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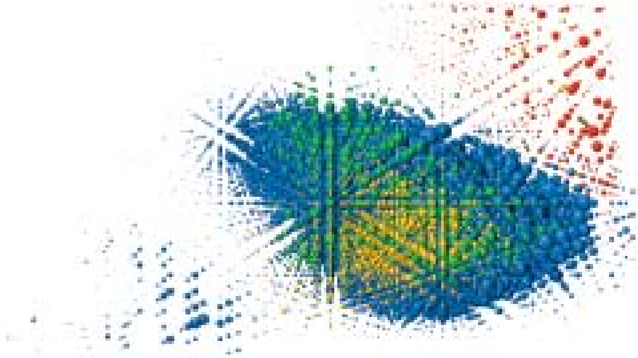

# From Molecular Analysis of Humic Substances – to Nature-like Technologies

Moscow
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# Invited Lectures

# Лекции приглашенных докладчиков

## The Study in the Model Experiment of the Effect of Biochar Introduction on the Intensity of Substrate-induced Respiration of Soils

K.G. Giniyatullin, E.V. Smirnova, R.V. Okunev, A.A. Valeeva, S.S. Ryazanov, I.A. Guseva
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The introduction of biochar to soils allows, in the opinion of most authors, to solve in the future the most important problems of our time: long-term improvement of soil fertility, waste utilization, and most importantly, the need for sequestration of atmospheric carbon. The possibilities of solving the latter problem began to be questioned to some degree in connection with the finding of a positive priming effect - increase in soil respiration (SR) due to the decomposition of soil organic matter (SOM) during the introduction of biochar. However there are research works in which a negative priming effect is detected or there is no significant effect on the SR. The discussion is also a question of the mechanisms of the effect of biochar on $CO_2$ emissions from soils.

In research works (Major et. al. 2010, Maestrini et. al. 2015) is expressed the opinion that the positive effect of biochars on SR is observed only in a short time due to the available of biochar OM. In the future the biochar can be considered as part of the SOM pool that resistant to oxidation. The aim of work: the assessment of the effect of the duration of soil-biochar mixtures incubation on the directivity, the intensity of substrate-induced respiration (SID) and evaluation of affecting factors.

For experiments used 10 biochar samples that prepared from various wood and grassy materials in different pyrolysis modes (400°C and in the range of 400-600°C). This biochars were characterized by 28 indicators including the content of organic carbon and nitrogen, the oxidability of biochar OM, ash content, pH, and the composition of acetate and water extracts. Model soil:biochar mixtures (20:1) were incubated at the optimum humidity and temperature. The SID value was determined after 3, 95 and 187 days of incubation. It was shown that the effect of incubation duration of 3 days operates in different directions, is observed a both decrease and increase in SID intensity and the direction of influence depends on the plant material. The incubation for 95 days leads to an increase in SID in all variants compared to the control. With further incubation (187 days) for some variants, there is a decrease in intensity, for some, on the contrary, an increase but for all variants the LED remains higher than in the control.

Regression analysis was used to evaluate the biochar properties affecting the intensity of SID. Since the data set refers to high-dimensional with a high degree of cross-correlation variables to evaluate relationships used ridge LASSO regression as well as multiple linear regression with step by step inclusion of variables. The inclusion of variables was carried out using the AIC criterion. At each stage of adding variables the VIF analysis was performed to diagnose the multicollinearity problem. It was revealed that the best results are obtained by the use of LASSO regression. The result of application of LASSO regression showed that during the short-term incubation a positively affects on SID the content of oxidized OM, $pH_{H2O}$ and negatively - the content of acetatsoluble sodium. During incubation of 95 days has a positive effect on the SID is the content of oxidizable OM, during 187 days - ash content. From the work it can be concluded that the duration of incubation of the soil:biochar mixture affects on SID in different directions and depends on its various properties.

The work was supported by the Russian Foundation for Basic Research – project № 17-04-00869.

# Biogeosystem Technique – Design of a Dispersed Soil System, Intra-soil Moistening, Intra-soil Waste Recycling – Priority Conditions for the Humic Substances Synthesis and Stability

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The significant role of humic substances in the provision of stable and high soil agronomic properties assumes the creation of prerequisites eliminating the climatic and anthropogenic stress of interaction in the system "environment – soil – organisms and biocosic matter of soil – technical means and technologies of agronomy, land reclamation, waste utilization".

The use of obsolete imitation principles of land use management leads to destruction of urban and agroecosystems, intensifies conflict between biosphere and humanity, increases a probability of modern biosphere evolution degradation scenario. The demand for new development vector is urgent in the world. It was proposed to develop nature-friendly technologies as a strategic reference point. The message is promising, but it assumes a creative approach to its comprehension and application, because "nature-similar" does not mean simple resemblance or copying – “nature-like”, but requires a much broader comprehension of the world development, especially given its current impasse in all directions.

Most modern technologies are built on unqualified imitation of natural phenomena, therefore they are not “nature-similar”, and the continuation of their application causes degradation of natural and anthropogenic biogeosystems, aggravates "ecosphere – technology" conflict. In aspect of humic substances this means a deterioration of of humic substances synthesis and stability in soil conditions, and soil fertility decrease.

In order to overcome the "ecosphere – technology" conflict, **Biogeosystem Technique** – the algorithm and technologies for controlling biogeochemical cycle in the gaseous, liquid, solid phase is proposed to create a biogeosystem with transcendental properties – not direct analogies with nature, but the search for a niche that nature has left for continuation of her intents. The approach allows, without contradiction to nature, to create a soil with highly disperse illuvial horizon; reduce the rate of fresh water consumption for bioproduction; Increase the productivity of environmentally safe recycling in disperse soil system. Biogeosystem Technique, in comparison to natural conditions and known standard imitation technologies, makes it possible to activate the biosphere process, have the priority conditions for humic substances synthesis and stability, healthy soil, increased soil biological production, save environment, resources and food at high production results, long-term economic benefits in a unified technological cycle.

The technical solutions and technologies of Biogeosystem Technique are developed that have no direct analogues in the world:

– milling processing of the soil inner layer of 20–50 cm allows to improve the conditions for humic substances synthesis and stability, increase the soil fertility by 30–80 % to the period up to 40 years, increase the profitability of farming technology by 2–3 times;

– recycling of industrial (including food waste) and domestic waste in the dispersed soil system in an amount up to 500 t/ha during milling the inner soil layer of 20–50 cm to stimulate the saprophyte activity and humic substances synthesis and stability;

– intra-soil pulsed continual-discrete irrigation watering provides water saving – a global deficit – by 5–20 times, eliminate soil over-moistening forms the optimal condition for soil biota, in particular for humic substances synthesis and stability;

– within soil recycling of hazardous chemical and biological waste in milling the inner soil layer of 20–50 cm and intra-soil pulsed continual-discrete irrigation watering provides the rupture of trophic distribution chains of poisons and infections, ensures the processing of biological material by soil destructors, improve the humic substances synthesis and stability, increase the veterinary, medical sanitary safety and fertility of soil.

Biogeosystem Technique provides:

– conditions for humic substances synthesis and stability;

– elimination of soil and landscape degradation;

– long-term improvement of soil fertility;

– waste recycling;

– enhancement the biospheric cycle of carbon, nitrogen, other elements and water through increased photosynthesis, accelerated growth of fresh organic matter, oxygen, free ions. biological diversity of biosphere;

– safety and quality of environment, terrestrial and aquatic ecosystems;

– stability of biosphere;

– stable earth's climate;

– reducing the desert area and increasing the area of land suitable for human habitation;

– accelerated environmentally sound technological development;

– growth of environmentally friendly food and raw materials;

– growth of environmentally friendly bio-fuels and biogas;

– restoration of resources;

– development of robotics;

– increase of employment in a knowledge-based environmentally safe production sector;

– decoupling – reduction of resources and energy consumption for production of an environmentally safe biomass of food and raw materials by 20–30 times.

Biogeosystem Technique has a world-class priority, ensures the development of domestic science-intensive industry, agriculture, environmental protection, the export orientation of domestic technological development, the priority conditions for the synthesis and stability of humic substances.

Long-term technological development of the Russian Federation on the principles of Biogeosystem Technique will ensure a high quality of life, a developed sustainable civil society of the Russian Federation, the possibility of Russia's influence the long-term processes of harmonious crisis-free world development in a high-quality sustainable biosphere on the technological platform of the noosphere.

Biogeosystem Technique provides a new quality humic substances beneficial effect on soils and plants.

# Биогеосистемотехника – конструирование дисперсной системы почвы, внутрипочвенное увлажнение почвы, внутрипочвенный рециклинг отходов – приоритетные условия синтеза и устойчивости гуминовых веществ

Валерий П. Калиниченко[1,2], Владимир Г. Систер[3], Андрей Н. Цедилин[3], Дмитрий А. Макаренков[3,4], Вячеслав И. Назаров[3]
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Знаковая роль гуминовых веществ в обеспечении стабильных высоких агрономических свойств почв предполагает создание предпосылок, элиминирующих климатический и антропогенный стресс взаимодействия в системе «окружающая среда – почва – организмы и биокосное вещество почвы – технические средства и технологии агрономии, мелиорации, утилизации отходов».

Применение устаревших имитационных принципов природопользования ведет к разрушению урбо- и агроэкосистем, усиливает конфликт биосферы и человечества, повышает вероятность реализации деградационного сценария в эволюции современной биосферы. В мире актуален запрос на новый вектор развития. Для того чтобы не воспроизводить одни и те же ошибки, в качестве стратегического ориентира предложено разрабатывать природоподобные технологии. Посыл многообещающий, но предполагает творческий подход к его осмыслению и применению, ведь «природоподобный» не означает простое подобие, копирование, требует значительно более широкого осмысления развития мира, особенно с учетом его текущей тупиковой ситуации по всем направлениям.

Современные технологии построены на неквалифицированной имитации природных явлений, потому не являются природоподобными, продолжение их применения обусловливает деградацию природных и антропогенных биогеосистем, усугубляет конфликт «экосфера – технология». В аспекте гуминовых веществ это означает ухудшение условий синтеза и устойчивости гуминовых веществ в почве, уменьшение плодородия почвы.

С целью преодоления конфликта «экосфера – технология» предложена **биогеосистемотехника** – алгоритм и технологии управления биогеохимическим циклом вещества биогеосистем в газообразной, жидкой, твердой фазе для создания биогеосистемы с трансцендентальными свойствами – не прямые аналогии с природой, но поиск ниши, которая природа оставила для возможности продолжения её замысла. Подход позволяет, без противоречия природе, создавать почву с высокой дисперсностью иллювиального горизонта; уменьшить норму потребления пресной воды на производство биопродукции; увеличить производительность экологически безопасного рециклинга вещества в дисперсной системе почвы. Биогеосистемотехника по сравнению с естественными условиями и известными стандартными имитационными технологиями позволяет активизировать биосферный процесс, что позволяет непротиворечиво решать производственные и экологические проблемы в едином технологическом цикле, получать прирост ресурсов и продовольствия с высоким производственным результатом и сниженными затратами, обеспечивает устойчивость и качество биосферы, климата и долгосрочную экономическую выгоду.

Разработаны не имеющие аналогов в мире технические решения и технологии:

– фрезерная механическая обработка внутреннего слоя 20-50 см позволяет увеличить плодородие почвы на 30-80% до 40 лет, повышение рентабельности технологии земледелия в 2-3 раза;

– рециклинг промышленных и бытовых отходов, отходов пищевых производств внутри дисперсной системы почвы в количестве до 500 т/га в процессе фрезерной механической обработки внутреннего слоя 20-50 см;

– внутрипочвенная импульсная континуально-дискретная парадигма ирригации обеспечивает экономию воды – глобального дефицита – в 5-20 раз;

– утилизация внутри почвы опасных биологических отходов за счет разрыва трофических цепей распространения инфекций обеспечивает переработку биологического материала почвенными деструкторами, повышение плодородия и ветеринарно-медицинскую санитарную безопасность.

Биогеосистемотехника обеспечивает:

– исключение деградации почв и ландшафтов;

– долгосрочное улучшение плодородия почв;

– рециклинг отходов;

– усиление биосферного цикла углерода, азота, воды и других элементов посредством усиления фотосинтеза, ускоренный прирост свежего органического вещества, кислорода, свободных ионов. биологическое разнообразие биосферы;

– безопасность и качество окружающей среды, наземных и водных экосистем;

– устойчивость биосферы;

– стабильный климат Земли;

– сокращение территории пустынь и увеличение площади суши, пригодной для проживания людей;

– ускоренное экологически содержательное технологическое развитие;

– прирост экологически чистой продовольственной и сырьевой базы;

– прирост экологически чистого биотоплива и биогаза;

– восстановление ресурсов;

– развитие роботизации;

– увеличение занятости населения в наукоемкой экологически безопасной производственной сфере;

– декаплинг ввиду уменьшения расхода ресурсов и энергии на производство экологически чистой единицы биомассы продовольствия и сырья в 20-30 раз.

Биогеосистемотехника имеет приоритет мирового уровня, обеспечивает развитие отечественной наукоемкой промышленности, сельского хозяйства, охрану окружающей среды, экспортную ориентацию отечественного технологического развития, приоритетные условия синтеза и устойчивости гуминовых веществ.

Долговременное технологическое развитие РФ на принципах биогеосистемотехники обеспечит высокое качество жизни, развитое устойчивое гражданское общество РФ, возможность влияния РФ на долгосрочные процессы гармоничного бескризисного развития мира в высококачественной устойчивой биосфере на технологической платформе ноосферы, обеспечить новое качество благотворного воздействия гуминовых веществ на почвы и растения.

# Understanding Natural Organic Matter at the Molecular Level: van Krevelen Diagram and Beyond

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Ultrahigh resolution mass spectrometry has become one of the important tools to study the natural organic matters. There has been a number of developments in analytical method and instrumentation that enabled the application. For an example, a graphical data interpretation method such as “van Krevelen Diagram” played an important role. With the van Krevelen Diagram, qualitative interpretation of high resolution mass spectra of natural organic matter is possible and it has been widely used since reported for the first time in 2003. In addition, recent instrumental developments such as “Paracell” and “quadrupolar detection” have enabled us to acquire data with higher resolution and mass accuracy. However, more works are needed to be done to achieve more complete understanding of natural organic matter at the molecular level. One of the key areas needing development is structural interpretation of natural organic matter. Understanding structures of the elemental formulae determined by ultrahigh resolution mass spectrometry is very important to elucidate the chemistry, transportation, and environmental influence of natural organic matter. To study chemical structures, we need to use hyphenated approach including ultrahigh resolution mass spectrometry, ion mobility mass spectrometry, chromatographic separation and new ionization technique. Especially, ion mobility mass spectrometry can be effectively combined with ultrahigh resolution mass spectrometry and theoretical calculation for structure identification.

# Mechanisms of Detoxification by Humic Substances. Bioluminescent Monitoring

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Humic substances (HS) are known to act as natural attenuators of toxicity of a lot of environmental pollutants – metals, surfactants, hydrocarbons, and organic oxidizers. Elucidation of detoxification mechanism is of great challenge for researchers. It is known that carboxyl, quinoid, phenolic, SH-, and other electron-donating groups are responsible for binding, and hence, decrease of content of free metal ions in water ecosystems. Hydrophobic HS fragments (aliphatic and aromatic) are able to deactivate organic pollutants by hydrophobic binding. Phenolic, SH-, and other groups of HS macromolecules are supposed to reduce toxic effects of organic and inorganic oxidizers in environment.

The mechanisms mentioned demonstrate 'chemical' approach to detoxification processes. However, this approach can hardly be applied to forecast toxic effects to living organisms in practice, due to non-additivity of effects of numerous toxic compounds (i.e. effect of sum is more or less than sum of effects of individual compounds). This problem is especially important for HS, as detoxifiers, due to their poly-functionality.

Integral effects of toxic compounds can be detected only by biological assays. It is supposed that combination of chemical and biological methods is able to provide with complex information on ecological state of environment.

In our study we used bioassays based on marine luminous bacteria. These types of bioassays are widely applied to monitor environmental toxicity for more than forty years. The tested physiological parameter here is the luminescence intensity; it can be easily measured instrumentally. The bacterial bioluminescent assays can be based on biological systems of different complexity – bacteria or their enzymes, with this providing study of effects of toxic compounds on cells or enzyme reactions, respectively.

The study aimed at classification of detoxifying mechanisms on chemical, biochemical, and cellular levels. The HS were used as detoxifying agents in model toxic solutions. A series of organic oxidizers and reducers (quinones and corresponding diphenols) and salts of metals (stable and radioactive) were applied as model toxic compounds.

Marine luminous bacteria *Photobacterium phosphoreum* and bioluminescent system of coupled enzymatic reactions were applied as bioassays to monitor toxicity of solutions. Ability of HS to decrease or increase toxicity of the solutions was demonstrated. Detoxification coefficients were calculated and HS detoxifying concentrations were determined. Antioxidant properties of HS were considered in detail. The detoxifying effects of HS were shown to be complex and regarded as 'external' (binding and redox processes in solutions outside the organisms) and/or 'internal' organismal processes. It was demonstrated that the HS can stimulate a protective response of bacterial cells as a result of (1) changes of rates of biochemical reactions and (2) stabilization of mucous layers outside the cell walls. Acceleration of auto-oxidation of NADH, endogenous reducer, by HS was suggested as a reason for toxicity increase in the presence of HS due to abatement of reduction ability of intracellular media. Role of Reactive Oxygen Species (ROS) in detoxification and toxic effects was studied and discussed.

## Theoretical Description of Photophysical Mechanisms Responsible for Similarity of Optical Properties of Humic Substances

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Optical methods are routinely used to characterize structural changes in humic substances (HS) in a wide range of applications including marine geochemistry, hydrology, soil science etc. The main advantage of this technique is its simplicity, cost, time of analysis and relative sensitivity. Based on the results of phenomenological studies, the so-called optical descriptors, which are parameters extracted from HS absorption and fluorescence spectra (e.g. SUVA and E4/E6), have been introduced to assess the origin of HS and its composition (e.g. the humification degree). However, there is no theoretical model, which describes the mechanism of HS optical properties formation and the reasons, which underlay similarity in the behavior of HS absorption and emission spectra regardless of their dramatic structural diversity. In this work, we report on the development of such model based on the results of investigation of heterogeneous set of HS, characterized by different origin, by a set of optical techniques and high resolution mass-spectroscopy (MS).

All HS are characterized by the following trends in optical properties: (i) exponential decay of absorption with wavelength (which is known in theoretical physics for disordered systems as an Urbach's rule), (ii) monotonous shift of the emission maximum and decrease of fluorescence quantum yield and fluorescence intensity with the excitation wavelength, (iii) decrease of fluorescence lifetime with the registration wavelength, (iv) spectral shifts and changes in the slope of the absorption spectrum accompanying chemical transformation of HS during oxidation, reduction, photobleaching, humification etc. We tried to develop a model which describes all these features of HS and is capable of predicting optical properties of HS on the basis of the knowledge about molecular composition of their components (provided by the MS data) and, visa versa, to predict major structural determinants of HS based on their optical properties. For this purpose, three major tasks were solved: (1) to determine the major molecular components responsible for HS optical properties formation due to interaction between them and (2) to show how intermolecular interactions (e.g. charge transfer) in disordered ensembles of chromophores/fluorophores result in the integral properties (i-iv) and (iii) what is the minimum set of components capable of providing properties (i-iv) for the system. The obtained results are of use for developing new optical descriptors of the HS structure and may help to reach a new level of understanding of molecular organization of HS, including the interaction of between molecular components in the ensemble.

## Molecular-level Insights into the Reactivity and Optical Properties of Dissolved Organic Matter in Aquatic Ecosystems

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The link between composition and reactivity of dissolved organic matter (DOM) is central to understanding the role aquatic systems play in the global carbon cycle; yet, unifying drivers of molecular composition have yet to be established. Data will be presented analyzing the source ($\delta$13C-dissolved organic carbon (DOC)), age ($\Delta$14C-DOC), and detailed molecular composition of DOM isolates from a diverse array of aquatic environments from headwaters to the oceans using absorbance and fluorescence spectroscopy and ultrahigh resolution mass spectrometry. The 37 isolates analyzed included end-members of allochthonous and autochthonous DOM from sites across the United States, the Pacific Ocean, and Antarctic lakes. Optical properties reflecting increased aromaticity and modern $\Delta$14C-DOC were directly related to polyphenolic and polycyclic aromatic compounds, whereas optical properties reflecting autochthonous production and enriched $\delta$13C-DOC were directly related to more aliphatic compounds as detected by ultrahigh resolution mass spectrometry. Furthermore, the two sets of autochthonous end-members (Pacific Ocean and Antarctic lakes) exhibited distinct molecular composition. However, across all sites and end-members studied, we find that aged $\Delta$14C-DOC is linked to composition, suggesting the formation of terminal degradation products given enough time in the majority of aquatic ecosystems.

One interesting and increasingly important exception to this apparent trend in DOM composition link to age is permafrost thaw derived DOM. Arctic permafrost soils contain vast quantities of ancient organic matter. Numerous studies have shown extensive permafrost thaw and degradation in the Arctic, but dissolved organic carbon (DOC) exported from the mouths of large Arctic rivers – which are expected to integrate processes and changes occurring through their watersheds – has been shown to be predominantly modern. This raises the question, where is the ancient DOC that is mobilized from permafrost thaw and the deepening of the active layer? Data encompassing DOC radiocarbon age, biolability, photolability, and DOM composition via FTICR-MS in permafrost thaw streams and the Kolyma River mainstem will be presented. Ancient permafrost thaw stream DOC is observed to be highly biolabile particularly in comparison to modern Kolyma River mainstem DOC, whereas it is not very photochemically active. In conjunction with this high biolability the permafrost thaw stream DOM exhibits large changes in molecular structure, loss of hydrogen rich (energy rich) aliphatic molecules, and production of molecules in the classical area in van Krevelen space associated with riverine DOM. Modern Kolyma River mainstem DOM conversely appears very stable in bioincubations in comparison to ancient permafrost thaw DOM. Thus the apparent offset between mobilization of ancient permafrost derived organic matter and the current predominantly modern age of DOC at the mouth of major Arctic rivers may be explained due to microbial degradation of permafrost derived DOC within the river's hydrologic residence time.

## Molecular Hysteresis of Riverine Organic Matter

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Rainfall-runoff processes have emerged as key controllers of the quantity and quality of terrestrial dissolved organic matter (DOM) exported from the landscape to inland waters. Hydrological events result in increased river discharge and a concomitant release of large amounts of DOM into fluvial networks. This study is part of a Macrosystems project which aims to test the Pulse-Shunt Concept: where rivers are converted from active to passive pipes during high discharge events ("pulse"), transporting labile, terrestrial DOM downstream ("shunt"), and relocating biogeochemical hotspots for DOM from the upper to the lower reaches of the watershed. The primary objective of our study was to track hysteretic changes in riverine DOM molecular composition over the course of storm events. Samples were collected from nested watersheds in the Passumpsic River catchment, a tributary of the Connecticut River (USA). High resolution monitoring (via in-situ sondes) and high frequency collection of discreet samples for FT-ICR/MS analysis captured short-term, hydrologically-driven variations in DOM concentration and composition. At the onset of the discharge event, we observed a unique DOM signature, enriched in aliphatic, and potentially biolabile, DOM. During peak discharge, and along the falling limb of the hydrograph, an aromatic, terrestrial-type DOM signature was more prevalent. Insights into the molecular hysteresis of fluvial DOM spotlights the impact of watershed hydrology on biogeochemical cycling in river networks and will be discussed within the intellectual framework of the Pulse-Shunt Concept.

## Immunotropic Activity of Peat Humic Acids

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The immunomodulating properties of humic acids (HAs) as are upcoming trend in the experimental pharmacology. According to V. Vetvicka [1], the HAs given a boost the interleukin-2 and antibodies secretion, the growth of tumor cells of experimental lung cancer Lewis inhibition and the manifestations of LPS-induced hepatotoxicity reduction. The HAs contribute to wound healing processes (in the HaCat cell model). In our opinion, HAs have influence in the immune system cells and, first of all, it have influence in the antigen-presenting cells-macrophages. Besides from the production of interleukins 1, 6, 12, tumor necrosis factor, the stimulation of inducible NO synthase is one of the fingerprint of the classical activation of macrophages towards enhancing the pro-inflammatory properties [2]. Therefore, the main goal of our paper is to study the capability of HAs the production of nitric oxide release by peritoneal macrophages of mice in vitro in the dynamics of various concentrations. This is allow to us to produce the treatments for correcting the immunity system in chronic, sluggish infectious inflammatory processes.

Materials and methods

The HAs isolated from nine samples of peat by 0.1 M solution of NaOH and 0.1 M solution of $Na_4P_2O_7$. The samples of peat were taken out from peat bogs of West Siberia (Tomsk oblast'). The biological activity of HAs was evaluated in a culture of peritoneal macrophages obtained from intact mice. In the experiment, 100 C57BL/6J mice of both sexes were used at the age of 8-12 weeks (1 conventional category according to the health certificate). Animals were kept in standard vivarium in an incomplete barrier system. In order to obtain the macrophages, the mice were washed by abdominal cavity with an ice isotonic sodium chloride solution. The resulting cell suspension was incubated during 2 hours (under conditions of 100% moisture and 5% $CO_2$) in plastic Petri dishes $2–2.5\times10^6$/ml in the culture medium after that the adhering to the plastics cells were collected. The macrophages were transferred to flat-bottomed 96-well plates and cultured ($3.0\times10^6$ cells/ml) during 48 hours in the presence of 1 μg/ml lipopolysaccharide (LPS) (serotype O111: B4, «Sigma», USA). The production of nitric oxide (NO) was evaluated by the content of nitrites in cell supernatants with the aid of the Grace reagent [3] which was mixed with an equivalent supernatant volume using a multichannel Titertek Multiskan® MCC spectrophotometer («Labsystems», Finland) under the wavelength of 540 nm. The concentration of nitrites was determined from a calibration curve constructed using standard solutions of sodium nitrite. The statistical processing was carried out using the software Statistica 6.0, using a single-factor analysis of variance and the Dunnett's t-test.

Results and Discussion

The HAs were tested by NO-stimulating ability with macrophages of mice at concentrations of 0.1, 1, 10, 50 and 100 μg/ml. Using the standard macrophage activator LPS resulted in an increase in NO production in 9 or more times in all series of experiments. Adding to the culture of HA cells with concentration of 0.1 μg/ml only four of the eighteen studied HAs given stimulatory properties. The same HAs were hold down and even though increased their stimulating properties with concentration of up to 1 μg/ml. There is four HAs with activating properties were revealed with concentration of 1 μg/ml.

The NO-stimulating properties of four HAs were reviled when macrophages were incubated with HAs in a concentration of 10 μg/ml. The activating properties in three samples of HAs were observed when concentration was increased to 50 μg/ml. Only two non-active HAs stimulation of nitric oxide production by macrophages were obseved when the cells were cultured with a maximum concentration 100 μg/ml.

Our data have good agreement with previous paper [4]. The HAs throwing to the culture of HUVEC cells leads to the dose-dependent enhancement of the production of nitric oxide which was suppressed by the addition of L-NAME (N (G)-nitro-L - argininemethylester) or L-NMA (N (G)-methyl-L-arginine), antioxidants (superoxide dismutase, vitamins C and E), and inhibitor of protein kinase H7.

According to our study, the HAs isolated from peats have the ability to stimulate the production of nitric oxide through peritoneal macrophages of mice. The NO-stimulating action degree depends on the HAs concentration as well as the peat species. Seven of the nine HAs isolated from 0.1 M solution of $Na_4P_2O_7$ from the upper sphagnum-moss, magellanicum, fuscum, as well as low-lying wood, grass-moss, and grass peats, activate macrophages at a concentration of 0.1 and 1 μg/ml with subsequent a dose-dependent enhancement of the effect increase in concentration. In this case the activating of HAs isolated by a 0.1 M solution of NaOH from the same peats is occurred with concentrations of 50 and 100 μg/ml. The HAs activity was observed from the pine-cotton and low-grass, grass-moss peat with concentrations of 50 and 100 μg/ml.

So, HAs isolated from different peat species of West Siberia have strong biological properties which be able to functional state of macrophages towards enhancing pro-inflammatory properties and, as a consequence, to regulate the immune response of Th1 and Th2 types. At the same time, higher activity of HAs by pyrophosphate extraction is noted. Therefore, the HAs extraction method has a great influence on their biological activity. This fact allows us to consider HAs as promising natural and low-toxic sources for the production of drugs, in particular pharmacological correctors of the immune response.

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## Section I
## Exploring molecular structures and properties of humic substances using advanced analytical and information technologies

## Секция I
## Исследование молекулярных структур и свойств гуминовых веществ с использованием передовых аналитических и информационных технологий

# Principles of a Humic Database Formation Based on the IR-EXPERT Information-Analytical System

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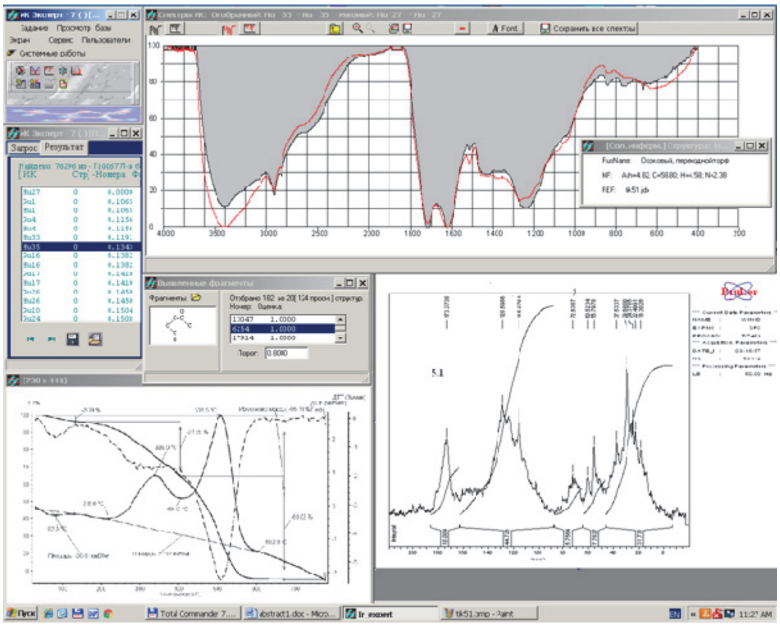
The importance of systematization of humic acids (HA) data is due to their extraordinary diversity and wide use in various fields. At present, a huge amount of factual material has been accumulated for humic acids, obtained with various analytical methods, IR spectroscopy being one of the most common and long-used. The outline of IR spectrum is one of the diagnostic features of HA.

The peculiarity of creating a humic acids database is that it is impossible to generate records of the "spectrum–structure" correspondence, since the HA structure varies depending on a large number of parameters, for example, on the formation time or territorial origin.

It is suggested to use the IR-EXPERT system created in NIOCH SB RAS for solving information-analytical problems on IR spectroscopy. The IR EXPERT system allows independently creating your own database "spectrum - structure - property" and replenishing existing databases. Unique representation of structures as a set of structural fragments makes possible to associate a spectrum with a combination of structural fragments rather than with the exact structure. And then you can work with such an original representation similarly to conventional structures: to perform a substructure search, to compare sets of structural fragments with each other. A quantitative evaluation of the structural similarity of two sets of structural fragments can be useful, for example, in a comparative analysis of humic acids of different territories or genesis.

Another interesting area of research on humic acids can be a test of the possibility of "obtaining" the IR spectrum by summing the IR spectra of compounds (a set of structural fragments), presumably entering the structure of the sample under study.



The database on humic acids could contain:

- name, soil-geographical zoning, type, soil horizon, depth of sampling, etc.
- elemental composition, ash content, water content;
- electronic, infrared, absorption spectra, NMR spectra, etc.
- thermogravimetric data;
- any other data – what you have at the moment

The creation of a database on humic acids - with digitized spectra, reflecting their composition by structural fragments, physico-chemical characteristics and other related information, would allow us to approach the fundamental problem of analysis of structure, modeling and forecasting of the structure of humic acids.

## Application of HPLC, HPLC-MS for Structural Analysis of Water-Soluble Fraction of Lignin

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Natural plant substances, due to their low toxicity and multiple pharmacological effects they exert, attract growing interest in the scientific community as candidates for designing novel medicinal products. Presently, small number of studies demonstrate potential of pharmaceutical application of lignin plant polymers and its transformation products. Heterogenic composition of lignin and its transformation products makes quality control tasks extremely complicated thus hindering their introduction in the pharmaceutical space.

BP-Cx-1 – a water-soluble fraction of plant lignin – is being developed for use as the starting material for pharmaceutical products. Structure of BP-Cx-1 has been elucidated with a wide range of analytical methods, comprising: dynamic light-scattering, molecular hydrodynamics, IR- and NMR spectroscopies, elementary analysis, gel-chromatography, reverse-phase HPLC and reaction GC-MS.

Results of the analyses suggest that BP-Cx-1 is a mixture of phenolic polymers, which, in aqueous solutions, form strong associates; hence isolation of individual components and elucidation of their structures are problematic and inefficient.

In order to cope with routine tests for BP-Cx-1 (identity and assay) and to obtain additional information on the nature and structure of this product, we have developed and tested a new HPLC/HPLC-MS method: BP-Cx-1 components are separated with the gradient elution method using a column packed with a cross-linked polymer with a defined pore diameter. Such method enables separation of BP-Cx-1 into two major fractions: hydrophilic low-molecular and more hydrophobic high-molecular.

As the ratio between these fractions is indicative and is reproduced across different batches of BP-Cx-1 this method can be used as both the identity test and as the IPC-test.

Use of high-resolution mass spectrometry detector enabled collecting data on unique mass composition of the low-molecular fraction, suitable for controlling identity of both the finished product and its starting raw material.

Based on the precise atomic masses, established with HPLC and high-resolution HPLC-MS methods, and data from obtained with other above-mentioned analytical methods BP-Cx-1 has been demonstrated to be a complex mixture of highly-substituted polyphenols with average molecular mass of 10’000 g/mol. Generalized empirical structural formula of the product has been proposed.

Thus, the developed HPLC/HPLC-MS method has made it possible to address BP-Cx-1 quality control problems and to establish structure of this product.

## FTIR Qualitative and Quantitative Analysis of Humic Substances in Aqueous Solutions and Dry Samples

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There is a problem of standardization of humic substance (HS) preparations as the properties of the final product depend on the HS source and parameters of the technological process. As humate-based preparations are used in such areas as medicine, pharmacology, cosmetology, veterinary medicine, plant growing, while HS are not molecules with a fixed composition, it is necessary to develop a technique for monitoring the production and controlling the final product.

FTIR spectroscopy is widely used to qualify the functional composition of HS. From the practical point of view, it is non-destructive and rapid analysis method that needs a small amount of the sample. State-of-the-art FTIR spectrometers are compact and could be used in field laboratories. However, techniques of FTIR spectroscopy and the interpretation of HS IR-spectra still need some research for accurate identification of individual absorption bands of HS to provide the comparative characteristics of the substances of various origins and establishing their identity. Also, existing techniques (KBr pellets, paraffin dissolution, or films) have long or complicated sample preparation and low reproducibility of spectra.

The aim of this work was the development of conditions for HS assessment in aqueous solutions and dry samples with the minimum sample preparation. First task lies in the identification of all characteristic bands and classification of humic samples. The second task is quantification of HS in aqueous solutions and the application of the quantification procedure to real samples and analysis of dry mixtures.

We used Aldrich, Powhumus, and Sila Zhizni [Life Force] commercial HS preparations and a collection of HS samples isolated from coal, peat, and soils. For commercial preparations, ATR-FTIR spectra of dry samples were recorded. The HSs under study have the same qualitative but different quantitative functional-group composition. HS Aldrich has a higher content of polysaccharides and aromatic radicals (1005–1035 and 910 $cm^{-1}$, respectively). Coal HSs mostly manifest intense bands of aliphatic groups, while bands corresponding to polysaccharides have low intensities.

For solid samples, the intensity of spectral bands is affected by the degree of dispersity of the sample and the contact between the ATR crystal and the sample, therefore, an internal standard is necessary. As the latter, we selected $K_3[Fe(CN)_6]$, which has only a single absorption band at 2115 $cm^{-1}$, which does not overlap with characteristic bands of HS. Relative intensities of the bands at 3690, 3620, 3330, 2922, 1560, 1380 and 1083 $cm^{-1}$ are linearly dependent on HS content from 4 to 35% w/w.

Aqueous solutions of HS in the range of 2–200 g/l show linear calibration dependences; the estimated limits of detection for the characteristic bands are 1 g/l for 1015, 1040, 1115, and 1388 $cm^{-1}$, 6 g/l for 1570 $cm^{-1}$, 11 g/l for 1388 $cm^{-1}$, and 15 g/l for bands of 2854, 2924, and 3692 $cm^{-1}$. Verification was made by the added-found method. The best sensitivity and accuracy are achieved when using calibration solutions prepared from the same test product. When determining HS using calibrations built for other HS preparations, the best results are obtained for bands 2924, 2852, 1560–1570, and 1380–1390 $cm^{-1}$.

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## Standard Samples of Humic Acids of Chernozem and Sod-podzol Soil of State Standard Level

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Until now, there were no humic acids (HA) standard samples of a high level of accreditation in the world. The most popular and famous are standard samples of International Humic Substances Society, IHSS (http://humic-substances.org/ihss-samples-a-retrospective).

The goal of our work was producing standard samples of chernozem and sod-podzolic soil HA, which can be further used for evaluating other samples of humic materials. Standardization of HA samples was performed in terms of carbon, nitrogen and hydrogen contents as determined by elemental analysis. Produced HA standard samples correspond to the state standard level.

Soil sampling: chernozem soil was collected in the field of long-term experiment (grain-and-fallow crop rotation since 1964) in Kursk region. Sod-podzolic soil was collected in Moscow region in spruce forest of more than 150 years old. About 50 kg of each soil was collected and used for HA isolation using IHSS protocol [1].

Samples of HA were attested in Russian Federal Agency on Technical Regulating and Metrology using standardized method of elemental analysis. Content of elements in chernozem soil HA was determined as: C 54.60±0.91%, H 3.16±0.38% and N 3.22±0.40%. For sod-podzolic soil HA elemental composition was determined as: C 48.9 ± 0.7%, H 5.05±0.25% and N 5.08 ± 0.26%. In addition standard samples of HA were characterized by molecular mass distribution using HPLS and structural fragment carbon distribution using liquid-state 13C-NMR and UV-vis spectrometry.

So, standard samples of soil HA were produced for the first time.

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## Quantitative Description and Classification Analysis of NOM Based on FTICR MS Data

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The use of van Krevelen (VK) diagrams to visualize information-rich FTICR MS data on natural organic matter (NOM), introduced by Kim with coworkers [1], has engendered substantial progress in understanding the molecular organization of NOM. This is because it allows to convert initial data sets into visually meaningful images. In this work we propose application of cell-based partitioning approach used in chemometrics studies [2] for converting VK plots into a set of numerical descriptors which can be used for quantitative comparison of different NOM samples analyzed with a use of FTICR MS. For realizing this approach, the H/C versus O/C VK diagram is first discretized into rectangular cells covering entire space occupied by NOM molecular formulas. Next, the resultant cell-based distribution of experimental points is used to calculate the relative number of stoichiometries found in each cell. The latter serves as a molecular descriptor. A set of these descriptors provides a unique identification for the sample.

FTICR MS data obtained for 37 NOM samples of different geographic regions, sources of origin and fraction compositions were used in this work. The mass lists obtained were treated with Transhumus software which was based on total mass difference statistics algorithm [3]. The molecular formulas were assigned on the basis of CHNO compounds and used for plotting corresponding VK diagrams.

Method of discriminant analysis in forward stepwise mode was used for the spectral data classification that was conducted according to source of NOM origin (coal, peat, soil, fresh water and shilajit) and fraction composition (humic acids, fulvic acids and non-fractionated NOM). The discriminant analysis was carried out with a use of Statistica software. To assess the classification ability of classification functions calculated, cross-validation procedure was realized.

For the purpose of this study, we have chosen two aqueous humic materials: fractions of fulvic (AFA) and humic (AHA) acids. The both NOM samples were analyzed using FTICR MS technique. The corresponding molecular assignments were, first, plotted in VK diagrams. Then the total space occupied by the data points in VK diagram was constrained by the H/C values from 0.2 to 2.2 and O/C values from 0 to 1. There were none of experimental data points observed which would lay off of these boundaries. This area was further binned into n cells. We have set n equal to 20, as it is shown in Figure 1A, but different values can be used. The cell-based distribution of experimental points was calculated by quantifying population density of each cell ($D_i$) according to the equation:

$D_i = N_i/N$, $i = 1, 2, \ldots n$,

where $D_i$ is the population density of the $i_{th}$ cell; $N_i$ is the number of data points within the $i_{th}$ cell; N is the total number of data points plotted in VK diagram.

To visualize how the descriptors obtained differ from one another with respect to the molecular composition of NOM, we have plotted them as corresponding histograms (Figure 1B). It can be seen that despite similar patterns characteristic of the VK diagrams of the both humic materials, clear differences can be discerned between them with respect to numerical values of the descriptors ($D_i$). For example, the AHA sample was characterized with the higher values of population densities of the cells with H/C < 1 (e.g., number 6, 7, 11) indicating higher unsaturation degree of AHA, whereas the AFA sample showed higher density values for the cells with H/C > 1 (cells number 8, 9, 13, 14) indicating depletion of aromatic structures intrinsic to the AFA sample. The comparable density values were observed for the both samples for the 12th cell that designated the center of distribution of molecular compositions over the VK diagram resulting in maximum population density values. The observed molecular trends are in line with the different behaviour of HA and FA fractions in acidic solutions: HA is more hydrophobic (enriched with aromatic units) and precipitate, while FA is more hydrophilic (enriched with less condensed oxidized units) and remains in solution.

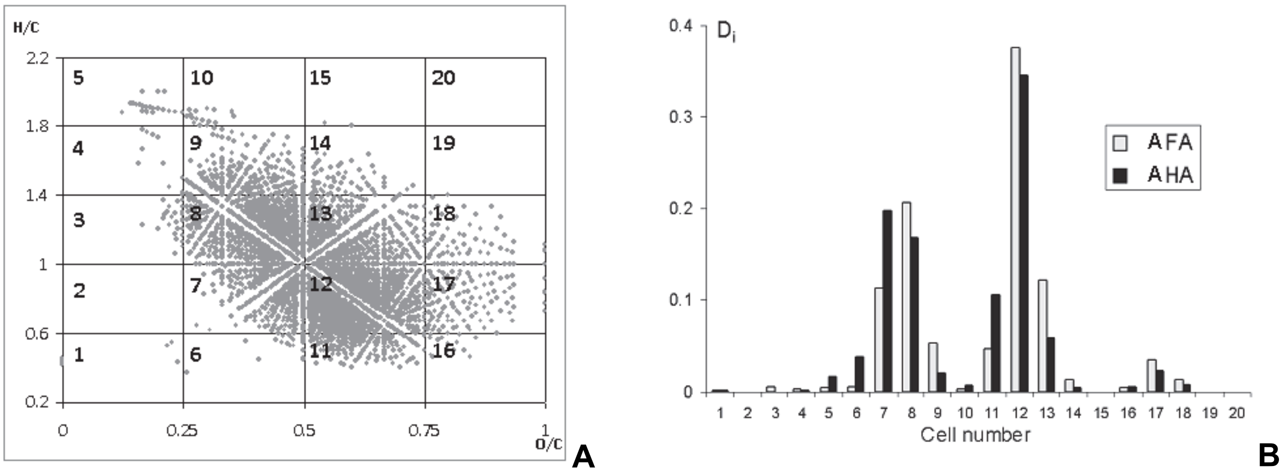


Figure 1. Translation of VK diagram plots (left) into histograms of numerical descriptors (right). VK diagram of NOM binned into 20 cells (A); the numerical values of the 20 descriptors calculated and plotted pairwise as comparison histograms for the samples studied (B).

To check if the quantitative descriptors obtained could be used for classification purpose, statistical classification technique of discriminant analysis was applied. Classification according to source of origin demonstrated 100% true classifications for coal, shilajit and soil NOM, while classes of aquatic and peat NOM showed 83% and 63% correspondingly. Percentages of true classifications according to fraction composition altered from 86% (for HA) to 89-90% (for FA and non-fractionated NOM samples, correspondingly). At that the value of $D_9$ descriptor attributed to non-oxidized aliphatic molecules (e.g. terpenoids) contributed the most to the discrimination between sources of NOM origin, while the value of $D_{11}$ (oxidized aromatic structures, such as hydrolyzable tannins) had the highest discrimination power in case of classes of NOM fractional composition.

Thus, the simple approach is proposed for quantitative description of chemical space occupied by different NOM samples using cell-based partitioning technique. Its advantage in comparison with pictorial images of VK plots is a possibility to describe the NOM sample by a set of numerical descriptors that can be written as a simple formula and plotted on a histogram. The numerical descriptors also can be used for classification analysis and quantitative structure activity relationship (QSAR) studies.

This research was partially supported by the Russian Foundation of Basic Research (grant # 13-04-01853).


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## Revealing Chemical Properties of Individual Compounds in Complex Mixtures Using in-ESI Source H/D Exchange Combined with FT ICR MS

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It was recently recognized that chemical and structural information about individual compounds of complex mixture can be revealed using isotope exchange combined with ultrahigh resolution mass spectrometry [1, 2]. This approach makes possible to enumerate active hydrogens and oxygens in thousands of compounds simultaneously. Here we present the application of the H/D exchange FT ICR MS for the investigation of humic substances, petroleum, bio-oil, wood pyrolysis produces etc. It was observed that compounds in regular crude oil have either 1 (naphthenic acids) or 0 (nitrogen containing compounds) labile hydrogen. Investigation of oil from ancient (V B.C.) Greek amphora revealed that ageing of oil in the presence of oxygen leads to the formation of compounds with 2 –OH groups. We have observed up to 3 labile hydrogens in wood pyrolysis products. We didn't observe labile hydrogens in positive ions of nitrogen containing compounds of the bio-oil produced by hydrothermal liquefaction, such results indicates that such compounds are present in form of onium compounds. For humic substances, the number of labile hydrogens changes from 1 to 6 in the mass range 200-600 Da and increases with the molecular weight.

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## Quantitative Determination of Humic Acids in Peat and Soils

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The soil organic matter has a complex structure, one of the parts which are especially interesting to specialists, is humic acids (HAs). Quantitative determination of total content of HAs in the peat and soils are still topical problem whose solution will identify the most promising areas – sources of biologically active substances.

Temperature the oxidation of HAs was determined by means of thermogravimetry and oxytermography. Experimentally found the range of chemical transformations, which is located in the region of 200-300°C, which corresponds to the minimum point on oxytermogram at 200 seconds (Fig. 1). The burning of soil, peat and flock have also observed peaks in this temperature region. The burning of peat moss (sphagnum) peak in this region was not observed.

The composition of HAs is known (%): carbon – 42.4±0.3, nitrogen – 0.82±0.02, the hydrogen – 4.65±0.06; ash content of HAs – 10% and hygroscopic moisture – 6%. The control determine the precision of HAs method of oxytermography was performed by the method of variation of the mounting samples (Tab. 1.).

Thus, the proposed rapid automated method for the determination of HAs in soils and peat. The method is characterized by a small of the mounting samples, low time of determination, and other advantages.

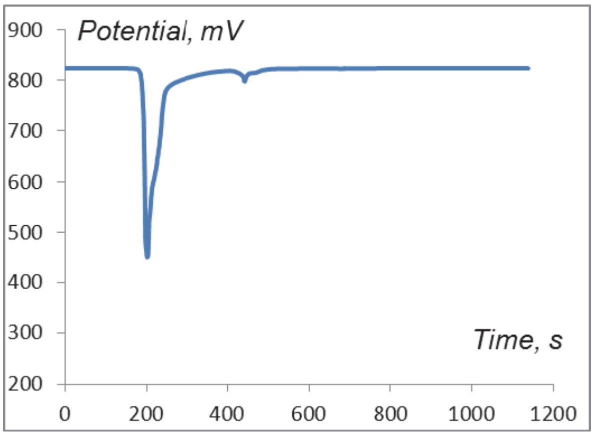


Figure 1. Oxytermogram of HAs.

Table 1. The results of quantitative determination of HAs organic carbon (dry weight)

| Hanging HAs, mg | $C_{org}$, mg | $C_{org}$, % |
|---|---|---|
| 1.5 | 0.64 | 42.7 |
| 2.7 | 1.14 | 42.2 |
| 4.5 | 1.91 | 42.4 |
| 6.6 | 2.79 | 42.3 |
| 11 | 4.66 | 42.4 |

## Study of the Structure of Macromolecules of the HA "Aldrich" in Aqueous Solutions at Concentrations < 20 mg/l by Absorption and Fluorescence Methods

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The purpose of this work was to obtain information on the structure of humic acids of HA in the concentration range < 20 mg/l in the absence of buffer. Interest in the study of the structural state of macromolecules of HA in this concentration range is due to the fact that in natural reservoirs the concentration of HA varies from 1 to 20 mg/l, and there is no detailed information on this issue in the literature. Within the framework of this interest, the concentration dependence of the absorption and fluorescence spectra of a standard sample of HA "Aldrich" was studied. Depending on the concentration, a different state of the structure of the HA macromolecules can occur and, accordingly, their accepting ability may change. This is one of the most important functions of the Civil Code, which determines the effectiveness of water purification from various pollutants. A priori, it could be assumed that if a change in the concentration causes a change in the structural state of the HA molecule, this change will be recorded in a change in the absorption and fluorescence spectra. In this paper, to obtain information on the concentration dependence of the spectral properties of HA, the shape of the contour (absorption, fluorescence) and the intensity of the spectrum (absorption, fluorescence) were studied. It should be noted that there was no information on the possibility of using absorption spectroscopy to obtain information on the dependence of the structural properties of HA on the concentration to date. There was also no information on the concentration dependence of the quantum yield of the fluorescence of the HA.

It is shown that the spectral parameters of the absorption and fluorescence spectra do not undergo changes when the concentration varies from 1 to 20 mg/l. The invariance of the spectral parameters of the HA macromolecules made it possible to conclude that in this concentration interval their structure does not undergo changes. Absorption experiments (Hewlett Packard 6041) were used to study the weighting coefficient of absorption and the ratio of absorption values at $\lambda$=250 and $\lambda$=400 nm, and in fluorescence experiments (Cary Eclipse Varian Fluorescence Spectrophotometr, $\lambda$exc=300 nm) the position of the maximum of the fluorescence line of the HA and its relative quantum yield were measured. To obtain correct emission spectra of HA fluorescence in the presence of fluorescence of a solvent, a relation is proposed that allows one to take into account the contribution of the fluorescence intensity of the solvent to the total fluorescence intensity of the HA solution.

## Изучение структуры макромолекул ГК “Aldrich” в водных растворах при концентрациях < 20 мг/л методами абсорбции и флуоресценции

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Целью настоящей работы было получение сведений о структуре гуминовых кислот (ГК) в области концентраций < 20 мг/л при отсутствии буфера. Интерес к изучению структурного состояния макромолекул ГК в этой области концентраций связан с тем, что в природных водоёмах концентрация ГК варьируется от 1 до 20 мг/л, а подробные сведения об этом вопросе в литературе отсутствуют. В рамках этого интереса было проведено изучение концентрационной зависимости спектров поглощения и флуоресценции стандартного образца гуминовой кислоты ГК “Aldrich”. В зависимости от концентрации может иметь место различное состояние структуры макромолекул ГК и, соответственно, может меняться их акцептирующая способность. Это одна из важнейших функций ГК, которая определяет эффективность очистки вод от различных поллютантов. Априори можно было полагать, что если при изменении концентрации будет иметь место изменение структурного состояния молекулы ГВ, то это изменение будет зарегистрировано в изменении спектров поглощения и флуоресценции. В данной работе для получения информации о концентрационной зависимости спектральных свойств ГК изучались форма контура (поглощение, флуоресценция) и интенсивность спектра (поглощение, флуоресценция). Следует заметить, что сведения о возможности применения абсорбционной спектроскопии для получения информации о зависимости структурных свойств ГК от концентрации до настоящего времени вообще отсутствовали. Также вообще отсутствовали сведения о концентрационной зависимости квантового выхода флуоресценции ГК.

Показано, что спектральные параметры спектров поглощения и флуоресценции при вариации концентрации от 1 до 20 мг/л не претерпевают изменений. Неизменность спектральных параметров макромолекул ГК позволило сделать заключение, что в этом концентрационном интервале их структура не претерпевает изменений. В экспериментах по абсорбции изучались весовой коэффициент поглощения и отношения величин поглощения на 250 и 400 нм, а в экспериментах по флуоресценции измерялись положение максимума линии флуоресценции ГК и её относительный квантовый выход. Для получения корректных спектров испускания флуоресценции ГК при наличии флуоресценции растворителя предложено соотношение, позволяющее учитывать вклад интенсивности флуоресценции растворителя в общую интенсивность флуоресценции раствора ГК.

## Chemoinformatic Approach to Identify Antiviral Components of Humic Substances

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Humic substance (HS) is a heterogeneous supramolecular ensemble of natural organic compounds formed by oxidative decomposition of biomacromolecules in soils, coals, waters, etc. It has a broad spectrum of biological activity, such as anti-inflammatory, antibacterial, and antiviral. Inhibitory effect of HS on reproduction of a wide range of DNA and RNA viruses reveals a possibility to apply HS against human pathogens with no approved therapeutics. However, the exact structural composition of HS can not be determined by modern analytical techniques and structure and the nature of HS bioactivity remains unknown, thus complicating the process of development HS-based drugs. In this study we investigated inhibitory effect of ten HS samples, obtained by standard protocols, on enteroviruses and flaviviruses in cell-based assays and developed a technique for an active component identification using high-resolution mass-spectrometry spectral data analysis and chemical database search.

All HS samples inhibited tick-borne encephalitis virus (TBEV) reproduction with EC50 values in range of 0.1-1 μg/ml and exhibited no cytotoxicity up to 10 μg/ml. The same HS samples did not inhibit reproduction of enteroviruses. Observed specificity of the samples was attributed to possible presence of compounds with a specific anti-TBEV activity. Molecular composition of all HS samples was explored by ESI FTICR MS, which allowed resolving thousands of exact molecular formulas in complex organic mixtures without preceding separation. More than 6000 unique formulae were identified in the studied HS samples. Spectral data were analysed using van Krevelen diagrams and heatmaps generated based on samples formulae fingerprints and similarities and differences in themolecular composition of the samples were revealed. Chemoinformatic approach was further employed to identify putative active components structures.

Similarity search in large bioactivity databases is an established strategy to predict biological targetfor compounds via comparison of similar structures with known bioactivity profiles. We developed a methodology for identifying active components of HS samples using publicly available data from ChEMBL version 23. At the first step all possible molecular formulae for molecular weight in range 200-800 g/mol were generated. Elemental composition restrictions were used to strip formulae that were not-relevant for humic substance. Formulae extracted from ChEMBL were merged with the generated formulae giving more than 4000 shared formulae. Structures (80K) related to these formulae were extracted and standardized using ChemAxon Standardizer. 800K bioactivity entries were found for these structures allowing to build activity profiles for HS subspace of ChEMBL chemical space. Chemical diversity of compounds was analyzed using principal component analysis, RBS, and scaffold analysis. Polyphenols and saponin analogs appeared to be dominant among tested individual compounds with formulas present in HS samples.

To retrieve antiviral activity data we used an in-house text-mining based approach. Antiviral activity profiles were built for each sample and data for flaviviruses and enteroviruses were carefully analyzed. Most antiflaviviral activities were attributed to dengue virus (DENV), a medically important pathogen from Flavivirus genus. We assess the activity of studied HS samples against DENV and found micromolar EC50 values in a cell-based assay, thus supporting the hypothesis that the HS activity could be at least partially attributed to the presence of these structures. According to ChEMBL, several compounds were tested against DENV protease NS3 in binding assays. This allows us to suggest that flavivirus NS3 protease may be the target for HS components. We constructed a homology model for TBEV NS3 on the DENV NS3 template and docked selected structures to the site on the NS3 surface. Docking results analysis revealed similar poses for the compounds in DENV NS3 and TBEV NS3 sites, explaining broad-spectrum activity of samples. The suggested approach pave the way for deeper understanding of nature of bioactivity of humic substances.

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# Mössbauer Diagnostics of Iron Compounds in Commercial Potassium Humate

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Iron-contain preparations based on humic substances (HS) are considered promising agrochemicals that make it possible to address the problems of increasing the stress-resistance of agricultural crops and providing them with nutrients. For the production of such fertilizers, it is intended to use commercial preparations of humic acids or humates of alkali metals. However, it is known that in addition to the main organic component, manufacturers often allow in their production the presence of a certain amount of ballast of inorganic origin. Perhaps the presence of water-soluble and water-insoluble ballast is due to the technology of production, the presence of impurities in the feedstock, or mechanical wear of the processing equipment. Obviously, some of the impurities include iron compounds, which is one of the most common elements. In any case, information on the presence, content and composition of impurity reagents in HS should be taken into account when planning for syntheses, using innovative agrochemicals based on HS that include both iron-containing nanoparticles and organoiron compounds of complex structure.

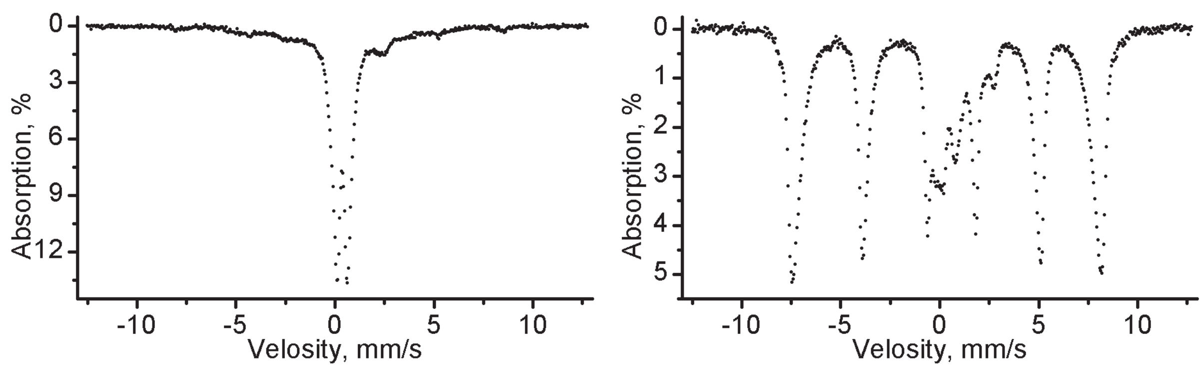


Figure 1. Mössbauer spectra for non-magnetic fraction of ballast after leaching with water recorded at 298 (left) and 78 (right) K.

In the present study, the samples isolated from the universal organomineral fertilizer with the stimulating effect of “Potassium humate Sakhalin”, containing 80% of humic acids (produced by Biomir-2000 Ltd., Moscow, Russia) were diagnosed by the Mössbauer spectroscopy method at $^{57}Fe$ atoms. In this case, the water-soluble organic part of the preparation itself, parts of insoluble ballast split by magnetic separation, the same samples after leaching with water, as well as sediments released from the aqueous solutions of the preparation by sedimentation (under the action of centrifugal and gravitational forces) were studied. By the method of Mössbauer spectroscopy at 78 and 298 K it was shown that iron-containing components, obviously of different composition, can be found in all the listed fractions of the organomineral preparation. At the same time, the organic part of the preparation basically contains complexes with iron (+3). From its aqueous extracts by sedimentation method precipitates enriched with iron compounds (+2) can be isolated. The magnetic part of the insoluble ballast is mostly a compound based on mixed iron oxide (+2, +3) of the magnetite type. The nonmagnetic part of insoluble ballast contains predominantly superparamagnetic particles based on iron oxide (+3) of maghemite (Figure 1).

Thus, on the example of one of the commercial organomineral preparations based on alkali metal humates by the Mössbauer diagnostics method it was shown that iron-containing components are contained in all fractions of the preparation (both soluble and not water-soluble), and their composition and form of existence depend on the properties of these fractions (solubility, magnetic properties).

*This work was supported by the Russian Science Foundation, project no. 16-14-00167.*

# Absorption Indexes and Derivative Spectroscopy as Tools for Rapid Classification of Humic Acids Isolated from Various Products

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The key factor influencing the chemical properties and biological activity of commercial humic products (HP) manufactured from various resources is the organic matter genesis; this determines the need for simple tools and diagnostic criteria to differentiate HP or humic acids (HA) isolated from various resources. The objective was to determine indicators using absorption spectra in combination with derivative spectroscopy that might serve as fast and relatively inexpensive tools to compare HA isolated from commercial HP of various origin: Chernozem soil and a number of industrially manufactured humates from varying raw source materials (brown coal, leonardite, peat, sapropel, lignosulphonate). Absorption measurements were recorded using Unico double-beam spectrophotometer for HA samples in aqueous phosphate buffer with pH 6.9.

Absorption spectra of HA isolated from different HP demonstrated similar wavelength-dependent character: monotonically decreasing absorbances along with wavelength increase (Figure 1).

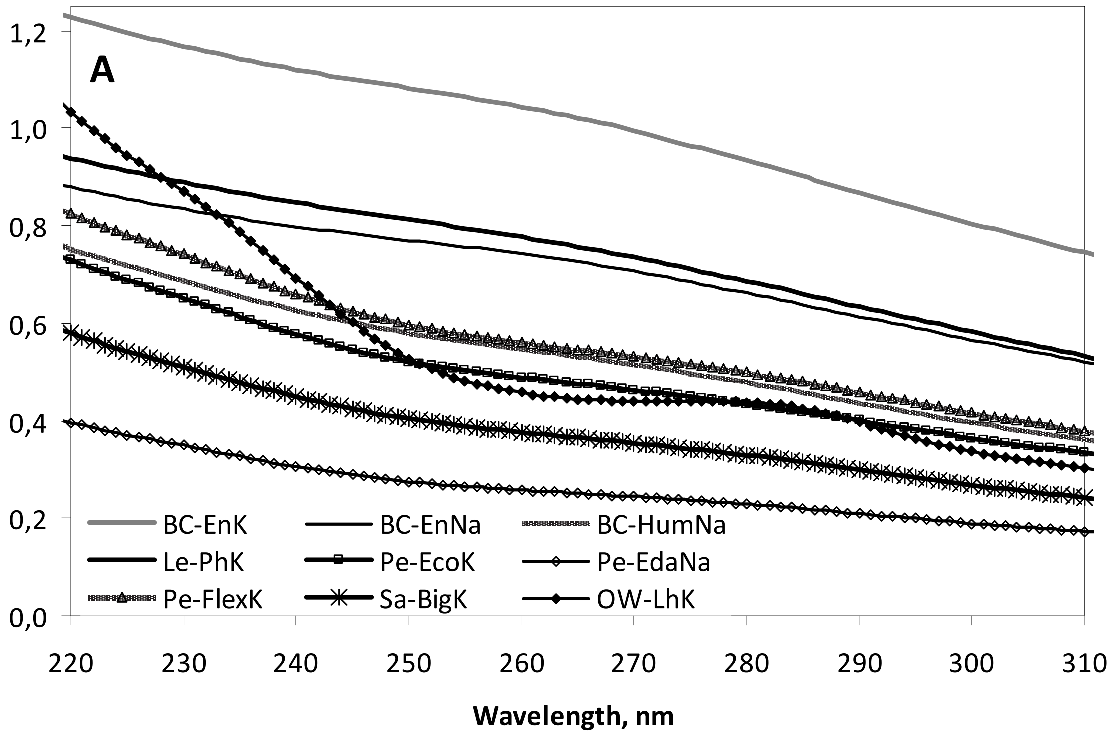


Figure 1. Absorption spectra for HA of various origin.

From absorption spectra the following indexes have been calculated: specific absorbances normalized by the HA content $SUVA_{254}$ (as an indicator of aromatic carbon content) absorbance ratios $E_2$:$E_3$, $E_4$:$E_6$, $E_{270/400}$, $E_{280/472}$, $E_{280/664}$, Δ log K and the slope ratio Sr. $SUVA_{254}$ values were determined as the absorbance at 254 nm divided by the carbon concentration measured in mg C $L^{-1}$. Absorbance ratios $E_2$:$E_3$ and $E_4$:$E_6$ were calculated as the ratio of absorbances at 250 and 365 nm, or 465 and 665 nm respectively. The $E_2$:$E_3$ corresponds to correlation of molecular size and aromaticity, and $E_4$:$E_6$ is believed to be related to the aromaticity and to the degree of condensation of the chain of aromatic carbons of the humic acids, and could be used as a humification index.

Absorbance ratios $E_{270/400}$, $E_{280/472}$, $E_{280/664}$ were calculated from absorbances taken at appropriate wavelengths. The value of Δ log K = log A400 – log A600, was calculated to determine degree of humification. Spectral slope ratio Sr was calculated as a ratio of spectral slopes for the intervals of 275–295 nm and 350–400 nm. Spectral slopes were calculated using linear regression of the log-transformed absorption spectra. Thus, steeper slopes indicate a more rapid decrease in absorption with increasing wavelength.

However, the most promising proxy seems to be indexes determined using derivative spectroscopy which more readily distinguish HA source. We used differentiation of absorption spectra versus wavelength to facilitate resolution of spectral bands and discriminate scattering components. A first-order derivative manifests the wavelength intervals with bigger spectral slopes in initial absorption spectra. The second-order derivative is useful to separate individual peaks of the overlapping bands in primary absorption spectrum. We calculated first- and second-order derivatives (Figure 2) from absorption spectra and implemented new indexes determined as ratios of amplitudes taken at certain wavelengths. For instance $SDR_{267/280}$ is the ratio of amplitudes taken at 267 and 280 nm in the second-order derivative spectrum.

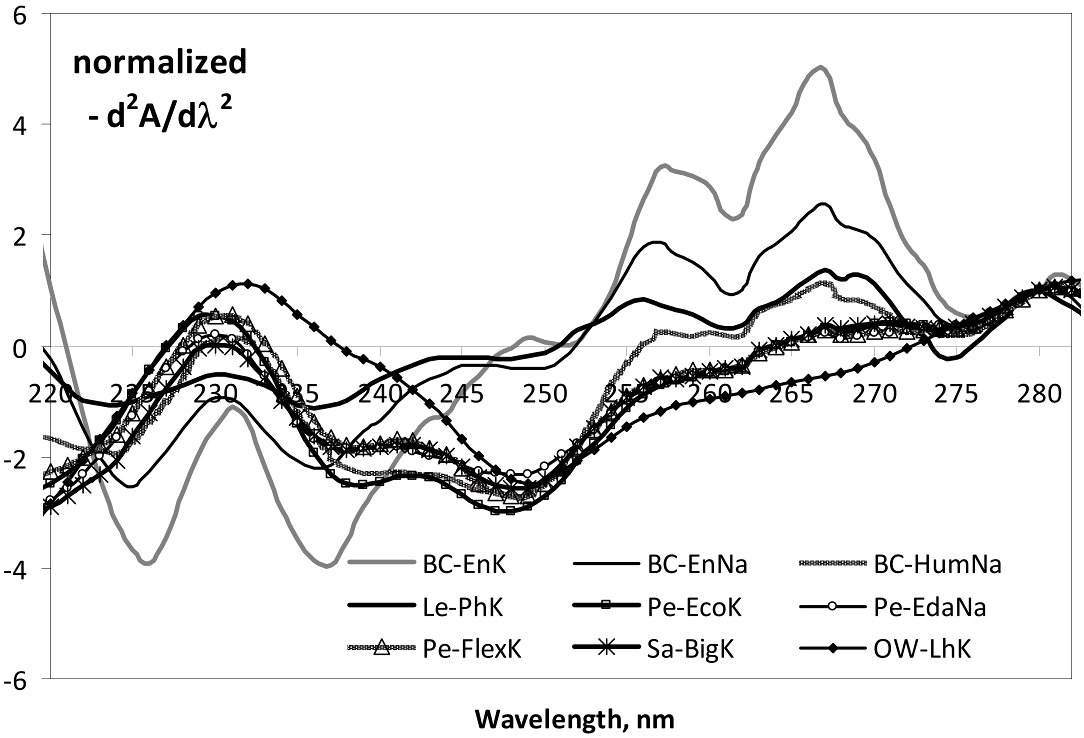


Figure 2. Second-order derivatives of absorption spectra normalized to amplitude at 280 nm for various HA samples.

According to the index $SDR_{267/280}$ calculated using second-order derivative of absorption spectra, all the studied HA can be segregated in 3 groups: (i) from soil and coal-derived HP, (ii) from peat- and sapropel- derived HP, (iii) from lignosulphonate–derived HP. This index showed good ability to classify the samples independently of HA concentration.

We resume that information extracted from absorption spectra and second-order derivative can be useful to discriminate organic matter source for HP from coalified materials, peat and lignosulphonate. Indexes with the highest descriptive ability are specific absorbance $SUVA_{254}$, absorbance ratios $E_{270/400}$, $E_{280/472}$, $E_{280/664}$, and derivative index $SDR_{267/280}$.

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# Selective Extraction Method of the Unconverted Humic Acids from the Peat Biomass

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Structural description and analysis procedure of biological polymers based on «green chemistry» principles cannot be provided without new approaches to selective extraction of the biological matrix components. Nowadays, most of the extraction methodologies are based on the liquid extraction, which have both the positive (simplicity, cheapness) and negative (time consuming, high reagents volume, incomplete extraction) sides.

In this paper, we proposed a selective extraction method of the unconverted humic acids (HA) from the peat biomass based on Accelerated Solvent Extraction (ASE) method. This is an express and high selectivity method (can use different solvents in one extraction) without any requirement to peat pretreatment.

We evaluated the advantages of the proposed method by matching physico-chemical characteristics of the HA extracted from high-moor peat by widely used nowadays liquid extraction methodology [1] and ASE – extraction. We used ASE 350 (Dionex, USA) extraction system for selective sequential extraction of HA and bitumen part from peat. Butyl acetate at 100°C was used for bitumen part extraction (1 extraction cycle) and for consequent humic acids extraction 0.1 N NaOH water solution at 150°C (5 extraction cycles). The characteristic of HA was determined using $C^{13}$ NMR spectrometry, FT-IR and molecular weight distribution (MWD). According the $C^{13}$ NMR spectra aromicity degree was 40%, aliphacity – 60%, carboxylic carbon percentage – 12.5%. MWD data showed that humic acids molecular weight was $M_w = 2.0 \cdot 10^3$ Da and polydispersity value – 2.2. Completeness of extraction was controlled by determination of the UV radiation absorption at wavelength of 250 nm and by gravimetric as paralleled method. The extraction ratio for liquid extraction was 12% (wt) for ASE-extraction - 30% (wt).

The FT-IR spectra of HA extracted by liquid and ASE – extraction revealed saving structural properties during the ASE-extraction procedure (fig 1). Obtained results of the comparative analysis showed the possibility of fast and high performance extraction of unconverted humic acids by ASE extraction method.

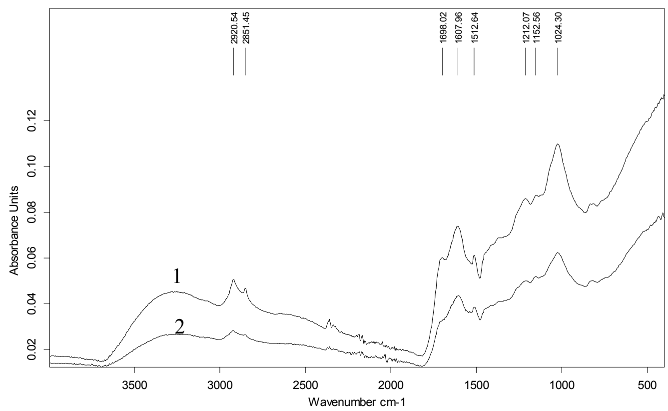


Figure 1. IR – spectra of liquid (1) and ASE – extracted (2) humic acids.

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# Spectral and Photochemical Properties of Humic Acids with Different Genesis of Organic Raw Materials

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Humic substances (including humic and fulvic acids) are naturally photoactive components, which are widely present in environment. Humic substances representing the main fraction of organic matter receive increased attention because of their reactivity as light absorbers. Depending on their origin and structure, humic substances have a remarkable ability to absorb light and transfer this energy to other substrates and in some cases strongly affect photolysis of xenobiotics. In water and in soils humic substances have been found to act as photosensitisers and they have also been reported to produce oxygen species upon irradiation, and be able to photoinduce the transformation of ecotoxicants. The photoquenching effects of humic substances on some chemicals are also known. Also, the possibility of an UV screening by humic substances on chemicals cannot be excluded since the energy-transfer and charge-transfer between the chemical and humic substances can deactivate the excited molecules. Excited singlet and triplet states of dissolved humic acids (HAs), the major component of humic substances, are important players for the transformation of organic chemical contaminants in natural waters. Our knowledge about these processes is still very limited.

This talk exhibits several examples of spectral and fluorescent study of humics acids and lignins with different genesis. Objects of the study were HAs and lignins from Arkhangelsk Region and HAs isolated from peat collected from the key site “Khanimey” of the Yamalo-Nenets Autonomous District. Peat samples used in the work were taken from different depths. Consequently, their botanical composition is different. A standard sample of humic acids isolated from brown coal was investigated for a comparison (a sample of the company “Fluka”; Code -130794430907051).

Fluorescence spectra of the studied samples show a faint glow in the range from 400 to 600 nm. The sample of humic acids “Fluka” has the highest intensity, as in absorption spectra. Such difference in the intensity and the shape of spectra is caused by different ratio of aromatic structures, as well as by the difference in functional groups of HAs samples.

For photochemical studies, an excilamps on working molecules $Xe_2$ and KrCl with $\lambda_{rad}$ ~ 172 and 222 nm, developed at the Institute of High Current Electronics of the SB RAS, were used as sources of UV radiation.

To study the impact of humic acids on processes of herbicide photodegradation to aqueous solutions 2,4-D (50 mg/l), the studied humic acids were added at various proportions of concentrations (from 25 to 100 mg/l), and the irradiation of solutions was carried out in steady state and in a flow photoreactor, registering the absorption and the fluorescence spectra. The irradiation time of aqueous solutions containing 2,4-D and humic acids was 1 – 150 minutes. The discussion includes comparative analysis of the direct and indirect photolysis.

The work was financially supported by Russian Foundation for Basic Research (Grant № RFBR-North № 17-45-290682).

# Express Method of Determining the Humic/Fulvic Acids Ratio in Humic Substances by Total Organic Carbon Analyzer

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Humic substances can be divided into fractions according to their solubility in various aqueous media - humic acids (soluble at pH > 2), fulvic acids (soluble at any pH) and humin - insoluble part [1]. The ratio of the content of humic acids to fulvic acids shows the degree of humification of substances, that characterizes humic products and can be useful for describing and predicting their properties. The most accurate method of determination is based on the fractionation of humic substances with subsequent weighing of individual components [2]. But this approach is very labor-intensive, especially if you perform a large series of samples. Methods based on spectrophotometric determinations [3] are specific to the sample and can not be recommended for a wide range of measurements. The content of carbon in the fractions is constantly within some limits for humic substances from various sources, and it is possible to determine the fraction ratio according to the carbon content in them. The popular method is based on the determination of the content of organic carbon in fractions under the influence of excess potassium dichromate, followed by titration of the residue [4]. The use of a total organic carbon analyzer (TOC) greatly simplifies this method and makes it possible to carry out rapid analyzes of a large batch of samples.

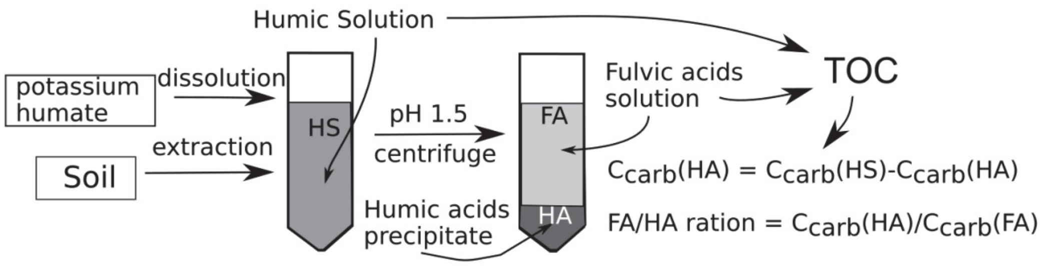


Fig. 1. Scheme of determination humic/fulvic acids ratio by TOC

For the study, two samples were chosen - commercial potassium humate and a soil sample. The isolation of humic substances from soil samples was carried out using the method of accelerated determination of humus by Kononova and Belchikova [5]. To do this, 2 ml of the sample was placed in a 50 ml beaker. A 40 ml solution of 0.1M ($Na_2P_2O_7$ + NaOH) was added to the beaker, left to stir at room temperature for 2 hours. Then the volume was adjusted to 50 ml with a saturated solution of $Na_2SO_4$ to coagulate the mud particles, allowed to stand for 20 minutes, poured into a centrifuge tube and centrifuged for 10 minutes at 10,000 r/c to separate the muddy portion. The supernatant was separated by decantation, which is a solution of humic substances.

A fraction of fulvic acids was extracted from the solutions of humic substances. For this purpose, 20 ml of a concentrated solution of humic substances were placed in a beaker and 5M HCl was added until pH 1.5, with the precipitation of humic acids. Then, the volume of the solution was adjusted to 25 ml, poured into a centrifuge tube and centrifuged for 10 minutes at 10,000 rpm. The supernatant was separated, which is a solution of fulvic acids.

The method for measuring organic carbon content in solutions using the TOC-L (Shimadzu, Japan) is based on determining the amount of carbon dioxide released during the catalytic combustion of an aliquot of a solution. The total content of organic carbon is determined by the difference in the values obtained total carbon and inorganic carbon. To obtain statistically significant concentrations, the instrument performs a multiple sample measurement until a convergent result is obtained. The procedure for determining the content of organic carbon with potassium dichromate is described in detail in the article [4].

The measurement of the ratio of humic acids to fulvic acids in commercial potassium humate was 7.4 using TOC and 7.6 using potassium dichromate. For soil samples, the results were 1.1 and 1.0 using TOC and dichromate, respectively. As can be seen, the results obtained with TOC are in good agreement with the traditional method, and this method can be recommended for widespread use.

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## Mineral Composition of Natural Humic Substances and Commercial Humics-Based Products

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Nowadays, it is known that humic substances (HS) consist of both organic and inorganic parts. Isolated humic materials may contain up to dozens percent of mineral compounds including both metals and nonmetals. Mineral impurities cannot be completely removed from a humic matrix even using harsh treatment with strong acids. This is indicative of the constitutive functions which the mineral part may play in supramolecular organization of HS by participating in different types of chemical linkages with organic functional groups. However, it has been so far much less investigated compared to organic part. Recently, the achievements in atomic spectroscopy have developed into versatile methods of elemental analysis.

This paper is devoted to the development of a reliable analytical approach to mutielement analysis of the mineral part of humic isolates from different environments. For reaching this goal, we have used state-of-the-art methods of atomic spectroscopy — atomic-emission analysis with inductively coupled plasma (ICP–AES). This method is *de facto* standard for various environmental, high-technology, clinical, and pharmaceutical applications.

The analysis of HS can be reliably implemented using the control is the obvious first step to render traceable humic substances and commercial humic-based products properties and to improve and advance the required technologies. This usually implies sensitive and selective multielement analysis because the impurities in humic substances have various nature and quantity levels.

This study deals with the analytical problems, possibilities, and results of ICP-AES quantitative multielement analysis of a wide range of humic materials and humus-based fertilizers using various types of sample preparation. Characteristic levels of elements in humic substances will be presented and discussed. In particular, the consideration will be given to the elemental composition of commercial humic products which can be very informative of how much HS are in these products. The examples will be given when we determine up to 65% of mineral compartments in the humic products, which raises a concern about the suitability of their positioning on the market as purely organic fertilizers. A much more suitable category in this case would be “organo-mineral” fertilizers.

On the other hand, humic substances have got wide acceptance not only as fertilizers, but as drugs in medicine, additives in drinks and food, etc. From this point of view, the role of assessment and standardization of humus composition at all the production stages is significantly increasing. Humus mineral composition is required to correctly estimate both the nutritional value and safety for plants, animals, and humans.

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# Достижения и проблемы в изучении взаимодействия живого и гумуса

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Взаимодействия и взаимосвязи в системе почва-растения всегда были одной из ключевых тем естествознания со времен античности. Наиболее ярко ее выразил В.И. Вернадский в своей идее о «единстве живого и гумуса». Очевидную связь продуктивности растений с содержанием гумуса пытались объяснить еще алхимики. Отголоском этого явилась чисто умозрительная, простая и «красивая» теория «гумусового питания» растений. На деле связь гумусовых веществ (ГВ) с урожаем оказалась гораздо более многофакторной и сложной. И на одном из первых мест в этой связи стоит физиологическая стимуляция ГВ процессов метаболизма биоты.

С момента открытия физиологической активности (ФА) ГВ прошло уже более 100 лет и все это время ученые предпринимают пока безуспешные попытки разгадать механизм этого взаимодействия. На роль активных структур предлагались различные участки макромолекул ГВ, но ни одна из этих структур не дала четкой зависимости. Единственной из обнаруженных нами зависимостей, показавшая сравнительно высокий уровень корреляции (на уровне 0,86), оказалась связь ФА ГВ с концентрацией в их препаратах свободных радикалов.

Не утихают попытки объяснить явление ФА ГВ их проникновением внутрь клетки через цитоплазматическую мембрану (по сути, частичная реинкарнация «гумусовой» теории питания растений) и прямым включением ГВ в сложнейшие процессы биосинтеза внутри живой клетки. Однако, прямых доказательств этого маловероятного (хотя бы с точки зрения мембранного транспорта или цитоплазматического гомеостаза) процесса до сих пор нет. С нашей точки зрения наиболее вероятной является гипотеза мембранотропного действия ГВ, которые, сорбируясь на поверхности мембран, с одной стороны оптимизируют минеральное питание, стимулируют мембранный транспорт, а с другой – оказывают биопротекторное действие, защищая мембрану от ксенобиотиков и неблагоприятных воздействий.

В наших исследованиях мы изучали действие ГВ на одноклеточную водоросль *Chlorella vulgaris.* Методика опыта позволяет в каждом варианте одновременно оценивать фотосинтез и окислительную деструкцию (дыхание) в ее клетках. Результаты показали, что *Chlorella vulgaris* достаточно активно реагируют на присутствие ГВ в растворе, но ответ на физиологическое воздействие ГВ различается. Установленный нами факт, что ГК разных почв по-разному стимулируют фотосинтез и дыхание может говорить о том, что механизмов воздействия, по-видимому, не один, а как минимум два или несколько. Большинство препаратов ГК стимулируют в разных соотношениях и фотосинтез и дыхание, но есть и стимулирующие только фотосинтез или только дыхание. Причем ГК, выделенные из разных частей гумусового профиля чернозема карбонатно-мицеллярного, могут совершенно по-разному действовать на физиологические процессы *Chlorella vulgaris.*

Нами также проводились прямые микроскопические наблюдения за взаимодействием ГК с мембраной клеток *Chlorella vulgaris*. Оценка количества и размеров клеток методом прямого счета в камере Горяева показала значимый рост клеток в присутствии ГК, стимулирующих процесс фотосинтеза. В случае активной стимуляции окислительной деструкции рост клеток не обнаруживался.

## Section II
## Ecosystem metabolomics: humic substances in soil and water ecosystems under conditions of changing climate and anthropogenic pollution and their impact on living organisms

## Секция II
## Экосистемная метаболомика: гуминовые вещества в почвенных и водных экосистемах в условиях изменяющегося климата и антропогенного загрязнения и влияние гуминовых веществ на живые организмы

## Humic Substances Development on the Initial Stages of Soil Formation Under the Reclamation practice on the Former mines of the Phosphorites, Leningrad Region

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Soil organic matter quality can be used as informative tool for assessment of soil development of soil biogenic-abiogenic system alteration in series of ecosystems restoration and development. Not only stocks and volumetric contents of soil organic matter could effective indexes of soil organoprofile development, but also the carbon species and distribution, derived form 13-C NMR spectroscopy could be applied for assessment of soils organic matter stabilization rates under various conditions and on the different age stages.

This study was devoted to assessment of humic acids quality on the monitoring plots of Kingisepp phoshporite mines (Leninrgad region). Three monitoring plots were established on reclaimed part of former quarry with a relatively flat relief. Parent materials were presented by semi artificial heaps of complicated composition: tills were mixed with local carbonate moraines and limestones boulders. Three mono species forest tree stands were studied: under larch (*Larix sibirica*), under Scotch pine (*Pinus sylvestris*) and under Norway Spruce (*Picea abies*). All soil were identified as Calcaric Regosols with total thickness of soil profile about 20-25 cm. Samples were collected from the same plots in 2004 and 2014. Humic acids were extracted according to standard IHSS procedure. Solid state 13-NMR spectroscopy were applied for characterization of molecular composition of humic acids. It was shown that the most characteristic feature of all the humic acids investigated is the dominance of aliphatic carbon on aromatic one. Portion of aromatic carbon showed variation between 28-36% in different ages soils. This could be related to stable conditions in plant communities and relevant stabilization of organic remnants fractional composition (i.e. composition of humification precursors) on different age stages. There were no any significant differences in carbon species composition of the humic acids under various types tree stands. These facts indicate that reclamation practice result in homogenization of vegetation cover, and, by this homogenization of humification conditions in superficial soil horizons. Relatively homogenous carbon species distribution and composition of humic acids became a result of mentioned environmental conditions of humification. As result of this work we can suggest that the humification rates are closely connected with the type tree stands and degree of organic layers homogeneity. Degree of humic acids unhomogenety is higher in those ecosystem compartments where the abandoned restoration appears.

This work was supported by Russian Scientific Foundation, project № 17-16-01030 *"*Soil biota dynamics in chronoseries of posttechnogenic landscapes: analyses of soil-ecological effectiveness of ecosystems restoration*".*

# Transformation of Dissolved Organic Matter and Its Organo-Mineral Complexes in Natural Waters during the Photodestruction

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Destruction of organic matter during its migration is the most important process which causes the existence of a biological cycle of elements. The main processes in transformation of dissolved organic matter (DOM) are bio- and photodestruction [2–4]. Photodestruction of DOM can also regulate the bioavailability of elements [5, 6] and the intensity of $CO_2$ emission from surface waters to atmosphere [1].

To study the influence of UV-irradiation on changes in composition and characteristics of DOM were made laboratory experiments on irradiation by UV-lamp samples of surface waters from river Senga (Vladimir oblast) for 26 days. These samples are characterized by high contents of dissolved organic carbon (DOC) and ferrum. Selected samples were filtered through 0.22-μm pores to remove particles and bacteria.

During the experiments pH in irradiated samples increased. This happened because of the process of mineralization of organic matter during the irradiation, which leads to increasing the part of bicarbonates in the solution.

It was shown that UV-irradiation causes changes in composition and characteristics of DOM. During the experiments the DOC content decreased in 3,5 times, such indicators as C/N and $SUVA_{254}$ were changed (which indicates transformation of DOM). The content of humic substances in the investigated samples decreased about in 6 times in comparison with its original content. The content of low molecular organic substances (<1 kDa) increased by more than 30%, in this case the emission of $CO_2$ was 1,7 mmol (20% from original content of DOC).

Was obtained a significant correlation of concentration of ferrum in solution and contents of DOC. Decreasing contents of Fe is due to destruction of DOM when part of the Fe released from complexes with organic ligands. Then the colloids of Fe hydroxide forms, stabilized with humic substances.

In our study, the destruction of DOM in samples of surface water occurs during the UV-irradiation, with forming low molecular organic substances as products of photodestruction. Concurrently with this process high molecular substances are forming, these are colloids of iron hydroxide, stabilized by organic substances.

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## Corrosion of Iron-containing Engineering Materials in the Presence of Humic Substances

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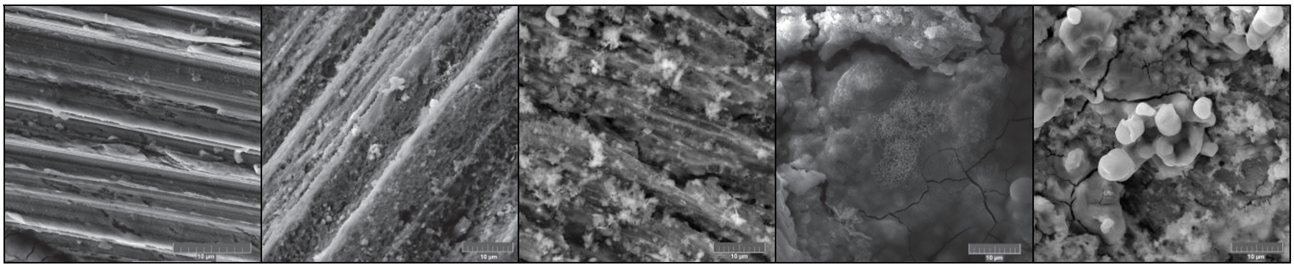
From the Iron Age to the nowadays, corrosion of metals stays an important technological problem for humanity. Chemistry of corrosion processes is one of the most serious branches of scientific knowledge to be developed. The most thoroughly studied mechanisms are that of corrosion of engineering materials under the influence of reagents of anthropogenic origin. However, a significant part of metallic structures contacts with ground and soils, containing natural polyelectrolyte of a multifactorial action (acid-base, redox, complexing, etc.) – humic substances (HS). On the one hand, due to their high reactivity, HS can directly interact, for example, with metallic iron [1]. On the other hand, it is known that archeologists often find metallic artifacts in very good condition in saturated HS waters (bogs, peat bogs, etc.). In addition, mixed corrosion inhibitors based on lignosulfonates, tannins and aminoalcohols are suggested for the protection of steel reinforcement in reinforced concrete. It is obvious that the mechanisms of interaction of HS with metals are complex, and require a comprehensive study.

The purpose of this work consists in revealing the role of HS in the corrosive destruction of iron-containing engineering materials with metallic protective coatings of various nature. In this work, corrosion of the following ferrous metals was considered: the low-carbon steel itself, its zinc, tin, nickel, chromium protective covers overlay in the industrial conditions, stainless steel, heat-resistant steel. On the surface of samples in the form of plates 0.5-1 $cm^2$ degreased and washed with tridistilled water a deep scratch up to the base metal was applied and the sample was completely immersed in an aqueous solution of the electrolyte. As the corrosive medium were used: a) tridistilled water (pH 6.5); b) 3% NaCl solution (pH 8.5); c) 0.01% HS solution (obtained by alkaline extraction from "Natural Humic Acids", LifeForce, Saratov, Russia) (pH 7.6); and d) a solution containing 3% NaCl and 0.01% of the above HS (pH 7.7). Samples of materials were kept in the indicated solutions at a temperature of 45°C within 48 hours. After the expiration of this time, the properties of solutions for practically all samples have changed significantly. So, solutions containing HS were almost completely discolored (except for solutions that contacted the material with chrome protective cover). For all samples, formation of brown sediment was observed (with the exception of the material with zinc protective cover for which a dense gray sediment was observed). Scratches on the surface of most of the samples were covered with a dense layer of corrosion products of brown color. Visually, the highest corrosion intensity was observed for solutions with NaCl. At the end of the experiment, the samples were carefully rinsed with tridistilled water, the traces of moisture were removed and dried at 45°C. The surfaces of the initial samples and the samples after treatment with these solutions were studied by optical microscopy ($5^x$ and $10^x$), and SEM (with resolution to 200 nm). The compositions of the metal substrate and the coatings of the materials were determined by the energy-dispersion analysis method.

The nature of the changes on the metal samples surface and in the depth of the "scratch" was controlled by comparison with similar data for untreated materials (Figure 1). From the data of a microscopic study, it follows that on the surface of a pure metal and some coatings of "active" metals (Zn), corrosion products are formed unevenly - distinct regions with more and less dense coatings of corrosion products are clearly distinguished, clearly differing in their morphology and, possibly by chemical composition. On the surface of the samples visually with a "passive" coating (Ni, Sn), thin layers of compounds are observed, but only under requirement of the presence of HS in the electrolyte. The most intense formation of corrosion products for all samples is observed in the area of "scratches" and natural defects, presented in the coating of the studied materials.

Moreover, products of corrosion are formed in water or NaCl solution as loose morphology. In the case of solutions containing HS, including the presence of NaCl, a dense coating is formed above the active (unprotected coating) surface of the sample, possibly from sparingly soluble iron compounds and HS. It is obvious that over the defects of protective coatings of black metal under the action of HS, an insulating protective cover had been created, which significantly slowed down the diffusion of reagents in the field of corrosion. Thus, using seven samples of iron-containing engineering materials, it was shown by direct experiment that despite its high chemical activity to many metals, and in particular to iron, HS can serve as shielding corrosion inhibitors even in such aggressive media as NaCl solutions.



A B C D E

Figure 1. Data of SEM for iron material with chrome protective cover before (A), and after contact with water (B), 0.3% NaCl solution (C), 0.01% HS solution (D) and 0.3% NaCl 0.01% HS solution (E).

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## Chernozems of the Kansk Forest-Steppe in a Virgin and Developed State: Chemodestructive Fractionation of Soil Organic Matter and Humus Condition

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Soil organic matter (SOM) is important for maintaining soil fertility and agroecosystems productivity. This is the starting material for the formation of a complex heterogeneous, dynamic system of humic substances that enters the surface (litter) and into the soil. Humic substances determine many genetic and agronomic soils properties of different natural zones, as well as their ecological and biogeochemical functions.

Bioclimatic indicators are the most important, largely influencing the composition and content of soil organic matter, on their humus state. The Kanskaya forest-steppe occupies the Kansk-Rybinsk depression, a vast depression between the Yenisei Ridge (in the west and north-west), the Middle Siberian Plateau (in the north and the northeast) and the Eastern Sayan (in the south and southeast). It is a hilly and steep plain, high and deeply divided, lowered in the central and northern parts, which causes the concentric zoning of natural zones and subzones. Vegetation is represented by a combination of forests and grassy steppes. Thus, the study area refers to the zone of grass forests and island forest-steppes of the Central Siberian province of light coniferous forests.

According to the structure of the soil cover, climatic indices and vegetation cover, the study area belongs to the southern forest-steppe, almost two-thirds of which are occupied by chernozems, with the absolute predominance of ordinary chernozems (Krupkin and Toptygin, 1976). Three full-profile sections of chernozems in virgin, arable and fallow state have been studied.

According to graduation L.A. Grishina and D.S. Orlova, the maximum content of humus (estimated as high) was recorded in the chernozem section (1978), the average content of humus was found in the chernozem of the fallow area. In plowed agrochernozem, humus is the least. The content of mobile humic substances released 0.1N NaOH at pH 7.0 (Grishina, Orlov, 1978) in determining the group and fractional composition of humus by Ponomareva-Plotnikova, characterizes the effective fertility of the soil.

In the dark-humus and agro-dark humus horizons of all three soils, their quantity is high and very high, while their maximum values are observed in virgin chernozem. In the plowed black soil, all the indicators of the humus state are somewhat worse than on the virgin soil. In the chernozem of the deposit, a gradual recovery is observed.

To assess the qualitative composition of soil organic matter, the method of chemodestructive fractionation of soil organic matter was used (Popov, Tsyplenkov, 1991). The method assumes an effect on soil samples with an oxidizer potassium dichromate with increasing oxidation potential, which allows to determine up to 11 fractions of soil organic matter (SOM). They can be combined into 3 groups: easily oxidized or labile (fractions 1-4); medium-oxidizable (5-7) and a difficult-oxidizing or stable part (8-11).

In the virgin chernozem the distribution of HDF fractions along the profile is irregularly. In the humus, and especially in the clay-illuvial horizon, the easy-oxidizable fraction prevails over the hardly oxidizable fraction, which indicates some mobility of humic substances. With agricultural use in arable soil in comparison with virgin land, there was a significant decrease (1.6-1.8 times) of the easily oxidized fraction of humus in the plow horizon.

The share of the medium-oxidized part also decreased, while the share of the difficult-oxidizing fraction increased noticeably. Moreover, in the clay-illuvial horizon and soil-forming rock, the ratio of fractions remained the same as in virgin soil, which indicates a significant transformation of humus in the upper part of the profile during swelling and its stable state in the lower part.

The CMI (C management index) by J. Blair (1995) was used to estimate the rate of change in total organic carbon in soils of agricultural and natural systems, as well as soil quality under anthropogenic influence. The index is calculated by the ratio of the forms of organic matter that are easy and difficult to oxidize by potassium permanganate. The higher the index, the better the quality of soils.

A.I. Popov and V.P. Chyplenkov (1991) noted that the effect of potassium permanganate is the closest to the HDF method of determining the active form of humus. In the authors' opinion, a stronger oxidizer (potassium dichromate), in contrast to a solution of potassium permanganate, fractionates more or less the entire amount of humus, which increases the information about its labile forms.

Based on this, we used data on easily and hardly hydrolyzable SOM groups obtained as a result of the HDF method for calculating C management index (CMI) in the soils of the Kansk forest-steppe. According to the C management index (CMI), the quality of chernozems on arable land is satisfactory (CMI = 39), and the reservoir (CMI = 51) is good.

In general, we can state a decrease in the real-energy exchange in agroecosystems, which is accompanied by an intensification of the processes of soil cover degradation. The content of the mobile, labile part of humus decreases, which ensures actual fertility, which, as a rule, indicates a deterioration in the quality of land.

However, with a high crop culture and saturation of crop rotations with perennial grasses under irrigated conditions, it is possible to increase the content of humus, the amount of humic acids and labile humic substances that is observed when soil is removed from active agricultural rotation to fallow mode.

# Structural Features of Humic Acids of Different Geographic Zones According to the IR EXPERT System Data

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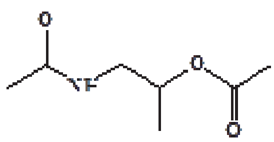
Humic substances have a pronounced ability to ion exchange, complexation with ions of polyvalent metals and ionic organic compounds. These useful properties are associated with the presence of functional groups and structural features of humic macromolecules.

The purpose of this work is to identify the most probable structural fragments of soil HAs of different geographical zones on the basis of their IR spectra analysis using the IR EXPERT information-analytical system, which is a combination of an extensive database (more than 50000 correlation records of "IR-spectrum–structure–structural fragments–accompanying information") and software modules that allow solving various spectro-structural tasks (i.e. search for spectral and structural analogs, determination of spectral feature of a structural fragment, creation of model spectra, determination of the difference degree or the similarity of spectral curves, etc.).

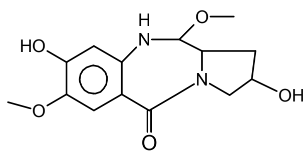
11 HA samples isolated from humus horizons of different northern soil types were used as the objects of the study. Geographical zones (soils) such as typical moss-lichen tundra (peaty-tundra gley, peat-tundra gley), northern forest-tundra (dry-peat mound soil, soil of frozen ceilings), northern taiga (gley podzolic soil, peaty-podzolic-gley soil), middle taiga (podzol illuvial-ferruginous), southern shrub tundra (sod-podzolic soil) were under studying.

To identify the most probable structural fragments of HAs, the following actions were performed:

- the nearest spectral analogs were searched for each HA spectrum by comparing full spectral curves. As a result, 11 search reports (SR) were obtained, each consists of 10 structures from IR EXPERT database. A close similarity of all search reports was found, with two compounds (I, II) being represented in all SRs with different degrees of similarity (from 0.2276 to 0.4772).



I



II

- all compounds of 11 received search reports were subjected to the decomposition procedure into their constituent fragments (from 3 to 10 vertex ones) and followed by analyses of ten-vertex fragments for which the non-randomness factor of appearance in IR-EXPERT database exceeds 0.9. It turned out that the number of such fragments varies from 5 to 196 from sample to sample

Analysis of the obtained ten-vertex fragments showed that all HA samples contain linear, slightly branched, conjugated chains of double C-C bonds, as well as fragments of aromatic amines and amides. It is likely that the structure of humic acids of these northern soils is predominantly aliphatic, which is confirmed by elemental analysis (H/C >1).In addition, HAs contain a large number of oxygen-containing groups, which allows to predict their high reactivity when interacting with ecotoxicants.

## Sporulation of Filamentous Fungi Under the Action of Humic Substances: Activity of Biogenic Fluorophores and Conidia Production

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The sensitivity to chemical contamination and virulence of pathogenic fungi strongly depends on the humus status, nutrient components, illumination and some other environmental parameters [2, 4, 5, 8]. Fungal resistance to stress is associated with the presence of melanin pigments in spores and mycelia, but the mechanisms of this phenomenon have not been studied. Fluorescence measurements performed on fungal particles, including spores, seems to be a useful tool for assessing of biomass, metabolism products, melanin production and ultimately associated virulence of Alternaria alternata. The significant biogenic fluorophores of fungi reflecting metabolic pathways are NADH, tryptophan, melanin and others which can be monitored using spectra; techniques [1, 3, 6].

The aim of the present study was to investigate the effect of HS on the sporulation of filamentous fungi: the conidia production and activity of biogenic fluorophores in fungi spores using spectral approaches.

In the model experiments, we investigated responses of *A. alternata* to HS of different origin (from leonardite and lignosulfonate) in two concentrations (0.1 and 0.02%) in liquid and agar Czapek medium with different concentrations of sucrose (0, 3, and 30 g/L). The strain was kindly provided by Marfenina O.E. and Ivanova A.E., Soil Science Faculty of MSU. Fluorescence emission spectra were measured using a luminescence spectrometer Solar CM2203 at several wavelengths of the exciting radiation (270, 310, 325 and 355 nm) for aqueous samples placed in quartz cuvettes. For comparasion the number of spores was counted in aqueous suspension by Goryaev counting chamber. The conidia production was calculated in units/cm$^2$ using the formula (Sanin, 2008). All obtained data were statistically analyzed by the one-way analysis of variance - ANOVA and factorial ANOVA.

Typical fluorescence spectra of fungal spores under the UV-excitation consist of two overlapping bands. The UV-band with a maximum at 300-350 nm is a protein-like fluorescence, and the band in the blue region with a maximum at 400-450 nm is emission of fungal chromophores like NAD(P)H or melanins. The intensity of protein-like fluorescence at 300-350 nm (excited at 270 nm) was directly correlated with the amount of biomass spores in water suspension.

The spores grown on sucrose-depleted medium (3 g/l of sucrose) did not emit fluorescence in the blue region of the spectrum, but only in the UV-region. NAD(P)H is sensitive to the changes of the cellular metabolism [1]. The lack of NAD(P)H fluorescence (emission at 460 nm with excitation at 310 nm) indicated the reduced intensity of energy processes. The spores of fungi grown on the sufficiency of nutrients (30 g/L sucrose) emit fluorescence in both wavelength regions. Free and enzyme-bound NAD(P)H may have different maximum in the excitation spectrum (at 340 and 280 nm, respectively) with the emission at 460 nm. The ratio of protein-bound and free NAD(P)H is an indicator for the metabolic activity [3]. The HS introduction changed the patterns of excitation spectra.

The results demonstrate that the introduction of HS was able to stimulate the sporulation intensity of *A. alternata*, especially when we introduced HS from lignosulfonate in the media with 30 g/L sucrose (Table 1). The sporulation intensity of *A. alternata* in a greater degree was depended on a combination of two factors, the HS type and concentration of sucrose.

Thus, in this research the HS introduction changed the patterns of excitation spectra and stimulate the spore production of dark-pigmented fungal culture *A. alnernata*. The fluorophores activity shows that the presence of HS of different origins in the fungal growth medium has a different effect on the synthesis of melanin.

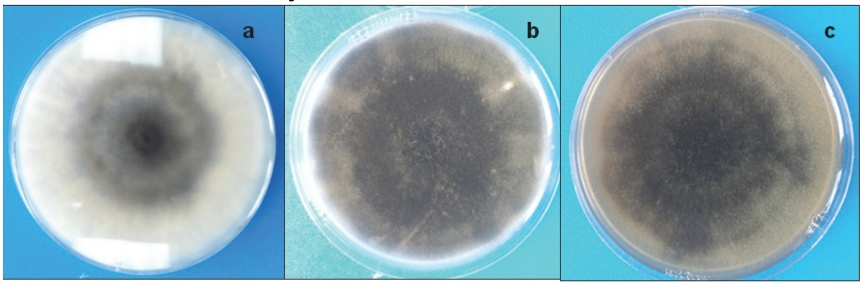


Figure 4. Fungal colonies of A. alternata with sporulation area on the 10th day of growth on the Czapek agar medium with: a) 30 g/L sucrose; b) 30 g/L sucrose and 0.02% HS from lignosulfonate; c) 30 g/L sucrose and 0.1% HS from lignosulfonate

Table 1. Results (F-value) of factorial ANOVA on effects of humic substances type (Type), added dose of HS (Dose) and sucrose content (Sucrose Dose) to spore production by A. alternata

| Source of variance | F-value |
|---|---|
| HS (Type) | – |
| Sucrose (Dose) | 37,9* |
| HS (Dose) | – |
| HS (Type) x Sucrose (Dose) | 163,5* |
| HS (Type) x HS (Dose) | – |
| Sucrose (Dose) x HS (Dose) | 19,2* |
| HS (Type) x Sucrose (Dose) x HS (Dose) | 26,5* |

*significant difference at $P < 0.01$;

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## Nitrogen Compounds of Labile Organic Matter of Long-term Experiments on Chernozems

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Nitrogen is the only essential plant nutrient that is not released by weathering of minerals in soil. Only a few microorganisms have the ability to use molecular $N_2$; all remaining living organisms require combined N for carrying out their life activities. Soil organic matter (SOM), acts as a storehouse and supplier of N for plant roots and microorganisms. Almost 95% of total soil N is closely associated with SOM. Major part of available N presents in rapidly turnover pools of SOM – labile organic matter. Thermolabile organic matter is a part of SOM which volatilized at heating up to $300^0$C. Content of thermolabile organic matter in soil closely is correlated with rapidly turnover C.

Goal of this work was to investigation of forms of N-content organic compounds in rapidly turnover pools of Chernozems of contrast organic matter regime by means thermodesorption with gas chromatography mass-spectrometry (TD-GC MS).

Objects of this work were Chernozems of long-term field experiments: 1. Steppe (virgin soil), 2. Permanent Black Fallow since 1964 and 3. Unmanaged Reservoir after Black Fallow since 1997.

Diversity of N compounds has decreased in the series “Steppe>Black Fallow>Reservoir”. All variants of Chernozems are characterized by presence pirazole and pyrimidine. There are observed nitrile-group and pyrrole in the Steppe and the Black Fallow. In the Steppe and the Reservoir imidazole was detected.

There were detected complexities compounds are contained at the same time a few N-groups: for example pyrozole and amine in the Black Fallow and the Reservoir.

In comparison with the Steppe in the Black Fallow there were detected molecular compounds of N with higher molecular weight. Only in the Reservoir carboxamide and carboximide were detected. These compounds can be markers of recovery processes.

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## Molecular Composition of Thermolabile and Thermostable Organic Matter in Aggregates of Chernozems

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A labile and stable part of soil organic matter (SOM) plays different roles in soil media. Wherein their closely link each other by genesis and processes of soil evolution.

Thermolabile organic matter is a part of SOM which volatilized at heating up to 300°C. Content of thermolabile organic matter in soil closely correlated with rapidly turnover C. In contrast thermostable one are volatilized at temperature from 300 to 600 °C and can be attributed to stable pool of SOM. In this connection molecular structure both thermostable and thermostable patrs of SOM can be obtained by double-shoot pyrolisys combain with gas chromatography mass-spectrometry (double-shoot Py-GC MS).

Goal of this work was to investigation of molecular composition of labile and stable SOM pools in Chernozems of long-term experiments by means double-shoot Py-GC MS Objects of this work were Chernozems of long-term field experiments: 1. Steppe (virgin soil), 2. Permanent Black Fallow since 1964, 3. Unmanaged Reservoir after Black Fallow since 1997 4. Permanent Potato, 5. Permanent Corn and 6. Grain-Fallow Rotation.

Experimental design included analyses of molecular composition for two factors: 1. Size soil aggregates and 2. Soil management. Two-way ANOVA was carried out.

Generally thermostable pool contained more substances in compare of thermolabile one for each classes: fragments of polysaccharides, aromatic, aliphatic, N-compounds and polyaromatic carbohydrates (PAH) – compounds. Size of soil aggregates depend on content of polysaccharides in thermostable SOM and PAH-compounds in thermolabile SOM. Soil management influenced on molecular composition practically all dependent factors except PAH-compounds in thermolabile SOM.

This result could suggest important role of PAH-compounds (black carbon?) in formation of aggregates of Chernozems.

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## Antioxidant Capacity of Soil Humic Substances: TEAC *vs.* ORAC Approach

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Humic substances (HS) play important functions in the environment by radical scavenging in biogeochemical redox reactions and thus influencing behavior of pollutants and preventing damage to cell membranes; this is due to antioxidant properties of HS. Phenolic moieties formed from more complex plant precursor molecules, such as lignin and tannins, have been suggested as the major electron-donating moieties of HS, and thus are thought to provide their antioxidant activity [1, 2]. However, due to difficulties in measuring the individual antioxidant components of a complex mixture such as HS, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid)-equivalency is mainly used as a measure for the antioxidant capacity (AOC) of HS. Trolox-equivalent antioxidant capacity (TEAC) is most often measured using the ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)). Nevertheless, the ABTS cation-radical is a synthetic one, so the antioxidant effect cannot be clearly interpreted in terms of biological systems. Besides, antioxidant interaction with ABTS both involves electro- and proton-donating mechanisms, whilst interaction of antioxidant with biologically relevant radicals such as the peroxyl radical could be realized only through proton donation. Consequently, it is important to compare data obtained by this method with data obtained by methods based on the use of the peroxyl radical such as oxygen radical absorbance capacity (ORAC) approach.

In our study we measured AOC of 9 soil HS both by TEAC and ORAC methods. A set of HS included 3 samples of fulvic acids (FA) extracted from sod-podzolic soils and 6 samples of humic acids (HA) obtained from sod-podzolic soils and chenozems. Both FA and HA were characterized by elemental analysis and 13C NMR spectroscopy. Evaluation of AOC using TEAC approach was conducted according [2] at pH 6.8 using 40 min as the end point. ORAC measurements were performed according to [3] at pH 7.4. To compare the obtained values of AOC, they were expressed in Trolox equivalents (TE) normalized to weight.

Values of measured AOC varied in the range 0.3–1.4 μmol/mg HS in case of ORAC and in the range 0.2–1.5 μmol/mg HS when TEAC was used. Antioxidant properties of FA were usually more pronounced than those of HA. Though similar ranges of AOC values for ORAC and TEAC methods were observed for the studied set of HS, AOC values obtained by ORAC were usually lower than those obtained using TEAC. The latter might be most likely explained by realization of two mechanism of radical scavenging (electron and proton donation) under TEAC conditions when only scavenging via electron donation could occur in case of ORAC. Surprisingly, there were two exceptions, namely, HA derived from chernozems when ORAC values were greater than TEAC. As compared to the other studied HS, chernozemic HA possessed the highest content of aromatic moieties (both non-substituted and oxygen-substituted carbon) and the lowest ratios O/C and H/C. As a short-term assay using the $ABTS^{\bullet+}$ is recommended for the evaluation of fast constituents, rather than for determination of the end-point AOCs of HS [2], our results allowed to conclude that humic materials of high aromaticity and low oxygen content have relatively low content of fast constituents demonstrating antioxidant activity.

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## Lignin Phenols as Biomarkers of Palaeoenvironments

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Biomarkers are organic molecules of the known structure and origin. In contrast to humic acids, whose formulas and chemical structures are still unknown, separate molecules of biomarkers can be reliably detected in living organisms, their residues, and waste, as well as in complex associates of humic substances in the soil. Lignin biomarkers are resistant to degradation and mineralization in soils under specific conditions, because of their specific structure and biochemical functions; therefore, they serve as molecular traces of land vegetation and as indicators of the rates of recent and past biochemical processes occurring in the biosphere. Individual organic compounds are well preserved not only in the normal profiles of postlithogenic soils, but also in their redeposition products, soil colluvial and alluvial complexes, pedosediments, pedoliths, derivatives of separate horizons, bottom sediments of water bodies, etc. The information role of biomarkers is important when the pool of molecular products of organic matter decomposition in soils is not identical to the sum of individual waste components because of the masking effect of the mineral matrix.

The objects of study included different dated paleosols detected in polygenetic profiles of recent soils and thoroughly studied by a set of physicochemical methods. The following soils were studied: (1) agrogray soils with a second humus horizon of the Bryansk Opolie and agrogray soils of the Kolomna Opolie (Moscow district); (2) mountain chernozems, chernozem-like soils, and mountain meadow soils of intermountain valleys and chestnut soils of Tien Shan; and (3) brown forest and mountain meadow soils of Caucasus. Zonal soil varieties containing no buried horizons were used for comparison in all cases. Lignin phenols were isolated in triplicate from living plant tissues (wood, roots, needles), waste, soil, and humic acids preparations. The alkaline oxidation of vascular pant tissues and their remains by copper oxide in the soil yields 11 phenols, which can be grouped in accordance with their chemical nature into three structural families: vanillyl (guaiacyl), syringyl, and cinnamyl. 13C NMR spectra were recorded for 25 humic acids preparations. The isotope composition of carbon in soil organic matter was determined on a Thermo V Plus isotope ratio mass spectrometer and a Thermo Flash 1112 elemental analyzer. The age of soils was determined by the radiocarbon dating of humus.

Analysis of the presented data on the composition of lignin phenols in plant tissues, as well as in the recent and buried horizons of different polygenetic soils, reveals that the composition of lignin phenols in soils serves as a molecular trace of land vegetation. The information role of the groups of compounds (chlorophyll, fungal melanins, entire lignin), in turn, reflects only the character of soil wetting and thus is less informative than the individual biological molecules, whose micro amounts are easily determined by modern methods.

The qualitative composition of lignin phenols in plants is reflected in the δ13C values of soil humus. The cinnamyl phenols make heavier the isotope ratios, which are very sensitive to C3–C4 changes. At the same time, the vanillyls and syringyls of woods and shrubs (C3 type of photosynthesis) lighten them.

In spite of the significant number of lignin peaks in the 13NMR spectra of humic acids, most of them allow only the qualitative identification of biomarker and the fixation of changes in the natural environment during the time intervals determined by radiocarbon dating. The comparison of the 13 NMR spectra of native lignin preparations isolated from different woody and herbaceous species with those of soil humic acids makes it possible to identify many characteristic shifts of lignin nature in humic acids at 56, 102, 115, 119, 131, 147, 151–152, 160, and 166 ppm.

The information role of biomarker has been tested at the reconstruction of paleovegetation in the uplands of the Russian Plain: an upward shift of the forest boundary has been recorded on the Northern Caucasus; the hypothesis about the steppe period of landscape development in the Tien Shan mountain valleys during the middle Holocene has been confirmed, and molecular traces of tropical flora have been revealed in the buried soils of Pleistocene age.

The obtained results and their use for paleosol studies show the suitability of the proposed biomarker for the diagnosis of paleovegetation and call for the further study of the pool of natural lignin phenols using their extraction from different plant tissues and the creation of database on the properties of lignin from specific plant species. The most important information role of biomarker is in the integrated studies of paleosols, along with the determination of the age, isotope composition of organic carbon, and other indicative properties.

# Investigation Colloidal Properties Depending on Composition of System of «Na-form of Humic Acids – Salt Solution of Heavy Metals»

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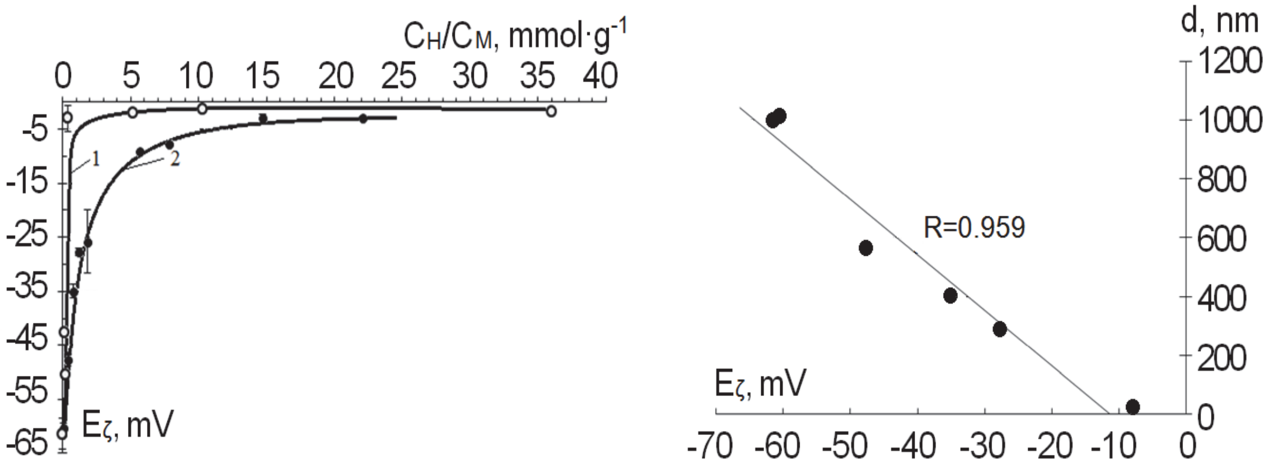
Binding process of ions of heavy metals (HM) by the Na-form of humic acids (HANa), generating colloidal system in solutions, proceeds by ion exchange and (or) complexation mechanisms [1-3]. A double electric layer (DEL) – a thin surface consists of spatially separated electric charges of the opposite sign arising at the particles with water environment interface. So the colloidal organic substances are steady due to the emergence of the DEL [4]. During the interaction of alkali solutions of HA with metal's ions, soluble or insoluble complexes may form depending on the ratio metal: HA [5, 6]. This can be explained by binding of a part of HA groups that provides elongated configuration of a macromolecule and high hydration with the HM. So due to compensation of the negative charge and weakening of repulsion the molecule coagulates [2, 7] ζ-potentials of HANa and metal-humic complexes were defined to confirm this interaction mechanism between HM and HA. Also colloidal particle's (HANa and HA-Cd) size was measured depending on different ratio HM: HANa (0.04-24 mmol/$Cd^{2+/}$g and 0.1-39 mmol $Pb^{2+/}$g).

Humic acids were isolated from the Oligotrophic bog of the Ilas peatland [8, 9].

Plot, illustrating dependence of ζ-potential of colloidal HANa on the relative content of ions HM in the system, is presented at figure a. This graph shows that decreasing of the colloidal particles' ζ-potential in presence of Pb (II) occurs more intensively than in presence of Cd (II). It might be caused by greater absorption of Pb (II) than Cd (II) at the same molar content of metals. The conclusion of the research is that HA have no another areas capable for binding of HM besides oxygen-containing groups. This follows from the observation that HA recharge does not occur even when the content of Pb (II) ions is several times more than total content of acidic groups.




a b

Figure - Changing of properties of the colloidal system:
dependence of the ζ-potential of colloidal HA on the relative content of ions HM:
1 – $Pb^{2+}$, 2 – $Cd^{2+}$;
b -dependence of the particles' size on the ζ-potential

Decreasing of colloidal particles' DEL naturally causes change of their size. Picture b shows dependence of the particles' size on the ζ-potential HA-Cd which is the most important characteristic of the DEL. The inversely proportional dependence between metals' concentration in solution and the ζ-potential of HA and their size can be seen on the graphs (Figure a, b). The change of the size of colloidal particles in the systems is associated with the coagulating effect of metal ions.

Thus, sorption by humic acids of HM is accompanied by a decrease of the ζ-potential of colloidal particles and violation of their sedimentation stability.

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## Structure and Properties of Humic Substances in Floodplain Soils of the European North-East

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The formation mechanisms of humic substances (HSs) in alluvial soils located within flood terraces of the Pechora, Vychegda, and Sysola Rivers in the European North-East of Russia were studied. Floodplain soils of the above rivers lie on non-carbonate, alluvial deposits. They are strongly acidic (pH 4.0-5.5), not-base-saturated; soil humus is dominated by fulvic acids (FAs). Humus resources in floodplain soils overlaid by grassy vegetation (at floodplain meadows) vary within 60-220 $t \cdot ha^{-1}$ depending on soil type and ecological soil forming conditions. Soils in the centre of floodplain possess the highest resources of soil organic matter. They are most mature and well-developed floodplain soils of the whole spectrum of mineral alluvial soils. Humus resources in soils of floodplain asp-birch forests are 1.6-1.9 times higher than those in soils of floodplain meadows.

Having studied the structure and properties of HSs preparations using up-to-date physical-chemical methods we have identified that alluvial soils and zonal podzolic soils have common features. In both alluvial and zonal soils, humus formation process results in a system of humic substances (HAs) which includes hymatomelanic (4-10%) and brown (90-96%) HAs. Regardless of soil genesis, all HAs are surely a reduction product (oxidation degree varies from -0.14 to -0.41). Excessive moisture content and low biochemical activity of alluvial soils (formation of alluvial soils in inter-ridge depressions of floodplain terraces) are accompanied by easing HAs molecular structure, decreasing the portion of aromatic components, and increasing the portion of periphery structures (amino acids (AAs); aliphatic and carbon components) in composition of HAs molecules. Neutral nonpolar and acidic AAs predominate in all HAs. In the humus-accumulative horizons of the all studied soils, in comparison with organogenic, an increase in the proportion of amino acids was noted with a decrease in the proportion of neutral nonpolar ones.

The fulvic acids of alluvial soils are close to HAs in terms of carbon content, but in contrast to the last are 1.4-1.6 times less nitrogen, 1.2-1.7 times less hydrogen and almost 2 times more oxygen. Amino acid composition of FAs is relatively higher (37-41% of the sum) and the proportion of basic amino acids (5-6% of the sum) is sharply reduced, compared to HAs, in which the content of these groups is 28-29 and 12 -14% of the sum of the identified amino acids.

At present, floodplain meadows are actively overgrown with aspen and birch. This contributes to a change in the ecological conditions of soil and humus formation. In the floodplain aspen-birch forests, the mineralization rate of plant litter decreases. In comparison with the soils of floodplain meadows, in the forest litter horizons the reserves of energy, carbon, nitrogen and biophilic elements are higher. In particular, in soddy forest soils, the reserves of SOC are 73-86 and 114-151 t $ha^{-1}$, respectively, which is 1.6-1.9 times higher in comparison with similar types of soils formed under grassy vegetation. At the same time, in comparison with the soils of floodplain forests, in the soils of floodplain meadows the humus compounds in the form of calcium humates and strongly bound complexes with clay minerals and minerals based on oxides and hydroxides of iron and aluminum are consolidated in the mineral layers of the soil profile.

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# Influence of the Nature of Organic Matter on the Formation of Soil Structure

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Soil structure is the basis of soil fertility. It is determined by different factors, in particular, by interaction between mineral particles. Soil organic matter (OM), a large part of which is provided by humic substances (HS), affects significantly formation of soil structure. Different approaches have been used to analyze the influence of HS on this process: soil structure was considered as HS gel network reinforced by clay particles or HS were also considered as hydrophobic modifiers of mineral particles increasing hydrophobic interactions between them. We have hypothesized that flocculation between HS adsorbed on mineral particles and soil polyelectrolytes (fresh OM released during the activity of soil biota) affects considerably the formation of soil structure.

The aim of this work was to show a combined role of different types of OM – labile polymers secreted by soil biota in situ (flocculants) and highly transformed HS adsorbed on mineral soil particles on their aggregation. The strength of model and real soils with various moisture contents was chosen as an indicator of soil transformation.

Montmorillonite and sand were used as model mineral components. Natural polysaccharide chitosan (Ch) was used as a model flocculant. Potassium humate isolated from leonardite was used as a model of HS.

As real soil systems, chernozem ($C_{org}$ 7.5%), virgin steppe ($C_{org}$ 6.1%), sod-podzolic ($C_{org}$ 5.0%) soils and long-term fallow plowed twice a year ($C_{org}$ 3.5%) were used.

Rheological parameters of clay pastes were determined by variations in shear stress at a given strain rate using a Reotest-2 rotation viscometer. A conical indenter plastometer was used for the study of soil strength of clay-sand model systems.

It was shown that strength of real soils as well as strength of model systems had an extremal behavior with increasing water content. The values of strength were higher for soil with a higher content of $C_{org}$ (7.5%) and reached the maximum value 900 g/cm$^2$ at a water content of 40%. Soil with lower $C_{org}$ (5.0%) had the lower maximum strength value of 600 g/cm$^2$ at the same water content. The maximum value of strength for model soil (10% – clay, 90% – sand) reached 1000 g/cm$^2$ at a water content of 20%. The addition of clay to sand systems led to hardening of the system up to 1900 g/cm$^2$ at 30% of clay. The modification of model clay systems by HS led to substantial increase of shear strength of the system after addition of Ch (>0.5 g/L). This fact may be explained by flocculation between HS covering mineral particles and chitosan as well as by the mineral structure of montmorillonite permitting HS molecules to penetrate into interlayer's space. HS play a role of an anchor which links mineral particles with a flocculant and facilitates the aggregation. The increase in Ch concentration and the decrease in molecular weight of Ch led to an essential increase in viscosity and shear strength. Sand systems were characterized by lower values of strength compared with clay-sand systems. The absence of the hardening effect in model sand systems without clay in the presence of Ch and HS may be explained by the formation of polyelectrolyte Ch-HS complexes not on the surface of sand particles having low surface area but in the space between them. That leads to the loosening of sand samples and decrease the strength values.

Shear strength of non-treated chernozem with $C_{org}$ 6.1% was significantly higher as compared to plowed soil with lower $C_{org}$ 3.5%. Intensive tillage results in the oxidation of soil organic matter and can lead to soil degradation. The decrease of shear strength values due to decrease in soil organic matter content may enhance the role of OM, especially its labile fractions, in the contact interactions in soils.

# The effect of Humic Substances on Productivity of *Solanum tuberosum* and *Hordeum vulgare* at Mineral Fertilizer Application in Conditions of Microfield Experience

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Humic products are actively used in agriculture because it helps to combat soil depletion, decrease in fertility and disturbance of the humus balance. However, scientific works on the optimizing the use of Humic products is not sufficiently done, although it is evident that such works are necessary to elaborate a strategy for the agricultural production and the rational use of the natural resources. This work is aimed at studying the effect of Humic Substances on productivity of potatoes and barley in conditions of microfield experience. In addition, the possibility of reducing doses of fertilizer when using humic preparations was evaluated.

Our scientific work consisted of two parallel experiments. The first part of the work the experimental field is azonal (Alluvial (floodplain) sod soil). The second part of the experiment was conducted in the north of the Moscow region. The soil type in this region is soddy podzolic. Effect of Humic products on the productivity and quality of cropst was estimated

The Life Force Humate Balance was used for growing barley. The considered variants of the experiment: 1 – control; 2 - Life Force Humate Balance; 3 – NPK; 4 - Life Force Humate Balance + NPK; 5 - Life Force Humate Balance + ½ NPK. The dose of humic preparation was 500 kilograms per hectare. The total fertilizer application rate was 60 kilograms of active ingredient (NPK) per hectare. Humic preparations and fertilizers were introduced into the soil in a dry form and were mixed in the upper soil horizon. Adding preparations performed once immediately before planting. The experiment was carried out on experimental plots. Barley was sown with a wide-row method of 15 rows per plot. The size of one experimental plot for the cultivation of barley has five square meters. Each variant of the experiment was performed in four replicates. Total twenty experimental plots there were in the Voronezh region, and the same in the Moscow region.

The Life Force Natural Humic Acids for the cultivation of potatoes was used. As well as in the cultivation of barley, there were five variants of the experiment. However, the dose of humic preparation was 1000 kilograms per hectare. Doses of mineral fertilizers were the same as for barley. The size of one experimental plot for the cultivation of potatoes has six square meters. The distance between the potato bushes in the row was 30 centimeters; the distance between the rows was 50 centimeters. The number of experiments varieties with potatoes and the way of introducing humic preparations and fertilizers remained the same as with barley.

At the end of the experiment, freshly harvested plant material was evaluated by morphometric characteristics. Determine the total length of barley plants, the length of the ear of barley, the number of grains in the ear of barley, total mass of barley plants. Further, the structure of the crop was determined (the mass of grains, the number of grains in the ear, the mass of thousands of grains). Potatoes were divided into three types by size (large, medium, small). The total crop yield and weight of each potato type were determined. Further, in the plant material, the physiological parameters of the plants were determined. For potatoes: nitrogen, phosphorus, potassium, starch, vitamin C. For barley: nitrogen, phosphorus, potassium, starch, protein.

Thus, the influence of two humic preparations on *Hordeum vulgare* and *Solanum tuberosum* grown in different climatic conditions with the use of fertilizers and without them was evaluated. The estimation of the possibility of reducing fertilizer doses with the use of humic preparations was investigated.

## Influence of Soil Minerals on Structural Features of Newly Formed Organic Matter During Transformation of Plant Residues

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Losses of soil organic carbon is considered to be the major problem in maintaining fertility in intensive agricultural systems. Plant residues are the main renewable source of organic matter (OM) in soils. The content and properties of soil organic matter are controlled mainly by the chemical composition of plant material entering the soil and the nature of the process of their decomposition. The mineralization processe is determined by the type of microbial community and stabilization mechanisms of OM. The mineral part of the soil, especially clay minerals, is one of the key factors affecting the processes of transformation of organic carbon in the soil. Soil mineral phases strongly influence soil dissolved organic matter and humic substances dynamics. The interaction of newly formed humic substances, as well as nonspecific organic compounds with an inorganic matrix makes them less accessible to microorganisms-destructors. The stabilization of organic carbon necessarily involves structural changes in organic matter in the chemical form that will be most stable under the given conditions. The role of various soil minerals in the formation and preservation of OM is still an open question.

The aim of this investigation is to study of humification process of plant residues in different mineral substrates in incubation experiment. Artificial organo-mineral substrates was prepared in the laboratory from plant residues, pure mineral components, and a microbial inoculum extracted from a gray forest soil. Model experiments were performed with corn (*Zéa máys*) and clover (*Trifolium praténse*), the aboveground mass of which was preliminarily dried and disintegrated to 3–5 mm. Pure quartz sand, carbonate–free cover loam (soil–forming rock of gray forest soil from the Experimental Field Station of our Institute), silica sand + 15 % of bentonite and silica sand + 30 % of kaolinite were used as the mineral substrates. These minerals were chosen because bentonite is an example for 2:1 layer phyllosilicate whereas kaolinite is 1:1 layer clay mineral commonly found in soils. The mineralogical composition of the loam includes quartz (59%), kaolinite (16%), mica (13%), feldspars (11%), and smectite (2%). The sand was preliminarily washed with a 10 % HCl solution. The minerals were thoroughly mixed with the plant biomass at a ratio of 1:10. The artificial organo-mineral substrates were incubated for 6 months under constant temperature and water content. We investigated the effect of mineral matrix on the structural and functional parameters of the newly formed dissolved organic matter (DOM) and humic-like compounds in the process of biodegradation residues. Fourier-transform infrared spectroscopy (FTIR), nuclear magnetic resonance spectroscopy (NMR), ultraviolet and fluorescence spectroscopy were used to characterize the newly formed OM.

The stabilization of newly generated humic substances in the forms of organo-mineral compounds is very important mechanisms of humus formation in soil. The content of newly formed humic substances is related to stability of their organo-mineral complexes and accessibility of OM to microorganisms. In the FTIR and solid-state $^{13}$C-NMR spectra clearly demonstrated the signals of the alkyl structures, polysaccharide, aromatic components, carboxyl, amide groups. The $C_{Alk}/C_{O\text{-}Alk}$ (according to NMR spectroscopy) and AR/AL (according to IR-spectroscopy) parameters were estimated. In the humification process of plant material increases the proportion of aromatic and unsubstituted aliphatic groups, decreases the proportion of the alkyl-substituted carbon. According to spectroscopic data mineral phase actively contributes to the accumulation of oxygen-, nitrogen-containing and aromatic fragments. Comparison with the original plant material shows that the organo-mineral substrates have increased aromaticity, i.e. occurs the process of the formation of humic substances. The surface-active soil mineral particles influences the structural composition of humic substances. Analysis of the FTIR- and solid-state $^{13}C$-NMR spectra shows that humic-like substances formed during incubation of plant residues enriched not only aromatic, but also alkyl groups. In the treatments with kaolinite occurs mainly the accumulation of compounds of a carbohydrate nature. The substrates with bentonite are characterized by a wide range of aromatic structures. The dissolved organic matter is the most labile and reactive fraction of the soil organic matter. The spectroscopic methods revealed selective sorption of water-soluble transformation products of plant residues. In the course of the incubation experiment the differences between all the extracted DOM were found. This study imply the direct participation of clay minerals in humus formation. Overall, our results suggest that both specific mineralogy and biochemistry of plant biomass affect the structural and functional properties of newly formed organic matter.

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## Binding of Phthalic Esters by Humic Acids in Water

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At the present time a huge amounts of xenobiotics penetrate into the most of water objects and phthalic acid esters (phthalates) are not the exception. Being the widespread plasticizing agents and detergents, which are using for producing many materials and consumables, phthalates are considered to be the ubiquitous environmental pollutants with rather high intake to water sources of urban and industrial areas. Humic substances, which contained in water in molecular and colloidal forms, are able to react complex formations with organic pollutants (including phthalates), such influencing redistribution of phthalates within the landscape.

The purpose of the work was to study the binding of phthalates with humic substances in water. An original method of determination of phthalates in water was developed. The analytical procedure allowed to investigate phthalate pattern and content in of 120-360ml water samples with a detection limit of 0.01 μg/l, using short-term liquid extraction and GC-MS detection. Two phthalic acid esters - dibutylphthalate and diethylphthalate - were studied for binding action with humic substances. The humate potassium PowHumus (Germany) was used as a model water soluble humic substance.

As a result isotherms were received which conformed to the second type of BET isotherm with polymolecular adsorption. Using linearization of the initial part of the isotherms the values of phthalate binding constants were obtained as well as the saturation capacity of the monolayer at the humic acid binding sites, which precedes the polylayer bonding, due to the adsorbate-adsorbate interaction.

Based on the research, the following conclusions have been made:

1. Type of binding isotherm of phthalic acid esters with water-soluble humic acid indicates the course of poly-adsorption at high concentrations of phthalate.
2. The apparent binding mechanism is determined by hydrophobic interactions, whose strength increases with the elongation of the alkyl radical chain of the phthalate.
3. The interaction of adsorbent-adsorbate in these systems is stronger than the interaction of adsorbate-adsorbate. It was also observed that the level of binding of humic acid-phthalate - the higher, the lower the concentration of the dissolved phthalic acid ester.
4. For diethylphthalate binding with humic acid was observed at a concentration level up to 300μg/l. The binding efficiency was significantly lower than for dibutylphthalate, that may be connected with the lower hydrophobicity of diethylphthalate.
5. For dibutylphthalate, the saturation capacity of the monolayer (Smax) is 14300μg/g (binding constant is 0.015). For diethylphthalate, binding with humic acid is much weaker - the saturation capacity of the monolayer is in the range 800-1000 μg/g.
6. Based on obtained results one can calculate, that for concentration range usually observed in natural surface waters only 10% of phthalates is detected as free form, up to 9 times higher quantity of phthalates may present in the undetectable form of complexes with humic substances.

## Study of Structural and Functional Composition of Soil Humus Acids of the Euro-Arctic Region by Molecular Absorption Spectroscopy (UV / visible)

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Formed under the influence of cryogenic processes, Arctic soils are very sensitive to the pollutants. Humus acids are able to bind pollutants, including heavy metals (HM), into slow-moving and poorly dissociating compounds. In this regard, the investigation of the structural and functional composition of humus acids in the Arctic soils can help in the study their eco-protective role.

Different types of soils of the Euro-Arctic region were chosen as research object. Soil profiles were laid by the participants of the complex scientific and educational expedition "Arctic Floating University-2013" in the following territories: light-loamy gley loam on medium-loamy moraine (Kanin Peninsula (Cape Kanin Nos)), humus-peat oligotrophic soil (Kolguev Island (Bugrino)), gleyzem is a typical calcareous medium-loamy (Vaigach Island), lithozem gray-humus illuvial-ferruginous sandy types (Archipelago Franz Josef Land (Hays Island)).

Humic (HA), fulvic (FA) and hyhromelanic (GMA) acids were extracted from the mixture of humus acids by an alkaline sodium pyrophosphate solution and following reaction on HA, FA and GMC by various extractants with additional fulvic acids extraction by adsorption chromatography with activated carbon as a sorbent.

The UV / visible spectra were recorded on a UV mini-1240 spectrophotometer (Shimadzu) using 0.005% alkaline solutions (0.1 N NaOH) of humus acids.

The humus acids UV / visible spectra of the studied samples have a maxima 213 ± 215 nm in the UV rang, which indicates phenolic and carboxyl groups in the molecules (Fig. 1).

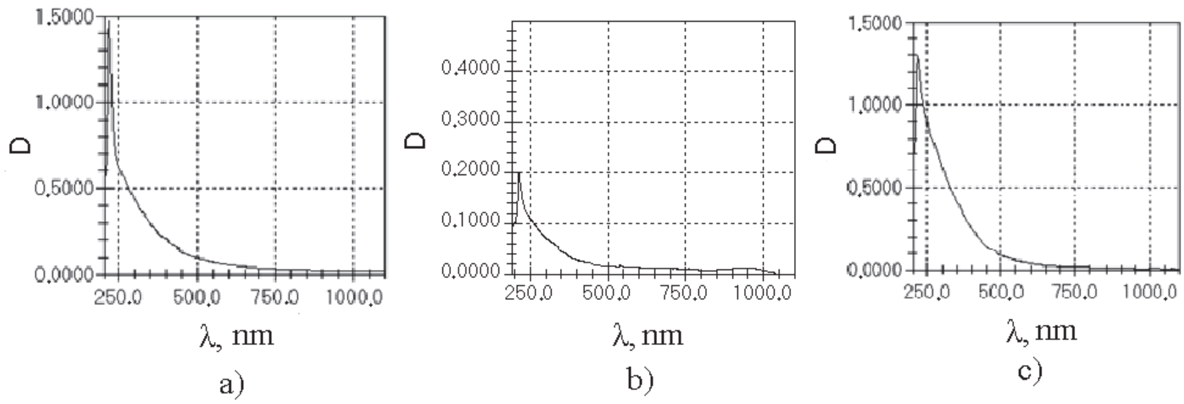


Figure 1. The humus acids UV / visible spectra extracted from humus-peat oligotrophic soil A) HA; B) FA; C) GMA

It was demonstrated that humic and gimatomelanoic acids of humus peat oligotrophic soil have more developed peripheral aliphatic component. These acids have more ability to bind heavy metals and show their eco-protective role. Other preparations have a more flat spectral curve, which indicates the development of aromatic structures in the nuclear part of the molecules

Such parameters as: calculated by the Pieravuori formula aromaticity, extinction coefficient $E^{0,005\%}_{1cm,465}$, adsorption ratio $D_{400}$ /$D_{600}$, characterizing the degree of humification and the adsorption ratio $D_{465}$ / $D_{650}$, characterizing the degree of aromatic nucleus condensation and conjugate fragments were used in the quantitative assessment of the nature of humus acids (table 1).

Table 1. Optical properties of humus acids of the studied soils

| Soil profiles | $E_{465} \cdot 10^{-3}$ | $\frac{D_{465}}{D_{650}}$ | $\frac{D_{400}}{D_{600}}$ | A |
|---|---|---|---|---|
| Humic acids | | | | |
| humus-peat oligotrophic soil | 26,0*± 0,4 | 2,60 ± 0,26 | 3,29 ± 0,18 | 31,56 ± 0,23 |
| gleyzem is a typical calcareous medium-loamy | 6,0 ± 0,3 | 3,00 ± 0,34 | 3,00 ± 0,25 | 25,39 ± 0,32 |
| lithozem gray-humus illuvial-ferruginous sandy types | 3,6 ± 0,5 | 2,57 ± 0,69 | 2,50 ± 0,65 | 36,41 ± 0,46 |
| light-loamy gley loam on medium-loamy moraine | 3,6 ± 0,1 | 4,50 ± 0,11 | 3,33 ± 0,93 | 34,04 ± 0,46 |
| Fulvic acids | | | | |
| humus-peat oligotrophic soil | 3,4 ± 0,3 | 1,54 ± 0,18 | 2,17 ± 0,17 | 32,90 ± 1,06 |
| gleyzem is a typical calcareous medium-loamy | 3,4 ± 0,1 | 2,12 ± 0,20 | 2,80 ± 0,09 | 31,34 ± 0,23 |
| lithozem gray-humus illuvial-ferruginous sandy types | 2,8 ± 0,3 | 1,75 ± 0,27 | 2,14 ± 0,33 | 29,91 ± 0,46 |
| light-loamy gley loam on medium-loamy moraine | 1,8 ± 0,1 | 1,12 ± 0,07 | 1,75 ± 0,04 | 29,80 ± 1,60 |
| Hyhromelanic acids | | | | |
| humus-peat oligotrophic soil | 24,0 ± 0,4 | 2,40 ± 0,16 | 2,77 ± 0,27 | 32,17 ± 0,15 |
| gleyzem is a typical calcareous medium-loamy | 6,0 ± 0,4 | 3,00 ± 0,23 | 3,00 ± 0,35 | 15,22 ± 0,26 |
| lithozem gray-humus illuvial-ferruginous sandy types | 12,4 ± 0,3 | 2,38 ± 0,07 | 3,16 ± 0,09 | 40,72 ± 0,03 |
| light-loamy gley loam on medium-loamy moraine | 3,8 ± 0,2 | 2,38 ± 0,11 | 2,00 ± 0,93 | 29,04 ± 1,31 |

Quantitative analysis of UV / visible spectra showed that the maximal barrier mechanism with respect to heavy metals will be humic and gimatomelanic acids of humus peat oligotrophic soil, due to the high content of phenolic and carboxyl groups in these molecules, the greatest degree of oxidation and the developed chains of conjugated bonds in comparison with other acids. It was established that the process of humus formation is mainly along the degradation type, in the direction of fulvic acids formation in all types of soils.

## On the Contribution of Minor Components to the Biological Activity of Humic Acids

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The stimulating effect of humic substances, especially humic acids and their salts, on a growth and development of plants, increase of their resistance to unfavorable environmental factors, stimulation of germination of seeds, increase of the productivity of cattle and poultry is stated in a number of studies [1].

Data of biological activity of humic acids, its correlation with structure of various fragments of humic acids macromolecule, nature and content of various functional groups are highly contradictory [2]. Probably, this can be explained by the reason investigation of humic substances of contaminated with components of wax and resins. At the same time, the significant contribution of minor components to the biological activity of various pharmaceutical preparations is discussing recently [3].

The effect of treatment with different fractions of peat (Table 1) on germination energy (%), seed germination (%) and the degree of defeat of salad shoots (according to GOST 12038-84) were studied to estimate the contribution of minor components to the biological activity of peat humic acids. The seeds were calibrated and soaked in a working solution at room temperature for 16 hours.

Table 1. Characteristics of working solutions

| № | Main fraction | Bitumen removal | Concentration of working solution,% | Solvent | pH | Content $K_2SO_4$, mg/l |
|---|---|---|---|---|---|---|
| 1 | Without additives | | | water | 7,1 | 120 |
| 2 | Humic substances | – | 0,01 | water | 7,2 | 120 |
| 3 | Humic substances | + | 0,01 | water | 7,9 | 120 |
| 4 | Fulvic acids | + | 0,01 | water | 7,5 | 120 |
| 5 | Humic acids | + | 0,01 | water | 7,6 | 120 |
| 6 | Bitumens | | 0,01 | etanol-water | 7,4 | 120 |
| 7 | Resins | | 0,01 | etanol-water | 7,6 | 120 |
| 8 | Waxes | | 0,01 | etanol-water | 7,7 | 120 |

The experiments showed that a wax fraction has the greatest agrochemical activity with a simultaneous minimum of seed lesions (2.7%). The main components of the wax fraction are paraffins and esters of alcohols (sterols, tocopherols). Humic acids have more positive influence on the germination processes than the peat wax (figures 1, 2). Fraction of resins (saturated hydrocarbons) inhibits germination process of seeds having the lowest degree of shoots damage at the same time. So, the conclusion is the resins perform a protective function against pathogens, but do not stimulate seed germination. Fractions of fulvic substances showed no agrochemical activity and had a comparative high degree of pathogen damage.

Therefore, it may be concluded that peat bitumen waxes can serve as a good defensive-stimulating agent and used, for example, for seed encapsulation.

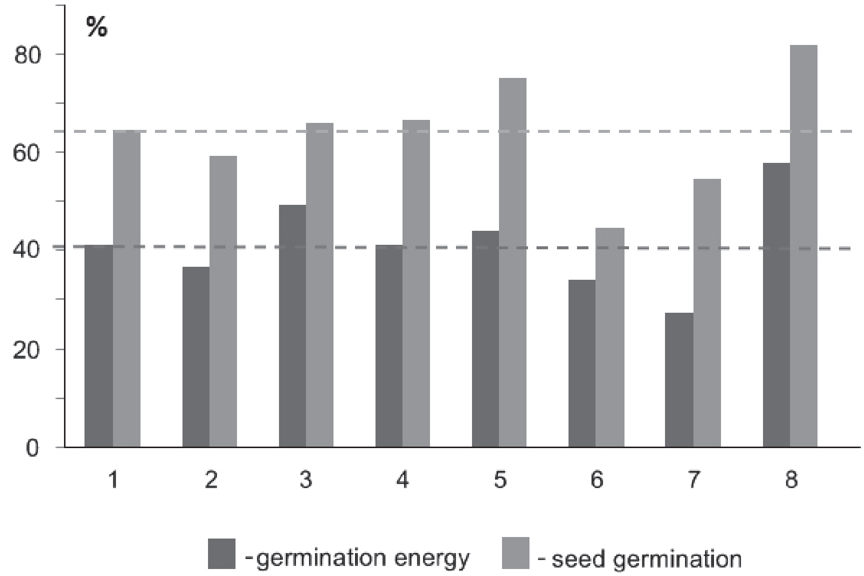


Figure 1. Effect of peat components on the stimulation of seed germination energy

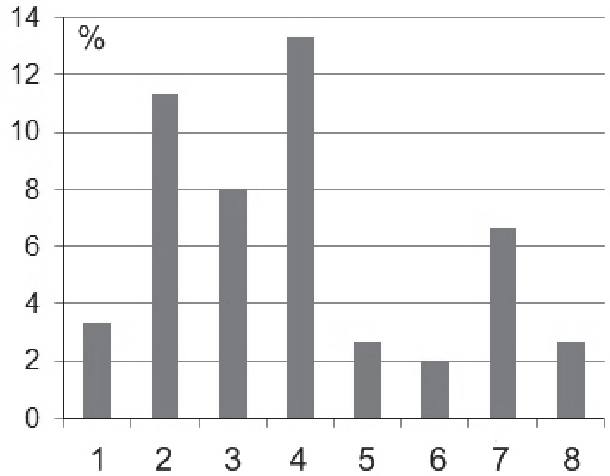


Figure 2. Effect of peat components on the defeat of salad shoots

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## Soluble Organic Compounds in Lichens and Mosses

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Complex study of Soluble organic compounds (SOC) in the ecosystems of Russian European Northeast revealed completeness, wide scale and versatility of this scientific problem. We studied lichens of two systematic groups from orders *Peltigerales* and *Lecanorales*, and mosses *Pleurozium schreberi*, *Racomitrium lanuginosum* and *Racomitrium canescens* collected at the Khibini Mountains (Kola Pepinsula).

In lichens, the total content of soluble acids, alcohols and hydrocarbons was from 0.3 to 12.3; in mosses, from 0.2 to 4.1 g/kg, that does not exceed 3% from the total C in SOC. In lichens, the minimal concentrations were found in Cladonia species (0.3 to 0.5). Cetraria species contained more soluble compounds (1.4 to 2.3), then followed *Peltigera canina, P. aphtosa* (5.3 to 6.1), *Nephroma* and *Solorina* (7.8, 12.3 g/kg respectively). In mosses, similar concentrations were found in *Racomitnium lanugonisum* and *R. canescens* (0.2 and 0.5 g/kg); concentrations in *Pleurozium schreberi* were significantly higher (4.1 g/kg).

Lichens had similar proportion of various compounds which was different from that of mosses. Alcohols prevailed (70-90%) in the lichens while hydrocarbons prevailed (80-90%) in the mosses. Total content of alcohols differed significantly in the different objects: in *Peltigerales lichens* it was 4.3-9.8, *Lecanorales* contained less alcohols – 0.2-1.9 g/kg. In all the objects, 90-100% of total alcohols were presented by mannitol and arabitol. 10 soluble hydrocarbons were found in all the objects: arabinose, ribose, fructose, glucose, saccharose, xylose, talose, turanose, galactose and mannose. First five hydrocarbons counted 80-100% of the total mass of this group that varies significantly in the different objects. The total concentration of carbonic acids in studied objects was between 4 and 400 mg/kg. In general, *Peltigerales* contained more acids than *Lecanorales* (0.2-0.4 and less than 0.1 respectively). Among mosses, *Pleuroziu schreberi* contained as many acids as *Peltigera*. In mosses from genus Racomitrium, acids concentration was 7-10 times lower. In the cyanolichens, the free amino acids content was about 10 times higher than in the chlorolichens and mosses that were determined by occurrence of the nitrogen-fixing cyanobacter. The remaining 18 identified components. Glutamic acid and glutamine accounted for 35 to 50% from the sum of identified compounds, about 20-30% was comprised by alanine and aminobutiroic acid. Asparaginic acid, asparagine and 2-phosphoserine accounted for 5 to 10%. The similar trend was found in the mosses, but concentrations of individual compounds were different: asparagine (35%) and aminobutiroic acid (25) prevailed in *Pleurozium schreberi* followed by aspraginic acid, 2-phosphoserine and alanine (totally 20%); amino acid profile in *Racomitrium lanuginosum* was similar with lichens. Glutamic and asparaginic acids and alanine prevailing in the objects under study suggested being the most stable in the soils.

Identified low molecular hydrocarbons and amino acids can participate in polycondensation reaction forming melanoidins – high molecular humic substances which are dark colored and stable against microbial destruction.

High water extractiveness of the studied organic compounds from plant samples allow to suggest that living lichens are important source of soluble acids (including hydroxy and amino acids), alcohols and hydrocarbons, which may be transported into the soil by precipitation and be transformed there including formation of humic substances.

This work was supported by the Project № 15-2-4-28 “Biogeochemical Processes as the Base of Stable Functioning of Arctic Soils under the Conditions of Changing Natural Environment (by the Example of Plain and Mountain Ecosystems)”, Program of Ural Division, Russian Academy of Science 2015-2017.

## Humic Substances as a Source of Novel Agents against TEM-1 beta-Lactamase

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The microbial resistance to antibiotics is a global threat. Effectiveness of well-known single-molecule beta-lactamase inhibitors (BLIs) has diminished with the evolution of bacteria [1]. Hence, new multi-target agents are in high demand. In this respect, humic substances (HS) deserve particular attention. HS are the products of abiotic combinatorial synthesis yielding supramolecular mixtures of natural organic compounds formed mostly by phenolic and carboxylic units. It was reported that they possess a broad spectrum of biological activity, such as anti-inflammatory and antiviral [2]. Here we present a new BLIs and HS combination as an agent against TEM-1 beta-Lactamase.

Inhibitory activity was determined by measuring rate of CENTA cleavage using UV-absorbance at 405 nm. Two HS samples (humic acids (HA) and hymatomelanic acids (HMA)) obtained by standard protocol showed moderate activity against TEM-1. In order to determine active compounds HMA was fractionated using SPE extraction followed by gradient $H_2O/CH_3OH$ elution. Molecular compositions of obtained fractions were determined by ultra-high resolution mass-spectrometry. Most hydrophobic fraction of HMA (hydrophFR) showed the highest inhibitory activity against TEM-1 close to commercial agent, e.g. sulbactam (Slb) and tazobactam (Tzb). Moreover, combination of HS samples with BLIs decreases the rate of CENTA cleavage product accumulation. Addition of hydrophFR of HMA showed a 55% increase of sulbactam inhibitory activity (Fig. 1). Therefore, using HS-related products may be a valuable option for patients infected with multidrug-resistant organisms.

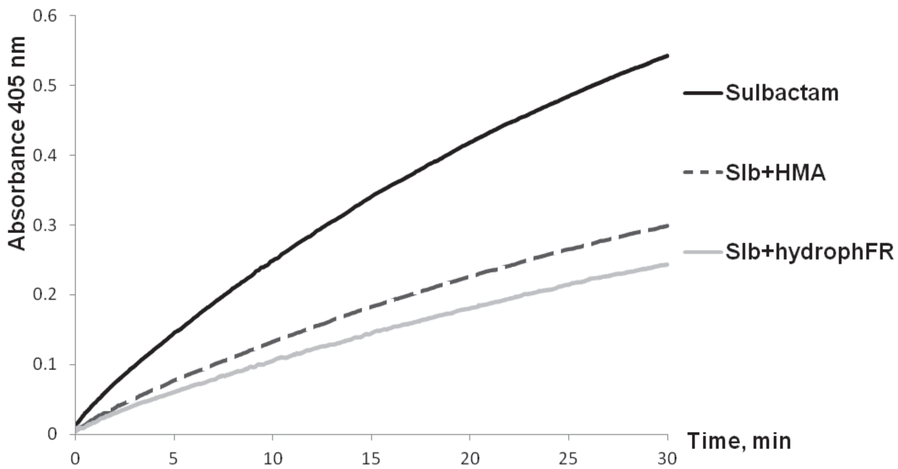


Figure 1. Kinetic curve of UV-absorbance at 405 nm of reaction mixture of TEM-1, CENTA and Sulbactam.

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## Degradation of Soil Humic Acid by Fungal, Bacterial and Lichen-derived Laccases: a Comparative *in vitro* Study

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Laccase (EC 1.11.1.14, benzendiol:oxygen oxidoreductase) is multicopper phenoloxidase which catalyzes one-electron oxidation of substituted phenols by $O_2$ which is reduced to water. Phenoxy-radicals and quinones produced from enzymatic oxidation of phenolic substrates can polymerize or initiate depolymerization reactions depending on substrate molecular weight. Laccase is ubiquitous in soils and is supposed to play important role in humus turnover [2]. However, degradation potentials of laccases from different producers have not been compared so far.

In this work we have compared fungal, bacterial and lichen-derived laccases by their ability to depolymerize humic acid from sod-podzolic soil (HAS) *in vitro*. We have used laccase of the white-rot fungus *Panus tigrinus* [1], homodimeric and monomeric laccases of the soil-stabilizing lichen *S. crocea* [3] and recombinant laccase from *Streptomyces anulatus*. Fungal and lichen-derived laccases were added to 1 mg $ml^{-1}$ HAS-$NH_4^+$ in Na-acetate buffer (pH 4.5) and mixtures were incubated for up to three days at 30ºC. The effect of *P.tigrinus* laccase on HA was additionally studied using submerged cultivation of the fungus under nitrogen rich conditions. Bacterial laccase was incubated with HA at pH 9.0 (20 mM Tris-HCl buffer) due to alkaline pH optimum. Molecular weight distributions were determined using gel-filtration on Sephadex G-25 gel with 0.025 M Tris-HCl buffer (pH 8.2) (experiments with fungal and bacterial laccases) or using HPLC on TSK 2000 SW column with 0.1 M Na-phosphate buffer (pH 7.0) as an eluent. In all cases 0.1% SDS was added to buffer solutions to avoid hydrophobic interactions.

Interaction of P.tigrinus laccase with HAS led to rearrangement in its molecular weight distribution most evident after 96 h of incubation. Initial HA consisted of high (>75 kDa) and low molecular weight fractions (13 kDa). After interaction with laccase the relative content of HA high molecular weight fraction decreased, while the amount and molecular weight of low molecular weight fraction increased. Under submerged cultivation conditions, laccase caused complete HA depolymerization within 15 days. Two laccases from the lichen *Solorina crocea* differed by their properties and oxidation potential. Small laccase is similar in its properties to the laccases of basidiomycetes. It is more resistant to elevated temperature and storage than the large form and showed a higher oxidation potential. Despite these differences, both laccases depolymerized humic acids from soils at comparable rates, with small laccase being slightly more effective. Recombinant bacterial laccase did not cause any changes in HAS.

Results of this study shows that HA degradation occurs in acidic conditions, depends on redox-potential of laccase and is much more efficient in presence of fungal cells than in *in vitro* system.

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# Molecular Composition of Permafrost Organic Matter Derived from Arctic Soil Cores

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Arctic permafrost is a large-scale source of bio-labile organic matter (OM), the decomposition of which would contribute significantly to the global carbon cycle. During global warming, the particular attention should be paid to its release and fate. In order to study the evolution of conserved molecular pool as well as the processes of its transformation in modern conditions, it is necessary to establish molecular markers that are characteristic for the structure of permafrost OM.

To achieve this goal, the most conservative fraction of permafrost OM - humic substances (HS) was extracted from six cores selected in the vicinity of Lake Shchuchye in the Kolyma River basin. Cores were sampled from different depths varied from 3 to 15 m. A total of 18 samples of humic (HA) and fulvic acids (FA) were obtained. Molecular compositions were determined by ultrahigh resolution 7T Fourier transform ion cyclotron resonance mass-spectrometry (FTICR MS) equipped with electrospray ion source (ESI).

Typical mass-spectra resolved 1900 and 1400 $C_cH_hO_o$ molecular compositions for HA and FA, respectively. Analysis of all HA revealed the high content of saturated compounds (Figure 1A). The contribution of oxidized species decreases with the depth. Otherwise, FA were characterized by the major contribution of oxidized aromatic compounds (Figure 1B). Such clear distinction of HA and FA molecular spaces is not typical for temperate regions.

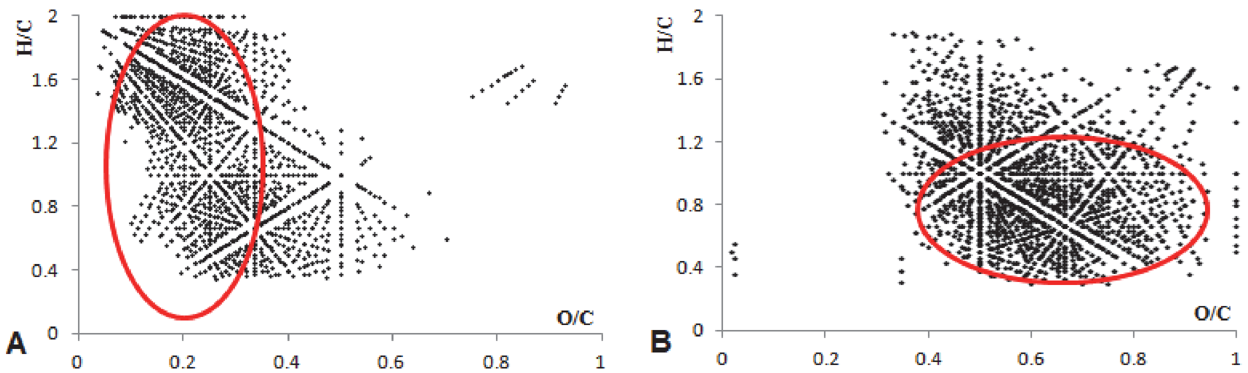


Figure 1. Typical Van Krevelen diagrams for $C_cH_hO_o$ molecular compositions of permafrost soil cores HA (A) and FA (B) determined by FTICR MS.

It is known that biolabile saturated constituents in permafrost soil are preserved due to low temperature of the region. At the same time global warming resulted in a significant role of oxidized FA in carbon cycling. Further changing of the climate will lead to fast release of bio-labile humic substances, which may cause crucial changes in environment of the Northern region and probably in the rest of the world.

This research was supported by the Russian Science Foundation grant 16-14-00167, the Russian Foundation for Basic Research grant 16-04-01753A, and the U.S. National Science Foundation funded Polaris Project (NSF 1044610).

## Изменение физиологической активности гумусовых веществ в процессе гумификации

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The peculiarity and specificity of the physiological activity manifestation of diverse humic substances formed during the transformation of organic matter into humus were investigated. The structural and physiological similarity of different fragments of humic substances with natural plant hormones, which are based on a single aromatic matrix, was analyzed. It was shown that lignin structures were basic for the organic matrix of the humic substances. When assessing the transformation of organic matter into humus three main stages of the process can be observed. In the process of transformation of organic matter into humus a change in the physiological activity manifestation of the products at the different stages with a diverse physiological effect of the type: gibberellins-auxins-cytokinins.

Исследована особенность и специфика проявления физиологической активности многообразных гумусовых веществ образующихся в процессе трансформации органического вещества в гумус. Проанализировано структурное и физиологическое сходство разных фрагментов гумусовых веществ с природными фитогормонами, имеющими в своей основе единую ароматическую матрицу. Показано, что основой органической матрицы гумусовых веществ являются лигниновые структуры. Оценивая трансформацию органического вещества в гумус можно условно выделить на три основных этапа. В процессе трансформации органического вещества в гумус происходит изменение проявления физиологической активности продуктов трансформации гумусовых продуктов с разноплановым физиологическим эффектом по типу: гиббереллины-ауксины-цитокинины.

Основой ароматической матрицы гумуса является лигнин (наиболее устойчивый к разложению биополимер). В процессе трансформации органического вещества в гумус можно выделить несколько этапов. На первом этапе в процессе окислительно-гидролитической трансформации лигниновые структуры все более ароматизируются, карбоксилируются и деметоксилируются, приобретая свойства аналогов гуминовых кислот (ГК). Образующиеся на этой стадии трансформации органического вещества в гумус модельные соединения впервые были названы лигногуминовыми кислотами (ЛГК)[1]. Выделенные ЛГК были близки к природным гуминовым кислотам по основным критериям оценки гумусовых веществ: элементному составу, наличию функциональных групп, молекулярно-массовому распределению, оптической плотности и характеру ИК-спектров. По особенностям химического и физико-химического состава сходство ГК и ЛГК было очевидным, исключение составляло почти полное отсутствие азота в составе ЛГК. Установлено, что ЛГК обладают высокой физиологической активностью, при этом новизна технического решения еще в 1985 г была подтверждена авторскими свидетельствами [1,2]. ЛГК структурно и физиологически схожи с природными гиббереллинами. Таким образом, в первой изученной модели было экспериментально показано, что основой гумуса может служить лигниновая матрица. Полученные в процессе трансформации лигнина соединения могут выступать в роли промежуточных модельных продуктов на пути трансформации лигнина в гумус. Демонстрируемый в модели механизм первичной гидролитической деструкции органических остатков с последующим трансформационным изменением скелетной структуры лигнина (без его расщепления до мономеров) подтверждает указанную Л.Н. Александровой [3] направленность процесса гумификации.

На втором этапе формирования гумуса происходит дальнейшая сополимеризация и поликонденсация фенольных фрагментов в ядерную основу гумуса (ароматическое ядро уплотняется) и начинает накапливаться азот, сначала на периферии, затем и в ароматической матрице. Идентифицированные ароматические фрагменты этой стадии трансформации фенольных соединений в почве близки к индольным структурам, свойственным природным ауксинам. Действительно, проведенные исследования показывают, что гумусовые вещества, полученные на этой стадии трансформиции, обладают ауксиноподобной физиологической реакцией на растения. Ауксиноподобный эффект ГВ отмечен в работах [4, 5].

Третьим этапом формирования гумуса является дальнейшая полимеризация и поликонденсация фенольных фрагментов ядра гумуса с включением азота уже не только на периферии, но в гетероцикле. Накопленный таким образом в матрице гумуса азот устойчив к разложению и является основным почвенным резервом, характеризующим хорошо окультуренные почвы. На этапе моделирования дальнейшего процесса гумификации путем окислительного аммонолиза были получены специфические продукты, обладающие цитокининовой физиологической реакцией. Разработана технология получения жидкого органо-минерального удобрения «Стимулайф» (Свидетельство о Государственной регистрации агрохимиката №1264-08-206-317-0-0-0-1; ТУ 2186-016-79850210-2007; санитарно-эпидемологическое заключение №77.99.30.099.А.000560.11.07). Гумусовые модельные соединения полученные на этой стадии уже используются в качестве средства управления ростом и развитием растений при возделывании сельскохозяйственных, лекарственных, декоративных и лесных культур [6].

Таким образом в процессе трансформации органического вещества в гумус происходит изменение проявления физиологической активности продуктов трансформации гумусовых веществ с разноплановым физиологическим эффектом по типу: гиббереллины-ауксины-цитокинины.

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## Section III
## Humics-based materials and nature-like technologies for ecosystem health, agriculture, and remediation

## Секция III
## Материалы на основе гуминовых веществ и природоподобные технологии для здоровья экосистем, сельского хозяйства и рекультивации

## Interaction of Metallic Iron and Humic Substances of Various Manufacturers

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Every year the role of "green technologies" in agriculture increases. In connection with this, the application of fertilizers based on natural compounds, for example, such as humic substances (HS), gets in demand. The popularization of environmentally friendly products, the absence of soil contamination with stable chelates, high bioavailability and biological activity of HS are the main advantages of natural compounds in comparison with synthetic drugs. Therefore, improving the consumer properties of HS is an actual agrochemical task. One of the most important directions of modification of HS is an increase in the content of such an important element as iron in them. Despite the fact that iron compounds are widely distributed in the earth's crust, in nature they are unavailable state for plants. Therefore, an important task is the development of new technologies for the production of HS preparations with a high content of biologically available iron. The simplest methods of producing preparations based on "humates of iron" are based on mixing solutions of water-soluble iron salts with HS solutions at constant pH. As a result of the synthesis, by similar techniques, complexes of iron with HS or iron oxohydroxides in the HS matrix can be obtained predominantly. Earlier in [1], we suggested a different approach to the synthesis of iron-containing HS preparations. This method is based on the natural oxidation of metallic iron in aqueous solutions of HS. A set of physicochemical methods showed that the products formed consist of iron (II) and (III) oxide nanoparticles stabilized by HS ($Fe_{3-\delta}O_4$@HS). The main advantage of this synthesis is the proximity to natural processes of the environment. A complex multicomponent system of HS independently determines the thermodynamically stable and beneficial forms of the existence of organoiron compounds, their composition and content. The biological availability of iron in the preparation, obtained in this way, was tested using the example of wheat *Triticum aestivum L*. [2]. In addition to the resulting products, a toxicological evaluation was conducted on the culture of green algae – *Scenedesmus quadricauda* (*Turp*.) *Breb*. However, we tested this method for the synthesis of iron-containing HS preparations using the example of only one natural reagent, namely, "Potassium humate Sakhalin" (Biomir-2000 Ltd., Moscow, Russia), which is obtained from the upper layers of highly oxygenated Solntsevskiy lignite deposits of Sakhalin Island.

The aim of this work was to establish the regularities of the interaction of metallic iron with HS of various nature, which would confirm the possibility of scaling the technique of oxidative synthesis of iron-containing HS preparations to HS of other manufacturers and of other origin. For this, the dynamics of the interaction of metallic iron with aqueous solutions of various commercial products of HS was compared. In particular, in addition to the data for this initial HS preparation, the data for HS "PowHumus", obtained by alkaline extraction from German leonardite - varieties of oxidized brown coal (Humintech, Düsseldorf, Germany), and "Natural Humic Acids", produced by low-temperature physico-mechanical treatment of lignites and subbituminous coals (Life Force, Saratov, Russia) were analyzed. In this case, the product Life Force "Natural Humic Acids" was previously transferred to the water-soluble form by leaching for 48 hours at room temperature with 10% KOH solution. To carry out the actual experiment, metal iron in the form of a powder was added to aqueous solutions of HS with a concentration of 250 mg/L, the container were hermetically sealed and stirred at room temperature by completely turning the container. Through known intervals of time, such parameters as pH, redox potential and optical density of solutions were controlled in solutions (Figure 1). The experiment lasted 13-17 days. There were no significant differences in the interaction dynamics for various HS preparations. The absence of significant differences in the dynamics of changes in the parameters, controlled during the experiment, testifies the unified mechanisms of the proceeding reactions in the "Fe - HS solution" system. It should be noted that the interaction with the preparation "Natural Humic Acids" was much faster than with other preparations HS. The observed differences in the rate of occurrence of processes in solutions of HS of various origins may be due to differences in the structural-group composition of the examined HS. In particular, the proposed scheme for the interaction of HS with Fe involves stage of the oxidation and complexing of metallic iron by HS [1]. Indeed, the data obtained in the framework of the present study indicate that the preparation obtained from the Life Force "Natural Humic Acids" products has the less value of permanganate oxidation index (PI), i.e. contains relatively large of number reactive groups.

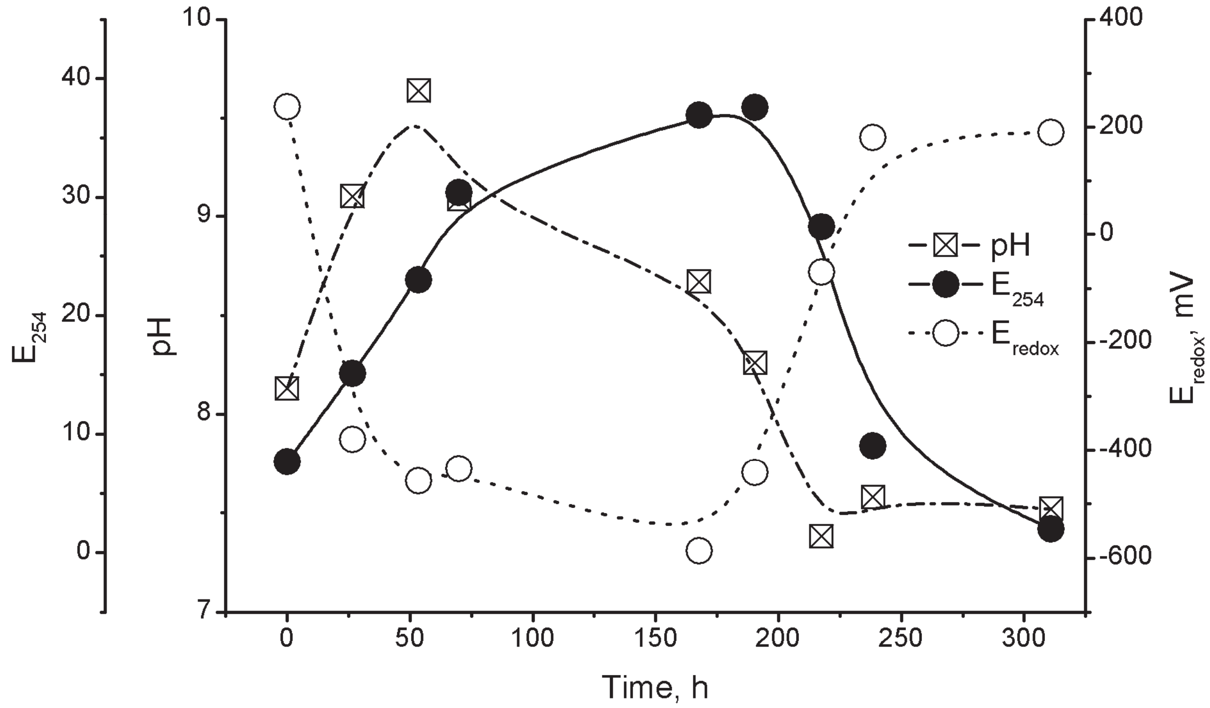


Figure 1. Change of absorbance at 254 nm, pH and redox potential during the interaction of Fe with aqueous solutions of product Life Force "Natural Humic Acids".

In this paper it has been shown that, regardless of the nature and origin of the HS, the process of interaction between HS and Fe is carried out by unified mechanisms. Despite this preparation of Life Force "Natural Humic Acids" interacts faster than other preparation studied due to the presence of a large number of reactive groups that are involved in the active interaction with iron.

*The authors are grateful to Life Force Group and to the head of the scientific group MGUMUS I.V. Perminova for the given preparations "Natural Humic Acids", "PowHumus" and "Potassium humate Sakhalin". The work was carried out in the framework of the competition of research works conducted by Life Force Group.*

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## Application of Polyelectrolytes for Production Ballastless Humic Substances

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Getting of ballastless humates is essential to production of humic organic and organic-mineral fertilizers of high quality. In this investigation we took an assessment of flocculating property of different polyelectrolytes during getting of humates from brown coal.

As humate-containing raw materials use earthy brown coal with natural humidity 50%, with contenting 12% of ash and 70% of humic substances on dry ash-free coal bassis.

As the flocculants is used anionic, cationic and nonionic polymers of label "Praestol" different molecular weight (copolymers of acrylamide): 2530, 2515, 2500, 851 TR 2540 N and 2300D. Also was used a promising polymer for using in agriculture, it is natural - occurring polymer – chitosan.

Selected the most effective flocculant, that promotes the deposition of ballast from the solution of humates, while maintaining them in solution. It was show with its influence on the deposition rate of the ballast and the save ability humates in solution. Poststimulus investigated the activity of the selected polymer.

## To the Problem of Prophylaxis of Diseases Caused Magnesium Deficiency

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The biological role of magnesium is proven and well known. An activity of all ATP dependent enzymes decreases in magnesium deficiency [1]. The magnesium deficiency leads to interruption of tissue energetic metabolism [2], connective tissue dysplasia [3]. Decreasing of magnesium admission into the organism with drinking water is considerate as most important factor of sudden death in cardiovascular diseases [4].

Magnesium level in healthy people blood serum and plasma is 18,7 – 21,8 mg/l. Replenishment of magnesium deficiency in the body is carried out, mainly, food. It is known that, for example, in green salads contains up to 40 mg of magnesium per 100 g of product.

The study used two varieties of green salad: "Dubrava" and "Nezhnyi", which were watered with two types of humic fertilizers: 1 – “Rostok” (“Eurika” Ltd., Tumen) and 2 – “Planta” (“Biotex” Ltd., Yekaterinburg). Concentration of fertilizer solutions, according to the recommendations of manufacturers, was 1.2 - 1.5% (vol.).

The magnesium content in green salads was determined trilonometrically after extraction of magnesium and calcium ions from the hinges of the green parts of plants into a solution of hydrochloric acid with heating. The complex-forming activity of humic fertilizers with respect to magnesium was estimated by the author's method, which is based on the formation of HDS in the presence and absence of ligands.

When watering fertilizer № 1, the best germination was observed for the "Neznyi" salad, and when fertilizing with fertilizer 2 - the best germination had a "Dubrava" salad. However, from the experimental data obtained, it follows that the use of fertilizers significantly reduces the magnesium content in plants of the variety "Dubrava": from 24.7 mg / 100 g to 9.6 mg / 100 g (fertilizer № 1) and 11.1 mg / 100 g (fertilizer № 2). While for the grade " Neznyi " magnesium reduction under the influence of fertilizers is less significant. The magnesium content without treatment with fertilizers is 31.9 mg / 100 g. After watering with fertilizers - 29.4 mg / 100 g (fertilizer № 1) and 24.0 mg / 100 g (fertilizer № 2).

The obtained data are consistent with the complexing properties of the aforementioned fertilizer solutions: when fertilizers are introduced into the system of the formed HDS, a faster decrease in light transmission is noted, which indicates the formation of poly-ligand insoluble complex magnesium compounds with humic substances contained in fertilizers.

Thus, we can conclude:

- ehe use of fertilizers increases germination, but can lead to a decrease in the content of magnesium in green salads. The degree of decrease for magnesium in lettuce leaves depends on the botanical characteristics of the variety;

- the complexing properties of organic substances that make up fertilizers must be evaluated in order to adjust the concentration of solutions used for watering plants.

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## К проблеме профилактики заболеваний, обусловленных дефицитом магния

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Биологическая роль магния доказана и хорошо известна. В условиях дефицита магния снижается активность всех АТФ-зависимых ферментов [1]. Недостаток магния приводит к нарушению энергетического обмена тканей [2], дисплазии соединительной ткани [3]. Уменьшение поступления магния в организм с питьевой водой рассматривается как важнейший экологический фактор внезапной смерти при сердечно-сосудистых заболеваниях [4]. Содержание магния в сыворотке и плазме крови у здоровых людей составляет 18,7 – 21,8 мг/л. Восполнение дефицита магния в организме осуществляется, в основном, продуктами питания. Известно, что, например, в зеленых салатах содержится до 40 мг магния на 100 г продукта.

В исследовании были использованы два сорта зелёного салата: «Дубрава» и «Нежный», которые поливали двумя видами гуминовых удобрений: 1 – «Росток» (ООО НПЦ «Эврика», Тюмень) и 2 – «Планта» (ООО НПЦ «Биотекс», Екатеринбург). Концентрация растворов удобрений, согласно рекомендации производителей, составляла 1,2 – 1,5 % (об.). Содержание магния в зелёных салатах определялось трилонометрически после экстракции ионов магния и кальция из навесок зелёных частей растений в раствор соляной кислоты при нагревании. Комплексообразующую активность гуминовых удобрений по отношению к магнию оценивали авторским методом, в основе которого лежит реакция образования ГДС в присутствии и отсутствии лигандов.

При поливе удобрением № 1 лучшая всхожесть наблюдалась для салата сорта «Нежный», а при поливе удобрением 2 – лучшую всхожесть имел салат «Дубрава». Однако, из полученных экспериментальных данных следует, что использование удобрений существенно снижает содержание магния в растениях сорта «Дубрава»: с 24,7 мг/100г до 9,6 мг/100 г (удобрение № 1) и 11,1 мг/100 г (удобрение № 2). В то время как для сорта «Нежный» снижение магния под воздействием удобрений менее значимо. Содержание магния без обработки удобрениями – 31,9 мг/100 г. После полива удобрениями – 29,4 мг/100 г (удобрение № 1) и 24,0 мг/100 г (удобрение № 2).

Полученные данные согласуются с комплексообразующими свойствами вышеуказанных растворов удобрений: при введении удобрений в систему формирующейся ГДС отмечается более быстрое падение светопропускания, что свидетельствует об образовании полилигандных нерастворимых комплексных соединений магния с гуминовыми веществами, содержащимися в удобрениях.

Таким образом, можно заключить:

– использование удобрений повышает всхожесть, но может приводить к снижению содержания магния в зелёных салатах. Степень снижения количества магния в листьях салата зависит от ботанических особенностей сорта;

– комплексообразующие свойства органических веществ, входящих в состав удобрений, необходимо оценивать с целью корректировки концентрации растворов, которые используются для полива растений.

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## Study of the Effect of the Humic Fertilizer Life Force Humate Balance on the Growing of an Ornamental Plant

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The universal urbanization results in increasing need for ornamental plants for urban greening. The container method, in which peat or compost amended with mineral fertilizers is used as a substrate, is one of the advanced and promising methods for ornamental plant growing. However, young plants are not always adequately supplied with all necessary nutrients, and a significant plant failure is observed at the seedling stage. The aim of this work was to study the applicability of a humic preparation for growing ornamental plants in container culture.

The humic preparation Life Force Humate Balance was used, which consists of 40% potassium humates and 60% natural humic acids. The preparation is marketed as an efficient ameliorant improving the physicochemical and biological properties of soil. Humate Balance is an active source of bioactivated humic acids from leonardite; it enhances the efficiency of the mineral nutrition of plants and favors the development of useful microflora.

Tests of the preparation were performed on 30 seedlings of bird berry (*Prunus padus*). The experimental design included 6 treatments (Table 1); experiment was conducted in 5 replicates. Seedlings of similar heights and root systems were planted into containers with the substrate, and morphometric observations were performed monthly. The following parameters were monitored: total increment, growth rate, plant height, and bole diameter.

Results are given in Table 1.

Table 1. Changes in morphometric parameters of bird berry seedlings during the period from April 15 to August 30, 2017, in experimental treatments with the humic preparation Life Force Humate Balance, %

| Treatment | Plant height change, Δh | Bole diameter change, Δd | Total increment, Δt | Average of three parameters |
|---|---|---|---|---|
| 1. Background-1 (peat) | 108 | 42.11 | 159.18 | 103.1 |
| 2. Background-1 + NPK and micronutrients | 69.1 | 22.81 | 117.03 | 69.6 |
| 3. Background-1 + Humate Balance | 90.61 | 39.13 | 186.6 | 104.4 |
| 4. Background-1 + NPK and micronutrients + Humate Balance | 63 | 30.91 | 138.88 | 77.6 |
| 5. Background-2 (compost) | 77.11 | 29.31 | 157.6 | 88.0 |
| 6. Background-2 + Humate Balance | 78.42 | 25.93 | 220.17 | 108.2 |

**Conclusions.** Combination of compost with the humic preparation Life Force Humate Balance is the most efficient substrate for the container growing of bird berry. An appreciable increase in total increment and bole diameter is also observed in treatment 3, where Humate Balance is used in combination with mineral fertilizers on peat substrate.

# The Effect of Humic Preparation on the Fertility of Chernozem and Interaction in the System Soil – Microorganisms – Plants

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The transition towards organic agriculture includes the concept of maximum use of biological factors in farming systems, as well as reducing the anthropogenic load on the soil. One of the components of biological farming is the use of humic fertilizers and preparations, which is due to their affinity to soil organic matter.

The research was carried out in the conditions of the Rostov region on chernozem ordinary. As a crop, winter wheat was used. In the Rostov region, winter wheat occupies a leading position, it is cultivated on an area of about 2 million hectares. The Rostov region is located in the zone of risky farming, so the use of biologics based on humic compounds is important, which are stimulants and adaptogens that increase the resistance of plants to unfavorable, including humid, environmental conditions.

The work was carried out with biohumus extract - humic drug BIO-Don, which is characterized by an alkaline reaction of the medium and contains humic acids, the sum of which is on average 2.24 g/l. They, as shown by numerous experiments, are growth stimulants and adaptogens, removing stress after application of plant protection products and from the influence of unfavorable weather factors. This preparation is diluted to an optimum concentration of 0.001% and the soil or plant is treated.

Microbiological composition of the preparation is characterized by the presence of bacteria growing on nutrient agar, and 78% of them are spore-forming bacteria of the genus Bacillus, which is associated with biological product production conditions and high pH values. Not less than three different species of this genus have been noted (presumably, on the basis of colony morphology). These bacteria are nonpathogenic representatives of normal soil microflora, active hydrolytics involved in the decomposition of fresh organic substances. However, their number is relatively small, so the preparation can not be classified as microbiological.

The effectiveness of the application of the biopreparation was evaluated by the dynamics of the nutrient elements, the yield and the quality of the grain. Soil sampling, determination of the content of nutrients and humus in the soil was carried out in accordance with GOST.

It is known that most of the stocks of plant nutrients in chernozems are in the form of compounds that are difficult to access or inaccessible to plants. Therefore, the importance of providing the soil with assimilable forms of N, P, K becomes important, the transformation of which is determined by the activity of microorganisms.

The interest in studying the effect of biologically active substances on soil fertility originates from the fact that the use of these substances may prove promising in terms of their effect on the availability of nutrition for plants. This is important for chernozems, where relatively low mobility of phosphorus is observed despite a significant gross amount of nutrient elements.

The study of microbiological activity has shown that the application of the humic drug BIO-Don on winter wheat crops provokes the activation of microorganisms in the root zone, which in turn contributes to the growth of the mineralization coefficient, which is calculated as the ratio of the number of bacteria capable to grow on mineral forms of nitrogen to the number of bacteria, using organic forms of nitrogen and reflects the dynamics of the processes of mineralization of nitrogen compounds. The maximum increase in this indicator is observed in the variant with the introduction of humic preparation in the soil, but also in those cases where the topical treatment of plants was applied, the positive dynamics of the mineralization coefficient was also noted.

The experiment has shown that the increase in the yield of winter wheat when using BIO-Don humic preparation was from 6.9 to 12.8 c/ha, that is, up to 35% compared to the background - mineral nutrition according to the recommendations for this zone (Tab. 1).

Table 1. The productivity of winter wheat treated with humic fertilizer BIO-Don on chernozem ordinary carbonate, c/ha

| Variants | | 2014 | | 2015 | | 2016 | |
|---|---|---|---|---|---|---|---|
| | | yield | increase | yield | increase | yield | increase |
| 1 | Background | 35,5 | – | 47,5 | – | 35,4 | |
| 2 | Background + soil treatment | 42,4 | +6,9 | 51,9 | +4,4 | 37,6 | +2,2 |
| 3 | Background + soil treatment + crop treatment | 46,0 | +10,7 | 57,0 | +9,5 | 39,4 | +4,0 |
| 4 | Background + soil treatment + 2-fold crop treatment | 48,3 | +12,8 | 55,3 | +7,8 | 42,4 | +7,0 |
| $LSD_{0,05}$ | | | | | 6,9 | | 2,7 |

The intensification of soil mineralization processes promotes the transfer of organic nitrogen into the mineral form and hard-to-reach forms of phosphorus into mobile forms, which we observe in this experiment (Fig. 1). In conditions of phosphorus deficiency inherent in carbonate chernozem, this is one of the leading factors in optimizing plant nutrition.

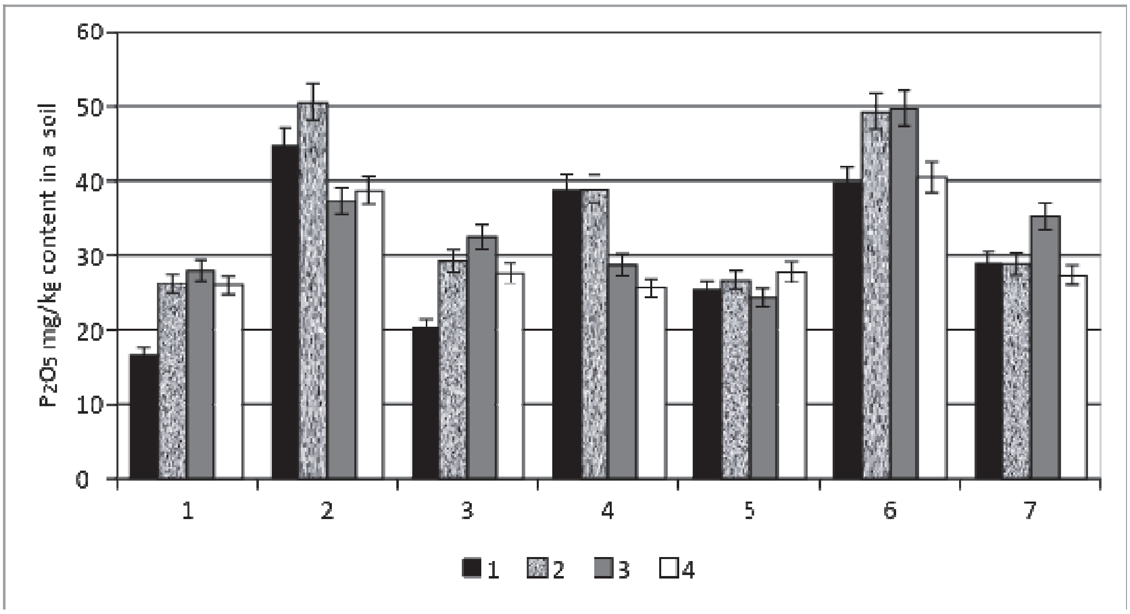


Figure 1. Dynamics of mobile forms of phosphorus in ordinary chernozem using humic fertilizer BIO-Don (2 l / ha).

Thus, humic preparation BIO-Don has a positive effect on the dynamics of elements of mineral nutrition, both in soil treatment and in the topical treatment of crop plants. With a shortage of nutrients, there is an increased removal of them from soil reserves, which is due to the activation of the microflora of the rhizosphere zone. One of the reasons for the positive effect of the humic preparation on agricultural plants is the adjustment of the content of available forms of nutrients.

## Chitosan as a Perspective Modifier for Nanodiamonds

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Chitosan is one of the most abundant polysaccharides in nature that consists of β-1,4-linked glucosamine with a high degree of N-acetylation. Since chitosan possesses such properties as good biocompatibility, high adhesion to the surface, a wide range of pH stability, an application area of this material is wide and includes pharmacy where chitosan is used as a direct tableting agent and as a vehicle for parenteral drug delivery devices [1]. This study is focused on the use of chitosan as a modifier of nanodiamonds to provide this nanomaterial additional functional activity and probably stability in the suspensions.

To this end we studied the adsorption chitosan on nanodiamonds surface from acetic solution. To determine the amount of chitosan in both solution after adsorption and adsorbed on nanodiamonds surface we used tritium labeled chitosan that was obtained by means of tritium thermal activation method [2]. Three types of nanodiamonds were used: (i) nanodiamonds produced by Sitna (Belarus) as an original material; (ii) nanodiamonds (Sinta) subjected to air annealing; and (iii) nanodiamonds (Sinta) subjected to nitration.

To the solution of chitosan in the acetic acid of known concentration and specific radioactivity the aqueous suspension of nanodiamonds was added. The suspensions were incubated at 25°C for 48 h followed by centrifugation and measuring radioactivity of supernatant for determination of the equilibrium concentration. Then the precipitate was then washed with water until pH reached neutral value, and radioactivity reached background. The suspension was then characterized by dynamic light scattering with determination of both particle size and zeta potential distribution. After that the suspension was centrifuged and the supernatant was replaced by liquid scintillator for direct measuring radioactivity of nanodiamonds with adsorbed chitosan for adsorption determination.

We have found that the value of chitosan adsorption is strongly depends on nanodiamonds surface properties: nitration and air oxidation of originally positively charged nanodiamonds results in the significant increase in chitosan adsorption. It has to be emphasized that only air annealing results in the changing in zeta potential of the original material. After chitosan adsorption zeta potential of all types of nanodiamonds converts to positive value close to 35 mV. Thus, chitosan modified nanodiamonds forms suspensions that possess higher stability in the aquatic media. However, the values of particle size of modified nanodiamonds in the aqueous suspension were two times higher than for the original material.

This work was supported by RFBR (grant # 17-03-00985).

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## Importance of Fulvic Acid in Agricultural and Industrial Applications

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Over the last decades, many research studies have been performed to elucidate the complex structure of humic substances and their utilities in various fields of application. Till now, the main focus was mostly on humic acids whereas the smaller humic compound, fulvic acid, was rather neglected. But recently, fulvic acids have aroused more curiosity and interest from industry, farmers (crop, chicken, dairy, etc.) universities and research institutes as well.

The most common use for fulvic acid is currently in agriculture. Due to its small molecular size distribution, it is a better biological activator and can be applied either in soil or directly on plants and foliar where it is able to penetrate right into the cell membranes and releases essential trace metals where required [1].

Recently, our company started a cooperation with one of the Netherlands' largest drinking water producer who is obtaining pure fulvic acid as a by-product during their water conditioning process. Since this fulvic acid is of food and feed quality (GMP$^+$ certified), the opportunities for its application has been highly increased. Presently, our company is investigating some new possibilities in collaboration with several research institutes, universities, farmers and industry partners. For instance, chicken farmers reported that cannibalism and fatal casualties among chickens was lowered by 17-18% after adding fulvic acid to the animals' drinking water for just three days. In addition, feather picking of hens was stopped almost completely. At the end of a period of 30-40 days, the total mortality of hens could be reduced by 23%.

Further observed positive effects of feeding fulvic acid was an increase in egg production while the feed usage was decreased about 5% compared to the control group. Additionally, similar positive effects have been reported for pig breeding farms using about 1.5% of dried fulvic acid as dietary supplement. Further ongoing research in animal feeding and breeding performed by Humintech and collaboration partners includes aquaculture (e.g. fish farming).

Another current research project regarding fulvic acid, is its utilization in the ceramic industry. Humic and fulvic acid have been reported to be used as an effective additive in handling and manufacturing “ceramic slurry” to enhance the stability during and after the production process [2]. In our studies, we strive to find out how exactly humic and fulvic acids influence the stability by comparing e.g. fractions obtained from various pH values and therefore their different chemical structures as well as chemical and physical properties.

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## Effect of Humic Product EDAGUM®SM on Soil Properties and Wheat Productivity in Pot and Field Experiments

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Effect of commercial humic product from peat "Edagum®SM" on soil chemical and physical properties, growth, yield and grain quality of wheat *Tríticum aestívum* L. was evaluated in pot and small plot experiments. In pot experiment effect of soil application of EDAGUM®SM (1.0; 1.5 and 2.0 L/ha) was compared with the one from farmyard manure (FYM, 2 t/ha). In small plot experiment the effectiveness of various methods of EDAGUM®SM application (soil, pre-plating seed treatment, foliar spray and their combination) was evaluated in comparison with FYM over the background of mineral fertilizers.

In pot experiment soil application of EDAGUM®SM in rates 1-2 L/ha showed no significant effect on soil chemical characteristics, whether provided the positive impact on soil physical properties and structure: amount of water-resistant and agronomically valuable soil aggregates increased by 4%, and porosity by 14%. Grain yield was not significantly different in the treatments with FYM and low doses of EDAGUM®SM, whether dose 2 L / ha provided the maximum yield.

In plot experiment acceleration of plant development was observed. The first positive effect was registered already on the 5th day after sowing and later EDAGUM®SM-treated plants developed 1-2 days earlier comparably to control at all stages of plant vegetation (tillering, stalking, earing, flowering and ripening). Grain yield increased by 7-12% compared to the treatment with application of FYM (Table 1). Grain quality showed no significant differences between treatments.

Table 1. Effect of EDAGUM®SM on wheat yield in plot experiment

| Treatment | 1000 gr weight, g | Grain yield, g/pot | Additional yield | |
|---|---|---|---|---|
| | | | g/pot | % |
| NPK+FYM | 41,3 | 41,0 | - | - |
| NPK+ Edagum®SM-2* | 40,5 | 44,3 | 3,3 | 7,9 |
| NPK+ Edagum®SM-3** | 42,0 | 45,8 | 4,8 | 11,6 |
| LSD 05 | | | 2,8 | |

*soil and seed treatment
**soil + seed + foliar spray

A combined soil, seed and foliar treatment provided the highest effectiveness of EDAGUM®SM. It accelerated seed germination of wheat; stimulated plant growth and development at all stages of vegetation; increased the yield of high quality grain (up to 12%) comparably with application of FYM.

Above-mentioned positive effects could be probably realized due to optimization of soil physical properties, as well as effects on physiological processes in plants. In turn, increase of root biomass promoted the development of soil biota (especially in rhizosphere), enhancement of biochemical activity and intensification of humus formation of as well as accumulation of non-humic organic compounds. Finally it resulted in increase of productivity of the test culture.

## Importance of Particle Size in Synthesis of Iron Humate Fertilizers

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Introduction

Iron fertilizers based on humic substances extracted from lignites, such as leonardite, are used in the Mediterranean area (as liquid concentrates) in drip irrigated fruit tree plantations This kind of iron complexes fertilizers are biodegradable and cheaper than synthetic iron chelates although less efficient for correcting iron chlorosis mainly because of their low stability in calcareous soils. Therefore, new technologies are necessary to improve iron humates and nanoparticulated fertilizers are been developed. Solubility, iron content and particle size are critical points of this technology that must be optimized. The aim of this work was to evaluate the importance of size particle in the synthesis of iron humates (1, 6 and 10% of Fe) prepared by complexation of leonardite HS with $Fe(NO_3)_3$ and $FeSO_4$ at three pH (7, 10 and 12).

Materials and methods

Potassium humater from leonardite (PowHumus) was kindly provided by Humintech Ltd.(Germany). Two products were obtained by complexation of the leonardite with $Fe(NO_3)_3$ 0.1M (Ferr-H at pH 7 and Goe-H at pH 12) and one by complexation with $FeSO_4$. $7H_2O$ 0.1M (FeH at pH 10). The products Ferr-H and FeH were prepared with 1, 6 and 10% of Fe and Goe-H with 1 and 10% of Fe. pH was adjusted with KOH 1M. The samples were dried by lyophilisation during three days. Two replicates were done for each product.

Iron concentration was measured by ICP-AES (Agilent Technologies 5110) and the particle size was determined using DLS (Nanobrook OMNI).

In order to determine the solubility, 0.1g of each product was dissolved in 1.0ml of distilled water in Eppendorf tubes and each solution was centrifuged for 5 min (100000 rpm). An aliquot of the supernatant was dried in a rotary evaporator and the solubility was calculated.

Results and discussion

Iron concentration increased with theoretical percentage of iron while the solubility decreased in the same way (Figure 1), mainly for FeH and Goe-H samples. Although the samples were grinded in a mortar, inhomogeneity was observed. According to DLS analysis (Figure 2), iron humate nanoparticles (< 50 nm) were observed in the products Ferr-H (10% of Fe) and FeH (6% of Fe). With respect to the product FeH (10% of Fe), it is probable that iron humate nanoparticles were forming aggregates at this pH.

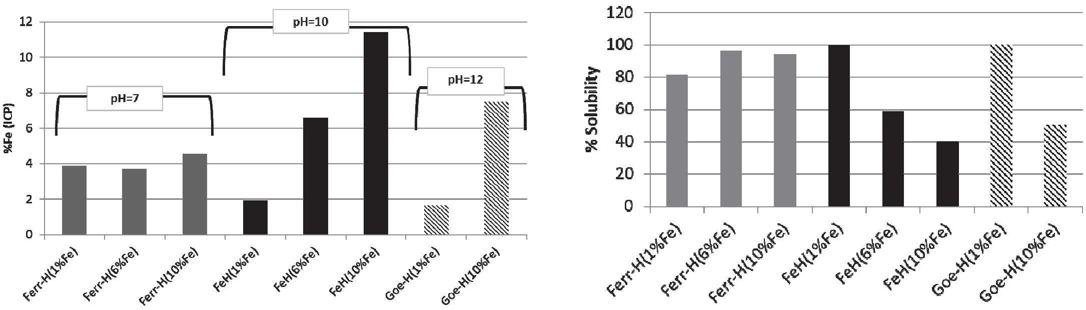


Figure 1: Percentage of iron measured by ICP-AES and solubility calculated for each sample.

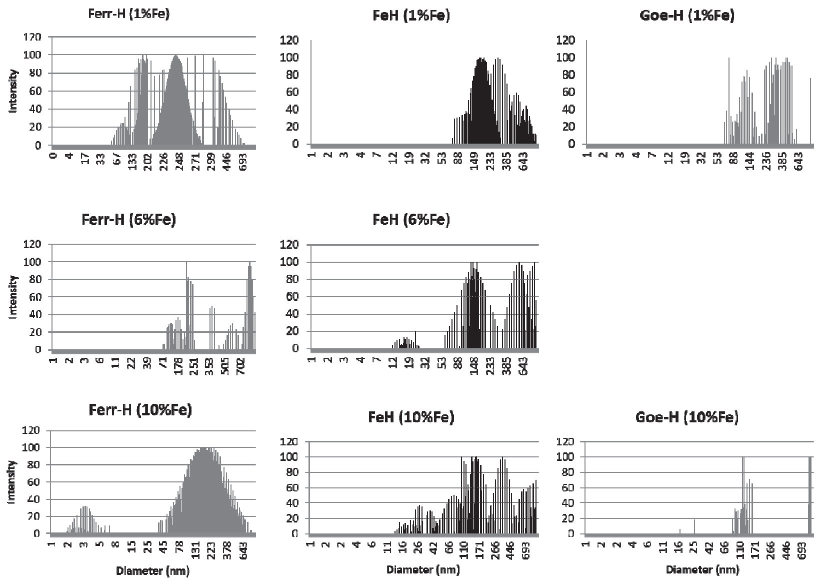


Figure 2: Size particle measured with DLS respect to the signal intensity in Ferr-H, FeH and Goe-H

High concentration of iron in the synthesis of iron humate fertilizers improves iron aggregates, inhomogeinity, low solubility and therefore, iron precipitation. High pH is not favorable for iron humate nanoparticles formation. Although this results must be corroborated, the product prepared with $FeSO_4$ with 6% of iron at pH10 seems to be the most efficient in provide iron nanoparticles.

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## Results of Simulation of Biological Activity of the Novel Polyphenolic Ligand of BP-C Family Drugs (*in vitro* and *in silico*)

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Natural substances, as safe and pluripotent candidates for designing medicinal products, draw interest of various scientific communities. By the present time methods for extraction and characterization of individual polyphenolic compositions and a solid evidence-based scientific database on potential molecular targets of these compositions have been developed.

Convincing nonclinical data, demonstrating, most of all, antioxidant properties of these compounds has been gathered; some of these compositions (e.g., Genistein; BP-C1) are being evaluated in clinical trials. At the same time, not much is known about pharmacological potential of derivatives of lignin, plant polyphenolic polymer. BP-Cx-1, novel polyphenolic ligand, is a water-soluble fraction of plant lignin and is the platform for a portfolio of innovative pharmacological products such as BP-C1 (antineoplastic agent), BP-C2 (radiomitigator) and BP-C3 (geroprotective composition).

Biological activity of BP-Cx-1 was assessed by Dr. R. N. Karapetian et al. (ChemRar Institute), using in-vitro Cerep Diversity Profile (P9) screening panel. It was demonstrated that BP-Cx-1 interacts with 40 of the 97 tested targets. Biological activity of BP-Cx-1 was also simulated in-silico using the following input parameters: results of HPLC/HPLC-MS, obtained by the scientific group headed by Dr. E. I. Savelyeva (Scientific-Research Institute of Human Hygiene, Professional pathologies and Ecology of Federal Medical-Biological Agency of Russia, Saint-Petersburg), results of CHN, 13C NMR and FTICR-MS, latter ones generated by the group of Prof. I. V. Perminova (Moscow State University n.a. M.V. Lomonosov, Moscow) and results of 1H NMR, generated by Dr. K. A. Krasnov (Scientific Research Institute of Toxicology, Saint-Petersburg). CHN analyses demonstrated that BP-Cx-1 does not comprise any nitrogen-containing components; NMR spectra indicate that BP-Cx-1 comprises predominantly highly substituted polyphenols.

For in-silico biological activity screening in ChemBL chemical database, components with molecular masses confirmed by both HPLC-MS and FTICR-MS methods were selected. The biological activity of BP-Cx-1 identified both in the in-vitro and in the in-silico test systems comprises 9 targets: Glucocorticoid receptor (NR3C1), Prostanoid EP2 receptor (PTGER2), Beta-2 adrenergic receptor (ADRB2), Vasopressin receptors (AVPR2, AVPR1A), Thyroid-stimulating hormone receptor (TRHR), Adenosine A1 Receptor (ADORA1), GABA trans-porter (SLC6A1). Identified interactions suggest BP-Cx-1 to have its own pharmacological activity (first of all, effect on inflammations and stress).

This work was supported by the RSF Grant # 16-15-00142.

## Mitigating Activity of Humic Substances under Water Deficiency Condition: Role of Phenolic Moieties

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A comparative evaluation of the mitigating activity of humic substances (HS) derived from coal and peat towards the seedlings of wheat *Triticum aestivum* L. under water deficiency conditions has been performed. The studied HS included humic (HA), fulvic acids (FA) and hymatomelanic acid (HMA) isolated from coal and peat as they are the main sources for industrial humates designed for use in agriculture as plant growth regulators. All the studied HS were characterized by the methods of elemental analysis, size-exclusion chromatography and 13C NMR spectroscopy.

Bioassay experiments was performed according to [1]. To create water deficiency, the hyperosmotic solution of polyethylene glycol with a molecular weight of 6,000 D (PEG 6000) with concentration 100 g/l (osmotic pressure –0.15 MPa) was used. Evaluation of the protective effect of HS was carried out at constant concentration of PEG-6000 and concentrations of HS varying in the range 5–100 mg/L. Distilled water was used as a blank solution. Wheat seeds were placed in the Petri dishes containing solutions under study and left in the dark at 24°C for 72 h. Then the length of roots and shoots of wheat seedlings was measured. Protective effect of HS was assessed based on comparison the lengths of roots and shoots of seedlings in the tested solutions to the blank one.

Experiments demonstrated that HS were able to reduce partially the inhibition of wheat seedlings' growth under water deficit caused by hyperosmotic solution of PEG-6000. The protective effect of HS was revealed to enhance along with increasing the content of oxygen-substituted aromatic fragments in HS structure. Thus, the obtained results allow suggesting about increasing the protective effect of HS in relation to plants under conditions of water deficit by increasing the content of phenolic fragments. The observed mitigating activity of HS could not be explained by their binding with PEG-6000, which is a negatively charged polyelectrolyte. Therefore, HS possess the ability to reduce the negative effects of water deficiency due to their direct effect on plants. As mitigating activity of HS enhanced by increasing the content of phenolic fragments that may indicate the antioxidant activity of HS as the main mechanism of their protective activity under conditions of water deficit. However, to confirm this assumptions, an additional research aimed at the study of membranotropic properties of humic materials should be conducted.

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# Improving Soil Properties by Silsesquioxane Humic Systems

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Soil humic substances directly and indirectly influence on wide range of properties that determine fertility. In particular they effect greatly on soil organic matter and soil structure. An introduction the humic acids (HA) modified with siloxanes (so called silsesquioxane humic systems, Si-HA) seems to be one of the most promising approach for improving soil properties, which are closely connected with soil fertility [1]. However, the development of this technology requires evaluation of interaction of silsesquioxane humic systems with wide range of soils.

The goal of our work was to evaluate effect of silsesquioxane humic systems on soil organic matter properties as well as soil structure of soils belonging to different soil-geographical zones in Russia.

To reach this goal, a model experiments with soil samples of humic horizons of Sod-podzolic, Grey forest, Chernozems and Chestnut soils in cultivated and no cultivated variants. Soils were saturated with HA or Si-HA 5 times, incubated at constant temperature and moisture during 21 d and dried. Then mean weight diameter (MWD) of water proof aggregates, microaggregates content (MAC) and ratio of $C_{HA}/C_{FA}$ were estimated.

Introduction to soil both HA and Si-HA increased MWD and MAC, and case of the Si-HA both MWD and MAC were significantly larger than in the soils treated with HA. $C_{HA}/C_{FA}$ changed insignificantly in presence of Si-HA as compared to the control values whilst in case with HA $C_{HA}/C_{FA}$ values considerably increased.

So, the obtained results demonstrated clearly improving soil properties by silsesquioxane humic systems.

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# Variation of Biological Properties under Influence of Humic Substances and in Conditions of Model Experiments with Sod-podzolic soils Contaminated with Heavy Metals

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In recent years, there has been growing interest in using humic substances for the purpose of reclamation of contaminated soils and increasing fertility [1]. The appearance of a large number of industrial humic substances (HS) of different in their properties and origin requires the creation of methods for their evaluation. In conditions of high anthropogenic influence, the soils are subjected to complex pollution, trace metals play a significant role in this pollution.

The purpose of our research was to evaluate the humic substances in model experiments using microbiological methods. The object of research was sod-podzolic soil, medium loamy. In research, we used three types of HS of various origins: Flexom, Lignohumate and purified humic acids. HS were added at a concentration of 10% and 30% in terms of carbon. Model contaminants for the experiment were lead (Pb) and zinc (Zn), which were introduced in the form of nitrates in a concentration of 540 mg/kg for lead, and 880 mg/kg for zinc.

After the incubation of the soil with heavy metals and HS for 3 weeks, microbiological indices and chemical indices of heavy metals in various forms of mobility were measured. To assess the functioning of soils, the characteristics of the soil microbial community were chosen: the carbon content of the microbial biomass and the $CO_2$ emission, due to microbial heterotrophic respiration. According to many researchers, these indicators give a good assessment of the state of microorganisms in the soil. For statistical data processing principal component analysis (PCA) was used.

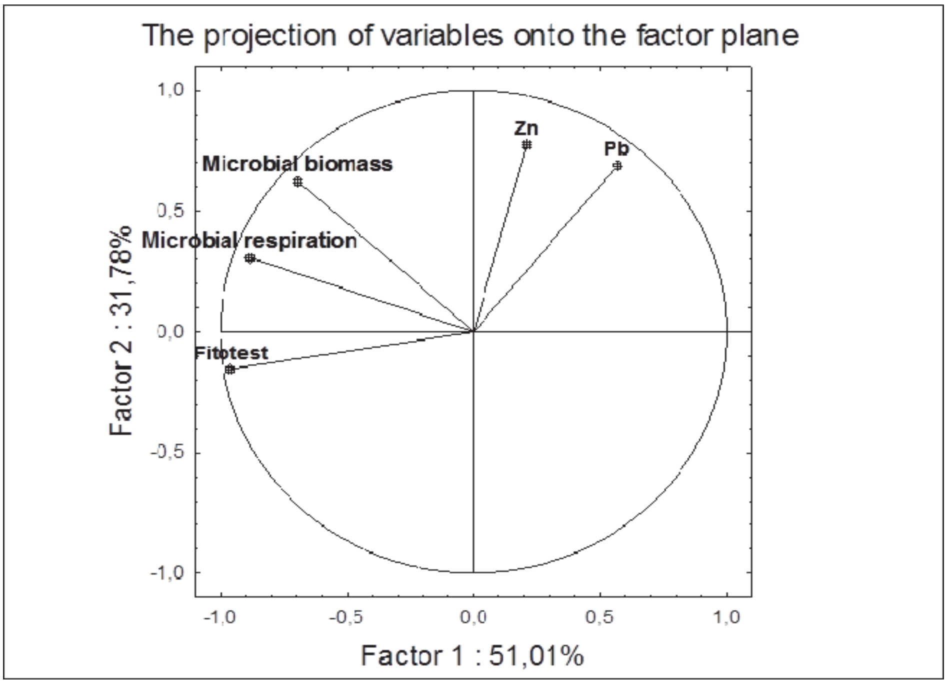


Figure 1. Diagram of results obtained by principal component analysis.

In research were obtained results:

1) The overall decrease in the content of mobile forms of trace metals under the influence of humic substances.

2) With the decrease in the content of active forms of lead, the indices of $C_{mic}$ and microbial respiration increased.

3) Purified humic acids are better than another HPs sorb lead.

4) Different doses (10% or 30%) of Lignohumate did not affect the concentrations of mobile lead forms.

5) The values of microbiological indices for Lignohumate are higher than for Flexom and HA, probably as a result of high content of mineral components and low molecular weight organic compounds readily accessible to microorganisms.

6) Analysis of data by the method of PCA allows us to conclude that it is possible to evaluate the effectiveness of humic acids by the values of microbiological indicators.

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## The Use of Humic Products on Agro-sod-podzolic Soils of the Udmurt Republic

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Agro-sod-podzolic soils are most common in the Middle Urals, which includes the Udmurt Republic. They are characterized by low natural fertility, acid reaction, low content of humus and accessible forms of nutrients. In connection with this, with intensive agricultural use, such soils require a long amelioration. Especially this is true for soils of light granulometric composition. For systematic amelioration it is possible to use organic and mineral fertilizers, as well as humic preparations.

The microfield experience in studying the use of the humic drug Life Forse Natural Humic Acids (NHA) for potatoes was conducted in 2017 on agro-sod-podzolic sandy loamy soil of the Udmurt Republic. The soil had the following indices: humus content 1.64%, pH(KCl) 5.95; the content of mobile phosphorus 165 mg / kg and exchange potassium 78 mg/kg. The studies were carried out on two backgrounds: without fertilizers and using N60P60K60. The experiment was carried out in five replicates. Results of accounting for yield are presented in Table 1.

Table 1 – Influence of a humic product of NHA on productivity of potatoes on the agro-sod-podsolic sandy soil, kg/sq.m (Udmurt Republic, 2017).

| Options | Total productivity, kg/sq.m | | | | Commodity productivity, kg/sq.m | | | |
|---|---|---|---|---|---|---|---|---|
| | Background I without fertilizers | | Background II NPK (60 kg/ha) | | Background I without fertilizers | | Background II NPK (60 kg/ha) | |
| | productivity | ± | productivity | ± | productivity | ± | productivity | ± |
| 1. Without NHA (control) | 1.12 | – | 1.64 | – | 0.89 | – | 1.27 | – |
| 2. NHA | 1.51 | 0.39 | 1.74 | 0.10 | 1.35 | 0.46 | 1.38 | 0.11 |

The humic product NNA showed higher efficiency at a background without fertilizers. The average increase in total and commodity yields relative to control was 34.8 and 52%, respectively. At the background of a full mineral fertilizer, the yield level is higher, but the increments to control are insignificant.

The research will continue, the conducting an analysis of the quality of the products obtained and soil samples is planned.

# Analyzing the Influence of Humic Acids on the Features of Urban Lawns

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In modern conditions, the city vegetation is experiencing considerable stress caused by pollution, acid rain and other anthropogenic factors. Lawn is an integral part of urban greening. Functions of lawn areas, significantly improve the ecological situation in the city, reducing the concentration of harmful gases in the atmosphere, dust and also adjusting temperature and humidity, hold excess stormwater runoff. The lawn functioning, in both ecological and aesthetic aspects, largely depends on their resilience to ongoing anthropogenic pressures in the urban environment.

Currently, the market is full of many different types of fertilizers (mineral and organic). From year to year new types of fertilizers are produced, representing as improved traditional fertilizer, and new brands. Great interest among them are Fertilizers, including in its composition of humic substances, have very high demand now. Some of these fertilizers are Humate K "Flexom", "Bioplant Flora Bio" and "Agro Nova Bio". In the structure they have in addition to humic and fulvic acids, different amino acids and are characterized by low content of NPK. As there is no extensive data on the effectiveness of the fertilizers applied to lawn grasses, as well as about the comparative characteristic of influence of mineral fertilizers and humic preparations, the present experiment was intended to obtain data about the effectiveness of humic substances applied to lawn grasses.

For our problems solution in June 2014, small-plot experiment was set in scientific-experimental landscape and soil-lysimetric park at the soil station of the faculty of soil science of Moscow state University. M. V. Lomonosov. The object of the study was the model of lawn ecosystem, representing constructsim with growing lawn grass mixture. In order to create organogenic horizon constructsim to the bulk soil, the corresponding PP-No. 514 of 2011 was used, which included 30% purified sand and 70% of lowland peat. For creation a grass cover a "Universal" type of lawn grass mixture of a brand "Izumrudnaya Polyana Lux" of agrocompany "Poisk" was used. The plot square is 2 $m^2$, with triple repetition. Before sowing the soil was treated with a solution of fertilizers "Agro Nova Bio" and Humate K "Flexom" in a dilution of 1:400, at the rate of 300 liters per 1 ha. Spraying of plant by "Agro Nova Bio" and Humate K "Flexom" was carried out 10 days after germination, and then after each research mowing (only 4 mowing during one vegetation period) solution dilution of 1:500, at the rate of 300 liters per 1 ha. In appropriate variants of the experiment a complete fertilizer NPK was made as the background, in the form of salts at a dose of N60P60K60 with presowing incorporation to a thickness of 0-10 cm.

As a result of three years research the following general regularities in the use of humic fertilizers were revealed. The application of humic substances increased the biomass of grasses compared to control. The greatest efficiency was observed when using of NPK the background in: applies to increase of biomass, and increased nitrogen content in plants and its' removal from biomass. Significant difference in the effect of the three investigated humates on grass biomass was not found.

The results of the study of projective cover using the program Surfer 11 show that the projective cover is also higher in the variants with the joint application of humic fertilizers and complete fertilizer. In the first year of the experiment more effectively the intensity of turfness is affected by humic fertilazer "Agro Nova Bio" together with NPK. Humic fertilizers themselves without mineral fertilizers significantly increase turfness of the experimental plots compared to the control without fertilizers (by 10-13%) and align them with options where only NPK was applied.

The combined use of humic preparations on the background of NPK allows you to achieve by the third year of vegetation projective cover close to 90 %. The content of total nitrogen in the grass biomass in the second year of study is growing in comparison with the first year and significantly differs on the plots with application of humic fertilizers, which shows the stimulating effect of humic substances on absorption of nitrogen from the soil by herbs.

Application of humic fertilizers leads to an increase in the proportion of disaccharides in the amount of saccharides that, together with the increase in the content of protein nitrogen indicates the best preparedness of the lawn to the conditions of temperature stress (winter survival) and lack of moisture (drought).

# Influence of Humic Preparations of LifeForce Group Company Study on Potato's Tuber Productivity in Moscow District

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Currently, the problem of production of ecologically clean products becomes more urgent. In such a case, application of different agrichemicals: artificial- synthesized pesticides, mineral fertilizers etc. is reduced or there is a total refusal of their application. But agriholdings need to use a lots of chemical products on theirs fields to enhance crops yield and quality, disease, pest and weed control. So they can't refuse of established cultivation technologies.

However, introducing of combined using humic preparations with traditional mineral fertilizers can be one of the way of increasing state of cultivation (improvement of soil consistency, increase the part of soil organic matter, improve microflora status, moistness and air supply etc.). As consequence of this producers can reduce fertilizer rate so it has positive effect on exacerbated ecological situation.

Year by year soil resources become more exhausted and it's difficult to reclamate them. Reduction in yields is associated with degradation of topsoil. Humic substances preparations in combination with appropriate agricultural methods promote to increase in productivity due to restoring soil fertility.

The aim of this study was to research the possibility of enhancing crops yield and quality of potato. Investigations were carried out in Voskresenskiy district of Moscow region in 2017. We applied new humic acids- based preparations: 1 – «Life Force Soil Conditioner Humate Balance for soil enrichment»; 2 – «Life Force Soil Conditioner Natural Humic Acids for soil fertility».

As a result there is the addition yield in «Life Force Soil Conditioner Natural Humic Acids for soil fertility» sample which amounted 12,5%. Addition yield in «Life Force Soil Conditioner Humate Balance for soil enrichment» sample amounted 8,4%. Yield structure analysis showed ambiguous data so we cannot assess specific characteristics of uniqueness of productions.

Thus, the findings suggest that yield can potentially increase in industrial scale. Whether we need to conduct more researches to argue that application of analyze humic. preparations can improve the quality of products.

# Synthesis of Nature-mimicking Silver Nanoparticles in the Presence Humic Substances of Different Origin

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At present, there has been a steady increase in the production of nanomaterials possessing unique properties such as thermal, optical, physical, chemical, magnetic etc. One of the most preferred nanoparticles are silver ones (AgNP) due to their active use primarily as a universal antiseptic agent inhibiting the growth of pathogenic microorganisms. The major drawback of AgNP fabrication is a use either high energy-consuming physical processes or toxic compounds (organic solvents, reducing agents and stabilizers) in their synthesis [1]. In the case of AgNP is especially critical, since the residual content of toxic reagents prevents the application of AgNP for medical purposes. Therefore, increasing attention is paid to the search for alternative ways of synthesis of AgNP using environmentally friendly technologies. Our work aimed at study of AgNP formation in the presence of humis substances (HS) of different origin. This approach is based on simulation of $Ag^+$ interaction with in the environment.

To reach this goal, synthesis of AgNP in the presence of HS was carried out at 25°C under 12-h photoperiod. Silver ions was introduced as $AgNO_3$ to reach final concentration 0.01 M, and concentration of HS was 1 g/L. Ten HS of different origin including soil, peat and coal humic acids (HA) and fulvic acids (FA) were used. HS were described using elemental analysis, gel permeation chromatography and $^{13}C$ NMR. AgNP formation was monitored using UV/Vis spectrophotometry, and the end $Ag^+$ – HS interaction was detected when peak corresponding AgNP at ca. 400 nm stopped its growth. In parallel, pH and Eh were monitored during all over the experiment.

Our results showed different rate of AgNP formation in the presence of HS depending HS properties reaching maximum in case with sod-podzolic soil FA and minimum in case with HA extracted from chernozem while peat and coal HS demonstrated intermediate rates. In general, higher rate was observed for HS with greater content of aliphatic moieties in their structure.

During AgNP formation values of Eh increased from ca. 0.2 to ca. 0.5 V indicating the oxidation of HS. Together with Eh growth, substantial pH decrease was detected. Therefore, carboxylic group formation in the HS structure could be hypothesized during AgNP formation.

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## Biotechnological Method of Applying "BIOMIN" in Increasing the Fertility of Soils of the Republic of Kazakhstan

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Currently, natural minerals of zeolites are a component for the production of organo-mineral fertilizers of prolonging action, since they contain significant amounts of elements of mineral nutrition of plants (Ca, Mg and K). However, the most valuable macronutrients such as nitrogen (N), phosphorus (P), as well as important trace elements of zinc (Zn) and manganese (Mn) are usually absent. In the Republic of Kazakhstan, there is a Chankaragai deposit of zeolites in the Almaty region, which are considered to be the best in the CIS by the content of clinoptilolite (up to 85%).

The purpose of scientific research since 2002 is the development of a new physicochemical meliorant and complex organo-mineral fertilizer of multifunctional and prolonged action.

Absorption by zeolites is associated with adsorption and sorption phenomena-the concentration of a substance from an aqueous salt solution or a gas phase on the surface of a solid (adsorbent) or in a volume (sorbent). The granule of any adsorbent is penetrated by channels, whose diameter in ordinary wide-porous adsorbents (aluminum or silicon oxides) reaches 10 nm or more, and in the volume during the sorption cavities of various configurations are formed. The combination of channels and cavities creates a system of pores whose surface (the inner surface of the adsorbent) can amount to hundreds of square meters per 1 kg, and the sorption capacity of zeolite pores is an order of magnitude higher. On this basis, the concept of modifying raw materials of zeolite by nutrients and microorganisms is based. But the absorption capacity of natural zeolites under ordinary conditions is low (2 mg-eq). We have developed a method for physico-mechanical processing of raw materials of zeolite and an increase in the sorption volume by a factor of 10 (know-how), which makes it possible to create volumes and reserves of absorption by nutrients, stimulant preparations and prolonged strains of effective microorganisms.

The first stage of the work consisted in developing a method for obtaining a modified zeolite, which had an increased cation-exchange capacity and contained missing macro- and trace elements. This allowed obtaining new material "BIOMIN" based on natural zeolite. The use of modified zeolite in field experiments for 10 years at a dose of 1 to 2 tons per hectare for rice, soybean and potato growing in arid zones of the Republic of Kazakhstan showed high efficacy of this preparation (yield increase of crops is more than 100%).

At the second stage of the research, a technology was developed using complex organo-mineral biofertilizer based on modified zeolite. To this end, beneficial soil microorganisms (nitrogen fixers, phosphate and potassium dissolving, petrochemicals, etc.), as well as humic substances and certain types of plant growth stimulants, have been inoculated on the modified zeolite matrix. This allowed to obtain new types of complex organo-mineral biofertilizers for ecology - bioremediation of oil contaminated lands in Western Kazakhstan in regions of extraction of oil raw materials.

At present, three patents of the Republic of Kazakhstan No. 20621 (2007), No. 27379 (2013), No. 31348 (2016) have been obtained on the methods of obtaining of a new type biomineral and bioorganic fertilizers.

Conclusions

1. A method for modifying zeolite has been developed with the aim of obtaining biomineralic and bioorganic fertilizers of a new type of prolonged action. To modify the zeolite, two-component mother liquor solutions of the type nitroammophosphate, humic and microbial preparations and stimulants of the new generation were used. The matrix of the modified zeolite at different levels of redistribution from 1 to 5 is more productive (3-7.5 times) and functional as a result of optimization of vital activity of nitrogen-fixing microorganisms for water supply and nutrition.

2. In the vegetative experiments, the greatest number of nitrogen fixers was detected using modified zeolites treated with humic and microbial biologics. When using biologics MERS, Gumi-K, Gumi-30 and Nanobiostimulator G, the number of microorganisms significantly increased several times.

3. Economically valid doses of biomineral and bioorganic fertilizers based on modified zeolite for soils of grain, vegetable crop rotations are a norm of 1-2 tons / ha.

4. Modified zeolites based on various nitrogen, bioorganic fertilizers and cultures of immobilized nitrogen-fixing microorganisms increase the effective productivity of soils up to 100%. The products of the modification possess the optimal physicochemical parameters, are technologically and economically useful for rice, vegetable, and technical crops.

# Биотехнологический способ применения «БИОМИНА» в повышении плодородия почв республики Казахстан

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В настоящее время природные минералы цеолиты являются компонентом для получения органо-минеральных удобрений пролонгирующего действия, так как они содержат в себе значительные количества элементов минерального питания растений (Ca, Mg и K). Однако, наиболее ценные макроэлементы как азот (N), фосфор (P), а также важные микроэлементы цинк (Zn) и марганец (Mn), как правило, отсутствуют. В Республике Казахстан в Алматинской области имеется Чанкарагайское месторождение цеолитов, которые по содержанию клиноптилолита (до 85 %) считаются лучшими в странах СНГ.

Целью научных исследований с 2002 года является разработка нового физико-химического мелиоранта и комплексного органо-минерального удобрения полифункционального и пролонгированного действия.

Поглощение цеолитами связано с явлениями адсорбции и сорбции-концентрированием вещества из водного солевого раствора или газовой фазы на поверхности твердого тела (адсорбента) или в объеме (сорбента). Гранула любого адсорбента пронизана каналами, диаметр которых в обычных широкопористых адсорбентах (оксиды алюминия или кремния) достигает 10 нм и более, а в объеме при сорбции образуются полости различной конфигурации. Совокупность каналов и полостей создает систему пор, поверхность которых (внутренняя поверхность адсорбента) и может составлять сотни квадратных метров на 1 кг, а сорбционная объемная емкость цеолитных пор на порядок выше. На этой основе основана концепция модифицирования цеолитного сырья элементами питания и микроорганизмами. Но поглотительная способность природных цеолитов в обычных условиях невелика (2 мг-экв). Нами разработана методика физико-механической обработки цеолитного сырья и увеличения сорбционного объема в 10 раз (ноу-хау), что позволяет создать объемы и резервы поглощения элементами питания, препаратами-стимуляторами и пролонгированными штаммами эффективных микроорганизмов.

Первый этап работы состоял в разработке способа получения модифицированного цеолита, который обладал повышенной катионно-обменной ёмкостью и содержал в себе отсутствующие макро- и микроэлементы. Это позволило получить новый материал «БИОМИН» основе природного цеолита. Применение в полевые вегетационных опытах модифицированного цеолита в течение 10 лет в дозе от 1 до 2 т/га при выращивании риса, сои и картофеля в аридных зонах Республики Казахстан показали высокую эффективность этого препарата (повышение урожайности культур более 100%).

На втором этапе исследований была разработана технология с использованием комплексного органо-минерального биоудобрения на основе модифицированного цеолита. Для этого на матрицу модифицированного цеолита были иннокулированы полезные почвенные микроорганизмы (азотфиксаторы, фосфат- и калийрастворяющие, ефтееокисляющие микроорганизмы и н другие), а также гуминовые вещества и некоторые виды стимуляторов роста растений. Это позволило получить новые типы комплексных органо-минеральных биоудобрений для экологии – биоремедиации нефтезагрязненных земель в Западном Казахстане в регионах добычи нефтянного сырья.

В настоящее время на способы получения биоминеральных и биоорганических удобрений нового типа получены три патента Республики Казахстан №20621 (2007), №27379 (2013), №31348 (2016).

Выводы

1. Разработан метод модифицирования цеолита с целью получения биоминеральных и биоорганических удобрений нового типа пролонгированного действия. Для модификации цеолита использовались маточные двухкомпонентные растворы удобрений типа нитроаммофосфата, гуминовые и микробные препараты и стимуляторы нового поколения.

Матрица модифицированного цеолите при различных уровнях передела от 1 до 5 является более производительной (3-7,5 раз) и функциональной в результате оптимизации жизнедеятельности азотфиксирующих микроорганизмов по водообеспеченности и питанию.

2. В вегетационных опытах наибольшее число азотфиксаторов обнаруживалось при использовании модифицированных цеолитов, обработанных гуминовыми и микробными биопрепаратами. При использовании биопрепаратов МЭРС, Гуми-К, Гуми-30 и Нанобиостимулятор Г достоверно увеличивалась численность микроорганизмов в несколько раз.

3. Экономически обоснованными дозами биоминеральных и биоорганических удобрений на основе модифицированного цеолита для почв зернового, овощного севооборотов является норма 1-2 т/га.

4. Модифицированные цеолиты на основе различных азотных, биоорганических удобрений и с культурами иммобилизированных азотофиксирующих микроорганизмов повышают эффективную продуктивность почв до 100%. Продукты модификации обладают оптимальными физико-химическими параметрами, технологичны и экономичны при использовании под рисовые, овощные, технические культуры.

## Effect of Humic Fertilizers on Crop and Quality of *Anethum graveolens* L.

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Humic fertilizers and preparations are widely represented in the agrochemical market. Our goal was to study their possible impact on the characteristics of the dill (Anethum graveolens L.). We compared the effectiveness of fertilizers, their forms and methods of use. The experiment was carried out with early ripe dill. Plant variety «Gribovskii». It has large, delicate and fragrant leaves. Seeds are produced by the company «Agrofirma AELITA». They were tested by the State Seed Inspectorate and met the requirements of international standards and GOST 32592-2013 in terms of quality.

The following products were used as growth stimulants:

1) «Liquid biohumus». Producer: company «Agrofirma «Polya russkie», Tambov region of Russia. The chemical composition: humic substances is not less than 3 g/l, microelements (Mg; Fe; B; Mn; Cu; Mo; Zn); pH is at least 7,5.

2) «Gumi-30». Producer: company «NVP» BashIncom», Russia, Republic of Bashkortostan. Ingredients: sodium humate not less than 60%, macroelements (nitrogen 0,5-2,0%, phosphorus 0,5-2,0%, potassium 0,1-1,0%), trace elements of natural origin. The Hazard class is Four (4) according to GOST 12.1 007. It is a low-hazard substance.

The small-scale experiment was laid in the territory of the Tula region of the Russian Federation in 3-fold repetition. The middle-cultivated leached chernozem was chosen as the soil for the experiment. Its characteristics were as follows: pH 7,4 and humus content 12,9%. The studies were conducted from August 1 to October 4, 2016, up to frosts.

Carrying out of experiments, sampling and their analytical processing were carried out according to classical methods. The area of the individual plot was 3 $m^2$. Fertilizers for dill have been applied in the concentration recommended by agrochemicals producers in three stages: soaking the seeds before sowing, watering young plants and sprinkling leaves after emergence. Sowing on natural soil without fertilizers was a control.

The results of the experiment showed that the preparation «Liquid biohumus» exerted a stronger influence on the yield. The overground biomass of dill in relation to the control increased by 85% when soaking the seeds before sowing, by 148% when watering during the plant growth and by 62% when sprinkling leaves after emergence. When applying «Gumi-30» the following results were obtained: 103% with soaking, 10% with watering and 152% with spraying.

The best way and optimal using of agrochemicals is soaking the seeds before sowing. When using «Gumi-30» plant biomass increased twice in relation to the control. When using «Liquid biohumus» - in 2,4 times. The application of «Gumi-30» increased the diameter of dill roots by 1,8 times, and the root length by 1,3 times.

Under the influence of «Gumi-30» and «Liquid biohumus», there is an increase in the accumulation of total nitrogen, protein nitrogen, total phosphorus, potassium, sodium in leaves and stems. The nitrogen content of the total in leaves increased by 2,5%, in stems by 0,4%, and the protein nitrogen content increases in leaves by 0,9-2,3%, in stems by 1,0-2,0%.

The concentration of potassium in the leaves increases with soaking «Gumi-30». Sodium from preparations was actively used by plants. The integral effect of the use of the humic fertilizers for dill of late sowing consisted in increasing the productivity (biomass), the intensity of physiological and biochemical processes in plants, and preserving the high quality of the crop.

## A Study of Crop Cultivation Effects; Technology and Agricultural Technology Development for Novel Preparations Based upon Humic Acid Complexes, Combining Adaptogenic and Growth Control Functions

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An intensive crop cultivation technology does not just include quality and timely execution of the series of interrelated procedures. It also requires being in compliance with a number of conditions, such as selection of the precursor crop, the quality and methods of soil preparation, sowing time and seed quality, fertilization and protective measures/ It should consider the level of available nutrients, as well as the selection of modern immunity boosting chemicals that increase the resistance of cultivated plants to the stress factors that include levelling effect of pesticides, particularly fungicides.

Therefore, perspective techniques for agricultural product quality improvement may include the method of pre-sowing seed and growing plants treatment with modern environmentally friendly growth regulators and biological products based on vermicular compost. The treatment should apply "green" chemistry methods, using physical influence of sonic hydrodynamic resonance.

During the technology development for "OrgaNIKALife" preparation the following technological points were addressed:

– vermicular compost preparation for later grinding combined with humic substances extraction with the formation of organomineral complexes under sonic hydrodynamic resonance conditions;

– optimization of the process conditions such as hydraulic module, temperature, reagent ratio, process time to increase the product yield;

– search for the environmentally friendly methods of product stabilization;

– optimization of existing concentrations and conditions of use.

During the experiments, the completion of these tasks led to an effective liquid preparation, containing no preservatives nor fungicides. The preparation has a shelf life of 2 years. It also exhibits adaptogenic and antioxidant properties.

The efficacy of the preparation for the agricultural crop seed and growing plats treatment has been studied for three years. The treatment was intended to stimulate germination energy, growth force, germination ratio, to strengthen the growth and formative processes, to increase the yield and quality of the agricultural products. The analysis of macro- and micronutrient content in dry and natural humidity source vermicular compost was carried on. The results show that the vermicular compost contains 49.7 % organic matter along with major macronutrients and three micronutrients (copper, zinc and manganese).

It has been found that under the preparation increases the yield of spring and winter wheat as well as sugar beets. The preparation was less effective for legumes. This can be attributed to the high seed quality for the studied legumes. Also, the weather was unfavorable for the legumes study: high ambient air temperature over 30 °C, low relative humidity and not a single drop of rain. However, even under these adverse conditions the application of vermicular compost increased the crop yield by 220-870 kg/ha with the seed treatment and 550-980 C/ha for foliar application. Both treatment methods fit into the cultivation technology for these crops.

## Structure of Nanocomposites Based on $Fe_3O_4$ and Humic Acids

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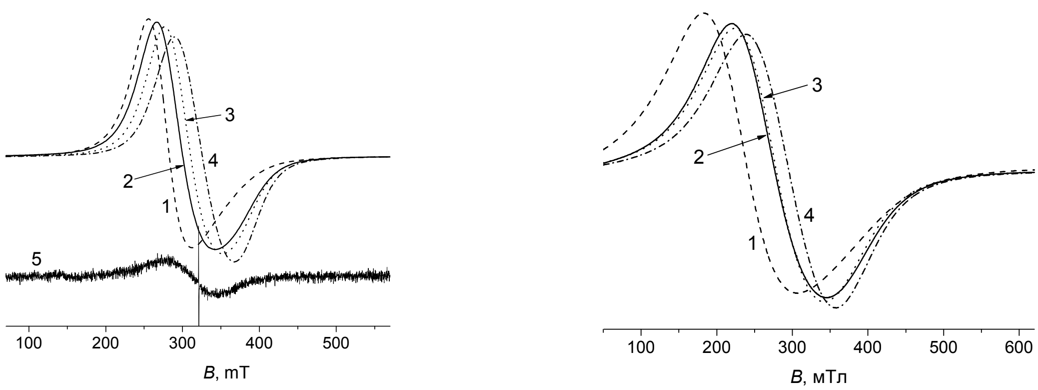
Composite materials based on $Fe_3O_4$ magnetite nanoparticles potentially can be used in various fields: from magnetic separation of harmful toxic technical waste to preparation of polymer suspensions for biology and medicine. $Fe_3O_4$ nanoparticles functionalized with humic acids (HA) can become an alternative to the currently used adsorbents for the highly efficient recovery of metal ions from wastewater and heavily polluted water. They are able to extract more than 95% of U (IV) and Zn (II) ions, and more than 80% of Cr (III) and Mn (II) ions [1, 2].

Samples of $Fe_3O_4$/HA nanocomposites were synthesized by chemical coprecipitation of $FeCl_2$ and $FeCl_3$ in a matrix of humic acids with a different initial ratio of components of $Fe_3O_4$/HA as 20:80, 50:50, 80:20 wt.%. They are gray-brown color of different intensity. Samples were studied by EPR, electron microscopy and XRD analysis.

According to electron microscopy, it can be assumed that $Fe_3O_4$ nanoparticles are located in the HA matrix discretely, and are stabilized by electrostatic interaction of iron ions of the surface of nano-$Fe_3O_4$ with oxygen ions of carboxyl groups and -OH of HA groups in the bulk of humic acids.

According to ultrasonic spectrometry, the nanocomposites formed have a smaller particle size compared to colloidal magnetite particles. The HA molecules prevent the aggregation of magnetite nanoparticles.

Typical EPR spectra of the studied iron-containing composites at 298 and 77 K are shown in Fig.1. All the spectra are wide asymmetric lines, characteristic for nonhomogeneously broadened spectra with unresolved anisotropy of the *g* tensor, and the electronic spins of $Fe^{3+}$ are bound by a strong ferromagnetic interaction. Paramagnetic centers are located in the volume of $Fe_3O_4$ nanoparticles, and on their surface. It can be stated that practically all iron ions contribute to the EPR spectra.




*a* *b*

Figure 1. The EPR spectra of $Fe_3O_4$ (1), $Fe_3O_4$/HA20 (2), $Fe_3O_4$/HA50 (3), and $Fe_3O_4$/HA80 (4) samples at 298 (a) and 77 K (b)

As it can be seen from Fig. 1, as the fraction of HA in the composite increases, the position of the maximum of the integral EPR spectrum of samples $B_0$ shifts to higher fields, while the width of the $\Delta B$ lines varies quite insignificantly with the increase in the amount of HA in the composite.

An increase in temperature from 77 to 298 K leads to a significant narrowing of the EPR line by 40-50 mTl and to a noticeable shift of the EPR line (value $B_0$) to a higher magnetic field (Fig. 1 a,b).

From a comparison of the spectra (2) and (5) in Fig. 2a clearly shows that less than 1% of the initial iron ions are washed off the surface of the composite nanoparticles: the integration of the spectra, taking into account the registration parameters of both spectra and the signal-to-noise ratio, gives an estimate of ~ 0.1% Fe (III) of the initial iron content in magnetite .

It should be noted that a significant increase in the parameter $B_0$ at both temperatures (by more than 50 mT) and some decrease in the width of the $\Delta$B line from the EPR spectrum (by about 15 mT at 77 K) with an increase in the HA fraction in the composite indicates structural changes taking place in system.

When the Fe (III) -COO$^-$ bonds are completely saturated, the iron ions of the nanoparticle surface begin to interact with the amino groups of heterocyclic HA fragments, as indicated by the appearance of peaks in the 2300 and 3000-3400 cm$^{-1}$ region.

The proposed types of complexes of HA with iron oxides, shown in Fig. 2, illustrate a large number of possible forms of such components and types of interaction for humic acids derived from coal. Probably, the functional groups of the HA occupy all the available places for coordination on the surface of the $Fe_3O_4$ nanoparticles, i.e. the latter are "enveloped" by the polyanionic, "dendrite" structure of the HA.

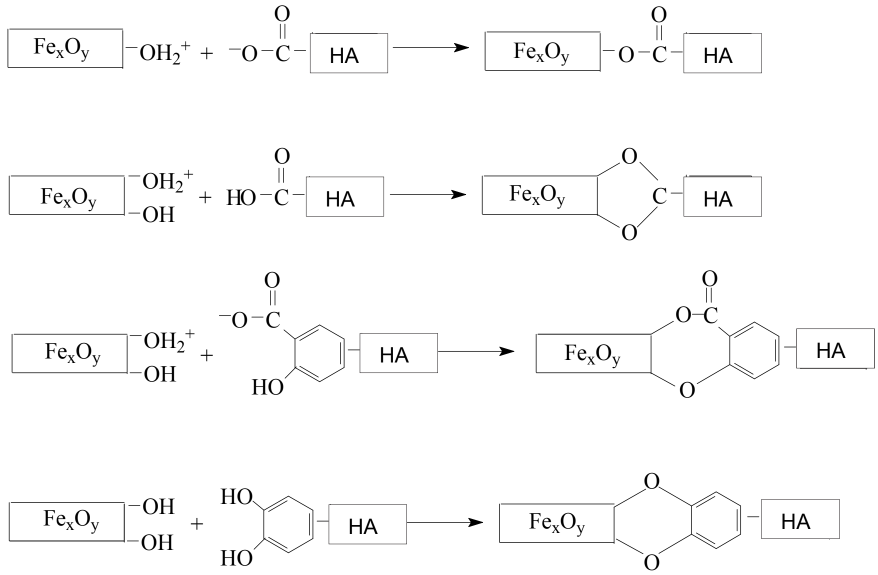


Figure 2. Possible types of interaction of iron oxides with the HA. Part of the scheme from [Gu B. et al, 1994] is adapted for our system.

According to XRD analysis, the adsorption of an increasing amount of HA to the ferromagnetic core of $Fe_3O_4$ leads to a decrease in the average size of <d> $Fe_3O_4$ nanoparticles from 9.2 to 5.7 nm. With such a decrease of <d>, the morphology of the nanoparticles should change. Part of the Fe (III) ions can pass from the surface of the ferromagnet to the HA layer in the form of metal complexes or clusters, and even to pass into solution when the samples are immersed in an aqueous medium.

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## Hybrid and Functional Humic-Based Materials: from Synthesis to Environmental Application

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Humic materials have the most striking feature in the context of environmental chemistry due to constellation of such unique properties as non-toxicity, biocompatibility, resistance to biodegradation, and polyfunctionality. As a result, diverse functional and hybrid materials can be derived on the basis of humic materials. These materials can be competitive on the market of biobased products, nominally, green special chemicals as dispersants, flocculants, chelators, etc.

A set of experimental approaches undertaken to produce nanocomposites based on $Fe_3O_4$ nanoparticles coating by humic acids (HA) for the sorption of ecotoxicants included: chemical precipitation methods in situ and ex situ. The small size of the nanoparticles (typically 7-16 nm in diameter) results in a very large exposed surface area for metal absorption, achieved without the use of porous materials that inevitably introduce a high mass transfer resistance. It has been evinced that $Fe_3O_4$-HA nanocomposite efficiently removed radioactive and heavy metal ions from contaminated water. Being magnetic, the nanocomposites are readily separable using an external magnetic field, while the humic acid coating effectively stabilizes the particles against aggregation.

The relatively new field of our research is formulation of novel magnetic imprinted nanoparticles ($Fe_3O_4$ @ Me(II) IIMs) with a high recognition for set of metal ions, incl. $UO_2^{2+}$ in the presence of competing ions prepared by cross-linking HA with aminocompounds. The thermodynamic and kinetic properties of the adsorption process were studied to explore the internal adsorption mechanism. Removal of the metal ions from the IIMs and creation of cavities for hosting metals was proved by comparison of the response of IIM/HA/APh to template with that of non-imprinted material (NIM/HA/APh). The relative selectivity coefficients of Me-IIM were determined.

Another part of research of HA in terms of environmental application is devoted to the design, development and application of a new generation of binders (non-stoichiometric interpolyelectrolyte complexes, NIPECs) for various dispersed systems, incl. soil. The universal binders are formed by interaction of oppositely charged polyelectrolytes (PEI/PDADMAC and PAA and HA), both chemically stable. Numerous results of laboratory experiments and field trials of the NIPEC formulations were obtained. In particular, large-scale tests have been done in the Kadzhi-Say uranium technogenic provinces where the NIPEC binders were shown to be effective means to suppress water and wind erosion thereby preventing a spread of radioactive particles (radionuclides) from contaminated sites. NIPECs are able to bind effectively a majority of toxic metals due to incorporation of metals inside hydrophobic NIPEC fragments generated by mutually neutralized cationic and anionic units.

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## Effects of Humic Acids of the Peat on the Coronary Reperfusion and the Contractive Activity of Isolated Heart of Rats

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The previous study [1] was performed toxicological studies of humic acids (HAs) by intraperitoneal injection of lethal doses. The effect of acute toxicity in mice and rats was similar: there was a weakening of muscle tone and poor coordination of movements, the sharp decline of physical activity, a pronounced cyanosis limbs, testes, ears and muzzle. The study of causes of mortality and identification of potential target organs, stricken with acute toxic effects, moderate perivascular and pericellular cerebral edema, hemorrhagic pulmonary edema, acute coronary and acute renal failure, dystrophic changes in the adrenal cortex were observed. As a result, the death of animals occurs from acute heart failure resulting from ischemic myocardium dystrophy was concluded. At the same time, acute cardiac abnormalities have led to stagnation in the pulmonary circulation with the development of hemorrhagic pulmonary edema, and general acute hemodynamic instability was a shock stimulus for the kidneys and caused acute renal failure. The manifestation of such mechanisms cardiotoxicity are characteristic for poisoning miotropnymi agents that affect ion channels (calcium channel blockers, potassium channel openers), nitric oxide donators. As a result, we have assumed the existence of direct kardiotropnyh (cardiovascular) effects when administered at therapeutic doses of HAs. Thus, the aim of this work is to investigate the influence of the native peat HAs on the performance of the pumping function and the rate of coronary perfusion of the isolated perfused rat hearts in a range of doses.

Materials and methods

Humic acids were isolated by alkaline extraction from samples of lowland wood-grass peats collected from "Tagan" deposit of Tomsk region. Bioactivity of HAs was tested on male rats “Wistar” 250-300 g. After thoracotomy, the heart was taken out and transferred into a cooled (+4°C) Krebs-Henseleit solution until spontaneous contractions cease. Retrograde perfusion of the heart was performed by the method of Langendorff with open circuit. The heart was perfused at a constant pressure of 52 mmHg. Krebs-Henseleit solution which was saturated with a gas mixture containing 95 % $O_2$ and 5 % $CO_2$ (solution temperature 37 + 0.5°C, pH = 7.5). To register cardiac contractility parameters were used device for electrophysiological studies of MP35 Biopac System Inc. (Goleta, United States). Effects were assessed HAs dissolved in the perfusion solution (0.001, 0.01, 0.1 mg/ml). And the effect on the contractile frequency, end-diastolic pressure (mmHg). And coronary perfusion of the heart rate. On the strength of heart contractions judged by the magnitude of pressure, developed by the left ventricle (mmHg), which is calculated as the difference between systolic and diastolic pressure. Coronary flow was measured by counting the amount of perfusate flowing through the heart for one minute (ml/min). The registration of all indicators conducted after a period of 20 minutes to adapt to the conditions of perfusion, on the 10$^{th}$ min perfusion with Krebs solution containing HAs at the 10$^{th}$ minute perfusion without the drug. The controls were isolated rat heart perfused in a similar way with Krebs solution containing no HAs.

Results and Discussion

Humic acids in concentration of 0.1 mg/ml have strong cardiovascular activity, an increase in coronary flow was observed after 10-min perfusion with Krebs solution of the isolated heart. This effect HAs were short-lived, because after 10 minutes of perfusion with no preparation coronary flow was indistinguishable from control. By reducing the concentration of HAs is 10 times the effect was less marked and disappear when using HAs concentration of 0.001 mg/ml. Thus, the HAs impact on the rate of coronary perfusion under in vitro conditions was reflected in a significant increase in this indicator, which may indicate a direct effect of vasoactivity.

Humic acids affect myocardial contractility. Against the background of the heart perfusion HAs solution a decrease in power cuts, which persisted and during subsequent perfusion without the drug. Reducing this parameter bore dose dependent, the maximum effect was observed at a concentration of 0.1 mg/ml.

Effect of HAs on the heart rate was expressed in a significant decrease of the indicator, with negative chronotropic effect was more stable at a dose of 0.1 mg/ml, than the negative inotropic effect of the test drug in the same concentration. Under the influence of the HAs occurred a statistically significant decrease in end-diastolic pressure. By reducing the concentration of HAs is 10 times the effect was less marked and disappear when using concentrations of 0.001 mg/ml.

Thus, in vitro experiments have established that the HAs have direct cardiovascular effects, are involved in the regulation of coronary tone in normoxic conditions, respectively, in the regulation of coronary heart circulation. It has been shown that HAs at a concentration of 0.1 mg/ml have pronounced vasodilating effect, indicating an improvement in coronary perfusion intact myocardium. Picked up the drug concentration of 0.1 mg/ml, in which it has the maximum vasodilator effect. It is known that crucial in the regulation of vascular tone belongs endothelial factors, which include nitric oxide [2]. Released from L-arginine, nitric oxide (NO) activates guanylate cyclase of smooth muscle cells with formation of cGMP, which leads to its relaxation [2]. Given the evidence of the ability of HAs to an increase in the generation of nitric oxide by endothelial cells [3, 4], we can assume that in the implementation of their vasodilating effect, the key role is played by the activation of this signaling pathway. Importantly, the presence of NO-synthase is proved in all parts of the heart, resulting in NO effect extends not only on the tone of the coronary vessels, but also on myocardial function [2]. According to some authors, under the influence of NO occurs decrease the severity and frequency of isolated cardiomyocytes contraction [2, 5]. Assuming that HAs stimulated NO generation in cardiomyocytes, the chrono detected and inotropic effects of these compounds may also have been due to the activation of this signaling mechanism.

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## The Productivity of Potatoes if Using Paste Fitogormonov Fertilizers

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Russia has rich natural resources for the production of various humic substances. Humic compounds included in the composition of organic substance of peat, soil, fossil coals, shales and sapropels. The raw material for production of humic fertilizers and drugs are a variety of underutilized natural materials, reserves of which are sufficient for experimental-industrial and industrial production of products based on the humates used in various fields of national economy.

Such fertilizers should be pasty fitogormony complex derived at the enterprise JSC "Buyskiy chemical plant" from peat, processed by way of cavitation. Refined pasty mass contains a number of macro – and microelements, biologically active substances, physiologically valuable groups of soil bacteria, and amino acids entering into the complex Association with humic acids. Wide application of complex fertilizers in agriculture is constrained by several factors (insufficient knowledge of the effects included in the fertilizer components in the physiological processes of plant organisms, their stability, productivity, etc.). This prompted us to conduct these studies. **The purpose of the research**. To study the effect of methods of application of an integrated pasty fitogormonov fertilizer on development and yield of potato cultivar Aurora. **Research methods**. Laboratory and field experiments were made in accordance with approved methods B. A. Dospekhova, 1985 [1]. For mathematical data processing was used software package Microsoft office.

The results of the research. To plant-soil complex functioned normally need to restore and replenish lost biogeocenosis links. For the real productivity of agricultural lands, along with management of soil fertility through physical and chemical actions, necessary to carry out biological treatment of crops [2]. Correction methods for biological productivity of crops are analogues of natural phenomena that can cause negative effects in the process of growth and development of plants. The very effective methods of correction of biological productivity of crops to take treatment of plants for planting and foliar treatment of crops with the use of humic complexes. The high biochemical activity of these substances plays an important role in maintenance of high biological productivity of the system plant – soil, and increase the sustainability of the system to adverse effects. It is known that the flowering stage of potato plants, the process of tuber formation is most intense. There is an outflow of products of photosynthesis to storage organs. Treatment of potato tubers before planting pasty ficopomatus contributed to the decline in consumption of the organic substances on the breath, which is reflected in the productivity of the culture. On the trophic level of security used types of fertilizers, provided the intensification of production process of potato plants. This circumstance directly affected the formation of the economically useful parts of potato plants (tab. 1).

Table 1 – Fractional composition of the harvest of potato tubers 2017.

| Option | The number of tubers with 1 Bush | | | The weight of tubers from 1 Bush, g | | | Yield t/ha |
|---|---|---|---|---|---|---|---|
| | small | average | large | small | average | large | |
| (background OMF 300kg/he) Control Spraying the tubers with water 300L/he | 4.83 | 2.00 | 2.67 | 151.60 | 133.03 | 348.81 | 25.34 |
| (background OMF 300kg/he) Control Spraying of plants with water 300L/ha | 7.67 | 1.89 | 2.78 | 235.99 | 124.78 | 269.80 | 25.22 |
| (background OMF 300kg/he)Pasty fatogoma. Spraying of tubers 100L/he | 6.67 | 3.67 | 4.89 | 191.67 | 173.05 | 500.71 | 34.62 |
| (background OMF 300kg/he) Pasty fatogoma. Spraying of tubers 100L/300L/he | 8.44 | 3.67 | 3.00 | 260.43 | 238.76 | 293.80 | 31.72 |
| (background OMF 300kg/ha)of Palestin. Spraying of tubers 2l/300L/he. MD | 7.78 | 2.33 | 2.33 | 250.53 | 158.17 | 248.98 | 26.31 |
| (background OMF 300kg/ha) of Palestin Spraying the plants with 2l/300L./he | 7.33 | 2.00 | 3.28 | 206.88 | 136.45 | 426.19 | 30.08 |
| LSD05 | 2.021 | 0.090 | 0.101 | 23.12 | 18.27 | 32.17 | 0.891 |

Potato yield when using paste hitogata for treatment of tubers were formed at the expense of the big food fractions and made up 34.62 t/ha, significantly exceeding the values of the control variant and the variant with the application of Palestine.

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## Results for the Use of Phytohormone-Humic Acid Compounds (PHCs) in Plant Production in Germany in the Past 12 Years

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Agriculture has not reached an environmental limit in Germany over the past 10 years. The potential of plant and soil aids to increase the productivity of agricultural crops with simultaneous decrease of environmental pollution is still controversially discussed in this context. Differently in Eastern Europe, where mineral fertilizer use and yields are traditionally much lower, and where have regularly reported increases in yields of 15-40% for the same fertilizer use since 1999.

In the past two decades, in the Ukraine, Russia and Belarus basic scientific work has been carried out on two classes of plant and soil aids - phytohormones and humic acids, mechanisms of action have been elucidated, and practical experience has been collected and published on several million ha. These knowledge is the starting point for the current international long-term program Tandem$^{12/21}$ to increase the soil fertility of the soil and to establish a sustainable biological nutrient reserve in the soil, using compositions from these two classes - so-called PhytoHuminCompounds (PHC).

In the long-term program Tandem$^{12/21}$ and the two upstream research projects Radostim A * B and future$^{9/12}$ , German farms with intensive economics have been involved since 2004. Over 170 feilds with a total area of approximately 4000ha were treated with PHC for up to 12 years.

For the increase of soil fertility and the biologically induced yield, the long-term development of the three soil parameters, which are closely interrelated: humus, air nitrogen binding bacteria and phosphormobilizing bacteria is of essential importance. A new empirical parameter - the biological soil index BSI * - was introduced for the first time on the basis of long-term measurement series to describe this interrelationship and to assess the efficacy of PHC applications in spring (plant application) and in autumn (soil application). The BSI * can be further increased by 8 to 32% as a result of soil application in autumn. The increase is dependent on the humic acid type used.

Changes in the CU (Cereal Unit) - standardized production functions were evaluated. Our conclusion is that depending on the location and SV (Soil value: 30 to 70), the yield maxima increased by 4 to 20 CU while reducing the nitrogen fertilizer use by 15 to 40 kgN/ha.

The results are discussed and explained within the framework of the YEN model. PHC will activate 1 to 3 kg of plant-available biological nitrogen per hectare from 1 million CFU / g of air nitrogen binding bacteria in soil. Their concentration increases to equilibrium values of 20-30 million CFU / g for several years by repeated PHC use. At the same time, the relative proportion of phytopathogenic bacteria in the soil is reduced. PHC applications are thus a reliable means of improving the N-balance of German agriculture by 15-40 kg N / ha, while reducing the environmental impact and meeting the requirements of the new Fertilizer Ordinance. The monetary effect is sustainable: 1 EUR PHC use, conservatively calculated, with 2 to 7 EUR profit to be booked.

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# Изучение при помощи оптических методов пролонгированного действия гуминового препарата Novihum на виноградниках Германии

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Novihum - органо-минеральное азотное удобрение (гранулят). Цель применения - стабильное обеспечение почвы субстратами с высококачественным, азотсодержащим устойчивым гумусом. Препарат ускоряет процесс трансформации питательных элементов в доступные для растения формы, что, в свою очередь, повышает эффективность удобрений и устойчивость растений к стрессовым ситуациям. Кроме того, улучшаются свойства почвы удерживать и передавать растению питательные элементы. Novihum получают из бурого угля посредством химического процесса оксидативного аммонолиза и содержит около 82% гуминовых веществ (гуматы,гуминовые кислоты, фульвокислоты) и от 5,5 до 6,0% азота, 33% которого в доступной для растения форме. Содержание $N_{min}$ до 4,2 г/кг СВ (сухого вещества). За пролонгированное действие отвечают гуминовые вещества. Novihum служит источником постоянного пополнения устойчивым азотсодержащим гумусом почв, обедненных или не содержащих гумус и препятствует вымыванию питательных элементов. Нормы применения: от 0,5 до 2,0 кг/м$^2$.

16 мая 2013 года в регионе Grossräschener See на рекультивированных терриконах на территории с/х предприятия Lindenfeld GbR был высажен виноградник по технологии Novihum - 2 сорта винограда - Cabernet и Solaris. Novihum вносился в объёме 470g/растение (лунка 16 литров). Контроль - стандартная технология посадки. Эксперимент сопровождался с момента высадки саженцев при помощи двух оптических методов - определение динамики флуоресценции хлорофилла CFD (Chlorophyll-Fluoreszenz-Dynamik) и определение динамики коэффициента хлорофилла C*/% (DPCA - Digital-Photo-Chrom-Analyse).

Оптические методы измерений доказали пролонгированное действие препарата Novihum. Заметное воздействие на активность процесса фотосинтеза (PHS), наблюдалось в 2014 - 2016 годах на протяжении всего вегетационного периода. Этот позитивный результат сопровождался более активным развитием саженцев, более высоким показателями витальности растений ($V_1$, $V_2$) и более высокими урожаями при сохранении качества продукции. Данные для сорта Cabernet обобщены в сводной таблице 1.

Таблица 1. Влияние препарата Novihum на рост винограда сорта Cabernet

| Вариант | Длина побегов 27.05.2015 | PHS | $V_1$ | $V_2$ | Средний урожай 12.10.2015 | | Шкала Эксле 12.10.2015 | |
|---|---|---|---|---|---|---|---|---|
| | см | % | % | % | г/растение | Станд. откл. | Oe | Станд. откл. |
| Контроль | 18,2 | 43,1 | 55,7 | 67,3 | 400 | 240 | 111,5 | 4,5 |
| Novihum | 25,0 | 47,1 | 55,9 | 71,4 | 1519 | 200 | 108,5 | 7 |

Источник: Сборник материалов XII международной научно-практической конференции daRostim2017, Одесский Национальный Университет им. И.И.Мечникова, 2016, с. 167. ISBN 978-617-689-179-6

## Diagnostic Features of Biological Activity of Humic Preparations in a Production Environment on the Example of Lignohumate

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In the work of any responsible industrial company engaged in the production of humic substances and plant growth stimulants, to control the product quality in addition to a wide range of physical and chemical analyses necessary to assess the biological activity of each manufactured batch. For this purpose different methods of bioassay, mainly used as test object plants. However, most of these methods are quite time-consuming, require a lot of time and effort in processing the experience, and not always objective. The specialists of "NPO "RET" to solve this problem based on standard techniques have been developed and widely used modified method for the comparative bioassay, which allows for a fairly short period of time to objectively assess the biological activity of the series and pilot production in terms of production.

The main features of the methodology is:

– the use of seed, characterized by good germination, speed of germination and ease of use in the course of the experiment without pre-soaking;

– the introduction of the rate of growth of absolutely dry biomass of roots and vegetative mass;

– the introduction of a standard reference sample of Lignohumate along with control (water).

This technique has been used successfully as for development, improvement of production technology and to control the current production batch, timing of their storage, as well as testing new grades and compositions on the basis of Lignohumate. Unique results have been obtained using this technique to create new grades of Lignohumate with biologics, allowing for a fairly short period of time to determine the ratio and the effective concentration of the selected components, and further significantly reduce the volume of agricultural trials.

As an example, the following is the result of a comparative bioassay of the compositions of Lignohumate and biostimulant plant growth Biolan, which was installed a stable synergistic effect obtained mixtures, and the optimal concentration and ratio of selected components. The maximum efficiency was shown by a variant with lower concentrations biolana, compared to the concentrations recommended by the manufacturer. Thus, the method allowed for a short period of time to create and offer consumers a new and effective lignohumate-BIO.

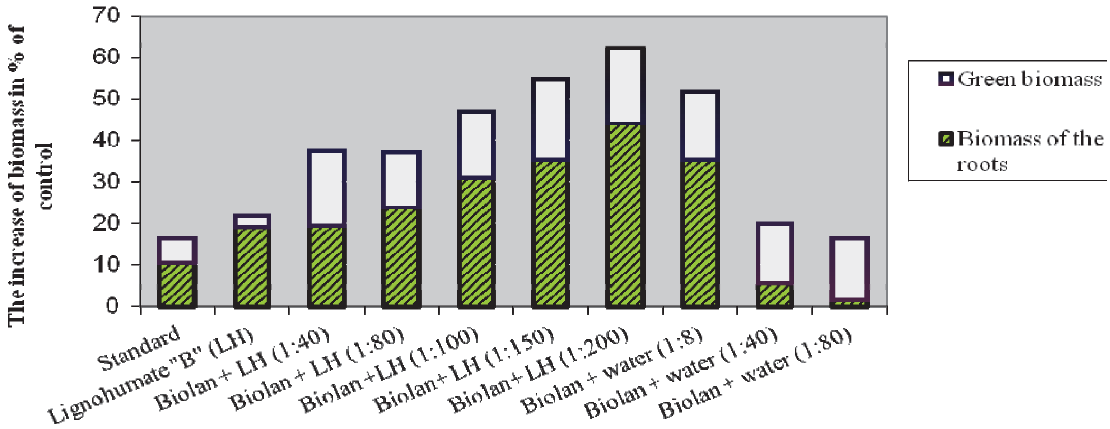


Figure 1. The increase in the biomass of radishes for biotesting compositions treatment with the growth regulator Biolan in comparison with the control

The method of comparative bioassay allows in terms of production:

– to adjust the quality of the products depending on obtained results;

– together with physico-chemical indicators to conduct a comprehensive monitoring of the shipments, and produced grades of Lignohumate as a whole;

– to carry out selection of the NPK components, microelements and biologically active substances (phytohormones, amino acids, growth regulators, biosurfactants etc.) to create effective systems on the basis of Lignohumate.

## The Effect of Humic Substances on Productivity and Quality of Radish Plants

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The aim was to study the influence of complex fertilizers (based on humic substances of Life Force Humic series) on productivity and quality of radish crops (variety Zhara). The substances were added to soil with different contents of mineral fertilizers. The vegetation experiment was carried out in triplicates; the area of a plot was 1 m$^2$; the seeds were sown in line (200 pieces per repetition). The complex fertilizers Humate Balance (HB) and Natural Humic Acids (NHA) were introduced into the soil at doses of 25 g/m$^2$ and 40 g/m$^2$, respectively (according to the information on product labels). The effects of the products included in the soil without fertilizers, with 2/3 of a fertilizer dose (N8 P13,4, K10,7 g/m$^2$) and a full fertilizer dose (N12 P20 K16 g/m$^2$) were compared. The doses were chosen according to the recommendations for growing radishes outdoors in Belarus. Control factors were the following: first, background control of the soil without any fertilizers; second, control of the soil with the application of a full dose of fertilizers (N12 P20 K16 g/m$^2$).

It was found out that the complex fertilizers based on humic acids in the soil which hadn't had any fertilizers before accelerated seed germination. Product HB increases seed germination of radish (compared to the background control and control 2) on average by 36.2% and 33.0% respectively. The use of NHA in conjunction with 2/3 of a dose of fertilizers significantly reduced seed germination (by 21.9% and 36.8%).

The maximum roots quantity to harvest was obtained in the variants with the introduction of the products HB and NHA in the soil which hadn't had any fertilizers before (35.2% and 20.3%, respectively, compared with the control 2); and the variant HB with a full dose of fertilizers, in which the roots quantity is more than in the control 2 by 14.8%. The minimum amount was 44.4% less than in the control 2 (obtained in the embodiment of the NHA with 2/3 dose of fertilizers). In other experimental variants the roots number on average were consistent with the control 2. It is noted that the number of roots (in cases when the product HB was added into the soil) was more than with the introduction of NHA. On the other hand, the analysis of commercial products showed that an average weight of a root is more in the experimental variants with the introduction of NHA into the soil with a full dose of fertilizers than in the options with the both products under consideration with 2/3 doses of fertilizers (by 70.2% and 45.2% and 37.6%).

Taking into account the GOST [All Union State Standard] R 55907-2013 "Radish. Technical conditions", we can say that the root mass was divided into 3 groups: non-standard (with a weight up to 15 g), standard-medium (from 15 g to 30 g) and standard-large (more than 30 g). The introduction of the products with fertilizers in the soil (2/3 of doses) allowed to obtain products with a minimal amount (10.7% and 11.2% respectively) of unconventional roots, compared to 16.2% in the control 2. In the experimental variant with the introduction of HB into the soil without fertilizers 42.9% of the marketable yield (in comparison with 29.1% in the control 2) was obtained. With the highest number of roots obtained this option was the most effective in terms of radish productivity. The greatest number of irregular roots were in variants with the addition of NHA into the soil without fertilizers (37.8%) and HB with the inclusion of a full dose of fertilizers (25.3% compared to control 2).

The addition of humic substances HB and NHA in the soil contributed to the increase of glucose content in the roots by 11.3% and 15.7%, and together with a full dose of fertilizers by 12.6% and 15.5%, respectively (in comparison with the background control); as for the control 2 no significant differences were detected. When the product NV was combined with 2/3 dose of fertilizers, ascorbic acid content increased by 10.2% in comparison with control 1; in other variants of the experiment such contents declined to different degrees comparing to the control 1.

Thus, the introduction of the complex fertilizer Humate Balance in the dose of 25 g/m$^2$ to the soil stimulates germination, increases viability, growth and development of radish plants, which leads to increasing plant productivity and getting a greater number of standard commercial products in comparison with the background control and the control with fertilizers. The addition of the humic substances HB and NHA in the soil increases the content of glucose in the roots by 11.3% and 15.7% in comparison with the background control. The level of accumulation of glucose with the introduction of the humic substances of Life Force series corresponds to its content with the application of a full dose of fertilizers.

# There's Plenty of Room… Between Humic Branches. Nanoparticles Synthesis Using Humic Substances

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Humic substances (HS) are unique multifunctional supramolecular ensembles resulted from postmortal transformations of living organic matter. The variety of functional groups and extreme structural diversity result in multiple useful properties and broad application of HS ranging from soil fertilization and remediation to biomedical technologies. Importantly, HS play a key role in formation and migration of nanoparticles in nature. Humics were also shown to be an effective platform for nanoparticles stabilization and, more recently, *in situ* nanomaterials synthesis.

In this report a number of recent works of our team on preparation of metal and metal-oxide nanoparticles using HS [1-4] are summarized: namely syntheses of various iron (hydr)oxide phases and gold plasmonic nanoparticles in eco-friendly aqueous systems. The revealed effects of humics include:

(i) redox interactions of humic phenolic constituents with metal ions;
(ii) entrapment of solid phase nuclei by HS hyperbranches;
(iii) changing the diffusion rate of ionic species consumed during the particle growth;
(iv) blocking of growing nanocrystal planes resulted in significant size reduction of some iron (hydr)oxide particles (e.g. feroxyhyte [1]);
(v) competitive substitution of low-molecular ionic species adsorbed on nanoparticle surfaces, which drastically changes the particle-particle interactions;
(vi) stabilization of the resulted nanoparticles in both aqueous suspensions and dry powders.

Careful combination of these effects resulted in preparation of valuable products including iron-rich humics-based fertilizers [3], highly stable biocompatible magnetic liquids and unique water-redispersible HS-stabilized gold nanoparticles with bright surface plasmon resonance [4]. Both the formation mechanisms and functional properties of the prepared nanomaterials were comprehensively studied by a powerful combination of instrumental methods including analytical transmission electron microscopy (TEM), Fourier-transform ion cyclotron resonance mass spectrometry (FTICR-MS) [4], Mössbauer spectroscopy of powders and frozen aqueous reaction mixtures [2], fluorescence spectroscopy, etc. We believe that these results open the opportunity for utilization of HS in novel eco-friendly nanotechnology products with a high added value.

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# Influence of Humic Substances Solutions Purification by Dialysis on the Mitotic Index of Apical Root Cells of Peas (*Pisum sativum* L.) and Onion (*Allium cepa* L.)

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Humic substances (HS) – dark-colored natural organic nitrogen-containing amphiphilic randomized redoks-heteropolymers of the arylglycoprotein nature. They are multifunctional polyampholytes too. A biological activity is one important property of HS (Guminski, 1947; Khristeva, 1953; Flaig, 1963; Vaughan, Ord, 1981; Gorovaya et al., 1995; Nardi et al., 2002; and many others). It is determined by: (i) presence the varied functional groups, (ii) colloidal characteristics and (iii) material constitution. The HS biological effect on green vascular plants is connected with that they, penetrating into plants (but unnecessary in cytoplasm), take part in different biochemical and biophysical processes (Popov, 2004).

Most often the biological activity of HS is estimated on the basis of definition the increase of a seedling, length of shoots and roots and also mass of sprouts of plants (Czerniawska-Kusza et al., 2006; Sujetovieny, Griauslyty, 2008; Voronina, 2012). However these methods demand the considerable time and rigorous standardization of conditions of cultivation of sprouts of plants. One of the fast and reproduced biotesting methods physiologically of the active materials is determination of the mitotic index (MI) value of plant cell root apexes (Fiskesjö, 1993; Dmitriyeva et al., 2004).

This work was devoted the assessment the biological activity of HS solutions which are isolated from the humus horizons of different soils. The biological activity of neutralized pyrophosphate solutions of HS and the same solutions that had been purified by dialysis was compared.

As an object of a research the HS solutions were used which were isolated from the humus horizons of Chernic Chernozems (State Nature Reserve “Belogorye”, Belgorod region) and its arable analog (Belgorod region, Mirotorg Ltd), of middle loamy Greyic Phaeozems (State Nature Reserve “Belogorye”, Belgorod region), and also of buried Chernozem and background hard loamy Greyic Phaeozems (Scythian fortification, urban-type settlement of Borisovka, Belgorod region).

The biological activity of HS solutions was estimated from the value of the mitotic index (MI) of root apical cells both peas (*Pisum sativum* L.) and onion (*Allium cepa* L.). Humic substances were isolated by alkaline pyrophosphate solutions (Kononova, Belchikova, 1961), after which solutions were neutralized (before pH ~ 7). One part of solutions was left without change, the second part of solutions before test was purified from mineral salts by dialysis through a cellophane membrane.

The biological activity of neutralized pyrophosphate HS solutions and the same solutions that were purified by dialysis was compared. In the course of the work, it was found that neutralized pyrophosphate extracts of humic substances of optimal concentration (0.001 mg C/ml) from all horizons of investigated soils and their dialyzed solutions, much increased the mitotic index in the root apical cells of onions (*Allium cepa* L.), and peas (*Pisum sativum* L.).

It was revealed that the dialysis of neutralized pyrophosphate extracts of HS had no significant effect on the size of the mitotic index of the apical root cells either in the case of onions or in the case of peas. The values of the mitotic index of apical root pea cells were larger than those of onions: 1.4–3.9 and 1.1–3.2 times higher than controls, so. The generalized index of Harrington’s desirability function demonstrated that the biological effect of HS isolated from all horizons was same.

## Benefits of Humic Products Compared to Biochar in Heavy Metal Polluted Soil Restoration

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Trace metal pollution of soils is increasingly exacerbated all over the world leading to ecosystems, water bodies, food safety and human health being threatened. *In situ* remediation techniques are of growing interest because they proved to be a promising low-cost alternative for landfilling and have been tested in pilot and full-scale field studies. Considerable attention has been paid to waste management option, and several materials have been proposed for soil reclamation, such as biochar, or variety humic products.

We conducted a greenhouse pot experiment with 0.5, 5% biochar, and 0.5, 1% humic product from lignosulfonate to compare their ability in creating favorable soil conditions in multy-polluted soils. In particular, we assessed their effects during 60-days on Zn, Cu, Cd and Pb mobility, enzyme activities influence on C, N, P, microbial biomass carbon, and ecotoxicity (*Eisenia foetida*, *Daphnia magna, Sinapis alba*). The soil used in this study was collected from the alluvium of the Litavka River in the village of Trhové Dušníky (60 km south of Prague). This area is characterized by exacerbating content of heavy-metals as a consequence of mining and smelting activities. The content of heavy metals in soil before study were: $Zn_{tot}$ 7595.65±194.76 ppm, $Cd_{tot}$ 80.82±4.58 ppm, $Cu_{tot}$ 62.51±6.45 ppm, $Pb_{tot}$ 4346.13±140.08 ppm.

Two materials, namely biochar (BC) derived from wood chips gasification (150 kW/h gas and 300 kW/h heat production) at the temperature range 700 - 900 °C, and commercial potassium humic product Lignohumate (Amagro, Czech Republic) produced by alkaline extraction from lignin (HP) were used as soil amendments in this study.

A strong effect of BC, and HP treatments on soil characteristics was revealed. All treatments induced liming effect (pH increased from 6.0 to 6.5 and 7.0 according to elevating dose) and changed in heavy metals mobility. Briefly, strong Cd immobilization was found only at BC 5 and HP 1 treatments while there was no change at smaller doses. The HP treatments were less effective in decreasing the Zn mobility compared to BC treatments, and both amendments markedly reduce Cu mobility.

Carbon dioxide production in the samples without the glucose additive (Basal respiration, BR) greatly varied across the treatments and had significant differences over the control. BC 0.5 increased BR during the first 30 days of incubation, but a higher dose (BC 5.0) significantly diminished BR even after 60d. HP considerably increased BR during the first 30d. The glucose induced respiration data testified to the stimulatory effects of HP and the inhibitory effect of BC. As a result, $qCO_2$ in BC treatments was increased by 16-42% over control, but in HP were characterized by the lowest metabolic quotient in compare to control. These results are reflected on soil enzymes activities.

BC positively affected urease, and β-glucosidase activities in compare with non-amended soil, and depressed acid phosphatase. The results of the experiments revealed a stimulating effect of HP on the enzymes activity, which is not surprising if we consider its positive influence on microbial populations.

A battery of simple, rapid and cost-effective soil and aquatic bioassays was performed for evaluating possible short-term soil remediation effects from amendments. At HP we detected significantly stimulated effect on plant growth (*Sinapis alba*) as compared to the NA soil and BC. The BC 0.5 and HP 0.5 treatments showed a more than 10% decrease in earthworm body weight, indicating that these soils are not supportive of normal earthworm growth. Mortality of adult earthworms during the exposure period was negligible in HP 0.5, 1 and BC 0.5 treatments in comparison with an artificial control (OECD soil), but we detected 100% avoidance of earthworms in BC 5 treatments.

Additionally, BC and HP significantly decreased the toxicity of leachates to *D. magna* to the low-toxic levels from high-toxic (NA).

The generalized data for microbiology and toxicology responses in presence of HP, and BC from this study are shown in the Table 1

Table 1. Summary table of effects observed in each biological parameter in soils with a presence of amendments

| Parameter | BC | | HP | |
|---|---|---|---|---|
| | 0.5 | 5 | 0.5 | 1 |
| *Sinapis alba*, root lenght | ▲ | ▲ | ▲ | ▲ |
| *Daphnia magna*, toxicity | ● | ▼ | ● | ● |
| Basal respiration | ● | ▼ | ● | ● |
| Microbial biomass carbon, $C_{mic}$ | $\underline{\blacktriangledown}$ | $\underline{\blacktriangledown}$ | ▲ | ▲ |
| Microbial quotient, $qCO_2$ | $\underline{\blacktriangle}$ | ● | ▼ | ▼ |
| Dehydrogenase activity | ● | ● | ▲ | ▲ |
| β-glucosidase activity | ▲ | ● | ▲ | ▲ |
| Acid phosphatase activity | $\underline{\blacktriangledown}$ | $\underline{\blacktriangledown}$ | ▲ | ▲ |
| Urease activity | ● | ▲ | ▲ | ▲ |
| FDA activity | ● | ● | ▲ | ▲ |
| *Eisenia foetida*, body weight | ▼ | n.d. | ▼ | ▲ |
| *Eisenia foetida,* No living earthworms | ● | $\underline{\blacktriangledown}$ | ● | ● |
| *Eisenia foetida,* No cocoons | ▲ | n.d | ▲ | ▲ |

Symbols ▲and ▼ stand for a corresponding decrease or increase of the parameter relatively to NA soil. Symbol ● is correspondingto zero-effect. Underlined symbols (__) represent changes in parameters that are considered deleterious. n.d. – not detected.

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Adding all together, results suggest that only HP 1 promoted good microbial performance and created suitable conditions to the organisms assayed (at least has no harmful affect). This is an advantage that cannot be overlooked or dismissed and brings the added value for sustainability and ecologically friendly management options.

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## Role of Fertilizer Products "LIFE FORCE®" in Stimulation of Productional Processes of Wheat and Maize Plantlets

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The role of humic acids in improvement of soil fertility is well-known. Fertilizers products "LIFE FORCE®" promote improvement of physical and chemical properties of the soil, activity of different groups of microorganisms, and as result, stimulate seeds germination, increase absorption of nutrients and activate plant growth.

The purpose of our researches was studying the use of fertilizers Life Force® Natural Humic Acids and Life Force® Humate Balance in stimulation of productional processes of wheat and maize plants.

The researches were made in laboratory of biotechnological researches of agriculture, agrochemistry and ecology department of Belgorod state agricultural university. The subjects of research were the wheat sort of Belgorod-16 and maize sort of Lakomka Belogorya, seed's viability and germinating energy, plantlet germination and growth dynamics. We have put the following variants of vegetative experiences in containers: variant 1 – the soil + fertilizer Life Force® Natural Humic Acids; variant 2 – the soil + Life Force® Natural Humic Acids + fertilizer Life Force® Humate Balance and variant 3 – control without humic fertilizers. The fertilizer was used according to the instruction.

During experience it has been found out that biological preparation increased laboratory viability and germinating energy of seeds on average per 4% and 30% according to control. Plantlet germination and accumulation of plants vegetative mass also were maximum in experiment variants. The most large increase of plant general vegetative mass of wheat was with the use of fertilizer Life Force® Natural Humic Acids, then with additional using of Life Force® Humate Balance and exceeded control variant on average per 22% and 15% in accordance ($p<0.05$).

There was found very high responsiveness on humic fertilizers products "Life Force®" in maize plant. The highest augmentation of vegetative herbage of maize plant is noted in combined using Life Force® Natural Humic Acids and Humate Balance. It exceeded control variant on average per 70% ($p<0.01$). The augmentation of general vegetative mass of maize plant with the use of fertilizer Life Force® Natural Humic Acids was higher control variant on average per 16% ($p<0.05$).

The important parameter which characterizes plants growth and development is the maintenance of chlorophyll. The maintenance of a chlorophyll in terms of the vegetative mass of wheat and maize plant was higher on average per 6 – 12% as against to control variant. Increase level of a green pigment says about the better development of the photosynthesize device of plants organism that promotes higher productivity and yield. The diagram figure 1 shows the dynamics of wheat and maize plants growth in experiment and control variants.

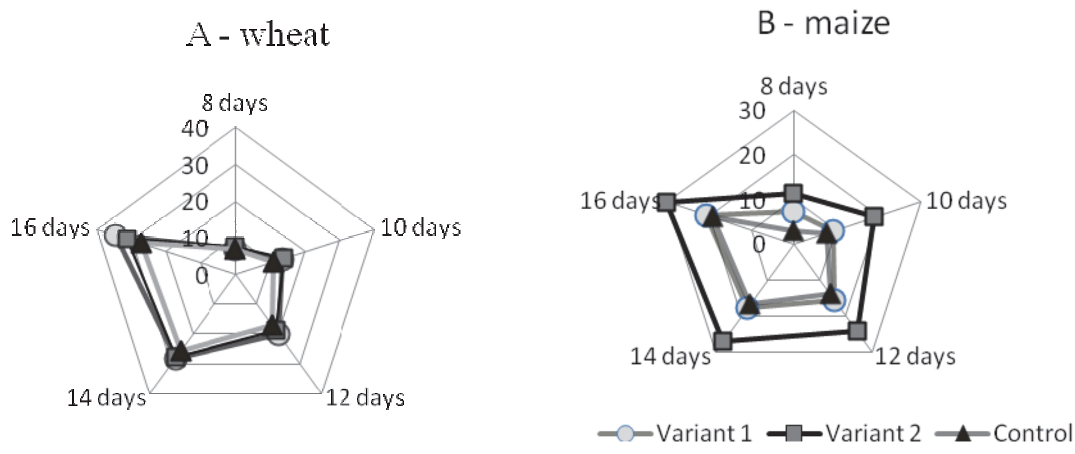


Figure 1. Dynamics of height (sm) of wheat (A) and maize (B) plants with the use of fertilizers: variant 1 – Life Force® Natural Humic Acids; variant 2 – Life Force® Natural Humic Acids and Humate Balance in comparison with control

The biometric characteristic of wheat plants shows that in the first and in the second experience variants the plant height exceeds control variant on average per 26% and 14% in accordance. The height of maize plants in the first and in the second experience variants is more on average per 8% and 38% in accordance.

The disperse data analysis, calculations of trend lines and regression equation of dynamics plants height were made.

Equation trend line of dynamics plants height of wheat plant is: for the first variant: $y=6.8784x$; for the second variant: $y=6.5273x$ and for control variant: $y=5.8273x$.

Equation trend line of dynamics plants height of maize plant is: for the first variant: $y=4.5215x$; for the second variant: $y=9.9173\ln(x) +11.916$ and for control variant: $y=4.0513x$.

For all variants the approximation coefficient R2 is within data 0.8816 – 0.9979 that corresponds to the steady increasing speed of growth plant. At the same time the coefficients (k) value is higher in the regression equations of experimental variants that demonstrates more considerable strengthening of plants growth and positive nutrient uptake from the soil under the influence of humic fertilizers.

Now therefore, fertilizers products "LIFE FORCE®" considerably stimulate productional processes of wheat and maize plantlet that will promote increase of crop yield.

## The Use of Lignohumates to Reduce Rates of Chemical Plant Protection Products

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Humic–based amendments are being increasingly used in agriculture. Growing population requires increasing food productivity especially rice since it of staple food for most people in the world. Bali (Indonesia) is a typical Asian rice growing region, but most of the paddy field is a shortage of organic matter so that the productivity of land for rice is very low. The first research objective was to determine the effect of commercial humic product Lignohumate AM® (LH) plant growth and rice yield at the farm located in Bali, since LH is a humic product, which could be a source of nutrients and biostimulant for rice growth. Besides, substantial amounts of chemicals for plant protection are used for rice cultivation on Bali. All of these plant protection products are usually toxic and can negatively influence grain quality. The second research objective was to investigate effect of LH in combination with insecticide on grain quality of rice, to reveal possible synergistic effects and to assess the possibility to decrease rates of chemical plant protection products.

Field trial was laid out in complete randomized design using traditional wet rice (*Oryza sativa* L., HYV Cigeulis) cultivation. Treatments included insecticidal sprays with beta-cyfluthrin at 100, 50 and 0% of recommended application rates, either alone or in combination with LH. LH was applied for seed pre-treatment before sowing (soaking in 0.5% LH solution) and as foliar spray (0.05%) together with insecticide. Plant height, leaf area, tiller and leaf number per hill, chlorophyll content in leaves, grain fresh weight and oven weight, 1000-grain dry and oven weights, numbers of seeds and panicles per hill were recorded. The levels of ash, protein, lipids, total carbohydrate and amylase in grain were determined. The data were variant analysed.

No significant effects on plant height, number of tillers, leaf number, leaf area or rice yield were recorded. LH stimulated root growth at early stages of plant development, and increased 1000-grain weight and number of grains per hill.

LH application promoted chlorophyll synthesis in leaves: treatment with LH and half-dose insecticide provided the highest content of chlorophyll. LH promoted also an increase in carbohydrate levels and amylose content in rice grain.

The experiment showed that when using Lignohumate, rice can be cultivated at significantly lower rates of pesticides than they are currently used.

Further experiments with LH, focused on the optimization of its application and considering local climate conditions and agricultural practices are worth conducting.

## Effect of Combined Application Humic and Selenium Fertilizers on Yield and Quality of Spring Wheat

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The most important issue in agricultural production is obtaining high good quality yields of grain. Application of micronutrients is one of the most perspective aspects of modern agricultural production.

Humic fertilizers can improve soil properties. These substances can react with the microorganisms in the soil and stimulate their reproduction.

Humic fertilizer not only increases the number of microorganisms, but also can increase the activity of the trace elements. Nitrogen and phosphorus become more available to plants with applying humic fertilizers. Minerals become more available for absorption form. In addition, these fertilizers is well absorbed with mineral and organic substances, reinforcement each other.

Selenium is also very important elements for plant. It is responsible for the formation of chlorophyll, synthesis of citric acid and metabolism of long chain fatty acids. It can also reduce the harmful impact of stress in the growth phase of plants.

To solve these issues, a series of experiments was engaged to study the effect of selenium and humic fertilizers on yield of spring wheat. In field and pot experiments research the effect of humic fertilizers and different methods of application of sodium Selenite. Not only productivity of plants was analyzed, also the plants structure was analyzed. Besides, selenium and humic fertilizers influence is researched under joint application for main quality characteristics of sping wheat main and by-product. The following humic fertilizers are researched: Life force Natural humic acids and Humate Life force balance, which were applied to the soil in recommended calculated amounts of Humate Life force balance 3 kg/100m$^2$, Life force Natural humic acid 4 kg/100m$^2$. Sodium Selenite was applied by pre-sowing seed treatment, foliar spraying and soil application.

## Humic Acids in Commercial Humic Products: Do They Reflect Organic Matter Genesis?

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Commercial humic products (CHP) are produced by manufactures from various organic raw materials, predominantly from lignite and peat. Humic acids (HA), which are considered to be the active ingredient in CHP, are different in chemical structure and, consequently, in their efficiency as plant growth promotors and soil conditioners, depending on organic matter genesis. The objective of this study was to determine properties of HA, extracted from lignite- and peat-derived CHP and to compare them with HA, extracted from corresponding raw materials in order: (i) to evaluate peculiarities of HA being formed in different environments and (ii) to reveal differences in HA composition occurring during the technological processes of manufacturing of CHP from raw material.

Materials and Methods. For lignite-derived CHP the raw materials from two deposits were analyzed: fossil coal with high degree of coalification (L1) and less coalified subbituminous coal (L2). Corresponding CHP are soluble potassium humates (HL1 and HL2) with pH 10 and 32-35% of ash. For peat-derived CHP (liquid potassium humate, HP1) low land peat was used. HA were extracted from raw materials and corresponding CHP and analyzed for elemental content, functional groups, carbohydrates and amino acids content, IR- and $^{13}$C-NMR spectra.

Results. Elemental content of HA extracted from lignites and corresponding CHPs is almost identical, but there are certain differences between L1- and L2-originated humics (Table 1). Both L1 and HL1 have the highest C content and H/C ratio with low O/C ratio, and high hydrophobicity, whereas HA from less coalified L2 and corresponding HL2 contain less C and more O. These facts, also supported by functional group analyses, IR and $^{13}$C-NMR data, prove higher condensation degree of HA molecule of fossil coal. Among carbohydrates and amino acid moieties, in HA from L1 and HL1 hexoses contribute 40% to the sum of sugars, pentoses and methylpentoses achieve 28-32% each. In L2-originated HA pentoses prevail.

Table 1. Elemental content and atomic ratio, sum of carbohydrates and amino acids of HA extracted from CHP and corresponding raw materials (wt% ash-free)

| Sample | C | H | N | H/C | C/N | O/C | Sugars | AA* |
|---|---|---|---|---|---|---|---|---|
| L1 | 63.1 | 5.1 | 1.6 | 1.10 | 47 | 0.35 | 11.0 | 8.8 |
| HL1 | 63.1 | 5.1 | 1.5 | 1.12 | 48 | 0.35 | 10.9 | 8.1 |
| L2 | 57.5 | 5.3 | 1.2 | 0.96 | 55 | 0.46 | 10.4 | 9.6 |
| HL2 | 57.6 | 5.4 | 1.3 | 0.96 | 51 | 0.46 | 10.4 | 9.3 |
| HP1 | 50.1 | 3.7 | 4.1 | 0.88 | 14 | 0.63 | 20.2 | 11.0 |

*AA amino acids

Unlike lignite-originated HA, the one from peat-derived CHP demonstrated the lowest C and highest N content, was enriched with acid functional groups and low molecular weight compounds (amino acids and carbohydrates).

Conclusions. Composition of lignite-derived HA and from corresponding CHP are almost identical, which indicates a weak transformation of organic matter in the technological process of manufacturing. Organic matter genesis is the key factor influencing composition and structure of HA in CHP. HA from fossil coal are more aromatic, hydrophobic, contain fewer functional groups and mobile low-molecular components comparably to the one from subbituminous coal. Peat-derived HA are the most aliphatic, hydrophilic and enriched with amino acids and carbohydrates.

# Testing a New Organic-Mineral Fertilizer with Restoreneuralnet "ARKSOIL" on Vegetable Cultures in the Conditions of the Rostov Region

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**Summary.** Vegetable growing is one of the main and most labor-intensive branches of agricultural production. To increase the yield of vegetable crops play an important role fertilizers. When growing vegetable crops of great importance is the application of new fertilizer, which contains not only macro - and microelements, but also having in its composition vitamins, amino acids and other biologically active substances with growth stimulating properties. These special fertilizers include “Arksoil”. Organic fertilizer is available in several formulations (CNE concentrate nano-emulsion, CSC – concentrate of colloidal solution, SP – wettable powder) and can be applied on some crops grown by farmers throughout the Russian Federation.

**Introduction.** Testing a new organic fertilizer “Arksoil”. in the forms of the CCS and the CNE was carried out in 2016 to Baraccudas vegetable breeding experimental station of all-Russian research Institute of vegetable growing (FGBNU "Baraccuda the OCOÑA VNIIO") in the climatic conditions of the Rostov region.

“Arksoil”. CNE (nanoemulsion concentrate) organic-mineral fertilizer with 40% of Lignohumate potassium, contains a set of macro - and microelements necessary for the growth and development of plants. It also contains Association inactivated by soil bacteria: *Pseudomonas aureofaciens* H 16 (3-indoleacetic acid, α-alanine, α-glutamic acid), *Bacillus megaterium* (poly-beta-hydroxybutyric acid), *Bacillus atrophaeus* (auxins). “Arksoil” CSC (colloid solution concentrate) – contains only mineral salts.

**The treatment effect "Arcola" on tomato plants non seedlings of the cultivar "Jane" F1 hybrid.** In 2016, the spring field works started at the optimum time. Foliar treatment of plants organic-mineral fertilizers “Arksoil”. CSC and the CNE on the background of mineral fertilizers N60 P60 K90 under drip irrigation had a significant effect on the weight characteristics of non seedlings of tomato fruits (Table 1).

Table 1. Weight characteristics of tomato fruits per plant depending on processing Arcola

| Option | Productivity of plants, g | Average fruit weight, g | The average weight of fruits per plant, g | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | the Central escape | on lateral shoots | | | | | | |
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| NPK-background | 2216,2 | 126,6 | 141,6 | 136,2 | 152,5 | 118,9 | 127,5 | 100,0 | 94,3 | 110,0 |
| Background+CSC | 2348,8 | 112,9 | 129,2 | 107,5 | 129,2 | 107,0 | 120,0 | 125,7 | 96,7 | 67,8 |
| Background+CNE | 2591,3 | 109,3 | 115,0 | 102,5 | 134,5 | 99,2 | 132,7 | 118,9 | 100,0 | 62,2 |

Apply fertilizer in foliar feeding during the growing season, the phases of growth and development of tomato plants has contributed to increased productivity of the Bush.

The highest productivity has provided a three-fold treatment of plants “Arksoil” CNE in the early phase of budding, beginning of flowering 1-2 and 3-4 of flower brushes, and made 2591,3 G. it Should be noted that “Arksoil”. CSC acted similarly, but to a lesser extent. The control on the background of fertilizers the average productivity of tomato is made up 2216,2 with Bush.

Table 2 presents information on the amount and quality of marketable and early yield of fruits of tomato depending on the processing plants “Arksoil” CSC and CNE during the growing season.

Table 2. Marketable and early yields, fruit quality of tomato nonseedlings

| Option | Marketable yield, t/ha | Increase to control | | Dry matter, % | Nitrates, mg/kg wet weight | The number of Mature fruits on the 20th day from the beginning of maturation, % |
|---|---|---|---|---|---|---|
| | | t/ha | % | | | |
| NPK-background | 68,74 | 0 | 0 | 5,96 | 46 | 72,9 |
| Background+CSC | 72,87 | +4,13 | +6,0 | 6,30 | 34 | 72,2 |
| Background+CNE | 75,51 | +6,77 | +9,8 | 6,37 | 32 | 69,4 |

Affected application “Arksoil” and average weight of fruit per plant. At three times the application “Arksoil” decreased the average fruit weight per plant. It mostly manifested itself in the option of using “Arksoil” CNE (109,3 g) for the greatest number of the ensuing fruits per plant. On the variant with application of “Arksoil” CSC average fruit weight was at 112,9 g. Similar results for the average weight of the fetus obtained on the shoots with drip irrigation on the background of fertilizers.

Application “Arksoil” in compliance with the relevant requirements of plants agro-technical measures allows to significantly increase the amount of marketable fruit yield of tomato nonseedlings culture in comparison with the background. Marketable yield increased with 68,74 t/ha to 75,51 t/ha in the variant with the use of “Arksoil” CNE

However, early yield (harvest August 22) in relation to a General commodity has decreased from 72.9% to 69.4%. Some are less apparent effect of “Arksoil” CSC in increasing marketable yield of tomato fruits nonseedlings. Assessed fruits of tomato on dry matter content and nitrate showed that the use of “Arksoil”. CSC and CNE dry matter increased from 5.96 to 6.30-6,37%, and the nitrates decreased slightly from 46 to 32-34 mg/kg wet weight, with MPC 150 mg/kg.

**The treatment effect “Arksoil” on plants of sweet pepper hybrid F1 variety "Temp".** Table 3 presents the results of assessing the impact “Arksoil” CSC on the processes of growth and development of sweet pepper.

Table 3. Biometric indicators of plants of sweet pepper depending on the level of nutrients before harvesting.

| Option | The height of the main stem, cm | The number of fruits per plant, units | The size of the fetus, cm | | | Average fruit weight,g | The productivity of plants, g |
|---|---|---|---|---|---|---|---|
| | | | length | diameter | the thickness of the pulp | | |
| NPK-background | 83,8 | 12,3 | 10,02 | 7,44 | 0,595 | 81,17 | 994,43 |
| Background+CSC | 84,7 | 12,7 | 10,26 | 7,44 | 0,610 | 84,83 | 1081,70 |

Foliar nutrition solution “Arksoil” CSC amid N120 P120 K120 under drip irrigation increased height of main stem, number of fruits per plant of pepper the size and average fruit weight in comparison with the background. Affected by the use of “Arksoil” CSC and the productivity of the plant, where it reached 1081,7 G.

In table 4 the data show a fairly high efficiency of “Arksoil” CSC on the culture of sweet pepper in the conditions of Rostov region.

Table 4. The yield and fruit quality of sweet pepper depending on processing plants “Arksoil” CSC

| Option | The total yield,t/ha | Increase | | Standard fruit,% | Patients Alternaria fruit,% | Dry substance, % | Nitrates, mg/kg wet weight |
|---|---|---|---|---|---|---|---|
| | | t/ha | % | | | | |
| NPK-background | 47,01 | 0 | 0 | 92,4 | 2,14 | 6,5 | 50,5 |
| Background+CSC | 54,54 | 7,53 | 16,0 | 90,9 | 1,22 | 6,6 | 38,8 |

Increase the total yield 7.53 t/ha (16,0%) with standard fruits of sweet pepper of 90.9%. The number of patients with Alternaria fruit made up 1.22%. It is almost two times lower than the background N120 P120 K120 (control).

Conducted laboratory analysis of the content in fruits of sweet pepper solids and nitrate showed that the action “Arksoil” CSC was an increase in dry matter and reducing nitrate to 38.8 mg/kg wet weight, with the MPC of 200 mg/kg.

Thus, the result of vegetation tests showed a positive effect of application of biofertilizers “Arksoil” on tomato plants and sweet pepper. It is the increase in productivity, increase in commercial quality of the fruit, reduces the incidence of early blight.

**Insights**

1. The use of bio-fertilizers “Arksoil” in the form of the CSC and the CNE during the growing season increases marketable yield fruit nonseedlings culture of tomato with 68,74 t/ha to 72,87-75,51 t/ha in comparison with the background (control).

2. Processing plants of sweet pepper solution “Arksoil” CSC gives the increase in the total yield of fruit – of 7.53 t/ha with the standard of 90.9 %.

3. The number of patients with Alternaria fruit decreased by almost half from 2.14 % in the control to 1.22 %.

4. Spraying of plants “Arksoil” on the background of mineral fertilizers under drip irrigation, optimum plant density, provide the best fruits set has been registered, the highest productivity of plants, increase the dimension of the fruit of non seedlings of tomato and sweet pepper seedlings compared to control.

5. In fruits of tomato and sweet pepper from the use of “Arksoil” dry matter increase and the amount of nitrates is reduced.

## Испытания нового органоминерального удобрения с ростостимулирующейактивностью «Арксойл» на овощных культурах в условиях ростовской области

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**Резюме.** Овощеводство является одной из основных и наиболее трудоемких отраслей сельскохозяйственного производства. В повышении урожайности овощных культур важная роль принадлежит удобрениям. При выращивании овощных культур большое значение занимает применение новых удобрений, содержащих не только макро- и микроэлементы, но и имеющих в своем составе витамины, аминокислоты и другие биологически активные вещества, обладающие ростостимулирующими свойствами. К таким специальным удобрениям относится «Арксойл». Органоминеральное удобрение выпускается в нескольких препаративных формах (КНЭ – концентрат наноэмульсии, ККР – концентрат коллоидного раствора, СП – смачивающийся порошок) и может быть применён на ряде культур выращиваемых сельхозпроизводителями по всей территории Российской Федерации.

**Введение.** Испытания нового органоминерального удобрения «Арксойл» в формах ККР и КНЭ проводили в 2016 году на Бирючекутской овощной селекционной опытной станции Всероссийского научно-исследовательского института овощеводства (ФГБНУ «Бирючекутская ОСОС ВНИИО») в климатических условиях Ростовской области.

«Арксойл КНЭ» (концентрат наноэмульсии) органоминеральное удобрение с 40% лигногумата калия, содержит набор макро- и микроэлементов, необходимых для роста и развития растений. В нём также содержатся ассоциации инактивированных почвенных бактерий: *Pseudomonas aureofaciens* Н 16 (3-индолилуксусная кислота, α-аланин, α-глутаминовая кислота), *Bacillus megaterium* (поли-бета-гидроксимасляная кислота), *Bacillus atrophaeus* (ауксины). «Арксойл ККР» (концентрат коллоидного раствора) – содержит только минеральные соли.

**Влияние обработки удобрением «Арксойл» на растения томата безрассадного сорта «Джейн» гибрид F1.** В 2016 году весенне-полевые работы начались в оптимальные сроки. Листовые обработки растений органоминеральными удобрениями «Арксойл ККР» и «Арксойл КНЭ» на фоне внесенных минеральных удобрений N60 P60 K90 при капельном орошении оказали существенное влияние на весовую характеристику плодов безрассадного томата (таблица 1).

Таблица 1. Весовая характеристика плодов томата на растении в зависимости от обработки «Арксойл»

| Вариант | Продук-тивность растения, г | Средняя масса плода, г | Средняя масса плодов на растении, г | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | на централь-ном побеге | на боковых побегах | | | | | | |
| | | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| NPK-фон | 2216,2 | 126,6 | 141,6 | 136,2 | 152,5 | 118,9 | 127,5 | 100,0 | 94,3 | 110,0 |
| Фон+ККР | 2348,8 | 112,9 | 129,2 | 107,5 | 129,2 | 107,0 | 120,0 | 125,7 | 96,7 | 67,8 |
| Фон+КНЭ | 2591,3 | 109,3 | 115,0 | 102,5 | 134,5 | 99,2 | 132,7 | 118,9 | 100,0 | 62,2 |

Применяемые удобрения при листовой подкормке в течение вегетации по фазам роста и развития растений томата способствовали повышению продуктивности куста.

Наибольшую продуктивность обеспечила трёхкратная обработка растений «Арксойл КНЭ» в фазе начала бутонизации, начала цветения 1-2 и 3-4 цветочных кистей, и составила 2591,3 г. Следует отметить, что «Арксойл ККР» действовал аналогично, но в меньшей степени. В контроле на фоне удобрений средняя продуктивность томата составила 2216,2 г. с куста.

В таблице 2 представлена информация о величине и качестве товарной и ранней урожайности плодов томата в зависимости от обработки растений «Арксойл ККР» и «Арксойл КНЭ» в течение вегетации.

Таблица 2. Товарная и ранняя урожайность, качество плодов безрассадного томата

| Вариант | Товарный урожай, т/га | Прибавка к контролю | | Сухое вещество, % | Нитраты, мг/кг сырой массы | Количество зрелых плодов на 20-е сутки от начала созревания, % |
|---|---|---|---|---|---|---|
| | | т/га | % | | | |
| NPK-фон | 68,74 | 0 | 0 | 5,96 | 46 | 72,9 |
| Фон+ККР | 72,87 | +4,13 | +6,0 | 6,30 | 34 | 72,2 |
| Фон+КНЭ | 75,51 | +6,77 | +9,8 | 6,37 | 32 | 69,4 |

Сказалось применение «Арксойл» и на средней массе плода на растении. При трёхкратном применении «Арксойл» снизилась средняя масса плода на растении. В наибольшей степени это проявилось в варианте с использованием «Арксойл КНЭ» (109,3г) из-за наибольшего числа завязавшихся плодов на растении. На варианте с применением «Арксойл ККР» средняя масса плода была на уровне 112,9 г. Аналогичные результаты по средней массе плода получены и на побегах при капельном орошении на данном фоне удобрений.

Применение «Арксойл» при соблюдении соответствующих требованиям растений агротехнических мероприятий позволяет заметно увеличить величину товарного урожая плодов томата безрассадной культуры в сравнении с фоном. Товарная урожайность возросла с 68,74 т/га до 75,51 т/га в варианте с использованием «Арксойл КНЭ».

При этом ранняя урожайность (сбор 22 августа) по отношению к общей товарной снизилась с 72,9% до 69,4%. Несколько в меньшей степени проявился эффект от действия «Арксойл ККР» в увеличении товарной урожайности плодов безрассадного томата. Проведенная оценка плодов томата на содержание сухих веществ и нитратов показала, что от применения «Арксойл ККР» и «Арксойл КНЭ» сухие вещества повысились с 5,96 до 6,30-6,37%, а нитраты незначительно снизились с 46 до 32-34 мг/кг сырой массы, при ПДК 150 мг/кг.

**Влияние обработки удобрением «Арксойл» на растения перца сладкого – гибрид F1 сорт «Темп».** В таблице 3 представлены результаты оценки влияния «Арксойл ККР» на процессы роста и развития перца сладкого.

Таблица 3. Биометрические показатели растения перца сладкого в зависимости от уровня питательных элементов перед уборкой.

| Вариант | Высота главного стебля, см | Число плодов на растении, шт. | Размер плода, см | | | Средняя масса плода, г | Продуктив-ность растения, г |
|---|---|---|---|---|---|---|---|
| | | | длина | диаметр | толщина мякоти | | |
| NPK-фон | 83,8 | 12,3 | 10,02 | 7,44 | 0,595 | 81,17 | 994,43 |
| Фон+ККР | 84,7 | 12,7 | 10,26 | 7,44 | 0,610 | 84,83 | 1081,70 |

Листовая подкормка раствором «Арксойл» ККР на фоне N120 P120 K120 при капельном орошении увеличила высоту главного стебля, число плодов на растении перца, размеры и среднюю массу плодов по сравнению с фоном. Сказалось применение «Арксойл ККР» и на продуктивности растения, где она достигла 1081,7 г.

Приведенные в таблице 4 данные свидетельствуют о достаточно высокой эффективности применения «Арксойл» ККР на культуре перца сладкого в условиях Ростовской области.

Таблица 4. Урожайность и качество плодов перца сладкого в зависимости от обработки растений «Арксойл ККР»

| Вариант | Урожайность общая,т/га | Прибавка | | Стандартные плоды,% | Больные альтернарией плоды,% | Сухое вещество, % | Нитраты, мг/кг сырой массы |
|---|---|---|---|---|---|---|---|
| | | т/га | % | | | | |
| NPK-фон | 47,01 | 0 | 0 | 92,4 | 2,14 | 6,5 | 50,5 |
| Фон+ККР | 54,54 | 7,53 | 16,0 | 90,9 | 1,22 | 6,6 | 38,8 |

Прибавка общей урожайности составила 7,53 т/га (16,0%) при стандартности плодов перца сладкого 90,9%. Количество больных альтернариозом плодов составило 1,22%. Это практически в два раза ниже, чем на фоне N120 P120 K120 (контроль).

Проведенный лабораторный анализ содержания в плодах перца сладкого сухих веществ и нитратов показал, что от действия «Арксойл ККР» наблюдалось повышение сухих веществ и снижение нитратов до 38,8 мг/кг сырой массы, при ПДК 200 мг/кг.

Таким образом, результат вегетационных испытаний показали положительный эффект от применения органоминерального удобрения «Арксойл» на растения томата и перца сладкого. Он заключается в увеличение урожайности, увеличение товарного качества плодов, уменьшает заболеваемость альтернариозом.

**Выводы**

1. Применение органоминерального удобрения «Арксойл» в форме ККР и КНЭ в течение вегетации повышает товарную урожайность плодов безрассадной культуры томата с 68,74 т/га до 72,87-75,51 т/га в сравнении с фоном (контроль).
2. Обработка растений перца сладкого раствором «Арксойл ККР» дает прирост общей урожайности плодов – 7,53 т/га, при стандартности 90,9 %.
3. Количество больных альтернариозом плодов снизилось практически вдвое с 2,14 % в контроле до 1,22 %.
4. Опрыскивание растений «Арксойл» на фоне внесения минеральных удобрений, при капельном поливе с оптимальной густотой стояния, обеспечивают лучшую завязываемость плодов, наибольшую продуктивность растения, повышают размерность плодов безрассадного томата и рассадного перца сладкого по сравнению с контролем.
5. В плодах томата и перца сладкого от применения «Арксойл» сухие вещества повышаются, а количество нитратов снижается.

## Universal Organic Fertilizers "EldORost"

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In the modern world it is increasingly arises the question about the ecological purity of products of plant origin. Pesticides, nitrates and other compounds harmful to the human body have become the norm, and each year their content in vegetables, fruits and cereals only increases. This occurs due to excessive application of mineral fertilizers and protection against pests with the artificial nature of origin. An alternative to the dominance of the chemicals are organic fertilizers that have been used by humans throughout the history of agriculture. They provide the restoration of the soil balance naturally, they have in their composition the entire spectrum of substances necessary for proper growth and development of plants. So, if there is not enough humus in the soil, most of the mineral fertilizers are not retained, "falls through" to groundwater and is washed away, not benefiting the plants and polluting water ponds. On the contrary, soils with a high humus content have more favorable water-physical, chemical, and other properties, they are less susceptible to the adverse effects of pesticides and the technical pollution. The rate of utilization of nitrogen fertilizers are increased with increasing the content of humus in the soil. Organic fertilizers develop humus layer of the soil by rotting in the ground, that significantly increase its soil fertility and content of nutrients. Organic fertilizers beneficially affect on the soil structure, improve such characteristics as air and water permeability, render a stabilizing effect on the soil structure. Furthermore, the introduction of organic fertilizers contributes to the regulation of biological processes in the soil and stimulates the activity of soil microorganisms. But most importantly, they do not harm the health of consumers of herbal products. Ecologically pure products are distinguished by their exceptional taste qualities and are very popular. This is the main argument tipping the scales in favor of the choice of organic fertilizers.

The process of production of organic fertilizers is based on the extraction of humic components of the organic part of substances from brown coal or peat with solutions of alkaline reagents by extraction method. This method provides full processing of peat and brown coal without waste. Universal organic fertilizer is a highly effective fertilizer, which intended for all types of agricultural crops. It is used for all types of soils, promotes to the rooting of seedlings and saplings, to increase of productivity of various crops, to increase of utilization ratio of mineral fertilizers and of humic substances in soil. It promotes to the cultivation of ecologically clean agricultural products.

"EldORost" is a universal organic fertilizer and natural plant growth regulator designed for all kinds of agricultural crops in all soil-climatic zones. It promotes to the cultivation of ecologically clean agricultural products, to the reducing the concentration of heavy metals, radionuclides and nitrates. It allows the producing of qualitative and more environmentally friendly products (with high content of carbohydrates, proteins, lipids and other valuable substances). "Eldorost" can be used in organic farming for the production of environmentally friendly products.

Contains: a concentrate of salts of humic and fulvic acids (12%), a complex of minerals and trace elements, amino acids, flavonoids and etc.

Application: For presowing treatment of seeds, foliar feed plants during the growing season, for post-harvest treatment of the soil.

Assurance: The increasing of productivity of different crops of 10-50% and more; the increasing of the root system and strengthening of immunity; the relieving stress and restoring normal growth of plants; the raising the penetration of fertilizers from soil; the improving the efficiency of mineral fertilizers and pesticides, reducing their use of 30-50%. The strengthening water-holding capacity of the soil, takes an active part in the formation of humus, accelerates the synthesis of chlorophyll and ripening by 10-12 days. It increases the quality and improves the safety of the crops.

Promote: to restore and improve soil fertility, including improving the mechanical, chemical and hygroscopic structure of the soil. The restoration of the biological characteristics of the soil, including the revival of bacterial communities, and activation of soil microorganisms. The improvement of protective properties of the soil. It has strong antioxidant properties, takes part in the neutralization and elimination of toxins. It protects plants against bacterial and fungal disease, increases resistance of plants to adverse environmental factors (drought, excessive moistur5e, frost); to enhance the development of the root system of plants and their breathing, to improve the survival rate of seedlings, saplings, and seedlings when transplanting.

The flow rate of the concentrate: pre-sowing seed treatment – 150-300 g/t, spraying of crops: grain, technical, vegetable, etc. – 150 g/ha, post-harvest treatment of soil – 400 g/ha. Compatible with most water-soluble fertilizers and pesticides.

In general, it is impossible to keep the ecological balance in nature without using organic fertilizers in agriculture.

## Ameliorating Properties of Silsesquioxane Humic Systems: a Model Experiment

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An increasing anthropogenic impact on agricultural land results in soil deterioration processes mainly due to soil structure degradation. Degradation of the soil structure of the arable horizon has been marked in the vast areas al over the world. The latter led to drastically reduced soil fertility due to poor conditions of crops growing. Therefore, searching for new methods of soil improvement aimed primarily at improving soil structure is of a great importance. An introduction the humic acids (HA) modified with siloxanes (so called silsesquioxane humic systems, Si-HA) seems to be one of the most promising approach. Despite the fact that the possibility of using Si-HA has already been shown earlier [1], there are still no studies aimed at comprehensive evaluate their ameliorative properties. So, the goal of our work was to conduct a comprehensive evaluation of Si-HA ameliorating properties including both evaluation of their effect on soil structure, their sorption on soil and their toxicity assessment towards higher plants and soil microflora as well.

To reach this goal, a model experiments with soil samples of arable horizon of sod-podzolic soil (pH 5.3, OC 1.83%, N 0.12%) were saturated with HA or Si-HA at different rates followed by estimation of HA or Si-HA desorption from soil, measuring soil substrate-induced respiration (SIR) according to [2], estimation of stable microaggregates using laser analyzer of particle size Microtrac Bluewave (Microtrac, USA) and bioassay of soil water extracts using wheat seedlings. To follow alteration of soil humus composition, a ratio of $C_{HA}/C_{FA}$ was also measured. Samples saturated with distilled water were used as blank ones. Si-HA was synthesized based on coal HA using (3-aminopropyl)triethoxysilane (APTES) according [1].

Our results demonstrated that additional stable microaggregates in the soil in the presence of Si-HA formed when the rate of Si-HA was 0.75% by weight of the soil or higher. That rate corresponded to humus content ca. 4% that is usually considered as optimum humus content in terms of humus' properties as"soil glue" [3]. Si-HA sorbed mainly irreversible by soil, and the amount of desorbed organic carbon did not exceed 10% of the input. That finding was consistent with the concept of adsorption Si-HA due to the formation of siloxane bonds. The latter was indirectly confirmed also by closeness of $C_{HA}/C_{FA}$ ratio in case with soil saturation with Si-HA to the blank values (0.76$\pm$0.03 for Si-HA *versus* 0.62$\pm$0.03 for blank), while soil saturation with unmodified HA resulted in significant increase in $C_{HA}/C_{FA}$ ratio (1.08$\pm$0.19). Si-HA stimulated SIR (probably, due to release of nitrogen of amino groups of APTES) and were not toxic for wheat seedlings up to the rate 1.25% by weight of the soil.

So, the obtained results allowed to conclude that further study of Si-HA as ameliorating soil agents is of great promise.

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# Гумусовые кислоты торфа. Физико-химические свойства и биологическая активность. Ранозаживляющие свойства фулиевых кислот

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Свойства полиэлектролитов смеси гумусовых кислот (гуминовой – HA, и фулиевой – FA) HPE, выделенных из торфа экстракцией спирт-вода, были изучены ИК-, УФ-, ААС – спектральными методами и титрованием. Емкость поглощения HPE, соответствующая концентрации карбоксильных и фенольных групп, установленная титрованием $Ca(OAc)_2$, NaOAc и NaOH, равна 4,4±0,1 ммоль-экв/г. Сорбционная емкость гумусовых кислот по отношению к биогенным металлам изменялась в ряду (ммоль-экв/г): Cu (7,6) > Zn (5,6) > Ca(4,3) > Fe(3,1). Фулиевую кислоту, характеризующуюся концентрацией кислотных групп, равной 6,5±0,2 ммоль-экв/г, получали дополнительной экстракцией HPE водой.

В исследованиях *in vitro* на крови крыс в эритроцитах установлено существенное улучшение энергетического метаболизма, катализируемого лактатдегидрогеназой, снижением интенсивности ПОЛ, отражающегося в дозозависимом уменьшении концентрации малонового диальдегида (на 30-70%), и повышение активности СОД.

Дерматологические гели, включающие фулиевые кислоты, показали хорошую ранозаживляющую активность.

# Применение гуминовых препаратов для биологической рекультивации

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Для рекультивации нарушенной территории (несанкционированной свалки бытовых отходов) изучали действие сухого (Humatebalance, производитель ООО НПО «Сила жизни») и жидкого (Росток, производитель ООО «НПЦ «Эврика») гуминовых препаратов на растения тест-культуры в вегетационном опыте. В качестве контроля взята почва пашни чернозём выщелоченный. Для сравнения дополнительно ввели вариант с сапропелью (грунт Дар земли марка А, производитель ООО «ТНП»).

Семена замачивали на сутки в чашках Петри в воде (варианты 1-4) и рабочем растворе препарата Росток (вариант 5). Отбирали для посева проросшие семена по 15 шт. на сосуд. Масса почвы в сосуде – 500 г. Доза Humatebalance соответствовала 1000 кг/га, сапропеля – 60 т/га. Сосуды помещали в климатостат марки КС-200 СПУ. Семена проращивались при температуре 200°С с заданной программой смены «дня» и «ночи». Подсчет энергии прорастания проводили на 4 сутки, всхожесть – на 7 сутки. На 14 сутки сосуды изымали, отмывали корневую систему и проводили биометрические замеры. Для статистической обработки результатов исследований применяли дисперсионный анализ данных вегетационного опыта с использованием программы Mucrosoft Excel 2010.

В почве свалки превышало ПДК содержание валовых и подвижных форм свинца и цинка. Это негативно повлияло на прорастание семян тест-культуры, снизились энергия прорастания и всхожесть на 22 и 12% соответственно по сравнению с контролем. При внесении удобрения Humatebalance в почву свалки энергия прорастания несущественно снизилась (на 1%), а всхожесть семян повысилась (на 5%) по сравнению с почвой свалки. Действие сапропеля и жидкого препарата повлияло на энергию прорастания, увеличение на 20 и 42% соответственно в сравнении с почвой свалки. Препарат Росток превышал и почву пашни на 11%.

При проращивании тест-культуры на почве свалки растения испытывали угнетение: существенно снизилось число корешков на 7%, длина и масса корней на 8 и 19%, высота и масса растений – на 22 и 30% по сравнению с контролем (почва пашни). Внесение органических удобрений Humatebalance и сапропеля не устранило отрицательное действие свалки на корневую систему растений. Показатели на этих трёх вариантах существенно не отличались. Удобрения Humatebalance и сапропель существенно повлияли на надземную часть тест-культуры. Высота и масса растений на вариантах с этими удобрениями превышали вариант с почвой свалки на 16 и 9%, 37 и 33% соответственно. Все биометрические показатели корневой системы и растений выше контроля (почвы пашни) были только при использовании препарата Росток: число корешков – на 7%, длина и масса корней – на 18 и 33%, высота и масса растений – на 6 и 27% соответственно.

Таким образом, предпосевная обработка семян жидким гуминовым препаратом Росток устраняла негативное действие свалки бытовых отходов на рост и развитие растений при биологической рекультивации. Внесение в почву свалки сухих удобрений Humatebalance и сапропеля не улучшило развитие корневой системы, но увеличило высоту и массу надземной части растений тест-культуры. Необходимо продолжить исследования по совместному применению жидких и сухих гуминовых препаратов.

# Эффективность гуминовых препаратов при возделывании картофеля в горных условиях Кыргызстана

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Аннотация. Исследования по изучению эффективности гуминовых препаратов при возделывании картофеля в горных условиях Кыргызстана позволили установить положительное влияние на урожай и повышение качества данной культуры.

Введение. Внесение гуминовых препаратов (ГБ) на черноземах Кеминской долины (плантации картофеля), по схеме опыта (см. схемы опыта) оказывает комплексное воздействие:

– как гуминовое удобрение;

– микробный консорциум бактериального препарата, который усиливает биологическую активность почвы;

– как стимулирующий бактериальный препарат улучшает фитосанитарное состояние орошаемой пашни и повышает качество продукции.

– Проводимая нами НИР, наряду с работами предшествующих авторов подтверждает агроэкологическую эффективность применения ГП стимулирующий почвенное плодородие и продуктивности растения низкоинтенсивными дозами эффективных удобрений

Научная новизна. Применение гуминовых препаратов на плантации картофеля в условиях поливного земледелия Кеминской долины Кыргызстана применяется впервые. Испытаны различные дозы применения ГП на урожайность и качество картофеля и на показатели плодородия орошаемой пашни черноземов.

Объект и методы исследований. Полевой опыт был заложен в горных условиях Чон-Кеминской долины Кыргызстана на высоте 1600-1700 м над уровнем моря. Климат долины характеризуется умеренным и сухим летом, относительно холодной зимой с выпадением 250-400 мм осадков в год с максимумом в весенне-летний период, а среднемесячные колебания температур составляют от 9,7°С до 16,0°С.

В долине распространены каштановые почвы, характеризующиеся следующими морфологическими признаками: пахотный слой имеет буровато-светлокоричневую и каштановую окраску, слабовыраженную непрочную комковатую структуру. Почва не засолена и не солонцевата, емкость поглощения составляет 22-25 мг.экв. на 100 г почвы. В составе поглощения оснований преобладает кальция и магний. Содержание гумуса в верхнем пахотном слое достигает 2,3-2,6%, а содержание азота колеблется от 11,8 до 21 мг/кг почвы, фосфора от 13,8 до 19,9 мг/кг почвы, обменного калия от 387 до 439 мг/кг почвы.

Полевой опыт был заложен 8 июня 2017 года в четырехкратной повторности на площади 2543 м$^2$ и включал следующие варианты (схема опыта):

1. Контроль – без внесения удобрений;
2. NATURAL HUMIK ACIDS- нормой 300 кг/га (NHA-300);
3. NATURAL HUMIC ACIDS – нормой 500 кг/га (NHA-500);
4. HUMATE BALANCE – нормой 200 кг/га (NB-200);
5. HUMATE BALANCE – нормой 400 кг/га (NB-400).

Площадь учетной делянки 28 м$^2$ (длина – 10, ширина 2,8 м).

Перед началом полевых работ была разработана технологическая карта, в которой учитывались аналогичные технологии по обработке почвы, уходу за посевами и уборкой картофеля с целью получения высоких урожаев. Согласно технологической карты была проведена весенняя обработка почвы, посадка картофеля сортом «Пикассо» проводилась картофелесажалкой нормой 4 т/га. Перед посадкой картофеля был проведен анализ почвы, который показал, что содержание азота достигает до 13,8 мг/кг почвы, фосфора – 17,6 мг/кг почвы, а калия – 397 мг/кг почвы, а содержание гумуса – 2,48%.

Внесение гуминовых препаратов проводилось вручную согласно схемы опыта, для чего делали бороздки в зоне междурядий на глубину до 12 см, в которые вносили препарат и закрывали почвой. В течение вегетации было проведено 4 полива из расчета 500-600 м$^3$/га каждый с последующим рыхлением и окучиванием. Для уничтожения сорных растений поле обрабатывали гербецидом «Зеллек-супер» из расчета 2,0 л/га. При заболевании растений фитофторозом всходы картофеля обрабатывали фунгицидом «Аскоцеб» из расчета 2,0 л/га. Постоянные наблюдения за появлением колорадского жука позволили вовремя обрабатывать растения инсектецидом «Энжио» нормой 0,2 л/га, что предотвратило их массовое размножение.

В течение вегетационного периода проводилось наблюдение за ростом и развитием ботвы картофеля, изменением агрохимических показателей почвы. При достижении технической спелости картофеля провели скашивание ботвы и приступили к его уборке. Сбор картофеля на учетной делянке, сортировка и транспортировка к месту взвешивания проводилась вручную.

## Влияние гуминовых препаратов на продуктивность картофеля

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В ГАУ Северного Зауралья в 2017 г. изучали влияние посадочного внесения сухого гуминового препарата Naturalhumicacids (содержание гуминовых кислот 95%) и некорневой обработки растворами регуляторов Изабион (содержание органического вещества 62,5 г/л) и Росток (содержание солей гуминовых кислот 10 г/л) на продуктивность картофеля сорт Тулеевский, выращиваемого на темно серой лесной тяжелосуглинистой почве. Схема опыта: 1. контроль (некорневая обработка инсектицидом Престиж Хамелеон, 0,1 мл/л), 2. посадочное внесение Naturalhumicacids (доза 500 кг/га, некорневая обработка инсектицидом), 3. некорневая обработка растений баковой смесью инсектицида с препаратом Изабион (3 мл/л), 4. некорневая обработка растений баковой смесью инсектицида с препаратом Росток (2 мл/л). Расход рабочего раствора 300 л/га. Учетная площадь 7 $м^2$, повторность трёхкратная, размещение делянок систематическое. Посадка вручную, глубина заделки клубней 6-8 см, схема посадки 30×70 см, норма посадки 45 тыс. шт./га. Всходы сорняков уничтожали культивацией междурядий КОН-2 и ручной прополкой.

На общее число клубней в гнезде существенно повлияли жидкие препараты: Изабион снизил на 32%, Росток повысил на 44%. Сухой препарат Naturalhumicacids снизил данный показатель на 12%, но различие с контролем не существенное.

Препараты Naturalhumicacids и Изабион по отношению к контролю существенно уменьшили число товарных клубней на 30 и 27% соотвественно, препарат Росток – увеличил на 20%.

Препарат Изабион не повлиял на общую и товарную массу клубней в гнезде, показатели на уровне контроля. Удобрение Naturalhumicacids существенно снизило массу товарных клубней на 12%. Положительное действие оказал только препарат Росток, увеличив общую массу на 36%, товарную – на 25%.

Масса товарного клубня существенно превышала (на 39%) контроль только при применении препарата Изабион.

Все препараты существенно повлияли на урожайность картофеля сорт Тулеевский. Изабион и Naturalhumicacids оказали отрицательное действие, Росток – положительное. Первые два препарата снизили общую и товарную урожайность на 2,1 и 2,6 т/га, 1,8 и 5,1 т/га соответственно в сравнении с контролем. Препарат Росток увеличил общую урожайность на 16,4 т/га, урожайность товарных клубней – на 10,5 т/га.

Решающую роль на двух вариантах в снижении урожайности,несмотря на увеличение массы товарного клубня, сыграло, то что растения картофеля при применении органических препаратов Изабион и Naturalhumicacids образовали меньшее число клубней в гнезде, особенно товарных. Для выяснения причины такого действия в настоящее время почва и клубни картофеля отданы на анализ в аккредитованную лабораторию.

## Новые гуминовые удобрения для сельского хозяйства

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Presents the results of the registration tests of fertilizer based on humic acids from different types of gumusosoderzhaschy raw materials. It is shown that the use of fertilizers single-component or mixture where the main component are humic acid promotes immunity and stress resistance of plants, increase the harvest by 5 to 30% and improve the quality of agricultural products.

Производство продуктов питания, импортозамещение, продовольственная безопасность – наверное самые широко используемые фразы нашего времени. Поиск путей решения этих задач заставляет наконец вплотную подойти к внедрению технологий, которые до этого времени вызывали немало скепсиса в среде товаропроизводителей. Широкое использование гуминовых удобрений способствует появлению на рынке как новых производителей, так и поиску новых форм самих удобрений. Наряду с традиционными гуминовыми и фульвокислотами, появляется много смесевых – с макро и микроэлементами, с органическими удобрениями, с аминокислотами.

Пришло понимание, что получение стабильно высоких урожаев качественной продукции, повышение иммунитета и стрессоустойчивости, невозможно без использования биоудобрений, особенно гуминовых. Так как главное их достоинство – это совместимость и удобство применения. Гуминовые удобрения полностью растворимы в воде, не требуют дополнительных затрат на внесение (совместимы с минеральными подкормками и обработками пестицидами).

В 2015-2016 годах для внесения в «Государственный каталог пестицидов и агрохимикатов разрешенных к применению на территории Российской Федерации» регистрационные испытания проходили более 10 наименований удобрений на основе гуминовых веществ, однокомпонентных или смесевых, где основным компонентом выступали гуминовые кислоты.

Отличительная особенность этих лет в том, что значительное количество регистрантов – это иностранные компании.

**Биокат-джи** – органоминеральное удобрение на основе комплекса аминокислот, фульво- и гуминовых кислот, производимое испанской компанией Атлантика Агрикола С.А. из мелассы тростниковой.

В условиях Рязанской области применение Биокат-Джи на культуре пшеницы яровой сорта Дарья способствовало повышению урожая зерна на 2,6-9,2 ц/га (8,2-29,2%) при урожайности в контроле 27,2 ц/га. (ФГБОУ ВО РГАТУ, 2016 г.).

На культуре томата сорта Дар Заволжья в условиях открытого грунта (Краснодарский край) применение удобрения Биокат-Джи способствовало повышению средней массы плода - на 9,1-19,8%, количества плодов на растении – на 8-14,1%. Общая прибавка урожая плодов составила 38,8-54,1 ц/га (20,8-29%) при урожайности в контроле – 186,6 ц/га (ФГБОУ ВПО КубГАУ, 2016 г.).

Удобрение на основе гуминовых кислот, производимое путем экстракции гуминовых и фульвокислот из леонардитов БЛЭКДЖЕК разработано компанией АМКОЛ Минералс Юроп Лтд. (Англия).

В условиях Ульяновской области некорневые подкормки пшеницы яровой сорта «Симбирцит» удобрением БЛЭКДЖЕК способствовали снижению поражения растений яровой пшеницы корневыми гнилями на 5,4-7,8% и интенсивности развития болезни на 2,3-3. Повышались основные показатели структуры урожая: длина колоса – на 4,6-12,3%, число зерен в колосе – на 4,3-8,1%, масса 1000 зерен – на 2,5-4,7%. Прибавка урожая зерна составила 1,6-4,2 ц/га (7,1-18,8%), при урожайности в контроле 22,4 ц/га. (ФГБНУ«Ульяновский НИИСХ», 2016 г.).

На культуре подсолнечника сорта Воронежский 638 в условиях Краснодарского края применение агрохимиката БЛЭКДЖЕК для некорневых подкормок растений обеспечило повышение размера корзинки на 3,1-5,1%, количество семян в корзинке - на 10,2-11,1%, масса 1000 семян –на 9,8-13,8%. Прибавка урожая семян составила 0,1-0,53 т/га (4,9-25,9%) при урожайности в контроле – 2,05 т/га. Масличность семян увеличилась на 2-2,2 (ФГБНУ НИИСХ ЦЧП, 2016 г.).

Удобрение на основе гуминовых кислот **Гидрогумин** разработано и производится ЧПУП «БИОХИМ» (Республика Беларусь) путем обработки торфа и биогумуса водно-щелочным раствором и обогащением полученного экстракта макроэлементами. По данным Кубанского ГАУ (2012 г.), применение удобрения Гидрогумин способствовало получению прибавки урожая зерна пшеницы озимой сорта Бригада - 6,6-9,2 ц/га (10,3-14,3%), при урожайности в контроле – 64,2 ц/га, гибрида кукурузы Мас 37 – 9,0-13,5 ц/га (12,2-18,3%), при урожайности в контроле – 73,7 ц/га, гибрида подсолнечника НК Неома – 2,5-4,3 ц/га (10,5-18,0%), при урожайности в контроле – 23,9 ц/га, риса сорта Диамант – 7,5-9,7 ц/га (10,6-13,7%), при урожайности в контроле – 70,8 ц/га, сои сорта Вилана – 1,6-2,3 ц/га (10,2-14,6%), при урожайности в контроле – 15,7 ц/га

Удобрение **Гумимакс** – продукт компании АО «Уралэкосоил» (г. Екатеринбург)., производится путем обработки торфа гидроксидом калия.

На культуре пшеницы яровой сорта Памяти Азиева в условиях Омской области применение Гумимакса способствовало прибавке урожая зерна 0,22 т/га (16,9%) при урожайности в контроле 1,3 т/га. Содержание клейковины в зерне повысилось – на 0,7%. На культуре ячменя сорта Омский 90 урожайность повысилась – на 0,09 т/га (6,4%) при урожайности в контроле 1,4 т/га. (ГНУ СибНИИСХ, 2014 г.).

В условиях Свердловской области применение Гумимакса для некорневой подкормки растений пшеницы яровой сорта Красноуфимская 100 способствовало повышению урожая зерна – на 0,49 т/га (12,3%) при урожайности в контроле 3,97 т/га (ГНУ Уральский НИИСХ, 2014 г.).

**Фульвогумат, марка Б** производится ООО НПО «Альфа-Групп» (Новосибирская область) путем щелочного гидролиза леонардита. В опытах Сибирского НИИ растениеводства и селекции в условиях мелкоделяночного опыта урожайность пшеницы яровой сорта Сибирская 12 повысилась – на 24% . На пшенице яровой сорта Омская 28 в условия Омской области прибавка урожая зерна под воздействием Фульвогумата, марка Б составила 8,4 ц/га (45,7%), при урожайности в контроле 18,4 ц/га. В опытах Сибирского НИИ кормов, в условиях Новосибирской области, на газонных травах и цветочных культурах установлено, что применение Фульвогумата, марка Б способствует повышению плотности травостоя газонных трав - на 17-19%; на петунье - увеличению количества цветоносов – на 3-6 шт. и размера цветков, повышению декоративных качеств.

В последние годы гуминовые и фульвокислоты стали неотъемлемой частью рецептуры смесевых удобрений в состав которых входят макро и микроэлементы, аминокислоты, различные органические соединения. Среди них: Гумат Грин ОК (18 марок), производитель – ООО «Латвийский институт гуминовых веществ» (Латвия); Удобрения комплексные «Здравень турбо» (18 марок), производитель - ООО «Ваше хозяйство» (г. Нижний Новгород); Агрикола-аква универсальное комплексное удобрение (31 марка), производитель - ЗАО «ТПК Техноэкспорт» (Московская область, г. Сергиев Посад); Комплексное водорастворимое минеральное удобрение с микроэлементами и гуматом «Ортон-Подкормка» (10 марок), производитель - ООО «Ортон» (Московская обл., г.Пушкино); Удобрение гуминовое комплексное жидкое марки Теллура-М, Теллура-Био, Феникс, производитель - ООО Научно-производственное предприятие «Теллура-бис» (Алтайский край); Нитроаммофоска с гуматами марка 16-16-16, производитель - ОАО «Минудобрения» (Воронежская обл., г. Россошь) и др.

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Под брендом Life Force® производятся продукты для почвы, растений, животных, птиц и рыб: почвенные кондиционеры, антистрессовые препараты, метаболические активаторы роста, микроэлементы, кормовые добавки, и многие другие продукты.

Группа компаний «Life Force» путем разработки современных продуктов на основе природных гуминовых кислот из леонардита в полной мере обеспечивает рациональные решения для производителей в сфере растениеводства и животноводства в России, СНГ, Украине, ЕС, Турции, Египте, Иране, Индии, Вьетнаме, Гане, Зимбабве, ЮАР, Нигерии и постоянно расширяет географию поставок своей продукции.

Понимая, что плодородная почва — это основа всей пищевой цепочки, необходимой для существования человечества, компания «Life Force Group» в начале 2017 года выступила организатором и спонсором международного конкурса научно-исследовательских работ «Life Force 2017» по оценке потенциала гуминовых продуктов и эффективности их применения в сельском хозяйстве и природоохранных технологиях на принципах органического, традиционного и интенсивного земледелия.

Целью этого конкурса является мобилизация интеллектуального потенциала перспективных молодых ученых для разработки стратегии и поиска решений актуальных проблем повсеместного истощения и дегумификации почв. Подведение итогов конкурса состоится 19 октября 2017 года.

**Имея на вооружении научную основу, мощную ресурсную базу и высокий интеллектуальный потенциал сотрудников и соратников, LIFE FORCE® участвует в решении глобальных экологических задач: поиск способов увеличения почвенного плодородия, выращивания животных и птиц без антибиотиков и гормонов роста, возможность производства экологически чистых продуктов растениеводства.**

ЛИГНО
ГУМАТ
КОНЦЕНТРИРОВАННЫЙ
ГУМИНОВЫЙ ПРЕПАРАТ

# НПО «РЭТ»

**РЕАЛИЗАЦИЯ ЭКОЛОГИЧЕСКИХ ТЕХНОЛОГИЙ**

Санкт-Петербург, 195112 Малоохтинский пр. 61 А,
офис 210 А, тел./факс: +7 (812) 655-00-89

## История создания

- 1984 Первые опыты ускоренной гумификации органического сырья на базе.
- 1992 Разработана промышленная технология синтеза гуминовых веществ из лигниносодержащего сырья.
- 2001 Начало опытно-промышленного производства.
- 2007 Строительство завода с непрерывным производственным циклом по выпуску 30 модификаций Лигногумата®
- 2017 Строительство новой очереди завода в рамках расширения производства и освоения новых рынков сбыта

## Производственная мощность

1 500 т. сухих препаратов, до 90% д.в.

7,5 млн. л. коллоидных растворов, до 20% д.в.

## 25 лет помогаем расти агробизнесу

Сегодня гуминовые препараты НПО РЭТ под маркой Лигногумат® успешно применяют для обработки 15 миллионов гектар посевных площадей. 56% нашей продукции импортируют Чехия, Германия, Нидерланды, США, Канада - всего более 20 стран мира.

Лигногумат® – высокотехнологичный продукт ускоренной гумификации органического сырья. В отличие от других стимуляторов роста, имеющих локальное воздействие, Лигногумат® оказывает комплексное влияние на все физиологические процессы, протекающие в растениях, существенно улучшает водно-физические и физико-химические свойства почвы.

От основной массы представленных на мировом рынке гуматов продукцию НПО РЭТ отличает уникальный комплекс гуминовых и фульвовых кислот с высоким содержанием биологически активной низкомолекулярной части.

### Факторы успеха

- 100% растворимость сухих модификаций, отсутсвие балласта в растворах
- Исходное сырьё — продукт переработки древесины;
- Постоянство состава препарата;
- Концентрация д.в. до 90%;
- До 20% фульвовых кислот от сухого вещества. Меньшая молекулярная масса фульвокислот оказывает яркий стимулирующий эффект на растение, и почвенную микрофлору;
- Хелатная форма микроэлементов обеспечивает ускоренное усвоение растениями питательных веществ;
- Высокое содержание серы в органически связанной форме оказывает существенное влияние на биологическую активность Лигногумата.
- Высокая концентрация лигнина в сырье позволяет обеспечить безотходный и экологически безопасный процесс получения гуминовых концентратов.

### Универсальное применение

Лигногумат не только отлично сочетается с большинством агропрепаратов, но и усиливает их эффективность.

Сочетание Лигногумата с минеральными удобрениями повышает коэффициент усвоения растениями минеральных компонентов. Это позволяет производителям снижать норму расхода удобрений.

Введение гуминовых препаратов в состав композиций химических средств защиты растений позволяет снизить нормы расхода пестицидов, снять токсическое воздействие на культурное растение и почву.

Многолетние исследования доказали способность Лигногумата к ремедиации почв, в том числе загрязненных экотоксинами и тяжелыми металлами.

Общий эффект от комплексного применения продукции НПО РЭТ, который отмечают все конечные потребители - значительное повышение рентабельности производства сельскохозяйственного производства.

Независимые исследования и полевые испытания показывают, что по комплексу показателей экономическая эффективность применения Лигногумата® выше традиционных гуматов и биогумуса.

### Приглашаем к партнерству

Долгосрочное вложение в сельскохозяйственные технологии - одна из самых выгодных и надежных инвестиций.

С нашей помощью вы участвуете в построении интенсивного сельскохозяйственного производства будущего, основанного на гармоничном сочетании ресурсосберегающих технологий, получении стабильных урожаев качественной продукции и бережного отношения к природе.

NATURAL
ORGANIC
MATTER
RESEARCH

IHSS was founded in Denver, Colorado, USA, on September 11, 1981 to bring together scientists in the coal, soil, and water sciences with interests in humic substances. IHSS has a membership of nearly 900 scientists. The Society is recognized as a world leader in fostering scientific education and research, and promoting public understanding of humic substances.

The motto of the International Humic Substances Society (IHSS) is:“To Advance the Knowledge and Research of Natural Organic Matter in Soil and Water”. The aims of the Society include the establishment and maintenance of a collection of standard samples of humic and fulvic acids from lignite, fresh water, a mineral soil, and an organic soil, and the assembling of characterization data. In addition, the Society has added reference samples that are a source of humic materials for research.

The IHSS convenes biennial international conferences, which bring together scientists from the soil, coal, freshwater, and marine sciences. These conferences are convened by leading scientists in collaboration with the IHSS Board.

http://humic-substances.org/

Land Green & Technology Co., Ltd.

NovaLand Formula-G1

About us

Land Green & Technology is a company based in Taiwan that is focused on innovating advanced technology, approaches and methods in organic farming.

Human minds are capable of formulating the most spectacular ideas possible. Every new idea brings progress, evolution, hope and a new vision. This is how we humans have developed, is developing and will develop. Taiwan is one of the world's leading countries where new innovating ideas and technologies are formed, created and implemented worldwide. Land Green & Technology follows this principle and dedicates itself in developing cutting-edge technologies, methods and products for the agricultural industry.

We believe that greenhouse farming is the solution for the food crisis that we soon, if not already, will all be facing. We plan to combine scientific and commercial approaches, using greenhouses and aquaponic systems, along with our organic nano-fertilizers, we are able to run experiments and carry out studies to discovering the ideal growth (nutrient) medium formulas for different kinds of crops.

The newest service we here at Land Green & Technology provide is a platform for distant gardening for individuals, researchers and parties around the globe. We believe that bringing all kinds of great minds together will speed the process of discovering new nutrient medium formulas and determine factors to increase plant growth speed and growth quantity. We intend to develop as the Green Technologies Center, to help, provide and bring together the scientific communities from different countries.

Contact in the US:
Controlled Environment Agriculture Center
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Tel: 520-626-9566
Fax: 520-626-1700

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2F-1, No. 65, Sec. 3, Hsin-Yi Road, Taipei, 10651, Taiwan (R.O.C)
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## ГУМИНОВОЕ УДОБРЕНИЕ ЭДАГУМ®СМ

ООО «Эдагум СМ Рус» (входит в группу компаний «СМ») специализируется на разработке и производстве гуминовых препаратов на основе торфа для применения в сельском хозяйстве, экологии, нефтегазовой отрасли.

Одним из известных продуктов, производимых компанией, является гуминовое удобрение ЭДАГУМ®СМ - натуральный биостимулятор роста и развития растений, которое выпускается с 2006г. Наукоемкая технология производства удобрения ЭДАГУМ®СМ позволяет извлечь из торфа и сохранить весь комплекс биологически активных веществ, созданных самой природой: гуминовые и фульвокислоты (40-50 г/л), макро и микроэлементы в форме биодоступных органических соединений, полезную микрофлору.

Высокая эффективность действия удобрения ЭДАГУМ®СМ подтверждена исследованиями 34 профильных российских и зарубежных НИИ, а также практикой применения в России, странах СНГ, Европы, Центральной и Юго-Восточной Азии, Латинской Америки. В результате применения удобрения при обработке вегетирующих растений достигнуто повышение урожайности: на пшенице до 29%, рисе – до 27,4%, картофеле - до 24%, томатах – до 34%, сое - до 33,7%, хлопчатнике - до 43% и мн. др. За высокое качество и уникальный состав ЭДАГУМ®СМ награжден 7 золотыми медалями и 24 дипломами российских и международных выставок.

Результаты исследований биологических особенностей гуминового удобрения ЭДАГУМ®СМ во Всероссийском НИИ сельскохозяйственной микробиологии РСХА (г. Санкт-Петербург, 2015г.) подтверждают высокую эффективность воздействия препарата на свойства почвы и способность стимулировать рост и развитие растений, что обусловлено высокой численностью физиологических групп микроорганизмов, содержащихся в препарате. Установлено, что при внесении в почву ЭДАГУМ®СМ возрастает дыхание микроорганизмов на 28,8%, ускоряется разложение органических соединений азота и фосфора и повышается их биодоступность. Улучшается питание растений и за счет самого препарата: в почве возрастает количество подвижных форм азота – на 7,4%, а также фосфора и калия на 22,0% и 10,5% соответственно.

Сравнительные исследования учёных кафедры химии почв факультета почвоведения МГУ им. М.В.Ломоносова на посевах пшеницы, проведенные в 2015г., показали, что эффект от обработки 1 литром препарата ЭДАГУМ®СМ 1-го гектара пашни и обработки семян перед посевом эквивалентен внесению 9-10 т навоза КРС 2-х летней выдержки. В результате влияния ЭДАГУМ®СМ ускоряются и усиливаются процессы формирования так называемого «молодого гумуса» (прогуминовых веществ и неспецифических органических соединений), препарат оказывает положительное комплексное воздействие на физические свойства почвы и ее структуру.

Учеными и специалистами компании разработана ресурсосберегающая технология возделывания сельскохозяйственных культур, основанная на комплексной обработке гуминовым удобрением ЭДАГУМ®СМ почвы (1-2 л/га), семян (0,4-0,8 л на 1 тонну) и вегетирующих растений (две обработки по 0,4 л/га) совместно с традиционными средствами химизации.

Данные научных исследований, проведенных в 2014-2016г.г. в разных регионах мира, свидетельствуют, что применение данной технологии позволило получить дополнительный урожай:

– сои 815 кг/га (21,5% к контролю) при одновременной экономии минеральных удобрений на 20% и пестицидов на 10% (НИИ СХ Технологий INTA, Аргентина);

– пшеницы 1290 кг/га (39% к контролю) при одновременной экономии минеральных удобрений на 30% и пестицидов на 10% (ДонГАУ, Россия);

– хлопчатника 320 кг/га (12% к контролю) при одновременной экономии минеральных удобрений на 20% и пестицидов на 10% (НИИ Земледелия, Туркменистан).

По результатам проведенных исследований можно сделать вывод, что системная, ежегодная обработка почвы гуминовым удобрением ЭДАГУМ®СМ позволит улучшить ее физические свойства и структуру, восстановить плодородие и экологические параметры.

Таким образом, **применение гуминового удобрения ЭДАГУМ®СМ в масштабах государства – это не только помощь в решении продовольственной проблемы, но и здоровье нации!**

www.edagum-sm.ru
info@edagum-sm.ru

НАУЧНО-ВНЕДРЕНЧЕСКОЕ ПРЕДПРИЯТИЕ

БашИнком

НАУЧНО-ВНЕДРЕНЧЕСКОЕ ПРЕДПРИЯТИЕ «БАШИНКОМ»

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Факс: 292-09-93.
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www.bashinkom.ru

Научно-внедренческое предприятие «БашИнком» создано в 1989 г. Компания занимается научными исследованиями, разработкой и производством биологических средств защиты и регуляторов роста и развития растений; антистрессовых, ростоускоряющих, иммуностимулирующих, биоактивированных препаратов и удобрений.

Свой первый продукт – удобрение Гуми – наше предприятие разработало и выпустило на рынок в 1992 г. Нам удалось получить препарат с уникальными характеристиками – биоактивированный, имеющий предельную концентрацию гуминовых веществ, с оптимальным молекулярным весом и микроэлементным составом. За Гуми последовали его модификации – Гуми-10, Гуми-20, Гуми-30, Гуми-90, отличающиеся препаративной формой и процентным содержанием основного действующего вещества

Накопленный опыт за 1995–1998 гг. по разработке и внедрению агропрепаратов привел нас к созданию биотехнологий антистрессового высокоурожайного земледелия АВЗ и Органического Живого Земледелия ОЖЗ.

Одним из ключевых моментов развития компании явилась разработка в 1999-2000 гг (совместно с БашНИИСХ) биофунгицида Фитоспорин-М. Данный препарат является высокоэффективным средством защиты растений от грибных и бактериальных инфекций. В настоящее время у нас в коллекции микроорганизмов находится 29 штаммов микроорганизмов, относящихся к различным родам бактерий и грибов: *Bacillus*, *Lactobacillus*, *Streptococcus*, *Bifidobacterium*, *Pichia*, *Trichoderma* и др.

Мы активно сотрудничаем с местными, региональными и центральными НИИ сельскохозяйственного профиля, многими институтами Российской Академии Наук, станциями защиты растений и производителями сельхозпродукции.

Сегодня мы уже достаточно зрелая фирма с солидным материальным и интеллектуальным капиталом. Мы находимся в постоянном поиске и продолжаем расти.

## Authors index / Авторский указатель

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Международное гуминовое общество (International Humic Substances Society, IHSS),
Международный союз чистой и прикладной химии (International Union of Pure and Applied Chemistry, IUPAC – ИЮПАК)
Группа компаний «Life Force» (генеральный спонсор конференции)
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