A TAXONOMIC STUDY ON NON-HETEROCYSTOUS FILAMENTOUS CYANOPROKARYOTES FROM SOIL OF YAZD PROVINCE, IRAN

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To investigate the non-heterocystous filamentous cyanoprokaryotes in terrestrial habitats of Yazd province, seven stations in Taft, Meybod and Ardakan counties were selected. In the present paper an attempt was made to identify cyanoprokaryotes using morphological characteristics. In this study 4 families including Microcoleaceae, Oscillatoriaceae, Pseudanabaenaceae and Leptolyngbyaceae, 18 species belonging to 9 genera including, Oscillatoria, Kamptonema, Phormidium, Lyngbya, Oxynema, Microcoleus, Jaaginema, Pseudanabaena and Leptolyngbya were identified. Ecological distribution and camera lucida drawing of species are presented. It should be noted that Phormidium arvense Gardne, Phormidium chlorinum (Kützing ex Gomont) Umezak and Microcoleus steenstrupii Petersen are the 3 new records from Iran.

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Key words: Cyanoprokaryotes; non-heterocystous filamentous; microflora; Yazd; Iran

مطالعه تاکسونومیک نمونههای خاکزی سیانو پروکاریوتهای رشتهای فاقد هتروسیست از استان یزد، ایران فریبا حکم اللهی: دانشجوی دکتری دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی حسین ریاحی: استاد دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی ندا سلطانی: استاد گروه میکروبیولوژی نفت، پژوهشکده علوم پایه کاربردی جهاد دانشگاهی دانشگاه شهید بهشتی، تهران، ایران زینب شریعتمداری: استادیار دانشکده علوم و فناوری زیستی، دانشگاه شهید بهشتی، تهران، ایران

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به منظور بررسی سیانوپروکاریوتهای خاکزی استان یزد، تاکسونهای موجود در خاک هفت ایستگاه از شهرستانهای تفت، میبد و اردکان مورد مطالعه قرار گرفت. شناسایی نمونهها بر اساس ویژگیهای مورفولوژیک صورت گرفت. در این تحقیق، در مجموع ۱۸ گونه متعلق به ۹ جنس Pseudanabaena "Jaaginema "Microcoleus "Oxynema "Lyngbya "Phormidium "Kamptonema "Oscillatoria و Leptolyngbyaceae شناسایی شدند. ادریابی نحوه پراکنش گونههای شناسایی شده در ایستگاههای مطالعاتی و ارائه تصاویر کامرا لوسیدای گونهها از دیگر نکات این مطالعه است. لازم Phormidium chlorinum (Kützing ex "Phormidium arvense Gardner یر این گونههای شده، ۳ گونه Microcoleus steenstrupii Petersen یر از میان گونههای شوند.

INTRODUCTION

Microalgae constitute an important group of soil microflora. Cyanoprokaryotes are arguably one of the most ancient groups of algae on earth, with some fossil representatives which are morphologically similar to present-day species (Schopf 1974, Knoll 2008). Cyanoprokaryotes have an important role in the nitrogen economy of soil (Nimbhore & Jadhav 2014). The presence of these microorganisms also stabilizes the soil surface and thus reduces erosion (Evans & Johansen 1999; Hu & al. 2004).

The Yazd province in Iran is located in a dry region, with limited water resources. Yazd soil is at risk of erosion. There are some mines in this area; hence the soil of Yazd province is contaminated with radioactive and heavy metal pollutants. Due to this fact, outbreak of different types of cancers in Yazd is high. There are several reports that show algae can function as bioindicators for soil quality, improve soil structure and reduce soil erosion. However, there is a general need to improve our knowledge about microalgae, to better appreciate the many benefits that human derive from their existence (Rahmani & Rezaei 2001, Rahmani 2012; Kannan & al. 2012, Bajguz,

Cyanoprokaryotes are an especially challenging group of algae to classify. Taxonomy of this group has been revised in the last decade according to several characteristics such as morphological, molecular and ecological evidences. For example, Komárek & al. (2014) revised classification of cyanoprokaryotes and suggested new orders and new families and elevated a few subfamilies to family level. According to new system of classification, these orders include Gloeobacterales. Synechococcales, Spirulinales, Chroococcales, Pleurocapsales, Oscillatoriales, Chroococcidiopsidales and Nostocales.

order Oscillatoriales. The now includes filamentous taxa with more complicated cytology, which are characterized with radial, fasciculate, or irregular thylakoid arrangement. This order includes the coccoid, phylogenetically close genus to Cyanothece. Overall, seven families are presented in this order of cyanoprokaryotes. These families include, Cyanothecaceae, Borziaceae, Coleofasciculaceae. Microcoleaceae, Homoeotrichaceae, Oscillatoriaceae Gomontiellaceae. The Oscillatoria, Phormidium and Lyngbya species belong to Oscillatoriaceae family (Oscillatoriales), Kamptonema, Microcoleus and Oxynema genera are located in Microcoleaceae (Oscillatoriales), and similar taxa such as Jaaginema spp., Pseudanabaena spp. (Pseudanabaenaceae) and Leptolyngbya spp. (Leptolyngbyaceae) are belonging to Synechococcales. It should be noted that some genera with parietal thylakoid arrangement recently located in Synechococcales (Komarek & al. 2014).

of Identification soil algae, cyanoprokaryotes, has been well documented in the world (Zancan & al. 2006, El-Gamal & al. 2008, Budel & al. 2009, Lin & al. 2013, Nimbhore & Jadhav 2014). Up to now, several species from different genera of filamentous cyanoprokaryotes were reported from terrestrial habitats of Iran but most of records related to species from paddy soils of our country. In general, there are a few floristic data available for cyanoprokaryotes of Iran (Abarkar & Riahi 1995, Moghtaderi & al. 2009, Saadatnia & Riahi 2009, Shariatmadari & al. 2011, 2013, Siahbalaei & al. 2008, 2010, 2011, Arman & al. 2014, 2015, Heidari & al. 2013. Hokmollahi & al. 2015). In addition, Jafari & al. (2014) have reported a new cyanoprokaryotes from the oil polluted soil in the south of Iran. With respect to the center of Iran, Moghtaderi & al. (2009) have reported Microcoleous vaginatus in soil crust of Chadormalu Desert area (Bafgh region in Yazd).

In the present study, we focused on filamentous species from different locations of Yazd provinces such as Desert area, rangelands and pistachio cultivated lands. With regard to the importance of this group of microorganisms, as well as considering the limited information about soil microflora of Yazd province, as a dry region of country, we decided to have a florestic study in this area using morphological emphasis on with non-heterocystous filamentous cyanoprokaryotes.

MATERIALS AND METHODS Field study, sampling and analyses:

Soil samples were collected according to Rangaswamy methodology (1966) from seven different regions (cultivated and barren lands) from Yazd province in Aug. 2013 (Table 1). The collected soil samples were cultured in BG11 medium (Rippka & al. 1979) and incubated at $28 \pm 2^{\circ}$ C with continuous illumination at 70 µmol photon m⁻²s⁻¹ prepared by several fluorescent lamps (Table 1). The physico-chemical characteristics of the soil including pH, EC, Na⁺ and K⁺ were determined by the Arian Fan Azma Company (Tehran, Iran).

Isolation identification of and cyanoprocaryotes

After isolation, determination of taxa was carried out by light microscopy (400-1,000×) and photographs were taken with a Canon camera (G10). Samples were purified by several subcultures on solid BG11 medium (Kaushik 1987). Identification of samples was carried out based on accepted references (Prescott 1962, Desikachary 1959, Komarek & Anagnostidis 2005). The most important vegetative and reproductive characteristics used in the taxonomic determination were: presence or absence of a sheath, filament shape and the color of cells, cell size and constriction at the cross walls, cellular granulation and cell shape, especially apical cell shape. The diversity of species is analyzed, and the diversity indices (Shannon's diversity index) were calculated using the standard formulae.

RESULTSSoil physico-chemical characteristics

The results of chemical characteristics showed that, stations 6 and 7 have high EC, and stations 3, 4, 5 and 6 have high pH. According to the result, the soil of studied stations can be divided to non-saline, saline and strong saline types. The correlation curves between pH and number of taxa and EC and number of taxa are presented in figs. 1, 2.

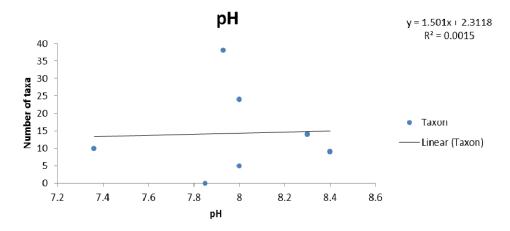


Fig. 1. The correlation curve between pH and number of taxa.

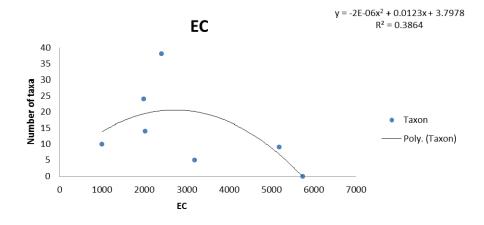


Fig. 2. The correlation curve between EC and number of taxa.

Species diversity

The species diversity was determined and highest number of species were observed in genus Phormidium (28%) followed by Oscillatoria (22.5%), Kamptonema (11%), Microcoleus (11%), Lyngbya 5.5%, Oxynema 5.5%, Jaaginema 5.5%, Pseudanabaena 5.5% and Leptolyngbya 5.5% (fig. 3).

The number of species at each site ranged from 1 to 8 with maximum at Panjdukan and Dehbala stations with 19% and 43% species diversity respectively and

minimum at Tezerjan, Barfkhane, Almas-e Kuhestan and Ardakan, Chah Afzal, pistatia population stations with 9.52%, 4.76% and 9.52% species diversity.

Taxonomic results

In this study, 18 species belonging to 9 genera,

Oscillatoria, Kamptonema, Phormidium, lyngbya, Oxynema, Microcoleus, Jaaginema, Pseudanabaena and Leptolyngbya were identified (fig. 3). All species and their distribution are listed in table 2. The photographs and camera lucida drawings can be seen in figs 5 to 8.

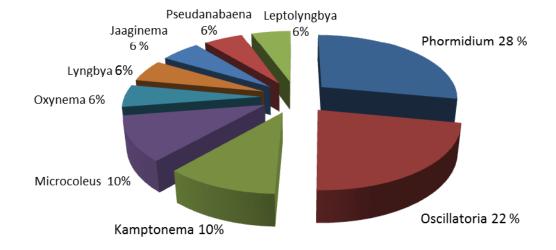


Fig. 3. The chart of species diversity in 9 genera of filamentous cyanoprokaryotes.

Description of the taxa

1. Oscillatoria curviceps Agardh ex Gomont 1892 (fig. 4b, fig. 7c)

Synonyms. No synonyms are currently included in AlgaeBase.

Occurrence. Oscillatoria curviceps was recorded from freshwater of Europe, America, Asia, Australia and New Zealand (www.algaebase.org).

Distribution in Iran. Hormozgan: Chah Ahmad hot spring (Arman & al. 2015). Yazd: Tezerjan, Barfkhaneh.

2. Oscillatoria tenuis Agardh ex Gomont 1892 (figs. 4c, d, fig. 6m)

Synonyms. Phormidium neotenue G.Hällfors 2004.

Occurrence. Freshwater, benthic and metaphytic, sometimes recorded also from wetted rocky walls; not very common, but distributed worldwide, possibly cosmopolitan. Several records are known from tropical regions (www.algaebase.org).

Distribution in Iran. Geno and Khamir hot spring (Heidari & al. 2013). Yazd: Dehbala, Almas-e kuhestan.

3. Oscillatoria ornata Kützing ex Gomont 1892 (fig. 6g).

Synonyms. Phormidium ornatum (Kützing ex Gomont) Anagnostidis & Komárek 1988.

Occurrence. Freshwater, usually in larger lakes, also on stony substrate and in benthos near the shore; known sporadically from the temperate and subtropical zones, Europe and N America, but numerous data exist from all over the world (www.algaebase.org).

Distribution in Iran. Yazd, Dehbala, Panjdukan.

4. *Oscillatoria* **sp.** (fig. 6i)

Thallus blue-green, in form of clusters to mats. Trichomes dark blue-green, ± straight, slightly constricted at cross-walls, 8 µm wide. Cells always shorter than wide, 1-1.5-2.5 µm long, not granulated at cross-walls. Apical cells rounded, without calyptra or thickened cell wall.

Distribution in Iran. Yazd, Dehbala, Panjdukan.

5. Kamptonema animale (C. Agardh ex Gomont) Strunecký, Komárek & J. Smarda 2014 (fig. 4e, f, fig. 6b)

Synonyms. Oscillatoria animalis C. Agardh ex Gomont 1892, Porphyrosiphon animalis (C. Agardh) Drouet 1968 and Phormidium animale (C. Agardh ex Gomont) Anagnostidis & Komárek 1988.

Occurrence. Warm, thermal and sulphur springs, wet soils, rarely in puddles, in stagnant cold water, walls of greenhouses, widely distributed in temperate and tropical zones, possibly cosmopolitan. *Kamptonema animale* was recorded from Europe, America, India, China, Australia and New Zealand and Russia (www. algaebase. org).

Distribution in Iran. Yazd: Tezerjan, Barfkhaneh.

6. *Kamptonema* **sp.** (fig. 4g, h & i, fig. 6k)

Filaments solitary without sheaths or with very fine, facultative and diffluent sheaths, sometimes with several to many trichomes associated more or less in parallel without common firm sheaths. The fascicles are not enveloped in slime. Continually narrowed trichomes towards ends, simple, cylindrical along their whole length (up to the end), uniseriate, blue-green, slightly constricted or unconstricted at cross-walls, motile, 2.5 μm wide. The cells are more or less isodiametric or a little longer than wide, the terminal cell is rounded and usually without calyptra and the very fine sheaths are developed only exceptionally.

Distribution in Iran. Yazd: Meybod, Shahva.

7. Phormidium arvense (Rao) Anagnostidis 2001 (fig. 4j)

Thallus thick, blue-green to greyish; sheaths firm, usually relatively thick \pm gelatinous, colorless, sometimes confluent, not layered; trichomes not constricted, neither uncinate nor capitate at the ends, 3.5-4 um wide, cells 1.5-3.6 um long, apical cells rounded.

synonyms. Phormidium mucosum var. arvense Rao 1937.

Occurrence. Stagnant rainwater pools in fields, India (www.algaebase.org).

Distribution in Iran. Yazd, Dehbala.

8. *Phormidium articulatum* (Gardner) Anagnostidis et Komarek 1988 (fig. 4k, l, fig. 6h)

Synonyms. Oscillatoria articulata.

Occurrence. Freshwater, benthic, later tychoplanktic, in stagnant waters, also on moist rocks; described from tropical regions (Chad, India, S Africa), USA, S Brazil and Europe (www.algaebase.org).

Distribution in Iran. Paddy fields of Iran (Shariatmadari & al. 2011); Yazd, Dehbala, Panjdukan.

9. Phormidium chlorinum (Kützing ex Gomont) Umezaki & Watanabe 1994 (fig. 5m, fig. 6c)

Thallus (fasciculated) fine, yellow-green, often in solitary filaments. Sheaths indistinct. Trichomes

straight or slightly flexuous, yellow-green, (3) 3.5-4 (6) um wide, not or very slightly constricted at cross-walls. Cells ± isodiametric or slightly longer or shorter than wide, (2.5) 3.7-8 um long, not granulated at cross-walls, without gas vesicles. Apical cells widely-rounded to slightly flattened, rarely slightly arcuated. **Synonyms.** No synonyms are currently included in AlgaeBase.

Occurrence. Benthic on organic mud, in sulphuretes, sometimes also in slight salty waters; recorded from many localities over the world, probably cosmopolitan, but morphologically variable: numerous deviations and morphotypes are described, Europe, Romania, China, Puerto Rico etc. (www.algaebase.org).

Distribution in Iran. Yazd, Dehbala.

10. *Phormidium formosum* (Bory ex Gomont) Anagnostidis & Komarek 1988 (fig. 6a)

Synonyms. Oscillatoria formosa Bory ex Gomont 1892.

Occurrence. Periphytic and benthic in stagnant waters, pools, lakes, also in brackish and salty waters, sewage polluted waters; data from thermal springs, moist cliffs and mineral springs must be revised; common, distributed worldwide (www.algaebase.org). Distribution in Iran. Yazd, Dehbala.

11. *Phormidium nigrum* (Vaucher ex Gomont) Anagnostidis & Komárek 1988 (fig. 5n, o & p, fig. 6d) *Synonyms. Oscillatoria nigra* Vaucher ex Gomont 1892; *Lyngbya nigra* [Vaucher] ex Hansgirg 1892.

Occurrence. Freshwater, benthic, attached to the substrate, later sometimes freefloating, Russia etc.; America, recorded also from India and Chile (www.algaebase.org).

Distribution in Iran. Chah Ahmad and Ramsar hot spring (Heidari & al. 2013), Chah Ahmad hot spring (Arman & al. 2015), Yazd, Dehbala, 31.64 N/ 54.15 E, Yazd: Panjdukn.

12. *Oxynema acuminatum* (Gomont) Chatchawan, Komárek, Strunecky, Smarda & Peerapornpisal 2012 (fig. 5q)

Synonyms. Oscillatoria acuminata Gomont 1892, Phormidium acuminatum (Gomont) Anagnostidis & Komárek 1988.

Occurrence. Thermal springs, often with higher levels of salinity or in sulphuretes, widely distributed; Europe: Austria, Greece, Hungary, Italy; outside Europe: Algeria, central Africa, Indonesia (Sumatra), Japan, Sri Lanka, America, USA (www.algaebase.org).

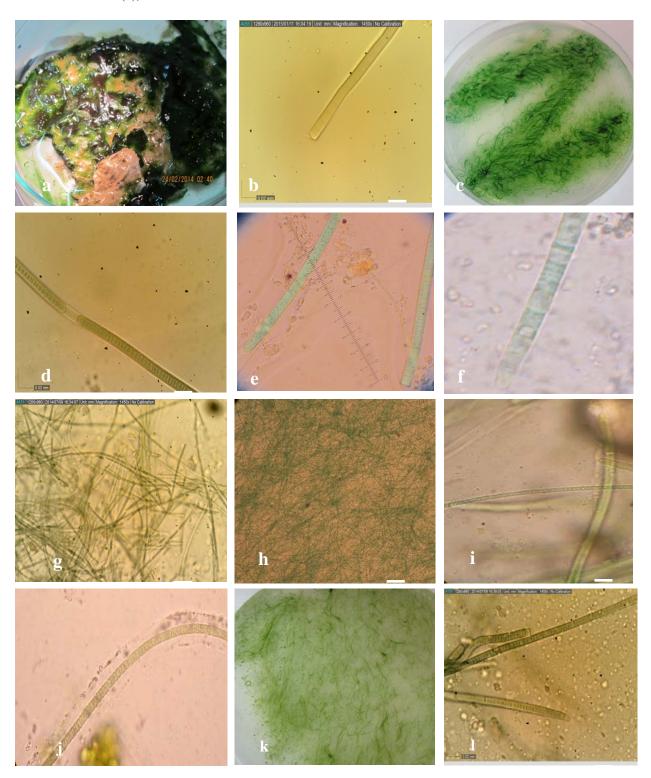


Fig. 4. a: Algae growth on soil plate, b: Oscillatoria curviceps, c & d: Oscillatoria tenuis, e & f: Kamptonema $animale, g,h \& i: \textit{Kamptonema} \ sp., j: *Phormidium \ arvense, k \& l: Phormidium \ articulatum \ (Bar = 0.02 \ mm).$

Distribution in Iran. Ardakan: Chah Afzal, Pistachia population; Geno, Lamzan and Toduluye hot spring, Arman & al. (2014).

13. *Lyngbya aestuarii* Liebman ex Gomont 1892 (Fig. 5r, s, t & u, fig. 6n, fig. 7b)

Synonyms. Conferva aestuarii Mertens 1816, Oscillatoria aestuarii (Mertens) Lyngbye 1818, Oscillatoria littoralis Carmichael 1833, Lyngbya littoralis (Carmichael) Cooke 1884, Oscillatoria aestuarii (Mertens) Lyngbye ex Gomont 1892, Oscillatoria aestuarii var. atrovirens Jürgens ex Gomont 1892, Lyngbya littoralis (Carmichael) Cooke ex Forti 1907.

Occurrence. Marine and halophilic (also in inland saline biotopes), benthic, periphytic, very common, attached on rocks, sandy bottom, loam, rarely on algae and other objects, forming widely expanded mats, occasionally floating, in the lower or upper littoral zones, usually associated with other filamentous cyanoprokaryotes, in salt pools and marshes; distributed worldwide along sea coasts, except for subpolar regions (www.algaebase.org).

Distribution in Iran. Yazd; Meybod, Shahva.

14. *Microcoleus lacustris* Farlow ex Gomont 1892 (fig. 5v)

Synonyms. No synonyms are currently included in AlgaeBase.

Occurrence. Freshwater, benthic, described from littoral of stagnant waters in USA, known also from Africa, Brazil and central Europe; recorded also from wet rocks (www.algaebase.org).

Distribution in Iran. Golestan province, North of Iran (Siahbalaei & al. 2011) and Yazd, Dehbala.

15. **Microcoleus steenstrupii* Petersen 1923 (fig. 5w, x, fig. 7a)

Filaments 30-65 μm wide; sheaths colourless, somewhat lamellated, containing numerous trichomes; trichomes 3.5-5 μm wide, not or slightly constricted at cross-walls slightly narrowed towards ends, cells 3.6-8 μm long, apical cells long-conical without calyptra.

Synonyms. No synonyms are currently included in AlgaeBase.

Occurrence. Described from Iceland, Greece, USA, Japan; morphologically similar populations (probably another species) were recorded from hypersaline desert lakes in Afghanistan, Libya, desert soils in USA (Nevada), and soils in India (Kolhapur) (www.algaebase.org).

Distribution in Iran. Yazd, Dehbala.

16. *Jaaginema woronichinii* (Anisimova in Elenkin) Anagnostidis et Komarek 1988 (fig. 6e)

Synonyms. Oscillatoria woronichinii Anisimova in Elenkin 1949.

Occurrence. In salty lakes and marine coastal pools, epiphytic on *Ruppia* or benthic, epilithic; Ukraine (www.algaebase.org).

Distribution in Iran. Yazd, Dehbala.

17. *Pseudanabaena limnetica* (Lemmermann) Komarek 1974 (fig. 6f)

Synonyms. Oscillatoria limnetica Lemmerman 1900. Occurrence. As pseudanabaena limnetica, described from Arctic, Europe, North and south America, Africa, Asia, Australia and New Zealand (www.algaebase.org).

Distribution in Iran. Yazd, Dehbala.

18. Leptolyngbya breviarticulata (Claus) Anagnostidis 2001 (fig. 6j)

Synonyms. Phormidium treleasei f. breviarticulatum Claus 1961.

Occurrence. As *Leptolyngbya breviarticulata*, North America: Arkansas (www.algaebase.org).

Distribution in Iran. Ardakan: Chah Afzal, Pistachia population.

DISCUSSION

Our knowledge about cyanoprokaryotes of Yazd province is limited. Research on cyanoprokaryotes is a new field of study in Yazd province and Iran as a whole. In this research we identified cyanoprokaryotes from Oscillatoriales and Synechococcales orders and described 9 genera: Oscillatoria, Kamptonema, Phormidium. Lyngbya, Oxynema, Microcoleus. Jaaginema, Pseudanabaena and Leptolyngbya belonging to these orders. Cyanoprokaryotes genera, with 18 taxa belonging to Microcoleaceae, Oscillatoriaceae, Pseudanabaenaceae Leptolyngbyaceae families are reported in Yazd soil. Budel & al. (2009) also reported these genera form southern African biological soil crusts.

In present study, also species diversity was determined and the highest species diversity was observed in *Phormidium* genus with five species.

The dominant species *Kamptonema animale*, *Phormidium articulatum* and *Phormidium nigrum* were found in two stations and the rest of species, there were only in one station.

Yazd province can be described as a huge and stressful area including a wide range of habitats: very strong saline, strong saline and non-saline habitats. Chah Afzal, pistatia and tamarix population stations had very strong saline soil, Meybod, Shahva, had

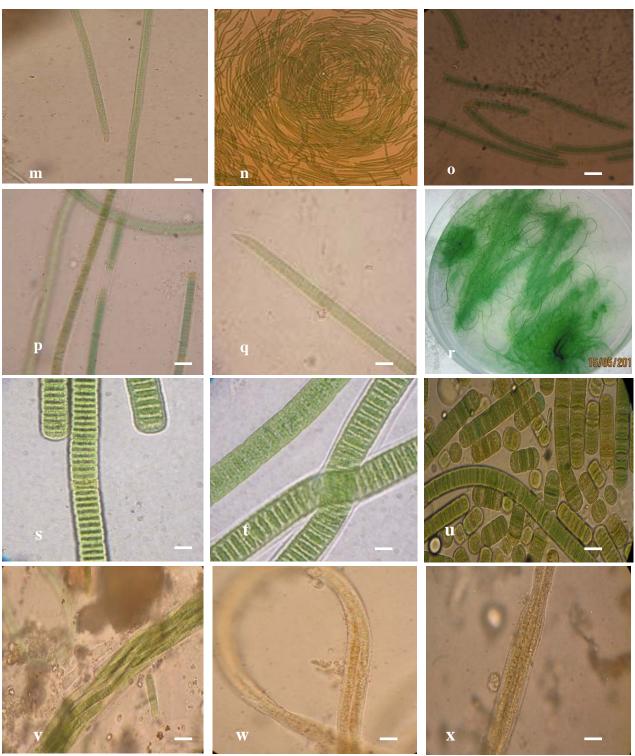


Fig 5. m: *Phormidium chlorinum, n, o & p: Phormidium nigrum, q: Oxynema acuminatum, r, s, t & u: Lyngbya $austerii \ , \ v: \textit{Microcoleus lacustris and} \ w \ \& \ x: \ *\textit{Microcoleus steenstrupii} \ (Bar = 0.02 \ mm).$

semi-saline soil and the rest of stations have non-saline soil. In station 7, no Algae were found, As the soil in this station had high EC, it was very salty (EC: 57400 µmhos.cm). Overall, it was observed that in our studied sites algal diversity decreases with increasing soil salinity and EC. However, some taxa can be seen in saline soils. For example, Singh & al. (2008) reported *Oscillatoria*, *Lyngbya* and colony of *Anabaena* from high saline soil.

In present study we reported Lyngbya austerii from saline soils, Meybod County, Yazd province. This genus was not observed in none-saline soil. Whitton & Potts (2000), reported that pH and EC can affect the species diversity. Siahbalaei & al. (2010) also represented that the lowest pH and highest EC can affect the cyanoprokaryotes variations. In our study, the stations with normal EC, had high algal diversity. We observed high numbers of taxa with tolerable EC increase, and higher EC amounts may simply had a negative effect on the development of the algal flora. This is in accordance with the results by Broady (1984). In this research no relationship was found between the amount of pH with the number of taxa. Gamal & al. (2008) reported some filamentous cyanoprokaryotes from different investigated soils, Egypt. With respect to all present salinity levels, the number of cyanoprokaryotes were not affected while, the numbers of chlorophytes decreased. The presence of numerous cyanoprokaryotes as compared to other types of soil algae is a matter of tolerance and adaptability (Metting, 1981).

Until now, several taxa of these genera were reported by several authors from different locations of Iran but there is a little information from cyanoprokaryotes in the center of Iran (Arman & al. 2015; Heidari & al. 2013). Our results showed distribution of these algae in seven stations of Taft, Meybod and Ardakan counties, Yazd province. In conclusion, our report emphasis the predominance of 9 genera, Oscillatoria, Kamptonema, Phormidium, lyngbya, Oxynema, Microcoleus, Jaaginema, Leptolyngbya Pseudanabaena and from nonheterocystous filamentous cyanoprokaryota different localities of Yazd province and introduce three taxa as new record for Iran. The three genera, Oxynema acuminatum, Leptolyngbya breviarticulata and Oscillatoria tenuis are dominant genera in the salty soils of Yazd province.

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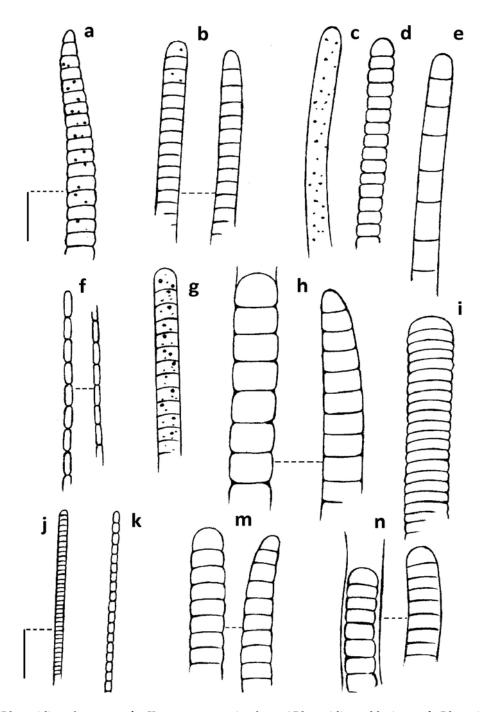


Fig 6. a: Phormidium formosum, b: Kamptonema animale, c: *Phormidium chlorinum, d: Phormidium nigrum, e: Jaaginema woronichinii, f: Pseudanabaena limnetica, g: Oscillatoria ornata, h: Phormidium articulatum, i: Oscillatoria sp., j: Leptolyngbya breviarticulata, k: Kamptonema sp., m: Oscillatoria tenuis, n: Lyngbya austerii (Bar= 10 μ).

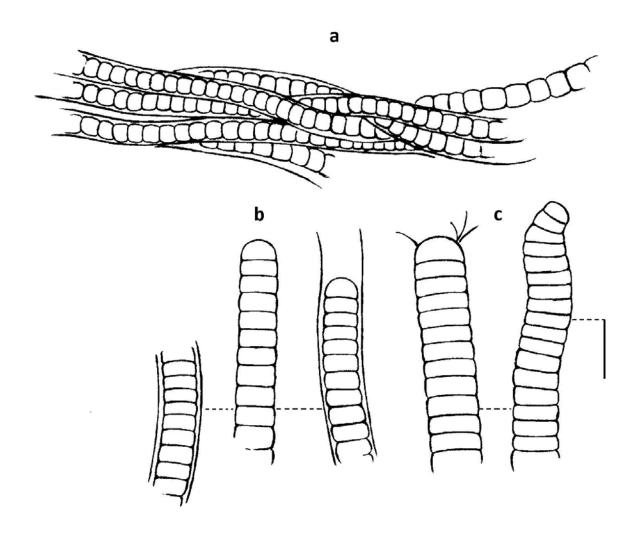


Fig. 7. a: *Microcoleus steenstrupii, b: Lyngbya austerii and c: Oscillatoria curviceps (Bar= $10~\mu$).