Pak. J. Bot., 55(1): 411-417, 2023. DOI: http://dx.doi.org/10.30848/PJB2023-1(34)

DIVERSITY AND DISTRIBUTION OF CYANOBACTERIAL COMMUNITY IN MANGROVE STANDS AND COASTAL WATERS ALONG THE COAST OF PAKISTAN

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Abstract

Cyanobacteria are a diversified group of microorganisms found in variety of habitats. For a better understanding of their diversity and distribution, these cyanobacteria epiphytic on pneumatophores of mangroves, adjacent surface sediments, and channel water were studied. A total of 47 taxa were observed belonging to five orders and fifteen families. The majority of the taxa were recorded in the order Oscillatoriales followed by Synechococcales and Chrococcales. Family Oscillatoriaceae contributed substantially (10.26%) in the order Oscillatoriales. While, in the order Synechococcales, Merismopediaceae had considerably contribution (17.95%) and the order Chroococcidiopsidales was represented by only one family. Diversity and distribution of cyanobacteria in the mangrove habitat was observed where fourteen species were recorded as epiphytes on pneumatophores and thirty seven species were distributed on the surface sediments. On the other hand, twenty one and fourteen species were present in adjacent channel waters of Manora and Sonmiani, respectively. Results showed that Manora channel water has greater cyanobacterial diversity compared to Sonmiani Bay waters. Diversity indices also reflect the same pattern. High species richness R_1 (9.36) and species diversity 0.97 (λ) and 3.53 (H') were recorded for surface sediment. Evenness (J') was similar (1.0) in all collected material from different sitesr however the dominance (D) was higher (0.07) for pneumatophore at Sandspit and channel water of Sonmiani Bay. Present study is initiated as preliminary survey to record the distribution and diversity of cyanobacteria which play a pivotal role in microbial food web. During the study it has been revealed that they have capabilities to act as nitrogen fixers, recyclers of nutrients and primary producers, thus further studies on significant contribution of cyanobacteria in the unique mangrove ecosystem are suggested.

Key words: Cyanobacteria, Species diversity, Distribution, Pakistan.

Introduction

Mangrove forests are very productive and dynamic ecosystem common in the tropical and subtropical regions (Vannucci, 1988; Agoramoorthy *et al*., 2008). The mangrove swamps have received much attention as they are important as natural barrier in the protection of coastline from wave erosion, coastal fisheries as well as in the sustenance of overall complex ecosystem (Alongi, 2008; Kathiresan, 2012; Kumar *et al*., 2021). These mangroves have capability to live in vary harsh environment and for their survival they develop some adaptations such as modified root system, salt glands on leaves and osmo-regulation, (Lovelock *et al*., 2015; Srikanth *et al*., 2016).

Mangroves in Pakistan are largely consisted of *Avicennia marina*, which covers about 95 % of its total cover (Siddiqui *et al*., 2008; Jabeen *et al*., 2021). Mangroves offer diverse habitat for assemblages of macro- & micro-fauna and flora (Siddiqui *et al*., 2000; Shafique *et al*., 2015a). A few reports are available on mangroves and their inhabitants along the coast of Pakistan. For example, literature is available on mangrove soil (Ibrahim *et al*., 1995) and forest structure (Rasool & Saifullah, 1996; Farooq & Siddiqui, 2020), leaf-litter production and decomposition (Farooqui *et al*., 2012; Shafique *et al*., 2013; Shafique *et al*., 2015b), benthic diatoms (Saifullah & Chaghtai, 1993), algal epiphytes (Saifullah & Taj, 1995, Saifullah *et al*., 1997; Saifullah & Rasool, 1998; Siddiqui *et al*., 2000), phytoplankton (Shoaib *et al*., 2017), zooplankton (Huda & Ahmed, 1988).

Among microalgae, cyanobacteria are major contributors (Carreira *et al*., 2015) and carry out photosynthesis thus provide organic rich matter and oxygen to other inhabitants. These cyanobacteria also play a pivotal role in carbon budget and capable to fix atmospheric nitrogen therefore serves as nitrogen fixer in the system (Kulasooriya & Magana-Arachchi, 2016). Cyanobacteria occupy several niches in mangrove habitat and can be found attached to pnumatophores (roots), floating as planktonic form in channel water, or benthic forms as cyanobacterial mats on sediment. Pnumatophores provide a considerable space for attachment of epipphytic cyanobacteria (Nedumaran *et al*., 2008; Estrada *et al*., 2012). On Sediment surfaces microalgal communities occupy and grows as thick carpet like structure or mats composed of various micro-organisms including cyanobacteria (Rothrock & Garcia-Pichel, 2005; Gaysina *et al*., 2019). Cyanobacteria are found growing as planktonic forms in the water column in channels and creeks (Caroppo *et al*., 2006; Andreeva *et al*., 2020).

The literature on marine cyanobacteria (blue-green algae) from Pakistan is obviously missing except for a few reports (Shameel & Tanaka, 1992; Bano, 1998). A few species of cyanobacteria from coastal and inshore waters have been reported (Bano & Siddiqui, 2003, 2004). The only data available for cyanobacterial association with mangroves is from tidal creeks (Saifullah & Taj, 1995; Siddiqui *et al*., 2000; Zaib-un-Nisa *et al*., 2000; Ahmed *et al*., 2016) and Sonmiani Bay (Saifullah *et al*., 1997; Mansoor, 2019). Owing to the scarcity of

available information on cyanobacteria, studies on the diversity and ecological significance of these phototrophs are required. The present study is an attempt to describe the species diversity of cyanobacteria in the two distinct mangrove stands. This preliminary survey would enhance the existing pool of knowledge of this neglected group and thus form a basis for future studies on their role in the unique mangrove ecosystem.

Materials and Methods

Sampling was done in mangrove habitat along the Karachi coast including Sandspit (SP; 24°49'21.83"N, 66°56'24.01"E), Manora Channel (MC; 24°48'7.71"N, 66°58'28.87"E) and Sonmiani Bay (SB; 25°30'53.42"N, 66°30'45.26"E) area (Fig. 1).

Sediment samples were collected from mangrove habitat at Sandspit backwaters. These samples were taken using a sterile plastic corer and the top first and second centimeter layers were separately stored in sterile tube for analysis. Pneumatophores, the respiratory roots of mangroves, were removed and cut into three portion, *i.e.* top, middle and bottom regions, with the help of sterilized scissor and secured in screw-caped tubes. Water samples were collected from surface using sterile water sampler at two different locations: a) Sonmiani Bay, along Balochistan coast is a location where three mangrove species occur naturally. This is a less polluted area with some sewage input from the small local community settlements; b) Manora backwater, a natural passage used for ship traffic to and from the port at Karachi. It receives Lyari River, essentially bringing untreated domestic and industrial waste, at the distal end and is open to open sea on the other end at a distance of about 8 Km.

Samples were brought to the laboratory under ice for further analysis. Cuttings of pneumatophores, sediments and water samples were inoculated in ASN III medium (Rippka *et al*., 1979). Any visible growth was aseptically transferred into other tube containing fresh media and final isolation was achieved using serial dilution technique or by streaking onto solidified (agarized) ASN-III medium (Appendix I). Isolates were kept under 12h/12h light/dark cycle with a light intensity of ca. 2000 Lux at room temperature (ca. 30±2°C). Direct observation of the field material was also done using Light microscopy (Olympus, Japan). Measurements of cells, trichome or filaments were taken averagely.

Taxonomic identification of cyanobacteria was carried out according to botanical mode of classification provided by Komarek and Anagnostidis (1986, 1989), Anagnostidis and Komarek (1985, 1988) and Desikachary (1959). Nomenclature for all given taxa were validated according to currently used names on Algae Base (Guiry & Guiry, 2018). Diversity indices were performed by using PRIMER v.7 (Clarke & Gorely, 2015).

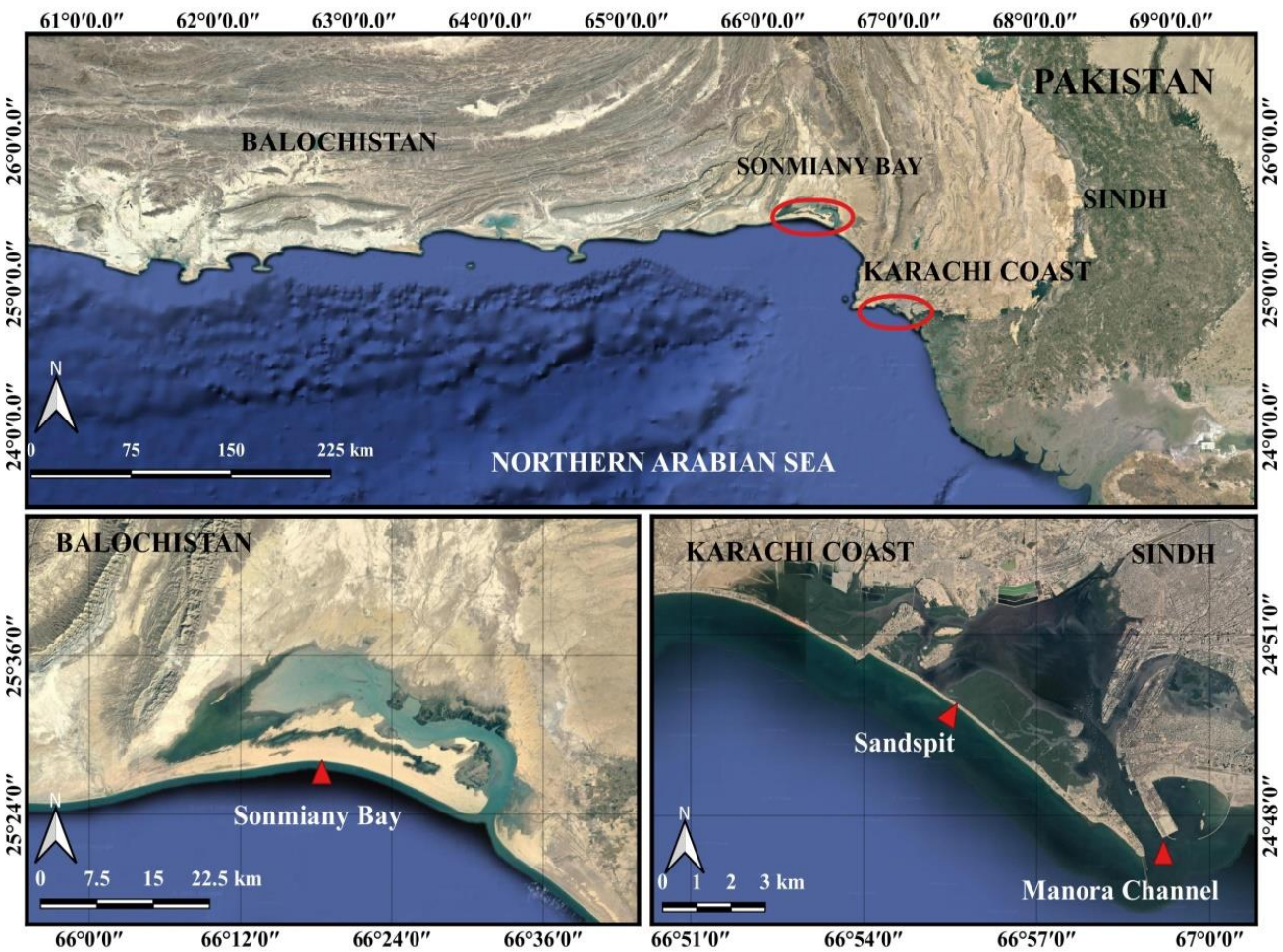

Fig. 1. Map showing study sites at Sandspit backwaters, Manora Channel and Sonmiani Bay.

Results

A total of forty seven taxa were recorded from mangrove environment. These cyanobacteria were member of five orders and fifteen families. The most dominating order was Oscillatoriales followed by Synechococcales and Chrococcales (Fig. 2). The order Oscillatoriales represents five families with highest species contribution by Oscillatoriaceae (10.26%). On the other hand, the order Synechococcales had four families recorded with Merismopediaceae showed substantial species contribution (17.95%), whereas only one family was recorded in the order Chroococcidiopsidales (Fig. 2).

Distribution on pneumtophores: The observed species of cyanobacteria associated with pneumatophores as epiphytes are listed in Table 1. A total of fourteen epiphytic species were identified belonging to eight families including eight unicellular and six filamentous species. On the top and bottom portion of pneumatophore, four unicellular species were found whereas, only two species was recorded from middle portion of pneumatophore (Table 1). It has also been observed that three unicellular species (*Cyanosarcina burmensis, Chroococcidiopsis indica* and *Synechocystis pevalekii*) were recorded only from top section of pneumatophore. *Synechocystis aquatilis* was exclusively found from middle section and *Gloeothece rupestris* and *Stanieria cyanosphaera* from bottom section of pneumatophore. In case of filamentous cyanobacteria each species was exclusively present on their repsctive proportion and out of these six species, only one was observed at top (*Hyella caespitosa*), two from middle (*Leptolyngbya tenuis* and *Phormidesmis molle*) and three from bottom (*Leptolyngbya angustissima, Pseudanabaena lonchoides* and *P. papillaterminata*) section of pneumatophore.

Distribution of cyanobacteria in water and surface sediments: Cyanobacterial diversity was considerably higher in water and surface sediments compared to pneumatophores. A total of 39 cyanobacterial species belonging to 11 families were recorded from mangrove sediments and channel waters (Table 1). Thirty four species were observed in sediment samples collected from rhizosphere; twenty nine spp in 1st cm and 15 spp in 2nd cm layers (Table 1). Ten species were common, ninteen occurred only in the 1st cm layer and five species in only 2nd cm layer. Manora waters harbor twenty one spp and Sonmiani Bay waters had fourteen spp. A total of fourteen and seven spp were exclusively present in Manora Channel and Sonmiani Bay, respectively. Most of the species observed from surface sediments were filamentous except for a few (12) unicellular species (Table 1). Table 2 and (Fig. 3) also reflect high diversity of cyanobacteria in sediments followed by water column and pneumatophore. High value Margalef (species richness; R1 9.36) and Simpson (λ 0.97) and Shanon (H' 3.53) were observed in sediment. Evenness (J') was similar (1.0) in all samples and the dominance (D) was higher (0.07) on pneumatophore at Sandspit and channel water of Sonmiani Bay (Table 2).

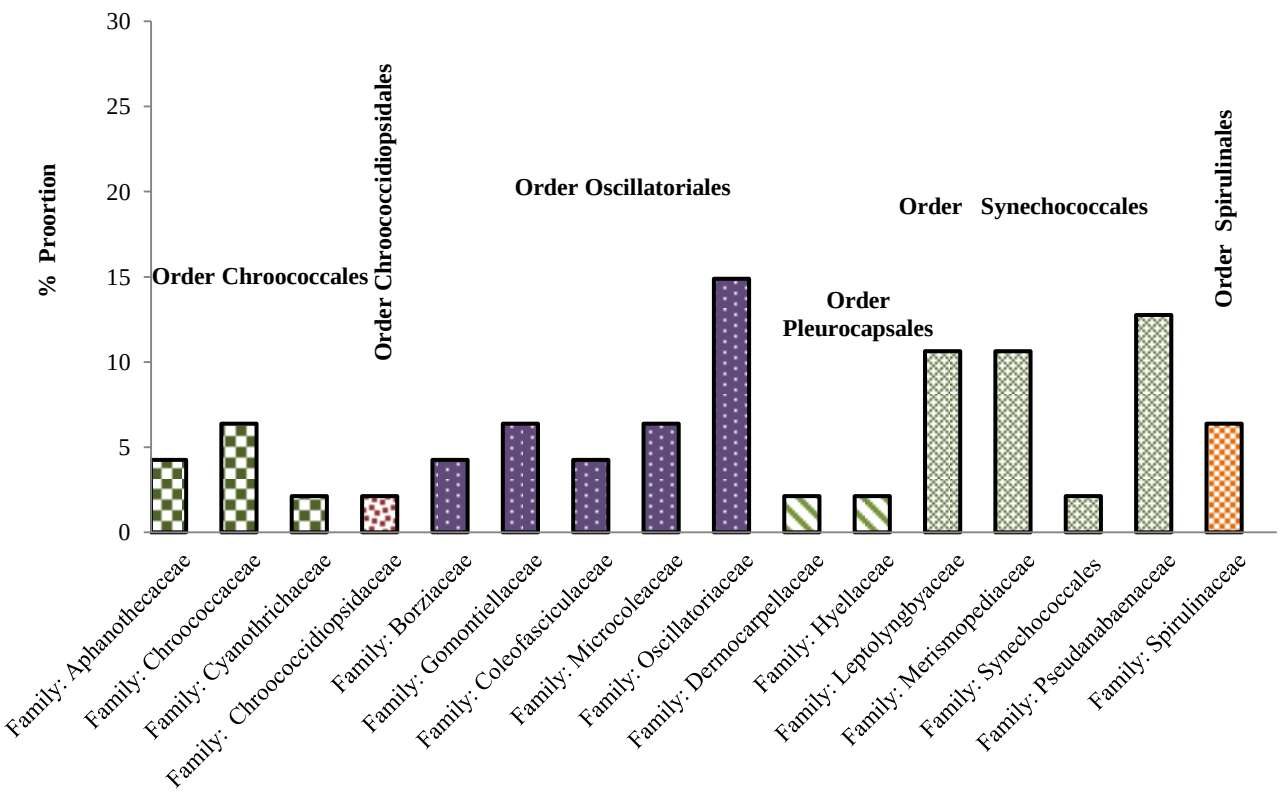

Fig. 2. Percent proportion of cyanobacterial orders and families recorded from Sandspit backwater and channel water of Manora and Soniani Bay.

Table 1. Distribution of cyanobacterial species observed from surface sediments pneumatophores of *Avicennia marina* at Sandspit (SP) backwaters and channel water of Manora (MC) and Sonmiani Bay (SB).

Cyanobacterial taxa			SP					MC	SB
			Sediments		Pneumatophore			Channel water	
			1st cm	2nd cm	Top	Middle	Bottom		
Order Chroococcales	Family: Aphanothecaceae	*Aphanothece nidulans*	+	-	-	-	-	-	-
		Gloeothece rupestris	-	-	-	-	+	-	-
	Family: Chroococcaceae	*Cyanosarcina burmensis*	-	-	+	-	-	-	-
		C. spectibilis	-	-	+	-	+	-	-
		Gloeocapsopsis crepidinum	-	-	-	+	+	-	-
	Family: Cyanothrichaceae	*Johannesbaptistia pellucida*	-	+	-	-	-	-	-
Order Chroococcidiopsidales	Family: Chroococcidiopsidaceae	*Chroococcidiopsis indica*	-		+	-	-		
Order Oscillatoriales	Family: Borziaceae	*Borzia susedana*	+	-	-	-	-	+	-
		B. trilocularis	+	-	-	-	-	+	-
	Family: Gomontiellaceae	*Komvophoron minutum*	+	-	-	-	-	-	-
		Hormoscilla pringscheimii	-	-	-	-	-	+	-
		Katagnymene accurata	-	-	-	-	-	+	-
	Family: Coleofasciculaceae	*Anagnostidinema accutissimum*	-	+	-	-	-	+	-
		A. pseudocutissimum	+	+	-	-	-	+	+
	Family: Microcoleaceae	*Arthrospira platensis*	+	-	-	-	-	-	-
		Kamptonema okenii	+	+	-	-	-	-	+
		Porphyrosiphon ceylanicus	-	-	-	-	-	+	-
	Family: Oscillatoriaceae	*Oscillatoria limosa*	+	-	-	-	-	-	-
		O. major	+	-	-	-	-	-	-
		O. nitida	+	-	-	-	-	+	-
		O. princeps	+	-	-	-	-	+	+
		Phordium corium	+	+	-	-	-	+	+
		P. schroeteri	+	-	-	-	-	-	-
		P. purpurascens	+	+	-	-	-	+	-
		Lyngbya spirulinoides	-	+	-	-	-	+	-
Order Pleurocapsales	Family: Dermocarpellaceae	*Stanieria cyanosphaera*	-	-	-	-	+	-	-
	Family: Hyellaceae	*Hyella caespitosa*	-	-	+	-	-	-	-
Order Synechococcales	Family: Leptolyngbyaceae	*Leptolyngbya angustissima*	+	+	-	-	+	+	-
		L. fragilis	-	+	-	-	-	+	+
		L. tenuis	+	-	-	+	-	+	-
		L. valderiana	+	-	-	-	-	+	-
		Phormidesmis molle	+	+	-	+	-	+	-
	Family: Merismopediaceae	*Aphanocapsa litoralis*	+	-	-	-	-	-	-
		Merismopedia tranquilla	+	-	-	-	-	-	-
		Merismopedia convulata	+	-	-	-	-	-	-
		Synechocystis aquatilis	-	-	-	+	-	+	+
		S. pevalekii	-	-	+	-	-	+	-
	Family: Synechococcales	*Jaaginema pseudogeminatum*	+	+	-	-	-	-	+
	Family: Pseudanabaenaceae	*Pseudanabaena amphigranulata*	-	-	-	-	-	-	
		P. galeata	-	+	-	-	-	-	+
		P. limnetica	+	-	-	-	-	-	-
		P. lonchoides	+	+	-	-	+	-	+
		P. mucicola	+	+	-	-	-	+	+
		P. papillaterminata	+	+	-	-	+	-	+
Order Spirulinales	Family: Spirulinaceae	*Spirulina labyrinthiformis*	+	-	-	-	-	-	+
		S. major	+	-	-	-	-	+	+
		S. subsalsa	+	-	-	-	-	-	+

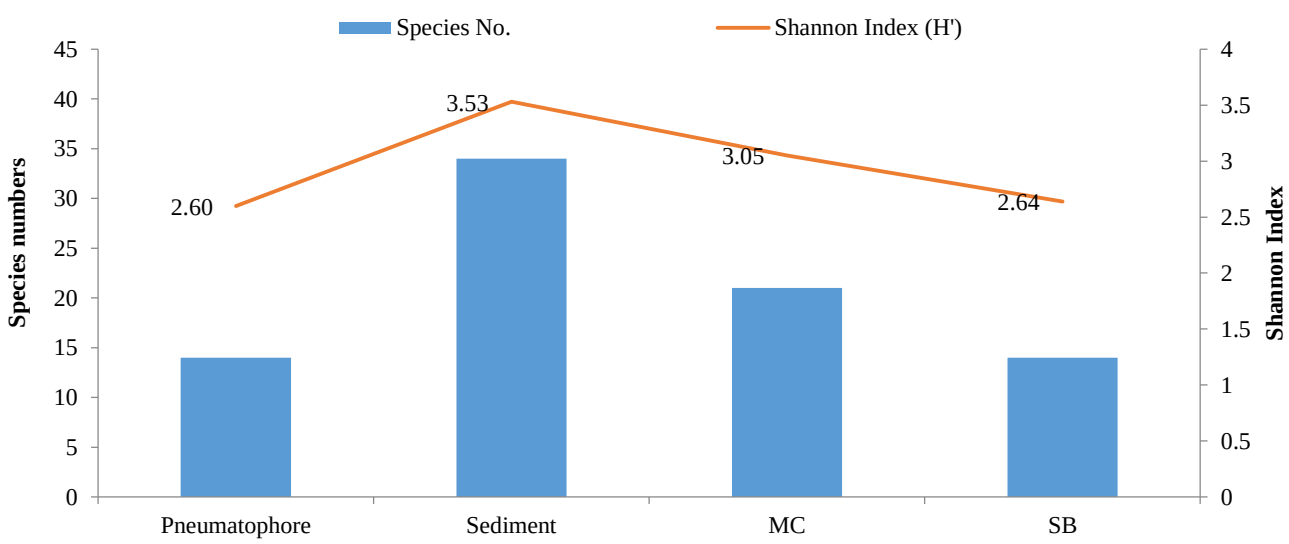

Fig. 3. Number of cyanobacterial species observed in various niches (pneumatophores, surface sediment and channel waters) in mangrove habitat are compared with Shannon diversity index.

Table 2. Variation in diversity indices of cyanobacterial species reported from Sandspit backwater, channel water of Manora and Sonmiani Bay.

Indices	Pneumatophore	Sediment	MC	SB
Dominance (D)	0.07	0.03	0.05	0.07
Simpson Index (λ)	0.93	0.97	0.95	0.93
Shannon Index (H')	2.64	3.53	3.05	2.64
Evenness (J')	1.00	1.00	1.00	1.00
Margalef (R_1)	4.93	9.36	6.57	4.93

Discussion

Cyanobacteria are significant component of the marine planktonic as well as benthic communities (Ferris & Palenik, 1998). They are generally found in broad range of niches in the environment including various water bodies (estuarine, coastal and open waters), sediments and as epiphytes on different part of mangrove plants (Singh & Elster, 2007; Comte *et al*., 2007; Perumal & Nedumaran, 2012; Oren, 2015; Sahoo *et al*., 2018). They form microbial mats on sediment surfaces in mangrove swamps (Alvarenga *et al*., 2015). They also live as epiphytes on pneumatophore of mangrove and thus provide additional nutrients and energy to the mangrove ecosystem (Pérez-Estrada *et al*., 2012; Hossain *et al*., 2014).

The cyanobacteria are also major food source for microplankton (heterotrophic flagellates and ciliates), as well as some other invertebrates such as micromolluscs, crustaceans. (Cannicci *et al*., 2008). Some unicellular organisms, for example, *Synechococcus* sp. and *Prochlorococcus* sp., are practically unique in all marine habitats where they comprise of substantial quantity of the chlorophyll biomass (Buitenhuis *et al*., 2012; Flombaum *et al*., 2013; Visintini*et al*., 2021). Several filamentous cyanobacteria also occupy pelagic and benthic habitat where they play a significance role in the ecological processes (Sahoo & Dhal, 2009; Ahmed *et al*., 2016) and they are preferred foods for consumers larger than their size, for example, mesograzers (O'Neil, 1999). Therefore, it may be concluded that a diverse group of organisms may depend on these cyanobacteria.

The identification of cyanobacteria belonging to different taxa is getting difficult due to their characterization only on morphological basis. The main identification guide still depends on Desikachary (1959) from Indian waters. However few studies done by Anagnostidis & Komárek, (1985; 1988; 1990) and Komárek & Anagnostidis, (1986; 1989) from temperate water were also helpful in the identification.

Despite their importance in several ecosystems, cyanobacteria have been generally ignored in most of the biodiversity studies (Rejmánková *et al*., 2004). The importance of cyanobacterial diversity and ecological role in mangrove ecosystem remain poorly understood (Barman *et al*., 2015). Comparatively few studies are currently available on diversity of cyanobacteria in mangrove ecosystems from Pakistan (Saifullah & Taj, 1995; Saifullah *et al.*, 1997; Zaib-un-Nisa *et al*., 2000; Ahmed *et al*., 2016). The epiphytic algal flora of pneumatophores of *A. marina* has already been described by Saifullah & Taj (1995). The data obtained in the present survey reflect that cyanobacterial species are highly diverse and that their distribution may be habitat specific. About fourteen different cyanobacterial taxa were found on the pneumatophore (Table 1). Similar study was done by Saifullah & Taj (1995) but they reported only few species from Pakistan. Santra *et al*., (1988) and Nedumaran *et al*., (2008) have reported some species of cyanobacteria associated with pneumatophore and aerial roots of *A. marina* and *Rhizophora* sp., respectively from other part of the world. However, the importance of relationship among epiphytic micro-organisms and pnumatophores of mangroves has also been described earlier (Naidoo *et al*., 2008).

All cyanobacteria recorded here are non-heterocystous filamentous or unicellular. Heterocystous species were not observed in this study and even in the previous reports from the coastal waters of Pakistan (Saifullah & Taj, 1995; Bano & Siddiqui, 2003 & 2004). This seems to have no relation with possible nitrogen loadings from Layari River in Manora channel as heterocyst forming genera were also absent in Sonmiani Bay a non-polluted site. Similar results were obtained in a study where mostly non-heterocystous genera were recorded from mats in an African mangrove stand (Mann & Steinke, 1993). However, only two heterocystous genera (*Anabaena* (2 spp) and *Rivularia* (1 sp.)) and 19 non-heterocystous species were recorded from Tanzanian mangroves (Kyaruzi *et al*., 2003). Few other studies have also reported presence of non-heterocystous cyanobacteria from coastal sediments (Sheridan, 2001; Sakthivel *et al*., 2013; Partensky *et al*., 2022).

Apart from direct observation of cyanobacteria from mangrove habitat, a significant number of cyanobacterial strains including both unicellular and filamentous were isolated through cultivation and morphologically characterized. The diversity of cyanobacteria, in the present study, inhabited in different niches in mangrove ecosystem is comparable to previous investigation from coastal waters of Karachi (Bano & Siddiqui, 2003 & 2004). In this study it has been observed that most diverse community was found on surface sediment at Sandspit backwater and channel water of Manora. The dominance of these species in backwater areas could be due to nutrients loadings through Lyari River and also from litter decomposition in the mangroves as describe earlier (Shafique *et al*., 2015b). Therefore, cyanobacteria constitute an essential component of the mangrove ecosystem and play an important role in the sustenance of biological diversity (Hoffmann, 1999; Lee *et al*., 2014).

The cyanobacteria are mostly unknown, especially with respect to their potential role as biofertilizers, new nitrogen fixer, and producers of bioactive compounds, such as, antibiotics, antitumor agents and related pharmaceuticals (Alvarenga *et al*., 2015; Kulasooriya & Magana-Arachchi, 2016; Rossi *et al*., 2016; Reis *et al*., 2017). Therefore, studies on the distribution of cyanobacteria, bacteria and their role in the productivity and nutrient cycling in the unique mangrove environment are recommended in Pakistan.

Acknowledgements

Authors wish to acknowledge support from ONR research project. We are also grateful to Marina Aboal (Department of Vegetal Biology, University of Murcia) for valuable suggestions to improve the manuscript.

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(Received for publication 25 April 2021)