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Study of the microbial biodiversity of the Lake La Izvor, Chisinau municipality

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Abstract

The microbial biodiversity of the lake "La Izvor" in Chisinau municipality was studied. Samples were taken from water, silt, and biofilm. The taxonomic groups of microorganisms were isolated from the samples: microalgae, cyanobacteria, actinobacteria, bacteria, yeasts, and micromycetes. Agarized nutrients specific to each taxonomic group were used to isolate microorganisms.

As a result of the research, 26 genera of microalgae and cyanobacteria from the phylum *Cyanophyta*, *Chlorophyta*, and *Bacillariophyta* were detected and studied. Most genera (21) were detected in water and 5 genera in biofilm. 8 genera of actinobacteria were also detected (*Actinomadura*, *Actinoplanes*, *Frankia*, *Geodermatophilus*, *Micromonospora*, *Nocardia*, *Rhodococcus*, *Streptomyces*). Representatives of the genus *Micromonospora*, *Actinoplanes*, *Geodermatophilus*, and *Streptomyces* are spread in the silt, and *Streptomyces* predominates in water and biofilm.

Out of 213 strains of bacteria isolated from water, silt, and biofilm, 148 strains were found to be pathogenic (*Escherichia*, *Klebsiella*, *Salmonella*, *Sarcina*, *Shigella*, *Staphylococcus*), and 65 strains (representatives of the genera *Bacillus*, *Brevibacillus*, *Micrococcus*, *Pseudomonas*) non-pathogenic. Most were isolated from water (120 strains), then from silt (54), and biofilm (39). 7 yeast strains were isolated in pure culture, 3 from water and 4 from silt (representatives of the genera *Saccharomyces*, *Schizosaccharomyces*, *Candida*).

As a result of the study of the fungal community in the water system "La Izvor", representatives of 18 genera were found: *Alternaria*, *Aspergillus*, *Acremonium*, *Ambrosiela*, *Arthrinium*, *Botrytis*, *Cladosporium*, *Chaetomium*, *Penicillium*, *Trichoderma*, *Fusarium*, *Monilinia*, *Mucor*, *Rhizopus*, *Trichocladium*, *Phoma*, *Stachybotris*, *Ulocladium*, *Talaromyces*. Also, fungi were detected, which could not be determined, because for this it is necessary to carry out more extensive research (biochemical, genetic). Representatives of the genera *Aspergillus* and *Penicillium* predominated in all samples.

Keywords: microorganisms; water; silt; biofilm; genera.

1. Introduction

Microbes play a central role in global ecological processes and in the biogeochemistry, with bacteria being the most important component of the microbial communities responsible in aquatic ecosystems for the processes of mineralization of organic matter and recycling of nutrients (Benson, 2019). The researchers analyzed the microflora present in the waters of lakes around the world and showed that, the most common types are *Proteobacteria*, *Actinobacteria*, *Cytophaga-Flavobacterium-Bacteroidetes*, *Cyanobacteria* and *Verrucomicrobia*. In fact, beta-proteobacteria are the most studied and often the most numerous (up to 60-70% of the total number of cells) bacteria in freshwater. This group of bacteria is mainly caused by high concentrations of different pollutants (nitrates) (Lliros, 2014).

As previous studies of aquatic microbial communities over several years show, as well as new data on the composition of freshwater bacterial communities, in addition to *Betaprotobacteria*, actinobacteria dominate (Methe, 1998; Eiler, 2005; Warnecke, 2005).

They dominate the freshwater lakes of Europe, Asia, Africa, especially the lakes of Egypt, Germany, Tanganyika, Finland (Methe, 1998; Hahn, 2003; Allgaier, 2006 De Wever, 2005; El-Naggar, 2015; Kolmonen, 2004; Benson, 2019).

Since 2000, works have been carried out to isolate actinomycetes from the bottom sediments (silt) and the water column of Lake Baikal. By 2002, a collection of 107 strains had been created, with strains of the genus *Streptomyces* predominating in water and the genus *Micromonospora* in water sediments (silt). Moreover, this collection was completed with representatives of 4 genera: *Streptomyces*, *Micromonospora*, *Nocardia*, *Arthrobacter*, which were found in water, in aqueous sediments and sponges (Terkina, 2002).

It is known that biofilm is one of the ways in which microorganisms can exist in the external environment. Biofilms are made up of different types of microorganisms, including bacteria, fungi, and single-celled algae (Markova, 2016). Using modern methods, the representatives of *Proteobacteria*, *Planctomyces*, *Actinobacteria*, *Verrucomicrobia*, *Fermicuts*, *Bacteroidetes*, as well as ammonium oxidizing bacteria, have been identified in the bacterial communities of biofilms. In identifying the biodiversity of *Nitrosomonas* plankton and freshwater lake biofilms, Russian scientists have identified representatives of oxygen phototrophic microorganisms - diatoms and green algae, cyanobacteria, and heterotrophic bacteria dominated by *Proteobacteria*, *Fermicutes*, *Actinobacteria* (Parfenova, 2013; Zaitsev, 2019).

Yeasts and yeast-like organisms can be found everywhere in an aquatic ecosystem. They are environmentally flexible, allowing them to tolerate a wide range of salinities, ambient temperatures, oxygen saturation levels and acidity in the environment (Harwell, 1974; Desnos-Ollivier, 2008). They were isolated from lake and marine sediments (Harwell, 1974; Lee, 2016), and at different depths of water. Furthermore, a wide variety of yeasts have been isolated from lakes with different organic compositions. They were found in the mesotrophic lakes of the United States (Uden, 1963), oligotrophic lakes in Patagonia, Argentina (Brandão, 2011), in lakes receiving sewage discharges and in lakes for recreational tourism in Brazil (Medeiros, 2008). In most cases, the dominant species were the genera *Rhodotorula*, *Candida*, and *Cryptococcus*. Pathogens of this genus have been found in lakes contaminated by human activities and can be used as biological indicators to determine the levels of contamination of these bodies of water. *Hortaea werneckii* was found in the sloping sediments of the Bay of Bengal on the east coast of the Indian Peninsula, India (Kuttys, 2013). *Candida*, *Cryptococcus*, *Williopsis*, *Hanseniaspora*, *Rhodotorula*, *Saccharomyces*, *Torulaspora*, *Trichosporon*, and *Yarrowia lipolytica* species were also recovered from sediment and water samples from two artificial lakes at Universidad del Valle (Cali, Colombia) (Silva-Bedoya, 2014).

Micromycetes – a permanent component of aquatic ecosystems. The first taxonomic studies of aquatic fungi began only in the early 19th century, and since then, knowledge of aquatic fungi is probably the least studied of the major taxonomic groups in aquatic organisms. (Kirilenko, 1977; Wu, 2013).

Of particular interest are the obligatory aquatic species, i.e. those that not only appear and develop actively in the water, but, most importantly, cannot reproduce outside the aquatic environment, but are also migrating micromycetes (Harwell, 1974; Farida, 1990; Rudenko, 2011; Savluk, 2013; Ittner, 2018; Damare, 2008).

In aquatic environments, filamentous fungi also belong to important microbial communities for organic decomposition, nutrient cycle, and energy flows (Abdulla, 2008; Canto, 2020; Moubasher, 2018; Dirisu, 2015). *Aspergillus*, *Penicillium*, *Curvularia*, *Alternaria*, *Cladosporium*, *Drechslera*, *Fusarium*, *Phoma*, and *Rhizopus* have been shown to be dominant

sedimentary members in a marine ecosystem off the east coast of Tamil Nadu, India (Saravanan, 2013) and in four stations in the Suq Al Shuyukh Swamps in the Thi-Qar government, Iraq. *Aspergillus, Eupenicillium*, and *Penicillium* were also common in the sediments of the Gulf of Aqaba, Jordan (Benson, 2019).
In freshwater environments, the diversity of fungi is very high, but in most cases fungi of the genera *Aspergillus* and *Penicillium* predominate (Das, 2007; Damare, 2008; Rudenko, 2011; Savluk, 2013).
The purpose of the research was to study the microbial biodiversity of Lake La Izvor, Chisinau.

2. Methodology

Sampling from the lake (water, biofilm, silt)
Water, silt, and biofilm samples were taken from the 3 lakes of the water basin. Each lake was divided into 3 sequences from each of which samples were taken. Each sample was collected from 6-8 different points. Samples were also collected from the canals that connect these lakes, as well as from the drainage of the last lake in the Bic River.
Sampling was performed in sterile vessels. The water was taken in 1 L bottles, 30 cm below the surface of the water, for which the bottle was placed face down in the water for deep sampling. The biofilms were taken from the surface of the bottled water. Silt samples were taken from the entire perimeter of the lake.
The samples were quickly presented in the laboratory for the isolation of microorganisms.
Isolation of microorganisms from samples taken.
The incorporation method was used for isolation: one milliliter of water, as well as the decimal dilutions (silt, biofilms) were distributed sterile with a graduated pipette in empty sterile plates, over which the molten medium was cooled and cooled to 45^{o}C. With slight rotational movements in one direction or another it mixed well. It was allowed to solidify and incubated in a thermostat at the required temperature depending on the taxonomic croup of the studied microorganism.

Nutrients used to isolate microorganisms:
Microalgae and cyanobacteria: medium 3NNB (for chlorococcal, volvox, filamentous green algae); Medium Danilov (for all algae); Medium Bristol; **Medium Gromov (universal**); **Medium Knop (for green algae); Z-8 Medium (universal)**; **Medium BG-11 and BG-11-N**.
Actinobacteria: For genus ***Actinomadura*** **–** soluble starch (20,0 g/l); K_2HPO_4 (0,5 g/l); $MgSO_4$ (0,5 g/l); KNO_3 (1,0 g/l); NaCl (0,5 g/l); $FeSO_4$ (10,0 mg/l); streptomycin (50 mcg/ml); nystatin (50 mcg/ml); agar - 20; pH=7,2-7,4. For genus ***Actinoplanes*** – oatmeal (2,5 g/l); K_2HPO_4 (1,0 g/l); KCl (0,5 g/l); $MgSO_4*H_2O$ (0,5 g/l); $FeSO_4*7H_2O$ (0,01 g/l); agar; streptomycin (50 mcg/ml); nystatin (50 mcg/ml); agar - 20pH=7,0. For genus ***Frankia*** – propionic acid (0,5 g/l); NH_4Cl (0,1 g/l); $CaCl_2*2H_2O$ (0,1 g/l); $MgSO_4*H_2O$ (0,2 g/l); $NaH_2PO_4*2H_2O$ (0,67 g/l); agar – 20; pH=6,8-7,2. For genus ***Geodermatophlius*** – yeast extract (1,0 g/l); glucose (1,0 g/l); soluble starch (1,0 g/l); $CaCO_3$ (1,0 g/l); streptomycin (50 mcg/ml); nystatin (50 mcg/ml); agar - 20 pH=7,0. For genus ***Micromonospora*** – soluble starch (20,0 g/l); K_2HPO_4 (0,5 g/l); $MgSO_4$ (0,5 g/l); KNO_3 (1,0 g/l); NaCl (0,5 g/l); $FeSO_4$ (0,01 g/l); gentamicin (1 mcg/ml); streptomycin (25 mcg/ml); agar – 20, pH=7,2-7,4. For genus ***Nocardia*** – $NaNO_2$ (2,0 g/l); Na_2CO_3 (1,0 g/l); K_2HPO_4 (0,5 g/l); gentamicin (1

mcg/ml); agar – 20, pH=7,0. For genus ***Rhodococcus*** – KNO_3 (1,0 g/l); K_2HPO_4 (1,0 g/l); KH_2PO_4 (1,0 g/l); NaCl (1,0 g/l); $MgSO_4*H_2O$ (0,2 g/l); $CaCl_2*2H_2O$ (0,2 g/l); $FeCl_3$ (0,0001 g/l); yeast extract (1,0 g/l); propionic acid (0,5 g/l); chloramphenicol (20 mcg/ml); agar – 20, pH=7,0. For genus ***Streptomyces*** – glucose (20,0 g/l); KNO_3 (1,0 g/l); NaCl (0,5 g/l); $MgSO_4$ (0,5 g/l); K_2HPO_4 (0,5 g/l); $CaCO_3$ (3,0 g/l); streptomycin (50 mcg/ml); agar – 20, pH=6,8-7,0.

Bacteria: meat peptonate broth agar; **King A**; **King B**;
Yeast: **Malt - agar**; **Sabourand**
Fungi: Czapek; **Sabourand; Malt - agar; Agar - nutritious**; **Raistrik**.

The identification of the specific belonging of the microorganisms from the samples taken was performed on optical microscopes (Lomo Mikmed – 2; B-292) using the determinants: **Algae** (Dobrojan S. 2016), **Actinobacteria** (Zenova, 2002), **Bacteria** (Zarnea, 1992), **Yeast** (Libkind, 2008); **Fungii** (Blagoveshenskaya, 2015).

3. Results and discussion

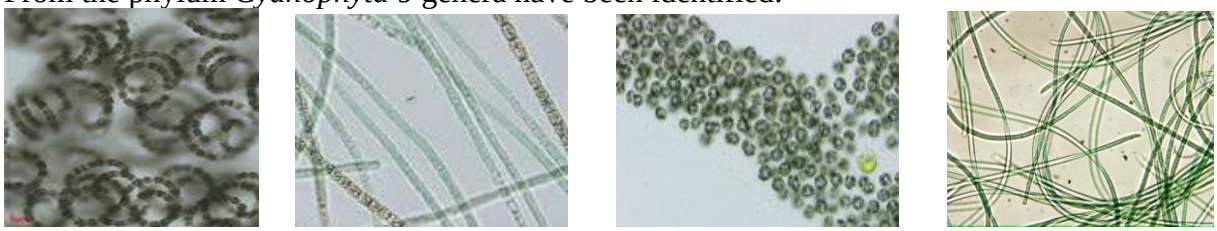
The diversity of microorganisms in the "La Izvor" lake basin was studied. The samples were taken from the 3 lakes, components of the water basin: water, silt, and biofilms. The isolation was performed on nutrient media according to the taxonomic group studied. The isolated microorganisms were examined according to the morpho-cultural properties visually, as well as under the microscope.

Thus, as a result of the investigations performed, microorganisms belonging to taxonomic groups were isolated and identified: microalgae and cyanobacteria, actinobacteria, bacteria, yeasts, and micromycetes. As a result of the investigations, 26 genera of microalgae and cyanobacteria from the thickness of water, biofilm and silt were isolated and identified. The data presented in Tab 1 show that, depending on the location of the detection, the number of isolates is different so that 21 genera of the phylum *Cyanophyta*, *Chlorophyta*, and *Bacillariophyta* were isolated from water. From biofilms 5 genera representative of the phylum *Cyanophyta* and *Chlorophyta*, namely the genera *Microcystis*, *Aphanizomenon*, *Anabaena*, *Oscillatoria*, and *Chlamydomonas*, which cause the phenomenon of hysteranthy of water, while in silt they were not detected, algae being photosynthesizing organisms.

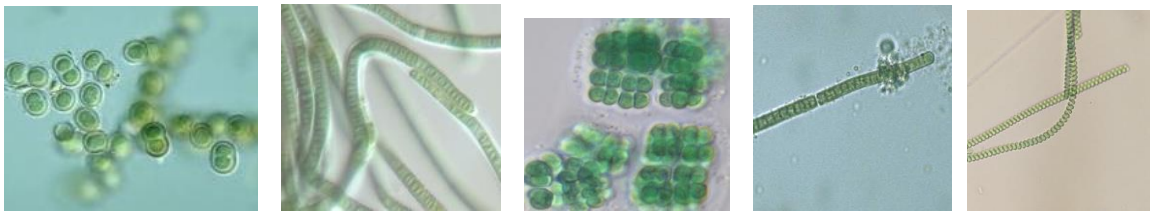
Table 1. Algae species identified and isolated from "La Izvor" lake.

Phylum	Number of genres isolated from		
	Water	Biofilm	Silt
Phylum *Cyanophyta*	**5 -** (*Gloeocapsa, Lyngbya, Merismopedia, Phormidia Spirulina*)	**4 -** (*Anabaena, Aphanizomenon, Microcystis, Oscillatoria*)	-
Phylum *Chlorophyta*	**9 -** *(Chlorella,Cosmarium Cladophora, Oocystis, Scenedesmus, Pandorina, Pediastrum, Ulothrix, Zygnema)*	***1*** *(Chlamydomonas)*	-
Phylum *Bacillariophyta*	***7 -*** *(Cymbella, Diatoma, Gomphonema, Navicula, Nitzschia, Pinnularia, Synedra)*		-

From the phylum *Cyanophyta* 9 genera have been identified:

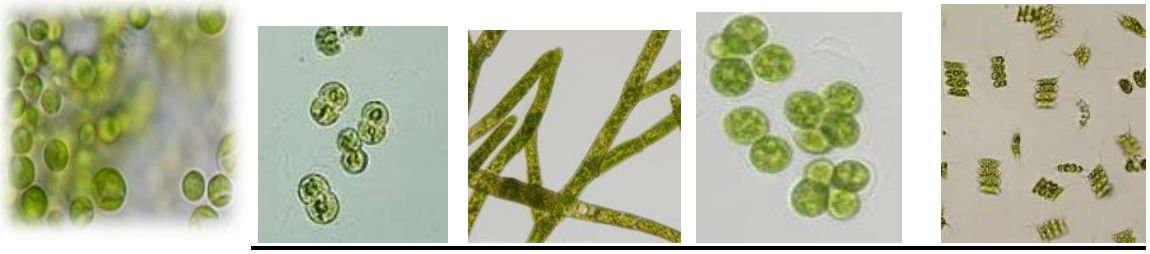


Anabaena sp. *Aphanizomenon sp.* *Microcystis sp.* *Oscillatoria sp.*



Gloeocapsa sp. *Lyngbya sp.* *Merismopedia* *Phormidia sp.* *Spirulina sp.*

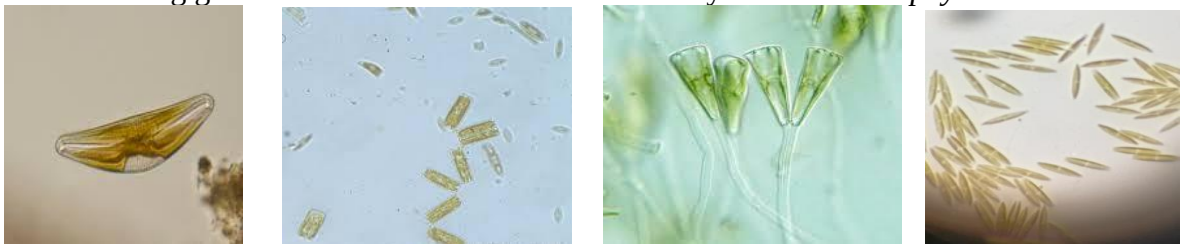
From the *Chlorophyta* Phylum, the following genera have been identified:



Chlorella sp. *Cosmarium sp.* *Cladophora sp* *Oocystis sp.* *Scenedesmus sp*

Pandorina sp *Pediastrum sp* *Ulothrix sp* *Zygnema sp* *Chlamydomonas sp.*

The following genera have been identified from the Phylum *Bacillariophyta*:



Cymbella sp. *Diatoma sp.* *Gomphonema sp.* *Navicula sp.*

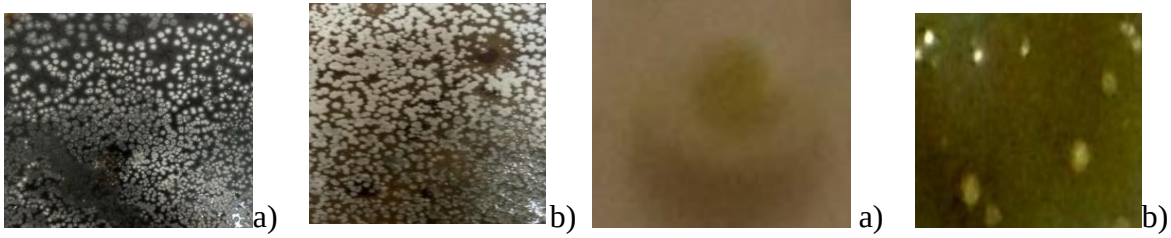
Figure 1. Microalgae and cyanobacteria isolated from La Izvor lake.

From the above it can be concluded that the algoflora of the lake "La Izvor" is particularly rich and varied. During the vegetation period, which coincided with the sampling period, we noticed that bacillaryophytes, chlorophytes, and cyanophytes algae predominate (Fig. 1). It is also important to note that in the warm period of the year in the lakes of our country develops the phenomenon of hysteranthy of the water, characteristic of the lake "La Izvor". *Aphanizomenon*, *Anabaena*, and *Chlamydomonas*. Filamentous green algae (*Cladophora*, *Rhizoclonium*, *Oedodonium*, *Mougeotia*, *Spirogyra*) grow abundantly in the coastal area of the lake, producing a biomass of up to 5-10 kg / m^2 (Nedbaliuc, 2016, 2019).
In order to identify and quantify actinomycetes, the method of inoculation on agar media of different compositions was used, which made it possible to identify new strains of actinomycetes - representatives of 8 genera, most often found in soil and aquatic microbial communities in Moldova.
In Tab. 2 shows the results of the determination of actinomycete strains in water, biofilm, and bottom sediments (silt of Lake La Izvor). It can be seen that the number of isolates in the samples is not the same and their belonging to one genus or another is different. Were found in the silt (36 strains), then in the water samples (25 strains), the fewest actinomycetes were found in the biofilm (9 strains), and the representatives of *Actinomadura* and *Rhodococcus* were not found in biofilm samples.

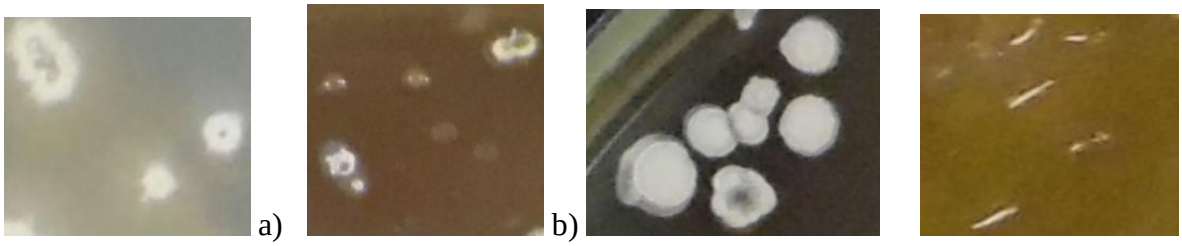
Table 2. Number of strains, representatives of the group actinomycetes isolated from the aquatic environment.

Genus	Water	Biofilm	Silt
Actinomadura	2	0	5
Actinoplanes	3	1	5
Frankia	1	1	2
Geodermatophilus	3	1	6
Micromonospora	3	1	6
Nocardia	2	1	2
Rhodococcus	2	0	4
Streptomyces	9	4	6
TOTAL	25	9	36

It has been established that the most common actinomycetes in the lake are the genera *Actinoplanes*, *Geodermatophilus*, *Micromonospora*, and *Streptomyces*. Representatives of the genus *Micromonospora*, *Actinoplanes*, *Geodermatophilus*, and *Streptomyces* are spread in the silt, and *Streptomyces* in water and biofilm (Fig. 2).



a) *S. massasporeus* b) *S. fradiae* *Geodermatophilus* ssp. (a), b)



Frankia ssp.: a), b) ***Micromonospora* sp.** ***Actinoplanes* ssp**

Figure 2. Colonies of actinobacteria isolated from the Lake La Izvor.

It has been established that the most common actinomycetes in the lake are the genera *Actinoplanes, Geodermatophilus, Micromonospora*, and *Streptomyces*. Representatives of the genus *Micromonospora, Actinoplanes, Geodermatophilus*, and *Streptomyces* are spread in the silt, and *Streptomyces* in water and biofilm.

Isolation of bacterial and yeast cultures was performed following successive dilutions, on the characteristic media described in the materials and methods. In order to select the pathogenic isolates of the enterobacteria type, all isolates were seeded on the Endo medium - characteristic for the isolation of the *Escherichia coli* strain (where the growth of a single culture appeared, so it was *E. coli*), after which they were re-seeded on the SS Agar medium - characteristic for the isolation of *Salmonella* and *Shigella* strains. Another specific medium used to isolate and select pathogenic cultures was the KIA medium. Bacteria of the genus *Brevibacillus* sp. were detected, also.

Of the total number of isolates in water (122 strains) cultured on KIA medium to eliminate pathogenic enterobacteria, only 25 remained. In the silt samples from the total number of 54 strains, the pathogenic enterobacteria were found to be 32 strains and 22 non-pathogenic. Of the 39 bacterial strains isolated from the biofilm samples, 21 strains were shown to be pathogenic enterobacteria, subsequently excluded from the study, and 18 strains of non-pathogenic bacteria

Planococcus sp. ***Bacillus sp.*** ***Pseudomonas sp.*** ***Micrococcus sp.***

Figure 3. Isolated bacterial colonies from Lake La Izvor (nutrient agar medium).

Thus, from the water, biofilm and silt of the lake "La Izvor", 213 new strains of bacteria were isolated in pure culture, of which 148 proved to be pathogenic as a result of identification on the KIA environment, and 65 were studied according to the properties colonial and cellular morphological and cultural characteristics. According to the morpho-cultural characteristics of the studied colonies we can see a large variety of isolated microorganisms belonging to different genera and species, which is confirmed by the great diversity of the shape of the cells found in the study, both morphologically and tinctorially. The genera: *Bacillus, Pseudomonas, Micrococcus, Paenibacillus, Planococcus* were identified (Figure 3).

Also, 7 yeast strains were isolated, 3 from water and 4 from silt (representatives of the genera *Saccharomyces, Schizosaccharomyces, Candida*) (Figure 4).

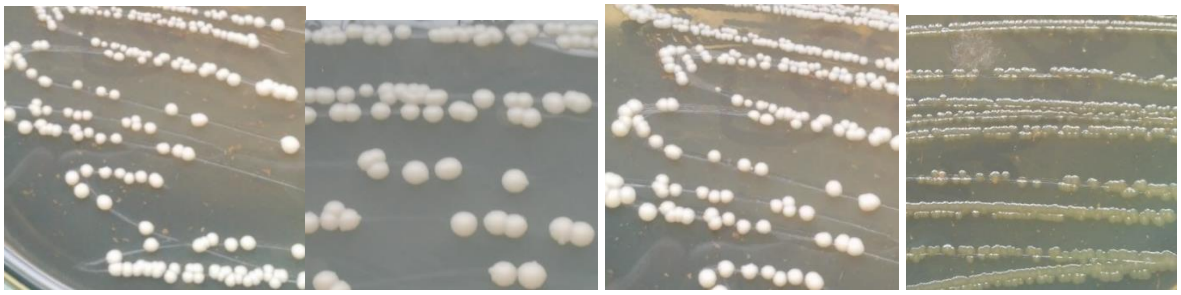
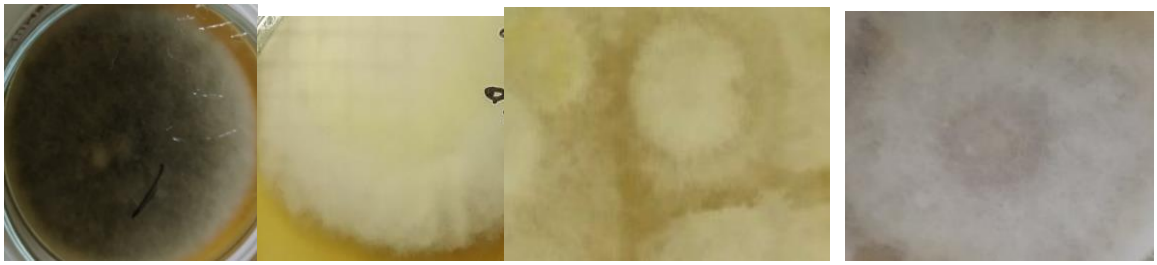


Figure 4. Isolated yeast colonies from Lake La Izvor (malt agar medium).

247 fungal strains were isolated and studied. Some of the fungi were identical, but the rest are representatives of different genera and species. Thus, most fungi were found in silt samples (87 strains), then in water samples (85 strains), and the least in biofilm (75 strains). The carried out result on the cultural and morphological characters, strains of the following genera were identified and isolated: *Aspergillus*, *Alternaria*, *Penicillium*, *Trichoderma*, *Fusarium*, *Botrytis*, *Monilia*, *Mucor*, *Rhizopus*, *Acremonium*, *Cladiosporum*, *Trichocladium*, *Phoma*, *Chaetomium*, *Stachybotrys*, *Ambrosiela*, *Talaromyces*. All kinds of micromycetes mentioned were detected in water, silt, and biofilms (Fig. 5). Stems that could not be determined were also found, as further research is needed.

Penicillium sp. ***Aspergillus sp.*** ***Trichoderma sp.*** ***Acremonium sp.***



Alternaria sp. ***Fusarium sp.*** ***Trichocladium sp.*** ***Ambrosiella* sp.**

Figure 5. Isolated fungal colonies from the Lake La Izvor (malt agar medium).

The results of the research show that a wide range of fungi belonging to different genera that are represented by various species coexist in the "La Izvor" water basin. Thus, depending on the place of detection of the fungus, they can be presented in the following series:
From water: *Penicillium → Aspergillus→ Trichoderma, Alternaria → Fusarium → Botrytis → Acremoniu → Monilia → Mucor, Rhizopus, Cladiosporum, Ambrosiella → Ulocladium, Chaetomium, Phoma, Trichocladium.*

- *From silt*: *Aspergillus, Penicillium → Alternaria → Trichoderma, Fusarium, Acremonium, → Mucor, Rhizopus →. Botrytis, Monilia, Talaromyces, Chaetomium, Cladiosporum, Phoma, Ambrosiella, etc.*

- From biofilm: *Aspergillus → Penicillium → Alternaria → Trichoderma → Mucor, Rhizopus → etc.*

Thus, it was found that in all the samples studied, representatives of the genera *Asprgillus* and *Penicillium* predominate. Representatives of the genera *Alternaria*, *Trichoderma*, *Fusarium*, *Acremonium*, *Mucor*, *Rhizopus*, etc. are less present.

4. Conclusions

As a result of the research, 26 genera of microalgae and cyanobacteria (from the phylum *Cyanophyta*, *Chlorophyta*, and *Bacillariophyta*), 70 strains of actinobacteria strains of 8 genera (*Actinomadura*, *Actinoplanes*, *Frankia*, *Geodermatophilus*, *Micromonospora*, *Nocardia*, *Rhodococcus*, *Streptomyces*) were isolated and studied. 213 strains of bacteria, of which 148 pathogenic strains (genera: *Salmonella*, *Klebsiella*, *Staphylococcus*, *Shigella*, *Escherichia*, *Sarcina*, *Brevibacillus*) and 65 non-pathogenic strains (*Bacillus*, *Pseudomonas* sp., *Planococcus* sp., *Micrococcus* sp.), 7 strains yeasts *(Saccharomyces*, *Schizosaccharomyces* sp., *Candida* sp.) and 247 strains of micromycetes of 18 genera (*Penicillium*, *Aspergillus*, *Trichoderma*, *Alternaria*, *Fusarium*, *Botrytis*, *Monilia*, *Mucor*, *Rhizopus*, *Acremonium*, *Cladosporium*, *Trichocladium*, *Phoma*, *Ulocladium*, *Ambrosiela*, *Talaromyces*), of which the genera *Aspergillus* and *Penicillium* predominate. Non-pathogenic strains were selected for further research.

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