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MICROBIOLOGICAL CHARACTERISTICS OF CURATIVE MUDS OF HAAPSALU BAY AND LAKE SUURLAHT, ESTONIA

Abstract. Mud-bath sanatoriums on Estonian mainland use curative mud from Haapsalu Bay and those on Saaremaa Island get mud from Lake Suurlaht. The authors investigated the microflora and the content of nitrogen compounds in these mud deposits. The same indices were used to characterize the upper water layers of curative muds.

Curative mud, a fine-grained lake or sea sediment which possesses certain curative properties, is an important natural resource in Estonia. It has developed over a long period of time under the impact of several geological, chemical, and biological factors. Curative mud is found mainly in the seabays off the western part of mainland Estonia (Läänemaa) and West-Estonian Archipelago (Kask, 1989). Estonian curative muds have been studied quite thoroughly. According to the data available, Grindel, a chemist from Riga, was the first to describe curative muds, and he also analysed them chemically in 1825 (Vadi, 1947). Schlossmann (1939) analysed curative muds for their chemical and physical properties. He also determined the abundance, species composition, and properties of bacteria in different curative mud deposits. In his monograph on Estonian curative mud Vadi (1947) deals with the physicochemical properties and structure of curative mud from the balneotherapic point of view relying on his long-term research experience and published data.

The research of the curative mud microflora has been mainly confined to evaluating the role of sulphuric bacteria in microbiological processes (Nadson, 1914; Priima and Tallmeister, 1938). Studying the sources of hydrogen sulphide formation in Estonian curative muds, Priima and Tallmeister differentiated 36 species of bacteria. All the 36 isolated strains belong to aerobic microbes of which 23 produce hydrogen sulphide. The most common bacteria in the muds are *Bacillius cereus*, *Bac. subtilis*, *Pseudomonas syncyanca*, *Achromobacter dentriticum*, *A. guttatum*, *A. geminum*, and *Serratia marcescence*.

Data on microflora are available only in a few papers. The reason is evidently that so far the research of curative mud microflora has served mainly for the purposes of mud treatment, and the investigators engaged in these studies have been interested in pathogenic microbes. The part of the microflora that is important in terms of mud formation and regeneration has deserved little attention.

The aim of the present work was to study the abundance and composition of microflora in different curative mud deposits and to evaluate on this basis their effect on the quality and ecological state of mud.

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Material and Methods

Water and mud samples were taken from the sites illustrated in Figs. 1 and 2. Water samples were collected from Haapsalu Bay and from the sites where water enters the bay. The aim was to elucidate the spread of ingredients and their effect on microbiological processes. The characteristics of water and mud samples are given in Table 1.



Fig. 1. Sampling sites in Haapsalu Bay.





Characteristics of water and mud samples

sampling	Description of sampling site	Sampling depth, cm	Sampling date	Sample code
	H	AAPSALU		
	Water			
1	Central part of Tagalahe Bay,			
	eastern part of the mud deposit		14. 05. 90	A1.1
1	**		19. 02. 91	A1.2
6	Jaama Stream, near a mill		14. 05. 90	A6
9	Outflow of municipal sewage treatment plant		14. 05. 90	A19
3	Lääne Kalur Collective Fishery,		14, 00, 50	AIU
	sewage water flow into Suur			
	Viik		14. 05. 90	A33
36	Väike Viik (a lagoon which has water exchange with Haap-			
	salu Bay through culverts)		14. 05. 90	A36
	Mud			
1				
1	Central part of Tagalahe Bay, eastern part of the mud deposit	0—10	14. 05. 90	A1.1
1	"	0-10	17. 02. 91	A1.2
1	"	10-20	14. 05. 90	A1.3
1	"	10—20	14. 05. 90	A1.3
3	Central part of Tagalahe Bay, western part of the mud deposit	0—10	14. 05. 90	A3.1
3	"		14. 05. 90	A3.2
0		10—20	14. 05. 90	A3.2
	SAARE	EMAA ISLAND		
	i like part they.			
	Mud			
1	Southern part of L. Suurlaht,		10 00 00	
	western part of the mud deposit	0—20	13. 09. 90	B1.1
1		0—20	13. 09. 90	B1.2
2	Southern part of L. Suurlaht, central part of the mud deposit	0—20	13. 09. 90	B2.1
9	"	0-20	13. 09. 90	B2.1 B2.2
2 2 2	**	20-40	13. 09. 90	B2.2 B2.3
	"	20-40	13. 09. 90	B2.4
4	Southern part of L. Suurlaht, eastern part of the mud deposit	0—20	13. 09. 90	B4.1
4	"	0-20	13. 09. 90	B4.2
4 4 4		0—50 50—100	23. 02. 91 23. 02. 91	B4.3 B4.4
	**	100-150	23. 02. 91	B4.5
5	Kuressaare Bay, estuary of the	and the second	10 00 00	
	Põduste (Meedla) River	0—10	13. 09. 90	B5.1
5	11	0-10	13. 09. 90	B5.2

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* See Figs. 1 and 2 for their location.

The depth of water in Haapsalu Bay is 1.2—1.5 m; in the upper layers the mud is blackish-grey, in the lower layers it is grey. In the coastal Lake Suurlaht the mud displays different shades of grey. The thickness of the water layer above the mud deposit ranges from 1.0 to 1.5 m. In addition a mud sample taken from the relatively polluted area of Kuressaare Bay was also analysed. The results obtained are presented in this paper. As a rule, the samples were analysed one or two days after sampling, except for B1.2, B2.2, B2.4, B4.2, and B5.2. These samples were stored for five months and on the day of analysis (February 19, 1991) they were completely frozen.

In all samples, the count of ammonifying, nitrifying, denitrifying, sulphate-reducing, and aerobic cellulose-decomposing and nitrogenfixing bacteria as well as of actinomycetes, fungi, and algae was determined. The frequency of the most widespread genera of fungi and the floristic composition of algae were also estimated. Microbiological analyses were carried out by the method of serial dilutions using selective media (Практикум по микробиологии, 1976). Counts of mircoorganisms are given in the tables as 10ⁿ cells per g absolute dry weight of the substrate or per ml water. To characterize the frequency of sulphate-reducing bacteria, instead of the count the efficiency of the H₂S excretion was used. The efficiency was determined as the degree of the darkening of Pb-acetate-treated filter-paper: weak, medium, strong or nil (Практикум по микробиологии, 1976).

In the mud and water nitrogen, ion-exchange ammonium (by distillation from KCl solution), fixed ammonium (with the mixture of 1n HF and 1n HCl; Bremner, 1959), and nitrates (colorimetrically with sodium salicylate; Marhnukuň et al., 1959) were estimated. The content of nitrogen compounds is presented in the tables as total nitrogen, mg per 100 g absolute dry substrate. The nitrate and nitrite reducing activity of curative mud was estimated colorimetrically by the concentration of reduced nitrate (with disulphophenolic acid) and of nitrite (Griess reagent), respectively. The values represent the activity of the enzymes per 100 g absolute dry substrate per 24 hours.

Results and Discussion

The data on the count of the functional groups of microorganisms are presented in Tables 2—5. The results obtained show that all the microorganisms which are found in the soil occur also in water and mud. Azotobacteria serve as an exception. These are free-living aerobic microorganisms capable of binding nitrogen from atmosphere. Azotobacteria usually occur in neutral or alkaline cultivated soils and in the temperature zone waters (Стейнер et al., 1979).

Ammonifying bacteria are capable of decomposing nitrogen protein compounds up to ammonium nitrogen. The count of ammonifying bacteria was determined simultaneously in two media: x_3 and LPA (Pärsim, 1969). In water samples the number of ammonifying bacteria was one or two orders of magnitude lower than in mud samples. They were more abundant in the mud of Haapsalu Bay than in the mud of Saaremaa Island.

Denitrifying bacteria reduce nitrate nitrogen to gaseous N-products (N_2, N_2O, NO) . This group of bacteria is an important indicator for evaluating the ecological state of water. Under normal conditions the ratio of denitrifying and nitrate respiratory bacteria is 0.2:0.8 in case

all agents of anaerobiosis are regarded as equal to 1. With man-made pollution the ratio decreases and the amount of faecal nitrate respiratory bacteria increases (partly on account of the family Enterobacteriaceae). In bottom sediments, at the depth of 10—20 cm, where the competition for nitrates between denitrificators and aerobic heterotrophs decreases, denitrifying bacteria are most active. On the whole, the number of denitrifiers is two orders of magnitude higher in sediments than in water.

Table 2

	983		an in		se 02	1 mariai		25		Fungi
Sample code	mon x ³	m- ifiers, LPA 0 ⁵	Denitri- fiers, 10 ³	Nitri- fiers, 10 ¹	Aerobic cellulose decomposers, 10 ²	Sulphate reducers	Actinomy- cetes, 10 ⁴	Algae, 10 ³	Total number, 10 ⁴	Dominating genera and their percentage
A1.1	0.3	5	3	1	11	weak	3	7	8	Penicillium, 16% Mucor, 83%
A1.2	7	10	1100	0	0	weak	0	0.06	0.7	Penicillium, 92%
A6	1	2	0.6	1	0.6	weak	3	0.6	13	Penicillium, 37% Mortirella, 37% Mucor, 25%
A19	10	13	11	110	3	strong	32	0.06	5	Penicillium, 63% Mucor, 18% Fusarium, 3%
A33	8	13	11	25	7	medium	92	0.03	13	Penicillium, 51% Mucor, 38% Torula, 4% Pullularia, 4%
A36	0.3	0.2	7	1	0.6	medium	3	11	2	Penicillium, 77% Mucor, 11%

The count of functional groups of microorganisms in water samples, ml-1

Tabel 3 The count of functional groups of microorganisms in the curative mud denosit

burn	bite			an in	Se 02	fier	art -		merin	Fungi
Sample code	An monif x ³ I 10	iers, PA	Denitri- fiers, 10 ³	Nitri- fiers, 10 ¹	Aerobic cellulose decomposers, 10 ²	Sulphate reducers	Actinomy- cetes, 10 ⁴	Algae, 10 ³	Total number, 10 ⁴	Dominating genera and their percentage
A1.1	84	140	640	350	3	medium	6	640	47	Penicillium, 31% Mucor, 56%
A1.2	20	15	3300	120	6	weak	2	3	0	
A1.3	29	120	460	250	3	medium	0.5	460	21	Penicillium, 67% Torula, 33%
A1.4	8	4	1200	30	3	nil	2	250	0.6	Penicillium, 82% Mortirella, 15%
A3.1	45	19	28	280	3	weak	5	510	30	Penicillium, 64% Torula, 16%
A3.2	137	24	3	260	3	medium	2	26	20	Penicillium, 70% Pullularia, 30%

This is in good agreement with the results obtained. The samples from the Kuressaare mud deposits were an exception: there denitrifying bacteria occurred in the same amount as in water.

An interesting fact was observed in the results of the analyses made in February. In all the mud and water samples (the frozen mud samples included), the number of denitrifying bacteria was three orders of magnitude higher than that obtained in the same samples in autumn. It may be supposed that, as a result of low winter temperatures and the freezing of mud, the concentration of oxygen in the medium decreases, and this promotes the development of denitrifying bacteria.

Table 4

The count of functional groups of microorganisms in the curative mud deposit of Suurlaht Bay, g^{-1}

					se 02					Fungi
Sample code			Denitri- fiers, 10³	Nitri- fiers, 101	Aerobic cellulose decomposers, 10 ²	Sulphate reducers	Actinomy- cetes, 10 ⁴	Ålgae, 10 ³	Total number, 10 ⁴	Dominating genera and their percentage
B1.1	8	67	30	620	0.6	weak	1	120	0.9	Penicillium, 38% Mucor, 62%
B2.1	7	36	64	300	3	weak	0.6	12	0.2	Penicillium, 97%
B2.3	14	46	28	280	0.3	weak	0.3	3	0.5	Penicillium, 98%
B4.1	8	53	30	300	0.2	weak	1	65	0.1	Penicillium, 9% Mucor, 81%
B4.3	21	27	1200	290	3	strong	3	5400	5	Penicillium, 83% Mucor, 12%
B4.4	23	28	3500	30	1	weak	2	440	0.2	Penicillium, 91%
B4.5	20	19	. 440	21	0.7	strong	1	350	0.07	Penicillium, 87%
B5.1	* 8	14	5	490	13	strong	1	510	45	Penicillium, 75% Aspergillus, 25%

* - curative mud of Kuressaare Bay

Table 5

The count of functional groups of microorganisms in frozen mud samples, g-1

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					se 02	1.36			· pillin	Fungi
Sample code	mon x ³	ifiers, LPA	Denitri- fiers, 10 ³	Nitri- fiers, 10 ¹	Aerobic cellulo: decomposers, 1		Actinomy- cetes, 10 ⁴	Algae, 10 ³	Total number, 10 ⁴	Dominating genera and their percentage
B1.2	28	23	3400	300	34	nil	0.9	0	0.7	Penicillium, 63% Mucor, 16%
B2.2	20	22	3000	250	45	nil	1.5	10	0.9	Penicillium, 89%
B2.4	12	12	1200	110	2	strong	0.7	230	0.3	Penicillium, 80% Mucor, 16%
B4.2	20	22	570	110	6	medium	3	4900	0.5	Penicillium, 87%
B5.2	5	8	1000	200	17	medium	0.6	1700	0.3	Penicillium, 92%

Nitrifying bacteria oxidize ammonium and other reduced nitrogen compounds to nitrates. They are characterized by a relatively slow life cycle. The count of nitrifying bacteria in water samples showed that their development is stimulated by the presence of municipal sewage regardless of its purification. In the water samples taken from sites 19 and 33, the number of nitrifying bacteria was two or three orders of magnitude higher; this is indicative of the occurrence of slowly decomposing organic nitrogen compounds in these samples. The count of nitrifying bacteria in different mud samples was almost equal, and the differences did not exceed one order of magnitude.

Aerobic cellulose-decomposing bacteria hydrolyse plant material with a complex structure to simpler C-compounds. The results obtained show that the abundance of this group of bacteria does not in principle differ in the mud and water samples. Only in the Kuressaare Bay mud the amount of aerobic cellulose-decomposing bacteria was an order of magnitude higher.

Sulphate-reducing bacteria reduce sulphates to gaseous hydrogen sulphide. The importance of sulphuric bacteria in microbiological processes becomes evident if we consider that sulphuric acid, which is a harmful and even poisonous substance for higher organisms, is oxidized by these bacteria over elemental sulphur to sulphuric acid. However, sulphuric acid, existing in the form of a sulphuric acid salt, is an important nutrient for higher plants.

In our study material a high hydrogen sulphide concentration was observed in one water sample which had been taken from the outflow of the Haapsalu municipal sewage treatment system, and in four mud samples (all from Saaremaa Island). According to Vadi (1947) the ability of H_2S formation is by no means dependent on the count of bacteria, but on the quality of the bacteria in the corresponding mud sample.

Actinomycetes are the microorganisms which are the least sensitive to environmental factors. Actinomycetes were the most abundant in the water samples taken from the treatment systems of the town of Haapsalu and from the sewage outflow of the *Lääne Kalur* Collective Fishery (presently joint-stock company *West*). This is indicative of the amount of carbohydrates in the water.

Fungi are primary decomposers of complex organic compounds. They have a mycelial structure, but they are considerably smaller than actinomycetes. The number of fungi was the lowest in the mud of the coastal Lake Suurlaht, followed by the water of Haapsalu Bay. Their count was the highest in the mud of Haapsalu and Kuressaare bays. The results obtained by comparing water analyses showed that the number of fungi was the highest in the water samples collected from the outflow of the Lääne Kalur Collective Fishery's sewage into Suur Viik inlet. The most abundant genus was *Penicillium*, which was found in all the samples. It was followed by *Mucor* (11 samples), *Pullularia* (3 samples), *Torula* (3 samples), and *Mortierella* (2 samples). The remaining genera (*Aspergillus*, *Fusarium*) occurred only once.

Microscopic algae are unicellular organisms, which in the daytime use light energy for their life activity; in the darkness they act as heterotrophic organisms. The number of algae differs to a great deal: it is the lowest in water samples and the highest in the mud samples taken in February. In the frozen mud the number of algae amounted to millions. In terms of species composition, blue-green and green algae and diatoms dominate in curative mud (Table 6). In the mud samples collected from Haapsalu Bay, the blue-green algae were represented only by the genus *Phormidium*, but the Saaremaa muds contained both blue-green and green algae and diatoms. The blue-green algae were especially abundant in the samples collected from the eastern part of the mud deposit in Lake Suurlaht. The genus *Phormidium* dominated, the next abundant was *Microcystis*. Green algae were dominated by *Chlorella*. The occurrence of green algae *Scenedesmus* sp. (indicator species) refers to a possible organic contamination. The species composition of algae is influenced by several ecological factors and also by increasing input of mineral substances and organic pollutants as a result of human activities.

Table 6

0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0 0 750 1300
0 0 0 0 0 1000 <i>Scenedesmus</i> sp.	0 0 0 750
0 0 1000 Scenedesmus sp.	0 0 750
0 0 1000 Scenedesmus sp.	0 750
0 0 1000 Scenedesmus sp.	0 750
0 0 1000 Scenedesmus sp.	0 750
1000 Scenedesmus sp.	10.10
1000 Scenedesmus sp.	10.10
Scenedesmus sp.	100
Chlorella sp.	
250 Chlorella sp.	1500
2000 Chlorella sp.	1800
ed assigns applying any	
250	0
Chlorella sp. 250	
	Chlorella sp. 250 Chlorella sp.

The count of systematic groups and floristic composition of algae in water and in mud samples

Nitrogen is easily transferred from one form to another, and therefore it is one of the most mobile elements in nature. Only this part of nitrogen that has been either bound in the process of biosynthesis by microbes or adsorbed by humic matter and clay particles remains in the soil.

The content of nitrate nitrogen revealed remarkable differences in the curative mud samples. In the samples collected from Haapsalu Bay, the nitrate content was seven times higher than in the samples from Lake Suurlaht on Saaremaa Island (Table 7). The results of ammonium determination showed that the curative mud of Lake Suurlaht contains more ammonium ions than nitrate ions. According to the published data, the content of ammonium nitrogen depends directly on the structure of

Table 7

The content of nitrogen compounds and enzymatic activity in water and mud, mg %

A beginned	NO LEVE	43 P. 112	Nitrogen	compounds	13 14 3 54	Enzymat	ic activity
Sample code	рНксі	NO ⁻ ₃	Ion- exchange NH+ 4	Fixed NH+	Total nitrogen	Nitrate reductase	Nitrite reductase
A1.2	io- goisia	Norden-		1 and the	REALING A		Residue a
(water)	7.2	0.98	1.12	2.52	4.62	not estin	nated
A1.2							
(mud)	7.9	4.34	23.99	10.66	38.99	136.21	10.98
A1.4	8.2	8.58	20.58	16.46	45.62	128.13	11.21
B1.2	7.9	3.16	27.16	19.01	49.33	111.55	11.32
B2.2	8.1	2.74	12.84	11.68	27.26	131.43	11.48
B2.4	8.3	0.61	4.98	5.98	11.57	87.69	2.12
B4.2	8.0	1.80	8.66	19.80	30.26	132.08	5.12
B4.3	7.9	1.60	46.65	9.60	57.85	140.12	11.20
B4.4	7.9	0.50	57.40	8.40	66.30	76.54	3.36
B4.5	7.7	0.52	34.37	9.89	44.78	60.34	5.19
B5.2	7.8	0.54	3.98	7.08	11.60	91.19	3.87

Table 8

Characteristics of mud and water samples

No. of sampling site	Characteristics	the count of

HAAPSALU BAY: water and mud samples

- 1 Aerobic processes prevail. Self-purification of water occurs. Mud formation negligible.
- 3 Mud formation in deeper sediments. Aerobic processes prevail in the topmost layer, this refers to organic matter decomposition.
- 6 Weak pollution. Higher plants favour self-purification of water.
- 19 Self-purification of water is modest. Heterotrophic processes dominate over autotrophic ones, i. e. the community of microbes capable of purifying water has not developed.
- 33 Water is cleaner than the sewage. Self-purification of water thanks to macrophytes.
- 36 Self-purification of water due to a microbe community. Aerobic processes prevail.

SAAREMAA ISLAND: mud samples

1, 2, 4

5

The organic matter released as a result of metabolic processes of microorganisms is utilized by the same microbial strains, i.e. the released organic matter does not leave the biological circulation. Mud formation barely noticeable.

The presence of aerobic and anaerobic microbial associations indicates absence of toxic substances in the mud. Decomposition of organic matter occurs. The decomposed organic matter is included in the autotrophic process.

the microflora of the count of denitrifying and nitrifying bacteria in soils. The greater the number of these organisms, the smaller the con-centration of ammonium ions. This kind of regularity was not observed in curative mud, and it has remained obscure why the processes of nitrification and denitrification tend to dominate in mud.

Nitrate reductase catalyses the first stage of denitrification, i.e. the reduction of nitrate nitrogen to nitrite nitrogen. Nitrite reductase catalyses the second reaction of denitrification process — reduction of nitrite to hydroxylamine. The data on the activity of enzymes (Table 7) show that the activity of nitrate and nitrite reduction decreases with the depth of sampling.

The above-presented data provide information on microbiological processes taking place in water and mud. Comparing the results one can obtain data on the actual state of environment. In terms of biological properties, the curative muds reveal great differences. With respect to three functional groups, the count of microorganisms was the highest in the Haapsalu curative mud deposit (Tables 3 and 4). The number of aerobic cellulose-decomposing bacteria and algae was higher in the Lake Suurlaht mud. This means that the functions which microorganisms fulfil in mud are more distinctly revealed in the Haapsalu Bay mud than in the Lake Suurlaht mud. Different biological properties of these muds characterize not only different mud deposits but also the changes which occur due to the ecological state of environment.

On the basis of bacteriobiological evidence, the ability of self-purification of water and the process of mud formation at different sampling sites are illustrated in Table 8.

The presented characterization of water and mud samples, based on the analysis of experimental data, may be somewhat speculative for the following reasons: (1) scantiness of data; (2) lack of published data on the occurrence of these microorganisms in curative mud; and (3) ecologically permissible limits for the groups of microorganisms under consideration have not yet been established.

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HAAPSALU LAHE JA SUURLAHE JÄRVE RAVIMUDA MIKROBIOLOOGILINE ISELOOMUSTUS

On uuritud Haapsalu ja Suurlahe ravimuda mikrofloorat ning lämmastikuühendite sisaldust. Mikroorganismide kolme funktsionaalse grupi (ammonifitseerivad, denitrifitseerivad ja nitrifitseerivad bakterid) arvukus Haapsalu ravimudas oli kõrgem kui Suurlahe ravimudas. Aeroobseid tselluloosi lagundavaid baktereid ja vetikaid oli siiski rohkem Suurlahe mudas. Ravimuda vetikaliikidest moodustasid põhiosa sini-, rohe- ja ränivetikad. Aktinomütseete ja seeni leidus kõige enam «Lääne-Kaluri» heitvees. Levinumaks perekonnaks osutus *Penicillium*. Nitraatlämmastikku oli rohkem Haapsalu ravimudas, ammooniumlämmastikku aga Suurlahe mudas.

Ravimuda mikrobioloogiliste protsesside uurimine võimaldab hinnata ümbritseva keskkonna ökoloogilist seisundit, muda juurdekasvu ja vee isepuhastusvõimet.

Эви ПЯРСИМ, Юри КАСК

ХАРАКТЕРИСТИКА МИКРОФЛОРЫ ЛЕЧЕБНОЙ ГРЯЗИ ЗАЛИВА ХААПСАЛУ И ОЗЕРА СУУРЛАХТ

Проведено исследование микрофлоры и содержания азота в лечебной грязи Хаапсалуского залива и озера Суурлахт. Численность микроорганизмов в трех изученных функциональных группах (аммонифицирующих, денитрифицирующих, нитрифицирующих) была выше в грязи Хаапсалуского залива, а численность аэробных целлюлозоразлагающих бактерий и водорослей — в грязи озера Суурлахт. В видовом составе водорослей преобладали синезеленые, зеленые и диатомовые. Актиномицетов и грибов содержалось больше всего в сточных водах колхоза «Ляэне калур». Самым распространенным оказался род *Penicillium*. Содержание нитратов в грязи Хаапсалуского залива превышало их содержание в лечебной грязи озера Суурлахт, а содержание аммония, наоборот, было выше в грязи озера.

Знание микробиологических процессов в лечебной грязи позволяет оценить экологическое состояние окружающей среды, изучить генезис грязи, а также определить способность воды к самоочищению.