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EFFECT OF SOIL TILLAGE INTENSITY ON THE COMPOSITION, PHYSICAL AND CHEMICAL PROPERTIES OF THE SECOND FRACTION OF HUMIC ACIDS EXTRACTED FROM THE TYPICAL BLACK SOIL**M.A. Popirny¹, O.T. Nikolov², E.V. Skrylnik¹**¹*National Scientific Center «O. N. Sokolovsky Institute for Soil Science and Agrochemistry Research»,
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The purpose of this work lies in studying the composition, physical and chemical properties of the second fraction of the humic acid (HA2) extracted from the typical black soil, depending on the intensity of the soil tillage. Physical and chemical properties of HA2 have been studied by using ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy and electron spin resonance spectroscopy (ESR). It has been demonstrated that increase of the tillage intensity of the typical black soil leads to decrease in the total carbon content and the HA2 content due to displacement of balance between the conjugated processes of humus synthesis and decay towards destruction. UV spectroscopy indicates intense absorption of humic acids in the near UV region, that corresponds to the donor-acceptor mechanisms π - π^* electrons of double bonds. Maximum absorption has been observed for the HA2 isolated from the black soil tilled by plowing. Moreover, the highest content of oxygen-containing functional groups and low quantity of aliphatic structures has been observed in the same sample. It has been shown that the nature of paramagnetic centers of the HA2 represented by chelate paramagnetic Fe^{3+} -complex formed by rearrangement π -electron system of double bonds, depending on the tillage intensity of the typical black soil. It has been found that plowing of the typical black soil enhances the number of paramagnetic centers in HA2.

KEYWORDS: organic soil matter, humic acid, soil tillage, supramolecular structure of the humic acid, aromatic system, carboxyl groups, chelate complexes.

ВПЛИВ ІНТЕНСИВНОСТІ ОБРОБІТКУ ҐРУНТУ НА СКЛАД, ФІЗИЧНІ ТА ХІМІЧНІ ВЛАСТИВОСТІ ДРУГОЇ ФРАКЦІЇ ГУМІНОВИХ КИСЛОТ, ЕКСТРАГОВАНИХ З ЧОРНОЗЕМУ ТИПОВОГО**M.A. Попірний¹, O.T. Ніколов², Є.В. Скрильник¹**¹*Національний науковий центр «Інститут ґрунтознавства та агрохімії імені О. Н. Соколовського»,
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Метою даної роботи було вивчення складу, фізичних та хімічних властивостей другої фракції гумінових кислот (ГК2), виділеної з типового чорнозему, в залежності від інтенсивності обробітку ґрунту. Фізичні та хімічні властивості ГК2 вивчено за допомогою ультрафіолетової (УФ) спектроскопії, інфрачервоної (ІК) спектроскопії і спектроскопії електронного парамагнітного резонансу (ЕПР). Було показано, що збільшення інтенсивності обробітку чорнозему типового призводить до зниження загального вмісту вуглецю і вмісту ГК2 через зсув рівноваги між сполученими процесами синтезу та розпаду гумусу в напрямку розпаду. УФ-спектроскопія вказує на інтенсивну адсорбцію гуміновими кислотами в ближній УФ-області, що відповідає донорно-акцепторним механізмам π - π^* електронів подвійних зв'язків. У зразку ГК2, виділеному з чорнозему після оранки, спостерігалось максимальне поглинання в ближній УФ-області. До того ж, в тому ж самому зразку ГК2 спостерігається найбільший вміст кисневмісних функціональних груп з низькою кількістю аліфатичних структур. Було показано, що природа парамагнітних центрів ГК2 представлена хелатними парамагнітними Fe^{3+} -комплексами, що утворюються за рахунок перегрупування π -електронної системи подвійних зв'язків, яке залежить від інтенсивності обробітку чорнозему типового. Було встановлено, що оранка типового чорнозему збільшує кількість парамагнітних центрів в ГК2.

КЛЮЧОВІ СЛОВА: органічна речовина ґрунту, гумінові кислоти, обробіток ґрунту, супрамолекулярна структура гумінових кислот, ароматична система, карбоксильні групи, хелатні комплекси.

ВЛИЯНИЕ ИНТЕНСИВНОСТИ ОБРАБОТКИ ПОЧВЫ НА СОДЕРЖАНИЕ, ФИЗИЧЕСКИЕ И ХИМИЧЕСКИЕ СВОЙСТВА ВТОРОЙ ФРАКЦИИ ГУМИНОВЫХ КИСЛОТ, ЭКСТРАГИРОВАННЫХ ИЗ ЧЕРНОЗЕМА ТИПИЧНОГО

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Целью данной работы было изучение состава, физических и химических свойств второй фракции гуминовых кислот (ГК2), выделенной из типичного чернозема, в зависимости от интенсивности обработки почвы. Физические и химические свойства ГК2 изучали с помощью ультрафиолетовой (УФ) спектроскопии, инфракрасной (ИК) спектроскопии и спектроскопии электронного парамагнитного резонанса (ЭПР). Было показано, что увеличение интенсивности обработки чернозема типичного приводит к снижению общего содержания углерода и содержания ГК2 через смещение равновесия между сопряженными процессами синтеза и распада гумуса в сторону распада. УФ-спектроскопия указывает на интенсивную адсорбцию гуминовыми кислотами в ближней УФ-области, что соответствует донорно-акцепторным механизмам π - π^* электронов двойных связей. В образце ГК2, выделенном из чернозема после вспашки, наблюдалось максимальное поглощение в ближней УФ-области. К тому же, в том же образце ГК2 наблюдается наибольшее содержание кислородсодержащих функциональных групп с низким количеством алифатических структур. Было показано, что природа парамагнитных центров ГК2 представлена хелатными парамагнитными Fe^{3+} -комплексами, образующимися за счет перегруппировки π -электронной системы двойных связей, которое зависит от интенсивности обработки чернозема типичного. Было установлено, что вспашка типичного чернозема увеличивает количество парамагнитных центров в ГК2.

КЛЮЧЕВЫЕ СЛОВА: органическое вещество почвы, гуминовые кислоты, обработка почвы, супрамолекулярная структура гуминовых кислот, ароматическая система, карбоксильные группы, хелатные комплексы.

The humic acids (HA) are a major component of the soil organic matter (humus substances) playing multifunctional role due to variable composition of the HA structure that results in a wide range of biotic and abiotic factors [1]. Physicochemical properties of the HA reflect the structural features of the HA. In the HA structure, the central structure (core) and peripheral parts are identified, each part has a variable composition depending on the type of soil and external environment. The variable composition of the HA depends on extracting methods from the soil (or another source of natural organic matter) and future purification, modification. The central structure (core) is formed by aromatic rings with various functional groups (the most important are carboxyl, phenol hydroxide, ketone, amide ones) and the peripheral parts by aliphatic polypeptide chains [2, 3]. Due to functional groups reactivity of HA is determined. Such structural features determine the chemical, physical and biological properties of the HA, specifying their redox activity, etc. [3]. The HA actively interact with different mineral components of the soil making complexes with mono-, di- and trivalent metals, thereby transforming the elements inaccessible to plants into the available state. In a certain range of pH, the humic organometallic complexes acquire high mobility and can be absorbed by the plant root hairs [2, 4]. It has been found that increasing complexation with the two- and trivalent metals (Ca^{2+} , Mg^{2+} , Fe^{3+} , Al^{3+}) is associated with increase of proportion of the oxygen-containing functional groups and decrease of the nitrogen-containing groups [5]. Iron ions can displace hydrogen in the carboxyl groups of the HA and form complexes such as chelates [1, 2, 4].

Current comprehension of the structural and functional features of the HA is based on the notions of self-organization of supramolecular complexes from smaller molecular associates of the HA. The main aspect of humic supramolecular structure is its stabilization by weak intermolecular interactions, such as hydrophobic, van der Waals, and hydrogen bonds, the formation of complex macrostructures providing for multifunctional properties of the soil

humic substances [6, 7, 8]. The HA are formed due to conjugated biosynthetic processes (decomposition and synthesis in bioorganic soils matter), mainly of the products of biodegradation of plant organisms, accumulating and stabilizing the HA, protecting the soil organic matter from total mineralization (destruction of organic soil matter to carbon dioxide and water) [6].

Biodegradation of plants crop residues and their subsequent participation in synthesis of the HA occurs by enzymatic activity of the soil microbial community, without which processes of humic acids formation and formation of the fertile humus layer of soil [1] are not possible. Soil fertility is determined by the content of humus, which, in its turn, is stipulated by the quantitative and qualitative features of the HA. The black soils, forming agronomically valuable soil structure, are the richest in organic soil matter content [1, 2, 8].

It is known that the intensive use of black soils of Ukraine gives rise to a significant deterioration of their fertility [8]. One of the most important diagnostic signs of the soil degradation is decrease of content of the soil organic matter, and its main component - humus substances [9]. Reduction of the amount of the soil organic matter and deterioration of its quality characteristics may be subject to a lack of constant compensation of organic compounds, and result in change of the ratio between mineralization of the soil organic matter and formation of the stabilized soil humic substances [9, 10]. An informational indicator of the agrogenic load on the soil quality is features of the HA fractions [10, 11].

According to the modern concepts, based on the results of the long-term field experiments with application of different agricultural technologies, the transformation of soil organic matter following the agronomic activity is observed predominantly in the labile fraction of the humic soil acids (the first fraction of the HA1) [12, 13]. Experiments on different soils have revealed a close correlation between the productivity of agricultural ecological systems and contents of labile fraction of the HA1 [10, 13]. Deviation from the optimal soil ecosystem (for example, from the virgin soil) is subject to increases of the process of the organic matter mineralization and impaired fertility, causing lower yields [11, 12]. Pursuant to some authors, for assessment of soil fertility it is important to consider not only the labile fraction of the HA1, but also a more stable and biochemically mature component of humus – the second fraction of the HA2 [10, 13]. Despite a vast amount of information on changes in the content of fractions of humic acid under the influence of the agricultural use of soil, the attention of researchers is attracted by mechanisms of responses at the molecular level of the HA. In our opinion, to establish responses at different levels to anthropogenic load it is necessary to study physical and chemical properties of the isolated HA fractions. Among the physical and chemical research methods UV/IR/ESR spectroscopy provides valuable information about the molecular structure of the HA. The main advantage of the above methods is to carry out an analysis without any prior sample preparation and the HA modifications bringing about to irreversible structural changes of the native nature of macromolecules.

The purpose of this work has been to study the content and physicochemical properties of the second fraction of the unmodified HA2 extracted from the typical black soil depending on intensity of the tillage technologies.

MATERIALS AND METHODS

Selection and laboratory-analytical analysis of soil samples

The samples of soil have been collected in the experimental field of the V.V. Dokuchayev Kharkiv National Agrarian University, the Department of Agriculture. The aim of experiments has been to determine the effect of intensity of the tillage technologies on

soil organic matter characteristics of the typical black soil (chernozem). The field experiment was started in 2006. The variants of the tillage investigated in the field experiment are as follows: 1) 40 year non-tilled typical black soil - virgin soil (control); 2) soil intensively tilled (traditional plowing depth of 20-22 cm); 3) minimally tilled soil (disks at the depth of 10-12 cm); 4) Non-tilled soil (provided by direct seeding into the non-tilled soil). Soil samples have been collected from the surface soil layer of 0-10 cm. The total carbon content in the soil has been determined by Tyurin [14], total nitrogen – by Kjeldahl method [14]. Mineralization (rate of biodegradation of the soil organic matter) has been calculated by the ratio of the total soil carbon content to the content of total nitrogen (C/N); increase of the parameter indicates a decrease in the degree of mineralization of the soil organic matter. The content of agronomically valuable fractions of HA in the soil has been determined by the method of humus fractionation [15].

Extraction of second fraction of the HA from the soil samples

Collected soil samples have been dried to the air-dry condition, crop residues have been removed and then the soil samples have been sieved through a sieve with 1 mm pore diameter. Extraction of HA2 fraction has been carried out by the method of Orlov [16]. The prepared soil samples have been subjected to decalcification by sulfuric acid, cleaned from calcium, and then washed with distilled water. Humic acid has been then dissolved in 0.1 N NaOH. After sedimentation for 24 hours, the centrifugation (3000 rpm, 10 min) for separation of clay minerals has been performed. Precipitation of humic acids has been carried out with 10% HCl, adjusting the solution to pH 1. Then the HA fractions have been washed with distilled water. The obtained gel of the HA has been crystallized in the water bath and dried in the oven at 80 C° (2 hours) to the air-dry state. A black crystalline powder – preparation of the second fraction of the HA of typical black soil has been the result.

Physicochemical methods of analysis of the second fraction of the HA

The Fourier Infrared Spectroscopy (FT-IR) of the second fraction of humic acids has been performed by the Agilent Technologies Cary Spectrometer 630 supplied with Diffuse Reflectance and Fourier Transformation in the standard measurement area of 1000-4000 cm⁻¹ (in a potassium bromide compressed tablet). Interpretation of the spectra has been performed on the characteristic absorption spectra of humic substances [1, 11, 16].

The electron spectra in the UV absorption of humic acids of the second fraction have been measured on the spectrometer Stellarnet BLACK-Comet in the range of 90 nm – 445 nm. To perform spectroscopic analysis, the HA have been dissolved in 0.1 N NaOH [16].

Analysis of the electron spin resonance (ESR) characteristics has been performed using spectrometer JES-ME-3x, which operates in the three-centimeter wavelength range (X-band) with a frequency modulation of 100 kHz. The weight of samples of humic acids for ESR spectroscopy is 100 mg, as placed in a quartz ampoule produced by JEOL Company. The sample of polycrystalline of diphenylpicrylhydrazyl (DPPH) has been used as a standard for assessment of the g-factors and intensities of the ESR spectra; the average value of the g-factor has equaled to 2.0036 ± 0.0003 . The samples in quartz ampoules have been placed in the antinode of the microwave magnetic field of the resonator of the ESR spectrometer. ESR has been performed at room temperature 12 hours after sample preparation. Concentration of paramagnetic centers in the HA2 samples has been determined by comparing the relative intensities of the signals (Tesla) with the value by the equation $S=(\Delta H)^2 I$, where ΔH is the width, and I is the amplitude of the line. The statistical error in assessment of the concentration of paramagnetic centers by ESR method is 10-20 %.

RESULTS AND DISCUSSION

Content of the fractions of humic acids and biological activity of the black soil

The total carbon content in the soil sample from virgin soil has been 3.1% (Table 1). It has been found that the total carbon content of the typical black soil has been significantly reduced with the intensive tillage (plowing) to 2.8%. The minimum tillage (disking) has reduced it to 2.9% and zero tillage (direct seeding) to 2.8% (Table 1). Therefore, the minimal tillage results in conservation of organic soil matter.

It has been shown that the intensive tillage results in an increase in the content of HA1 (18.8%) in the soil, at the same time the direct seeding results in reduction of the movable HA1 (7.0%) (Table 1). The minimal tillage (disking) results in the HA1 content (16.1%) that is close to the value of HA1 after plowing (18.8%). It has been found that minimization of processing (disking and direct seedling) results in accumulation of the HA2 on the level close to its content in the virgin typical black soil (39.8%). Plowing causes a significant reduction in the HA2 (22.7%).

Table 1

Total carbon content (%) in the first fraction of humic acids (HA1) and the second fraction of humic acids (HA2) in the typical black soil depending on the soil tillage intensity

Values	Variants of tillage technology			
	Virgin soil (Control)	Intensive tillage (Plowing)	Minimal tillage (Disking)	No tillage (Direct seeding)
C _{total} , %	3.1±0.5	2.8±0.4	2.9±0.5	2.8±0.4
HA1, %	12.4±2.0	18.8±3.2	16.1±5.3	7.0±1.6
HA2, %	39.8±6.2	22.7±5.4	33.9±5.9	39.9±6.3

The biological parameters of the typical black soil of the experimental field show that the tillage of the typical black soil causes changes in the diversity of the soil microbial populations (Table 2), [18].

Table 2

Indexes of biodegradation (C/N) assessed in our work, and biochemical activity of organic soil matter of the typical black soil [18]

Values	Plowing	Disking	Direct seeding
C/N	10.5	11.9	11.7
Biogenic diversity, % [18]	31.6±1.6	26.30±1.2	98.0±4.0
Invertase (mg/100 g of soil) [18]	290.0±5.0	305.0±5.0	435.0±6.5
Polyphenoloxidase (mg/100 g of soil) [18]	4250±80	4317±75	4342±75

The total index of biogenic diversity shows that the typical black soil, after direct sowing, is less susceptible to changes in the microbial community [18]. In our work, the rate of biodegradation of humus in the typical black soil by microbial populations has been estimated (Table 2, C/N index). It has been found that the minimal tillage reduces the intensity of decay of soil organic matter in the typical black soil. The intensive tillage, on the contrary, increases it, thereby shifting the conjugated processes of synthesis and decay of humus towards

destruction (Table 2). The indicators of biochemical activity of the typical black soil depend on the intensity of tillage in the experimental field (Table 2), [18]. It is known that content of the soil humic acids correlates with the activity of enzyme invertase that hydrolyzes carbohydrates, peptides and aliphatic structures, and with the polyphenoloxidase activity, that is involved directly in the oxidative polycondensation of the aromatic system of the humic acids [1]. It has been shown that the activity of invertase and polyphenoloxidase is reduced with the intensification of the typical black soil tillage (Table 2), [18]. We have used physicochemical methods to investigate processes of formation and change of the HA2 of the typical black soil, depending on the tillage intensity.

UV spectroscopy of the second fraction of humic acid (HA2), depending on intensity of the typical black soil tillage

Fig. 1 shows results of the analysis of UV spectroscopy of the HA2 isolated from the typical black soil under different tillage intensities. The HA2 of the typical black soil absorb in the near UV range (275 nm – 350 nm). After the intense plowing, the intense absorption at bands 280 nm, 310 nm, 340 nm is observed.

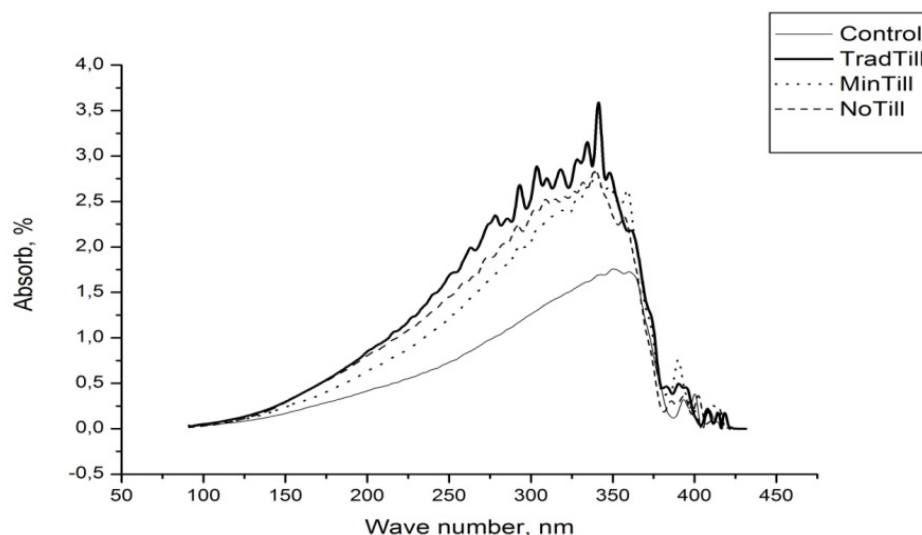


Fig. 1. UV absorption spectra of HA2, depending on the tillage intensity of the typical black soil: Control – virgin soil; No Till – direct seeding; Min Till – disking; Trad Till – plowing.

It is known that intense absorption and appearance of the "shoulders" at the spectra of the HA (in the area of 270 nm – 300 nm) are caused by quinones and chromophore functional groups (oxygen and nitrogen-containing) – polyconjugated bonds of aromatic system of HA2, having $\pi-\pi^*$ electronic transitions. The intense absorption band (at 310 and 340 nm) of the HA2 isolated from the typical black soil after plowing corresponds to the donor-acceptor mechanisms as caused by $\pi-\pi^*$ electronic transitions in the ring system of the conjugated bonds, in chelate complexes with transition metals.

FT-IR spectroscopy of the second fraction of humic acids (HA2)

In the FT-IR spectra of the humic acids similar characteristic absorption bands that show similar structural elements and functional groups co-existing in these macromolecules are identified (Fig 2).

The hydroxyl group (-OH), hydrogen bonds (H-bonds) absorb in range of 3600 cm^{-1} – 3400 cm^{-1} ; doublet of aliphatic structural components (-CH₂, -CH₃) – at 2900 cm^{-1} and 2800 cm^{-1} ; oxygenated aromatic carboxyl group of (-COOH) and ketone (C=O) at 1710 cm^{-1} ,

1715 cm^{-1} and given by quinon $-\text{OH}$ group; aromatic double bond system with $\text{C}=\text{C}$ – at 1625 cm^{-1} , and an absorption band corresponding oxygen-containing groups substituted with metal ions – at 1333 cm^{-1} . The appearance of 3137 cm^{-1} band is based on the interaction of the OH -group with metals (for example Fe^{3+} or Al^{3+}) forming the various complex compounds. This wave number is also associated with presence of the H-bonds, stabilizing individual macromolecular structure of the HA2 depending on the dimensional configuration of such structures [4].

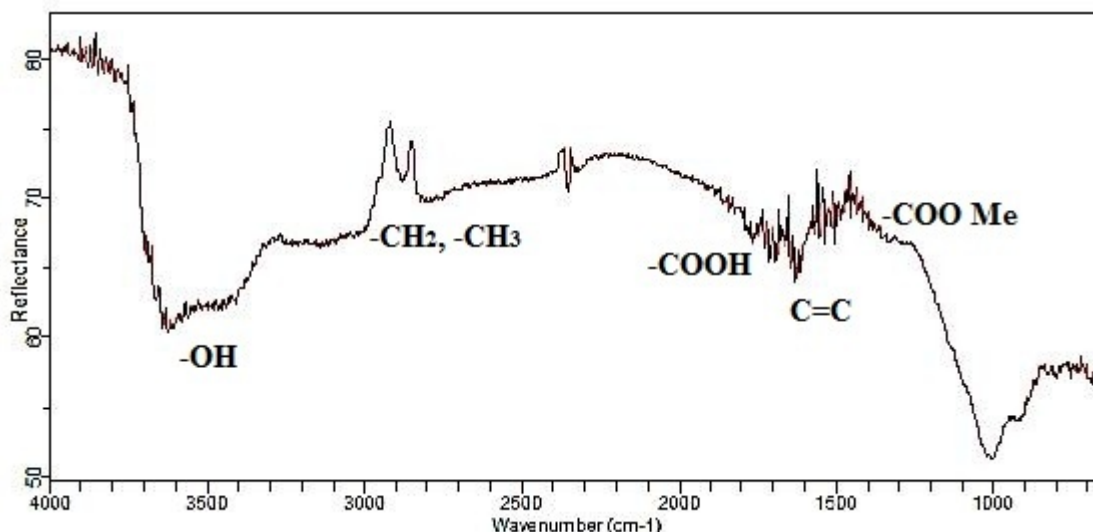


Fig. 2. FT-IR spectrum of HA2 isolated from the typical black soil under intensive tillage (plowing).

Analysis of the structural elements in the HA2 isolated from virgin soil indicates a high content of aliphatic and aromatic structures. Intense absorption of the carboxyl group is shown in aromatic system of the HA2 as extracted from the black soil after plowing. The intense band 1333 cm^{-1} corresponds to formation of metal complex, which is caused by a complete replacement of the carboxyl group and a greater degree of ionization of the oxygen-containing functional groups. The HA2 fractions isolated from the disked black soil are characterized by a greater degree of aliphatic groups and a lower degree of oxygen-containing groups in the aromatic system. The HA2 fractions isolated from the typical black soil under zero tillage technology are characterized by a lower diversity in the structural and functional elements.

ESR spectroscopy

Results of examining the HA2 through ESR are shown in Fig. 3. The resulting ESR spectra have the g -factor of 2.0036 close to the g -factor of DPPH, and the broad line between the wide range of absorption curves (inflection points of the absorption curve are distanced from antinodes approximately at 0.1 Tesla). Components of the fine and hyperfine structure are not available in the obtained spectra. In the area of the g -factor of DPPH 2.0036 there is a slight narrow "shoulder", corresponding to the signal of semiquinone radicals of the HA2 as a result of imposition of the broad line of signal that has been shown in some studies by ESR spectroscopy of humic acid [2, 11, 17, 19]. Availability of the narrow signal indicates to the HA2 aromatic structure being formed due to oxidative condensation of quinones (lignin of the plant tissue residue) by semiquinone radicals. Formation of the aromatic structures is catalyzed by the enzyme polyphenoloxidase.

Form of lines of all the ESR spectra is independent from the tillage technology. The said spectra differ only by intensity, and accordingly to the number of paramagnetic centers (Fig. 4). We have found that plowing causes a significant increase in paramagnetic signal of the HA2, due to increase in the number of paramagnetic centers (paramagnetic chelate complexes) (Fig. 4).

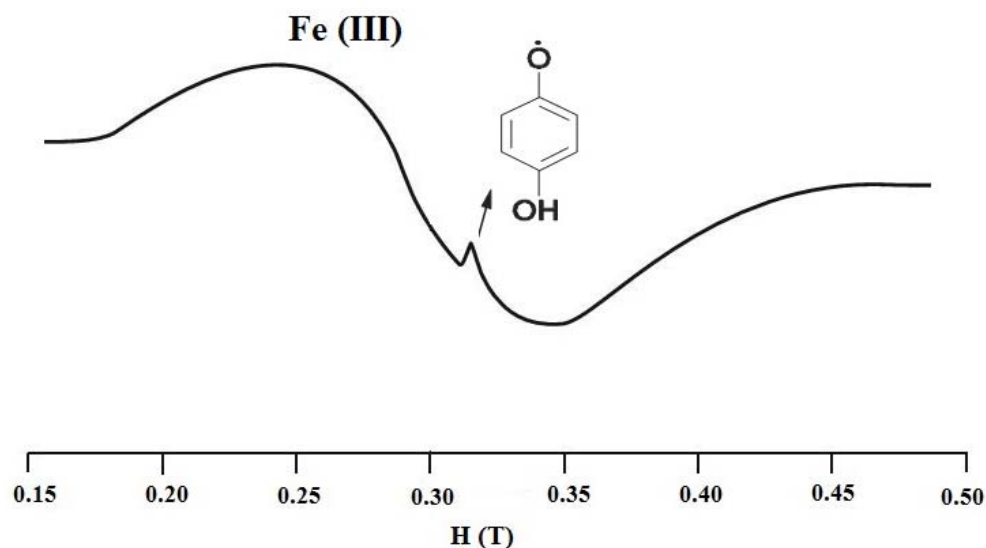


Fig. 3. ESR spectrum of HA2 from virgin soil. H(T) is measured in Tesla.

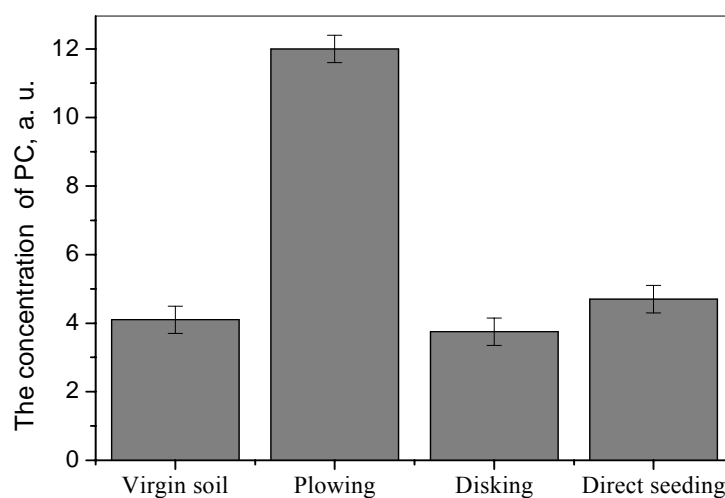


Fig. 4. Concentration of paramagnetic centers (PC) in arbitrary units in HA2 depending on the tillage intensity of the typical black soil.

Dynamics of the content of total carbon in the HA fractions of the typical black soil (Table 1) point out to a formation of mobile humic compounds (increase of content the HA1 in humus) and reduction of total carbon at intensification of tillage of the typical black soil, that is caused by a more intense biodegradation of the soil organic matter. The rate of decay process of organic matter by enzymes depends on the overall ratio of carbon to nitrogen (C/N) [20]. The maximum rate of the biodegradation of the humus typical black soil is observed after plowing (Table 2, C/N ratio). Thus, plowing causes displacement of the coupled processes of synthesis and decomposition of humus to the decay as associated with the change of microbial community (Table 2, index of diversity), which gives rise to a change in the

enzymatic activity of important enzymes like polyphenoloxidase and invertase performing the formation of humic acids (Table 2).

Less intensive tillage of typical black soil (disking) has caused a lower mineralization and some increase in total carbon accumulation of the HA2 and some smaller quantity of the HA1 as compared to plowing (Table 1). Importantly, a low content of the HA1 and a high content of the HA2 at direct seedling (Table 1) point out to a formation of more inert humic acids that reduce the overall mobility of the humic system and results in formation of structures of the soil available to plants.

The virgin typical black soil, which is a natural ecological system, is characterized by a more balanced state, caused by the shift of equilibrium between the synthesis and decay of humus in soil, to the accumulation of humus (total carbon). Minimizing of tillage (disking and direct seeding) causes a decrease of humus decay. At direct seeding an accumulation of inert humus is observed. At disking, its quality indicators are more favorable for development of plants. It is known that tillage induces changes of physical properties of the soil (contents of the air and moisture) and leads to a redistribution in population of microorganisms by mechanical tillage of the black soil [21]. Plowing leads to the improved aeration and causes activation of aerobic microorganisms that mineralize the soil organic matter, that activate the HA formation processes, as indicated by an increase of the HA1 after plowing and disking.

It has been found that intensification of tillage of the typical black soil induces strong absorption of aromatic structures of the HA2 in the UV region (Fig. 1), caused by electronic π - π^* transitions. It is known that the π - π^* electronic transition is associated with the transition between the main and excited states of humic acids molecules, featuring the energy transition [22]. As a result, the unpaired electrons are moved throughout the system of double bonds. Hyperchromic effect in the HA2 after plowing is caused by a more active mobility of the π -electron system, which is due to the donor-acceptor interactions between the HA structure and coordinated paramagnetic transition metals [22]. According to the results of FTIR spectroscopy, appearance of a clear signal in the 1330 cm^{-1} and absence at $1720\text{-}1710\text{ cm}^{-1}$ (Fig. 2) bear evidence of the formation of complexes of carboxylic acid/ketone groups with metals. The OH groups in the HA2 react with metal ions Fe^{3+} and Al^{3+} (Fig. 2). The absorption ratio of the structural elements in the HA2 testifies to a decrease in the proportion of aliphatic structures with the tillage intensification, due to the increased mineralization process, bringing about to an increase in the content of carboxyl and ketone groups. The content of aliphatic structures and oxygen-containing groups of the aromatic system increases after disking. A smaller variety of structural elements is detected in the HA2 of typical black soil after the non-tillage technology, due to the formation of inert humus substances. Subject to the fact that the general shape of the ESR spectrum's line is not affected by the soil treatment intensity, and differs only by the spectrum intensity (Fig. 3), we can conclude that paramagnetic centers of the same structure (one type of paramagnetic centers) in the HA2 tested samples are formed. For example such a structure can be represented by paramagnetic centers formed by complexes with paramagnetic metals of intermediate group. It may be (Fe^{3+}), since it is known that at room temperature the ESR signal of Fe^{2+} is not detected [23].

The broad line in ESR spectrum of humic acids with g-factor of 2.0023 is associated with the paramagnetic centers, which are represented by complexes with paramagnetic metals. It is known that paramagnetic Fe^{3+} refers to groups with the electron configuration of the ions that are in the S-state (spin moment of 6 (5/2) for Fe^{3+} and Mn^{2+}) [23, 24]. In the above ions, the orbital angular momentum is zero, so the main levels in the crystal lattice should not be split, but the paramagnetic activity is shown. S-state ions in the complexes have the g-factor close to the value of unpaired electron (2.0023) that is found in the spectra [23]. Thus, a broad ESR line of the HA2 with the g-factor of the free unpaired electron clearly corresponds to the "non-

free" unpaired electron in chelate complex with the ion Fe^{3+} . Aromatic systems of humic acids are represented by different cyclic structures with the oxygen-containing functional groups, the spatial configuration forming various complexes due chelate (and macrocyclic) effects. Complexity of the organization of supramolecular structure increases the stability and multifunctional properties of paramagnetic complexes and of the entire supramolecular structure [24].

We have proposed a generalized structure scheme of the chelate supramolecular complex of the HA2 (Fig. 5) subject to the data obtained by the UV, FTIR, and ESR spectroscopy. Configuration of the supramolecular structure is also stabilized by the hydrogen bonds (like a dative bond), and the latter may be united in cooperative paramagnetic clusters in the HA2 [27-29].

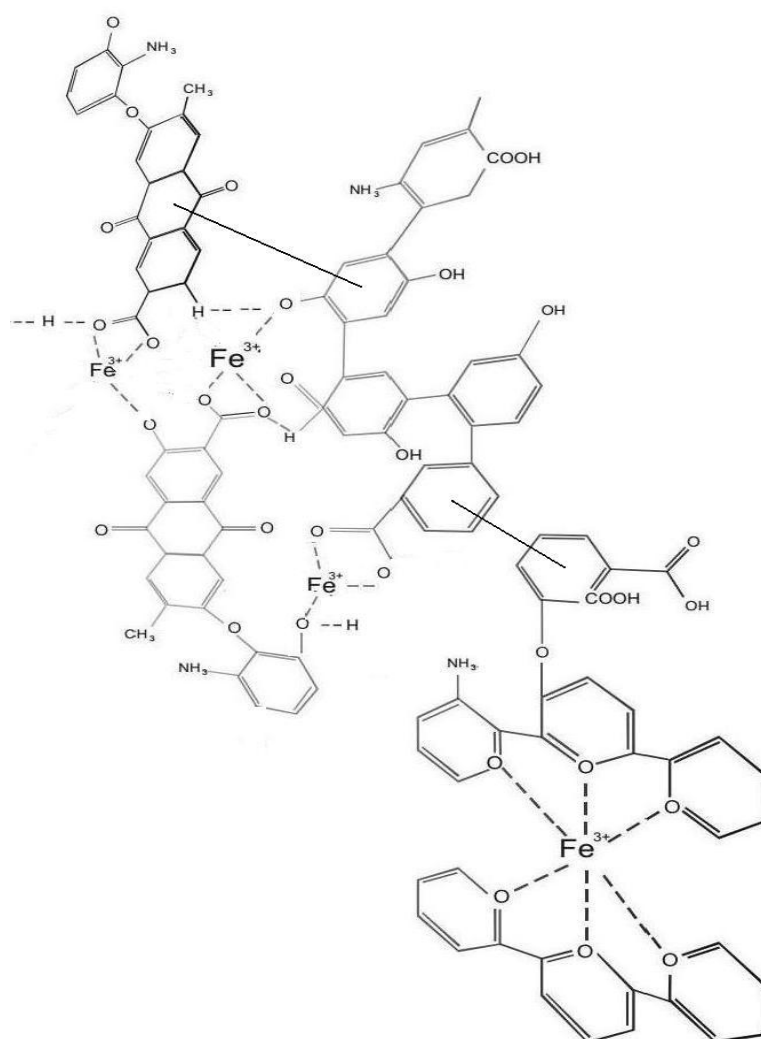


Fig. 5. Scheme of the fragment of supramolecular structure of HA2 from the black soil tillage by plowing, including the cooperative cyclic interaction with Fe^{3+} ions also stabilized by H-bond.

The ESR spectra of the HA2 isolated from the black soil after plowing tillage have the highest intensity of signal (Fig. 4), due to a high content of paramagnetic centers represented by paramagnetic chelate complexes with Fe^{3+} . In the aromatic system Fe^{3+} chelate, the complexes are formed owing to oxygen-containing groups due to the electrostatic interactions. In the macroscopic approach, such complexes can be regarded as a cluster structures with cyclic delocalized cooperative interactions because the electron-donor-acceptor interactions with the formation of Fe^{3+} complexes [24, 25-27] are observed in the

HA2. Such complexes are paramagnetic due to overlap of the electron clouds of the paramagnetic ion and ligands of π -aromatics system and the unpaired electron can move throughout the system of double bonds of the complex [26-28].

The demonstrated intense mineralization after plowing of the typical black soil is accompanied by a more active formation of the so-called "immature" humic formation of associates of low molecular weight complex structure in the soil [5, 21]. This process is connected with emergence of paramagnetic complexes with Fe^{3+} in the HA2. Presumably, subject to changes of external conditions (for example, redox potential), the supramolecular complexes can dissociate into low molecular weight compounds [29, 30], especially in the root zone of plants (rhizosphere) due to the plant exudation [6, 21]. It is known that in rhizosphere, the biofermentation activity varies, which fact consequently impacts the metabolic redox processes and pH, causing a change of the valence of the metals of the intermediate group, in this case, of ions of Fe^{2+} into Fe^{3+} , with the intensification of mechanical processing the soil, thereby affecting the physicochemical properties of the soil [1, 2, 19, 20].

CONCLUSIONS

It has been found that the methods of tillage of the typical black soil affect the redistribution and content of agronomically valuable fractions of the HA1 and the HA2 in the soil. Such effect is connected with the change of microbial and enzymatic activity in the soil microbial community. It is shown that an increase in the tillage intensity causes a reduction in the overall carbon content in the typical black soil and a decrease of the HA2 content through a shift of equilibrium between the humus synthesis and decay in the direction of humus degradation (mineralization). It is found that direct seeding results in low content of the HA1 and high content of the HA2 in the soil, testifying to formation of inerte humus in the soil, and affecting fertility of the soil.

It is shown that the intense absorption of the HA2 in the near-UV region is due to the active electron mobility in the π - π^* electronic system of double bonds of the aromatic system, caused by formation of the complexes with metals of the intermediate group. The HA2 that are isolated from the black soil after plowing are characterized by the most intense absorption in the UV area. It is shown that direct seeding gives rise to a lower diversity in the structural and functional elements of the HA2. It is found that the soil disking increases the content of aliphatic elements in the HA2 by reducing the mineralization process. It is proved that the highest content of the metal-oxygen-containing groups in the HA2 of the typical black soil is observed after intensive tillage. It is demonstrated that the broad line of the ESR spectrum of the HA2 corresponds to a paramagnetic chelate complexes with Fe^{3+} . It has been revealed that plowing provides an intramolecular rearrangement in aromatic system of the HA2, as evidenced by the increase in number of the paramagnetic Fe^{3+} complexes.

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REFERENCES

1. Orlov D. S. Humic substances and general theory of humification / D.S. Orlov; Moscow: MSU, 1990. – 325 p.
2. Stevenson F.J. Humus chemistry: Genesis, composition, reactions / F.J. Stevenson; 2nd ed. John Wiley & Sons, Ltd, 1994. – 512 p.
3. Giovanela M. Elemental compositions, FT-IR spectra and thermal behavior of sedimentary fulvic and humic acids from aquatic and terrestrial environments / M. Giovanela // *Geochemical Journal*. – 2004. – N. 38. – P. 255-264.

4. Ishakov H.A. / Humic complexes / H.A. Ishakov // Chemical technology. Vestnik Kazansky STU. – 2010. – N. 6. – P. 126-129.
5. Physiological effects of humic substances on higher plants / S. Nardi, D. Pizzeghello, A. Muscolo, A. Vianello // Soil Biology & Biochemistry. – 2002. – N 34. – P. 1527-1536.
6. Nebbioso A. Advances in Humeomic: enhanced structural identification of humic molecules after size fractionation of a soil humic acid / A. Nebbioso, A. Piccolo // Analytica Chimica. – 2012. - N 720. - P. 77-90.
7. Piccolo A. The supramolecular structure of humic substances / A. Piccolo // Soil Sci. - 2001. – N. 166. - P. 810-832.
8. Kravchenko Y. Amount and dynamics of soil organic matter in typical chernozem of Ukraine under different soil tillage systems / Y. Kravchenko // China: Northeast Forestry University. – 2010. – 109 p.
9. Skrilnik E.V. Effect of tillage system and fertilization on humic condition and nutrition in typical black soil / E.V. Skrilnik, O.S. Perebikovska, V.P. Moskalenko // Agrohimiya i Gruntoznavstvo. – 2008. – N. 68. – P. 90-94.
10. Ovchinnikova M.F. Symptoms and Mechanisms of nutrient transformation of humic substances sod-podzolic soil / M.F. Ovchinnikova // Agrohimiya. – 2012. – N. 1. – P. 3-13.
11. Characterization of humic acids from a Brazilian Oxisol under different tillage systems by EPR, ¹³C NMR, FTIR and fluorescence spectroscopy / M. Gonzalez, P. Ladislau, C. Sergio [et al.] // Geoderma. - 2004. – N. 118. – P. 181-190.
12. Haynes R.J. Labile organic matter fractions as central components of the quality of agricultural soils: an overview / R.J. Haynes // Adv. Agron. – 2005. – N. 85. – P. 221-268.
13. Bakina L.G. Labile humus sod-podzolic loamy soils of the North-West of Russia at liming / L.G. Bakina // Agrohimiya. – 1997. – N. 6. – P. 27-31.
14. DSTU 7926:2015. Method for determining total carbon and nitrogen in organic samples. Soil quality. – Kiev: DP UkrNDCN. – 12 p.
15. DSTU 7828:2015 Determination of the group and fractional composition of humus by I. V. Tyurin methods in modification by V.V. Ponomareva and T.A. Plotnikova. Soil quality. – Kiev: DP UkrNDCN. – 14 p.
16. Orlov D. S. Practicum work on humus biochemistry / D.S. Orlov, L.O. Grishina. – Moscow: MSU. – 1981. – 272 p.
17. Effect of no-till cropping systems on soil organic matter in a sandy clay loam Acrisol from Southern Brazil monitored by electron spin resonance and nuclear magnetic resonance / R.L. Parfitt, B.K. Theng, J.S. Whitton, T.G. Shepherd // Soil & Tillage Research. – 2000. – N. 53. – P. 95-104.
18. Vilnyiy R.P. Effect of minimization soil tillage on ecobiological condition of black soil / R.P. Vilnyiy // Pochvovedenie i agrohimiya. – 2015. – N. 1. – P. 104-114.
19. Investigation of the structure of humic acid by EPR-spectroscopy / V.P. Strigutskiy, Yu.Yu. Navosha, T.P. Smychnik, N.N. Bambalov // Pochvovedenie. – 1992. – N. 1. – P. 147-151.
20. Schapov L.N. Microbe succession at organic soil matter transformation / L.N. Schapov // Pochvovedenie. – 2004. – N. 8. – P. 967-975.
21. Kravchenko Y. Quality and dynamics of soil organic matter in a typical chernozem of Ukraine under different long-term tillage systems / Y. Kravchenko, N. Rogovska, L. Petrenko // Canadian Journal of Soil Science. – 2012. – N. 92. – P. 429-438.
22. Kudayarova A.Yu. About informativeness of the electronic spectra of humic substances / A.Yu. Kudayarova // Pochvovedenie. – N 11. – 2001. – P. 1323– 331.
23. Altshuler S. A. ESR-spectroscopy the elements of the transition groups / S.A. Altshuler, B.M. Kozyrev. –M.: Science. – 1972. – 366 p.
24. Konstantinova E.A. ESR spectroscopy of disordered and low-dimensional solid-state systems / E.A. Konstantinova, Yu.V. Kashkarov. – M.: MSU. - 2002. – 74 p.
25. Gubin S.P. Cluster chemistry / S.P. Gubin – M.: Science. – 1987. – 262 p.
26. Melson G.A. Coordination chemistry of macrocyclic compounds / G.A. Melson. – New York: Plenum Press. – 1979. – 325 p.
27. Len Zh.-M. Supramolecular chemistry. Concepts and Perspectives / Zh.-M. Len. – Novosibirsk: Science. – 1998. – 333 p.
28. Ionenko V.I. Crown clathrate concept of humus structure / V.I. Ionenko // Melioratsiya i himizatsiya zemledeliya Moldavii: tez. dokl. konf. – Kishinev, Ch. 1. – 1988. – P. 5-8.
29. Phenomenology of functional structural models of natural and synthetic humic substances / V.I. Ionenko, E.A. Golovachev, A.M. Besckrovnuy [et al.] // Sibirskiy Bulletin of Agriculture. – 1988. – N 5. – P. 91–104.
30. Tsapko Yu.L. Structure of humic acids in aspects of effect on the acidic soil functions / Yu.L. Tsapko // Bulletin of V.V. Dokuchayev Kharkiv National Agrarian University. – 2014. – N 1. – P. 12-18.