Amphiphilic properties of humic substances in soils of the southern Vitim Plateau (Transbaikalia, Eastern Siberia)

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Abstract. Humic substances of soils are considered as a multicomponent system of amphiphilic (exhibiting both hydrophilic and hydrophobic properties) substances. Humic substances from soils of different genesis in the south of the Vitim Plateau (quasigley chernozem – Turbic Chernozem Molliglossic and brown soil – Stagnic Phaeozem Molliglossic) were studied. Using the hydrophobic interaction chromatography on Octyl-Sepharose® CL-4B, we separated a mixture of humic (HAs) and fulvic acids (FAs) due to their different ability to enter into hydrophobic interactions with the gel matrix. A higher percentage of hydrophobic compounds was revealed in HAs of quasigley chernozem, compared to HAs of brown soils, and FAs. The fulvic acid sample showed a lower capacity for hydrophobic interactions than the humic acid sample.

1. Introduction
The fate of natural organic matter (OM), and especially humic substances (HSs), has attracted increasing interests of scientists representing various disciplines over recent decades. HSs are the main constituents of natural organic matter, found in both aquatic and terrestrial environments. HSs are natural dark-coloured compounds, which comprise the major part of stable organic matter in soils, sediments, and waters. HSs are a complex, dispersed, and heterogeneous mixture of various organic compounds synthesized from organic matter residues, decomposed by microorganisms [1, 2]. HSs are now envisioned as supramolecular associations of small heterogeneous molecules associated with hydrogen bonds and hydrophobic interactions that can be disrupted in the presence of organic acids [3, 4].

These organic molecules are composed of a heterogeneous mixture of biochemical substances (proteins, carbohydrates, lipids) destructed to a greater or lesser extent by microorganisms, as well as partially formed by the condensation of small organic molecules [5, 6]. As a result, HSs represent a polydisperse mixture of complex molecules that differ in their structure and chemical composition. These compounds are generally described as consisting of aromatic rings and aliphatic chains with O-, N-, and S-containing functional groups and nonpolar fragments. This combination of polar and non-polar fragments, gives them amphiphilicity, i.e. the ability to exhibit both hydrophobic and hydrophilic properties [7].

The presence of humus components differed by capacity for hydrophobic interactions confirmed by the results of salting-out [8] and the fractionating humus substances by hydrophobic interaction chromatography (HIC) [9]. This method was taken from biochemistry, where it is used for the purification of proteins [10].

The salting-out effect depends on surface characteristics of separated molecules, i.e., on the surface charge density and distribution of hydrophobic and hydrophilic fragments. At low salt concentrations,
molecules with high hydrophobic fragment content aggregate first, whereas molecules with low hydrophobic fragment content remain in solution even when very large amounts of salt are added. The results of work [11] have shown that it could also be true for humic acids (HAs).

The work aimed to study the heterogeneity amphiphilic (hydrophobic–hydrophilic) fractions of HAs obtained by hydrophobic interaction chromatography on Octyl-Sepharose® CL-4B.

2. Objects and Methods
The interpretation of results of the fractional precipitation of humic and fulvic acids (FAs) as an indicator of their amphiphilic properties requires experimental data confirming the differences in hydrophobicity among the fractions separated. The study area is located in a permafrost zone with a thickness of 100-120 m in the south of the Vitim plateau. The average annual air temperature is −4.1°C with the average temperature of the warmest month (July) +17.1°C, the coldest month (January) −25.4°C, and the absolute minimum temperature reaches −50...−54°C. This leads to deep (3 m or more) soil freezing. The sum of biologically active temperatures is 1,330°C.

The object of research was quasigley chernozems (52°30.894’ N, 111°32.738’ E), under meadow-forb phytocenoses, and brown soil (52°51.793’ N, 111°43.497’ E), formed under sparse larch-birch forest. Both soil types are formed under conditions of permafrost in the forest-steppe landscape. Morphological description, physical and chemical characteristics of soils, and a more detailed description of the humus state were published earlier [12, 13].

The following procedure was used for the separation and purification of preparations. HSs were extracted with 0.1N NaOH from a preliminarily decalcified soil sample. The extract was purified from mineral colloids by long centrifugation at 25,000 rpm and separated by fractional precipitation into the three following fractions: (I) HSs precipitated from the solution with 10% ammonium sulphate; (II) HSs precipitated from the saturated ammonium sulphate solution; and (III) HSs remaining in the saturated ammonium sulphate solution. HAs were isolated from the obtained fractions and precipitated twice, purified from soluble salts, and dried [14]. Fulvic acid preparation, presumably the most hydrophilic humus fraction, was prepared by the Forsyth method.

Chromatographic separation was carried out on a 1x10 cm column (Amicon) filled with Octyl-Sepharose® CL-4B gel (Pharmacia, Sweden) on a low-pressure liquid chromatograph BioLogic LP (BioRad, USA), using 0.05M Tris-HCl buffer (pH 7.2) and 1 ml of HAs and FAs solution (0.2 mg/ml). Decreasing linear gradient of 10% ammonium sulphate and increasing step gradient of 0.3% sodium dodecyl sulphate (DDS-Na) were used. Adding salt to the initial buffer increased the polarity of the eluent and allowed hydrophobic fragments of separated molecules to interact with the hydrophobic gel matrix [7]. Using 0.3% DDS-Na allowed the most hydrophobic fragments of HAs to be completely eluted. The first two fractions eluted from the column in the presence of ammonium sulphate had predominantly hydrophilic properties (hydrophilic fractions), and the following fractions were hydrophobic.

3. Results and Discussions
Initial HAs and FAs preparation consisted of 5 fractions, eluted from the column in order of their hydrophobicity (figure 1). The most hydrophilic HAs’ components (fractions I and II) were eluted from the column first, in absence of DDS-Na ionic detergent. The most hydrophobic components (fraction V) were eluted last together with 0.2% DDS-Na.

The FAs sample showed weaker hydrophobic interactions than the HAs sample: the amount of non-adsorbed compounds was 80 and 90% of FAs for the brown soils and quasigley chernozem, respectively, whereas for the corresponding HAs it was 28 and 41%. Furthermore, HSs of meadow-chernozem (HAs + FAs) are more hydrophobic than those of brown soils. These observations are in accordance with the investigations on surface-active properties of sedimentary HSs [15]. The high solubility of components of these fractions combined with their capacity to enter in strong hydrophobic interactions suggests a diphilic structure of the surfactant-like type.

We attribute the profile differentiation of hydrophilic and hydrophobic components of HAs for quasigley chernozem and for brown soil to climatic features and peculiarities of plant residue...
input into the soil. In quasigley chernozem, with a moisture coefficient close to unity, annual root litter is 2-3 times higher than the aboveground litter. An increase in the amount of plant residues together with an improvement in the hydrothermal regime promotes humification, which increases the proportion of HAs, and their mobility also increases.

During humification of plant residues on the surface of brown earth, only the hydrophilic components of the HAs mixture enter the mineral horizons with the downward water flow. Whereas in quasigley chernozem, the humification of OM (primarily root debris) proceeds directly in the mineral horizons of soils, hydrophobic fractions predominate in the composition of HAs here.

Chromatographic analysis of HSs of genetic horizons of soils of different genesis (figure 1) showed the main patterns of differentiation of amphiphilic components of HAs and FAs along the profile.

![Chromatograms of humic and fulvic acids from quasigley chernozem (a) and brown soil (b) for different soil horizons.](image)

Figure 1. Chromatograms of humic and fulvic acids from quasigley chernozem (a) and brown soil (b) for different soil horizons.

In brown soils and quasigley chernozems, the multicomponent composition of HSs persists along the profile (table 1). Within the humus-accumulative horizon there is an increase in the relative content of hydrophobic components with depth. Hydrophilic compounds absolutely dominate in the composition of FAs regardless of their genetic association. In the HAs’ composition in the upper horizons of the profile, the whole range of amphiphilic fractions is present, with some prevalence of hydrophobic components. The dominance of hydrophobic fractions is clearly pronounced with depth.
A somewhat different pattern is observed in the hydrophilic group of HSs, where the content of the fractions decreases with depth, but the entire component set of amphiphilic HSs’ fractions present above is retained. In all likelihood, the more hydrophilic properties of the HSs are expressed, the more these substances will be mobile in a soil profile, the more they will act as agents for acid hydrolysis of minerals and will be eluted. On the contrary, hydrophobic HSs will be fixed at the place of formation, forming accumulative characteristics of the profile. This seems to be the significance and role of amphiphilic properties in the formation of the humus profile of soils.

Table 1. Relative content of chromatographic fractions in humic substances.

| Soil, type of HSs     | Depth, cm | Fraction, % |
|-----------------------|-----------|-------------|
|                       | 1         | 2           | 3           | 4           | 5           |
| Brown soil, HAs       | 0-10      | 15.4 ± 3.2  | 21.5 ± 2.7  | 34.6 ± 6.8  | 27.1 ± 5.5  | 1.5 ± 0.6   |
|                       | 10-20     | 10.5 ± 2.8  | 22.5 ± 3.5  | 32.7 ± 5.9  | 33.1 ± 6.9  | 1.1 ± 0.5   |
|                       | 20-30     | 11.0 ± 5.5  | 17.1 ± 2.8  | 37.9 ± 7.0  | 33.1 ± 5.0  | 0.9 ± 0.1   |
| Quasigley chernozem, HAs | 0-10    | 19.6 ± 0.9  | 21.9 ± 2.2  | 34.9 ± 4.1  | 23.0 ± 4.3  | 0.7 ± 0.2   |
|                       | 10-20     | 16.4 ± 2.2  | 22.8 ± 3.6  | 39.9 ± 5.0  | 20.2 ± 0.9  | 0.7 ± 0.1   |
|                       | 20-30     | 14.7 ± 3.8  | 21.4 ± 5.5  | 44.6 ± 6.1  | 19.0 ± 3.7  | 0.3 ± 0.1   |
| Brown soil, FAs       | 0-10      | 89.2 ± 15.9 | 5.9 ± 1.7   | 1.2 ± 0.3   | 2.5 ± 0.6   | 1.2 ± 0.4   |
|                       | 20-30     | 81.4 ± 19.0 | 12.5 ± 4.6  | 2.0 ± 0.5   | 2.7 ± 0.6   | 1.5 ± 0.2   |
| Quasigley chernozem, FAs | 10-20    | 87.8 ± 20.8 | 7.3 ± 2.0   | 2.3 ± 0.1   | 1.8 ± 0.2   | 0.9 ± 0.3   |
|                       | 20-30     | 89.7 ± 11.7 | 3.4 ± 1.1   | 2.3 ± 0.2   | 3.3 ± 0.6   | 1.3 ± 0.1   |

The distribution of amphiphilic fractions in soils of different genesis indicates the existence of different mechanisms for the formation of the humus profile of these soils. Active mineralization of OM with excess of oxygen leads to oxidized OM forming with primarily hydrophilic properties. These conditions usually occur in the upper soil horizon. With mineralisation in the lower horizons, humus formation apparently proceeds in a different way, resulting in a relative accumulation of hydrophobic organic molecules. This tendency is confirmed by the chromatographic fractionation of HSs.

This result once again confirms that the humification process, regardless of soil formation factors and soil type, has one direction and consists in the selection and accumulation of thermodynamically stable products of the transformation of organic material. The set of experimental data on chromatographic fractionation of HSs showed that, in the framework of the traditional classification of humic acids, hydrophilic components of HSs are close to FAs, and hydrophobic ones – to HAs. The question of the nature and nativeness of HAs and FAs as a part of humus residues is a hotly debated issue [5].

The variation in the content of amphiphilic components in the composition of the HSs of soils of different genesis is determined by the nature of OM release into the soil and local conditions in the humification zone. Under oxidizing conditions, processes of chemical and microbiological mineralization predominate. Under anaerobic conditions, along with the slowed mineralization of OM, its conservation and, possibly, relative accumulation of microbiologically resistant to further transformation of more hydrophobic components take place.
4. Conclusion

Humus acids from soils of different genesis differ in the ratio of amphiphilic components. Hydrophobic interaction chromatography of preparations of humus acid shows a higher proportion of hydrophobic compounds among the HAs of quasigley chernozem compared to the HAs and FAs of brown soil. Apparently, the more expressed hydrophilic properties of HSs, the higher mobility of these substances in the soil profile, the stronger ability to dissolve minerals. On the contrary, hydrophobic HSs are fixed at the place of formation providing accumulative characteristics of the profile. It seems that in the process of formation of the humus profile of soils these functions of HSs are realized due to their amphiphilic properties. HAs of brown soil are more hydrophilic (due to the predominance of side groups in their molecules) compared to those of quasigley chernozem – these compounds include many hydrophobic aromatic structures.

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