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Д.В. Сокольский атындағы
«Жанармай, катализ және электрохимия институты» АҚ

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ИЗВЕСТИЯ

НАЦИОНАЛЬНОЙ АКАДЕМИИ НАУК
РЕСПУБЛИКИ КАЗАХСТАН
АО «Институт топлива, катализа и
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Қазақстан Республикасы Ұлттық ғылым академиясы «ҚР ҰҒА Хабарлары. Химия және технология сериясы» ғылыми журналының Web of Science-тің жаңаланған нұсқасы Emerging Sources Citation Index-те индекстелуге қабылданғанын хабарлайды. Бұл индекстелу барысында Clarivate Analytics компаниясы журналды одан әрі the Science Citation Index Expanded, the Social Sciences Citation Index және the Arts & Humanities Citation Index-ке қабылдау мәселесін қарастыруда. Webof Science зерттеушілер, авторлар, баспашылар мен мекемелерге контент тереңдігі мен сапасын ұсынады. ҚР ҰҒА Хабарлары. Химия және технология сериясы Emerging Sources Citation Index-ке енуі біздің қогамдастық үшін ең өзекті және беделді химиялық ғылымдар бойынша контентке адалдығымызды білдіреді.

НАН РК сообщает, что научный журнал «Известия НАН РК. Серия химии и технологий» был принят для индексирования в Emerging Sources Citation Index, обновленной версии Web of Science Содержание в этом индексировании находится в стадии рассмотрения компанией Clarivate Analytics для дальнейшего принятия журнала в the Science Citation Index Expanded, the Socia1 Sciences Citation Index и the Arts & Humanities Citation Index. Web of Science предлагает качество в глубину контента для исследователей, авторов, издателей и учреждений. Включение Известия НАН РК в Emerging Sources Citation Index демонстрирует нашу приверженность к наиболее актуальному и влиятельному контенту по химическим наукам для нашего сообщества.

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PHYSICOCHEMICAL AND ANTIOXIDANT PROPERTIES OF HUMIC ACIDS OF LOW-MINERALIZED PELOIDS OF THE TUZKOL DEPOSIT

Abstract. As a result of studies it was found that the peloids of the deposit of Tuzkol Lake (Republic of Kazakhstan) are high quality medium-sulfide, weakly alkaline sludge muds. It was found that peloid-derived humic acid contains 49.63 % oxygen, 22.37% carbon, 9.65 % silicon and 6.26% calcium; it also contains Na, Mg, Al, Fe and an insignificant amount of S, Cl, K, F. Electron microscopy and IR spectroscopy analysis of the molecular structure of humic substances of peloids indicated that their macromolecule consists of framework olefinic and aromatic fragments with a substantial proportion of unoxidized aliphatic groups, as well as aromatic structures containing carboxyl groups. The findings of amperometric detection of the antioxidant properties of humic acids suggest that they have antioxidant activity, which will make it possible to use them as a biologically active substance for medicines.

Key words: Low-mineralized sludge sulphide mud (peloids), humic, fulvic, hymatomelanic, humic acids, antioxidant activity.

Introduction. Nowadays, Kazakhstani market of medicines is rather filled with expensive antioxidant medicines mostly of foreign manufacturers. The country needs domestic drugs and medicines that are able to compete with foreign analogues. In this context, the development studies on innovative import-substituting pharmaceutical products on the basis of humic substances of peloids and coals are of particular relevance and practical value.

In terms of economic and therapeutic effectiveness, peloids are one of the most interesting and promising sources of biologically active substances (BAS), in particular, low-mineralized suphide mud representing a complex of both inorganic and organic substances. The predominant constituent of such mud, responsible for biological activity, are humic acids, which currently gained wide use in veterinary medicine, animal breeding, plant growing, both as effective biostimulants and protectors.

From a scientific perspective, the redox properties of humic substances of soils, peat, brown coal, obtained through different chemical and physical-chemical methods, have been the subject of investigation for a long time. But the redox properties of humic substances of peloids derived from low-mineralized sludge sulphide mud from the deposits of Kazakhstan have not been studied extensively or have not been adequately justified for their pharmaceutical applications.

Peloids are complex substances that are formed in the natural conditions of biocenoses under the influence of hydrogeological processes. The formation of therapeutic mud takes place under the influence of a complex of natural factors. Peloids formed by sedimentation in bodies of water and marshes are the most common [1]. The annual reduction of peloid reserves is explained not only by their stable production, but also by the degradation of mud deposits owing to environmental contamination. The most frequently deposits of therapeutic mud are contaminated with solid metals [2].

Sulphide sludge mud is characterized by a low degree of mineralization and is a source of environmentally safe natural medicinal substances. Studies in the development of new biologically active compounds have demonstrated that humic substances of different genesis have reparative, immunomodulatory, anti-inflammatory, antioxidant effects, as well as constitute a universal matrix, which allows introducing the essential microelements into the human body in an active form [3-13].

Research and experimental work on the development of technologies for obtaining and using both natural antioxidants (AO), since they are less toxic compounds, as well as humic substances contained in therapeutic muds are assuming greater practical importance over the last few years in Kazakhstan along with the investigation of the properties of synthetic products [14]. Currently, developing innovative medicinal substances based on domestic natural raw materials to produce modern, competitive pharmaceuticals is a primary objective of

pharmacy. Humic substances are the most promising from this point of view, since their therapeutic effect involves the realization on the subcellular and molecular level. The pharmaceutical use of humic substances of peloids formed under reductive conditions with the participation of suphate-reducing microorganisms which is reflected in their structure and chemical properties that determine the biological activity of the compounds is topical [15].

The main therapeutic muds of Almaty region are spread on small lakes drying up in summer, located along the shores of large lakes (Balkhash, Alakol). But there are small deposits of therapeutic muds and brine, such as Arasan-Kundyzdy, Tuzkol lake, etc. Tuzkol Lake is located in the Raiymbek district of Almaty region, 330 km southeast of Almaty and 35 km northeast of the administrative center Narynkol. The maximum depth of the lake reaches 0.9 m (average - 0.5 m), length - 6.3 km, maximum width - 2.8 km.

This paper presents the results of experimental studies to define the physico-chemical and antioxidant properties of humic substances isolated from low-mineralized sulfide mud of Tuzkol Lake.

Materials and methods. The characteristic of the mud sample from the deposit of Tuzkol lake is given in table 1. The mud outwardly is a homogeneous soft-plastic mass dark gray in color with black interlayers, with a hydrogen sulphide odor and with a small residual matter of the liquid phase.

Table 1. Physicochemical characteristics of the therapeutic mud of the Tuzkol lake deposit

No.	Indicator	Measurement unit	Requirements for therapeutic sulphide sludge muds
1	Appearance		Soft, pasty mass
2	Color		Dark gray to black
3	Smell		Hydrogen sulphide odor
4	Volume weight	g/cm^3	1.300-1.900
5	Mass fraction of volatile matters and moisture	%	25-75
6	Shear resistance	Pa	2500
7	Contamination bi mineral impurities > 0.25 mm in size, max	%	0.20
8	Hydrogen index, pH	pH	5.0-8.5
9	Weight percentage of sulphides	%	0.11
10	Free iron	%	0,35
11	Hydrotroillite	%	0,20
12	Mineralization of mud solution	g/dm^3	110-250
13	TMC (total microbial count), not more	CFU / 10 g	500000
14	LPBC (lactose-positive Bacillus coli) titre, not more than	g	10
15	Clostrolium titer, not more	g	0.1
16	Pathogenic staphylococci	CFU / 10 g	no
17	Pseqdomonas aeruginosa	CFU / 10 g	no

The average moisture content of the mud is 48.0 %, volume weight is 1.73 g/cm^3, shear resistance is 2500 Pa at the rate of 1500-1400 Pa. Heat capacity, which depends on the moisture content, is small and amounts to 0.60 cal/g. deg., the reaction of the medium is neutral (pH - 7.75), density - 0.048 g/cm^3. Contamination by mineral particles 0.25-5.0 mm in size is 0.20 % at the rate of no more than 3 %. Active ingredients were found in the mud in significant quantity: hydrogen suphide - 0.11 %, free iron - 0.35 %, hydrotroillite - 0.20 %. Peloids of the Tuzkol Lake deposit belong to the high quality medium-suphide, weakly alkaline sludge muds.

Samples of the original peloid from the Tuzkol deposit and their humic substances were studied with the use of a Quanta 3D 200i electron microscope (FEI Company, USA) at the National nanotechnology Open Laboratory at the Al-Farabi Kazakh National University.

IR spectra were obtained using a Bruker (USA) Tensor II IR spectrometer at frequencies of 600-3600 cm-1 with a diffraction grating. The spectra were interpreted and analyzed according to the data [16-18].

The elemental composition of the original peloid and humic acids were defined with a Micro XRF elemental analyzer. Antioxidant properties - by using chromatograph Tsvet Yauza AA-01 under the following conditions: direct current mode -dc.; working electrode potential (Up) -(+) 1.3 V; eluent; orthophosphoric acid solution (0.0022 mol/L); eluent flow rate 1.2 ml/min; injection volume 1.0 ml. Registration and statistical analysis of the results were performed using the universal computer system Z-lab for data collection and processing and the "AD&CD Data Processing System".

For plotting the calibrated diagram of quercetin recommended as a standard for this instrument in the Research Institute of Metrological Service, the signals of standard solutions of quercetin were recorded sequentially in order of increasing concentration. The results were used to plot a graph of the dependence

of the peak area (signal value) on the concentration of quercetin.

Results and discussion.

Elemental composition of humic acids and low-mineralized sulphide mud of the Tuzkol deposit.

According to the results of elemental analysis (Table 2) of the original peloid from the Tuzkol deposit and humic acids (HA) in their composition, respectively, a decrease in the carbon content from 13.66 to 8.36%, oxygen - from 40.38 to 32.12 %, aluminum - 4.8 times, silicon - 24.6 times, potassium from 0.96 to 0.45 %, iron from 5.09 to 3.74% was observed. At the same time, the content of sodium increased from 1.86 % in the peloid to 6.52 % in the HA, magnesium from 3.60 to 4.58 %, chlorine from 1.31 to 9.15 % and calcium from 12.75 to 13.35 %. The obtained results meet the criteria for the elemental composition of humic substances adopted by the International Humic Substances Society (IHSS).

Table 2. Elemental composition of the initial peloid and the resulting humic acids (on ash-free basis)

No.	Elements	Peloid		Obtained HA	
		At. %	Wt. %	At. %	Wt. %
1	C	12.89	13.66	22.37	8.36
2	O	37.90	40.38	49.63	32.12
3	Na	0.33	1.86	1.59	6.52
4	Mg	2.02	3.60	2.91	4.58
5	Al	4.89	4.56	3.32	0.94
6	Si	12.33	13.79	9.65	0.56
7	S	4.67	0.72	0.44	0.22
8	Cl	2.31	1.31	0.73	9.15
9	K	2.02	1.96	0.99	0.45
10	Ca	13.90	12.75	6.26	33.35
11	Fe	6.73	5.09	1.79	3.74
12	F	–	0.30	0.31	–

* At. - atomic percentage; Wt - weight percentage.

Method for isolating humic substances from peloid

For destroying mineral complexes and removing sulphides, carbonates and other mineral components, the native mud was treated with Hydrochloric acid solution 2 M without thermal action so as to prevent the destruction of organic substances.

More pure preparations were obtained by extraction of specific organic substances with 0.5 M sodium hydroxide solution at the ratio peloid: solvent = 1:10 to a maximum of three times, since in the subsequent portions the amount of mineral components increased considerably. At this stage, an extract containing the complex of fulvic, hematomelanic, and humic acids was obtained.

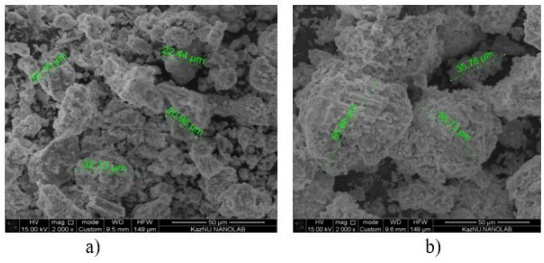
The fulvic acid fraction was isolated with 0.5 M sodium hydroxide solution and converted to the H-form with the use a cation exchange resin and pH constancy was monitored with the use of an ionometer. The obtained preparation was dried at a temperature not exceeding 35°C.

The filter residue after isolation of the fulvic acid fraction was washed with water until a negative test against sulphate ions and exhaustive extraction with ethanol was performed until the extractant (the hymatomelanic acid fraction) turned pale yellow in color.

The filter residue after alcohol extraction is humic acids, which constitute the major portion of humic substances. It was dissolved in a minimal amount of 0.02 M sodium hydroxide solution, then precipitated with sulphuric acid. For the purpose of obtaining a low-ash preparation, the reprecipitation was carried out twice, and then the alkaline solution was passed through a cation exchange resin and dried.

Electron microscopic analysis of samples.

Figure 1 shows electronic images of the examined samples of the original peloid and humic acid of the peloid, which differ from each other by grains of different size. The structure and appearance of the original peloid (Figure 1a) differs markedly from similar indicators of humic acid of the peloid (Figure 1b), specifically: samples of the original peloid consist of the smaller particles mostly of irregular shape. They contain particles with sizes of 20.15; 20.98; 22.44; 32.73 nm, and their mineral constituent is represented by clay minerals, carbonate products of degradation of microorganisms, and suphide formations. Sample of humic acid of the peloid in appearance are rare large particles of different sizes 35.78; 55.73; 65.60 nm.



a) b)

Figure 1. Electronic image of a) initial peloid, b) humic substance of the peloid.

Infrared spectroscopy of humic substances

Infrared spectroscopy has a number of advantages over visible and ultraviolet spectroscopy, as it makes it possible to trace changes in all major types of bonds in the molecules of the substances under investigation. By the number and position of absorption peaks in infrared spectra it is possible to estimate the nature of functional groups of substances (qualitative analysis), and by the intensity of absorption bands - about their relative quantitative ratio [19].

IR spectra of the original peloid and humic acid

are given in Figure 3. The spectrum of the original peloid (Figure 2a) shows 13 absorption peaks in the 3392.10-628.27 cm-1region , and the spectrum of humic acid (Figure 2b) shows 19 peaks in the 364132-610.63 cm^{-1}region.

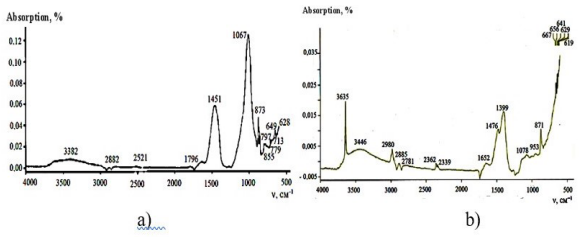


Figure 2. IR spectra of a) original peloid and b) humic acid

According to the IR spectra of the original peloid and humic acid isolated from the peloid, a common absorption band in the 3446-3392 cm^{-1} region was found, which is attributable to the presence of primary amides. Absorption bands appeared in the spectrum of humic acid in the 3612 cm-1 region, corresponding to the valence vibrations of the hydroxyl group, the absorption band in the 1625 cm-1 region, indicating the presence of the C = C bond conjugated with phenyl, and also 2 peaks in the 2362-2339 cm-1 region were detected, which confirms the presence of secondary amines R_2NH_2 +. In the spectrum of the original peloid, an absorption band appeared in the 1796 cm-1 region, corresponding to the presence of the RR'C=CH_2 bond conjugation with the terminal methylene group. The common absorption bands in the 2882 cm^{-1}, 2521 cm^{-1}, 2980 cm^{-1}, 2885 cm^{-1}, 2781 cm-1 regions correspond to the valence vibrations of CH_2, the absorption bands in the 1451-1457 cm-1 confirm the presence of CH_3 absorption in alkanes. Also, the common absorption bands in the 1078-953 cm-1 region correspond to the valence vibrations of aliphatic compounds. The bands of the average intensity of the carbon skeleton vibrations are also in this region. Detection of 1 peak in the 871 cm^{-1} region and 5 peaks in the 873-713 cm^{-1} region correspond to R-NO_2 nitro compounds in 920-830 (10.88-12.05) region and R-O-NO_2 nitrate (bands of variable intensity) and in the ~660 (~15.15) s region to R_2N-N=O nitrosamines, in the ~620 (~16.13) δO=C-N region to IV amides. The remaining 2 peaks related to peloids as well as 6 peaks related to humic substances in the 667-610 cm^{-1} region confirm the presence of alkynes ≡C-H.

Analysis of the molecular structure of humic substances of peloids by IR spectroscopy demonstrated that their macromolecule consists of framework olefin and aromatic moieties with a substantial proportion of unoxidized aliphatic groups, and also includes aromatic structures containing a considerable amount of carboxyl groups.

Identification of the antioxidant activity of humic acid by the amperometric method

One of the methods to measure directly the total antioxidant capacity of the sample is the amperometric method [20], which measures the electric current arising as a result of electrochemical oxidation of the substance under investigation on the surface of the working electrode at a certain electrode potential.

The result is the arithmetic mean value of five measurements (relative mean-square deviation is not more than 5 %), and the calibration graph is plotted (Figure 3).

In the studied HA samples according to the calibration graph of quercetin (Figure 4), the antioxidant mass concentration equivalent to quercetin was determined by calculating the peak area based of the obtained chromatograms. The total content of antioxidants was determined in solutions of humic acids of peloids at concentrations (mass.%): 0.001; 0.010; 0.100 and 1.000.

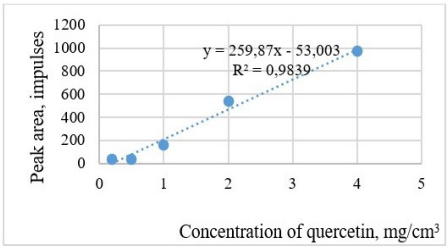


Figure 3. Dependence of the peak area on the concentration of quercetin

The humic acid signal value of 516 nAs with a concentration of 0.001 % indicates that the total antioxidant capacity is less than 0.09 mg/ml. When the concentration of humic acids is 0.01 %, the total antioxidant capacity is 0.93 mg/ml, at a concentration of 0.1 % - 5,37 mg/ml, and when the concentration of humic acids is equal to 1 %, the value of the total antioxidant capacity reaches 49,81mg/ml. The obtained data suggests that humic acids have antioxidant activity, which value increases, if the concentration is increased.

The data presented in Table 3 demonstrate that the total amount of antioxidants in humic substances gradually increases as their concentration increases.

Table 3. Quantification of humic substances of peloids by total antioxidant capacity

Indicators	Total antioxidant capacity (TAC), 10-2 mg/ml			
Solution concentration (wt%)	0.001	0.010	0.100	1.000
Humic acid of peloid	0.09	0.93	5.37	49.81

Conclusion. The results of amperometric determination of the antioxidant properties of humic acids of the studied low-mineralized sulphide muds from the Tuzkol deposit suggest that they have antioxidant activity, and this will make it possible to use them as a natural biologically active substance for medicines.

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ФИЗИКО-ХИМИЧЕСКИЕ И АНТИОКСИДАНТНЫЕ СВОЙСТВА ГУМИНОВЫХ КИСЛОТ НИЗКОМИНЕРАЛИЗОВАННЫХ ПЕЛОИДОВ МЕСТОРОЖДЕНИЯ ТУЗКОЛЬ

Аннотация. В результате проведенных исследований установлено, что пелоиды месторождения озера Тузколь (Республика Казахстан) относятся к высококачественным среднесульфидным, слабощелочным иловым грязям. Определено, что получаемая из пелоидов гуминовая кислота в своем составе содержит 49,63 % кислорода, 22,37 % – углерода, 9,65 % – кремния и 6,26 % – кальция, также в ее элеметном составе при-сутствуют Na, Mg, Al, Fe и в незначительном количестве S, Cl, K, F. Анализ молекулярной структуры гуминовых веществ пелоидов, выполненный с помощью электронной микроскопии и ИК-спектроскопии, показал, что их макромолекула состоит из каркасных олефиновых и ароматических фрагментов со значительной долей неокисленных алифатических группировок, а также ароматических структур, содержащих карбоксильные группы. Полученные результаты амперометрического определения антиоксидантных свойств гуминовых кислот свидетельствуют о том, что они обладают антиоксидантной активностью, наличие которой позволит использовать их в качестве биологически активной субстанции для лекарственных препаратов.

Ключевые слова: низкоминерализованные иловые сульфидные грязи (пелоиды), гуминовые, фульвовые, гиматомелановые, гумусовые кислоты, антиоксидантная активность.

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ТҮЗКӨЛ КЕН ОРНЫ АЗ МИНЕРАЛДАНҒАН ПЕЛОИДТАРДЫҢ ГУМИН ҚЫШҚЫЛДАРЫНЫҢ ФИЗИКА-ХИМИЯЛЫҚ ЖӘНЕ АНТИОКСИДАНТТЫ ҚАСИЕТТЕРІ

Аннотация: Жүргізілген ғылыми-зерттеу жұмыстарының нәтижесінде Тұзкөл көлінің (Қазақстан Республикасы) полоидтары жоғары сапалы орташа сульфидті, әлсіз сілтілі лай сульфидті батпақ екендігі анықталды. Пелоидтардан бөліп алынған гумин қышқылдарының құрамында 49,63% оттегі, 22,37% көміртек, 9,65% кремний және 6,26% кальций, сонымен қатар Na, Mg, Al, Fe және аз мөлшерде S, Cl, K, F бар екендігі анықталды, Пелоидты гуминдік заттардың молекулалық құрылымы электронды микроскопия және ИҚ-спектроскопияның көмегімен анықталды. Олардың макромолекуласы қаңқалы олефиндік және тотықпаған алифатты топтардың үлес салмағы бар ароматты фрагменттерден, сонымен қатар карбоксил топтары бар ароматты құрылымнан тұратындығы анықталды. Гумин қышқылдарының антиоксиданттық қасиеттерін амперометриялық анықтаудың алынған нәтижелері олардың антиоксиданттық белсенділік қасиеті бар екендігін көрсетті, оларды дәрілік заттар үшін биологиялық белсенді зат ретінде қолдануға мүмкіндік береді.

Түйін сөздер: аз минералды лай сульфидті батпақ (пелоид), гумин, фульво, гематомелан, гумус қышқылдары, антиоксиданттық белсенділік.

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