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RESEARCH INTO BIOLOGICAL CHARACTERICTICS OF DRIED SAPROPEL

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Abstract. **Microbiological characteristics of dried sapropel of Lake Rušona and Lake Ubagova and concrete containing sapropel and hemp sheaves (Ubagova Lake) have been studied. The antimicrobial activity was studied by the reference test cultures *Staphylococcus aureus ATCC 25923, Salmonella enteritidis ATCC 13076, Enterococcus faecalis ATCC 29212, Bacillus cereus ATCC 10876, Escherichia coli ATCC 25922, Candida albicans ATCC 10231*. Antibacterial activity on *Staphylococcus aureus* reference test cultures is stated in Rušona Lake sample before treatment with UV rays. Antibacterial activity is not stated in Ubagova Lake sapropel sample. 20 minutes' long UV ray treatment is not long enough to prevent the growth of sapropel materials. Both Rušona Lake and Ubagova Lake samples contain microorganisms that start growing and intensively reproducing in favourable conditions. When in contact with humidity, at the temperature from 18 to 37± 1 ^{0}C, mold colonies form on concrete containing sapropel and hemp sheaves, for this reason this material should not get in contact with humidity when used in construction.**

Keywords: **bacteria, fungi, mold.**

I INTRODUCTION

Sapropel is the sediment of freshwater lake mud, which is made up of more than 95% organic substances and is formed on the bottom of the body of water as a result of incomplete decomposition (oxidation) of dying and sunken biomass (different aquatic plants, phytoplankton and zooplankton, living organisms, pollen and spores of highest plants) under conditions of oxygen deprivation. The sapropel is strategic natural resources, numerous studies have shown the possibility of the effective use of sapropels in bio-energy, food industry, chemical industry, agriculture, cattle-breeding, forestry, construction industry, medicine (balneology, pharmacology, mud therapy) and cosmetics [1] – [9]. There are noticeable resources of sapropel in Latvia but insufficient study prevents the use of it. Latvia has 2256 lakes with a total area of 1001 km^2 or 1.5 % of the country. The total area of mires is 6401 km^2 or 9.9 % of Latvia. Most lakes and bogs contain sapropel deposits. The State Geology Office of Latvia states that there are more than 750 million m^3 of lake sapropel resources and about 1.5 billion m3 of sapropel reserves. There are about 2 billion m^3 of joint sapropel resources in Latvia [10]. Nowadays issues concerning ecological construction and production of environmentally friendly materials are getting even more important. A noticeable part of social resources has to be invested in construction materials, thus it is very important to produce effective and environmentally friendly materials by using local resources. Development of ecological construction materials and increasing application of them in construction expands because it gives an opportunity to save resources during production and does not pollute environment. These materials are fully recyclable and decompose in relatively short period of time after the exploitation. As the interest in economics that uses local natural resources increases, the study of sapropel use in construction material production is getting more topical. Several scientists [8], [10], [11], [12] point on use of sapropel in construction. Gružāns [13] economical calculations show that sapropel-concrete could become one of the cheapest and most available construction materials in the whole Latvia. But solid sapropel is a new, almost unused material yet. To find maximally effective application for sapropel, its mechanical, physical, chemical and biological

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characteristics should be known. The aim of this study is to determine the biological activity of solid sapropel materials.

II MATERIALS AND METHODS

In experiments Nr 1 to Nr 5 two samples were tested – the 1st sample is sapropel from Diunoklis gulf of Lake Rušona, collected from upper layer (0.3 - 0.5 m in depth), the 2nd sample is Lake Ubagova sapropel, collected from deeper layer. The samples were dried at the temperature of 20 - 23 ^{0}C and preserved in laboratory conditions for 3 months. Experiments were carried based on CLSI M100-S23 Performance standards for antimicrobial susceptibility testing [14]. In assessing of the antibacterial agents were used the following criteria: absence of zones around the wells for microbial growth inhibition or occurrence of zone areas.

For the evaluation of antimicrobial activity in experiments 1 to 4 were used the following test strains: *Staphylococcus aureus ATCC 25923*, *Salmonella enteritidis ATCC 13076*, *Enterococcus faecalis ATCC 29212*, *Bacillus cereus ATCC 10876*, *Escherichia coli ATCC 25922*, *Candida albicans ATCC 10231*. Reference test cultures were planted on PCA (Plate Count Agar) and incubated for 24 h in 37 ± 1 ^{0}C temperature.

In Experiment Nr 1 sapropel samples are divided into small pieces. MHA (Mueller-Hinton agar) is melted in boiling water bath, then cooled to the temperature of ~ 40 ^{0}C, obtained broth is poured in sterile 90 mm Petri dishes. The mixture is allowed to solidify for 20 minutes. Reference cultures are inoculated in TSB (Tryptone Soya Broth), to 0.5 McFarland (the turbidity is evaluated by comparing to 0.5 McFarland standard). Sterile cotton swab is soaked in inoculant, pressed against the inner wall of test tube, then densely draw stripes on the surface of MHA, this action is repeated two times turning Petri dish for 60 degrees each time. The Petri dish is left to dry for 15 minutes. Then small sapropel material sample is put and pressed in the dish using sterile pincers.

In Experiment Nr 2 after incubation reference cultures from PCA are inoculated in TSB, till 0.5 McFarland (the turbidity is evaluated by comparing to 0.5 McFarland standard) and incubated in thermostat for 1 h at 37 ^{0}C temperature in order to get ”refresh” substance. Sapropel samples are milled put into laminar and treated with UV rays. Sterile test tubes filled with MHA are melted in boiling water bath, then cooled to the temperature of ~ 40 ^{0}C, add reference cultures 1 ml (incubated in TSB), mixed with electrical mixer (Vortex), obtained broth is poured in sterile 90 mm Petri dishes. The mixture is allowed to solidify for 20 minutes. Using sterile instrument a hole in the broth is made. Using sterile instrument the hole is filled with sapropel.

In Experiment Nr 3 reference cultures are disseminated on PCA. Sapropel samples are milled put into laminar and treated with UV rays for 20 minutes. MHA is melted in boiling water bath, then cooled to the temperature of ~ 40 ^{0}C, obtained broth is poured in sterile 90 mm Petri dishes. Agar is allowed to solidify for 20 minutes. Reference cultures are inoculated in TSB, 0.5 McFarland (the turbidity is evaluated by comparing to 0.5 McFarland standard). Sterile cotton swab is soaked in inoculated TSB, pressed against the inner wall of test tube, then densely draw stripes on the surface of MHA, this action is repeated two times turning Petri dish for 60 degrees each time. The Petri dish is left to dry for 15 minutes. Using sterile instrument a hole in the broth is made. Using sterile instrument ½ of the hole is filled with sapropel.

In Experiment Nr 4 broth and reference cultures are made as in experiment Nr. 3. Preparatory procedure of sapropel material is different - after treating with UV rays for 20 minutes, milled sapropel sample is poured out in sterile Petri dishes, saline is added, mixed, put in thermostat at 37 ^{0}C and is allowed to “maturate” for 30 minutes. Using sterile instrument a hole in the broth is made. Using sterile instrument the hole is filled with maturated in the thermostat sapropel.

In experiment Nr 5 two microorganisms reference cultures have been used: *Staphylococcus aureus ATCC 25923*, *Escherichia coli ATCC 25922*. Reference cultures were planted on PCA and incubated for 24h at 37^0 C temperature. Sapropel samples are milled, put into laminar and treated with UV rays for 20 minutes. Reference cultures are inoculated in TSB, 0.5 McFarland (the turbidity is evaluated by comparing to 0.5 McFarland standard). 9 ml of inoculant with sterile pipette is taken into 3 test tubes. To the 1st test tube 1g of the 1st sapropel sample is added, to the 2nd test tube 1g of the 2nd sapropel sample is added, the 3rd test tube – control. All test tubes substance is mixed. Test tubes are incubated in for 24 h at 37 ^{0}C temperature. Dilution from incubated TSB is made till 10^{-10} degree. 90 mm Petri dishes are filled with 1 ml dilution from 10^{-6} till 10^{-10}. It is melted on PCA, cooled to the temperature of ~ 40^0 C. Dilution is poured with PCA, mixed and agar is allowed to solidify for 20 minutes. It is incubated for 24 h at 37 ± 1 ^{0}C temperature. Then colonies are counted.

In experiments Nr 6 – 9 concrete containing sapropel and hemp sheaves have been tested.

Experiments are carried out based on standard LVS EN ISO 4833-1:2014 – enumeration of microorganisms.

In experiments Nr 6 and 7 10 ± 0.01 g of sample in a sterile bag is weighed out, then add 90 sodium chloride peptone solution. Substance is being mixed with electrical mixer Stomaher™ for 3 minutes, then

allow the biggest parts to settle. From the obtained solution dilution from 10^{-3} - to 10^{-10} is made (with sterile pipette suspensions are taken into another test tube with 9 ml sodium chloride peptone solution). 1 ml of tested sample dilution (from 10^{-2} -10^{-10}) is taken on each of 2 sterile Petri dishes, then about 12 - 15 ml PCA is poured on each dish, when cooled till 44 - 47 ^{0}C, is carefully mixed and allowed to solidify. Incubate for 72 ± 4 h at 30 ± 1 ^{0}C and 22 ± 1 ^{0}C. Calculate colonies (for calculating are chosen the dishes which contain less than 150 colonies).

Calculate the number N of mesophilic aerobic and facultative anaerobic microorganisms using the equation:

$$N = \frac{\Sigma C}{V \cdot (n_1 + 0.1 \cdot n_2) \cdot d} \quad (1)$$

where C- sum of the colonies counted, V- the volume of inoculum applied on each dish (1 ml), n_1- the number of dishes retained at the first dilution, n_2 - the number of dishes retained at the second dilution, d - the dilution factor – 1.

In experiment Nr 8 2 ± 0.01 g of sample is weighed out on 4 sterile Petri dishes. 2 ml of sterile water is poured on each dish, then mixed. Incubate at 22 ± 1 ^{0}C, 30 ± 1 ^{0}C, 37 ± 1 ^{0}C, at room temperature which fluctuates from 18 ^{0}C till 23 ^{0}C. Every 24 h growth control is made, in order to avoid material desiccation, 1ml sterile water is added.

In experiment Nr 9 2 ± 0.01 g of sample is weighed out on 4 sterile Petri dishes. 2 ml of sterile water is poured on each dish, then mixed. Dishes are taken into ”Whril-pack” sterile bags, closed hermetically. Incubate at 22^0 ± 1 ^{0}C, 30 ± 1 ^{0}C, 37 ± 1 ^{0}C, at room temperature. Growth control is made every 24h.

III RESULTS AND DISCUSSION

In experiment Nr 1 checking the antibacterial activity of sapropel materials on *Staphylococcus aureus, Salmonella enteritidis, Enterococcus faecalis, Bacillus cereus, Escherichia coli , Candida albicans* it has been noticed that Rušona Lake sapropel materials have *Staphylococcus aureus* 2 mm growth delay, in other reference cultures - *Salmonella enteritidis, Enterococcus faecalis, Bacillus cereus, Escherichia coli, Candida albicans* growth delay has not been stated. In its turn, the development of growth delay zone has not been noticed in any of Ubagova Lake sapropel material of applied reference cultures. Intense growth on broth of both studied materials has been noticed, colonies are large, with rugged edges, tarnished, white and cream-coloured.

In experiment Nr 2, in order to evaluate growth delay and suppress the growth of sapropel natural microflora, sapropel was pulped and treated with UV rays; as a result, the development of delay zone has not been noticed neither in Rušona Lake sapropel materials, nor in Ubagova Lake sapropel materials with any of applied reference cultures. Findings are difficult to be read and interpreted, as sapropel has maturated and increased its volume ~2 times. Part of materials has been electrified and stuck to Petri dishes covers. Growth of both studied materials on broth has been noticed, colonies are large, with rugged edges, tarnished, white and cream-coloured.

In experiments Nr 3 and 4, in order to prevent volume increasing, which disturbs to evaluate results effectively, moistened material has been used and the hole ½ filled with studied material. As a result the development of delay zone has not been noticed neither in Rušona Lake sapropel materials, nor in Ubagova Lake sapropel materials with any of applied reference cultures. Findings are clearly to be read. Growth of both samples on broth has been noticed, colonies are large, with rugged edges, tarnished, white and cream-coloured.

In experiment 5, adding Rušona Lake sapropel material and Ubagova Lake sapropel material to *Staphylococcus aureus* and *Escherichia coli* suspensions, after 24 h period of incubation a number of microorganisms has not decreased, is observed the decreasing of the number of microorganisms comparing to control sample. Results of the study of antibacterial activity of test samples, presented in the Table 1.

TABLE 1.

ANTIBACTERIAL ACTIVITY OF TEST SAMPLES

Reference test cultures	Rušona Lake sapropel sample (kvv/1ml)	Ubagova Lake sapropel sample (kvv/1ml)	Control (kvv/1ml)
Staphylococcus aureus	14 x 10^8	15 x 10^8	10 x 10^8
Escherichia coli	16 x 10^8	14 x 10^8	12 x 10^8

In experiment Nr 6, studying concrete containing sapropel and hemp sheaves after 72 h of incubation at 30 ± 1^{0}C temperature, the number of mesophilic aerobic and facultative anaerobic microorganisms is 7.5 x 10^6 kvv/1g (Table 2).

In experiment Nr 7, after 72h of incubation at 22 ± 1^{0}C temperature, the number of mesophilic aerobic and facultative anaerobic microorganisms is 6.3 x 10^6 kvv/1g (Table 2).

In experiment Nr 8, incubating concrete containing sapropel and hemp sheaves without

broth with added water, at all checked temperatures (22 ± 1 ^{0}C, 30 ± 1 ^{0}C, 37 ± 1 ^{0}C, room temperature, which fluctuates from 18 ^{0}C till 23 ^{0}C), after 7 days is observed the increasing of mold colonies (Table 2).

In experiment Nr 9, incubating concrete containing sapropel and hemp sheaves without broth with added water in a sterile polyethylene bag, the increasing of mold colonies is observed at all checked temperatures (22 ± 1 ^{0}C, 30 ± 1 ^{0}C, 37 ± 1 ^{0}C), room temperature, which fluctuates from 18 ^{0}C till 23 ^{0}C after 5 days (Table 2).

TABLE 2.

BIOLOGICAL CHARACTERISTICS OF CONCRETE CONTAINING SAPROPEL AND HEMP SHEAVES

Microorganisms	22 ± 1 ^{0}C	30 ± 1 ^{0}C	37 ± 1 ^{0}C	Room temperature 18 ^{0}C – 23 ^{0}C
Number of mesophilic aerobic and facultative anaerobic microorganisms	6.3 x10^6	7.5 x10^6	not checked	not checked
Mold colonies (Pates "Whril-pack")	positive	positive	positive	positive
Mold colonies (Plate)	positive	positive	positive	positive

Platonov *at al*. [2] states that sapropel protective antibiotic activity against *E.coli, St.aureus, C.diphterie* and fungi Candida was demonstrated. Strus *at al.* [15] studies have shown a slight antibacterial activity of sapropel against selected test cultures (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 4636, *Candida albicans* ATCC 885/ 653). However, in this study, antibacterial activity of Rušona Lake sapropel sample has been observed only on *Staphylococcus aureus,* but Ubagova Lake sapropel sample has not shown anti-bacterial effect on any of applied microorganisms reference cultures. Stankeviča, Kļaviņš [10] point out that anti-bacterial effect is characteristic of fresh sapropel. Also Marčenko and Gurinovich [16] mention that microorganisms that educe antibiotics, which are antagonistic chain of pathogenic saprophilic microorganisms have been found in fresh sapropel. In this study dried sapropel has been studied.

The research shows that sapropels contain an extremely large amount of microorganisms – in 1 g of fresh sapropels there are 12 billion microorganisms in its top layer. In the lower layers, starting from around 0.6 - 1.0 m from the lake bed surface the amount of microorganisms decreases [17]. In Latvia, in 1965 Stūris [18] in his research of Kaņiera Lake and Babītes Lake stated that microorganisms distribution and biochemical activity directly depend on the depth of sapropel layer and season.

In this study sapropel samples have been taken from different lakes and depths, as a result its biological activity is different.

Also Stankeviča and Kļaviņš [10] point out that content and characteristics of sapropels from various deposits are very different and depend on the productivity of the water body, characteristics of the aboveground water flow, and climatic conditions. Before using concrete containing sapropel in construction, its biological characteristics and chemical content must be studied. Specific features of biological activity correlate with chemical content of substances [2]. Antibiotics and sulphanilamide in sapropels are synthesized by fungus and actinobacteria.

Possibly, chemical characteristics of fresh and dried sapropel are different, natural microflora changes and it does not show typical antibacterial characteristics. In order to approve this hypothesis, chemical characteristics of fresh and dried sapropel at different depths from several lakes must be studied.

While incubating Rušona and Ubagova Lakes sapropel samples on broth the growth has been noticed, which points out that not only fresh sapropel, but also dried samples have microorganisms that are able to grow. This characteristic has been observed after treatment with UV rays, so 20 minutes' treatment with UV rays do not eliminate all natural microflora of samples.

Studying concrete containing sapropel and hemp sheaves construction materials it has been stated that they contain a big number of microorganisms. When in contact with humidity, at the temperature from 18 ^{0}C to 37 ^{0}C, mold has developed on sapropel and hemp sheaves construction materials. The mold is a significant risk factor for health, especially in relation to the diseases affecting the human respiratory and immune system. Overall four types of health problems are observed in relation to moisture and mold exposure - allergic diseases, respiratory irritation, infections and toxicological effects [19].

IV CONCLUSION

1. Both Rušona Lake and Ubagova Lake dried sapropel samples contain microorganisms, which begin to grow and reproduce intensively in enabling circumstances.

2. 20 minutes' treatment with UV rays is not enough to eliminate the growth of sapropel made samples.
3. Rušona Lake sapropel test sample has antimicrobial activity on *Staphylococcus aureus* reference culture before it is treated with UV rays.
4. Ubagova Lake sapropel test sample does not have antimicrobial activity.
5. When in contact with humidity, at the temperature from 22 ± 1 ^{0}C, 30 ± 1 ^{0}C, 37 ± 1 ^{0}C, room temperature (18 ^{0}C till 23 ^{0}C), mold colonies form on concrete containing sapropel and hemp sheaves, for this reason this material should not get in contact with humidity when used in construction.

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