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MICROCHAETE GOEPPERTIANA KIRCHNER, A NEW MORPHOSPECIES OF NOSTOCALEAN CYANOPHYTA FOR ALGAL FLORA OF IRAN

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A new morphospecies, *Microchaete goeppertiana* Kirchner (Nostocales, Microcheataceae) has been reported for algal flora of Iran. Collection was done at 2011 from oil polluted regions of Khuzestan province. Identification performed using light, epifluoresence and phase contrast microscopy, in addition of behavioral analysis both in liquid and solid cultures. Observation and description were done in a multidisciplinary way including morphological variations in relation to pH and salinity concentration fluctuations at limited irradiance (2 µE m^{-2} s^{-1}). Regarding to biological versatility of cyanophyta, it has been tried to emphasize on most prominent traits for identification and determination. A new description of the species has been presented regarding to morphological characterization.

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Key words: Cyanophyta, *Microchaete*, morphospecies, Iranian Algal Flora

گزارش جدید گونه Microchaete goeppertiana Kirchner از سیانوفیتای نوستوکال برای فلور جلبکی ایران

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گونه سیانوفیت *Microchaete goeppertiana* Kirchner (راسته Nostocales و تیره Microchaetaceae) برای اولین بار برای فلور جلبکی ایران گزارش می گردد. نمونه برداری در طول یک دوره یک ساله از مناطق آلوده نفتی استان خوزستان انجام گرفت. شناسایی بعد از کشت و تخلیص با استفاده از میکروسکوپ نوری، فلورسانس، فازکنتراست و نیز رفتار شناسی در محیط جامد و مایع انجام شد. روش چند وجهی ارزیابی تنوع مورفولوژیکی شامل بررسی رفتارشناسی در ارتباط با تغییرات اسیدیته و قلیاییت و شوری در شرایط محدود نوری (۲ µE $m^{-2}s^{-1}$) صورت پذیرفت. نظر به تنوع بیولوژیکی سیانوفیتا، سعی بر تاکید بر ویژگی‌های غالب برای شناسایی بوده است. توصیف تکمیلی از گونه *Microchaete goeppertiana* Kirchner با استفاده از مهم ترین و ثابت ترین شاخص ها ارایه می گردد.

INTRODUCTION

It seems that in the oil polluted soil of south of Iran, especially the Khuzestan province, some strains of nostocales are common and even dominant (Soltani et al. 2012) but there is no clear report about their morphological characterizations and taxonomic situations. Both morphological variability and long time effect of oil pollution on behaviors of cyanophyta (and other microalgae), may cause some problems in studying of organisms of such special habitats. The proposed experiments in the study have shown that using the famous common manuals for determination of the genus and species of microchaete like what that are used for the other nostocalean cyanophyta is not applicable. These manuals need to be revised or even new identification keys are needed regarding to special

morphological variations of specimens with emphasize on local conditions (Soltani et al. 2010).

In recent years, molecular identification by partial sequence of 16S rRNA gene has helped regrouping of cyanophyta in some cases, corresponding to phylogenetically coherent taxa. In other groups in the traditional classification the extant diversity is underestimated (Gugger & Hoffman, 2004). Using physiological characteristics including pigment composition and photosynthetic characteristics have been introduced as more or less strong diagnostic features directly or beside morphological and molecular characterization especially since last two decades. (Komárek & Anagnostidis 1989; Soltani et al. 2010; Shokravi & Soltani 2011). In bacteriology, in particular the tolerances to the salinity of the environment have been recognized as an important phenotypic properties correlating with phylogeny (Gugger & Hoffman, 2004). Light is evidently one of the other important factors for natural distribution of cyanophyta (Valiente & Leganes, 1989). Additionally pH is the other factor that clearly affects the distribution of cyanophyta. Most cyanophyta grow in environments that are neutral to alkaline, and in laboratory cultures the optimal pH ranges from 7.5 to 10. Generally, a wide range of adaptation to pH has been observed not only among different genera but also between different isolates of the same species (Poza-Carrion et al. 2001; Soltani et al. 2006).

The aim of this research was to characterize the exact taxonomical position and also provide a comprehensive description of common but previously unexplored edaphic and periphytic taxon of Khuzestan province, *Microchaete goeppertiana* Kirchner. However, a comparative study on the morphology needs more consideration of the variations caused by illumination and combined effect of pH and salinity fluctuations. We proposed the clearly revised description at species level of the mentioned cyanobacterium in an oil-polluted habitat.

Up to now, except some disperse reports, we have no published data about microchaeteaceae of Iran. Soltani et al. (2009) have reported one species (*Microchaete tenera* Thuret) from Tehran province. Most of the published data about nostocalean cyanophyta of Iran have been restricted to paddy-fields and mainly floristic, physiological and ecophysiological studies (Siahbalaei et al. 2008; Pakzad et al. 2012). Some species of the genus *microchaete* have been reported in floristic investigations of paddy-fields of Northern provinces by Siahbalaei et al. (2008). Shariatmadari et al. (2011) recently identified five species of *nostocalean* from paddy-fields of Northern provinces. Pakzad et al. (2012) have investigated the morphological features of *Anabaena* and *Nostoc* strains from paddy-fields of Golestan province. Polyphasic investigation of *Fischerella* sp. FS 18 has been studied by Soltani et al. (2010). *Hapalosiphon fontinalis* (C. Agardh) Bornet is another stigonematalean cyanophyta which has been characterized morphologically by Shokravi et al. (2012). The combination effects of pH and limited irradiance on *Hapalosiphon* sp. FS 56 has been studied by Shokravi et al. (2012). So this is the first paper about morphological characterization of *microchaete* from south of Iran as a whole.

MATERIAL AND METHODS

Epidaphic, endaphic and periphytic samples were obtained from stations of Khuzestan province. A complete description about stations and their geographical situations and environmental conditions have been reported in Soltani et al. (2012). The collected samples were cultured by usual methods (Andersen 2005). After colonization and isolation, the blue- green algae *Microchaete* sp. was purified and incubated in axenic condition (Andersen 2005). At this phase it has been coded as *Microchaete* sp. ISC13 and preserved in algal culture collection of Research Institute of Applied Science, ACECR. Stock cultures were grown in BG110 solid and liquid medium. The cultivation was incubated under limited illumination (2 $\mu E\ m^{-2}\ s^{-1}$) in a gradient of pH (5, 6, 7, 8, 9) by using Tris and Hepes tampons in different salinity (0, 0.25, 0.5, 0.75 and 1%). The temperature was adjusted on 30 ± 1 ºC. Illumination was supplied with 40W cool white fluorescent tubes. Plates were placed at different distances from the light source to obtain a linear gradient of irradiance (Poza-Carion et al. 2001). Light measurements were made with a Licor LI-1000 Datalogger equipped with a quantum sensor. Alternatively, other experiments were carried out in batch cultures, using 100 ml of inoculated medium in 250 ml. Erlenmeyer flasks stoppered with cotton plugs. Culture was maintained without aeration or stirring and buffered and illuminated as above. After 48h of inoculation, when cells were fully adapted to culture condition, light regime, pHs and carbon dioxide concentrations, aliquots were taken and used for determinations (Shokravi et al. 2012). Morphological observations were made in liquid as well as on solid media. Form and color of aggregations, filament form and sheath presence, thallus growth, trichome structure and biometrical characteristics were recorded (Gugger & Hoffmann, 2004). Colony formation and cells shapes were evaluated by binocular and light microscope (in addition to phase contrast and epifluorescence microscopy) daily for two weeks. The growth curves

Table 1. Biometrical Analysis (µm) at different pHs of *Microchaete goeppertiana* Kirchner (Min- Max)

Cells	pH			
	11	9	7	5
F (L)	3.5-4	3.6-5	4.3-6	5.3-8.4
F (W)	4.5-7.2	4.4-4.8	6.3-8.2	4.5-6.5
T (L)	3.5-4	3.6-5	4.3-6	5.3-8.4
T (W)	4.5-8	4.5-8	6.3-8.3	4.5-6.5
H (L)	6.4-10.3	6.5-8	6.5-8.5	4.5-10.5
H (W)	6.2-10.5	6.3-8.3	6.4-10.5	6-8.4
S (L)	7.5-8.5	8-8.2	7.6-9.4	-
S (W)	7.5-8.4	8-8.2	7.6-9.3	-

L: Length; W: Width; S: Spore; H: Heterocyst; T: Trichome; F: Filament.

Table 2. Biometrical Analysis (µm) at different salinities of *Microchaete goeppertiana* Kirchner (Min- Max)

Cells	Salinity (%)			
	1	0.5	0.25	0
F (L)	6.1-7.9	6.5-7.9	4.8-7.4	4.5-7.5
F (W)	5.7-6.6	5.8-7.2	5.3-6.5	5.2-7
T (L)	6. -7.8	6.5-7.7	4.1-7.2	4.5-7.5
T (W)	5.7-6.5	5.8-7	5.3-6.5	5.2-6.8
H (L)	4.8-8.8	4.5-8.2	4.4-6.4	6.5-8.8
H (W)	6.8-10.1	6.7-8.3	4.9-8.3	6.3-8.8
S (L)	9.8-8.2	8-8.8	7.8-9.4	8.4-9.8
S (W)	9.8-8.2	8-8.8	7.8-9.4	8.4-9.8

L: Length; W: Width; V: Vegetative Cell; S: Spore; H: Heterocyst; T: Trichome; F: Filament.

were obtained via measurement of chlorophyll change daily using Jensen method (1978). Identification at the species level and morphological trait comparison was done according to John et al. (2003), Komárek & Anagnostidis (1989), Tiffany & Britton (1971), Prescott (1962), Desikachary (1959)

RESULTS

Aggregations of the strain tend to make a creeping manner of growth especially in solid cultures (Fig. 1). In liquid cultures tendency to make a clump shape form may be dominated but irregular expanding forms depending on pH and salinity. Centrifugal aggregation behaviours seem dominant but at extremely alkaline (pH11) and saline conditions (1%), centripetal forms may also be seen. In both cases intensive tendency to produce microbial mats seems noticeable (Stal 1995). This behaviour seems interesting and may be regarded as a constitutive trait. We saw both free floating and attached form especially at one side at liquid culture conditions that are important taxonomical features for microchaete species (John et al. 2003). Green (especially light green) was the most prominent color of aggregations but acidic and alkaline (not extremely) conditions at both DIC (dissolved inorganic carbon) availability and limitation cause dark green and even slightly yellow which may depend to chromatic adaptation (Bennet & Bogorad, 1973), (**Fig. 1**).

For studying the sheath, epifluoresence techniques cause better understanding. Light microscopy shows a very thin sheath and distinguishable only at free space of the filaments, but epifluoresence and phase contrast techniques reveal the sheath which is hyaline and not laminate (Fig. 3). The form of filaments may be remained straight both at liquid and solid cultures and may not be influenced by pH. Five or six day after inoculation at salinity (1%), slightly tapering toward the base may be seen. It may be regarded as a constant trait for identification. Tapering is quite different from *Anabaena* groups and seems near to straight groups of *phormidium* and *planctothrix*.

Fluorescence microscopy analysis, showed basically heterocysts. Intercalary heterocyst could not be distinguished using both phase contrast and light microscopy techniques at different salinity and pH conditions (Fig 3). So this trait may be constant at this strain and may not be affected by environmental fluctuations. The form and size of heterocyst may be depended on environmental conditions but usually it tends to keep cylindrical or oval-cylindrical form. Spores (akinetes) have more or less spherical form but the numbers of spores and distance from heterocysts may be seriously affected by combination effect of pH and salinity. Acidic conditions (pH 6) and lower

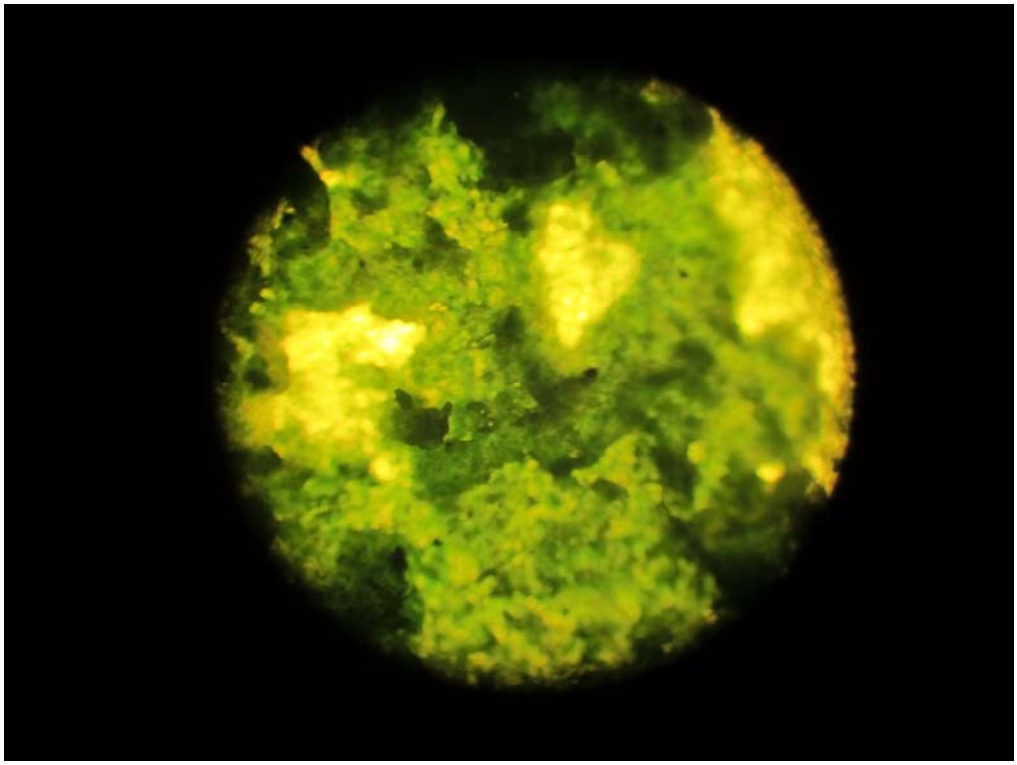

Figure 1. Aggregation behaviors of *Microchaete goeppertiana* Kirchner in Solid culture

salinities (0.25%) cause disperse spore production but moving toward alkaline and higher salinities short string of spores increase which are far from heterocysts (Fig. 2). A thin brown layer may be seen around spores only at pH 11 (**Figs 2, 3, 4).**

Biometrical analysis (Tables 1and 2) showed that the length and diameter of the vegetative cells and spores seem slightly more than that have already been recorded by John et al. (2003) and less than Desikachary (1959). At this species tendency to make spherical and rarely sub-spherical spores seems dominant and possibly constitutive trait, but in contrast, vegetative cells showed a wide flexibility and changed their form from rectangular to oval and oval-cylindrical (and even spherical) (Fig. 4, Tables 1 and 2).

DISCUSSION

Research on cyanophyta is a new field of study in Khuzestan province and Iran as a whole. So just a few Nostocalean and Stigonematalean morphotypes have been cultured and investigated like *Fischerella*, *Nostoc*, *Anabaena*, *Hapalosiphon*, *Phormidium*, *Lyngbya and Oscillatoria*. It should be admitted that the high variability of morphotypes found in nature is not represented in culture (Soltani et al. 2009; Soltani et al. 2010; Zarei-darki, 2009; Siahbalaei et al. 2008; Siahbalaei et al. 2010; Shokravi et al. 2012 . Results

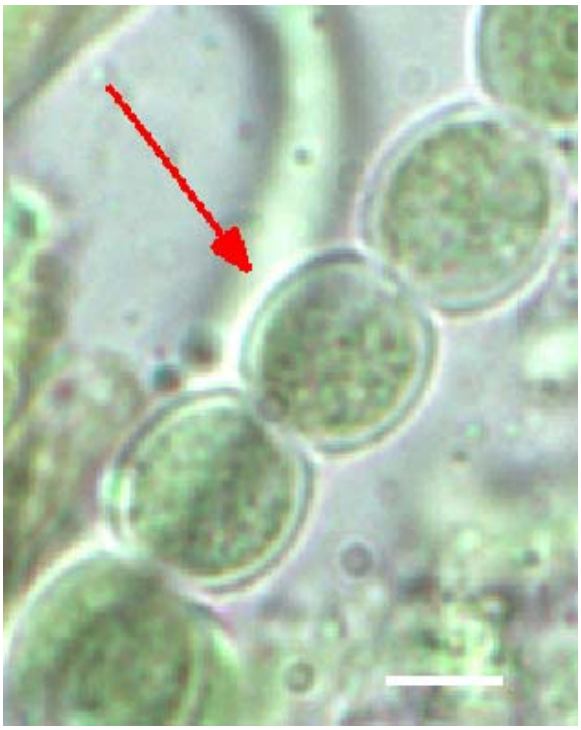

Figure 2. Spores (short strains) in *Microchaete goeppertiana* Kirchner (Scale: 7 μm)

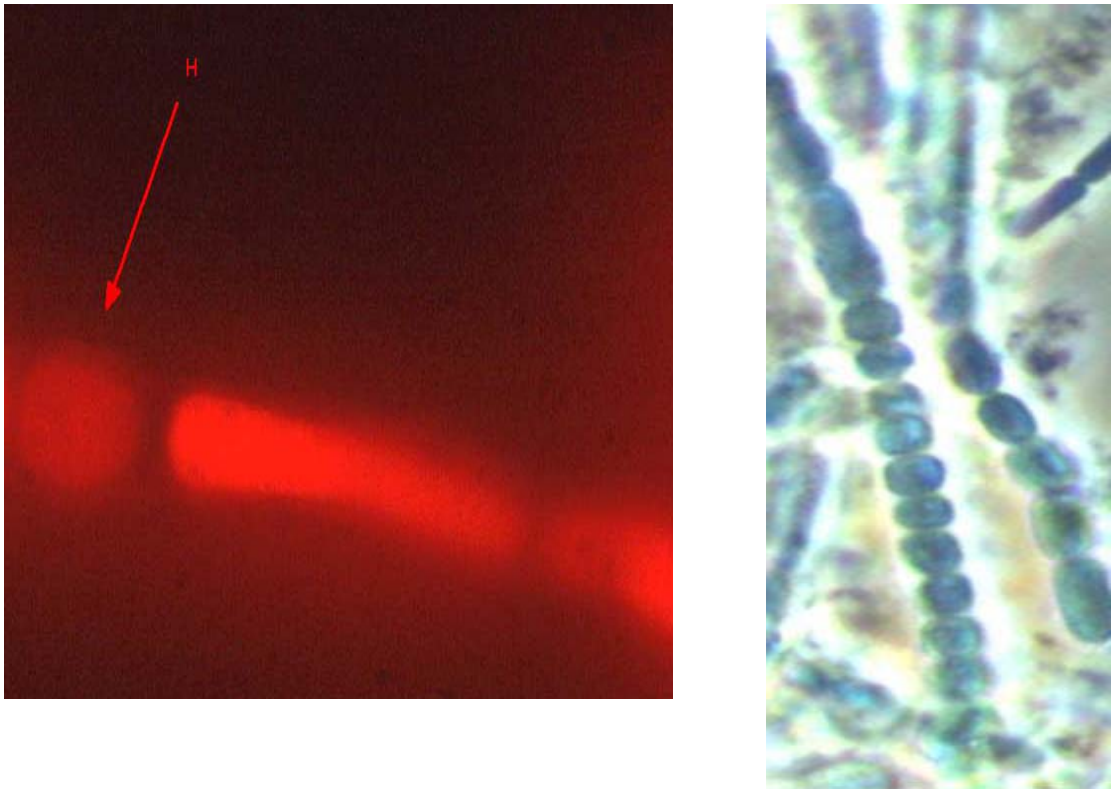

Figure 3. Filament (sheath presence and situation) using phase contrast microscopy (right) and heterocyst situation using fluorescence microscopy (left) in *Microchaete goeppertiana* Kirchner

seem not enough and are more local to show a primitive picture of the morphological and taxonomical situation of nostocalean cyanophyta in oil polluted soils. Our results were compared with four famous botanical and world wide identification keys (John et al. 2003, Prescott 1962, Tiffany & Britton 1971 Desikachary 1959). All mentioned identification keys have used morphological traits without studying morphological variations caused by environmental fluctuations. In Bergeys manual of systematics bacteriology (Castenholz 2001) *Microchaete* strains have been studied as form-genera of Subsection IV, not so multidisciplinary to help Iranian researcher to get exact information compatible to native organisms, especially epidaphic, endaphic and periphytic ones that show the highest variety of polymorphism and versatile metabolic behaviours. As a result, a new description based on native characters, worldwide descriptions, and new morphological data achieved from long time morphological characterization seems necessary.

pHs above 9 and below 5 affected the aggregation attachment. On solid medium, all isolates had a creeping growth. This was in agreement with the manuals (John et al. 2003; Desikachary 1959). In liquid culture we saw both attachment at one side and free floating aggregations. This was in agreement with the description of John et al. (2003) who emphasized on one side attachment of *Microchaete tenera* Thuretex Born. et Flah and *M. diplosiphon* Gomont ex Bornet & Flahault. Desikachary (1959) included many species in *M. tenera* and emphasized on one side attachment too. In Tiffany (1970) and Prescott (1962) one side attachments have been emphasized for species of microchaete but as said above both researchers have no report about laboratory culture conditions and so their results need to be regarded with cautious. The dominant color of aggregations was green but possibly chromatic adaptations caused light to dark and rarely gray greens. In extremely acidic conditions (pH 5) we saw yellow –green to even yellow colors but it may be related to death and lysis of the filaments caused by acidic shocks.

All of the world wide documents have emphasized on the straightness of the filaments of microchaete.

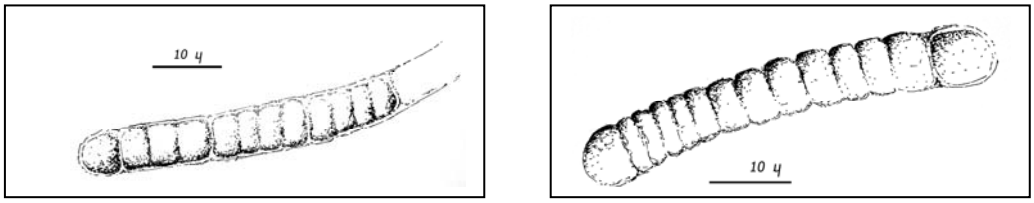

Figure 4. Filaments (left) and trichomes (right) in *Microchaete goeppertiana* Kirchner

Results of this study were in agreements with previous works but exact observations showed that high salinity (1%) and alkaline pH (11) may change the topology of filaments and cause trichomes to bend slightly, especially near the apices. By statistical analysis, it is difficult to reach a unique pattern in morphological variation in vegetative cells of this strain. However, with minimum light intensity (2 µE m^{-2} s^{-1}) and pH 9 cross enlargement of the trichome may be observed. Constriction of the cross walls may be nearly complete at 0.5-1% salinity and alkaline and extremely alkaline (pHs 9 & 11) conditions. Growth analysis showed that these organisms can be considered as an alkalophilic organism. Optimal growth rates were observed at pH 9 which is nearly equal to pH that usually in which the cyanophyta isolates were found (Soltani et al. 2009; Soltani et al. 2010).

From bacteriological point of view, it seems hard to reach certain taxonomical results even with using famous manuals. Basal-apical polarity of trichomes may be the most prominent diagnostic trait. In addition, special morphology of apical region of trichome (slightly enlarged terminal cells) together with the characteristics of the chain form of the spores may keep it in form-genus Microchaete (Castenholz 2001). There is not any precise description of the species (strains) of the form-genus and it has been restricted to the briefly discussed form-genus Tolypothrix. So it seems logical to emphasize on botanical references for determination and description at the species level. *Microchaete goeppertiana* Kirchner and *M. tenera var. tenuis* Barhadwaja are two candidates that may be considered using identification keys for determination. In *M. tenera* var. *tenuis*, we must see intercalary heterocysts. In addition the main filament is uniseriate, lateral branches show turf like appearance and filaments are30-40 µ wide. It seems that sheath production in this species may be affected completely by environmental fluctuations. However the width of sheath is not as wide as what have been reported by John et al. (2003). Accordingly more accurate polyphasic description of the species may be as the following:

***Microchaete goeppertiana* Kirchner** 1900

Aggregations depend on pH, irregular to clump shape, in liquid culture mostly centrifugal and only centripetal at extreme alkaline conditions (pH 11), at different salinities clump shape is predominant; aggregation color is mostly green, rarely light green (pHs 5 and 7) and yellow (pH 5), dark green aggregations maybe seen at alkaline conditions and different salinities (0.25% and 1%); filaments mostly straight, slightly bent toward the base, at alkaline conditions the lengths of filament decrease; lengths of the cells of filament completely depend on pH, but less depend to salinity, from 3.5 (pH11) to 8.4 (pH5), at different salinities, 4.5µ (0%) - 7.9µ (0.5 % and 1%), cell width is less variable than length, 5 to 7.2µ (8.2 at pH7); sheath present but very narrow and only distinguishable at free space; spores spherical (rarely sub-spherical) with a thick outer layer at extremes alkaline conditions (pH11),7-10 µ; hetrocyst spherical to sub spherical and oval, oval-cylindric at alkaline conditions and different pHs, apical at different conditions, with no intercalary position, 4.4- 8.4-10µ in length,4.9-6.2-10.5 in width depend salinity and pH.

Studied Site: Oil polluted regions of Khark Island (Khuzestan province).

Synonym(s): No synonyms are currently included in AlgaeBase

ACECR Cultures: Cultures from Culture Collection of Algae at Research Institute of Applied Science, ACECR.

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