

## Assessing the Complexing Properties of Soil Organic Matter by IMAC (Case Study of Copper Ions)

A. A. Dymov<sup>a, \*</sup> and E. Yu. Milanovskii<sup>b, c</sup>

<sup>a</sup>*Institute of Biology, Komi Scientific Center, Ural Branch, Russian Academy of Sciences, Syktyvkar, 167982 Russia*

<sup>b</sup>*Lomonosov Moscow State University, Moscow, 119991 Russia*

<sup>c</sup>*Dokuchaev Soil Science Institute, Moscow, 119017 Russia*

\*e-mail: aadymov@gmail.com

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**Abstract**—Immobilized metal affinity chromatography (IMAC) has been used to fractionate organic compounds in alkaline extracts from forest soils of the Komi Republic by their ability to form complexes with copper ions. An algorithm for chromatographic fractionation of substances into the groups of homogeneous molecular compounds is proposed by selecting the separation conditions; this algorithm comprises sequential change in pH and ionic strength of the eluent. As is shown, the alkaline extract contains three fractions, the components of which differ in their ability to form complexes with copper. The humic substances of iron-illuvial podzol (Albic Podzol), podzolic (Glossic Retisol), and peaty-podzolic gleyic (Histic Retisol) soils are shown to differ in their relative contents of ligand-forming organic compounds. The maximum content (according to  $C_{\text{org}}$ ) of copper complexones is recorded in the organic matter of forest litters of the examined soils (14–21 g/kg). The corresponding values of the organic matter content in the eluvial horizons are 0.5–1.5 g/kg. The highest content of the carbon compounds able to bind copper ions is characteristic of podzolic soils, while the organic matter components of podzols and peaty-podzolic gleyic soil display somewhat lower affinity for copper. The proposed algorithm can be used to assess the soil vulnerability to heavy metal pollution.

**Keywords:** forest soils, soil organic matter, SOM fractionation methods

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### INTRODUCTION

Soils are the strongest geochemical barrier for pollutants. Heavy metals (HMs) are among the major environmental pollutants. Various types of anthropogenic activities bring pollutants to the environment [13, 19]. The fate of pollutants—accumulation in soil or migration to subordinate landscapes and water bodies—depends on the properties of soils. Soil organic matter plays the leading role in HM accumulation [1, 3, 7, 16, 30]. The mechanisms of HM accumulation and migration in soils and geochemically associated landscapes have been studied mainly by pH titration and ion exchange equilibrium [15]. The types of HM compounds in soils have been shown to influence their fate considerably [9, 14]. The advent of chromatographic techniques allowing for fractionation of organic compounds by their complex-forming capacity significantly expands our understanding of the mechanisms underlying natural chemical differentiation of the profile, as well as the accumulation and migration of pollutants for further regulation and control of their content.

Development of the toolkit of analytical chemistry determines a gradual reinterpretation of the role and

function of individual organic compounds and their groups with close functional features. One of the promising methods for assessing the functional properties of organic matter is metal affinity chromatography [11, 25].

The term ligand exchange chromatography was introduced in 1961 by Helfferich [24]. The concept of immobilized-metal affinity chromatography (IMAC) was formulated and published by Porath et al. [31]. This approach utilizes the ability of biological macromolecules to bind metal ions (such as  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Fe}^{2+}$ ) immobilized on a chromatography matrix. The specific binding is determined by the presence of free electron-donor groups on the surface of molecules. Under IMAC conditions (neutral pH and high salt concentration), the imidazole group of histidine ( $\text{pK} \sim 6.7$ ), thiol group of cysteine ( $\text{pK} \sim 8.5$ ), and indole group of tryptophan ( $\text{pK} \sim 9.41$ ) are the potential ligands. C-terminal amino acids ( $\text{pK} \sim 7.7$ ), as well as aspartic acid and glutamic acid residues ( $\text{pK} \sim 3.9$ ), can take part in such interaction. The amino acids are ordered as follows according to the stability of the formed complexes: His, Cys > Asp, Glu  $\gg$  other amino acids. The interaction between the sorbent and

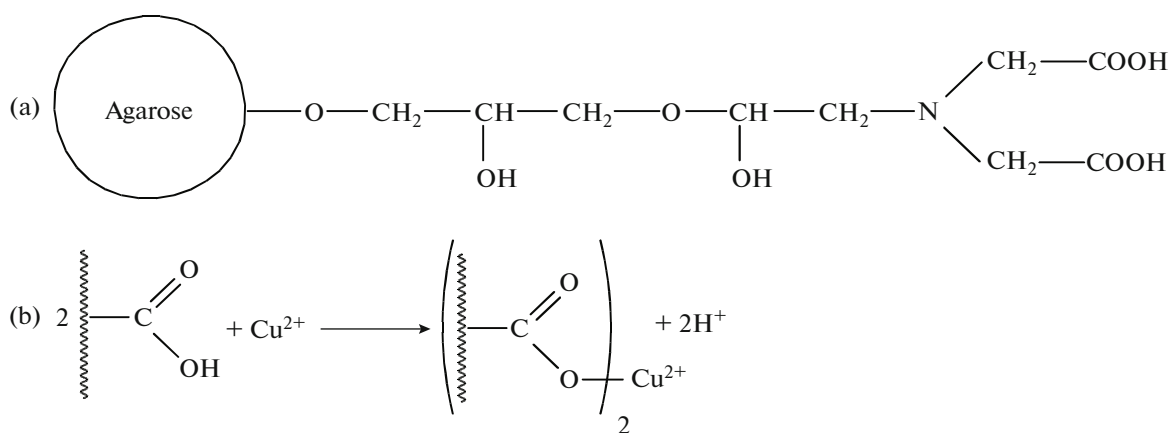


Fig. 1. (a) Structure of fast flow agarose gel and (b) scheme of interaction (charging) of the column with copper ions.

analyte is pH-dependent. Correspondingly, the bound substances can be eluted by decreasing the ionic strength of the buffer, changing pH, or using other chelating agents [20, 21, 31].

Currently, IMAC is used for pretreating samples assayed for drugs [10], for complexing properties of organic compounds in seawater [27], and for fractionation and purification of proteins and amino acids in biochemical studies [2, 8, 18, 32]. Recent decades brought about the first papers on the use of IMAC for assessing the complexing properties of organic compounds in water objects, humic substances (HSs), and soil extracts [23, 25, 28, 29].

The goal of this work was to assess the potential of IMAC in studies of the complexing properties of organic matter in the forest soils of European North.

The study included (i) estimation of the IMAC potential for identification of complex-forming compounds in labile soil organic matter; (ii) characterization of the specific features of homogeneous molecular fractions in organic matter using individual organic compounds carrying different ionogenic groups; and (iii) IMAC assessment of the specific features in HS complexing properties in the prevalent soil types of the taiga zone.

## OBJECTS AND METHODS

The upper genetic horizons of the soil types typical in the Komi Republic were the objects of this study, namely, (I) iron-illuvial podzol (Albic Podzol) developed from sandy material under lichen pine stands in the middle taiga [22]; (II) podzol developed from covering loam under dwarf shrub spruce stands in the middle taiga (Glossic Retisol) of the southern Timan Range [5]; and (III) peaty-podzolic gleyic soil (Histic Retisol) developed from moraine loams under myrtillus-sphagnum spruce stand in the northern taiga of the Subpolar Urals [4].

Humic substances were extracted from soil specimens with 0.1 mol/dm<sup>3</sup> NaOH at a soil-to-solution ratio of 1 : 10 for 20–24 h. The HS extract was purified from mineral impurities by centrifugation (10000 rpm, 20 min). The supernatant (0.5 cm<sup>3</sup>) was supplemented with 1.0 cm<sup>3</sup> of the starting solution (0.02 mol/dm<sup>3</sup> phosphate buffer containing 0.5 mol/dm<sup>3</sup> NaCl, pH 7.0).

Isolated humic substances were fractionated by chromatography in agarose gel (Fig. 1) with covalently linked chelate groups (IMAC Sepharose 6 FF-GE Healthcare) in a 1 × 10 cm Bio-Rad column; gel dynamic binding capacity, 25 mg (Cu<sup>2+</sup>)/cm<sup>3</sup> [26]. The scheme of complex formation is shown in Fig. 1b.

Copper ions were immobilized on the chromatography matrix using a 1 × 10 cm Bio-Rad column filled with IMAC Sepharose 6 FF gel by pumping 10 cm of 0.5 mol/dm<sup>3</sup> CuSO<sub>4</sub> solution using an Econopump. Uniform blue staining of the gel made it possible to visually control the qualitative state of the column (Fig. 2).

To remove the copper ions that did not react with the gel, the column was washed with 40 cm<sup>3</sup> of distilled water and 20 cm<sup>3</sup> of starting buffer (0.02 mol/dm<sup>3</sup> phosphate buffer with 0.5 mol/dm<sup>3</sup> NaCl, pH 7.0). An increase in the ionic strength of the buffer by adding NaCl minimizes the nonspecific interaction of HSs with affinity matrix. A BioLogic LP (United States) chromatography system was used for separation. Optical density was recorded at 280 nm; the feed rate of eluents was 2 cm<sup>3</sup>/min.

The solution of HSs (440 μL) was loaded on the column equilibrated with starting solution. The components of the sample that did not react with copper ions were eluted in the first chromatography fraction. The affinity interaction of organic compounds with chromatography matrix was weakened via decreasing pH of the eluent. Fraction 2 of weakly bound organic compounds was eluted with 0.05 mol/dm<sup>3</sup> acetate buf-

fer supplemented with 0.5 mol/dm<sup>3</sup> NaCl (pH 4). Fraction 3 (stably bound organic compounds) was eluted with 0.2 mol/dm<sup>3</sup> NaOH supplemented with 0.5 mol/dm<sup>3</sup> NaCl (pH 12). After fractionation, copper ions were removed from the column with 0.2 mol/dm<sup>3</sup> EDTA solution.

For regeneration, the gel was placed into a Buchner funnel and successively washed with distilled water, 25% propanol, 50% propanol, 100% propanol, 100% butanol, 50% propanol, 25% propanol, and distilled water, 15–20 mL each. The regenerated gel was loaded into the chromatography column and saturated with copper ions. All used solutions were filtered through 0.22- $\mu$ m membrane Millipore filters, degassed, and controlled for pH. Several compounds were used as standards: glycine (0.5 mol/dm<sup>3</sup>), oxalic acid (0.5 mol/dm<sup>3</sup>), histidine hydrochloride (0.1 mol/dm<sup>3</sup>), aspartic acid (0.1 mol/dm<sup>3</sup>), phenylalanine (0.1 mol/dm<sup>3</sup>), etc. MultiChrom 2.0 software was used for integration and computation of chromatography fraction areas.

## RESULTS AND DISCUSSION

Comprehensive descriptions of morphological and physicochemical properties of soils have been published earlier [4, 5, 22]. Table 1 consolidates the most general physicochemical properties of the examined soils. The studied soils have close physicochemical properties. The soils are acidic: the pH of water extracts varies from 3.3 in the organic horizon of peaty-podzolic gleyic soil to 4.3 in the litter horizon of podzol. A low degree of base saturation is characteristic of the soils. The organic horizons accumulate raw humus. Note that the organic matter in podzolic and peaty-podzolic gleyic soils is richer in nitrogen as

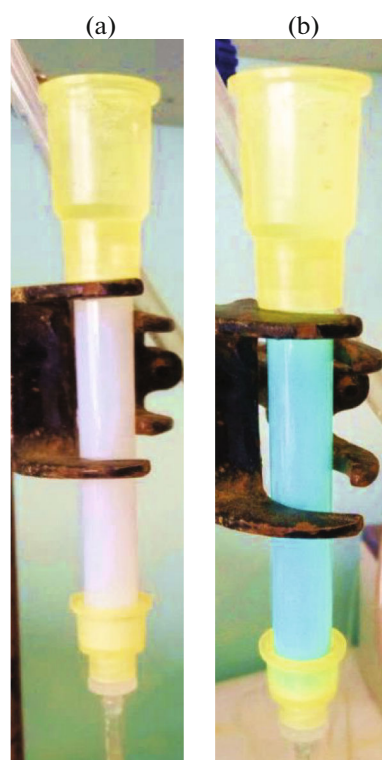


Fig. 2. Column with IMAC Sepharose 6 FF (a) before and (b) after “charging” with copper cations.

compared with that in iron-illuvial podzols. The content of carbon compounds extractable by decinormal alkali varies from 67–102 g/kg soil in organic horizons to 1.3–4.5 g/kg soil in mineral horizons. The fraction of alkali-soluble compounds amounts to 17–25% of  $C_{total}$  in litters and 18.4–32.5% in the upper mineral horizons. The maximum  $C_{alkaline}$  (relative to  $C_{total}$ ) is

Table 1. Physicochemical and chemical properties of examined soils

Horizon	Depth, cm	pH		HA*	Exchangeable cations		V**	$C_{total}$	$N_{total}$	$\frac{C}{N}$	$C_{alkaline}, \%$	$\frac{C_{alk}}{C_{org}}, \%$
		H <sub>2</sub> O	KCl		Ca <sup>2+</sup>	Mg <sup>2+</sup>						
		cmol(+)/kg					%					
Podzol (I)												
O(H)	1–3	4.3	3.3	45.9	8.8	1.4	18	43.7	0.69	54	7.48	17.1
E	3–13	4.2	3.3	3.6	0.3	0.1	10	0.87	0.02	37	0.16	18.4
Podzolic (II)												
O(H)	6–8	4.0	2.9	73.7	11.4	3.0	16	35.8	1.58	26	6.67	18.6
EL	8–11	3.9	2.9	14.9	0.6	0.3	6	2.2	0.15	17	0.45	20.4
Peaty-podzolic gleyic (III)												
O(H)	7–13	3.3	4.2	82.5	2.1	1.2	4	40.2	1.56	29	10.2	25.4
ELhi, g	13–30	3.8	5.0	7.8	0.3	0.1	5	0.4	0.03	15	0.13	32.5

\* Hydrolytic (total) acidity.

\*\* Base saturation, %.

observed in the horizons with morphological features organic matter migration in peaty-podzolic gleyic soil. According to the earlier data [6], the carbon content in the examined soils to a depth of 1 m is 2.9 kg/m<sup>2</sup> in podzols, 9.2 kg/m<sup>2</sup> in podzolic soils, and 12.1 kg/m<sup>2</sup> in peaty-podzolic gleyic soils. Although these soil types belong to boreal forests, they have several differences in the conditions of their formation, as well as accumulation and decomposition of organic matter. Two horizons—lower part of the litter and upper mineral horizon—were selected for the analysis, as these layers in most cases are most affected by chemical pollution.

The choice of fractionation algorithm involved testing of different combinations of conditions for HS alkaline extract fractionation into homogeneous molecular fractions utilizing the following practices of ligand exchange chromosome of proteins:

(i) The ligand exchange using histidine, imidazole, ammonium chloride, or other substances that form more stable complexes with metal ions (i.e., with the affinity for chelate metal ions);

(ii) Destruction of chelate compounds with a strong chelating agent (EDTA), thereby releasing compounds and desorbing metal; and

(iii) Linear or stepwise pH gradient or eluent providing protonation of HS donor groups.

The two first fractionation variants gave a low reproducibility and ambiguous data interpretation for the complex mixtures of HS alkaline extracts. Presumably, the approaches involving ligand exchange and destruction of chelate compounds with EDTA are better applicable to the mixtures that are more uniform in their molecular composition and require prepurification and removal of low molecular weight organic compounds.

The variant of HS separation with pH gradient displayed a high reproducibility; it included 10 min using buffer 1 (0.02 mol/dm<sup>3</sup> phosphate buffer in 0.5 mol/dm<sup>3</sup> NaCl, pH 7); 15 min, buffer 2 (0.05 mol/dm<sup>3</sup> acetate buffer in 0.5 mol/dm<sup>3</sup> NaCl, pH 4); and 20 min, buffer 3 (0.2 mol/dm<sup>3</sup> NaOH in 0.5 mol/dm<sup>3</sup> NaCl, pH 12). Examples of chromatograms are shown in Figs. 3 and 4.

The selected algorithm was used to fractionate the alkali-soluble organic matter of the examined soils (Fig. 5) and to assess the relative share of individual fractions (Table 2). The proposed algorithm allowed for separation of the HSs into three homogeneous molecular fractions according to their ability to interact with copper ions. The relative share demonstrates that the peaty-podzolic gleyic soil displays the minimal ability to bind copper ions and podzolic soil, the maximum ability. As for the mineral horizons, the HSs in the peaty-podzolic gleyic soil contain the largest share of organic compounds that interact with copper ions; the smallest share of these compounds is in the podzolic soil.

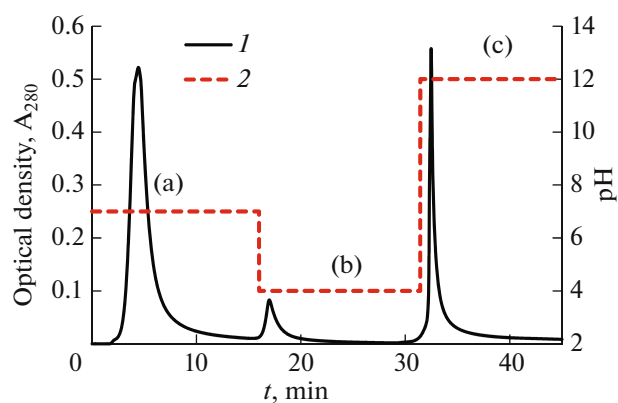


Fig. 3. IMAC fractionation of 0.1 mol/dm<sup>3</sup> NaOH extract: 1, optical density; 2, pH; (a) 0.02 mol/dm<sup>3</sup> phosphate buffer in 0.5 mol/dm<sup>3</sup> NaCl (pH 7); (b) 0.05 mol/dm<sup>3</sup> acetate buffer in 0.5 mol/dm<sup>3</sup> NaCl (pH 4); and (c) 0.2 mol/dm<sup>3</sup> NaOH in 0.5 mol/dm<sup>3</sup> NaCl (pH 12).

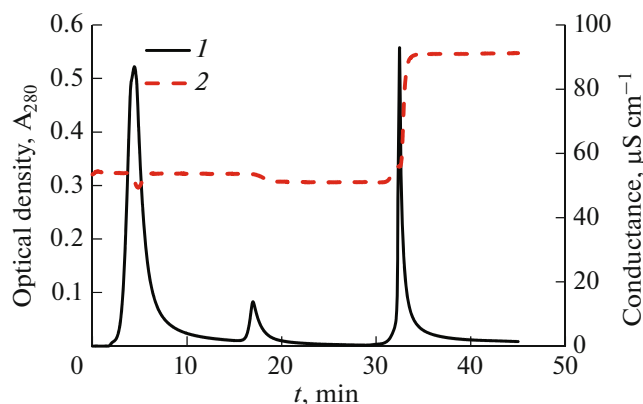


Fig. 4. Changes in the (1) optical density and (2) conductance of the eluate with time.

The estimates of the content of individual fractions with due account for the carbon content in alkaline extract and the relative content of chromatography fractions are listed in Table 3. Among the organic horizons, the minimum content of fractions 2 and 3 (14.4 g/kg soil) is observed in podzol; the litter of peaty-podzolic gleyic soil has similar values. The content of carbon of the bound and stably bound compounds in podzolic soil amounts to 21 g/kg soil with a minimum carbon content extractable by alkali. At a first glance, the complexing ability of the HS components in the mineral horizons is significantly lower. The maximum content of fractions 2 and 3 is observed in podzolic soil versus podzol and peaty-podzolic gleyic soil, where these values are two–three times lower. In part, this is associated with a smaller amount of organic carbon in the horizon. On the one hand, it is necessary to take into account that up to 50% HSs in the alkaline extracts from the eluvial horizons of

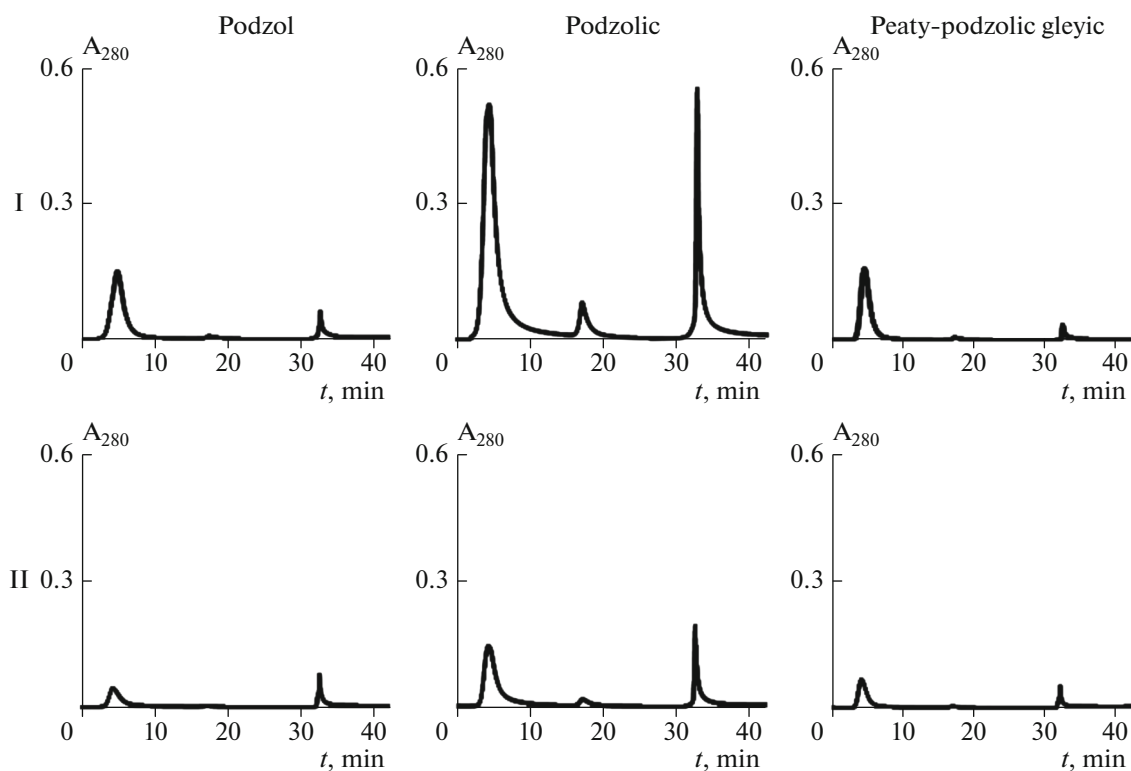


Fig. 5. Chromatograms of 0.1 mol/dm<sup>3</sup> NaOH extract of HSs from (I) litter and (II) eluvial horizons.

podzol are represented by stable complex compounds with iron and aluminum [6, 12], which are unable to interact with immobilized gel matrix.

Table 4 lists the stability constants ( $pK_a$ ) for copper ions in individual compounds with different func-

**Table 2.** Relative content of fractions in 0.1 mol/dm<sup>3</sup> NaOH extract from organic and mineral horizons (mean of three measurements), %

Soil type	Chromatography fraction*			Sum 2–3
	1	2	3	
Litter				
I	81 ± 6	3.2 ± 0.4	16 ± 5	19.2
II	68.7 ± 0.9	5.6 ± 0.6	25.8 ± 1.4	31.4
III	86 ± 3	4.4 ± 0.9	10.0 ± 2.4	14.4
Eluvial horizon				
I	60.8 ± 3.0	3.0 ± 0.9	36.3 ± 2.0	39.3
II	65.9 ± 3.5	5.5 ± 0.5	27 ± 3	32.5
III	60 ± 7	10 ± 4	30 ± 3	40

Here and in Table 3: 1, weakly bound organic compounds; 2, bound organic compounds; and 3, organic compounds stably bound to copper ions; I, iron-illuvial podzol; II, podzolic soil; and III, peaty-podzolic gleyic soil.

tional groups for approximation of the results to the soil properties. The obtained chromatograms and  $pK_a$  logarithms suggest that the first chromatographic peak contains the substances that either do not interact with the copper ions immobilized on gel surface or poorly interact with the ligand immobilized on the matrix. Correspondingly, they are eluted by starting buffer passing through the system. The range of  $pK_a$  values for individual compounds eluted with the retention time falling in the range of the first fraction is 4.4–8.6 (Table 3). Note that the individual compounds of the first fraction somewhat differ in their retention times (Fig. 6). Presumably, the first peak contains the substances with the properties similar to oxalic acid, aspartic acid, and glycine. This fraction mainly contains low molecular weight compounds with amino ( $-NH_2$ ) and carboxyl ( $-COOH$ ) functional groups. The elution time of the fractions of organic compounds that did not interact with copper ions corresponds to the elution time of model substances.

The second chromatography fraction contains the organic compounds bound to copper; they are eluted from the column in the presence of acetate buffer with a low pH. The range of the  $pK_a$  of such individual compounds is assessed at 8.6–10.2 (Fig. 7). The elution time of the second HS fraction of the soil alkaline extract also matches the elution time of model substances, namely, histidine hydrochloride and 8-hydroxyquino-



**Table 3.** Carbon content in 0.1 mol/dm<sup>3</sup> NaOH extract and chromatography fractions, g/kg

Soil type	C <sub>0.1 NaOH</sub>	Chromatography fraction			Sum 2–3
		1	2	3	
Litter					
I	75	60.8	2.4	12.0	14.4
II	67	46.0	3.8	17.3	21.0
III	102	87.7	4.5	10.2	14.7
Eluvial horizon					
I	1.6	1.0	0.0	0.6	0.6
II	4.5	3.0	0.2	1.2	1.5
III	1.3	0.8	0.1	0.4	0.5

Assumptions in calculations: all organic compounds of alkaline extract are eluted from the column, absorb light at  $\lambda = 280$  nm, and do not differ in extinction coefficient.

line. Presumably, this fraction contains the compounds with N-heterocyclic aromatic structures.

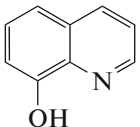
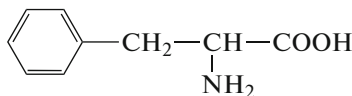
The elution time of 8-hydroxyquinoline peak suggests that the  $pK_a$  range for the organic compounds stably bound with copper ions is over 12.2. Taking into account that copper cations are completely eluted from the column with 0.2 mol/dm<sup>3</sup> EDTA solution, the upper limit of their  $pK_a$  is most likely 18. The chro-

matographic behavior of individual organic compounds suggests that they are analogous to soil HSs in their complexing characteristics (similarity of ionogenic groups). The third peak in chromatograms of model compounds is associated with the second stability constants, which have been described for most of the studied individual substances. Presumably, the compounds contained in the third fraction play the most significant role in long-term stabilization of copper cations. Most likely, these are the most complex molecular structures containing both aromatic structures and carboxyl groups. Conceivably, the components of this fraction are represented by the soil-specific biopolymers with the highest molecular weight.

The chromatographic peaks of soil samples were wider than those of the model organic substances, which points to a mixed composition of closely related ligands with very similar affinity for copper.

Note that ranking of the compounds contained in individual fractions according to  $pK_a$  is rather conditional, since  $pK_a$  values can somewhat change depending on pH and conductance. As has been demonstrated earlier [28], the  $pK_a$  of metal compounds with individual substances insignificantly influence the retention ability.

**Table 4.** Organic substances used in the work and their stability constants at pH 7 and 25°C (according to [17])

Substance	Structural formula	$pK_a$ of complex compounds with copper ions	Elution time, min
EDTA	$\begin{array}{c} \text{HOOCCH}_2 \\ \diagdown \\ \text{N} - \text{CH}_2 - \text{CH}_2 - \text{N} \\ \diagup \\ \text{NaOOCCH}_2 \end{array} \begin{array}{c} \text{CH}_2\text{COONa} \\ \diagdown \\ \text{N} \\ \diagup \\ \text{CH}_2\text{COOH} \end{array}$	18.8 15.5 16.5	—
8-Hydroxyquinoline		12.2 23.4	19.0 32.5
Histidine hydrochloride	$\begin{array}{c} \text{N} - \text{C} - \text{CH} - \text{COOH} \cdot \text{HCl} \cdot \text{H}_2\text{O} \\ \parallel \quad \parallel \quad   \\ \text{HC} \quad \text{CH} \quad \text{NH}_2 \\   \\ \text{N} \\   \\ \text{H} \end{array}$	10.2 18.3	18.2 32.6
Aspartic acid	$\text{HOOC} - \text{CH}_2 - \underset{\text{NH}_2}{\text{CH}} - \text{COOH}$	8.6 15.4	5.3 32.4
Phenylalanine		8.3 14.7	6.0 32.5
Glycine	$\text{H}_2\text{N} - \text{CH}_2 - \text{COOH}$	8.1 15.2	4.5 32.3
Oxalic acid	$\text{HOOC} - \text{COOH}$	4.4	4.4

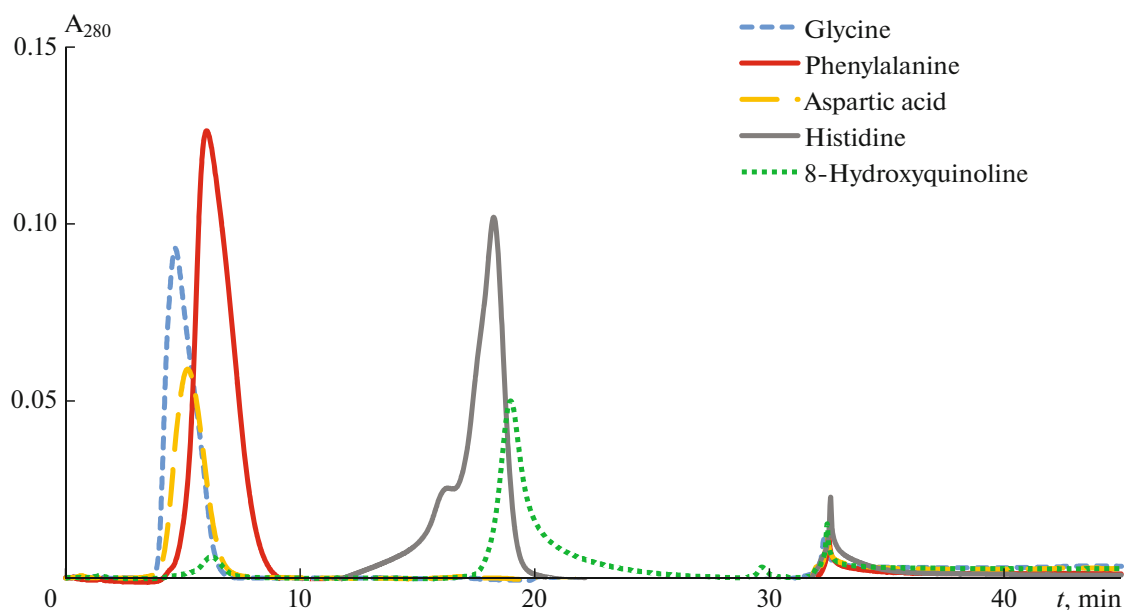


Fig. 6. IMAC patterns of individual chemical compounds according to specified separation algorithm.

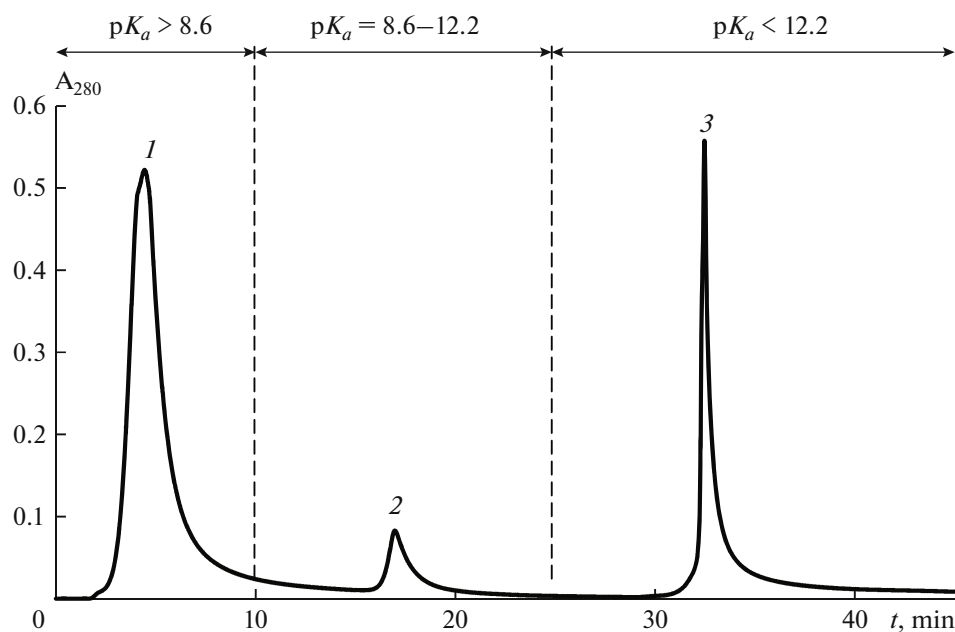


Fig. 7. IMAC pattern of  $0.1 \text{ mol/dm}^3$  NaOH extract (podzolic soil, litter): 1, weakly bound organic compounds; 2, bound organic compounds; and 3, organic compounds stably bound to copper ions.

## CONCLUSIONS

Different practices of HS separation into homogeneous molecular fractions according to the affinity for copper ions have been tested. Successive change in the pH of eluent leading to protonation of donor groups appeared to be the most effective IMAC approach. The proposed algorithm for fractionation of HS alkaline extract allows for isolation of three HS fractions,

the components of which differ in their ability to bind copper ions (weakly bound organic compounds, bound organic compounds, and organic compounds stably bound to copper ions). The use of individual model substances with known functional groups and  $pK_a$  values for copper ions has shown that the first HS fraction contains low molecular weight compounds failing to bind copper ions and displaying low  $pK_a$  val-

ues (up to 8.6), mainly, the low molecular weight compounds with amino and carboxyl functional groups. The second HS fraction contains the compounds with N-heterocyclic aromatic structures, and the third fraction—the organic compounds stably binding copper ions—comprises the most high molecular weight substances with  $pK_a > 12.2$ .

This fractionation algorithm has been used to assay the HSs of the litters and eluvial horizons of iron-illuvial podzol, podzolic soil, and peaty-podzolic gleyic soil. A quantitative estimate of the carbon contribution of individual fractions has shown that the HSs of podzolic soil display the highest ability to bind copper ions. The tested fractionation technique can be used for assessing the soil vulnerability to heavy metal pollution.

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#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

#### REFERENCES

1. V. A. Beznosikov, E. D. Lodygin, and S. N. Chukov, "Landscape-geochemical evaluation of the background content of heavy metals in taiga soils," *Vestn. St.-Peterb. Univ., Ser. 3: Biol.*, No. 2, 114–128 (2010).
2. V. A. Davankov, J. D. Navratil, and H. F. Walton, *Ligand Exchange Chromatography* (CRC Press, Boca Raton, 1988; Mir, Moscow, 1990).
3. A. A. Dymov, E. M. Lapteva, A. V. Kalashnikov, and S. V. Deneva, "Background content of heavy metals, arsenic, and hydrocarbons in soils of Bol'shezemel'skaya tundra," *Teor. Prikl. Ekol.*, No. 4, 43–48 (2010).
4. A. A. Dymov, E. V. Zhangurov, and V. V. Startsev, "Soils of the northern part of the Subpolar Urals: morphology, physicochemical properties, and carbon and nitrogen pools," *Eurasian Soil Sci.* **46**, 459–467 (2013).
5. A. A. Dymov and E. Yu. Milanovskii, "Changes in the organic matter of taiga soils during the natural reforestation after cutting in the middle taiga of the Komi Republic," *Eurasian Soil Sci.* **46**, 1164–1171 (2013).
6. A. A. Dymov, Doctoral Dissertation in Biology (Moscow, 2018).
7. A. Kabata-Pendias, and H. Pendias, *Trace Elements in Soils and Plants* (CRC Press, Boca Raton, 1984; Mir, Moscow, 1989).
8. O. A. Kel'tsueva, V. D. Gladilovich, and E. P. Podol'skaya, "Metal-affine chromatography: principles and use," *Nauchn. Priborostr.* **23** (1), 74–85 (2013).
9. D. V. Ladonin, "Heavy metal compounds in soils: problems and methods of study," *Eurasian Soil Sci.* **35**, 605–613 (2002).
10. A. A. Lugovskoi, A. S. Yakovleva, and A. V. Shcherbakov, "Synthesis of a sorbent for metal-chelate affine chromatography and its use for the isolation of recombinant proteins," *Tr. Fed. Tsentra Okhr. Zdorov'ya Zhivotn.* **5**, 248–255 (2007).
11. E. Yu. Milanovskii, "Amphiphilous components of soil humic substances," *Eurasian Soil Sci.* **33**, 617–625 (2000).
12. E. Yu. Milanovskii, *Soil Humic Substance as the Natural Hydrophobic-Hydrophilic Compounds* (GEOS, Moscow, 2009) [in Russian].
13. T. M. Minkina, G. V. Motuzova, O. G. Nazarenko, V. S. Kryshchenko, and S. S. Mandzhieva, "Forms of heavy metal compounds in soils of the steppe zone," *Eurasian Soil Sci.* **41**, 708–716 (2008).
14. D. S. Orlov, O. I. Min'ko, V. V. Demin, V. G. Sal'nikov, and N. B. Izmailova, "Nature and mechanisms of formation of metal-humic complexes," *Pochvovedenie*, No. 9, 43–52 (1988).
15. D. L. Pinskiy, *Ion Exchange Processes in Soils* (Institute of Soil Science and Photosynthesis, Russian Academy of Sciences, Pushchino, 1997) [in Russian].
16. V. M. Semenov and B. M. Kogut, *Soil Organic Matter* (GEOS, Moscow, 2015) [in Russian].
17. R. M. C. Dawson, D. C. Elliot, W. H. Elliot, and K. M. Jones, *Data for Biochemical Research* (Oxford University Press, Oxford, 1986; Mir, Moscow, 1991).
18. L. Andersson and J. Porath, "Isolation of phosphoproteins by immobilized metal ( $Fe^{3+}$ ) affinity chromatography," *Anal. Biochem.* **154**, 250–254 (1986).
19. T. Bauer, D. Pinskiy, T. Minkina, D. Nevidomskaya, S. Mandzhieva, V. Chaplygin, and Y. Popileshko, "Time effect on the stabilization of technogenic copper compounds in solid phases of haplic chernozem," *Sci. Total Environ.* **626**, 1100–1107 (2018). <https://doi.org/10.1016/j.scitotenv.2018.01.134>
20. H. Block, B. Maertens, A. Spriestersbach, N. Brinker, J. Kubicek, R. Fabis, J. Labhan, and F. Schäfer, "Immobilized-metal affinity chromatography (IMAC): a review," *Methods Enzymol.* **463**, 439–473 (2009). <https://doi.org/10.1016/j.pep.2007.09.019>
21. R. C. F. Cheung, J. H. Wong, and T. B. Ng, "Immobilized metal ion affinity chromatography: a review on its applications," *Appl. Microbiol. Biotechnol.* **96**, 1411–1420 (2012). <https://doi.org/10.1007/s00253-012-4507-0>
22. A. A. Dymov and D. N. Gabov, "Pyrogenic alterations of podzols at the North-East European part of Russia: morphology, carbon pools, PAH content," *Geoderma* **241–242**, 230–237 (2015). <https://doi.org/10.1016/j.geoderma.2014.11.021>
23. R. Halko, T. Neurocny, and M. Huttai, "Utilization of immobilized aluminum(III) metal ion affinity chromatography for analysis of humic acids," *Pol. J. Soil Sci.* **43** (2), 149–157 (2009).
24. F. T. Helfferich, "Ligand exchange: a novel separation technique," *Nature* **189**, 1001–1002 (1961). <https://doi.org/10.1038/1891001a0>



25. M. Hutta, R. Gora, R. Halko, and M. Chalanyova, "Some theoretical and practical aspects in separation of humic substances by combined liquid chromatography methods," *J. Chromatogr. A* **1218**, 8946–8957 (2011). <https://doi.org/10.1016/j.chroma.2011.06.107>
26. *Instruction 28-4046-21, AD Affinity Media. IMAC Sepharose TM 6 Fast Flow* (GE Healthcare, Chicago, 2010).
27. R. L. Nixon and A. R. Ross, "Evaluation of immobilized metal-ion affinity chromatography and electrospray ionization tandem mass spectrometry for recovery and identification of copper(II)-binding ligands in seawater using the model ligand 8-hydroxyquinoline," *Front. Mar. Sci.* **3** (246), (2016). <https://doi.org/10.3389/fmars.2016.00246>
28. I. Paunovic, R. Schulin, and B. Nowack, "Fractionation of dissolved organic carbon from solution with immobilized metal ion affinity chromatography," *Eur. J. Soil Sci.* **59** (4), 198–207 (2008). <https://doi.org/10.1111/j.1365-2389.2007.00975.x>
29. I. Paunovic, R. Schulin, and B. Nowack, "Evaluation of immobilized metal-ion affinity chromatography for the fractionation of natural Cu complexing ligands," *J. Chromatogr. A* **1100**, 176–184 (2005). <https://doi.org/10.1016/j.chroma.2005.09.055>
30. A. Piccolo, "The supramolecular structure of humic substances: a novel understanding of humus chemistry and implications in soil science," *Adv. Agron.* **75**, 57–134 (2002). <https://doi.org/10.1097/00010694-200111000-00007>
31. J. Porath, J. Carlsson, I. Olsson, and G. Belfrage, "Metal chelate affinity chromatography a new approach to protein fractionation," *Nature* **258**, 598–599 (1975). <https://doi.org/10.1038/258598a0>
32. *Protein Purification: Principles, High Resolution Methods, and Application* Ed. by J.-C. Janson and L. Ryden (Wiley, New York, 1998).

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