Sanitary and microbiological researches of therapeutic muds of the deposit "Kossor" of the Almaty oblast

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Abstract: Deposits of therapeutic mud (peloids) are formed in natural environment under the influence of geological, physical and chemical and biological processes. There are 6 genetic types of peloids differing from each other in the formation conditions, initial material, chemical composition: sulfide silt deposits of therapeutic mud of salty reservoirs, silt deposits of fresh reservoirs and sapropel; peat formations of bogs (peat mud), mound mud, clay mud and hydrothermal mud. Research of sanitary and microbiological characteristics of therapeutic mud is topical and gives scientific foundation at constructing seasonal health resorts specializing on mud cure and development of medical and health-improving recreation.

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1.Introduction

The deposit "Kossor" is located in 42 km to the northeast from Uch-Aral town. The lake of suffusion origin takes the lowest hypsometric position in the closed lake basin that characterizes the lake as the final zone of mineral salts migration, carried out by ground waters. Besides ground waters the lake basin is also fed by waters of the pressure water-bearing horizons. The hydrologic and hydro-chemical regime of the lake is notable for its stability. Even in the period of summer low water the lake never loses its water cover. The structure of the lake brine is sulfate sodium at general mineralization of 68 g/l (August, 2013).

The area of distribution of mud deposit in "zero" borders is 5,594 sq.km, and in balance borders is (0,1 m) 4,866 sq.km. The balance mud stocks are 986,6 thousand sq.m, including those of category "A" -138,7 thousand sq.m and 847,9 thousand sq.m of category "B". Silt is of black color, soft, plastic, in the top part it is liquefied, uniform, salt saturated with the smell of hydrogen sulfide. In the extreme parts attached to the coastal strip there is mixed silt with the power up to 2 cm. The horizon of dark gray silt is soft, more condensed, uniform, also with the smell of hydrogen sulfide. Silts of both horizons are very similar in their physical and chemical characteristics and are the same kind of therapeutic mud [1].

Research methods

The research was conducted with use of the methods given below at the Regional State Enterprise with the Right for Economic Activity "Institute of Microbiology and Virology" of the Ministry of Education and Science of the RK, Microbiology Department, in Almaty.

We determined organoleptic properties of mud (color, smell, consistence, structure) and its physical and chemical properties (pH, humidity, contamination with mineral particles of 0,25-5,0 mm in size and firm mineral inclusions more than 5,0 mm in size). Humidity was determined by weight method at the temperature of 105ºC. pH was measured at pH-meter MettlerToledoMP220.

Detection and assessment of the number of ammonification bacteria was carried out on starch-and-ammonia agar of the following composition (g/l): mannitol-15,0; $K_2HPO_4 \times 3H_2O$ – 1,0; $MgSO_4 \times 7H_2O$ – 1,0; NaCl – 1,0; $CaCO_3$ – 1,0; $(NH_4)_2SO_4$, starch – 10,0, agar-agar – 20,0; pH 7,0.

Petri dishes with inoculations of soil suspension were kept in the thermostat at the temperature $(29\pm1)^0$C within 3 days for determination of presence of bacterial microorganisms, 7-14 days for determination of actinomycete and fungi. All researches were repeated three-five times. The accounting of the results was carried out as follows: the number of colonies on the dishes was summarized, then it was divided into the number of replications and multiplied by the potency. The result was expressed with the number of colony-forming unit (CFU in 1 gram of the soil) [2].

The results of the research

The received sample represents silt mud of black color. The smell is characteristic for mud; there is the smell of hydrogen sulfide. The sample has viscoplastic, pasty consistence, soft to the touch and colloid uniform mass.

According to the organoleptic properties the sample of mud corresponds to the standards sulfide silt mud.

pH, humidity, contamination with mineral particles of 0,25- 5,0 mm in size and firm mineral inclusions more than 5,0 mm in size were studied. The received data are in table 1.

Table 1 – Physical and chemical properties of the mud sample

Sample	Humidity, %		Clogging with mineral particles 0,25 - 5,0 mm in size, % of natural matter		Clogging with hard mineral inclusions more than 5,0 mm in size, % of organic matter		pH
	Norm for sulfide-silt mud	Indicator	Norm for sulfide-silt mud	Indicator	Norm for sulfide-silt mud	Indicator	Indicator
Mud sample	25 - 75	42,9	Not more than 3,0	0,3 – particles 0,25mmin size and smaller	Absence	absent	9,2

Humidity of the sample is 42,9%, at the norm for silt mud of 25 - 75%. In the sample there was no norm excess for therapeutic mud in the characteristic of particles infestation 0>0,25 mm (0,3% at the norm up to 3%). There are no hard mineral inclusions of 5,0 mm and more in size in the sample. The degree of the environment acidity estimated by pH 9,2 corresponds to the alkaline reaction of the environment. According to mud classification this mud can be included into the group of alkaline mud, pH>9,0.

According to the sanitary and microbiological properties the mud sample corresponds to the standards approved for all mud groups. In the sample there are no microorganisms testifying to existence of fecal pollution with lactose-positive colon bacillus (LCB), and also potentially pathogenic (*S. aureus*) and pathogenic (*Pseudomonas aeruginosa*) bacteria for people [3]. The total microbial number of cells (33 thousand/g of mud) does not exceed the standards (500 thousand/g of mud), Table 2.

Table 2 - Sanitary - microbiological properties of assessment of quality of the mud sample

Indicator	Dimension,	corm for all groups of mud,KOE/g of mud	Indicator
General microbal number of cells (GMN) in 1 gram of natural matter	Bacteria in 1 g	33000	Not more than 500000
Titre of general coliform bacteria (coli - titre)	For 1g of bacteria	10 and more	10 and more
Pathogenic coccus microflora (staphylococci) in 10 g of natural matter	Bacteria in 1 g	absent	absence
Pathogenic coccus microflora (streptococci) in 10 g of natural matter	Bacteria in 1 g	absent	absence
Pathogenic coccus microflora (enterococci) in 10 g of natural matter	Bacteria in 1 g	absent	absence
Blue pus bacillus *(Pseudomonas aeruginosa)* in 10 g of natural matter	Bacteria in 1 g	absent	absence

Table 3 - Natural structure of the microflora of the mud sample (bacteria, fungi, actinomycete)

Ecologic-trophic groups of microorganisms	Indicator, KOE/g of mud
Bacteria (general number)	$3,6x10^2$ KOE/g of mud
Actinomycete	$2,0x10^1$ KOE/g of mud
Microscopic fungi	$1,0x10^1$ KOE/g of mud

In the microbial cenosis of the studied mud sample there are the following groups of microorganisms (growth on oat agar): bacteria – 92,3%, actinomycete – 5,1%; micromycete – 2,6% (Table 3).

Table 4 - Physiological groups of bacteria present at the mud sample (bacteria, fungi, actinomycete)

Physiological groups of bacteria	Indicator (characteristic), KOE/ g of mud
Genus *Bacillus*	$4,7x10^2$ KOE/ g of mud
Oligotrophic bacteria	$3,8x10^2$ KOE/ g of mud
Ammonifying bacteria	$1,5x10^5$ KOE/ g of mud

Presence of the following physiological groups of bacteria was studied: bacilli, oligotrophic bacteria and ammonifiers processing the compounds of nitrogen in the soil (Table 4). Low level of presence of bacilli and oligotrophs and high level of presence of ammonifiers (10^5 KOE/g) was noted. As a whole, microflora activity in the mud sample is typical for silt sulfide mud.

1.Determination of antimicrobial action of the mud sample concerning a laboratory strain of *Staphylococcus aureus* 209P. When using dilution of test microorganism 10^5 - 10^6 KOE/ml influence of the mud sample on growth of *Staphylococcus aureus* 209P was not revealed. On Petri dishes plentiful growth of test microorganism was observed [4]. When using dilutions 10^3-10^4 KOE/ml inhibition of growth of *Staphylococcus aureus* 209P was noted. The mud sample suppresses growth of *Staphylococcus aureus* 209P in dilution 10^4, KOE/ml in 30 minutes of exposition by 20,0%, in 2 hours of exposition by

30,0%, in 4 hours by 42,5%. When using bigger dilution of test microorganism (10^3 KOE/ml) the increase in inhibition of growth of *Staphylococcus aureus* 209P was observed - in 30 minutes of exposition by 27,2%, in 4 hours by 54,5%, Table 5.

Table 5 – Research of influence of the mud sample on growth of a laboratory strain of *Staphylococcus aureus* 209P

The name of the studied sample	Dilutions	Number of colonies, KOE/ml			
		Exposition time with test-microorganism			
		30 min	60 min	120 min	240 min
Sample of silt mud	10^6 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^5 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^4 KOE/ml	160	155	140	115
	10^3 KOE/ml	40	40	32	25
Control	10^6 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^5 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^4 KOE/ml	200	200	200	200
	10^3 KOE/ml	55	55	55	55

2. Determination of antimicrobial action of a laboratory strain of *Escherichiacoli*. At use of dilution of test microorganism 10^5 - 10^6 KOE/ml influence of the mud sample on growth of *Escherichiacoli* was not revealed. On Petri dishes plentiful growth of test microorganism was observed. When using dilutions 10^3-10^4 KOE/ml was noted inhibition of growth of *Escherichiacoli [5]*. The mud sample inhibits growth of *Escherichiacoli* in dilution 10^4, KOE/ml in 30 minutes of exposition by 7,6%, in 2 hours of exposition by 28,8%, in 4 hours by 36,5%. When using bigger dilution of test microorganism (10^3 KOE/ml) the increase in inhibition of growth of *Escherichiacoli* was observed - in 30 minutes of exposition by 14,2%, in 4 hours by 37,1%, Table 6.

Table 6 – Research of influence of the mud sample on growth of a laboratory strain of *Escherichiacoli*

The name of the studied sample	Dilutions	Number of colonies, KOE/ml			
		Exposition time with test-microorganism			
		30 min	60 min	120 min	240 min
Sample of silt mud	10^6 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^5 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^4 KOE/ml	240	200	185	165
	10^3 KOE/ml	30	25	25	22
Control	10^6 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^5 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^4 KOE/ml	260	260	260	260
	10^3 KOE/ml	35	35	35	35

Table 7 – Research of influence of the mud sample on growth of a laboratory strain of *Candidaalbicans*

	Dilutions	Number of colonies, KOE/ml			
The name of the studied sample		Exposition time with test-microorganism			
		30 min	60 min	120 min	240 min
Sample of silt mud	10^6 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^5 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^4 KOE/ml	220	180	160	120
	10^3 KOE/ml	30	30	20	15
	10^6 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^5 KOE/ml	≥ 300	≥ 300	≥ 300	≥ 300
	10^4 KOE/ml	280	280	280	280
	10^3 KOE/ml	40	40	40	40

3. Determination of anti-microbial action of a laboratory strain of *Candidaalbicans*. When using dilution of test microorganism 10^5 - 10^6 KOE/ml influence of the mud sample on growth of *Candidaalbicans* was not revealed. On Petri dishes plentiful growth of test microorganism was observed.

When using dilutions 10^3-10^4 KOE/ml inhibition of growth of *Candidaalbicans* was noted.

The mud sample inhibits growth of *Candidaalbicans* in dilution 10^4, KOE/ml in 30 minutes of exposition by 21,4%, in 2 hours of exposition by 42,9%, in 4 hours by 57,1%. When using bigger dilution of test microorganism (10^3 KOE/ml) the increase in inhibition of growth *Candidaalbicans* was observed – in 30 minutes of exposition by 25,0%, in 4 hours by 62,5%, Table 7.

Conclusions

Influence of the mud sample on growth of laboratory strains of gram-positive and gram-negative opportunistic pathogenic bacteria and yeast-like fungi was studied. Bactericidal action of the mud sample at exposition from 30 minutes to 4 hours on the studied test microorganisms (*Staphylococcus aureus 209P, Escherichiacoli, Candidaalbicans*) in dilution 10^3-10^4, KOE/ml was established. When using dilutions of test microorganisms 10^5 - 10^6 KOE/ml influence of the mud sample on their growth was not established.

The inhibition of growth of test microorganisms at exposition of 30 minutes in dilution 10^4 KOE/ml by 7,6-21,4%, in dilution 10^3 KOE/ml by 14,2-25,0 % was noted. At increase of exposition time of the mud sample with microbial suspensions the inhibition of growth of test microorganisms increases: at exposition for 2 hours in dilution 10^4 KOE/ml by 36,5 – 57,1%, in dilution 10^3 KOE/ml by 37,1-62,5%. It was established that the mud sample inhibits growth of *Escherichiacoli* at least, growth of *Candidaalbicans* – the most.

There are no gangrenous bacillus and pathogenic coccus microorganisms. The Total Microbial Number (TMN) is in norm. The received results show compliance of sanitary and microbiological properties of the studied mud sample to the standard values for the mud used in balneology and physical therapy.

The natural structure of the microflora of the mud sample was presented by bacteria, actinomycetes and fungi in the ratio 36:2:1, the level of bacteria presence on oat agar is 10^2, KOE/g of mud, fungi and actinomycetes - 10^1, KOE/g of mud. High level of presence of ammonifiers 10^5 KOE/g was noted. As a whole, microflora activity in the studied mud sample is typical for silt sulfide mud.

By the results of the conducted research and according to the requirements imposed to the assessment of quality of therapeutic mud, the studied mud sample is conditioned for medical purposes.

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