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Structural Variations of Humic Acids in Overphosphatized Soils

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Abstract—Participation of phosphate ions in specific (donor–acceptor) interactions with humic acids in forest soils was found to change the structure and properties of the acids. Chelate entities containing aluminum and iron atoms were detected in humic acids molecules occurring in overphosphatized soils. The behavior of phosphorus-modified humic acids in polar media is discussed.

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INTRODUCTION

Ecological consequences of over-accumulation of phosphorus in soils are generally considered to be limited to eutrophication of natural water bodies. However, such consequence as impaired quality of a unique natural entity, soil humus, is at least equally important. Although there are published data [1–3] indicating possible transformations of soil humic substances (HS) by the action of exogenous inorganic compounds, studies on intermolecular interactions involving humic acids have not received due attention. It is generally accepted that the key role in intermolecular interactions is played by nonspecific (van der Waals) interactions that do not affect the properties of humic molecules to an appreciable extent. However, the contribution of specific (donor–acceptor) interactions which may be regarded as chemical bonding, is usually underestimated, though it may dominate under certain conditions. In this case, properties of molecules of humic substances, in particular their optical properties, may change considerably.

Electronic spectroscopy makes it possible to reveal specificities of electronic structure of molecules, which are responsible for their electronic absorption spectra and efficiency of interactions of different origins [4, 5]. Electronic spectroscopy is widely used to compare optical properties of HS in various natural soils [6, 7]; however, it has almost not been used to study their transformations in a particular soil.

Soil humic acids (HA) consist of polyatomic macromolecules, but their variations involve most frequently only someparticular structural fragments. Electronic absorption spectroscopy is used to detect in organic molecules chromophoric fragments containing conjugated C=C and multiple heteroatom bonds [4, 8]. Multiple bonds (main functional groups in organic molecules) give rise to absorption in the regions λ 210–230 (C=C) and 250–280 nm (mostly C=O bonds, as well as N=O, P=O, C=N, etc.). If metal complexes with organic ligands are present in solution, the above chromophoric groups belong to the ligands [9].

The formation of a chemical bond between two elements with different electronegativities (i.e., the ability to attract electrons) is accompanied by appearance of partial (fractional) effective charges[1] on the atoms as a result of electron density displacement [10, 11]. For example, in a multiple bond between heteroatoms or in an ordinary bond like C–OH, more electronegative atom (O) acquires an effective negative charge, whereas the carbon atom attached thereto acquires a positive charge. Coordination of a metal ion to electronegative oxygen atom leads to increase in the positive charge on the carbon atom. Non-uniform distribution of electron density is responsible for the polarity of such bonds. Phosphorus–oxygen bonds are more polar than carbon–oxygen bonds [10].

The presence of polar bonds in an organic molecule determines the possibility for its interaction with other

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[1] Partial (fractional) effective charge is a part (fraction) of an elementary unit charge. It arises on atoms of organogenic elements due to asymmetric electron density distribution in a chemical bond formed by two elements with different electronegativities [10, 11].

organic and inorganic molecules. Cations and anions derived from inorganic compounds can add (coordinate) to negatively and positively charged organogenic atom, respectively. The concomitant electron density redistribution involves the entire molecule, leading to structural transformation of chromophoric groups. If the electron density redistribution is insignificant, the chromophoric fragment remains almost intact. However, if the electron density distribution is considerable, the coordinated atoms may constitute the "base atoms" of the chromophore [8]. This means qualitative change of the chromophore or, more precisely, the formation of a new chromophore, which should be reflected in the electronic absorption spectra and reactivity of a compound.

In routine electronic absorption spectra of modified humic substances (using the solvent as reference), relatively small structural variations may be obscured by absorption of intact fragments of macromolecules. Therefore, differential electron spectroscopy is used to reveal fine structural variations [4, 12, 13]. Difference electronic spectra of modified humic acids are recorded with respect to the corresponding unmodified humic acids from soil (reference) rather than with respect to the solvent [14]. In this case, the absorption intrinsic to both the solvent and common fragments of modified and unmodified humic acids is subtracted automatically. As a result, the accuracy in the determination of optical density increases, the shape of absorption bands is refined, and new features of their fine structure (invisible in routine spectra) can be revealed.

The goal of the present work was to examine (1) spectral properties of phosphate-modified humic acids from forest soil, (2) the relation between the spectral and chemical properties of P-modified humic acids; and (3) phosphate-initiated transformation of humic macroligand in iron complex.

MATERIALS AND METHODS

As subjects for the study we used (1) samples withdrawn from humic and arable horizons of, respectively, virgin (pH_{KCl} = 4.57) and arable (rN_{KCl} = 5.15) grey forest soils at the experimental station of the Institute of Physicochemical and Biological Problems of Soil Science, Russian Academy of Sciences (Pushchino, Moscow oblast); (2) synthesized iron humate complex. A sample of arable soil was taken from a field which was not treated with any fertilizer over a period of 10 years (a control version of stationary field test [15]). The main chemical parameters of the initial soil samples are given in [16].

The effect of $NH_4H_2PO_4$ on the structure and properties of humic acids in virgin and arable soils was studied. The virgin soil samples were incubated over a period of three years without addition (control) and with addition of $NH_4H_2PO_4$ (100 mg of P_2O_5 per 100 g) in wet state (55% of the total soil water capacity) at room temperature. The arable soil samples were incubated under analogous conditions. Experiments included three versions (1) without any addition (blank), (2) with addition of $NH_4H_2PO_4$, and (3) with addition NH_4NO_3. The amount of added phosphorus was the same as in experiments with virgin soil samples. The amount of nitrogen added with NH_4NO_3 corresponded to its concentration in $NH_4H_2PO_4$ and was 20 mg per 100 g of soil. The incubation duration (3 years) was selected taking into account the results of previous studies [17], according to which a fairly long period is necessary to clearly observe variations in the structure of soil humic acids.

Soil samples were dried in air, and humic substances were extracted therefrom (without preliminary decalcification) with a 0.1 N solution of NaOH, the soil–alkaline solution ratio being 1 : 50. Soil suspensions were shaken for 2 h in capped flasks, left overnight, and filtered. No other reagents were added to facilitate filtration. The alkaline extracts were acidified with 1 N aqueous HCl or 1 N H_3RO_4 (the extract-to-inorganic acid ratio was 3 : 1) to precipitate HA (HCl) and HA (H_3PO_4), respectively. Intentionally, humic acids were not purified from inorganic impurities. To determine the yield of HA, four aliquot samples of the alkaline extracts were treated with the corresponding acid. After addition of inorganic acid, the mixture was kept for 15 min at 50–55°C and left overnight. The precipitate of HA was separated by filtration through a dense ash-free filter paper ("blue band"). The precipitate was washed with water on a filter and dried in air until constant weight. The amount of HA was calculated by subtracting the weight of the filter. Humic acids were dissolved in 0.1 N. NaOH and analyzed.

The concentration of carbon in humic acid solutions was determined by dichromate oxidation [18]. The concentrations of metals (Al, Fe) and phosphorus were determined by ashing with a 3 : 1 mixture of concentrated sulfuric acid and 57% perchloric acid, followed by plasma spectroscopic analysis (for metals) or standard photocolorimetric procedure (for phosphorus) using ascorbic acid (Murphy–Riley method) [19]. The same procedure was used to quantify phosphorus after addition of ethanol to neutralized solutions of ashed humic acids.

The carbon content of humic acid solutions used for recording electronic absorption spectra ranged from 6.6 to 7.6 mg/50 ml for virgin soil, and from 4.8 to 5.2 mg/50 ml for arable soil. The routine and difference electronic spectra of humic acids solutions were recorded in the range λ 190–450 nm on a Hitachi-557 spectrophotometer (Japan). The routine spectra were recorded against the solvent (0.1 N NaOH), and the difference spectra, against an alkaline solution of humic acids from control soil sample, using 0.5-mm cells [14].

A dry humic acids specimen HA(HCl) with an ash content of 22% was isolated from the initial arable soil according to [20]. Humic acid, 50 mg, was treated with 50 ml of deionized water (control) or with 50 ml of 1 M KH_2PO_4 (pH values 4.04 and 3.99, respectively). The mixture was shaken for 1 h, and the liquid phase was analyzed for Al, Fe, and C.

In other experiments, humic acid, 50 mg, was dissolved in 50 ml of 0.1 N NaOH, and a solution of KH_2PO_4 was added to the alkaline solution at a ratio of 1 : 1 so that the concentration of P_2O_5 in the system attained 0.25 mg per ml of HA solution. The electronic absorption spectra of the control and phosphate-containing solutions were recorded within an hour on a Specord UV VIS spectrophotometer (Germany; 20-mm cell path length).

The iron–humate complex was synthesized as follows; iron(III) chloride was added to an alkaline solution of humic acids isolated from the initial arable soil to a ratio of 300 mg Fe per 50 mg C (pH 5.1). The precipitate (iron complex) was filtered off, washed with water, and dried at room temperature. The C/Fe ratio in the isolated complex was 1 : 3.2. The complex was treated with a solution of KH_2PO_4 (pH 4.01) over periods of 3 and 5 weeks. The concentration of phosphorus in the system iron complex–phosphate corresponded to C/P and Fe/P ratios of 1 : 18 and 1 : 5. It simulated phosphate load of the complex in a thin layer of soil surrounding a grain of water-soluble phosphate fertilizer [21, 22]. Immediately after addition of phosphate solution, the liquid phase turned brown. Simultaneously, white turbidity and dark brown flakes appeared.

After 3 or 5 weeks, distinct phase separation was observed: a light brown liquid and two solid products (crystalline and amorphous phosphorus which can be readily separated from each other) were present. All phases were analyzed for C, Fe, and P. The solid dark brown phase (amorphous P) containing more carbon was analyzed by IR spectroscopy (KBr). The IR spectra were interpreted according to [23, 24].

All analytical data given in this paper were obtained from 3–4 parallel runs. The corresponding variation coefficients are given in the notes to tables.

RESULTS AND DISCUSSION

The routine electronic spectra of alkaline solutions of HA(HCl) from the initial virgin and arable soils (recorded against 0.1 N NaOH; Fig. 1a) displayed absorption bands in the region λ 210–230 nm and much weaker absorption in the region λ 250–280 nm, which were assigned to conjugated C=C bonds and multiple heteroatom bonds (mostly C=O) [4]. The absorption intensities of HA from the initial virgin and arable grey forest soils differed considerably. The optical density (*D*) of HA solution from the virgin soil (Fig. 1a, curve *1*) was appreciably higher as compared to HA from the arable soil, so that curve *3* lies considerably lower.

The optical density of HA solutions is generally related to the concentration of carbon therein [6, 7]. In fact, a direct dependence is observed between the optical densities of solutions of HA from the initial virgin and arable soils (Fig. 1a; curves *1*, *3*) and the corresponding concentrations of carbon (507 and 320 mg per 100 g of soil, respectively; Table 1). However, no such dependence was found for phosphate-rich (doped with $NH_4H_2PO_4$) virgin and arable soils. The optical density of a solution of HA(HCl) from the over-phosphatized virgin soil was higher (Fig. 1a, curve *2*) as compared to the initial soil, though the concentration of carbon therein is considerably lower (346 against 507 mg per 100 g of soil; Table 1). The optical density of a solution of HA(HCl) from the over-phosphatized arable soil increases even more significantly (Fig. 1a, curve *4*) while the carbon content of HA solution is almost the same (Table 1).

As follows from Table 1, solutions of HA(HCl) from over-phosphatized soils contain larger amounts of Al and Fe than those from the initial samples. This is seen especially clearly with account taken of the yield of HA (Table 2), i.e., when the concentration of metals

Table 1. Concentrations of carbon, metals, and phosphorus in HA(HCl) from the initial and overphosphatized virgin and arable soils (mg per 100 g of soil and mg per gram of HA)[a–c]

Soil	C	Al	Fe	P		C/(Al + Fe)
				without ethanol	with ethanol	
Virgin soil:						
initial	507 (230.5)	60.1 (27.3)	29.2 (13.3)	4.5 (2.1)	6.9 (3.1)	5.7
overphosphatized	346 (227.6)	63.4 (41.7)	31.7 (20.9)	6.7 (4.4)	Not determined	3.6
Arable soil:						
initial	320 (367.8)	27.6 (31.7)	25.4 (29.2)	2.9 (3.3)	18.2 (20.9)	6.0
overphosphatized	320 (238.8)	54.2 (40.5)	42.1 (31.4)	5.7 (4.3)	Not determined	3.3

[a] Here and in Tables 2 and 4, overphosphatized soil refers to the soil with addition of $NH_4H_2PO_4$ (100 mg of P_2O_5 per 100 g of soil).

[b] Here and in Table 4, the concentration of chemical elements calculated per gram of HA is given in parentheses (see Table 2).

[c] Here and in Table 4, the coefficient of variation of the concentrations of carbon, metals, and phosphorus did not exceed 4% ($n = 3$), 5% ($n = 3$), and 2% ($n = 3$), respectively.

is calculated with respect to humic acids. Correspondingly, the C/(Al + Fe) ratios decrease, indicating negative change of the HA quality due to overphosphatization (Table 1).

The data in Table 3 show that a considerable amount of metals and carbon is eliminated from humic acid to the liquid phase by the action of P-solution. The electronic spectra of P-modified HA (Fig. 2, curve *2*) displayed strong reduction of the absorption intensity throughout the examined wavelength interval, especially in the visible region ($\lambda > 350$ nm) typical of absorption of metal complexes. Presumably, this is the result of elimination of peripheral metal-containing fragments at multiple bonds, which contributed much to the electronic spectrum of the initial HA.

The data characterizing primary P-initiated changes in the structure of HA (Table 3, Fig. 2), in combination with those presented in Table 1, suggest that metal phosphate complexes transferred to solution could be incorporated directly into chromophoric fragments of humic macroligands, favoring their subsequent transformation.

Metal ions located in peripheral fragments of soil humic molecules can be coordination centers toward exogenic phosphate anions having electron-donating oxygen atoms. The coordination can produce differently charged metal phosphate complexes [16, 25, 26]. Negatively charged complexes (metal phosphate anions) like $[Me^{3+}(HPO_4)_2]^-$ are capable of being transferred to the liquid phase in the system soil–

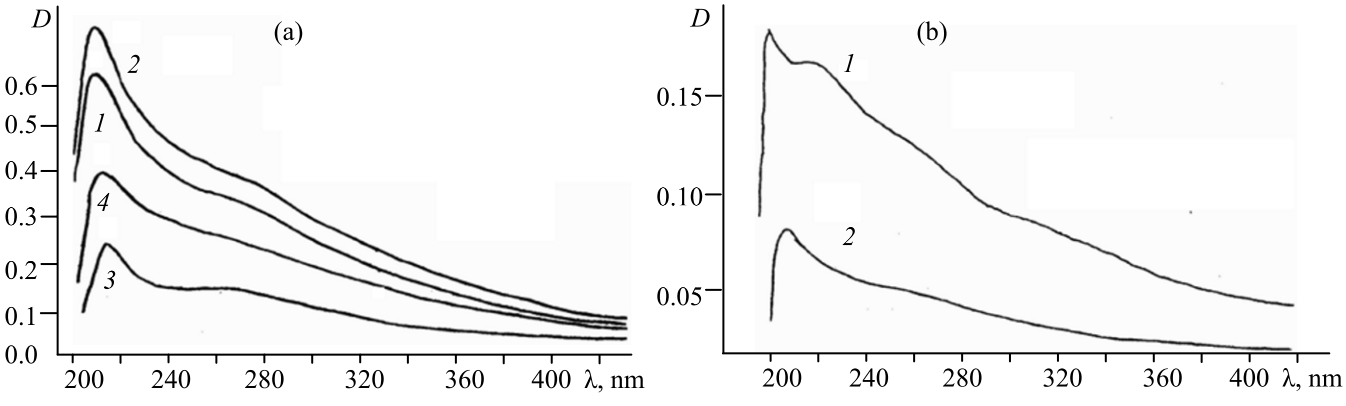


Fig. 1. (a) Routine (against 0.1 N NaOH) and (b) difference [against HA(HCl) from the corresponding initial soil] electronic absorption spectra of humic acids [HA(HCl)] isolated from the initial and overphosphatized soils. (a): (*1*) initial virgin soil, (*2*) overphosphatized virgin soil, (*3*) initial arable soil, (*4*) overphosphatized arable soil; (b): (*1*) arable soil, (*2*) virgin soil.

Table 2. Yields of humic acids (HA) from the initial and overphosphatized soils (g per 100 g of soil)[a]

Soil	Yield of HA	
	precipitated with 1 N HCl	precipitated with 1 N H_3PO_4
Virgin soil:		
initial	2.20	3.98
overphosphatized	1.52	2.57
Arable soil:		
initial	0.87	1.53
overphosphatized	1.34	1.95

[a] The coefficient of variation did not exceed 4% ($n = 4$).

phosphorus-containing solution, which was confirmed experimentally [16].

The presence in P-modified HA of metal phosphate fragments that enhance absorption of heteroatom chromophores (Fig. 2, curve *2*) follows from red shift of absorption bands originating from multiple bonds of the two types due to their increased polarity.

Metal phosphate anions are stronger electron-donating (nucleophilic) reagents than simple phosphate anions, and they are capable of adding to positively charged (electrophilic) atoms, in particular to carbon atoms at multiple bonds [10, 11, 27]. Therefore, metal phosphate chelate entities may be formed in the structure of humic macroligands.

Chelate entities in organic polymer molecules generally give rise to absorption in the region λ 290–390 nm [28–30]. No absorption in that region was observed in routine electronic spectra (solvent as reference) of HA(HCl) from both initial and P-rich soils (Fig. 1a), for it was obscured by absorption of common organic chromophores (multiple bond-containing groups). By contrast, the difference electronic spectra recorded against HA(HCl) from the initial soil (Fig. 1b) made it possible to detect P-modified fragments in humic acids from over-phosphatized arable and virgin soils. The spectra of P-modified HA(HCl) from the arable soil (Fig. 1b, curve *1*) displayed absorption in the region λ 310–370 nm, which may be attributed to chelate entities. This absorption was seen especially clearly after abstraction of the contribution of the nitrogen component of the phosphate salt (Fig. 3). A broad absorption band in the region λ 230–430 nm (Fig. 3) indicates participation of heteroatom multiple bonds (λ 230–280 nm) in chelation.

Table 3. Metal and carbon release into the liquid phase (% of the initial concentration in HA)

System	Al	Fe	C
HA–H_2O	Traces	Traces	Traces
HA–H_2O–P_2O_5	31	33	14

No absorption in the region 310–370 nm was observed in the spectra of P-modified HA(HCl) from the virgin soil (Fig. 1b, curve *2*), but the absorption intensity in the region λ 370–440 nm somewhat increased; this pattern was more pronounced in the spectra of HA precipitated with 1 N H_3PO_4 (Fig. 4, curve *1*). The absorption band at λ 340–440 nm (Fig. 4, curve *1*) is usually assigned to inner-sphere electron transitions induced by coordination of a new ligand (in our case, phosphate) [9, 29]. Apart from that band, the spectrum of HA(H_3PO_4) from the P-rich virgin soil (Fig. 4, curve *1*) contained the second absorption band in the region λ 270–350 nm, which is likely to arise from metal phosphate fragments simultaneously incorporating into HA molecules. Insofar as phosphate treatment almost did not affect the structure of organic

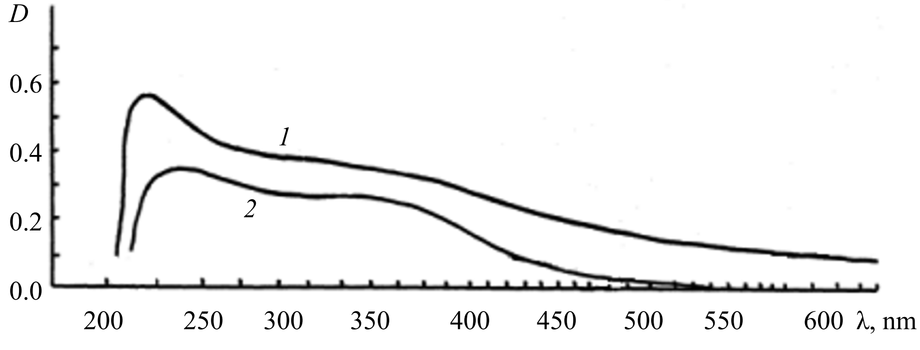


Fig. 2. Electronic absorption spectra of alkaline solutions of (*1*) initial HA (against 0.1 N NaOH) and (*2*) after addition of a phosphate solution (against 0.1 N NaOH + P-containing solution, 1 : 1).

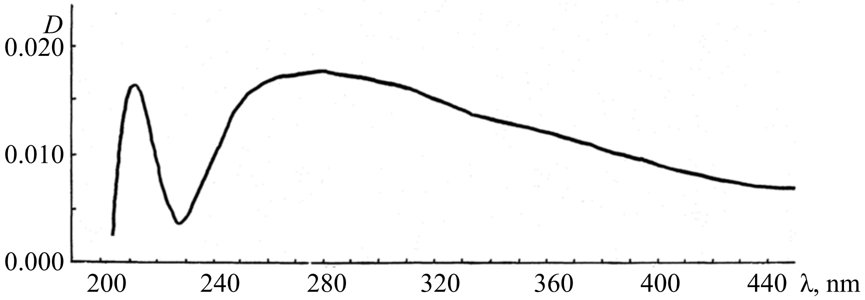


Fig. 3. Variation of the difference electronic spectra of HA(HCl) isolated from the arable soil by the action of phosphate component [$NH_4H_2PO_4$; against HA(HCl) isolated from soil doped with NH_4NO_3].

chromophores (Fig. 4, curve *1*, λ 200–260 nm), the reaction of HA from virgin soil with phosphate anions is limited mainly to metal coordination and addition of the resulting metal phosphate complexes to positively charged organogenic atoms in humic macroligands.

The spectral parameters of HA(H_3PO_4) from overphosphatized arable soil (Fig. 4, curve *2*) revealed essential transformations of the structure of organic chromophores, which may be induced by metal-containing fragments present initially in the humic macroligand. It is seen that additional phosphate treatment of the initially P-modified humic macroligand from the arable soil resulted in more profound transformation of bond system in HA(H_3PO_4).

As shown in Figs. 1b and 4, P-modified humic acids precipitated from alkaline extracts of soils of both types with different mineral acids (1 N solution of HCl and H_3PO_4) have different electronic spectra and hence different structures. Analogous data were obtained previously [14] by comparing the structures of HA precipitated with 1 N HCl and 1 N H_2SO_4. In both cases, the effect of mineral acid anion on the spectral parameters of HA from the virgin soil was weaker than on the spectral parameters of HA from the arable soil.

The effect of mineral acid anion was also significant in the yield of HA from soils and their elemental composition (Tables 1, 2, 4). The yield of HA from the arable soil was lower than from the virgin soil, but phosphate treatment of the arable soil increased the yield of HA due to accumulation of aluminum, iron, and phosphorus. Stronger effect of inorganic anion on the structure of HA from the arable soil is likely to be related to previous agricultural usage implying phosphate fertilization. In keeping with the data in Table 1, humic acids from the arable soil

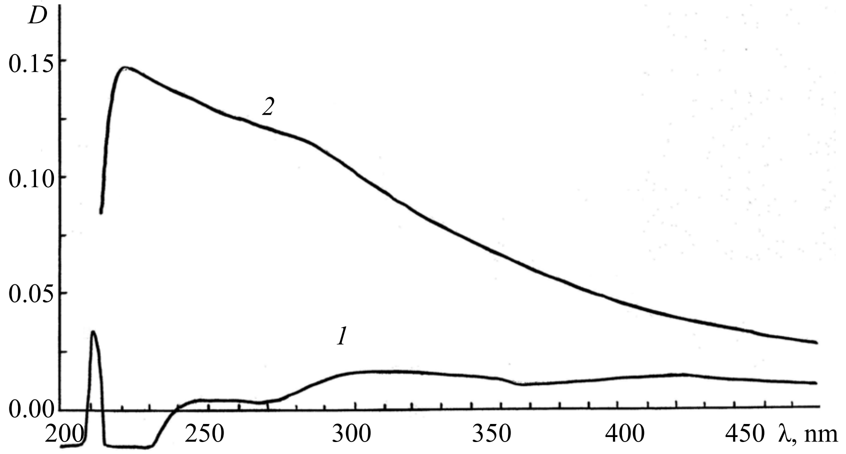


Fig. 4. Difference electronic absorption spectra of HA(H_3PO_4) isolated from overphosphatized (*1*) virgin and (*2*) arable soils [against HA(H_3PO_4) from the corresponding initial soil].

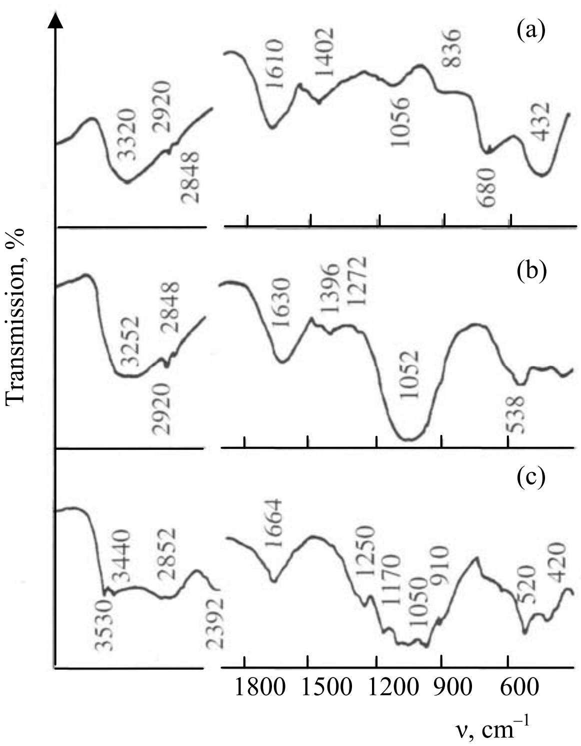


Fig. 5. IR spectra of (a) initial iron humate complex and the same complex after treatment with a phosphate solution for (b) 3 and (c) 5 weeks.

were initially enriched with phosphorus to a greater extent as compared to HA from the virgin soil, but a considerable part of phosphorus in the former was not detected by the Murphy–Riley standard procedure without preliminary cleavage of phosphorus bonds with ethanol.

As shown above, further phosphate treatment of initially P-modified humic macroligands from the arable soil induced their more severe transformations. Insofar as humic acids are products of decomposition of different metal humate complexes in alkaline solution, it was reasonable to study the mechanisms of transformation of humic macroligands directly in complexes with a particular metal.

Table 4. Concentrations of carbon, metals, and phosphorus in HA(H_3PO_4) from the initial and overphosphatized virgin and arable soils, mg per 100 g of soil and mg per gram of HA (in parentheses)

Soil	C	Al	Fe	C/(Al + Fe)
Virgin soil:				
initial	600 (150.8)	19.4 (4.9)	7.4 (1.9)	22.4
overphosphatized	560 (217.9)	36.1 (14.1)	13.2 (5.2)	11.4
Arable soil:				
initial	422 (275.8)	18.2 (11.9)	3.4 (8.8)	19.5
overphosphatized	360 (184.6)	26.1 (13.4)	8.2 (4.2)	10.5

The transformation sequence of humic macroligand in phosphate solution was studied using a specially synthesized iron humate complex. The initial step was decomposition of the complex with formation of soluble and two solid products. About 45% C and 7% Fe present in the initial iron humate complex were detected in the liquid phase of the iron complex–phosphate solution system. This means that coordination of phosphate anions to iron atoms was accompanied by substitution of the initially coordinated organic fragments in the humic macroligand, and these fragments entered into the liquid phase together with newly formed iron phosphate complexes. Compounds transferred to the liquid phase could favor further transformations of the solid decomposition products of the initial complex (Fig. 5).

The two solid decomposition products (Table 5) were iron complexes with ligands containing phosphorus and carbon. The concentration of phosphorus therein exceeded the concentration of carbon by a factor of 2 and 7, respectively, which indicated incorporation of phosphorus atoms into organic ligands. The transformation sequence of the macroligand in the dark brown complex (with a higher carbon content; Fig. 5a) in phosphate solution can be traced by the IR spectra. Figures 5a and 5b show that considerable difference in the macroligand structures after treatment with a phosphate solution for 3 and 5 weeks.

The spectrum of the 3-week treatment product contained a strong absorption band at 1052 cm^{-1}, indicating addition of phosphate groups (P–O bonds give rise to absorption at 538 cm^{-1}) to positively charged carbon atoms of C–O bonds in alcoholic,

Table 5. Composition of the iron humate complex and its transformation products in phosphate solution, % (mean ± SD)

Complex	C	Fe	P
Initial	21.1± 1.1	66.7± 2.3	0.25±0.02
P-Modified:			
dark brown	5.1± 0.3	15.8± 0.9	11.8±0.27
light grey	2.2± 0.2	30.1± 1.6	13.9±0.31

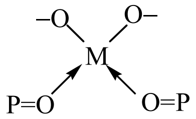
phenolic, polysaccharide, and other fragments of the macroligand) with formation of new C–O–P moieties in which the effective positive charge is localized on the phosphorus atom [22, 23]. It whould be noted that the IR spectrum of the 3-week treatment product (Fig. 5b) retained absorption bands typical of the initial complex due to OH stretching vibrations, as well as to stretching vibrations of carbonyl groups coordinated to metal (Fig. 5a, 3320–1402 cm^{-1}) [22].

Quite strong changes were observed in the same regions and at lower frequencies in the IR spectrum of the 5-week treatment product (Fig. 5c). Appearance of additional bands in the region 1100–1000 cm^{-1}, corresponding to P–O–C vibrations [23], is likely to result from addition of free organic ligands present in solution to positively charged phosphorus atoms in C–O–P groups formed previously. Presumably, phosphate fragments occurring in solution also add to phosphorus. This follows from the absorption band at 910 cm^{-1} which is typical of pyrophosphate (P–O–P) functionality in organic molecules [22]. Therefore, organic and phosphate ligands in solution could be embedded into the macroligand of the solid complex via addition to positively charged (electrophilic) phosphorus atoms in the P-modified macroligand.

Reactions at electrophilic phosphorus atom always lead to the formation of P=O bond [31, 32]. This corresponds to the presence in the IR spectrum (Fig. 5c) of two bands assignable to stretching vibrations of a free P=O group (1250 cm^{-1}) and P=O group involved in additional interactions, including hydrogen bonding (1170 cm^{-1}). The presence of a broad absorption band at 2392 cm^{-1} indicates that P=O groups are involved in strong intramolecular hydrogen bond with OH groups [23]. The IR spectrum of the 5-week treatment product (Fig. 5c) lacked absorption bands at 1630 and 1396 cm^{-1}, which were present in the spectrum of the 3-week product (Fig. 5b), while a broad band appeared at 1664 cm^{-1}. This pattern corresponded to formation of chelate rings with iron coordination to not only P=O but also C=O ligand fragments [22].

The above data suggest that the P-modified humic macroligand in the iron complex is successively transformed by the action of free potential ligands present in the liquid phase, which include (in addition to phosphate ligands) metal phosphate and organic ligand moieties released upon coordination of phosphate anions. Reactions occurring at positively charged phosphorus atom underlie the mechanism of qualitative transformations leading to the formation of new ligand fragments having a P=O bond.

On the basis of the IR spectral data (Fig. 5) in combination with the data given in Tables 1 and 4, the formation of the following metal phosphate chelate centers in P-modified humic acids from the arable soil may be presumed:



If supplemented with hydrogen bonds, such fragments have cyclic structure which extends the conjugated bond system [9, 22]. This is likely to be reflected in the shape of spectral curves of P-modified humic acids (Fig. 1b, curve *1*; Fig. 4, curve *2*). The electronic spectrum of HA(H_3PO_4) (Fig. 4, curve *2*) features a broad absorption band which overlaps absorption of all chromophores. This band may arise from a donor–acceptor complex (charge-transfer complex), a particular case of which is intramolecular hydrogen bonding [4, 9]. Presumably, increased absorption intensity of C=O chromophores and blue shift and broadening of the corresponding band are determined by the presence of neighboring HO–P=O group which strongly tends to form intramolecular hydrogen bonds [23, 32]. Here, the OH group acts as proton donor, and P=O, as proton acceptor.

The ability of P=O groups to form strong hydrogen bonds [which is reflected in the IR spectrum (Fig. 5c)] favors stabilization of metal chelates in polar media [23, 32, 33]. An example is HA(H_3PO_4) (Table 4). Despite phosphate assistance to accumulation of Al and Fe in humic acids (Tables 1, 4), the metal content of HA(H_3PO_4) (Table 4) is lower than that of HA(HCl) (Table 1). Apparently, H_3PO_4 stabilizes those HA fragments which contain metal phosphate moieties. Chemical reactions with the latter can occur at the positively charged phosphorus atom with participation of various electron-donating groups, including phosphate groups. These reactions could lead (see above) to the formation of P=O-containing groups in the P-modified macroligand.

Stabilization in polar media is related to a high degree of charge (electron density) separation in P-containing organic molecules. The degree of charge separation increases in parallel with the concentration of phosphorus [5, 33]. Increased number of polar

Table 6. Solubility of soil phosphorus compounds, mg P per 100 g of soil

Initial soil	0.2 N HCl	0.1 N NaOH	
		total (inorganic + organic)	P_{org}
Virgin	0.8	34.7	31.4
Arable	2.2	15.0	10.3

bonds may be responsible for the higher ability of HA from the arable soil to undergo specific donor–acceptor interactions with inorganic molecules. This is associated with the originally higher P content of HA from the arable soil (Table 1).

I previously noted [17, 34] that structural variations of molecules of humic substances may be judged by change of acid–base properties of the P-containing fragments. The data in Table 6 show that extractability of phosphorus from the initial arable soil with 0.1 N NaOH is considerably lower than from the initial virgin soil despite its higher total content (0.14% against 0.11% in the virgin soil). By contrast, the extractability of phosphorus with 0.2 N HCl from the arable soil was higher. In keeping with these data, P-containing fragments in humic compounds in the arable soil possess a larger number of basic (electron donor) groups as compared to acid groups (electron-acceptor). This may be due to variation of the ligand environment of metal atoms, which ensures saturation of their outer electron shells.

Change of the ligand properties are related to change of the properties of metal complexes on the whole [35]. As shown in [36], at a certain overphosphatization level of arable soil, the extractability of both P and Fe with 0.1 N NaOH strongly decreased, and their extractability with 0.2 N HCl somewhat increased, as compared to control. On the whole, the extractability of iron and phosphorus decreased upon overphosphatization of soil, which indicated a tendency for stabilization of P-containing compounds in arable soil by polar media.

Thus the differences in the spectral and chemical properties of humic acids isolated from virgin and arable soils are determined by their different abilities for specific interactions with inorganic anions of compounds both added to the soil and used for the isolation of humic acids. Higher ability of HA from the arable soil for specific interactions, which is enhanced upon addition of phosphates, is related to their stronger polarization due to accumulation of P, Al, and Fe in their molecules.

To conclude discussion of the experimental data, it should be noted that the transformation of humic substances in soil is generally believed to be induced by the activity of microbiota. The role of abiotic chemical reactions is strongly underestimated. However, taking into account impact of anthropogenic compounds on HS in microsites of their accumulation in soil, the significance of abiotic reactions may be quite considerable. This was shown using phosphates as an example; their effect on humic substances was approximated to the conditions intrinsic to the location of a water-soluble phosphorus fertilizer granule.

CONCLUSIONS

The results showed that difference electronic absorption spectra are helpful in detecting fine structural variations in chromophore fragments of humic acid molecules, initiated by interactions with inorganic anions. Intermolecular interactions affect the structure of humic acids from arable soil to a considerably greater extent as compared to humic acids isolated from virgin soil. The effect directly depends on the concentration of phosphorus in humic acids from arable and virgin grey forest soils. A large contribution of inorganic anions to the interactions with humic acids was revealed and shown to change their spectral properties, as well as the yield and chemical composition. The importance of specific (donor–acceptor) interactions with phosphate anions as a factor determining properties of humic acids was especially emphasized. According to the spectral and chemical data, chelate entities containing tervalent metal (Al, Fe) and phosphorus atoms are formed in humic acid molecules isolated from overphosphatized soil. It is presumed that these entities originate from incorporation of anionic metal phosphate complexes generated by coordination of simple phosphate anions to Al and Fe atoms in humic molecules. Phosphorus-modified fragments of humic molecules were found to undergo profound transformations in further reactions with phosphate anions. The presence of P=O groups strongly enhances the ability of humic macroligands to form metal chelates and their stabilization in polar media. This should be taken into account while analyzing overphosphatized soils for metals and phosphorus using standard analytical procedures.

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