

DIVERSITY OF FUNGI ASOCIATED WITH AQUATIC BASINS OF CHISINAU MUNICIPALITY, REPUBLIC OF MOLDOVA

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Abstract: Fungi in freshwater ecosystems constitute an extremely diverse and ecologically important group, but aquatic filamentous fungi continue to be relatively poorly studied. Despite the recognition of their essential role in the decomposition of organic matter, in the recycling of nutrients and in maintaining ecological balance, current knowledge regarding the diversity, distribution and functions of these fungi remains limited. In this context, this paper aims to contribute to expanding knowledge on the diversity of filamentous fungi from 3 lakes of Chisinau municipality (La Izvor, Valea Morilor and Valea Trandafirilor). The research focused on the study of 476 strains of filamentous fungi, isolated from water, sediment and biofilm samples of the mentioned lakes. The density and diversity of filamentous fungi, in all studied lakes, varied depending on the location of water basins, the highest being identified in the sediment samples. As a result of the study of morpho-cultural characteristics, representatives of 18 genera were identified, which constitute 85% of the total number of isolates. The most frequently encountered were representatives of the genera: *Penicillium*, *Aspergillus*, *Trichoderma*, *Alternaria*, *Fusarium*.

Keywords: aquatic fungi; biotechnological potential; classification; diversity; isolation.

INTRODUCTION

Filamentous fungi are essential for ecosystems, contributing to the decomposition of organic matter and the recycling of nutrients, meeting in all environments, including terrestrial, marine and freshwater. Most live in terrestrial environments, but there are also aquatic species that develop on submerged organic substrates. Aquatic basins are favorable environments for microorganisms, due to more stable living conditions, compared to those on land, such as the main environmental factors: temperature, mineral and gaseous composition, protection from solar radiation, nutrient-rich diversity, etc. In aquatic ecosystems, they represent a taxonomic group of the most diverse and widespread. Therefore, the investigation of aquatic fungi is a promising research direction, with relevant applications in areas such as biotechnology, medicine and environmental protection [1, 12, 17, 20, 21, 28, 32].

In freshwater ecosystems, a significant number of fungi species have been described, which account for a considerable percentage of the decaying microbial biomass of plant detritus. They play a crucial role in ecological processes such as carbon processing, nutrient cycle and energy transfer to higher trophic levels, thus contributing to the balance of aquatic ecosystems. Fungi are major decomposers of organic matter, parasites, pathogens or mutualists and participate in the denitrification of sediments [5, 6, 9, 10, 26, 30, 59].

In aquatic ecosystems filamentous fungi play important roles in the nutrient cycle and decomposition of organic compounds, and some species are promising sources of bioactive compounds for medicine and industry. They can also contribute to the bioremediation of pollutants [4, 22, 24, 38, 45, 53].

The origin of aquatic filamentous fungi is still uncertain, but it is estimated that they evolved into the late Proterozoic 900-570 million years ago. However,

fossils from the Ongeluk Formation suggest a much older origin, about 2,400 million years old, indicating that fungi lived in submarine volcanoes. Most current marine species have links to terrestrial ones, and transitions between marine and terrestrial environments have occurred several times in evolution. During the Pre-Cambrian period, events such as water cooling and the increase in dissolved oxygen facilitated the adaptation and migration of fungi between the two media [18, 27].

In 2022 Calabon *et al.* conducted a classification of fungi (filamentous fungi) which includes 13 classes, 3,870 species, which come from various substrates and geographical locations. Of these, from 2,968 species (1,018 genera) belong to Ascomycota, from 333 species (97 genera) belong to Chytridiomycota, from 221 species (105 genera) belong to Rozellomycota, and from 218 species (in 100 genera) belong to Basidiomycota. Other less represented phyla include Blastocladiomycota, Monoblepharomycota, Entomophthoromycota, Mucoromycota, Aphelidiomycota, and others, with fewer than 50 species each [15, 35].

Taxonomically, hyphomycetes are associated with the Ascomycota phylum, and only a small percentage is affiliated with Basidiomycota, and aero-aquatic ones are classified as Ascomycota, and four species as Basidiomycota and one species as Oomycetes. The Ascomycota phylum includes genera that live exclusively in freshwater habitats, fungi that live both in freshwater habitats and in terrestrial habitats, species of fungi that live in freshwater and marine habitats, and species that are found in freshwater, terrestrial and marine habitats. From a microscopic point of view, they are characterized by inoperculated unified asci and perithecial ascomata [48, 61].

In recent years, filamentous fungi from aquatic environments have become the subject of intense research, due to their essential role in the nutrient cycle, carbon processing and maintaining ecological balance. Although their metabolic and ecological

functions are still partially understood, the study of new strains is necessary to elucidate these processes and harness the biotechnological potential [52, 56, 57, 60].

The diversity of aquatic fungi is influenced by factors such as the composition of the biological community and salinity, the latter causing major differences between freshwater and marine fungi. In freshwater ecosystems, the Ascomycota phylum species predominate, recognized for their adaptability and high metabolic potential [8, 11, 19, 37, 47, 51, 55]. These species can also produce a wide range of extracellular enzymes and bioactive compounds with biotechnological applications. In this context, the study of aquatic fungi contributes both to the understanding of biodiversity and to the identification of new resources of practical interest [14, 34, 42, 50].

Based on the reported facts, we can conclude that the study of the diversity of fungi in aquatic basins is a topical issue and of great scientific importance. It contributes not only to the deepening of knowledge on the structure and functioning of aquatic ecosystems, but also to the understanding of the ecological role of these microorganisms. At the same time, research in this field may have important applicative implications, including in monitoring the quality of the environment, in bioremediation and in identifying new sources of bioactive compounds with biotechnological potential.

MATERIALS AND METHODS

Object of the study

As the object of study served filamentous fungi isolated from the aquatic basins „La Izvor” (247 strains), „Valea Morilor” (124 strains) and „Valea Trandafirilor” (105 strains) in Chisinau municipality. These are some of the largest lakes in the municipality. All three are located in the park area and represent recreation areas for the inhabitants of the capital (Fig. 1).

Lake "La Izvor" is located in the Sculeni area, within the park of the same name, which covers an area of 150 ha and consists of three interconnected lakes. Lake "Valea Morilor" is located in the Buiucani area, within Valea Morilor Park, which covers an area of 100 ha, with the lake itself occupying 34 ha. Lake "Valea Trandafirilor" is situated in the Botanica area, within the park of the same name, which covers an area of 145 ha, of which the lake occupies 9 ha.

Methods of achieving research

Samples of water, sediment (silt) and biofilm were taken from the mentioned lakes. Sampling was carried out in sterile vessels. The water was taken in bottles with a capacity of 1 liter each, 30 cm from the water surface. The sediment samples (silt) were taken from the bottom of the lake all over the lakes. The samples were taken using a sediment shovel and placed in sterile vessels. After the sediments were stored on the bottom of the pot, the water was removed, and from the

pot was taken 1 gram of sediment and carried out by successive dilutions. Biofilm samples were taken from the water surface using hydrophobic plates and placed in sterile vessels. All samples taken were presented in the laboratory for immediate isolation of microorganisms [13, 40, 43, 44, 54].

From samples taken or prepared successive dilutions to decrease the density of cells of microorganisms. A maximum of 5 decimal dilutions were made depending on the density of the suspension cells. Of the last 3 dilutions were performed inoculations on 5 agar media (Malt agar, Czapek, Sabourand, nutritional agar, Raistrick) specific to the growth and development of filamentous fungi with different pH value. Media were prepared according to standard protocols. For inhibiting the growth and development of bacteria and highlighting filamentous fungi in agar nutrient media, levomycetin (chloramphenicol) solution was added at a concentration of 0.1% / L.

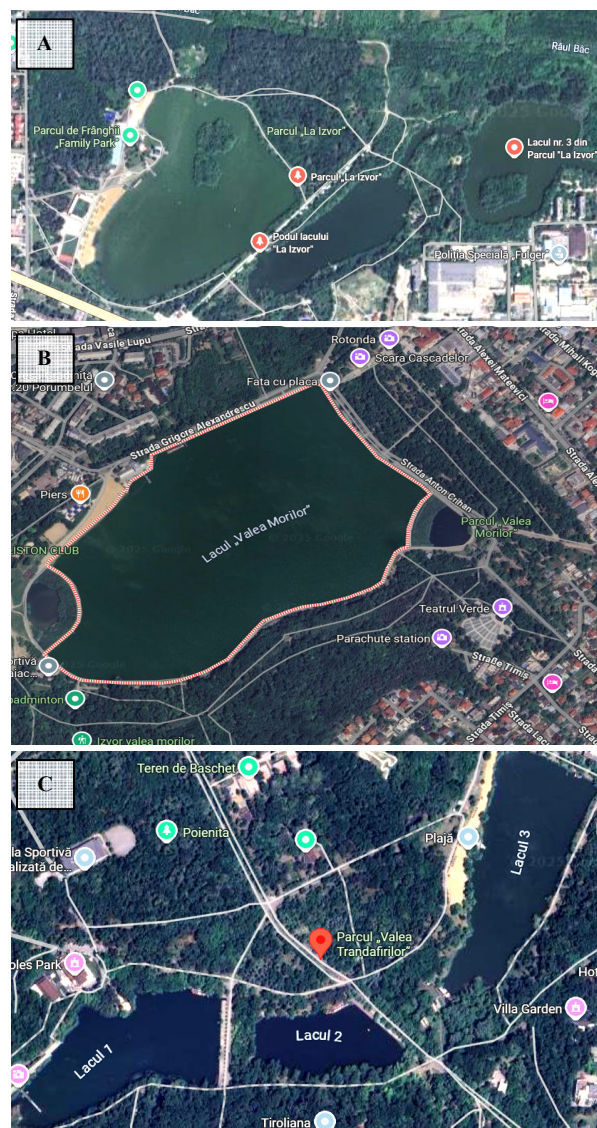


Figure 1. Location of the lakes A. La Izvor, B. Valea Morilor, C. Valea Trandafirilor on the map Chisinau city, Republic of Moldova (source: Google Maps)

The incorporation method was used for fungal isolation. One milliliter of the initial sample and of each selected dilution was aseptically transferred into sterile Petri dishes, followed by the addition of molten agar medium cooled to 40–45°C. After solidification, the plates were incubated at 28–30°C for 3 to 10 days. All inoculations were performed in triplicate. Sterile agar plates without inoculum served as negative controls.

After incubation, visible fungal colonies were counted. The density of fungi was expressed as colony-forming units (CFU) per milliliter of sample, calculated according to the dilution factor used.

Pure cultures were obtained by transferring individual colonies from primary isolation plates onto fresh Malt Agar plates using sterile inoculation needles. Malt Agar was selected as a universal medium suitable for the growth and maintenance of a wide range of fungi. The purified isolates were incubated at 28–30°C and periodically checked for purity [1,36].

All fungal isolates were examined macroscopically and microscopically. Macroscopic characteristics included colony morphology, growth rate, texture, pigmentation of the surface and reverse, and margin structure. Microscopic observations were carried out using an Optica B-292 microscope and focused on hyphal structure, septation, conidiophores, conidia, asci, ascospores, and other reproductive elements.

The identification of fungal strains was carried out to the genus level using taxonomic keys, fungal identification manuals, and standard monographs, based on data obtained from macroscopic and microscopic examinations. Taxonomic assignment was performed by comparing the observed characteristics with published descriptions in fungal identification guides [33, 39, 64].

The experimental data were subjected to descriptive statistical analysis, including calculation of arithmetic means, standard deviations, and coefficients of variation. Statistical analyses were performed using Microsoft Excel 2020 and PAST software version 4.16c.

RESULTS

The research carried out consisted of the sampling of water, biofilm and sediment (silt) from the water basins of the Chisinau municipality: "La Izvor", "Valea Morilor" and "Valea Trandafirilor". From the lake "La Izvor" 11 samples were taken each (water, sediments, biofilm), from the lake "Valea Morilor" 10 samples, and from the lake "Valea Trandafirilor" 9 samples. The samples were transported to the lab. From the samples were taken 1 mL of water, 1 gram of sediment (silt) and 1 gram of biofilm and carried out successive dilutions.

In order to establish the density of fungi, the colonies that appeared in the Petri dishes with the malt-agar medium, being the most numerous, were counted and established the density of the colony-forming units (UFC)/mL (Tab. 1). According to the results obtained, the average density of UFC/mL varied from one lake to another, the highest density being detected in Lake "La Izvor" regardless of the place of insulation (water, sediments, biofilm). In all the studied water basins the highest density being detected in sediments, ranging from 8×10^4 in lake "Valea Morilor" to 2×10^6 in lake "La Izvor". In water and biofilm, the density of fungi was also higher in Lake "La Izvor" compared to the other lakes. Abundant spore was observed in sediment isolates suggesting increased metabolic activity.

Table 1. Density of colony-forming units (CFU/mL) of samples taken from lakes "La Izvor", "Valea Morilor" and "Valea Trandafirilor"

Lake	Place of isolation	Density, UFC/mL
"La Izvor"	Water	6×10^4
	Sediment	2×10^6
	Biofilm	5×10^3
"Vale Morilor"	Water	5×10^4
	Sediment	8×10^4
	Biofilm	2×10^3
"Valea Trandafirilor"	Water	3×10^4
	Sediment	5×10^4
	Biofilm	1×10^3

According to the data presented in Table 1, we can note that in the lakes "Valea Morilor", "La Izvor", "Valea Trandafirilor" the abundance of fungi was different depending on the place of isolation, those and the number of isolates was different. Many of the isolated strains in the lakes studied were identical according to the morpho-cultural peculiarities. This indicates that many strains can be different variants of the same species. This can be explained by the natural variability of fungi, which indicates that morpho-cultural peculiarities may vary depending on the nutrient medium and living conditions.

At the next stage of each morphologically distinct fungal colony, aseptic subcultivation of mycelium was carried out in Petri dishes on the malt-agar medium and then incubated at 25–28°C. Thus, the strains that differed according to the morpho-cultural peculiarities were isolated in pure culture and studied the morphological and cultural peculiarities. In total from the lakes "Valea Morilor", "La Izvor", "Valea Trandafirilor" were isolated and studied 476 strains of fungi, represented by different genera and species. Thus, from the samples taken from "La Izvor" lake were isolated 247 strains, from "Valea Morilor" lake - 124 strains, and from "Valea Trandafirilor" - 105 strains. The number of strains varied depending on the lake from which the samples were collected, but also depending on the place of isolation (Tab. 2).

According to the data presented in Table 2, it can be observed that in the studied lakes, the highest fungal diversity was detected in sediments, followed by water, and to a lesser extent in biofilms.

Table 2. Number of isolated strains from lakes La Izvor, Valea Morilor and Valea Trandafirilor in dependence on the place of isolation

Lake	Total number of isolated strains	Place of isolation	Number of isolated strains
"La Izvor"	247	Water	85
		Sediment	87
		Biofilm	75
"Vale Morilor"	124	Water	49
		Sediment	45
		Biofilm	30
"Valea Trandafirilor"	105	Water	36
		Sediment	49
		Biofilm	20

Microorganisms, especially filamentous fungi, play an important role in the decomposition of organic matter in aquatic basins, which serves as their nutrient source. They can also be considered indicators of pollution with substances of various origins: plant, animal, or wastewater inputs. Thus, it is generally assumed that the higher is the amount of organic matter, the greater is density and diversity of fungi [7, 41, 49].

In this study, the highest fungal density was identified in Lake "La Izvor", which suggests that it is subject to the highest degree of pollution. This finding highlights the necessity of implementing urgent measures for monitoring and reducing pollutant inputs into this ecosystem. The study of the morphological and cultural peculiarities of the isolates in pure culture allowed to establish their genera belonging.

From lake "La Izvor" were isolated 247 strains, representatives of 18 genera of which 40 strains were representatives of the genus *Penicillium*, and 41 belonged to the genus *Aspergillus*. The frequency of occurrence for the mentioned fungal strains constituted 16.19% and 16.6% of the total number of isolates, respectively. A significant number of strains are representatives of the genera: *Alternaria* (23), *Trichoderma* (17) and *Fusarium* (12), the frequency of occurrence of which constituted respectively 9.31%, 6.88% and 4.86% of the total number. Genera: *Mucor*, *Rhizopus* and *Acremonium* were represented by 9 strains each, the frequency of occurrence of which is 3.64% each, and the genus *Botrytis* (8 strains) - 3.24%, of the total number of isolates. The incidence of strains in the remaining identified genera was less than 3% each, varying within the range of 1.21-2.43%. Unidentified strains constituted 15% of the total number of isolates (Fig. 1).

Depending on the place of isolation in the samples taken from "La Izvor" lake, representatives of the genera *Aspergillus* and *Penicillium* predominated. In the water, representatives of the genus *Penicillium* predominated, and in sediments and biofilm, representatives of the genus *Aspergillus* predominated.

From the lake "Valea Morilor" were isolated 124 strains, representatives of 15 genera. Of the number of isolated strains, representatives of the genus *Aspergillus* 29 strains, 27 strains of the genus *Penicillium*, the frequency of which constitutes 27% and 21% were identified, respectively. The genera *Trichoderma* were represented by 10 strains each, the

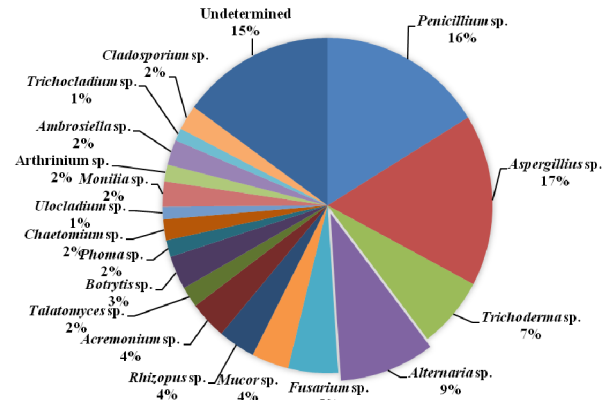


Figure 2. Frequency of occurrence of fungi genera in samples taken from "La Izvor" lake

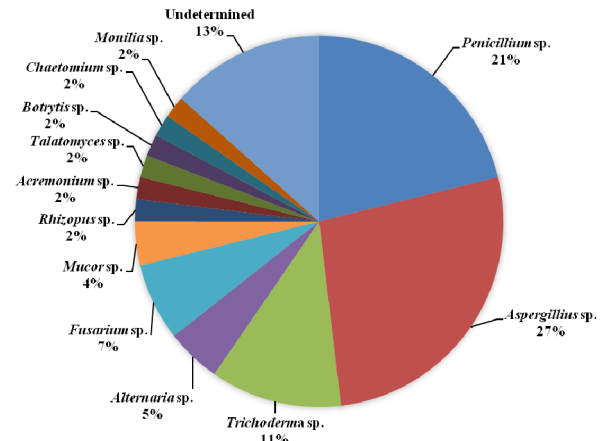


Figure 3. Frequency of occurrence of fungi genera in samples taken from the "Valea Morilor" lake

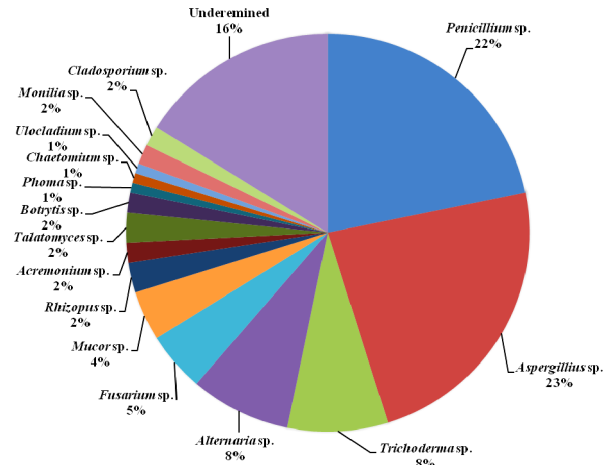


Figure 4. Frequency of occurrence of fungi genera in samples taken from the "Valea Trandafirilor" lake

frequency of occurrence being 11%, and the genera *Fusarium* (6 strains) and *Mucor* (4 strains) have a frequency of 6.84 and 4.03% of the number of isolates, respectively. Representatives of the other detected genera had an insignificant frequency that varied within 0.8-2.42% of the total number of isolated strains. The number of unidentified strains was 20 and constituted 13% of the total number (Fig. 2).

In this lake, representatives of the genus *Aspergillus* predominated in water and sediments, and representatives of the genus *Penicillium* in biofilm.

From the lake "Valea Trandafirilor" were isolated 105 strains, representatives of 14 genera, more representative were of genera *Penicillium* (22 strains), *Aspergillus* (28 strains) and *Trichoderma* (12 strains), the frequency of which constituted 20.96%, 22.68% and 8.43 % of the total number of isolates, respectively. Also, a significant frequency in this lake have strains which represent genera: *Alternaria* - 7.76%, *Fusarium* - 4.75% and *Mucor* - 3.81%. Representatives of the other identified genera have an insignificant frequency of less than 2% each, of the total number of isolated strains. Fourteen strains of the total number of 105 isolates in this lake have not been identified, the frequency of which constitutes 16.34% (Fig. 3).

In all samples taken from the "Valea Morilor" - water, sediments and biofilm predominated representatives of *Aspergillus* genus, followed by representatives of the genus *Penicillium*.

From the results obtained, it can be stated that "La Izvor" lake hosts the most diverse fungal community, indicating that it is the most polluted among the three studied lakes. Notably, this lake has never been cleaned since its formation.

Based on the findings, it can be concluded that the lakes in the municipality of Chisinau represent a valuable source of fungi, with 476 strains isolated, belonging to 18 genera. Among these, *Aspergillus* and *Penicillium* were the most frequently encountered,

confirming their adaptability to aquatic environments. A comparative analysis of the three lakes revealed that "La Izvor" lake provided the highest number of isolates, suggesting the presence of favorable trophic and ecological conditions for fungal proliferation. The composition of fungal communities reflects the adaptation of species to the variability of the physicochemical parameters of water and to the degree of anthropogenic influence, highlighting their major ecological role in the decomposition of organic matter and in the dynamics of local biogeochemical cycles. [16, 41]. Some of the most representative strains found are shown in figure 4.

Most of them belong to the Ascomycota phylum, which is consistent with data from the literature. Numerous studies show that fungi in the phylum Ascomycota and Basidiomycota are the most abundant fungal groups, even in deep marine ecosystems, where they account for about 70-80% and 10-20% of fungal communities, respectively. Among the genera of high-frequency filamentous fungi is noted *Penicillium*, *Aspergillus*, *Cladosporium* and *Fusarium* [32, 47, 61]. Species belonging to the genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria* and *Acremonium*, and super-saline waters have also been identified [38]. These strains are well known as major producers of extracellular enzymes (cellulases, amylases, proteases, ligninases) [46, 58]. These genera contribute significantly to the degradation of organic substances in water. Through their intensive metabolic activity, they not only accelerate the recycling of organic matter but also influence the structure of microbial communities, playing a key role in maintaining the functionality of aquatic ecosystems. In the context of pollution and anthropogenic changes, the predominance of these groups indicates not only a high adaptive capacity but also a potential utility in bioremediation processes and environmental quality monitoring.

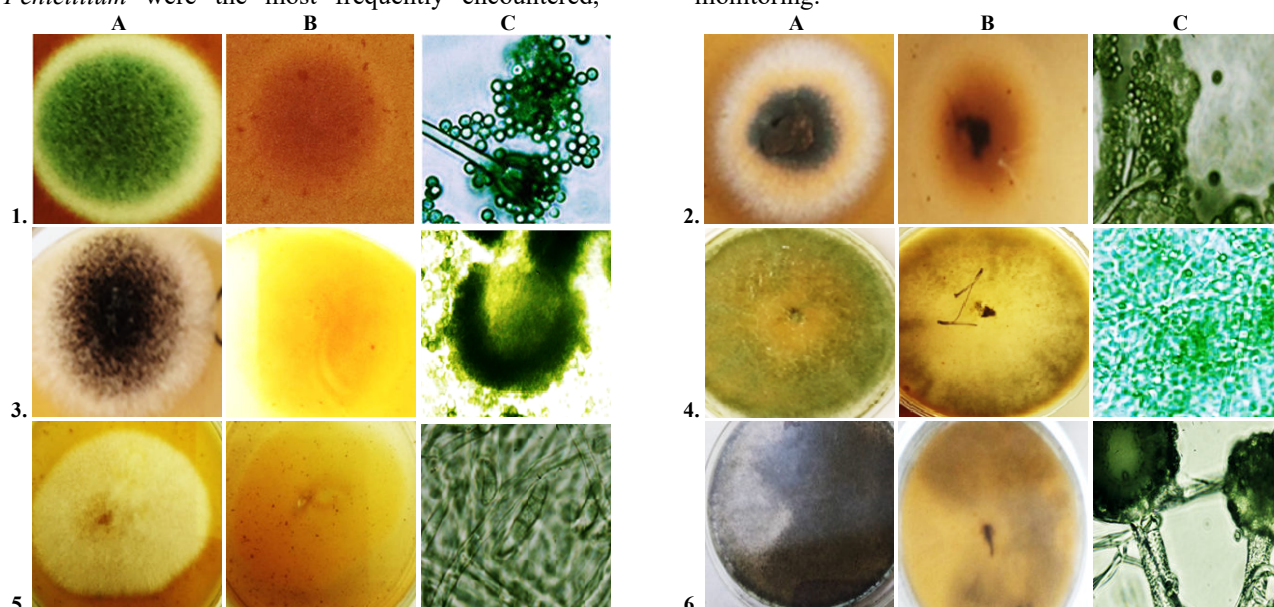


Figure 4. Micromycete strains isolated from the aquatic basins of Chisinau municipality: A - front, B - back, C - microscopic image scale bar = 20 μ m; 400 $\times$, of genus: 1. *Penicillium*, 2. *Talaromyces*, 3. *Aspergillus*, 4. *Trichoderma*, 5. *Fusarium*, 6. *Mucor*.

DISCUSSION

Aquatic basins represent favorable ecosystems for the development of filamentous fungi, which possess a diverse and well-developed enzymatic system. These fungi benefit from optimal conditions of humidity, organic matter, and nutrients, which confer upon them an essential role in the decomposition of woody debris and leaves that fall into freshwater from the adjacent riparian vegetation. This was also demonstrated in the study of microbial diversity in the aquatic basins of Chisinau municipality, from which 476 strains of filamentous fungi were isolated, representing different genera, with the greatest diversity detected in sediment (silt) samples, where plant and organic debris accumulate. Based on the analysis of morphological and cultural characteristics of the strains isolated from the three studied lakes, 18 fungal genera were identified, accounting for 85% of the total number of isolates. It was found that in the aquatic basins of Chisinau, the dominant representatives were genera *Aspergillus* and *Penicillium*, whose frequency ranged between 17-26% and 16.2-21.8% of the total fungal isolates, respectively. A considerable number of isolates also belonged to the genera *Trichoderma*, *Alternaria*, *Fusarium*, and *Mucor*. Although these fungi are not true aquatic species, they are cosmopolitan and reach lakes through rainwater or plant debris. These results are consistent with data reported in the literature, which indicate that the aforementioned fungal genera are characteristic of freshwater ecosystems. Similarly, in microbial diversity studies of Lake Nam Co on the Tibetan Plateau, it was shown that the dominant fungal genera were *Lecanicillium*, *Alternaria*, *Penicillium*, and *Mucor* [63], similarly, in the waters of Dal Lake, Kashmir, 23 fungal species were identified, belonging to six genera: *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizopus*, *Acremonium*, and *Mucor*, with *Penicillium* and *Aspergillus* being the dominant genera [52]. In Big Tambukan Lake, located in the Caucasus region of Russia, representatives of the genera *Acremonium*, *Alternaria*, *Aspergillus*, *Chordomyces*, *Emericellopsis*, *Fusarium*, *Gibellulopsis*, *Myriodontium*, *Penicillium*, and *Pseudeurotium* were detected [25].

Fungal strains representing the genera *Aspergillus*, *Penicillium*, *Trichoderma*, *Fusarium*, *Mucor*, and *Rhizopus* are known for their significant enzymatic potential and are often involved in organic matter decomposition, making them valuable in biotechnology [2, 3]. Additionally, strains from the genera *Trichoderma*, *Penicillium*, *Aspergillus*, and *Fusarium* are used in the production of biopreparations for agricultural applications [2, 25, 29, 31].

Within aquatic ecosystems, these fungi, as major decomposers, represent a promising avenue for environmental monitoring, offering a sensitive and multifactorial approach to assessing freshwater health. Their ability to respond rapidly to a wide range of environmental stressors – such as pollutants, climate

change, and habitat degradation –combined with their essential roles in nutrient cycling, establishes them as efficient bioindicators capable of providing early signals of ecological imbalances. Moreover, aquatic fungi can serve as valuable bioindicators in less-studied ecosystems, such as temporary rivers, brown water lakes, and small ponds [8, 23, 54, 49, 62].

This observation was also confirmed by our research, where the density and diversity of fungal communities indicated varying degrees of pollution among the studied lakes. Lake "La Izvor", which has never been cleaned, exhibited the highest density and diversity of fungal communities, indicating the most severe level of pollution compared to the other lakes studied, and therefore requiring urgent remediation.

Furthermore, it can be noted that the studied aquatic basins harbor a wide diversity of filamentous fungi, which could serve as an inexhaustible and readily accessible source of microorganisms with biotechnological potential. The identification and characterization of these microbial resources can contribute to the development of new sustainable products and technologies applicable in agriculture, industry, environmental protection, and even the pharmaceutical sector. In an era where innovative biological resources are increasingly sought to address global challenges related to food security, climate change, and pollution remediation, the fungal diversity of these aquatic ecosystems emerges as a strategic element of both scientific and economic interest.

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