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Influence of the Paratunka geothermal deposit of thermomineral waters on the ecological characteristics of the formation and state of the therapeutic mud deposit

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Abstract. The present work describes the physico-chemical, sanitary and microbiological properties of the peloid and feeding waters of Lake Utinoye (Kamchatka), investigated over 50 years of operation of the mud deposit "Lake Utinoye". An increasing presence of thermomineral waters of the Paratunsky hydrothermal deposit in the composition of the feeding waters of the lake has been established. The content of microelement components in the surface waters and in the ecological structure of the peloid is determined. It discovers the ratio of micronutrient concentrations in the ecological structure of the peloid, the accumulation of toxic substances in the solid phase of the peloid. The degree of non-compliance of peloids with medical conditions is established, the description of the method of substandard medical mud activation and its comparison with the efficiency of passive regeneration are given. The structure of a specific microbial community involved in the formation of therapeutic mud is determined. The action mechanisms of thermal water chemical factors at the level of microelements on the community of bottom microorganisms of therapeutic mud have been established. The paper describes the physicochemical, biochemical and microbiological mechanisms of peloid regeneration. The dynamics of sanitary and bacteriological parameters of therapeutic mud in the process of their regeneration in the mud storage is established. The level of the most important manifestations of the peloid biological activity based on the dynamics of the survival of the potentially pathogenic flora in the activation of therapeutic mud has been established.

Keywords: thermal water, peloid, microflora, abundance, heavy metals, geochemical activity, ecological structure.

1. INTRODUCTION

The therapeutic mud of the only in Kamchatka proved and exploited deposit "Lake Utinoye" providing with therapeutic mud Paratunka resort zone of Kamchatka region has been insufficiently studied up to the present moment. There have been geological exploration activities specifying the resources and defining the conditions of the lake-spring sulfide silt mud for more than 50 years of the deposit exploitation. During last 40 years it has been reported some negative change in the conditions of therapeutic mud "Lake Utinoye" associated with pollutants.

The purpose of the study is to assess the ecological state of therapeutic mud and surface waters of the deposit "Lake Utinoye". The monitoring of the ecological state of the spring silt sulfide therapeutic mud deposit was carried out according to physical, physico-chemical, biochemical, microbiological properties, content and level of abiotic and biotic pollution factors established during its exploitation; the formation process of Lake Utinoye therapeutic mud, its conditioning, biological activity and regenerative properties by physical, chemical and microbiological characteristics were studied.

2. RESEARCH METHODS



The basis for this study was the long-term monitoring of the ecological state of the therapeutic mud deposit "Lake Utinoye" from 1991 to 2013.

The object of research was Lake Utinoye as a reservoir, forming the eponymous deposit of therapeutic mud, located 1.5 km to the north of the village Paratunka, Elizovsky district of the Kamchatka region. Near the village Paratunka used in heat exchangers waters of Paratunka thermal springs with salinity of 2.0 g/l, chloride-sulfate sodium-calcium composition flow into the streams Paratunsky and Korkin.

Paratunka geothermal deposit has been exploited since 1967. Useful resources of the deposit as of 2003: 24.8 thousand m³/day with weighted average temperature of 77°C. The deposit waters are chemically nitrogen siliceous highly thermal low-mineralized ($M = 1,1-2,2 \text{ g/dm}^3$), sulphate, chloride-sulphate calcium-sodium, sodium-calcium, alkaline mineral. The studies of lake Utinoye therapeutic mud were carried out in accordance with "Criteria for assessing the quality of therapeutic mud during its exploration, application and protection, 1987", as well as in accordance with the guidelines [4]. Physico-chemical properties of the mud solution were studied by the methods: [3], [7], [14]. Alkaline, alkaline-earth metals and microelements were determined by atomic absorption spectrophotometer AAS-6300 Shimadzu with flame and electrothermal atomization.

Anionic composition of liquid media was determined by high-performance liquid chromatography (HPLC) using a liquid chromatograph Shimadzu LC-20 equipped with a conductometric detector. Hydrocarbonate ions were determined potentiometrically using "Expert-001".

Sanitary-bacteriological indicators of the coliform bacteria titre are determined by the fermentation method with the use of the universal enrichment lactose-peptone broth (LPB), medium of Endo, and semi-solid medium with glucose; titre-perfringens defined by inoculation of mud dilution into Kitt-Tarozzi medium and skimmed milk. Medium LPB, "Shine", king-A and SF were used successively to determine the titre of *Pseudomonas aeruginosa*. *Staphylococcus aureus* was determined on a peptone broth with sodium chloride and subsequent passage to bile-salt agar.

The total number of aerobic saprophytes was determined by inoculation into meat-and-peptone agar (MPA). The study of sanitary and microbiological properties of the lake surface waters was carried out in accordance with Sanitary Regulations and Norms 2.1.5.980-00. NDT was used for test methods MU 4.2.1884-04, MU 4.2.2723-10. The number of microorganisms involved in nitrogen, carbon, sulfur, iron cycle and peloid formation was studied. Determined putrefactive aerobes and anaerobes, nitrifying and denitrifying, butyric-acid, cellulose decomposing aerobes and anaerobes, sulfate-reducing and thionic, iron-oxidizing, actinomycetes and mold fungi.

3.RESULTS AND DISCUSSION

3.1. *Physical and chemical characteristics of therapeutic mud and surface waters*

Physical and chemical properties of therapeutic mud of lake Utinoye were evaluated by the results of works carried out in 1962, 1991, 1996, 2010, 2012 and the studies of therapeutic mud particular properties, carried out in 2004, 2007, 2008, 2011, 2013.

The chemical composition of the surface waters is characterized as mineralized, chloride-sulphate, sodium-calcium, fresh (800-954 mg/dm³), with a total hardness of 4.6–6.5 mg-EQ/dm³, it is slightly acid-alkaline (pH 6,0–8,71) on the level of acid-alkaline balance.

A significant share of thermal water in the lake surface water (10–40 %) causes the accumulation of chemical elements: Li, F, B, As, Mn, which concentrations not exceeding the MRL were established in the studies of bottom sediments in 2012 (Fig. 1).



Fig. 1 – Distribution of chemical elements in the mud deposit scheme of lake Utinoye.

The results of our studies of lake Utinoye therapeutic mud emphasize the high quality of peloid by the level of hydrogen sulfide (0.159% for wet mud), negative oxidation-reduction potential (−146 mV), organic carbon content (16.31% for absolute dry weight). The ratio C / N is 12.74. Summing up the variation analysis of the characteristics of lake Utinoye mud deposit since 1962–2012, we note the decrease in its physical size.

Mechanical pollution by particles > 0.25 mm, % (from 2.0 to 3.04) continues and increases. There is a certain degradation of the deposits: covering with turf, drainage (Fig. 1) and thermal water supply (up to 40%), as indicated by the increased volume of run-offs, their mineralization, total production of thermal waters. Parameter analysis of the long-term studies should take into account the season of observations. From this point of view, the data of 2010 and 2012 (winter) differ in a number of parameters, although they are stable in most parameters.

Biochemical studies of therapeutic mud showed that the content of organic carbon in terms of an absolute dry weight is 16.31%; nitrogen – 1.28%. The ratio of carbon to nitrogen is 12.74, which is typical for organic substrates. The content of humic acids in terms of an absolute dry weight is 8.03%; lipids – 0.99%. Carotenoids are determined in the composition of lipids in an amount of 20.99 mg%. 100 g abs. dry mud contains 8.43 µg of chlorophyll "a", 0.80 µg of chlorophyll "b", chlorophyll "c" is not found. Low pigment index (1.68) indicates the predominance of carotenoids and a relatively small variety of pigment sources in the mud. Chlorophyll in the mud is quite stable, as showed by the low content of pheophytin "a" – 18,01% of the chlorophyll content.

Humic acids are characterized by a high ratio of $E_{465} / E_{665} = 5,48$, which may indicate a relative "youth" and a weak condensation of the aromatic ring of humic acids. It was found that the content of fulvic acids is 2.15% of abs. dry weight of mud. In parallel, the content of iron was determined in the centrifuge. It was found that 1 g of fulvic acid binds 4.42 mg of iron.

In the summer-autumn season of 2013, the degree of anthropogenic and technogenic pollution of the therapeutic mud of the lake Utinoye deposit with toxic metals contained in the

feeding waters of the lake was investigated. Studies were conducted in 4 samples of therapeutic mud and 6 samples of water.

The content of microelements in the therapeutic mud samples is – in the centrifuge: Cu (0.006–0.028 mg/l); Ni (<0.001–0.007 mg/l); Zn (0.043–0.087 mg/l); Mn (0.084–0.089 mg/l); Co (<0.005 mg/l); in the sediment: Cu (0.15–12.0 mg/kg); Ni (3.7–4.0 mg/kg); Zn (11.0–18.4 mg/l); Mn (32.2–60.0 mg/l); Co (<0.005 mg/l). MAC of these elements is: Cu – 1,000 mg/kg; Ni – 0.100 mg/l; Zn – 1,000 mg/l; Mn – 0.100 mg/l; Co – 0.100 mg/l.

Concentrations of metals in the mud are characterized by the following series: point No. 3 – Mn > Zn > Cu > Ni > Co (centrifugate), Mn > Zn > Ni > Cu > Co (solid phase); point No. 4 – Zn = Mn > Cu > Ni > Co (centrifugate), Cu > Mn > Zn > Ni > Co (solid phase); point No. 5 – Mn > Zn > Cu > Ni > Co (centrifugate), Cu > Mn > Zn > Ni > Co (solid phase); point No. 6 – Mn > Zn > Cu > Ni > Co (centrifugate), Mn > Zn > Cu > Ni > Co (solid phase). The content of toxic metals in all samples of therapeutic mud does not exceed MPC.

The contents of toxic elements in the samples of the lake Utinoye surface waters is: Cu – 0,002–0,009 mg/l; Ni <0,002–0,008 mg/l; Zn – 0.005 s–0,084 mg/l; Mn – <0,005–0,036 mg/l; Co – <0,005–0,020 mg/l. MPC of surface waters is the same as of the bottom sediments.

Concentrations of metals in samples of the surface waters of the lake are characterized by the following series: point No. 3 – Co > Mn > Ni > Cu > Zn; point No. 6 – Zn = Mn > Ni > Cu > Co; point No. 4 – Zn > Mn Cu > Ni > Co; point No. 7 – Zn > Co = Mn > Cu = Ni; point No. 5 – Zn > Mn > Co > Cu > Ni; point No. 12 – Zn > Cu > Mn > Ni.

The analysis of these data shows that runoff waters of the health resort are of thermal and sewage origin in the ratio of the studied elements. Runoff water is characterized by higher mineralization and content of calcium ions, thermal water contains micro and macro components, characteristic of the Paratunka nitrogen-silicon thermal waters (Muradov, 2013). It has been recently observed that when the share of thermal water reaches 40% in the feed waters of lake Utinoye, the concentration of lithium, zinc, arsenic, etc. create antibacterial background, hindering the development of the autochthonous microflora, but do not exceed the maximum permissible concentration of toxic metals in therapeutic mud.

The results of the research establish that the therapeutic mud of Lake Utinoye does not contain harmful substances and heavy metals in quantities exceeding the MPC for natural substrates, and therefore can be used for medical procedures under the condition of sanitary and bacteriological safety. Biochemical studies of therapeutic mud characterize the high content of humic and fulvic acids, lipids, carotenoids. Conducted during the deposit exploitation and modern studies research has shown that Lake Utinoye is a stable deposit of high-quality silt sulfide therapeutic mud on the physical, physical-chemical, biochemical properties. The increasing share of thermal mineral waters of Paratunsky geothermal deposit in the feed waters of lake Utinoye determines the exceptional conditions of therapeutic mud formation on the content of trace elements that enlarge the balneological properties of the peloid, but creating a factor of toxic effect on the autochthonous microflora.

3.2. Sanitary and microbiological characteristics of therapeutic mud and surface waters

The sanitary condition of the "Lake Utinoye" mud deposit was assessed as unsatisfactory over the years at different times: 1962, 1990, 1996, 2004, 2007, 2010, 2012, 2013. The sources of pollution of the reservoir are mainly sewage discharge into the streams Paratunsky and Korkin (Fig. 2), which are the main source of hydro-mineral supply of the lake and have a negative impact on the sanitary condition of the lake and its bottom sediments.

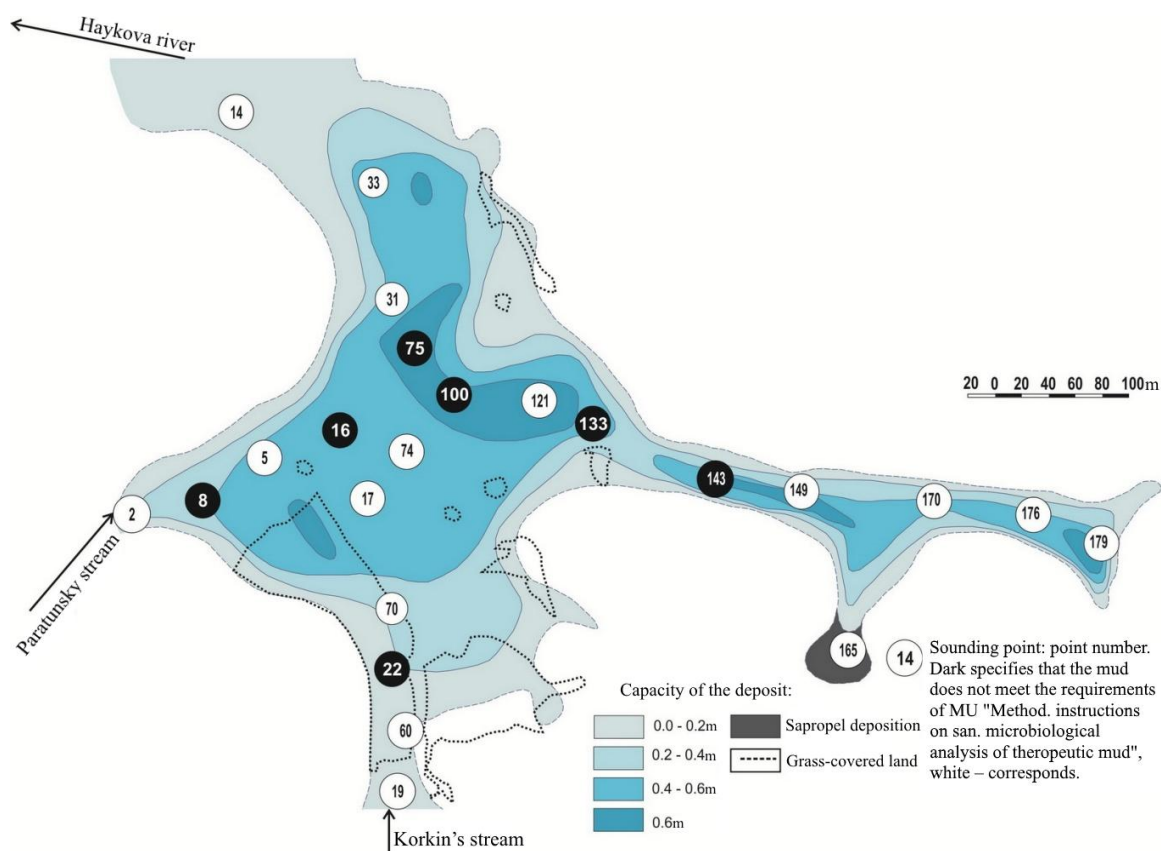


Fig. 2 – Plan of the lake Utinoye mud deposits in isopachytes

In 2013, while studying sanitary and microbiological state of the therapeutic mud deposit, the sampling of bottom sediments was not made during the flood. According to the bacteriological index of the sediment and surface water of the entire observation period it was found that the samples of mud deposits, selected 13.09.2013, were substandard (75% of samples – lactose-positive *Bacillus* titre, the titre of enterococci and clostridia titre); the samples selected 11.10.2013, were conditional on 75% of the samples (one sample was substandard for the titer of *Clostridium*), which corresponds to the results of many years observations.

It was found that the surface water during the flood (2 samples dated 28.06.13) contains a greater number of coliform bacteria-3336 and 2572 CFU/100 ml. According to the requirements for the purity of reservoirs 1 and 2 categories (this is the position of the lake Utinoye), the norm of sanitary-indicatory microbes in water should be respectively no more than 1000 and 500 (CFU/100 ml). Surface water, studied in two samples dated 13.09.13 does not contain coliform bacteria. Surface water in two samples selected 11.10.2013 contains coliform bacteria, which is significantly below the established norms: sample № 1 – 21 CFU/100 ml; sample №2 – 20 CFU/100 ml.

In summer-autumn observations in 2013, during the flood period there was a sanitary pollution of lake waters by thermotolerant coliform bacteria (CFU/100 ml). In the low streamflow period, there is a normal level of sanitary indicators and the absence of pathogenic microflora, this is due to the fact that foreign polluting objects are not brought into the lake because of the maintenance of its rim.

Thus, during the period of studies the sanitary condition of the surface waters has improved, which may be associated with water protection measures, but the share of thermal waters in the lake feed waters has increased. It can be noted that the titer of the *E. coli* increased according to the results of studies in 2010–2013 and the absence of *Clostridium perfringens* in the samples of therapeutic mud with full absence of pathogenic microflora. The unsatisfactory sanitary condition of the deposit was confirmed by the results of sanitary and bacteriological analyses of lake water

and therapeutic mud in 2004 and in subsequent studies in 2007, 2010, 2012, 2013. As a result of long time observations of the changes in sanitary and microbiological indicators of therapeutic mud of the lake Utinoye, nonconformity to the standards of the content of opportunistic (*Escherichia coli* and *Clostridium perfringens*) flora (in the absence of pathogenic microorganisms in the mud deposits and surface waters) was found.

Samples of mud solution in the initial (lake) mud and activated in aerobic and anaerobic conditions were also studied using molecular biological approaches.

The development of physiological activity of microbes involved in the formation of therapeutic mud leads to the displacement of the introduced microflora, purification of the peloid [8], [15].

The natural composition of bottom sediments microflora of lake Utinoye is represented by physiological groups of microorganisms involved in the process of mineralization of organic and mineral compounds of lake sediments and in the formation of their therapeutic properties (Fig. 3). The diagram shows the initial level of presence of physiological groups and changes in their activity in the processes of regeneration and activation. Domination of putrefactive aerobes (NH_3), butyric bacteria, denitrifying (N_2O) was registered. Specific silt microflora of therapeutic mud is diverse and sufficient in count, but cleaning power of the lake therapeutic mud is lowered in connection with the anthropogenic impact of exploitation of the Nizhne-Paratunsky geothermal field.

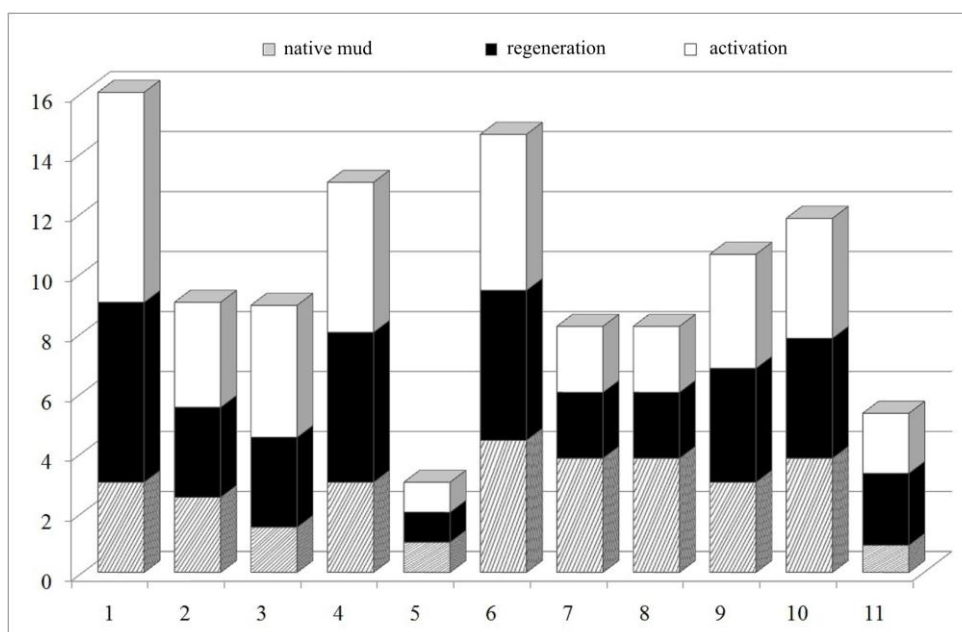


Fig. 3 – Microbiological parameters of the lake Utinoye therapeutic mud in the processes of regeneration and activation. Abscissa axis – physiological groups of microorganisms: 1 – putrefactive aerobes (NH_3); 2 – putrefactive aerobes (H_2S); 3 – putrefactive anaerobes; 4 – denitrifying (N_2O); 5 – denitrifying (N_2); 6 – butyrate; 7 – cellulose fermenting aerobes; 8 – cellulose fermenting anaerobes; 9 – sulfate-reducing; 10 – thionic; 11 – mold fungi. Ordinate axis – lgN (N-number of microorganisms in 1 g of therapeutic mud)

3.3. Ecological state and conditions of the therapeutic mud deposit

Assessment of the mud appropriateness for medical procedures is made by physical, physico-chemical and bacteriological parameters [2].

The level of requirements for therapeutic mud by physical and chemical, sanitary and bacteriological and toxicological parameters corresponds to the average results of long-term research of the lake Utinoye therapeutic mud and similar deposits of silt sulfide mud. The exception is the mechanical pollution of the lake Utinoye peloid by particles of 0.25–5 mm, the

level of which increases to 3.5%, as well as the decreasing bacterial contamination of the peloid by the *E. coli*, the titer of which corresponds to the norm of 75% in the samples (2012), while in 1990 and 1996 100% of the samples did not meet the sanitary standards.

The main idea of the basic requirements for the ecological state of therapeutic mud is to ensure the development of autochthonous microflora of the peloid, which forms its biological activity. The parameters of the lake Utinoye therapeutic mud do not contradict these conditions, except for mechanical (sand) pollution, which changes the thermal properties of the peloid, and the level of sanitary-indicative microorganisms *E. coli* and *C. perfringens*, the composition and number of which vary by seasons.

The ecological state of therapeutic mud is determined by the state of the abiotic component of the peloid: mud solution, crystalline skeleton and colloidal complex, which largely determines the conditions of existence, and, consequently, the composition and biological activity of the microbial complex. These components of the peloid interact with pollutants with different involvement depending on the type of the pollutants and change the total state, expressed in the peloid appropriateness for therapeutic use [8], [10].

Three types of environmental situations correspond to the types of deposits according to the levels of pollution: a) satisfactory; b) unsatisfactory; C) dangerous [1].

The main physical and chemical parameters of the lake Utinoye therapeutic mud correspond to the established conditions. The results of physical and chemical studies of the lake Utinoye therapeutic mud made in 1963, 1990 and 1996 indicate the stability of their parameters. The increase in the content of Ca^{2+} ions has been associated with the flow of waste and in a greater degree thermomineral water into the lake feed system recently. High level of organic matters content is characteristic of transitional type of silt sulfide mud to the sapropel. Among the characteristic properties of the Lake Utinoye mud should be noted more acidic reaction and lower content of iron sulfides, as well as involvement in the genesis of mud deposits of thermal waters, bringing microelements into the peloid (within the MPC), enriching its balneological effect similarly to the mount therapeutic mud.

Various researchers [2], [4], [6], [5], [12] specify the technical requirements for therapeutic muds ready for treatment procedures. The data in table 1 reflect the most important physical parameters of the mud (humidity, impurity, shear resistance) and their compliance with the approved standards according to the results of our studies (1990–2007) as the most typical according to season. When these parameters correspond to the established standards, there is a tendency to increase clogging (sand), increase in shear resistance, and a higher level of mud moisture (74–78%), as well as to reduce TBC (< 500 thousand/g), coli-titer and titer-perfringens in 60% of the studied samples.

Table 1 – Compliance of therapeutic mud quality of the lake Utinoye deposit with the accepted standards (Muradov, 2000)

Parameters	Dimension	Norm	1990	1996	2007
Moisture	%	25-75	74	78	78,56
Impurity with the particles 0,25–0,5 mm in size	% from natural material	Not more 3	3,4	3,5	2,79
Hard mineral spots not more 0,5 mm in size	—”—	absent	absent	absent	absent
Shear resistance	dyne/cm ²	1500–4000	2600	2700	2305
Sanitary-and-bacteriologic parameters					
Aerobes	Bacteria in 1 gr	Not more 500 thous.	Less than 500 thous.	Less than 500 thous.	Less than 500 thous.
Coli-titer	gr. per 1 bacterium	10 and more	in 40 % samples	in 40 % samples	in 40 % samples

Titer-perfringens	gr. per 1 bacterium	0,1 and more	in 80 % samples	in 80 % samples	in 80 % samples
Pathogenic coccal microflora	Bacteria in 1 gr.	absent	absent	absent	absent

Environmental assessment of the "Lake Utinoye" deposit is recognized as unsatisfactory – corresponding to the contaminated deposits, its resources can be used for medicinal purposes only after preliminary preparation (self-cleaning, pasteurization or sterilization of mud), as well as developed by us activation processing of the peloid and obtaining product of controlled conditions.

Conditions as stable properties of the therapeutic mud are based on the geological exploration results and analytical studies conducted on the deposit in 1962, 1990, 1996, 2008, 2010. Chemical composition of the surface waters is characterized as mineralized water, slightly acidic slightly alkaline, chloride-sulphate, sodium-calcium, fresh (800–954 mg/dm³) with a total hardness 4,6–6,5 mg-EQ/dm³, the level of acid-alkaline balance is slightly acidic–slightly alkaline (pH 6.0–of 8.71). According to the "Criteria for assessing the quality of therapeutic mud during its exploration, application and protection" of the Ministry of health of the USSR (1987), the mud of the lake Utinoye deposit can be classified as therapeutic silt, low-mineralized, medium-sulphide.

According to the classification of mineral waters and therapeutic muds for the purposes of their certification [13], the lake Utinoye peloid is recognized as highly sulphide, low mineralized, labeled as Paratunsky type, 1,0–1,5 g/l mineralization, >0,500 g/l sulfides, > 90% ash content, 7,0–9,0 pH, -500–0 Eh, 45–75% moisture. In general, according to its physical and chemical parameters the mud of the lake Utinoye deposit corresponds to the conditions developed for this type of peloids, except for high clogging (the norm is not more than 3%) and slightly increased moisture (the norm is not more than 75%). The closest analogue of the considered muds are the peloids of lake Maly Shirlan and Lunevsky lake of Pskov region.

Balance reserves of the lake Utinoye deposit is 70 560 m with an average power of 0.44 m, which allows to place it to the medium category capable to ensure proper functioning of the mud baths of the region for more than 50 years at the stated needs of 500 m³/year.

3.4. Rehabilitation of the mud sanitary conditions and effectiveness of application

Analysis of the mud samples from lake Utinoye, selected in 2013, showed their non-compliance with sanitary-bacteriological requirements of peloids. Low mineralization of the peloid in combination with the high content of organic substances of domestic origin in it creates the conditions for long-term preservation of potentially pathogenic and pathogenic microorganisms in the mud substrate. Investigation of the mud self-purification timing from the mentioned above bacteria was carried out by incubation in the mud storage and activation, including heating, stirring, aeration, liquefaction with water. For this purpose, the collected mud stored at a temperature of 20–22°C was analyzed every 30 days before the normalization of its sanitary and bacteriological parameters.

According to the data in figure 4, the number of coli-titers and titer-perfringens reached simultaneously the required level after 4 months. The same results were obtained by activating the mud by heating, stirring, liquefying with water in another time scale – 5, 10, 15 hours. After artificial contamination of the incubated mud experiment I (passive regeneration, Fig. 4) with sanitary-indicative microorganisms, their coli-titers decreased from 10 to 10⁻³, titer-perfringens – from 10⁻¹ to 10⁻³, the size of the titers of *Pseudomonas aeruginosa* and *Staphylococcus aureus* reached 10⁻¹, the total number of aerobic saprophytes increased 60 times. The initial level of *E. coli* and *C. perfringens* corresponds to the norms.

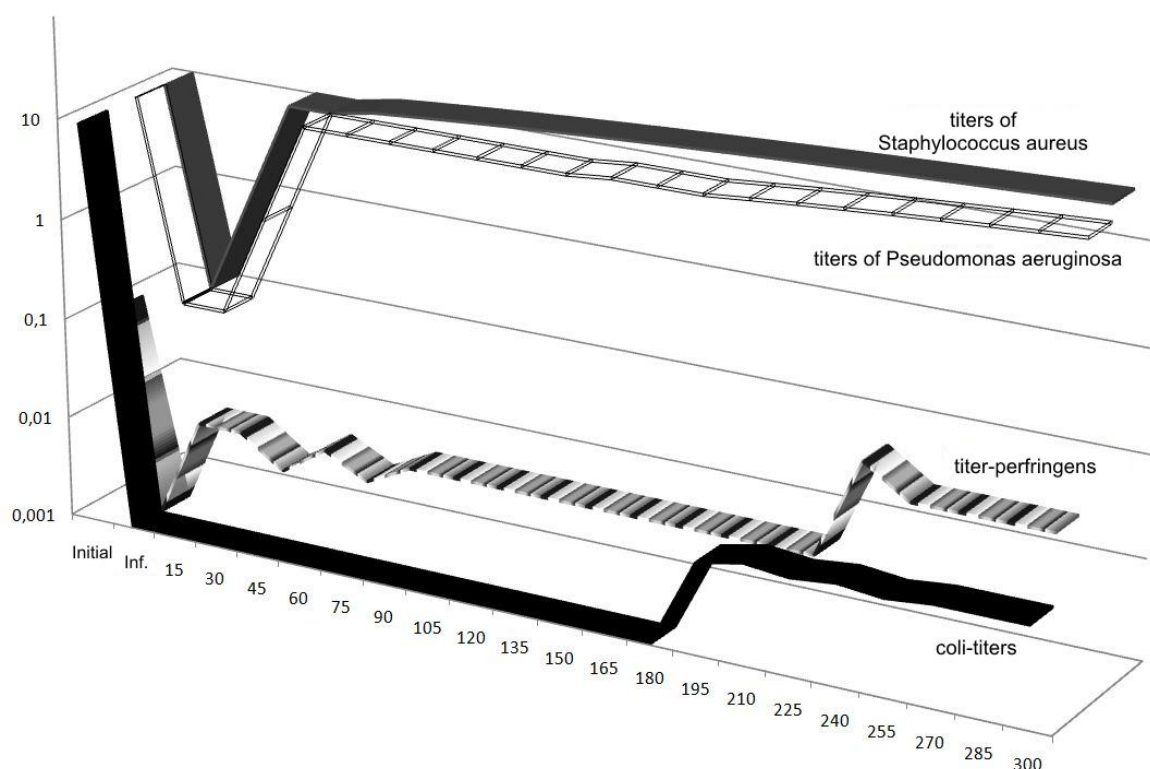


Fig. 4 – The dynamics of sanitary and bacteriological parameters of the lake Utinoye therapeutic mud in the process of its regeneration after infection. On the x-axis – time, days; on the y-axis – titer.

Further observations showed a gradual increase in the titer of *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their normalization by titer, correspondingly by 30 and 45 days of experience. Another pattern was observed with respect to the inoculation of *E. coli* and *C. perfringens*. For seven months of mud regeneration its number had increased only by one order (from 10^{-3} to 10^{-2}), and in the next three months remained at the same level (Fig. 4). Similar data were obtained after inoculation of *C. perfringens*. By day 15 of the experiment titer-perfringens had increased by one order (from 10^{-3} to 10^{-2}), and then it had not changed up to day 300 of observations. Exactly the same patterns were observed in relation to the dynamics of sanitary-indicative microorganisms survival in the experiments on regeneration in unheated and heated muds. So, both heated and unheated muds were purified from *Staphylococcus* to 30-day and from *Pseudomonas aeruginosa* — to 45-day experiments, whereas a coli-titer and titer-perfringens had not reached the norm for 10 months of observations.

Improving the efficiency of the process of self-cleaning with the introduction of long-term heating, mechanical activation, liquefaction with water, creating conditions for aerobic or anaerobic incubation are closely related to the biological activity of therapeutic mud, due to the accumulation of byproducts of the autochthonous microflora. The most pronounced of them according to antibacterial effect on the imported pathogenic and opportunistic pathogenic microorganisms are: pH, Eh, content of ammonia, hydrogen sulfide. The total microbial number in these processes is closely related to the amount and activity of the peloid-forming autochthonous microflora. Mud with natural contamination and with the introduction of sanitary-indicative and pathogenic microorganisms (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) is guaranteed to be cleaned with prolonged heating, activation, deliquation of mud with water and change of extractant. The minimum duration of this process is 10 hours.

In our experiments, the duration of the activation process of substandard mud was 18 hours. Probing of the total number of microorganisms in this process shows its increase in 2–3 hours of activation (Fig. 5). Patterns of changes in the ratio and number of physiological groups of

microorganisms correspond to the process of regeneration during mud incubation, but in a different, accelerated time scale. After 18 hours of activation, the total number of microorganisms is reduced to the level of therapeutic mud conditions (Fig. 3, 5). The comparative efficiency of regeneration by incubation in the mud storage and activation is shown in table 2.

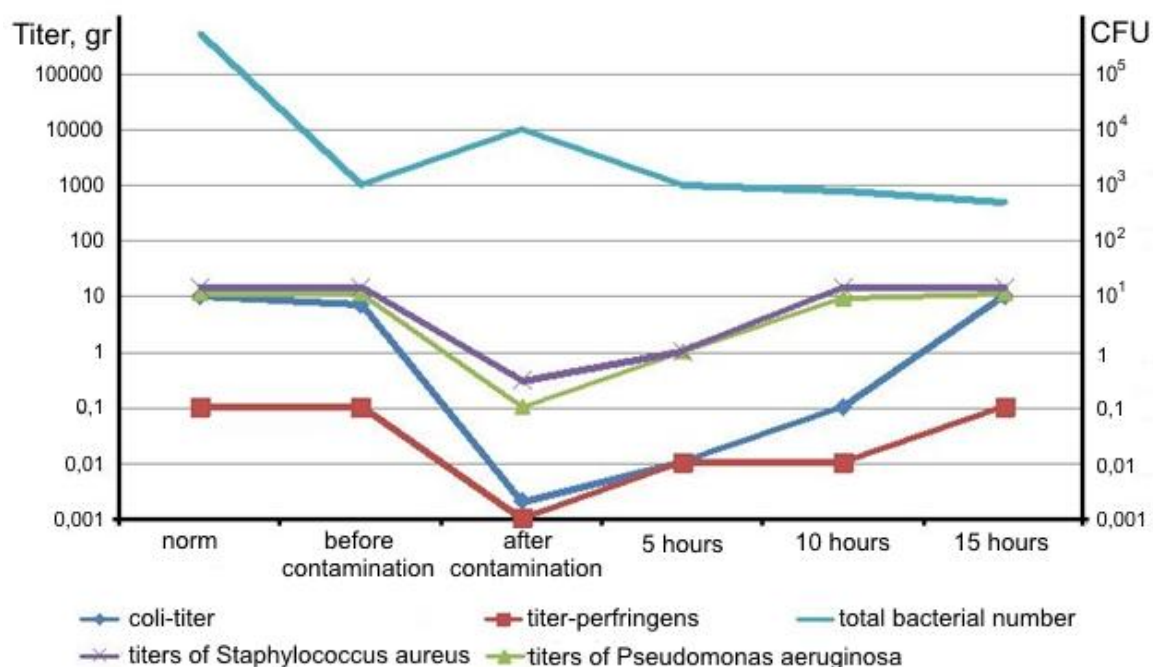


Fig. 5 – Dynamics of *Escherichia coli* and *Clostridium perfringens* survival, *Staphylococcus aureus*, *Pseudomonas aeruginosa* during activation of therapeutic mud of lake Utinoye

Table 2 – Dynamics of survival of *E. coli* and *C. perfringens* in the peloid of lake Utinoye during incubation in the mud storage and activation by temperature, stirring and deliquation

Term of mud selection	Coli-titers (norm 10 and more, cm ³)	Titer-perfringens (norm 10 and more, cm ³)
Incubation in the mud storage		
1 month	10 ⁻²	10 ⁻²
2 month	10 ⁻²	10 ⁻²
3 month	10 ⁻¹	10 ⁻²
4 month	10	10 ⁻¹
Activation		
5 hours	10 ⁻²	10 ⁻²
10 hours	10 ⁻¹	10 ⁻²
15 hours	10	10 ⁻¹

Our developed method of therapeutic mud ecological activation provides fast purification from the introduced sanitary-indicative – *E. coli* and *C. perfringens* and pathogenic microorganisms of various systematic groups.

As established in our study, the activation of the mud silt mixture by deliquation, stirring, warming up at 45°C after 5 hours of the process activates the microbial mud community in the total number up to $3 \cdot 10^3$, corresponding to 15 days of incubation of mud in the mud storage.

Next, 10-hours mud processing increases the total number of microorganisms to $8 \cdot 10^2$, corresponding to almost 300-days incubation in the mud storage.

Fifteen-hours activation of therapeutic mud provides not only a high number of microorganisms, but also a change in the biochemical properties of the mud solution and

purification from opportunistic pathogenic and pathogenic microorganisms, experimentally introduced into the mud medium.

Predominance of ammonium and denitrifying bacteria in microbial cenosis, significant amount of sulphates (70–80 mg/EQ.%) in the liquid phase of lake Utinoye mud in the presence of organic matters enable anaerobic conditions to develop rapidly another group of microorganisms, which are sulphate-reducing, and they principally determine the status of the studied peloid as the medium sulphide variety.

Turning to the consideration of physical and chemical parameters of mud before their contamination and after 300 days of storage and correspondingly 5, 10, 15 hours of activation, it should be immediately said that in both experiments, these data differ slightly from each other.

The chemical composition and physico-chemical parameters of therapeutic mud in the activation process vary due to the physiological activity of a specific autochthonous microflora. Among other changes that have occurred in the parameters of the mud in the regeneration and activation experiments, there is a decrease in the sulfate ion content in the mud solution and a corresponding increase in the amount of ion-hydrocarbonate due to the intensive life of sulfate-reducing bacteria.

The decrease in the total mineralization of the mud can probably be attributed to liquefaction, substrate watering, shifting, stirring, contamination, sampling. In general, the main parameters of the mud before and after the experiments correspond to the original type of peloid (table. 3).

Table 3 – Physico-chemical parameters of the lake Utinoye therapeutic muds before and after regeneration and activation processes (heating, stirring, deliquation)

Parameter	Before regeneration	After regeneration	Before activation	After activation
Shear resistance, dyne/cm ²	7198	300	2700	400
Eh of a solution	-85	-140	-90mV	-135 mV
pH of a solution	7,5	7,5	5,6	7,6
Total mineralization, g/ l	4,8	2,7	5,0	2,6
The chemical composition of the mud solution	$\frac{\text{SO}_4}{82}$ $\frac{\text{Cl}}{17}$ Ca 55 (Na+K) 38	$\frac{\text{SO}_4}{65}$ $\frac{\text{Cl}}{27}$ (Na+K) 52 Ca 43	$\frac{\text{SO}_4}{82}$ $\frac{\text{Cl}}{17}$ Ca 59 (Na+K) 35	$\frac{\text{SO}_4}{57}$ $\frac{\text{Cl}}{28}$ $\frac{\text{HCO}_3}{15}$ (Na+K) 53 Ca 39

Therapeutic mud with natural contamination and with the introduction of sanitary-indicative and pathogenic microorganisms (*E. coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) is guaranteed to be cleaned with prolonged heating, activation, deliquation of mud with water and change of extractant. The minimum duration of this process is 10 hours. In our experiments, the duration of the activation process of substandard mud was 18 hours. Probing of the total number of microorganisms in this process shows its increase in 2–3 hours of activation. Regularity of changes in the proportion and number of physiological groups of microorganisms correspond to the process of regeneration during mud incubation, but in a different, accelerated time scale.

After 18 hours of activation, the total number of microorganisms is maintained at a high level with the normalization of therapeutic mud conditions according to the content of sanitary-indicative flora. The chemical composition and physico-chemical parameters of therapeutic mud in the activation process regularly change due to the directions of physiological activity of specific autochthonous microflora in therapeutic mud.

The structural and functional characteristics of the microbial community naturally change during the activation of therapeutic mud, being the main processor of changes in its physical, chemical and biological properties [9]. When the share of thermal water reaches 40% in the feeding waters of Lake Utinoye, which has been observed in recent years, there is an increase in the concentration of Li, Zn, As, etc. (table 4).

Table 4 – content of trace elements in the water extract of the lake Utinoye therapeutic mud (WETM) and thermal water of Nizhne-Paratunsky springs

Trace elements	content, mcg/l		
	WETM	Thermal water	WETM on thermal water
Lithium	25,01	140,04	66
Bohr	680,33	770,12	440
Aluminum	510,03	100,03	1000
Scandium	3,11	4,14	3,0
Manganese	890,31	17,03	1300
Cobalt	60,02	41,21	38
Zinc	30,04	280,02	45
Arsenic	13,13	69,01	64
Bromine	240,43	800,32	480
Rubidium	11,21	30,12	15
Molybdenum	15,32	18,01	15
Silver	0,49	0,99	1,2
Antimony	2,31	0,76	0,76
Iodine	3,30	37,00	27
Bismuth	0,20	0,20	0,2
Tungsten	2,00	18,00	2,0

Dilution of therapeutic mud during activation reduces the concentration of trace elements of mud solution and thus stimulates the activity of microflora and biological activity of the peloid. Long-term re-extraction of therapeutic mud withdraws trace elements combined with the solid substance of the peloid. It explains the higher level of manganese, aluminum, cobalt, antimony in extracted solutions, compared with the content of trace elements in thermal water (table 4).

Historically, waters with similar physical and chemical properties from the Nizhne-Paratunsky springs flowed into the mud deposit, but their volume was not comparable with the volume of feed surface and spring waters.

The content of individual trace elements in the product WETM depends on the effect of thermal water on the mud formation and technology of product formulation. The composition of the controlled trace elements in the product WETM includes: Al, Fe, Mn, Mo, Cu, As, Ti, Sr, Si, Ag (table 4). As a factor of positive balneological action, these metals inhibit a specific microbial community of therapeutic mud.

Series of trace elements in WETM, put in descending order, are as following: Mn > B > Al > Br > Co > Zn > Li > Mo > As > Rb > Sc > Sb > W > Ag > Bi. Series of trace elements in thermal water are as follows: Br > B > Zn > Li > Al > As > Co > I > Rb > Mo > W > Mn > Sc > Ag > Sb > Bi.

A significant decrease in the content of toxic elements in the process of activation of therapeutic mud reduces their overwhelming influence on the autochthonous microflora of therapeutic mud, stimulates the cleaning ability of the peloid, increases its biological activity and includes trace elements in the balneological factors of therapeutic action similarly to the mount therapeutic mud [9].

Physical and chemical factors affecting biological activity of the peloid, can reach significant levels with prolonged activation of therapeutic mud in the reactor, providing the conditions of incubation. The activation process lasted 30 days in both aerobic and anaerobic incubation variants. The observed changes in the physical and chemical properties of extracts in the activation processes are explained by the development of the corresponding physiological groups of the microbial community of therapeutic mud.

In anaerobic conditions, achieved by the insulating layer of paraffinic oil, stabilization and accumulation of organic matter, intermediate products of delayed anaerobic dissimilation occurs. Mineralized extracts have a high level of antibacterial activity in relation to the introduced microflora, and also have a reflex-irritant effect. Extracts after anaerobic activation have a high content of BAS, including protein substances, amino acids, vitamins, humates, which have anti-inflammatory, wound-healing effect.

Aerobic activation processes, which are intensive in their nature, expending organic substances, cause significant mineralization of mud solution, acidify it and make the process terminal, after which the extracted liquid fraction has a pronounced antibacterial and reflex-irritant effect.

Balancing of the change directions of the mud substrate happens in anaerobic processes while accumulating gidrotroilit, mud becomes dark in color, contains a large number of biologically active substances. Anaerobic reverse recovery is possible after the aerobic process of peloid transformation, requiring organic nutrition and the removal of the final products by dilution with distilled water.

Microbiological properties of mud solution were analyzed for the content of microorganisms in the product itself, as well as by its impact on the development of cultures of pathogenic and opportunistic pathogenic bacteria of various systematic, morphological, physiological, biochemical groups – cocci, *Bacillus* (rods), capsule – forming, spore-bearing, gram-positive, gram-negative.

In the initial concentration, the solution of activated therapeutic mud inhibits the growth of *Staphylococcus aureus* and *Kl. pneumoniae* (capsule microorganism). The product had the most significant effect on gram-negative microorganism *E. coli*. The studied substance of an aqueous extract of therapeutic mud (product WETM) in the in vitro system has a depressing effect on *S. aureus*, *Kl. pneumonia* and has bactericidal effects on *E. coli* [8], [11].

Antibacterial action of mud solutions is revealed using discdiffusion method (impact on the monolayer of bacteria grown on 3% MPA). The extracts form the clarifying zone in a monolayer culture of *Escherichia coli*. The most pronounced antibacterial effect of the action is observed in aerobic activation of the peloid [9]. The total microbial contamination of mud solution (mesophilic aerobes and optional anaerobes) was not higher than 300 colony-forming units per milliliter, which corresponds to the purity of the spring water. Polymerase chain reaction diagnostics of the sanitary-indicative microflora presence in the process of activation and production of peloid preparations show their purity and conditioning for therapeutic use. Thus, the solution of activated therapeutic mud is not only deprived of the contaminating parameter, but also has a pronounced antibacterial effect due to extractive substances.

Activated mud solution of native mud is sulphate, sodium-calcium, slightly mineralized with a weakly acid or neutral medium reaction in composition. It has a valuable set of properties: wound healing from mechanical and burn injuries, antibacterial properties in relation to opportunistic pathogenic and pathogenic microorganisms, as well as devoid of toxicity and irritant effects on the skin and mucous membranes [9].

4.CONCLUSION

The conducted research is a significant addition to the monitoring observations of the therapeutic mud of lake Utinoye associated in its formation with the Paratunsky geothermal deposit. The degree of bacterial contamination of mud deposits and surface waters of lake Utinoye for more than 40 years of observations is shown. For the first time, experimental studies on the bacterial-chemical transformation of therapeutic mud in connection with its bacterial contamination were conducted.

The contamination of the lake surface waters with sanitary-indicative microorganisms was

identified. There is a decrease in the contamination of surface waters with the content of sanitary-indicative bacteria above the norm and in the contamination of mud-silt deposits to the permissible value of bacterial contamination.

The initial regenerative (cleaning) ability of therapeutic mud of lake Utinoye in relation to pathogenic and opportunistic pathogenic microflora was revealed. The regenerative activity of therapeutic mud was established. The method of biological testing revealed antibacterial activity of therapeutic mud of lake Utinoye in relation to opportunistic pathogenic and pathogenic microorganisms.

The study of physical and chemical properties of therapeutic mud in connection with the study of the trace element composition of mud solutions and feed water of the mud-forming reservoir, the content and quality of organic matter of therapeutic mud is significantly supplemented.

Different levels of bacteriostatic and antibacterial action of mud extracts obtained in significantly different incubation conditions have been established. The results of the therapeutic mud activation are experimental and theoretical basis for the production of peloid preparations from solutions and extracts of lake Utinoye therapeutic mud. The dynamics of changes in the number and proportion of physiological groups of microorganisms in the processes of regeneration of the peloid were established.

The methodology of integrated environmental assessment of the mud deposit was developed to enable its use for predicting adverse ecological situations on the background of anthropogenic and technogenic impact, minimize or eliminate their effects.

The scientific results of the study allow to clarify the MPC of heavy metals and other toxicants entering the water bodies of various economic importance, as well as determine the ecological ways that improve the sanitary condition of water bodies. The results of the work allow to supplement the existing measures of nature protection with methods that restore the ecological conditions of natural substrates, generalize the importance of natural microbial communities in the trends of changes and the formation of the peloid.

Kamchatka region is the main carrier of thermal mineral water resources of modern Russia; their successful balneological application includes mud treatment, and in this regard the preservation and successful operation of the only therapeutic mud deposit in the region – "lake Utinoye".

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