Research Article **ISSN: 2349 – 7114**

Asian Journal of Research in Pharmaceutical Sciences and Biotechnology

Journal home page: www.ajrpsb.com

https://doi.org/10.36673/AJRPSB.2021.v09.i01.A02

LARGER DIATOM FLORA ISOLATED AND IDENTIFIED FROM THE NORTHEAST PELAGIC SEACOAST OF MARAKKANAM, TAMIL NADU, INDIA

Vishnu Kiran Manam*[1] and G. Sumathi[2]

[1*]Department of Research and Development, BMR Group, Aquaculture Division, Chennai, Tamil Nadu, India.
[2]Institute of Microbiology, Madras Medical College, Chennai, Tamil Nadu, India.

ABSTRACT

Diatoms representing aquatic phototrophs play major role in biogeochemical cycle of silica and fixes global carbon. In the present study the larger marine diatoms were isolated and identified from the Marakkanam sea coast, North East of Tamil Nadu, India. The diatoms were identified and classified using both traditional and morphological methods. The isolation of micro-algae was done using agar plating technique (2% agar in F/2 medium).The diatoms isolated from the coast were dominated by three classes belonging to Bacillariophyceae, Mediophyceae and Coscinodiscophyceae. About 12 genera and 14 species of larger diatoms were isolated and identified in the current study which emphasis the species abundance of the Marakkanam coast and their rich nutritional profile.

KEYWORDS

Diatoms, Isolation, Identification, Classification and Applications.

Author for Correspondence:

Vishnu Kiran Manam,

Department of Research and Development,

BMR Group, Aquaculture Division,

Chennai, Tamil Nadu, India.

Email: dna.vishnu@gmail.com

INTRODUCTION

Diatoms play a major component and dominant life form of the phytoplankton community. Diatoms are made up of complex silica cell wall and microscopically single-celled autotrophic eukaryotic algae belonging to the class Bacillariophyceae and kingdom Protista[1]. The tiny algae diatom range from 2 and 500µm in length or diameter and their habitation vary from ocean to freshwater systems exhibiting seasonal variations during monsoon seasons[2,3]. They are usually yellowish to brown colour in nature and found among the surface of plants, moist soils and in the

oceans[4]. They primarily form a salient link between autotrophic and heterotrophic productions which constitutes a major component of aquatic ecosystem. They constitute an important role in the food chain and are useful as valiant bio indicators of the system where their presence is enhanced in unpolluted waters[5]. Diatoms generate greater organic carbon in the sea which is equivalent to all the terrestrial rainforests together thus contributing major portion of the photosynthesis in the earth's atmosphere[6,7]. Diatoms are considered as the key vectors for biological carbon in the oceans[8-10] and the various recent studies in the wide distribution of diatom morphology, elemental composition, and community structure play major factors in understanding the carbon export efficiency[11-13]. The importance of diatom research has been extensively studied in the areas of taxonomy, ecology, bio-monitoring and algal-biotechnology over the last few decades[14]. The diatom taxonomy was initially started with microscopic observations[15] and its identification was based on two parameters either with ribosomal sequences[16] or with the morphology, shape of frustules and the external silica cell wall[17]. The Phylogeny, morphometric[18] and teratological structures in diatoms[19,20] forms the basis for culturing the diatoms. Diatom culturing for obtaining axenic cultures can be done using the manual isolation technique or automated isolation technique as well as the combination of both[21-24]. In the present study, the isolated diatoms were cultured using both manual and automatic isolation procedures and the identification was based on the morphological, shape of frustules and the silica cell wall of the diatoms using a Trinocular microscope attached to the monitor.

MATERIAL AND METHODS

Sampling Site

The pelagic regions of the coast at Marakkanam, Villupuram district, Tamil Nadu, India [Figure No.1] were selected for the collection of the samples. The sampling was done during June to September 2019 at the latitude of 12.2ºN and longitude of 79.95ºE.

Sample collection

The water samples were collected manually using plastic water bottles volume 2 Litres capacity by using a plankton net mesh size 25µM. About 50 liters of seawater was collected from four different locations which were filtered through plankton net and transferred to 100mL glass bottles. Collected samples were enriched with nutrients (Sodium Nitrate, Sodium Dihydrogen Orthophosphate, Sodium Silicate, Ferric chloride, EDTA, Trace Metals and Vitamins based on f/2 medium stock solution[25,26] Table No.1. The enriched samples were incubated in the laboratory with ambient illumination using 20-watt white fluorescent tube lights and the temperature was maintained between 26ºC to 28ºC for 12:12 hour light and dark cycles. The sample from the plankton net was prepared directly for the isolation and identification process.

Culturing and isolation

The culturing procedures were done using the samples from the plankton net which were subjected to general isolation methods. Techniques like centrifugation, rarefaction and filtering methods were employed to enhance the cell numbers and in order to separate the mixed samples they were primarily set for centrifugation process at 500rpm for about 15 to 20 minutes[27]. The centrifugation process results in the precipitation of the contaminants and zooplankton and also eliminates the tiny organisms such as bacteria tom the uppermost layer of the aqueous solution[28]. The bottom water column was carefully withdrawn using a micropipette and subjected to a serial dilution process[29,30]. The serial dilution was performed using 10mL test tubes enriched with F/2 medium and the dilutions were done from 10^{-1} to 10^{-10} and were incubated at 26ºC to 28ºC for 24 hrs under illumination. The serially diluted samples containing the diatoms were then plated using agar plating technique[31] via micro capillaries for the isolation process. The agar medium was prepared by using 2% agar (Hi-media) and then autoclaved at 120ºC for 15 min at 15 lbs. After autoclaving, the semi cooled agar medium was enriched with F2 medium and poured into Petri dishes of 20ml

capacity per plate. The samples from serial dilution (10^{-1} to 10^{-10}) of 0.1mL were spotted in the middle of the agar plates and spread evenly on the surface using a glass spreader. The plates were sealed with parafilm to avoid drying out and incubated in the culture shelve under proper illumination at an ambient temperature ranging between 26°C to 28°C. The emerged colonies on the plates were and re-streaked onto fresh agar medium[32]. Pure unialgal colonies were obtained after repeated streaking on fresh agar media and confirmed by microscopic observation. The pure colonies were then transferred into the liquid medium by inoculating a single colony into each well of the 24 and 96-wells micro titer plate containing 1mL and 200µL of f/2 medium, respectively.

Diatoms preservation

The cleaned diatoms were preserved in 4% formalin added with glycerin.

Morphological Identification and characterization

The cultured species were examined using Trinocular microscopy (Labomed LX 400) for the identification of diatoms in the genus and species level. The identification of the larger diatom isolates was based on the morphological characteristics and phytoplankton identification books[33-37]. The isolated diatoms were also identified with the help of eminent algologist from India and Belgium.

RESULTS AND DISCUSSION

The diatoms from the pelagic seacoast of Marakkanam were isolated and identified belonging to 12 genera [Figure No.3D] namely *Trieres*, *Chaetoceros*, *Eupyxidicula*, *Odontella*, *Coscinodiscus*, *Guinardia*, *Lauderia*, *Skeletonema*, *Conticribra*, Asterionellopsis, *Nitzschia*, *Craticula*, 9 orders [Figure No.3C] namely Eupodiscales, Chaetocerotales, Stephanopyxales, Coscinodiscales, Rhizosoleniales, Thallasiosirales, Rhaphoneidales, Bacillariales, Naviculales, 6 subclass [Figure No.3B] namely Thallasiosirophycidae, Chaetocerotophycidae, Archaegladiopsophycidae, Coscinodiscophycidae, Urneidophycidae, Bacillariophycidae, 3 class [Figure No.3A] namely Mediophyceae, Coscinodiscophyceae, Bacillariophyceae and 14 species were identified as *Trieres mobiliensis*, *Trieres chinensis*, *Chaetoceros calcitrans*, *Eupyxidicula turris*, *Odontella aurita*, *Coscinodiscus radiatus*, *Guinardia striata*, *Lauderia annulata*, *Skeletonema marinoi*, *Skeletonema costatum*, *Conticribra weissflogii*, *Asterionellopsis glacialis*, *Nitzschia acicularis* and *Craticula cuspidata.*

Trieres mobiliensis

Valves elliptical or lanceolate (bipolar). An elevation with an ocellus at each pole. Cells in straight (united by both elevation). Two or more labiate processes per valve, usually with long external tubes. Numerous small chloroplasts observed lying against valve wall with prominent elevations and valve face flat or concave or bulging in the middle. The middle part of valve face was flat or slightly concave, external tubes of processes and elevations diverging[38,39] [Figure No.2A and Table No.1].

Trieres chinensis

Valves elliptical or lanceolate (bipolar). An elevation with an ocellus at each pole. Two or more labiate processes per valve, usually with long external tubes. Numerous small chloroplasts lying against valve wall. Cell wall weakly silicified, middle part of valve face shaped in various ways, Processes close to slender elevations, valve face between processes flat or concave[39,40] [Figure No.2B and Table No.1].

Chaetoceros calcitrans

Cellulae solitariae, cylindricae. Valvae ellipticae, planae vel convexae a centro, 5-16µ longae. Zona connectivales fere major quam 1/3 alti frustuli. Setae tenues, rectae, 4-5 tanto longior prae axe longo valvae, a margine valvae at ad zonam connectivaleum frustulorum diagonaliter directae[41] [Figure No.2C and Table No.1].

Eupyxidicula turris

Cylindrical, sometimes nearly spherical, capsule-shaped cells. Valves are domed with large hexagonal areolae. Cells have numerous discoid or

lobed chloroplasts. Some species form resting spores[42, 43] [Figure No.2D and Table No.1].

Odontella aurita

Heavily silicified cells form curving or spiraling chains, joined by mucous pads on ends of elevations (horns). Numerous small chloroplasts. Cells can be solitary[44] [Figure No.2E and Table No.1].

Coscinodiscus radiatus

Frustule discoid. Cingulum with three bands, valvocopula more than double the width of one of the two other bands. All bands with hyaline broad marginal borders, slightly rounded ends, areolae in regular rows, 5-6 in µm, broad ligulae with more irregular structure. The band in abvalvar direction with a horizontal marginal flange. Valve flat with rounded margins. Mantle shallow. Valve diameter 90-40µm. Central hyaline area with varying number of branches and a small circular hole externally. Locular areoloe 4-6 in 10µm on valve face, 6-8 in 10µm on valve mantle in radial rows[45] [Figure No.2F and Table No.1].

Guinardia striata

Cylindrical cells that form straight, curving and sometimes spiraling chains. Valves flat but with rounded edges. External process is marginal, and fits into a shallow depression in adjoining valve. Girdle bands appear as collars but hard to see. Small numerous chloroplasts was observed[46] [Figure No.2G and Table No.1].

Lauderia annulata

In valve centre an area set off from rest of valve by a silicified ring. Irregularly shaped areolae, more than 30 in 10µm, in marginal zone. Radial, dichotomously branched costae, more than 30 in 10µm, separated by usually 2 rows of minute pores, present on rest of the valve. Structured tubuli usually more in marginal zone than in central part of the valve with long exernal tubes and short internal tubes. One large labiates process at some distance from valve margin[47] [Figure No.2H and Table No.1].

Skeletonema marinoi

Cells in girdle view are rectangular, with rounded corners, 2.0-7.0µm wide and 2.0-10.0µm high. The colonies are chain like, long, consisting of 15-30 cells, strait or slightly curved. The chloroplasts (one or two) are large, plate like, located near the cell wall. The valves are rounded, slightly convex[48] [Figure No.2I and Table No.1].

Skeletonema costatum

Short and cylindrical cells connected by undulated chains which are long and with spines of marginal rings straight and undulated Convex to flat valve face observed. A dotted ring was observed between adjacent cells with midway length interlocking spines. A central with two chloroplasts was observed. Tubular and semicircular strutted processes in cross section were observed. One labiate process is present near the center of the valve inside the ring of processes[47] [Figure No.2J and Table No.1].

Conticribra weissflogii

Valves are round, flat, with short mantles. The frustules are relatively lightly silicified. Fine areolae were observed and the light microscopic view of the structure was not clearly visible. A small external opening with a ring of marginal fultoportulae was present. The center of the valve face was observed with central fultoportulae between three to six numbers. The margin of the valve was observed with a single prominentrimoportula[49,50] [Figure No.2K and Table No.1].

Asterionellopsis glacialis

It is a Pennate diatom. The cells are connected by star-shaped or spiraling chain valve faces. A single cell with two chloroplasts was observed. The symmetric valves towards apical axis and asymmetric valves towards transapical axis were observed. Linear-lanceolate shaped valves with capitate ends were observed in valve view. Stellate colonies are formed with living cells attached by mucilage pads at the foot poles or basal ends. Living colonies present cells in girdle view shoes the presence of living colonies in the cells whereas the processed samples contain single valve with break up the colonies in the valve view. The light microcopy revealed the presence of small spines on the valve margins[51] [Figure No.2L and Table No.1].

Nitzschia acicularis

Valves are lightly-silicified. The valve with parallel sides and sharp tapering to narrow apices was observed at the central part. The evenly spaced fine fibulae which are restricted towards the margin through the central valve was observed with a density 15-20 in 10µm. The light microscopy revealed the absence of central nodule with unclear striae[52] [Figure No.2M and Table No.1].

Craticula cuspidata

Wide rhombic-lanceolate valves at the center and tapering to narrow apices were observed. The entire valve was observed with parallel and equidistant striae throughout. An orthostichous pattern was observed with thin transapical costae intersecting perpendicular to the longitudinal forming striae. Small, elliptic areolae, approximately 11-15 in 10µm in the transverse direction and 23-25 in 10µm longitudinally were observed in striae. Wide central area with narrow axial area and slightly concave margins were observed. Filiform raphe observed. Expanded proximal raphe ends which are straight, or slightly hooked in the same direction and hooked distal raphe ends which are deflected in the same direction was observed[37,51,53]. [Figure No.2N and Table No.1].

In the present study, the isolated and identified diatoms from the Marakkanam Sea coast determine the rich nutritional profile for the growth of the organisms and as bio-indicators for the water quality. The diatoms isolated from the coast may serve multiple purposes including as a feed for shrimp larvae, post larvae, oysters and copepods as well as for fuel precursors in liquid. The dead diatoms in the sea bed along with organic matter forms diatomite which is used as a porous material in filtration of sugars and alcohols[54-57]. The diatoms *Chaetoceros calcitrans*, *Skeletonema costatum* and *Conticribra weissflogii* are widely used in aquaculture as live feed[58-60]. The diatom *Skeletonema marinoi* are rich in phenolic compounds and the most available antioxidant substance in photosynthetic organisms making it valiant in the pharmaceutical industry[61,62]. The diatoms belonging to the genera *Trieres*, *Coscinodiscus*, *Nitzschia* may be used as experimental foods for larvae of grey mullet species[63]. The biosynthesis of gold nanoparticles using diatom *Eupyxidicula turris* plays a crucial role in nano-biotechnology applications[64]. *Odontella aurita* which is widely used as a food supplement due to its rich EPA content and fucoxanthin may help in the management of obesity especially abdominal fat[65]. The diatom genera such as *Navicula*, *Lauderia* and *Asterionellopsis* can be tapped for possible biofuel production and as a source for EPA[66-69]. Thus the isolated diatoms find their applications in aquaculture as a live feed, as a food supplement in the pharmaceutical industry, possible biofuel production, biosynthesis of nanoparticles in the nanotech industry, used as experimental foods and as antioxidant substances as well as for various bioactive substances.

Table No.1: Composition of the F/2 Medium (Guillard and Ryther 1962, Guillard 1975)

Solution I	**Stock Solution [g/L] dH2O**	**Quantity [ml/L]**	**Final Medium Concentration [M]**
Sodium Nitrate	75	1	8.83×10^{-4}
Sodium Dihydrogen Orthophosphate	5	1	3.63×10^{-5}
Solution II	**Stock Solution [g/L] dH2O**	**Quantity [ml/L]**	**Final Medium Concentration [M]**
Sodium Silicate	35	1	1.05×10^{-4}
Solution III	**Stock Solution [g/L] dH2O**	**Quantity [ml/L]**	**Final Medium Concentration [M]**
Ferric chloride	-	3.17	1.18×10^{-5}
EDTA	-	4.38	1.18×10^{-5}
Solution IV [Trace Metals]	**Primary Stock Solution [g/L] dH2O**	**Quantity [ml/L]**	**Final Medium Concentration [M]**
Manganese Chloride	180	1	9.10×10^{-7}
Zinc Sulphate	22	1	7.66×10^{-8}
Cobaltous Chloride	10	1	4.20×10^{-8}
Copper Sulphate	9.8	1	3.94×10^{-8}
Sodium Molybdate	6.3	1	2.60×10^{-8}
Solution IV[Vitamins]	**Primary Stock Solution [g/L] dH2O**	**Quantity [ml/L]**	**Final Medium Concentration [M]**
B_1	20	5	2.98×10^{-7}
H	1	5	$2.07 \times 10^{-}$
B_{12}	1	5	3.68×10^{-10}

Table No.2: Identification and classification of isolated diatoms from north east pelagic sea coast of Markkanam, Tamil Nadu, India

Figure	Empire	Kingdom	Phylum	Subphylum	Class	Subclass	Order	Family	Genus	Species
2A	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Thallasiosirophycidae	Eupodiscales	Parodontellaceae	Trieres	mobiliensis
2B	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Thallasiosirophycidae	Eupodiscales	Parodontellaceae	Trieres	chinensis
2C	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Chaetocerotophycidae	Chaetocerotales	Chaetocerotaceae	Chaetoceros	calcitrans
2D	Eukaryota	Chromista	Bacillariophyta	Coscinodiscophytina	Coscinodiscophyceae	Archaegladiopsophycidae	Stephanopyxales	Stephanopyxidaceae	Eupyxidicula	turris
2E	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Thallasiosirophycidae	Eupodiscales	Odontellaceae	Odontella	aurita
2F	Eukaryota	Chromista	Bacillariophyta	Coscinodiscophytina	Coscinodiscophyceae	Coscinodiscophycidae	Coscinodiscales	Coscinodiscaceae	Coscinodiscus	radiatus
2G	Eukaryota	Chromista	Bacillariophyta	Coscinodiscophytina	Coscinodiscophyceae	Coscinodiscophycidae	Rhizosoleniales	Rhizosoleniaceae	Guinardia	striata
2H	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Thallasiosirophycidae	Thallasiosirales	Lauderiaceae	Lauderia	annulata
2I	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Thallasiosirophycidae	Thallasiosirales	Skeletonemataceae	Skeletonema	marinoi
2J	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Thallasiosirophycidae	Thallasiosirales	Skeletonemataceae	Skeletonema	costatum
2K	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Mediophyceae	Thallasiosirophycidae	Thallasiosirales	Thallasiosiraceae	Conticribra	weissflogii
2L	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Bacillariophyceae	Urneidophycidae	Rhaphoneidales	Asterionellopsidaceae	Asterionellopsis	glacialis
2M	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Bacillariophyceae	Bacillariophycidae	Bacillariales	Bacillariaceae	Nitzschia	acicularis
2N	Eukaryota	Chromista	Bacillariophyta	Bacillariophytina	Bacillariophyceae	Bacillariophycidae	Naviculales	Stauroneidaceae	Craticula	cuspidata

Figure No.1: Location map of the study area for the isolation and identification of diatoms

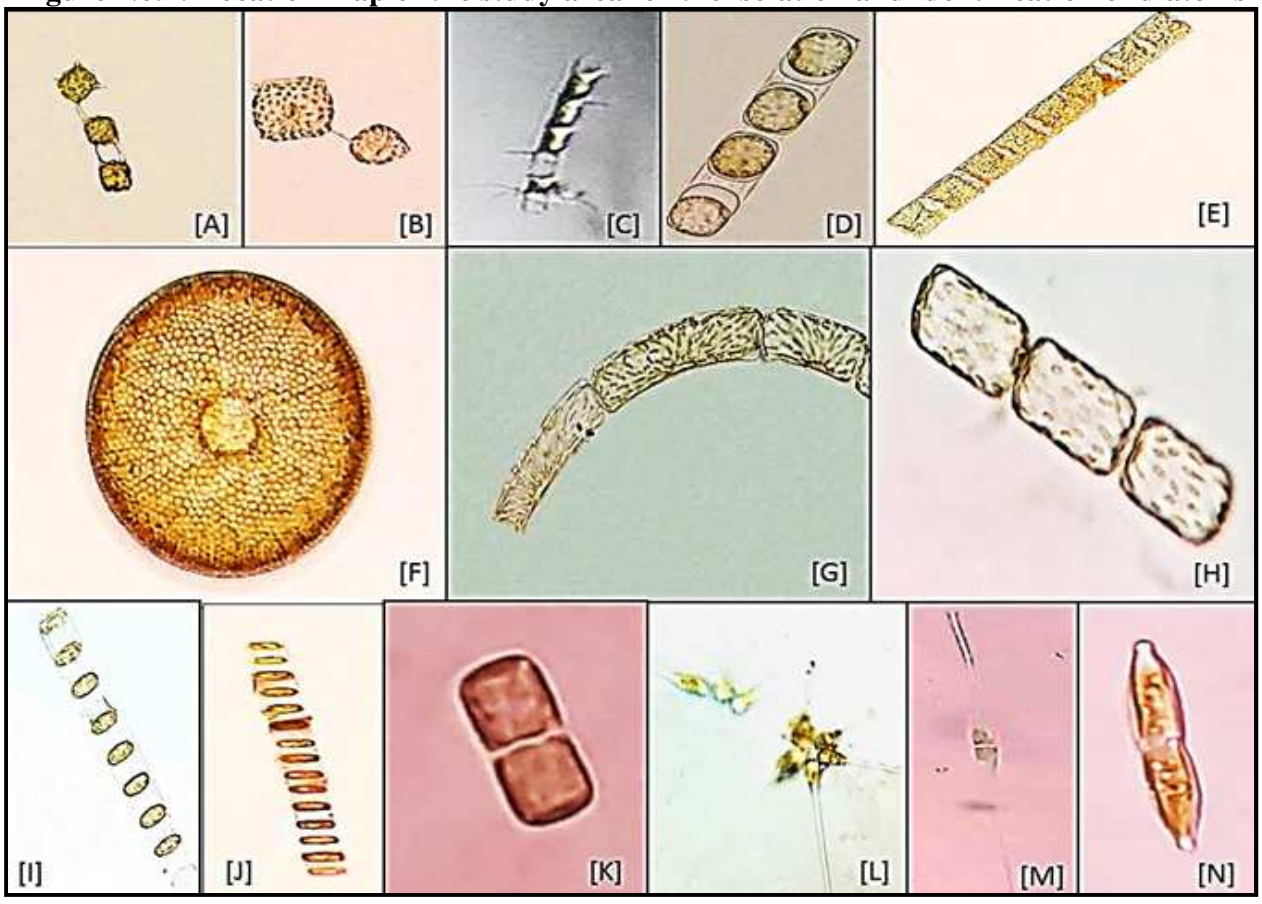

Figure No.2: [A] ***Trieres mobiliensis*** **[B]** ***Trieres chinenesis*** **[C] C*haetoceros calcitrans*** **[D] E*upyxidicula turris*** **[E] O*dontella aurita*** **[F] C*oscinodiscus radiates*** **[G]** ***Guinardia striata*** **[H]** ***Lauderia annulata*** **[I]** ***Skeletonema marinoi*** **[J]** ***Skeletonema costatum*** **[K]** ***Conticribra weissflogii*** **[L]** ***Asterionellopsis glacialis*** **[M]** ***Nitzschia acicularis*** **[N]** ***Craticula cuspidata***

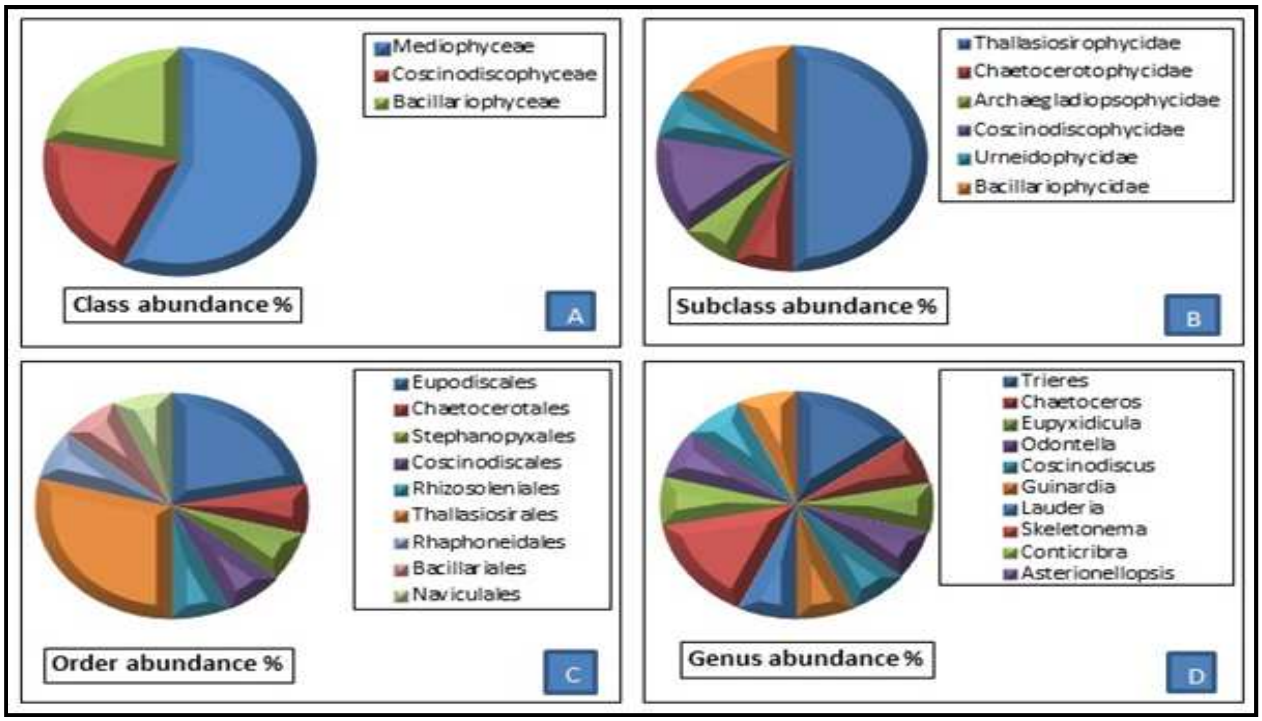

Figure No.3: Abundance percentage of [A] Class, [B] Subclass, [C] Order and [D] Genus present from the north east pelagic seacoast of Marakknam, Tamil Nadu, India

CONCLUSION

The diversity of diatoms from the northeast pelagic coast of Marakkanam was studied by isolation and identification techniques. The twelve genera with nine orders of diatoms confirm the rich diatom community present in the Marakkanam coast. The isolated diatoms are economically important and find their applications in various fields such as aquaculture, nanotechnology, food industry, pharmaceutical industry, biofuel production and medicinal properties. The culturing techniques employed in the study enables the diatoms to be cultured in mass scale production for the above mentioned applications. The remarkable properties and applications of diatoms can be tapped and studied further for the economic development and new compound identification with useful properties for the aquatic and human ecosystems. Thus the diatoms isolation and identification from the Marakkanam coast portrays the rich diversity and forms the basis for their further advanced research beneficial to mankind and in water quality indicator of the coast.

ACKNOWLEDGEMENT

The author would like to thank Mr. Ravisekhar, Technical director, BMR Group, Aquaculture Division, Chennai Tamil Nadu, India for providing the laboratory support and Mr. Trinadu Rao Kola who assisted in the sample collection.

The author would also like to thank the following algologists for the identification of the genus and species of the isolated diatoms Dr. Victor A. Chepurnov, Ghent University, Belgium, Dr. Jagadeesan Loganathan, CSIR-NIO, Vizag, Andhra Pradesh, India and Mr. Sathish Kumar, Assistant Professor in Botany, Sri Vidya Mandir College, Krishnagiri, Tamil Nadu.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR'S FUNDING

The research work performed by authors by a self-financial scheme and no financial assistance was received from any funding agencies.

BIBLIOGRAPHY

1. Davis R B. Paleolimnological diatom studies of acidification of lakes by acid rain: An application of Quaternary science, *Quaternary Scie Revi,* 6(2), 1987, 147-163.
2. Bentley K, Cox E J, Bentley P J. Nature's batik: A computer evolution model of diatom valve morphogenesis, *Journal of Nanoscience and Nanotechnology,* 5(1), 2005, 25-34.
3. Armbrust E V, Berges J A, Bowler C, Green B R, Martinez D, Putnam N, Zhou S, Allen A E, Apt K E, Bechner M, Brzezinski M A, Chaal B K, Chiovitti A, Davis A K, Demarest M S, Detter J C, Glavina T, Goodstein D, Hadi M Z, Hellsten U, Hilldebrand M, Jenkins B D, Jurka J, Kapitonov V V, Kroger N, Lau W W Y, Lane T W, Larimer F W, Lippmeier J C, Lucas S, Medina M, Monstant A, Orbornik M, Parker M S, Palenik B, Pazour G J, Richardson P M, Rynearson T A, Saito M A, Schwartz D C, Thamatakoln K, Valentin K, Vardi A, Wilkerson F P, Rokshar D. The genome of the diatom Thalassiosira pseudonana: Ecology, evolution and metabolism, *Science,* 306(5693), 2004, 79-86.
4. Mishra S, Gupta A K, Mishra M K Mishra V. Study on variation of diatoms in Gomati River at Jaunpur for forensic consideration, *Journal of Pharmacy and Biol. Sciences,* 11(4), 2016, 59-62.
5. Round F E. Diatoms in river water-monitoring studies, *Journal of Applied Physiology,* 3(2), 1991, 129-145.
6. Nelson D M, Treguer P, Brzezinski M A, Leynaert A, Queguiner B. Production and dissolution of biogenic silica in the ocean: Revised global estimates, comparison with regional data and relationship to biogenic sedimentation, *Glob. Biogeochem. Cycles,* 9(3), 1995, 359-372.
7. Field C B, Behrenfeld M J, Randerson J T, Falkowski P. Primary production of the biosphere: Integrating terrestrial and oceanic components, *Scie,* 281(5374), 1998, 237-240.
8. Buesseler K O, Andrews J E, Pike S M. Particle export during the Southern Ocean iron experiment (SOFeX), *Limnol Oceanogr,* 50(1), 2005, 311-327.
9. Romero O E, Armand L K. Marine diatoms as indicators of modern changes in oceanographic conditions, In: The Diatoms, Applications for the environmental and earth sciences, *Cambridge University Press*, *Cambridge,* 2nd Edition, 2010, 373-400.
10. Rigual-hern´andez A S, Trull T W, Bray S G. Seasonal dynamics in diatom and particulate export fluxes to the deep sea in the Australian sector of the southern Antarctic Zone, *J Mar Syst,* 142, 2015, 62-74.
11. Assmy P, Smetacek V and Montresor M. Thick-shelled, grazerprotected diatoms decouple ocean carbon and silicon cycles in the iron-limited Antarctic circumpolar current, *Proc Natl Acad Sci,* 110(51), 2013, 20633-20638.
12. Qu´eguiner B. Iron fertilization and the structure of plank tonic communities in high nutrient regions of the Southern Ocean, *Deep Sea Res Part II Top St Ocea,* 90, 2013, 43-54.
13. Sackett O, Armand L, Beardall J. Taxon-specific responses of Southern Ocean diatoms to Fe enrichment revealed by synchrotron radiation FTIR micro spectroscopy, *Biogeosciences,* 11(20), 2014, 5795-5808.
14. Supriya G, Ramachandra T V. Chronicle of marine diatom culturing techniques, *Indian Journal of Fundamental and Applied Life Sciences,* 1(3), 2011, 282-294.
15. Muller O F. Diatomaceen (Vibrio paxillifer, V. bipunctatus, V. tripunctatus, Gonium pulvinatum). The small animals of the sea and the lips on each of the things that has unfolded to fluviatilia, systematically, according to the writing and to make a living to sketch the cured, *Animalcula infusoria*, *Havniae, Nauturque curiosol, Berlin,* 1786.
16. Medlin L K, Kooistra W H C F, Gersonde R, Wellbrock U. Evolution of the diatoms (Bacillariophyta). II, Nuclear-encoded small-

subunit rRNA sequences comparisons confirm paraphyletic origin for centric diatoms, *Molecular and Biological Evolution,* 13(1), 1996, 67-75.

17. Karthick B, Taylor J C, Mahesh M K, Ramachandra T V. Protocols for collection, preservation and Enumeration of diatom from Aquatic habitats for water quality monitoring in India, *The IUP Journal of Soil and Water Sciences,* 3(1), 2010, 25-60.
18. Mann D G, Thomas S J, Evans K M. Revision of the diatom genius: Sellaphora: A first account of the larger species in the British Isles, *Fottea,* 8(1), 2008, 15-78.
19. Falasco E, Bona F, Badino G, Hoffmann L, Ector L. Diatom teratological forms and environmental alterations: A review, *Hydrobiologia,* 623(1), 2009, 1-35.
20. Hakansson H, Chepurnov V. A study of variation in valve morphology of the diatom Cyclotella meneghiniana in monoclonal cultures: Effect of auxospore formation and different salinity conditions, *Diatom Research,* 14(2), 1999, 251-272.
21. Miquel P. De la culture artificielle des diatomees, Cultures pures des diatomees, *Le Diatomiste,* (1), 1890, 149-156.
22. Price N M, Harrison G I, Hering J G, Hudson R J, Nirel P M V, Palenik B, Morel F M M. Preparation and chemistry of the artificial culture medium, *Aquil. Biological Oceanography,* 6(5-6), 1989, 443-461.
23. Keller M D, Bellows W K and Guillard R R L. Microwave treatment for sterilization of phytoplankton culture media, *Journal of Experimental Marine Biology and Ecology,* 117(3), 1988, 279-283.
24. Zengler K. Central role of the cell in microbial ecology, *Microbiology and Molecular Biolo Revi,* 73(4), 2009, 712-729.
25. Guillard R R L. Culture of phytoplankton for feeding marine invertebrates, In: Culture of marine invertebrate animals (Ed. by Smith W.L. and Chanley M.H.) *Plenum Press, New York, USA,* 1975, 26-60.
26. Guillard R R, Ryther J H. Studies on marine plank tonic diatoms. Cyclotella nana Hustedt and Detonula confervacaea (Cleve) Gran, *Cana J Microbiol,* 8(2), 1962, 229-239.
27. Battarbee R W. Diatom Analysis. In: Handbook of holocene paleoecology and paleohydrology (Ed. by Berglund B.E.) John Wiley and Sons Ltd, Great Britain, *Journal of Quaternary Science,* 1(1), 1986, 86-87.
28. Andersen R A, Kawachi M. Traditional Microalgae Isolation Techniques, Chapter 6. In: Algal culturing techniques (Ed. by Andersen R.A.), *Elsevier,* 2005, 83-100.
29. Cullen J J, Macintyre H L. On the use of the serial dilution culture method to enumerate viable phytoplankton in natural communities of plankton subjected to ballast water treatment, *J Appl Phycol,* 28(1), 2016, 279-298.
30. Stein E D. Handbook of Phycological Methods, Culture methods and growth measurements, *Cambridge University Press, Cambridge,* 1973-1985.
31. Barker K. At the Bench: A laboratory navigator, Cold spring harbor, *Cold Spring Harbor Laboratory Press, New York, USA,* 1998.
32. Knuckey R M, Brown M R, Barrett S M, Hallegraeff G M. Isolation of new nanoplanktonic diatom strains and their evaluation as diets for juvenile Pacific oysters (Crassostrea gigas), *Aquaculture,* 211(1-4), 2002, 253-274.
33. Yamaji I. Illustration of the marine plankton of Japan, *Hoikusha Publishing Co. Ltd, Osaka, Japan,* 1976, 369.
34. Newell G E, Newell R C. Marine plankton, A practical guide, *Hutchinson of London,* 5th Edition, 1977, 244.
35. Tomas C R. Identifying marine phytoplankton, *Academic Press, London,* 1997, 858.
36. Barsanti L, Gualtieri P. Algae: anatomy, biochemistry and biotechnology, *Taylor and Francis, Boca Raton, FL,* 2006, 301.

37. Guiry M D. Guiry G M. Algae Base, World-wide electronic publication, *National University of Ireland, Galway,* 2020.
38. Bailey J W. Microscopical observations made in South Carolina, Georgia and Florida, *Smithsonian Contributions to Knowledge,* 2(8), 1851, 1-48.
39. Ashworth M P, Nakov T and Theriot E C. Revisiting Ross and Sims (1971), toward a molecular phylogeny of the Biddulphiaceae and Eupodiscaceae (Bacillariophyceae), *Jour of Phyco,* 49(6), 2013, 1207-1222.
40. Greville R K. Descriptions of new and rare diatoms, Series XIX, Transactions of the microscopical society, *New Series,* 14, 1866, 77-86.
41. Takano H. On the diatom *Chaetoceros calcitrans* (Paulsen) emend and its dwarf form *pumilus* forma nov, *Bulletin Tokai Regional Fisheries Research Laboratory,* 55(7), 196, 1-7.
42. Pritchard A. *A history of infusoria*, including the Desmidiaceae and Diatomaceae, British and foreign. Fourth edition enlarged and revised by Arlidge J T, Lond B A, W Archer ESQ, J Ralfs M R CSL, W C Williamson ES Q, F R S and the Author, Whittaker and Co, *Av Ma La, Lon,* 4th Edition, 1(98), 1861, 1-12.
43. Blanco S, Wetzel C E. Replacement names for botanical taxa involving algal genera, *Phytotaxa,* 266(3), 2016, 195-205.
44. Agardh C A. *Conspectus criticus diatomacearum, Lundae* [Lund], *Literis Berlingianus,* 4, 1832, 49-66.
45. Ehrenberg C G. Uber noch zahlreich jetzt lebende Thierarten der Kreidebildung und den Organismus der Polythalamien Preprint of Abhandlung der Konglichen Akademie der Wissenschaften zu Berlin aus dem Jahre 1839 [1841] with different pagination, *Berlin,* 4, 1840, 1-94.
46. Hasle G R, Syvertsen E E. Marine diatoms, In: Identifying marine phytoplankton, *(Tomas, C.R. Eds, San Diego: Academic Press,* 1996, 5-385.
47. Cleve P T. Examination of diatoms found on the surface of the Sea of Java, *Bihang till Kongliga Svenska Vetenskaps-Akademiens Handlingar,* 1(11), 1873, 1-13.
48. Sarno D, Kooistra W H C F, Medlin L K, Percopo I, Zingone A. Diversity in the genus *Skeletonema* (Bacillariophyceae): II. An assessment of the taxonomy of *S. costatum*-like species, with the description of four new species, *Journal of Phycology,* 41(1), 2005, 151-176.
49. Fryxell G A, Hasle G R. The genus *Thalassiosira*: Species with a modified ring of central strutted processes, In: Proceedings from the fourth symposium on recent and fossil marine diatoms, Oslo, August 30 - September 3, 1976. (Simonsen, R. Ed.), *Beihefte Zur Nova Hedwigia,* 54, 1977, 67-98.
50. Stachura Suchoples K, Williams D M. Description of *Conticribra tricircularis*, A new genus and species of Thalassiosirales, with a discussion on its relationship to other continuous cribra species of *Thalassiosira* Cleve (Bacillariophyta) and its fresh water origin, *European Journal of Phycology,* 44(4), 2009, 477-486.
51. Round F E, Crawford R M. Mann D G. The diatoms biology and morphology of the genera, *Cambridge University Press, Cambridge,* 1990, 1-747.
52. Smith, W. *A synopsis of the British Diatomaceae*; with remarks on their structure, function and distribution and instructions for collecting and preserving specimens, The plates by Tuffen West, John van Voorst, *Paternoster Row, London,* 1(2), 1853, 1-89.
53. Kutzing F T. *Die Kieselschaligen Bacillarien oder Diatomeen*, Nordhausen: Zu finden bei W, *Kohne,* 1(7), 1844, 1-30.
54. Muller-Feuga A, Moal J. Kaas R. The microalgae of aquaculture, In: Live feeds in marine aquaculture (Ed. by J.G. Stottrup and L.A. Mc Evoy), *Black Scie,* 2003, 206-252.

55. Benemann J. Microalgae for biofuels and animal feeds, *Ener,* 6(11), 2013, 5869-5886.
56. Victor A C, Claas G S, Phil S. Diatoms as hatchery feed: On-site cultivation and alternatives, *Hatchery feed,* 6(3), 2018, 23-27.
57. James M G, Linda E G, Shahrizim B Z, Brian F P, Spencer W H, Jun Y. Freshwater diatoms as a source of lipids for biofuels, *J Ind Microbiol Biotechnol,* 39(3), 2012, 419-428.
58. Mayavan V, Rajeswari Thangavel B. Comparative study on growth of Skeletonema costatum: Amicroalga as live feed for aquaculture importance, *International Journal of Research in Fisheries and Aquacul,* 4(3), 2014, 117-121.
59. Soliman H, Abdel R Abdel Razek F A,Abou Zeid A E, Mohamed A. Optimum growth conditions of three isolated diatom species; Skelatonema costatum, Chaetoceros calcitrans and Detonulla confervacea and their utilization as feed for marine penaeid shrimp larvae, *Egyptian Journal of Aquatic Research,* 36(1), 2010, 161-183.
60. Ortega-salsa A A, Pedro Flores N. Cultivation of the Microalga Thalassiosira weissflogii to feed the Rotifer Brachionus rotundiformis, *J Aquac Mar Biol,* 6(5), 2017, 00169.
61. Goiris K, Muylaert K, Fraeye I, Foubert I De Brabanter J, De Cooman L. Antioxidant potential of microalgae in relation to their phenolic and carotenoid content, *J. Appl. Phycol,* 24(6), 2012, 1477-1486.
62. Goiris K, Van Colen W, Wilches I, Leon-Tamariz F, De Cooman L, Muylaert K. Impact of nutrient stress on antioxidant production in three species of microalgae, *Algal Res,* 7, 2015, 51-57.
63. Nash C E, Kuo C M. Hypotheses for problems impeding the mass propagation of grey mullet and other finfish, *Aquaculture,* 5(2), 1975, 119-134.
64. Pytlik N, Kaden J Finger M, Naumann J, Wanke S, Machill S, Brunner E. Biological synthesis of gold nanoparticles by the diatom *Stephanopyxis turris* and *in vivo* SERS analyses, *Algal Res,* 28, 2017, 9-15.
65. Haimeur A, Ulmann L, Mimouni V, Gueno F, Pineau-Vincent F, Meskini N, Tremblin G. The role of Odontella aurita, a marine diatom rich in EPA, As a dietary supplement in dyslipidemia, platelet function and oxidative stress in high-fat fed rats, *Lipids in health and disease,* 11, 2012, 147.
66. Selva Kumar P, Athia S, Umadevi K, Ajith H. Checklist, Qualitative and quantitative analysis of marine microalgae from offshore Visakhapatnam, Bay of Bengal, India for biofuel potential, In book: Microalgal biotechnology, *Intechopen,* 2018, 1-27.
67. Thomas K, Marella A, Itzel Y, Lopez-Pacheco B, Roberto parra-saldivar B, Sreenath dixit A, Archana T . Wealth from waste: Diatoms as tools for phycoremediation of wastewater and for obtaining value from the biomass, *Science of the Total Environment,* 724, 2020, 137960.
68. Wen Z Y, Chen F. Heterotrophic production of eicosapentaenoid acid by the diatom Nitzschia laevis: Effects of silicate and glucose, *J Ind Microbiol Biotechnol,* 25(4), 2000, 218-224.
69. Wen Z Y, Chen F. Heterotrophic production of eicosapentaenoic acid by microalgae, *Biotechnol Adv,* 21(4), 2003, 273-294.

Please cite this article in press as: Vishnu Kiran Manam and Sumathi G. Larger diatom flora isolated and identified from the northeast pelagic seacoast of Marakkanam, Tamil Nadu, India, *Asian Journal of Research in Pharmaceutical Sciences and Biotechnology*, 9(1), 2021, 7-18.