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## **Invited speakers**

## Biocomposition based on humic acids and oil-degrading microorganisms in the relation to petroleum products

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Keywords: humic acids, oil, petroleum products, oil-degrading microorganisms, *Rhodococcus*

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Humic acids are the nutrient substrates for oil-degrading microorganisms involved in the decomposition of petroleum hydrocarbons, which, after applying them to the surface being cleaned, are attached to the oil film at the oil-natural phase section and are involved in the petroleum biodegradation. The purpose of this work is to study the detoxifying ability of biocompositions based on humic acids and oil-degrading microorganisms of the genus *Rhodococcus* in the relation to petroleum products.

The objects of this study were peats humic acids (HAs) of the Tula region: red fen peat (RFP), black alder fen peat (BFP), sphagnum high-moor peat (SHP) and sphagnum transition peat (STP) [1]. Bacterial strains of *Rhodococcus erythropolis* S67, *Rhodococcus erythropolis* X5 [2]. The remediation efficiency of biocompositions was analyzed on two samples of oil-contaminated soils from the territories of an oil refinery and a metallurgical enterprise with a high ( $3321 \pm 1162$  mg / kg) and very high ( $9180 \pm 2300$  mg / kg) levels of contamination. The initial and residual content of oil products at the end of the remediation measures was determined on a fluid analyzer "Fluorat 02-3M" according to the method of PND F 16.1: 2.21-98. The effectiveness of biocompositions was assessed by vegetation experiments with a watercress test object according to a complex of biological indicators before and after remediation. The treatment of oil-contaminated soil samples from the territories of industrial enterprises with biocompositions can significantly reduce the content of oil products in the soil system, which was confirmed by chemical analysis. Maximum oil recovery efficiency of biocompositions "HAs of RFP + *Rh. erythropolis* S67" and "HAs of RFP + *Rh. erythropolis* X5": the amount of petroleum products in the soil sample with a very high level of contamination is reduced to a low level ( $1160 \pm 290$  mg/kg). As a result of exposure to the soil sample with a high level of contamination of the biocomposition "HAs of RFP + *Rh. erythropolis* S67", the content of oil products corresponds to the permissible level of pollution ( $948 \pm 102$  mg/kg); "HAs of SHP + *Rh. erythropolis* X5" reduces the amount of petroleum products in the soil by 2 times. An increase in the level of biodegradation of soils oil pollution is due to the complex action of humic acids, which consists both in the stimulating effect of humic acids on microorganisms of the genus *Rhodococcus* - components of biocompositions and the native microflora of the soil, and the detoxifying effect of HAs in the relation to soils oil pollution.

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## **The influence of Kazakhstan potassium humate on the productivity of beef cattle**

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Keywords: emulsion, liniment, paste, salve, potassium humate

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The analysis of the current state and development of cattle breeding in the Republic of Kazakhstan shows that there is a tendency to reduce the number of livestock, and, consequently, to reduce beef production. Therefore, increasing the efficiency of beef cattle breeding, obtaining high-quality raw materials of animal origin is one of the main problems of the modern agro-industrial complex [1]. The use of domestic preparations based on humic acids in meat farming would increase weight gain, reduce the incidence of infectious diseases, and activate metabolic processes. The results of the analysis of antimicrobial and bioregulatory properties of drugs of domestic origin based on humic acids showed their antimicrobial activity, biological activity and harmlessness [2].

The aim of the research is to develop an effective feed additive to increase productivity and normalize the metabolism of meat breeds of animals, using a local and cheap natural source of mineral and biologically active substances. A non-toxic domestic preparation of potassium humate obtained from brown coals of Kazakhstan is used as a feed additive.

The analysis of potassium humate showed the content of a wide range of biologically active substances: the mass fraction of humic acids in terms of dry matter is 56%, additionally contains more than 70 mineral compounds. The method of feeding farm animals and poultry was characterized by the fact that the following is additionally introduced into the diet: 1% solution for animals, 1.5% solution of potassium humate for poultry, 1 time a day during morning or evening feeding for 10 to 15 days with feed or water.

It has been established that the domestic preparation of potassium humate has a positive effect on the general condition of animals, normalizes metabolic processes in the body, increases the natural resistance, stimulates growth and development, and improves the conversion of feed in the rumen, which has a positive effect on the productivity of beef cattle and poultry. Regardless of age, on the 7th day of taking a feed supplement of potassium humate, calves have a noticeable shine and a bright color of wool, molting is completed within a month, calves become mobile and active, chewing gum is activated, average daily weight gain increases by 7.6-8.9%. Clinical signs of metabolic disorders and beriberi completely disappeared in animals after 20-30 days from the start of the feed additive application. In pigs, calmer animal behavior, a decrease in stress response, and a decrease in piglet waste by up to 20% were noted. Potassium humate has a positive effect on the increase in live weight of broilers-quails and goslings: in quails – on average by 5%, in goslings-by 7%. The use of the drug to laying quails contributes to an increase in egg production of poultry by 3%.

In production experiments, it was found that the intake of potassium humate increases the monthly weight gain in fattened bulls weighing 350-450 kg from 28 to 36 kg or on average  $30.76 \pm 2.5$  kg, which allowed us to recommend it as a feed additive.

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## **Molecular composition of humic acids in the permafrost peatlands of the zonal series of soils as paleorecord of climatic change**

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**Keywords:** Arctic, permafrost peat mounds, humic acids, <sup>13</sup>C NMR

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High latitude peat ecosystems of the Arctic are especially sensitive to climate change. The composition of peatlands organic matter serves as a characteristic measure reflecting climatic conditions of its formation period. It is connected with the content of botanic and palynological material in the Holocene periods. The study was conducted in key testing areas of five large clusters of the Arctic wetland ecosystems from the Far North Taiga to Northern Tundra on two soil types – Hemic Folic Cryic Histosol and Hemic Folic Cryic Histosol (Turbic). A quantitative assessment of the content of functional groups and molecular fragments of studied humic acids (HAs) (<sup>13</sup>C-NMR CP/MAS data) indicates the difference in their molecular composition both in seasonal thawed (STL) and permafrost layers of peat, and in the zonal series of soils. The structural and functional parameters of HAs in the peatlands under study are determined through a combination of natural selection of aromatic structures in the humification process, species composition and decomposition degree of peat, and hydrological conditions, which reflect the peat-forming climatic conditions in the Holocene. The climatic parameters of the Atlantic period shaped the character of the vegetation composition, with sedge and tree communities – consequently HA precursors with higher lignin content – dominating. Present-day peat deposits formed in the Mid and Late Holocene mainly out of bryophyte residues contain HAs with a significant share of carbohydrate and paraffin structures. A certain trend towards decreased concentration of aromatic (*fa*) and higher content of unoxidised aliphatic fragments in HAs from STL in high latitude peatlands is observed, which is related to values of bioactive temperatures of soils (Figure).

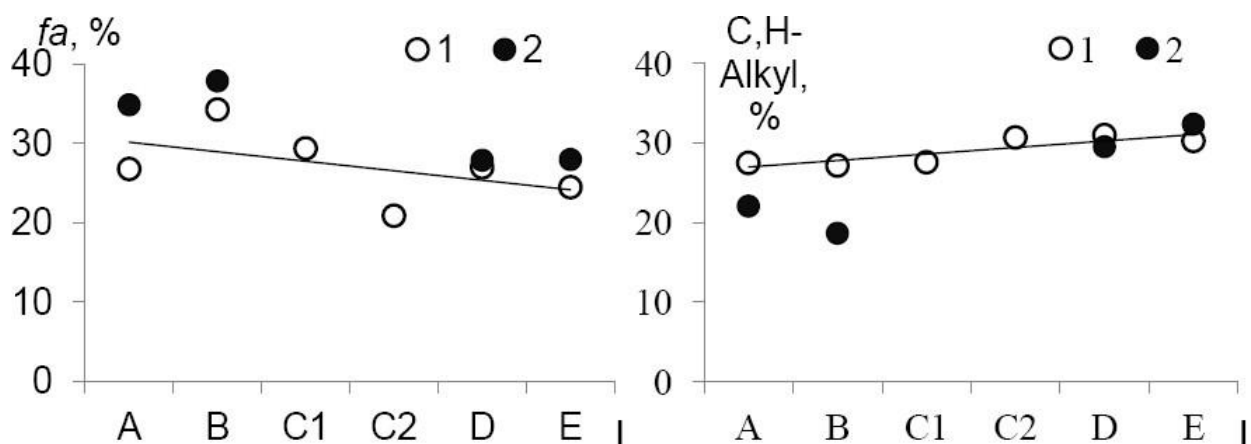


Figure. Content of HA aromatic (I) and C,H-Alkyl fragments (II) from seasonal thawed layers of Hemic Follic Cryic Histosol (1) and Hemic Follic Cryic Histosol (Turbic) (2): A – the far north taiga; B – the forest-tundra; C – the south tundra; D – the ecotone south tundra and north tundra; E – the north tundra (Barents Sea coast).

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## Humic-based polyelectrolyte complex: effect on soil properties and ecotoxicity

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Keywords: soil conditioner, cationic polymer, anionic biopolymer, interpolyelectrolyte complex, bioassay, mitigation

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Interpolyelectrolyte complexes (IPECs), the products of interaction between two oppositely charged polyelectrolytes (PEs), have been suggested for soil structure stabilization. An ability of IPECs to improve soil hydrophysical properties, prevent soil erosion and act as soil conditioners have been studied in our previous works [1]. The efficacy of IPECs was dependent both on chemical composition of IPEC and soil properties. Using humic substances as the polyanionic ingredient of IPEC is a prospective approach to develop environmental-friendly and effective formulations.

In this study we used a positively charged IPEC prepared from the commercial polymers: cationic poly(diallyldimethylammoniumchloride) (PDMA) and anionic biopolymer lignohumate (LH), a humic-based plant growth stimulator. The objective of the study was to assess the effects of the three PEs on soil properties and toxicity to a number of standard test-species. The results obtained for cationic IPEC were compared with the ones for anionic LH and cationic PDMA.

Soil experiments were conducted in a small-plot trial. The results showed, that humic-based IPEC drastically reduced the share of large soil aggregates and prevented the formation of small aggregates, thereby significantly increasing the content of agronomically valuable aggregates (0.25-10 mm). Application of IPEC in concentrations of 1-5% did not lead to a significant change in the amount of exchangeable cations, except for potassium. Soil treatment with IPEC promoted plant growth and development in the extend comparable to the effect of LH, humic plant growth stimulator.

The toxicity was evaluated in bioassays using bacteria *Escherichia coli*, ciliates *Paramecium caudatum*, mammalian cell (bull spermatozoa *Bos taurus*) *in vitro*, and three plant species *Sinapis alba*, *Raphanus sativum* and *Triticum durum*. Test responses were assessed (1) in an aqueous matrix at direct contact with PEs and (2) after polymer interaction with a soil matrix. In water matrix, PDDA and IPEC were highly toxic to bacteria and ciliates in all tested concentrations and less toxic to the mammalian cells. Higher plants were less sensitive to the polymers with a progressive decrease of the toxicity in a row PDDA-IPEC-LH. In soil matrix, the phytotoxicity of PDDA and IPEC significantly decreased. In environmentally relevant concentrations, used for the anti-erosion polymer-soil crust formation, no toxicity of both polymers was detected in phytotests. Being introduced in soil matrix, cationic polymers bound to organic and mineral components that resulted in mitigation of the polycation's toxicity.

The results of the work show that LH in the polycomplex retains its biostimulant properties and a humic-based IPEC can be used to create productive artificial soils.

Acknowledgements. The study was performed according to the State Assignment No. 121040800154-8.

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## Optics of humic substances: recent advances

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Keywords: humic substances, DOM, fluorescence, optics

DOI:10.36291/HIT.2021.062

The origin of optical properties of DOM and humic substances (HS) is still a matter of active debates in the literature. The key question is why are the optical spectra of dramatically different (in terms of molecular composition) samples so similar. Recently, we've uncovered ultrafast decay component in fluorescence decay of DOM and HS and interpreted it as an evidence of fluorophores interaction within the system [1], thus favoring the interaction rather than the pure superposition model. The next step was the detailed analysis of artificial systems exhibiting humic-like optical properties. Using this framework,

the mechanisms of optical properties formation in DOM and HS can be extended for a broad range of natural systems.

Acknowledgements. The work was supported by be Young Investigator Research Grant from IHSS.

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## Preparation of composite adsorbents based on humic acid and silicon oxide

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Keywords: humic acid, silicon oxide, composite, heavy metals, sorption

DOI:10.36291/HIT.2021.064

The paper presents the results of research on the preparation and study of the physicochemical properties of a composite based on humic acid and silicon oxide.

To obtain humic acid (HA), brown coal from the «Maikuben» deposit was used as a feedstock, pre-crushed to a particle size of less than 0.3 mm and having the following characteristics (wt%): Ad 25.8; Wr 9.3; Vd 46.6; Std 0.71. The yield of free humic acids was 56%.

To obtain the modification of HA / SiO<sub>2</sub> and its carbonized form, the dried sample (HA) is crushed to a powdery state and mechanically mixed with SiO<sub>2</sub> obtained from ash-and-slag waste from «Bogatyr» coal in a ratio of 10/1 (HA / SiO<sub>2</sub>). The resulting powder was carbonized in a tube furnace in an argon atmosphere of 800°C for 1 hour at a heating rate of 5°C per minute. The elemental composition of the carbonized composite based on HA/SiO<sub>2</sub> = 1:1 is (wt%): C-27.46, O-31.32, Na-0.07, Mg-0.14, Al-2.2, Si-8.67, S-0.76, Cl-1.1, K-8.89, Ca-0.53, Cu-0.69, Fe-8.18. SEM images of the sample are shown in Fig. 1, where one can observe flocculent formations and spherical silicon oxide with sizes ranging from 223.1 nm to 515.8 nm. Raman results are signals with characteristic peaks D

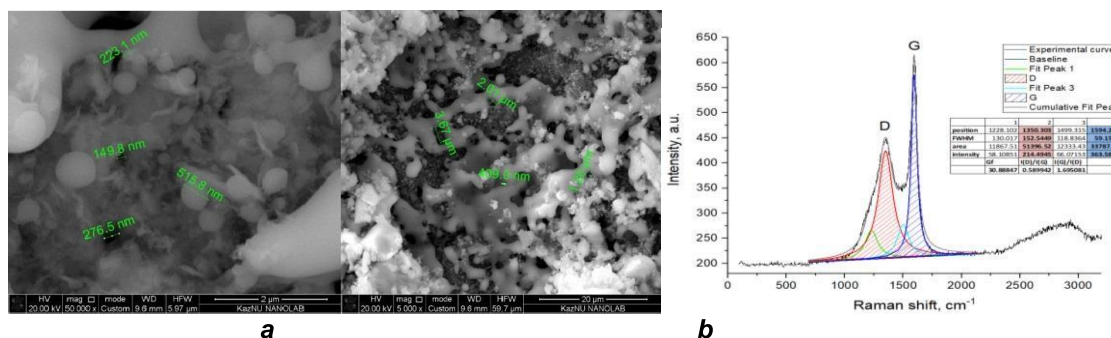


Figure 1. Results of SEM images (a) and Raman images (b) of a composite based on HA /SiO<sub>2</sub>.

and G (1350.08 cm<sup>-1</sup> and 1594.5 cm<sup>-1</sup>). The intensity ratio ID/IG is 0.59, which confirms a large number of sample defects. The degree of graphitization (Gf) is 30.88%. The resulting composite material has been tested for the purification of heavy metals under dynamic conditions. As a result of sorption, the resulting composite showed a high degree of purification (%): Zn-100.0, Cd-91.57, Pb-91.66, Cu-100.0, which may be due to the significant ability of HA to form complex and intracomplex (chelate) compounds with heavy metals and high sorption capacity of the composite with silicon oxide nanoparticles.

Acknowledgements. The work was carried out within the framework of project No. IRN AP09260096 on the topic "Development of technology and organization of pilot production of modified organic biological products based on humic polyelectrolyte acids obtained from the coals of Kazakhstan", funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

## **Humic substances – what is behind this term?**

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Keywords: humic matter, humus, soil organic matter, organic carbon stabilization, humification

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Humic substances (HS) are considered as a special category of natural compounds ubiquitous in soils, sediments and waters. They have long time been described as a series of polydisperse dark-colored macromolecules that are resistant to biodegradation and have no analogues in living organisms. According to hypotheses, HS are formed during degradation of organic debris and by secondary synthesis reactions (a process called humification). On the basis of solubility characteristics HS are divided into humic acids (HA, soluble, precipitated at pH <2), fulvic acids (FA, soluble at all pH values) and humin (insoluble residue). It was estimated that HS comprise about 85-90% of soil humus and about 50% of dissolved organic matter in natural waters. However, there is still much confusion around the term "HS" and around all humic paradigm. Recent experimental evidence, based on non-destructive spectroscopic methods led a number of scientists to a conclusion, that alkali-extractable HS are plant and microbial components on the different stages of decomposition. It has been suggested to abandon all humic terminology as well as a concept of secondary synthesis. Such criticism is not new and dates back to the beginning of XX century. We are going to discuss the problems of humic terminology and nomenclature, the paradigm of secondary synthesis, the concept of stability of alkali-extractable HS and their macromolecular organization. The old definition of HS is ambiguous and uncertain: specific chemically unique and newly synthesized compounds from the one hand and a sum of operational fractions of humus (humic matter) from the other hand. The first definition turns the term HS into hypothetical abstraction, making almost impossible their identification in already formed humus. Operational definition is inconsistent with humic nomenclature, which divides humus into identifiable molecules (non-HS) and substances with poorly defined structure (HS). It is obvious that the alkali extracts and the residue (humin) contain both types of compounds. It is under the debate whether to include humin into the group of HS or not. The definition of HS requires revision. Identifiable molecules and compounds with uncertain structure form the dynamic system which determines the functioning of natural organic matter in the environment and

separation of these compounds is unreasonable from the functional and practical points of view. One of the solutions might be re-definition of HS as compounds comprising humified organic matter as a whole (substances of humus or humic matter). Another solution is exclusively operational definition of HS as alkali-extractable components of humus because in this sense the term is used in most studies. The terms HA, FA, humin should be retained as the names of preparations obtained in a certain way. Alkaline extraction is effective method for the isolation of hydrophilic polar substances, acid precipitation allows their concentration for further study. Description of HS as macromolecular products of secondary synthesis or recalcitrant compounds should be avoided. We will show that macromolecular fractions of soil HA are unstable once separated from the mineral matrix. We will also show that under the environmentally relevant concentrations and dynamic conditions no macromolecular compounds are formed from monomeric precursors in the presence of the biocatalyst and the mineral phase. However, free-radical reactions favor stabilization of certain phenolic compounds on the mineral phase and determine the composition of organo-mineral complexes. Contribution of free-radical reactions to the formation of humic matter need further investigations.

Acknowledgements. The work is supported by RSF grant 17-14-01207 (experimental section) and by State assignment 121040800154-8. The research was performed within the MSU program «Future Planet and Global Environmental Change».

## **Bionanomaterials with antibacterial properties based on humic substances**

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Keywords: humic substances, silver nanoparticles, bionanomaterials, antibacterial activity, pharmacological properties

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For today, infectious diseases occupy a leading position in the world. The deficiency of effective therapeutic methods in the treatment of chronic non-healing wounds induces to infection, and against the background of the existing problem of antibiotic resistance - to sepsis and death. That's why, any potentially perspective molecules with antimicrobial and wound-healing properties have to be detailed studied and introduced into medical practice.

In the Natural Humic System Lab MSU by Professor I.V. Perminova the methodology that includes targeted synthesis of bionanomaterials based on humic substances (HS) by polyelectrolyte assembly and template synthesis of nanoparticles was developed. The original humic substances samples (HS) and the samples silver nanoparticles ultradispersed in humic substances (HS-AgNPs) were tested in this study: HS of high-moor peats (Russia), and also commercial HS by Humintech (Germany), Genesis

(Russia), etc. There were a total of 16 tested samples. All experiments were performed in comparison with the colloidal silver preparation Protargol®.

The study of the cytotoxicity of the tested substances (HS/HS-AgNPs) on two lines of cell cultures was done and the limits of toxic effects in the concentration range were established. The study of the cell membranes permeability on the lines of cell cultures in relation to the HS/HS-AgNPs was done and the parameters of their intracellular distribution were established, and also their ability to penetrate through the cell membranes into the cytosol of the cell and the nucleus. The study of the prooxidant and antioxidant / antiradical activity of the HS/HS-AgNPs on the cell culture line was done and their redox reactivity were established, and also their ability to reduce the intracellular level of reactive oxygen species in the prooxidants introduced into the incubation medium ( $\text{H}_2\text{O}_2$ , tert-butyl hydroperoxide,  $\text{Fe}^{2+}$ ). The study of immunomodulatory properties (activity balance of NO-synthase / arginase) of the HS/HS-AgNPs in the cell culture of peritoneal macrophages, including relative to LPS-stimulated control was done.

The antibacterial activity of the samples according to the development of periodic bacterial culture response to the samples was studied. Also, we determined the minimum inhibitory concentrations (MIC) for substances with established antibacterial activity. In this study the control test-strains of *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* was used. The study of changes in the permeability of the cell wall and cytoplasmic membrane (example by *Staphylococcus aureus*) was done and the changes in the activity of resistance genes under the conditions of the tested substances action were determined. The study of the antibacterial and wound-healing properties of the HS-AgNPs to *in vivo* experiments on a model of purulent-inflammatory soft tissues in experimental animals was done.

Based on research, the pharmacological properties base for the HS-AgNPs was formed and the perspective samples with antibacterial and wound-healing properties were selected.

This work was supported by the financial RSF (Grant No. 20-65-47052).



## **Abstracts from conference sponsors**

## **Chemical and pharmacological study of the coal humic substances by the Genesis company from the Kansk-Achinsky deposit (Pereyaslovsky open-pit)**

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Keywords: humic substances, biological activity, pharmacological, microbiological and chemical safety

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Today, a difficult moment in the study of humic substances (HS) is the impossibility of non-experimental prediction of their biological activity, because HS are characterized by a multifaceted pharmacological activity and a nonavailability of the strict constancy by the chemical composition. There are many examples in the literature when HS can be sources of new diverse biologically active molecules depending on the origin of raw materials (peat, coal, sapropel, mumiyo) and methods of production. It is this fact that determines the need for constant biological and chemical standardization of each specific humic product.

Chemical-pharmacological study, testing of the chemical, microbiological and pharmacological safety of the coal HS by the Kansk-Achinsky deposit (by Genesis company, Russia) were done.

The structural parameters of HS were studied by ICP-MS, electronic, fluorescence, infrared, <sup>13</sup>C-NMR spectroscopy, elemental C,H,N,O-analysis, gel chromatography. The 10 essential elements were established - K, Ca, Mg, Mn, Fe, Cu, Zn, Mo, Co, Se. Microbiological (pathogenic microorganisms, molds and yeasts) and chemical (heavy metals, arsenic, radionuclides) safe parameters were corresponded to requirements of the Russia State Pharmacopoeia.

According to the results of the toxicity testing with the introduction of a single dose (acute toxicity) and the introduction of repeated doses (28-day toxicity) were established that administered intragastrically HS didn't have a toxic effect on the organs and systems of the experimental animals, and belong to the V hazard class - substances low-hazard (according to GOST 32644-2014). The irritant effect and sensibilization tests were established that HS didn't have the allergenic properties. According to the toxicological testing on cell culture in vitro was established that HS also didn't have a cytotoxic effect in a wide range of doses.

According to the results of the intracellular distribution study on cell culture, the HS ability to penetrate cell barriers and accumulate in the intracellular compartment, mainly in the perinuclear compartment and the cell nucleus was established.

The results of testing the specific pharmacological activity in various experimental models were established the antioxidant, immunotropic, cardiotropic, cytoprotective and antihypoxic properties of HS. The cytoprotective properties of HS were established due to

they reduced the intracellular reactive oxygen species level after induction of oxidative stress by prooxidants (tertbutyl hydroperoxide, hydrogen dioxide, and  $\text{Fe}^{2+}$ ), which point at the HS stimulation of the antioxidant cell self-defense system.

Therefore, the coal HS by the Kansk-Achinsky deposit (by Genesis company, Russia) are safe and promising substances for dietary supplements with protective, antihypoxic, antioxidant and immunomodulatory properties.

## **Influence of a humic and fulvic stimulator on indicators of productivity and quality of grapes in Mediterranean climate conditions**

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DOI:10.36291/HIT.2021.029

Keywords: lignohumate; growth regulator; yield; grape; combined action; variety; productivity

### **Research methodology**

Field trials regarding the effectiveness of the application of Lignohumate product were carried out in the period of 2018-2020. The tests were carried out at Abrau-Dyurso LLC (Krasnodar region, Russia) Merlot and Cabernet varieties. Trial plot - 3x1.5, planting age – year 1996 of planting.

Table 1. Effect of Lignohumate product on the yield of Merlot and Cabernet varieties in 2018-2020.

Variants	Average mass of a grape cluster, g	Yield				
		From a bush, kg	From 1 Ha, tons	Increase in comparison to control		Shoot productivity index
				t/ha	%	
Merlot control	90	5,0	11,0	-	-	150
2.Merlot Lignohumate	+119	<b>5,58</b>	<b>12,4</b>	<b>1,4</b>	<b>12,6</b>	162
3. Cabernet Control	97	5,4	11,1	-	-	150
4.Cabernet Lignohumate	+119	<b>5,67</b>	<b>12,7</b>	<b>1,6</b>	<b>14,8</b>	167

Lignohumate product significantly reduced the number of pea-like and diseased berries in relation to control. The introduction of the product contributed to an increase in the average size of berries, which led to an increase in the average mass of a grape cluster up to 24% for Cabernet and 18% for Merlot.

For the varieties Merlot and Cabernet, the sugar content increased by 2.6-3.4 g / 100 cm<sup>3</sup> with a decrease in acidity by 0.8-0.9 g / dm<sup>3</sup> according to the trial variants conducted.

As a result of field trials, it was demonstrated that the application of Lignohumate had a positive effect on the quality of Merlot and Cabernet grapes. The number of inflorescences naturally increased with a threefold application.

## **The role of Reasil® Humic Health feed additive in the elimination of antibiotics from the body of broilers**

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Keywords: humic acids, broilers, elimination, antibiotics

DOI:10.36291/HIT.2021.031

The feed additive of humic acids Reasil® Humic Health, made from natural leonardite, a product of humification of ancient plants, contains more than 70 names of biologically active substances, which explains its diverse use in animal husbandry, agronomy and medicine.

Numerous experiments in the poultry industry have shown that the inclusion of Reasil® Humic Health in the composition of mixed feed for broilers of the Cobb-500 cross in the amount of 1,5 kg per 1 ton of mixed feed increases the productivity of poultry by 9,1 – 9,5 %, improves the safety of livestock and reduces the mortality of young poultry during the growing period by 1,9-5,3 %. Feeding the preparation of humic acids to laying hens of the High-line cross in the same concentration contributed to the improvement of all the main economic indicators. Scientific and production testing of the drug in the diets of turkeys from 1 to 42 days of life indicated an increase in the live weight of poultry by 20,57% and a decrease in feed costs per unit of production by 9,6 %.

The analysis of physiological experiments showed that the inclusion of the dry feed additive Reasil® Humic Health in the diet of broiler chickens in the amount of 1.5 g per 1 kg of mixed feed leads to an improvement in the digestibility of feed nutrients, increases the digestibility of nitrogenous and mineral substances, the availability of amino acids and increases the intensity of live weight gain of broilers.

It has been experimentally established that the use of a preparation of humic acids improves the anatomical and morphological characteristics of carcasses and their parts obtained from the slaughter of broiler chickens and contributes to an increase in the yields of the most valuable parts and the formation of a larger mass fraction of the edible part in comparison with the in-line cultivation technology. The organoleptic evaluation of poultry meat products that received the humic acid preparation showed that they have acceptable organoleptic characteristics that are not inferior to the control group that did not receive the additive.

The results of studying the effect of the Reasil® Humic Health feed additive on the elimination rate of the antibacterial drug "Florfenicol" and the coccidiostatic "Pulcox 40 %" from the body of broiler chickens showed that the addition of Reasil® Humic Health in a dose of 0,5 g/kg removes trimethoprim, sulfadiazine, doxycycline, oxytetracycline and gentamicin, which allows them to be sold as gherkins. The combined use of the antibiotic "Florfenicol" and the feed additive Reasil® Humic Health does not prevent the accumulation of the antibacterial drug in the muscle tissue in therapeutic concentrations, and after stopping the evaporation, the antibiotic begins to be actively excreted. Feeding Reasil® Humic Health at a dose of 1,5 and 2.0 g/kg of feed leads to an acceleration of the elimination of "Florfenicol" from muscle tissue by 4 times compared to the control.

## A new strategy in agriculture from LLC "NVP" BashInkom

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Key words: eco-adaptive technologies, bio-assay, farming technologies

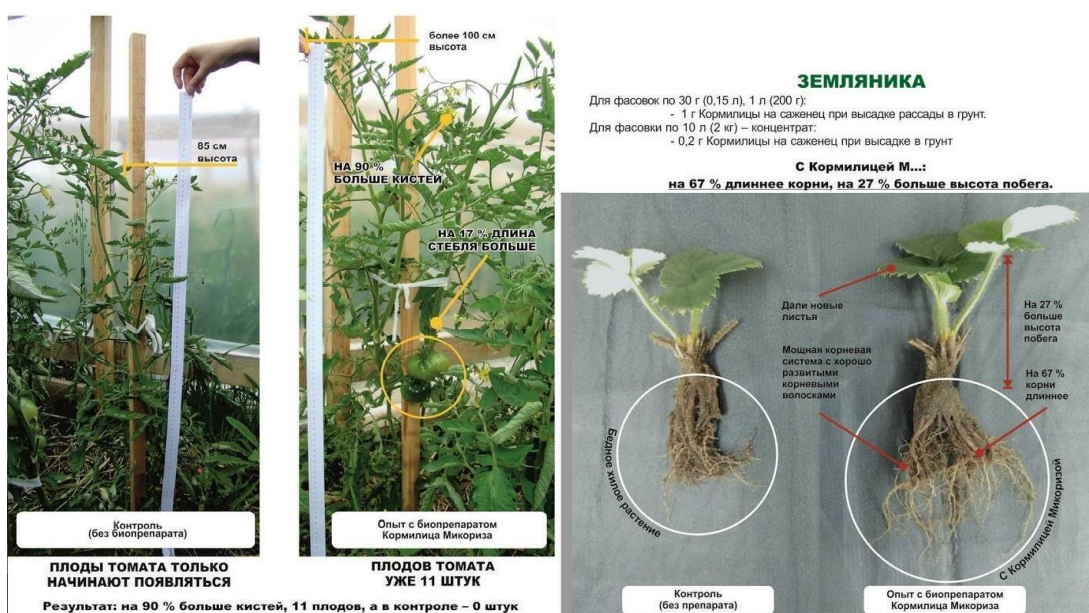
DOI:10.36291/HIT.2021.078

The company LLC "NVP" BashInkom" is developing a new direction in its product line related to eco-adaptive farming technologies.

Preparations containing humic acid salts, phyto sporin, endomycorrhizal fungi of the genus *Glomus* and more than 40 strains of useful soil microorganisms have been developed and tested.

The tests were carried out on vegetable and berry crops, as well as on agricultural crops. The tests were carried out in the phytolaboratory, in greenhouses and on plots in the open ground.

The growth indicators of the root system and the green mass of plants were studied and analyzed. It was found that the use of our drugs allows you to increase the root system of plants by 20-45%, and the green mass up to 90% relative to the control.



## **Abstracts (alphabetical order)**

## Humic substances in municipal water management

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Keywords: humic substances, municipal management

DOI:10.36291/HIT.2021.001

Humic substances (HSs) are products of organic matter decomposition process, mostly of plant and animal origin. HSs are present in soil and in aquatic environment (surface and groundwater). Research conducted for over a dozen years, including these at the Cracow University of Technology, shows that municipal management is an important source of HSs formation. They were found in the water intake for municipal drinking water purposes, but also in leachate of municipal landfills, activated sludge, leachate of excess and digested sludge, as well as raw, treated and treated wastewater. Leachate from municipal landfills, due to the high concentration of organic and inorganic pollutants, is discharged to a biological treatment plant. Since the HSs contained in them are not biodegradable, they are partially adsorbed on the activated sludge, while the light fractions remain in the treated wastewater and are discharged mostly to rivers, from which water is intake for municipal purposes. In water treatment plants, HSs generate carcinogenic, mutagenic and teratogenic compounds during the oxidation process. At the same time, HSs are essential for plants, contribute to soil fertility, transport organic and inorganic substances to the root mass, and maintain an optimal level of soil moisture. Statistical data show that in Poland there is almost 65,000 hectares of land requiring reclamation. For example, according to the Central Statistical Office, the greatest number of such land is in the Greater Poland (10.9 thousand ha) and Lower Silesia (8.1 thousand ha) voivodeships, as well as in Silesia, Warmia-Masuria and Lodzkie (approximately 4.9 thousand ha each). Therefore, the work includes isolation of humic substances from many sources of the municipal management. Qualitative analysis confirmed the presence of HSs in the given sources - they were mainly fulvic acids due to their high mobility. For this purpose, the extracts were analyzed for the UV-VIS and IR spectrum as well as the qualitative analysis of the elemental composition and inorganic micropollutants. The obtained test results confirmed that the main organic substances are HSs, occurring depending on the source in various concentrations. Phytotoxicity tests were carried out for selected humic substances, which showed their high suitability for plant cultivation. The analysis of the number of biological wastewater treatment plants and the amount of sewage sludge produced in Poland and municipal waste landfills generating leachate with a high concentration of HSs indicates that municipal management is an attractive source of humic substances that can be used for the revitalization of wastelands and reclamation of degraded areas.

## **The humus aspect in the cheapening of the technology of mining uranium-molybdenum-rhenium deposits in the Moscow basin**

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Keywords: ore mining, humic acids, eco-adaptivity

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Source: uranium-molybdenum-rhenium ore formations of the brown coal basin near Moscow (Briketno-Zheltukhinskoe and others), containing up to 85% humic acids. To increase the content of the productive solution intended for the extraction of final target metals, it is possible to use a cheaper geotechnological method, instead of traditional expensive methods of their extraction, taking into account the value of the components of ore formation and the region of its location.

The essence of the approach: activation of humic acids in the composition of brown coals, including fulvic acids - the most mobile and playing a key role in the dissolution of mineral substances to ionic forms; safe and economically feasible use of groundwater in comparison with traditional sulfuric acid leaching.

The method of providing is a selective, mobile and regulated water-alkaline effect on the coal mass of a working reagent that takes into account the anionogenicity of uranium, molybdenum, rhenium and provides an increase in their migration properties. The main qualitative characteristic of fulvic acids, the product of decomposition of humic acids, is biological activity due to low molecular weight; easy binding of minerals and elements in their molecule, which leads to their dissolution and mobilization; they react more to negatively charged electrons; they are well soluble in water, alkali, gas.

Advantages of the Method: eco-adaptivity; exclusion of the danger of gas explosions and rock impacts; use of the state of natural limitation of ore formations with a "lime foundation", significant humidity of the host rocks (up to 40%); the presence of porous tubular-fibrous forms of pyrite formations; the possibility of effective conversion of valuable minerals into a soluble state; providing the cheapest way to increase the content of fulvic acid salts in groundwater.

## **Protective function of humic substances of peloids in the acute intoxication model by polychlorinated biphenyls**

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Despite constant increased attention to pollution problems, its severity does not decrease. The industry has often used substances that are naturally non-decomposing and so dangerous that its production and use are prohibited by the 2001 Stockholm Convention, but they are nevertheless present in the environment. The eco-toxicants of high priority include polychlorinated biphenyls (PCB), which tend to cumulate in humans.



The aim of this work was a research of humic substances of peloid of protective action in model of acute intoxication caused by PCB.

The experiment was made on white outbred rats. Acute intoxication was simulated by single introduction via the probe of PCB mix to the stomach in a dose of 0,1 LD50. To control group of animals (n=6) 0.9% chloride sodium solution was entered. As group of comparison were animals (n=6) to which after PCB intoxication daily entered 0.9% chloride sodium solution. With the medical purpose to animals (n=24) daily subcutaneously entered solutions of peloid drugs on the basis of the humic acids (HA), the hymatomelanic acids (HMA), fulvic acids (FA) and also initial humic substances extract (HFA) emitted from the low-mineralized silt sulfide muds (pH=7.4) by the patented techniques. The experiment was carried out within 10 days.

The therapeutic effectiveness of humic peloid drugs was evaluated by activity of enzymes of aspartate aminotransferase (AST) and alanine aminotransferase (ALT), change of bilirubin content, cholesterol and albumin on the 3rd and 10th day of the experiment in blood serum.

On the 3rd day of the experiment in comparison group under the influence of PCB there was a significant increase in activity of AST and ALT for 27% and for 19.7% respectively in comparison with healthy animals, the content of bilirubin increased by 10 times, cholesterol - by 1.5 times, at the same time the content of albumin decreased by 17%.

The use of peloid drugs has been resulted in smoothing of toxic action of PCB in various degree of intensity: on the 3rd day under the influence of all drugs the AST and ALT level has been sharply decreased; bilirubin content under the influence of HA and HMA decreased in comparison with comparison group more than twice – from 50 mkmol/L to 25 and 21  $\mu\text{mol/L}$  respectively; action of FA and HFA decreased bilirubin slightly – till 31 and 44  $\mu\text{mol/L}$ . Level of cholesterol decreased to physiological norm under the influence of all drugs, and the content of albumin was normalized only under the influence of HMA. On the 10th day all biochemical indicators of animals were normalized under the influence of humic substances.

Protective effect of all studied peloid drugs is established, at the same time the most effective was HMA on extent of influence on activity of enzymes, protein and lipidic metabolism.

## **Estimation of influence of hymatomelanic acids from peloids on pro- and antioxidative systems condition in model of an immune inflammation**

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Keywords: hymatomelanic acids; Freund's adjuvant; superoxide dismutases; malondialdehyde; unsaturated higher fatty acids; catalase

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Alongside with traditional mud cure recently there is popular use various peloidpreparations in a mode of physiotherapy that allows to make treatment accessible to the extensive categories of patients, to make it's dosed out, to increase efficiency.

Therefore, there is actual an allocation and studying of biological activity of separate components of medical muds. Object of our research were peloidpreparations based on hymatomelanic acids (HMA) – alcohol-soluble fractions of humic substances, allocated of low mineralized sludge muds from «Sergiyevsk mineral waters resort» by the patented technique which was developed at the Department of Medical Chemistry. For definition of biological activity white not purebred rats in model of a chronic immune inflammation which was caused by subplantar introduction in the right hinder leg of 0,1 ml full Freund`s adjuvant have been used. On the 14th day after an inflammation induction started to enter HMA as 0,1 % (weight) water solution (pH=7,4). Injections were carried out daily within 10 days (table 1).

Table 1. Changes in blood pro - and antioxidant parameters under the influence of HMA

Enzyme	Indicator	3 day	7 day	12 day
Catalase, nmol/ml × min	M±m	120,24 ± 8,35	112,19 ± 6,44	89,48 ± 7,68
	P	0,47	0,14	0,002
SOD, conl. units.	M±m	2,06 ± 0,10	2,04 ± 0,12	1,87 ± 0,09
	P	0,69	0,6	0,7
Unsaturated higher fatty acids, mol/l	M±m	0,37 ± 0,04	0,91 ± 0,05	0,46 ± 0,07
	P	0,02	0,07	0,09
Malondialdehyde, nmol	M±m	79,83 ± 10,27	70,08 ± 11,76	55,72 ± 6,19
	P	0,0006	0,02	0,02

Functioning pro- and antioxidative systems estimated on activity of catalase and superoxide dismutase, expressiveness of diene conjugation (DC) of the highest nonsaturated fat acids and concentration of malonic dialdehyde (MDA) for 3,7,12 days after the beginning of HMA injection. Under Freund`s adjuvant`s action, superoxide dismutase activity has decreased on 38,30 % - 61,69 % in various terms of supervision while catalase activity has decreased insignificantly. Activity of prooxidative systems, on the contrary, has considerably increased - DC has increased on the average for 60 %, and MDA concentration has increased in 3,5 times. The application of HMA has led to normalization of superoxide dismutase activity for 3 days; MDA concentration has decreased for 35,58 % - 40,68 %. Expressiveness of a DC under action of HMA for the third day has decreased a little bit below an initial level, but by the end of treatment was normalized. Thus, application of peloidpreparations on a basis of hymatomelanic acids promotes normalization of oxidation-reduction systems in an organism at diseases in which basis lay autoimmune processes.

## The relationship of magnetic induction of soil with the content of heavy metals and humus in the soil

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Keywords: magnetic induction, humus, heavy metals, soil

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Magnetic characteristics of soil are closely related to soil properties and can serve as indicators of soil condition in natural and anthropogenic environments. It is known that magnetic properties of soils depend on the content and distribution of certain iron oxides and hydroxides in soils horizons [1]. The profile of sod-podzolic soil is strongly differentiated by the content of magnetic fractions with their decreases in the lower horizons. The predominance of iron compounds in the upper horizons can be explained by the action of organic matter: humus fixes particles containing iron in the soil and prevents them from leaching to the lower horizons.

The purpose of our study was to identify the relationships of the magnetic field induction with the functional soil parameters responsible for the soil quality in the urban ecosystem. This allows low-cost tracking of soil degradation and changes in soil quality under anthropogenic influence.

The induction of the soil magnetic field (TI) was studied in situ using samples of urban-podzolic soil (UP) on fluvioglacial deposits and urban-sod-carbonate podzolic soil (USCP) on fluvioglacial deposits taken in the park of the Novokosino district (the Eastern Administrative District of Moscow) in 2019.

The dependence of the intensity of magnetic induction, on the content of iron, cobalt, nickel, copper, zinc, lead, and the total content of heavy metals (HMe), as well as humus content in the soil layers of the test areas was studied.

Studies of magnetic soil properties were carried out using the author's device and created programs for studying magnetic induction of soils [2]. Analytical studies were conducted in the Testing Laboratory Center (ILC) of the V. V. Dokuchaev Soil Institute.

Large values of magnetic field induction in the layers of the soil profile of UP soil (from 60 to 63  $\mu\text{TI}$ ) were determined in comparison with the values of magnetic field induction in the soil layers of USCP soil (from 59.29 to 58.28  $\mu\text{TI}$ ). The total content of soil HMe was maximum in USCP soil at lower values of the magnetic induction (57.8 - 60  $\mu\text{TI}$ ), while HMe were not detected in these soils at high values of the magnetic field induction. The decrease in the total content of HMe (including iron) with depth was revealed in USCP soil. A positive correlation of the content of heavy metals: iron, copper, lead, nickel, cadmium with the content of humus was determined.

A nonlinear dependence of the magnetic field induction on the humus content was found in the range from 57.8 to 63  $\mu\text{TI}$  for samples of both soils (UP and USCP soils) with extreme regions of the magnetic induction value of about 60  $\mu\text{TI}$  and humus content of 7.75 % and 7.73%.

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## **Antiradical activity of compositions with antimicrobial and wound-healing properties based on humic substances in the cell culture *in vitro***

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Keywords: humic substances, silver nanoparticles, antiradical activity, prooxidants

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Oxidative stress is one of the main reasons of disruption in the process of wound healing, especially if a high level of reactive oxygen species (ROS) persists for a long time. Therefore, investigation of antiradical activity (ARA) for new wound healing substances is a relevant task. The synthesis of nanoparticles containing biologically active silver ultradispersed in the matrices of humic substances and their derivatives (HS-AgNPs) was carried out in the laboratory of natural humic systems (Medicinal Chemistry and Fine Organic Synthesis Division, Lomonosov Moscow State University).

The aim of the current study was to investigate the effect of the initial humic matrices (HS), as well as HS-AgNPs, on the basal and prooxidant-stimulated production of ROS in 3T3-L1 and HepG2 cells *in vitro*.

Intracellular ROS production was assessed using a 2,7-dichlorofluorescein diacetate (DCFDA) fluorescent probe (final concentration 5  $\mu$ M) as described [1]. Briefly, hydrogen peroxide at a final concentration 100  $\mu$ M was used to stimulate the production of ROS. After 60 minutes of incubation fluorescence was determined in the wells of the culture plate ( $\lambda_{\text{ex}}$ =485 nm,  $\lambda_{\text{em}}$ =530 nm) using a Tecan® Infinite 200 PRO multifunctional microplate reader (Tecan, Switzerland).

To identify the significance of differences, one-way ANOVA was used. The differences were considered significant at  $p < 0.05$ .

As a result, it was found that studied HS and HS-AgNPs have an ability to reduce the ROS level stimulated by hydrogen peroxide *in vitro*. Prooxidant properties were not shown for any sample of HS and HS-AgNPs. In general, all samples demonstrated a significantly greater effect on the stimulated ROS level compared with the basal one. The studied samples of HS-AgNPs have a more pronounced ARA in comparison with the initial HS.

Depending on the decrease in their ARA (basal and stimulated ROS levels), all studied HS samples can be arranged in the following row: FA  $\rightarrow$  CHP-K  $\rightarrow$  CHE-K  $\rightarrow$  CHS-K  $\rightarrow$  PHF-T3  $\rightarrow$  Peat1  $\rightarrow$  Peat2. When constructing a similar row for the studied HS-AgNPs, the following sequence was obtained: FA-AgNPs  $\rightarrow$  CHP-AgNPs  $\rightarrow$  CHS-AgNPs  $\rightarrow$  CHE-AgNPs  $\rightarrow$  PHF-T3-AgNPs  $\rightarrow$  Peat2-AgNPs  $\rightarrow$  Peat1-AgNPs.

FA sample has the most pronounced ARA (stimulated level of ROS was decreased in 3T3-L1 by 27.9% ( $p < 0.05$ ), in HepG2 - by 32.1% ( $p < 0.05$ )). FA-AgNPs reduces the basal ROS level in the 3T3-L1 by 20.5% ( $p < 0.05$ ), in HepG2 - by 43.3% ( $p < 0.05$ ), the stimulated level of ROS in 3T3-L1 - by 48.7% ( $p < 0.05$ ), in HepG2 - by 55.9% ( $p < 0.05$ )).

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## Energy consumption of mechanochemical equipment being used for treatment of humic acids sources: brown coal and peat

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Mechanochemical treatment of brown coal and peat is widely used in industry and is under investigation for decades. Mechanical treatment of brown coal with sodium percarbonate allows one to oxidize its organic matter, which leads to the increasing of the yield of humic acids and the content of oxygen-containing moieties [1, 2].

For mechanochemical processing of brown coal and peat, mechanochemical equipment with a shock-shear type of action is most often used: planetary ball mills (laboratory scale), centrifugal roller and vibrocentrifugal mills (industrial scale). It is known that for all types of mechanochemical equipment a significant part of the energy is spent directly on the movement of grinding bodies and turned into heat, and only a small part is spent directly on the mechanochemical process. In this work we search for a possibility for energy efficiency improvement by the example of laboratory planetary ball mill AGO-2 and semi-industrial centrifugal roller mill RM-50.

The obtained for brown coal and planetary ball mill data is summarized in Table 1. The effect of mechanochemical treatment is clearly seen: the yield of water-soluble humic acid increased more than ten times for all samples. Variation of ball/reagent loading ratio have proved to be a useful tool for energy efficiency optimization for mechanochemical-assisted extraction of humic acids from brown coal. Rational selection of conditions for mechanochemical treatment allows near the same increasing of the yield of humic acids, with the half of the specific energy consumption.

Table 1. Characterization of samples treated with different ball/reagent mass ratio

Treatment mode, ball/reagent ratio	E, W*h/g	HA yield, %	EPR, a.u.
Init. coal (Itatskoe dep.)		< 1%	592.9
200/10	23.0	12 ± 3	696.3
200/20	11.5	14 ± 2	670.0
175/10	22.0	15 ± 4	611.0
150/10	21.0	21 ± 3	785.1
150/20	10.5	15 ± 2	547.8
125/10	19.6	20 ± 3	721.5
100/10	17.9	19 ± 1	742.6

(E, W\*h/g – specific energy consumption, HA yield – the yield of water-soluble humic acids, EPR – amount of paramagnetic centers, a.u.)

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## **Analysis of fulvic acid – protein complexes by radiochemical and spectroscopic techniques**

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**Keywords:** Biopolymers; Fulvic acids, Lysozyme, Albumin Co-adsorption; Enzymatic activity

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In the present work the interaction between fulvic acid separated from Suwannee River (SRFA) that is IHSS standard and two model globular proteins (lysozyme and albumin) was studied at the liquid-liquid interface as well as in the bulk of an aqueous solution. We used tritium labeled compounds, obtained by means of tritium thermal activation method, for determination direct amount of both SRFA and protein at the aqueous-p-xylene interface and in the solid precipitate. Note that p-xylene was used as model of cellular membrane. Aqueous-air interface was characterized by tensiometry.

It was found that both lysozyme and albumin substitute SRFA from both liquid-liquid and aqueous-air interfaces. Moreover, lysozyme also formed a precipitate with SRFA. Using tritium labeled compounds it was found that molar ratios SRFA:lysozyme in the adsorption layer and in the precipitate were similar, when SRFA concentration was lower than 20 mg/L. At high SRFA concentrations molar ratios SRFA:lysozyme reached 3:1 in the adsorption layer and 2:1 in the solid phase. However, when the mass ratio was higher than 1:2 (SRFA:albumin) albumin loses its secondary structure, while lysozyme preserves its structure as well as an enzymatic activity even increases it being encapsulated by SRFA.

To analyze the interaction in the bulk of an aqueous phase the fluorescent spectra of proteins as well as its mixtures with SRFA was recorded in the range from 290 to 540 nm at excitation wavelength 280 nm to determine the intrinsic fluorescence of tryptophan. Tryptophan fluorescence quenching in the presence of SRFA indicates the interaction between SRFA and both proteins. Formation of complex between albumin and natural organic matter via hydrogen and van der Waals bonds results in the reduction of  $\alpha$ -helix of protein, while lysozyme has more stable structure that is stabilized by disulfide bridges. We can suggest that lysozyme preserves its structure being encapsulated by SRFA. Therefore, preservation of the enzymatic activity can be expected.

Enzymatic activity of lysozyme was measured in the relationship to *Micrococcus luteus* cells by means of turbidimetric assay. It was found that SRFA preserves and even increases the enzymatic activity of lysozyme. When SRFA concentration is about 10-20 mg/L, the enzymatic activity reaches the highest value and decreases at SRFA concentration of 50 mg/L, remaining one and a half times that for lysozyme in the absence

of fulvic acid. Note that Trp108 is in van der Waals contact with Glu35, one of two catalytic carboxyl groups and hydrophobization of Trp108 enhances the substrate binding ability of lysozyme. The decrease in activity at SRFA concentration of 50 mg/L is probably due to the steric hindrance of the interaction between the enzyme and the substrate because of the possible adsorption of free SRFA on the cell surface. Although, it is important to emphasize that free SRFA did not lead to *Micrococcus luteus* cells lysis.

Therefore, our results suggest that interaction of SRFA with lysozyme does not lead to significant changes in the protein structure as compared with albumin that probably lose its secondary structure being involved in the complex with SRFA.

## **Trace elements (Cu, Zn, Ni, Pb) in humic acids of Western Transbaikalia soils**

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Keywords: trace elements, gray forest soil, chernozem, chestnut soil, quasi-clay chernozem, Western Transbaikalia

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The studied elements are involved in all soil biochemical processes of accumulation, transformation, transfer of substances and energy in the biosphere. Minerals concentrators of the elements determine their amount in parent material. Humic substances in soil are both the initial and the final link in their cycle.

The aim of the research was to determine to what extent humic acids (HA) bind biophilic elements such as Cu and Zn, conditionally biophilic Ni, as well as toxic to plants Pb, in steppe (chernozem and chestnut) and forest-steppe (gray forest and quasi-gley chernozem) soils of the region.

The parent material in steppe landscapes is proluvial-deluvial sandy loam deposits, and in forest-steppe it is proluvial-deluvial-alluvial loamy deposits. The basic minerals are granitoids, primary minerals such as feldspar and mica.

Plant samples were selected by mowing and taking monoliths, where trace elements were determined. The HA was selected according to Grimm. Trace elements were determined with the help of atomic absorption spectrophotometer.

Copper belongs to the class of strong complexing agents and is fixed in the soil in the form of strong organic chelates (Vozbutskaya, 1964; Orlov et al., 1991). Copper bound by organic matter is difficult to wash out; therefore, its availability for plants is significantly reduced (Peive, 1980). The amount of copper in steppe soil was found as 13.2-22.8 mg/kg, in forest-steppe as 14.5-23.1. It is significantly lower than MPC rate as the parent material lacks it badly;  $22.6 \pm 7.61$  was on chernozem and  $10.5 \pm 1.28$  on chestnut. HA traps copper in steppe soil in the range of 1.43-1.47 mg/kg, and in forest-steppe it does slightly more – 2.1-2.8 mg/kg.

In steppe soil there is 38.5-73.0 zinc, chernozem contains  $68.4 \pm 30.74$  zinc, and chestnut soil contains  $31.3 \pm 5.93$  mg zinc. Zinc is accumulated in the HA of steppe soil as much as 6.3-14.7, while in forest-steppe soil the amount of the element is almost 7-10 times higher. The latter is explained by the similarity of zinc and calcium chemical

properties as of the elements of the same (second) group. Apparently, with insufficient calcium content HA is additionally saturated with zinc, especially in gray forest soil.

Steppe soil contains nickel in significantly lower amount than MPC (24.9-34.4 mg /kg, while forest-steppe soil contains even less nickel (16.6-17.4), although its content in parent material is much higher (41.8-62.6 mg). In the HA of both studied soils it is found in equally small amounts: 5.24 and 5.3 - 5.5 mg.

Lead, toxic to plants heavy metal is found in soil in quantities higher than its MPC (20). In steppe soil its content is on average 32.2 mg, in parent material it is on average 27.9, with Clark 10 mg. Lead content in forest-steppe soil is on average 20.9: a) with 12.5 in quasi-clay chernozem; b) with 10.2 in gray forest soil. Thus, we can observe aerogenic pollution on these soils.

Thus, soil humic acids are able to bind a significant amount of heavy metals, which depends on the quantitative and qualitative composition of humus, soil conditions, and the chemical properties of the metals. Humic acids bind an insignificant amount of trace elements from soils, even despite their high content in soils and underlying rocks, which is apparently due to their evolutionarily selective ability, as well as due to individual properties of each specific element.

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## **Humic substances as a natural biotic adaptogen**

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Keywords: adaptogenic activity of humic substances, free radicals, physiological activity, bioprotective properties.

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The mechanism of the adaptive effect of humic substances (HS) on plants and biota was formed and tuned over millions of years of the bio- and humosphere joint evolution. HS significantly increase the adaptation (nonspecific resistance) of plants and animals to chemical and climatic stresses and other unfavorable environmental conditions.

Nowadays, the adaptive properties of HS are used in plant growing, animal husbandry and various ecoprotective technologies, as well as in medicine and veterinary medicine. In plant growing and animal husbandry, using preparations based on HS, it is possible to achieve an increase in yield and weight gain up to 20%.

We record this phenomenon in the form of physiological and bioprotective activity of HS in relation to biota, including animals and humans. The phenomenon of physiological activity of HS was studied from different angles and at different levels of biota organization - from plant tissues (coleoptiles) to unicellular and more complexly organized plant and animal organisms, including animals and humans. But the mechanism of the biological action of HS on living organisms is still unclear. On the one hand, this is due to the variability and polydispersity of the HS structure. In other hand, this is due to biodiversity and multifactorial physiological reactions of living organisms to the effect of HS preparations in laboratory experiments.



The hypothesis of membranotropic activity assumes that HS are adsorbed on the membranes of living cells (root hairs, unicellular organisms, etc.) and activate the entry of mineral nutrients and water into cells. In addition, HS may act through membrane receptors on the expression or depression of certain regions of the genome of living organisms, performing a trigger function. Perhaps this explains the adaptive and bioprotective activity of HS.

In our studies, it was possible to trace the relationship between physiological activity and the concentration of HS free radicals. This relationship is consistently traced at different levels of organization of plant organisms - on isolated plant tissues (coleoptiles), and on whole unicellular and multicellular organisms.

HS of virgin soils favorably affected the growth of corn coleoptiles and metabolic processes in unicellular organisms. HS of arable and buried soils "worked" much worse. Moreover, HS of buried soils, which have the lowest content of free radicals, weakly stimulated photosynthesis and sharply increased the oxygen consumption by cells. In this case, this can be considered as a stress reaction of the plant to the action of these HS. 'This indicates a decrease in the physiological activity and adaptive potential of soil HS under anthropogenic impact.

In experiments with pollution of the environment with heavy metals, it was found that HS stimulate the internal nonspecific adaptive mechanisms of plants, which allow them to overcome the negative effect of chemical stress. The results obtained allow us to put forward a generalizing hypothesis of the systemic response of a living organism to the effect of HS, depending on their molecular structure, which will allow us to take a fresh look at their adaptive role in the soil – plant system.

## **Organic matter of Haplic Chernozem under contrast land use: chemical vs physical fractions (<sup>13</sup>C CP/MAS NMR comparative study)**

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Keywords: soil organic matter, humic acids, fulvic acids, humin, granulodensitometric fractions, <sup>13</sup>C CP/MAS NMR

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Despite a flood of reports on the use of modern high-resolution methods to investigate soil organic matter (OM) nearly in situ, various chemical and physical fractionations (and their combinations) coupled with physico-chemical methods of analysis remain the most common. Fractionation makes it possible to isolate more homogeneous preparations by their solubility in acids and alkalis (the classic version of humic substances analysis), or by density, or by density and particle size both, i.e. by degree of association with soil minerals (densitometric and granulodensitometric methods). The parallel use of these two approaches to the same soils provides complementary data, but such studies are scarce. Here we aimed to investigate the relationship between the distributions of OM components in chemical and physical fractions by comparing the contributions to the soil TOC, hydrolyzability and structural features of humic acids (HA), humin, and fraction "acid

soluble OM + fulvic acids (FA)", separated by alkaline extraction with the same characteristics of the OM of granulodensitometric fractions: light fraction occluded inside the aggregates (LF<sub>occ</sub>), clay (Clay) and residue after separation of light fraction and clay (Res). Haplic Chernozem of two contrasting land use were chosen for the study: steppe and long-term permanent bare fallow. The variants are characterized by maximum differences in C content. Due to the territorial proximity and high spatial homogeneity of the Chernozem of the study area, the virgin soil can be considered as the initial/native soil condition for the fallow one. The study of long-term bare fallow allows the properties of the stable OM prevailing in it to be investigated and the direction of degradation changes in the OM structure (transformation of mature OM) to be identified separating them from the transformation processes of fresh and/or intermediate OM. The results of the study revealed that for the virgin soil the proximity of the OM structure of the following pairs of fractions is observed: HA - LF<sub>occ</sub>; "acid-soluble fraction + FA" - Clay; humin – Res. For OM of the indicated pairs of chemical and physical fractions, the contributions to C<sub>org</sub> and C/N ratios are also close. For the fallow soil sample the structural composition of OM of the above chemical and physical fractions is also close, but their contributions to C<sub>org</sub> and C/N value are notably different. A comparison of C content of the chemical fractions of the two studied soils showed that all fractions are lost during soil degradation, but the greatest losses are observed for humin. At the same time LF<sub>occ</sub> is most wasted among the granulodensitometric fractions. Thus, there is a preferential association of the chemical fractions in concern with the corresponding granulodensitometric ones in undisturbed steppe soil, whereas in degraded soil of long term bare fallow, whose OM is distinctively more hydrophobic and aromatic, this distribution is disturbed.

## **Humic acids use to reduce the tailing dump toxicity in Primorsky region, Russia**

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Keywords: tailing dumps, toxic elements, humic acids, methods of use, doses, Primorsky region, Russia

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Samples from four toxic tailing dumps in the Primorsky region were used to assess the humic acids (HA) ability to bind toxic elements and to determine the optimal methods for their application and doses. The possibility of improving the conditions for the plants growth (using oats as an example) on the tailing dump upper layer under the humic acids action isolated from low-lying peat was studied. In laboratory experiments the possibility of binding toxic elements of HA, a decrease in the general toxicity of tailing dumps, as well as the efficiency of HA use for plant growth were determined. We used both aqueous solutions of HA preparations when watering planted seeds, and adding their dry powder

into the surface layer before or after planting plants. The toxic elements were determined by the XRF-SR (storage ring VEPP-3, BINP SB RAS).

The laboratory experiment had six options for growing oats on dump samples: I - non-germinated seeds (control 1, without HA); II – the seeds with sprouted roots (control 2, without HA); III – the seeds with sprouted roots and watering with a solution with a HA concentration of 0.01%; IV - the same, with a HA concentration of 0.005%; V – the seeds with sprouted roots with the addition of HA dry powder to the dump after planting oats; VI - the same before planting oats.

It was revealed that the surface layer of tailings, among a wide range of microelements, contains a number of highly toxic elements, such as arsenic, copper, nickel, cobalt, cadmium, the concentration of which exceeds the permissible norms by 1-3 orders of magnitude. Only an insignificant part of the pollutant total pool can be inhibited by HAs located directly in the sediments. Without exception, all HA from peat, introduced into the tailing dump sediments, increased their saturation with toxic elements several times (from 1.5 to 5 and more times). The duration of exposure to HA had a directional effect on the amount of sorbed elements in the case of chromium, nickel, copper, and cobalt, but did not have a clear direction in the case of arsenic, zinc, and molybdenum. However, in both cases, the concentration of all elements increased significantly.

When HA interact in the form of different concentration solutions with tailings sediments, no pronounced relationship between the content of bound toxic elements and the concentration of HA solutions was revealed for the options considered.

It was shown that the toxic effect on oat plants when added to the surface layer of HA in the form of a powder was significantly reduced in both variants (5 and 6) even after exposure for 7 days; however, the best effect was obtained by adding HA in the form of a dry powder before planting hatched oat seeds (Variant 6).

Our study confirmed the perspective for the development of the direction associated with the use of HA, obtained from the lowland peat soils, to enhance the protective functions of tailings dumps, which leads to a decrease of their toxicity and increase the positive effect on the plant growing.

## **Synthesis of new O-alkyl and O-acyl derivatives of peat**

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Peat is a unique natural composite, a source of humic substances and raw materials for industry and agriculture. The aim of this work is to develop a new acylated thermoplastic binder from peat to study the possibility of its application in the industry of plate materials [1]. The influence of duration mechanochemical treatment of peat on the process of benzylolation under the action of benzyl chloride in the presence of NaOH (tabl. 1) [2]. The esterification of peat was carried out in the processing of lowland peat acetic anhydride by mechanochemical method with different molar ratio of reagents from 1:1:0.5

to 1:1:2.5 for 30 min at 25 °C. The chemical composition of products of esterification of peat given in the table 2.

Table 1. Benzylation of peat

Duration benzylation of peat, min	Content of benzyl groups, %	Solubility in chloroform, %
10	3.8	17
20	4.7	25
30	5.9	36
40	6.8	45
50	7.7	53
60	8.6	60

It is shown that the increase in the duration benzylation of peat is the increase in the content and solubility products in chloroform (tabl. 1).

Table 2. The influence of the molar ratio of OH:Ac<sub>2</sub>O in the acetylation of peat in conditions of intensive mechanical grinding on the properties of the obtained products\*

Sample	Mole ratio OH:Ac <sub>2</sub> O	Content of acetyl groups, %	Solubility in acetone, %	Conversion OH-groups, %
Peat	-	-	5	-
1	1:0,5	10.5	20	42
2	1:1	11.7	30	47
3	1:2	15.2	47	61
4	1:3	17.8	60	71

\*the mass of peat is 10.0 g, the temperature of the acetylation – 50 °C, the duration of the acetylation – 60 min.

It was found that with an increase in the molar ratio there is an increase in the content of bound acetyl groups in products and their solubility in acetone (tabl. 2).

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## Suppression of methane generation during methanogenesis by humic substances modified with Fe(III)

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Keywords: methanogenesis, humic substances, Fe(III)

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Global warming can cause a significant increase in methane emissions from vast wetlands into the atmosphere as a result of melting permafrost. However, it was shown that presence of humic substances (HS) and Fe<sup>3+</sup> in these territories can inhibit the release of CH<sub>4</sub>. This effect is associated with HS and Fe (III) used by anaerobic methanogenic microorganisms as a terminal electron acceptor [1]. However, it was revealed that the donor/acceptor capacity of HS, extracted from various sources, varies greatly due to differences in their chemical composition [2]. When studying the efficiency of biogas accumulation under the action of natural anaerobic consortia in the presence of different HS, it was found that fulvic acids samples introduced into the nutrient medium at a concentration up to 10 g/L stimulated the accumulation of biogas production, but 10 g/L of potassium humate modified with naphthoquinone led to reduction of CH<sub>4</sub> synthesis [3].

The introduction of various concentrations of chemically modified humic compounds (HS) with Fe(III) into the media with free and immobilized anaerobic consortia accumulating landfill gases was studied. The following composite materials were used: Fe(III) hydroxide fulvate, Fe(III) hydroxide 1,4-naphthoquinone potassium humate, Fe(III) hydroxide potassium humate.

The maximum decrease (up to 40%) of the methanogenic parameters (the efficiency of biogas production and the proportion of methane in biogas composition) under the action of anaerobic cell consortia being in suspended and in immobilized form, was observed when 1,4-naphthoquinone potassium humate enriched with Fe(III) hydroxide was used. The introduction of Fe(III) hydroxide fulvate led to a slight decrease in the amount of accumulated biogas and the percent of CH<sub>4</sub> in it. At the same time, the yield of biogas accumulated under the action of immobilized cells of the anaerobic consortium at the same concentrations of composite HS with Fe(III) was 5-20% higher than the yield of biogas accumulated under the action of suspended cells. In general, the additional enrichment of HS with Fe (III) did not lead to a significant decrease in the efficiency of methanogenesis in comparison with native HS. This may be due to the fact that the additional enrichment of HS by Fe(III) leads to a decrease in the acceptor properties of HS by formation of a stable complex with Fe(III). In this regard, it may be necessary to use other combinations of substances with HS to enhance the suppression of CH<sub>4</sub> synthesis.

The obtained results constitute important information that can be taken into account in the further development of scientifically grounded approaches to effective and directionally controlled suppression of gas emissions at real landfills.

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## **Molecular composition of thermal fractions of organic matter of typical chernozems under different land uses**

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Keywords: analytical pyrolysis, PY-GC/MS

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Chernozems are the most fertile soils in the world, so they are actively used in agriculture. The land use has a noticeable effect on the transformation processes of soil organic matter (SOM) and directly affects its molecular composition. Analytical pyrolysis is one of the methods for evaluating the composition of SOM. The aim of the study was to evaluate the molecular composition of thermal fractions of typical chernozems of different land uses. The objects of the study were typical chernozems of long-term field experiments of various land uses, including undisturbed variants, variants at the stage of restoring stocks of organic matter, as well as arable soils. Double shot pyrolysis with gas chromatography and mass spectrometry was used to evaluate the molecular composition.

Double shot pyrolysis of typical chernozems of different types of land use allowed us to divide the variants of chernozems according to the degree of transformation of the SOM. It is shown that the molecular composition of the thermolabile SOM of undisturbed variants of typical chernozems is characterized by a high proportion of lignin derivatives and long-chain alkanes, and for arable soils, short-chain alkanes, nitrogen-containing compounds, and some pyrolyzates of polysaccharides (furfural and methylfurfural). The molecular composition of the thermally stable SOM of chernozems is characterized by a high content of components of a cyclic structure, the largest proportion of which was observed for arable soils, acyclic hydrocarbons were characteristic of undisturbed soils.

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## **Protective function of the humic preparation in relation to the antifungal activity of nystatin**

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Keywords: humic substances, nystatin, antifungal activity, soil filamentous fungi

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Fungicides of various actions are widely used to struggle with pathogenic fungi. The advantages of polyene antibiotics, which include nystatin, are a wide spectrum of activity against pathogenic filamentous fungi and effectiveness against ergosterol-containing fungi [1]. Fungicidal activity along with protective properties is also characteristic of humic

preparations (HPs) [2]. The experimental work was devoted to the study of the effect of HP (synergistic or antagonistic) on the antifungal activity of nystatin.

The strains of *Alternaria alternata*, *Ulocladium septosporum*, *Acremonium roseolum*, *Geomyces pannorum*, *Penicillium citreonigrum*, *Purpureocillium lilacinum*, *Trichoderma viridae* were used. We measured the fungal growth endpoints: the accumulation of mycelium biomass, the colony growth, and the conidia production. Fungi were cultivated on Chapek medium (C). Nystatin was added at two concentrations: 0.05 g/L (N1) and 0.01 g/L (N2). HP (potassium humate "Energy") was added at 0.2 g/L (HP). We created the variants of combined action (N1+HP and N2+HP).

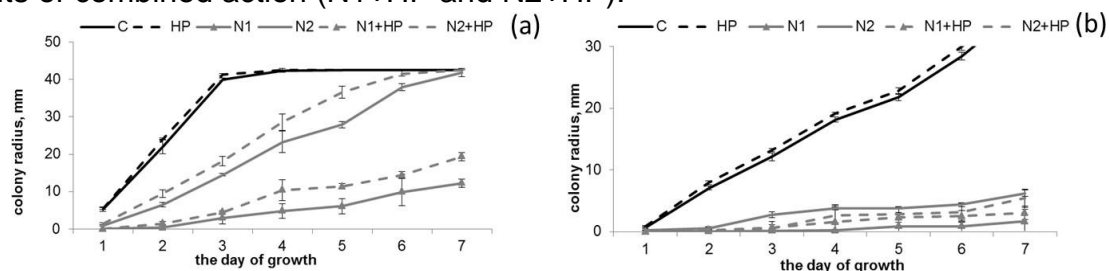


Figure 1. Colony radius of a) *T. viridae*; b) *A. alternata* (explanations are in the text)

The addition of HP had a stimulating effect on the fungal growth endpoints (a significant increase in the colony radius, conidia production, and biomass accumulation were noted). At the same time, the positive effect of HP was more noticeable on the background of the introduction of nystatin, which inhibited the growth of fungi. Two species *P. lilacinum* and *G. pannorum* proved to be immune to either nystatin or HP, either separately or together in the medium. Thus, in the experiment, we revealed the protective function of HP, which reduces the susceptibility of soil filamentous fungi to nystatin.

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## **Geostatistic analysis of humus stocks in fallow soils taking into account spatial heterogeneity**

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Keywords: fallow soils, spatial heterogeneity of humus stocks, geostatistics

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The danger of global climate change on the Earth associated with the accumulation of greenhouse gases in the atmosphere is one of the most serious challenges of our time. According to Batjes (1996), soils contain about 1500 Pg organic carbon. This is about three times the amount of carbon in the Earth's vegetation cover and twice the amount of carbon in the atmosphere. Naturally, an increase in the content of soil organic matter (SOM) is considered one of the most important potential sinks of atmospheric carbon in terms of preventing global climate change. Since the removal of land from arable turnover is a modern trend in the development of agricultural production in the world, including Russia, the accumulation of SOM under fallow vegetation can be considered as a very significant sink of CO<sub>2</sub> from the atmosphere. At the same time, a realistic assessment of the stocks of accumulated humus in fallow soils can hardly be given without an assessment of their spatial heterogeneity. The purpose of the work is to assess the total and accumulated reserves of SOM using interpolated maps of spatial heterogeneity of soil properties. The purpose of the work is to assess the total and accumulated stocks of SOM using interpolated maps of spatial heterogeneity of soil properties.

We studied two fallow areas (15-20 years old) under different fallow vegetation (birch forest and meadow grassland overgrown with forest). Samples were taken using a stratified randomized design in layers from the entire depth of the old arable horizon. The humus content and the bulk density were determined in the samples. For each site, three maps of heterogeneity of soil properties (the content of SOM in each layer, bulk density, and thickness of the old arable horizon) were constructed using various interpolation methods. These maps were used to create a map of total SOM stocks in the old arable horizon, as well as a map of accumulated stocks by the difference in humus content in the upper and lower parts of the old arable horizon. Assessment of SOM stocks in the old arable layer in territories occupying from several tens to hundreds of hectares is characterized by strong spatial heterogeneity, and in some areas, these stocks differ by more than two times. It was shown that the accumulated stocks of humus under the fallow of 15-20 years old form more than 20% of the total humus stock in the old-arable horizon. Studies have shown that the type of fallow vegetation (deciduous forest and meadow vegetation) had an insignificant effect on the accumulated stocks of humus. The qualitative composition of SOM accumulated under fallow vegetation was studied using various spectroscopic, analytical, and incubation methods. It was shown that the organic matter newly formed in the old arable horizon is represented by labile (unstable) fractions that are easily subject to oxidation and microbial decomposition.

The area of fallow lands in modern Russia, according to various estimates, ranges from 20 to 40 million hectares. It is obvious that an unregulated return to arable turnover of such lands can lead to an abrupt emission of CO<sub>2</sub> into the Earth's atmosphere due to the mineralization of SOM accumulated under fallow vegetation over the past decades.

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## Statics and kinetics of dissolution of tricalcium phosphate in a membrane mini-reactor (MR) in the presence of humic acids

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Keywords: tricalcium phosphate, solubility, kinetics, membrane mini-reactor, humic acid.

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The problem of increasing the efficiency of mineral phosphate fertilizers is largely determined by the physicochemical processes of the interaction of molecular-soluble forms of their dissolution products with inorganic constituents of soils. To study the processes

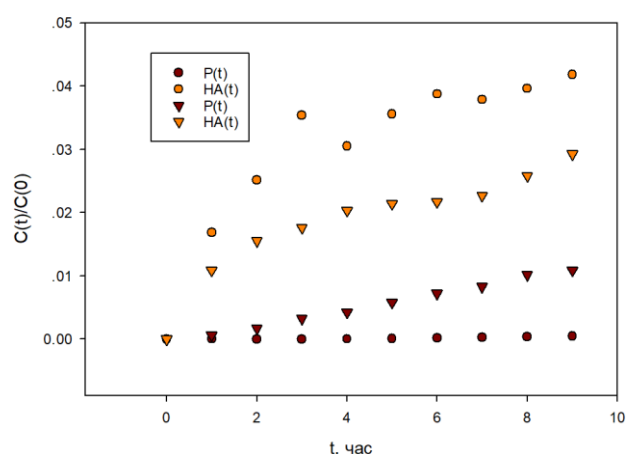


Fig. 1. Kinetics of dissolution of TCP and HA components from MR into a 0.1 M NaNO<sub>3</sub>, 22°C solution. Circles - TCP and HA - in one MR; triangles - TCP and HA - in different MRs.

with the participation of soluble phosphates and oxide phases, we experimentally analyzed the heterogeneous equilibria and the kinetics of dissolution of the most sparingly soluble calcium phosphate -  $\beta$ -modification of tricalcium phosphate (TCP) in the background electrolyte, depending on the pH of the solution and the concentration of humic acids in it. The theoretical analysis of equilibria was carried out using the HSC Chemistry and MathCad programs. Experimental studies were carried out by the limited

volume method using the membrane mini-reactor technique [1]. This made it possible to observe the mass transfer of  $\text{Ca}^{2+}$ ,  $\text{PO}_4^{2-}$ ,  $\text{OH}^-$  ions into an external electrolyte solution (0.1 M NaNO<sub>3</sub>) from the solid phase of TCP, placed inside one or two different mini-reactors (MR), the walls of which were a semipermeable membrane made of a lavsan track membrane with pore size 0.1 or 1.0  $\mu\text{m}$ , or it was found that the joint presence of TCP and HA in the volume of one MR reduces the rate of dissolution of phosphate, but does not affect the rate of dissolution of solid HA, Fig.1. The reason for this is the large difference in the solubility of HA and TCP. If TCP and HA are in separate MRs, the accumulation of HA in solution decreases due to dissolution as well as the rate of dissolution of TCP. The presence of the  $\text{PO}_4$ -ion in the solution reduces the rate of dissolution of solid HA. The report discusses the problems of controlling the solubility of phosphates in soil, of using them as sorbents for removing strontium radionuclides using the MR membrane technique.

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## **Biogeosystem Technique methodology for biological reversible carbon sequestration and priority humic substances functioning in soil**

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Keywords: Biogeosystem Technique, carbon sequestration, climate system, humic substances

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Humic substances functioning in the "environment – soil – soil organic matter – soil mineral matter – technical means and technologies of agronomy, land reclamation, and waste utilization" interfaces is linked to the current conflict "Ecosphere – Technology". Copying the Nature fragmentarily in the current outdated imitative land use technological platform leads to the climate and biosphere uncertainty, soil degradation, water scarcity, and unsafe waste management. These limits the humic substances soil improving and fertilizing function.

To overcome the fundamental shortcomings of current industrial technological platform we propose a new field of science and technology as a platform for the world strategic technological development in the Noosphere – Biogeosystem Technique (BGT\*). BGT\* transcendental (uncommon for Nature) approach allows to face the large-scale calls: sustainable evolution of healthy soil, water saving, high soil fertility, high quality environment to reduce current "organic matter – mineral – water" interfaces elusiveness and stabilize the Climate system and Biosphere. BGT\* provides environmentally and economically sound interdisciplinary institutional innovative integrated solutions. BGT\* increases the Earth biogeochemical flux and biogeosystem's stability.

BGT\* provides:

Micro-, mezzo-, and macro-aggregate multilevel stable architecture and improved structure of the soil illuvial horizon via intra-soil milling of the 20–50 cm. The high rate long-term development of the rhizosphere and soil biome ensures soil fertility and better plant organogenesis.

For 5–20 times reduced fresh water consumption and higher rate plant growth via intra-soil pulse continuously-discrete watering. The soil solution matrix potential is provided circa –0.2 MPa to –0.4 MPa. The stomatal apparatus of plant operates in a regulation mode. Plant transpiration is reduced. Higher soil solution concentration provides higher rate plant organogenesis and productivity. This decreases off-target transport of nutrients, pesticides, organic matter.

Environmentally safe dispersed municipal, industrial, biological (including agricultural, slaughterhouse, hazardous) waste and gasification byproduct recycling for soil structuring and plant nutrition via intra-soil milling and/or intra-soil pulse continuous-discrete watering (chemigation).

BGT\* enhances soil health and high productivity, priority humic substances functioning as an agent of the soil organic matter synthesis, and plant resistance to stress

and phytopathogens. The abundant biogeochemical cycle ensures expanded biological reversible carbon sequestration in the soil and in the additional biological product. Higher biological productivity provides Biosphere and Climate stability.

## **Determination of lignosulfonates and humic components in humate fertilizers by ATR-FTIR-spectroscopy**

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Keywords: lignosulfonates, humate, fertilizers, FTIR-spectroscopy

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Currently, there is a problem of controlling the production of fertilizers based on humic substances (HSs) since the properties of the final product depend on the HS source and the characteristics of the technological process. The content of additional components, including lignosulfonates (LSs), is also not controlled. Since humus is formed during the lignin decomposition in nature, the structure and properties of HSs and LSs are similar, and, therefore, the methods for determining each component are the same. However, the functions of LSs and HSs in fertilizers differ; therefore, it is necessary to analyze and control each component individually in preparations based on HSs.

IR spectroscopy is already used to characterize the functional group composition of both HSs and LSs. However, since HSs are present in fertilizers in relatively high concentrations, their solutions have a high absorbance and strongly absorb radiation, thus complicating the use of IR spectroscopy in the transmission mode. FTIR spectroscopy allows the analysis of both solid objects and solutions. Therefore, this work aimed to develop a method for directly determining (1) LSs in individual solutions and (2) HSs and LSs in aqueous solutions of fertilizer preparations based on HS by ATR-IR spectroscopy with minimum sample preparation. Several commercial preparations of HS, LS, and fertilizers were investigated. A Bruker Vertex 70 spectrometer with a diamond-crystal ATR attachment was used.

Model mixtures show that without preliminary sample preparation, only qualitative determination of LS in a mixture with humate at the band of  $1266\text{ cm}^{-1}$  is possible, even with a 4-fold excess of humate. To quantify LS in a mixture with humate, it is necessary to first separate silicate impurities in humate by centrifuging the solutions for 30 min at a rate of  $40,000\text{ min}^{-1}$ . Spectral information processing affects the error of determining LS in the mixture. The most accurate results are achieved by processing the band without using a baseline and taking into account the content of the second component in the mixture.

The most accurate determination of LS in the concentration range of 10–100 g/L is possible using 1093 and  $1042\text{ cm}^{-1}$  bands with an error of 0.5% in individual samples and up to 5% in humate mixtures. Determination of humate content in the concentration range of 10–150 g/L by 1570 and  $1383\text{ cm}^{-1}$  bands is possible with an error of up to 10% at HS : LS ratios of 2 : 1 or higher.

Thus, it is possible to simultaneously determine the content of lignosulfonate and humate in their mixtures.

## Organic fertilizer based on basic potassium humate and their prospects

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In modern conditions in Kazakhstan the development of ornamental plant breeding is noted, the need for ornamental plant species has increased, the work with the range of ornamental crops resistant to abiotic and biotic factors is strengthened. The latter includes the use of fertilizers of organic origin "Kazuglegumus" with regard to the needs and biology of a particular species. Research is carried out by scientists of the "Institute of Coal Chemistry and Technology" LLP and Kazakh Agrotechnical University named after S. Seifullin together with producers of the preparation "SPA "Kaztechnougol" LLP during last three years. The preparation was used for treatment of planting material and root treatment during plant vegetation, Kazuglegumus fertilizer was produced by non-thermal method, by cold synthesis method by electrophysical effect, from oxidized coal of Maikuben basin. The base component of the obtained humate contains the following micro and macroelements presented in table 1. The use of humate increases immunity and stress resistance, adaptive properties and enhances the physiological mechanisms of plant protection against harmful organisms.

Table 1. Content of macro and micro elements in "Kazuglegumus"

Name of the preparation	C,%	H,%	N,%	S,%	O,%	K,%	P,%	Mg,%	Humic substances content,%
Kazuglegumus (Potassium humate)	22.21	1.90	3.5	0.68	20.44	9,16	0,01	0,24	56

The objects of the experiment are all groups of ornamental crops: trees, shrubs, perennials and annuals in open ground and plants in closed ground. When growing seedlings of flower crops, for example, zinnia (*Zinnia*): the studied drug was used as a root top dressing to enhance the growth of biomass, obtain a large leaf surface and large buds and a stable stem mass (Fig. 1). With the same planting dates, the seedlings treated with humate in terms of biomass and constitutional parameters were an order of magnitude higher than the control variant.



Figure 1. Growth and development of seedlings of zinnia (*Zinnia*): a) using basic humate; b) control.



Figure 2. a) mandarin plant (*Citrusreticulata*) damaged by the pest mealybug to the loss of leaf surface up to 80%; b) plant restored when feeding with basic humate and diatomite treatment.

Optimum results are shown by the use of basic humate in combination with diatomaceous earth for plant recovery after active damage by a pest, for example, mandarin (*Citrusreticulata*) (Fig. 2). Thus, the basic humate "Kazuglegumus" shows itself as a promising, multifactorial organic preparation for use in agricultural technology and the protection of the previously listed groups of ornamental crops.

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## Nitration of humic acids in water-dioxane or water-ethanol

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The functionalization of natural polymers is a significant trend of targeted changing the properties and expanding the possibilities of their practical application. Among the methods of chemical functionalization, nitration is distinguished. A distinct advantage of nitration is that NO<sub>2</sub> groups increase the polarity of macromolecules, surface activity, and complexing properties. In addition, NO<sub>2</sub> groups can be converted or replaced by other functional groups (halogens, amino, alkoxy, nitrile, etc.) using simple chains of transformations [1].

The research aimed to study the nitration of humic acids (HA) with nitric acid in dioxane, ethanol, and water.

The HA of the high-moor peat were used for the study. Nitration was carried out in a boiling water bath for a specified time (5 ... 60 min). The consumption of nitric acid was 28 mmol / g HA; the solvent was 8 ml / g HA. After the completion of the reaction, the liquid was separated from the insoluble part. The filtrate was evaporated in a vacuum, and the filter residue was washed with water until neutral and dried in a vacuum desiccator. The elemental composition of the nitration products was determined, and electronic ionization spectra were recorded.

It was found that reaction products with a nitrogen content of up to 5.3% are formed during the nitration of humic acids. An absorption band with a maximum at 430 nm, characteristic of nitro compounds, appears in the ionization spectra. A feature of the nitration of HA in an aqueous-dioxane medium is that the reaction proceeds quickly, and HA react to a soluble form (see Fig.).

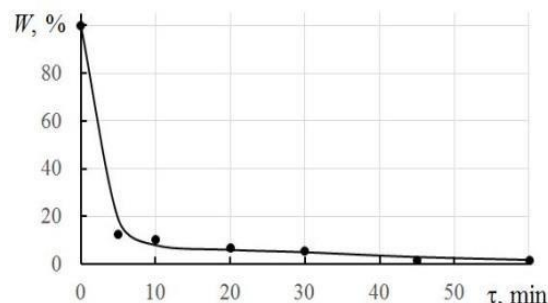


Figure. Dependence of the yield of the insoluble part of HA ( $W$ , % of the mass of HA) on the duration of nitration ( $\tau$ , min) in dioxane.

For example, after 10 min of nitration, the yield of the insoluble part was 10, 45, and 75% using dioxane, alcohol, and water, respectively. Thus, the possibility of obtaining soluble nitrated HA has been shown.

Acknowledgements. The reported study was funded by RFBR according to the research project № 18-05-70087.

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## Large-scale SPE-filtration system based on a use of Bondesil-PPL bulk sorbent for isolation of gram quantities of DOM from marine water

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Keywords: dissolved organic matter, isolation, ultra large filtration, molecular composition

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Dissolved organic matter (DOM) comprises large pool of organic carbon in the World Ocean. Its typical concentrations in the marine environments do not exceed 5 mgC/L. The most widespread approach to DOM isolation is solid phase extraction (SPE) using pre-packed cartridges, such as Bond Elut PPL. The closest bulk sorbent is Bondesil PPL, but it has the different basis (silicagel versus polymers in BondElut). Prior to its use for large-scale filtration, it was necessary to compare molecular composition of DOM extracted with a use of Bond Elute PPL cartridges and bulk sorbent Bondesil PPL.

The goal of this work was to estimate suitability of bulk sorbent - Bondesil PPL (Agilent) for preparative extraction of large quantities of DOM, which would be comparable in composition to DOM samples extracted by prepacked cartridges Bond Elut PPL.

Firstly, the model experiments were conducted on isolation of peat DOM (Fulvagara) using BondElut PPL cartridges, and self-packed columns of Bondesil PPL sorbent. Secondly, the large-scale filtration system was assembled using the self-packed columns with Bondesil PPL. It was used for isolation of marine DOM from 500-100 L. From the same station, marine DOM was isolated using the Bond Elut PPL cartridges to enable direct comparison of molecular composition of the DOM isolated by different sorbents.

The lab experiment was conducted using 3L of 70 mg/L Fulvagra solution, which was passed through the sorbents under study. Close similarity was revealed for sorption capacity of both sorbents:  $60.1 \pm 2.1\%$  and  $64.3 \pm 2.4\%$  respectively, for Bondesil PPL and Bond Elut PPL, and extraction efficiency:  $42.5 \pm 1.4$  and  $45.4 \pm 2.3\%$ , respectively. The extracted materials were analyzed using optical spectroscopy and FT-ICR mass-spectrometry (15 T Solarix mass spectrometer located at the Zelinsky Institute of Organic Chemistry of RAS). The samples revealed close similarity in molecular composition with respect to the main peaks with the range of  $m/z$  300-500 zone.

Large-scale SPE filtration set up was assembled aboard research vessel Ac. Keldysh during International Siberian Shelf Study (ISSS) Expedition in October 2020. Surface sea water was collected into 1000 L HDPE tank (about 500 L) after filtration through pre-combusted GF/F filters conducted by team of University of Stockholm led by O. Gustaffsson. It was acidified until pH 2 and discharged through two sets of self-packed 60 mL cartridges filled with 15 g Bondesil-PPL sorbent each. The cartridges were unloaded in the lab using methanol elution. The extracts were also investigated using FT-ICR MS. The extracted fractions revealed an abundance of tannins and phenylisopropanoids. The similarity of fractions was proved by estimating of correlations between the fractions using mathematical models of comparison such as tanimoto, cosine and estimation of correlations.

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## Simulation-dynamic model for estimation of the physiological activity of humic preparations

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Keywords: humus preparations, physiological estimation, viability, germination energy, germination, biotest of Grodzinsky, length of seedlings and roots

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To estimate the biological effect of humic substances on plants, various methods are currently used, based mainly on the determination of the viability (germination energy) and on the germination of seeds. Various biotests are also used. One of such testing systems



is the biotest of Grodzinsky (BG), which makes it possible to determine the  $LD_{50}$  index by germination of 50 out of 100 tested seeds in the control variant. In relation to this indicator, seed germination is estimated in the studied variants. The indicator BG is more informative than the estimation of the viability and of the seed germination. However, all these methods for estimation of the biological activity have static indicators at a fixed point in time. At the same time, all biological objects develop in dynamics; therefore, to assess their state, it seems expedient to move from static indicators to dynamic ones.

The objects of research were model humic substances extracted from peat (preparation No.1) and from kraft lignin (preparation No.2) by using of various technologies.

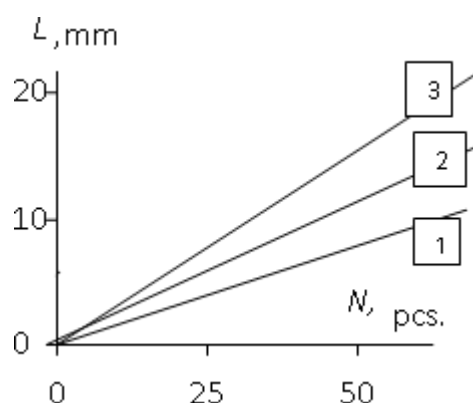


Figure 1. Seed germination model according to the length of seedlings (linear trend)  
1 - control group, 2 – preparation №1, 3 – preparation № 2.

Fig. 1 shows the results of estimation for a sample of seed germination according to the length of seedlings. It can be seen that preparation No.1 provides more significant results than preparation No.2. The sampling is made in Excel. Sampling results were sorted in row from lowest to highest. The resulting dataset was transferred to the histogram. A simple linear trend has been added to the histogram. This trend is acceptable only for the initial stages of seed germination, when only growth reserves in the endosperm are used. When switching to nutrition from the soil and to photosynthesis, there will be another curve.

All seeds with a linear trend then "line up" in terms of germination intensity, covering the entire sample. It turns out the simulation dynamics of seed germination under the influence of the studied factor (or preparation).



## Soil biomarkers as indicators of paleoenvironments

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Keywords: lignin, n-alkans, amino sugars, fatty acids, isotopes

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Unlike humic acids, the formulas and chemical structure of which still remain unknown, biomarkers are organic molecules of known structure and origin. Individual molecules of phenols, amino acids, amino sugars, lipids can be reliably found in living organisms, in their remains and litter, in soil, in complex associates, in humic substances. Many biomarkers, due to their specific structure and biochemical functions at certain conditions turn out sustainable to degradation and mineralization in soils and, therefore, will be served by molecular traces of the paleobiota and terrestrial vegetation, as well as an indicator of the intensity of biochemical processes occurring in biosphere not only in the present but also in the past. At the same time, individual organic compounds are well preserved not only in the "normal" profiles of post-lithogenic soils, but also in products of their redeposition, in soil-colluvial and soil-alluvial complexes, in pedosediments, pedoliths, derivatives of individual horizons, in bottom sediments of water bodies, etc. Organo-mineral soil particles or soil concretions contribute to the preservation individual organic molecules in time, marking the conditions of paleoenvironments.

The objects of the study were polygenetic gray forest soils that form typical geochemical catena in Moscow (Kolomenskoe opolye), Vladimir and Bryansk (Trubchevskoe opolye) areas. Research methods included: group and fractional analysis of humus, gas-liquid chromatography to determine the fractional composition of fatty acids, amino sugars, lignin phenols, n-alkans. The isotopic composition of carbon was measured on a Thermo-Finnigan Delta V mass spectrometer Plus IRMS and elemental analyzer Thermo Flash1112. The age of the soils was determined by the method radiocarbon dating.

The content in the discussed soils of the total amount of lignin (VSC), chlorophyll and fungal pigment Pg reflects the degree of soil hydromorphism, naturally accumulating in Gley soils and in the second humus horizons. The samples have enriched with biomarkers of woody vegetation (n-alkanes C27 and C29).

Analysis of the presented data on the composition of lignin phenols in buried horizons of different polygenetic soils reveals that the compositional sets of lignin phenols in soils serve as molecular traces of terrestrial vegetation. However, the interpretation of the signal is largely complicated by the superposition of biochemical processes initiated by alternating vegetable associations. Informational role of organic compound groups (chlorophyll, fungal melanins, lignin in general) is less informative than individual biological molecules, trace amounts of which are easily determined by modern research methods.

The qualitative composition of lignin phenols, n-alkans, amino sugars, fatty acids is naturally reflected in  $\delta^{13}\text{C}$  values of soil humus. At the same time, cinnamyl phenols increase the values of isotopic relationships that are very sensitive to C3-C4 changes. Vanilla and syringyl phenols of trees and shrubs (C-3 type of photosynthesis) facilitate them.

The results obtained and an attempt to apply them in paleosoil studies reveal the promise of using biomarkers and the need for further studying the pool of natural organic

compounds of soils. In any case, the informational role biomarkers are found to be the highest in complex studies of organic matter daytime and paleosoils.

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## **Cure for treatment of purulent-necrotic diseases of the animals limbs**

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Keywords: emulsion, liniment, paste, salve, potassium humate, purulent-necrotic disease, limbs

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All age and sex groups of animals are susceptible to infectious diseases of the extremities. According to some data, in economically developed countries of Western Europe, the prevalence of purulent-necrotic diseases of animal extremities, such as necrobacteriosis, hoof rot, Mortellaro's disease, purulent pyodermatitis, purulent and purulent-necrotic pododermatitis, alarinitis, Rustergolz ulcers, etc., ranges from 27 to 74 %; in farms of the Russian Federation, it ranges from 12 to 88%, in Kazakhstan - from 15 to 50%. Various veterinary drugs have been developed to combat diseases of the extremities of animals, and methods for treating purulent-necrotic diseases have been proposed [1].

The research aims to develop an effective agent for treating purulent-necrotic diseases of the extremities of animals of various etiologies with low cost, high efficiency, and a broad spectrum of antimicrobial action.

As a result of the research, the "Humik-Salve" product was developed, which uses potassium humate from brown coals of Kazakhstan (with a mass fraction of humic substances up to 56% in terms of dry matter). The preparation contains potassium humate, talc, vitamins, lanolin, glycerin, and the base: linseed or vegetable oil, petroleum jelly, depending on the dosage form (liniment, emulsion, paste, ointment) with a specific content of components.

The determination of the drug activity was carried out in relation to *Fusobacterium necrophorum* and *Escherichia coli* isolated from the contents of purulent-necrotic lesions, as well as pure cultures of *Streptococcus spp.*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*. Ichthyol ointment was used as a control. It was found that the zone of no growth of *F. necrophorum* was 2.8-3.5 cm, the area of no growth of other bacteria - 3.0-3.7 cm, around the control - 1.2-2.4 cm, which indicates the high antimicrobial effect of the developed product.

Production tests of the effectiveness of the means and method of treating animals with purulent-necrotic diseases of the extremities were carried out in various farms of the Akmola, Karaganda, South Kazakhstan, Almaty regions of the Republic of Kazakhstan. Cows (361 animals) were selected according to the method of pairs-analogs with necrobacteriosis, Mortellaro's disease, purulent pyodermatitis, purulent and purulent-necrotic pododermatitis, alarinitis; sheep affected by hoof rot (68 heads), horses with purulent-necrotic lesions of the limbs, scuffs, burns, lacerations, biting midge (57 heads). The treatment results showed that the "Humik-Salve" agent is highly effective for the

above lesions, helps to reduce the treatment time by 2-3 times, and has a high penetrating ability. This allows us to recommend it for implementation in veterinary practice.

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## The role of humic acids in the transport of riverine suspended matter

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Keywords: humic acids, chitosan, suspended matter

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The study of the mechanisms of riverine suspended matter (RSM) transport is an important topic due to the increasing anthropogenic load on aquatic ecosystems. The developed surface area of suspended and colloid particles contributes to the sorption of substances of different nature on the mineral surface, which influence the RSM behavior under changing salinity in the river-sea barrier zone. The loss of aggregate stability of RSM results in their sedimentation in coastal zones, while the stabilization of mineral particles leads to their transport to marine environment [1]. The aim of our research was to study the behavior of mineral components of river runoff under changing salinity and the influence of humic acids (HA) on this process.

The mineral components of RSM were modeled by clay substances (kaolinite, montmorillonite) and calcite. HA potassium salt separated from brown coal (leonardite) (Powhumus, Humintech, Germany) served as a model of transformed dissolved organic matter of river water, and cationic polysaccharide chitosan (Ch) (83 kDa, 82% degree of deacetylation in chloride form, Bioprogress, Shchelkovo, Russia) was used as a model of a labile organic flocculant. The salinity (1-30‰) was modeled by adding sodium chloride to the suspension.

The mineral suspensions (2 g/L) were prepared by adding weighted clay or calcite samples in distilled water at room temperature. Modification of mineral particles by HA (10 mg/L) was carried out by their adsorption on minerals for 3 days. The suspensions were shaken for several minutes (after three days of swelling for clays) and the absorbance was measured at 535 nm using an Agilent spectrophotometer (USA) and the turbidimeter HI 93703 (Romania).

It was shown that the modification of mineral particles by HA leads to the stabilization of mineral suspensions over the whole range of investigated salinity. Conversely, a sharp decrease in the stability of such suspensions is observed in the presence of Ch, compared with unmodified minerals. The study of adsorption of phenols (the substances that are included in HA) showed that phenol is better absorbed on activated carbon (BAU) —

7.6 x 10<sup>-5</sup> mol/g, while its adsorption on kaolinite, limestone and calcite were 1.8 x 10<sup>-5</sup>, 1.2 x 10<sup>-5</sup> and 1.1 x 10<sup>-5</sup> mol/g, correspondently. Comparison of the adsorption of phenols showed that the increasing number of phenolic hydroxyl groups leads to an increase in the phenol adsorption on minerals [2].

Thus, the transport function of HA in the barrier zone and the ability of HA to increase the efficiency of the mineral flocculation process in the mixing zone were shown.

The work was performed in the framework of the State Assignment "Colloidal Chemistry", number CITIS 121031300084-1.

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## Antihypoxic and cardiotropic properties of humic substances

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Keywords: humic substances, cardioprotectors, antihypoxants

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According to statistics, every year an average of about 17 million people on the planet die from pathologies of the cardiovascular system. In view of this, the more increasing preventive measures role is taken, consisting in the use herbal cardioprotective drugs that do not have a systemic toxic effect from prolonged use. The perspective cardioprotective substances also are the humic substances (HS) due to they have high protective properties.

Cardioprotective properties of the 1% aqueous solution HS (by Novoland company, Russia) were tested on 24 rats "Wistar" (n=12, 2 groups) as described in [1, 2]. The animals were divided on 9 groups of 10 animals each for the study of normobaric hypercapnic hypoxia, tissue and hemic hypoxia. Mexidol® was used as a reference drug.

The direct cardiovascular effects were establish for the tested HS. The preventive HS use in the period preceding ischemia-reperfusion was increased the resistance of the isolated myocardium to hypoxia. This fact was confirmed by the repair of its contractility (end-diastolic pressure (EDP) and pressure developed by the left ventricle (LVDP) decreased) (Table 1).

The antihypoxic HS effect at the 500 mg/kg concentration was comparable to that of Mexidol® only at the 1000 mg/kg concentration (Table 2), which indicates about 2 times higher efficiency of the tested HS in comparison with Mexidol®.

Table 1 Influence of HS on contractility of the isolated myocardium

Time	LVDP (mmHg) (n = 12)		EDP (mmHg) (n = 12)	
	Control	Test sample HS	Control	Test sample HS
10 min of adaptation	92,75±2,5	93,7±2,1	15±0,1	15±0,1
20 min of perfusion HS	94,6±2,3	**73,6±2,4	14,45±0,2	**12,2±0,28 <sup>s</sup>
Reperfusion, min				
5 min	###8,2±0,8	###9,1±1,7	###76,3±2	**###64±1,9
15 min	###9,6±0,9	**###17,2±2	###75,6±2,3	**###61,2±1,9
30 min	###25,2±2,4	**###29,7±1,2	###68,25±3,9	**###59,8±1,9

\*reliability relative to similar values in the control  $p < 0,05$ ; \*\* $p < 0,01$ ;

#reliability relative to baseline values in the group  $p < 0,05$ ; ## $p < 0,01$ ; ### $p < 0,001$ .

Table 2 Influences of HS on the course of hypoxia

Injected substance	Latent time of hypoxia, min		
	Normobaric hypercapnic hypoxia	Tissue hypoxia	Hemic hypoxia
NaCl, 0 mg/kg (control)	20,9±1,1	12,9±0,4	7,4±0,4
Mexidol®, 1000 mg/kg	24,8±0,9	12,9±0,6	7,2±0,3
HS, 500 mg/kg	25,8±1,1	13,3±0,4	8,3±0,6

\*differences with the "control" group are statistically significant,  $p < 0,05$ .

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## Development of a reference standard based on natural humic acids for the determination of organic carbon

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Keywords: organic carbon, determination, reference materials of composition, peat, soil, humic preparation Rostok, oxythermography

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Determining the content of total organic carbon (OC) in natural objects (soil, peat, vegetation) is an important and topical issue. It is one of the most widely used analyses in the world. Currently, an oxythermography device has been created, which is equipped with two sensors for registering an oxidizer, i.e. air exiting a high-temperature reactor. Programmed oxidation of the analyzed natural sample is conducted in the reactor. The device can simultaneously determine the oxidizability of the analyzed sample and the OC content in it. The analysis requires a small weighed amount of a dry sample of humic acids (soil, peat, HAs) ~0.03 g. The device can be used both in laboratory conditions and in the

field. For metrologically correct measurements and reliable interpretation of the results obtained, reference standards are required [1, 2]. The authors propose using the commercial Rostok humic agent as such a standard. It is produced by the LLC SPC Evrika SIE (a small innovative enterprise) of the SAU of the Northern Trans- Urals [3]. The agent's composition, its homogeneity, stability of its properties and composition over time (>10 years) were determined, and the optimum conditions for thermal oxidation were selected. The authors assume that the stability of the dry natural HA agent is due to the absence of microbiological processes. The preparation contains OC (%):  $44.7 \pm 0.3$  ( $n = 25$ ,  $P = 0.95$ ),  $s_r = 0.5\%$ .

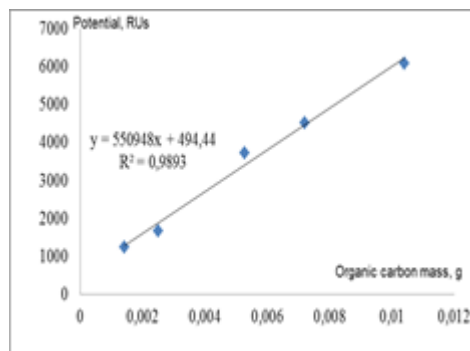


Figure 1. Calibration graph of the HAs preparation obtained by oxythermography at 570-670 K.

HAs fully correspond to the composition of peat and its flammable properties. The method is reliably reproducible; inorganic carbon does not interfere with the analysis.

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## Study of the cytotoxicity of bionanomaterials based on humic substances and silver nanoparticles in cell culture

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Keywords: humic substances, silver nanoparticles, cytotoxicity.

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The problem of treatment of chronic wounds due to the high incidence of ulcers and pressure ulcers is of great concern in society. For example, Ag / Zn nanocomplexes are described covalently grafted to an organic matrix [1], and their ability to suppress the

growth of both gram+ and gram- microorganisms has been shown. The dressings with various compositions of silver has their antibacterial effect and is using to treat acute aseptic and infected wounds [2]. Humic substances (HS) have a wide spectrum of biological activity, therefore with the creation of bionanomaterials based on HS as a matrix containing silver and zinc nanoparticles is of great interest today for the treatment of bacterial infections and for wound healing.

Research the safety profile of new bionanomaterials based on humic substances and silver nanoparticles as their cytotoxic effect in cell culture.

The cytotoxic effect of 8 different initial HS samples and samples of silver nanoparticles ultradispersed in the corresponding HS matrices (HS-AgNPs) was investigated by the colorimetric method on cell cultures of normal fibroblasts (3T3-L1) and human hepatocellular carcinoma (HepG2) in vitro. We used a method for assessing cell viability using a neutral red dye in the concentration range from 7.8 µg/ml to 1000 µg/ml. The reference drug was the colloidal silver preparation "Protargol". The significance of the differences was significant at  $p < 0.05$ .

Studying the cytotoxic properties of samples of the initial HS matrices, we found that did not possess significant toxic properties in cell culture. Toxic effects were manifested only at concentrations of 500 and 1000 µg/ml for most of the tested substances of the starting HS matrices, therefore, IC<sub>50</sub> values were not achieved in the studied concentration range for all cell lines except for two samples of peat HA Peat1 (250 µg/ml) and Peat2 (125 µg/ml). When studying the cytotoxic properties of the reference drug "Protargol" containing colloidal silver, we established the IC<sub>50</sub> values of  $165.7 \pm 17.6$  µg/ml and  $517.8 \pm 71.9$  µg/ml for 3T3-L1 and HepG2, respectively.

In the cell culture of normal fibroblasts (3T3-L1), all tested AgNP / HS samples showed less cytotoxicity in comparison with the colloidal silver preparation "Protargol". The following samples have a higher safety profile in tests on both cell cultures: PHF-T3-AgNPs, Peat1-AgNPs, CHE-AgNPs, and CHI-AgNPs.

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### **Molecular composition – inhibition activity relationships for humic substances narrow fractions sets obtained by solid-phase extraction**

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Keywords: SPE fractionation, FTICR MS, humic substances, inhibition of beta-lactamase activity

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Humic substances (HS) have a wide spectrum of biological activity including inhibitory activity against β-lactamases.<sup>1</sup> The latter are capable of hydrolyzing beta-lactam antibiotics and represent one of the main pathways of bacterial antibiotic resistance. HS

are characterized by low toxicity and good solubility in water. A use of HS for therapeutic purposes is hindered by extreme molecular heterogeneity and high level of isomeric complexity. Solid-phase extraction (SPE) fractionation in combination with ultra-high resolution mass spectrometry (FTICR MS) is a promising method to simplify this molecular system and isolate the most active components of HS. The aim of this work was to test various SPE fractionation schemes as an approach to directed isolation of the components with the given activity from HS.

The sample of coal humic acids (CHA-G) was isolated from the commercial sodium humate "Genesis" and separated using SPE cartridge according to gradients in polarity<sup>1</sup> and acidity<sup>2</sup> inherent within the molecular components of HS. Inhibitory activity against  $\beta$ -lactamase TEM-1 and its mutants was measured using chromogenic substrate CENTA. Molecular composition of fractions was determined using FTICR mass spectrometer 15 T solariX (Bruker Daltonics) located at the Collective Use Center of Zelinsky Institute of Organic Chemistry of RAS. Molecular assignments were plotted into van Krevelen diagrams. The diagrams were binned into 20 cells are assigned to seven chemotypes, and occupational densities for each chemotype were calculated after Perminova.<sup>3</sup>

For the fractions separated by polarity, a substantial difference in the molecular composition was observed. Inhibitory activity grew along with an increase in hydrophobicity. The HS activity increased along with an increase in contribution of condensed tannins and phenylisopropanoids ( $O/C < 0.5$ ,  $H/C < 1.4$ ) and decreased along with contribution of hydrolyzed tannins ( $O/C > 0.5$ ,  $H/C < 1.4$ ). The similar analysis was conducted for the fractions separated with regard to pKa value of the dominating functional groups. The most isomeric complex molecular components were defined, which can be found in different HS fractions, but they are identical in elemental composition. The data obtained make it possible to choose the most efficient fractionation method that effectively lowers the molecular complexity of HS and makes it possible to isolate the most active HS fractions.

SPE-fractionation in combination with 2D chromatography is going to be used in our future studies to achieve high resolution separation and more reliable "molecular composition-activity" relationships. Further research might bring substantial advance in the field of directed design of biologically active humic-based materials and compositions.

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## Assessment of formula-based structural annotation of humic substances by mild chemical derivatization and mass spectrometry

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Keywords: NOM, FTICR MS, aromaticity index, derivatization, bromination, structure

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Natural organic matter (NOM) plays an important role in the environment and its chemical properties and molecular composition reflect balance between mineralization and sequestration of organic carbon. Ultrahigh resolution mass spectrometry (e.g., FTICR MS) provides essential molecular information about NOM. However, NOM molecular heterogeneity prevents application of tandem MS experiments and direct structural information is ultimately missing leaving opportunities to only ambiguous formula-based annotation. The main aim of this work was to develop a chemical workflow to reliably examine the accuracy of several FTICR MS-derived structural indices with the focus on aromaticity and O-functional groups, which greatly impact compound properties.

Four NOM samples of different origin (coal, oxidized lignin, river, and permafrost thaw) were brominated by NBS in acetonitrile for 24 hrs. at RT. Carboxylic groups in all samples were determined by selective deuteromethylation using CD<sub>3</sub>OD/SOCl<sub>2</sub> reaction and by HATU amidation with <sup>15</sup>N labeled glycine. Carbonyl groups were reduced by NaBD<sub>4</sub>. All parent and labeled mixtures were analyzed by ESI FTICR MS. Custom python scripts were developed to treat spectra and enumerate specific structural moieties in individual components. Obtained data was used to assess reliability of exact aromaticity indices (AI)<sup>1</sup> and aromaticity equivalents (X<sub>c</sub>)<sup>2</sup>.

Lignin- and coal-derived samples turned out to be the most sensitive to bromination which corroborated with the model phenolic structures. On contrary, permafrost thaw, which is enriched with labile species, was mostly resistant to bromination - 22% of molecular ions were brominated. Moreover, unlike oxidized riverine sample, coal NOM included polybrominated species, which implies that reaction efficiency depends on reactivity (i.e. substituents) of aromatic fragments. Samples were characterized by drastically different bromine distributions on van Krevelen diagrams, which correlated with the distribution of non-carboxylic oxygen atoms. Further, we compared AI and X<sub>c</sub> aromaticity indices in terms of the proportion of correctly assigned aromatics. The data on brominated molecules were in good agreement with the AI values; however, apparently AI tends to overestimate the number of non-aromatics in the sample since it describe averaged aromaticity rather than the factual presence of aromatic ring. On the other hand, X<sub>c</sub> perfectly recognized non-aromatics. In general, a higher proportion of correctly attributed aromatics was observed for the aromaticity equivalent X<sub>c</sub> (up to 68%), which tends to find aromatic moieties in non-aromatic molecules assigned by AI. Still, we observed a number of aromatic- and condensed aromatic-assigned compounds, which were resistant to bromination or included lesser Br-atoms than the evaluated number of aromatic rings. Reaction with NaBD<sub>4</sub> and enumeration of labeling series revealed the presence of carbonyl groups in these species, which in case of multiple reducing could be reliably assigned to quinone – condensed non-aromatic compounds. The approach may be of great importance in biogeochemical and medicinal studies of NOM.

Acknowledgements. This work was supported by the Russian Science Foundation gran № 21-47-04405.

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## The use of gel made of silanol derivates of humic substances for long time storage of alive microorganisms

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Keywords: silanol derivates of humic substances, gel, bacteria, survival, long time, petroleum bioremediation

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Liquid biopreparations for bioremediation are convenient and economically attractive, but microbial survival under standard environmental conditions is poor. A novel way to increase survival of various hydrocarbon oxidizing microorganisms (HOM) during long-term storage was developed and tested. A new type of biocompatible gel based on humates modified with organosilanes has been recently developed (Volikov et al. 2016 a, b). Gel was prepared with the use of humate of Na and K "Powhumus" (Humintech, Germany) and the 3-aminopropyltriethoxysilane (APTES) (AGM-9, Penta 91, Russia) according to the previously modified procedure. This gel was used for immobilization of HOM cells of different types: bacteria *Rhodococcus qingshengii*, *Acinetobacter seifertii*, *Pseudomonas aeruginosa*, *P. extremaustralis* and yeast *Yarrowia lipolytica*. Microbial cultures of stationary phases were mixed (1:3 of total volume) with gel and left at room temperature for few months. After storage for up to 12 months, titers of alive cells (colony forming units, CFU) of HOM in the gel were ten to one hundred times higher than in the control (as shown in fig. 1 for *A. seifertii* and *P. aeruginosa*). Hydrocarbon degradation by the stabilized cultures began earlier and was faster and more complete than in the control after few month storage.

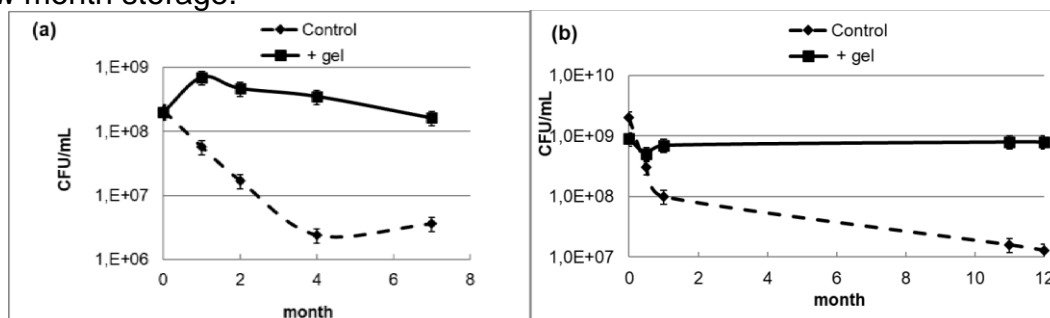


Figure 1. Effect of immobilization of *A. seifertii* (a) and *P. aeruginosa* (b) into the silanol-humic gel on their survival during storage.

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## Membrane fractionation of water-extractable organic matter of typical chernozem

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**Keywords:** soil organic matter, fractionation, ultrafiltration, track-etched membranes, fluorescence spectrometry, infrared spectrometry, inductively-coupled atomic emission spectrometry

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Soil organic matter (SOM) is a highly heterogeneous and heterophase complex system comprising high-molecular, low-molecular, and colloidal particles. Membrane filtration is a promising fractionation method to simplify this system. It allows studying particles ranging in size from 10 Da to several hundred micrometers [1]. Nowadays, there is a growing interest in using this separation method for organic matter, predominantly dissolved organic matter (DOM) of water.

This study used cascade filtration through track-etched membranes with pore size from 0.2 to 5  $\mu\text{m}$  to fractionate water-extractable organic matter (WEOM) from typical chernozem obtained at room temperature and by 80 °C water extraction. By definition, soil WEOM is the DOM obtained by extracting a given mass or volume of soil with an aqueous solution [2]. The filter cut-off used is somewhat arbitrary, but 0.4 to 0.6  $\mu\text{m}$  pore size is usually considered the boundary between dissolved and particulate materials [2]. Thus, membrane filters with the specified pore size used in this work covered not only dissolved but also particulate material. The main focus of the work was on narrow fractions obtained by washout from the corresponding membrane filters.

Based on an analysis of fractions, it was found that fractions differ significantly from each other both in organic and mineral constituents. According to element analysis, element ratios vary significantly between fractions for both macro- and microelements. Al, Fe, and Ti are primarily observed in the fractions with particle size larger than 0.2  $\mu\text{m}$ , while Ba, Mn, Si, Zn, Ca, K, S, and Mg, to a more significant extent are linked with particles of less than 0.2  $\mu\text{m}$ . By fluorescence spectrometry, we found that all fractions contain intensively fluorescing organic components with changing compositions, in

particular humic-like and tyrosine-like substances. All obtained fractions contain clay minerals with also changing compositions. Thus, organic matter of different compositions is sorbed on clay minerals of different compositions. Thus, the developed method represents a new approach to isolating WEOM fractions preparatively and expands knowledge about WEOM chemical composition and its relationship with the mineral phase. In the future, we plan to continue fractionation using membrane filters with smaller pore sizes to isolate soil WEOM fractions.

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## Humic substances mitigate the radionuclide effects on marine luminous bacteria

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**Keywords:** Humic substances, hormesis, bioluminescence

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It is known that microorganisms of soils and aqueous media are sensitive to the presence of humic substance (HS), products of natural decomposition of organic matter, which play a role of natural attenuators of environmental toxicity. We study the effects of alpha- and beta- emitting radionuclides (americium-241 and tritium, respectively) in the presence of HS under the conditions of low-dose exposures (<0.1 Gy). Luminous marine bacterium *Photobacterium phosphoreum* was applied as a model unicellular water microorganism to monitor toxicity and activation ability of the radionuclide solutions. To imitate the marine environment for bacterial cells and to balance osmotic processes, the 3% NaCl solutions were used.

The bioluminescence response of the marine bacteria to americium-241 and tritium corresponded to the "hormesis" model: it included stages of bioluminescence inhibition and activation, as well as the absence of the effect. HS were shown to decrease the inhibition and activation effects of the radionuclides on the bacterial luminescence. The changes were shown to be related with the accumulation of americium-241 in the bacterial cells [1]. The correlations between the bioluminescence intensity and the content of Reactive Oxygen Species (ROS) were found in the radioactive bacterial suspensions in the presence of tritiated water [2].

The results demonstrate an important role of HS in natural processes in the regions of low radioactive contaminations: HS can mitigate radiotoxic effects and adaptive

response of microorganisms to low-dose radioactive exposure of alpha- and beta- types. The involvement of ROS to these processes was demonstrated.

The results can provide a basis for predicting a response of living organisms to radiation at large territories infected with low-intensity radiation after accidents, discharges of nuclear plants, or underground mining of natural resources.

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## Analysis of the biological anti-HIV activity of humic substances based on data on the molecular composition

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Keywords: FTICR MS, HIV, peloid hyalomelanin acid, humic substances, Van Krevelen diagram

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The problem of finding new sources and types of biologically active natural compounds is the most important task of modern medicinal chemistry. Humic substances are known to be a carrier of antiviral activity [1]. This study aimed to study the anti-HIV activity of the peloid hyalomelanin acid narrow fractions and to analyze its relationship with the molecular composition obtained by FTICR MS.

Solid-phase extraction was used to obtain narrow fractions followed by gradient elution on three Bond Elut cartridges: C8, C18 and CNE. The eluent was various mixtures of methanol and water. A total of 33 fractions were obtained, 11 from each of the 3 cartridges. Each fraction was characterized by FTICR mass spectrometry. Cytotoxicity and anti-HIV activity were measured.

The most active fractions for cartridges C8 and C18 were obtained by elution with mixtures of methanol:water from 60:40 to 80:20, and 70:30 to 90:10 for CNE.

Signals of impurities were removed from the obtained spectra. For each group of spectra, Tanimoto similarity matrices were calculated and Van Krevelen diagrams were plotted. Correlation diagrams were constructed for all 3 sets of fractions based on the 2d binning of Van Krevelen diagram. The correlation was calculated between the population densities of the squares on the van Krevelen diagram and the inverse decimal logarithm EC50 (Fig. 1).

The correlation analysis suggests that tannins are associated with high anti-HIV activity.

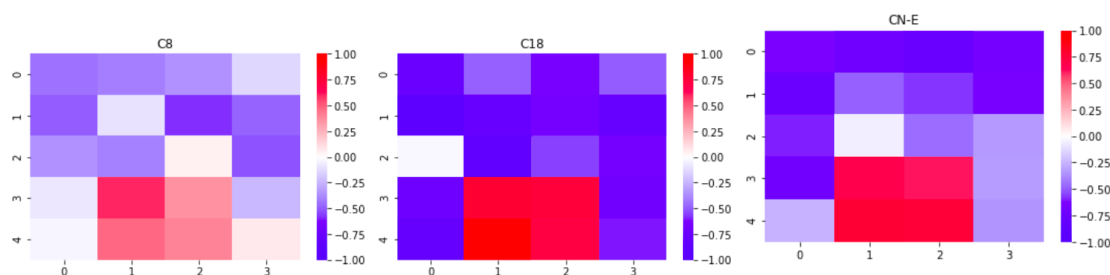


Figure 1. The data of the correlation analysis that was carried out on the data of the descriptors of the population densities of the Van Krevelen diagram for the fractions of the HMA of peloids obtained from three different cartridges and the data of the anti-HIV activity. From left to right fractions got on cartridges C8, C18, CNE.

**Acknowledgements.** This work was supported by the Russian Science Foundation grant № 21-73-20202

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## Application of humic substances in intensification of anaerobic biotransformation of oxidized sulfur compounds

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**Keywords:** humic compounds, methanogenesis

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The specificities of the functioning of some natural biosystems with the participation of microorganisms and humic substances (HS) are increasingly attracting the attention of researchers [1-3]. The effect of HS on the activity of different bacteria and consortia of microorganisms was studied separately [4-5]. It was found that potassium humate and chemically modified HS are inhibit the metabolic activity of bacterial cells, while the fulvic acids and sapropel are stimulate the metabolic activity of natural methanogenic consortia [4-5].

Anaerobic methanogenic consortia are widely used not only for the production of biogas, but also for the decomposition of various wastes and for wastewater treatment. In this work, we used HS for stimulating metabolic activity of anaerobic cells during the biocatalytic stage of the hybrid chemical-biological process of oil desulfurization [6-7]. This process is a sequential combination of chemical stages (oxidation of sulfur-containing substances and their extraction) and a biocatalytic stage (transformation of an extract containing oxidized sulfur compounds under anaerobic conditions with simultaneous production of biogas and inorganic sulfide). Sapropel (Ruskiyi ogorod) and fulvic acids

(Greenway) were introduced in methane tank. The liquid phase of the methane tank also was contained ethanol and dibenzothiophenesulfone (Table 1).

Table 1. Process characteristics of ethanol extract containing dibenzothiophenesulfone transformation by anaerobic consortium

HS	Methanogenesis efficiently, %	Methane content in biogas, %	Transformation of sulfur into inorganic sulfide, %
-	64.3±2.6	49.7±2.2	72.5±3.7
Fulvic acids	64.5±2.6	72.5±2.8	92.8±5.2
Sapropel	65.8±2.8	62.8±2.7	87.9±4.3

It was shown that the efficiency of methanogenesis in this process practically did not change with the introduction of different HS into the medium. Increasing the methane proportion in the biogas composition and the degree of sulfur conversion into inorganic sulfide in the process was significant (by 15-20%). It is possible that these HS stimulate the metabolism of methanogenic archaea and sulfate-reducing bacteria being components of anaerobic consortium.

The work was supported by the RFBR (grant number 18-29-05064).

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## Synthesis and characterization of combined silver nanoparticles/zinc humate preparation for antibacterial and wound healing applications

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Keywords: humic substances, silver nanoparticles, zinc, wound healing, antimicrobial activity.

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Antimicrobial activity of silver nanoparticles (Ag-NP) against antibiotic-resistant bacteria strains led to an active application of Ag-NP in medical practice and festering wound treatment. The long-lasting wounds such as bedsores, burns, purulent wounds are an enabling environment for pathogenic flora. To increase the rate of wound healing of such wounds various Zn compounds are currently used. In this regard, a combination of Ag-NP and Zn in one treating complex could increase the productivity of wound healing and reduce the possibility of its infection. However, in addition to antibiotic activity, Ag-NP may show the cytotoxic effect due to an elevation of oxidative stress in human cells. To avoid this negative effect the utilization of humic substances (HS) both as a reduction agent for the formation of Ag-NP and an antioxidant to reduce the cytotoxicity of

synthesized Ag-NPs is a promising technique. Besides the ability to reduce Ag HS is also a natural complexing ligand that could form stable humate complexes with metal ions including Zn. For this reason, the aim of the present study was to optimize the synthesis of the combined preparation of Ag-NP/Zn humate as a promising agent for wound healing with intensive antimicrobial activity.

In the present study, we analyzed influence of pH, initial concentration of AgNO<sub>3</sub> and presence of Zn(NO<sub>3</sub>)<sub>2</sub> on degree of Ag<sup>+</sup> into Ag-NP conversion. The degree of conversion was estimated by UV/Vis-spectroscopy using position of maximum and intensity of Ag surface plasmon resonance (SPR) peak at 400-420 nm. The size of the synthesized nanoparticles was investigated using transmission electron microscopy (TEM).

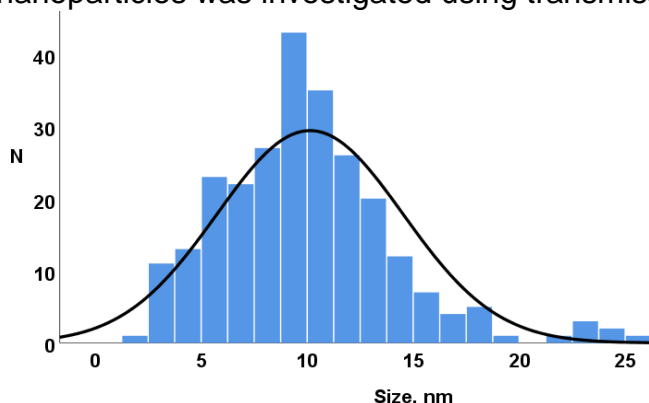


Figure 1. Size distribution of Ag-NPs.

The maximum degree of conversion was achieved after 3 h at the initial concentration of HS 10 g/L, AgNO<sub>3</sub> 42 mM and pH 11. According to TEM results, fine nanoparticles with an average diameter of 10±4 nm were formed (Figure 1). More than 90% of Ag<sup>+</sup> was converted into Ag-NP. It was also found that addition of 10 mM Zn(NO<sub>3</sub>)<sub>2</sub> solution at the initial stage of the synthesis decreases the conversion rate of Ag-NP.

However, addition of separately synthesized Zn humate with concentration of HS of 10 g/L, and of Zn - 10 mM in the ratio of 1:1 (v:v) did not significantly affect the Ag-NP formation. The concentration of Ag-NPs stabilized at the same level as in the absence of Zn<sup>2+</sup> in the solution for 3.5 h and remained stable.

As a result, we have shown that the preferable way of synthesis of combined silver nanoparticles/zinc humate preparation is separate synthesis of the components followed by their mixture in 1:1 volume ratio.

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## Specificity of determining of the organic matter content in the Rostov agglomeration natural soils

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Keywords: soil organic matter, total organic carbon, analytical procedures.

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Determination of the organic matter content is an important stage in the comprehensive study of the soil, which makes it possible to understand its genesis and properties, as well as ways for fertility increasing. Now there are several soil organic matter determination procedures, or rather, their modifications. Analysis speed, results reproducibility, and accuracy are key aspects in finding the optimum procedure for laboratory tests.



Therefore, the purpose of this work is to identify the features of determining the organic matter content of natural soils of the Rostov agglomeration applying two different analytical procedures. These methods are based on qualitatively different approaches to the organic carbon compounds determination. One of the procedures is the B.A. Nikitin's modifications of methods that involve a wet combustion of the organic matter with a mixture of potassium dichromate and sulfuric acid at about 150 °C in a drying oven. The residual dichromate is determined by the optical density measured on a spectrophotometer at a wavelength of 590 nm [1]. Other procedure is high temperature catalytically assisted combustion on an analyzer of total organic carbon TOC-L CPN Shimadzu. The TOC (total organic carbon) value is found by a differential method, which is described by the following equation:  $TOC = TC - IC$ , where TC is total carbon and IC is inorganic carbon [2].

Calcic Chernozem were the object of this study. The soil sampling was carried out in tenfold repetition from the upper 10 cm for each of the five monitoring plots. Such sampling procedure enabled the performance of statistical processing of the data and to exclude the vary factor of the content of many indicators in the surface samples.

Using of both procedures have shown that there is no significant difference in total carbon content with a sample of  $N = 100$  and a significance level of 0.05. The average organic carbon content determined by Nikitin's method was  $3.54 \pm 0.98$ . The average organic carbon content determined by carbon analyzer was  $3.90 \pm 1.01$ . These tests were shown the equivalence of the compared procedures and high results reproducibility of it. However, the carbon analyzer procedure provides a more complete combustion of carbon. Also the high-temperature catalytically assisted combustion allows observing the principles of an ergonomic approach: the amount of reagents and time for examining the sample are significantly reduced, errors associated with the analyst's actions are minimized. There is no negative effect of the vapors of the sulfuric acid on a person as well.

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## The humic acids impact on the photodegradation process of alkylphenols

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Keywords: humic acids, photodegradation, excilamps, alkylphenols

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Humic acids (HAs) play an important role in the process of migration of pollutants by controlling their flows in the environment and reducing toxicity. HAs cannot be synthesized from other substances, it can be found only in natural sources: soil, peat or brown coal. However, modification of humic acids properties is possible under the influence of, for instance, mechanochemical or photochemical actions or other chemical methods. HAs can absorb light and transfer light energy to other components of aqueous solutions, in some cases strongly influencing the photolysis of xenobiotics. Research on the spectral-luminescent properties of different HAs samples is relevant since it determines the influence of optical radiation of natural and technogenic origin on biogeosystems.

Alkylphenols are world widely detected in aquatic environments, sediments, soil, air and organisms tissues [1]. Alkylphenols are released into aquatic environment as major metabolites of non-ionic surfactants widely used in a variety of industrial, household and commercial application [2]. Moreover, the usage in the production of phenol/formaldehyde-based resins also contribute to alkylphenols occurrence in environment.

The absorption and fluorescence spectra of different samples of humic acids (HAs) are investigated. The samples of HAs were prepared from peat of Arkhangelsk Region. The comparison of this HAs with the samples of humic acids obtained from Aldrich Chemical Co is carried out. The effect of UV radiation from excilamp (KrCl, 222 nm) on the spectroscopic properties of humic acids has been investigated. 2,6-Bis(hydroxymethyl)-4-methylphenol (Aldrich Chemical Co) was selected as a alkylphenolic compound. The presence in the water solution of phenol compound the humic acids has a noticeable effect on the processes of photochemical degradation under the action of UV radiation. The fluorescence intensity of the mixture drops sharply after irradiation, to a much greater extent than in substituted phenol. This allows us to conclude that the addition of Aldrich humic acid significantly increases the photodegradation of the compound under study. The addition of peat humic acid leads to much weaker changes in the absorption and fluorescence spectra of the mixtures, though in the same direction as the addition of the Aldrich humic acid.

HAs were found to have an enhancing effect on the degradation of substituted phenol, which could be due to various processes. First of all, humic substances are important photosensitisers in soil and water environments, due to their ability to produce reactive species under irradiation. This effect may be also caused by energy transfer and by formation of charge-transfer complexes.

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## Regulation of methane emission in various biocatalytic systems

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Keywords: methanogenesis, humic substances, biocatalytic systems

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The problem of CH<sub>4</sub> emission into atmosphere is attend increasing ecological attention, since its concentration has raised significantly over the past 40 years and it is a strong greenhouse gas with a heat absorption potential 30 times greater than that of CO<sub>2</sub>. The largest anthropogenic sources of CH<sub>4</sub> emissions on the planet are swamps, bottom sediments of water resources, agriculture, animal husbandry and landfills.

The analysis of various biological systems revealed mechanisms that allowing reduction of CH<sub>4</sub> proportion in the resulting biogas produced by methanogenic archaea, due to syntrophic communication with other bacteria, for example, sulfate-reducing and denitrifying, and the use of such alternative electron acceptors (AEA) as humic substances (HS), Fe<sup>3+</sup>, SO<sub>4</sub><sup>2-</sup>, NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup> [1]. The most pronounced anaerobic oxidation of CH<sub>4</sub> (0.80 ng C /g dry soil/h) was noted in rice soils when pig manure and NaNO<sub>3</sub><sup>-</sup> (22,3 µg/g soil) were used as fertilizers.

In the marine environment the inhibiting effect of SO<sub>4</sub><sup>2-</sup> on CH<sub>4</sub> emission was noted, as far as SO<sub>4</sub><sup>2-</sup> the most common AEA due to its high concentration (~3 g/l). Decrease of CH<sub>4</sub> synthesis up to 10% was observed in the anaerobic zone when a mixture of 3.28 mg SO<sub>4</sub><sup>2-</sup>/g soil and 1.84 mg Fe<sup>3+</sup>/g soil was introduced into the soil used for landfills covering [2].

The addition of HC derivatives enriched with quinonones at concentrations above 1 g/l notably reduced the energy status of cells and the efficiency of methanogenesis catalyzed by various anaerobic consortia [3].

The influence of addition of various fatty acids to animal feedings on CH<sub>4</sub> emissions during the functioning of consortia in the rumen was studied. The inhibition of methanogenesis by long-chain fatty acids (30 mg/l) is based on the solubilization of bacteria lipid layer, which leads to cell lysis, inhibition of enzyme activity and destruction of the electron transport chain [4]. Under large-scale conditions, a similar quenching of CH<sub>4</sub> can be achieved by using long-chain surfactants.

Strong inhibition of acetoclastic methanogens has been shown by treatment with NaCl in concentrations over 20 g/l [5].

Thereby, this analysis shows the possibility of directed regulation of methanogenesis by introducing various natural compounds into biocatalytic systems that can reduce the synthesis of CH<sub>4</sub>, for example, in landfills.

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## Determination of organic carbon in solutions of humic substances

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Keywords: humic acids, fulvic acids, total organic carbon

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The ratio of the content of humic acids (HA) to the content of fulvic acids (FA) can be used as an index of the degree of humification of humic substances (HS). It is possible to determine the HA/FA on the basis of determining the amount of organic carbon contained in the composition of humic acids or fulvic acids - CHA/CFA. The classical method is the Tyurin method, which consists in the oxidation of all organic substances of the studied fraction of humic substances with an excess of a solution of potassium dichromate in dilute sulfuric acid with further determination of the amount of dichromate remaining in the solution. Now there are more advanced methods, for example, the method of determining TOC (Total Organic Carbon). The method of determining TOC allows analyzing large batches of samples faster.

Potassium humates CHP-K Powhumus (Humintech, Germany), CHS-K "Sakhalin Humate" (Biomir2000, Russia) and PHA-K "Kostroma humate" were used as the studied samples. For the analysis, the samples were dissolved on distilled water, the ash part is separated by centrifugation. After centrifugation, a supernatant (HS) was decanted into a conical flask. To separate humic acids from fulvic acids, an aliquot of a 20.00 ml supernatant was taken into a 50.0 ml beaker. pH of solution was adjusted to 1 by 5 M hydrochloric acid solution, after that the solution was transferred to a 25.0 ml measuring flask and the solution was brought to the mark with a 0.1 M hydrochloric acid solution. The solution was poured into a falcon and left for 3 hours, during which time there is a complete precipitation of humic acids. After three hours, the falcon was centrifuged and centrifugate (FA) was poured into pure falcon. The carbon content of HS and FA was determined by the TOC determination method using the TOC-L carbon analyzer (Shimadzu, Japan). The determination of CHA/CFA was carried out according to GOST [26213-91], by the Tyurin method with two endings. The first is the titration of the remaining dichromate by the solution with a Mohr salt in the presence of phenylanthranilic acid, the second is the spectrophotometric analysis of solutions. CHA defined as the difference between CHS and CFA. Values of CHA/CFA determined by different methods are presented in the table below

Table 1. Values of CHA/CFA determined by different methods

Sample	$C_{HA}/C_{FA}$ , titration.	$C_{HA}/C_{FA}$ , UV-vis	$C_{HA}/C_{FA}$ , TOC
CHP-K	$7,7 \pm 2,1$	$8,0 \pm 1,9$	$8,1 \pm 1,6$
CHS-K	$10,5 \pm 2,0$	$10,1 \pm 1,3$	$9,9 \pm 1,1$
PHA-K	$1,4 \pm 0,1$	$1,4 \pm 0,1$	$1,4 \pm 0,1$

It has been shown that the use of the TOC method has a number of advantages over the method for determining organic matter according to GOST due to its greater reproducibility and rapidity.

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## Humic acids of bog profiles – Bog condition matters

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Keywords: peat bogs, humic acids, humification, H/C, O/C

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The data has been reported concerning the physical and chemical parameters of humic acids (HAs) isolated from two cores of ridge-hollow complex in West Siberia. One of the cores was formed at the Ridge while the second at the Hollow. The Ridge and Hollow are considered to be ombrotrophic, but they significantly differ in hydrological conditions, vegetation cover, structure of peat deposits. Sampling was carried out for the entire depth of the peat column with an interval of 10 cm, which made it possible for us to trace the process of transformation of the structural features of HAs over a very long time period. HA were analyzed with respect to elemental (C, H, N, O) analysis, analyzed using Fourier transform infrared spectroscopy (FT-IR) and UV–Vis spectroscopy (E4/E6 ratio).

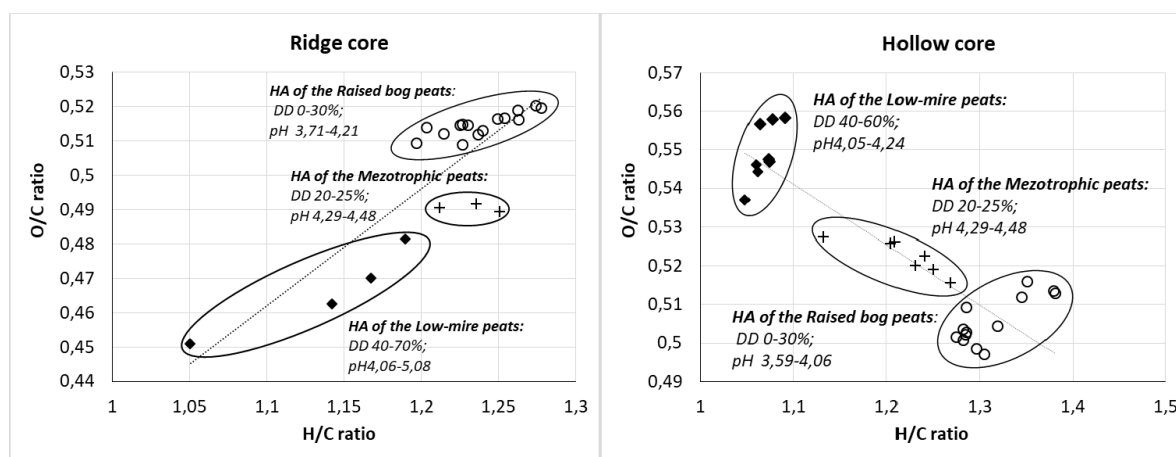


Figure 1. Van Krevelen graph (H/C vs O/C atomic ratio) of humic acids elemental composition change isolated from peat samples from ridge-hollow bog complex (Ridge core and Hollow core) in Western Siberia; DD – decomposition degree of peat

The Van Krevelen diagram (Fig. 1) demonstrates that, the HAs of peats of both peat columns form three separate groups, each of which is characterized by clear boundaries of changes in the H/C and O/C ratios. The first group corresponds to the HA of high-moor peats, the second group - the HA of transitional peats, and the third - the HA of lowland peats. However, the multidirectionality of the processes of HA formation and transformation in each core is evident. On the Ridge, where the acrotelm zone is clearly expressed the HAs of the raised peats are the most oxidized compared to those of low-mire peats. which showed a greater degree of aromatic condensation in the deeper layers than the surface ones. At the same time, we see an active dehydrogenation process in Has of low-mire peats. In the Hollow, where the humification processes is fraught with high humidity, the situation has the opposite tendency. Here, the HAs of low-mire peats are more oxidized in comparison with the HAs of the raised peats. Elemental analysis data are supported by both UV-spectroscopic (E4 / E6) and IR spectroscopic data. In general, our

data showed that the conditions (particularly high watering) of the bog environment can be one of the main drivers of the humification processes and transformation of HA in peat sections.

## Molecular modelling of interactions of the probable individual components of humic substances with $\beta$ -lactamase

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Keywords: humic substances, TEM-1, ChEMBL, molecular dynamics, allosteric inhibitors

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$\beta$ -lactam antibiotics have proven their efficiency against a wide variety of bacterial infections. However, antibiotic resistance spreads and renders known antibiotics less effective. The main reason of this phenomenon is cleavage of a  $\beta$ -lactam ring by serine- $\beta$ -lactamases [1]. This is why inhibitors of serine- $\beta$ -lactamases capable of irreversible binding to active center of beta-lactamases (such as sulbactam, tazobactam) were introduced into the clinical practice to combat antibiotic resistance. Due to emergence of resistant mutants of serine- $\beta$ -lactamases, these beta-lactam inhibitors lose effectiveness as well. New non beta-lactam inhibitors are needed. According to our previous work [1], humic substances (HS) from coal possess inhibition activity with respect to beta-lactamase TEM1. However, the mechanism of their inhibition activity is not known.

The goal of this work was to model interactions of probable individual components of HS with beta-lactamase TEM1 using molecular dynamics and molecular docking.

For identification of the probable molecular components of HS capable of beta-lactamase inhibition, dereplication procedure was used. For this purpose we used the data reported in [1] on inhibitory activity of narrow fractions of coal humatmelanic acid against TEM1- $\beta$ -lactamase, and the data on molecular composition of the same fractions as determined by Fourier transform ion cyclotron resonance mass spectrometry (FT ICR MS). Then, the data mining was conducted in ChEMBL database in search of the molecules with the reported inhibition activity against beta-lactamases and the same molecular formulae as those occurring in the molecular composition of HS.

Dereplication procedure thus gave 66 molecular formulae of substances corresponding to those in the molecular composition of HS. In accordance with their respective SMILES-descriptors, those 66 structures were drawn and their logP coefficient was estimated. Given the usual structural and chemical composition of HS, number of investigated probable HS-like structures was reduced down to 3 (Fig. 1).

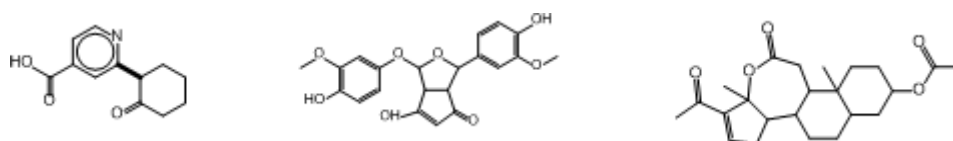


Figure 1. Probable individual HS components that show highest activity against serine- $\beta$ -lactamases.

During molecular dynamics simulation, it was found that HS-like ligands were capable of forming supramolecular aggregates in explicit solvent (water). Moreover, it was

shown that HS-like structures could interact with the known allosteric site of beta-lactamase TEM 1. This allows us to suggest that HS act as allosteric inhibitors.

This work was funded by the Russian Scientific Foundation (grant no 21-73-20202).

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## Application of silsesquioxane-humic complexes as dust suppressants

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Keywords: dust, pm2.5, pm10, sorption, humics

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The particulate matter (PM) contained in the dust, when weathered, pose a serious danger to the health of citizens and the ecology. Modification of humic substances with silanol derivatives leads to the possibility of their immobilization on various mineral particles, which can be used to bind dust and prevent wind erosion. Due to their lack of toxicity and resistance to biodegradation, they can be safely used on city streets using sprinklers. Previously, our laboratory developed methods and approaches for the synthesis of highly adhesive derivatives of humic substances - silsesquioxane-humic complexes (SSHC). The resulting complexes in the aqueous medium gradually condense with the formation of more curly and crosslinked structures. Spraying these complexes can lead to the binding of particulate matter especially coarse particles ( $\leq 10 \mu\text{m}$  – PM10) and fine particles ( $\leq 2.5 \mu\text{m}$  – PM2.5).

To synthesis SSHC 20 ml of a 10 g/l solution of humic substances (CHP-K, Powhumus potassium humate) were prepared. Then the aliquot of APTES (3-aminopropyltriethoxysilane) was added (50, 100, 200 mg for CHP-APTES-25, -50, -100, respectively). To prepare dust models the dried soil was sieved with 100  $\mu\text{m}$  mesh. For the experiment, 10 grams of model dust was placed in Petri dishes, after which 5 ml of derivatives or distilled water as control were added to dust and dried in the electric oven at 50 °C. To study of binding particulate matter by SSHC lab-scale air-blowing tests were used. In this test after treatment of model dust with SSHC and water as control, the samples were placed in a plastic container equipped with a compressed air source and a particle matter sensor. The determined concentration of particulate matter after exposure of compressed air on the samples is shown bellow

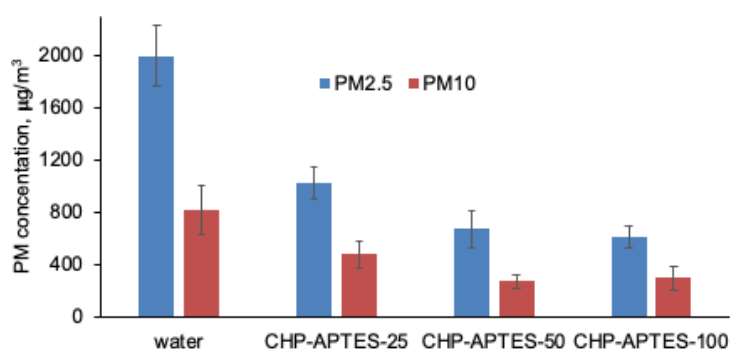


Figure 1. Determined concentration of particulate matter after exposure of compressed air to dust samples.

As can be seen from the above results, treatment with SSHC leads to a decrease the content of particulate matter in the air. Treated samples are more resistant to wind erosion. It can also be seen that this effect increases with an increase of organosilane content in the complex. This can be explained by the binding of small particles in the dust by silanol groups in the complexes. Thus, the complexes can be considered as promising dust suppressants.

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## Temperature influence on infrared spectra of brown coal humic substances

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Keywords: humic substances, FTIR, infrared spectroscopy, temperature infrared spectra, temperature-induced evolution of band frequencies

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IR spectroscopy is a developed method to study humic substances (HS). However, the bands of different HS functional groups overlap, and a resulting HS IR spectrum shows a small number of relatively uninformative broad bands. Changes in the vibrational spectra of solid samples upon heating or cooling provide information on polymorphic transformations, structural phase transitions, intramolecular interactions, and second-order phase transitions. However, even if such changes are absent, temperature-dependent IR spectra may reveal alterations in the band intensities, positions, shape, and width [1]. According to Hooke's law, the decreased nearest neighbor distances imply larger force constants and higher frequencies. Transitions between higher levels are less energetic than transitions between lower, which causes a redshift of band maxima with temperature



and band broadening [2]. Thus, the temperature dependence of band parameters in the IR spectrum of a solid has a fundamental nature that is mainly associated with the structural deformation of its crystal lattice. This effect has long been observed and studied in various minerals. According to our data, no similar studies with humic substances have been carried out.

Humic acid sodium salt (Sigma-Aldrich) and potassium humate from German leonardite and Sakhalin leonardite were used. The analysis by IR spectroscopy was carried out on an FTIR spectrometer with a single attenuated total internal reflection attachment with a diamond crystal heated in the range from room temperature to 215 °C.

It was shown that when heated in the range of 25–215 °C in air, the IR spectra of coal humic substances undergo several significant changes. Almost all bands, both belonging to mineral fragments and purely organic, shifted in frequency; this effect is reversible and, when cooled, the bands return to their original frequency. When comparing the samples, some bands demonstrate an almost complete identity of the temperature-induced change, while other bands behave differently. This approach provides a more detailed HS structure analysis without total HS decomposition or destruction.

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## Problems of modelling of trace adsorption phenomena in humic acid solutions

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**Keywords:** humic acids, complexation, sorption, colloids, microelements, radionuclides

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The current state of research in the field of complexation and sorption processes in systems with humic acids is characterized. Although humic acids are crucial for physicochemistry of heterogeneous transformations with participation of microelements and radionuclides, there is still little information about the stability of their humate complexes and the mechanisms of interaction. This is one of the main obstacles for modeling of mass transfer phenomena for humate complexes of radionuclides/microelements in complex technogenic objects and natural aquatic habitat. The information about the composition and stability of humate complexes is an element of humate reactivity assessment and is necessary for the description of their sorption and

biosorption behavior with respect to colloids, inorganic and organic suspensions. It can be obtained using highly sensitive up-to-date analytical techniques, first of all, chromatomass-spectrometry, nuclear magnetic resonance spectroscopy, X-ray absorption fine structure, modifications of the distribution method, including sorption, ultrafiltration, extraction, chromatography and electrochemistry methods.

Important, but yet poorly known is the role of photocatalytic transformations, occurring under the action of visible light, X-ray and gamma irradiation from both radiation background and radionuclide atoms – complexing agents, ligands ( $^3\text{H}$ ,  $^{14}\text{C}$  etc.), in the reactivity of humic acid and humate complexes. Further development of the theory of structure, functional properties and the nature of humic acids complexation selectivity will make it possible to describe the acid-base characteristics, redox properties of molecular and colloidal forms of humic acid and humate complexes in aqueous solutions. Some insight into the correlation between the type of humic acid functional groups and the stability of complexes can be gained by comparing them with the stability constants of lower carboxylic acids. The variations in the stability of these complexes are qualitatively similar, therefore the conclusion about the important role of the interaction of the central atom with carboxylic groups can be considered to be true for all known cationic humate complexes. The higher stability of humate complexes compared with acetate complexes is likely to be determined by the conditions of coordination of ions with humic acid carboxylic groups [1].

Years-long dynamics of the number of studies shows that the development of notions in the field of physicochemistry of heterogeneous transformations involving humate complexes of microelement ions, first of all *f*- and *d*-elements, is a pressing radiochemical challenge in the reactivity of humic acids. As natural high-molecular or colloidal compounds with unique functional properties and complexity, humic acids are able in a large measure to provide control in future over radionuclide mass transfer conditions in natural and technogenic environments.

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## Synthesis of zinc-containing hybrid materials for medical use

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Keywords: Zinc, complexes, metal-organic frameworks, humic substances, wound healing

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Zinc is a bioactive element, which takes part in cell division, differentiation, reparation, and in formation of T-cell immunity. It is included in different enzymes and plays an important role in the processes of skin regeneration, hair and nail growth. There is a growing interest in designing delivery agents of this element with high bioavailability

for human organism. In particular, slow release forms are needed. The purpose of this work was to synthesize zinc-containing hybrid materials capable of slowly releasing zinc.

We have synthesized Zn-MOF-5 from terephthalic acid and zinc acetate using optimized technique. This included optimization of the ratio and concentrations of the precursors selected. The reproducibility of the synthesis was confirmed. The synthesis was carried out in a Milestone Ultraclave III unit with microwave heating at the argon pressure of 40 atm. The resulting crystalline product was characterized by XRD and SEM methods. (Fig.1,2)

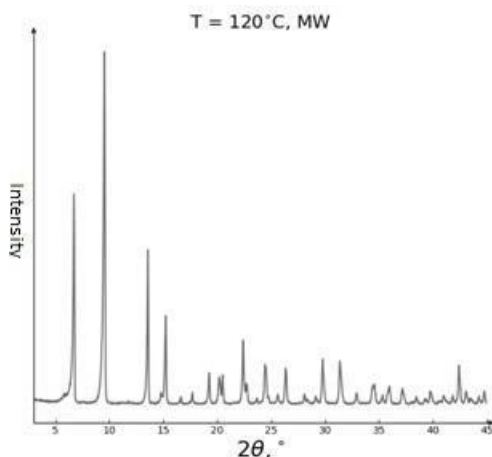


Figure 1. XRD of Zn-MOF-5

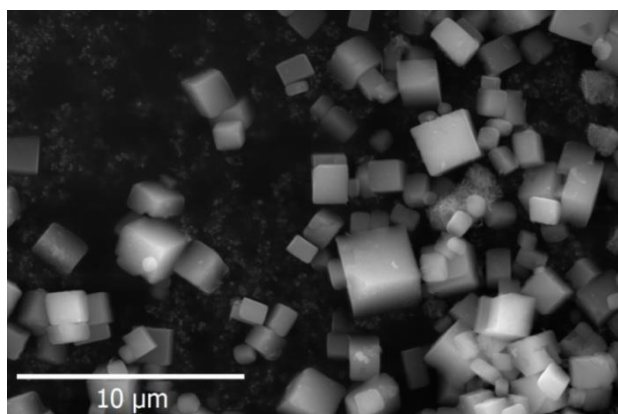


Figure 2. SEM of Zn-MOF-5

As the second form of slow release Zinc, complexes with humic substances were synthesized: zinc nitrate and sulfate were used as precursors. The Zn-humics complexes were synthesized by mixing the precursors at room temperature. The combination of XRD and EXAFS data confirmed the formation of zinc humate complexes.

The obtained Zn-containing materials could become a source of slow-release Zn ions for wound healing and other medical applications alternative to a use of zinc oxide. In the future, experiments will be carried out on zinc release rate determination from the obtained preparations. Also, experiments to identify the wound-healing properties of these preparations are planned.

**Acknowledgments.** XAS data were measured at the Kurchatov Synchrotron Radiation Source (project code RFMEFI61917X0007). This research was partially financially supported by Russian Science Foundation, project no 20-63-47070.

## Electrophysical characteristics of an activated composite based on humic acid and microspheres

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Keywords: composite, humic acid, microspheres, electrophysical characteristics

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Electrophysical properties are the main indicator of carbon materials used in electrothermal processes, as well as for the manufacture of superconducting materials, capacitors and fuel cells from them. Measurements of the electrophysical properties were carried out according to the technique [1].

In this work, the electrophysical characteristics of an activated composite based on humic acid and a microsphere (10/1) have been investigated. The porous carbon material was obtained by carbonization and activation in argon and water vapor. The study of the electrical properties (dielectric constant ( $\epsilon$ ), electrical resistance (R)) was carried out by measuring the electrical capacity of the samples on a serial device LCR-800 (Taiwan) at an operating frequency of 1 kHz, 5 kHz and 10 kHz continuously in dry air in a thermostatic mode with a holding time at each fixed temperature (293–483 K). Based on the data obtained, the band gap ( $\Delta E$ ) of the samples was calculated.

The electrophysical characteristics of the obtained composite material were investigated to study the possibility of using it as electrode materials for a supercapacitor. According to the results of the study, it was found that the activated HA: microsphere = 10:1 at all studied frequencies of 1, 5 and 10 kHz in the range of 293 - 483 K shows very high, and at 1 kHz - colossal values of the dielectric constant:  $2.27 \cdot 10^7$  (1 kHz),  $1.41 \cdot 10^6$  (5 kHz),  $4.14 \cdot 10^5$  at 293 K,  $7.20 \cdot 10^8$  (1 kHz) at 423 K,  $4.0 \cdot 10^8$  (5 kHz) at 483 K and  $1.27 \cdot 10^8$  (10 kHz) at 483 K. It should be noted that the dielectric constants of this relatively cheap carbon material can compete with a similar characteristic of the new material  $\text{La}_{15/8}\text{Sr}_{1/8}\text{NiO}_4$ , which has a gigantic dielectric constant of  $10^5$ - $10^6$ . The composite exhibits semiconductor conductivity in the range of 293-333 K, metallic conductivity at 333-363 K, and semiconductor conductivity again at 363-483 K. The band gap at 293-333 K is 0.60 eV, and at 363-483 K - 0.34 eV can be attributed to narrow-probe semiconductors. The data obtained demonstrate the prospects for using this product in the electrochemical industry, having already at 293 K a colossal dielectric constant, especially 1 and 5 kHz, is of interest for microcapacitor, and as a semiconductor is also important for semiconductor technology.

Acknowledgements. The work was carried out within the framework of project No. IRN AP09260096 on the topic "Development of technology and organization of pilot production of modified organic biological products based on humic polyelectrolyte acids obtained from the coals of Kazakhstan", funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

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## Obtaining fulvic acid from oxidized coal of Kazakhstan

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Keywords: oxidized coal, fulvic acid, humic acid, functional groups

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The paper presents the results of obtaining fulvic acid (FA) from oxidized coal in Kazakhstan and the study of its functional groups.

Oxidized brown coal from the «Maikuben» deposit with the following characteristics (wt%) was used as a feedstock:  $A^d$  - 25.8;  $W^r$  - 9.3;  $V^d$  - 46.6;  $S^{td}$  - 0.71. The content of humic substances is 56%.

Humic acids (HA) and FA are obtained on the basis of potassium humate, by acidification with 5% hydrochloric acid solution to pH 1.0-1.5. All organic substances remaining in the acidic solution after the isolation of HA were considered as fulvic acids. HA was separated by centrifugation, transferred to a filter, washed with distilled water until neutral pH, and dried at 60-70 °C to constant weight. FA was purified by passing through ion exchangers. The functional groups of the obtained sample were studied on an IR Fourier spectrometer (Nicolet iS 10) (Fig. 1).

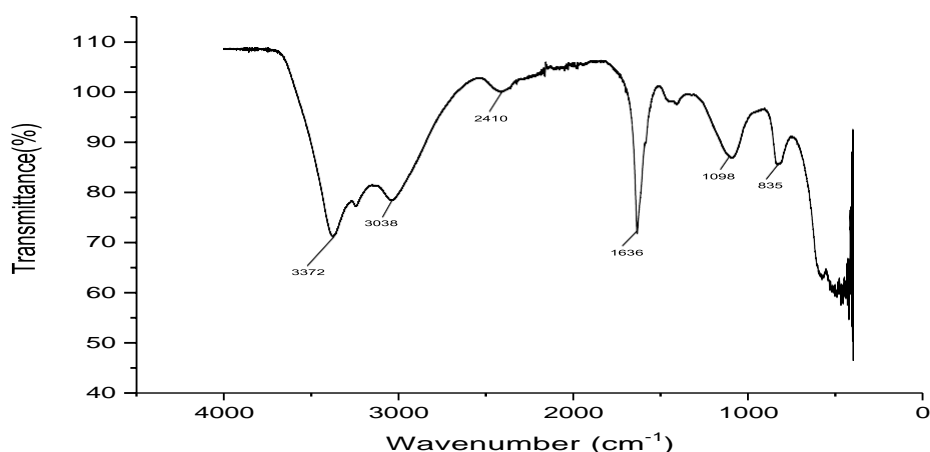


Figure 1. IR spectrum of fulvic acid.

According to the results of the study, a peak was found at 3372 cm<sup>-1</sup>, corresponding to the vibrations of N-H/O-H groups in the structures of amide, amines, carboxyl compounds linked by hydrogen bonds. In the region of 3100–3000 cm<sup>-1</sup>, vibrational vibrations of aromatic C–H bonds are observed. The less intense absorption peak at 2400 cm<sup>-1</sup> is probably due to the presence of aliphatic CH bonds. An intense absorption band at 1636 cm<sup>-1</sup> is characteristic of the carboxyl group. The absorption peaks at 1098 cm<sup>-1</sup> and 835 cm<sup>-1</sup> refer to vibrations of the -C-O bonds of phenols, polysaccharides and/or aliphatic groups. Thus, with the help of IR spectroscopy, it was found that the composition of PA contains both acidic and basic groups, which indicates the amphotericity of the sample.

Acknowledgements. The work was carried out within the framework of project No. IRN AP09260096 on the topic "Development of technology and organization of pilot production of modified organic biological products based on humic polyelectrolyte acids

obtained from the coals of Kazakhstan", funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

## Synthesis of carbon nanofibers based on humic acid and polyacrylonitrile by electrospinning method

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Keywords: carbon nanofibers, humic acid, polyacrylonitrile, electrospinning

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The article describes a method for producing carbon nanofibers from humic acid from oxidized coal and polyacrylonitrile (PAN) by electrospinning in laboratory conditions, the value of the interelectrode voltage was 20-25 kV. The elemental composition was determined and the surface morphology of the studied sample was studied, the type of modification of the carbon fiber was revealed. As a result of energy dispersive X-ray spectroscopy and SEM microscopy, the chemical composition of the initial CNF was revealed (C - 89.16%, O – 8.22%, Na – 0.11%, Mg – 0.02%, Al – 0.20%, Si – 0.18%, Cl – 0.11%, K – 1.11%, Ca – 0.09%, Fe – 0.55%, Cu – 0.24%), the diameter of carbon fibers was from 123.5 nm to 781.9 nm. Using the workover method, the degree of graphitization was calculated, which was - 23.97% and the ratio  $I(D) / I(G) = 0.7$ ,  $I(G) / I(D) = 1.4$

Humic acid and polyacrylonitrile are dissolved in N,N-dimethylformamide in an oven at 60 °C until homogeneous. Composition: humic acid-5%, polyacrylonitrile (PAN)-3%, N,N-dimethylformamide - 92%.

At the subsequent stages of high-temperature treatment - carbonization at 800 °C, accompanied by the removal of hydrogen and heteroatoms in the form of volatile compounds, the final formation of carbon fibers occurs (Fig. 1).

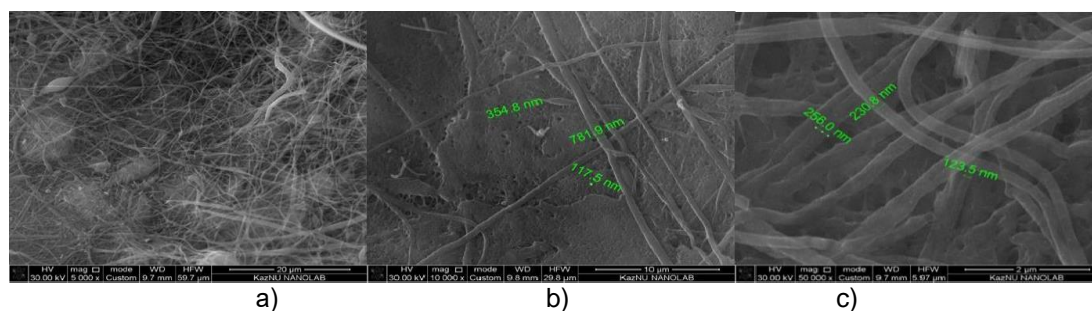


Figure 1. Electron microscopic images of carbonized carbon nanofiber based on humic acid and PAN: a) x5000, b) x10000, c) x50000.

Thus, the proposed method for producing CNFs is based on the electrospinning method, which is the most promising method of industrial production and provides a product with a relatively high fraction homogeneity, which determines the achievement of strength characteristics for structural materials. These characteristics make it possible to use CNF obtained from HA and PAN as an electrode material for batteries and

supercapacitors. Batteries with high energy density and long service life are widely used in our daily life, such as electric vehicles, mobile phones, laptops, etc.

**Acknowledgements.** The work was carried out within the framework of project No. IRN AP09260096 on the topic "Development of technology and organization of pilot production of modified organic biological products based on humic polyelectrolyte acids obtained from the coals of Kazakhstan", funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan.

## **Pharmacokinetic study of natural polyphenols and new insights on the mechanism of their action**

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**Keywords:** tissue distribution, mass spectrometry, structure, chemoinformatics, molecular target

DOI:10.36291/HIT.2021.071

Molecular complexity of natural polyphenols prevents description of molecular mechanisms of their biological action and hampers pharmacokinetic studies. They are composed of thousands of unknown compounds and routinely, tandem MS experiments are hardly applicable. Still, FTICR MS combined with chemical and isotopic labeling can be used to obtain various structural information. Additionally, incorporation of deuterium tags enables to explore tissue distribution of majority of compounds. Molecular and structural information can be further elucidated by using chemoinformatics approaches. Here, we report a first successful attempt to apply a comprehensive approach consisted of determination of individual components in mouse liver, structural characterization and full-structure generation by deep learning and application of chemical similarity network analysis (CSNAP) to predict exact molecular targets.

An algorithm has been developed for fully automatic calculation of the series of isotopic or chemical labeling. For each reaction, a set of heuristic rules was formulated which were taken into account in the computer code to remove possible analysis artifacts. Applied to liver extracts it resulted in detection of 600 and 450 components after intravenous and oral administration, respectively. For the determined components 100 thousand relevant structures were generated and recorded in the form of SMILES. The distribution of Bemis-Murcko scaffolds was calculated. The most common scaffolds of compounds corresponded to known natural compounds: flavonoids, coumarins, and steroids. Application of CSNAP method was adapted to create a weighted graph with vertices corresponding to joints and edges corresponding to chemical similarities between them. The generated CSNAP topology was used to find probable targets for compounds action using Schwikowski and Hishigaki scores. As a result, for 657 compounds there was at least one structural analogue found in ChEMBL. A total of 11 target proteins involved in the pathogenesis and progression of non-alcoholic fatty liver disease (NAFLD) were predicted. From the predicted biological targets, the three most probable were selected that would be associated with NAFLD and structures that would be active in relation to

these diseases: Kelch-like ECH-associated protein 1 (KEAP1), which is responsible for binding to Nrf2, Peroxisome proliferator-activated gamma receptor (PPAR $\gamma$ ) and AMP-activated protein kinase (AMPK). To examine the predicted results top-down proteome analysis was performed. Accordingly, alkylation of KEAP1 in the presence and absence of phenol mixture was performed followed by tryptic digestion and LC-MS analysis. The significant decrease of alkylation degree of three cysteine residues supported results of CSNAP prediction. Additionally, fraction enriched with the liver-detected compounds was obtained and tested against NAFLD model on mice showing normalization of biochemical blood parameters and increase of survival.

Acknowledgements. Mass spectrometry experiments were supported by RSF grant no 21-47-04405.

## **Effect of humic nature preparation on seed quality and growth processes of vegetable crops**

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Keywords: vegetable crops, sowing qualities, growth stimulator, humates, cropping capacity (yield).

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Currently, the world has sharply increased interest in organic fertilizers. This is primarily due to the fact that environmental problems caused by the total agriculture chemicalization have attracted the attention of the world community, which has become increasingly concerned about the harm caused to human health by pesticides, agrochemicals and other products of soil, water and air pollution. Consumers began to pay more and more attention to the quality of food products and their environmental friendliness. The use of environmentally safety products of natural origin is a global trend today. This is due to the fact that more and more data is being accumulated on the positive impact of humic substances on plant growth and development, as well as on the quality of agricultural products and soil fertility. Humates, being physiologically active substances, regulate and intensify metabolic processes in plants and soil, and contribute to the active intake of nutrients in a form that is digestible for plants.

We obtained a universal plant growth and development stimulator "KazGumates" from highly oxidized brown coal by extraction with alkaline reagents with the addition of a complex of amino acids, macro - and microelements, which is intended for all types of agricultural crops for growing environmentally friendly agricultural products in any soil and climatic zones.

"KazGumates" is a new – generation humic-free preparation with a high humification degree. The preparation composition includes easily soluble physiologically active salts of humic and fulvic acids (humates and fulvates), a complex of amino acids, macro - and microelements in an accessible form for plants. Eco-friendly product, completely soluble in water, which is extremely convenient for drip irrigation system. It is intended for all types of agricultural crops in any soil and climatic zones, acts as plant hormones: the optimal drug



concentration is 0.0001%. The preparation drug is used for pre-sowing seed treatment, foliar treatment during the growing season as an independent fertilizer and post-harvest soil treatment. Its use does not require changing existing agricultural technologies – it is compatible with any plant protection products and other fertilizers. It is used as a working solution both independently and in tank mixtures with plant protection products and soluble fertilizers as part of planned treatments. Increases the efficiency of mineral fertilizers and pesticides, reducing their use by 20-40%. It is characterized by economical production and use: pre-sowing seed treatment - 200 g/t, spraying of crops: cereals-150 g/ha, post-harvest soil treatment – 400 g/ha.

Laboratory and field tests conducted on potatoes and vegetable crops (tomatoes, cucumbers, cabbage, carrots, onions, beets) have shown high effectiveness of the drug "KazGumates", which significantly improves the sowing quality of vegetable seeds: increased germination and seed germination energy, intensive stimulation of root formation in plants, accelerated growth processes (formation of biomass and food organs), increased fruiting period, reduced maturation, higher yield of potatoes and vegetable crops. The results obtained in laboratory and field experiments on potatoes and main vegetable crops showed high humic growth stimulator efficiency by "KazGumates". This confirms that the use of humic compounds is the most promising agricultural technology direction. Their use is actively expanding, especially in connection with the desire to make agriculture as eco-friendly, efficient and economical as possible.

## **Iron (III) complexes with humic and polysaccharide macroligands for correction of iron deficiency anemia**

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Keywords: humic substances, iron (III), bioavailability, cytotoxicity.

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More than 2 billion people worldwide suffer from iron deficiency anemia (IDA) [1]. Despite the variety of drugs produced, the incidence rate is not decreasing. The most promising are Fe(III) preparations stabilized by polysaccharides, but the bioavailability of these preparations is 5 times lower than that of Fe(II) preparations [2]. We considered natural humic substances (HS) as new ligands capable of increasing bioavailability of Fe(III) and of reducing the number of side reactions. Humic macroligands contain phenolic and carboxyl groups, possess complexing and redox properties, which determines the prospects of their use for obtaining bioavailable complexes with Fe(III).

The following precursors were used: humic acids of coal (HA), fulvic acids of peat (FA), polymaltose (PM). We have synthesized 5 preparations: mixed-ligand (HS:PM = 1:1) and monoligand (HS=100%) preparations of iron (III) hydroxide humates with an iron content of 12-13%; mixed-ligand and monoligand fulvates of iron (III) hydroxide with an iron content of 7-8% and a monoligand reference preparation of iron (III) hydroxide polymaltose - 6% by weight of the dry preparation.

These preparations were characterized by X-ray phase analysis (XPA) and transmission electron microscopy (TEM). Diffraction patterns confirmed that all samples

are characterized by low crystallinity of the iron-containing phase, presented in the form of ferrihydrite. The phases are NaCl in fulvate and KCl in humic samples. According to the TEM data for the preparations, discrete nanoparticles with a size of 2-5 nanometers are observed.

To assess the bioavailability of iron in the composition of the preparations obtained, the ferrozine method was used.

According to the results of the ferrozine study, it was shown that the highest percentage of the content of bioavailable iron in the sample was shown by mixed-ligand preparations, about 6%. In monoligand preparations, the percentage of bioavailable iron was found to be lower within the range of 1-4%.

Cytotoxicity was assessed on two cell lines, 3T3-L1 and HepG2, by the MTT test.

The results of cytotoxicity assessment showed the absence of pronounced cytotoxicity in the entire line of drugs, IC<sub>50</sub> was not achieved.

The conclusions are that the largest percentage of iron and bioavailable iron is contained in the preparation of iron (III) hydroxide. In view of its non-cytotoxicity and bioavailability of iron, we can talk about the prospects for further study of mixed-ligand preparations as a potential active pharmaceutical substance (APS).

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### **Correction of iron deficiency anemia by Fe(III) hydroxocomplexes stabilized with humic ligands in rats**

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Keywords: humic ligands, iron deficiency, acute blood loss, Fe(III) hydroxocomplexes

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Iron deficiency is the most common cause of anemia. This kind of anemia (iron deficiency anemia, IDA) is usually associated with blood loss. Searching for substances with high iron bioavailability based on alternative ligands stabilizing Fe(III) hydroxocomplexes continues. In this regard, humic substances are promising source of new active pharmaceutical substances (APS).

C100 (Fe(III) hydroxide complex with potassium humate), C50 (Fe(III) hydroxide complex with potassium humate and polymaltose (1:1)), F100 (Fe(III) hydroxide complex with

fulvic acids) and F50 (Fe(III) hydroxide complex with fulvic acids and polymaltose (1:1)) samples were used in the investigation of humic iron-containing APSs pharmacodynamic activity. A Wistar rats (♀) model of acute blood loss (1.5% of body weight) was chosen [1]. Blood was taken from the tail vein under anesthesia. The results are presented in table 1.

All studied APSs demonstrate antianemic activity. The most pronounced activity was shown for C100 sample. Less activity was observed for F100 sample. C50 and F50 showed the least activity. It can be assumed that the lowest activity of C50 and F50 is probably due to the synthesis method applicable for these APS (combined matrices). Iron disrupts polymaltose cycles, which reduces the stability of the latter.

Table 1. Influence of acute blood loss and daily (5 days) intragastric administration of C100, C50, F100, F50 samples and reference drug Ferrum Lek® on hematological parameters and serum iron level in rats ( $X \pm SE$ )

Group of animals	Hematological indicators							
	Before IDA modeling				After course of intragastric administrations			
	HGB, g/L	HCT, %	RBC, $\times 10^{12}/L$	Fe, $\mu M$	HGB, g/L	HCT, %	RBC, $\times 10^{12}/L$	Fe, $\mu M$
Water (n=5)	193,5 $\pm$ 6,4	55,0 $\pm$ 1,8	9,6 $\pm$ 0,6	58,9 $\pm$ 3,6	147,6 $\pm$ 2,1*	44,3 $\pm$ 0,7*	7,2 $\pm$ 0,1*	37,4 $\pm$ 4,0*
Ferrum lek® (n=5)	195,0 $\pm$ 8,7	59,0 $\pm$ 2,8	10,4 $\pm$ 0,4	43,9 $\pm$ 6,3	156,0 $\pm$ 3,6*	47,2 $\pm$ 4,5*	7,8 $\pm$ 0,2*	46,5 $\pm$ 4,5
C100 (n=5)	185,0 $\pm$ 6,4	56,0 $\pm$ 2,3	10,4 $\pm$ 0,4	52,3 $\pm$ 5,9	176,4 $\pm$ 7,7#	52,7 $\pm$ 2,3#	8,8 $\pm$ 0,4#	50,8 $\pm$ 2,4#
C50 (n=5)	191,0 $\pm$ 13,3	43,6 $\pm$ 1,6	7,8 $\pm$ 0,3	56,3 $\pm$ 7,0	146,0 $\pm$ 2,9*	42,6 $\pm$ 0,8	7,1 $\pm$ 0,3	38,8 $\pm$ 7,3
F100 (n=5)	177,0 $\pm$ 4,7	52,8 $\pm$ 1,5	9,3 $\pm$ 0,2	51,4 $\pm$ 2,9	169,6 $\pm$ 2,0*#	51,7 $\pm$ 0,6#	8,6 $\pm$ 0,1#	51,8 $\pm$ 1,7#
F50 (n=5)	172,1 $\pm$ 3,0	43,5 $\pm$ 2,8	7,9 $\pm$ 0,4	58,7 $\pm$ 3,9	143,6 $\pm$ 6,8*	41,4 $\pm$ 1,9	7,1 $\pm$ 0,4	51,1 $\pm$ 4,4

Notes: 1. \* differences with the group "Before IDA modeling" are statistically significant,  $p < 0.05$  (Friedman's test). 2. # differences with the "Water" group are statistically significant,  $p < 0.05$  (Kruskal-Wallis's criterion).

The work was carried out with the financial support of the Siberian State Medical University (Regulation No. 34 dated 04.09.2020).

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## Influence of water-soluble humic substances on the accumulation of $^{241}\text{Am}$ and $^{242}\text{Pu}$ by submerged macrophyte *Elodea canadensis*

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Keywords: *Elodea canadensis*,  $^{241}\text{Am}$ ,  $^{242}\text{Pu}$ , humic substances, accumulation

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Aquatic plants are perennial (sometimes annual) plants whose necessary habitat is fresh (mostly), salty or briny water. Biological availability of radionuclides from global particles is determined by the time of their formation, composition and dispersion. Leaching of

radionuclides is, as a rule, higher in fine particles than in coarse ones. Particles containing radionuclides from the global fall out are extremely small (of up to about 1  $\mu\text{m}$ ) and can almost completely be dissolved in water. The biological mobility of radionuclides is commensurable with their mobility in aqueous solutions.

In the course of the experiments without humic substance, it was revealed that most of the radionuclides was absorbed by the biomass during the first 24 hours of the contact, i.e. about 33-50 % of the added activity, to be followed by further accumulation of radionuclides by the biomass. A larger content of the accumulated  $^{241}\text{Am}$  was found in the exchange and absorption fractions, i.e., on the plant surface (Figure 1, a). The surface of plants taken from the natural environment is known to be covered with a sediment which consists both of mineral (carbonates, sulfates, particles of bottom sediment, etc.) and biological components, including the surfaces of the plant itself, i.e. epidermal tissue. In the medium where only the solution of  $^{241}\text{Am}$  is added, with time, there occurs the radionuclide redistribution into the biomass. However, for  $^{242}\text{Pu}$  binding with the exchange and adsorption fractions is not dominant (Figure 1, b). This is likely to be caused by the fact that in the case of  $^{242}\text{Pu}$ , due to its physicochemical properties different from those of Am, there appear competing processes of sorption and oxidation-reduction.

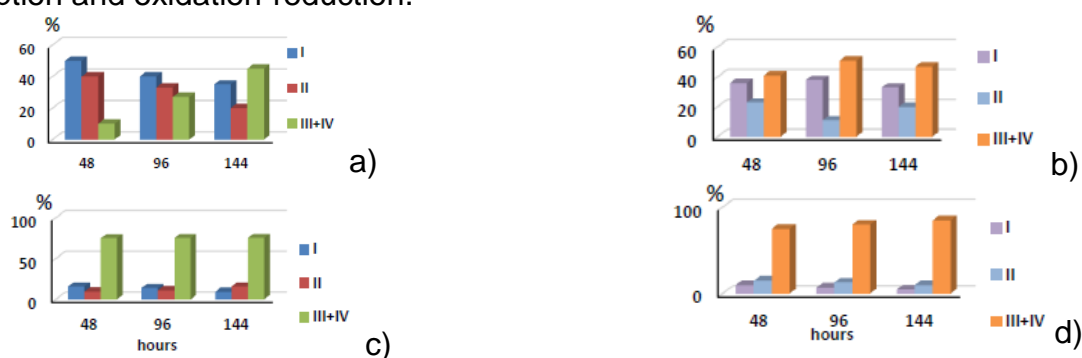


Figure 1. Dynamics of the distribution among the chemical fractions for  $^{241}\text{Am}$ ,  $^{242}\text{Pu}$  accumulated by the *Elodea* in the course of the experiment: I – exchange fraction; II – absorption fraction; III+IV – organic fraction+mineral residue upon without addition (a, b) and the addition of humic acids (c, d)

In the system with the addition of sodium humate the americium content in the plant biomass after as few as 48 hours from the beginning of the experiment reached the potential maximum and amounted to ~75 % from the accumulated amount (Figure 1, c,d). As in the case with americium, when salts of humic acid were added into the experimental system the fraction of plutonium in the plant biomass considerably increased (85 %), i.e. in both cases one can observe the process of hyperaccumulation of the radionuclides under study by the plant biomass; these radionuclides exist in the aqueous medium bound to an organic ligand, in this case, to a ligand of humic acid.

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## **Advertisement**

GENERAL SPONSOR



**Relict Organics® is a brand born in Novosibirsk Akademgorodok, the scientific center of Siberia. The manufacturing company "Genesis" develops and produces organic products for agriculture based on humic and fulvic acids.**

The product line includes complex humic products for crop farming, livestock farming and soil restoration applicable to both large producers and smaller private farms. The production uses innovative technologies, advanced equipment and high-quality raw materials.

All products and raw materials pass through several stages of quality control, which allows to consistently produce high-class products. The main ingredient for our products is Leonardite (an oxidized form of brown coal), which has a unique organic composition and a high content of humic acid, fulvic acid and other humic substances that have high biological activity.

Our team consists of specialists in the field of chemistry, physics and agronomy, continuously engaged in expansion of our product line, introduction of new technologies and optimization of the production process. Relict Organics® sales staff has extensive knowledge in crop and livestock production, thereby providing customers with competent advice on any issues and offering comprehensive solutions for their professional needs.

The main goal of Relict Organics® is ecologization of agricultural industry in the whole world, this is why our products are non-toxic and eco-friendly. Relict Organics® – SINCE THE BEGINNING...





**Contacts:**

**Manufacturer:** OOO NPP "Genesis"

+7 (905) 938 67 56 - sales department

+7 (905) 938 67 56 - for potential distributors

Working hours: 9 a.m. to 6 p.m. monday to friday (GMT +7)

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<https://en.relictorganics.com/>

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**ЛИГНОГУМАТ**  
КОНЦЕНТРИРОВАННЫЙ ГУМИНОВЫЙ ПРЕПАРАТ

**LIGNOHUMATE company being a composite part of RET Company Group is one of the oldest horticultural and livestock humic product market leaders. We have spent 25 years manufacturing and promoting the products of Lignohumate trade mark on both domestic and foreign markets of Europe, America, Asia.**

**Lignohumate® - helps agribusiness to grow since 1992**

Lignohumate® is the brand name for the humic products which are successfully nourishing 15 million hectares of crops. 56% of our products are imported by more than 20 countries, including the Czech Republic, Germany, the Netherlands, the United States and Canada.

**Key features of Lignohumate®**

- 100% solubility of dry modifications, lack of insoluble ballast in solutions
- Formulation constancy;
- Up to 90% DM active substances concentration;
- Up to 20% organic are fulvic acids. The smaller molecular weight of fulvic acids renders a bright stimulating effect on plants and soil microflora;
- A high sulfur in an organically bound form has a significant effect on the biological activity;
- The high concentration of lignin in the raw materials provides waste-free and environmentally safe production of humic concentrates.

Lignohumate® is a high-tech product of plant raw materials accelerated humification. Unlike other soil and plant amendment products with a local effect, Lignohumate® affects all physiological processes in plants, and significantly improves the water-physical and physicochemical soil qualities.

Contacts:

LIGNOHUMATE Ltd., St. Petersburg e-mail: [ksk@lignohumate.ru](mailto:ksk@lignohumate.ru)

Phone: +7 (812) 600-46-01 +7 (921) 767-10-40 website: <https://lignohumate.ru>

**Large-scale application**

Lignohumate® not only perfectly couples with fertilisers, pesticides and agrochemicals, but it also improves their efficiency.

Combined with mineral fertilisers Lignohumate® enhances the absorption of nutrients by plants. This allows to reduce the rate of fertilisers consumption.

Lignohumate® also works as an adhesive for chemical plant protectors. It makes seeds dressings and plant treatment more effective, and also relieves stress caused by the toxic effect on plants and soil.

Long-term studies also shows the ability of Lignohumate to remediate soils contaminated with ecotoxins and heavy metals.

**Benefits of partnership**

Working with us you get the benefits of direct deliveries from the manufacturer, scientific and practical 'from sale to the field' support by our specialists. We can assist with recommendations on complex treatment best suit the particular soil-climatic factors and culture.

We can also supply Lignohumate® as a component for your private label fertilisers, and agrochemical products.

Get involved in building the future based on a harmonized combination of nature friendly and resource-efficient technologies, and high quality agricultural production.



**Sakhalin Humates Group of Companies was established in 2000 and exclusively produces and distributes environmental friendly natural organo-mineral fertilizer Humate Sakhalinsky. The goal of Sakhalin Humates GC is manufacture and development of highly effective and environmental friendly products based on leonardite.**

Leonardite mining is carried out on the Sakhalin island, Russian Federation. All stages of extraction and selection of raw materials are carefully monitored by our specialists.



Sakhalin Humate GC products have a high content of humic substances and are available in various formulations (powder, aqueous solution, granules).

Especially for agriculture, the SAKHALIN HUMATES Group of Companies developed Potassium Humate and Sodium Humate.

These preparations are produced under the brand name AGRO and are positioned as a line of professional agrochemistry.

Distinctive features of humates of this line are high degree of oxidation of hydrocarbon chains and increased content of low molecular weight humic acids.

Humate "Sakhalinsky" under the AGRO trademark contain an optimum set of microelements in chelated form: silicon, iron, magnesium, sulfur, zinc, cobalt, copper, manganese, etc.

It goes well with fungicides, herbicides, growth regulators, with urea and alkaline microelement solutions.

The preparations have successfully passed a full range of agrotechnical tests and are recommended for widespread implementation, both for reclamation and restoration of depleted and infected lands, and as an effective accelerator of plant growth and development.



The use of humates significantly increases uptake of mineral nutrition and productivity of plants.

The high effectiveness of their use is confirmed both with the facts and figures of scientific reports of the best agricultural chemists and soil scientists of Russia, as well as by the positive reviews of agricultural enterprises that already use Potassium Humate and Sodium Humate ® AGRO.

Humate "Sakhalinsky" is fully suitable for organic farming.

Contacts:

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**LLC BashInkom Research and Development Enterprise was established in 1991. The RDE BashInkom has its own modern certified scientific and production laboratories: agrochemical, microbiological, physiological and breeding activities of microorganisms and phytopathology. The company is equipped with modern production and optical bases for the production of humic and bioactive fertilizers, as well as microbiological laboratories for the production of biofungicides, probiotics and feed additives for animal husbandry.**



The main activities of the company:

- Production of natural fertilizers and plant protection products, which can increase the yield several times without the use of pesticides;
- Introduction of effective biotechnologies for crop cultivation;
- Creation of multi-strain biologics for veterinary medicine, feed production, stubble processing, manure and storage of agricultural products;
- Development of high-performance drilling reagents for the oil and gas industry.



The RDE BashIncom LLC actively cooperates with local, regional and central research institutes of agricultural profile, many institutes of the Russian Academy of Sciences, plant protection stations and agricultural producers.

The RDE BashIncom LLC welcomes any mutually beneficial cooperation in the development, production and marketing of agro-technical products that meet our concept of Organic Live Farming, Anti-Stress High-Productivity Farming, Anti-Stress High-Productivity Livestock.

Our main products:

Integrated microbiological bioproducts for protecting plants from diseases and pests of the Fitosporin series.

Complex bioactivated humic preparations enriched with micro-, meso- and macroelements.

Contacts:

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## Life Force

**Life Force is an international group of companies including a research center for humic substances studying and modern manufacturing enterprises.**

For 18 years we have been developing feed additives for animal health and nutrition, as well as products and fertilizers for plants and soil based on humic and fulvic acids from leonardite.

We mine leonardite in ecologically clean regions of Siberia, using our own technologies to turn it into high-quality and effective products.

Our new development is the range of feed additives and a litter conditioner with a high content of humic substances under the brand name Reasil®. These products can significantly increase the profitability of any poultry and livestock enterprises by increasing productivity, improving quality and ensuring veterinary safety of products, as well as improving the environmental and hygienic conditions of livestock and poultry farming.

In addition to ready-for-use products for the livestock, poultry and crop farming, we offer various types of leonardite as a raw material with a content of humic and fulvic acids from 50 to 90%. We also provide the scientific organizations with leonardite for research and the development of new

products.



Life Force's leonardite is actively used by the companies around the world for the production of: medicines for people, feed additives and materials for livestock, poultry and aquaculture, fertilizers and soil conditioners for effective organic farming and the fight against desertification of soils.

Contacts:

Learn more about the company: [lifecycle.pro](http://lifecycle.pro)

Learn more about Reasil® Technology: [reasil.com](http://reasil.com)





**Land Green and Technologies Co., Ltd. (LGT)** - is Taiwanese company which is focusing on development of the new technology-advanced organic farming methods based on application of Nano and micro biotechnologies.

Taiwan is one of the world's leading countries with being constantly created, developed and worldwide implemented innovating ideas and technologies. Land Green & Technology follows this strategy and dedicates itself in developing of cutting-edge technologies, approaches and products applicable in agricultural industry.



Our main task is to help farmers to overcome daily challenges and help them to increase overall production efficiency and quality. The strategy of the company is to develop nanotechnologies applied in agriculture as well as the design of the first scientific cybernetic system of plant growth factors management and control named "Formula-G1". We focus on the world leading technology-advanced organic farming methods and application of Nano and micro biotechnologies.

One of the latest developments from the company is a smart LED lamp with mobile control. The lamp has 17 spectra of sunlight with automatic regulation of up to 250 kinds of light for each spectrum. Special lamp for research work on the role of light in plant growth

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SM GROUP OF COMPANIES  
 EDAGUM SM RUS LLC

## EDAGUM®SM HUMIC PRODUCTS

"Edagum SM Rus" LLC since 2006. specializes in the development and production of premium-class humic products from environmentally friendly raw materials - peat.

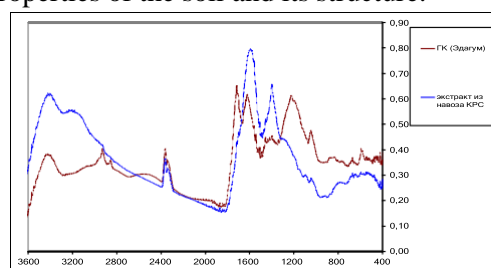
The high efficiency of the EDAGUM® brand products has been confirmed by studies of more than 30 specialized Russian and foreign research institutes. More than 80% of the products are exported to Europe, Central and Southeast Asia, Africa, North and South America.

As a result of the application of humic fertilizer EDAGUM®SM when processing vegetative plants, an increase in yield was achieved: on wheat up to 33.6%, rice - up to 27.4%, potatoes - up to 122%, tomatoes - up to 34%, soybeans - up to 33.7% %, cotton - up to 43%, etc.

The results of studies of the biological characteristics of the EDAGUM®SM humic fertilizer at the All-Russian Agricultural Microbiology Research Institute of the Russian Agricultural Academy (St. Petersburg, 2015) confirm the high efficiency of the product's impact on soil properties and the ability to stimulate plant growth and development, which is due to the high number of physiological groups of microorganisms contained in the fertilizer. It has been established that when EDAGUM®SM fertilizer is introduced into the soil, the respiration of microorganisms increases by 28.8%, the decomposition of organic compounds of nitrogen and phosphorus is accelerated and their bioavailability increases.

Studies by scientists of the Faculty of Soil Science of Lomonosov Moscow State University on wheat crops (2015) showed that the effect of processing 1 liter of EDAGUM®SM fertilizer on 1 hectare of arable land is equivalent to the introduction of 5-10 tons of cattle manure, and the IR spectra of

humic substances of fertilizer and manure show high similarity in the nature of functional groups. As a result of the influence of EDAGUM®SM fertilizer, the processes of the formation of young humus are accelerated and intensified, the product has a positive complex effect on the physical properties of the soil and its structure.



3-year scientific field trials at the Kursk State Agricultural Academy have shown the superiority of humic fertilizer EDAGUM®SM over specialized biological products in almost all tested indicators:

- 1) stocks of available moisture
  - 2) microbiological soil activity
  - 3) the density of the soil
  - 4) the number of earthworms in the soil
  - 5) nutrient regime of the soil
  - 6) phytosanitary state of crops
  - 7) plant growth and development
  - 8) barley yield and grain quality
  - 9) the content of heavy metals in soil and plants
- These and other scientific studies carried out in 2006-2021. allow us to conclude that systemic soil cultivation with EDAGUM®SM fertilizer is capable of restoring its fertility, improving environmental parameters, physical properties, structure, and making the products of higher quality and environmentally friendly.

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## MERC

The Life science business of Merck has brought together the exceptional knowledge, products, and capabilities of Merck Millipore and Sigma-Aldrich, it became one of the global leaders in the field of Life Science. Our purpose is to solve the toughest problems in life science by collaborating with the global scientific community – and through that, we aim at accelerating access to better health for people everywhere. We provide scientists and engineers with best-in-class lab materials, technologies and services. With the combination of Merck Millipore and Sigma-Aldrich, we now have a broad portfolio of 300,000 products, an expanded global footprint and the industry-leading eCommerce

platform - SigmaAldrich.com. We are dedicated to making research and biotech production simpler, faster and safer. Our three life science business areas are designed to best address our customers' needs:

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We are the Regional Chapter of the Commonwealth of Independent States of the International Humic Substances Society (CIS IHSS). The chapter was founded in Zvenigorod, Moscow Region, Russia at the Open Meeting of the Russian IHSS - Chapter held on September 25, 2002. As such, CIS-IHSS is a successor of the Russian IHSS Chapter founded in 1994. The main goal of the CIS IHSS is consolidation of the efforts of the CIS scientists in the field of basic and applied humic research. At present, the CIS IHSS has more than 100 members and is the largest chapter of the IHSS. The membership includes scientists, students, engineers, practitioners, and business representatives from Belarus, Kazakhstan, Kyrgyzstan, Russia, and Ukraine. The scientists and engineers are both from research institutes and industrial firms. The scope of scientific interests includes structure, molecular properties, genesis of humic substances, interactions of humics with heavy metals and organic ecotoxins, application of humics for remediation technologies, biological activity of humics; technologies of manufacturing and agricultural applications of humics, and others. We are glad to be a part of the world-wide humic research community. We are open for cooperation and any kind of HUMIC activities.

For more information about the CIS IHSS visit our website at <http://www.humus.ru/ihss/>.  
Information about the IHSS you can find at the website: <http://www.humicsubstances.org>

You are very welcome to join us!

Irina Perminova  
Regional Coordinator of the CIS IHSS

Olga Yakimenko  
Secretary of the CIS IHSS

Конференция проводится при финансовой поддержке следующих организаций:

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ООО «Научно-производственное объединение «Генезис», г. Новосибирск, Россия [www.relictorganics.ru](http://www.relictorganics.ru)

**Серебряный спонсор конференции**

ООО «Лигногумат», Санкт-Петербург, Россия  
<http://www.lignohumate.com>

**Спонсоры конференции:**

Land Green&Technology Ltd, Тайвань  
<https://www.lgt.tw>

ООО «Эдагум СМ Рус», Москва, Россия  
<http://www.edagum-sm.ru>

ООО «Научно-внедренческое предприятие «БАШИНКОМ», Уфа, Россия <https://bashinkom.ru>  
Международное гуминовое общество (International Humic Substances Society, IHSS), <https://humic-substances.org>

**круглых столов и цифровизации:**

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<http://www.humate-sakhalin.ru/>

Группа компаний «Life Force», Москва, Россия <https://lifeforce.pro>

Компания «Merck», Москва, Россия  
<https://www.sigmaaldrich.com>

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