

Mycological assessment of the waters of the Araz River in the territory of the Republic of Azerbaijan

 **Muradov P.Z^{1*}, Bakhshaliyeva K.F^{1,2}, Isayeva V.K.¹, Muradova S.M³,  Azadaliyeva S.F.⁴**

¹Institute of Microbiology, Ministry of Science and Education of the Republic of Azerbaijan, Baku, Azerbaijan; mpanah@mail.ru (M.P.Z.) isayevavusale33@gmail.com (I.V.K.).

²Institute of Fruit and Tea Cultivation of the Ministry of Agriculture of the Republic of Azerbaijan, Guba, Azerbaijan; konul.baxsh@mail.ru (B.K.F.).

³Azerbaijan State Pedagogical University, Baku, Azerbaijan; muradovasevda1961@gmail.com (M.S.M.).

⁴Sumqayit State University, Republic of Azerbaijan, Sumgait, Azerbaijan; azadeliyevaseadet@gmail.com (A.S.F.).

Abstract: The purpose of the presented work was to analyze the fungal biota of the Araz River, which is the largest tributary of the Kura River and is considered a transboundary river, from the point where it enters the territory of the Republic of Azerbaijan to the point where it joins the Kura River. It has become clear that fungi are found in river waters in any season, and their number varies between 500 and 1900 CFU/ml. It was determined that 41 fungal species participated in the formation of the mentioned number, which are characterized by wide diversity in terms of their taxonomic structure and forms of manifestation of ecotrophic specialization (conditionally pathogenicity, toxigenicity, phytopathogenicity, etc.). Despite the fact that fungi participate in a wide range of functions in any ecosystem, there are still no normative indicators regulating the use of fungi in the biomonitoring of aquatic ecosystems. The results of studies conducted in a specific area allow us to note the importance of preparing such a document, especially in a situation where biological pollution of waters is inevitable.

Keywords: Transboundary river, fungal biota, number composition, species composition, pollution degree.

1. Introduction

Water, one of the most widespread and unique substances in nature, is figuratively considered one of nature's anomalous substances, which is explained by the fact that some of its properties do not even comply with physical laws in some cases. There is no life without water, and the amount of water in the bodies of all living things varies between 50-90% [1]. In short, the existence of any living being, the realization of any technological process, and more precisely, the creation of a natural environment for life on Earth, occurs with the participation of water.

The quantitative and qualitative indicators of water, which are of such great importance and are the basis for its use, are determined by various criteria. These criteria are generally physicochemical and biological in nature [2]. The physicochemical characteristics of water include its taste, smell, pH, temperature, density, amount of cations and anions, transparency, degree of mineralization, hardness, etc. Biological indicators of water are related to the presence of living organisms in the water, primarily microorganisms and their number.

Currently, people use water for domestic purposes (drinking, preparing food, meeting personal hygiene needs, etc.), industry, and agriculture. The total water resources on Earth are 1.4 billion km³, with only 2.5% (35 million km³) being freshwater. Most of the world's freshwater is stored in glaciers (Arctic, Antarctic, and Greenland), 69%, groundwater and groundwaters, 30%, and a small part in rivers, lakes, and reservoirs [3].

The world's population is increasing year by year, which in turn leads to an increase in water demand. Increased water use also leads to a number of problems. Examples of these include the decline in water resources, pollution of water bodies, deterioration of drinking water quality, reduction in the water levels of large rivers, lack of water resources in drought zones, and so on. Solving these issues is currently a global concern that is on everyone's mind. Currently, 40% of the world's population lives in water scarcity [4].

If we look at the Republic of Azerbaijan in the context of problems related to water resources in the world, we can clearly see that the situation is not so encouraging, and it is worth recalling just one fact to confirm this. Thus, up to 70% of the country's surface waters are formed by transboundary rivers, and two of the three countries through which these rivers flow (Armenia and Georgia) have not joined the 1992 Helsinki Convention on the Protection and Use of Transboundary Watercourses and International Lakes [5].

A complete solution to water-related problems, both globally and in Azerbaijan, does not seem realistic, but it is possible to minimize the negative impacts of these problems, increase the efficiency of water resource use, and make many problems manageable. For these reasons, the methods and approaches used to calculate the amount of water resources and increase the efficiency of their use must be updated in accordance with the principles of sustainable development of society [6], and it is extremely important, or rather necessary, to prepare a monitoring system that meets modern requirements.

Biological safety of surface water is one of the issues that are not given due attention in their work aimed at solving water-related problems. Thus, the World Health Organization (WHO) has identified more than 90 criteria for drinking water quality indicators, which can be summarized under the headings of microbiological, chemical, and radiological [7]. Microbiological criteria mainly concern the absence of disease-causing bacteria and viruses in water, with studies primarily focusing on bacteria [8]. Regulatory documents on drinking water do not include indicators related to fungi, and the main emphasis remains on fecal contaminants [9]. Although fungi have the ability to spread anywhere that has organic matter (water, soil, air, plants, animals, etc.), among them are both pathogens, toxins, and allergens [10]. Any of the mentioned can be transmitted by water and aquatic organisms, and as a result of this, serious consequences for human health are quite real, and these cases have already encountered in practice [11]. Because 80% of the polluted water produced by human activities is discharged into rivers, seas, and oceans without purification, the environment is polluted, and more than 50 diseases are created. Additionally, 80% of diseases and 50% of child deaths worldwide are caused by poor water quality [12].

There is some literature on the role of fungi in the deterioration of water quality indicators, but it is not sufficient to assess water quality deterioration based on fungi's role in water and the damage they cause.

Therefore, the aim of the presented work was to assess the fungal biota of the Araz, one of the largest transboundary rivers of Azerbaijan, in terms of number and species composition.

2. Materials and Methods

General characteristics of the sampled river. The studies were conducted in the section of the Araz River from its entry into Azerbaijani territory to its confluence with the Kura River (Figure 1). It should be noted that the Araz is the largest tributary of the Kura, the largest river in the Republic of Azerbaijan. The total length of the river is 1072 km, of which 364 km belongs to the territory of Turkey.



Figure 1.

General view of the sampled areas of the Araz River (●).

Its basin area is 101.9 thousand km². It originates from the northern slope of the Bingöl Mountain Range in Turkey (2990 m). After the confluence of the Akhura branch, the Araz forms the state border of Armenia and Azerbaijan with Turkey and Iran for a distance of approximately 600 km, up to the vicinity of the Bahramtepe water junction. The last 80 km of the river flow through the territory of Azerbaijan and flow into the Kura River in the area of the city of Sabirabad. It is the second-largest river in Transcaucasia in terms of water volume. The Araz is divided into two parts (mountainous and lowland) according to its relief and flow characteristics. In Turkey, the Araz is a typical mountain river. The speed of the river in this section is relatively slow. Here, the Sevjur, Razdan, Arpachay, Nakhchivan, and other tributaries join the Araz from the left, and the Garasu, Maku, and other tributaries from the right. In this section, the Araz receives the Alinja, Ordubad, Mehri, Ohchu, etc., tributaries from the left, and the Gotur, Girsi, etc., tributaries from the right. After the mouth of the Hakari River, the Araz gradually rises to the plain and the Kur-Araz lowland. Below the mouth of the Hakari River, the Araz River is joined by the Guruchay, Kondalanchay, and other tributaries on the left, and the Selin, Garasu, and other tributaries on the right. The Araz River has no tributaries in the section from the Bahramtepe water junction to its mouth. The active erosion process of the river in the mountainous area is replaced by accumulation in the plains. The Araz is fed by mixed sources. 44% of its flow is made up of rivers, 38% of snow waters, and 18% of rainwater. The average annual water flow near the mouth of the Araz is 279 m³/sec, and the flow volume is 8.8 billion m³. Its water is hydrocarbonate-calcium. The average mineralization is 560-880 mg/l during the low-water period and 260-400 mg/l during the high-water period. The Araz has great irrigation and hydropower importance. To use its water efficiently, the Bahramtepe water junction was built on the river, and according to the

Soviet-Iranian agreement, the Araz water junction and the Mil-Mugan water intake junction were built. The Araz is unsuitable for navigation [13, 14].

Sampling and analysis. Samples were taken from 5 points along the Azerbaijani territory of the Araz River, with the distance between the sampling points being 12-20 km (depending on the availability of appropriate conditions for sampling). For sampling, sterile glass flasks (500 ml) were used, and at least 4 samples were taken from each point. Transferring the collected samples to the nutrient medium (agarized malt juice, Saburo agar, agarized Chapek, etc.) was done either at once or by diluting to 1 ml and incubating at 28°C for 3 days. Determination of the number of colonies formed after 3 days was calculated according to the following formula:

$$N(\text{CFU/ml}) = \frac{a \cdot b \cdot c}{d}$$

where N is the number of fungi, a is the number of colonies formed in a Petri dish, b-is the amount of dilution, c is the number of drops in 1 ml of suspension, and d-is the amount of water taken for analysis (ml).

The species composition of the fungi in the samples was determined according to known classical mycological methods [15], and their microscopic and macroscopic features were studied. The results obtained were identified with the data in known identifiers [16, 17] and atlases [18-20] to determine the species composition.

3. Results and Discussion

Fungi are cosmopolitan organisms that are permanent components of any ecosystem containing organic matter, which is facilitated by their easy adaptation to environmental conditions. This was confirmed by the results of the analysis of the fungal biota of water samples taken from the Araz River, which was chosen as the object of the study, both in terms of number and species composition. Thus, it was shown that fungi were present in each sampled location and that they had a certain taxonomic diversity (tab.1). Apparently, fungi have been found in the Araz River even in winter. After the Araz River enters Azerbaijan from Armenia, the number of fungi in the water decreases up to a certain distance, and after the second point, an increase in the number of fungi is observed. The highest level of this increase occurs at the last point, where the river joins the Kura. In our opinion, the reason for this is that points 1 and 2, where the sample was taken after the Araz entered the country from Armenia, were territories occupied by Armenia between 1993 and 2020, and the population there was extremely low. In points 3-5, polluted water generated as a result of population use is often discharged into the river without treatment, which results in an increase in organic matter there. The abundance of substances that constitute the food of fungi, among them, ultimately leads to an increase in the number of fungi.

It should be noted that when assessing the degree of water pollution according to microbiological indicators, the main indicator is the amount of bacteria in the water (CFU/ml) [21], and the amount of bacteria for category I (completely clean) and category II (less clean) waters is 0.5 CFU/ml and 0.51- 5.0×10^3 CFU/ml, respectively. If we characterize the results obtained in the studies according to these indicators, the water of the Araz River along the studied areas can be classified as low-purity water, that is, category II. Looking deeper into the matter, it can be noted that the issue is not at all as stated, and it is not correct to apply the same approach to the numbers of fungi and bacteria. Thus, one of the most common groups of biota in any ecosystem that includes water, soil, plants, and animals is bacteria and fungi, the former of which differ from other living things in terms of their numerical composition and the latter in the amount of biomass they produce. Studies have also shown that the number of bacteria in most biotopes, even in the human body, is many times higher than that of fungi [22], but the fact that fungal cells are larger in size than bacteria and have a mycelial structure [23] casts doubt on the numerical superiority of bacteria. On the other side, the biologically active metabolites of fungi, including those with toxic effects, are broad-spectrum compared to other organisms, including bacteria [24, 25].

Table 1.

Variation in the number composition of fungal biota along the Araz River.

Indicatores	Number of fungi, CFU/ml				
	1*	2	3	4	5
Spring	1600	1100	1300	1500	1600
Summer	1800	1400	1500	1700	1900
Autumn	1600	1200	1300	1400	1600
Winter	900	600	600	700	900

Note. *1-Jabrayil, 2- Fizuli, 3-Beyləqan, 4-İmishli, 5-Sabirabad (Sugovushan).

It should be noted that various criteria are used to assess environmental quality, including mycological factors such as taxonomic diversity, the proportion of melanin-synthesizing micromycetes, opportunistic micromycetes, eurytrophic micromycetes, and the proportion of widespread and rare species [16]. Taking this into account, the data obtained from the studies conducted in the studied section of the Araz River were analyzed based on some of the mentioned criteria.

The primary criterion is related to the taxonomic structure of the fungi recorded in the studied areas, and according to classical mycological methods, the distribution of 41 species (*Acremonium charticola*, *A. spinosum*, *Alternaria alternata*, *A. tenuissima*, *Aspergillus carneus*, *A. fumigatus*, *A. flavipes*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. sydowii*, *A. terreus*, *A. ustus*, *A. versicolor*, *Aureobasidium pullulans*, *Botrytis cinerea*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *C. herbarum*, *Fusarium oxysporum*, *F. solani*, *Mucor circinelloides*, *M. hiemalis*, *M. racemosus*, *Penicillium aurantiogriseum*, *P. brevicompactum*, *P. canescens*, *P. citrinum*, *P. commune*, *P. expansum*, *P. funiculosum*, *P. granulatum*, *P. restrictum*, *P. rubrum*, *P. spinulosum*, *Phoma herbarum*, *Ph. medicaginis*, *Rhizopus stolonifer*, *Stachybotrys chartarum*, *Trichoderma koningi* and *T. viride*) of fungi was determined in that part of the Araz River, and information about their taxonomic structure was given in table 2. As seen, the recorded fungi do not belong to the *Basidiomycota* division, and the majority of fungi belong to the *Ascomycota* division.

Table 2.

Taxonomic structure of fungi recorded in studies.

Kingdom	Subkingdom	Division	Class	Order	Family	Genus (species)
Mycota	Mucormycota	<i>Mucormycota</i>	1	1	1	2(4)
	Dikarya	<i>Ascomycota</i>	3	5	9	12(37)
Total	2	2	4	6	10	14(41)

More precisely, they belong to the anamorphs of sac fungi. Thus, all 37 recorded species of sac fungi belong to anamorphs. The abundance of anamorphic fungi, their high adaptability, and the presence of melanin-synthesizing fungi, more precisely, those that form dark-colored colonies, eurytrophs, opportunists, toxigenic fungi, and other characteristics cast doubt on the characterization of the Araz River as having less clean water. Thus, only 5 (*Acremonium charticola*, *A. spinosum*, *Botrytis cinerea*, *Fusarium oxysporum*, and *F. solani*) out of the 41 recorded fungal species have colonies that are white or close to it, and the rest form colonies with shades of color from gray to dark black. According to the principles [26] of using microorganisms in environmental biomonitoring, a high number of dark-colored mycelial fungi is considered an indicator of pollution. On the other hand, among the recorded fungi, both phytopathogens (*Acremonium charticola*, *A. spinosum*, *Alternaria alternata*, *A. tenuissima*, *Botrytis cinerea*, *Fusarium oxysporum*, *F. solani*, *Phoma herbarum*, *Ph. medicaginis*, *Rhizopus stolonifer*, etc.) and toxigenics (*Alternaria alternata*, *A. tenuissima*, *A. fumigatus*, *A. flavipes*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. sydowii*, *A. terreus*, *A. ustus*, *A. versicolor*, *Botrytis cinerea*, *C. herbarum*, *P. citrinum*, *P. commune*, *P. expansum*, *P. funiculosum*, etc.) are found, which constitute a significant part of the total fungi recorded there.

In the sanitary regulations of some countries, not a specific species belonging to a particular genus, but all species belonging to genera such as *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Fusarium*, *Penicillium*, *Phoma*, *Mucor*, *Rhizopus* and *Trichoderma* are classified as group 4 -opportunistic pathogens

[27] then the presence of such fungi in one or another biotope is a serious reason to note the presence of biological pollution of the environment.

As mentioned above, bacteria are mainly used to determine categories for assessing the degree of pollution of waters, including freshwater, so that in our previous studies, the Araz River, until it joins the Kura, passes from category II to category III ($5.1-10 \times 10^3$ CFU/ml), which is characterized as polluted waters. In determining the categories of pollution, neither the number nor the species composition of fungi is taken into account, and in our opinion, it is incorrect to assess the state of the waters, especially from the point of view of biological safety, as it generally indicates a minimal level of pollution. Thus, the fact that fungi are not taken into account in this issue does not allow for real conclusions to be drawn regarding the dangers posed by water, namely the transmission of this or that disease, the occurrence of toxicoses, and the determination of biological risk levels in general. For this reason, it is important to take fungi into account when determining the pollution category of water in the future; that is, it is very important to prepare regulatory documents that reflect this, and to take into account the ratio of bacteria and fungi when determining pollution categories. More specifically, the indicators of the bacteria/fungus ratio for water pollution categories should be clarified. Because freshwater ecosystems are characterized by complex food relationships [28], while some of the living organisms that live here (algae) participate in the formation of primary organic matter (as producers), some are consumers (fish, arthropods, etc.), and some are reducers (bacteria and fungi). Although aquatic fungi have not been widely studied, they play important roles in freshwater ecosystems, which are manifested in the degradation of organic residues, material cycling, and other processes [29]. In addition, fungi are also important in the population dynamics of phytoplankton [30]. Today, it can be said with certainty that the role of fungi in freshwater ecosystems is not limited to those mentioned above, and this should be clarified in future research. In addition, fungi are currently the focus of research as active producers of secondary metabolites [25] and studies aimed at the practical use of the secondary metabolites [31] they synthesize are increasing.

4. Conclusion

Thus, during the assessment of the part of the Araz, the transboundary river of Azerbaijan, where it joins the Kura, for its fungal biota, it became clear that today there are no perfect normative documents for the assessment of waters for their fungal biota, neither in the world nor in the Republic of Azerbaijan. Therefore, today it is important to develop a new monitoring system to determine the levels of pollution of waters, including river waters, and to prevent threats to waters, primarily biological pollution. For this system to be perfect, it is extremely important to consider the number of fungi, as well as their ratio to bacteria.

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Transparency:

The authors confirm that the manuscript is an honest, accurate, and transparent account of the study; that no vital features of the study have been omitted; and that any discrepancies from the study as planned have been explained. This study followed all ethical practices during writing.

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