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Full Length Research Paper

The population abundance, distribution pattern and culture studies of isolated microalgal strains from selective sampling sites along the south east coast of India

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The present study was conducted to understand the microalgal dynamics and surveillance in the selective sites along the south east coast of India. Algal isolation was carried out in 61 sampling stations characterized by different ecological features. In total 10 microalgal species were isolated under laboratory condition from the collected samples. The composition of microalgal distribution and their surveillance were related to the environmental factors are discussed in the present paper. From the results it was observed that *Isochrysis galbana* [MA1] has the maximum surveillance at 37 spots [60.7%]. It was also observed that 25.7% of the collection spots may share same microalgal dynamics and surveillance. In order to understand the better background information about the importance of culture condition in the optimal growth of microalgal strains, experimental setup were designed using modified Walne's and Guillard f/2 medium. Studies were also carried out to understand the relation between the growth conditions and environmental factors including salinity, temperature, pH and dissolved oxygen. The growth study was further designed by providing the culture setup with 2 different light : dark illustration of 24:0 with 1000 lux setup and 16:8 with 1000 lux. The results show 70% of the isolated samples grown in Walne's medium and 60% of samples grown on guillard's f/2 medium prefer to grow optimally under 16:8 light: dark illustration. It was also observed that Walne's medium encourages better growth for the collected microalgal samples when compared with the Guillard's medium.

Key words: Microalgae, inoculum, phytoplankton, dissolved oxygen, GPS.

INTRODUCTION

Marine phytoplankton comprises a complex community of several thousands of floating microalgae in the sea ranging in size from about 1 µm upto a few millimeters. Based on their size, phytoplankton can be classified as macroplankton (more than 1 mm), microplankton (less than 1 mm, retained by nets of mesh size 0.06 mm), nanoplankton (between 5 and 60 micrometers) and ultraplankton (less than 5 micrometers). Many phyto-plankton species belong mainly to the nanoplankton and microplankton fractions.

The scientific approach towards the selective studies on microalgae is significant because of its dynamic growth characteristics, potent reproducibility, easily available *in vitro* culture method, distinguished taxonomy, hidden medicinal properties and possible gene recom-bination ability (Parker et al., 2008). Studies on the various aspects of species composition, density, distri-bution and seasonal variations of marine phytoplankton relating to coastal waters have been carried out from various parts of the world by Ignatiades and Mimicos (1977) from the Sarnikos gulf waters, Figueiras (1989) from the Spanish waters of Atlantic coast, Jae Sam Yong (1990) from Maxiwell bay of George Island and Yamaguchi et al. (1994) from Thale Sap Songkhia of Thailand.

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Singh (1942), and Parukutty (1940) made effective contribution to the Indian flora of algae. Publications by Iyengar and Desikachary (1944, 1946a, 1946b, 1953, 1954) and Desikachary (1945, 1946a, 1946b, 1953) added consisderably to the existing knowledge of Indian algae, particularly the blue green algal flora of south India.

In India, such studies have been carried out from the east and west coasts. Preliminary and systematic accounts on the phytoplankton of Madras coast (Subrahmanyan, 1946) and the shore waters of Gulf of Mannar (Prasad, 1958) are found to be very useful and significant. In recent years, microalgal culture technology is a business oriented line owing to their different practical applications. Innovative processes and products have been introduced in microalgal biotechnology to produce vitamins, proteins, cosmetics and health foods. For most of these applications, the market is still developing and the biotechnological use of microalgae will extend into new areas. With the development of sophisticated culture and screening techniques, microalgal biotechnology can meet the challenging demands of both food and pharmaceutical industries (Raja et al., 2008).

Algal cultural profile is a relative conservative property of the laboratory conditions maintained during the experiments. It explains the relative property of particular algal strains. Many factors which affect the culture status of the culturing microalgae such as genetic characteristics, role of symbiotic microbes, environmental factors such as light intensity (lux), temperature and pH. It has been previously reported that in a batch culture experiments under laboratory conditions, the growth status of particular species can vary with growth stages and physical conditions (Ref). Studies were also carried out to understand the relation between the growth conditions and environmental factors including salinity, temperature, lux and nutritional values. It is noteworthy that the nutrients (including NPK sources) are the most notable factors affecting the growth conditions.

The microalgal isolation was carried out in 61 sampling stations characterized by different ecological features. The composition of microalgal distribution and their surveillance were related to the environmental factors and discussed. This paper also provides better background information about the importance of culture condition in the optimal growth of microalgal strains.

MATERIALS AND METHODS

Marking of collection spots

61 sampling sites were selected along the south east coat of Tamil Nadu India to understand the habitat biology and the distribution pattern of microalgae. The selection and marking of the spots were achieved by the “mapsource” software version 2002. The topography of the sampling site including the hydrodynamics were taken into account during the collection. The identified collection spots were marked as destination points in the GPS which was stored as (Position 1, 2, 3, 4, 5) in the way points list.

GPS based survey method

The identified collection spots using map source were Physically surveyed (Figure 1). Global positioning system (GPS) was used for the spotting of the area. The GPS is a remote sensing instrument which is a site investigating instrument controlled by 24 satellites. The GPS provide information about the latitude and longitude position of the sample collection spot. It also provides information about the level of the collection spot from the mean sea level. On each sample collection spot a way point was recorded on the GPS providing separate name or symbol. Later the way point data were transferred to arc-GIS explorer mapping software in which the mappings of the spots were performed.

Sample collection

Algal samples were collected from the shore using microalgal net cone shaped of mesh 20 µm in size. 4 buckets of sea water of 14 L capacity each, was poured into the algal collection net. The water samples were collected in the cup which was tied in the bottom of the algal net. The collected microalgal samples were transferred to 0.1 N HCl pretreated, steam sterilized screw cup bottles. The collected samples were preserved instantly into the ice bucket which maintained 5 ± 1°C and transferred to the laboratory.

Measurement of hydrodynamics

The hydrodynamic characteristics of the sampling sites such as pH, temperature, dissolved oxygen and salinity were measured using Rickley hydrological meter and the obtained results were tabulated and discussed.

Isolation and separation of microalgal strains

Methods of isolation and maintenance of microalgae in axenic cultures are based on serial dilution culture techniques and agar plate method as described by Gopinathan (1996). The sample which was collected on screw cap bottles were transferred to the laboratory from the collection spot, 50% of sea water (filtered sea water and double distilled water 1:1) infused agar plates were prepared on autoclave petriplates and allowed to solidify. After solidification, 100 µL of serially diluted (10^{-4}) sea water sample were spreaded over the prepared agar plates and incubated at room temperature under 1000 lux light until the formation of algal colonies.

From the 48 - 96 h old agar plates, the formed algal colonies were scrapped out using sterile inoculation needle and inoculated into 10 ml of freshly prepared culture medium.

Isolation of microalgae

Culture medium (Walne’s medium) was taken in a series of test tubes and each inoculated with the formed algal colonies in various concentrations. These tubes were kept under sufficient light (1000 lux) and incubated in the algal culture room under room temperature (22 - 28°C). After 15 - 18 days, some discoloration was seen in the culture tubes due to the growth of microalgae. These were examined under the microscope and successful cultures were diluted and sub cultured in 20 mL and subsequently to 250 mL Erlenmeyer flask and maintained as stock culture under a luminosity of 1000 lux. Further culture and growth studies were performed in the isolated microalgal strains and the results were tabulated.

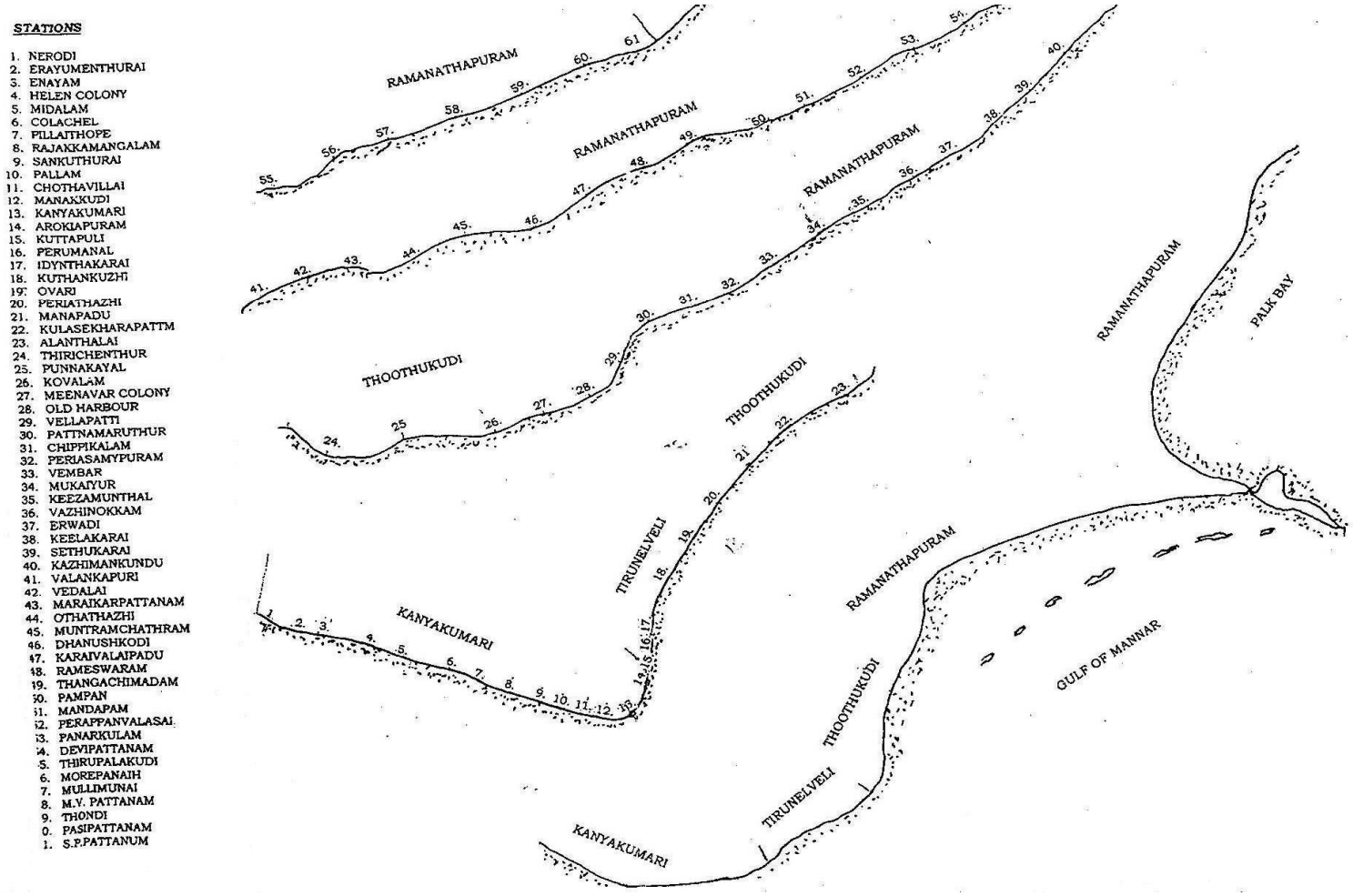

Figure 1. Collection spots using map source.

Table 1. A generalized set up conditions for the microalgal culture.

Parameter	Range
Temperature (°C)	22 - 28°C
Light intensity	1000 lux
Photo period	1 Batch - 24:0 maximum 2 Batch - 16:8 minimum
pH	8.2 ± 1

Culture conditions

A generalized set up conditions for the microalgal culture was maintained for the entire study (Table 1).

Media/ nutrients used for the growth studies

i) Walnes medium (modified from Lainy, 1991)
ii) Guillards f/2 medium (modified from Smith et al., 1993a)

Culture studies

Two sets of experiments were maintained for the culture studies. The culture setup differs in the phenomenon of light intensity (Light: Dark illustration, 24:0 of 1000 lux setup and 16:8 of 1000 lux set up). Proper experimental conditions were maintained and isolated algal samples were allowed to grown on 2 selective medium and the growth status were regularly monitored. Samples were taken once in 48 h and cell concentration of the algal biomass was determined using hemocytometer and the results were tabulated.

Scaling up of culture

100 ml culture (10 -12 days old) was taken as inoculums for culturing algae in 1 litre flask. The experiments were carried out in duplicates for each algal species. The 1 litre sterile conical flasks were filled with 900 ml of filtered sea water (salinity 28 ppt). The required nutrients for the growth of the algae were added and mixed thoroughly. To this 100 ml of inoculum were added, mixed and incubated. All cultures were maintained in at temperature (22 - 28°C) and illuminated with (24:0 of 1000 lux setup and 16:8 of 1000 lux set up). Shaking of the cultured flasks thrice a day was done to ensure proper growth. Inoculum density and initial cell count was taken immediately. Cell count was taken for alternative days and

recorded.

Algal cell count

The samples were taken from each flask and fixed with formalin in order to kill cells. After thorough mixing 0.1 ml of sample was placed on the counting chamber and a cover slip was placed over it and the cells were counted under compound microscope. The total cell density was calculated using the formula: Total number of cells/ml = (No. of cell counted / No. of squares counted) x Total no. of squares in that particular type x 10^4. Following the above procedures, exponential phase of the algal cultures were determined.

RESULTS AND DISCUSSION

Collection studies

Microalgal samples were collected from 61 sampling site across the south east coast of Tamil Nadu, India with an average sampling distance of 5.42 km. Algal blooms in the sea have occurred throughout recorded history but have been increasing during recent decades (Anderson, 1989). Ten microalgal species were isolated under restricted laboratory conditions from the 61 sampling sites as described in the isolation procedure (Tables 2a and 2b). In the present study, minimal fluctuations in the population density of phytoplankton were observed. Similar observations were reported by Regini (2004) in Rajakkamangalam, Kadiapatinam and Manakkudy estuaries. It is evident from several works that diatoms and dinoflagellates are the predominant forms found almost throughout the year in most of the Indian waters (Devassy and Bhatajiri, 1974). Microalgal distribution frequency was analyzed in the present study and it was observed that *Isochrysis galbana* [MA1] has the maximum surveillance at 37 spots [60.7%] followed by *Chlorella marina* [MA6] with the surveillance at 36 spots [59%] followed by *Chromulina freibergensis* [MA4] with the surveillance at 33 spots [54.1%] followed by *Dicrateria inornata* [MA3] with the surveillance at 29 spots [47.5%] followed by *Chaetoceros calcitrans* [MA7] with the surveillance at 23 spots [37.7%]. Interestingly the microalgae's *Pavlova lutheri* [MA5], *Dunaliella salina* [MA8], *Platymonas* sp [MA9] and *Synechasystic salina* [MA10] register its surveillance at 18 spots [29.5%]. *Nannochloropsis oculata* [MA2] shows least surveillance with a presence at 17 spots [27.9%].This observation was supported by the findings of (Perumal et al. (1999) absorb the presence of *I. galbana*, *D. inornata*, *C. marina* were abundant in all the seasons along the south east coast of the country. Perumal et al. (1999) carried out studies on the bloom across the south east coast of India and reported that no equal representation of the species and population density across the collection spots. Some of the algae that represented more in number on one season and collection spots could not be counted as the maximum in the other seasons and spots (Sobha et al., 1997). These findings are partly substantiated with the observations made by Kannan (1980) on the diatoms species diversity of Porto Novo, Tamil Nadu and De et al. (1994) in the species diversity of Hugli estuary, north east coast of India. Kannan (1996) has reported a total number of 126 species of phytoplankton from Manoli and Hare islands of the Gulf of Mannar. But in the present study only 10 species could be isolated from 61 collection spots along the south east coast of the country.

The Sorensen's Similarity Index of algal species in various collection spots were calculated. The similarity between alga taxa found in different collection spots ranges from 0 to 100%. Highest similarity index of 100% was recorded between water samples collected from sites S_1 and S_{15} (3.2%); S_3 and S_{33} (3.2%); S_6 and S_{14} (3.2%); S_{10} and S_{57} (3.2%); S_{11} and S_{35} (3.2%); S_{53} and S_{55} (3.2%). Interestingly sites S_9, S_{30}, S_{27} and S_{44} (6.5%) shows 100% similarity with algal distribution w.r.t the isolated microalgal strains. From the obtained results it is evident that 25.7% of the collection spots may share same microalgal dynamics. 9 collection spots (14.75%) shows variations between other sites ranges between 18.5% and 90.9% similatiry. No similarity (0%) was recorded between 85.2% of collection spots, where S48 shows 0% similarity with 26.2% of collection spots followed by S21 (21.3%), S8 and S41 (19.6%), S1 (18%), S15 (16.3%), S18 (14.7%), S54 (13.1%). It was also observed that rest 44 (72.1%) collection spots out of 52 collection spots with 0% similarity index shows less than 10% of no similarity between collection spots. Rohani et al. (2006) explains the similarity of the microalgal species across the collected spots in the research work which is supporting the present study. The collected microalgal samples from sand beaches from 12 separate islands shows a wide range of variation in the similarity. A total of 24 microalgal species were identified from the sand samples collected from the study sites.

It was observed that the physical characteristics of the collection spots also influencing the growth and surveillance of the microalgal species. It is evident from the results that the growth and abundance of particular microalgal species is proportionate with the level of dissolved oxygen at a particular spot. It was observed from the results that 25 collection spots (40.9%) with the dissolved oxygen level of > 9 mg/L shows significant growth dynamics of not less than 5 microalgal strains with the maximum isolation of 6 algal strains at sites S17 and S31 where the dissolved oxygen level was 12.43 mg/L and 12.64 mg/L respectively. One sample statistics T-test shows the total mean of dissolved oxygen across the collection spot is 8.63 ± 1.967 and with SEM of 0.252. The T-test analysis of pH shows the mean of 8.30 ± 0.107 with SEM of 0.014; temperature shows the mean of 30.75 ± 0.662 with SEM of 0.085 and salinity shows the mean of 33.69 ± 0.882 with SEM of 0.113.

Culture studies

Four experimental setups with 2 culture mediums

Table 2a. Hydrodynamic information of the sampling sites and the isolated microalgal strains.

S/N	Name of the site	Latitude/ Longitude	Topography	Physical parameters of the collected spot				Isolated microalgal Strains (MA)									
				Mean pH	Mean Temperature	Mean Salinity (PPT)	Mean Dissolved oxygen (mg/L)	1	2	3	4	5	6	7	8	9	10
1	Nerodi	N:0817328 E:07706120	No sand dunes,sea wall present; houses within 100 m of the coast	8.13	29.6	32.6	5.68				1	1				1	
2	Erayumenthurai	E:0814636 N:0770977	Thenkapatanam kayal is about 150 km wide estuary.	8.16	29.6	32.6	7.32			1	1	1				1	
3	Enayam	E:0813019 N:07711276	Sea wall present, rocks present	8.13	29.6	32.6	7.13	1		1		1			1		
4	Helen colony	N:0812755 E:07711842	Sandy shore, coconut plantations	8.13	29.6	32.3	6.88				1	1	1	1			
5	Midalam	N:0812328 E:07712796	Sand mining by IRE. Sandy shore	8.23	30.3	32.3	6.72		1				1	1			1
6	Colachel: Muramadi	N:0810327 E:07715283	Sandy shore, jetty 300m away	8.26	30.3	32.6	5.43	1			1		1		1		
7	Pillai thope	N:0807502 E:07720159	Sandy shore, fishing activity identified	8.26	30	32.3	9.12	1		1	1				1		1
8	Rajakamangalam	N:0806908 E:7722350	Sandy shore, Sand dunes present , sea wall present	8.26	30.3	32.3	5.26		1	1				1			
9	Sankuthurai	N:0805988 E:07725541	Within 60m from the shoreline a coastal road is laid. Sand dunes were removed. Sandy shore. Slight primary productivity noticed	8.2	30.6	32.3	9.88	1		1		1	1		1		
10	Pallam	N:0805931 E:07725847	It is a fishing village. Sandy shore.Slight primary productivity noticed	8.16	29.6	32.6	6.64	1			1		1	1			

Table 2a. Continued.

11	Chothavillai	N:0805605 E:07725847	Many sand dunes were identified. Sandy shore. Slight primary productivity noticed	8.16	29.6	32.6	6.63	1		1	1		1				
12	Manakkudi (melamnakudi)	N:0805392 E:07738535	Sand dunes present. Sandy shore ,Medial wave action	8.23	29.6	31.6	9.85	1		1		1	1				1
13	Kanyakumari	N:0805098 E:07733158	Houses very close to the high tide limit. Seawall is laid. Groins present, clear water.	8.23	29.6	31.6	10.12	1			1		1	1		1	
14	Arokiapuram	N:0807191 E:07733549	Fishing village. A hook shaped seawall is made. Sandy shore. Slight primary productivity noticed	8.23	29.6	31.6	8.76	1			1		1		1		
15	Kuttapulli	N:0808719 E:07736005	Seacoast is narrow; houses are within 30m from the coast and are in elevated area. Less productivity of planktons.	8.23	30.3	32	5.66				1	1				1	
16	Perumanal	N:0809504 E:07738739	No sea front modification sand mining with in 50m from the shore,sand dunes present.	8.23	30.3	32.3	10.11	1			1		1			1	1
17	Idyinthakarai	N;0810667 E:07744786	Sand dunes moderate: houses sandy shore, no sand mining	8.26	30.3	32.6	12.43	1		1		1	1		1		1
18	Kuthankuzhi	N:0813047 E:07946963	Less plankton productivity, sandy shore, Neem, storm water drain land, within 100m, coral reef found, sand mining.	8.16	30.3	32.6	7.88		1			1		1			1
19	Ovari	N:0816664 E:07753661	Sandy shore, show little primary productivity, human activity, sand dunes present, coral reefs found.	8.26	30.3	33	9.93		1		1			1	1		1
20	Periathazhai	N:08020009 E:07758326	Land use, coconut, Palmyra, casuarinas equisetitolia, sand dunes present coral reefs present	8.23	30.6	33.3	7.78		1		1		1			1	
21	Manapadu	N:0822105 E:0780336	Land use: coconut, Palmyra, seawall present, coral reef present, sand dunes present	8.33	30.6	33.3	8.23		1				1		1		1
21	Manapadu	N:0822105 E:0780336	Land use: coconut, Palmyra, seawall present, coral reef present, sand dunes present	8.33	30.6	33.3	8.23		1				1		1		1
22	kulasekharapattanam	N:0824637 E:07803796	Sand dunes present, sand mining, coral reef present.	8.33	30.6	33.3	8.29	1		1		1				1	
23	Alanthalai	N: 0827743 E:07805912	Land use within 200m sand dunes present, coral reef present.	8.33	30.6	33.3	7.96		1				1		1		

Table 2a. Continued.

23	Alanthalai	N: 0827743 E:07805912	Land use within 200m sand dunes present, coral reef present.	8.33	30.6	33.3	7.96		1				1		1		
24	Thirichenthur	N:0829598 E:07807690	Land use within 100m. Sand dunes,coral reefs present	8.23	30.6	33.3	9.86	1		1		1		1		1	
25	Punnakayal	N:0838078 E:07807282	Seawall present. Sand dunes present	8.23	30.6	32.3	9.88	1		1			1	1		1	
26	Kovalam	N:0843023 E:07809378	Coral reef present, sand dunes present	8.2	30.6	33.6	11.76		1	1			1	1			1
27	Meenavar colony (Karavalaipadu)	N:0844631 E:07810224	Casuarinas, neem, sea wall present, sandy shore.	8.23	31.3	33.3	11.82	1		1		1	1		1		
28	Old Harbour	N:0847866 E:07809476	Sea wall, primary productivity observed.	8.26	31	34.3	11.98	1			1		1		1		1
29	Vellapatti	N:0851419 E:07810001	Land use, Vellapatti river mouth, sandy shore.	8.2	31	34.6	12.08	1		1		1		1		1	
30	Pattna Maruthur	N:0855356 E:07811162	Sand dunes present, sandy shore, less plankton productivity.	8.16	31.6	33.6	12.16	1		1		1	1		1		
31	Chippikalam (Vaipar)	N:0859641 E:07815164	Land use within 200m sand dunes present, coral reef present.Ship breaking unit. Contaminted water body, more Human activity	8.13	31.3	34	12.64	1	1		1	1			1		1
32	Periasamypuram	N:0902664 E:07819619	Sand dunes present, Coral reefs present	8.23	31.3	33.6	12.53		1		1			1	1		1
33	Vembar	N:0904584 E:07821910	Land use Casuarina, Thespesia palm within 200m. coral reef present	8.3	31.3	33.6	8.46	1		1		1			1		
34	Mukaiyur	N:0907653 E:07835103	Sand dunes present, palmyra within 200 m. coral reef present	8.26	31.3	33.6	9.75	1		1		1			1		1
35	Keezamunthal	N:0908265 E:07835103	Land use, palmyra,neem within 200 m, coral reefs present	8.36	31.3	34	8.33	1		1	1		1				
36	Vazhinokkam	N:0909893 E:07838938	Barmouth land use with in 200 m, sea wall ,sand dunes, coral reef present, boat yard present, ship breaking yard within 200m, less productivity	8.46	31.6	34	8.97		1		1	1	1				
37	Erwadi	N:0911680 E:07843140	Sand dunes present, coral reef present	8.46	31.6	34.3	9.61	1		1			1		1		1
38	Keelakarai	N:0913697 E:07847219	Sewage mix with sea, Sand dune moderate, seawall, boat building yard, Coral reef present	8.46	31.3	33.3	7.78	1		1			1		1		
39	Sethukarai	N:0914885 E:07850595	Sand dune moderate, Coral reef present, land use; coconut , Palmyra	8.4	30.6	32.3	8.23	1			1	1			1		

Table 2a. Continued.

40	Kazhimankundu	N:0915396 E:07852449	Sand dune moderate, Coral reef present, land use; coconut , Palmyra, povarasu	8.36	30.6	33.3	8.29	1			1	1		1				
41	Valankapurai	N:0916391 E:07858250	Coral reef present, land use; coconut	8.36	30.3	33.6	5.32				1		1					
42	Vedalai	N:0915896 E:07906497	Sandy shore, less productivity, plantations like Casuarina, Thespesia, Palmyra.	8.43	31	34	5.76		1		1		1					
43	Maraikarpattanam	N:0917249 E:07907973	seawall present, no sand mining,land use; Casuarina equisetifolia coconut.	8.23	31.3	34	9.33	1		1	1			1		1		
44	Othathalai	N:0913827 E:07919798	land use; Casuarinas, Palmyra, sandy shore, less productivity	8.23	31.6	34.3	9.73	1		1		1	1		1			
45	Muntramchathram	N:0911927 E:07922232	land use; Casuarina equisetifolia Accasia Arabica, Less plankton productivity, sandy shore.	8.23	31.3	34.3	9.66	1			1		1	1				1
46	Dhanushkodi	N:0909188 E:079026695	Sand dunes, no vegitation but for grass & Casuarina equisetifolia: migratory birds, Flamingoes, 1964 cyclone damages can be seen.	8.23	31.6	34.3	9.83	1				1	1		1			1
47	Karaivalaipadu (Ramarkoil)	N:0914296 E:07921120	Land use; Casuarina equisetifolia, lake, coral reef present, rocky bed & sandy shore.	8.43	31.6	34.3	5.78	1		1			1					
48	Rameshwaram	N:0913977 E:07929867	Land use; Casuarina equisetifolia, Coral reef present, sandy shore with rocky pebbles.	8.43	31.6	34	5.52		1	1					1			
49	Thangachimadam (Palk bay)	N:0917530 E:07914413	Casuarina equisetifolia, Palmyra, marshy land, sewage, fish export company, sand dunes present, good plankton productivity	8.46	31.6	34.6	5.77	1					1		1			
50	Pampan	N:0916994 E:07912669	Land use: Casuarina equisetifolia, seawage mix with sea, shown less plankton productivity.	8.43	31.6	34.3	7.38	1		1			1			1		
51	Mandapam	N:0916610 E:7908964	Land use: Casuarina equisetifolia, seawage mix with sea, high fishing activity, coral reef present.	8.46	30.6	33.6	8.42	1				1		1		1		
52	Perappan valasai	N:0918575 E:07902585	Land use: Casuarina equisetifolia, seawage mix with sea, coconut, Palmyra, casuarinas plantations, sand dunes present.	8.46	31.3	34.3	8.19		1		1		1					1

Table 2a. Continued.

53	Panaikulam	N:0922994 E:0785768 8	Land use: Casuarina equisetifolia, palmyra, fishing village, sandy shore, rocky bed off shore 100 meters.	8.46	31.3	33.6	8.22	1			1			1		1	
54	Devipattanam	N:0928634 E:0785391 4	Land use: Casuarina equisetifolia, palmyra, neem trees, less palnkton productivity.	8.46	31	34	8.12	1		1					1		1
55	Thirupaiakudi	N:0932668 E:0785514 4	Land use: Casuarina equisetifolia, palmyra, neem trees, sewage mixed in sea.	8.46	30.6	34.6	8.34		1		1			1		1	
56	Morepanaih	N:0936589 E:0785616 9	Land use; Accasia arabica, Casuarina equisetifolia, neem trees, sewage mixed in sea.	8.43	30.6	34.3	8.77		1		1	1		1			
57	Mullimunai	N:0939466 E:0785825 5	Land use; Accasia arabica, Casuarina equisetifolia, forest, coconut farm. Sewage mixed in sea, good plankton productivity.	8.36	31.6	34.6	8.86	1			1		1	1			
58	M.V. Pattanam	N:0942530 E:0785965 9	Land use:; Casuarina equisetifolia,Thespesia populnea, neem, rocky bed shore, less planktonic productivity.	8.33	31.3	34	8.83			1	1		1	1			
59	Thondi	N:0944574 E:0790132 3	Land use; Casuarina equisetifolia, Thespesia populnea, neem. Manimutharu river mouth.	8.36	31.3	34.3	8.65			1	1			1		1	
60	Pasipattanam	N:0948595 E:0790482 3	Land use; Casuarina equisetifolia, neem, pasumanai river mouth, sandy shore, good productivity.	8.4	30.6	34.6	8.43			1			1	1		1	
61	S.P. Pattanum	N:0950132 E:0790616 2	Land use; Casuarina equisetifolia, neem, chittar& Pampanar river mouth, storm water drain found.	8.33	31.3	34.6	8.33		1		1		1			1	

Isolated microalgal strains: **MA1**:*Isochrysis galbana*, **MA2**:*Nannochloropsis occulata*, **MA3**:*Dicrateria inornata*, **MA4**:*Chromulina freibergensis*, **MA5**:*Pavlova lutheri*, **MA6**:*Chlorella marina*, **MA7**:*Chaetoceros calcitrans*, **MA 8**:*Dunaliella salina*, **MA9**:*Platymonas Sps*, **MA10**:*Synechasystic salina.*

Table 2b. Sorenson index showing the percentage similarity of isolated microalgal stain across the 61 sampling sites.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20	S21	S22	S23	S24	S25	S26	S27	S28	S29	S30	S31	S32	S33	S34	S35	S36	S37	S38	S39	S40	S41	S42	S43	S44	S45	S46	S47	S48	S49	S50	S51	S52	S53	S54	S55	S56	S57	S58	S59	S60	S61
S1	100																																																															
S2	85.7	100																																																															
S3	28.5	50	100																																																													
S4	28.5	50	25	100																																																											
S5	0	0	25	50	100																																																											
S6	28.5	25	25	50	50	100																																																											
S7	25	44.4	44.4	44.4	66.6	66.6	100																																																									
S8	0	28.5	28.5	28.5	28.5	0	25	100																																																							
S9	25	22.2	88.8	44.4	44.4	66.6	66.6	25	100																																																						
S10	28.5	25	25	50	75	75	66.6	28.5	44.4	100																																																					
S11	28.5	50	50	50	50	75	66.6	28.5	66.6	75	100																																																			
S12	25	44.4	66.6	44.4	66.6	44.4	88.8	25	88.8	44.4	66.6	100																																															
S13	50	44.4	22.2	44.4	66.6	66.6	60	25	40	88.8	66.6	40	100																																													
S14	28.5	25	50	50	50	100	66.6	0	66.6	75	75	66.6	66.6	100																																													
S15	100	85.7	28.5	57.1	0	28.5	25	0	25	28.5	28.5	25	50	28.5	100																																												
S16	25	22.2	22.2	44.4	44.4	66.6	80	0	40	66.6	66.6	60	80	66.6	50	100																																												
S17	22.2	40	80	40	60	60	72.7	22.2	90.9	40	60	90.9	36.3	60	22.2	54.5	100																																											
S18	28.5	25	25	50	50	0	22.2	28.5	22.2	25	0	44.4	22.2	0	28.5	22.2	40	100																																											
S19	25	22.2	22.2	44.4	22.2	44.4	40	25	20	44.4	22.2	20	40	44.4	25	40	36.3	66.6	100																																										
S20	57.1	50	0	50	25	50	22.2	28.5	22.2	50	25	22.2	66.6	50	57.1	66.6	20	25	44.4	100																																										
S21	0	0	25	25	50	50	44.4	28.5	44.4	25	25	44.4	22.2	50	0	44.4	60	50	66.6	50	100																																									
S22	57.1	75	50	25	25	25	44.4	28.5	66.6	25	50	66.6	44.4	25	28.5	44.4	80	25	0	25	0	100																																								
S23	28.5	25	25	50	25	75	44.4	28.5	44.4	50	50	22.2	44.4	75	28.5	44.4	40	25	66.6	75	75	0	100																																								
S24	50	66.6	66.6	44.4	44.4	22.2	40	50	60	44.4	44.4	60	60	22.2	50	40	54.5	44.4	20	44.4	0	88.8	0	100																																							
S25	25	44.4	44.4	44.4	44.4	44.4	60	50	60	66.6	66.6	60	60	44.4	25	60	54.5	22.2	20	44.4	22.2	66.6	22.2	80	100																																							
S26	0	22.2	22.2	44.4	66.6	22.2	60	75	40	44.4	44.4	60	40	22.2	0	40	54.5	66.6	60	44.4	66.6	22.2	44.4	40	60	100																																							
S27	25	44.4	88.8	44.4	22.2	66.6	60	25	100	44.4	66.6	80	40	66.6	25	40	90.9	22.2	20	22.2	44.4	66.6	44.4	60	60	40	100																																						
S28	25	22.2	44.4	44.4	66.6	88.8	80	0	75	60	66.6	66.6	60	80	22.2	80	72.7	22.2	60	44.4	66.6	22.2	66.6	20	40	40	60	100																																						
S29	50	44.4	66.6	44.4	44.4	22.2	40	50	60	44.4	44.4	60	60	22.2	40	40	54.5	44.4	20	22.2	0	88.8	0	100	80	40	60	20	100																																						
S30	25	44.4	88.8	44.4	22.2	66.6	60	25	100	44.4	66.6	80	40	66.6	25	40	90.9	22.2	20	22.2	44.4	66.6	44.4	60	60	40	100	60	60	100																																						
S31	44.4	40	60	40	40	60	54.5	40	54.5	40	40	54.5	36.3	60	44.4	54.5	66.6	40	72.7	40	60	40	60	36.3	18.8	36.3	54.5	72.7	36.3	54.5	100																																						
S32	25	22.2	22.2	44.4	44.4	44.4	40	50	20	44.4	22.2	20	40	44.4	25	40	36.3	66.6	100	44.4	66.6	0	66.6	20	20	60	20	60	20	20	72.7	100																																					
S33	28.5	50	100	25	25	50	66.6	28.5	88.8	25	50	66.6	22.2	50	28.5	22.2	80	25	22.2	0	25	75	25	66.6	44.4	22.2	88.8	44.4	66.6	88.8	60	22.2	100																																					
S34	25	44.4	88.8	22.2	44.4	44.4	60	25	80	22.2	22.2	80	20	44.4	25	40	90.9	44.4	40	0	44.4	66.6	22.2	60	40	40	80	60	60	80	72.7	40	88.8	100																																					
S35	28.5	50	50	50	25	50	88.8	28.5	66.6	50	100	66.6	66.6	75	28.5	66.6	60	0	22.2	50	25	50	50	44.4	66.6	44.4	66.6	66.6	44.4	66.6	40	22.2	50	44.4	100																																					
S36	57.1	50	25	75	25	50	44.4	28.5	44.4	50	50	44.4	44.4	50	57.1	44.4	40	50	44.4	75	50	25	75	22.2	22.2	44.4	44.4	44.4	22.2	44.4	40	44.4	25	22.2	50	100																																					
S37	0	22.2	66.6	22.2	66.6	66.6	80	25	80	44.4	66.6	80	40	60	0	60	90.9	22.2	40	22.2	66.6	44.4	44.4	40	60	60	80	80	40	80	90.9	40	66.6	80	66.6	22.2	100																																					
S38	0	25	75	25	50	75	66.6	28.5	88.8	50	75	66.6	44.4	75	0	44.4	80	0	22.2	25	50	50	50	44.4	44.4	44.4	88.8	66.6	44.4	88.8	40	22.2	75	66.6	75	25	88.8	100																																					
S39	57.1	50	75	50	25	75	44.4	0	66.6	50	50	44.4	44.4	75	57.1	44.4	60	25	44.4	25	25	50	50	44.4	22.2	0	66.6	66.6	44.4	66.6	80	44.4	75	66.6	50	50	44.4	50	100																																					
S40	57.1	50	50	75	50	50	44.4	28.5	44.4	75	50	44.4	66.6	50	57.1	44.4	40	50	44.4	25	0	50	25	66.6	44.4	22.2	44.4	44.4	66.6	44.4	60	44.4	50	44.4	50	50	22.2	25	75	100																																					
S41	80	66.6	33.3	66.6	0	33.3	28.5	0	28.5	33.3	33.3	28.5	28.5	33.3	80	28.5	25	33.3	28.5	33.3	0	33.3	33.3	28.5	0	0	28.5	28.5	28.5	28.5	50	28.5	33.3	28.5	33.3	66.6	0	0	66.6	66.6	100																																					
S42	33.3	28.5	28.5	57.1	28.5	85.7	75	0	50	85.7	85.7	50	75	85.7	33.3	75	44.4	0	25	28.5	28.5	28.5	57.1	25	50	25	50	75	25	50	44.4	25	28.5	25	85.7	57.1	50	57.1	57.1	57.1	40	100																																					
S43	50	66.6	22.2	44.4	44.4	44.4	60	50	40	66.6	66.6	40	80	44.4	50	60	36.3	22.2	40	44.4	0	66.6	22.2	80	80	40	40	40	80	40	36.3	40	44.4	40	66.6	22.2	40	44.4	44.4	66.6	28.5	50	100																																					
S44	25	44.4	88.8	44.4	44.4	66.6	60	25	100	44.4	66.6	80	40	66.6	25	40	90.9	22.2	20	22.2	44.4	66.6	44.4	60	60	40	100	60	60	100	54.5	20	88.8	80	66.6	44.4	80	88.8	66.6	44.4	28.5	50	40	100																																					
S45	25	22.2	22.2	66.6	88.8	66.6	80	25	40	88.8	66.6	60	80	66.6	25	80	54.5	44.4	60	44.4	44.4	22.2	22.2	40	60	60	40	80	40	54.5	60	22.2	40	66.6	44.4	60	44.4	44.4	66.6	28.5	75	60	40	100																																					
S46	25	22.2	66.6	44.4	66.6	66.6	60	0	80	44.4	44.4	80	40	66.6	25	60	83.3	44.4	40	22.2	66.6	44.4	44.4	40	40	40	80	80	40	80	66.6	40	66.6	80	44.4	44.4	80	66.6	66.6	44.4	28.5	50	20	80	60	100																																					
S47	0	28.5	57.1	28.5	57.1	57.1	75	33.3	75	57.1	85.7	75	50	57.1	0	50	44.4	0	0	28.5	28.5	57.1	28.5	50	75	25	75	50	50	75	22.2	0	57.1	50	85.7	28.5	75	85.7	28.5	28.5	0	66.6	50	75	50	50	100																																					
S48	0	28.5	57.1	0	0	28.5	25	66.6	50	0	28.5	25	0	28.5	0	0	44.4	28.5	50	28.5	57.1	28.5	57.1	25	25	50	50	25	25	50	44.4	50	57.1	50	28.5	28.5	50	57.1	28.5	0	0	0	25	50	0	25	33.3	100																																					
S49	0	0	57.1	28.5	57.1	85.7	50	0	75	57.1	57.1	50	50	85.7	0	50	66.6	0	25	28.5	57.1	28.5	57.1	25	50	25	75	75	25	75	44.4	25	57.1	50	57.1	28.5	75	85.7	57.1	28.5	80	66.6	25	75	50	75	66.6	33.3	100																																					
S50	28.5	50	50	25	50	50	75	28.5	66.6	50	75	66.6	66.6	50	28.5	44.4	60	0	0	50	25	75	25	66.6	88.8	44.4	66.6	44.4	66.6	66.6	20	0	50	44.4	75	25	66.6	75	25	25	0	57.1	66.6	66.6	44.4	44.4	85.7	28.5	57.1	100																																					
S51	57.1	50	50	50	25	25	25	28.5	44.4	25	25	44.4	66.6	25	57.1	44.4	40	50	22.2	25	0	75	0	88.8	66.6	22.2	44.4	22.2	88.8	44.4	40	22.2	50	44.4	25	25	20	25	50	75	33.3	28.5	66.6	44.4	44.4	44.4	28.5	0	28.5	50	100																																					
S52	0	25	0	25	50	50	75	28.5	22.2	50	50	57.1	44.4	75	28.5	44.4	40	50	44.4	75	75	75	0	66.6	22.2	66.6	22.2	66.6	0	22.2	60	66.6	0	22.2	50	75	44.4	25	25	25	33.3	67.1	22.2	22.2	44.4	44.4	28.5	28.5	28.5	25	0	100																																					
S53	57.1	50	25	50	50	50	50	28.5	22.2	75	50	22.2	88.8	50	57.1	66.6	20	25	44.4	50	0	50	25	66.6	66.6	22.2	22.2	44.4	66.6	22.2	40	44.4	25	22.2	50	25	22.2	25	50	75	33.3	57.1	88.8	22.2	66.6	22.2	28.5	0	28.5	50	75	25	100																																					
S54	0	0	50	0	50	50	50	28.5	44.4	25	25	44.4	22.2	50	0	44.4	60	50	66.6	25	75	25	50	22.2	22.2	44.4	44.4	66.6	22.2	44.4	80	66.6	50	66.6	25	25	66.6	50	50	25	0	28.5	22.2	44.4	44.4	66.6	28.5	57.1	57.1	25	25	50	25	100																																					
S55	57.1	50	25	50	50	50	50	28.5	22.2	75	50	22.2	88.8	50	57.1	66.6	20	25	44.4	50	0	50	25	66.6	66.6	22.2	22.2	44.4	66.6	22.2	40	44.4	25	22.2	50	25	22.2	25	50	75	33.3	57.1	88.8	22.2	66.6	22.2	28.5	0	28.5	50	75	25	100	25	100																																					
S56	57.1	50	25	75	25	25	25	57.1	22.2	50	25	22.2	44.4	25	57.1	22.2	20	50	66.6	50	25	25	50	44.4	22.2	44.4	22.2	22.2	44.4	22.2	60	66.6	25	22.2	25	75	0	0	50	75	66.6	28.5	44.4	22.2	44.4	22.2	0	28.5	0	0	50	50	50	25	50	100																																					
S57	28.5	25	25	75	75	75	75	28.5	44.4	100	75	44.4	88.8	75	28.5	66.6	40	25	44.4	50	25	25	50	44.4	66.6	44.4	44.4	66.6	44.4	44.4	40	44.4	25	22.2	75	50	44.4	40	50	75	33.3	85.7	66.6	44.4	88.8	44.4	57.1	0	57.1	50	50	50	75	25	75	50	100																																					
S58	28.5	50	25	75	50	50	75	57.1	44.4	75	75	44.4	66.6	50	28.5	44.4	40	25	44.4	50	25	25	50	44.4	66.6	66.6	44.4	44.4	44.4	44.4	40	44.4	25	22.2	75	50	44.4	50	25	50	33.3	57.1	66.6	44.4	66.6	22.2	57.1	28.5	28.5	50	25	50	50	0	50	50	75	100																																					
S59	57.1	75	25	50	25	25	50	57.1	22.2	50	50	22.2	66.6	25	57.1	44.4	20	25	44.4	50	0	50	25	66.6	66.6	44.4	22.2	22.2	66.6	22.2	20	44.4	25	22.2	50	25	22.2	25	25	50	33.3	28.5	88.8	22.2	44.4	0	28.5	28.5	0	50	50	25	75	0	75	50	50	75	100																																					
S60	28.5	50	25	50	50	25	50	57.1	44.4	50	50	44.4	66.6	25	28.5	44.4	40	25	22.2	50	25	50	25	66.6	88.8	66.6	44.4	22.2	66.6	44.4	0	22.2	25	22.2	50	25	44.4	50	0	25	0	28.5	66.6	44.4	44.4	22.2	57.1	28.5	28.5	75	50	25	50	0	50	25	50	75	75	100																																					
S61	57.1	50	25	50	50	75	75	0	44.4	75	75	44.4	88.8	75	57.1	88.8	40	0	22.2	75	25	50	50	44.4	66.6	22.2	44.4	66.6	44.4	44.4	40	22.2	25	22.2	75	50	44.4	50	50	50	33.3	85.7	66.6	44.4	66.6	44.4	57.1	0	57.1	75	50	50	75	25	75	25	75	50	50	50	100

[Walne's medium and Guillards f/2 medium] also differ in the phenomenon of light intensity (24:0 of 1000 lux setup and 16:8 of 1000 lux set up) were experimented over a period of 16 days under controlled laboratory condition to determine the optimal growth of the isolated algal strains (Tables 3, 4, 5 and 6). Barnabé (1990) observation in his research work substantially supporting the present study, which explains about the optimal physical parameters and hydrodynamics, may enhance the growth and surveillance of microalgae under controlled laboratory conditions. The difference in the phenomenon of light intensity and its impact on microalgal growth in the isolated microalgal strains cultured in Walne's medium was observed

Table 3. Growth phase of isolated microalgal strains cultured in Walne's medium under 16:8 h light: dark illustration.

MA Species	Walne's Medium (16/8) cell count reading (cells/l) X10^4								
	1st day	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
Algae 1	41.8 ± 1.303	61.6 ± 1.816	94.2 ± 1.923	242.8 ± 1.095	297.2 ± 1.643	431.4 ± 1.203	519.8 ± 1.043	583.2 ± 0.948	522.4 ± 1.023
Algae 2	67.2 ± 0.838	121.4 ± 1.223	239.6 ± 1.235	383.6± 1.076	472.8 ± 1.764	640.6 ± 0.543	788.2 ± 1.234	888.4 ± 1.768	864.2 ± 1.222
Algae 3	63.2 ± 0.139	111.4 ± 1.284	229.6 ± 1.136	413.6 ± 1.017	598.6 ± 1.245	824.6 ± 0.214	815.6 ± 0.235	818.6 ± 1.709	734.2 ± 1.003
Algae 4	17.2 ± 1.034	41.4 ± 1.202	77.6 ± 1.031	113.6 ± 0.192	198.4 ± 1.012	221.4 ± 1.140	358.6 ± 0.905	418.4 ± 1.023	334.2 ± 1.343
Algae 5	62.2 ± 0.130	117.4 ± 1.114	219.8 ± 1.136	355.6 ± 1.077	520.6± 1.040	750.8 ± 0.213	811.8 ± 0.836	718.6 ± 1.609	694.2 ± 0.832
Algae 6	34.2 ± 0.933	53.6 ± 1.216	81.2 ± 1.920	162.8 ± 1.003	260.2 ± 1.683	262.2 ± 1.003	377.8 ± 0.243	459.2 ± 0.908	413.4 ± 0.323
Algae 7	11.4 ± 1.224	20.4 ± 0.992	37.8 ± 1.022	53.4 ± 0.792	98.4 ± 1.012	127.4 ± 1.072	178.4 ± 0.905	168.6 ± 1.023	121.4 ± 1.343
Algae 8	27.4 ± 1.024	51.4 ± 1.092	81.2 ± 0.871	133.2 ± 0.992	208.4 ± 1.812	291.4 ± 1.109	297.4 ± 0.525	378.4 ± 1.903	370.6 ± 1.023
Algae 9	09.6 ± 1.224	12.4 ± 0.992	23.4 ± 1.022	52.8 ± 0.792	48.4 ± 1.612	67.4 ± 0.935	98.2 ± 0.249	128.6 ± 1.013	119.4 ± 1.110
Algae 10	61.2 ± 0.839	91.4 ± 0.284	199.8 ± 1.239	323.6 ± 1.217	579.6 ± 1.265	577.6 ± 0.274	685.4 ± 0.935	768.4 ± 1.709	732.6 ± 1.083

Table 4. Growth phase of isolated microalgal strains cultured in Walne's medium under 24:0 h light: dark illustration.

MA Species	Walne's Medium (24:0) cell count reading (cells/l) X10^4								
	1st day	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
Algae 1	44.8 ± 1.113	81.6 ± 1.206	91.2 ± 1.093	93.8 ± 0.954	194.2 ± 1.643	231.2 ± 0.913	319.8 ± 1.843	223.2 ± 0.922	172.2 ± 1.913
Algae 2	17.2 ± 0.238	25.4 ± 1.093	39.8 ± 1.635	62.4 ± 1.876	82.4 ± 0.760	140.4 ± 0.947	116.4 ± 0.834	92.8 ± 0.983	86.2 ± 1.092
Algae 3	67.2 ± 0.139	121.4 ± 1.084	210.8 ± 0.136	313.4 ± 0.317	498.8 ± 0.245	524.4 ± 0.984	675.8 ± 1.335	618.8 ± 1.229	434.2 ± 1.123
Algae 4	27.4 ± 0.434	71.4 ± 1.452	117.8 ± 1.731	283.4 ± 0.112	291.4 ± 1.042	298.4 ± 1.330	345.8 ± 0.905	418.6 ± 1.023	194.4 ± 1.093
Algae 5	12.2 ± 0.130	17.4 ± 1.094	21.4 ± 1.096	35.8 ± 0.067	52.6± 1.049	132.6 ± 0.213	111.4 ± 0.246	68.6 ± 0.609	42.2 ± 1.872
Algae 6	69.2 ± 0.939	129.4 ± 0.894	218.4 ± 0.146	342.4 ± 0.842	488.4 ± 1.240	514.6 ± 0.986	688.8 ± 1.465	598.8 ± 1.086	534.2 ± 0.126
Algae 7	22.4 ± 1.224	28.4 ± 0.892	48.8 ± 1.802	113.4 ± 0.862	118.8 ± 1.082	225.4 ± 1.072	168.4 ± 0.905	94.6 ± 0.933	51.2 ± 1.343
Algae 8	67.4 ± 1.112	91.4 ± 0.872	181.8 ± 0.692	253.2 ± 1.292	398.4 ± 1.082	591.4 ± 1.076	597.4 ± 0.224	373.4 ± 0.922	317.6 ± 1.873
Algae 9	29.6 ± 1.224	25.4 ± 0.972	42.4 ± 1.046	82.8 ± 0.762	198.4 ± 1.612	218.8 ± 0.235	108.2 ± 0.149	88.6 ± 0.713	49.4 ± 0.870
Algae 10	65.2 ± 0.630	116.4 ± 1.286	209.8 ± 1.054	423.6 ± 1.216	582.6 ± 0.807	572.6 ± 0.274	446.4 ± 0.982	468.4 ± 1.226	432.6 ± 1.094

for a period of 16 days. It was observed70% of the isolated microalgal species shows significant increment and delivers optimal growth of MA1 (45.2%) on day 14, MA2 (53%) on day 14, MA3 (18.1%) on day 10, MA4 (66.5%) on day 12, MA5 (72.2%) on day 14, MA7 (25.8%) on day 12 and MA10 (24.2%) on day 14 with the light intensity of 16:8 h when compared with the 24:0 h light intensity (Tables 3 and 4). However 24:0 h culture setup shows optimal growth for MA6 (66.7%) on day 12, MA8 (63.9%) on day 10 and MA9 (58.7%) on day 10.

Similarly the difference in the phenomenon of light intensity corresponds to microalgal growth in the Guillards f/2 medium was observed for a period of 16 days. It was observed 60% of the isolated microalgal species shows significant increment and delivers optimal growth of MA1 (59.8%) on day 14, MA2 (27.4%) on day 14, MA3 (10.5%) on day 14, MA4 (40.2%) on day 12, MA5 (30.1%) on day 14 and MA9 (50.4%) on day 12 which favors the optimal growth with the light intensity of 16:8 h when compared with the 24:0 h light intensity (Tables 5 and 6). However 24:0 h

Table 5. Growth phase of isolated microalgal strains cultured in Guillard's f/2 medium under 16:8 h light: dark illustration.

MA Species	Guillard's Medium (16/8) cell count reading (cells/l) X10^4								
	1st day	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
Algae 1	36.2 ± 0.639	41.2 ± 0.282	76.8 ± 1.232	130.6 ± 0.768	289.6 ± 0.865	317.6 ± 0.274	355.4 ± 0.824	468.4 ± 1.704	432.6 ± 0.052
Algae 2	57.2 ± 0.938	118.4 ± 1.033	230.6 ± 1.024	318.6 ± 1.078	446.8 ± 1.764	622.6 ± 0.543	678.2 ± 1.144	728.4 ± 0.768	664.2 ± 1.372
Algae 3	63.2 ± 0.139	117.4 ± 1.264	192.6 ± 1.036	263.6 ± 1.116	378.6 ± 1.264	424.6 ± 0.814	515.6 ± 0.235	578.6 ± 1.709	534.2 ± 1.823
Algae 4	27.2 ± 1.634	32.4 ± 1.202	66.6 ± 1.031	104.6 ± 1.192	198.4 ± 1.062	212.4 ± 1.140	348.6 ± 0.906	278.4 ± 0.823	214.2 ± 1.284
Algae 5	44.2 ± 0.988	58.6 ± 1.116	80.2 ± 0.980	132.8 ± 1.603	233.2 ± 1.683	292.2 ± 0.983	378.8 ± 0.268	518.2 ± 0.908	413.4 ± 0.320
Algae 6	42.8 ± 0.308	68.6 ± 1.786	94.2 ± 1.923	182.8 ± 1.095	277.2 ± 1.843	331.4 ± 0.204	428.8 ± 1.043	544.2 ± 0.748	422.4 ± 0.828
Algae 7	37.4 ± 1.024	58.4 ± 1.092	62.2 ± 0.871	93.2 ± 0.992	128.4 ± 1.812	192.4 ± 1.109	228.4 ± 0.525	266.4 ± 1.203	172.6 ± 0.086
Algae 8	19.6 ± 1.224	38.4 ± 0.952	43.4 ± 1.022	92.8 ± 0.792	116.4 ± 1.692	167.4 ± 0.935	188.2 ± 0.289	128.6 ± 1.013	119.4 ± 1.110
Algae 9	18.4 ± 1.824	28.4 ± 0.242	67.8 ± 1.022	83.4 ± 0.792	118.4 ± 0.922	147.4 ± 1.882	218.4 ± 0.905	168.6 ± 1.823	121.4 ± 1.322
Algae 10	52.2 ± 0.130	98.4 ± 1.114	188.8 ± 1.136	354.6 ± 1.677	420.6 ± 1.660	582.8 ± 0.283	612.8 ± 0.866	618.6 ± 0.668	494.2 ± 0.226

Table 6. Growth phase of isolated microalgal strains cultured in Guillard's f/2 medium under 24:0 h light: dark illustration.

MA Species	Guillard's Medium (24:0) cell count reading (cells/l) X10^4								
	1st day	2nd day	4th day	6th day	8th day	10th day	12th day	14th day	16th day
Algae 1	37.2 ± 0.778	55.4 ± 0.882	59.8 ± 1.635	52.4 ± 0.116	112.4 ± 0.220	188.4 ± 1.027	112.4 ± 0.114	82.8 ± 0.332	76.2 ± 0.292
Algae 2	23.6 ± 0.224	34.4 ± 0.222	62.4 ± 1.346	88.2 ± 1.212	138.4 ± 0.612	174.8 ± 0.235	198.2 ± 0.239	208.6 ± 0.712	149.4 ± 0.340
Algae 3	77.4 ± 0.612	121.4 ± 0.882	177.8 ± 1.192	263.2 ± 1.092	328.4 ± 0.782	423.4 ± 0.876	517.4 ± 0.294	473.4 ± 0.222	367.6 ± 1.273
Algae 4	61.2 ± 0.362	136.4 ± 1.280	194.8 ± 0.254	283.6 ± 1.668	382.6 ± 0.834	392.6 ± 0.374	476.4 ± 0.902	528.4 ± 1.226	422.6 ± 0.694
Algae 5	32.4 ± 1.284	68.4 ± 0.812	98.8 ± 1.802	142.4 ± 0.860	178.8 ± 0.382	236.4 ± 1.172	362.4 ± 0.326	220.6 ± 0.526	146.2 ± 1.343
Algae 6	37.2 ± 0.166	81.4 ± 0.824	167.8 ± 0.196	233.4 ± 0.624	378.8 ± 0.842	484.4 ± 0.334	472.8 ± 1.208	568.8 ± 0.902	482.4 ± 0.823
Algae 7	38.8 ± 0.614	92.6 ± 0.286	198.2 ± 0.293	278.8 ± 0.554	366.2 ± 0.648	356.2 ± 0.823	418.8 ± 1.843	326.2 ± 0.922	272.2 ± 0.928
Algae 8	46.4 ± 1.124	86.4 ± 0.472	147.8 ± 0.722	276.4 ± 0.712	395.4 ± 1.242	398.4 ± 0.330	436.8 ± 0.346	424.6 ± 1.223	292.4 ± 0.293
Algae 9	22.2 ± 1.130	47.4 ± 0.094	41.4 ± 1.556	45.8 ± 0.237	52.6 ± 0.449	72.6 ± 0.413	108.4 ± 0.246	108.6 ± 0.628	102.2 ± 1.872
Algae 10	64.2 ± 0.134	112.4 ± 1.094	178.4 ± 0.946	242.4 ± 0.641	388.4 ± 0.872	484.6 ± 0.382	568.4 ± 0.465	618.8 ± 0.186	474.2 ± 1.126

culture setup shows optimal growth for MA6 (04.2%) on day 14, MA7 (36.3%) on day 12, MA8 (56.8%) on day 12 and MA10 (0.64%) on day 14. Interestingly MA6 and MA8 registers good growth in the 24:0 h light intensity compares with 16:8 h light intensity. Further MA7 which favors 16:8 light intensity pattern in the Walne's medium setup shows optimal growth in 24:0 h light intensity setup in Guillards f/2 medium. It was also observed that MA9 which favors 24:0 light intensity pattern in the Walne's medium setup shows optimal growth at 16:8 h light intensity setup in Guillards f/2 medium. Laboratory scale-up process prior to the cultivation of marine unicellular algae in the field was studied by Laing (1991) and theresults are supporting the culture setup of the present study.

The growth status of isolated microalgal species cultured at 16:8 h light intensity in both Walne's medium and Guillards f/2 medium were analyzed. It was observed that most of the isolated algal species (70%) shows optimal growth in the

Walne's culture medium when compared with the Guillard f/2 medium. The optimal growth of the isolated microalgal species cultured in Walne's medium was registered as MA1 (19.7%) on day 14, MA2 (18%) on day 14, MA3 (29.8%) on day 10, MA4 (16.7%) on day 14, MA5 (36.1%) on day 12 MA8 (50.2%) on day 14 and MA10 (20%) on day 14. However MA6 (15.6%) on day 14, MA7 (33%) on day 14 and MA9 (41.2%) on day 12 shows optimal growth on guillard f/2 medium when compared with Walne's medium.

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