# PHYTOPLANKTON COMMUNITY ASSOCIATED WITH MARINE SPONGE LIOSINA PARADOXA THIELE, 1899 AT SANDSPIT, KARACHI

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## Abstract

The phytoplankton community associated with marine sponges was studied seasonally at Sandspit backwater during January to December 2013. The sponge samples were collected from pneumatophores of *Avicennia marina* and thoroughly washed with seawater. The samples were retained in 200 ml plastic bottles and 4% formalin was added as a preservative. The phytoplankton communities were sorted from the samples and their members were identified using light microscopy. Twenty species, representing three classes were recorded. Ammong them Bacillariophyceae was observeed with highest diversity (14 species) while four species were belonged to Cyanophyceae and two to Chlorophyceae. The highest number of individuals were also recorded for Bacillariophyceae (157 individuals) which indicated that *Pinnularia* spp. (20%) was the most dominant genus followed by *Surirella ovata* (17%) and *Nitzschia palea* (13%) whereas minimum abundance recorded for Chlorophyceae (6 individuals). Highest numerical abundance was observed in summer and lowest during winter season. Physicochemical parameters of water recorded were temperature (27-35 ± 4.6°C), salinity (35-39 ± 1.47 PSU), dissolved oxygen (0.11-3.44 ± 1.15mgL<sup>-1</sup>) and pH (7.04-7.69 ± 0.19). The results indicated that phytoplankton diversity is greatly influenced by environmental factors. This is the first study of the phytoplankton community associated with marine sponges in Pakistan. Further study is needed to determine the communities associated with *Liosina paradoxa* in mangrove area and other sponge species from different regions to understand the interaction between host-sponge and its inhabitants from coastal waters of Pakistan.

Key words: Mangrove sponge, Symbiotic interaction, Avicennia marina, Pakistan.

# Introduction

Mangrove forests create distinct ecological environment that supports highly diverse biological communities (Shafique et al., 2010; Farooqui et al., 2012). The forest provides a nurturing and stable ground that protects its habitants from variability and harshness in environmental factors such as sunlight, temperature, salinity, tidal action and sedimentation etc. (Nagelkerken et al., 2008). The presence of submerged mangrove roots in shallow water enables marine benthic communities with diverse meiofauna, macrofauna (infauna, epifauna) and planktonic species to become embellished (Rützler, 1995; Morrisey et al., 2010). Among benthic communities of marine ecosystems, sponges are dominant and most diversified epibionts on mangrove roots because of the availability of suitable substrate for attachment (Rützler et al., 2000; Wulff, 2000; Diaz et al., 2004; Diaz, 2012). The association of sponges with bacteria, cyanobacteria, unicellular and multicellular algae (like diatoms and dinoflagellates) and facultative anaerobes in mangroves occurs at both intra and extra-cellular levels (Althoff et al., 1998). Marine sponge benefits from supplemental provision of photosymbiotic organisms, obtaining more than 50% of their respiratory oxygen output by photosynthetic oxygen production (Steindler et al., 2002).

Sponges harbor unicellular or filamentous endosymbiotic cyanobacteria and eukaryotic microorganisms (diatoms, dinoflagellates, fungi, zoochlorellae and some cryptomonads) either inside their vacuoles or extracellularly beneath their outer surface of sponge (Haygood *et al.*, 1999). The photosynthetic species provide oxygen, shade and protection from the damaging effects of light while the other organisms, such as bacteria and facultative anaerobic symbionts, provide with nutrients (such as glycerol, nitrogen) from (Becerro & Paul, 2004; Giamate, 2007). They also maintain nitrogenase activity and sponges get benefit through their nitrogen fixation from atmosphere (Carpenter & Foster, 2002).

Marine cyanobacterial symbionts can make up as much as 40% volume of sponge tissue (Vacelet, 1975). This allows optimum light reception for photosynthetic symbionts (Wilkinson *et al.*, 1992). Common sponge specific cyanobacteria genera are *Proclorococcus*, *Synechocystis*, *Oscillatoria*, *Phormodium* and *Cyanobacterium* (Carpenter & Foster, 2002; Taylor *et al.*, 2007). The food source for sponges are the micro and pico-plankton species which they ingest through active suspension feeding (Savarese *et al.*, 1997).

The coastline of Pakistan is about 1050 km long and lies in Sindh (250 km) and Balochistan (800 km) provinces (Ahmed et al., 2016; Shoaib et al., 2017). Four species of mangrove have found in Pakistan, the most frequently encountered being Avicennia marina followed by Rhizophora mucronata, Ceriops tagal and Aegiceras corniculatum (Siddiqui et al., 2008). The major mangrove forests, which occupy about 867.27 km<sup>2</sup> along the Indus deltaic region at South west of Karachi (Anon., 2005) and few pockets of 0.2 km<sup>2</sup> along Makran coast of Balochistan (Saifullah & Rasool, 1995; Khan & Aziz, 2001; Tariq et al., 2006). Previous studies on mangrove phytoplankton in Sandspit backwaters have revealed the presence of cyanophytes Phormidium tenue, Oscillatoria brevis, O. subbrevis, O. limosa, O. princeps and Spirulina labyrinthiformis (Ahmed et al., 2016; 2020) and Chroococcidiopsis sp. and Microcystis sp (Bano et al., 2021) as well as several species of diatoms (Shoaib et al., 2017)) and diatoms Cyclotella cf. meneghiniana, Cylindrotheca closterium, Navicula sp., Nitzschia sp., Pleurosigma sp. and Gyrosigma sp., whereas Chaetocerose affine, Navicula directa, Nitzschia longissima, N. closterium, Pseudonitzschia fraudulenta, P. subfraudulenta, Rhizosolenia setigera and

*Thalassionema nitzschiodes* have recorded from Manora channel (Naz *et al.*, 2010; 2012; 2014). There are no data on the phytoplankton community structure from marine sponges in Pakistan. The goal of this study was to explore the phytoplankton associated with marine sponge *Liosina paradoxa* Thiele 1899 that attaches to pneumatophores of *Avicennia marina* at Sandspit backwater, Karachi coast.

# **Materials and Methods**

Marine sponge Liosina paradoxa Thiele, 1899 was collected from pneumatophores of Avicennia marina from intertidal region of Sandspit backwater (66°54'25" E, 24°49'20" N), Karachi coast in 2013 (Fig. 1). The collected samples were washed thoroughly with seawater, retained (200 ml) triplicate water subsamples in screw tap polythene bottles and preserved with 4% buffered formalin solution. During collection, physicochemical parameters (temperature, salinity, pH and dissolved oxygen) were observed in situ from channel water. The sponge specimen was identified through morphological features (shape, texture and color and skeletal framework) illustrated in previous taxonomic literature (Hooper, 2000; Hooper & Van Soest, 2002; Morrow & Cárdenas, 2015; Jabeen et al., 2018) and World Porifera Database (Van Soest et al., 2017).



Fig. 1. Map of study site Sandspit, Karachi coast, Pakistan (Google Earth Pro version 7.1.4.1529).

For qualitative assessment of phytoplankton, the preserved samples were sieved through plankton net (45  $\mu$ m mesh size) and the residue was put onto a glass slide, mounted with canada balsam and photographed under light microscope (Nikon Eclipse 50i, Japan). The species found were identified using recent literature and confirmed their names via AlgaeBase (Guiry & Guiry, 2017). Statistical analyses were conducted using PRIMER version 6 (Lambshead *et al.*, 1983), PAST version 2.13 (Hammer *et al.*, 2001) and MINITAB version 17.1.0 (2013).

# Results

A total of twenty species, representing 11 genera and three classes of phytoplankton (Cyanophyceae, Bacillariophyceae and Chlorophyceae) were found in association with *L. paradoxa* in the Sandspit backwater. The species and their characteristics are presented below.

#### **Morphological features**

### Cyanophyceae Schaffner, 1909

Oscillatoria perornata Skuja 1949: 47 (Guiry & Guiry, 2017). Trichomes erect, with attenuated and curved apices, well constricted at cross wall. Cells broad and granular with depressed ends. Size of filament: about  $65 \times 5 \mu m$  (Fig. 2A).

*O. princeps* Vaucher ex Gomont 1892: 206, pl. VI/6: fig. 9 (Guiry & Guiry, 2017; Ahmed *et al.*, 2016). Trichomes straight and shorter than wide cylindrical cells attenuated at their ends. Unbranched and smooth filaments. Akinetes and heterocysts are absent. Filament size: about  $55 \times 15 \mu m$  (Fig. 2B).

*O. tenuis* Agardh ex Gomont 1892: 220, pl. VII/7: figs. 2, 3 (Guiry & Guiry, 2017). Trichomes are thin, straight, curved at ends, not capitate and not attenuated at apices. Filaments are thin, well-branched, elongated. Cells are hemispherical. The size of filament: about  $55 \times 02 \ \mu m$  (Fig. 2C).

*Phormidum tenue* Gomont 1892: 169, pl. IV/4: figs. 23-25 (Guiry & Guiry, 2017; Ahmed *et al.*, 2016). Filaments long, solitary, finely thin and coiled consisting of thin cylindrical trichomes slightly attenuated at the ends and with apical rounded cells. Cells cylindrical, longer than wide without akinetes, heterocytes and calyptras. The filament size: about  $110 \times 1.5 \mu m$  (Fig. 2D).

#### Bacillariophyceae Haeckel, 1878

Diploneis smithii (Brébisson) Cleve, 1894: 96, pl. 5 (Guiry & Guiry, 2017). Elliptical with small central nodule and terminal nodules close to end. Furrows narrow, costae indistinctly punctate, 8 to 10 in 10  $\mu$ m, alternating with alveoli rows arranged in oblique lines. Size: about 25 × 18  $\mu$ m (Fig. 3A).

*Gyrosigma* sp. Hassall, 1845 (Guiry & Guiry, 2017). Elongated and sigmoid valves with small central nodule. The ends of median line directed contrary. Central and axial areas small, not distinct. Areolae arranged in longitudinal rows. Size: about  $55 \times 09 \ \mu m$  (Fig. 3B).

Gyrosigma wansbeckii (Donkin) Cleve, 1894: 119, pl. 5 (Guiry & Guiry, 2017). Linear and slightly curved cell with tapered and oblique rounded ends. Median line is sigmoid and eccentric. Size: about  $45 \times 06 \mu m$ .

*Halamphora coffeaeformis* (Agardh) Kützing, 1844: 108, pl. 5: fig. 37 (Guiry & Guiry, 2017). Valves lanceolate, delicate, obtuse rounded ends with strong marginal longitudinal lines. Size: about  $20 \times 07 \mu m$  (Fig. 3C).

*Halamphora proteus* Gregory, 1857: 518, pl. 13: fig. 81 (Guiry & Guiry, 2017). This is elliptical, truncate, narrow and long. Acute valves with obtuse ends, inner lines are curved and nodules are distinct. The cell size is about  $25 \times 10 \mu m$ . The striations in inner lines are longitudinally arranged while transverse striations are fine moniliform (Fig. 3D).

*Navicula* sp. Bory, 1822 (Guiry & Guiry, 2017). Oblong cell with median longitudinal line. Frustules are free, valves are convex and nodules present at center. Striations arranged in circular dots. Cell size: about  $10 \times$  $1.5 \ \mu m$  (Fig. 3E).



Fig. 2. Light microscopic images of species belong to Cyanophyceae; scale =  $10 \mu m$ , (A) *Oscillatoria princeps*, (B) *O. perornata*, (C) *O. tenuis* and (D) *Phormidium pepperitima*.

*Navicula derasa* Grunow, 1880: 39, pl. 2, fig. 46 (Guiry & Guiry, 2017). The striations on both sides of median line separated by a linear area, which is not strongly transverse. Cell size: about  $35 \times 05 \mu m$ .

*Nitzschia longissima* (Brébisson) Ralfs in Pritchard, 1861: 783, pl. 4: fig. 23 (Guiry & Guiry, 2017). Cell is much elongated, slightly attenuated with capitate edges and valves have striations. Size: about  $35 \times 2.3 \mu m$ .

*Nitzschia palea* (Kützing) Smith, 1856: 89 (Guiry & Guiry, 2017). This is linear, valves are linear-lanceolate with acute edges. Size: about  $40 \times 05 \ \mu m$  (Fig. 3F).

*Nitzschia sigma* (Kützing) Smith, 1853: 39, pl. 13: fig. 108 (Guiry & Guiry, 2017). Lanceolate and linear cell with acute, blunt edges and striations of keel present in a double row. Cell size: about  $65 \times 07 \mu m$  (Fig. 3G).

*Pinnularia* sp. Ehrenberg, 1843 (Guiry & Guiry, 2017). Cell is lanceolate and ribbed with distinct costa, not aligned into striate. Frustules are free, valves are convex with median line and nodules present at center and ends. Cell size: about  $40 \times 12 \ \mu m$  (Fig. 3H).

Surirella fastuosa (Ehrenberg) Ehrenberg, 1843: 388 (Guiry & Guiry, 2017). Ovate cell with small alae, few canaliculi, turgid median line and inflated towards margin. Cell size: about  $18 \times 10 \ \mu m$  (Fig. 3I).

Surirella linearis Smith, 1853: 31, pl. 8: fig. 58 (Guiry & Guiry, 2017). Cell is ovate with distinct canaliculi. Frustules are free, valves with longitudinal median line and margins produced into linear, parallel and acuminated alae. Cell size: about  $15 \times 11 \mu m$ .

Surirella ovata Kützing, 1844: 62, pl. 7: figs 1-4 (Guiry & Guiry, 2017). The cell is minute, ovate with small alae and marginal canaliculi. Cell size: about  $25 \times 16$  (Fig. 3J).

#### Chlorophyceae Wille, 1884

*Rhizoclonium tortuosum* (Dillwyn) Kützing, 1845: 205 (Guiry & Guiry, 2017). Thalli, soft, composed of a cluster of unbranched filaments, green, cells, barrel-shaped, not constricted at nodes, rhizoids absent; chloroplast parietal. Filament length: about 150  $\mu$ m, cell size: about 15 × 03  $\mu$ m (Fig. 3K).

Ulothrix tenuissima Kützing, 1833: 518 (Guiry & Guiry, 2017). Cylindrical barrel-shaped cells have distinct pyrenoids which distinguished by surrounding chloroplast. Curved and unbranched filaments in old stages contains uniseriate cells with girdle-shaped parietal chloroplast. Filament length: about 110  $\mu$ m, cell size: about 10 × 04  $\mu$ m (Fig. 3L).



Fig. 3. Light microscopic images of species belong to Bacillarioophyceae, (A) *Diploneis smithii* (scale = 10  $\mu$ m), (B) *Gyrosigma* sp. (scale = 10  $\mu$ m), (C) *Halamphora coffeaeformis* (scale = 5  $\mu$ m), (D) *H. proteus* (scale = 10  $\mu$ m), (E) *Navicula* sp. (scale = 5  $\mu$ m), (F) *Nitzschia palea* (scale = 10  $\mu$ m), (G) *N. sigma* (scale = 15  $\mu$ m), (H) *Pinnularia* sp. (scale = 10  $\mu$ m), (I) *Surirella fastuosa* (scale = 5  $\mu$ m), (J) *S. ovata* (scale = 5  $\mu$ m) and species of Chlorophyceae; scale = 10  $\mu$ m, (J) *Rhizoclonium tortosum* and (K) *Ulothrix tenuissima*.

**Seasonal diversity:** The seasonal diversity of Cyanophyceae was similar during pre-monsoon and monsoon, Bacillariophyceae diversity was maximum in monsoon while Chlorophyceae in post-monsoon (Table 1). The percent species composition of diatoms was dominated by 14 species (90%) among all other communities, along with 4 species of cyanobacteria (6%) and 2 species of green algae (3%) (Fig.

4). The seasonal variation between cyanophytes, bacillariophytes and chlorophytes were observed during premonsoon, monsoon and post monsoon periods and overall highest density was observed in monsoon season in which cyanophytes and chlorophytes proliferated (64% and 50%, respectively) while diatoms were most abundant in the premonsoon season (42%) (Fig. 5).

Species	Seasons					
	Pre-monsoon	Monsoon	Post monsoon			
	Cyanophyceae					
Oscillitoria priceps		+	+			
O. perornata	-	+	-			
O. tenuis	+	-	-			
Phormidium pepperitima	+	-	-			
		Bacillariophyceae				
Diploneis smithii	+	+	-			
Gyrosigma sp.	+	+	+			
Gyrosigma wansbeckii	+	+	+			
Halamphora coeffoeformis	+	-	-			
H. proteus	+	+	-			
Navicula sp.	+	+	+			
<i>Nitzschia</i> sp.	-	+	+			
Nitzschia palae	+	+	+			
N. sigma	+	+	+			
N. longissima	-	+	-			
Pinnularia sp.	+	+	+			
Surirella fastuosa	+	+	+			
S. linearis	+	+	+			
S. ovata	+	+	+			
	Chlorophyceae					
Rhizoclonium tortosum	+	-	+			
Ulothrix tenuissima	-	-	+			

 Table 1. The seasonal occurrence of phytoplankton species associated with Liosina paradoxa Thiele, 1899 at

 Sandspit backwater mangroves, Karachi.

+ Presence of species, - Absence of species

Table 2. Seasonal variation in diversity indices	s of
phytoplankton species at Sandspit backwater. Ka	rachi

Indices	Cyanophy- ceae	Bacillario- phyceae	Chloro- phyceae
Margalef (R <sub>1</sub> )	0.83	0.40	0.56
Menhinick (R <sub>2</sub> )	0.90	0.24	0.82
Simpson (λ)	0.51	0.65	0.28
Shannon-Weiner (H')	0.86	1.07	0.45
Evenness (J')	0.79	0.97	0.78
Dominance (D)	0.49	0.35	0.72



Fig. 4. Percent composition of phytoplankton groups associated with *Liosina paradoxa* at Sandspit backwater, Karachi.

The physicochemical factors of water in mangroves have shown variation with season. Temperature shown decreasing point seasonally from pre-monsoon to post monsoon period while salinity value was found minimum (35 PSU) during monsoon (Fig. 6). The concentration of pH was observed alkaline throughout study whereas, the trend line of dissolved oxygen in water also indicated decreasing trend from pre-monsoon (3.45 mgL<sup>-1</sup>) to post monsoon (0.11 mgL<sup>-1</sup>) as depicted by temperature (Fig. 6). The range of temperature was 27-35  $\pm$  4.6°C, salinity was 35-39  $\pm$  1.47 PSU and pH was 7.04-7.69  $\pm$  0.19, respectively.

The cumulative dominance (K dominance curve) of phytoplankton community has shown highest species rank during monsoon followed by pre-monsoon whereas slight lowest rank was observed during post-monsoon (Fig. 7). The maximum species richness was observed 0.53 Margalef (R<sub>1</sub>) index, 0.46 Menhinick (R<sub>2</sub>) index. Species diversity was 0.25 Simpson ( $\lambda$ ) index and 0.48 Shannon-Weiner (H') index during post monsoon. The range of species evenness (J') was 0.42-0.70 in which maximum value was observed during monsoon. The maximum dominance (D) was recorded 0.89 during pre-monsoon (Table 2).

Pearson correlation coefficient between phytoplankton community associated with *L. paradoxa* and physicochemical variables in mangroves at Sandspit backwater set-out in (Table 3). The significant positive correlation was indicated between Cyanophyceae and Bacillariophyceae. Temperature, pH and dissolved oxygen was observed significantly positive correlation with Bacillariophyceae and salinity with Chlorophyceae.



Fig. 5. Seasonal composition of phytoplankton groups associated with Liosina paradoxa at Sandspit backwater, Karachi.



Fig. 6. Seasonal variation in physicochemical parameters (temperature °C, salinity PSU, pH and dissolved oxygen mgL<sup>-1</sup>) at Sandspit backwater, Karachi.

Table 3. Pearson correlation coef	fficient matrix betweer	n phytoplankton o	community and	physicochemical
pai	rameters at Sandspit b	ackwater, Karac	hi.	

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	Cyanophyceae	Bacillariophyceae	Chlorophyceae	Temperature	Salinity	pН
Bacillariophyceae	*0.510					
Chlorophyceae	-0.866	-0.872				
Temperature	-0.075	*0.819	-0.434			
Salinity	-0.996	-0.430	*0.817	0.165		
pH	0.287	**0.970	-0.727	**0.934	-0.198	
Dissolved Oxygen	0.172	**0.935	-0.641	**0.970	-0.081	**0.993

\*= Significant, \*\*= Highly significant at p value>0.05



Fig. 7. The K dominance curves (cumulative dominance) for seasonal abundance of phytoplankton community associated with L. paradoxa at Sandspit backwater, Karachi.

# Discussion

Mangrove sponges are usually considered to live in submerged habitat due to their sensitiveness with air and light exposure (Osinga et al., 1999). In submerged condition, their mode of nutrition is filter feeding from channel water where they capture food particles (Steindler et al., 2002). In intertidal shallow water zone of Sandspit backwater, the only reported sponge species Liosina paradoxa on pneumatophores of Avicennia marina (Jabeen et al., 2018) have strong association with photosynthetic symbionts and their filtering capability may interrupt during exposure at low tide (Steindler et al., 2000; Jabeen et al., 2018). Photosymbionts of marine sponges, specifically cyanobacteria produce mycosporine-like amino acids which provides protection from UV light for both host and symbiont (Gröniger *et al.*, 2000), therefore, photosymbionts are additional source of nutrition during air exposure for intertidal sponges which may related to cellular adaptation and response for desiccation (Rützler, 1995).

Several phytoplankton species such as Oscillatoria princeps, Navicula sp., Nitzschia sp., Nitzschia longissima, Gyrosigma sp. and Halamphora sp. have recorded earlier from mangrove region of Sandspit backwater (Ahmed et al., 2016; Shoaib et al., 2017) but this study represents the first exploration of phytoplankton community assemblage with marine sponge L. paradoxa from A. marina in Pakistan, North Arabian Sea. The results have shown both bacillariophytes and cyanophytes were found numerous in sponge mesohyl extracellularly. Diatoms were pennate and mostly occurred in pairs. Cyanobacteria, particularly Oscillatoria spp. and Phormidium tenue as sponge symbiotic association in mangrove ecosystem are largely temperature tolerant and may resist to high temperature than other species (Usher, 2008). They are faster overgrowing and space competitive organisms among other benthic communities within host sponge (Diaz et al., 2007). Sponge provides large access to nutrient concentration ( $NH_4^+$  and  $PO_4^{-3}$  ions) and more likely shelter from predation of zooplanktons and zoobenthos to photosymbionts that proliferate within host tissue or grow as free-living species in mangrove habitat (Furnas & Crosbie, 1999; Partensky et al., 1999). Alternately, these photosymbionts are responsible for nitrogen fixation, particularly, Oscillatoria sp. fixes nitrogen by utilizing ammonia for sponges, shelters the sponge body to give protection from sunlight exposure and extreme temperature, provides high oxygen level to sponges through photorespiration and produce toxic biologically active secondary metabolites for their defensive purpose (Erwin & Thacker 2007). Thus, the autotrophic and heterotrophic mutualistic combination maximizes the benefits of photosynthetic output and acceptable environment to grow (Usher, 2008). The freshwater species of Chlorophyceae (Rhizoclonium tortuosum and Ulothrix tenuissima) in mangrove region with sponges may proliferate with subject to high nutrient availability at study site through effluents of Lyari river (Shoaib et al., 2017). During monsoon, rainfall is another chief factor that influence on diversity and abundance of phytoplankton community in mangrove habitat. The excessive precipitation during this season causes rising of pH level and decreasing salinity range which ultimately creates a phytoplankton bloom in channel water (Ahmed et al., 2016). The diversity of phytoplankton has varied with the season which shown maximum values during monsoon. The inter-relation of phytoplankton density with season may attribute to increase in temperature during premonsoon with respect to low salinity and high concentration of dissolved oxygen and other nutrients in channel water. The minimum density of phytoplankton has found in postmonsoon which may attribute to low temperature.

The diversity indices, particularly Shannon-Weiner (H') and Simpson ( $\lambda$ ) with and evenness (J') have indicated maximum values for Bacillariophyceae whereas, the values of species richness (Margalef and Menhinick index) have found to be high for Cyanophyceae which clearly indicated the pollution status in mangrove channel water (Brraich & Kaur, 2015). From the above estimated results, it can be

concluded that the present study is the first assessment of phytoplankton community from marine sponge *L. paradoxa* which is first recorded sponge species found attached with pneumatophores of *A. marina* at Sandspit backwater, Karachi coast, Pakistan. The dominance order in terms of phytoplankton diversity in the present study was Bacillariophyceae > Cyanophyceae > Chlorophyceae. The physicochemical factors were highly influenced on their diversity pattern. They are active bio-indicators of water quality in dense contaminated environment in Sandspit mangrove ecosystem.

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