BLUE-GREEN ALGAE INHABITING COASTAL BACKWATERS OF MANORA, KARACHI, PAKISTAN

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ABSTRACT

Altogether nine species of algae belonging to five genera, three families, two orders, one class and one phylum (division) were collected from Manora, Karachi. The blue-green algal species have been found growing on the backwaters of Manora. High tidal water floods the Mangrove area that brings up abundant amount of nutrients enriching sub littoral regions for the growth of phytoplankton and blue-green algae, which include members of Chroococcales having two species of *Aphanothece* and one species *Microcystis* genera and members of Nostocales having three species of *Lyngbya,* two species of *Oscillatoria* and one species of *Gloeotrichia* genera. These species were systematically investigated and periodically recorded during the year 2017.

Keywords: Blue-green algae, Karachi, *Aphanothece* sp., *Lyngbya* sp., *Microcystis* sp., *Oscillatoria* sp., *Gloeotrichia* sp.

INTRODUCTION

Blue-green algae are classified in phylum Cyanophycota by Shameel (2001, 2007, 2008). These are found in tropical and subtropical environments with an ability of photosynthesis. Blue-green marine algae are a group of organisms that are found in freshwater, marine or brackish environments comprising of more than 95 % of the flora (Baig *et al.,* 2002; Valeem and Shameel, 2005). Approximately 740 species were documented in India of which around 60 species are of commercial importance (Ramalingam *et al.,* 2000). The blue-green algae are involved in nitrogen fixation in light as well as in dark conditions. They contribute a significant total nitrogen input into the ecosystem of mangrove (Potts, 1979). The blue-green algae are also considered as bio indicator of pollution besides its commercial and industrial value (Moore, 1981; Patterson, 1996) but very few and less information are available regarding their availability in Manora backwaters. The coast of Manora is situated strategically under the control of Pakistan Navy and Coast Guards due to which it is saved from anthropogenic activities. The blooms of blue-green algae were recorded from sandy portion of upper littoral area indicating increasing rate of pollution. The members of cyanophycota found in mangrove area were reported by Saifullah and Taj (1995), Shameel *et al.* (1996), Saifullah *et al.* (1997) and Bano and Siddiqui (2003). In the present paper blue-green algae collected from mangrove area of Manora backwaters are collected, microscopically examined and systematically arranged according to Shameel (2001). Leaf litter of mangroves hosts several blue-green algae to play important ecological processes, including a significant number of undescribed taxa (Alvarenga *et al.,* 2015; Oliveira *et al.,* 2016). In order to investigate and access unknown taxa, the present study was undertaken with the purposes of isolating and characterizing blue-green algae inhabiting the phyllosphere of *Avicennia marina* trees from mangrove forest of Manora.

MATERIALS AND METHODS

Samples of seawater and leaves of *Avicennia marina* containing blue-green algae, plankton and soil of Mangrove habitat were collected during September 2017 by horizontal scoop net (mesh size 50 μ) from sandy muddy area (Altitude, -48.0 m; Latitude, 24º 83′ 35″ N; and Longitude, 66º 92′ 32″ E) during low tidal condition. The samples were preserved in 4 % seawater: formalin mixture soon after collection in plastic bottles and brought to the laboratory for exploration of marine resources (LEMR) for further identification and microscopic examinations. Salt-excreting leaves of the mangrove *i.e. Avicennia marina* are the habitat of blue-green algae, which were isolated and characterized ecologically, morphologically and genetically. Seawater samples were collected for studying physico-chemical features through Nansen bottle and analysed by using standard methods (APHA, 1985). Lund *et al.,* (1958) used Utremohal Method to determine species composition. Compound microscope of Nikon Labophot (Japan, 10x40) was used for anatomical studies of algal species, which were later on used literature like (Tilden, 1910; Hustedt, 1930; Abdul-Majeed, 1935; Smith, 1950; Desikachary, 1959; Prescott, 1962; Siddiqi and Faridi, 1964; Patrick and Reimer, 1966, 1975; Philpose, 1967; Tiffany and Briton, 1971; Vinyard, 1979; Akiyama and Yamagishi, 1981; Whitton, 2011; Oliveira *et al.,* 2016) for identification.

RESULTS

CLASS CHROOCOPHYCEAE

The members of this class are found in colonial or palmelloid or unicellular form.

Order Chroococcales

In this order cells form an irregular colony, which is loosely bounded with gelatinous sheath. Cell division or endospores formation is the result of reproduction; No exospores and nannocytes present. The blue-green algae comprising of the members of Chroococcales having two species of *Aphanothece* and one species *Microcystis* genera; of Nostocales three species of *Lyngbya*, two species of *Oscillatoria* and one species of *Gloeotrichia* genera (Table I; Figs. 1-9).

Family Chroococcaceae Nägeli (1849)

Usually colonial, ensheathed brackish, marine or freshwater blue-green algae that reproduce by colonial fragmentation and simple cell division and are usually isolated in a distinct order. The cells are lamellated with mucilage forming amorphous and shapeless colonies or occur singly in spherical, ellipsoidal, cylindrical, tubular or hemispherical and spindle shaped having thick membrane. Elongated cells are divided in only one direction but usually in two or three directions; single parent sheath may contain cells of many generations. Cells multiply occasionally through nannocyte. Planococci are present with firm membrane. Genera, which were collected might be distinguished as followed:

1.	A few cells or a colony …..….	1*
	A colony of cells without any definite arrangement ……..…….	2
2.	Cells ellipsoidal to cylindrical ……	*Aphanothece*
	Cells packed into microscopic colonies ……..	*Microcystis*

Aphanothece Nägeli

The organism is single celled or in colony of two or more than two cells enveloped concentrically, towards the outside of the thallus particularly. Cells are many roughly shapeless, cylindrical or ellipsoidal, slightly bent or straight, lamellated, mucilage homogenous, found individually enveloped and gelatinized. Nannocytes present divide transversely. Species collected might be distinguished as:

1.	Ovoid cells with diameter of 3.5 - 5.0 μm ……..……	*A. pallida* (1)
	Cells having 4 μm width and 6.4 μm length ……...	*A. stagnina* (2)

1. *Aphanothece pallida* (Kützing) Rabenhorst 1863: 76

Basionym: *Palmella pallida* Kützing

References: (Cotton, 1912: 178; Huber-Pestalozzi, 1938: 342; Hirose *et al.*, 1977a: 933; Komárek & Anagnostidis, 1989: 548; Whitton, 2011: 158).

General characters: Extensive colonies are terrestrial, gelatinous and olive-green. Blue-green to olive grainy cells, ovoid cells become spherical after division freely found in the main matrix and; enveloped concentrically closely packed at margins having 7.0 - 10.5 μm length, 3.5 - 6.0 μm diameter. Transparent or yellowish envelopes at outsides, closely packed in concentric manner measuring 9.5 - 12 μm crosswise. Nannocytes absent (Fig. 1).

Geographical distribution: it is cosmopolitan in temperate regions. Wet rocks, Lochbuie, Isle of Mull; Pakistan.

Local distribution: Freshwater/ terrestrial species; found growing on damp soil or sand along creek banks of Mangrove swamp of Manora.

Remarks: The soil of Manora Mangrove swamp is damped with sewerage and industrial water inflow throughout the year, where temperature may rise up to 42° C making hot surroundings. Material mainly collected from swampy areas of Manora backwaters during September 2017.

Table I. Locality and date of collection of the investigated algal species (taxonomically arranged according to Shameel (2001; 2008).

S. No.	Algal Taxa	Locality	Date
A	**KINGDOM MONERA**		
1	**PHYLUM CYANOPHYCOTA**		
I	**CLASS CHROOCOPHYCEAE**		
1	**ORDER Chroococcales**		
1.	**Family Chroococcaceae**		
1.	*Aphanothece pallida* (Kützing) Rabenhorst	Manora	September 2017
2.	*Aphanothece stagnina* (Sprengel) A. Braun	Manora	September 2017
3.	*Microcystis aeruginosa* (Kützing) Kützing	Manora	September 2017
II	**CLASS NOSTOCOPHYCEAE**		
2	**ORDER Nostocales**		
2.	**Family Oscillatoraceae**		
4.	*Lyngbya hieronymusii* Lemmermann	Manora	September 2017
5.	*Lyngbya majuscula* (Dillwyn) Harvey	Manora	September 2017
6.	*Lyngbya martensiana* Meneghini *ex* Gomont	Manora	September 2017
7.	*Oscillatoria princeps* Vaucher	Manora	September 2017
8.	*Oscillatoria sancta* (Kützing) Gomont	Manora	September 2017
3	**Family Rivulariaceae**		
9.	*Gloeotrichia raciborskii* Woloszynska	Manora	September 2017

2. *Aphanothece stagnina* (Sprengel) A. Braun in Rabenhorst 1863: no. 1572

Basionym: *Coccochloris stagnina* Sprengel

References: (Rabenhorst, 1865: 66; Tilden, 1910: 32; Geitler, 1932: 164; Desikachary, 1959: 137; Humm and Wicks, 1980: 49; Olenina *et al.*, 2006).

General characters: Thallus generally subspherical, 1.0-2.5 μm. Colourless or yellowish, lamellated sheath up to 15.5 μm thick; Oblong or polygonal, cylindrical cells 4.0 μm broad and 6.4 μm long, single or colonies, embedded or enveloped in a common mucilage (Fig. 2).

Geographical distribution: Wet rocks, Lochbuie, Isle of Mull. Inhaea Island near the northern bay. Chidenguele: (Rino, 1972); Bela Vista: Inhaca Island (Rino, 1972); marine, on rocks and sand (Silva *et al.*, 1996); Namaacha (Rino, 1972); Sul do Save: (Rino, 1979), Shallow artificial basin, France, Paris, 1990, Pakistan.

Local distribution: Karachi District: Manora.

Remarks: It is being recorded for the first time from the marine or brackish habitat of Manora backwaters, mostly water logged with toxic water of tanneries, Pakistan.

***Microcystis* Kützing**

Cells are ellipsoidal or irregularly overlapping or net-like colony; cells are colourless or homogenous, free swimming, diffluent mucilage, elongated, spherical, mostly very densely arranged, attached with daughter colonies; individual envelope absent, elongated cells divide transversely but generally in all directions; gas filled vacuoles and nannocytes present. Only one species has been collected that may be distinguished as:

1.	Colonies several times longer than broad …….	1*
	Colonies not several times longer than broad ……...	2
2.	Clathrate colony……..	*M. aeruginosa*
	No clathrate colony ……	1

3. *Microcystis aeruginosa* (Kützing 1833) Kützing 1846

Basionym: *Micraloa aeruginosa* Kützing 1833

References: (Crow, 1923: 61; Frémy, 1929: 18, 1933: 10; Desikachary, 1959: 93; Vasishta, 1960: 582; Starmach, 1966: 78; Gupta, 1972: 483; Nizamuddin and Gerloff, 1982: 138; Day and Wiskich, 1995: 203; Aftab and Shameel, 2006).

General characters: Spherical, irregularly lobed and clathrate colony of numerous spherical cells; within a gelatinous matrix, several colonies embedded together in a matrix and forming a sheath or rope-like structure colonial mucilage homogeneous, maintaining a definite shape for the colonies; cells 3.5-5.0 μm in diameter, contents blue-green, highly granular and with pseudovacuoles (Fig. 3).

Geographical distribution: Australia: New South Wales (Day and Wiskich, 1995); Queensland (Day and Wiskich, 1995); South Australia (Day and Wiskich, 1995); Victoria (Day and Wiskich, 1995); South America: Brazil (Bicudo *et al.,* 2002), Pakistan: Miani Hor, Balochistan (Aftab and Shameel, 2006).

Local distribution: Karachi District: Manora backwaters.

Remarks: It is being recorded for the first time from the marine or brackish habitat of Manora backwaters, while previously it has been reported form Miani Hor, Balochistan, Pakistan by Aftab and Shameel (2006).

Family Oscillatoriaceae

The trichomes are unbranched, which consist of uniformly broad cells arranged in a single row and tapering at the ends; they are with or without lamellated or diffluent or homogeneous mucilage, having strong sheath; growth apical and intercalary; filaments regularly or irregularly straight or spirally coiled; many hormogonia present along the longitudinal axis showing a spiral movement by rotation. Following two genera of this family were collected that might be distinguished as:

1.	Sheath present ……..	2
	Sheath absent ……	3
2.	Cells rounded…….....	*Lyngbya*
	Cells isodiametric……..	3
3.	Filaments not coiled into spirals……	*Oscillatoria*
	Filaments coiled into spirals……..	1

Lyngbya* C. A. Agardh 1824: 25 *ex* Gomont 1892: 118, *nom. cons.

Trichomes straight; sheath forward and colourless, cells in a row, end-cell rounded. Following two species were collected, which may be distinguished as:

1.	Sheath firm, colourless, homogenous, ……..…….....	*L. hieronymusii* (4)
	Sheath firm, macroscopic diameter up to several cm ……..	2
2	Sheath colourless, lamellated ……	*L. majuscula* (5)
	Trichomes isopolar, straight or slightly waved …….	*L. martensiana* (6)

4. *L. hieronymusii* Lemmermann 1905: 146

Basionym: *Lyngbya hieronymusii* Lemmermann 1905: 146.

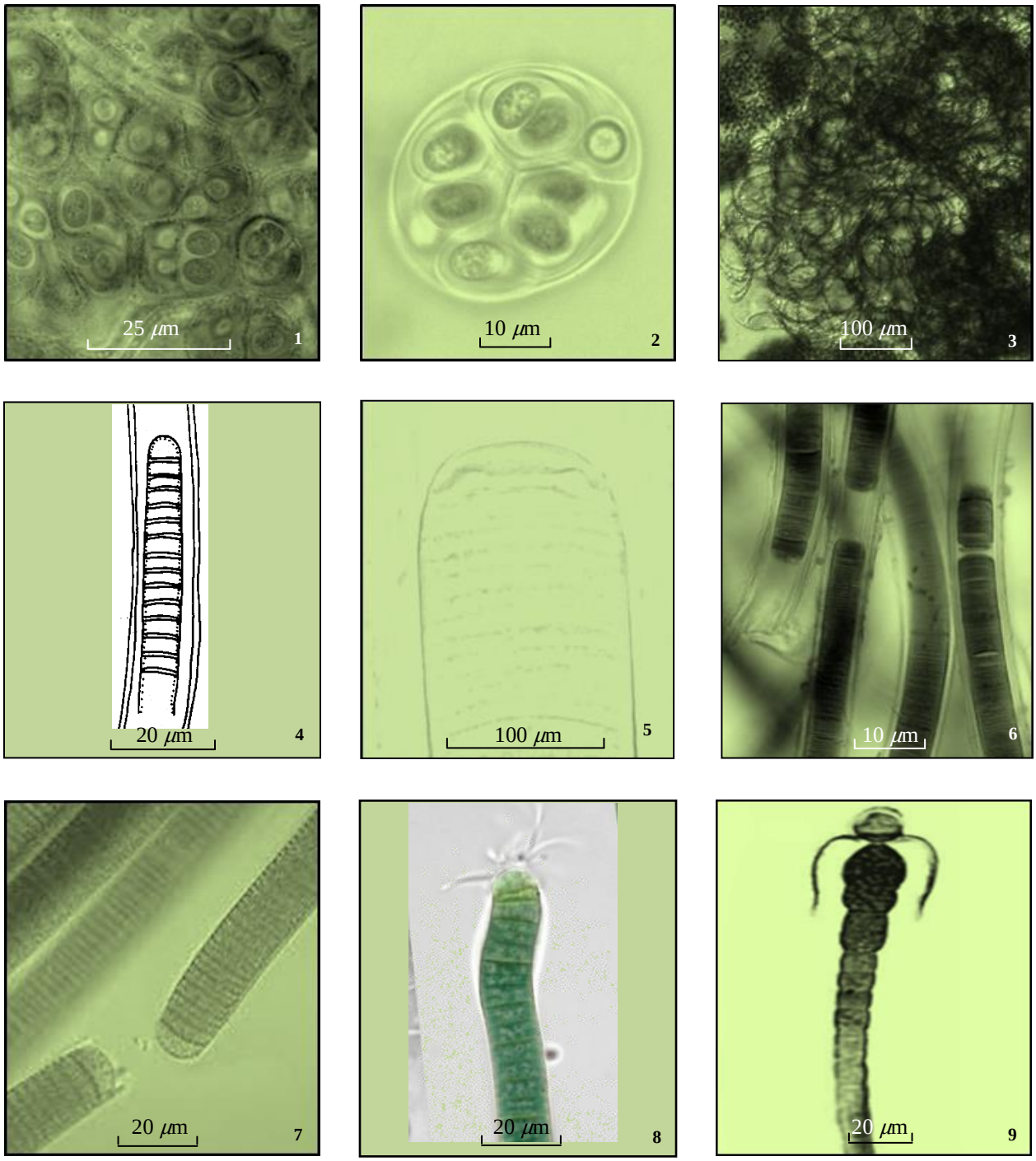
References: (Lemmermann, 1910: 139; Frémy, 1930: 192; Geitler, 1932: 1047; Desikachary, 1959: 297; Hirano, 1964: 176; Prescott and Vinyard, 1965: 501; Islam, 1976: 71; Masud-ul-Hasan, 1980: 76; Masud-ul-Hasan and Batool, 1987: 347; Masud-ul-Hasan and Yunus, 1989: 104; Naz *et al.,* 2004: 468).

General characters: Filaments single, free-floating, straight, 16-18 μm broad; sheath firm, homogenous, colourless; cells 11-13 μm broad and 3 - 4 μm long, not constricted at the cross-walls, granulated; end-cell broadly rounded (Fig. 4).

Geographical distribution: India: Allahabad; Pakistan: Karachi District, Manora.

Local distribution: District Karachi: Manora backwaters.

Remarks: The collection was made during summer. It was found in stagnant water channel in free-floating state.



Figs. 1-9. Blue-green algae collected from Manora backwaters, Karachi: 1. *Aphanothece pallida* (Kützing) Rabenhorst; 2. *A. stagnina* (Sprengel) A. Braun; 3. *Microcystis aeruginosa* (Kützing) Kützing; 4. *L. hieronymusii* Lemmermann; 5. *L. majuscula* (Dillwyn) Harvey; 6. *L. martensiana* Meneghini *ex* Gomont; 7. *Oscillatoria princeps* Vaucher; 8. *O. sancta* (Kützing) Gomont; 9. *Gloeotrichia raciborskii* Woloszynska.

5. *Lyngbya majuscula* (Dillwyn) Harvey *ex* Gomont: 151 (1892)

Basionym: *Lyngbya confervoides* C.Agardh *ex* Gomont.

References: (Tilden, 1910: 123; Geitler, 1932: 1060; Desikachary, 1959: 313; Humm and Wicks, 1980).

General characters: It has long filaments, 50.0 - 52.0 μm broad flexuous. Colourless, lamellated and thick 3 - 4 μm sheath. Trichome neither constricted at the cross-walls nor attenuated at the ends, cross-walls not granular. Width of cells 9-10 times greater than length that is ranging from 5.0 - 10.9 μm and breadth from 39.0-45.0 μm. Calyptra absent, protoplasm homogeneous dark green (Fig. 5).

Geographical distribution: Channel Islands (Lyle, 1920); France (Feldmann, 1954); Greece (Gkelis and Panou, 2016); Ireland (Adams, 1908, Cotton, 1912), Pakistan: Karachi District, Manora.

Local distribution: District Karachi: Manora backwaters.

Remarks: The collection was made at the end of summer. It was found in stagnant water channel.

6. *Lyngbya martensiana* Meneghini *ex* Gomont

Basionym: *Lyngbya martensiana* Meneghini *ex* Gomont

References: Meneghini (1837); Gomont, [1892 (1893)]; Geitler (1932); Etheredge and Pridmore (1987).

General characters: It is filamentous, macroscopic, up to several cm in diameter, layered, connected with thick and firm sheaths. Trichomes are isopolar, straight or slightly waved, uniserial, composed of shortly cylindrical or barrel-shaped discoid tells, 5.5-60 μm thick, cross walls are constricted or unconstricted (Fig. 6).

Geographical distribution: It is a ubiquitous species. India: Kolkata, Lucknow, Pakistan: Karachi District, Manora.

Local distribution: Sialkot District: Head Marala (1986), Karachi District South, Manora.

Remarks: The material was collected from stagnant water channel after summer. The pH of stagnant water channel was alkaline (8.0).

***Oscillatoria* Vaucher 1803: 165 *ex* Gomont 1892: 198**

Thallus filamentous, cells in a narrow row, commonly differentiated filaments; filament free, no exospores and endospores formed; reproduction by the formation of hormogonia, akinetes or resting spores; cross-walls dim and often granulated. The present collection included two species, which may be distinguished as follows:

1. Trichomes not constricted at the cross-walls …… *O. princeps* (7)
   Trichomes very thin, forming mats of yellow green colour ……..… *O. sancta* (8)

7. *Oscillatoria princeps* Vaucher

Basionym: *Oscillatoria princeps* Vaucher

References: Brühl and Biswas, 1923: 3; Geitler, 1932: 978; Desikachary, 1959: 240, Hirose *et al.,* 1977b; Masud-ul-Hasan and Zeb-un-Nisa, 1986: 234; Naz *et al.,* 2004: 506.

General characters: Trichomes solitary, not constricted at the cross-walls, quite straight, narrow towards ends, subobtuse, non capitate; non calyptrate apex, which is more often bent at sides; cells 5 - 7 μm broad, 3 - 4 μm long, contents bluish-green (Fig. 7).

Geographical distribution: India: Kolkata; Pakistan: Sialkot, Manora.

Local distribution: Sialkot District: Sambaraal Road.

Remarks: It was collected during autumn from stationary water ponds in free-floating state.

8. *Oscillatoria sancta* (Kützing) Gomont

Basionym: *Oscillatoria sancta* (Kützing) Gomont

References: Gomont, 1892: 229; Geitler, 1932: 977; Desikachary, 1959: 241; Starmach, 1966: 327; Drouet, 1968).

General characters: Dense mucilaginous yellowish green mat having bubbles, trichomes very thin, forming mats of yellow green colour, attenuated, not constricted or granulated, conical relatively bent, not capitates, 4 - 5 μm broad; cells broader than length, 2.0-3.5 μm long, obtuse at the end. Nercridia and any other reproductive structures not recorded. Habitat ecology: Thick mats from bloom at the surface of pool at sandy area.

Geographical distribution: India, Pakistan: Buleji.

Local distribution: Buleji Leg, Manora.

Remarks: It was collected during autumn from quiet water ponds in free-floating state.

Rivulariaceae

It is a filamentous family; filaments are simple, heteropolar, distinguished into basal and apical parts, joined parallel at irregular strata, diameter is several mm to cm thick; several layers in strata gelatinous to leathery or intensely covered by calcium carbonate, trichomes agglomerated densely, oriented by the apical hair-like parts to the surface of the colony covering the substrate. Trichomes are cylindrical at the cross walls, constricted or unconstructed, dividing at intercalary heterocytes. Trichomes remain divided located parallel in the colony within the mother sheaths. Hyaline, narrow, long cells form hairs. Firm sheath, coluorless and lamellated. Aerotopes and akinetes lacking. Trichomes dissociate to reproduce within colonies and form heterocytes. Several species are known from littoral region of marine environment preferably in clear, unpolluted, stagnant or streaming waters (Komárek *et al*., 2014).

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***Gloeotrichia* J. Agardh ex Bornet & Flahault**

A genus of numerous species, found throughout the world. Thallus spherical, longer than broad cells at the base shorter than broad. The present collection included only one species that might be distinguished as:

1. Trichomes shorter than broad ……. (1*)
   Sheath covers the basal heterocyst ……...……... *G. raciborskii* (8)

9. *Gloeotrichia raciborskii* Woloszynska

Basionym: *Gloeotrichia raciborskii* Woloszynska

References: Brühl & Biswas 1923: 3, Geitler 1932: 978, Desikachary, 1959, p. 563, Masud-ul-Hasan and Zeb-un-Nisa 1986: 234; Naz *et al.,* 2004: 506.

General characters: It has soft and spherical thallus having broader filaments with breadth of 30 - 40 μm and length up to 500 μm; trichomes have breadth 9 - 10 μm, end up in a long hyaline structure; heterocyst are up to 10 - 13 μm in diameter; akinete is 10 - 16 μm broad and 24 - 40 μm long; at the base of the trichome cells are thinner than broad, pale blue-green in colour; the basal heterocyst is covered with sheath and thinning out from base to apex, exhibiting conical shape with dull brown in colour (Fig. 9).

Geographical distribution: Australia: Day and Wiskich, 1995; Entwisle and Nairn, 1999; India: Jodhpur, Rajasthan (Vishnoi *et al.* 2008); Pakistan: Miani Hor, (Aftab and Shameel, 2008).

Local distribution: Pakistan: Miani Hor, (Balochistan), Manora (Sindh)

Remarks: During young stage, they were found attached with debris and leaf litter but later occurred free-floating in the form of pale and brown masses.

DISCUSSION

The members of Cyanophycota are found on mangrove trees of *Avicennia marina* and *Ceriops tagal* at Inhaca Island. They are associated with several species of red algae on pneumatophores of and on the base and trunks of afore-mentioned two species of mangroves, which provide suitable habitat for the development of the members of Cyanophycota that can be observed on the pneumatophores. It can be explained that pneumatophores are less vulnerable to extreme environmental conditions like high light intensity, high temperature resulting into desiccation than on the base of *C. tagal*. Siva, (1991) identified sixteen taxa of Cyanophycota including 12 genera (*Aphanothece*, *Arthrospira*, *Calothrix*, *Chamaecalyx*, *Chroococcus*, *Hydrococcus*, *Lyngbya*, *Microcoleus*, *Nodularia*, *Oscillatoria*, *Stichosiphon* and *Xenococcus*. Members of cyanophycota in the form of bloom were observed from the sandy pool of the Buleji coast Karachi. The study revealed presence of two species of *Oscillatoria* and one species of *Lyngbya*, out of which *Oscillatoria brevis* was first time reported from the coast of Karachi (Munawar and Aisha, 2017).

The other species were previously reported by Bano (1998); Bano and Siddiqui (2003); Shameel *et al.* (1996); Saifullah *et al.* (1997); Saifullah and Taj (1995), but not in the form of blooms. The species of *Oscillatoria* described in this paper were identified basically on the measurement of their width and the apical cell (Anagnostidis and Komárek, 1988); Desikachary, 1959). Suda *et al.* (1998) reported *Oscillatoria brevis* and *O. tenuis* the width of the cell 4-5 μm broad while on the other hand had much more broader cells in comparison with *O. brevis*, up to 11 μm while the apical cells were also found attenuated. The diagnostic feature of calyptra may be inconstant as its' absence or presence depend on different environmental conditions or the developmental stage of individual filaments in *Lyngbya hieronymusii* var. *hieronymusii* (Suda *et al.*, 1998).

High tidal water floods the Mangrove area of Manora that brings up abundant amount of nutrients enriching sub-littoral regions for the growth of phytoplankton and blue-green algae, which include members of Chroococcales having two species of *Aphanothece* and one species *Microcystis* genera and members of Nostocales having three species of *Lyngbya*, two species of *Oscillatoria* and one species of *Gloeotrichia* genera. These species were systematically investigated and periodically recorded during September 2017. It was noticed during the collection that algal organisms were associated with the oil pollution and leaf litter. Algal species contaminated with oil exhibited proliferated growth of blue-green algae especially *Oscillatoria* sp. (Table I; Figs. 7 & 8), which was thought provoking to initiate research on the growth of oil eating blue-green algae. The prolific growth of blue-green algae is a sign of pollution in seawater of mangrove habitat, which may create toxicity and choke the pores of pneumatophores causing anoxic conditions to produce cyanotoxins.

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