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BIODIVERSITY AND TAXONOMIC CHARACTERIZATION OF PHOTOTROPHIC MICROORGANISMS ISOLATED FROM EXTREME ECOSYSTEMS OF KAZAKHSTAN

Extreme ecosystems represent natural models for studying the mechanisms of microbial adaptation to a complex of abiotic stress factors, including elevated mineralization, thermal stress, pH fluctuations, radiation exposure, and limited nutrient availability. Kazakhstan is characterized by a wide diversity of such unique natural environments; however, the biodiversity of phototrophic microorganisms inhabiting these ecosystems remains insufficiently studied to date. Contemporary climate challenges and the demand for sustainable bioenergy solutions have increased interest in extremophilic phototrophic microorganisms demonstrating adaptation to harsh environmental conditions.

The goal of the present study was to assess the biodiversity and taxonomic structure of microalgae and cyanobacteria isolated from extreme aquatic and soil ecosystems in various regions of Kazakhstan. To achieve this objective, field expeditions were conducted at designated monitoring sites, and microbiological methods were employed to isolate axenic cultures.

As a result of the study, 171 species and subspecies of phototrophic microorganisms were identified in soil samples, and 14 species in aquatic ecosystems, predominantly belonging to the classes Chlorophyceae, Cyanophyceae, and Bacillariophyceae. Axenic cultures representing six genera Chlorella, Anabaena, Synechocystis, Coelastrum, Leptolyngbya, and Phormidium were successfully obtained. The identified taxa exhibited morphological and ecological plasticity, reflecting their adaptation to thermal, saline, highly mineralized, and radiation-active environmental conditions.

The obtained results indicate a high adaptive potential of phototrophic microorganisms inhabiting extreme ecosystems and highlight their prospects for the development of sustainable biotechnological processes, including biomass and lipid production for biofuel generation.

Keywords: biodiversity, extreme ecosystems, phototrophic microorganisms.

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Қазақстанның экстремальды экожүйелерінен бөлініп алынған фототрофты микроорганизмдердің биоәртүрлілігі мен таксономиялық сипаттамасы

Экстремалды экожүйелер микроорганизмдердің абиотикалық стресс факторларының кешенді әсеріне бейімделу механизмдерін зерттеуге арналған табиғи модельдер болып табылады. Мұндай факторларға жоғары минералдану, термиялық стресс, pH көрсеткішінің ауытқуы, радиациялық әсер және қоректік заттардың шектеулі болуы жатады. Қазақстан осындай бірегей табиғи нысандардың кең алуандығымен сипатталады, алайда аталған экожүйелердегі фототрофты микроорганизмдердің биоалуантүрлілігі бүгінгі күнге дейін жеткілікті деңгейде зерттелмеген. Қазіргі климаттық өзгерістер мен тұрақты биоэнергетикалық шешімдерге деген сұраныстың артуы экстремалды орта жағдайларына бейімделген экстремофильді фототрофты микроорганизмдерге деген ғылыми қызығушылықты күшейтуде.

Зерттеу жұмысының мақсаты – Қазақстанның әртүрлі аймақтарындағы экстремалды су және топырақ экожүйелерінен бөлініп алынған микробалдырлар мен цианобактериялардың биоалуантүрлілігі мен таксономиялық құрылымын бағалау. Қойылған мақсатқа жету үшін зерттеу нүктелеріне экспедициялық сапарлар ұйымдастырылып, таза (аксеникалық) дақылдарды бөліп алу үшін микробиологиялық әдістер қолданылды.

Жүргізілген зерттеулер нәтижесінде топырақ үлгілерінен фототрофты микроорганизмдердің 171 түрі мен түр тармағы, ал су экожүйелерінен 14 түрі анықталды. Олар негізінен *Chlorophyceae, Cyanophyceae* және *Bacillariophyceae* кластарына жатады. *Chlorella, Anabaena, Synechocystis, Coelastrum, Leptolyngbya* және *Phormidium* туыстарының аксеникалық дақылдары алынды. Анықталған таксондар морфологиялық және экологиялық икемділігімен сипатталады, бұл олардың термалды, тұзды, жоғары минералданған және радиациялық белсенді орта жағдайларына бейімделуін көрсетеді.

Алынған нәтижелер экстремалды экожүйелердегі фототрофты микроорганизмдердің жоғары бейімделу әлеуетін дәлелдейді және олардың тұрақты биотехнологиялық процестерді, соның ішінде биожанармай өндіруге арналған биомасса мен липидтер алуды дамытудағы перспективалылығын айқындайды.

Түйін сөздер: биоалуантүрлілік, экстремалды экожүйелер, фототрофты микроорганизмдер.

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Биоразнообразие и таксономическая характеристика фототрофных микроорганизмов, выделенных из экстремальных экосистем Казахстана

Экстремальные экосистемы представляют собой природные модели для изучения механизмов адаптации микроорганизмов к воздействию комплекса абиотических стрессовых факторов, включая повышенную минерализацию, термический стресс, колебания pH, радиационное воздействие и ограниченную доступность питательных веществ. Казахстан характеризуется широким разнообразием подобных уникальных природных объектов, однако биологическое разнообразие фототрофных микроорганизмов в данных экосистемах до настоящего времени остается недостаточно изученными. Современные климатические вызовы и потребность в устойчивых биоэнергетических решениях усиливают интерес к экстремофильным фототрофным микроорганизмам, демонстрирующим адаптацию к экстремальным условиям среды. Целью данной работы является оценка биоразнообразия и таксономической структуры микроводорослей и цианобактерий, выделенных из экстремальных водных и почвенных экосистем различных регионов Казахстана. Для достижения поставленной цели были осуществлены экспедиционные выезды в мониторинговые точки обследований, а также использованы микробиологические методы для выделения чистых культур.

В результате проведенных исследований, в почвенных образцах был выявлен 171 вид и подвид фототрофных микроорганизмов, в водных экосистемах – 14 видов, преимущественно относящихся к классам *Chlorophyceae, Cyanophyceae* и *Bacillariophyceae*. Получены аксенические культуры представителей шести родов *Chlorella, Anabaena, Synechocystis, Coelastrum, Leptolyngbya* и *Phormidium*. Выявленные таксоны характеризуются морфологической и экологической пластичностью, что отражает их адаптацию к термальным, солоноватым, высокоминерализованным и радиационно активным условиям среды.

Полученные результаты свидетельствуют о высоком адаптационном потенциале фототрофных микроорганизмов экстремальных экосистем и подчёркивают их перспективность для разработки устойчивых биотехнологических процессов, включая получение биомассы и липидов для производства биотоплива.

Ключевые слова: биоразнообразие, экстремальные экосистемы, фототрофные микроорганизмы.

Introduction

Extreme ecosystems represent key natural systems for investigating the mechanisms of microbial adaptation to a complex of environmental stress factors, including salinity, pH fluctuations, thermal stress, elevated mineralization, radiation exposure, and limited nutrient availability (Matarredona et al., 2020). The territory of Kazakhstan is characterized by substantial diversity of such natural environments; however, their microbiological diversity remains insufficiently studied.

The present study encompasses a number of extreme natural sites across Kazakhstan: the Chundzha hot springs (Almaty Region); Barlyk-Arasan and Lake Alakol (Abai and Jetisu Regions); Rakhman Springs and the Sibin Lakes (East Kazakhstan Region); the saline lakes and therapeutic muds of Lake Solyanka and Erkenkush (Aktobe Region); the radon hot springs near Aktau (Mangystau Region); and Lake Sabyndykol (Pavlodar Region). These territories are characterized by a spectrum of abiotic stress factors, including hypersalinity, elevated temperatures, radon activity, high mineralization, and a specific ionic composition of water and soils, which collectively create unique ecological niches.

Although extreme factors have traditionally been regarded as limiting conditions for microbial growth, recent studies demonstrate that stress may also perform an activating function. Under conditions of salinity, temperature fluctuations, or nutrient limitation, microalgae are capable of enhancing the synthesis of lipids and secondary metabolites, thereby increasing their energetic value and biotechnological significance. Several studies have demonstrated (Shi et al., 2020; Song et al., 2022) that stress should not be regarded as a limiting factor but rather as a trigger for the activation of biosynthetic pathways that promote the accumulation of compounds relevant for bioproduct production.

The relevance of this study is determined by the urgent need for alternative energy sources in the 21st century, associated with the depletion of fossil fuel reserves, rising greenhouse gas emissions, and ongoing global climate change (Kumar et al., 2023). The intensification of aridization processes and the growing demand for renewable bioenergy sources have heightened interest in extremophilic phototrophic microorganisms as potential producers of biomass and lipids for biofuel production.

According to previous studies (Rojas-Villalta et al., 2024; Sharma et al., 2024), cyanobacteria and microalgae exhibit broad ecological plasticity, demonstrate tolerance to various stress factors, maintain efficient photosynthetic activity under extreme conditions, and possess valuable metabolic pathways, making them key candidates for biofuel production, CO_2 mitigation, biomaterial synthesis, and the development of sustainable biotechnological applications.

Furthermore, a number of studies conducted in Kazakhstan have addressed various aspects of phototrophic microorganisms in extreme environments (Zayadan et al., 2014; Sandybayeva et al., 2023), however, previous investigations have been predominantly local or fragmentary in nature. Moreover, these studies typically focused on individual extreme factors or isolated natural sites, without encompassing the broad spectrum of stress conditions and the extensive geographical coverage represented in the present study.

The novelty of the present study resides in a comprehensive taxonomic assessment of phototrophic microorganisms inhabiting diverse extreme ecosystems of Kazakhstan, with a focus on evaluating their functional potential. Particular attention is given to the identification of axenic strains exhibiting broad adaptive capacity.

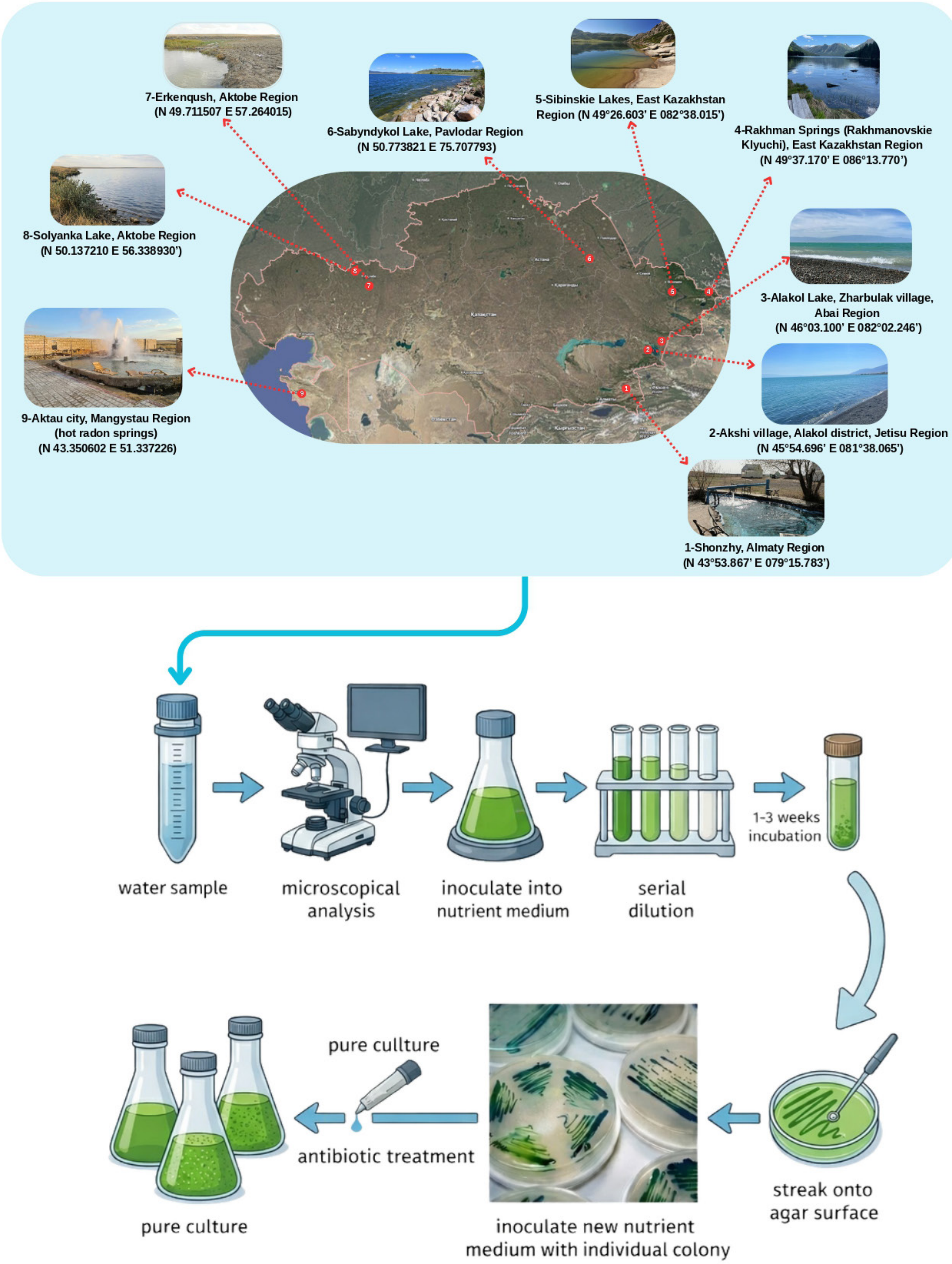
The goal of this study is to investigate the biodiversity and characterize the taxonomic structure of phototrophic microorganisms inhabiting extreme ecosystems of Kazakhstan, as well as to identify their metabolic features relevant for biofuel-oriented biotechnological applications. It is expected that the obtained results will provide a scientific foundation for the development of environmentally oriented technologies for renewable energy production, in line with the principles of the green economy and the United Nations Sustainable Development Goals, including Goal 7 (Affordable and Clean Energy).

Materials and methods

Study sites and sampling. Water and soil samples were collected during the summer of 2025 across the eastern, southeastern, northeastern, western, and southwestern regions of Kazakhstan (Figure 1).

In the area of the Chundzha hot springs (Almaty Region), water samples were collected at the Shonzhy site (43.53.867° N, 079.15.783° E). The monitoring location included five sub-sites for soil sampling, ranging from 43.5308° N / 79.2415° E to 43.5304° N / 79.2423° E. The Chundzha hot springs represent extreme thermal ecosystems characterized by elevated temperatures (20–100 °C) and specific hydrochemical conditions that impose persistent stress on microorganisms. Strains isolated from such environments frequently exhibit enhanced lipid synthesis under stress conditions, indicating their significant biotechnological potential (Sandybayeva et al., 2026).

Figure 1
Monitoring sites of extreme ecosystems in Kazakhstan and schematic diagram of axenic culture isolation of phototrophic microorganisms



In the Katon-Karagay District (East Kazakhstan Region), water samples were collected in the area of Rakhmanovskie Klyuchi: the first sampling point was located at 49°37.170′ N, 86°13.770′ E, with five sub-sites for soil sampling ranging from 49.619782° N to 49.620318° N and from 86.229547° E to 86.229814° E. The second sampling point was located at the Sibin Lakes (49°26.603′ N, 82°38.015′ E), where five soil sub-sites were established within the coordinate range of 49.443665° N to 49.444152° N and 82.633649° E to 82.633902° E. The Rakhman Springs (Rakhmanovskie Klyuchi), characterized by sulfate–bicarbonate sodium–magnesium type waters, represent thermal radon springs with elevated temperatures, natural radioactivity, and a specific ionic composition, forming a complex of extreme environmental factors (Nunes et al., 2023). In contrast, the Sibin Lakes are freshwater oligotrophic tectonic water bodies with low mineralization and slightly alkaline pH; calcium (Ca^{2+}) and bicarbonate (HCO_3^-) ions dominate their ionic composition, allowing the waters to be classified as hydrocarbonate–calcium type (Samarkhanov et al., 2025).

Two sampling sites were selected at Lake Alakol, the only deep endorheic lake and the second largest lake in Kazakhstan. Lake Alakol is classified as an extreme aquatic ecosystem due to the combination of elevated mineralization (1.2–11.6 g/kg), alkaline pH values (7.2–9.1), the predominance of sodium–sulfate and chloride ions, and its closed hydrological regime. The combined effects of osmotic stress, specific ionic composition, semiarid climatic conditions, and high evaporation rates create substantial environmental pressure, favoring stress-tolerant phototrophic microorganisms (Yapiyev et al., 2021). In the Barlyk-Arasan area (Abai Region, Makanchi District), water samples were collected at 46°03.100′ N, 82°02.246′ E. Soil samples were obtained from five sub-sites ranging from 46.051948° N to 46.052463° N and from 82.037512° E to 82.037721° E. In the Alakol District (Jetisu Region), near the settlement of Akshi, water sampling was conducted at 45°54.696′ N, 81°38.065′ E, and soil samples were collected from sub-sites within the coordinate range of 45.911880° N to 45.912250° N and 81.634620° E to 81.634720° E.

In the Alga District (Aktobe Region), two monitoring sites were selected for the study. The first site was Lake Solyanka, a saline lake characterized by elevated mineralization resulting from its endorheic regime and intense evaporation under the region's arid climatic conditions. The high concentration of dissolved salts imposes pronounced osmotic and ionic stress, creating extreme environmental conditions and restricting biological communities to halophilic and halotolerant forms. Water sampling at this site was conducted at 50.137210° N, 56.338930° E, and five soil sub-sites were established within the coordinate range of 50.137482° N to 50.137701° N and 56.338998° E to 56.339214° E. The second site was located near the settlement of Erkenqush (therapeutic mud deposits) at 49.711507° N, 57.264015° E, with five soil sub-sites ranging from 49.711782° N to 49.712154° N and 57.264081° E to 57.264302° E. Therapeutic muds in this area are formed under conditions of elevated mineralization, high organic matter content, limited aeration, and unstable hydrological regime, which together create a complex of extreme physicochemical factors (Karakaya et al., 2010).

In the Munaily District near the city of Aktau (Mangystau Region), water samples were collected from hot radon springs at 43.350602° N, 51.337226° E. Five soil sub-sites were established within the coordinate range of 43.350623° N to 43.350812° N and 51.337215° E to 51.337956° E. Radon springs are classified as extreme natural environments due to the presence of natural radioactivity (elevated Rn-222 concentrations), associated with radon migration from uranium-bearing rocks, as well as the specific hydrogeochemical conditions of the arid region. Elevated mineralization, high water temperature, and moisture deficit create distinct physicochemical conditions that significantly limit biological diversity and require pronounced adaptive mechanisms (Nunes et al., 2023).

In the Bayanaul District (Pavlodar Region), water samples were collected from Lake Sabyndykol at 50.773821° N, 75.707793° E. Five soil sub-sites were established within the coordinate range of 50.774086° N to 50.774312° N and 75.707512° E to 75.707842° E. Hydrochemically, the lake is classified as a freshwater body of the sodium–bicarbonate type, with dominance of HCO_3^- and Na^+ ions. This ionic composition generates osmotic and ion-specific stress, potentially shaping microbial metabolism and the structural organization of phototrophic communities (Boros et al., 2017).

Water and soil samples were collected in triplicate following standardized protocols to ensure sample preservation and analytical reliability. Sampling was performed at designated monitoring sites (Figure 1) in accordance with national standards (GOST 17.1.5.05-85 for surface waters and GOST 17.4.4.02-2017 for soils).

At each monitoring site, water samples were collected from both surface and bottom layers to determine dominant species of microalgae and cyanobacteria. All water and soil samples were placed into sterile 60 mL plastic containers and stored under refrigeration until transfer to the laboratory. Water samples were filtered through membrane filters with a pore size of 0.45 µm and transported at 4°C prior to laboratory processing. Soil samples were collected using sterile forceps and spatulas and placed in sterile containers.

Water temperature was measured in situ using a thermometer, and pH values were determined with a digital pH meter (HM Digital PH-80, USA) (APHA, 2017).

Species Identification and Morphological Analysis of Microalgal and Cyanobacterial Cultures

Following sampling and preliminary processing, water and soil samples were transferred into appropriate culture media to stimulate the growth and enrichment of cyanobacteria and microalgae. Samples collected from different regions of Kazakhstan were inoculated into liquid media including Zarrouk, BG-11, Gromov, Artari, Tamiya, and Bold's Basal Medium (BBM). Each sample was cultured in 250–500 mL flasks supplemented with 10–20% of the original water or soil suspension to preserve the natural microelement composition and physicochemical characteristics. Vitamin B_{12} was added to each medium at a concentration of 1×10^{-5} g/L to enhance growth and metabolic activity (Alanis, 2013).

Axenic cultures were obtained using agar-based solid media combined with repeated serial dilution and antibiotic treatment (ampicillin and streptomycin, 10–50 mg/L) to eliminate contaminating bacteria and non-target algae (Badr & Fouad, 2023).

Cultures were incubated at 25 ± 1 °C under a 16:8 h (light:dark) photoperiod with a light intensity of 50–70 µmol photons m^{-2} s^{-1} for 14–21 days.

Species identification was performed using a light microscope (Microscopes TB-5W Optika, Italy) equipped with digital imaging. Each strain was labeled and photographed at 40× and 100× magnifications. Morphological assessments were conducted at different growth stages in both liquid and solid media, including evaluation of cell shape, size, division patterns, and the presence of mucilaginous sheaths (Badr & Fouad, 2023). Cell counts were determined using a Goryaev counting chamber and an automated cell counter (NanoEntec EVE-MC2), ensuring quantitative control for further analyses.

Statistical Analysis. All experiments were performed in 3–5 independent replicates. Data were analyzed using one-way analysis of variance (ANOVA). Differences were considered statistically significant at $p < 0.05$.

Results and discussion

Structure of Microalgal and Cyanobacterial Communities in Aquatic and Soil Extreme Ecosystems of Kazakhstan

Kazakhstan encompasses a number of unique natural territories characterized by extreme ecosystems with specific physicochemical conditions. Particular attention is drawn to the eastern, southeastern, and northeastern regions of the country, where thermal springs, mineral lakes, and diverse soil ecosystems are concentrated, including the Chundzha hot springs, Rakhmanovskie Klyuchi, the Sibin Lakes, Barlyk-Arasan, and Lake Alakol. Moreover, extreme environmental conditions are also characteristic of western and southwestern Kazakhstan, including Lake Solyanka and the therapeutic mud deposits near the settlement of Erkenqush.

The presence of such factors as elevated water mineralization, temperature fluctuations, variations in pH, and increased concentrations of dissolved salts and microelements creates unique conditions for the detection of rare, endemic, and potentially valuable strains of phototrophic microorganisms. Soil ecosystems in these regions are characterized by high contents of macro- and microelements, including nitrogen (N), phosphorus (P, as PO_4^{3-}), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), as well as iron (Fe^{3+}/Fe^{2+}), manganese (Mn^{2+}/Mn^{4+}), zinc (Zn^{2+}), and copper (Cu^{2+}/Cu^+). A distinctive feature of these soils is their elevated mineralization and salinity, the presence of sulfates (SO_4^{2-}) and carbonates (CO_3^{2-}), alkaline conditions (pH 7.5–9.5), and the occurrence of trace elements such as lithium (Li^+), strontium (Sr^{2+}), and boron (B, as BO_3^{3-}) (Issanova et al., 2017).

The presence of algal flora was detected in various soil samples based on morphological characteristics, relative frequency, growth rate, and colony formation patterns. A total of 171 species and subspecies of algae and cyanobacteria were identified in soil samples, belonging to the divisions Ochrophyta (59 species), Chlorophyta (51 species), Xanthophyta (8 species), Eustigmatophyta (1 species), and Cyanobacteria (52 species).

Among the classes, the highest diversity was observed within Bacillariophyceae, Chlorophyceae, and Cyanophyceae, which were considered polymorphic and dominant (Table 1).

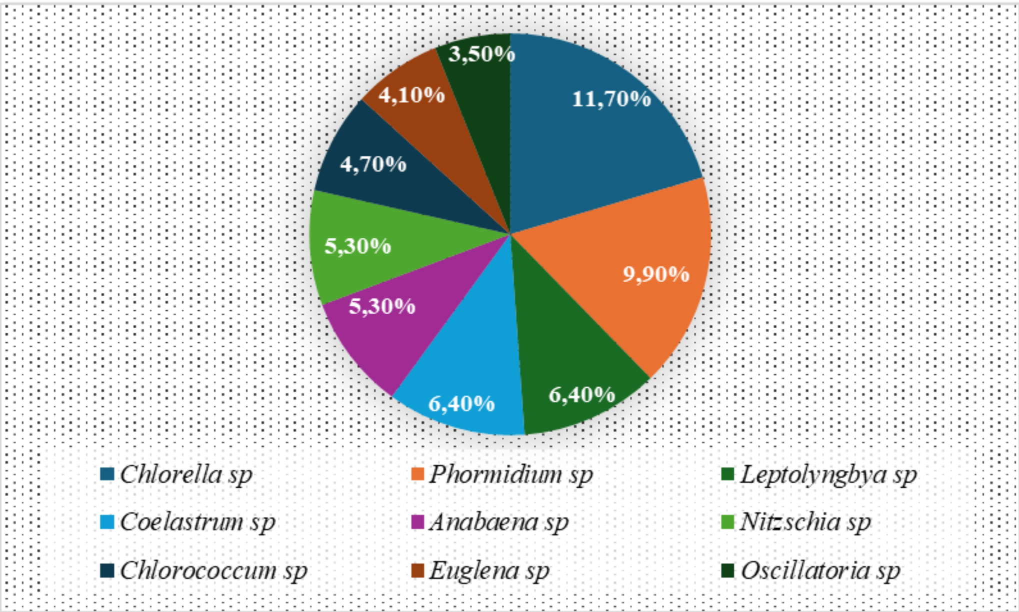
Table 1
Genera of microalgae and cyanobacteria identified in water and soil samples collected from the studied regions of Kazakhstan

No	Sample Type	Location / Sampling Site	Coordinates	Sampling Month	Genera Identified
1	Water	Chundzha hot springs (Uygur District , Almaty Region)	43.53.867° N 79.15.783° E	August	*Ankistrodesmus, Chlorella, Coelastrum, Klebsormidium, Scenedesmus*
	Soil		43.5308° N / 79.2415° E – 43.5304° N / 79.2423° E.		*Chlorella, Coelastrum, Dictyochloropsis, Kirchneriella, Phormidium, ScenedesmusLyngbya, Phormidium*
2	Water	Rakhman Springs (Katon -Karagay district, East Kazakhstan Region)	49°37.170 ° N 086°13.770°E	August	*Chlorella, Oscillatoria*
	Soil		49.619782°N / 49.620318°N – 86.229814°E / 86.229547°E		*Chlorella, Oscillatoria*
3	Water	Sibin Lakes (Katon -Karagay district, East Kazakhstan Region)	49°26.603° N, 082°38.015°E	August	*Chlorella, Oscillatoria*
	Soil		49.443665° N/ 49.444152° N – 82.633902° E/ 82.633649° E.		*Chlorella, Oscillatoria*
4	Water	Barlyk-Arasan, (Makanchi district, Abai Region)	46°03.100°N 082°02.246°E	August	*Chlorella, Leptolyngbya,*
	Soil		46.051948°N/ 46.052463° N – 82.037721° E/ 82.037512° E		*Anabaena, Hydrodictyon, Coelastrum, Oocystis, Scenedesmus, Cosmarium, Scenedesmus, Chlorococcum, Monoraphidium, Scenedesmus, Uronema, Chlorella, Scenedesmus*
5	Water	Alakol Lake (Akshi village, Alakol district, Jetisy Region)	45°54.696°N 081°38.065°E	August	*Phormidium, Ulothrix*
	Soil		45.911880° N/ 45.912250° N – 81.634720° E/ 81.634620° E		*Anabaena, Hydrodictyon, Coelastrum, Oocystis, Scenedesmus, Cosmarium, Chlorococcum, Monoraphidium, Scenedesmus, Chlorella*
6	Water	Lake Solyanka, Alga Distric,t Aktobe Region	50.137210° N 56.338930° E	August	*Anabaena, Chlorella,Cosmarium*
	Soil		50.137482° N/ 50.137701°N – 56.339214° E/ 56.338998° E		*Oocystis, Chlorella*
7	Water	Erkenqush village (therapeutic mud deposits) Aktobe Region	49.711507°N 57.264015°E	August	*Chlorella, Coelastrum, Scenedesmus, Lyngbya, Phormidium*
	Soil		49.711782° N/ 49.712154°N – 57.264302°E/ 57.264081° E		*Dictyochloropsis, Kirchneriella, Phormidium,*
8	Water	Hot radon springs (Aktau city, Munaily District, Mangystau Region)	43.350602° N 51.337226° E.	August	*Chlorella, Coelastrum, Scenedesmus*
	Soil		43.350812° N / 43.350623° – 51.337215° E/ 51.337956° E.		*Chlorella, Coelastrum, Phormidium*
9	Water	Lake Sabyndykol, (Bayanaul District, Pavlodar Region)	50.773821°N 75.707793° E	August	*Ankistrodesmus, Chlorella, Coelastrum,*
	Soil		50.774086° N/ 50.774312° N – 75.707512° E/ 75.707842° E		*Chlorella, Coelastrum, Dictyochloropsis, Lyngbya,*

The dominant genera comprised 108 species, accounting for approximately 63% of the total species diversity. Their distribution by genus was as follows: *Chlorella* – 20 species (≈11.7%), *Euglena* – 17 species (≈9.9%), *Phormidium* – 11 species (≈6.4%), *Coelastrum* sp. – 11 species (≈6.4%), *Synechocystis* sp. – 10 species (≈5.8%), *Leptolyngbya* sp. – 9 species (≈5.3%), *Nitzschia* sp. – 9 species (≈5.3%), *Chlorococcum* sp. – 8 species (≈4.7%), *Anabaena* sp. – 7 species (≈4.1%), and *Oscillatoria* sp. – 6 species (≈3.5%) (Figure 2).

Figure 2

Genera of phototrophic microorganisms identified in soil samples from extreme ecosystems

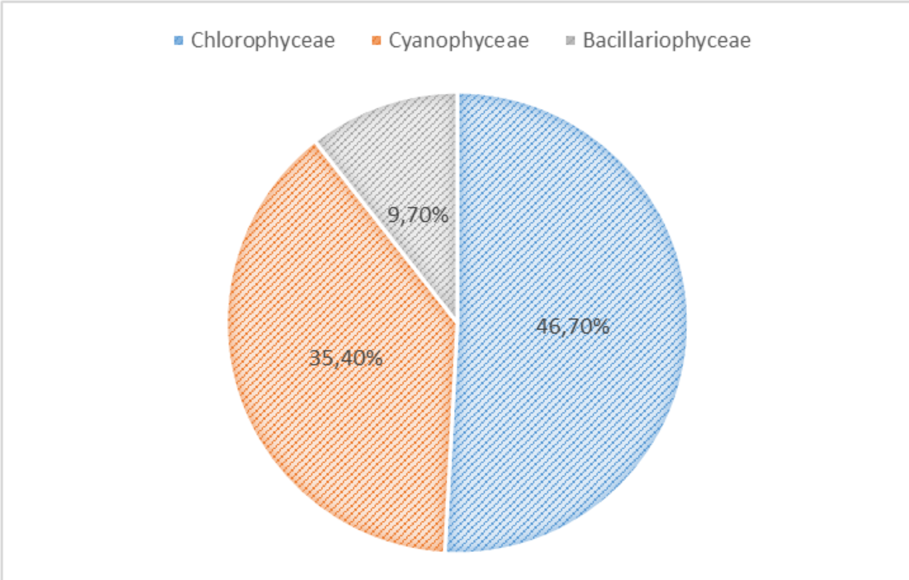


In water samples collected from the Chundzha hot springs (Almaty Region), Rakhmanovskie Klyuchi and the Sibin Lakes (Katon-Karagay District, East Kazakhstan Region), Barlyk-Arasan, Lake Alakol (Abai Region, Makanchi District; Zhetysu Region, Alakol District), Lake Solyanka and the therapeutic mud deposits near the settlement of Erkenqush (Aktobe Region, Alga District), as well as the hot radon springs in the Munaily District (Mangystau Region), a diverse phototrophic microflora was identified, including both microalgae and cyanobacteria. In total, 14 species of phototrophic microorganisms were identified, distributed among three major taxonomic groups: *Chlorophyceae* – 6 species (≈43%), *Cyanophyceae* – 5 species (≈36%), and *Bacillariophyceae* – 2 species (≈14%) (Figure 3).

Unicellular taxa were predominantly represented by the genera *Chlorella, Chlorococcum*, *Cosmarium, Monoraphidium*, and *Euglena,* whereas colonial morphotypes included Scenedesmus, *Coelastrum*, and *Ankistrodesmus*. Filamentous and branched taxa comprised *Cladophora*, *Hydrodictyon*, *Klebsormidium*, *Anabaena*, *Nostoc*, *Leptolyngbya*, *Lyngbya*, *Oscillatoria*, *Phormidium*, and *Ulothrix*.

Thus, the extreme soil and aquatic ecosystems of the studied regions of Kazakhstan are characterized by high taxonomic and morphological diversity of phototrophic microorganisms. The predominance of representatives of the genera *Chlorella*, *Phormidium*, *Synechocystis*, *Leptolyngbya*, and other stress-tolerant forms indicates pronounced adaptation of microalgae and cyanobacteria to conditions of elevated mineralization, temperature fluctuations, and ionic imbalance, underscoring their ecological significance and biotechnological potential.

Figure 3
Distribution and relative abundance of algal taxonomic groups in water samples from extreme environments



Isolation of axenic cultures of microalgae

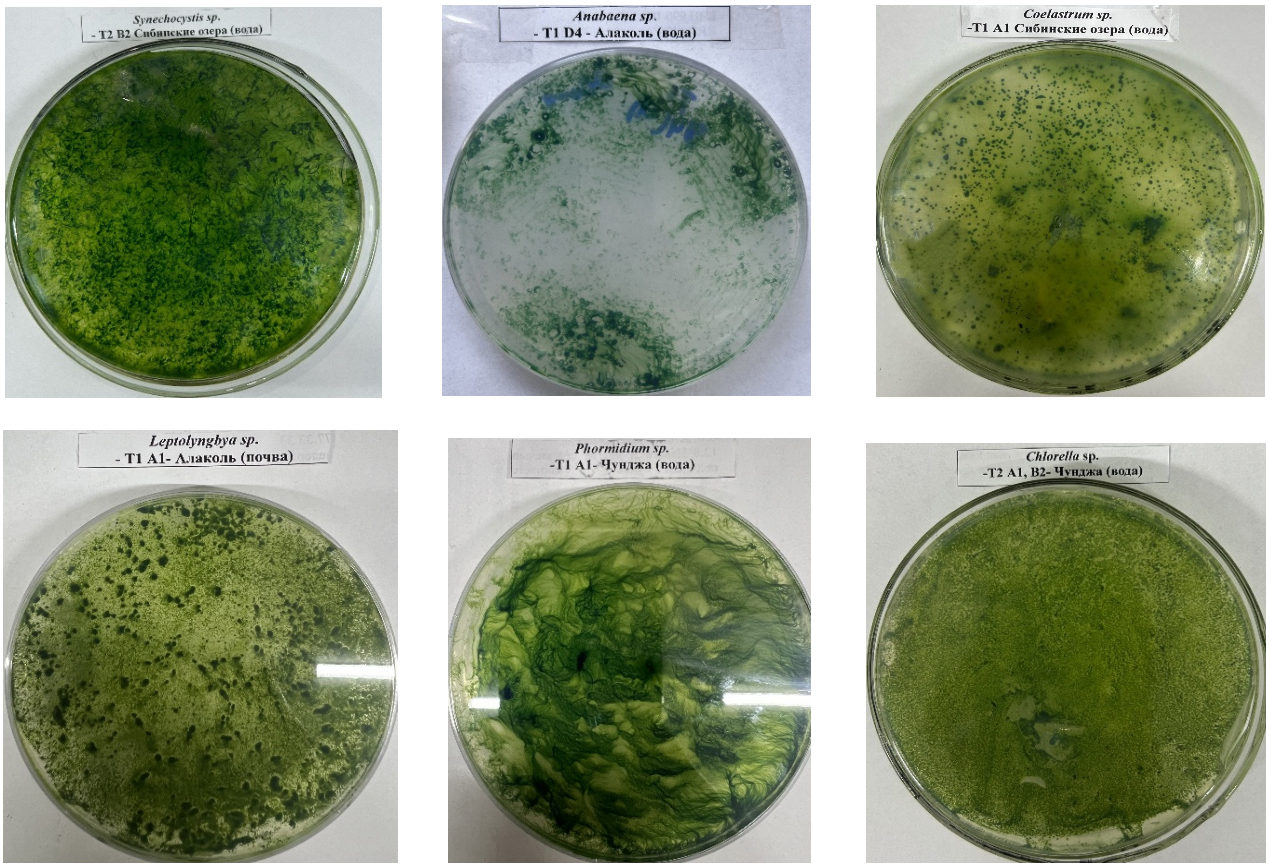
Axenic cultures were obtained from enriched water and soil samples collected at monitoring sites No. 1–9 using standard microbiological isolation techniques. Initially, samples were subjected to enrichment cultivation under controlled temperature and continuous illumination to promote active growth of microalgae and cyanobacteria.

Subsequently, the cultures were transferred onto both liquid and solid media (supplemented with 1.0–1.5% agar), including Zarrouk, BG-11, Gromov, Artari, Tamiya, and Bold's Basal Medium (BBM). Cultivation was performed in Petri dishes and culture tubes under illumination (Figure 4). Petri dishes were incubated at 22–25 °C under continuous light (40–60 µmol photons m^{-2} s^{-1}) until visually distinguishable colonies developed (5–14 days).

Individual pure strains were obtained using the streak plate method. A small aliquot of culture was transferred onto the surface of agar medium with a sterile inoculation loop and sequentially spread across the plate. With each successive streak, the number of cells decreased, allowing the formation of isolated colonies derived from single cells. After colony emergence, isolates were subcultured several times onto fresh media until fully pure cultures were obtained.

Culture purity was verified microscopically and by additional plating on bacteriological media to exclude contamination. As a result, axenic cultures of phototrophic microorganisms were established, predominantly represented by the genera *Chlorella* sp., *Anabaena* sp., *Synechocystis* sp., *Coelastrum* sp., *Leptolyngbya* sp., and *Phormidium* sp., which are characteristic of thermal waters and soils of the studied regions of Kazakhstan.

Figure 4
Growth of microalgal and cyanobacterial colonies on agar-solidified medium in Petri dishes after 10 days of incubation



Morphological Assessment of Isolated Cyanobacterial and Microalgal Strains

From water and soil samples collected at the Chundzha hot springs (Almaty Region), Rakhmanovskie Klyuchi and the Sibin Lakes (Katon-Karagay District, East Kazakhstan Region), Barlyk-Arasan, Lake Alakol (Abai Region, Makanchi District; Zhetysu Region, Alakol District), Lake Solyanka and the therapeutic mud deposits near the settlement of Erkenqush (Aktobe Region, Alga District), as well as the hot radon springs in the Munaily District (Mangystau Region), axenic cultures of microalgae and cyanobacteria were isolated, including *Chlorella* sp., *Anabaena* sp., *Synechocystis* sp., *Coelastrum* sp., *Leptolyngbya* sp., and *Phormidium* sp. (Figure 5).

Phormidium sp. (Cyanophyceae) – filamentous cyanobacteria characterized by cylindrical trichomes 3–6 µm in diameter and extending up to several millimeters in length. Vegetative cells are straight or slightly curved and enclosed within a mucilaginous sheath. Reproduction occurs via hormogonia formation, and the organisms typically develop thin mats on soil and water surfaces. Recent studies indicate the ability of certain strains to produce bioactive compounds (Berrendero et al., 2016).

Synechocystis sp. (Cyanophyceae) – unicellular, spherical cyanobacteria measuring 1.5–3 µm in diameter, surrounded by a dense mucilaginous capsule. Cells exhibit gliding motility and may occur as solitary cells or form small aggregates. Strains isolated from hot spring environments demonstrate high photosynthetic efficiency and enhanced UV tolerance, highlighting their potential for bioengineering and ecological applications (Tripathi et al., 2025).

Leptolyngbya **sp. (Cyanophyceae)** – thin filamentous cyanobacteria with trichomes 1–3 µm in diameter and up to 5–10 mm in length. Cells are cylindrical, lack heterocysts, and exhibit limited motility. These taxa are commonly found in moist soils enriched with organic matter. Recent studies have shown that strains from thermal and saline ecosystems possess enhanced capabilities for organic compound biodegradation and the synthesis of bioactive peptides (Takahashi et al., 2021).

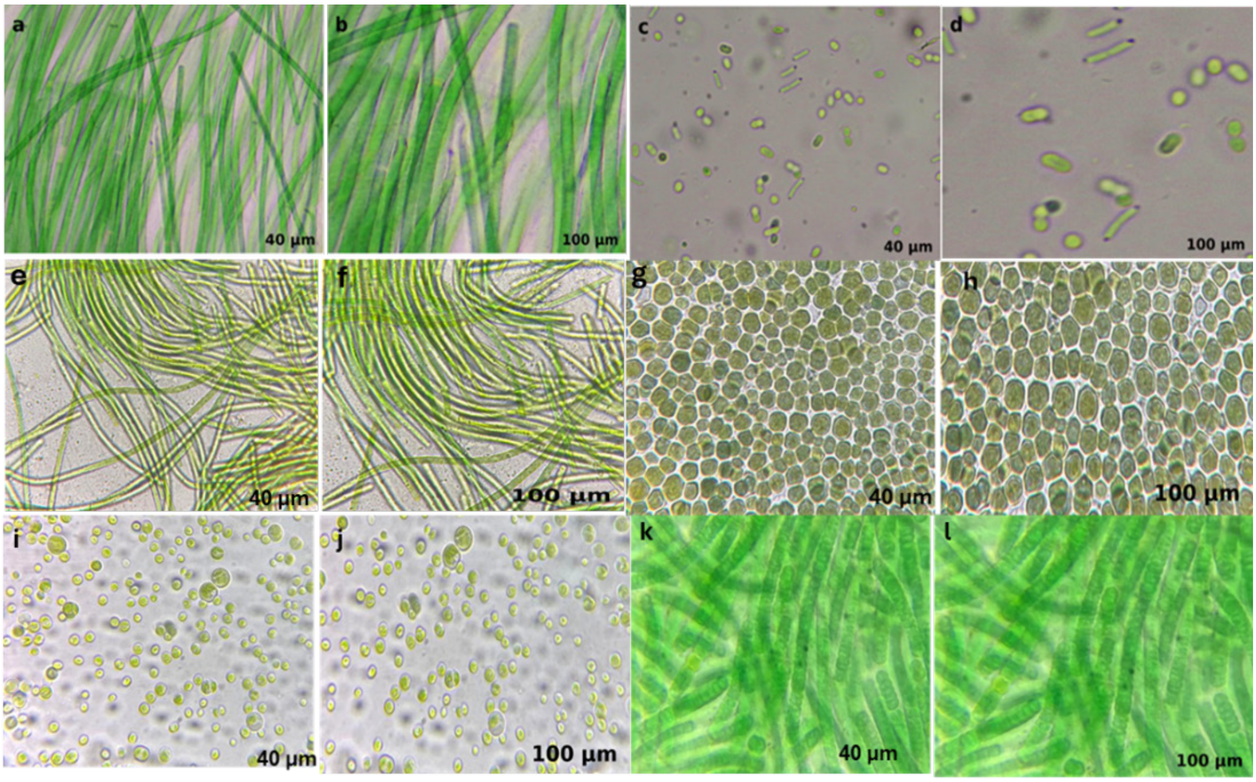
Coelastrum sp. (Chlorophyceae) – colonial green algae composed of cylindrical cells measuring 10–30 µm in length and 2–5 µm in width, arranged in parallel to form linear colonies. Reproduction occurs vegetatively. Recent studies have demonstrated tolerance to elevated temperatures and pH fluctuations, as well as the capacity for heavy metal bioremediation in aquatic and soil systems (Xu et al., 2025).

Chlorella sp. (Chlorophyceae) – unicellular green algae 2–10 µm in diameter, spherical to ellipsoidal in shape. Cells contain a single large chloroplast occupying most of the cell volume. Reproduction occurs by autosporulation (mitotic cell division). In liquid culture, cells are evenly distributed, do not form colonies, and exhibit minimal motility. Recent studies indicate that *Chlorella* strains isolated from thermal environments display enhanced tolerance to temperature and salinity stress, along with high biomass productivity and lipid accumulation suitable for biofuel production (Chou et al., 2019).

Anabaena sp. (Cyanophyceae) – filamentous cyanobacteria characterized by alternating vegetative cells and heterocysts. Trichomes are straight or slightly curved, with cells 3–6 µm in diameter. Heterocysts are larger, colorless, and typically occur at intervals of 5–10 vegetative cells. These taxa are capable of atmospheric nitrogen fixation and reproduce via hormogonia formation. Recent research has demonstrated that *Anabaena* strains from thermal and brackish environments are capable of synthesizing exopolysaccharides and antioxidant compounds, highlighting their ecological significance and biotechnological relevance (Bhakat et el., 2024).

Figure 5

Light microscopy images of newly isolated microalgal strains at 40× and 100× magnification: (a, b) Phormidium sp, (c, d) Synechocystis sp., (e, f) Leptolyngbya sp., (g, h) Coelastrum sp, (i, j) Chlorella sp, (k, l) Anabaena sp.



Overall, the isolated strains display considerable morphological and ecological plasticity, reflecting their adaptation to thermal and saline environments and underscoring their biotechnological relevance for environmental monitoring, biomass generation, lipid accumulation, and the synthesis of bioactive metabolites.

Conclusion

This study presents comprehensive data on the biodiversity and taxonomic characteristics of phototrophic microorganisms isolated from diverse extreme ecosystems of Kazakhstan, including thermal springs, radon waters, saline and mineral lakes, therapeutic mud deposits, and highly mineralized soils.The extensive geographic coverage of the research, spanning eastern, southeastern, northeastern, and western regions of Kazakhstan — namely the Chundzha hot springs (Almaty Region), Rakhmanovskie Klyuchi and the Sibin Lakes (Katon-Karagay District, East Kazakhstan Region), Barlyk-Arasan, Lake Alakol (Abai Region, Makanchi District; Zhetysu Region, Alakol District), Lake Solyanka and the therapeutic mud deposits near the settlement of Erkenqush (Aktobe Region, Alga District), as well as the hot radon springs in the Munaily District (Mangystau Region) significantly enhances the representativeness, reliability, and ecological breadth of the obtained data, thereby reinforcing the overall scientific value and integrative significance of the study.

The conducted study demonstrated that extreme aquatic and soil ecosystems of Kazakhstan are characterized by high taxonomic diversity of microalgae and cyanobacteria adapted to elevated mineralization, pH fluctuations, temperature variability, and specific ionic composition of the environment.

A total of 171 species and subspecies of phototrophic microorganisms were identified in soil samples, belonging to the divisions Ochrophyta (59 species), Chlorophyta (51 species), Xanthophyta (8 species), Eustigmatophyta (1 species), and Cyanobacteria (52 species). In aquatic ecosystems, 14 species of phototrophic microorganisms were identified, predominantly representing the polymorphic and dominant classes *Chlorophyceae* (46.7%), *Cyanophyceae* (35.4%), and *Bacillariophyceae* (9.7%). The dominant genera included *Chlorella, Euglena, Phormidium, Coelastrum, Synechocystis,* among others. The isolation and identification of six axenic cultures confirm the tolerance of representatives of the genera *Chlorella, Anabaena, Synechocystis, Coelastrum, Leptolyngbya*, and *Phormidium* to diverse extreme environmental conditions.

The isolated cultures exhibit pronounced morphological diversity and broad ecological plasticity, reflecting their adaptability to thermal, radon-influenced, saline, and highly mineralized environments, which indicates the substantial adaptive potential of phototrophic microorganisms inhabiting extreme ecosystems.

Therefore, extreme aquatic and soil ecosystems of Kazakhstan should be regarded not only as natural models for investigating adaptive mechanisms under environmental stress, but also as promising reservoirs of robust phototrophic strains with lipid-accumulating capacity and potential applicability in industrial renewable biofuel production.

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Conflict of interest

All authors have read and are familiar with the content of the article and have no conflict of interest.

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A.A. Dauletova – Investigation, Methodology, Resources, Writing – original draft.

S.A. Ismailova – Investigation, Methodology, Validation, Writing – original draft.

A. Askar – Investigation, Visualization, Validation, Writing – original draft.

Silvano Piazza – Consultation, Methodology, Writing – review & editing.

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