

APPLICATION OF SPECTROPHOTOMETRY TO QUANTIFICATION OF HUMIC SUBSTANCES IN PERMAFROST SEDIMENTS

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The application of spectrophotometry to determining the content of chromophoric humic substances in permafrost sediments is discussed. Strong linear correlation between the content of chromophoric humic substances and the content of organic carbon in the humic fractions isolated by a sequential resin-alkali extraction procedure has been revealed. It has been shown that a rapid non-destructive spectrophotometric method can be used when monitoring the state of humic substances stored in permanently frozen strata.

Permafrost sediments, Arctic, thawing, humic substances, spectrophotometric measurements

INTRODUCTION

The modern analytic equipment in the area of environmental monitoring presupposes development of operative, inexpensive and sensitive techniques ensuring large arrays of data to be obtained. Stable functioning of biocenoses is largely controlled by humic substances (HS), which constitute a large part of the organic matter of terrestrial and aqueous ecosystems. For the northern territories of Russia, the information about the quantity and quality of the humic substances stored in frozen strata is quite essential. As the permafrost continues to degrade, the HS will be getting involved into the bio-geochemical circulation and become transformed, which may affect the environmental situation in the adjacent territories.

Due to extreme complexity of the molecular structure of the humic compounds, their description in strictly chemical terms is still missing, nor is there a direct analytical method for identifying the content of HS in the samples of natural and anthropogenic origins. Time-tested is only the classical notion of HS as a system of polyphenol polymers of different degrees of chemical maturity: from labile low-humified compounds with a relatively simple aromatic structure to well-formed stable species with highly conjugated aromatic systems. Humic substances are referred to the class of polyfunctional polyelectrolytes, where the acid functional groups (carboxylic and phenolic groups), the concentration of which naturally changes in the process of biochemical transformation of humic compounds, are the main ones. Traditional description of the quality of HS includes evaluation of the ratio between the fulvic acids (FA), which unite low-humified components, and humic acids (HA), which include chemically mature material [Humic..., 1985; Orlov, 1990; Stevensen, 1994; Swift, 1999].

Over the recent decades, the methods of optical spectroscopy have been widely used to evaluate the amount and structural characteristics of the humic

material of different origins. Humic substances are chromophoric materials, and certain optical parameters reflect the degree of their chemical maturity [Bloom and Leenheer, 1989; Orlov, 1990; Senesi et al., 1991; Domeizel et al., 2004; Sierra et al., 2005; Fuentes et al., 2006; Shirshova et al., 2006, 2009; Birdwell and Engel, 2010]. To assess the concentration of HS in solutions and of dissolved organic matter (DOM) from water sources, two indirect methods are commonly used, based on measuring: 1) the content of organic carbon (the traditional technique); and 2) optical density at a fixed wavelength.

The advantage of spectrophotometric registration of HS is the selectivity of this method, due to the fact that detection of chromophoric humic substances takes place. At the same time, spectrophotometry opens up an essential possibility of measuring the molar concentration of HS using the molar absorbance coefficient, the definition of which is a topical issue for the modern chemistry of humic compounds. However, in modeling the processes of mass and energy exchange in biogeocenoses, data are required on the content of organic carbon in the composition of certain fractions of the natural organic matter, including HS. As a rule, close linear correlation is revealed between the content of organic carbon and the optical density measured in the analyzed samples of HS/DOM, allowing a rapid sensitive spectrophotometric method to be used in monitoring HS/DOM in solutions and in natural water [Wang Gen-Shuh and Hsieh Shu-Ting, 2001; Chen and Gardner, 2004; Ghabbour and Davies, 2009]. To assess the content of HS in solid objects, the authors proposed, in their previous study, a novel optical parameter obtained on the basis of spectrophotometric measurements [Shirshova et al., 2013]. This study is focused on analysis of the relationship between the value of this optical parameter and the content of organic carbon in the samples of humic fractions stored in permafrost strata.

OBJECTS AND METHODS OF STUDY

The objects of the study were samples of permafrost sediments of the tundra zone in the eastern Arctic sector (Table 1). For comparison, air-dry samples of humic-accumulative horizons of modern soils were used: permafrost-affected alluvial soil (Kolyma lowland, the lower course of the Bolshaya Chukochya River) and the sodic haplustert of the south-steppe zone (Stavropol region). Some of the samples of the permafrost sediments were analyzed in two conditions: naturally frozen samples (designated as F) and thawed air-dried samples stored at a positive temperature during two months (these samples, as well as the air-dried soil samples, are designated as T). The information about the place of sampling and description of the samples' storage and preparation conditions are contained in [Shirshova *et al.*, 2009, 2013], devoted to analysis of the fluorescent behavior of HS, the spectrophotometric parameters of which are discussed in this paper.

The humic substances were isolated from samples of sediments and soils using the method of 3-step sequential resin-alkali extraction [Shirshova, 1991; Shirshova *et al.*, 2009]: 1) first the mobile HS-S fraction was isolated in distilled water using ion-exchange Na^+ -sulfocationite (RSO_3Na); 2) then the strongly cation bound HS-C fraction was isolated using Na^+ -carboxylate resin (RCO_2Na); 3) finely the solid residue was treated by 0.1 M NaOH to extract the free HS-A fraction, the components of which are linked in the polymer complexes. The HS-C and HS-A fractions are analogous to the 2 and the 1 fractions, accordingly, in the commonly used method of humus analysis [Ponomareva and Plotnikova, 1980]. The advantage of resin-alkali extraction consists in more selective and mild extraction of material, tightly bound with mineral part (the HS-C fraction) and associated with organic components (the HS-A fraction).

The HS extracts obtained were purified from the mineral particles by filtering them through the 0.45- μm membrane filter, then the pH value of each of the isolated humic fractions was adjusted to 7.5–8.0; the conditions of HS sample preparation and storage were described in [Shirshova *et al.*, 2013].

The concentration of chromophoric humic substances in the HS-S, HS-C, HS-A extracts was determined by the optical density of the samples of the respective extracts at the wavelength $\lambda = 337$ nm, and for some samples it was also determined by the optical density at the wavelength $\lambda = 254$ and 465 nm. The measurements were conducted with a HITACHI 557 spectrophotometer at room temperature using quartz cells (1 cm \times 1 cm). To evaluate the content of the chromophoric humic substances in the samples under study, the measured values of optical density (A_{337} , cm^{-1}) were normalized to 1 g of a sample of

Table 1. The characteristic of the permafrost sediments studied

Sample number	Sediment type	C_{org} , mg/g
1	Holocene Q_{IV} , peaty loam	54.5
2	Late Pleistocene Q_{III} , loam	16.0
3	Late Pleistocene Q_{III} , loam	14.9
4	Middle Pleistocene Q_{II} , loam	5.9
5	Late Pliocene–Early Pleistocene N_2-Q_I , sandy loamy sediments	4.9
6	Permafrost-affected alluvial soil, horizon A	9.4
7	Sodic haplustert, horizon A	30.2

sediment/soil by multiplying them by the volume of the isolated HS extract (ml) and by dividing them by the weight of the sample (g) on a dry weight basis. The optical parameter obtained was designated as CHS and represented as arbitrary units (a.u.): A_{337} , $\text{cm}^{-1}\cdot\text{ml}/\text{g}$. The same principle was used to calculate the values of CHS using the optical density values A_{254} , A_{465} , cm^{-1} . The relative standard deviation in determining the CHS in triplicate did not exceed 3 %. The absorption spectra in the region of 200–600 nm were recorded for solutions of the humic fractions normalized by optic density at $\lambda = 337$ nm [Shirshova *et al.*, 2013].

The content of organic carbon (C_{org}) in the air-dry samples of sediments and soils was determined with an express analyzer for AN-7529 carbon, and in the liquid samples of humic fractions the wet combustion method was used [Tyurin, 1965]. The relative standard deviation associated with determining C_{org} (in triplicate) did not exceed 5 %.

RESULTS AND DISCUSSION

Fig. 1 shows data on the content of humic fractions in the permafrost sediment and soil samples obtained by the spectrophotometric methods (in terms of CHS) and by the wet combustion method (in terms of C_{org}). In total, both parameters provided similar descriptions of the fraction composition of the HS, although certain differences were observed in the ratio of the fractions. For example, the relative content of the HS-C fraction was somewhat higher in the evaluation in terms of CHS than in terms of C_{org} . This is likely to be related to the structural organization of the HS-C and HS-A materials. In the HS-C fraction tightly bound with the mineral components, chemically mature forms are accumulated [Shirshova and Ermolaeva, 2001]. In accordance with this, concentration of chromophoric groups and (or) chromophoric humic macromolecules in the HS-C fraction may be higher, compared to the other fractions, which is registered in terms of CHS. The HS-A fraction is enriched by the fragments of biomolecules [Shirshova *et al.*, 2006a], which, on the one hand, may increase

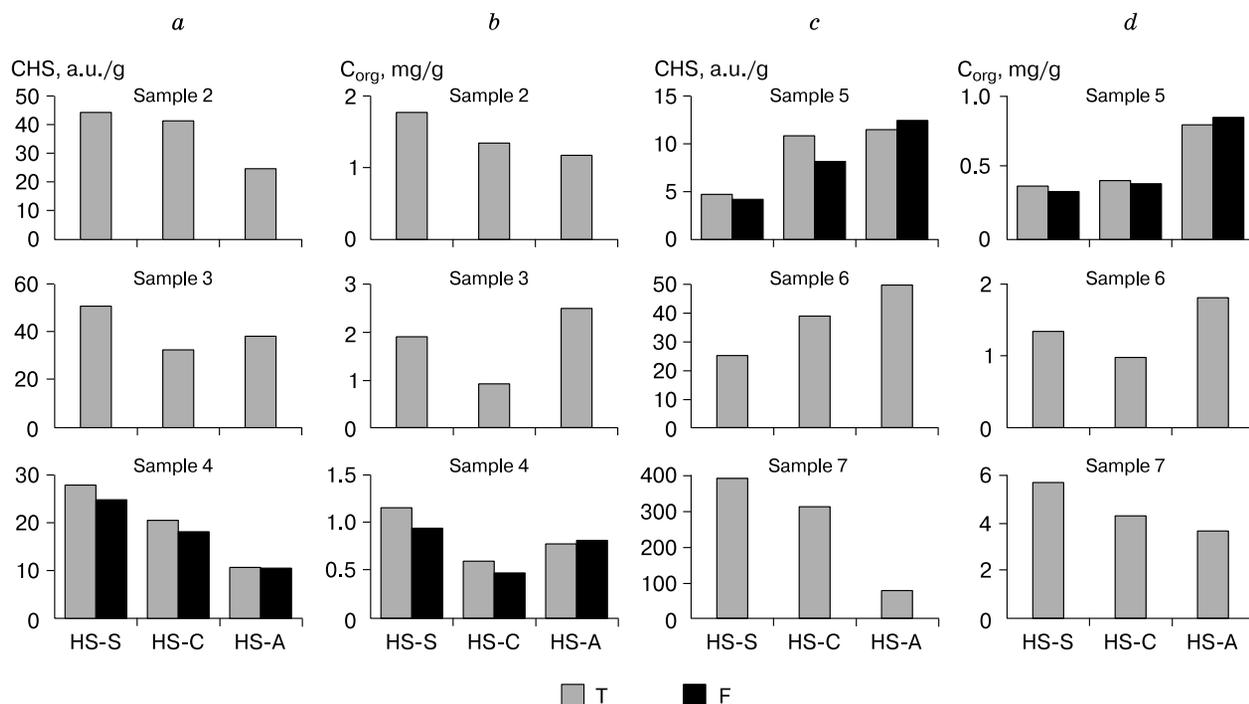


Fig. 1. The content in permafrost sediments (samples 2–5) and modern soils (samples 6, 7):

a, c – the chromophoric humic substances (CHS); *b, d* – organic carbon of humic substances (C_{org}). The HS fractions were isolated sequentially: HS-S – with sulfonate resin, HS-C – with carboxylate resin, HS-A – 0.1 M NaOH; F – naturally frozen samples; T – thawed air-dry samples stored at a positive temperature for two months.

the amount of C_{org} , and on the other hand, may dilute the chromophoric groups and (or) influence their condition. In other words, the relationship between CHS and C_{org} will depend on the quality of the humic material under study (Fig. 2).

As seen from Fig. 2, *a*, in each of the three humic fractions isolated from the samples of permafrost sediments (including F and T), close direct linear corre-

lation is observed between the values of C_{org} and CHS (coefficient of determination $R^2 = 0.99, 0.93, 0.92$). This suggests the possibility of using the value of CHS to determine the content of organic carbon in certain humic fractions and HS in total (by the sum of fractions). The linear correlation between C_{org} and CHS holds for the totality of all fractions (Fig. 2, *b*), but here it is less close ($R^2 = 0.68$) than

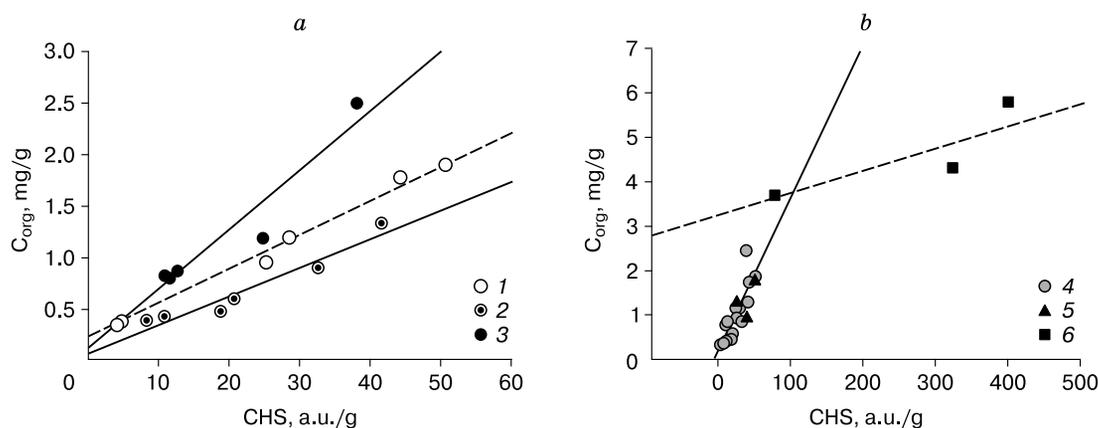


Fig. 2. Relationship between the values of CHS and C_{org} in humic fractions isolated from samples of permafrost sediments (samples 2–5) and modern soils (samples 6, 7).

a (samples 2–5): 1 – fraction HS-S ($R^2 = 0.99, p < 0.0001$); 2 – fraction HS-C ($R^2 = 0.93, p < 0.0017$); 3 – fraction HS-A ($R^2 = 0.92, p < 0.0024$); *b* – the totality of the above humic fractions ($R^2 = 0.68, p < 0.0001$): 4 – samples 2–5; 5 – sample 6; 6 – sample 7.

for each fraction separately. Inclusion of the fractions of modern permafrost-affected alluvial soil does not practically change the situation, as opposed to sodic haplustert, indicating dependence of the measured parameters on the specific features of the structural organization and the chemical environment of HS formed and functioning in different bioclimatic conditions. It should be emphasized that the results of analyzing the relationship between C_{org} and CHS shown in Fig. 2 confirm reasonability of fractioning HS by the method of sequential resin-alkali extraction, allowing effective differentiation between different forms of HS [Shirshova, 1991; Shirshova and Ermolaeva, 2001; Shirshova et al., 2006a]. The character of fixation of each of the isolated humic fractions on an organomineral matrix influences the arrangement of the humic macromolecules and, as a result, their electronic properties. In [Wang Gen-Shuh and Hsieh Shu-Ting, 2001] the authors, based on analysis of literature data and on their own experimental data, come to a conclusion that the search for universal linear correlation between C_{org} and the spectrophotometric parameter is hardly productive, and it is necessary for each source to have its own correlation plot built between the indicated parameters. The choice of the wavelength to register optical density in the HS samples depends on the target of the study and on the source of the humic fractions being analyzed. In this study, when the CHS value was determined, the optical density in the samples of the humic fraction extracts was measured at $\lambda = 337$ nm, which is the length of the excitation wave in the fluorescence spectra that minimized the number of measurements during spectral analysis [Shirshova et al., 2013].

Despite the lengthy period of investigations, the connection between the electronic absorption spectra and the molecular structure of HS remains a subject of discussion. This refers to the number and nature of the chromophoric groups in the humic compounds, the electronic spectra of which are represented by a smooth curve of intensity reduction in transition from the ultraviolet (UV) region to the visible region of the spectrum with a small maximum value/shoulder in the short-wave region of the spectrum. In accordance with the commonly used concept, absorption at $\lambda = 254$ nm may be attributed to carboxyphenols, according to the terminology of [Bloom and Leenheer, 1989], whereas absorption at $\lambda = 436, 465$ nm is related to chromophores responsible for the black and brown color of the chemically mature humic material. Fulvic acids absorb intensively in the UV-range, as their low-humified components are enriched with carboxylic and phenolic groups; for HS characteristic is absorption in the visible (part) region of the spectrum [Bloom and Leenheer, 1989; Korshin et al., 1997; Abbt-Braun and Frimmel, 1999; Del Vecchio and Blough, 2004; Domeizel et al., 2004; Fuen-

tes et al., 2006]. In the previous studies [Shirshova et al., 2006b], the authors measured optical densities at $\lambda = 254, 337,$ and 465 nm and calculated the respective values of CHS: CHS_{254}, CHS_{337} (referred to as CHS above), and CHS_{465} . In accordance with these data, there is close direct correlation between CHS_{254} and CHS_{337} ($R^2 = 0.998$, significant probability $p < 0.0001$), and between CHS_{337} and CHS_{465} ($R^2 = 0.982$, $p < 0.0001$). It is logical to assume that experimental determination of CHS_{254} and CHS_{465} would allow information to be obtained about the ratio in the composition of the isolated humic fractions of the material of different degrees of chemical maturity. Initial measurements of optic density at $\lambda = 254, 337, 465$ nm are taken in the same cells, therefore determination of CHS_{254}, CHS_{337} and CHS_{465} does not take much time.

The problem is that all the humic components are connected in complex macromolecular and(or) macroaggregate structures, where optical properties do not obey the rule of additivity [Parker, 1972]. Yet, measuring CHS_{254} and CHS_{465} , it is possible to identify the fractions where low-humified or chemically mature material prevails. In monitoring the state of HS of permanently frozen strata, information about low-humified forms is especially important, as during degradation of permafrost they will be the first to be subject to biochemical transformation, which may be accompanied by formation and emission of greenhouse gases. First of all, the state of the mobile potentially water soluble fraction HS-S should be controlled, the components of which may infiltrate into conjugated water ecosystems. The results of the previously held fluorescence spectroscopy led us to conclude that accumulation of low-humified material in the HS-S is characteristic of permanently frozen sediments, and this process progresses with the time [Shirshova et al., 2009, 2013].

Shown in Fig. 3 are the absorption spectra of the humic fractions isolated from the sediments most distinguished by age: sample 1 (Q_{IV}) and sample 5 (N_2-Q_I). As seen, the most intense absorption in the UV-region of the spectrum is characteristic of HS-S, proving the concept of concentration of low-humified material in the composition of this fraction. In addition, it is the HS-S spectra from the samples F and T of each of the sediments clearly differ, indicating qualitative differences in the humic material [Shirshova et al., 2013].

Fig. 4 demonstrates data on the fraction composition of HS in terms of CHS_{254}, CHS_{337} and CHS_{465} , obtained for sample 1 (Q_{IV}) and sample 5 (N_2-Q_I). It follows from comparison of the fraction composition of HS in terms of CHS_{465} and CHS_{337} that measurement of CHS_{465} does not provide new information for both sediments. Some humic extracts from the samples investigated in this study had very low

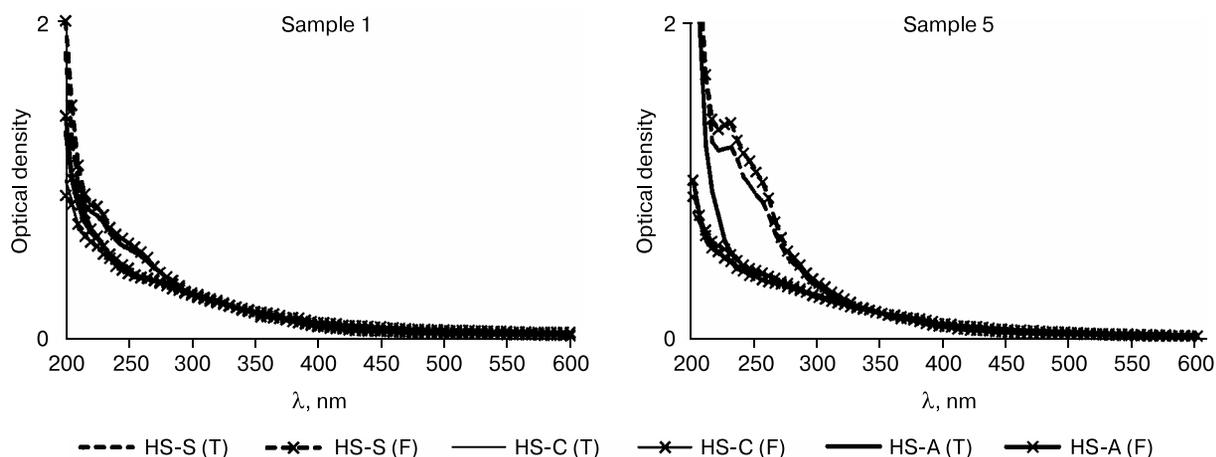


Fig. 3. The UV-visible spectra of humic substances isolated from samples 1 and 5 of permafrost sediments (the spectra are normalized at $\lambda = 337$ nm).

See the symbols in Fig. 1 and in the text.

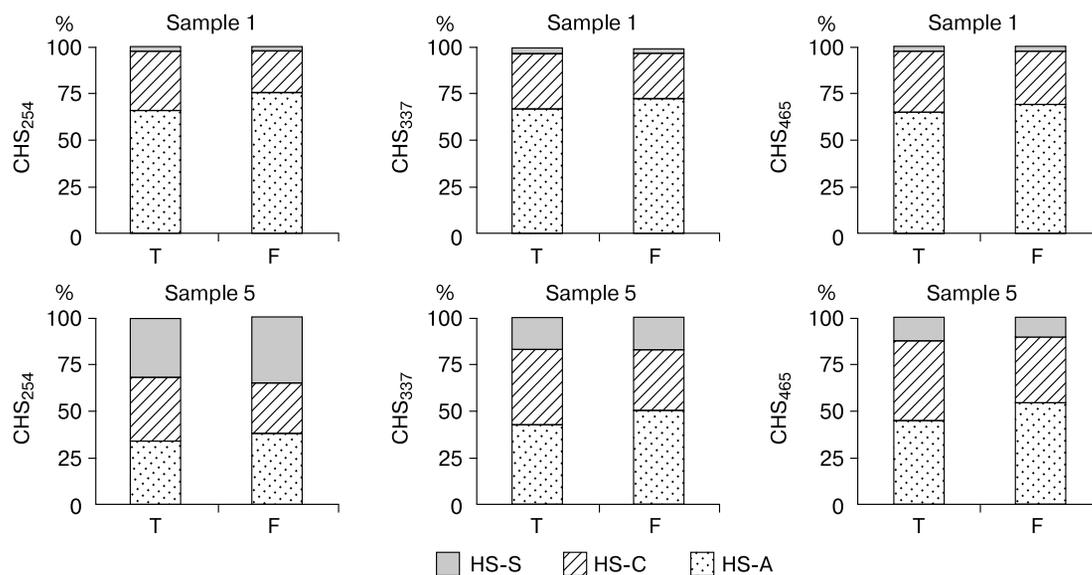


Fig. 4. The fraction composition of the humic substances isolated from samples 1 and 5 of permafrost sediments (in terms of CHS_{254} , CHS_{337} (in the text CHS), CHS_{465}).

See the symbols in Fig. 1 and in the text.

absorption in the visible region, therefore it was not reasonable to measure optical density at $\lambda = 465$ nm. In sample 1 (Q_{IV}), no significant difference was revealed in the percentage of the HS fractions in terms of CHS_{254} and CHS_{337}/CHS_{465} , only a trend for an increase in the share of the HS-S fraction in evaluation in terms of CHS_{254} . In sample 5 (N_2-Q_1), the ratio of the humic fractions in terms of CHS_{254} differs from that in terms of CHS_{337}/CHS_{465} (the share of HS-S is nearly two times higher in terms of CHS_{254}). This indicates a high content of this se-

diment in HS-S, low-humified material enriched in carboxylic and phenolic groups, which is consistent with the results of fluorescence analysis, complementing them. Thus, a comparison of the fraction composition of HS in terms of CHS_{337} and CHS_{254} allows accumulation of mobile low-humified forms in them to be revealed. To ensure more complete analysis of the components of different chemical maturity in the composition of the humic material, it is necessary to carry out its preliminary fractioning [Shirshova et al., 2009, 2013].

As shown in Fig. 1 and Fig. 4, the fraction composition of HS from naturally frozen samples F has certain distinctions from that of the thawed samples T (stored at a positive temperature for two months). A higher content of the HS-C fraction is typical of the HS from samples T, whereas the HS-A fraction is more characteristic for samples F. The observed distinctions may be attributed to reversible transformation during the freezing-thawing cycle of the macromolecular structure of the HS components and(or) of their binding with the organomineral matrix, which is accompanied by a change in the solubility of its components. For HS-C, where complex chemically mature components tightly bound with the mineral part are concentrated, these transitions will take longer time; therefore, sample F contains a certain quantity of "frozen" HS-C structures of reduced solubility. Under conditions of alkaline extraction, additional ionization of acid functional groups takes place in the "frozen" material of HS-C, which contributes to its isolation as part of HS-A. It should be added that in the laboratory experiments on the influence of freezing-thawing on the fraction composition of HS of soil samples changes in the extraction lability of individual fractions during freezing of soil samples, with subsequent recovery of the soil after keeping the samples at a positive temperature, were observed [Polubesova *et al.*, 1994; Polubesova and Shirshova, 1997]. In characterizing the state of the HS of permafrost sediments, it is recommended to correlate the results obtained with the time of exposure of the original samples to the positive temperature.

CONCLUSIONS

To determine the content of the chromophoric humic substances in the samples of permafrost sediments, an optical parameter is proposed, obtained on the basis of spectrophotometric measurement of the concentration of humic extracts. It has been shown that there was close linear correlation ($R^2 = 0.92-0.99$) between the optical parameter and the content of organic carbon in the humic fractions isolated by the method of sequential resin-alkali extraction. Spectrophotometric detection of humic substances allows accumulation of mobile low-humified forms to be revealed. The rapid non-destructive spectrophotometric method may be used for monitoring the state of humic substances conserved in frozen strata.

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