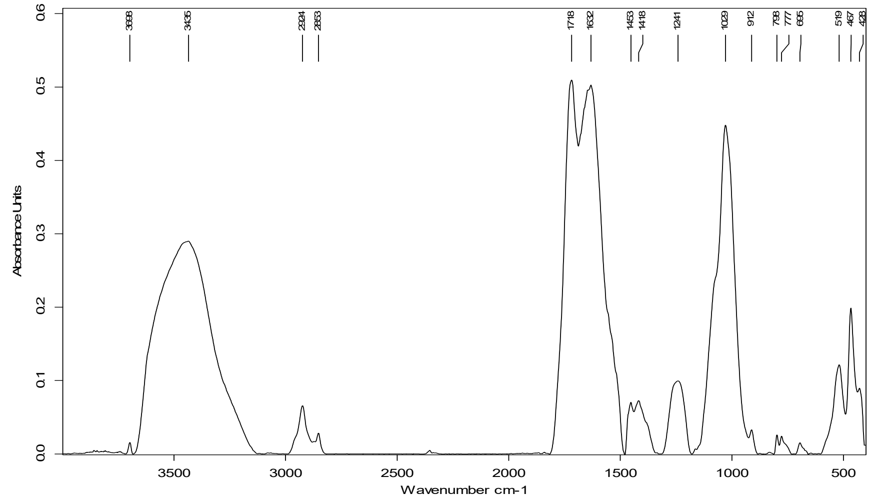


Figure 5. Infrared spectrum of humic acids in sod-podzolic soil under seven-course crop rotation

( 28.6 percent legumes)

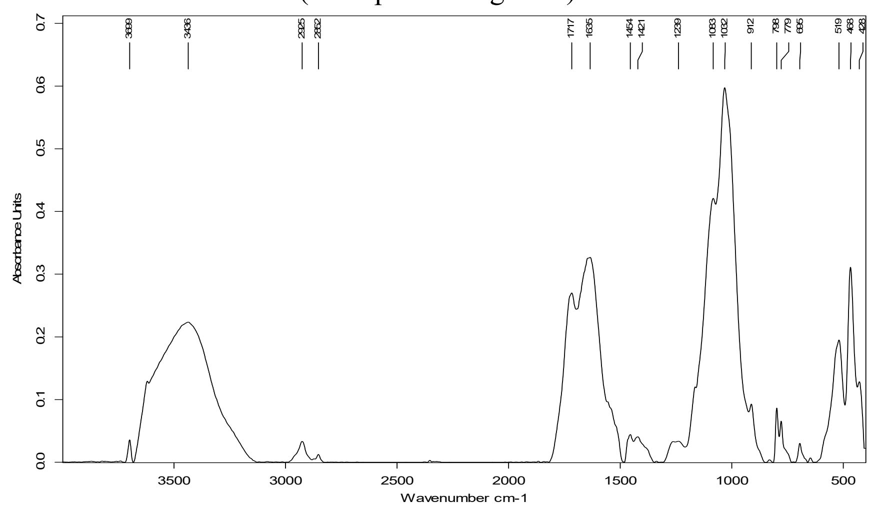


Figure 6. Infrared spectrum of humic acids in sod-podzolic soil under seven-course crop rotation ( 42.9 percent legumes)

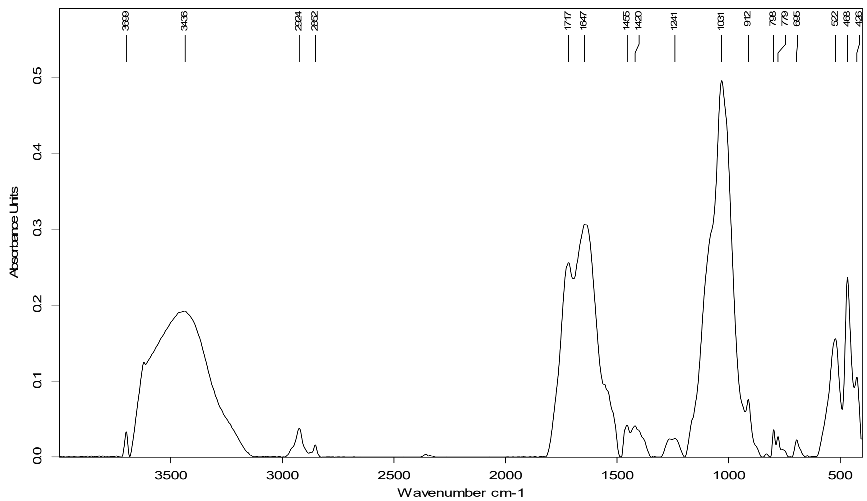

Figure 7. Infrared spectrum o f humic acids in sod-podzolic soil under neglected field

In the range of wave numbers 1800-1300 $cm^{-1}$, the most clear and intense were the absorption bands 1718-1719 and 1632-1647 $cm^{-1}$, which were present in humic acids in all variants. That may be explained by the presence of carboxylate ions and bending vibrations of $NH_2$ amides (Amide II band). The band at 1719–1717 $cm^{-1}$ was owing to >C=O bond vibrations of carboxylic acids (Orlov, 1990).

The severity degree of this band affirmed that soil humic acids under the conventional crop rotation and continuous barley have the largest number of carboxyl groups in their content. The weakest fluctuations of the >C=O group of carboxylic acids were expressed in soil humic acids under crop rotation with a high (42.8 percent) share with legumes and in neglected field soil. The rest of the absorption bands can be associated with deformation vibrations of $NH_2$ amides (bands Amide I; Amide II) – compounds of amino acids type.

Based on the general form of the spectra, it can be assumed that the composition of humic acids of the studied variants contains nitrogen-containing structures of the amino acid type. The absorption band in the range of 1647-1632 $cm^{-1}$ was owing to valence wavering of aromatic structures C=C group in supramolecular aggregates of humic acids. That band was most intensely expressed in the IR spectrum of conventional crop rotation, and the least in the spectrum of humic acids in neglected field soil. In the range of wave values 1300–500 $cm^{-1}$, the interpretation of absorption bands is rather difficult. In this area, oxygen-containing groups of various nature (alcohols, ethers, phenols), that is, OH groups, can appear. It can be assumed that within the wave numbers of 1000-1300 $cm^{-1}$, the absorption bands showed the presence of oxygen-containing groups of HA.

## CONCLUSION

Different types of land using caused changes of different intensity and direction in condition of studied sod-podsolic soil. The humic acids separated from arable soil and its virgin analogs in long-term experiment, in terms of the content of basic elements (C, H, N, O, S), corresponded to the average values for humic acids in sod-podzolic soils. The carbon content in the HAs of arable and virgin sod-podzolic soil was 28.3-33.5, hydrogen 40.1-47.5, oxygen 19.6-27.9, nitrogen 2.1-2.5 and sulfur 0.1-0.2 at. percent. Aliphatic fragments predominated in the HA structure (H / C ratio - 1.20-1.67). Differences in the HA in arable and virgin soils were resulted from the qualitative content of organic matter input to the soil, its quantity and the intensity of anthropogenic impact. It was found that the saturation of field crop rotation with legumes up to 42.9 percent make it possible to increase the share of aliphatic groups in the structure of soil humic acids (the maximum H/C ratio of 1.67 was noted). The use of five-course grain crop rotation promoted to increase the ratio of stable aromatic structures (the minimum H/C ratio 1.20 was noted), that indicates the predominance of organic matter mineralization processes over the accumulation of humic substances. The depletion of the HA structure with nitrogen was noted in the soil under grain crop rotation, (the maximum C/N ratio was noted - 16.2). The O/C ratio of HA in the experiment increased with a decrease in the anthropogenic impact on the soil and was maximum in the soil HA under neglected field 0.95. There high degree of oxidation of humic acids (W=+0.53) was noted, what may indicate the most favorable conditions for the humification of organic matter. Humic acids separated from the soil in different treatments of long-term experiment had the same type of IR spectra. Based on the presence of absorption bands in the range 1718–1719 $cm^{-1}$ (wavering of the C=O group of carboxylic acids) and 1632–1647 $cm^{-1}$ (valence vibrations of conjugated double bonds), it can be assumed that aromatic structures were presented in supramolecular aggregates, what was more intensely expressed in the spectra of soil humic acids under conventional crop rotation and continuous barley.

The aggregates of soil humic acids in crop rotation soil with high saturation with legumes and soil under mixed forest had more branched structure, apparently, they consist from a larger number of individual monomers of various molecular weights with large number of aliphatic groups. That was confirmed by intense absorption in the range 2929-2849 and 1400-1470 $cm^{-1}$, caused by valence vibrations of methyl and methylene groups.

The predominance of organic matter mineralization processes over the accumulation of humic substances was noted in the soil under grain crop rotation and under continuous barley, where the loss of carbon over 41 years of the experiment was 7-9 percent compared with the initial level. The most favorable conditions for the humification of organic matter was noted in the soil under neglected field and mixed forest. The soil under conventional seven-course field rotation with legumes and under poaceous-forb meadow was characterized by relatively favorable parameters, these variants occupy an intermediate position among all studied treatments.

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