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Regularities of Extracting Humic Acids from Soils Using Sodium Pyrophosphate Solutions

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Abstract—Regularities of extracting humic acids from different soil types (soddy-podzolic soil, gray forest soil, and all chernozem subtypes) with sodium pyrophosphate solutions at different pH values (from 5 to 13) have been studied. It is found that, regardless of soil type, the process occurs in two stages through the dissociation of carboxylic groups and phenolic hydroxyls, each of which can be described by a logistic function. Parameters of the logistic equations approximating the extraction of humic acids from soils at different pH values are independent of the content and composition of humus in soils. Changes in the optical density of humic acids extracted from soils using sodium pyrophosphate solutions with different pH values are described in the first approximation by the Gaussian function. The optically densest humic acids are extracted using sodium pyrophosphate solutions at pH 10. Therefore, it is proposed to use an extract with pH 10 for the characterization of organic matter with the maximum possible degree of humification in the given soil.

Keywords: organic matter, humic acids, Umbric Retisols (Abruptic), Greyzemic Phaeozem, Haplic Chernozems (Pachic)

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INTRODUCTION

The study of soil organic matter is based on the concepts of heterogeneity of its composition and properties. It follows two main lines of inquiry: the chemical composting of humus depending on forms and strengths of bonds with mineral soil components and the biokinetic fractionation with the separation of organic pools with different thermodynamic stabilities depending on their mineralization capacities. Despite the actively developing methods for the biokinetic fractionation of soil organic matter [18, 19], chemical methods remain the most common and are widely used in the study of humus quality, not only in agronomical practice, but also in ecological investigations [9, 14, 22, 23].

Sodium pyrophosphate is one of the main chemical reagents whose solutions are used for extracting humic acids from soils. The over half-century-long practice of using sodium pyrophosphate solutions to study humic substances in soils, peats, and brown coals was described in detail earlier [4]. In the opinion of most researchers, the action mechanism of sodium pyrophosphate reduces to the degradation of organic–mineral and humus–mineral compounds due to the irreversible exchange reaction with the formation of insoluble salts with calcium, aluminum, and iron, as well as the formation of soluble sodium humus and aluminum–and iron–humus salts, which pass into the solution (extract) [2, 4, 6, 24, 29].

The use of pyrophosphate solutions and the interpretation of results obtained at the extraction of humic acids (HAs) using sodium pyrophosphate solutions are directly related to the alkalinity of the solutions used. It is generally accepted that a neutral sodium pyrophosphate solution (pH 7) extracts labile humic substances [10, 17, 21], while a strongly alkaline extract (pH 13) is almost analogous to that obtained after soil decalcification and, hence, also contains stable fractions, which is confirmed experimentally [25, 27]. A strongly alkaline solution of sodium pyrophosphate is used for the fractionation of humic substances according to the accelerated Kononova–Belchikova procedure for their maximum extraction from the soil.

It should be noted that the use of a strongly alkaline pyrophosphate solution for the complete extraction of HAs from different substrates never drew objections, while not all authors recommend the use of a neutral solution for the extraction of labile substances. Many researchers think that the use of sodium pyrophosphate at pH 7 in chernozemic soils is not advisable and

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† Deceased.

recommend it only for soddy-podzolic soils [5, 16, 17]. However, it is still unclear what this opinion is based on: principal differences in the humus properties or in the extractions processes of humic substances depending on the soil type. In this context, it is important to study regularities of the extraction of humic substances from different soil types using sodium pyrophosphate solutions in a wide pH range for determining the main regularities of this process.

OBJECTS AND METHODS

Regularities of extracting humic substances from different soils were studied in two series of experiments. Objects of study included samples of different soil types collected from upper humus-enriched horizons. The first series included three soil types: soddy-podzolic soil (Umbric Retisol (Abruptic) according to WRB-2014), gray forest soil (Greyzemic Phaeozem), and ordinary chernozem (Haplic Chernozem (Pachic)). The detailed characterization of these soils, including humus composition, was described earlier [3]. The second series included four reference samples: soddy-podzolic soil (Umbric Retisol (Abruptic)) and three chernozem subtypes: leached chernozem (Luvic Chernozem (Pachic)), typical chernozem (Haplic Chernozem (Pachic)), and southern chernozem (Haplic Chernozem).

The experimental procedure for the extraction of humic substances from soils using sodium pyrophosphate solutions at different pH values was as follows. Decimolar solutions of sodium pyrophosphate were brought to the target pH by adding NaOH or H_2SO_4 with an accuracy of ±0.05 pH units. The initial pH value was a weakly acid solution reaction (pH 5), and the final was pH 13. The combination of humic acids passing into solution at pH 13 characterizes the maximum recovery of humic acids from the soil (except the strongly bound acids of the third fractions, which are extracted from the soil only after long alkaline hydrolysis). The sample/solution ratio is 1 : 10; the exposure time is 18–20 h at room temperature. In the first series of experiments, extracts were filtered through a paper filter using a saturated sodium sulfate solution as coagulant, which was added to the extract 30 min before the filtration, as recommended by Ponomareva and Plotnikova [14]. At the second stage of the study, the filtration of extracts was performed without coagulation of mineral colloids in accordance with recommendations of Aleksandrova [2], Kononova and Belchikova [6], and D'yakonova [17], who deliberately omitted this operation and emphasized that the use of a Na_2SO_4 solution can result in the coprecipitation of HAs with colloids and thus reduce their content. HAs were precipitated with a 1.0 N sulfuric acid under short-term heating to 60–70°C, as recommended in the classical works by Tyurin [20], Kononova and Belchikova [6], Aleksandrova [1], and Ponomareva and Plotnikova [15]. In extracts, C_{tot} was also determined after the precipitation of HAs (C_{HA}). The optical density index of HAs, $E_C^{mg/mL}$, was measured at 440 nm using a 1-cm cell according to the Plotnikova–Ponomareva procedure [12, 13].

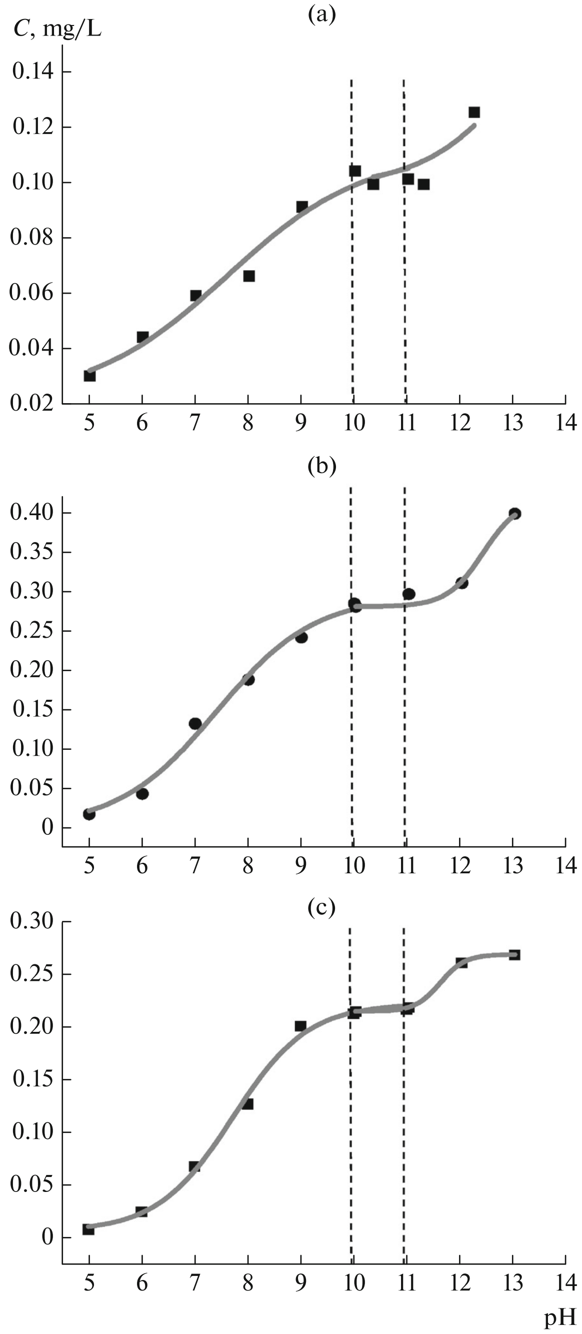

Fig. 1. Extraction of HAs by a 0.1 M $Na_4P_2O_7$ solution at different pH values with salting out from the soils: (a) soddy-podzolic soil; (b) gray forest soil; (c) chernozem.

From the results of two series of experiments on the extraction of humic substances from different soil

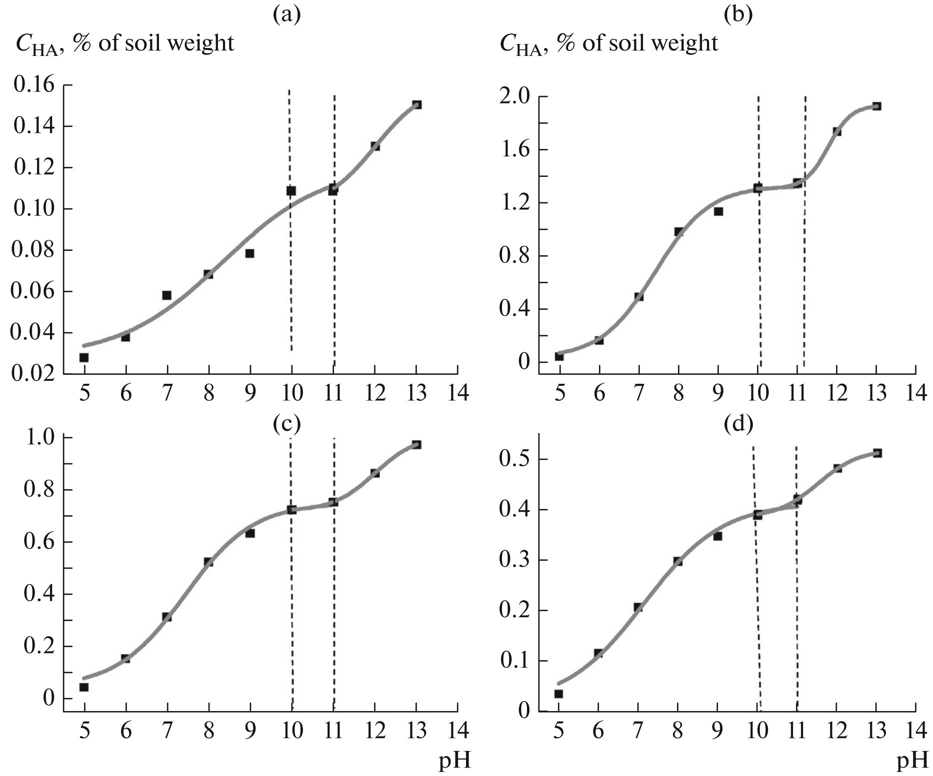

Fig. 2. Extraction of HAs with a 0.1 M $Na_4P_2O_7$ solution from the soils—(a) soddy-podzolic soil, OSO 10801; (b) leached chernozem, OSO 29107; (c) ordinary chernozem, OSO 29801; (d) southern chernozem, OSO30501—at different pH values without salting out.

types (with and without salting out), mathematical simulation was performed to select functions most adequately reflecting the chemical processes and to calculate the equation parameters. It is known that mathematical simulation, i.e., the formalized description of real objects or processes as a combination of mathematical concepts (formulas, equations, inequations, etc.), can reveal the most significant properties of objects or processes. Analysis was performed for HAs as a group of compounds, to which the most important and informative role is conventionally assigned in the assessment of the fertility and ecological stability of soils. Mathematical simulation was performed using Origin-Pro 7.5.

RESULTS AND DISCUSSION

From the experimental results, the extraction of HAs from soils can be described as a two-stage process, regardless of soil type and humus content and composition. Stage I, at which HAs are extracted from the soil mainly due to the dissociation of carboxylic groups, is observed in the pH range from 5 to 11. At stage II, the dissociation of phenolic hydroxyls is added, which occurs in the pH range between 10 and 13. Thus, there is a pH range from 10 to 11, which belongs to both the stages. Studies showed that the both stage are described by logistic functions, which overlapped with each other in the pH range from 10 to 11

$$C_{(\mathrm{pH})} = C_{\min} + \frac{C_{\max} - C_{\min}}{1 + \exp[(\mathrm{pH} - \mathrm{pH}_0)/\mathrm{pH}_C]}, \qquad (1)$$

where $C_{\min}$ is the content of carbon at the initial pH value for each experimental stage (pH 5 for the first stage and pH 11 for the second stage); $C_{\max}$ is the maximum possible content of C_{HA} in the extract at the given stage (pH 10 for the first stage and pH 13 for the second stage); pH_0 is the pH value in the inflection point, where $C = (C_{\max} + C_{\min})/2$; pH_C is the constant, which characterizes the slope of transition from $C_{\max}$ to $C_{\min}$ at each reaction stage.

The experimental relationships for the experiments with and without salting out are presented in Figs. 1 and 2, respectively. Parameters of Eq. (1) for both stages of HA extraction from soils are given in Table 1. It should be emphasized that the developed model ensures a very high approximation quality of experimental data, because the determination coefficient R^2 varies in the range from 0.976 to 0.996.

The following conclusions can be drawn from the experimental data, mathematical simulation, and analysis of calculated equation parameters. Extraction of HAs using sodium pyrophosphate solutions occurs similarly to different soil types. The content of HAs passed into solution at different pH values directly depends on the capacity of acid functional groups (carboxylic and/or phenolic hydroxyl ones) to dissociate at these pH values. The dissociation of HA functional groups occurs in some pH range rather than at a specific pH value, which determines regularities of their gradual solubilization, as visible in the plot (Figs. 1 and 2).

The content of HAs extracted from soils using sodium pyrophosphate solutions, which is characterized by parameters $C_{\min}$ and $C_{\max}$, significantly varies depending on the soil type and depends on the total content and qualitative composition of humus. However, the relationship between the extraction of HAs and the solution pH, which is characterized by parameters pH_0 and pH_C, is similar for all of the studied soils.

First, the pH_0 values for both experimental stages, which are mainly related to the dissociation of carboxylic groups (stage I) or phenolic hydroxyls (stage II), are very similar in different soils, regardless of the presence or absence of salting out of mineral colloids, which results in a partial loss of HAs due to coprecipitation. For the first step of the experiment with salting out, pH_0 was 7.57 ± 0.33 at stage I and 12.40 ± 0.80 at stage II. For the second step of the experiment without salting out, pH_0 was 7.58 ± 0.61 at stage I and 11.83 ± 0.31 at stage II. When these parameters are averaged for two experiments, it may be stated with a high degree of certainty that the pH_0 value characterizing the content of HAs extracted from the soil mainly due to the dissociation of carboxylic groups is 7.6 ± 0.3; the pH_0 value characterizing the content of HAs extracted from the soil mainly due to the dissociation of phenolic hydroxyls is 12.1 ± 0.5.

Second, for all of the studied soils, the rate of HA extraction into solution due to the dissociation of carboxylic groups, i.e., below pH 10, is lower than that due to the dissociation of phenolic hydroxyls (pH from 10 to 13), because the constant pH_C, which characterizes the slope of transition from $C_{\min}$ to $C_{\max}$, is always higher (by 1.6–3.1 times) at stage I than at stage II.

Thus, the regularities of extracting humic substances with sodium pyrophosphate solutions at increasing pH values do not depend on the soil type and the content and fractional–group composition of humus; they are determined by the pH ranges in which the dissociation of carboxylic and phenolic hydroxyl groups occurs.

The nature of HAs was characterized using the optical density index $E_C^{\text{mg/mL}}$ proposed by Plotnikova and Ponomareva, which is one of the most informative parameters for assessing the chemical nature of humic acids and is successfully used in soil science. It indirectly characterizes the degree of humification of organic matter, because the values of $E_C^{\text{mg/mL}}$ are directly and almost

Table 1. Parameters of Eq. (1) approximating the extraction of humic acids from soils by $Na_4P_2O_7$ solutions at different pH values

Parameter	Stage I	Stage II
Soddy-podzolic soil		
$C_{\min}$, g/L	0.020 ± 0.021	0.102 ± 0
$C_{\max}$, g/L	0.111 ± 0.014	0.150 ± 0
pH_0	7.59 ± 0.60	13.21 ± 0.3
pH_C	1.28 ± 0.75	0.75 ± 0
R^2	0.976	0.790
Gray forest soil		
$C_{\min}$, g/L	0.001 ± 0	0.284 ± 0
$C_{\max}$, g/L	0.294 ± 0.002	0.420 ± 0
pH_0	7.40 ± 0.22	12.41 ± 0.10
pH_C	0.93 ± 0.16	0.34 ± 0.08
R^2	0.991	0.976
Ordinary chernozem		
$C_{\min}$, g/L	0.007 ± 0	0.214 ± 0
$C_{\max}$, g/L	0.223 ± 0.006	0.268 ± 0.001
pH_0	7.72 ± 0.09	11.59 ± 0.01
pH_C	0.71 ± 0.07	0.23 ± 0.01
R^2	0.996	1
Soddy-podzolic soil, OSO 10801		
$C_{\min}$, g/L	0.03 ± 0	0.10 ± 0
$C_{\max}$, g/L	0.12 ± 0.02	0.16 ± 0
pH_0	8.34 ± 0.59	12.00 ± 0
pH_C	1.23 ± 0.36	0.62 ± 0
R^2	0.966	1
Leached chernozem, OSO 29107		
$C_{\min}$, g/L	0.04 ± 0	1.32 ± 0
$C_{\max}$, g/L	1.33 ± 0.02	1.95 ± 0
pH_0	7.41 ± 0.10	11.80 ± 0
pH_C	0.68 ± 0.08	0.29 ± 0
R^2	0.994	1
Ordinary chernozem, OSO 29801		
$C_{\min}$, g/L	0.04 ± 0	0.71 ± 0
$C_{\max}$, g/L	0.75 ± 0.04	1.01 ± 0
pH_0	7.43 ± 0.10	12.00 ± 0
pH_C	0.82 ± 0.08	0.51 ± 0
R^2	0.995	1
Southern chernozem, OSO 30501		
$C_{\min}$, g/L	0.02 ± 0	0.38 ± 0
$C_{\max}$, g/L	0.42 ± 0.01	0.52 ± 0
pH_0	7.15 ± 0.14	11.51 ± 0
pH_C	0.98 ± 0.11	0.52 ± 0
R^2	0.993	1

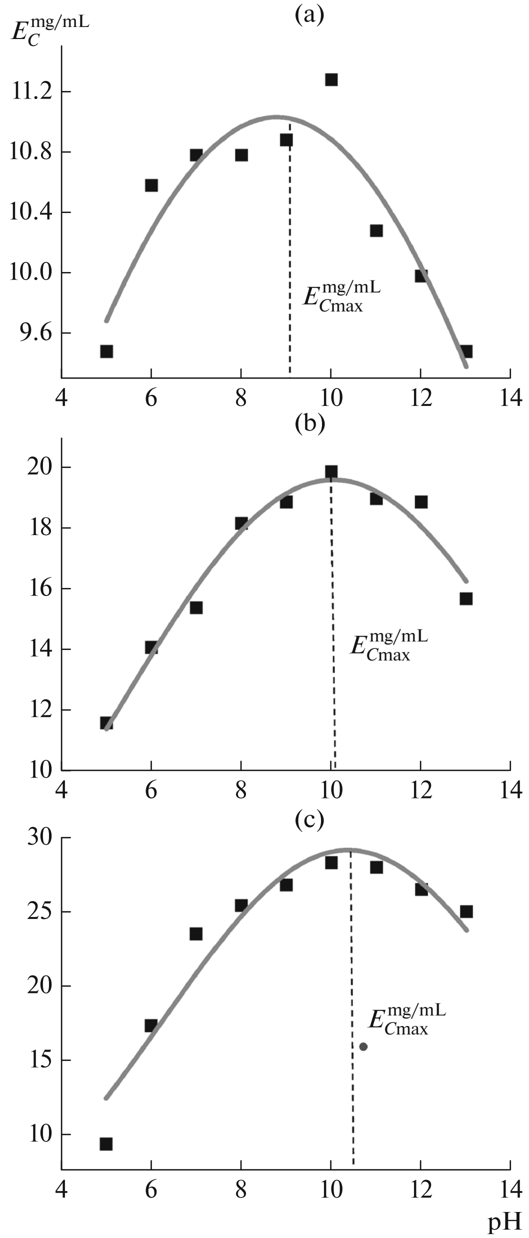

Fig. 3. Indices $E_C^{mg/mL}$ of HAs from the soils—(a) soddy-podzolic soil; (b) gray forest soil; (c) chernozem—as functions of sodium pyrophosphate solution pH, an experiment without salting out.

functionally related to the share of aromatic structures in HA molecules. Numerous recent works devoted to the study of the chemical structure of humic substances also confirm the direct relationship between the content of aromatic structures in HA molecules (i.e., their degree of humification) and the absorption of electromagnetic radiation in the visible spectral region [7, 8, 11, 26, 28], although some authors oppose the use of optical density as a single criterion for the degree of humification of humic substance [21].

Mathematical analysis of the results of determining the optical density indices of HAs in both experiments revealed the following. The value of change in $E_C^{mg/mL}$ for HAs extracted from the soil using sodium pyrophosphate solutions depending on pH can be described in the first approximation by the Gaussian function

$$E_C^{mg/mL} = E_{C_0}^{mg/mL} + E_{C\max}^{mg/mL} \times \exp\left[-2\frac{(\mathrm{pH} - \mathrm{pH}_{\max})^2}{\omega^2}\right], \tag{2}$$

where $E_{C_0}^{mg/mL}$ is the initial value of HA optical density index independent on pH; $E_{C\max}^{mg/mL}$ is the maximum value of HA optical density index; $\mathrm{pH}_{\max}$ is the pH value at which HAs are characterized by $E_{C\max}^{mg/mL}$; and $\omega = \frac{\sigma}{\sqrt{\ln 4}} = 1.177\sigma$ is the parameter characterizing the peak width (pH), σ being equal to the peak width at the half-height.

Results of both experiments are graphically shown in Figs. 3 and 4. The main parameters of Eq. (2) approximating the index $E_C^{mg/mL}$ as a function of pH are given in Table 2.

The approximation quality of the developed model is found to be slightly lower than that of the model for the quantitative extraction of HAs (Table 1); however, it is also very high (R^2 varies from 0.864 to 0.974).

The presented data show that the maximum value of HA optical density index, which is determined by the parameter $E_{C\max}^{mg/mL}$, significantly varies among soil types. The values of $E_{C\max}^{mg/mL}$ for HAs from soddy-podzolic soils are low (11.1–14.2), but those of HAs from gray forest soil and especially chernozems increase to 20–30 and higher. The optical density index apparently depends only on the chemical structure of HAs. However, the regularities of changes in the $E_C^{mg/mL}$ value depending on the pH of the sodium pyrophosphate solution used for extraction are common for all soil types, as evidenced by Eq. (2) parameters calculated for $\mathrm{pH}_{\max}$, i.e., the pH at which HAs have the maximum optical density index $E_{C\max}^{mg/mL}$.

For all of the soils studied in the experiment, despite the significant variation of the index $E_C^{mg/mL}$ characterizing HAs, its maximum value in any soil is reached at pH of about 10. When the $\mathrm{pH}_{\max}$ values are averaged for the entire set of data, it may be stated that the optically densest HAs, which are characterized by the highest $E_C^{mg/mL}$ values in the given soil, are extracted at pH 10.0 ± 0.6.

Thus, the sodium pyrophosphate solution at pH 10 extracts HAs with the maximum optical density indices from soils. This suggests that HAs characterized by

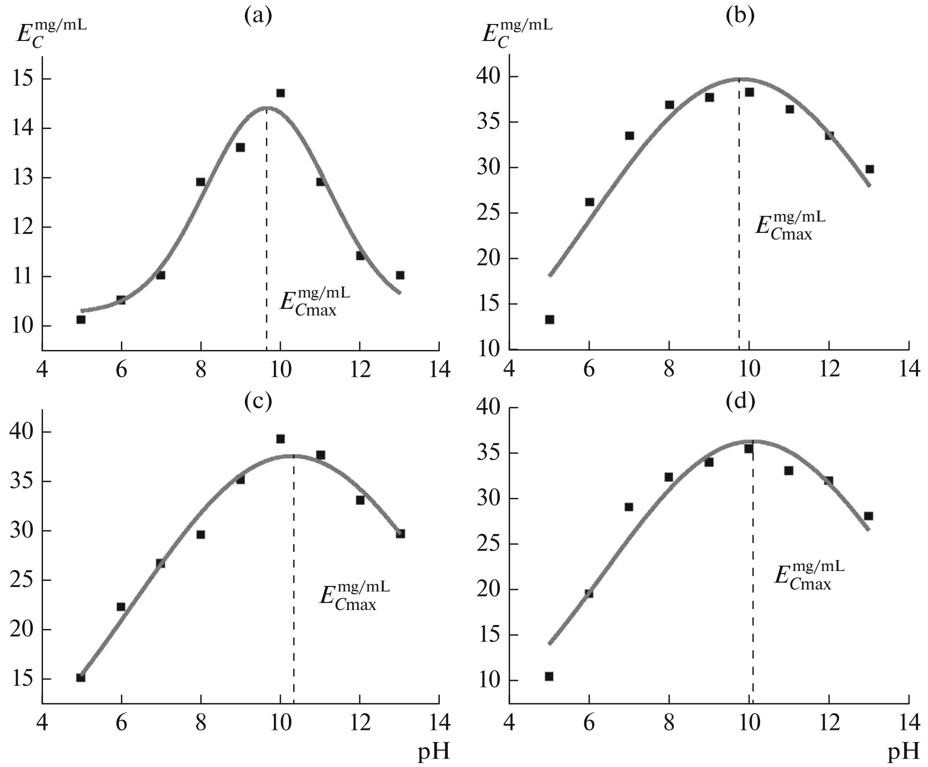

Fig. 4. Indices $E_C^{mg/mL}$ of HAs from the soils—(a) soddy-podzolic soil, OSO 10801; (b) leached chernozem, OSO 29107; (c) ordinary chernozem, OSO 29801; (d) southern chernozem, OSO30501—as functions of sodium pyrophosphate solution pH, an experiment with salting out.

the maximum possible degree of humification in the given soil are extracted at pH 10 due to the dissociation of carboxylic groups. In the alkaline pH region from 11 to 13, in which phenolic hydroxyls are the major reacting groups, there occurs the extraction of HAs with lower degrees of humification, as evidenced by their optical density indices.

The reveled regularities in the extraction of humic substances using sodium pyrophosphate solutions, which are common for all soil types, allow a question to be raised as to the suitability of a differentiated approach to the selection of reagents in the study of labile humic substances. Different reagents are proposed to be used in this study: neutral and (more rarely) weakly alkaline solutions of sodium pyrophosphate for soddy-podzolic soils and a 0.1 N NaOH solution for chernozems [5, 16, 17]. However, in our opinion, the extraction of the same groups of humic substances (mobile, labile, etc.) from soils by different

Table 2. Parameters of Eq. (2) approximating the index $E_C^{mg/mL}$ of humic acids extracted from different soils as a function of sodium pyrophosphate solution pH

Soil	Parameter		
	pH_{max}	$E_{C\,max}^{mg/mL}$	R^2
Soddy-podzolic soil	9.91 ± 0.22	11.1 ± 1.6	0.864
Gray forest soil	10.03 ± 0.12	19.6 ± 1.8	0.970
Ordinary chernozems	10.41 ± 0.32	29.2 ± 4.4	0.931
Soddy-podzolic soil, OSO 10801	9.66 ± 0.12	14.2 ± 1.3	0.964
Leached chernozem, OSO 29107	9.80 ± 0.21	39.7 ± 4.0	0.908
Ordinary chernozem, OSO 29801	10.28 ± 0.14	37.7 ± 3.4	0.974
Southern chernozem, OSO 30501	10.08 ± 0.21	36.4 ± 4.4	0.934

reagents depending on soil type is theoretically incorrect and introduces uncertainty in the study of intermediate and transitional soil types, e.g., gray forest soils. One would argue that the differentiated approach is widely used in agrochemical practice depending on soil type, e.g., to study mobile forms of nutrients (phosphorus and potassium) using different extractants. So, mobile potassium forms are extracted with 0.2 N hydrochloric acid according to the Kirsanov method from acid noncalcareous (podzolic, soddy-podzolic, and forest) soils and by the Machigin method involving potassium extraction with a 1% ammonium carbonate solution from calcareous soils (chernozems, chestnut soils, and serozems). However, the main task in the selection of reagent for the extraction of mobile, plant-available phosphorus and potassium from soils is to imitate soil solutions (weakly acid in acid soils and weakly alkaline in calcareous soils). On the other hand, the main purpose of reagents used in the study of soil organic matter is to break bonds of humic substances with mineral components and to make these substances soluble. In our opinion, such reagents should be used for all soils with consideration for the properties of humic substances necessary for interaction with some or other reagent with the formation of soluble compounds extractable from the soil. Thus, both alkali and sodium pyrophosphate solutions should be used for all soils, and the same chemical (biochemical, ecological) interpretation of results should be used depending on the mechanism of chemical reactions. The decisive criterion in the estimation of the availability of humic substances for biochemical decomposition should be primarily the degree of their chemical maturity and humification rather than the amount of substances passed into solution. The chemical reactions occurring in all soils (sediments, peats, brown coal) under the effect of sodium pyrophosphate solutions are obviously of the same nature, and the difference will be in the initial content of humic substances capable of interaction with the extractant. This conclusion is illustrated by the revealed regularities in the extraction of humic substances from different soil types using sodium pyrophosphate solutions, which are common for all soil types and do not depend on the content and qualitative composition of their humus.

CONCLUSIONS

(1) The extraction of HAs from soils under increasing pH of sodium pyrophosphate solution from 5 to 13 proceeds in two stages, and each of them can be described by a logistic function. Stage I, at which HAs are extracted mainly due to the dissociation of carboxylic groups, occurs at pH of 5 to 11; stage II, at which HAs are extracted due to the dissociation of phenol hydroxyls, occurs at pH of 10 to 13.

(2) Parameters of logistic equations approximating the extraction of HAs from soils at different solution pH are independent of the content and composition of soil humus.

(3) The optically densest HAs characterized by the maximum values of $E_C^{\mathrm{mg/mL}}$ are extracted using sodium pyrophosphate solutions at pH 10.0 ± 0.6. Therefore, a sodium pyrophosphate solution with pH 10 is suitable for characterizing the maximum possible degree of humification of organic matter in soils.

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