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## DATA ON ANTIOXIDANT ACTIVITY OF HUMIC SUBSTANCES OF LOW-MINERALIZED SLUDGE SULPHIDE MUD (PELOIDS) AND THEIR CLASSIFICATION

(Review)

Abstract. In this work, an overview of the antioxidant activity of humic substances of low-mineralized sludge sulphide muds (peloyds). Though these substances show the greatest biological and therapeutic activity, in comparison with similar substances of other sources (keonardits, coals, etc.) they are insufficiently studied. Formed in the reduction conditions, molecules of humin substances ma low-mineralized silt sulphidic mud possess a unique structure, and excludes a possibility of education in the course of receiving carcinogenic the alkilnic the halogenderivants and thereof urgent is a research in given the directions.

Key words: antioxidatic properties, activity, humin substances, peloida.

Peloid therapy or mud cure is one of the earliest methods of therapy using natural components, which have the biggest adaptogene potential Being customary stimulants, they have an expressed curative influence [1,2].

The peloid therapy can be considered as a unique method for sanitation, since the range of deceases, on which it has positive influence, is quite wide [3]. Peloids have immunomodulatory effect, they cause natural adaptive reactions of human body [4,5], produce anti-inflammatory, desensitizing, antineoplastic [2,6,7], anaesthetic and absorbable effect, improve hemodynamics and lymphdynamics, reduce the activity of exudative and infiltrative processes, soften commissural structures [9]. The mud cure is applied when treating various pathologies: arthritis, respiratory illness, dermatological deceases, gastrointestinal problems, gynaecological deceases, trophic ulcers, hepatitis, inflammatory diseases of lungs, paradontium [10].

The therapeutic effects of mud are based on the combination of actions of closely related thermal, mechanical, chemical and biological factors. Depending on physical and chemical composition of the mud, the severity of the biological reactions of each of them is different. The literature sources describe studies devoted to the study of the peloid composition, but they all characterize mineral component of the mud, and almost do not include functional ability of the organic component. The insufficient knowledge of the organic substances of peloids caused their underestimation in the mechanism of effect on the body [11].

The peloids are divided into the following groups basing on the content of organic substances: peat, sapropelic, sludge sulphide [12]. A number of studies [10] has showed a high biological effect of sludge sulphide mud, which is associated with the components of the composition of the organic fraction. Ë

Sludge sulphide mud are dividing into the following categories basing on the content of sulphides: weakly sulphide (0.01–0.15 % of wet mud), medium sulphide (0.15–0.5 %), heavy sulphide (exceeding 0.5 %). Depending on the salinity of mud solution, low-mineralized (1.0–15.0 g/l), medium-mineralized (15.0–35 g/l), highly mineralized (35–150 g/l), saline-saturated (exceeding 150 g/l) sulphide muds [13] are distinguished.

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The therapeutic significance of sludge sulphide mud is related to high viscous-plastic and thermal properties, content of large amounts of sulphides (exceeding 0.2 %) and organic substances (approximately 5 % on dry basis), including humic acids and their salts, lipids and hemicellulose, and presence of vitamins, enzymes and hormones [14]. Groups of nonspecific and specific compounds are distinguished from the composition of organic substances of peloids. Non-specific compounds include lipids, carbohydrates, amino acids, enzymes, keratinoid pigments, vitamin complexes, and others [10, 15-17].

A higher content of organic substances in peloids is represented by specific organic components – humic substances [16, 18, 19], which content is equal to 45–90 %.

Scientists of all countries are making attempts to isolate and study properties of organic components of peloids to use them independently as biologically active natural compounds and introduce them into public health practice. The study of fractions of humic substances of peloids: himatomelanic, humic, fulvic acids is a considerable interest for creating innovative pharmacotherapeutic drugs based on them, including those with high antioxidant activity [20-24].

Humic substances are a combination of biothermodynamically stable compounds that form, plant and animal residues having no analogues in living organisms in the process of decomposition and biotransformation, which are distinguished by dark color, polydispersity, high molecular weights [25-27]. All humic substances are formed as a result of the postmortal transformation of organic humification residues, process of transformation of decomposition products having different composition and origin of organic residues into humic substances [28].

The difference of this group of natural organic substances from others lies in their pestoichiometric structure and stochastic nature related to the peculiarities of formation resulting from the selection of biothermodynamically stable structures. The variaty of molecular forms of humic substances is determined by the mechanism of biochemical reactions that accompany formation and conditions of geochemical transformation [28, 29].

Classification of humic substances is based on various solubility of these substances in acids and alkalis. According to this classification, they are divided into the following fractions: fulvic, himatomelanic, humic, humin acids and humin-conglomerate of esters of acids and their organo-mineral sorption complexes with clay minerals, insoluble in most solvents [28-30].

Humic acids are insoluble at pH <2, in a dissolved state they have a dark brown colour, in dry state – black powder or flakes [27]. They consist of aromatic core and peripheral part formed by specific chains.

The himatomelanic acids are an alcohol-soluble fraction, which have a cherry-red colour in solution and were first isolated in 1889 and described by the German biochemist, doctor Ernst Felix Hoppe-Zeiler [25].

Fulvic acids are soluble in the whole range of medium acidity, have a variable colour in the range from straw-yellow to orange colour. They vary from humic and himatomelanic acids by greater oxidation and lower carbon content, as well as greater hydrophilicity [27].

Humus acids are the sum of humic, hymatomelanic, and fulvic acids. They have more folded, complex, stochastic structure. Presumably, being supramolecular structures, humus acids are destroyed during classic fractionation of humic substances. Besides, mechanical mixing of already obtained fractions following separation does not have the same set of properties, that humus acids have [14, 25].

Free radical activity is a fundamental property of humic substances [31, 32]. Studying the structure of various natural biopolymers, we can judge the reactivity of the specified substances and their possible transformations in one or another environment. The nature and properties of stable organic free radicals of humic substances has been investigated in a large number of works [32-33]. The content of aromatic structures in the composition of humic substances determines their low solubility in water and high stability during transformation. Electrophilic substitution reactions occur mainly at benzene nuclei, and side chain alkyl are primarily subjected to oxidation.

It is known that one of the most important characteristics of humic substances is high content of paramagnetic centers in them, which presence may be related to free radicals. Presence of paramagnetic centers increases, if the aromaticity of the humic series compounds increases [26]. Paramagnetism of humic saids and their fractions consists of at least three types of paramagnetic components: radicals with a predominant localization of the unpaired electron on the heteroatomic peripheral groups; high-molecular

systems with a sufficiently developed poly-conjugation chain with structural defects; as well as complexes of metals with variable valence [26].

The effect of humic substances on the intensity of biochemical processes is explained by their participation in reductive-oxidative reactions due to the presence of polyphenolic and quinoid groups. During the enzymatic oxidation of polyphenols, quinones are generated through seven quinones-type intermediate compounds [28].

In a similar manner, the electrons of reactive oxygen species and electrons in the molecules of humic substances are blocked.

The study of the antioxidant activity of humic substances obtained from various sources, today is an important task. The reductive-oxidative properties of soil humic substances [26], peat [34] under the influence of various chemical and physical and chemical factors have already been determined. At the same time, the study of the reductive-oxidative properties of humic substances of peloids is not well understood. Although these substances exhibit the greatest biological and therapeutic activity, compared with similar substances of other sources (keonardites, coals, etc.). Formed under reductive conditions, molecules of humic substances of low-mineralized sludge sulphide have a unique structure, and it excludes the possibility of generation when obtaining carcinogenic alkyl halogen derivatives. It means that the research in this field becomes imprortant.

The methods for determining the antioxidant activity are based on the principles of direct or indirect measurement of the rate or completeness of reaction of antioxidants with appropriate reagents, among which amperometry and manometry have the greatest interest. For the quantitative determination of antioxidants, the amperometric method seems to be the most reliable, since it allows directly measuring the content of all antioxidants in a sample. It is based on the measurement of the electric current that is generated during the electrochemical oxidation of the investigated substance (or mixture of substances) on the surface of the work electrode at a certain potential. Under conditions of amperometric detection, compounds containing a hydroxyl group are well oxidized, the limit of their detection lies in the range of  $10^{-9}$ – $10^{-12}$  g, and under favourable conditions, some compounds are determined at a level of  $10^{-15}$ .

The antioxidant activity of humic acid solutions with various concentrations of 1 %, 0.1 %, 0.01 %, 0.001 % was measured in works [35-39] (Table 1). Quercetin was used as a reference material ( $C_{15}H_{10}O_7$ ). To build an analytical curve of quercetin, recommended as a standard for this device, the All-Russia Scientific Research Institute of Metrological Service sequentially recorded signals of standard solutions of quercetin in the order of their concentrations increasing. According to the obtained results, curves of the peak area (signal value) dependence on quercetin concentration were built.

An arithmetic average of five measurements was taken as a result (relative standard deviation no more than 5 %), and a calibration curve was built (Figure 1).

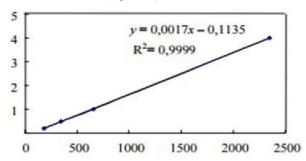


Figure 1 - Dependence of the peak area on the concentrations of quercetin

	Table 1 - The value of the	total content of antioxidants.
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Name	Concentration	Signal value (area), нАс	CCA, mg / ml (standard
			quercetin)
Humic acid	0.001%	516	Менее 0.0001
Humic acid	0.01%	3602	0.00058
Humic acid	0.1%	14571	0.00387
Humic acid	1%	44177	0.043

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The value of the humic acid signal of 0.001 %, concentration – 516 nAc indicates that the total content of antioxidants is less than 0.0001 mg/ml. Increasing the concentration of humic acids, the value of digital signals, and total content of antioxidants increases. When the concentration of humic acids is 0.01 %, the total content of antioxidants is 0.00058 mg/ml, at a concentration of 0.1 % – 0.00387 mg/ml, and when the concentration of humic acids is equal to 1 %, the value of the total content of antioxidants reaches 0.043 mg/ml. The obtained data suggests that humic acids have antioxidant activity, which value increases, if the concentration is increased.

If several years ago humic substances were used mainly in agriculture and animal farming, today their use has proven to be in demand in pharmacy. The research of their physical, chemical and pharmacological properties will improve the effectiveness and accessibility of peloid therapy for patients, and will allow making dosed treatment. Peloid preparations are easier packed and transported, do not require special conditions for their storage. Waste material (mud) is disposed in the mud baths, but it still keep valuable substances. The active components isolated from these "wastes" can later be used to isolate humic substances and produce peloid drugs.

The Kazakhstan market of medicines is filled with foreign antioxidant drugs, and this affects their final price paid by the consumer. The country needs domestic drugs that can generate high competition. Therefore, the research related to the creation of innovative medicines based on humic substances of peloids has particular importance and relevance.

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## ЛАЙ СУЛЬФИДТІ БАТПАҚТАН АЛЫНҒАН ГУМИНДІК ЗАТТАРДЫҢ АНТИОКСИДАНТТЫ БЕЛСЕНДІЛІГІ МЕН ОЛАРДЫҢ КЛАССИФИКАЦИЯСЫ ТУРАЛЫ

(Шолу)

Аннотация. Бұл мақалада лай сульфидті батпақтан алынған (пелоид) гуминді заттардың антиоксиданттық белсенділігіне шолу жасалды. Бұл заттар басқа да көздерден алынған ұқсас заттармен салыстырғанда (кеонардит, көмір және т.б.) ішіндегі ең биологиялық және терапевтік белсенділікке ие болғанымен, олар аз зерттелген. Қалыптасу жағдайында туындаған лай сульфидті батпақтардағы гуминді заттардың молекулалары бірегей құрылымға ие және оларды алу барысында канцерогенді алкил галоген туындыларының пайда болу мүмкіндігін жоққа шығарады, нәтижесінде осы саладағы зерттеулердің маңыздылығы зор екендігі қалыптасады.

Түйін сөздер: антиоксиданттық белсенділік, гуминді заттар, пелоид.

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### О АНТИОКСИДАНТНОЙ АКТИВНОСТИ ГУМИНОВЫХ ВЕЩЕСТВ НИЗКОМИНЕРАЛИЗОВАННЫХ ИЛОВЫХ СУЛЬФИДНЫХ ГРЯЗЕЙ (ПЕЛОИДОВ) И ИХ КЛАССИФИКАЦИЯ

(Обзор)

**Аннотация.** В данной работе сделан обзор об антиоксидантной активности гуминовых веществ низкоминерализованных иловых сульфидных грязей (пелойдов). Хотя данные субстанции проявляют наибольшую биологическую и терапевтическую активность, по сравнению с аналогичными субстанциями

других источников (кеонардитов, углей и др.) они недостаточно изучены. Образованные в восстановительных условиях, молекулы гуминовых веществ низкоминерализованных иловых сульфидных грязей обладают уникальным строением, и исключает возможность образования в процессе получения канцерогенных алкильных галогенопроизводных, и вследствие этого актуальным становится исследование в данном направлений.

Ключевые слова: антиоксидантная активность, гуминовые вещества, пелоиды.

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