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SEASONAL ABUNDANCE OF SIX DOMINANT FILAMENTOUS CYANOBACTERIAL SPECIES IN MICROBIAL MATS FROM MANGROVE BACKWATERS IN SANDSPIT PAKISTAN

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Abstract

Microbial mats in the mangrove forests of Sandspit coast area, south of Karachi, Pakistan were studied between Jan 2012-Jan 2014. Six major filamentous cyanobacteria belonging to three genera *Oscillatoria* (3), *Spirulina* (2) and *Phormidium* (1) were identified in the backwaters mat samples. One of the cyanobacterium species *Spirulina labyrinthiformis* is reported from this site for the first time in Pakistan. The most dominant species was *Oscillatoria brevis* (22% abundance), closely followed by *Phormidium tenue* (21%). These filamentous forms were present in all seasons and tolerated varying physico-chemical ranges such as temperature (15-28ºC), pH (6.8-7.5), salinity (36-42‰) and dissolved oxygen (0.201-0.543ppm). Chlorophyll *a* levels in mat area sediments were ranged between 0.039 up to 5.050 mg/g. The minimum and maximum biovolume was 0.174 and 1.649mm$^{3/}$l respectively. We observed a strong positive correlation ($p<0.05$) between observed filamentous form of cyanobacteria and field parameters such as water-soil-air temperatures, pH and dissolved oxygen. The outcome suggests a potential for detailed molecular microbial mat study. Further studies are required to understand the interactions of microbial mat with soil and water components.

Key words: Mangrove, Cyanobacteria, Microbial mat, Taxonomic assessment.

Introduction

Pakistan has a coastline of 1050 km in the Arabian Sea (Amjad *et al.*, 2007; Siddiqui *et al*., 2008). The mangrove population in Pakistan thrives in the Indus delta. This fan shaped delta affects the coast of the Sindh and Baluchistan provinces where mangrove forests are present at Sandspit, Korangi, Keti Bundar, Miani, Kalmathor Jiwani and Gawadar bay respectively. Mangrove swamps are considered significant in terms of coastline protection; they provide economic and cultural support through food, fodder and fuel wood for local populations. Mangroves are also ecologically significant, as they serve as breeding grounds and sustain various species of flora and fauna. This habitat is influenced by tidal height and variations in temperature, salinity, pH, oxygen content, organic and inorganic nutrients and humidity, among others.. The forest floor is covered by conspicuous microbial mats constituted by decomposers and primary producers including bacteria, cyanobacteria, fungi and protista. Benthic cyanobacteria, photoautotrophic, gram negative bacteria, (Prescott, 1998; Hoiczyk & Hansel, 2000) constitute a significant proportion of these microbial communities (Cole *et al*., 2014) as free–living, in associative or endo-symbiotic relationships (Puyana *et al.*, 2015). In aquatic systems, cyanobacteria and some eukaryotic microorganisms (e.g. diatoms) can form microbial mats and contribute to the local primary productivity. Heterocystous and some non-heterocystous cyanobacteria fix nitrogen (Zehr *et al*, 2001) reducing it to ammonia that becomes available to other organisms including mangrove plants which then proliferate in otherwise nitrogen-limited conditions. Cyanobacteria have a significant potential in terms of nutrient recycling (Hegazi *et al*., 2010), prevention of soil erosion (Sugumar *et al*., 2011), increased tolerance to extreme variations in temperature and pH levels, phosphorous and nitrogen storage (Özbay, 2011), and increased oxygen concentrations (Mandal *et al*., 1998). Cyanobacteria can obtain carbon dioxide where it is limited, decreased concentration sites, supporting low water retention times in nitrogen limited sites (Miyachi *et al*., 2003; Wu *et al*., 2008; Gamze, 2014) Although there has been a significant number of studies on the coastal flora of Pakistan including macro and micro algae (Shameel & Tanaka, 1992; Mansoor *et al*., 2000; Bano & Siddiqui, 2004; Rizvi *et al*., 2004; Saifullah & Ahmed, 2007; Siddiqui *et al*., 2009; Shafique *et al*., 2013; Faroouqi *et al*., 2014; Sarwat *et al.*, 2014), benthic filamentous cyanobacteria have not been studied yet in the country´s mangrove swamps. Only one report describing in detail the systematic characterization of mangrove associated microalgae, including cyanobacteria, is available (Zaib-un-Nisa *et al*., 2000). Therefore, the objective of this investigation was to assess the the abundance and seasonal variation of filamentous cyanobacteria growing at the Sandspit mangrove forest, and identify some of the dominant species.

Material and Methods

Sandspit backwaters are located approximately at 24º 49´ N and 66º 56´ E between Manora and Hawksbay waters and adjacent to a Navy dockyard (Fig. 1).

Sampling took place every three months between January 2012 to January 2014. The collection period was broadly divided into pre-monsoon (winter, NE monsoon season), monsoon (spring, SW monsoon season) and post monsoon seasons (autumn). Microbial mats from different forest floor areas were collected in triplicate using glass slides and transferred into sterile plastic containers. These were marked (culture code, site, date, and color) and kept in an ice cooler, then transferred to the laboratory for further analysis. 3.0 mm fractions of samples were observed in triplicate within 24 hours using artificial seawater without fixatives and kept at ambient temperature under light source (2000lux) during analysis. Cells were observed under light microscope (Olympus, Japan) to record morphological characteristic and measure morphometric features of interest. Despite molecular taxonomic assessment which have facilitated a more detailed assessment of cyanobacterial species (Engene *et al*., 2013), classic taxonomic assessment is still relevant for some preliminary evaluation (Puyana *et al*., 2015). Hence for our initial observations, cyanobacteria were taxonomically identified following Komarek (1992), Komárek & Hauer (2013) and Guiry & Guiry (2016). Environmental variables were recorded at every collection. Air, sediment and water temperature (ºC) were measured with a (mercury thermometer), salinity was measured with a ATAGO 0161633 refractometer (Japan) and pH was determined with an ELEMETRON CP-401 pH meter., Chlorophyll *a* (benthic samples using 30 mm polyethylene corers) and dissolved oxygen (channel water flowing over mat area, sampled in ambient 80ml bottles) determinations were performed in triplicate following Strickland & Parsons (1972). Pearson correlations were calculated to determine the relationship between field environmental variables and relative abundance of species. Bio-volume, which is a measure of cyanobacterial biomass, was calculated using the standard formulae (Hillebrand *et al*., 1999; Türkmen & Kazanci, 2010). To determine the predominate filamentous cyanobacterial species present in mat samples, the abundance of individual species was calculated by the methods of Roger *et al.* (1991) and Laslett *et al.* (1997). Statistical analyses were performed using the Microsoft Excel 2013 and Minitab statistical software Version 17.1.0.

Results

Experimental variables noted showed seasonal fluctuations (Table 1). Slight differences in temperature range were noted for air (19-24ºC), water (17-24.5ºC) and sediments (15-24ºC). Lower values were obtained during the pre-monsoon (Jan) and higher during the monsoon (Apr, Jul) and post-monsoon (Oct) seasons. Both pH (6.8-7.5) and salinity (36-42‰) varied with seasons and lower values were observed during the monsoon season. The dissolved oxygen concentration in water samples ranged from 3.70 to 7.18 ppm, showing greater values during the monsoon season. Chlorophyll *a* (0.038 to 5.05 mg/g) had greater values during the pre- monsooand post-monsoon seasons, and lower values were observed during the monsoon season.

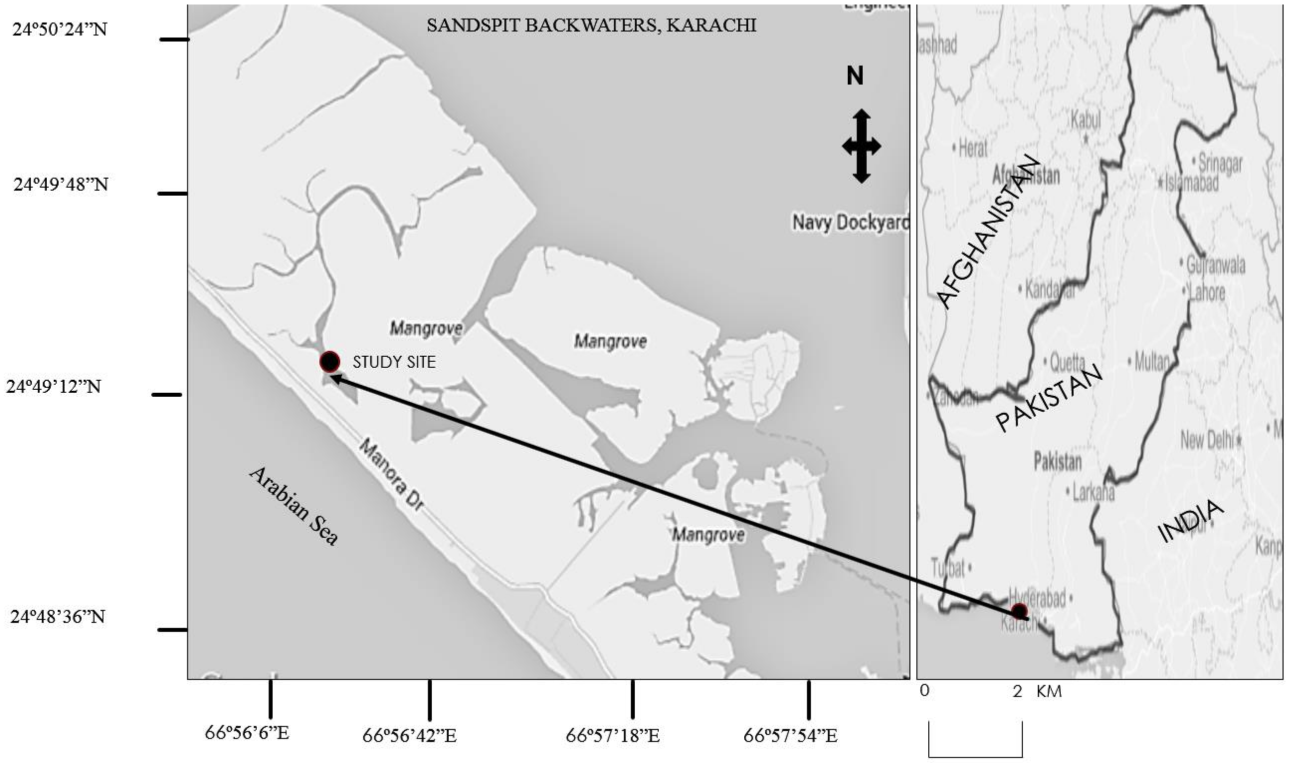

Fig.. 1. Map showing the location of Sandspit Mangrove forests.

Table 1. Seasonal variations in physical and chemical parameters of water and sediments from Sandspit backwaters mangroves (Mean± S.D., N=6).

Seasons	Months	DO	Temperature ºC			pH	Sal ‰	Chl *a* mg/g
		ppm	Air	Water	Soil			
Pre monsoon	January	3.85 ± 1.74	20 ±2.22	17 ±3.33	15 ±5.50	7.2 ±0.33	42 ±3.00	1.13 ±2.34
Monsoon	April	6.25 ±1.80	24 ±2.65	23 ±2.29	24 ±2.31	7.5 ±0.40	36 ±3.06	0.24 ±2.84
	July	7.18 ±2.46	19 ±0.71	20 ±3.18	28 ±2.83	6.8 ±0.50	42 ±2.83	0.04 ±3.54
Post monsoon	October	3.70 ±1.72	20 ±2.65	24.5 ±3.00	24 ±6.66	7.5 ±0.35	38 ±3.46	5.05 ±0.58

where, DO = Dissolved oxygen, Chl a= Benthic chlorophyll a content, Sal ‰ = Salinity

Six filamentous cyanobacterial species were dominant throughout the sampling periods at Sandpit backwaters. The characteristics of these cyanobacterial species are outlined below:

1) *Phormidium tenue* Gomont, 1892. Ann. Sci. Nat. Bot., ser. 7, 16: 91-264, pls 1-7. (Komárek, 1992; Guiry & Guiry, 2016) Observed characters: Filamentous; filaments long, solitary or coiled into clusters, intensely coiled, thin, fine, 2 µm wide. Trichomes fine, cylindrical, slightly attenuated, with rounded apical cells, slightly constricted at the cross walls, jerky motility. Cells longer than wide, cylindrical, with homogeneous content, pale green, end cells without thickened cell walls or calyptras, heterocytes and akinetes are absent. Filaments of *Phormidium tenue* in microbial mat remained alive and active for 5 days in plain seawater (without addition of nutrients at ambient temperature). Observed in bubbly, dry and fine mats samples (Fig. 2A).

2) *Spirulina labyrinthiformis* Kützing ex Gomont 1892. Ann. Sci. Nat. Bot., ser. 7, 16: 255. (Komárek, 1992; Komárek & Hauer, 2013) Observed characters: Filamentous; unbranched, no sheaths, screw- like coiled along the whole trichome length, screws are very densely tight. Trichomes, 2 µm wide, not attenuated towards the ends, intensely motile having screw like and jerky motility, usually with homogeneous content, olive green end cells widely rounded, without thickened cell walls or calyptras. Heterocytes and akinetes absent. Always found with *Spirulina major.* Filaments remained active for 2 days in plain seawater. Found in bubbly and smooth mat (Fig. 2B).

3) *Spirulina major* Kützing ex Gomont 1892. Ann. Sci. Nat. Bot., ser. 7, 16: 251. (Komárek, 1992; Komárek & Hauer, 2013) Observed characters: Filamentous; unbranched, no sheaths. Screw- like coiled along the whole trichome length, screws are not densely tight and broad. Trichomes 2 µm wide and 4-6µm distant, not attenuated towards the ends, motile screw like rotation, usually with homogeneous content, dark green end cells widely rounded, without thickened cell walls or calyptras. Heterocytes and akinetes absent. Found with or without *Spirulina labyrinthiformis*. Filaments viable for 2 days in plain seawater. Found in smooth and bubbly mat (Fig. 2C).

4) *Oscillatoria limosa* Agardh ex Gomont 1892. Ann. Sci. Nat. Bot., ser. 7, 16: 210. (Komárek, 1992; Komárek & Hauer, 2013) Observed characters: Filamentous; unbranched. Trichomes slightly waved, 22 µm wide, composed of shortly discoid cells, slightly constricted at the cross walls, shortly attenuated to the ends, exhibited gliding motility. Cells with fine granulation, dark green to light brown. End cells widely rounded, heterocytes and akinetes absent. Filaments present in mat sample remained alive for up to 3 days in plain seawater. Usually found in smooth and bubbly mats along with *Oscillatoria princeps* (Fig. 2D).

5) *Oscillatoria brevis* Kützing ex Gomont 1892. Ann. Sci. Nat. Bot., ser. 7, 16: 229 = *Phormidium breve* (Komárek, 1992; Komárek & Hauer, 2013) Observed characters: Filamentous; unbranched, smooth, layered. Trichomes straight, 5 µm wide, composed of shortly barrel-like cells (always shorter than wide), unconstricted at the cross walls, shortly attenuated to the ends, showed jerky gliding motility. Cells olive green. End cells widely rounded, heterocytes and akinetes absent. Filaments viable up to 5 days in plain seawater. Found in leathery, dry and bubbly mat (Fig. 2E).

6) *Oscillatoria princeps* Vaucher ex Gomont 1892. Ann. Sci. Nat. Bot., ser. 7, 16: 206. (Komárek, 1992; Komárek & Hauer, 2013) Observed characters: Filaments simple, unbranched, smooth. Trichomes straight, 16 µm wide, composed of shortly cylindrical cells (always shorter than wide), unconstricted at the cross walls, shortly attenuated to the ends, having gliding motility. Cells greenish brown, end cells widely rounded, heterocytes and akinetes absent. Filaments in mat samples remained active for 2 days in plain seawater. Found in bubbly and dry mat (Fig. 2F).

The distribution and biovolume of the six most dominant cyanobacterial species are shown in Figs. 3 and 4, respectively. *Spirulina major, S. labyrinthiformis, Oscillatoria brevis, O. princeps, O. limosa,* and *Phormidium tenue* were the major constituents of cyanobacterial mats throughout the year. Among them *O.limosa* and *O.princeps* had the greater bio-volume compared to the other dominant species (Fig. 3). On the other hand, *Phormidium tenue and O. brevis* were the most abundant (Fig. 4) among the dominant species.

The dominance of cyanobacterial species in microbial matsat Sandspit mangroves remained fairly constant thoughout the study. Some species varied in their abundance depending on the season. *Spirulina* sp. and *O.limosa* were not observed in the pre monsoon season. By the end of the post monsoon season, *Spirulina labyrinthiformis* and *S. major* started to decline. The growth pattern of *Spirulina* species was low in July (second half of monsoon season) (Fig. 5). Other microorganisms which were observed frequently along with major filamentous forms are listed in Table 2.

The mats that were present all over the sediment displayed an array of colors. Although light intensity is one of the main factors which exhibit these shades but we also observed that these mat colors were stable under 4ºC temperatures for up to 72 hours in dark. This may be due to the abundance or scarcity of certain observed diatoms and cyanobacterial species (Delfano *et al*., 2012; Taj *et al*., 2014). They had different tones of brown, yellow or green in different intensities, sometimes attaining very dark shades. *Oscillatoria brevis* and *O.princepes* appears to have cal poly (artichoke) green, *O.limosa* displayed an olive green color and *Phormidium tenue* revealed a lighter shade of forest green whereas, both *Spirulina labyrinthiformis* and *S. major* exhibited a dark apple green shade. Often, *O. brevis, O. princepes* and *O. limosa* turned into black colonies. Biofilms of *Navicula* sp. diatoms were observed during the winter and displayed dark brown colour. Yellow and light brown mats usually contained more diatoms and less cyanobacteria. Protista were occasionally found in mixed mats.

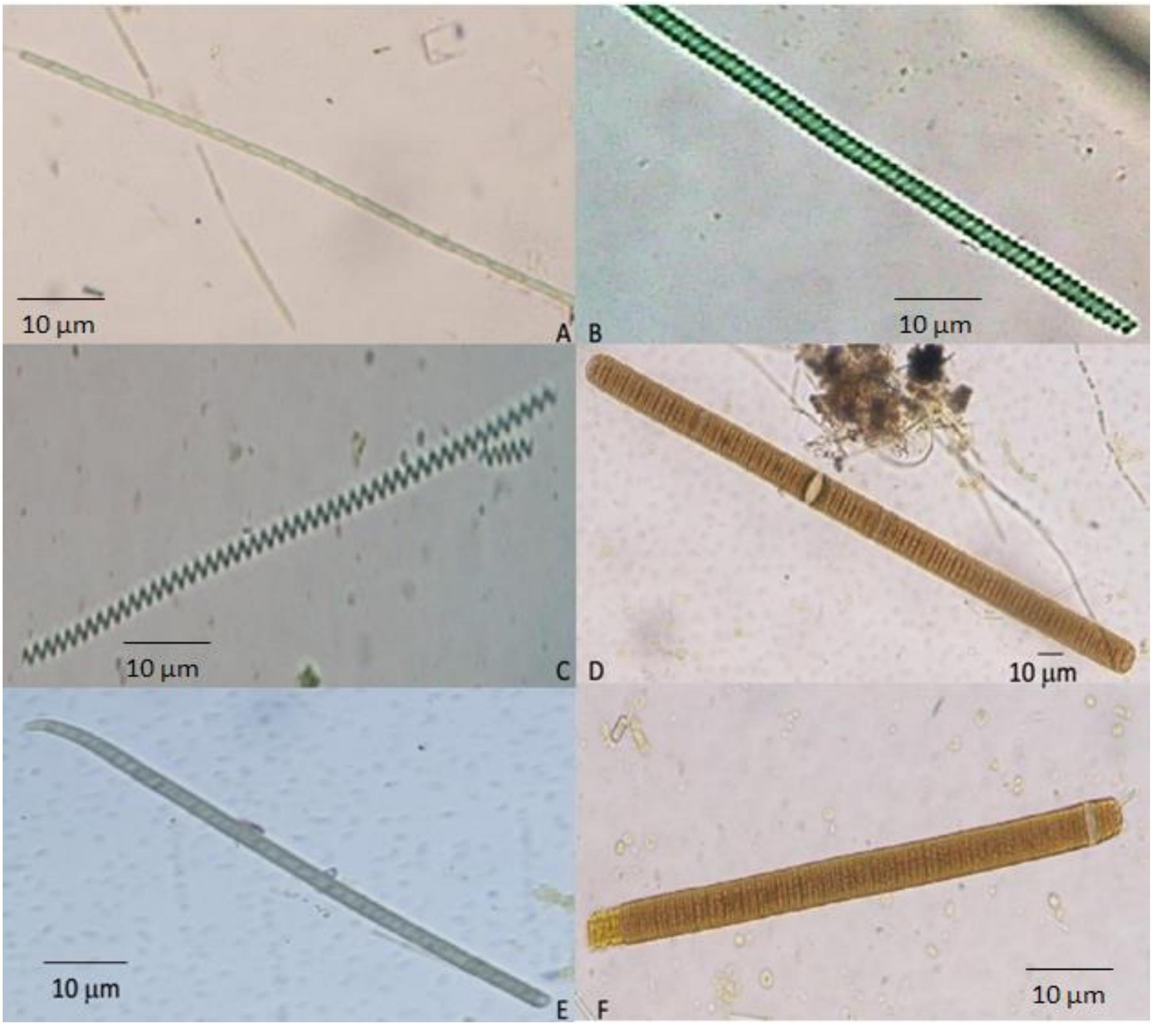

Fig. 2. Filamentous cyanobacteria most abundant at Sandspit mangrove backwaters during the study period. (A) *Phormidium tenue* (B) *Spirulina labyrinthiformis* (C) *Spirulina major* (D) *Oscillatoria limosa* (E) *Oscillaoria brevis* and (F) *Oscillatoria princeps.* All the pictures except Fig. 2 (D) (200x) were taken at 400x.

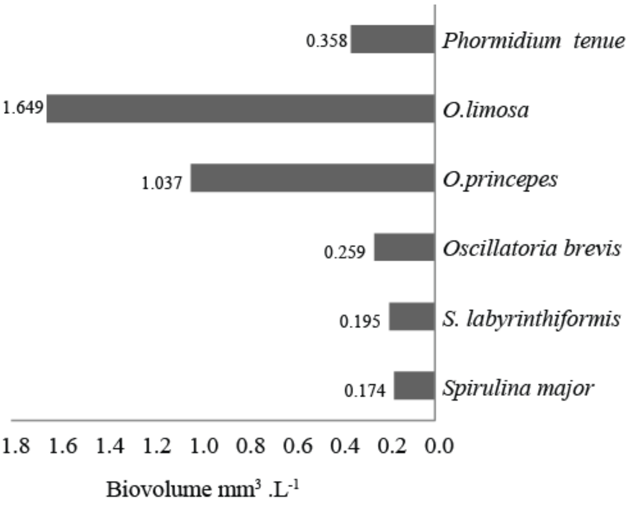

Fig. 3. Biovolume of the six dominant benthic cyanobacteria at Sandspit mangrove backwaters.

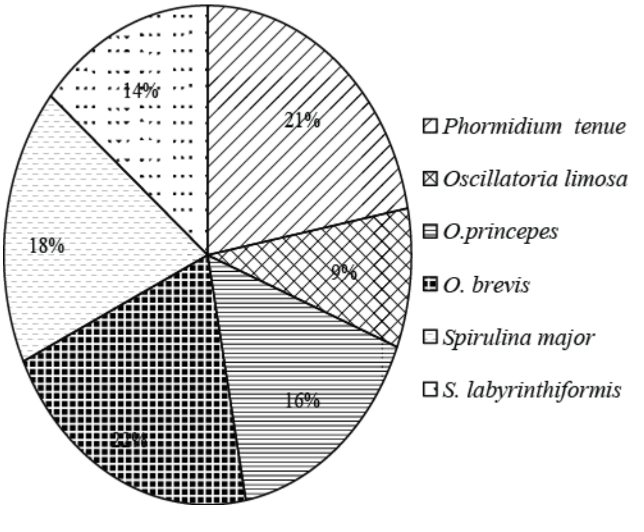

Fig. 4. Average proportional distribution of the six most dominant cyanobacterial species at Sandspit mangrove backwaters for the period 2012-2014.

Table 2. List of organisms observed in microbial mats during the seasonal study period of Sandspit mangrove area.

Organisms	Seasons		
	Pre monsoon	**Monsoon**	**Post monsoon**
Cyanobacteria			
Phormidium tenue	++	++	++
Spirulina major	+	+	++
S. labyrinthiformis	+	+	++
Oscillatoria brevis	++	++	++
O.limosa	-	++	++
O.princeps	++	++	++
O. subbrevis	+	+	+
Microcystis sp.	+	+	+
Synechococcus sp.	+	+	+
Diatom			
Navicula sp.	++	++	++
Nitzschia sp.	+	+	+
Cymbella sp.	+	+	+
Amphora sp.	+	+	+
Cocconeis sp.	+	+	+
Pinnularia sp.	+	+	+
Gyrosigma sp.	+	+	+
Pleurosigma sp.	+	+	+
Hantzschia sp.	+	+	+
Protista			
Ciliates	-	+	+

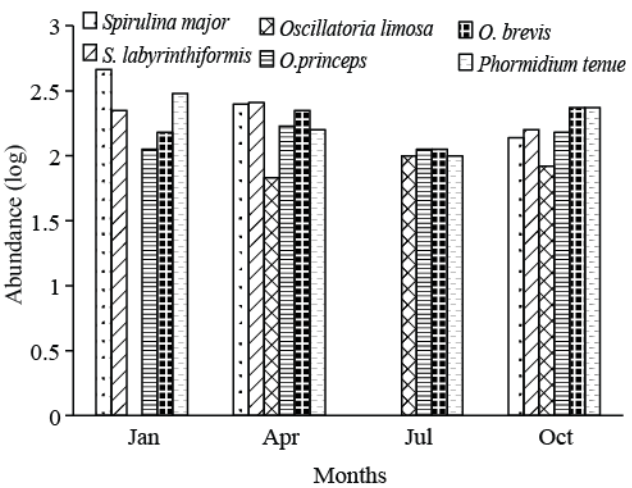

Fig. 5. Abundance of the dominant cyanobacterial species in pre- monsoon (January), monsoon (April, July) and post monsoon (October) at Sandspit mangrove area.

The Pearson correlation analyses (Table 3) showed a positive relation between major filamentous cyanobacterial species and physico-chemical parameters. *Oscillatoria* sp. were positively correlated with salinity (0.500), chlorophyll *a* (0.513), soil-water temperatures (0.999, 1.000) and pH (0.999). *Phormidium* sp. was positively correlated with soil-water temperature (0.971, 0.999), pH (0.971) and chlorophyll *a* concentration (0.558) whereas, *Spirulina* spp. were positively correlated with dissolved oxygen (0.993), air-soil temperature (0.993, 0.596) and pH (0.596) respectively. Field variables such as Dissolved oxygen was positively correlated with air temperature (0.999), air temperature was correlated with soil and water temperatures (0.500, 0.500), water temperature was correlated with soil temperature, pH and chlorophyll a concentration (0.982, 0.982 and 0.513) and finally soil temperature was positively significant with pH (1.000). We also observed a positive correlation among major species. The presence of *Oscillatoria brevis* influenced by other filamentous forms except *O.princepes. O.limosa* was positively correlated with *Phormidium tenue* (Table 3).

Discussion

Sandspit backwater mangrove forests consist exclusively of stands of *Avicennia marina* . The forest sediments are covered with dense microbial mats. Different microbial communities are involved in these mats, where cyanobacteria are the dominant group. It was observed in earlier studies (Seckbach, 2007; Rigonato *et al.*, 2012; Shafique *et al.*, 2013) that microalgae (including the genera from our study) associated with mangrove ecosystems enrich the soil by releasing nutrients, fix carbon and nitrogen, and control soil moisture content. Thus they play an integral part in sustaining the primary productivity of related habitat. Under the same set of environmental conditions, *Phormidium tenue* and *Oscillatoria brevis* were the hardiest of the six dominant cyanobacterial species. They tolerated extreme temperature, salinity and pH ranges during all the seasons. *Phormidium tenue* is known to be halotolerant (Thajuddin & Subramanian, 1992). *Oscillatoria* and *Spirulina* species have been known to thrive in acidic and alkaline soil substrates (Nayak & Parsana, 2003).

Table 3. Pearson correlations between dominant filamentous cyanobacterial species at Sandspit mangrove and environmental field parameters.

Parameters	*Oscillatoria brevis*	*O.limosa*	*O.princepes*	*Phormidium tenue*	*Spirulina labyrinthiformis*	*S.major*	DO ppm	Air °C	Water °C	Soil °C	pH	Sal
O.limosa	**0.990											
O.princepes	-0.786	-0.866										
P. tenue	**0.981	**0.999	-0.891									
S. labyrinthiformis	*0.519	0.392	0.121	0.343								
S.major	*0.558	0.434	0.075	0.386	**0.999							
DO ppm	0.412	0.277	0.240	0.227	**0.993	**0.986						
Air °C	0.459	0.327	0.189	0.277	**0.998	**0.993	**0.999					
Water °C	**0.990	**1.000	-0.866	**0.999	0.392	0.434	0.277	0.327				
Soil °C	**0.999	**0.982	-0.756	**0.971	*0.559	*0.596	0.454	*0.500	**0.982			
pH	**0.999	**0.982	-0.756	**0.971	*0.559	*0.596	0.454	*0.500	**0.982	**1.000		
Sal ‰	-0.929	-0.866	*0.500	-0.839	-0.799	-0.826	-0.721	-0.756	-0.866	-0.945	-0.945	
Chl *a* mg/g	0.385	*0.513	-0.874	*0.558	-0.589	-0.551	-0.682	-0.643	*0.513	0.342	0.342	-0.015

($p<0.05$, *Significant,**Highly significant, DO=Dissolved oxygen, Sal=Salinity)

There were different physical and chemical variables that correlated with the abundance of the observed cyanobacterial species. When forming mats, these organisms when have a greater assimilation efficiency when compared to phytoplankton (Fong *et al*., 1993). Cyanobacteria can tolerate a broad range of pH levels and salinity (Bano & Siddiqui, 2004). The pH was lower (6.8) during the monsoon season and greater during the pre monsoon (7.2) and post monsoon (7.5), probably due to the uptake of carbon dioxide from photosynthesis processes by cyanobacteria and phytoplankton (Chen & Durbin, 1994). Our site is affected by moderate to high temperatures which are favorable for cyanobacterial growth (Miyazono *et al*., 1992). Rainfall is also one of the main features which brings about various hydrographical changes in the mangrove environment. The monsoon season with flash flooding started in July and continued until September (approx.137.5 mm, Pak. Met. Data 2012-2013). The rainfall rate was below average ranges, resulting in a late cyanobacterial bloom. Our observations (similar to the earlier studies of Redekar & Wagh, 2000; Steidinger *et al*., 2002) showed that the level of chlorophyll *a* was high in the pre monsoon season (1.13 mg/g, ± 2.34), low during the monsoon (0.24 mg/g, ± 2.84 and 0.04 mg/g, ± 3.54) and higher in the post monsoon (5.05mg/g, ± 0.58). Although we found a significant positive correlation ($p<0.005$) between physical and chemical parameters and predominate filamentous cyanobacteria, there was no consistent positive relation between Chlorophyll *a* and other physical parameters (except for water temperature) (Odate *et al*., 1990). Dissolved oxygen values were greater during the monsoon due to increased biological activity, turbid conditions near the microbial mat and fresh rainwater input (Satpathy *et al*., 2011). Oxygen concentrations were low in the remaining seasons. Tidal height, duration as well as wave movement and turbulence, these factors also affects development of benthic cyanobacteria (Steinberg & Hartmann, 1988). Other reasons that might explain their abundance and dominance can be the strong chemical defense system (Pajdak-Stos *et al*., 2001) toxin production (Graham *et al*., 2008; Frazão *et al*., 2010) and less copasetic or unpalatable attribute (Repka *et al*., 1999; Okogwu & Ugwumba, 2008) which keeps the organisms away from grazing type feeders like some zooplanktonic organisms along with the lack of organisms which do prefer it (Rautio & Warwick, 2006) but might not be able to survive in polluted waters.

The six filamentous cyanobacterial dominant species did not perish under extreme conditions rather, it diminished its growth then seem to adjust and acclimatize to the new set of conditions such temperature, salinity, pH, dissolved oxygen and others. As a result of this apparent adaptation, it started to bloom again and maintain its presence throughout the year. Dominant cyanobacteria can thrive in oxidizable organic matter and in low levels of dissolved oxygen. These species can therefore be found in polluted habitat and can be considered good pollution indicators (Vijayakumar *et al*., 2007; Okogwu & Ugwumba, 2008). Cyanobacteria also have a great potential to accumulate certain hazardous materials like heavy metals and industrial dyes (Rawat *et al*., 2011; Vijayakumar & Manoharan, 2012). Further research is required to understand the survival mechanisms, and the bioaccumulation and bioremediation potential of these microorganisms. We observed less cyanobacterial diversity compared with an earlier study of the same site by Zaib-un-Nisa *et al*. (2000) suggesting that minor cyanobacterial species are indifferent to waste water pollution that may have compromised this site (author, unpublished data). Further molecular research is required to explore microbial mat which may facilitate detailed observation of species interaction and their biochemical aspects.

Conclusion

Microbial mats were observed and taxonomically identified using preliminary microscopic technique. The dominant filamentous cyanobacteria were able to tolerate considerable differences of various physico-chemical parameters. There was no negative impact of seasons on the observed cyanobacteria.

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