DOI: 10.22092/BOT.J.IRAN.2023.360711.1336

Diversity and distribution of heterocystous cyanobacteria across solar radiation gradient in terrestrial habitats of Iran

Received: 27.11.2022 / Accepted: 31.12.2022

Pardis Irankhahi: PhD Student, Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C., Tehran, Iran

Hossein Riahi⌧**:** Prof., Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C., Tehran, Iran (h-riahi@sbu.ac.ir)

Zeinab Shariatmadari: Assistant Prof., Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C., Tehran, Iran

Zahra Aghashariatmadari: Assistant Prof., Irrigation and Reclamation Engineering Department, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Abstract

Cyanobacteria are important part of microflora in terrestrial ecosystems. Due to the presence of protective mechanisms in these microorganisms, they have potential to tolerate abnormal ecological conditions especially in arid and semi-arid habitats. In the present study, the diversity, distribution and community's structure of the heterocystous cyanobacteria isolated from natural habitats of Iran with different solar radiation gradient were investigated. In total, 41 heterocystous morphospecies were isolated from soils of 21 studied sites. The isolated taxa were belonged to eight genera including *Nostoc* (54.68%) followed by the *Calothrix* (13.63%), *Cylindrospermum* (9.76%), *Anabaena* (7.32%), *Trichormus* (7.32%), *Wollea* (2.43%), *Nodularia* (2.43%), and *Hapalosiphon* (2.43%), respectively. According to the results, ecological factors such as solar radiation, relative humidity, and soil salinity can affect the diversity and distribution of these cyanobacteria in terrestrial ecosystems. The results also showed that, some taxa were dominant in stations with high radiation levels. Among the identified taxa, *Nostoc* was found to be the dominant genus at all stations, especially in sites with higher solar radiation levels. In addition, the presence of the brown *Nostoc* species in arid areas confirming their resistance due to their high amount of carotenoids content and other protective mechanisms that protect them from high light intensity.

Keywords: Carotenoid, ecological factors, morphology, semi-arid habitats, solar radiation intensity

تنوع و پراکنش سیانوباکتری‌های دارای هتروسیست در طول شیب پرتوی خورشید در زیستگاه‌های خشکی ایران*

دریافت: ۱۴۰۱/۰۹/۰۶ / پذیرش: ۱۴۰۱/۱۰/۱۰

پردیس ایرانخواهی: دانشجوی دکتری دانشکده علوم و فناوری‌های زیستی، گروه علوم و زیست‌فناوری گیاهی، دانشگاه شهید بهشتی، تهران، ایران

حسین ریاحی⌧**:** استاد دانشکده علوم و فناوری‌های زیستی، گروه علوم و زیست‌فناوری گیاهی، دانشگاه شهید بهشتی، تهران، ایران (h-riahi@sbu.ac.ir)

زینب شریعتمداری: استادیار استاد دانشکده علوم و فناوری‌های زیستی، گروه علوم و زیست‌فناوری گیاهی، دانشگاه شهید بهشتی، تهران، ایران

زهرا آقاشریعتمداری: استادیار گروه مهندسی آبیاری و آبادانی، پردیس کشاورزی و منابع طبیعی، دانشگاه تهران،کرج، ایران

خلاصه

سیانوباکتری‌ها از اجزای اصلی و مهم میکروفلور اکوسیستم‌های خشکی به شمار می‌آیند. این میکروارگانیسم‌ها به دلیل داشتن مکانیسم‌های دفاعی مختلف توانایی مقاومت در مقابل شرایط سخت بوم‌شناختی نظیر زیستگاه‌های خشک و نیمه‌خشک را دارند. در مطالعه حاضر، تنوع زیستی، پراکنش و ساختار جمعیتی سیانوباکتری‌های دارای هتروسیست در زیستگاه‌های طبیعی ایران براساس شیب پرتوی خورشید مورد بررسی قرار گرفت. به طور کلی، ۴۱ ریخت گونه از ۲۱ ایستگاه مطالعاتی جمع‌آوری و جداسازی شد. این آرایه‌ها به ترتیب به هشت جنس، با سطوح تنوع زیستی متفاوت متعلق بودند که عبارتند از: *Nostoc* (54.68%)، *Calothrix* (13.63%)، *Cylindrospermum* (9.76%)، *Anabaena* (7.32%)، *Trichormus* (7.32%)، *Wollea* (2.43%)، *Nodularia* (2.43%) و *Hapalosiphon* (2.43%). بر این اساس، عوامل بوم‌شناختی مانند میزان تابش نور خورشید، رطوبت نسبی و شوری خاک روی تنوع و پراکنش سیانوباکتری‌های مورد مطالعه اثرگذار بود. همچنین نتایج نشان داد، برخی آرایه‌ها در مناطق با سطح پرتوی زیاد پراکنش وسیع‌تری دارند. در میان آرایه‌های مورد بررسی، جنس *Nostoc* در تمام ایستگاه‌های مطالعاتی، به ویژه در ایستگاه‌هایی با میزان تابش بالای نور خورشید به عنوان جنس غالب شناخته شد. همچنین در بررسی حاضر مشخص گردید که مقاومت پرتوی اعضای این جنس می‌تواند به دلیل حضور مقادیر زیاد کاروتنویید و سایر مکانیسم‌های حفاظتی باشد که از آن‌ها در برابر تابش شدید نور خورشید محافظت می‌کند.

واژه‌های کلیدی: ریخت‌شناسی، زیستگاه‌های نیمه‌خشک، شدت تابش خورشیدی، عوامل بوم‌شناختی، کاروتنویید

* مستخرج از رساله دکتری نگارنده نخست به راهنمایی دکتر حسین ریاحی و دکتر زینب شریعتمداری ارایه شده به دانشگاه شهید بهشتی

Introduction

Cyanobacteria are photosynthetic prokaryotes and colonize in various habitats including terrestrial and aquatic ecosystems. These microorganisms tolerate harsh habitats, and adapting to environmental changes such as high solar radiation, desiccation, salinities, and high or low temperature (Garcia-Pichel & Belnap 1996). Their existence in extreme conditions shows their flexibility in different habitats and their high adaptability. Generally, growth and distribution of cyanobacteria are strongly influenced by various environmental factors such as temperature, salinity, and solar radiation (Liopis *et al.* 2022). Suitable light intensity is an essential factor for their optimum growth where high solar radiation causes damages to their photosynthetic machinery, DNA and proteins structure, and photo-bleaching of their cells (Rastogi *et al.* 2010). Due to the ozone layer depletion in the atmosphere, the deleterious effects of solar ultraviolet radiation (UV) wavelengths increases on the earth surface (Pathak *et al.* 2017). Cyanobacteria have acquired strategies to respond to intense solar radiation, and with the help of these evolutionary mechanisms, they maintain their survival in ecosystems with intense radiation. Synthesis antioxidant compounds, mat formation, and accumulating UV-absorbing pigments such as carotenoids, mycosporine-like amino acids (MAAs), and scytonemin are some of their strategies against UV exposure (Hartman *et al.* 2015).

Climatically, a large area of Iran (approximately 70% of the 164 million hectares), is located in the arid and semi-arid regions with low annual rainfall between 50–250 mm (Soltani *et al.* 2011, Feizi *et al.* 2014, Etemadi-Khah *et al.* 2017). Iran is among the countries with high received solar radiation, which makes it possible to provide a significant part of its energy demands through this clean energy source (Agha-shariatmadari 2011). This country with 280 sunny days/year is located within the global solar belt, where solar radiation is at its high level in compare to other countries. It has been reported that, the annual solar radiation level in Iran is approximately between 1800–2200 kWh m^2 which is higher than the world average (Ahmad 2018).

Solar radiation is the basic source of energy for all biochemical and physical processes on the earth surface and its values have special significance in many environmental and agricultural studies. The high level of solar radiation and its changes in different regions of the country have great impact on the biodiversity of soil photosynthetic microorganisms including cyanobacteria. Relative humidity is an important weather parameter, which influences the amount of solar radiation and affects the received solar intensity in each area (Tasie *et al.* 2018).

The arid and semi-arid regions are characterized by high solar radiation and soil salinity, as well as water scarcity, where in such environmental condition; formation of plant communities is quite slow where microalgae communities are well-adapted (Llopis *et al.* 2022). In recent years, the diversity of soil cyanobacteria has been investigated in different regions of the world. Several studies have also been conducted on the algal flora of terrestrial ecosystems in Iran, but these reports are not sufficient, especially in stressful areas. Moghtaderi *et al.* (2009) investigated cyanobacteria in soil desert crust of Chadormalu in Bafgh region (Yazd province, Iran). Hokmollahi *et al.* (2016) described the cyanobacteria present in the soils of Yazd province (Iran) using morphological, molecular and physiological characteristics. The morphological and molecular studies on the algal flora of Kavir National Park is also reported in recent years (Etemadi-Khah *et al.* 2017).

Regarding importance of cyanobacteria and lack of information about distribution of their populations according to environmental gradients such as solar radiation gradient, the present investigation is aimed to study soil microflora, with emphasis on heterocystous cyanobacteria, in regions with different solar radiation levels. This study, therefore, provides a better understanding about the effects of solar radiation on algal flora and part of their adaptation strategies.

Materials and Methods

- Field study, sampling, and analyses

Soil samples were collected from 21 sites of Iran with different climatic characteristics (Table 2), based on the methodology given by Rangaswamy (1966). Soil sampling was done in the summer of three consecutive years between 2017–20. Daily weather data of selected synoptic stations were collected from the Iranian Meteorological Organization (IRIMO). The climate of stations varied from arid to semi-arid areas. At this point, the long-term statistics of the meteorological stations on a daily basis were first collected from the above-mentioned organization and a yearly-classified database was formed. This bank includes data on total solar radiation (R_S), sunshine hour fraction, air temperature, precipitation, and relative humidity. Data quality control was performed according to Moradi *et al.* (2009) quality control algorithm. Only the variables related to radiation (total radiation and number of hours of sunshine) of all meteorological data received showed statistical deficiencies. Therefore, as one of the aim of this study, the quality control of radiation statistics was performed with high accuracy. The distribution maps of solar radiation and climate types of Iran in the study area were drawn using the ArcGIS 8.1 software (Figs 1–2).

To determine the climatic conditions of different regions, the De Martone aridity-humidity index (De Martonne 1925) was implemented. This climate classification index is one of the oldest aridity indices that indicate the degree of climate dryness at a given location. The index is calculated by the following equation:

$$AI = \frac{P}{T + 10}$$

AI = aridity index, P = annual mean precipitation (mm), and T = annual mean air temperature (°C).

The classification of the De Martonne aridity climate is given in Table 1.

Table 1. De Martonne aridity classes

Climate type	AI value
Arid	$AI \langle 10$
Semi-arid	$10 \leq AI \langle 20$
Mediterranean	$20 \leq AI \langle 24$
Semi-humid	$24 \leq AI \langle 28$
Humid	$28 \leq AI \langle 35$
Very humid	$35 \leq AI \leq 55$
Extremely humid	$AI \rangle 55$

According to De Martonne climate classification of Iran (Khalili & Bazrafshan 2022), among our selected localities (i.e., Gilan and Mazandaran provinces) which are located on the southern shore of the Caspian Sea with a perhumid climate; Qom, Isfahan, Yazd, and Kerman provinces are located in the central and southern parts of Iran with arid and semi-arid climate conditions, while other stations that located in central regions, are areas with temperate conditions (Fig. 1). In addition, soil-sampling sites were located along gradient solar radiation with different levels of radiation (Fig. 2). Therefore, northern region of the country located in an area with low solar radiation, and the central region located in an area with medium to high solar radiation (The highest solar radiation belonged to the southern regions).

In terms of soil salinity level, based on remote sensing data from Google Earth Engine (GEE), sampling sites were categorized into three levels of low, medium, and high salinity (Fig. 3). Among studied sites, 14 sites were located in regions with medium and high, and seven sites were in low soil salinity regions.

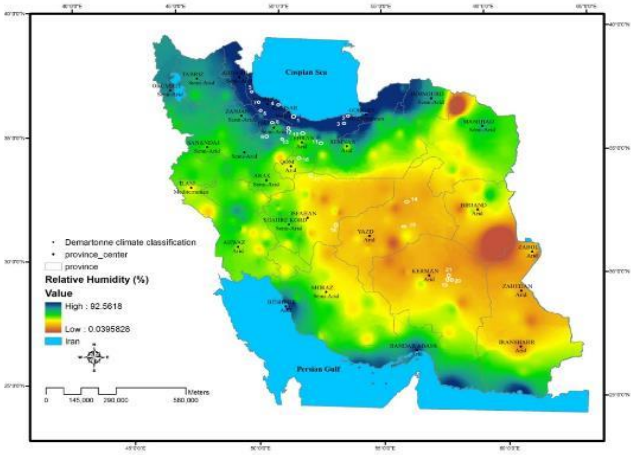

Fig. 1. Geographical distribution and climate classification based on De Martonne (studied sites are shown in the map).

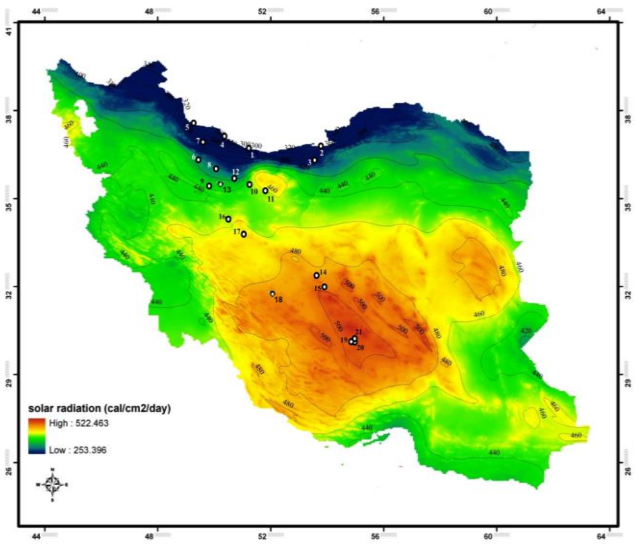

Fig. 2. Solar radiation gradient based on average annual radiation (studied sites are shown in the map).

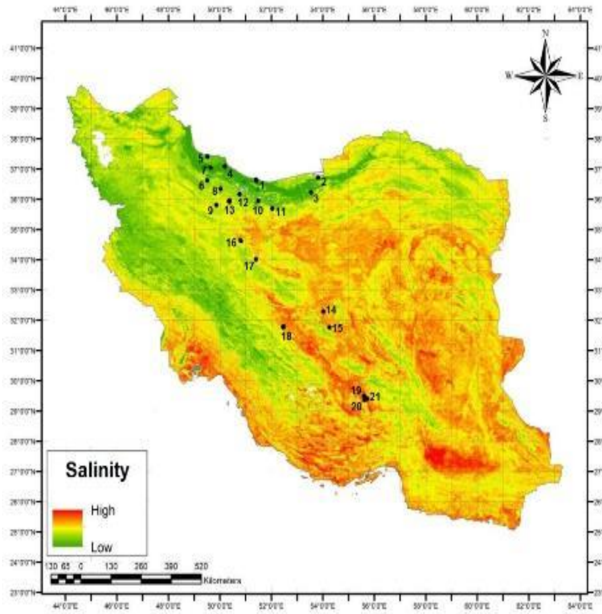

Fig. 3. Soil salinity gradient in different regions based on 2016 GEE data (studied sites are shown in the map).

- Isolation and purification of cyanobacteria

The collected soil samples were first sieved and 10 g of each sample transferred into sterile petri dishes containing sterile liquid nitrate-free BG-11 medium and then incubated at 25±2 °C with artificial illumination at 74 µmol photons m^{-2} s^{-1} with a 12/12 h light/dark cycle for three weeks. For purification, after the appearance of cyanobacterial colonies, each colony were transferred into plates containing solid nitrate-free BG-11 and incubated for ten days at 25±2 °C with artificial illumination at 74 µmol photons m^{-2} s^{-1} with a 12/12 h light/dark cycle (Andersen 2005). For pigments extraction, all purified samples were cultivated in 500 mL Erlenmeyer flasks containing 200 mL sterile liquid nitrate-free BG-11 medium.

- Morphological identification and diversity of morphospecies

The identification of cyanobacterial taxa based on morphology is the basic method in this group of photosynthetic prokaryotes. The higher orders such as *Nostocales* can show some outstanding features such as presence of specialized cells, heterocytes or akinetes, as these structures along with their diversity help morphometric identification (Komárek *et al*. 2014). Therefore, in many species of heterocystous cyanobacteria, morphometric identification is a suitable method, although in complex taxa, the polyphasic method facilitates identification process (Komárek 2016).

In order to perform the taxonomic determinations, in the present investigation, the semi-permanent slides of purified colonies were prepared and identification of taxa was performed up to species level (wherever possible) by light microscopy (Olympus, Japan), based on the available references (Wehr *et al*. 2002, Komárek 2013, Komárek & Hauer 2013). Several morphological characteristics were used to identify heterocystous cyanobacteria including form and color of colony, thallus form, shape, and size as well as position of heterocytes and akinetes, presence or absence of gelatinous sheath, shape of apical and terminal cells, shape, as well as size of vegetative cells (Rajaniemi *et al*. 2005, Martineau *et al*. 2013). The cyanobacterial morphospecies were documented by photomicrography.

The diversity percentage of each genus in studied sites was calculated by the following formula (Tong *et al*. 2021):

$$D_G = \frac{N_{mg} \times 100}{N_{tm}}$$

D_G = diversity of each genus, *Nmg* = number of identified morphospecies in each genus, and *Ntm* = total number of studied morphospecies.

- Measurement of photosynthetic pigments

Chlorophyll-a and total carotenoids contents were determined according to Han *et al.* (2016) with some modifications. For this purpose, 5 mL aliquot of wet biomass was centrifuged at 4000×g for 8 minutes. The supernatants were discarded and 5 mL cold methanol (4 °C) was added. The samples were stored in dark at 4 °C for 45 minutes. After incubation, samples were centrifuged at 10,000 g for 10 minutes. The supernatant was transferred into a cuvette and measured at 470 and 665 nm in a UV-visible spectrophotometer (Hitachi 2900, Japan), using methanol as a blank. Contents were calculated using following equations:

$$\text{Chlorophyll–a}(\text{mg L}^{-1}) = 13.43 \times \text{A}_{665\text{nm}} \times \text{Volume}_{\text{methanol}} / \text{Volume}_{\text{cell}}$$

$$\text{Carotenoids}(\text{mg L}^{-1}) = (1000 \times \text{A}_{470\text{nm}} - 44.76 \times \text{A}_{665\text{nm}}) / 221 \times \text{Volume}_{\text{methanol}} / \text{Volume}_{\text{cell}}$$

- Statistical analysis

The data were analyzed by one-way analysis of variance (ANOVA) using SPSS 20.0 (SPSS Inc., Chicago, IL, USA). For determination of data significance at $p < 0.05$, the Duncan test was used. The statistical results were expressed as the mean ± standard deviation and finally histograms were drawn using GraphPad Prism 8.4.2 (GraphPad Software Inc., San Diego, CA, USA).

Table 2. Geographical data of the sampling locations

Code	Province	Site	Latitude/Longitude	Altitude (m)	pH
1	Mazandaran	Chalous	36°38′51″ N 51°24′06″ E	73	7.28
2	"	Galougah	36°43′11″ N 53°48′57″ E	37	7.33
3	"	Kiasar	36°14′21″ N 53°32′37″ E	1264	7.26
4	Gilan	Amlash	37°05′24″ N 50°11′58″ E	22	7.33
5	"	Chukam	37°25′12″ N 49°31′12″ E	-9	7.31
6	"	Loushan	36°37′46″ N 49°30′24″ E	354	7.41
7	"	Saravan	37°03′05″ N 49°38′48″ E	84	7.35
8	Qazvin	Esmaeilabad	36°20′55″ N 50°01′47″ E	1479	7.64
9	"	Ebrahimabad	35°48′55″ N 49°52′05″ E	1240	7.61
10	Tehran	Fasham	35°57′19″ N 51°29′50″ E	2150	7.40
11	"	Firouzkooh	35°41′50″ N 52°02′14″ E	1983	7.37
12	Alborz	Taleghan	36°10′31″ N 50°46′08″ E	1855	7.23
13	"	Dasht-e-Qazvin	36°38′51″ N 51°24′06″ E	1411	7.38
14	Yazd	Ardakan	32°17′30″ N 54°01′15″ E	1054	7.73
15	"	Taft	31°46′21″ N 54°15′29″ E	1444	7.65
16	Qom	Qom	34°37′26″ N 50°49′25″ E	957	7.55
17	Kashan	Kashan	34°01′17″ N 51°24′01″ E	947	7.66
18	Isfahan	homeini-shahr	36°38′51″ N 51°24′06″ E	1693	7.44
19	Kerman	Nosratabad	29°30′33″ N 55°36′01″ E	1727	7.45
20	"	Najafshahr	29°24′25″ N 55°43′40″ E	1736	7.62
21	"	Baesfahrjan	29°26′47″ N 55°38′24″ E	1718	7.92

Results

The studied sites showed the variations and differences of ecological parameters. The pH values were slightly neutral to alkaline ranging between 7.26–7.92. Solar radiation showed an increasing in its gradient with respect to the altitude. The highest values were observed in southern regions of Iran, with 500 cal/cm^2/day in sites 19, 20, and 21, while northern regions of the country showed the minimal value (300–360 cal/cm^2/day). Indeed, the solar radiation value in site 5 (Chukam), was lower in compared with other sites. In studied provinces, the relative humidity ranged from 5–67%, which showed a significant difference between different sites. The relative humidity of the northern regions of the country was significantly higher than other regions. In addition, the highest relative humidity (67%) was observed in site 5 (Chukam).

Soil salinity is another factor that effects on distribution of cyanobacteria. Some studied stations including sites 19, 20, and 21 were located in the area with the highest soil salinity in compare with other sites (Figs 1–3).

In the present investigation, 41 heterocystous taxa were identified and recorded from the studied sites (Table 3, Fig. 4), and also several species of the genera, viz. *Nostoc*, *Anabaena*, *Cylindrospermum*, *Calothrix*, *Trichormus*, *Wollea*, *Nodularia*, and *Hapalosiphon* were determined. More than half of the isolates belonged to *Nostoc* (54.68%) followed by *Calothrix* (13.63%), *Cylindrospermum* (9.76%), *Anabaena* (7.32%), *Trichormus* (7.32%), *Wollea* (2.43%), *Nodularia* (2.43%), and *Hapalosiphon* (2.43%), respectively (Fig. 6).

Table 3. The list of identified taxa with their characters and distributions in the studied sites

No.	Taxon	Filament and colony color	Family	Site
1	*Nostoc linckia*	Blue-green, brown	*Nostocaceae*	2,7, 11, 21
2	*N. punctiforme*	Blue-green, olive-green	"	10, 13
3	*N. passerinianum*	Blue-green, brown	"	3, 10
4	*N. commune*	Olive-green	"	13
5	*N. paludosum*	Blue-green	"	1, 6, 9, 13, 17
6	*N. calcicola*	Blue-green	"	13
7	*N. sphaericum*	Brown	"	19
8	*N. ellipsosporum*	Olive green, brown	"	8, 18
9	*N. verrucosume*	Brown	"	17
10	*N. carneum*	Blue-green	"	2, 5
11	*N. microscopicum*	Olive-green, blue-green	"	3, 13,16
12	*N. flagelliforme*	Blue-green	"	3
13	*Nostoc* sp.$_1$	Blue-green	"	8
14	*Nostoc* sp.$_2$	Brown	"	15
15	*Nostoc* sp.$_3$	Blue-green	"	5
16	*Nostoc* sp.$_4$	Brown	"	20
17	*Nostoc* sp.$_5$	Blue-green	"	12
18	*Nostoc* sp.$_6$	Brown	"	13
19	*Nostoc* sp.$_7$	Blue-green	"	16
20	*Nostoc* sp.$_8$	Blue-green	"	4
21	*Nostoc* sp.$_9$	Brown	"	18
22	*Nostoc* sp.$_{10}$	Blue-green	"	6
23	*Anabaena torulosa*	Blue-green	"	12
24	*A. iyengarii*	Blue-green	"	9
25	*Anabaena* sp.	Blue-green	"	11
26	*Wollea vaginicola*	Blue-green	"	8
27	*Cylindrospermum majus*	Blue-green	"	8
28	*Cy. alatosporum*	Olive-green	"	3
29	*Cy. longisporum*	Blue-green	"	12
30	*Cylindrospermum* sp.	Brown	"	9
31	*Calothrix capitularis*	Blue-green	*Rivulariacea*	8
32	*C. marchica*	Olive-green	"	5
33	*C. parietina*	Brown	"	1
34	*Calothrix* sp.$_1$	Blue-green	"	4
35	*Calothrix* sp.$_2$	Olive-green	"	11
36	*Calothrix* sp.$_3$	Blue-green	"	9
37	*Trichormus variabilis*	Blue-green	*Nostocaceae*	11, 13
38	*Trichormus* sp.$_1$	Blue-green	"	8
39	*Trichormus* sp.$_2$	Blue-green	"	2
40	*Nodularia harveyana*	Blue-green	"	14
41	*Hapalosiphon welwitschii*	Olive-green	*Stigonemataceae*	3

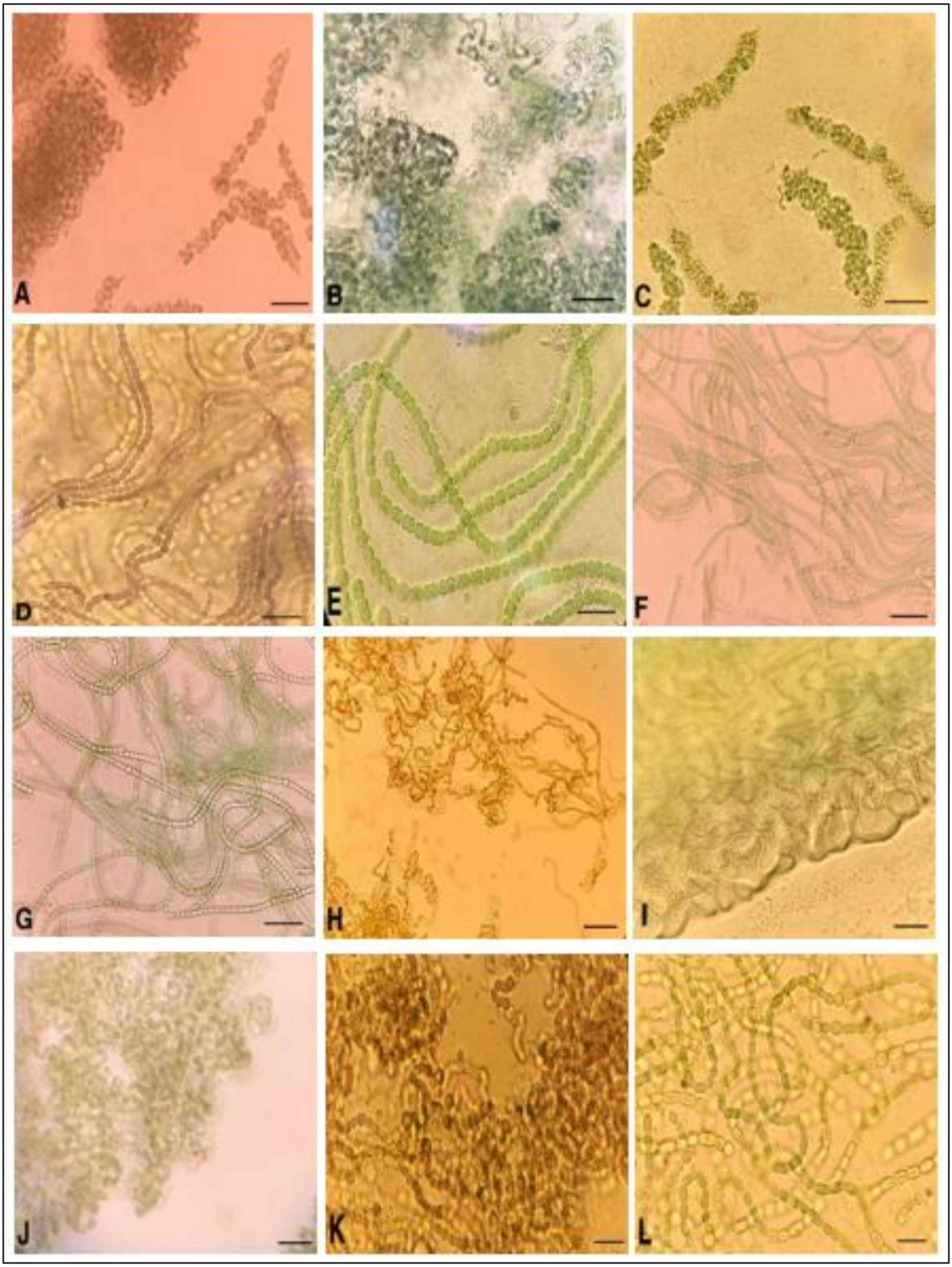

Fig. 4. Cont. in the next page.

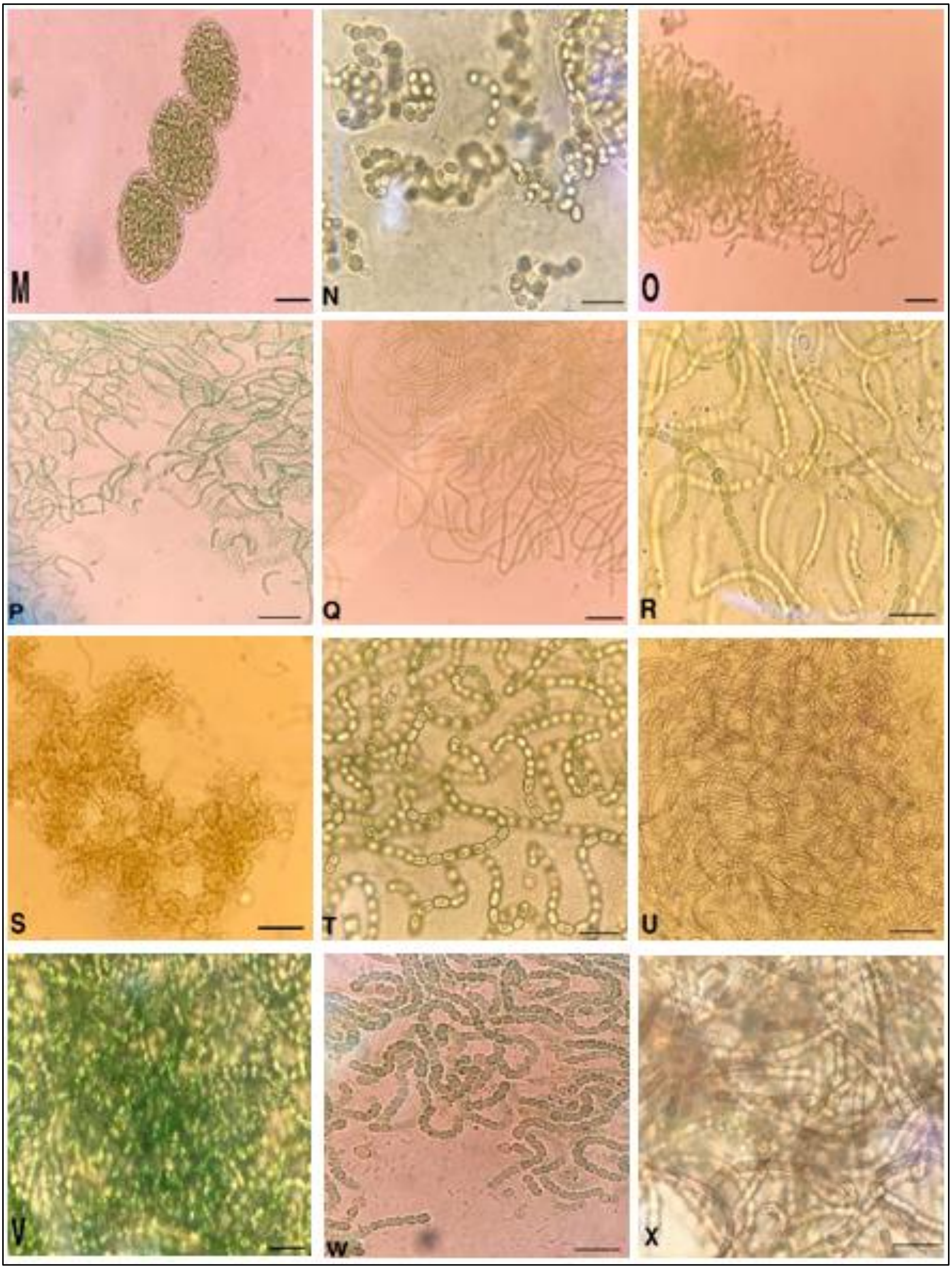

Fig. 4. Cont. in the next page.

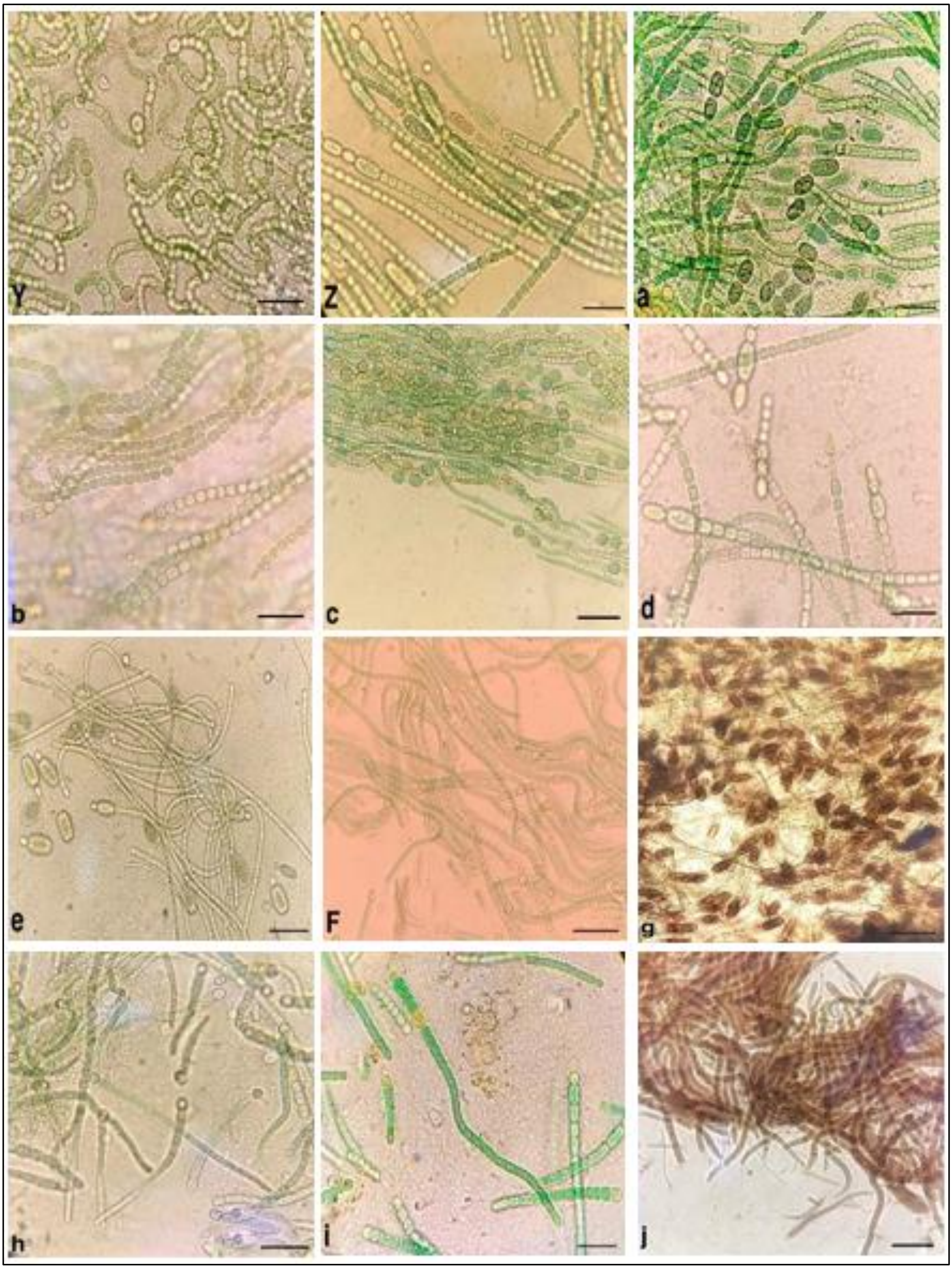

Fig. 4. Cont. in the next page.

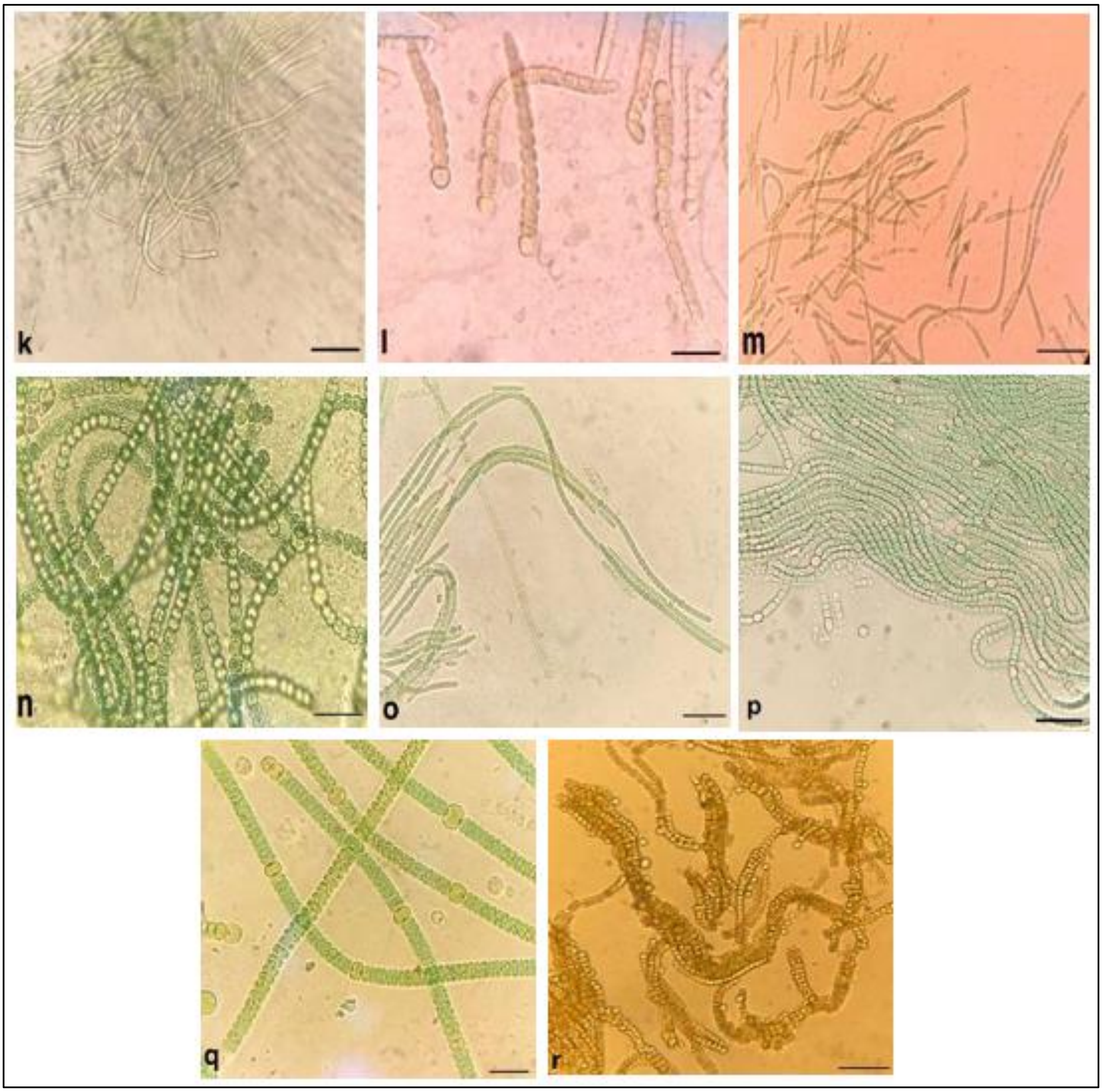

Fig. 4. Micrographs of identified taxa under the light microscopy: A, B. *Nostoc linckia*, C. *N. punctiforme*, D. *N. passerinianum*, E. *N. commune*, F. *N. paludosum*, G. *N. calcicola*, H. *N. sphaericum*, I, J. *N. ellipsosporum*, K. *N. verrucosume*, L. *N. carneum*, M, N. *N. microscopicum*, O. *N. flagelliforme*, P. *Nostoc* sp.$_1$, Q. *Nostoc* sp.$_2$, R. *Nostoc* sp.$_3$, S. *Nostoc* sp.$_4$, T. *Nostoc* sp.$_5$, U. *Nostoc* sp.$_6$, V. *Nostoc* sp.$_7$, W. *Nostoc* sp.$_8$, X. *Nostoc* sp.$_9$, Y. *Nostoc* sp.$_{10}$, Z. *Anabaena torulosa*, a. *A. iyengarii*, b. *Anabaena* sp., c. *Wollea vaginicola*, d. *Cylindrospermum majus*, e. *C. alatosporum*, f. *C. longisporum*, g. *Cylindrospermum* sp., h. *Calothrix capitularis*, i. *C. marchica*, j. *C. parietina*, k. *Calothrix* sp.$_1$, l. *Calothrix* sp.$_2$, m. *Calothrix* sp.$_3$, n. *Trichormus variabilis*, o. *Trichormus* sp.$_1$, p. *Trichormus* sp.$_2$, q. *Nodularia harveyana*, r. *Hapalosiphon welwitschii* (Bars = 10 µm).

In order of occurrence, the common species present in most sites were *Nostoc paludosum* (5 sites), *N. linckia* (4 sites), and *N. microscopicum* (3 sites). Furthermore, the highest diversity of taxa was observed in the northern regions such as Mazandaran and Gilan provinces with low solar radiation, low soil salinity, and high relative humidity (Table 4). In particular, the greatest genus diversity was observed in the Mazandaran province, while only two genera (*Nostoc* and *Calotrix*) were observed in Gilan province. The lowest diversity of cyanobacteria (each site with only one species), was found in those sites that were located in regions with high solar radiation (500 cal/cm^2/day), high soil salinity, and low relative humidity (Figs 1–3). Although, the dominant genus was *Nostoc* in all regions, but diversity and distribution of all species of this genus were different along climatic conditions based on their filaments and colonies color (Table 4). Considering that, in sites with high solar radiation such as 14, 19, 20, and 21, only brown *Nostoc* species were appeared, whereas in site 5 (Chukam), that located in northern regions with highest relative humidity and lowest solar radiation, only blue-green *Nostoc* species were observed. Our observations indicated that, in northern and central regions that have low or medium solar radiation, blue-green and olive-green *Nostoc* species with gelatinous sheath were dominant (Fig. 5 b & d). The most famous species of *Nostoc* i.e. *N. commune*, with a massive gelatinous sheath around filaments, was found in site 13 with low solar radiation, low soil salinity, and high relative humidity; while the brown *Nostoc* species, in sites with high solar radiation, had thin layers of gelatinous sheathe around their filaments (Fig. 5 a & c).

Table 4. Regions categorized based on ecological conditions and *Nostoc* species distributions

Region	Site No.	Solar radiation (cal/cm^2/day)	Salinity	Relative humidity (%)	Distribution
Northern provinces	1–7	300–440	Low	41–67	Species with blue-green or olive-green filaments and colonies
Central provinces	8–18	440–480	Medium to high	5–44	Species with blue-green, olive-green and brown filaments and colonies
Southern provinces	19–21	480–500	High	34–37	Only species with brown filaments and colonies

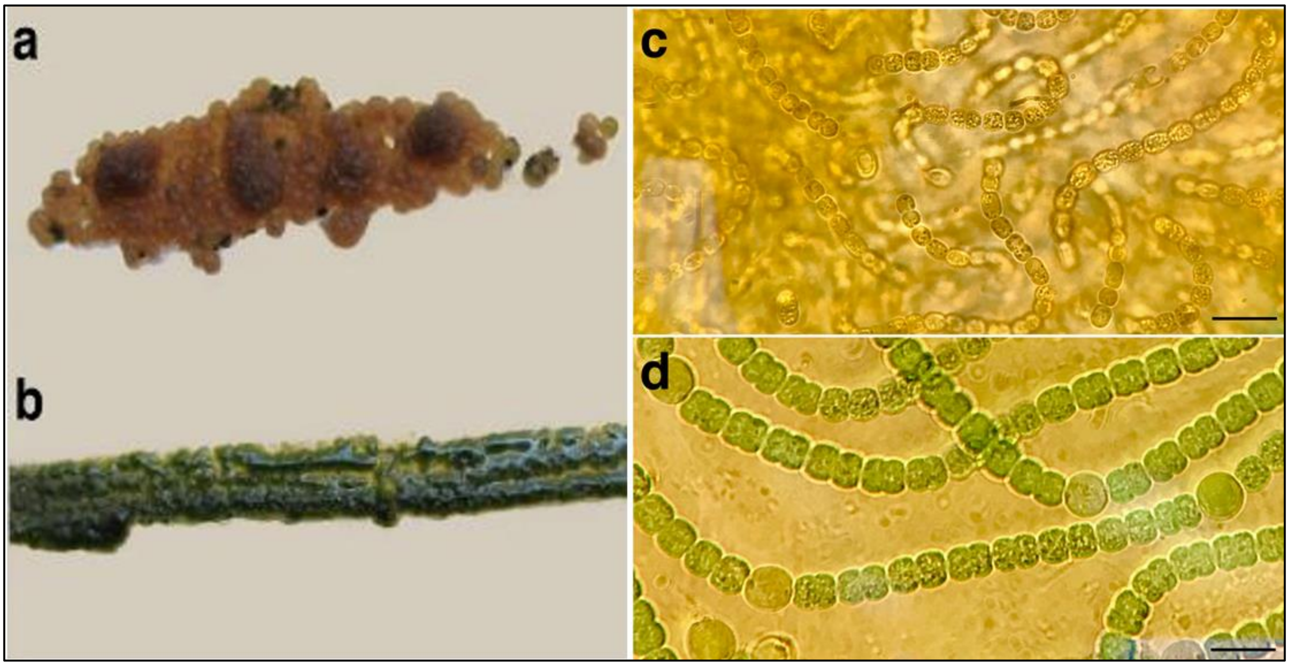

Fig. 5. Patterns of brown and green (olive-green or blue-green) colonies and filaments in two types of heterocystous cyanobacteria: a & c. *Nostoc sphaericum* (brown cyanobacteria), b & d. *N. commune* (green cyanobacteria) (Bars = 10 µm).

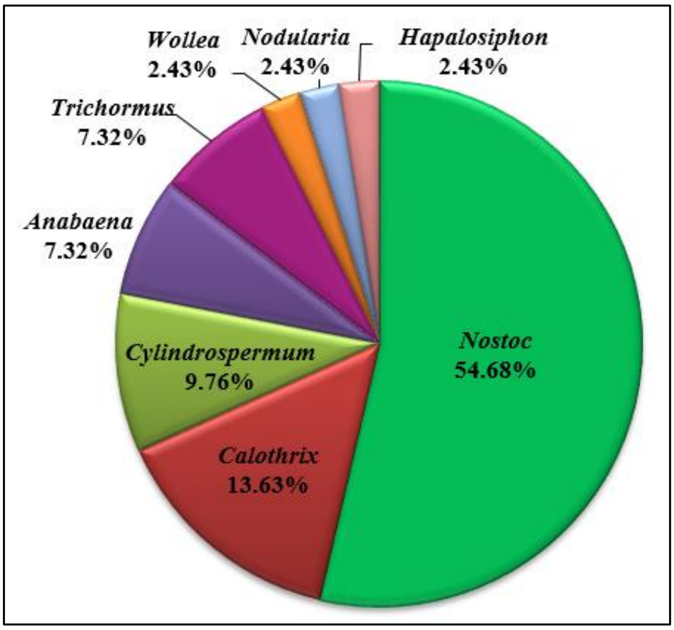

Fig. 6. Diversity of the isolated genera in the studied regions.

According to the results of this study, photosynthetic pigments content of taxa was various. The contents (carotenoids and chlorophyll-a) of isolates is shown in a heatmap graph (Fig. 7). According to this chart, the cyanobacterial chlorophyll-a content along the solar radiation gradient significantly decreased and carotenoids content increased. In northern and central regions, the amount of photosynthetic pigments of taxa were similar where higher chlorophyll-a content observed in compare with the southern regions. In addition, in southern regions that only brown *Nostoc* species were observed, these taxa have the highest amount of carotenoids and medium chlorophyll-a content. These results showed that, carotenoids as an important photosynthetic pigment have a protective role against solar radiation and protect cyanobacteria against the environments with high solar radiation.

Discussion

Cyanobacteria are important component of soil microflora. These microorganisms colonize in different terrestrial habitats due to their ecological tolerances (Garcia-Pichel & Castenholz 1991). Based on the present study, environmental factors such as solar radiation, relative humidity, and soil salinity showed some influences on diversity and distribution of cyanobacteria in terrestrial ecosystems. This research revealed that, the cyanobacterial diversity in regions with high solar radiation, high salinity, and low relative humidity was less than other studied sites. This study also showed the existence of heterocystous cyanobacterial distribution and diversity of 41 taxa from eight genera under three families in the soils of 21 sampling sites with different solar radiation levels around Iran. Based on the results derived here, heterocystous cyanobacteria were present in all studied sites, even though in habitats with high intensity of solar radiation. Among the studied provinces, Mazandaran province with ten genera and Kerman province with one genus (*Nostoc*) possess the highest and the lowest genus diversity, respectively. Wang *et al.* (2022) reported that, the amount of light, affects the microbial structure of cyanobacterial communities in natural ecosystem. Ultimately, it is concluded that, various cyanobacteria had different ecological distribution in habitats with high or low solar radiation.

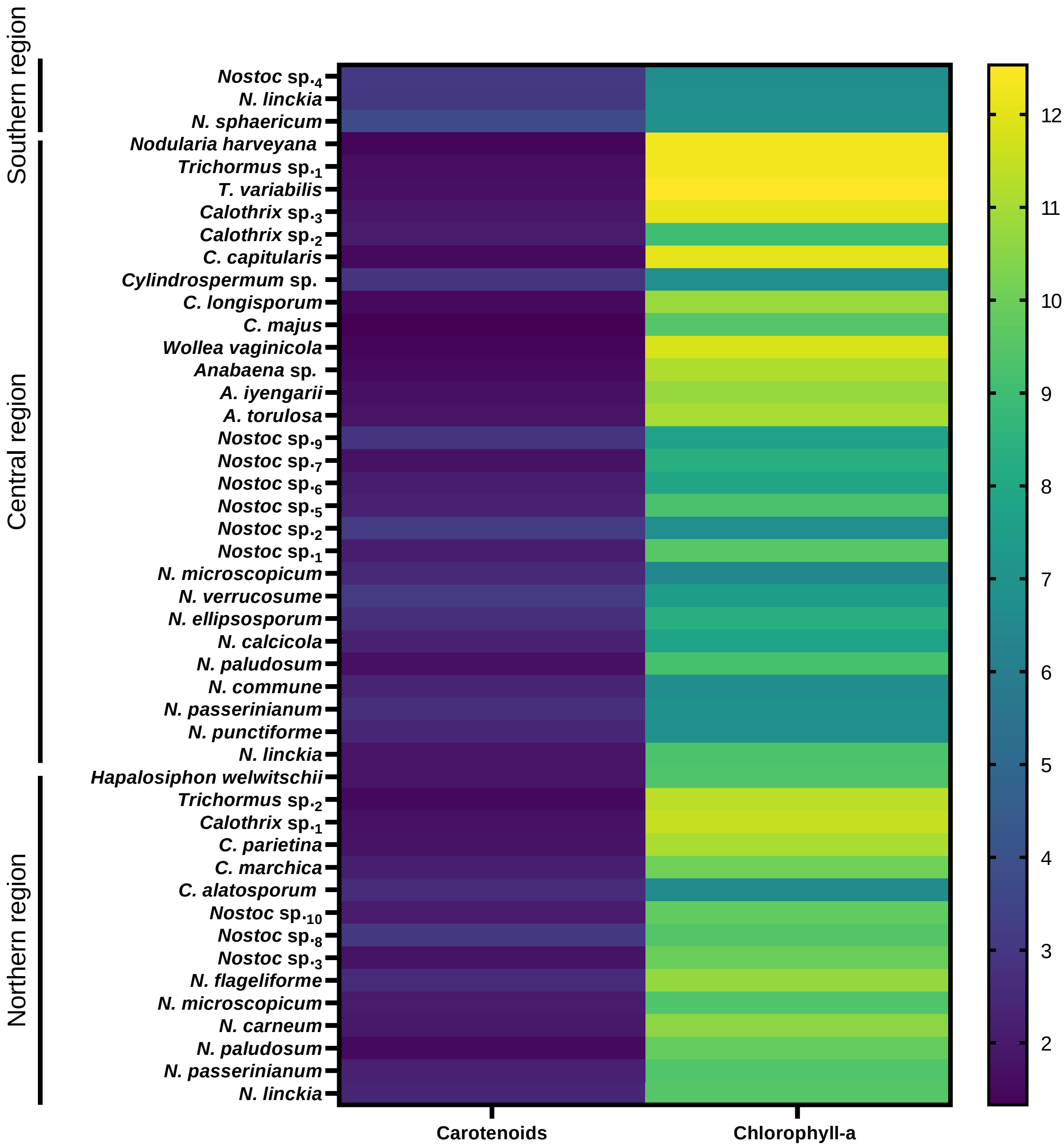

Fig. 7. The heatmap graph for chlorophyll-a and carotenoids in the studied species. Data are means ± standard deviation (n = 3).

In this study, among the identified taxa, *Nostoc* species were dominant in all the studied sites and had the highest diversity in the soil samples of all sites as well. These results indicated the resistant of *Nostoc* species in compare with other taxa, even though species with brown colonies that were present in arid regions with high solar radiation. These results confirmed that, brown *Nostoc* species have an extraordinary resistance both in arid and semi-arid regions. The main reason of *Nostoc* species success for better distribution in terrestrial ecosystems, is the presence of defense mechanisms in response to intense solar radiation and other abnormal conditions, especially synthesis brown pigments such as carotenoids and scytonemin (De Chazal & Smith 1994). In addition, amount of carotenoids in *Nostoc* species that belonged to provinces with high solar radiation were higher than other provinces. Carotenoid pigments in photosynthetic organisms act as an antenna to absorb light energy and play an important protective role against irradiation damages that cause with reactive oxygen species (Sözer 2011, Khanipour Roshan *et al*. 2015). Therefore, intense light induces increasing synthesis carotenoids in habitats with high solar radiation considering as an adaptation mechanism to protect chlorophyll in these organisms (Han *et al.* 2003, Gao *et al.* 2007). Amount of light can, therefore, alter the structure of cyanobacterial communities, their distribution in terrestrial ecosystems, and effects on their amount of photoprotective pigments to enhance the resistance of these microorganisms.

Řeháková *et al.* (2011) indicated that, *Nostoc* species were adapted to high altitude and extreme conditions due to their well-developed defense strategies including synthesis gelatinous sheath. Results of the present study suggested that, in addition to the presence of gelatinous sheath, high amount of carotenoids compounds or other photoprotective pigments are important as fast mechanisms to protect cells from damages against solar radiation in terrestrial ecosystems. Previous studies also indicated that, carotenoids with other protective pigments such as yellow-brown color scytonemin in cyanobacteria play an effective role as sunscreens against intense radiation damages (Sinha & Häder 2008, Rosic 2019).

Another key factor that influencing the distribution of cyanobacterial communities is humidity (Hakkoum *et al.* 2020). In this study, the highest variety of cyanobacteria was recorded in northern province of Iran with low solar radiation and high humidity. Soil enrichment and moisture condition in northern provinces plays an important role in the distribution and diversity of soil microalgae (Kooch *et al.* 2008, Lin *et al.* 2013). Tasie *et al.* (2018) showed that, there is an inverse relationship between humidity and solar radiation intensity; hence increasing in average relative humidity causes a decrease in solar radiation and vice versa.

Furthermore, soil composition affects distribution of soil cyanobacteria, and soil salinity is an inhibition factor in the cyanobacterial growth and diversity (Rejmánková *et al.* 2004). However, in this study diversity and distribution of cyanobacteria in sites with low soil salinity was higher in compare with other sites. Hence, in sites with high soil salinity only cyanobacteria that belong to *Nostoc* genera were observed. In some cases, it was reported that, salinity induces the synthesis of exopolysaccharides and may help to increase the resistance of cyanobacteria in high salinity (Singh *et al.* 2016, Llopis *et al.* 2022). Some studies reported that, macroscopic structure and densely aggregated trichomes of *Nostoc* species with the presence of extracellular gelatinous sheath around filaments are mechanisms that protect these microorganisms against osmotic pressure in environments with different levels of soil salinity (De Caire *et al.* 1997, Stal 2007, Srivastava *et al.* 2009). Therefore, these species have better salinity adaptation than other cyanobacterial taxa. Furthermore, Obana *et al.* (2007) results indicated that, among several taxa of *Nostoc*, those species with spherical-shaped cells showed a higher tolerance to dryness and salinity in compare with other species.

In conclusion, this study constitutes the first attempt to investigate heterocystous cyanobacteria diversity and distribution in terrestrial ecosystems of Iran based on several environmental factors such as solar radiation gradient. It is concluded that, solar light intensity affects the diversity, distribution, and photosynthetic pigments content in cyanobacteria. In addition, the distribution of cyanobacteria in terrestrial ecosystems with different environmental conditions is not the same, due to their resistance and adaptability. The study of soil microflora and the presence of *Nostoc* in all sample sites indicated that, it is a dominant genus in different habitats in Iran. The presence of the brown *Nostoc* species in ecosystems with high solar radiation confirming their resistance in compare with the green *Nostoc* species.

Acknowledgments

The authors wish to thank Shahid Beheshti University (Tehran, Iran) for funding this research. Thanks also to Dr. Ms. S. Sayadi for her valuable guidance.

References

Aghashariatmadari, Z. 2011. Evaluation of model for estimating total solar radiation at horizontal surfaces based on meteorological data, with emphasis on the performance of the angstrom model over Iran. PhD Dissertation, University of Tehran.

Ahmad, F.A. 2018. Valuation of solar power generating potential in Iran desert Areas. Journal of Applied Sciences and Environmental Management 22(6): 967–972.

Andersen, R.A. 2005. Algal Culturing Techniques. 1st. edn., Elsevier Academic Press. London.

De Caire, G.Z., De Cano, M.S., Zaccaro de Mulé, M.C., Palma, R.M. & Colombo, K. 1997. Exopolysaccharide of *Nostoc muscorum* (Cyanobacteria) in the aggregation of soil particles. Journal of Applied Phycology 9: 249–253.

De Chazal, N.M. & Smith, G.D.1994.Characterization of a brown *Nostoc* species from Java that is resistant to high light intensity and UV. Microbiology 140: 3183–3189.

De Martonne, E. 1926. Aerisme, et índices d'aridite. Comptesrendus de L'Academie des Sciences 182: 1395–1398.

Etemadi-Khah, A., Pourbabaee, A.A., Noroozi M., Alikhani, H.A. & Bruno, L. 2017. Biodiversity of isolated cyanobacteria from desert soils in Iran. Geomicrobiology Journal 34(9): 784–794.

Feizi, V., Mollashahi, M., Frajzadeh, M. & Azizi, G.H. 2014. Spatial and temporal trend analysis of temperature and precipitation in Iran. Ecopersia 2(4): 727–742.

Garcia-Pichel, F. & Belnap, J. 1996. Microenvironments and microscale productivity of cyanobacterial desert crusts. Journal of Phycology 32: 774–782.

Garcia-Pichel, F. & Castenholz, R.W. 1991. Characterization and biological implications of Scytonemin, a cyanobacterial sheath pigment. Journal of Phycology 27: 395–409.

Gao, K., Yu, H. & Brown, M.T. 2007. Solar PAR and UV radiation affects the physiology and morphology of the cyanobacterium *Anabaena* sp. PCC 7120. Journal of Photochemistry and Photobiology B 89: 117–124.

Hakkoum, Z., Minaoui, F., Douma, M., Mouhri, K. & Loudiki, M. 2020. Diversity and spatial distribution of soil cyanobacteria along an altitudinal gradient in Marrakesh area (Morocco). Applied Ecology and Environmental Research 18(4): 5527–5545.

Han, P.P., Shen, S.G., Wang, H.Y., Yao, S.U., Tan, Z.L., Zhong, C. & Jia, S.R. 2016. Applying the strategy of light environment control to improve the biomass and polysaccharide production of *Nostoc flagelliforme*. Journal of Applied Phycology 29: 55–65.

Han, T., Sinha, R.P. & Häder, D.P. 2003. Effects of intense PAR and UV radiation on photosynthesis, growth and pigmentation in the rice-field cyanobacterium *Anabaena* sp. Photochemical and Photobiological Sciences 2(6): 649–654.

Hartmann, A., Albert, A. & Ganzera, M. 2015. Effects of elevated ultraviolet radiation on primary metabolites in selected alpine algae and cyanobacteria. Journal of Photochemistry and Photobiology B 149: 149–155.

Hokmollahi, F., Riahi, H., Soltani, N., Shariatmadari, Z. & Hakimi, M.H. 2016. A taxonomic study of blue-green algae based on morphological, physiological and molecular characterization in Yazd province terrestrial ecosystems (Iran). Iranian Journal of Botany 16(2): 152–163.

Khalili, A. & Bazrafshan, J. 2022. A comparative study on climate maps of Iran in extended de Martonne classification and application of the method for world climate zoning. Journal of Agricultural Meteorology 10(1): 3–16.

Khanipour Roshan, S., Farhangi, M., Emtyazjoo, M. & Rabbani, M. 2015. Effects of solar radiation on pigmentation and induction of a mycosporine-like amino acid in two cyanobacteria, *Anabaena* sp. and *Nostoc* sp. ISC26. European Journal of Phycology 50: 173–181.

Komárek, J. 2013. Süßwasserflora von Mitteleuropa, Bd. 19/3: Cyanoprokaryota 3. Teil/3rd part: Heterocytous Genera, Springer Spektrum.

Komárek, J. & Hauer, T. 2013. CyanoDB.cz-on-line database of cyanobacterial genera. World-wide Electronic Publication. University of South Bohemia & Institute of Botany AS CR. http://www.cyanodb.cz.

Komárek, J., Kaštovsk, J., Mares, J. & Johansen, J.R. 2014. Taxonomic classification of cyano-prokaryotes (cyanobacterial genera), using a polyphasic approach. Preslia 86: 295–335.

Komárek, J. 2016. A polyphasic approach for the taxonomy of cyanobacteria: principles and applications. European Journal of Phycology 51: 346–353.

Kooch, Y., Jalilvand, H., Bahmanyar, M.A. & Pormajidian, M.R. 2008. The use of principal component analysis in study of physical, chemical and biological soil properties in southern Caspian forests (north of Iran). Pakistan Journal of Biological Sciences 11(3): 366–372.

Lin, C.S., Chou, T.L. & Wu, J.T. 2013. Biodiversity of soil algae in the farmlands of mid-Taiwan. Botanical Studies 54(41): 1–12.

Llopis, P., García-Abad, L., Pretel, M.T., Montero, M.A., Jordán, M.M. & Asencio A.D. 2022. Effects of climate change on the production of polysaccharides and phycobiliproteins by *Nostoc commune* Vaucher ex Bornet et Flahault. International Journal of Environmental Research 16: Doi: 10.1007/s41742-022-00401-0.

Martineau, E., Wood, S.A., Miller, M.R., Jungblut, A.D., Hawes, I., Webster-Brown, J. & Packer, M.A. 2013. Characterization of Antarctic cyanobacteria and comparison with New Zealand strains. Hydrobiologia 711(1): 139–154.

Moghtaderi, A. Taghavi, M. & Rezaei, R. 2009. Cyanobacteria in biological soil crust of Chadormalu area, Bafq region in central Iran. Pakistan Journal of Nutrition 8(7): 1083–1092.

Moradi, I., Mueller, R., Alijani, B., Ga, K. 2009. Evaluation of the Heliosat-II method using daily irradiation data for four stations in Iran. Solar Energy 83(2): 150–156.

Obana, S., Miyamoto, K., Morita, S., Ohmori, M. & Inubushi, K. 2007. Effect of *Nostoc* sp. On soil characteristics, plant growth and nutrient up take. Journal of Applied Phycology 19(6): 641–646.

Pathak, J., Sonker, A.S., Richa, R., Rajneesh, R., Kannaujiya, V.K., Singh, V., Ahmed, H. & Sinha, R.P. 2017. Screening and partial purification of photoprotective pigment scytonemin from cyanobacterial crusts dwelling on the historical monuments in and around Varanasi, India. Microbiological Research 8(1): 4–12.

Rajaniemi, P., Hrouzek, P., Kaštovská, K., Willame, R., Rantala, A., Hoffmann, L., Komárek, J. & Sivonen, K. 2005. Phylogenetic and morphological evaluation of the genera *Anabaena*, *Aphanizomenon*, *Trichormus* and *Nostoc* (Nostocales, Cyanobacteria). International Journal of Systematic and Evolutionary Microbiology 55(1): 11–26.

Rangaswamy, G. 1966. Agricultural Microbiology. Asia Publishing House. India.

Rastogi, R.P., Richa, Kumar, A., Tyagi, M.B. & Sinha, R.P. 2010. Molecular mechanisms of ultraviolet radiation-induced DNA damage and repair. Journal of Nucleic Acids. Doi: 10.4061/2010/592980.

Řeháková, K., Chlumská, Z. & Doležal, J. 2011. Soil Cyanobacterial and microalgal diversity in dry mountains of Ladakh, NW Himalaya, as related to station, altitude, and vegetation. Microbial Ecology 62(2): 337–346.

Rejmánková, E., Komárek, J. & Komárková, J. 2004. Cyanobacteria: a neglected component of biodiversity: patterns of species diversity in inland marshes of northern Belize (Central America). Diversity and Distributions 10(3): 189–199.

Rosic, N.N. 2019. Mycosporine-like amino acids: making the foundation for organic personalized sunscreens. Marine Drugs 17. Doi: 10.3390/md17110638.

Singh, S., Verma, E., Niveshika, Tiwari, B. & Mishra, A.K. 2016. Exopolysaccharide production in *Anabaena* sp. PCC 7120 under different $CaCl_2$ regimes. Physiology and Molecular Biology of Plants 22(4): 557–566.

Sinha, R.P. & Häder, D.P. 2008. UV-protectants in cyanobacteria. Plant Science 174(3): 278–289.

Soltani, S., Saboohi, R. & Yaghmaei, L. 2012. Rainfall and rainy days trend in Iran. Climatic Change 110(21): 187–213.

Sözer, O. 2011. Carotenoids assist in assembly and functions of photosynthetic complexes in cyanobacteria. Dissertation, University of Szeged.

Srivastava, A.K., Bhargava, P., Kumar, A., Rai, L.C. & Neilan, B.A. 2009. Molecular characterization and the effect of salinity on cyanobacterial diversity in the rice fields of Eastern Uttar Pradesh, India. Saline Systems 5. Doi: 10.1186/1746-1448-5-4.

Stal, L.J. 2007. Cyanobacteria: Diversity and versatility, clues to life in extreme environment. Pp. 659–680. *In*: Seckbach, J. (ed.), Algae and Cyanobacteria in Extreme Environment. Springer. Netherland.

Tasie, N., Israel-Cookey, C. & Banyie, L. 2018. The effect of relative humidity on the solar radiation intensity in Port Harcourt, Nigeria. International Journal of Research 5(21): 128–136.

Tong, Y.J., Yang, H.D., Shaw, J.J., Yang, X.K. & Bai, M. 2021. The relationship between genus/species richness and morphological diversity among subfamilies of jewel beetles. Insects 12. Doi: 10.3390/insects12010024.

Wang, Z., Li, G., Huang, H., Zhang, W., Wang, J., Huang, S. & Zheng, Z. 2022. Effects of solar radiation on the cyanobacteria: diversity, molecular phylogeny, and metabolic activity. Frontiers in Ecology and Evolution 10. Doi: 10.3389/fevo.2022.92881.

Wehr, J.D., Sheath, R.G. & Thorp, J.H. 2002. Freshwater algae of North America: ecology and classification. Aquatic Ecology Press. California.

Zancan, S., Trevisan, R. & Paoletti, M.G. 2006. Soil algae composition under different agro-ecosystem in north-eastern Italy. Agriculture, Ecosystems and Environment 112: 1–12.