

The research of the influence of heavy metal ions on the aggregation of humic substances on the mica by atomic force microscopy

E.D. Dmitrieva^{(a)}, M.M. Leontyeva^(a)*

(a)Laboratory of Chemistry, Tula State University, Tula, Russian Federation

* Corresponding author:

mani.leontyeva@gmail.com

Received 15 Oct 2018,

Revised 27 Dec 2018,

Accepted 28 Dec 2018

Abstract

The conformation of molecules of humic substances of peats sorbed on hydrophilic mica in the absence and presence of heavy metal ions was studied by atomic-force microscopy in semi-contact mode in an aqueous medium. A model of the lignin-carbon complex is proposed, which ensures the conformation of the three-dimensional ring structure of humic substances depending on pH and allows accurate description of the surface structure of humic substances. The sizes of single globular molecules of humic substances were determined: 140-320 nm in diameter and 5-25 nm in height. Possible ways of the interaction of humic substances of peats with metal cations (Pb^{2+} and Zn^{2+}) with account of conformational changes of humic substances have been established. It has been proven that lead cations are sorbed mainly on the surface of humic molecules, and sorption of zinc cations proceeds with reorientation of humic substances around zinc ions.

Keywords: Humic substances; himatomelanic acids; molecular conformation; heavy metals; atomic force microscopy; lignin-carbohydrate complex.

1. Introduction

Chemical and physicochemical behavior of humic substances in natural soil and water environments is a function of their molecular structure, and determines the mobility of organic matter of humic substances and their aggregation in natural environments [1]. They are also chaotic polydisperse planar discs forming compact soft spheroids [2]. The size, shape, and charge of humic substances molecules can change depending on solution pH [3]. Humic substances can be described by supramolecular aggregation of relatively small molecules held together by weak dispersion forces. The Van der Waals forces and hydrogen bonds are responsible for the large size of molecules of humic substances, which becomes more significant with increasing pH. Despite much research being carried out over the last decade, the details of the conformation of HS have still not been fully understood. Although the main structural units of the HS molecule have long been identified [4,5], little is known about how these macromolecular components are related and how they affect on the molecular conformation as a whole. The interaction of humic substances with various heavy metal ions with the formation of insoluble humates is quite fully described in the literature, however, the question of the conformational changes occurring during the interaction of humic substances with heavy metal ions, as well as the structure of the insoluble humates formed, remains open. Since the last 20 years, atomic force microscopy (AFM) is being used to observe the micromorphology of organic molecules and humic substances in the aqueous media [6,7]. Compared with electron microscopy, AFM has advantage in sample preparation, as there is no need for dehydration, coating the sample, or strong vacuum conditions. This makes it possible to probe the microstructure of organic and mineral colloids by surface analysis at different pH values, and the results can be easily visualized as three-dimensional images [8]. Using AFM, depending on the concentration of HS and pH, aggregates of globular units, chain assemblies, perforated sheets and spongy ring structures were observed in colloids of humic substances [9,10]. However, due to the complex nature of HS and the possibility of aggregation or conformational changes, the exact structure of the HS remains uncertain. In addition, many researchers have reported that the determination of the molecular size of HS by AFM measurements is difficult for several reasons related to the geometry and force generated by the tip of the microscope, which may reduce the accuracy of the technique and increase the error rate in the topographic data [11, 12]. In this paper, we compared the observations of a semi-contact regime (AFM-C) in an aqueous medium with the aim of studying the conformation of HS adsorbed on hydrophilic mica in the absence and presence of heavy metal ions. The description of the nanoscale three-dimensional structure of HS and metal humates is potentially useful for understanding the interactions of HS with each other and with metal ions, as well as constructing a more realistic model of HS structure. Consequently, the purpose of this work was to establish the structure of the surface of humic substances that are in direct contact with the surface of the hydrophilic medium and possible ways of interaction of humic substances with metal ions (Zn^{2+} and Pb^{2+}) by atomic force microscopy.

2. Materials and methods

Humic substances of peats of the Tula region were used in this research: reed fen peat (RFP), sphagnum highmoor peat (SHP), black alder fen peat (BFP), sphagnum transitional peat (STP) isolated by the method described in earlier published work [13] and humic acids (HMA), which were extracted from humic substances by hot ethanol extraction in the ratio of 1 g of the sample per 200 cm³ of alcohol. The solution was boiled for 3 to 4 hours. The extract was filtered, and the filtrate was subjected to distillation. The filtrate residue was dried 24 hours at room temperature [14]. The main substrate used for AFM bioobjects is mica. Mica has a hydrophilic surface with atomically flat areas larger than 100 μm. For AFM studies of biopolymers to mica substrates, the following general requirements are advanced: the surface must be enough fairly and adsorb the objects under investigation. Solutions of humic substances concentration of 1 g/l was obtained by dissolving a sample of humic substances in a 0.1 M solution

of NaOH, followed by dilution of the solution with a background electrolyte (0.1 M NaNO₃). Working solutions of metal salts with concentrations of 0.2 mM and pH = 4 were prepared by diluting 0.1 M solutions of Pb(NO₃)₂, Zn(NO₃)₂. The required volumes of working solutions were transferred to a 25 ml volumetric flask and diluted to a mark of 0.01 M HNO₃. The pH values of the solutions were monitored by pH - Meter Anion 4154. Solutions of lead and zinc humates were prepared by adding to the solutions of humic substances (1 g/l) solutions of Pb(NO₃)₂, Zn(NO₃)₂ with concentrations of 0.2 mM in a 1: 1 ratio. Samples for AFM analysis were obtained by precipitation of 200 µl of a suspension of HS or metal humates on freshly peeled mica plates, which were then placed in a muffle furnace heated to 360°C for 3 minutes. The particles, which were loosely bound to the surface of the mica, were blown with a stream of nitrogen. The samples were analyzed by means of an atomic force probe microscope using a semi-contact method. The measurements were carried out using a SolverPRO scanning probe microscope in a contact atomic-force regime. Characteristics of the microscope: cantilever shape - rectangular; working frequency 0,2-2 Hz; accuracy of the microscope 500x500x3 nm; the minimum scanning step is 0.006 nm; the cantilever size is 1.6 × 3.6 mm; cantilever thickness 0,4 mm; the size of the tip (needle) is 10-20 µm. The images were processed using Nova 1.0.26 RC1 Build 914.

3. Results and discussion

The method of atomic-force semi-contact microscopy was used to obtain images of the relief of surfaces of humic substances of peats and hymatomelanoic acids (Figure 1 - 2).

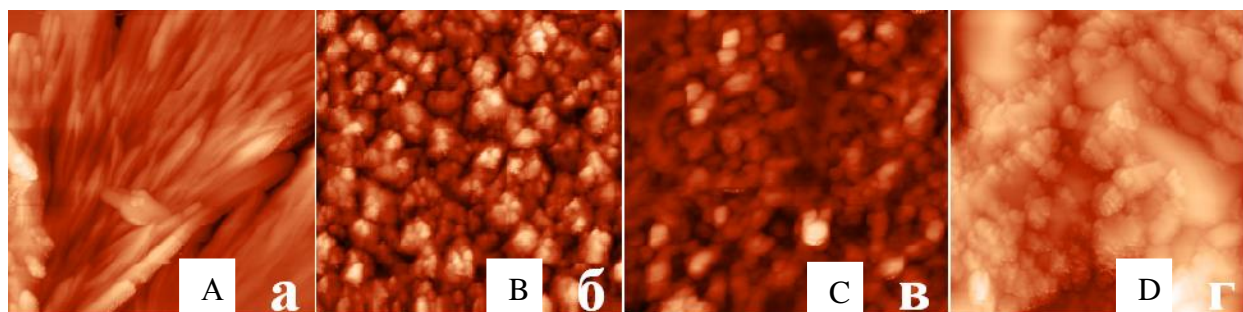


Figure 1. Images of the surface relief (5x5 µm scan) of humic substances of peats on hydrophobic mica: A - black alder fen peat, B - sphagnum transitional peat, C - reed fen peat, D - sphagnum highmoor peat

The surface of sorbed molecules of humic substances on hydrophilic mica depends on their origin. Filling of the mica surface with humic substances occurs unevenly: areas with a high, medium and low level of filling, as well as free areas are observed. The difference in elevation is 150 - 250 mkm. Molecules of humic substances of BFP (Figure 1A), retained by van der Waals forces and hydrogen bonds, form a dense, flat surface without inclusions. The molecules of humic substances STP and RFP, unlike the humic substances of BFP, do not form a single structure, but are combined into spherical agglomerates with a diameter of 500-700 nm and a thickness of 200-400 nm with an inhomogeneous surface (Figure 1B,C). They represent spheres with diameter of 230-330 nm and thickness of 19-25 nm. Individual HS molecules of RFP can be combined into spherical agglomerates with a diameter of 300-500 nm and a thickness of 100-300 nm. The surface of the agglomerates is not homogeneous. The HS molecules of SHP (Figure 1D) are spheres with a diameter of 160-220 nm and a thickness of 10-12 nm.

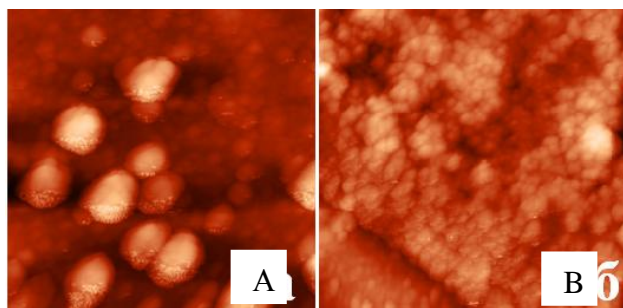


Figure 2. Hyatomelanolic acids: A - black alder fen peat, B - sphagnum transitional peat, 5x5 mkm

Molecules of HMA of BFP (Figure 2), in contrast to the initial molecules of HS BFP (Figure 1), are spheres with a diameter of 280-420 nm and a thickness of 17-23 nm. Individual molecules are combined into spherical agglomerates with a diameter of 500-700 nm and a thickness of 200-300 nm. The difference from the original molecule can be caused by the fact that during the process of ethanol extraction, one large molecule of HS BFP breaks down into several smaller fragments of the HMA BFP. Molecules of the HMA STP (Figure 2B), as well as the molecules of the initial HS of the STP, are spheres with a diameter of 120-160 nm and a thickness of 5-9 nm. Individual molecules are combined into spherical globules with a diameter of 300-400 nm and a thickness of 100-150 nm. Like the molecules of the HMA BFP, the molecules of the HMA STP are smaller than the molecules of the initial HS of the STP.

In terms of their nanomorphology, humic substances and hyatomelanolic acids are separate spherical molecules with a small deepening in the center. The subunits of molecules of humic substances are self-assembled together and are held together by weak dispersion forces: van der Waals, π - π -stacking, and hydrogen bonds responsible for a larger size of aggregates of humic substances. Humic substances can be deposited on clay surfaces either in the form of spiral molecules-units of the lignin-carbon complex, or as molecules of humic substances of black alder fen peat enriched with lignin, in the form of filaments [15]. The chemical structure of the HS structure can be described from the point of view of the lignin-carbon complex or LCC model (Figure 3).

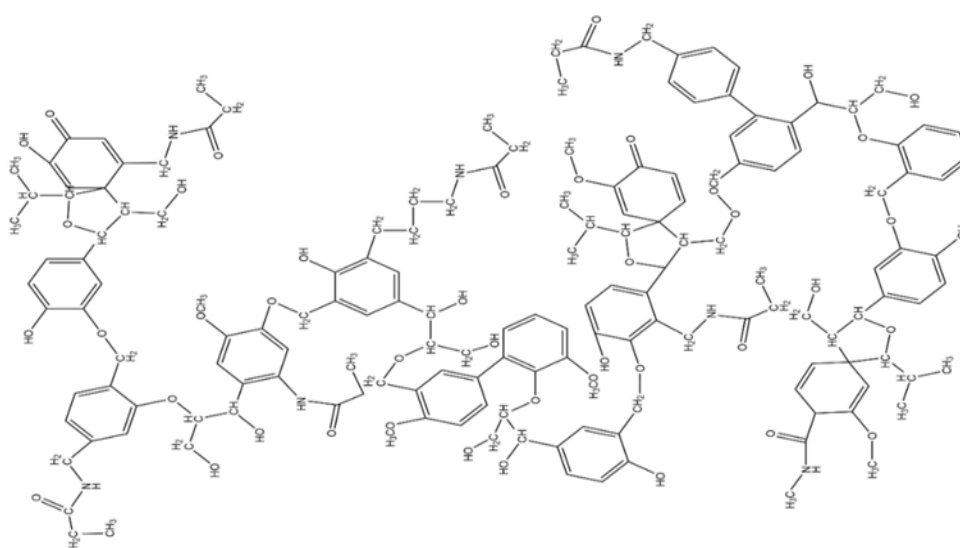


Figure 3. Model of the spiral structure of humic substances of the lignin-carbon complex.

The LCC model provides a possible conformation of the three-dimensional ring structure of humic substances, depending on the pH. The ring structure is open for the formation of linear chain configurations, which contributes to the formation of unfoldable structures. Hydrogen bonds can also play an important role in the stabilization of oligomeric chains in a helical structure, while dipole-dipole and van der Waals interactions support a ring conformation with the formation of a supramolecular structure. The three-dimensional ring structure observed on the AFM corresponds to the conformation of HS and other lignin-like molecules. Both the primary and secondary structures are important for the binding of metal ions. The sterile location of phenolic and carboxyl groups has shown that they play a key role in the aggregation of humic substances on clay minerals. Possible mechanisms of binding humic substances with mineral surfaces, including polyvalent cation bridge, ligand exchange, anion exchange, bath-der-Waals interaction and hydrogen bonding have been proposed and discussed in the literature. The interaction between particles of HS and the surface of hydrophilic mica leads to orientations that maximize the contact area at the solid-liquid interface. Covalent and hydrogen bonds stabilize the oligomeric chain of the helical structure, while dipole-dipole and van der Waals interactions support an annular conformation. Aggregation of annular macromolecules and polymers of natural lignin is observed in soils and aquatic environments. The morphology of the HS structure, obtained by us by the AFM method, confirmed this fact. The lignin-carbon complex model was optimized by use of ChemOfficePro 16.0 (Figure 4).

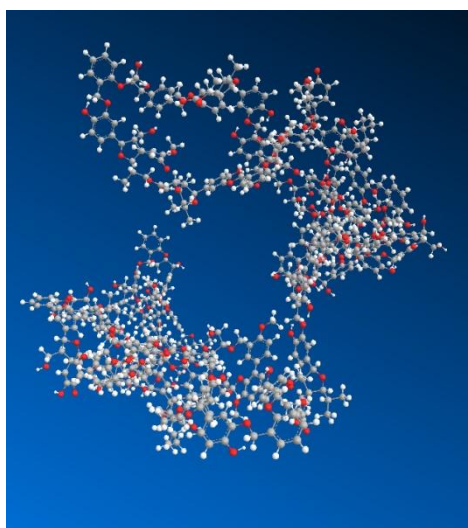


Figure 4. The ball-rod structure of HS is the result of molecular modeling; optimized 3D view of the lignin-carbon complex model

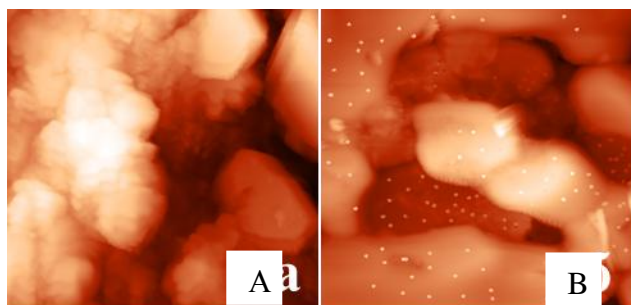


Figure 5. Humic substances: A - black alder fen peat, B - sphagnum transitional peat, with Pb^{2+} ions, 5x5 mkm

Chains of molecules of humic substances are able to connect to each other and form two-, three- and six-helical structures like a DNA molecule and then fold in the form of rings; stable supramolecular structures are formed on the

surface of clay minerals in the soil. The interaction of humic substances with various heavy metal ions with the formation of insoluble humates is quite fully described in the literature, however, the question remains of the conformational changes taking place in this interaction, as well as the structure of the resulting insoluble humates.

We obtained images of the structures of lead and zinc humates sorbed on the surface of hydrophilic mica by atomic force microscopy (Figure 5).

When Pb^{2+} ions introduced into the solution of humic substances of black alder fen peat, aggregation of the HS molecules takes place and the "sticking" of Pb^{2+} ions on the humic ensembles occurs. Various structures with a diameter of 1-2 nm are observed on the surface being scanned, which were not found in the initial molecules of the humic substances of black alder fen peat, which are linear elongated structures. Humic substances of sphagnum transition peat retain their globular structure in the presence of Pb^{2+} ions, however, the diameter of the aggregates increases approximately 5-fold compared to the initial molecules of HS and it is 2-3 nm. The surface of globular aggregates of the humates of HS of STP is covered by various inclusions with a diameter of 1 nm, commensurate with the dimensions of Pb^{2+} ions. We assume that during the interaction of humic substances with Pb^{2+} ions, sorption proceeds throughout the surface of humic substances, with the use of carboxyl and phenolic groups, which correlates with a high degree of binding of Pb^{2+} ions with HS [16]. The analysis of the surface of lead humates showed that the lead ions adhere to the hydrophilic sections of the HS molecules. Non-fragmented accumulations of the complexes "HS-lead ions" were obtained. It is shown that lead ions interact mainly with the hydroxyl fragments of the sorption center of HS molecules (Figure 6).

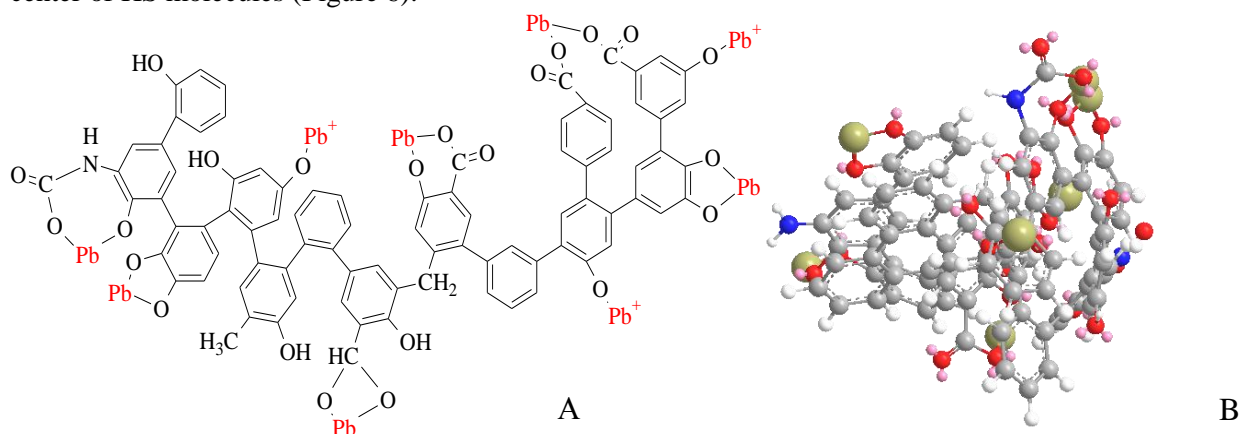


Figure 6. A -conformational changes in humic substances in the presence of Pb^{2+} ions; B -model of lead humate optimized by ChemOffice Pro 16.0

Zinc ions substantially change the conformation of the molecules of humic substances on the surface of hydrophilic mica (Figure 7A). While the initial humic substances are linear molecules closely adjacent to each other, humic substances, together with Zn^{2+} ions, are disk structures 450-500 nm in thickness and 5-7 mm in diameter.

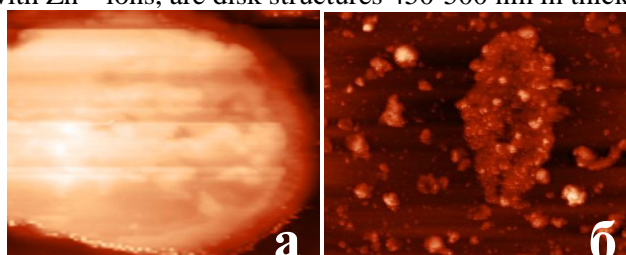


Figure 7. Humic substances: A -humic substances of black alder fen peat; B -humic substances of sphagnum transitional peat with Zn^{2+} ions, 5x5 mkm

The addition of Zn^{2+} ions to humic substances of sphagnum transition peat changes the morphology of the molecules of initial humic substances (Figure 7B). In addition to spherical aggregates of initial HS of STP with a diameter of 500-700 nm and a thickness of 200-400 nm, presumably being the initial molecules of HS of STP, aggregates of a spherical or ellipsoidal structure with a diameter of 3-7 μm and a height of 340-420 nm are observed. The diameter of aggregates of humic substances with Zn^{2+} ions increases approximately in 10 times, and the height - by 2 times, which indicates self-assembly of molecules of humic substances around Zn^{2+} ions.

When Zn^{2+} ions are added to the solution of HS, a reorientation of individual molecules of humic substances in space that surround the cations of zinc occurs, creating a macro-complex "metal-HS" with a change in the conformation of the agglomerates of the initial humic substances (Figure 8)

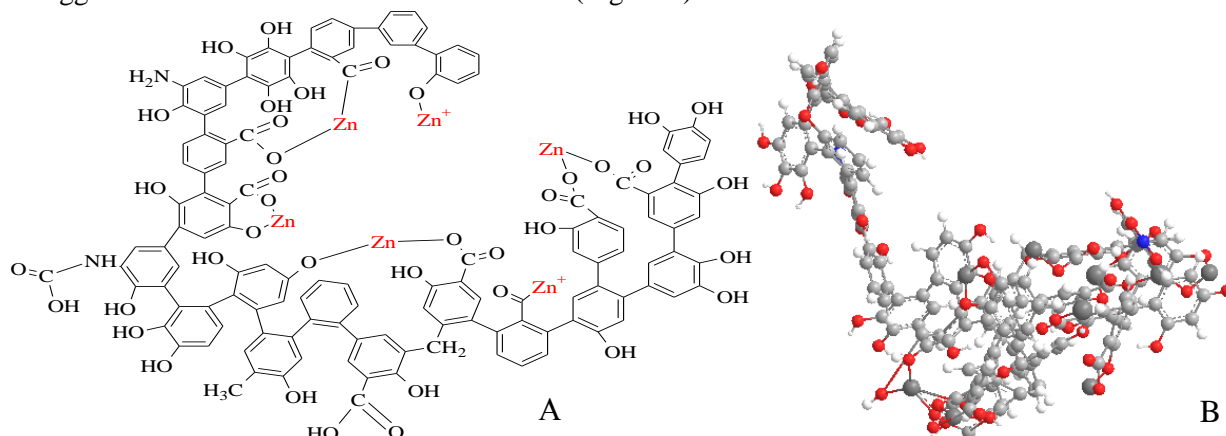


Figure 8. A - conformational changes in humic substances in the presence of Zn^{2+} ions; B - optimized by ChemOfficePro 16.0 model of zinc humate

The 3D models (Figure 6B, 8B) show how Pb^{2+} and Zn^{2+} ions are embedded in all sorption centers both on the surface of supramolecular structures of humic substances (Pb^{2+} cations) and inside molecules of humic substances with the formation of chelate complexes (Zn^{2+} cations). Separate molecules of humic substances are capable, surrounding Zn^{2+} ions, to form with them, due to ionic and donor-acceptor bonds, structures similar to the structure of hemoglobin (Figure 9)

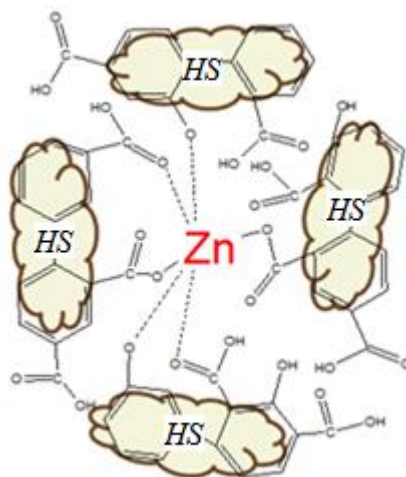


Figure 9. Supramolecular structure of "Zn²⁺ - HS molecule" fragment

4. Conclusion

Method of atomic-force microscopy determined the structure of the surface of humic substances of peats. The dimensions of single globular molecules were 140-320 nm in diameter and 5-25 nm in height. To describe the structure, a lignin-carbon complex model is proposed. Humic substances of black alder fen peat are the artifact and are elongated molecules 1-3 mm long, 20-50 nm wide and 10-30 nm high. This structure is explained by the fact that the basis of these humic substances are lignin residues. Possible ways of interaction of humic substances of peats with metal cations (Pb^{2+} and Zn^{2+}) are considered taking into account conformational changes in humic substances. It is proved that the sorption of lead ions proceeds on the surface of humic substances, and of zinc ions with the reorientation of humic substances and the formation of supramolecular structures in the type of hemoglobin.

References

- [1] J. Wiley, S. Bernhard, *Rep. of Dahlem workshop*, (1988) 133- 148.
- [2] A.I. Popov, 2004 248.
- [3] J.A. Rice, *Org. Geochem*, 17, (1991) 635.
- [4] E.M. Perdue, *Geochim. Cosmochim. Acta*, 48, (1984) 1435.
- [5] M. Plaschke, J. Römer, R. Klenze et al., *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 160, (1999) 269–279.
- [6] M. F. Barriquello, F. L. Leite, D. K. Deda, *Materials Sciences and Applications*, 3, (2012) 478-484.
- [7] C. Chen, X. Wang, H. Jiang, W. Hu, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 302, (2007) 121-125.
- [8] C. Colombo, G. Palumbo, R. Angelico et al., *Chemosphere*, 138, (2015) 821-828.
- [9] B.C. Donose, A.V. Nguyen, G.M. Evans, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 329, (2008) 100-105.
- [10] X. Meng, W. Tang, L. Wang, *Journal of Membrane Science*, 487, (2015) 180-188.
- [11] F. L. Leite, D. K. Deda, M. L. Simões et al., *Science and Technology*, 2, (2012) 61-65.
- [12] Y. Sugimoto et al, *Nature*, 66, (2007) 446.
- [13] E.D. Dmitrieva, A.A. Goryacheva, M.M. Leont'eva [and others], *Izvestiya TulGU. Natural Sciences*, 3, (2015) 205-219.
- [14] E.D. Dmitrieva, A.A. Goryacheva, K.V. Syundyukova [and others], *Izvestiya TulGU. Natural sciences*, 2, (2015) 132 - 137.
- [15] E.D. Dmitrieva, M.M. Leont'eva, K.V. Syundyukova, *Chemistry of plant raw materials*, 4, (2017) 187-194
- [16] K.V. Siundiukova, E.D. Dmitrieva, A.A. Goryacheva, E.N. Muzafarov, *Sorption and chromatographic processes*, 6, (2016) 788-796.