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ABSTRACT

of the dissertation for the degree of Doctor of Philosophy

SPECIES COMPOSITION AND ECOBIOLOGY OF MICROMYCETES IN SOILS EXPOSED TO SALINATION

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Field of science:	Biology
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Baku – 2024

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INTRODUCTION

Relevance and degree of development of the topic. The soil plays a vital role within the biocenosis, serving as a primary factor in maintaining its genetic and species diversity, as well as contributing to the overall ecosystem richness. The soil's productivity correlates directly with its quality, making it a crucial determinant of organic richness alongside its mineral composition. This constitutes the organic wealth of the soil, encompassing living organisms and their residues that contribute to soil biodiversity. "*Bacteria, fungi, and other primitive organisms, which have their own place and role in the composition of soil biodiversity, have a high quantitative index in terms of species and number composition."*[1]. These organisms, which have a special role in soil biomass, have an important and undeniable role in soil formation and soil mineralization. These organisms play a vital role not only in soil formation but also in the cycling of substances, elements, and energy within the ecosystem.

This highlights the necessity of examining the microorganisms within the biotic structure of soil, alongside analyzing its chemical composition, to accurately assess soil quality.

Global change, disruption of ecological balance, climate variations, and heightened anthropogenic impacts have resulted in more extreme conditions and the intensification of various stressors. These changes have prompted organisms to either perish or adapt by acquiring new traits suited to their environment.

The examination of organisms, including microorganisms, that have adapted to thrive in altered or particularly extreme environments, is of paramount importance from both a scientific and practical standpoint. Therefore, investigating diverse organisms capable of thriving in such environments, particularly from an ecophysiological perspective, can spur the development of biopreparations with novel qualities and enhance technologies for extracting a broad array of biologically active substances.

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[1] Fierer, N. Embracing the unknown: disentangling the complexities of the soil microbiome. Nature Reviews Microbiology, 2017, 15, 579–590.

Alternatively, viewed from the perspective of restoring altered environments, understanding the processes occurring therein and comprehensively studying their participants are the realities unveiled by the modern era.

"Soil salinization is a serious and prominent problem" in numerous regions worldwide, resulting in the accumulation of salt in the topsoil, particularly due to evaporation.[2] . This issue primarily arises in arid and semi-arid regions, "*with over 20% of the world's irrigated areas currently affected by salt stress. Projections indicate that this figure could escalate to 50% by 2050*"[3].

The issue of land salinization, particularly affecting agriculturally suitable areas, is not unfamiliar in the Republic of Azerbaijan. Reports indicate that "43.8% of Azerbaijan's irrigated lands have experienced some degree of salinization."[4].

As interest in studying fungi within soil biodiversity, particularly those thriving in extreme conditions, continues to grow, research efforts in this area have also intensified.

Given that saline soils present an extreme environment for organisms, exploring the species composition, distribution patterns, and other characteristics of microscopic fungi in such environments is of significant interest both scientifically and practically. While mycological research has been ongoing in various habitats in the Republic of Azerbaijan for some time, there has yet to be a comprehensive study of the mycobiota in extreme environments, particularly saline soils. Therefore, there is an undeniable importance in conducting research in such biotopes.

Purpose and objectives of the research. The aim of this study is to compare the mycobiota of the coastal soils of Lake

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[2] Chele, K.H., Tinte, M.M., Piater, LA. Et al. Soil Salinity, a Serious Environmental Issue and Plant Responses: A Metabolomics Perspective.//Metabolites, 2021, 22;11(11):724. doi: 10.3390/metabo11110724.

[3] Hussain, S., Shaukat, M., Ashraf, M. et al. Salinity Stress in Arid and Semi-Arid Climates: Effects and Management in Field Crops.//IntechOpen, 2019 doi: 10.5772/intechopen.87982.

[4] Aliyev Z.H. Aliyev Z.G. and Aliyeva Kh.Z. Problems of Irrigated Farming in Azerbaijan and Prospects of its Development.// Acta Scientific Agriculture 2.3 (2018): 42-45.

Masazir, an area with high salinity in Azerbaijan, in terms of both the abundance and species composition of micromycetes, as well as their sensitivity to salinity.

To accomplish the stated objective, the following tasks are outlined for resolution:

1. Determination of the physicochemical parameters of the coastal soils of Lake Masazir, Absheron Peninsula;
2. Determination of the species and numerical composition of fungi involved in the formation of the mycobiota of Masazır Lake;
3. Characterization of the fungi involved in the formation of the mycobiota of Masazir Lake based on their frequency of occurrence and ecotrophic relationships;
4. Studying the influence of salinity on the biomass yield of fungi contributing to the formation of the mycobiota of Masazir Lake.

Research methods. The study used mycological, microbiological and biochemical methods and approaches used in similar studies. Sampling for the study of the mycobiota involved the route method, obtaining pure fungal cultures through dilution, and determining the salt tolerance index (SDI) based on biomass extraction. Statistical analysis was conducted using the SPSS 23 IBM program, while ArcGIS 10.4.1 was utilized for setting GPS field codes, database creation, and mapping. The experiments were repeated a sufficient number of times to allow for statistical analysis of the results. The data used in the dissertation met the reliability criterion (i.e., compliance with the formula $m/M \leq 0.05$), ensuring the accuracy of the findings.

The main clauses brought for protection.

1. Soil salinization significantly affects the growth and abundance of organisms;
2. Despite the strong impact of salinity, each mycobiota is unique in its species composition;
3. Different levels of salinity in nutrient media influence the biomass yield;
4. The high halotolerance index of microfungi species from highly saline areas reflects their adaptation to altered conditions;

The scientific novelty of the research. During the research, the coastal soils of Lake Masazir were thoroughly examined to assess the type and abundance of mycobiota, their frequency of occurrence, ecotropic relationships, and salt tolerance.

The mycobiota of Masazir Lake comprises 25 species from 12 genera within the Ascomycota branch. Among these, 56% of the species (14 species from 3 genera) belong to the Eurotiomycetes class, while 16% (4 species from 3 genera) belong to the Dothideomycetes class, and the remaining 28% (7 species from 6 genera) are representatives of the Sordariomycetes class.

Among the fungi identified in the study area, *Aspergillus arenarioides* Visaqie, Hirooka & Samson, *A.transcarpathicus* A.J.Chen, Frisvad &Samson, *Cordyceps farinosa* (Holmsk)Kepler, B.Shrestha & Spatafora(=*Paecilomyces farinosus* (Holmsk)A.H.S.Br &G.Sim), *Fusarium equiseti* (Corda)Sacc, *F.xylarioides* Steyaert, *Parengyodontium album* (Limber) C.C.Tsang, J.F.W.Chan, W.M.Pong, J.H.K.Chen, A.H.Y.Nigan, M.Cheung, C.K.C.lai, D.N.C.Tsang, S.K.P.Lau, &P.C.Y.Woo(=*Beauveria alba* (Limber), Saccas), *Penicillium subericola* Barreto, Frisvad & Samson, *Purpurocillium lilacinus*(Thom)Luangsa-ard, Houbraken, Hywel-Jones & Samson(= *Paecilomyces lilacinus* (Thom) Samson) stands out as a novel addition to the typical mycobiota of Azerbaijan.

Out of the 25 fungal species recorded during the study, 6 exhibit a frequency of occurrence typical of dominant species, 8 are frequently encountered species, while 11 are considered random or rare species.

A total of 22 out of the 25 fungal species documented during the study exhibit salt tolerance, indicating their halotolerant nature. It has been observed that these species can thrive even under conditions where the concentration of NaCl in the medium exceeds 10%.

Establishing that species such as *Aspergillus ochraseus, A.terreus*, *A.niger*, *Aspergillus sp.*, *Penicillium subericola*, *P.citrinum*, *P.resticulosum, Cladosporium cladosporioides* and *Fusarium xylarioides* are halotolerant, it was found that their lethal dose exceeds 20% due to NaCl.

The theoretical and practical significance of the research. The obtained results represent factual material that contributes to expanding knowledge about fungi involved in the formation of mycobiota in saline soils.

The obtained results serve as important indicators that confirm the usefulness of using microfungi in the biological cleaning and restoration of areas affected by environmental pollution in the future.

Determining the salt tolerance index can provide essential data for identifying genes that regulate this index in genetic studies and for further research in this field.

The results acquired highlight the potential benefits of the symbiotic relationship between endomycorrhizal and ectomycorrhizal forms with agricultural plants.

Approbation and application of dissertation. The dissertation has resulted in 9 publications, comprising 5 scientific articles and 4 conference papers and dissertations. The materials of the dissertation were presented at the jubilee conference on Microbiology and mycology (Moscow, 2018), at the scientific-practical conference on "Actual problems of modern biology" (Baku, 2019), at the "IV international scientific conference of young researchers" (Baku, 2020), at the "II international science and technology conference" (Baku, 2021) was reported.

Organization where the dissertation is performed. The research was conducted in the microbiological biotechnology and experimental microbiology laboratories of the Institute of Microbiology under the Ministry of Science and Education of the Republic of Azerbaijan.

The total volume of the dissertation. The dissertation comprises an introduction, four chapters, a concluding research analysis, main conclusions, a list of references, appendices, and an abbreviation list used in the dissertation. The dissertation spans 145 pages, encompassing tables and figures, along with a bibliography, totaling 215,400 characters.

CHAPTER II
MATERIALS AND METHODS

2.1. General characteristics of the research areas

The research focused on the coast of Masazir Lake, situated in the Absheron region and renowned as a natural salt source. Absheron region is located on the west coast of the Caspian Sea. Absheron district borders with Sumgayit city from the north, Khizi district from the north-west, Gobustan district from the west, Hajigabul district from the south-west, and Baku city from the east. In the territory of the district there is 1 city, including the city of Khyrdalan, 8 settlements, including the city of Djeyranbatan, Saray, Mehdiabad, Nubar, Digah, Gobu, Guzdek, Hokmeli, and Ashagi Guzdak, and 6 villages, including Masazır, Fatmayi, Mammadli, Göradil, Pirakeshkul, and Novkhani. The soil is mainly saline and sandy. The Masazır Lake, situated in the Masazır village of the Absheron region, is positioned approximately 21 km northwest of Baku city. It lies between coordinates 49°45'15"E and 49°47'45"E longitude, and 40°29'21"N and 40°31'40"N latitude, nestled amidst Novkhani and Masazır villages and the Saray settlement. The lake sits at an elevation of 4 meters above sea level. The lake, characterized by its elliptical shape, comprises stagnant saltwater with no inflow or outflow. Stretching along a coastline of 14 km, it covers an area of 10 km2, with a salinity level of 33 ppm (>200 mS/s). Since 1813, the lake has been a regular source of salt extraction, with a salt factory situated on its shoreline. Its water source primarily comprises rainfall, supplemented partly by underground water.

2.2. A comprehensive overview of the methods and approaches employed during the analysis of the gathered samples

The research was conducted between 2017 and 2022. Masazır Lake was divided into four directions: North-East, North-West, South-East, and South-West. Wells of 100-200 m depth and 50-80 cm depth were dug along the shore, approximately 2-3 m away from the lake, and determined diagonally from the vertical part. A total of 216 soil samples were collected from various depths (surface, 10 cm, 20 cm, 40 cm, 60 cm, 80 cm). To determine the degree of salinity of the collected soil samples, 10 g of each of the soil samples taken using the “*known methods*”[5]. is weighed and mixed with 50 ml (1:5) distilled water in a sterile flask for 30-60 minutes and a soil suspension is prepared. The prepared suspension is left undisturbed for a specific duration, after which the soil's salinity is assessed by measuring the solution's specific electrical conductivity using a qcond 2200 cϵ electrical conductometer (EC). Concurrently, the suspension's acidity (pH) is measured using the “Hanna Instruments HI 2221” instrument.

The resulting suspension is transferred, either directly or after dilution (up to 10-100 times), onto a Petri dish containing 1 ml of solid nutrient medium. The suspension obtained is transferred either directly or by dilution (up to 10-100 times) onto a Petri dish containing 1 ml of solid nutrient medium. Afterward, the Petri dishes are sealed and placed in a thermostat set at a temperature of 27±1°C. From the third day of growth, the colonies formed in the nutrient medium are diluted and transferred to a new Petri dish, and the process is continued with a pure culture medium. The purity of the culture is monitored using a microscope (OMAX 40X-2500X LED Digital Trinocular Lab Compound Microscope).

Agarized malt juice (ASH), rice (DA), starch (NA), potato [6](KA) agars, and agarized Capek environment were used as nutrient media for pure culture of fungi. Preparation of media, sterilization and pouring into Petri dishes, as well as their inoculation, were carried out according to "*known methods*"[7,8] accepted in microbiology and mycology.

The numerical composition of the mycobiota in the study area was determined using the formula “*N(CFU/g) = (bvq/d)*”[9].

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5 Rayment G.E. and Higginson F.R. Electrical conductivity, in Australian laboratory handbook of soil and water chemical methods, Inkata Press, Melbourne, 1992, -p.15-16.

6 Методы экспериментальной микологии/Под. ред. Билай В.И. Киев: Наукова думка, 1982, 500 с.

7 Нетрусов А.И., Егорова М.А.,Захарчук Л.М. и др. Практикум по микробиологии. М.:Издательский центр «Академия», 2005, 608 с.

8. Мирчник, Т.Г. Почвенная микология./Т.Г.Мирчник. -М.:Из-во МГУ, -1988, -220 с.

9. Герасимов А. О., Поляк Ю. М. Оценка влияния засоления на аллелопатическую активность микромицетов в дерново-подзолистой почве//Агрохимия, 2021, № 3, с. 51–59 51.

The macroscopic and microscopic characteristics of each fungal colony transferred into pure culture were examined, and the species composition was determined based on the "known determinants".

The frequency of occurrence and prevalence rate of micromycetes recorded in the studies were determined using the formula: 100RT= (n/N) x100, where *RT* epresents the frequency of occurrence (or prevalence rate) of micromycetes in percentage, *N* is the total number of research areas, *n* is the number of research areas where micromycetes were detected.

According to the obtained results, the fungi isolated from the research area were categorized as follows: dominant if the frequency of occurrence was 50% or more, frequent if it ranged between 10-40%, and rare or random species if it was less than 10% [Borrego, S. et, al 2016, Shearer.C et, al 2007, Hyde.K et, al 2007].

In addition to calculating RT, the territorial distribution of the species isolated from the area was analyzed using the ArkGIS 10.4.1 program. This software was also utilized for specifying the GPS codes of the isolated areas, creating a database, and mapping the distribution.

To assess the biomass yield of each species on a dry nutrient medium relative to varying salt concentrations in laboratory settings, 100 ml of NaCl solution with different concentrations (ranging from 0.25% NaCl to 30% NaCl) were added to separate 250 ml flasks. Subsequently, the flasks were sterilized under pressure at 0.5 atm for 45 minutes.

The selected strains were cultivated in a dry nutrient medium containing various concentrations of NaCl and incubated in a thermostat at a temperature of 27 ± 2°C for 7-10 days. Following the incubation period, the samples were filtered through filter paper and subsequently dried at a specific temperature (105°C) to measure the dry biomass. After drying, the dry biomass was weighed using an analytical balance. A KERN ABS 220-4 N analytical balance equipped with an automatic timer and offering an accuracy of 0.2 mg was employed for this measurement.

The salt tolerance index in environments with different concentrations for each species was calculated using the formula: **STI= (TDW at Sx/ TDW at S1)x100** where *STI* represents the salt tolerance index, *TDW* at *S1* indicates the total dry weight of the control, and *TDW* at *Sx* denotes the total dry weight of the sample [Bağci, S.A et al. 2003].

Throughout the research, experiments were conducted with 4-5 repetitions, and the obtained results were "statistical". Results satisfying the criterion $m/M = P \leq 0{,}05$ (where *M* represents the average value of repetitions, *m* denotes the mean square deviation, and *P* signifies the student’s criterion) were deemed reliable and included in the dissertation.

CHAPTER III
GENERAL CHARACTERISTICS OF MASAZIR LAKE AND ITS MYCOBIOTA

3.1 Physical and chemical parameters of the soil of Masazır lakeŞ

A total of 216 soil samples were collected from 42 sampling sites situated along the coastline of Masazır Lake, the focal area of this study. These sampling sites were categorized into four distinct sections: North-East, North-West, South-East, and South-West. The soil characteristics of the study site can be described as predominantly gray-brown, with a composition that includes elements of sand, gravel, and clay. The salinity levels range from 1.08 mS/cm to 61.12 mS/cm, while the pH levels vary between 7.4 and 9.03. Table 3.1 summarizes information about the determined parameters of the samples taken from the land plots selected in the research.

Table 3.1
Description of soil samples collected from the coastline based on specific indicators.

№	Conditionally divided territory	Soil charachteristics	Average salinity concentration mS/cm	Average pH value
1	North – East coast	Sandy, gravel, mostly clayey	20.49	8.03
2	North-West coast	Sandy, gravelly, clayey	14.42	7.87
3	South – East coast	Sandy, gravelly, mostly clayey	12.6	8.03
4	South - West coast	Sandy, gravelly, clayey	14.26	7.83

It is important to recognize that soil serves as a habitat for various organisms and is a crucial component of biodiversity. Therefore, the suitability of the soil environment is regarded as a key factor. Salinity stress, like other stressors, significantly impacts soil fertility and biodiversity.

3.2. Evaluation of fungi contributing to the mycobiota formation in Lake Masazir based on their abundance and species composition.

It should be noted that, "*soil microbiota is the first to respond to the effects of various types of pollution*"[10] and at the initial stage of pollution its number and species abundance, taxonomic structure, metabolic activity and activity of soil enzymes change. In saline soils, the activity of several "*microbiological processes intensity tends to*

[10] Якутин М.В., Анопченко Л.Ю., Андриевский В.С. Влияние засоления на биомассу микроорганизмов в разновозрастных почвах в лесостепной зоне Западной Сибири // Почвоведение. 2016. № 12. С. 1500–1505.

decrease"[11].

Considering these factors, investigations were carried out to analyze the distribution of fungi in the study area, focusing on both species and numerical composition

The species composition of micromycetes comprising the mycobiota of Masazır Lake was determined from 216 soil samples collected at 42 points during the study. Table 3.2 presents the overall structure of the recorded microscopic fungi.

Overall, the fungi isolated from the area predominantly belong to the Ascomycota division. Specifically, 56% of the identified species, representing 3 genera, belong to the Eurotiomycetes class, while 16% of the species, spanning 3 genera, belong to the Dothideomycetes class.

Additionally, 28% of the identified species, distributed across 6 genera, belong to the Sordariomycetes class.

In the formation of this mycobiota, 8 species belonging to the genus (*Aspergillus transcorpathicus, Aspergillus arenarioides, Aspergillus ochraseus, Aspergillus terreus, Aspergillus niger*, *Aspergillus flavus, Aspergillus versicolor, Aspergillus sp*.), 1 species belonging to the genus Parengyodontium (*Parengyodontium album = Beauveria alba*), 2 species belonging to the genus Cladosporium (*Cladosporium cladosporioides, Cladosporium sp*.), 1 species belonging to the genus Curvularia (*Curvularia prasadii*), Epicoccum cinsinə aid 1 növ (*Epicoccum nigrum*), 2 species belonging to the genus Fusarium (*Fusarium xylarioides*, *Fusarium equiseti*), 1 species belonging to the genus Paecilomyces (*Paecilomyces sp*.), 5 species belonging to the genus Penicillium (*Penicillium subericola, Penicillium citrinum, Penicillium glabrum, Penicillium roseopurpureum, Penicillium resticulosum*), 1 species belonging to the genus Phomopsis (*Phomopsis cinerascens*), 1 species belonging to the genus Verticillium (*Verticillium sp*.), 1 species belonging to the genus Cordyceps (*Cordyceps farinosus=Paecilomyces farinosus*), 1 species belonging to the genus Purpureocillium (*Purpurocillium lilacinus= Paecilomyces lilacinus*) in total, 25 species belonging to 12 genera are involved.

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[11]. Артаманова В.С., Дитц Л.Ю., Елизарова Т.Н., Лютых И.В. Техногенное засоление почв и их микробиологическая характеристика//Сибирский экологический журнал, 2010, №3, с.461-470.

Table 3.2

Taxonomic structure of fungal species recorded in the studies

Division	Class	Order	Family	Genus
Ascomycota	Eurotiomycetes	Eurotiales	Aspergillaceae = Trichocomaceae	Aspergillus (8) Penicillium(5)
			Thermoascaceae	Paecilomyces (1)
	Dothideomycetes	Pleosporales	Pleosporaceae	Curvularia (1)
			Didymellaceae	Epicoccum (1)
		Capnodiales	Cladosporiaceae = Davidiellaceae	Cladosporium (2)
	Sordariomycetes	Glomerellales	Plectosphaerellaceae	Verticillium (1)
		Hypocreales	Nectriaceae	Fusarium (2)
			Ophiocordycipitaceae	Purpureocillium (1)
			Cordycipitaceae	Cordyceps (1)
				Parengyodontium (1)
		Diaporthales	Diaporthaceae	Phomopsis (1)
Total (1)	**3**	**6**	**10**	**12(25)**

14

A certain part of the fungi recorded in the researches is from those recorded in the researches conducted in Azerbaijan at different times. Nevertheless, there is no information about the registration of some fungi, first of all *Aspergillus arenarioides* Visaqie, Hirooka & Samson, *A.transcarpathicus* A.J.Chen, Frisvad & Samson, *Cordyceps farinosa* (Holmsk) Kepler, B.Shrestha & Spatafora(=*Paecilomyces farinosus* (Holmsk)A.H.S.Br &G.Sim), *Fusarium equiseti* (Corda)Sacc, *F.xylarioides* Steyaert, *Parengyodontium album* (Limber) C.C.Tsang, J.F.W.Chan, W.M.Pong, J.H.K.Chen, A.H.Y.Nigan, M.Cheung, C.K.C.lai, D.N.C.Tsang, S.K.P.Lau, &P.C.Y.Woo(=*Beauveria alba* (Limber), Saccas), *Penicillium subericola* Barreto, Frisvad & Samson, *Purpurocillium lilacinus*(Thom)Luangsa-ard, Houbraken, Hywel-Jones & Samson(=*Paecilomyces lilacinus* (Thom) Samson), in Azerbaijan, that is, the nature of Azerbaijan can be registered as a new area for them.

The resilience and sustainability of the ecosystem rely not only on species diversity but also on population numbers within the system. Hence, alongside investigating the mycodiversity of the studied area, the numerical composition was also examined. Thus, the north-east coast of the conditionally divided territory is $1.58x10^2$, the north-west coast is $1.25x10^2$, the south-east coast is $1.6x10^2$, the south-west coast is $1.43x10^2$ CFU/g.

During the analysis of factors influencing numerical composition, it is evident that aside from salinity stress, the physical parameters of the land plot also play a significant role in shaping the composition and persistence of mycobiota, as outlined in Table 3.1.

3.3. The occurrence frequency and spatial distribution of fungi contributing to the formation of Lake Masazir's mycobiota

The occurrence frequency of species isolated from the study area was calculated, identifying *Aspergillus niger, Penicillium citrinum, Cladosporium cladosporioides, Cladosporium sp, Fusarium xylarioides, Paecilomyces sp* dominant, *Aspergillus ochraseus, Aspergillus sp, Aspergillus flavus, Parengyodontium* *album = Beauveriya alba, Curvularia prasadi, Purpureocillium lilacinum=Paecilomyces lilacinus, Cordyceps farinosa=Paecilomyces farinosus*, *Phomopsis cinerascens* tez – tez rast gəlinən, *Aspergillus transcorpathicus, Aspergillus arenarioides, Aspergillus terreus, Aspergillus versicolor*, *Epicoccum fungus, Fusarium equiseti, Penicillium subericola, Penicillium glabrum, Penicillium roseopurpureum, Penicillium resticulosum, Verticillium sp.* within Lake Masazir's mycobiota as rare and randomly occurring species.

Hence, among the 25 fungal species identified from the coastal soils of Masazır Lake during the study, 24% exhibit a dominance characteristic in their occurrence frequency, 32% are common species, and 44% are classified as random or rare species. Throughout the study, the taxonomic composition of Lake Masazir's mycobiota, along with the frequency of occurrence of genera and species, as well as the spatial distribution of isolated species and their corresponding GPS coordinates, were established. Subsequently, a comprehensive database was constructed and visualized using the ArkGIS 10.4.1 program (Fig. 3.1).

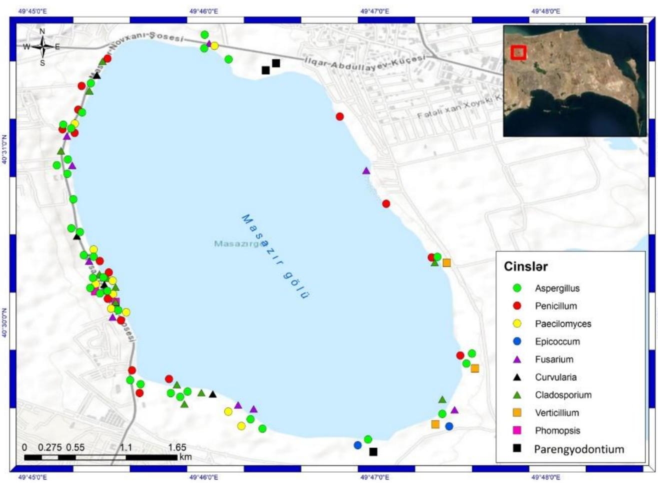

Figure 3.1. The spatial distribution of genera constituting the mycobiota of Masazır Lake

During the research in the study area, it was observed that fungal species are present even at depths of 80 cm in the soil. Dominant species belonging to the genera Aspergillus, Penicillium, and Cladosporium were also identified at this depth.

3.3. Annotated list and description of fungi that constitute the microbiota of Lake Masazir

An annotated list of 25 fungal species recorded in the studies is compiled based on the following data.

1. The current name and systematic affiliation of the fungi recorded in research at different times on the official website() of the International Mycological Association;
2. The place where the sample was taken according to the conditional distribution of Lake Masazir;
3. Cultural-morphological indicators and illustrative materials are considered during the identification of mushrooms.

CHAPTER IV

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF THE SPECIES THAT MAKE UP THE MYCOBIOTA OF MASAZIR LAKE

4.1 Assessment of the resistance of species within the mycobiota of Masazir Lake to salinity stress.

The study investigated the growth and biomass production of fungi isolated from the research site in Capek-Doks dry nutrient medium with varying concentrations of NaCl. Additionally, the salt tolerance index was determined based on the biomass of the mycobiota species (Table 4.1).

Table 4.1
The Salt Tolerance Index (STI) was calculated based on the biomass of fungi contributing to the mycobiota of Lake Masazir.

№	Species isolated from the area	The concentration of NaCl in the Capek-Doks dry nutrient medium in (%), and the corresponding pH indicator					
		2% pH 6.54	5% pH 6.33	10% pH 6.3	15% pH 6	20% pH 5.6	21% pH 5.3
1	2	3	4	5	6	7	8
1	*Aspergillus arenarioides*	2.04	2.03	2.03	1.60	1.46	-
2	*A.flavus*	1.49	1.32	1.43	0.95	0.44	-
3	*A.niger*	2.64	3.59	4.15	3.49	-	-
4	*A.ochraseus*	1.25	1.51	1.77	1.89	2.00	1.47
5	*A.terreus*	2.71	2.20	2.18	1.75	1.70	-
6	*A. transcorpaticus*	1.25	1.01	0.75	-	-	-
7	*A. versicolor*	3.44	2.42	2.04	-	-	-
8	*Aspergillus sp.*	1.58	1.60	2.07	1.64	-	-
9	*Paecilomyces sp*	0.89	-	-	-	-	-
10	*Penicillium citrinum*	1.58	1.58	2.04	1.73	-	-
11	*P.glabrum*	1.30	1.31	1.28	1.18	1.16	-
12	*P.resticulosum*	1.51	2.16	3.55	3.55	-	-
13	*P.roseopurpureum*	1.39	1.23	1.06	-	-	-
14	*P.subericola*	1.24	1.24	1.34	1.55	1.36	-

Table 4.1-continued							
15	*Curvularia prasadii*	2.79	2.21	2.14	-	-	-
16	*Epicoccum nigrum*	0.97	0.64	-	-	-	-
17	*Cladosporium cladosporioides*	1.86	2.01	2.28	2.26	-	-
18	*Cladosporium sp*	0.69	-	-	-	-	-
19	*Phomopsis cinerascens*	4.94	7.88	4.40	-	-	-
20	*Fusarium equiseti*	1.89	3.36	-	-	-	-
21	*F. xylarioides*	1.61	1.89	3.81	5.14	-	-
22	*Purpureocillium lilacinum*	0.72	0.70	0.67	-	-	-
23	*Cordyceps farinosa*	1.08	0.94	0.82	-	-	-
24	*Parengyodontiu m album*	1.47	1.16	-	-	-	-
25	*Verticillium sp*	0.72	-	-	-	-	-

Total 4 species of the genus Aspergillus (*Aspergillus ochraseus, Aspergillus terreus, Aspergillus niger, Aspergillus sp*.), 3 species of the genus *Penicillium* (*Penicillium subericola, Penicillium citrinum, Penicillium resticulosum*), 1 species of the genus *Cladosporium* (*Cladosporium cladosporioides*), 1 species of the genus *Fusarium* (*Fusarium xylarioides*) was observed to grow optimally even in medium with 10% NaCl concentration.

4.2. General statistical analysis and evaluation of fungi for response to salinity stress

Statistical analysis of the results of the response of species isolated from the study area to salinity stress was carried out in the SPSS 23 IBM program. Histogram graphs of the Q-Q plot test showing the results obtained in dry nutrient environments with NaCl concentration of 0.25%, 2%, 5%, and 10% are depicted (Figure 4.1).

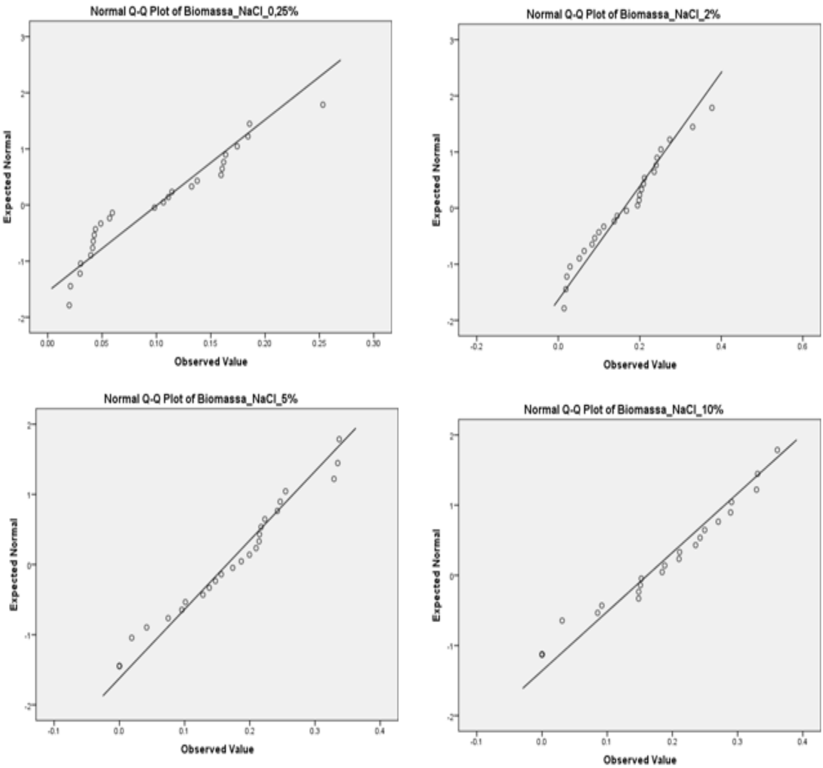

Figure 4.1 Q-Q Plot of the normality test results of biomass dependence on salinity in nutrient media with 0.25%, 2%, 5%, and 10%

The correlation analysis of the obtained results was conducted using the Pearson test. The results indicate a strong correlation between the salinity of the environment and the biomass, as shown in Table 4.2.

The statistical analysis proceeded with Levene's Test, enabling the determination of both homogeneity and heterogeneity within the results. To analyze this difference, the One-Way ANOVA Scheffe Post Hoc test was applied (Table 4.3).

Table 4.2
The Pearson analysis results of fungi contributing to the formation of Lake Masazir's mycobiota

Correlation		
		Biomass
Salinity in %	r	-.808**
	p	.005
	N	10
**. Significant correlation at 0.01		

Based on the analysis of the obtained results, it can be concluded that salinity stress induced significant changes in the microscopic fungi. According to the indicators, the substantial biomass yield observed in nutrient media with NaCl concentrations of 2%, 5%, and 10% implies that these organisms exhibit a high level of tolerance to saline environments, likely due to their adaptation to such conditions.

FINAL ANALYSIS OF THE RESULTS

Soil serves not only as an environment where living organisms interact but also plays a crucial role in nutrient cycling and carbon storage, particularly in accumulating organic biomass. To comprehend the multifaceted role of this intricate structure, it is crucial not only to assess the soil's physical and chemical parameters but also to understand the diversity of its bacterial, fungal, and other organisms. Understanding both the living organisms, or biotic components, and the non-living factors, or abiotic components, within this structure is essential for assessing soil fertility, which serves as a key indicator of soil productivity.

Table 4.3

The Scheffe Post Hoc test was conducted to analyze the differences in biomass yield among fungi contributing to the mycobiota of Lake Masazir

NaCl concentration in dry Czapek-Dox nutrient medium, %	N	Subset for alpha = 0.05			
		1	2	3	4
25%	25	.000000			
21%	25	.009267	.009267		
20%	25	.051954	.051954	.051954	
0.25%	25	.100698	.100698	.100698	.100698
15%	25		.114029	.114029	.114029
0.5%	25			.123246	.123246
1%	25			.142412	.142412
2%	25				.161423
10%	25				.161533
5%	25				.164854
Sig.		.078	.053	.183	.699

Soil, recognized as a dynamic and open system, plays a pivotal role in the cycling of carbon, nitrogen, oxygen, phosphorus, water, and other substances. Its mineral content and water potential are also key indicators of productivity.

As industrialization accelerates globally and anthropogenic influence increases, accompanied by noticeable global climate change, ecosystems face a multitude of negative environmental factors. Soil, being a crucial component of ecosystems, is significantly impacted by various environmental stressors. Natural disasters like floods and landslides, along with erosion, compaction, pollution, reduced water permeability, salinization, and mineralization of organic matter, pose significant threats to soil health. These degradation phenomena contribute to a decrease in biodiversity and have far-reaching consequences for agriculture. The escalating severity of these threats exacerbates the risk of food shortages and other related challenges.

Soil salinization is one of the processes that is considered as a serious and noteworthy environmental problem in the world. In many parts of the world, particularly in arid and semi-arid areas, evaporation is a serious problem that results in the formation of salt deposits in the upper layers of the soil and is developing rapidly.

It should be noted that salinization occurs not only as a result of natural processes, but also as a result of man-made pollution, and among the sources of this pollution are, "*chemical and hydrotechnical reclamation, oil extraction and processing, construction of roads, creation of artificial geological objects, irrigation with mineralized water*"[12] etc.

Soil salinization, a multifactorial process, occurs for various reasons. Climatic factors such as temperature, humidity, precipitation, wind, and duration of sunlight influence the physical and chemical parameters of soils. "*For instance, the speed and impact of wind, the type and quantity of annual precipitation, groundwater depth and mineralization level, local vegetation, anthropogenic activities, and soil structure are factors that accelerate salinization*"[13]. Alongside the physical and chemical properties of the soil, these factors also significantly influence the growth and biodiversity of organisms that contribute to its biomass.

Microorganisms thrive in extreme environments such as hot, cold, dry, highly saline, acidic, and alkaline conditions. "*Over the past few decades, these microorganisms inhabiting extreme environments have garnered attention as potential sources of novel bioactive compounds and as subjects of research to elucidate*

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[12] Q.Məmmədov.Torpaqşünaslıq və torpaq coğrafiyasının əsasları. Bakı 2007.Səh 378-383.

[13] Chavez, R., Fierro, F., Garcia-Rico, R.O., and Vaca, I. 2015. Filamentous fungi from extreme environments as a promising source of novel bioactive secondary metabolites. Front. Microbiol. 6, 903.

evolutionary mechanisms"[14]. Recent research efforts focused on studying extreme environments and the organisms inhabiting them have underscored the significance and priority of such endeavors. However, it's worth noting that the findings obtained are context-specific. This is because each research site possesses unique soil and climatic conditions, and organisms isolated from these areas develop adaptive traits tailored to their environment. Therefore, the diversity of results obtained emphasizes the importance of maintaining an individualized approach to each biotope.

The Republic of Azerbaijan boasts a diverse and vibrant natural landscape, distinguished by its soil and soil biodiversity. However, the effects of climate change and the rapid growth of the country's economy, driven by both industrial and agricultural sectors, are tangible realities that exert increasing pressure on the ecosystem. Consequently, research on soil microbiota and the alterations occurring in extreme environments, particularly saline soils, due to these and similar influences, has become a focal point for numerous studies conducted in Azerbaijan. Considering these factors, it was deemed pertinent to conduct research in this field in the presented work.

Lake Masazir, situated in the Absheron peninsuila of Azerbaijan, stands out as a natural extreme saline environment and was selected as the focal point of the research.

Throughout the study, 216 soil samples were collected from 42 research sites, revealing that the soil composition of the area comprised gray-brown, sandy, gravelly, and clayey components. Analysis of the soil samples indicated salinity concentrations ranging from a minimum of 1.08 mS/cm to a maximum of 61.12 mS/cm, with corresponding pH values ranging from 7.4 to 9.03.

A total of 25 types of microscopic fungi, spanning 12 genera, were isolated from the soil samples collected in the research area. These fungi constitute the mycobiota of Masazir Lake and are categorized under the Ascomycota division. Notably, during the

[14]. Səfərəliyeva, E.M. Antropogen təsirə məruz qalmış bitotopların mikobiotasının formalşamasında iştriak edən göbələklərin növ tərkibi və ekofiziologiyası:/b.ü.f.d. dissertasiyasının avtoreferatı/-Bakı, 2021, -31s.

study, 56% of the isolated micromycetes belonged to the Eurotiomycetes class, 16% to the Dothideomycetes class, and 28% to the Sordariomycetes class.

The annual average numerical composition of fungi comprising the mycobiota in the study area was determined to be $1.47 x 10^2$ CFU/g. This figure stands relatively low even when compared to biotopes affected by anthropogenic influences. For instance, in studies conducted in Absheron aid, the fungal count in oil-contaminated soils reached $2.2 x 10^3$ CFU/g, while in soils contaminated with chemical production by-products, it rose to $3.2 x 10^3$ CFU/g. In relatively clean soils, this indicator surged to $5.7 x 10^3$ CFU/g. Such findings suggest that the area is unfavorable for fungi or, more precisely, constitutes an extreme environment for living organisms. This underscores the importance of exploring the adaptation mechanisms of organisms thriving in such conditions.

Throughout the study, one of the objectives was to investigate the spatial distribution of fungi composing the mycobiota across the territory, along with their frequency of occurrence and randomization in soil depth. Out of the 25 fungal species isolated from the coastal soils of Masazir Lake, 24% were characterized as dominant species, 32% were classified as common, and 44% were categorized as random and rare species based on their occurrence frequency. Notably, species affiliated with the genera Aspergillus and Penicillium were prevalent both along the coast and across various soil depths, as observed in the distribution patterns.

Aspergillus niger, identified during the research, was deemed a ubiquitous species, particularly notable due to the coexistence with Penicillium citrinum fungi in the central region of the study area, comprising 8% of the fungal species within the mycobiota of Masazir Lake. Furthermore, 12 species, accounting for 48% of the overall mycobiota species diversity, were isolated from the shores designated as 2-3, based on conventional division, whereas 11 fungal species, constituting 44%, were exclusively isolated from a single shore segment as per the same division criteria.

During the study, 8 species of *Aspergillus transcorpathicus*, *Aspergillus arenarioides*, *Parengyodontium album* = *Beauveria* *alba, Fusarium xylarioides, Fusarium equiseti, Penicillium subericola, Cordyceps farinosus=Paecilomyces farinosus, Purpurocillium lilacinus= Paecilomyces lilacinus* isolated from the area were recorded as new species for the mycobiota of Azerbaijan. This observation underscores the significance of studying the characteristic mycobiota of Azerbaijan's natural habitats, highlighting the current research trend. It also suggests that the fungi documented in various studies fall short in adequately representing the full spectrum of fungal species known to science today.

The composition of micromycetes in saline soils remains inadequately explored within research conducted in Azerbaijan. During the study, the biomass yield of 25 species isolated from the area was assessed in laboratory conditions using nutrient media with varying NaCl concentrations. Of the studied fungi, 22 were classified as halotolerant, with no halophilic species identified. Interestingly, the naturally high salinity of the area demonstrated optimal biomass yield in nutrient media with a salinity concentration of 5-10% for the species isolated from this region.

Despite this, fungi such as *Aspergillus ochraseus, A.terreus, A.niger, Aspergillus sp., Penicillium subericola, P.citrinum, P.resticulosum, Cladosporium cladosporioides* and *Fusarium xylarioides* exhibit a lethal dose of salinity above 20% suggests that these fungi have acquired specific characteristics as a result of adapting to low conditions. This finding not only underscores the significance of further research into this phenomenon but also broadens our understanding of fungal traits and their adaptive capacities. It opens up avenues for exploring the mechanisms underlying fungal tolerance to extreme environmental conditions, thereby expanding the scope of scientific inquiry in this area.

Studying the fungi that form the composition of the microbiota of natural extreme environments, determining their distribution in the world, and modeling biotic relationships is also important.

Thus, the conclusion of the operational research was expressed by the following 5 final findings and 2 practical recommendations.

RESULTS

1. The research revealed that the mycobiota of Masazır Lake comprises 25 species belonging to 12 genera. Among these, 56% of the species, representing 3 genera, are classified under the Eurotiomycetes class, while 4 species from 3 genera fall within the Dothideomycetes class. Additionally, 28% of the species, encompassing 6 genera, are categorized under the Sordariomycetes class.

2. It was determined that species such as *Aspergillus arenarioides* Visaqie, Hirooka & Samson, *A.transcarpathicus* A.J.Chen, Frisvad & Samson, *Cordyceps farinosa* (Holmsk)Kepler, B.Shrestha & Spatafora(=*Paecilomyces farinosus* (Holmsk) A.H.S.Br &G.Sim), *Fusarium equiseti* (Corda) Sacc, *F.xylarioides* Steyaert, *Parengyodontium album* (Limber) C.C.Tsang, J.F.W.Chan, W.M.Pong, J.H.K.Chen, A.H.Y.Nigan, M.Cheung, C.K.C.lai, D.N.C.Tsang, S.K.P.Lau, &P.C.Y.Woo(=*Beauveria alba* (Limber), Saccas), *Penicillium subericola* Barreto, Frisvad & Samson, *Purpurocillium lilacinus*(Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson(=*Paecilomyces lilacinus* (Thom) Samson) were recorded in the nature of Azerbaijan for the first time.

3. The analysis revealed that out of the 25 fungal species identified in the research, 6 species exhibited a dominance frequency, 8 species were frequently encountered, and 11 species were classified as random and rare. This indicates that the frequency of occurrence for dominant species ranged between %, for frequently encountered species, and % for random and rare species.

4. It was evident that 22 out of the 25 fungal species identified in the research exhibited salt resistance, characterized as halotolerant species. These species demonstrated the capability to thrive even in environments with NaCl concentrations exceeding 10%.

5. It became clear that species such as *Aspergillus ochraseus, A.terreus, A.niger, Aspergillus sp., Penicillium subericola, P.citrinum, P.resticulosum, Cladosporium cladosporioides* and *Fusarium xylarioides* recorded in the studies are characterized as halophiles and the lethal dose for NaCl is above 20%.

PRACTICAL RECOMMENDATIONS

1. In light of global climate change, desertification, salinization, and other environmental challenges, it is crucial to conduct comprehensive research focused on harnessing fungi from organisms adapted to extreme conditions, addressing emerging ecological issues.

2. In forthcoming studies, it is imperative to elucidate the molecular mechanisms underlying adaptation to extreme conditions and explore their potential utilization in the development of recombinant organisms.

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The defense of the dissertation will be held on 04 april 2024 at 14:00 at the meeting of the Supreme Attestation Comission under the President of the Republic of Azerbaijan FD. 1.07 Dissertation Council operating at the Institute of Microbiology under the Ministry of Science and Education of the Republic of Azerbaijan

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The dissertation is available in the library of the Institute of the Microbiology at the Azerbaijan National Academy of Sciences

Electronic versions of the dissertation and abstract are aviable on the official website (https://www.azmbi.az/index.php/az/) of the Microbiology at the Azerbaijan National Academy of Sciences.

The abstract was sent to the necessary addresses on 01 march 2024

Signed for print: 27.02.2024

Page format: A5

Volume: 37504

Number of hard copies: 20