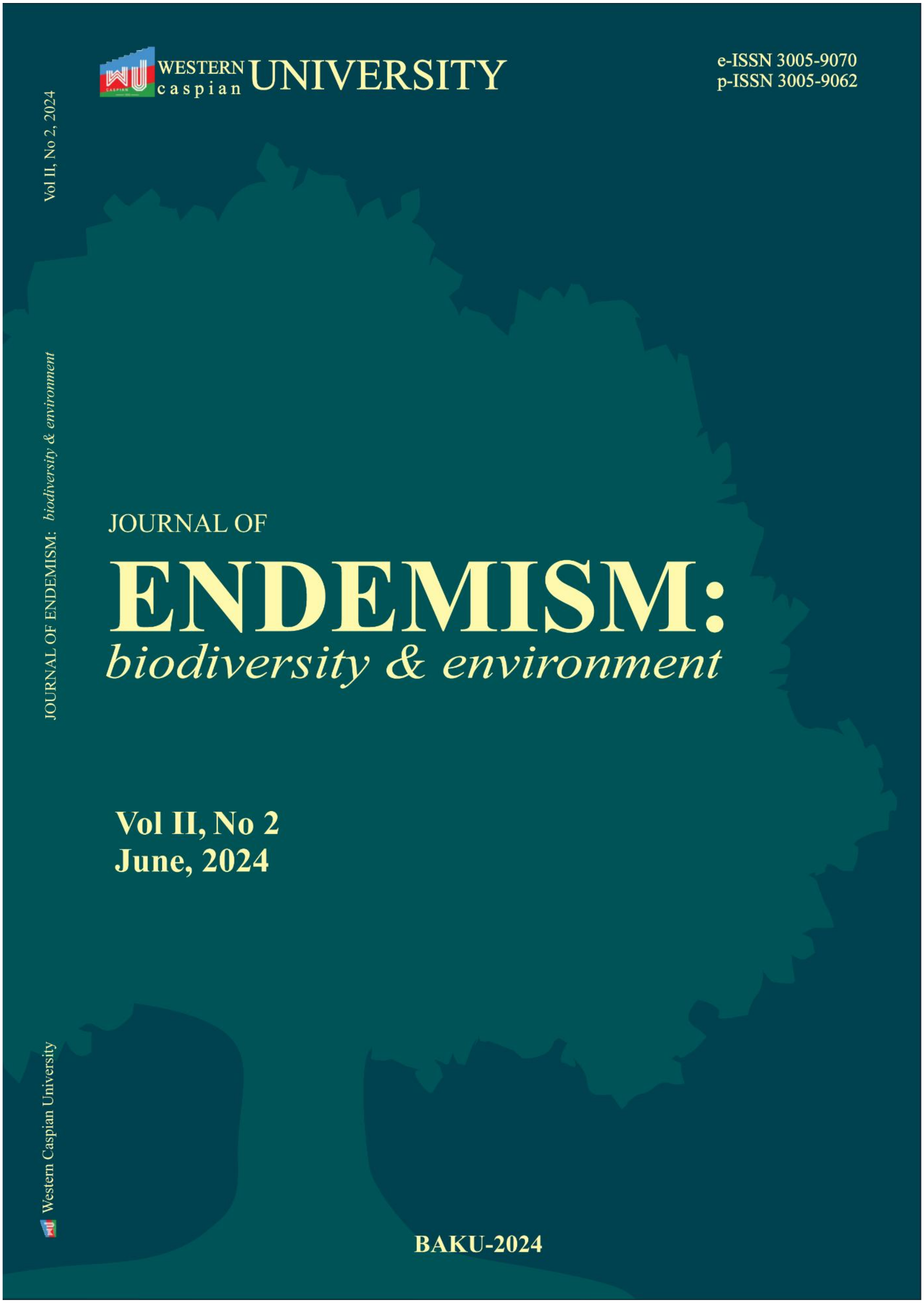

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DETERMINATION OF FOREST COVER CHANGE IN SHAKI-OGHUZ DISTRICTS

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Abstract: This article investigates the changing trends in the territory of Shaki and Oghuz districts of Azerbaijan, especially in the forest cover, and studies the floristic composition of the forests. The research includes the registration of the species composition of forests from various points in the regions and collection of literary information. To study the changing processes, the chlorophyll index method based on the reflection of chlorophyll in leaves (CIgreen) was applied. For this re- search, multispectral images from 1987 to 2022 were obtained from the Landsat satellite database of the United States. Additionally, comparing the suitability of weather conditions and the vegeta- tion period suitable for mountainous terrain, a comparison of July months was conducted using the ArcGIS Pro platform. The results of the analysis show that the density of forests in the territory of Shaki region has decreased (Figure 1). During the desert research in the forest ecosystems of Shaki and Oghuz regions, a total of 147 species belonging to 45 families and 118 genera were identified in the area. In Shaki region, approximately 2969 ha of surface covered by water or ice (-1), 5169 ha of open land cover (0), 10437 ha of dense forests (3), and 1106 ha of high-density forests have de- creased, while medium-density or sparse forests, woodlands, and shrubs have increased by 19684 ha. 11543 ha of dense forest vegetation was replaced by medium-density forest or shrubs. The den- sity of forests in the territory of Oghuz region has also decreased (Figure 2). Approximately 2719 ha of surface covered by water or ice (-1), 22047.84 ha of dense forests (3), and 1106 ha of high- density forests have decreased, while open land cover (0) has increased by 4290 ha, and medium- density or sparse forests, shrubs, and vineyard areas have increased by 20951 ha. 22521 ha of dense forest vegetation was replaced by medium-density forest or shrubs. Approximately 1500 ha of for- est area has been lost as land over the years 1987-2022.

Keywords: landsat, forest density, vegetation cover, chlorophyll reflection

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Introduction:

The Caucasus region, renowned for its rich biodiversity and unique ecosystems, harbors a diverse array of flora and fauna, making it a global biodiversity hotspot (Abiyev, 2020a). Among the countries within this region, Azerbaijan stands out with its remarkable natural landscapes, including the Oghuz-Shaki region, which is characterized by its lush forests and unique biodiversity (Mehdiyeva & Mursal, 2022).

Over the past decades, however, the Oghuz-Shaki region has experienced significant changes in its forest cover, influenced by both anthropogenic activities and natural factors. These changes have raised concerns about the preservation of biodiversity and the sustainable management of natural resources in the region (Mehdiyeva & ets, 2022).

Understanding the dynamics of forest cover change in the Oghuz-Shaki region requires an analysis of various factors, including land use, land cover changes, and the impacts of human activities. Anthropogenic factors such as deforestation, agricultural expansion, and infrastructure development have played a significant role in altering the natural landscape of the region. Additionally, natural factors such as climate change and natural disasters have also contributed to changes in forest cover.

Studying changes in forest ecosystems using aerospace remote sensing is crucial for the sustainable management of biodiversity (Fescenko & Wohlgemuth, 2017). The analysis of plant spectra is important for evaluating plant health (Brown & ets, 2019). The process of satellite image processing relies on the differential absorption, transmission, and reflection of the electromagnetic spectrum using passive sensors, including red, blue, green, and infrared (Wu & ets, 2012). The biochemical and biophysical properties of green leaves affect their reflection spectrum (Hunt & ets, 2013). Monitoring chlorophyll content in leaves (green biomass) is not only useful for assessing plant physiology, production, and nutritional status, as well as detecting aging and stress due to drought and disease spread, but also for measuring changes in forest cover boundaries and density (Darvinzadeh & ets, 2019). Vegetation indices are now considered a simple, rapid, and cost-effective method by researchers (Le Marine & ets, 2008). The "Chlorophyll Index Green" (CIgreen) is commonly used to estimate chlorophyll content in leaves (Croft & ets, 2013). "CIgreen" is directly related to the difference between the reflectance of broad green and near-infrared wavelengths (Yin & ets, 2016).

This article aims to provide a comprehensive analysis of the dynamics of forest cover change in the Oghuz-Shaki region, focusing on the interactions between anthropogenic and natural impacts. By examining historical trends and current patterns of forest cover change, this study seeks to identify the key drivers of change and their implications for biodiversity conservation and sustainable land use practices.

Material and Methods:

Research was conducted in the territories of Shaki and Oghuz regions of Azerbaijan (Figure 1). The structure of these forests is similar to each other, mainly represented by 27 species that mainly constitute the forest community. *Quercus petraea* subsp. *polycarpa* (Schur) Soó, *Carpinus betulus* L., *Fagus orientalis* L., *Alnus incana* (L.) Moench, and *Carpinus orientalis* L. are the dominant species in the forests. Additionally, *Crataegus monogyna* Jacq., *Crataegus pentagyna* Waldst. & Kit. ex Willd., *Cotinus coggygria* Scop., *Mespilus germanica* L., *Populus alba* L., and *Fraxinus excelsior* L. are the main components of the forests.

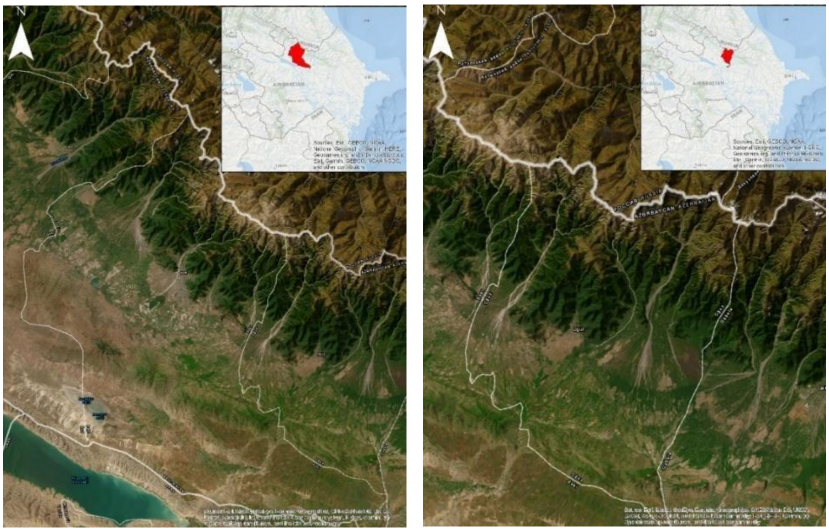

Figure 1. Study area.

This research utilized multispectral images obtained from the Landsat satellite database between 1987 and 2022. Additionally, a comparison of June and July was conducted based on the suitability of weather conditions and vegetation periods for mountainous terrain. The research was carried out using the ArcGIS 10.8 platform. Infrared (Landsat 5 - Band 4; Landsat 8 - Band 5) and green (Landsat 5 - Band 2; Landsat 8 - Band 3) bands in the images taken with the Landsat sensor are standard bands applied in chlorophyll indexing. Calculations are based on the ratios of values owned by each pixel in the images (Gitelson et al., 2003).

$$\mathrm{CIgreen} = (\mathrm{NIR}/\mathrm{GREEN}) – 1$$

"CIgreen" - chlorophyll index green, NIR - near-infrared wave, GREEN - green wave.

The values of the chlorophyll index increase from -1. The spatial expression of the values obtained in our results is as follows: -1 - water, ice, stone, etc., 0 - non-vegetated, dead vegetated (e.g., harvested crop area) soil areas, steppe and semi-desert zones, 1 - normal density forests, forest-shrub vegetation, sparse floodplain forests, etc., 2 - dense forest cover, healthy or in the best condition of vegetation period, 3 - very dense forest or vegetation.

The areas covered by pixel values were calculated by multiplying the number of pixels by the area of each pixel:

$$\text{Area} = \text{cell count} \times \text{cell area}$$

"Cell count" - number of pixels on the value

The change was calculated according to the following rule (Badamasi & Yelwa, 2010):

$$\text{Change rate} = D_{\text{last}} - D_{\text{first}}$$

"D_{first}" and "D_{last}" respectively represent the beginning (1992) and end (2017) of the research year.

The pixel dimensions in Landsat images are 30 m.

Cell area = 30 m × 30 m = 900 m^2 = 0.09 ha

"Cell area" - area covered by one pixel.

Results and discussion:

During desert studies conducted in the forest ecosystems of Shaki and Oghuz regions, a total of 147 species belonging to 118 genus and 45 families were identified. Among the families, the most represented in terms of genera and species are *Poaceae* Barnhart (9 genera, 11 species), *Boraginaceae* G. Don. (7 genera, 8 species), *Rosaceae* Juss. (15 genera, 18 species), and *Asteraceae* Bercht. & J.Presl (5 genera, 6 species). Other families are represented by fewer genera and species. Based on life forms, out of the 147 species, 27 are trees, 16 are shrubs, 1 is a shrub-liana, 12 are herbaceous monocots, and 91 are herbaceous dicots. Of these, 11 are Caucasus endemics, and 11 are rare species included in the 3rd edition of the Red Book of the Republic of Azerbaijan.

Currently, approximately 19.7% of the district's territory is covered by forests. The analysis results show that the density of dispersed forests in Shaki district has decreased (Figure 1). In July, the area covered by water or glaci- ers (-1) decreased by approximately 2969 ha, open soil cover (0) by 5169 ha, dense forests (3) by 10437 ha, and high-density forests by 1106 ha, while medium-density or sparse forests, shrub vegetation, and intensive vineyards increased by 19684 ha. 11543 ha of dense forest vegetation has been replaced by medium-density forest or shrub vegetation (Table 1). The decreases mainly cover the high mountain- ous zone. Between 1987 and 2022, approxi- mately 8000 ha of tree-based greenery has in- creased in the area, the majority of which con- sists of dense groves of apple, almond, hazel- nut, and other trees in the northwest direction of the district. Overall, 68000 ha of forests, shrubbery, and vineyards are recorded in the district, of which 48000 hectares are forested areas, 28000 hectares are riverbanks and other shrubberies, fragmented small groves, and tree-based intensive vineyards.

Table 1. Comparison of Shaki region for July of 1987-2022.

Index	Pixel count (July 1987)	Area (ha)	Pixel count (July 2023)	Area (ha)	Change (ha)
-1	36670	3300.3	3682	331.38	-2968.92
0	1976297	177866.73	1918860	172697.4	-5169.33
1	453898	40850.82	672606	60534.54	+19683.72
2	204626	18416.34	88655	7978.86	-10437.48
3	12287	1106.91	0	0	-1105.83

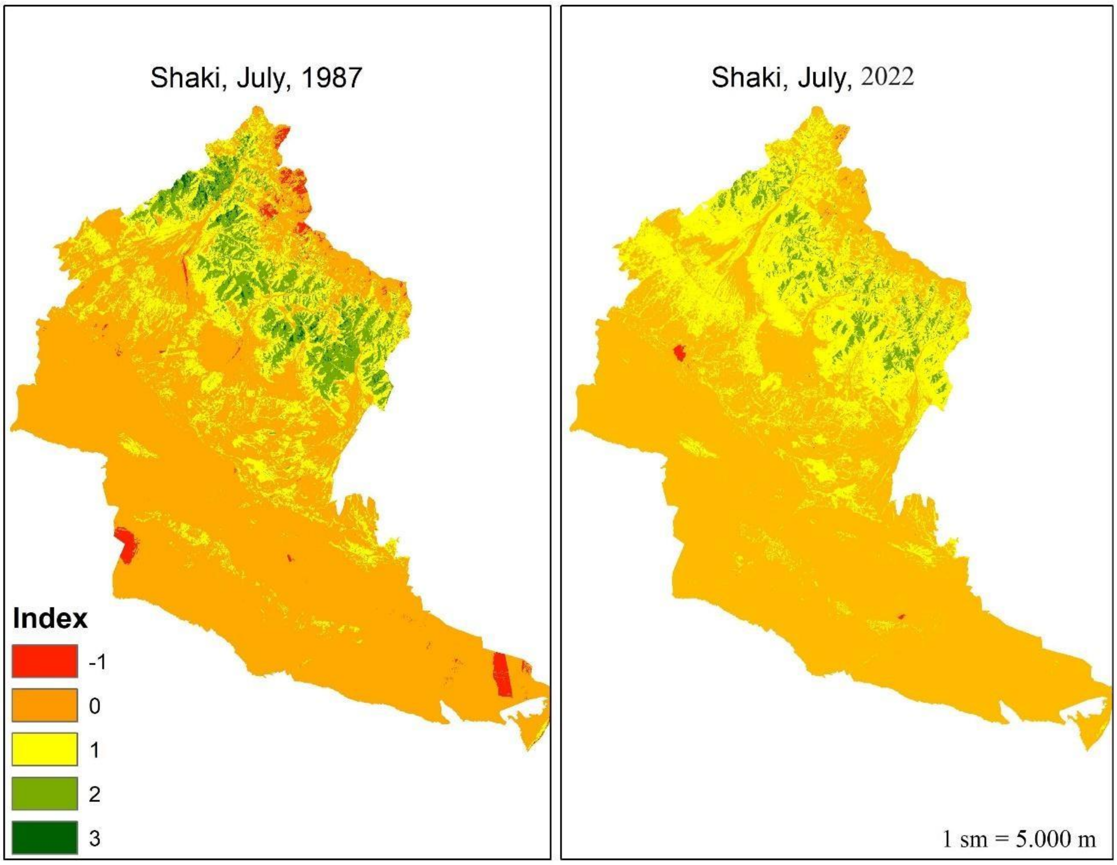

Figure 2. Surface and vegetation changes in Shaki district between 1987 and 2022.

Currently, approximately 26.9% of the region is covered with forest. The results of the analyzes show that the density of the forests spread in the territory of Oghuz region has decreased (Figure 2). As of July, the approximate surface covered by water or glaciers decreased by (-1) 2,719 ha, dense forests (3) by 22,047.84 ha, and high-density forests by 1,106 ha, dense forests (3) by 10,437 ha, and high-density forests by 473.4 ha has decreased, on the contrary, open land cover (0) has increased by 4290 ha, medium density or sparse forest, shrub vegetation and garden areas have increased by 20951 ha. 22,521 ha of dense forest vegetation was replaced by medium-density forest or shrub vegetation (Table 2). Between 1987 and 2022, approximately 1500 ha of forest area was destroyed. In total, approximately 55,000 ha of forest, fragmented groves, shrub vegetation, and tree-based gardens were registered in the region. 29,000 ha of these green areas are covered by primary forest.

Table 2. Comparison of Oghuz district in July of 1992-2017.

Index	Pixel count (July 1987)	Area (ha)	Pixel count (July 2013)	Area (ha)	Change (ha)
-1	32985	2968.65	2770	249.3	-2719.35
0	500316	45028.44	547979	49318.11	+4289.67
1	351167	31605.03	583956	52556.04	+20951.01
2	278119	25030.71	33143	2982.87	-22047.84
3	5260	473.4	0	0	-473.4

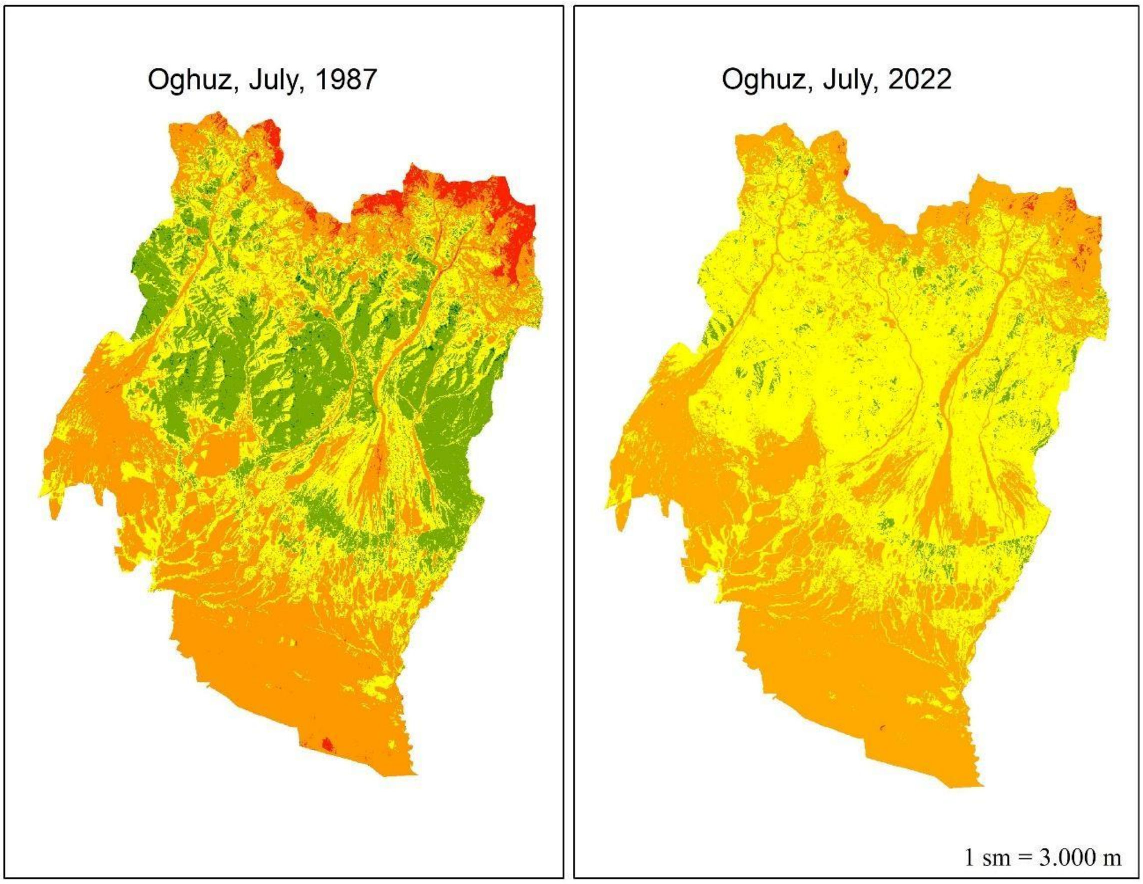

Figure 3. Surface and vegetation changes in Oghuz region between 1987 and 2022.

Conclusion:

Research conducted in the Samur-Yalama National Park in Khachmaz has shown a similar decrease of 12% in density. However, unlike in Sheki and Oguz regions, borders have remained stable (Abiyev & ets, 2020b). Research in Zangilan has shown a decreasing trend in density, but an increase in patches of natural vegetation has been observed due to the area being unused as a result of occupation (Abiyev, 2021).

Illegal logging and forest fires in the past in Oguz and Sheki regions have contributed to the decrease in forest cover. Furthermore, the development of intensive horticulture in Sheki, especially the expansion of vineyards, has had a positive impact on increasing tree cover.

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BIOINFORMATIC STUDY OF GENES RESPONSIBLE FOR FAMILIAL MEDITERRANEAN FEVER

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Abstract: This article is a bioinformatics study of the MEFV gene. Bioinformatics involves the study of genetic variations and their association with diseases using computational tools used to analyze biological data. To understand what diseases the MEFV gene causes due to mutations, the gene was analyzed using various bioinformatics programs. By comparing MEFV sequences from different species using BLAST and Clustal Omega programs, the extent to which the gene is conserved across organisms was studied. The study performed a bioinformatics analysis of the MEFV genes from *Homo sapiens* (humans), *Mus musculus* (house mouse), and *Prionailurus bengalensis* (Bengal cat). The gene length, number of nucleotides, and percentages were analyzed for all three species, and although these values are different, it was found that the gene is orthologous, meaning it performs the same functions.

Keywords: MEFV gene, nucleotide sequence, mutation analysis, orthologous genes, bioinformatics analysis.

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Introduction:

Familial Mediterranean Fever (FMF) is a group of hereditary inflammatory diseases and is widespread mainly in the Mediterranean region (Ozdogan & Ugurlu, 2019; Onen, 2006; Bakkaloglu, 2003). The disease is accompanied by fever, abdominal, chest and joint pain, which seriously impairs the quality of life. FMF is an autosomal recessive hereditary disease, that is, the disease is transmitted from parents to offspring by transmitting mutations in the FMF gene. The FMF gene provides the synthesis of the pyrin protein, which plays an important role in the regulation of inflammation in the body (Richards & ets, 2001; Bertin & DiStefano, 2000; Srinivasula & ets, 2002). When the function of the pyrin protein is impaired, abnormal inflammatory reactions occur in the body, which leads to FMF.

Different mutations of the MEFV gene can lead to different phenotypes of FMF disease with different symptoms. Determining the location and effect of these mutations is important for understanding the disease at the molecular level and developing treatment strategies. For this reason, research on the MEFV gene is one of the main areas of research in the diagnosis and treatment of FMF disease. Bioinformatics serves as an important tool for better understanding the genetic basis of diseases.

Bioinformatics is an interdisciplinary field that uses mathematical and computational methods to collect, store, analyze, and interpret biological data ((Luscombe & ets, 2001; Morgan & ets, 2024; Yamashita & ets, 2023)). This field is particularly concerned with the analysis of genetic and protein sequences, prediction of structure and function, study of genetic variations, and understanding of disease mechanisms at the molecular level. Bioinformatic analysis of the MEFV gene involves the use of various programs and methods to better understand the structure and function of this gene and its association with disease through mutations.

The MEFV gene is studied not only in humans but also in other species, studying how the gene is preserved during evolution and the functional similarity of orthologous genes. In this study, a comparative analysis of the MEFV gene was conducted in species such as *Homo sapiens* (humans), *Mus musculus* (house mouse) and *Prionailurus bengalensis* (Bengal cat). Comparison of the nucleotide sequences of the MEFV gene, the regions susceptible to mutations, as well as the structure and function of the encoded protein pyrin among these species revealed which organisms have the most common mutations associated with the disease.

The bioinformatic analyses performed may provide more information about the molecular basis of FMF disease and contribute to the development of modern methods, such as gene therapy, for the treatment of this disease in the future. In this article, based on the analysis of the MEFV gene mutations, we consider the role of this gene in the development of FMF disease, how it is conserved in the evolutionary process in different species, and how the results obtained by bioinformatics methods can be used in the study of methods for treating the disease.

Materials and Methods:

In this study, a bioinformatic analysis of the MEFV gene responsible for Familial Mediterranean Fever (FMF) was performed. Various computational methods and bioinformatics tools were applied to study the structure, function and association of the MEFV gene with diseases due to mutations. The study conducted a comparative analysis of the MEFV gene in three major species - *Homo sapiens* (human), *Mus musculus* (house mouse) and *Prionailurus bengalensis* (Bengal cat).

Gene Sequences Obtained DNA and protein sequences of MEFV gene were obtained from databases such as NCBI (National Center for Biotechnology Information) and Ensembl. These data served as the main source for analyzing gene length, nucleotide sequence, and genetic variations among species.

Software for comparative analysis of nucleotide and protein sequences, such as Clustal Omega and BLAST (Basic Local Alignment Search Tool), were used to compare the nucleotide sequences of the MEFV gene between different species. Using these tools, similarities and differences between gene sequences of different species were visualized, and whether a gene was orthologous or not was determined. The results were used to analyze how disease-causing mutations are maintained throughout evolution.

Identification of genetic variations. Genetic variants of the MEFV gene, including single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variations, were identified using programs such as GATK (Genome Analysis Toolkit), SAMtools, and BCFtools. These analyses identified potential disease-causing mutations.

Gene expression analysis. Transcriptomic data were obtained from databases such as GTEx (Genotype-Tissue Expression), TCGA (The Cancer Genome Atlas) and ArrayExpress to study how gene expression changes in different tissues, cell types and conditions. These data were used to understand under what conditions and to what extent the MEFV gene is active in FMF disease.

Homology modeling or AB initio modeling methods were used to predict the three-dimensional structure of the pyrin protein. Structural bioinformatics tools such as Swiss-Model and PyMOL were used for this process. In this way, it was analyzed how the identified mutations affect the structure and function of the pyrin protein. Evaluation of the impact of mutations. Programs such as PyMOL and Swiss-PdbViewer were used to evaluate the impact of mutations on the pyrin protein. These tools played an important role in investigating how disease-causing mutations affect protein stability and function.

Phylogenetic Analysis and Population Genetics MEGA (Molecular Evolutionary

Genetics Analysis) software was used to construct a phylogenetic tree, calculate FST, and analyze allele frequencies. This analysis allowed us to study the evolutionary history of the MEFV gene and the prevalence of mutations in different populations.

Using these methods, a detailed study was conducted to understand how the MEFV gene is evolutionarily conserved, genetic variations in different species, and the role of this gene in the development of FMF disease.

Results and discussions:

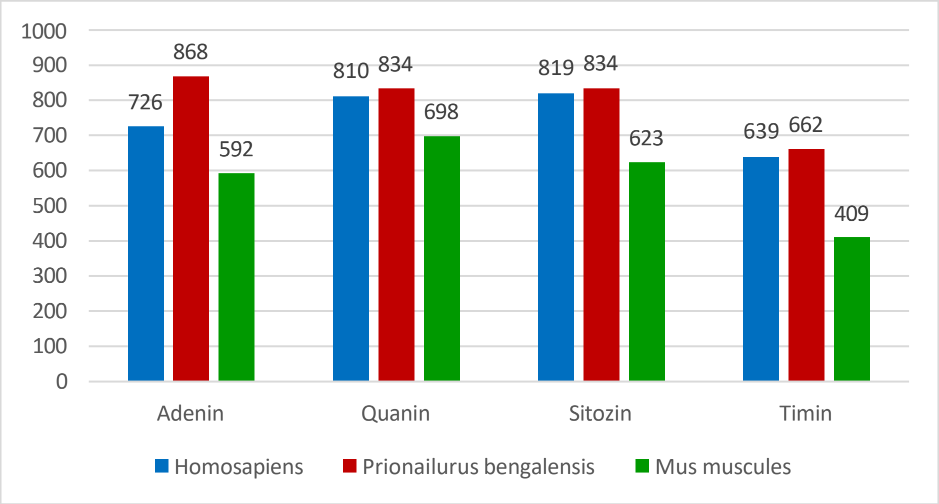
Bioinformatics analysis of the MEFV gene. Bioinformatics is an interdisciplinary field that combines biology, computer science, mathematics and statistics to analyze and interpret biological data. The main focus of bioinformatics is the development of methods and tools for understanding biological processes at the molecular level. This includes the analysis of DNA, RNA and protein sequences, prediction of protein structures and functions, the study of genetic variations and their association with diseases, modeling of biological pathways, etc. includes.

Bioinformatic analysis of the MEFV gene involves a number of computational methods to understand its structure, function, and association with diseases caused by gene mutation. For this purpose, the DNA or protein sequence of the MEFV gene was first obtained from databases such as NCBI or Ensembl and visualized using programs such as BLAST or Clustal Omega, which will be necessary for comparing MEFV sequences between different species. Genetic variants of the MEFV gene, including single nucleotide polymorphisms (SNPs), insertions, deletions, and structural variations, can be identified using programs such as GATK, SAMtools, or BCFtools. In addition, the frequency and distribution of MEFV gene variants, genetic diversity, population structure, and evolutionary dynamics can be estimated using methods such as allele frequency analysis, FST calculation, or phylogenetic tree construction. Using transcriptome data from databases such as GTEx, TCGA or ArrayExpress, it is possible to analyze MEFV gene expression patterns in different tissues, cell types and conditions, and generate a 3D structure of pyrin using protein structure prediction programs such as homology modeling or AB initio modeling and simultaneously determine that we can analyze how variants may affect protein structure, stability and function using structural bioinformatics programs such as PyMOL or Swiss-PdbViewer.

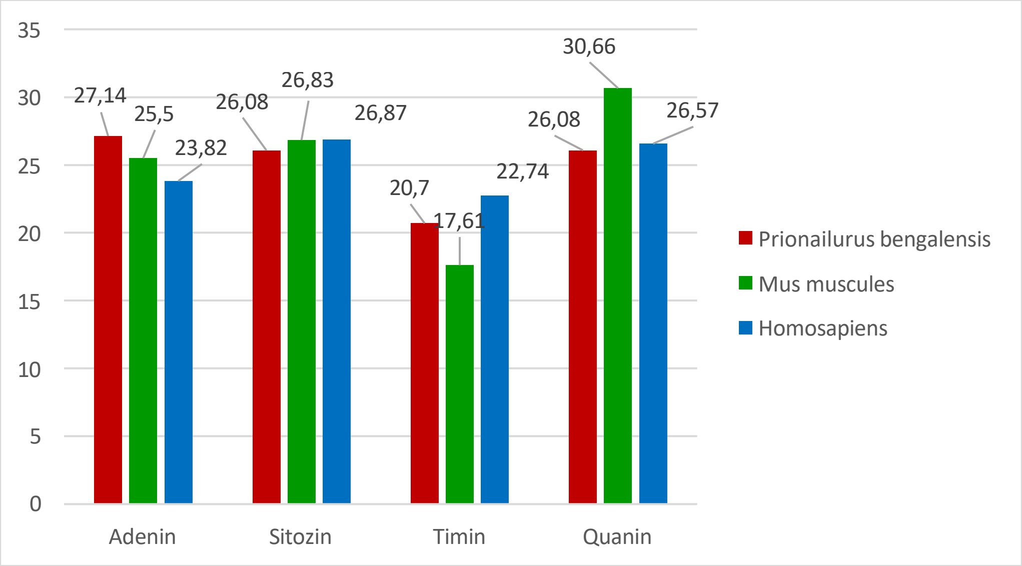
Bioinformatic analysis of the MEFV genes of *Homo sapiens* (human), *Mus musculus* (house mouse) and *Prionailurus bengalensis* (Bengal cat) revealed differences in gene length, nucleotide sequence, nucleotide count, nucleotide content and codon arrangement, and clearly showed promoter regions (Tables 1, 2; Schemes 1, 2, 3). Comparison of the MEFV gene will tell us whether these organisms are orthologous, which will form the basis for the use of methods such as gene therapy to eliminate mutations that cause AAA disease.

Table 1. Gene length, number of nucleotides and percentage values.

Name	Lenght	A	C	T	G	A%	C%	T%	G%
Prionailurus bengalensis	3198	868	834	662	834	27.14	26.08	20.70	26.08
Mus musculus	2322	592	623	409	698	25.50	26.83	17.61	30.66
Homo sapiens	3048	726	819	693	810	23.82	26.87	22.74	26.57



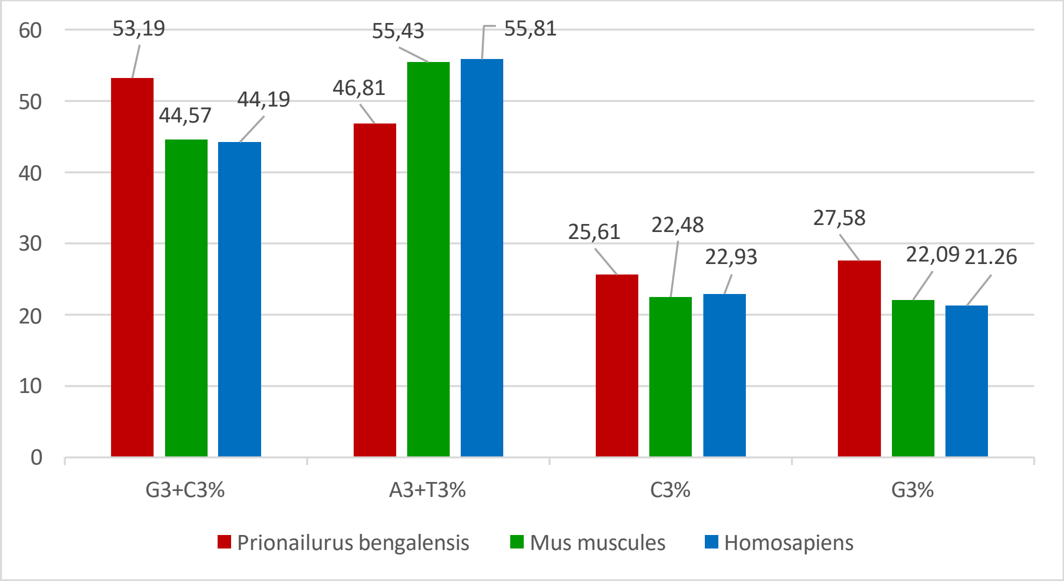
Scheme 1. Schematic estimation of the number of nucleotides.



Scheme 2. Schematic assessment of nucleotide percentages.

Table 2. Percentage of mutable nucleotides.

Name	G+C %	G+A %	G+T %	A+T %	A+C %	C+T %	G3+C 3%	A3+T 3%	C3%	G3%
Prionailurus bengalensis	52.16	53.22	46.78	47.84	53.22	46.78	53.19	46.81	25.61	27.58
Mus musculus	56.89	55.56	47.67	43.11	52.33	44.44	44.57	55.43	22.48	22.09
Homo sapiens	53.44	50.39	49.31	46.56	50.69	49.61	44.19	55.81	22.93	21.26

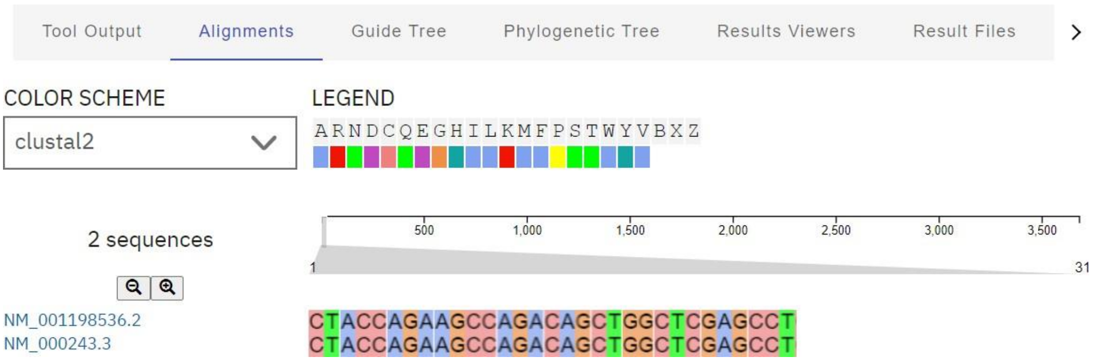


Scheme 3. Percentage of mutable nucleotides.

As can be seen from the diagrams, there were no significant changes in the number of nucleotides in these organisms. This indicates that the gene has not changed much over the years in orthologous organisms. These visualized results allow us to determine which organism is more prone to mutations. will be the basis for the application of methods such as gene therapy.

The studies and bioinformatics analysis show that the nucleotides in the 3rd position of the codon are more prone to mutations. If the disease is caused by a point mutation, then most likely it is associated with cytosine and guanine in the last position of the nucleotides. This can affect the structure and function of the pyrin protein. The specific effects of each mutation may vary, but they usually lead to increased inflammation and characteristic symptoms of FMF.

Visualization of the nucleotide sequence showed that these organisms do not have DNA with the same nucleotide sequence, but are composed of different codons encoding the same amino acid, and that although these compositions are different, they cause slight changes in the structure and function of the protein. . There are codons that code for the same amino acid, although they have different nucleotide sequences, indicating that the organisms are orthologous. Studying orthologs of the MEFV gene in different species helps researchers understand the evolutionary conservation of this gene and its importance in health and disease.

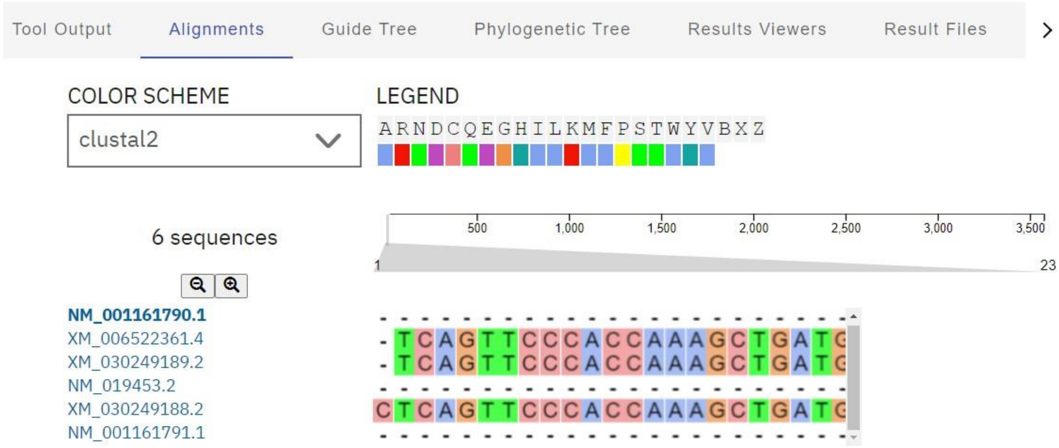


COLOR SCHEME: clustal2

2 sequences

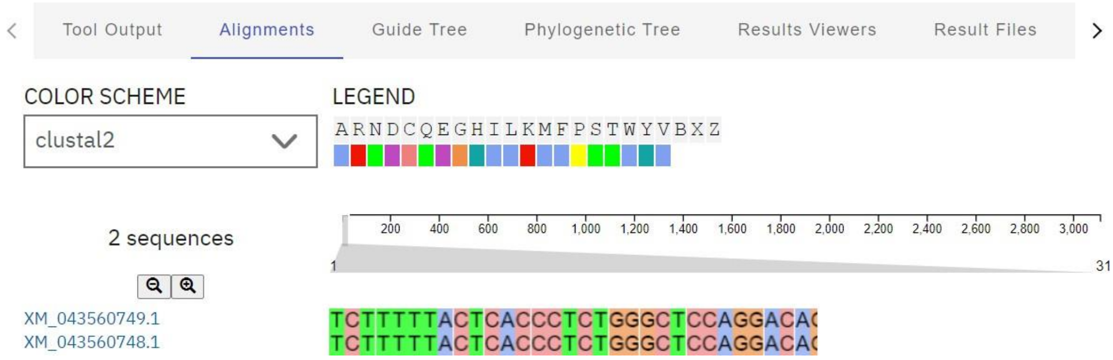
```
NM_001198536.2    CTACCAGAAGCCAGACAGCTGGCTCGAGCCT
NM_000243.3       CTACCAGAAGCCAGACAGCTGGCTCGAGCCT
```

Nucleotide sequence of the *Homosapiens* MEFV gene.



```
NM_001161790.1   -----------------------
XM_006522361.4   -TCAGTTCCCACCAAAGCTGAT
XM_030249189.2   -TCAGTTCCCACCAAAGCTGAT
NM_019453.2      -----------------------
XM_030249188.2   CTCAGTTCCCACCAAAGCTGAT
NM_001161791.1   -----------------------
```

Nucleotide sequence of the *Mus muscules* MEFV gene.



```
XM_043560749.1   TCTTTTTACTCACCCTCTGGGCTCCAGGACA
XM_043560748.1   TCTTTTTACTCACCCTCTGGGCTCCAGGACA
```

Nucleotide sequence of the *Prionailurus bengalensis* MEFV gene.

Conclusıon:

According to the results of this study, the bioinformatic analysis of the MEFV gene has great significance for understanding the genetic basis of FMF disease. The ortholog of the gene in Homo sapiens, Mus musculus and Prionailurus bengalensis and the conservation of genetic variations indicate that the gene plays important functions in the evolutionary process. The fact that the third nucleotides of codons are more prone to mutations plays an important role in the development of the disease. The obtained results indicate that mutations in the MEFV gene can cause AAA disease by changing the structure and function of the pyrin protein. Such bioinformatic analysis can lead to a more accurate understanding of the relationship between genetic variations and diseases, as well as to the development of treatment methods such as gene therapy. Thus, research conducted in this area opens up new prospects for the treatment of FMF disease, and it may be possible to apply potential genetic approaches to the treatment of this disease in the future.

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ECOLOGICAL ASSESSMENT OF RIVERS FLOWING IN THE KARABAKH AND EASTERN ZANGAZUR REGIONS

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Abstract: Due to limited research in the Karabakh and Zangezur regions until 2021, indirect methods were used to assess river samples, revealing concerning levels of manganese (Mn), aluminium (Al), and iron (Fe). While most metals are below WHO safety thresholds, the variability in concentrations highlights the need for regular monitoring. Acts of sabotage have damaged agriculture, the environment, and irrigation systems, necessitating purification efforts and prevention measures. Elevated levels of nickel and copper also require attention. Ensuring water safety involves treatment to remove heavy metals and further studies to evaluate its suitability for crops or blending with cleaner sources.

Keywords: Water Quality Analysis, Heavy metals in river waters, Fish species, Dynamics of Heavy Metal Concentrations.

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Introduction:

Water quality and the preservation of aquatic ecosystems are crucial concerns for any region, and Azerbaijan is no exception. Despite its rich natural resources, a significant portion of the population—approximately 75%—relies on river water that does not meet basic sanitary and hygienic standards for drinking. This alarming statistic underscores the urgent need for effective water management and pollution control to safeguard public health. The Kharabakh and Eastern Zangazur regions are particularly important in this regard, not only due to their significance as freshwater sources but also because of their unique biodiversity, which includes various fish species critical to the region's ecosystem (Rustamov and Kashkai, 1989).

The diverse ichthyofauna found in these areas—ranging from carp and goldfish to loaches and catfish—serves as a vital indicator of the health of these freshwater systems. The preservation of these species, many of which are listed in the "Red Book" of Azerbaijan, is vital for maintaining ecological balance and ensuring sustainable fisheries. However, decades of interrupted hydrometric research, especially since 1988 due to regional conflicts, have limited our understanding of the dynamics of water resources in these areas. This lack of continuous monitoring, particularly concerning water discharge and quality, highlights the need for renewed efforts in environmental research (Eminov et al., 2023; Hasanov et al., 1973).

This study aims to address these gaps by analyzing the quality of water in key rivers such as the Tartar, Hekari, and Oxchu, focusing on the mineralization, cation and anion flows, as well as the concentrations of metals and pollutants. Through a series of chemical analyses, this report aims to assess the current state of water quality in these rivers and recommend solutions for improving the sustainability of aquatic resources in the region. The findings of this study are crucial for the continued protection of both the natural environment and the health of the population dependent on these water sources (Junfang Liu, et al., 2020).

Materials and Methods:

This study focused on assessing the water quality of rivers in the Karabakh and Eastern Zangazur regions, specifically the Tartar, Hekari, and Oxchu rivers. Water samples were collected from various points along these rivers to analyze parameters such as mineralization, ion composition, and metal content.

Water sampling followed standard protocols, with samples collected in sterile containers to avoid contamination. A total of 10 samples were gathered from different upstream, midstream, and downstream locations. Key parameters measured included cations (calcium, magnesium, sodium, potassium), anions (chloride, sulfate, bicarbonate), and trace metals (lead, mercury, cadmium). Mineralization was determined through conductivity, and ion concentrations were analyzed using ion chromatography. Metal concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS).

Physical properties like temperature, pH, and turbidity were also recorded with field meters. The results were compared to water quality standards to evaluate the suitability of the water for drinking, agriculture, and ecosystem health. Data analysis was performed to identify trends and potential pollution sources in the region (Samah Al-Jabari, et al.,).

Results and discussions:

One of the most alarming issues in Azerbaijan is that 75% of the population uses the waters of these rivers, which do not meet sanitary and hygienic requirements, as drinking water. If urgent measures are not taken in this area, it is not difficult to imagine the consequences that may arise.

The information you provided about the ichthyofauna in the Karabakh and Eastern Zangazur regions is very important. Analyzing the fish species living in these regions is crucial for the conservation of water resources and the protection of fish species.

Fish Species and Distribution Areas

1. Fish Families and Species:

- Carps: 17 species (approximately 75% of all species)
- Goldfish: River goldfish, naked angora, naked Kura
- Loaches: Caucasian loach, golden loach
- Catfish: Caucasian catfish

2. In the Karabakh Region:

- Kondelenchay: 14 species of fish
- Qarqarchay: 8 species of fish
- Terterchay: 14 species of fish

3. In the Eastern Zangazur Region:

- Besitchay, Oxchuchay, and Hekerichay: 22 species of fish
- Hekerichay: 10 species of fish
- Terterchay: 6 species of fish

4. Species Listed in the "Red Book" of the Republic of Azerbaijan:

- River Goldfish: Found in Zangilan, Lachın, and Qubadlı districts, as well as in Kelbecer
- Zerdeper: Found in Zangilan, Lachın, and Qubadlı districts, and in Xocalı and Fuzuli

This information reflects the richness and diversity of the region's ecosystems. Additionally, it is essential to implement special measures for the protection of species listed in the "Red Book." The conservation of these species, along with the protection of their habitats and the sustainable use of water resources, will ensure the ongoing development of both the region's ecosystems and its fisheries.

Since 1988, the operation of hydrometric stations regarding the rivers of the region has been halted by Armenia. The last station to cease operations was the Kelbajar station on the Tartar River. Continuous stationary observations of water discharge at this station were conducted for 44 years, covering the period from 1949 to 1992. Due to the lack of research in the Karabakh and Zangezur regions over a long period, the presented studies were conducted using indirect methods till 2021 years.

In hydrochemical studies, correlation, linear interpolation, and analogy methods are primarily used for the restoration of mineralization series. Through these methods, the mineralization series of the studied rivers has been restored based on previous research.

To study the cation and anion flow of rivers, the restored series was used to calculate the long-term average values of the main ions. By summing the quantities of the main ions given in mg/l, cation and anion flows were determined for each river station.

Table 1: Calculated cation and anion flow based on the restored chemical flow series rivers

Test station	Multiannual average water consumption, Q (m^3/s))	Flow volumeW (million m^3)	Multiannual average ion current	Cation flow, thousand tons			Total Cation flow	Anion flow, thousand tons			Total Anion flow
				Ca	Mg	Na+ K		HCO_3	SO_4	Cl	
Incachay-Gulistan	1,1	35	8,8	1,3	0,5	0,34	2,13	4,48	2	0,15	6,63
Tartar-Kalbajar	5,2	164	50	8,4	1,3	4,1	13,8	23	11,6	1,6	36,2
Magavuz	18,2	573	218	36,6	4,5	21,3	62,4	94,2	47,4	13,7	155,3
Tartar-Suguvung	20	631	223	30,5	9	20	59,5	105,4	33,6	24	163
Levchay-Kamishli	5,74	181	55	8	1,9	5	14,9	27	10,6	2,37	40
Gloomy-mansab	3,95	124	44	5,5	3	1,8	10,3	28,8	4	0,95	33,75
Khachinchay-Vankulu	4,02	127	40	5,6	1,4	3,53	10,57	22,4	5,57	1,83	29,8
Gargarchay Ashagikorpu	2,09	66	33	3,26	0,8	4,9	8,96	8,5	15	0,77	24,27
Okchuchay-Gafan	9,76	308	226	25	9	34,5	68,5	70	60,3	27,8	158,1
Bazarchay-Eyvazlar	22	693	206	32,4	6,8	14,8	54	109	32,2	10,7	151,9
Hekarichay-Lachin	10,1	319	97	15,8	2,8	7,6	26,2	50,6	16,5	3,6	70,7
Zabukhchay-Zabukh	4,97	157	46	7,5	2	1,87	11,37	30,9	2,34	1,5	34,74
Guruchay-Togh	1,3	41	14	2,2	0,7	0,47	3,32	9,49	0,3	0,5	10,3
Kondalanchay-Kırmızı Bazar	0,58	18	8,7	1,28	0,3	0,82	2,38	3,8	1,6	0,87	6,27

Analysis of Water Quality Monitoring Results (2021-2023)

During the monitoring of Zabuxchay, Khachinchay, and Kondelenchay throughout the year, water samples were analyzed, and the quality indicators were found to be within permissible limits. Notable findings include:

- In the Tartar River, the concentration of iron (Fe) was 1.2 times higher.
- In the section of the Hekari River passing through the Zangilan region, cadmium (Cd) levels were 1.3 times higher.
- In Tutqunchay, cadmium (Cd) levels reached 2.9 times higher than permissible limits.
- In the section of Quruchay passing through Fuzuli, iron (Fe) levels were 1.5 times higher, while in the section near Shukurbayli village, cadmium (Cd) levels were 4.0 times higher than allowable limits.

Throughout 2022, monitoring of Hekari, Zabuxchay, Tartar, Tutqunchay, Quruchay, and Kondelenchay resulted in water sample analyses that indicated quality indicators remained within permissible limits.

In 2023, monitoring was conducted quarterly on Zabux, Quruchay, Khachinchay, Tartar, Hekari, and Kondelenchay. Analysis of samples from Zabuxchay and Quruchay confirmed that the quality indicators were within allowable limits (Imanov et al., 2021).

Additionally, four monitoring sessions were conducted in the Balasoltanlı area of the Hekari River in the Qubadli region, resulting in the collection of four water samples. "Azelab" LLC performed 70 relevant physicochemical analyses on these samples. The results indicated that only the concentration of iron (Fe) was 2.9 times higher than permissible limits in the second quarter, while other determined indicators remained within acceptable ranges.

Table 2. Sample Water Quality Indicators:

River	**pH**	**Conductivity μS/cm**	**(TDS) mg/L**	**Salinity %**	**DO mg/L**	**DO %**	**T °C**	**Resistance μs/h**
Oxchu River	7.87	508	254	0.03	8.89	96.2	19.2	0.047
Zangilan, 3rd Aghali	8.04	320	160.7	0.02	9.59	103.6	19.1	0.043
Hekari River	7.93	506	253	0.03	8.44	91.3	17.2	0.047
Zangilan, entrance bridge, Oxchu River	7.96	228	114.1	0.01	9.46	101.9	18.9	0.043

The pH values of the water samples range from 7.87 to 8.04. This indicates that the water is neutral to slightly alkaline. A neutral pH (7) reflects the overall water quality of the environment. Conductivity values range from 228 μS/cm to 508 μS/cm. This indicates the ion content of the water. Higher conductivity values suggest a greater presence of minerals and ions. TDS values range from 114.1 mg/L to 254 mg/L. Higher TDS values increase the mineral content of the water. Water with high TDS levels contains more pollutants and mineral substances. Salinity values range from 0.01% to 0.03%. This indicates that the water is slightly saline, but these values are very low, suggesting that the water is clean. DO values range from 8.44 mg/L to 9.59 mg/L. High DO values indicate that the water is rich in oxygen, which is essential for the health of aquatic ecosystems.DO % values range from 91.3% to 103.6%. This indicator reflects the degree of saturation of oxygen in the water. Values above 100% indicate that the water contains more oxygen than typical levels. Water temperature ranges from 17.2°C to 19.2°C. Temperature affects the physical state of the water and chemical reactions. Resistance values range from 0.043 μs/h to 0.047 μs/h. This indicator shows the conductivity resistance of the water. A higher resistance may indicate a lower ion content in the water.

Table 3. Measured Concentration of Metals in the Analyzed Samples (μg/L)

River	**Cr**	**Mn**	**Ni**	**Zn**	**Al**	**Fe**
Hekari River, 3rd Aghali	0.1	7.592	<0.20	23.18	34.74	137.26
Best River	1.96	3.485	<0.20	3.47	46.86	111.9
Spring	6.03	3.477	0.27	7.16	15.1	29.82
Oxchu River 1	1.09	181.8	<0.20	1508.8	725.7	1823
Oxchu River 2 Bridge	1.45	170.84	0.53	732	629	2241
Hekari River	0.57	21.644	2.19	17.35	48.1	274.41
WHO	50	100	70	5000	200	300

All measured **Cr** concentrations are below the WHO guideline of 50 μg/L. The highest concentration (6.03 μg/L) is found in the Spring sample, while the Hekari River, 3rd Aghali has the lowest (0.1 μg/L). Overall, Cr levels appear to be within safe limits.

Mn concentrations show significant variability, with the highest concentration observed in Oxchu River 1 (181.8 μg/L) and Oxchu River 2 Bridge (170.84 μg/L), both exceeding the WHO guideline of 100 μg/L. The presence of high Mn levels in these rivers suggests potential pollution or natural mineral deposits that require further investigation.

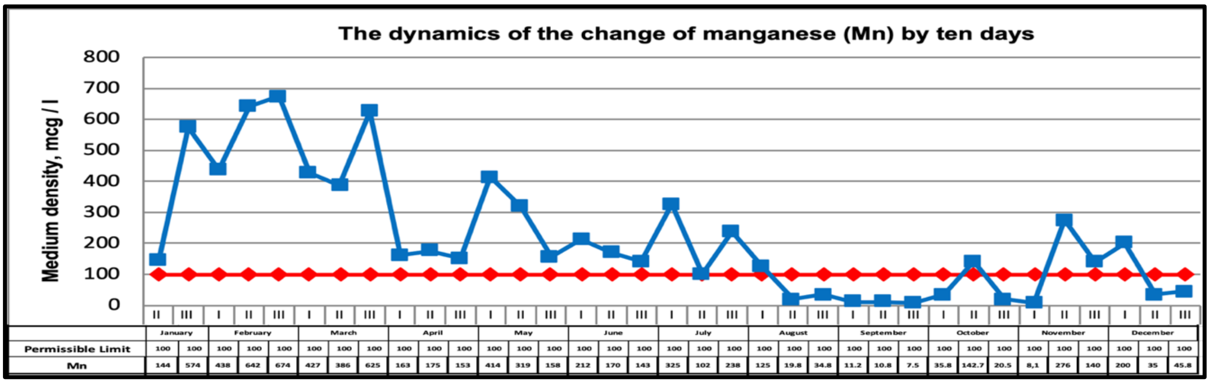


Figure 1. The dynamics of the change of Mn in Oxchu River Sayifli region by ten days of 2023 year

Ni concentrations are generally low across the samples, with values below the WHO guideline of 70 µg/L. The highest measured concentration is 2.19 µg/L in the Hekari River. This indicates that Ni levels are not a concern in these water bodies.

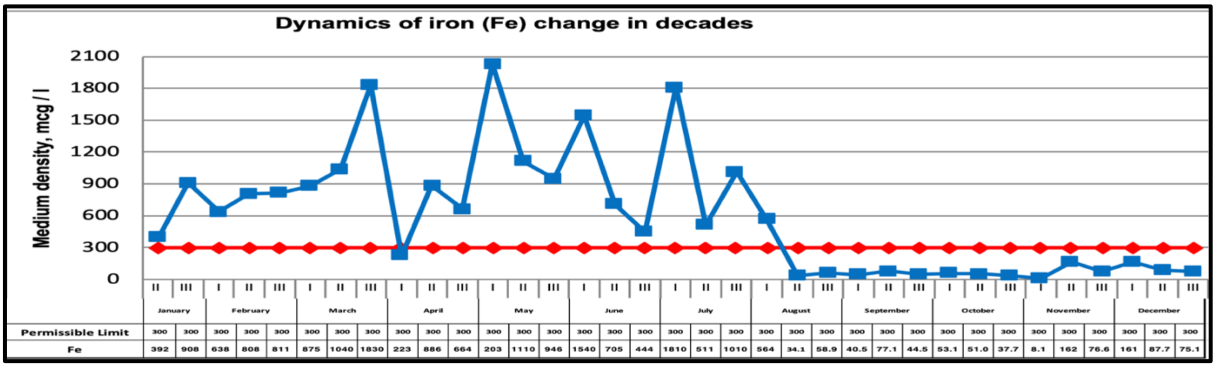


Figure 2. Dynamics of Fe change of Oxchu river Sayifli region by ten days of 2023 year

Fe concentrations are notably high in Oxchu River 2 Bridge (2241 µg/L) and Oxchu River 1 (1823 µg/L), both far exceeding the WHO guideline of 300 µg/L. The elevated Fe levels could be attributed to mining activities, industrial runoff, or natural geological factors.

Zn concentrations vary significantly, with a maximum of 1508.8 µg/L in Oxchu River 1, which is still well below the WHO guideline of 5000 µg/L. However, such high levels may indicate localized pollution sources. Other samples, like the Besit River, have much lower concentrations (3.47 µg/L).

Al levels vary widely, with the highest concentration (725.7 µg/L) also found in Oxchu River 1, exceeding the WHO guideline of 200 µg/L. This suggests possible contamination and warrants monitoring, especially in rivers with elevated aluminum levels.

Concerns for Pollution: The Oxchu River samples (1 and 2 Bridge) show concerning levels of Mn, Al, and Fe, indicating potential environmental issues that may need immediate attention and further investigation.

Overall Water Quality: While many metals remain below the WHO safety thresholds, the variability in concentrations, especially for Mn, Al, and Fe, suggests a need for regular monitoring to ensure water quality and address potential pollution sources. However, in the Oxchu

River, which flows into the Zangilan region of Azerbaijan from the territory of the Republic of Armenia, the concentration values for the element Mn are 1.7-1.8 times higher, for Al are 3.1-3.6 times higher, and for Fe are 6-7.5 times higher than the permissible limits. It should be noted that the maximum allowable conductivity value for drinking water is determined by the WHO as 400 µS/cm. The conductivity values of the samples taken from the Oxchu River (508 and 506 µS/cm) are above this limit, indicating that the river cannot currently be used as a source of drinking water. All of this suggests that the Oxchu River has been subjected to the influence of anthropogenic pollutants before entering the country's territory. The impact of such heavy pollution on the ecosystem of the river basin is also inevitable. Given that the pollution process likely continued during the long years of occupation of Zangilan, it is essential to carry out research and monitoring of water and sediment samples in the riverbed to thoroughly study and control the pollution

Oxchu River

Based on the analysis results of water samples taken from the Oxchu River in 2023, the dynamics of pollutant variations by ten-day periods in the water environment are as follows:

Table 4. Summary of water quality parameters (Sayifli region)

	Hardness			**NH_4**			**Mn**			**Mo**			**Fe**			**SO_4**		
	PCL = 7			**PCL = 0.5**			**PCL = 100**			**PCL = 250**			**PCL = 300**			**PCL = 500**		
	I	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**	**I**	**II**	**III**
January		16.4	16.5		0.0	1.3		144	574		164	308		392	908		372	586
February	17.1	14.0	10.0	1.3	1.2	2.4	438	642	674	233	247	674	638	808	811	498	476	277
March	10.6	10.8	9.9	1.5	1.6	1.6	427	386	625	234	170	233	875	104	183	372	381	247
April	11.8	8.4	7.6	0.0	0.0	0.0	163	175	153	95.4	202	219	223	886	664	205	104	200
May	8.8	8.4	6.0	0.0	0.0	0.0	414	319	158	234	204	122	203	111	946	212	294	171
June	3.1	3.89	4.2	0.0	0.0	0.0	212	170	143	38.5	44.4	65	154	705	444	93	133	141
July	4.86	6.0	6.9	0.0	0.0	0.0	325	102	238	135	143	245	181	511	101	129	205	217
August	7.3	7.76	8.3	0.0	0.2	0.0	125	19.8	34.8	221	248	237	564	34.1	58.9	213	225	253
September	8.2	8.1	8.0	0.1	0.0	0.0	11.2	10.8	7.5	263	141	94.9	40.5	77.1	44.5	251	257	372
October	9.69	8.33	10.1	0.0	0.0	0.0	35.8	142.7	20.5	153	126	170	53.1	51	37.7	359	390	403
November	10.45	12.5	12.4	0.0	0.0	1.1	8.1	276	140	73.5	273	114	8.1	162	76.6	348	476	498
December	11.4	9.23	11	0.0	0.4	0.0	200	35	45.8	295	237	277	161	87.7	75.1	400	272	365

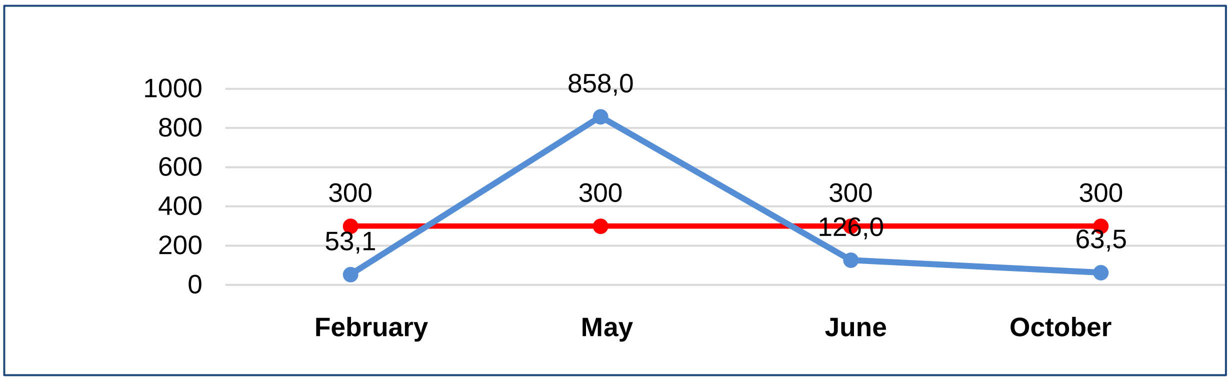
Observations:

- Hardness levels vary, with the highest in February.
- NH_4 levels are consistently low, mostly at 0.0, except for February and March.
- Mn shows significant variation, peaking in March.
- Mo remains mostly low, with peaks in March and May.
- Fe levels fluctuate significantly, especially high in March and August.
- SO_4 levels also vary, with notable peaks in March and October.

Hakari river

The dynamics of iron (Fe) in Hakari River's water environment were variable in 2023. In May, the analysis results showed a rise to 858 µS/cm, while the permissible conductivity limit (PCL) for the river water is 300 µS/cm.

Figure 3. CHANGE DYNAMİCS OF İRON (FE) İN HEKARİCHAY'S WATER ENVİRONMENT FOR 2023



Tartar River

In recent years, water samples have been periodically taken from the Tartar River (up until August 2020). These samples underwent on-site rapid analyses and radiometric measurements, as well as organoleptic, physico-chemical, and microbiological analyses conducted in the laboratory of the Azerbaijan National Academy of Sciences (ANAS). The results of the comprehensive analyses revealed that the quality indicators of the Tartar River water do not meet the relevant regulatory requirements.

Radioactive background levels were measured in the surrounding areas along the Tartar River, and it was determined that the water samples were contaminated with anthropogenic xenobiotics and pathogenic microorganisms.

According to the latest data from January to August 2024, three water samples were taken from the Kelbajar region station of the Tartar River and three samples from the Vang village station, totalling six water samples. These samples were subjected to 101 relevant physical-chemical analyses conducted by “AzeLab” LLC.

Based on the analysis results, it was determined that only the concentration of iron (Fe) among the heavy metals exceeded the permissible limit by 1.1 times in the Kelbajar region station during the second quarter of this year.

Pollution of water resources from serseng and suqovushan reservoirs:

Until August 2020, the waters artificially discharged from the Serseng and Suqovushan (formerly known as “Madagiz”) reservoirs in mountainous areas have been contaminated with organic waste and microorganisms. This is one of the ongoing violations.

Sabotage and Problems:

- Wastewater Issues: The prevention of water flow has resulted in chaotic discharges and artificially created floods in rivers, aimed at hindering agricultural development.
- Damage in Surrounding Areas: During cold periods when irrigation is not needed, disruptions in the technological operating regime have caused soil erosion in agricultural fields and damage to residential communication lines (Mona Fathi, et al., 2018).

These acts of sabotage have had serious impacts on the region’s agriculture, the environment, and irrigation systems. The implementation of appropriate purification methods and the prevention of such violations are crucial.

Microbiological Contamination

Until August 2020, the microbiological contamination of the Tartar River was characterized by serious issues. Analyses indicated that:

- The number of Escherichia coli was 3-4 times higher than allowable limits.
- Unacceptable cocci (such as *Staphylococcus aureus*), microscopic fungi (including *Candida* and *Aspergillus*), and pathogenic bacteria (like *Salmonella*) were detected.

These results indicated that Tartar River water was unfit for direct consumption. The microbiological contamination was linked to the discharge of organic waste into the river from the Suqovushan reservoir, located in an area occupied by Armenian armed forces.

Purification Stages

The contamination of Tartar River with organic waste required the implementation of both mechanical and biological purification stages. The application of a multi-stage complete purification cycle could help make this water safer.

Changes After the Patriotic War

Following the victory of the Azerbaijani Army in the 44-day Patriotic War, positive changes were observed in the microbiological indicators of Tartar River water:

- The number of pathogenic microorganisms sharply decreased.
- Alpha and beta radiation were not detected.

Table 5. Radioactivity of the Tartar River compared to Baku City

Natural Radioactive Radiation Indicators	**Tartar river**		**Baku city**
Dose Rate (μR/h):	0,018-0,030		0,011-0,045
Alpha Radiation Intensity (Bq/cm²):	0-0,02		0-0,04
Concentration of Isotopes:	Surface:	Bottom:	Surface:
Na-22 Isotope (Bq/L):	0,30-0,40	0,31-0,42	0,28
K-40 Isotope (Bq/L):	0,08-0,10	0,09-0,11	0,16

Conclusıon:

This study provides a comprehensive analysis of water quality in the Tartar, Hekari, and Oxchu rivers, illustrating the significant impact of both natural factors and anthropogenic activities on these vital water bodies. The observed variations in pollutant levels across different regions highlight the growing environmental challenges that threaten water resources, particularly in the Karabakh and Eastern Zangazur regions. Elevated concentrations of specific pollutants in certain areas serve as a warning about the potential consequences of unsustainable practices. These findings underscore the critical need for continuous monitoring, effective resource management, and the implementation of sustainable practices to ensure the long-term preservation and quality of water resources in the region.

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MOLECULAR INVESTIGATION OF CYANOBACTERIA IN THE CASPIAN SEA

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Abstract: This study examines the presence of cyanobacteria in the Caspian Sea and their potential for microcystin production. DNA from water samples collected across the sea was analysed using CC-CG PCR, revealing positive amplification in 9 of 14 samples, indicating active cyanobacterial presence. However, attempts to detect microcystin-producing genes (mcyB) were unsuccessful, likely due to low DNA concentrations and sample quality. Regular monitoring of physicochemical parameters highlighted the influence of nutrient changes on cyanobacterial communities. These findings underscore the need for systematic monitoring to prevent the proliferation of toxic species and inform strategies to mitigate their spread, contributing to understanding the Caspian Sea's ecosystem and conservation.

Keywords: Cyanobacteria, Microcystin, PCR, DNA extraction, Ecosystem monitoring

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Introduction:

Cyanobacteria, commonly known as blue-green algae, are prokaryotic organisms capable of photosynthesis due to the presence of chlorophyll-a pigment. These microorganisms are found across a wide range of environments, including aquatic ecosystems, soil surfaces, and in symbiotic relationships with certain plant roots (Berman-Frank & ets, 2003). As integral components of aquatic ecosystems, cyanobacteria play a crucial role in maintaining ecological balance. However, in recent decades, various environmental changes, particularly those driven by human activities, have led to the excessive proliferation ("blooming") of cyanobacteria. This phenomenon not only negatively impacts water quality and aquatic organisms but also results in the production of toxins that pose serious risks to human health (Nuriyeva, 2018; Nuriyeva, 2019).

The Caspian Sea is one of the regions significantly affected by this issue. Rapid growth of cyanobacteria in the sea has been accompanied by an increase in cyanotoxin levels. Industrial and domestic waste pollution creates favourable conditions for cyanobacterial blooms, with nutrient-rich discharges - especially nitrogen compounds - acting as primary drivers. The proliferation of cyanobacteria and their toxins has a detrimental impact on the survival of fish, molluscs, plants, and other aquatic organisms. Additionally, humans are exposed to these toxins directly or indirectly, jeopardizing public health (Akhundova, 1996; Berman-Frank & ets, 2003).

The cyanotoxins produced by excessive cyanobacterial blooms are biologically active and hazardous, capable of affecting human health in various ways. Therefore, molecular-level studies of these toxins are critical to restoring the ecological balance of the Caspian Sea and safeguarding aquatic and human health. Advanced molecular techniques such as ELISA and PCR are widely used to analyze the composition and concentration of cyanotoxins, providing precise detection and forming the foundation for control strategies.

Comprehensive research is needed to identify the causes of cyanobacterial blooms in the Caspian Sea, assess the associated risks, and develop effective control measures. Such studies not only improve our understanding of cyanobacterial impacts but also help evaluate the effectiveness of physical, chemical, and biological methods for controlling blooms. By creating a robust knowledge base, it becomes possible to implement cost-effective and sustainable solutions for preventing cyanobacterial proliferation. These efforts are vital for improving the ecological condition of the Caspian Sea and ensuring environmental protection.

Materials and Methods:

This study utilized various methods to determine the presence of cyanobacteria and microcystin production in the aquatic environment of the Caspian Sea. A systematic approach, including water quality assessment, DNA extraction, and amplification procedures, was applied.

During the study, 14 water samples were collected from the Caspian Sea outlet on November 17, 2023, and March 5, 2024. The samples were collected in 1-litre glass containers and immediately transported to the laboratory. The samples were stored at 0-4 °C. Universal primers were used to determine the presence of oxyphotobacteria.

For DNA extraction, 0.5 L of the water sample was filtered. During filtration, nitrocellulose filters with a pore size of 0.45 µm were used. The filters were dissolved in 1 mL of PBS (phosphate-buffered saline) solution. The Qiagen DNeasy PowerWater Kit was used for DNA extraction. DNA quality and quantity were measured using a spectrophotometer (NanoDrop).

For PCR operations, CC-CG primer pairs were selected. Each PCR reaction was conducted with 12.5 µL of 2X PCR mixture, 1 µL of universal primer, 1 µL of DNA sample, and 9.5 µL of sterile water. The reactions included an initial denaturation step at 95 °C for 5 minutes, followed by 30 seconds of denaturation at 95 °C, 30 seconds of annealing at 55-65 °C, and a 1-minute extension step at 72 °C. The PCR products were separated using 1% agarose gel electrophoresis.

To determine microcystin production, genus-specific primer pairs (mcyB for Microcystis sp.) were used. The concentration of microcystin was assessed using the ELISA (Enzyme-Linked Immunosorbent Assay) test (Burrell & ets, 2016).

The results of the study provide monitoring data for cyanobacteria and microcystin production in the Caspian Sea's aquatic environment. These findings present important information for future research and are significant for assessing ecosystem health.

Results and discussions:

The first and crucial step in conducting molecular research on cyanobacteria is the isolation of pure cultures. For this purpose, the area where the samples will be collected must be determined initially, and the samples should be gathered in accordance with the required protocols. The cyanobacteria samples used in this study were collected from the shores of the Shirvan National Park on the Caspian Sea during the summer months of 2023.

To prevent contamination during the collection process, sterile equipment was used, and each sample was stored in specialized containers. These containers were maintained under proper conditions, with the appropriate temperature and lighting, until they were transported to the laboratory.

The samples were collected from the same source at various depths (5-10 cm from the surface of the shore) and taken multiple times. During the transportation of the collected material to the laboratory, all necessary requirements were adhered to. Upon arrival at the laboratory, the samples were processed under suitable conditions to ensure the isolation of pure cultures (Figure 2).

Figure 1. Cyanobacteria collected from the shores of the Shirvan National Park area on the Caspian Sea.

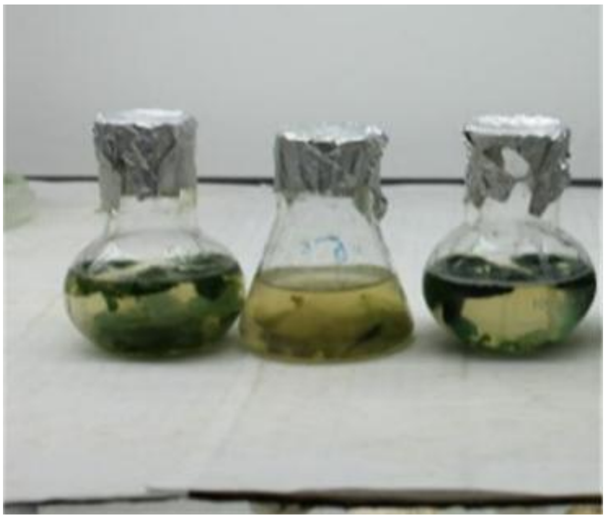

Figure 2. Enriched cultures of cyanobacteria.

The composition of the culture medium was prepared by the expected algological composition of the samples. The process of isolating pure cultures typically consists of three main stages: obtaining enriched (cumulative) culture (Figure), isolating the pure culture, and confirming the purity of the isolated culture. Individual pure cultures were isolated using the streak plate method. For this purpose, a small amount of the sample was taken using a microbiological loop and spread over the surface of the culture medium.

Initially, the streaks contained a large number of microalgal cells. However, with the movement of the loop, the number of these cells gradually decreased, eventually reaching a single-cell level. After the distribution of cyanobacteria in the culture medium, the plates were incubated to allow for colony growth (Figure 3).

The purity of all three isolated cyanobacteria cultures was verified using several methods: visual microscopic inspection and the use of different culture media to confirm the culture's purity.

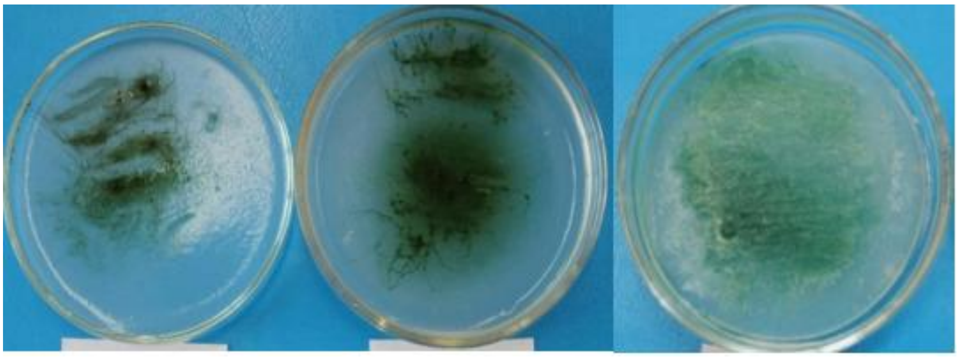

Figure 3. Growth of cyanobacteria on agar medium.

The results of the study conducted to analyze the cyanotoxins of cyanobacteria collected from the shores of the Shirvan National Park on the Caspian Sea are presented in Table 1. In all samples, the concentration of microcystin in the Caspian Sea was 0.2 µg/L. In June and July, the concentrations of saxitoxin in two samples were near the detection limit of 0.03 µg/L, while the sample collected in August was approximately 0.02 µg/L.

Table 1. Concentrations of microcystin, saxitoxin, and anatoxin-a in samples from the Caspian Sea during June-August 2023:

	24.06.2023		15.07.2023	05.08.2023	Negativ
Microcystin µg/l	0.2	0.2	0.2		< 0.10 µg/l
Saxitoxin µg/l	0.01	0.03	0.02		< 0.02 µg/l
Anatoxin -a µg/l	53.6	9.5	25.1		< 10 µg/l

Results for anatoxin-a showed higher concentrations in the samples compared to microcystins and saxitoxin. In the sample collected from the Caspian Sea in June, the average concentration of anatoxin-a was 53.6 µg/L, 25.1 µg/L in August, and 9.5 µg/L in July.

ELISA kits are widely used by several laboratories for the detection of cyanotoxins. This method allows for rapid and relatively inexpensive detection of cyanotoxins in environmental samples. However, this method only estimates the total amount of toxin and has difficulty distinguishing between different analogs of the toxin. Additionally, due to high cross-reactivity, false positives may occur, and therefore, it is recommended to verify the results with other methods (Salmaso & Zignin, 2020). Since the analysis of saxitoxin and anatoxin-a with ELISA kits is relatively new, there is limited research available on this topic.

The risk of exposure to cyanotoxins in humans and animals is a cause for concern. Although the concentrations of microcystins and saxitoxin in the Caspian Sea are low, the concentration of anatoxin-a is sufficiently high. This exceeds the guideline value of 20.0 µg/L for recreational waters (Flombaum & ets, 2013). The high concentration of anatoxin-a is dangerous for both wild and domestic animals; in the United States, several dogs have died after drinking water contaminated with anatoxin-a (Flombaum & ets, 2013).

Fish are more susceptible to cyanobacterial toxins, affecting their embryonic development and growth rate. The composition of the fish community in the Caspian Sea has decreased with changes in the hydrological regime. The

Caspian Sea sustains the biodiversity of fish, which are a food source for the local population. However, the consumption of meat from organisms that accumulate microcystins is risky (Durai & ets, 2015).

The airborne transmission of microcystins and cyanotoxins could be problematic during recreational activities in lakes (Backer & ets, 2009). The fate of cyanotoxins in the aquatic environment is also of significant importance. Among biodegradation mechanisms, the biodegradation of these compounds appears to be the most effective. Heterotrophic bacteria use cyanotoxins as a carbon source (Nybom, 2013).

Molecular methods, particularly PCR (Polymerase Chain Reaction)-based techniques, are widely used in the study of cyanotoxins. These methods enable the identification of the presence of cyanobacteria and their potential for toxin production (Salmaso & Zignin, 2020). Spectrophotometric results of DNA samples from the Caspian Sea, when extracted by various methods, were characterized by low concentrations and purity indicators.

CC-CG PCR successfully amplified 606 bp fragments of cyanobacterial DNA. Positive amplification was observed in 9 out of the 17/11/2023 samples, providing significant information regarding the presence of cyanobacteria (Salmaso & Zignin, 2020).

Table 2. Concentration and purity of DNA extracted from the Caspian Sea (X) from June to August 2023. Extraction dates: 17/11/2023 and 05/03/2024, and positive samples (CC-CG) for Oxyphotobacteria-specific primers.

Sampling Location and Date		Extraction on 17/11/2023		CC-CG primers for positive samples	Extraction on 05/03/2024		CC-CG primers for positive samples
		Concentration ng/µl	**Purity A260/A280**		**Konsentrasiya ng/µl**	**Saflıq A260/A280**	
X1	24.06.23	15.9	1.6		19.6	1.5	+
	15.07.23	18.7	2.1	+	34.2	2.4	+
	05.08.23	8.1	1.6	+	40.1	1.4	
X2	24.06.23	23.3	1.7		27.1	1.9	+
	15.07.23	13.8	3.9		58.9	2.1	
	05.08.23	4.4	-2.1	+	22.3	1.7	+

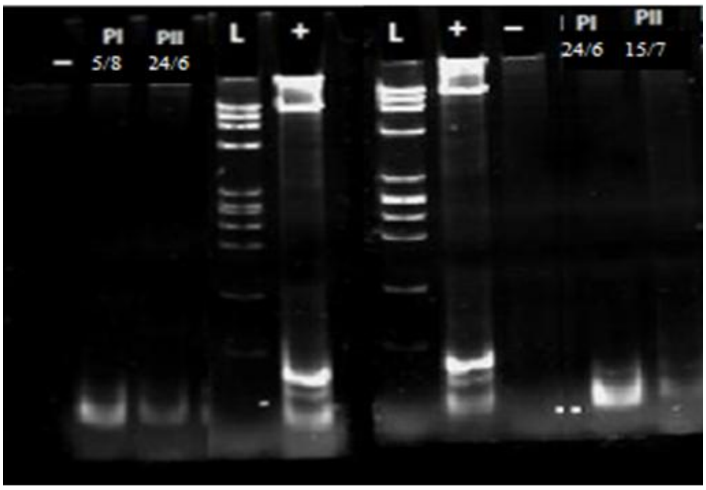

Figure 4. PCR results for the detection of toxic Microcystis in the Caspian Sea using primers specific to the Microcystis sp. mcyB gene. (+) Positive control, (-) Negative control, (L) Ladder.

At the PI sample collection station located at the mouth of the Caspian Sea, using universal primers for oxyphotobacteria, two positive amplifications were observed in PCR for the months of June, July, and August. This sampling station is characterized by the highest salinity throughout the study period. The sample taken on 05/08/23 tested positive after the PCR operation for both extractions. A sample collected at the same station in June showed a positive band, but no amplification was observed in the July extracts.

PCR reactions were performed several times using genus-specific primer pairs to amplify the regions of the gene responsible for the synthesis of microcystin (mcyB in Microcystis sp.). A nested PCR reaction was carried out with 1 µl of PCR product, but no amplification was detected. Microcystis sp., used as a positive control, showed good amplification in all tests, indicating that the PCR reaction was well optimized. The failure of genus-specific primers to amplify bands in the PCR is related to the low quantity and quality of the DNA extracted on 17/11/23.

Negative PCR results with genus-specific primers can be consistent with the low microcystin concentration measured by ELISA in the Caspian Sea. This may indicate that the cyanobacterial cells detected in microscopic analyses and CC-CG PCR do not produce microcystin. It is thought that blooms in the same water column may show variations in toxicity from year to year.

Further research on the cyanobacterial profiles of the Caspian Sea may be needed in the future. PCR optimization could also be effective. The multilayered cell wall of cyanobacteria and the mucous layer around the cells hinder effective DNA extraction. Additionally, environmental samples are often associated with humic acids, sands, and other materials that accompany DNA. Spectrophotometric results have detected protein contamination in several samples with a purity of 2 A260/A280 or higher.

Long-term analyses may help explain the nature of cyanobacterial communities in these lakes. Low nutrient concentration, especially nitrogen, promotes the growth of non-toxic Microcystis cells. Similarly, the low concentration of nitrogen compounds in the Caspian Sea corresponds to the characteristics of cyanobacteria in these waters.

Microscopic identification and PCR amplification detected the presence of cyanobacterial cells. The absence of PCR bands corresponding to mcy gene regions may indicate that the cyanobacteria in the Caspian Sea do not produce microcystin. Toxin analyses demonstrated low concentrations of microcystin and saxitoxin in the Caspian Sea, but a high concentration of anatoxin-a.

The continuous monitoring of physical-chemical parameters, along with the abundance of blue-green algae and cyanotoxin concentration in the Caspian Sea, can help improve the current state of the lakes. Since there is frequent displacement of toxic and non-toxic cyanobacteria in the water environment, restoration strategies should be implemented in the future to prevent the development of toxic cyanobacteria species.

Conclusıon:

This study investigated the presence of cyanobacteria and microcystin production in the Caspian Sea water environment. DNA analysis amplified by CC-CG PCR method yielded positive amplification results in 9 out of 14 samples. This indicates that cyanobacteria are active in this area and could potentially produce toxins. At the same time, PCR reactions conducted using genus-specific primers (mcyB) to detect microcystin production did not yield positive results. This was associated with the possible low DNA concentration and the quality of the samples.

Monitoring the environmental conditions in the Caspian Sea revealed that the cyanobacterial flora may show changes. Physical-chemical parameters, nutrient concentrations, and ecosystem health should be regularly assessed. Furthermore, the study results are crucial for developing strategic plans to prevent the future development of toxic cyanobacteria species. Systematic monitoring and research are necessary to protect the

Caspian Sea's ecology, which will help to better understand the factors affecting human health and local ecosystems. The results provide an important foundation for the implementation of scientifically and ecologically relevant measures.

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