J. Algal Biomass Utln. 2013, 4 (3): 26–34 Cyanobacterial distribution in estuary region of southeastern coast of Tamilnadu, India.

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Studies of Cyanobacterial distribution in estuary region of southeastern coast of Tamilnadu, India.

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Abstract:

Cyanobacteria are photosynthetic autotrophs, which almost covers the world prominently by exhibiting different adaptations to their locations and perform important functional asset to that ecosystem. Such tendencies help mankind in various ways such as energy fuel, food, medicine and also in bioremediation process. Marine cyanobacterial distributions are start from the estuary region to epipelagic zone of seas and ocean. Estuaries are transitional ecosystems between the ocean and freshwater biome, the benthic and picoplanktonic cultures in it. Estuarine cyanobacteria can alter phytoplankton succession, competition and bloom formation. The biodiversity of cyanobacteria were investigated in two estuaries regions such as Periyasamipuram and Keelzhavaippar in Ramanathapuram district, Tamil Nadu, India. The cyanobacterial mats were collected in three different seasons and cultivated in different media such as ASNIII and BG11 marine broth. Morphological characterization was done. Among the two regions, cyanobacterial diversity study at Periyasamipuram site showed about fourteen genera of cyanobacteria such as *Oscillatoria, Lyngbya, Microcystis, Spirullina, Chroococcus* and *Calothrix* were dominant with 36 cyanobacterial species under five families were reported when compared to other region studied. Simultaneously their environmental factor roles as biofertilizer were observed in *Triticum aestivum* (Wheat plants).

Keywords: algal mats, diversity, plant growth, *Triticum aestivum.*

Introduction:

Of the total estimated area of 150 million sq. kms of the earth, about 70.68% is occupied by oceans. India is bestowed with an exclusive economic zone (EEZ) extending to 2.02 million km^2. Such marine environment exhibits various types of settings such as shallow sea, benthic regions, estuaries, lagoons and saltpan (Kesava Das & Desai, 1988; Nedumaran, 2009).

Estuaries can be defined as the regions were fresh water mixes with sea water bodies, exhibiting oligohaline to mesohaline (Schuchardt, 1999). Vembar and Vaippar are two non perennial rivers come from Western Ghats and run towards southeastern coastal region of Tamilnadu, India. Tides are of semidiurnal type, showing substantial range and time. The average tidal range near the mouth of the estuary is -0.6m.

Cyanobacteria are Gram-negative prokaryotes but cell wall contains peptidoglycan like eubacteria. They exist in different morphologies including unicellular and filamentous forms (Castenholz, 2001). Recently, cyanobacteria in estuary region during summer seasons is abundant than other environments and simultaneously gained interest by its significant potential bio-active compounds. On account of their immense applied potentials, they are being explored widely (Murrell & Lores, 2004; Sincy Joseph, 2005).

Cyanobacteria are contains a wide range of habitat leads to wide biodiversity studies according to various environments. Samit Ray, 2006 stated that many cyanobacterial species are capable of growing on the soil and other terrestrial habitats where they play an important role in the ecosystem especially with regard to nutrient cycling.

Such cyanobacteria are presumably related to alkaline conditions and an ability to tolerate high salt concentrations. According to salinity and the location of saltpans, enrich some species of cyanobacteria. Inspite of biofertilizer ability, saline tolerant cyanobacteria can be implemented in reclamation of saline or alkaline rich soil (Sugumar et al., 2011).

The cyanobacteria have high stimulation growth, seed productions of common bean plant and simultaneously increased the biological activity of soil (Hegazi et al., 2010). The present studies appraised the ecological distribution of

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marine cyanobacteria in two estuary sites of southeast coastal region of Tamilnadu and simultaneously checked their influence on salt tolerance on wheat plants treated with seawater.

Materials and Methods

Sampling sites:

The sample was carried out at two different estuary region near Periyasamipuram ($9^0$03'05.16" N, $78^0$17'54.95" E) and Keelzhavaippar ($8^0$32'05.65" N, $78^0$06'36.60" E) are arised by mixing of non perennial rivers Vembar and Vaippar comes under southeastern coastal region of Tamilnadu, India. Both river basins are experiences both south-west and north-east monsoons. The samples were collected during pre-monsoon, monsoon and post-monsoon.

Sample collection:

Samples such as water, soil and visible cyanobacterial mats two sites of each estuary regions were bimonthly collected from August 2011 to September 2012. Such samples were maintained in sterile polythene bags and plastic containers with code markings of place and area of collections. Such collections were made in triplicate and preserved temporarily in ice bags.

Cultivation and Purification:

Cyanobacterial mats were maintained in ASN III, MN II and BG 11 marine broth (Rippka et al., 1979). The cultures were incubated at 25^oC with an illumination of white fluorescent lamps of 2500 lux for 6 hrs till 20 days. The cultures were made pure by repeated and frequent subculturing in BG 11 medium. Then the samples were plated on solid cyanophycean agar medium. Inoculated plates were incubated in culture room maintained at 25 ± 2^oC fitted with cool white fluorescent tube emitting 2500lux for 18hrs a day (Vijayakumar et al., 2007).

Identification:

Microscopic observations were carried out at 40X magnifications using light microscope. Cyanobacterial specimens were identified using the publications of Desikachary (Desikachary, 1959). Enumerations and seasonal distribution of marine cyanobacteria in various estuaries sites were observed.

The statistical observations were performed on both occurance and habitat. Diversity indices like Simpson's index, Margalef index, McIntosh index, Pielou evenness & McIntosh evenness of different season on estuary sites were calculated periodically using standard procedures (Gencer Turkmen & Nilgun Kazanci, 2010). The physicochemical parameters of soil samples from the sampling sites were calculated using standard procedures (Vijayakumar et al., 2011). The correlationship parameters of soils samples to the cyanobacterial count during Post- monsoon season (April) were calculated.

Effect of salt tolerance improvement in plants

Algal Spray of 1 Litre containing homogenized algal cells of about 0.5%, 0.1% Tween 20 and plant bioregulators (Ascorbic acid, Benzyl adenine) 220 pm. The algal spray was sprayed from the germinative stage of wheat (*Triticum aestivum*) plants irrigated with sea water. Calculate their growth upto 40^{th} day of plants (Hanaa et al., 2008).

Results and Discussion:

In this present investigation, a total number of 36 species of cyanobacteria from 14 genera belonging to 5 families were recorded of which, 13 were unicellular colonial forms and 23 nonheterocystous filamentous forms. Among the two estuaries studied, maximum number of species about 23 species of 12 genera was recorded from Periyasamipuram and minimum in Keelzhavaippar site. Different seasonal habitation of cyanobacteria were observed and its mean were expressed in Table 1. Interestingly during Post monsoon season, highest numbers of cyanobacterial species are acquired.

Cyanobacteria from estuaries belong to the following genera: *Aphanocapsa*, *Aphanothece*, *Chroococcus, Microcystis, Synechococcus, Synechocystis, Myxosarcina, Stichosiphon, Spirulina, Oscillatoria, Phormidium, Lyngbya, Microcoleus* and *Calothrix.* Among these 14 genera, *Oscillatoria* and *Lyngbya* were represented by seven species followed by *Phormidium* and *Spirulina* were represented by four species, *Microcoleus, Calothrix* and *Stichosiphon* genera shown by single species and others were observed as two species. The cyanobacterial species such as *Chroococcus minutus, Oscillatoria subbrevis* and *Lyngbya aestuarii* were observed in both estuaries which are considered as versatile species (Fig.1).

The diversity indices such as species richness, evenness and diversity of different seasonal estuaries sites were calculated (Table.2). Diversity value in Periyasamipuram estuary (0.96) site is highest than the other. Interestingly, cyanobacterial species were evenly distributed in both estuaries site.

The physico-chemical parameters of soil were observed and its mean value was tabulated (Table 3). The correlation ships between physic-chemical parameters with the cyanobacterial count from the estuaries sites were calculated (Table 4). The correlation coefficient results showed that the soil parameters were almost positively correlated with the cyanobacterial count. Among the physico-chemical parameters, phosphorus (0.939), Manganese (0.76) and pH (0.573) were significantly positive correlationship with cyanobacterial count.

Under invitro condition, the three versatile cyanobacterial species from the two estuaries sites were found that the salt tolerant of wheat plants irrigated with sea water (Table 5). When compared with the control (T2), the marine cyanobacterial extract treated plants have the higher growth and higher amount of fresh and dry weight. Among the different treatment, P3 treatment (*Lyngbya aestuarii* extract + BRGs) shows the maximum effect on the growth of wheat plants.

When compared to the unicellular and non heterocystous filamentous forms, the heterocystous forms were very poor in numbers in the east coast of India and this might be due to the high levels of combined nitrogen in the sea (Thajuddin & Subramanian, 1990). Selvakumar and Sundararaman (2009) observed twelve species of unicellular and filamentous species of cyanobacteria belonging to either *Chroococcaceae* or *Oscillatoriaceae* families in estuarine water. Similar observation could be made in the present study also.

The remarkable adapting ability of cyanobacteria to wide ranges of environmental factors or to the salinity is however well known (Desikachary, 1959; Carr & Whitton, 1982). Thajuddin and Nagasathya (2008) observed that 61 cyanobacterial species recorded from different saltpan region of south eastern coast of India. Subramanian and Thajuddin (1995) reported that the maximum diversity of the cyanobacterial flora in the Gulf of Mannar region correlated well with the higher salinity, pH and nutrient content of the water.

Salinity and pH was found to be positively correlation with cyanobacterial numbers. The significant positive correlation between the cyanobacterial diversity and micronutrients was observed (Muthukumar et al., 2007). Chellappa et al., (2003) reported the collective dominance by the species of cyanobacteria was due to their capacity to grow in turbid water and low light intensity to maintain buoyancy and the capacity to grow exponentially in wet period in which nitrogenous nutrients were high.

Cyanobacteria can enhance the plant growth by both directly and indirectly. Nanjappan-Karthikeyan et al., (2007) concluded that cyanobacterial species have growth promoting activity as inoculants of wheat. Rodriguez et al., (2006) stated that the improvement of rice seedling salt tolerance was probably due to the presence of hormones in the extracellular products produced by the cyanobacterium *Scytonema hofmanni*. Hanaa, (2008) observed that the extracts of *Spirulina maxima* take over the salinity stress induced in wheat crops while treated with brackish water containing 20% (v/v) sea water.

The present studies showed that all diversity indices and evenness results of cyanobacterial species in a particular region were almost similar and highly resemble to each others, interestingly few exhibiting soil reclamation applications. Likewise cyanobacterial growth is highly correlated with the medium (sea water and soil) compostition. Apart from the nitrogen fixation, marine cyanobacterial species act as bioferitlizers for the growth of plants in the semi-arid regions. Further studies in these marine cyanobacteria are needed to find out its phylogenetic relationship with each other and explore its potential.

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Table1. Cyanobacterial species observed in different seasonal estuary sites.

Name of the organism	Collection sites					
	Pre monsoon		Monsoon		Post monsoon	
	PS	KS	PS	KS	PS	KS
Order: Chroococcales Wettstein **Family: Chroococcaceae** Nageli						
Aphanothece microscopica Nag	+	+	+	+	-	++
Aphanocapsa littoralis Hansgirg	+	+	+	+	+	+
Aphanocapsa marina	-	-	-	-	+	-
Chroococcus minutus (Kutz.) Nag	+	++	+	++	+	++
*Chroococcus turgidus (*Kutz.*)* Nag	-	-	+	+	-	+
Microcystis litoralis (Hansg.) Forti	-	-	-	+	-	+

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Microcystis aeruginosus Kutz	-	-	-	-	-	-
Synechocystis salina wislouch	-	+	+	-	+	+
Synechocystis pevalekii Ercegovic.	-	-	-	-	-	-
Synechococcus aeruginosus Nag	-	-	+	+	+	+
Synechococcus elongatus Nag	+	+	+	-	+	++
Order: Pleurocapsales Geitler **Family: Pleurocapsaceae** Geitler						
Myxosarcina burmensis skuja	-	+	-	-	-	+
Myxosarcina spectabilis Geitler	-	-	-	-	-	+
Order: Nostocales Geitler **Family: Oscillatoriaceae** Kirchner						
Lyngbya aestuarii Liebm. Ex Gomont	+	+	++	++	+	++
Lyngbya ceylanica v. *constricta* Fremy	-	-	+	-	+	-
Lyngbya sps	-	-	-	+	-	-
Lyngbya lutea (Ag.) Gomont	+	+	-	-	+	-
Lyngbya tayloriae Forti	+	+	-	-	+	-
Oscillatoria brevis (Kutz.) Gomont	-	-	-	-	-	+
Oscillatoria chlorina Kutz. ex Gomont	+	+	-	-	+	+
Oscillatoria limosa Ag. Ex Gomont	-	-	+	+	+	-
Oscillatoria lemmermannii Wolosz	+	+	-	-	-	-
Oscillatoria subbrevis Schmidle	++	+	+	+	+	++
Oscillatoria salina Biswas	-	-	+	+	-	+
Oscillatoria willei Gardner em. Drouet	-	-	+	-	+	-
Oscillatoria tenuis Ag. ex Gomont	-	-	-	-	-	+
Microcoleus chthonoplastes Thuret ex Gomont	-	-	-	-	-	+
Spirulina major Kutz. Ex Gomont	-	-	-	-	-	-
Spirulina laxissima forma *major*	+	+	+	+	-	-
Spirulina subsalsa Oerst. Ex Gomont	-	-	+	+	+	+
Spirulina labyrinthiformis (Menegh.) Gomont	+	+	-	-	-	+
Phormidium fragile (Meneghini) Gomont	+	+	-	+	-	+
Phormidium corium (Ag.) Gomont	+	+	-	-	+	-
Phormidium tenue (Menegh.) Gomont	-	-	+	-	-	+
Phormidium valderianum (Delp.) Gomont	-	-	+	+	+	+
Family: Rivulariaceae Rabenhorst						
Calothrix geitonos Skuja	-	-	-	+	-	+
PS-Periyasamipuram estuary site, KS-Keelzhavaippar estuary site,						

Table 2. Diversity indices of cyanobacteria in both estuaries sites.

Diversity Indices		Estuary Site					
		Pre monsoon		Monsoon		Post monsoon	
		PS	KS	PS	KS	PS	KS
Richness	Margalef index (d)	6.5	6.4	8.8	8.5	8	10.7
	McIntosh Index (Mc)	0.63	0.74	0.86	0.83	0.79	0.98
Evenness	Pielou Evenness (J’)	0.75	0.67	0.99	0.96	0.93	0.99
	McIntosh Evenness (McE)	0.87	0.85	0.999	0.97	0.95	1.0
Diversity	Simpson’s Diversity	0.91	0.92	0.96	0.94	0.95	0.96
	Shannon‘s Diversity (H’)	2.6	2	2.9	2.8	2.6	3.5

PS-Periyasamipuram estuary site, KS-Keelzhavaippar estuary site

Table 3. Physico-chemical parameters of soil samples in both estuaries sites;

Station	Physico-chemical parameters									
	pH	EC (dSm^{-1})	N ($Kgha^{-1}$)	P ($Kgha^{-1}$)	K ($Kgha^{-1}$)	C (%)	Fe ($mg.Kg^{-1}$)	Mn ($mg.Kg^{-1}$)	Zn ($mg.Kg^{-1}$)	Cu ($mg.Kg^{-1}$)
PS^{*}	7.62	15.68	229	10	370	0.55	7.25	6.10	0.98	0.52
KS^{*}	8.15	8.48	90	19	180	1.14	7.15	5.62	0.73	0.56
PS^{**}	7.57	14.12	101	18	370	0.75	5.75	5.95	0.84	0.49
KS^{**}	7.19	14.27	131	14	471	0.25	8.50	5.25	0.85	0.42
PS^{***}	7.96	13.36	210	12	330	0.45	5.68	4.87	0.69	0.41
KS^{***}	8.23	12.45	156	11	370	0.64	7.90	6.45	0.86	0.45

PS-Periyasamipuram estuary site, KS-Keelzhavaippar estuary site, EC- Electric conductivity, N- Available Nitrogen, P-Phosphorus, K- Potassium, C- Carbon, Fe-Iron, Mn-Manganese, Zn- Zinc and Cu-Copper. * - Pre-monsoon, ** - Monsoon, *** - Post-monsoon.

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Table 4. Correlation coefficient of physico-chemical parameters of soil against total cyanobacterial count

	pH	**EC**	**N**	**P**	**K**	**C**	**Fe**	**Mn**	**Zn**	**Cu**	**TCS**
pH	1										
EC	-0.662	1									
N	0.004	0.596	1								
P	0.309	0.204	-0.028	1							
K	-0.722	0.830	0.275	0.138	1						
C	0.620	-0.781	-0.538	0.155	-0.891	1					
Fe	-0.196	-0.035	-0.096	-0.202	0.335	-0.243	1				
Mn	0.267	0.059	-0.077	0.779	0.014	0.325	0.26	1			
Zn	-0.421	0.643	0.301	0.357	0.529	-0.281	0.43	0.67	1		
Cu	0.262	-0.446	-0.315	0.027	-0.683	0.829	-0.04	0.44	0.19	1	
TCS	0.573	0.062	0.141	0.939	-0.069	0.251	-0.22	0.76	0.26	0.09	1

TCS-Total cyanobacterial count, EC- Electric conductivity, N- Available Nitrogen, P-Phosphorus, K- Potassium, C-Carbon, Fe-Iron, Mn-Manganese, Zn- Zinc, Cu-Copper

Table: 5 Effect of Cyanobacteria extracts to improve salt tolerance of wheat plants irrigated with sea water

Treatment	**Height of plants (cm)**				**Weight of plants (g) after 40 days**	
	10 days	**20 days**	**30 days**	**40 days**	**Fresh weight**	**Dry weight**
T1	9.96±0.15	19.90±0.20	24.96±0.15	28.06±0.20	2.72±0.05	1.42±0.01
T2	6.03±0.10	17.00±0.10	21.60±0.10	23.40±0.2	2.21±0.03	1.15±0.01
T3	9.03±0.25	18.60±0.15	22.73±0.15	26.62±0.15	2.50±0.01	1.21±0.03
C1	15.10±0.30	23.61±0.15	27.00±0.10	29.41±0.25	2.80±0.01	1.45±0.01
C2	13.00±0.20	22.15±0.15	26.00±0.15	27.93±0.20	2.54±0.01	1.38±0.01
C3	17.00±0.30	27.53±0.15	30.51±0.10	32.00±0.25	2.90±0.03	1.60±0.01
D1	15.90±0.15	24.00±0.15	27.43±0.15	29.96±0.25	2.81±0.03	1.56±0.04
D2	13.53±0.25	21.96±0.15	26.63±0.15	28.33±0.20	2.75±0.01	1.40±0.01
D3	17.20±0.10	27.82±0.10	31.16±0.15	33.00±0.20	3.41±0.03	1.82±0.02
P1	16.13±0.32	24.96±0.15	29.26±0.15	32.16±0.20	3.51±0.04	1.61±0.01
P2	15.50±0.20	23.11±0.32	27.46±0.15	31.93±0.20	3.33±0.03	1.52±0.03
P3	18.00±0.15	28.36±0.41	31.23±0.20	33.95±0.26	3.72±0.05	1.96±0.01

T1 = Tap waters + BRGs, T2 = Sea water (control), T3 = Tap water, BRGs = Plant BioRegulators, C1 = Sea water + *Chroococcus minutus* extract; C2 = Sea water + BRGs; C3 = Sea water +*Chroococcus minutus* extract +BRGs, D1 = Sea water + *Oscillatoria subbrevis* extract; D2 = Sea water + BRGs; D3 = Sea water + *Oscillatoria subbrevis* extract + BRGs, P1 = Sea water + *Lyngbya aestuarii* extract; P2 = Sea water + BRGs; P3 = Sea water + *Lyngbya aestuarii* extract + BRGs

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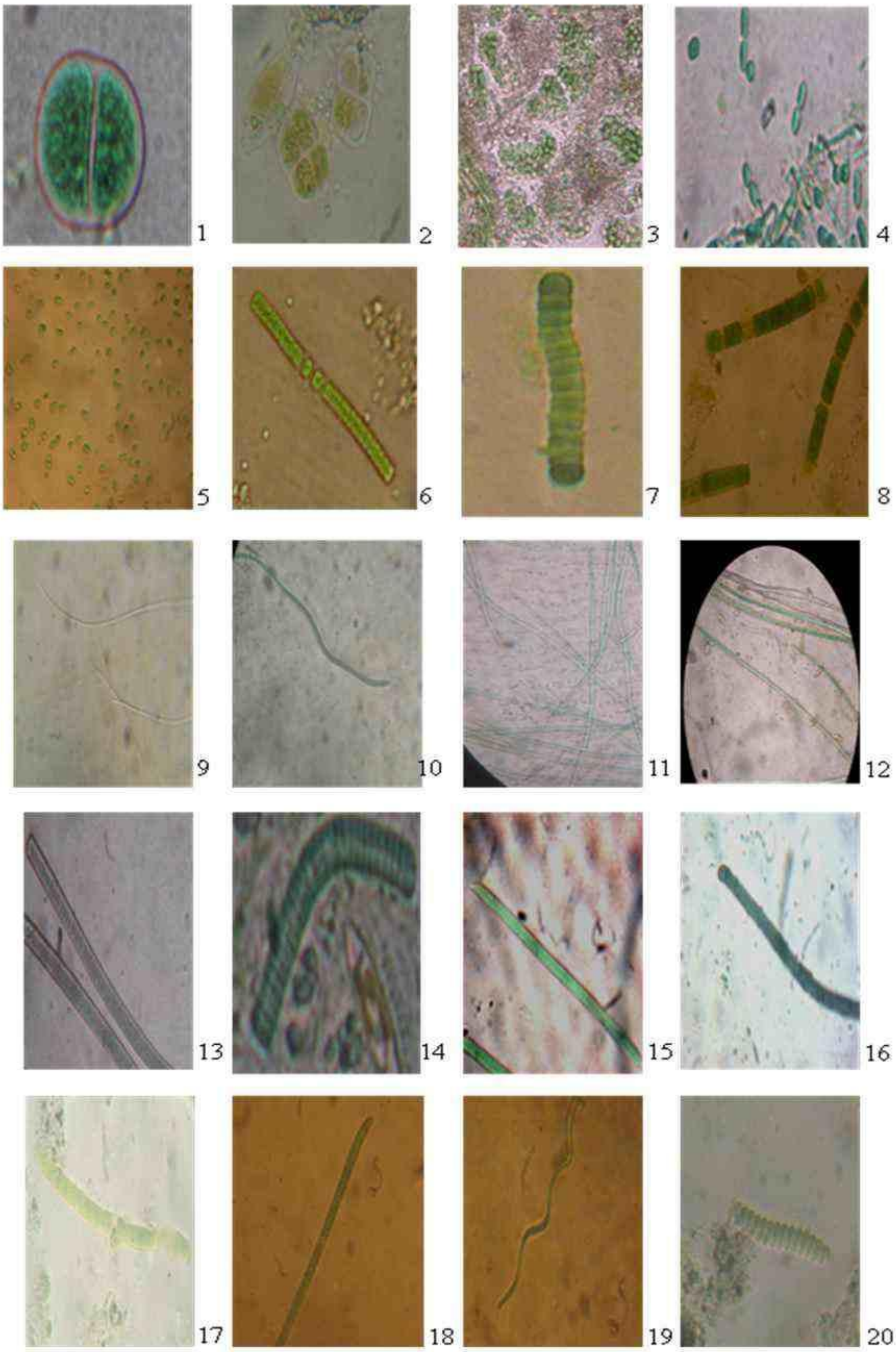

Fig. 1 Microscopic photograph of cyanobacteria from estuaries sites of southeast coast of Tamilnadu: 1. *Chroococcus minutus* (Kutz.) Nag, 2. *Myxosarcina spectabilis* Geitler along with diatom, 3. *Myxosarcina burmensis* Skuja, 4.*Synechococcus aeruginosus* Nag, 5.*Synechocystis pevalekii* Ercegovic, 6.*Lyngbya* sps, 7.*Lyngbya polysiphoniae* Fremy, 8.*Lyngbya ceylanica* v. *constricta* Fremy, 9. *Phormidium valderianum* (Delp.) Gomont, 10.*Phormidium tenue* (Menegh.) Gomont, 11. *Lyngbya lutea* (Ag.) Gomont, 12. *Lyngbya aestuarii* Liebm ex Gomont, 13. *Oscillatoria limosa* Ag. ex Gomont, 14.*Oscillatoria subbrevis* Schmidle, 15. *Oscillatoria salina* Biswas, 16.*Oscillatoria lemmermannii* Wolosz 17. *Oscillatoria chlorina* Kutz. ex Gomont, 18.*Oscillatoria willei* Gardner em. Drouet, 19.*Spirulina laxissima* forma *major* and 20. *Spirulina subsalsa* Oerst. ex Gomont.

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