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PHYTOPLANKTON DIVERSITY OF VEMBANAD ESTUARY: A SEARCH FOR NUTRACEUTICALLY POTENT MEMBERS

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Phytoplankton plays an important role in primary organic production in the estuaries and also a source of biologically active components. In order to evaluate the phytoplankton diversity an investigation of eight stations representing central to southern end of Vembanad estuary was conducted. Studies were carried out during the pre-monsoon and monsoon seasons of 2019.In the present study, a total 42 species of phytoplankton were recorded, among which 18 genera and 28 species belongs to Bacillariophyceae, 7 genera and 9 species to Chlorophyceae, 2 genera and 3 species to Cyanophyceae, and 2 genera to Euglenophyceae. Bacillariophyceae formed the dominant group in both the seasons. Palmer's algal general pollution index is employed in all the sampling stations. Station S6 (Vayalaar) was found to be with high organic pollution in both pre-monsoon and monsoon with index value of 22 and 21 respectively. From the present study the Palmer's algal pollution index value represent probable high organic pollution in Vembanad estuarine system. The agricultural fertilizers from the Kuttanad rice fields, solid wastes and sewage wastes might be the major source of pollution in Vembanad estuary. Economically important and nutraceutically valuable members like *Chlorella vulgaris, Scenedesus* sp, *Oscillatoria* sp and *Euglena* sp were identified during the study period.

Key words: Nutraceutical, Phytoplankton, Vembanad estuary.

Introduction

Phytoplanktons play an important role in primary organic production in any aquatic bodies including the estuaries and form an important link in the food chain. Phytoplankton contributes approximately 45% of the earth's annual primary production[1]. The phytoplanktonic study could also be a really useful tool for the assessment of water quality in any kind of water body and also contributes to understanding of the essential nature of lake[2].

Vembanad estuary has been designated as the Ramsar site No.1214 has a unique environmental habitat which supports rich and diverse species of flora and fauna. Estuaries are the transition zone between fresh water and saline water play a vital role as they serve as areas of interaction between fresh and salt water[3]. A total of 73 genera of phytoplankton were recorded in a study of phytoplankton abundance and distribution from the Vembanad estuary[4].

Microalgae are excellent reservoirs of wide range of nutraceuticals. Although many compounds of high biological value and their health benefits have already been reported, microalgae still remain one of the most unexplored groups of organisms in the world, as around 97% of marine microalgal compounds are yet to be isolated and characterized[5]. There is a need for more research in the area of bioprospecting of microalgal strains. This investigation is an attempt to study the phytoplankton diversity of Vembanad estuary inorder to screen the algae with nutraceutical potential, so that it can lead to the discovery of novel metabolites with nutraceutical benefits[6].

Material and Methods

Study Area: Vembanad estuary:

Vembanad estuary (09˚00' -10˚40'N and 76˚00'-77˚30'E), situated in Kerala, is the largest tropical wetland ecosystem along the south-west coast of India. This tropical estuary, fed by ten rivers draining into the Arabian Sea, covers an area of 21,050 ha and spreads across three districts of Kerala – Ernakulam, Kottayam and Alappuzha (Figure.1). A unique feature of the estuary is the Thaneermukkom salt water barrier which separates the water body into two zones.One zone with freshwater fed by the rivers at the southern side, and the other on northern side with brackish water by rivers and Arabian Sea. The estuary has two permanent openings into the Arabian Sea one at Cochin and the other at Azhikode[4].

Field Sampling and Analysis:

Field Sampling was performed in two distinct seasons viz., Pre- Monsoon (February to May), and Monsoon (June to September) of 2019 to record the phytoplankton diversity of Vembanad estuary. Total of 8 Stations representing central to southern zones of Vembanad estuary were selected. The study stations:S1-Thevara, S2-Kumbalam, S3-Kumbalangi, S4- Ezhupunna, S5-Pallippuram, S6- Vayalaar, S7-Thanneermukkom South and S8-Thanneermukkom North.

The surface water sample of 0.1 to 0.5 meter depth was collected in a well cleaned polythene bottle. Field data like temperature, salinity and p^H were noted. Collection was done in a time between 7am to 10 am. 1L sample was collected from each sampling site, filtered using a phytoplankton net (sieve size 20µm), concentrated to 100 ml and preserved in 4% formalin. Numerical estimation of phytoplankton was done using Sedgewick Rafter counting cell[7]. Identification of algal forms was made with the help of relevant and available literature[8,9]. Photomicrographs were prepared with the help of Biolinkz microscope with attached photographic camera. Water quality assessment was done using Palmers algal pollution index[10]and species diversity indices were calculated by using Simpson index and Shannon–Wiener index. The collected samples from the above location were also inoculated into different artificial culture media for further studies.

Figure 1: Map of Vembanad estuarine system indicating study stations

Results and Discussion

The algal taxa consisted of 42 forms belonging to Chlorophyceae, Cyanophyceae, Bacillariophyceae and Euglenophyceae. The Bacillariophyceaen members were dominant in both the pre-monsoon and monsoon seasons and represented by 28 genera. Most abundant diatoms were represented by *Coscinodiscus marginatus, Skeletonema costatum* and, *Nitzschia* sp. Chlorophyceaen members were represented by 9 genera, Cyanophyceae with 3 genera and Euglenophyceae with 2 genera.The highest number of phytoplankton was recorded in pre- monsoon period (36 species) than the monsoon period (32 species). A check list of phytoplankton sampled in the present study is presented in Table 1.The maximum value for Shannon Wiener index (H) was observed in Vayalar(1.16) during pre-monsoon period and in Pallippuram(0.86) during the monsoon period (Figure.2). Highest value for Simpson Diversity Index (D) was obtained in Thanneermukkom South (3.92) during the pre-monsoon period and in Thanneermukkom North (3.8) during the monsoon period (Figure.3).

The hydrogen ion concentration remains alkaline during the pre-monsoon period with the maximum p^H of 8.3 at Kumbalangi and lowest p^H was reported in monsoon period with 7.3 at Vayalaar. The maximum salinity of 22ppt was observed in Thevara during pre-monsoon period and minimum salinity of 14ppt was observed in Vayaalar in the monsoon period. The highest temperature of 32°C was noted in Thevara during the pre-monsoon period and lowest temperature of 27.8°C at Pallipuram in the monsoon period.Palmer's Algal Pollution Index was applied in all the eight study stations and Vayalaar was found with highest index value (22 – pre-monsoon and 21- monsoon) that indicates high organic pollution in both the seasons. Ezhupunna and Pallippuram are reported with moderate organic pollution and rest of the stations with probable high organic pollution in both the seasons (Table 4). Pollution tolerant genera like *Oscillattoria, Chlorella, Navicula, Cyclotella* and *Nitzschia* were found in both the seasons. Nutraceutically valuable members like *Chlorella ellipsoidea, Chlorella vulgaris, Chaetoceros* sp, *Nitzschia* sp, *Scenedesmus* sp, *Oscillatoria* sp and *Euglena* sp were reported during the study period (Figure.4).

Bacillariophyceae
1. *Achanthes* sp
2. *Amphiprora* sp
3. *Asterionella japonica*
4. *Chaetoceros* sp.
5. *Coscinodiscus centralis*
6. *Coscinodiscus granii*
7. *Coscinodiscus marginatus*
8. *Cyclotella* sp.
9. *Cymbella* sp.
10. *Fragilaria* sp.
11. *Gomphonema* sp.
12. *Gyrosigma acuminatum*
13. *Gyrosigma attenuatum*
14. *Melosira moniliformis*
15. *Navicula* sp1.
16. *Navicula* sp2.
17. *Nitzschia closterium*
18. *Nitzschia longissima*
19. *Nitzschia paradoxa*
20. *Nitzschia reversa*
21. *Pinnularia* sp.
22. *Pleurosigma angulatum*
23. *Pleurosigma* sp.
24. *Rhizosolenia alata*
25. *Rhizosolenia* sp.
26. *Skeletonema costatum*
27. *Surirella* sp1.
28. *Surirella* sp2.
Chlorophyceae
29. *Chlorella ellipsoidea*
30. *Chlorella vulgaris*
31. *Chlorococcum* sp.
32. *Closterium* sp.
33. *Cosmarium* sp.
34. *Scenedesmus acutus*
35. *Scenedesmus quadricauda*
36. *Staurastrum* sp.
37. *Tetraspora* sp.
Cyanophyceae
38. *Microcystis* sp.
39. *Oscillatoria* sp
40. *Oscillatoria* sp
Euglenophyceae
41. *Euglena* sp.
42. *Phacus* sp.

Table 1 : Checklist of phytoplankton identified during the study period

A total of 42 species of phytoplankton were recorded during the present study from different classes viz, Chlorophyceae, Bacillariophyceae, Cyanophyceae and Euglenophyceae. Bacillariophyceae formed the dominant group in both the Pre- Monsoon and monsoon seasons.

Previous reports suggests that Bacillariophyceae formed the dominant group in all seasons in Vembanad estuarine system[4]. They reported 73 genera of phytoplankton for a period of two years. Studies conducted in Cochin estuary also reported the dominance of diatoms (85 species) in all the seasons[11,12]. During the present study diatomslike *Coscinodiscus marginatus, Skeletonema costatum* and *Nitzschia* sp were dominant in the pre-monsoon season. The maximum value for p^H, temperature and salinity were also recorded in the pre-monsoon period. The phytoplankton abundance in pre-monsoon season may be due to increased salinity, temperature, p^H and high intensity of light penetration[13,14]. The maximum value for Shannon Wiener index (H) was recorded at Vayalaar (1.16) during pre-monsoon period than the monsoon period. The higher value of Shannon index (H) indicates high planktonic diversity[15]. The low value of Shannon index of phytoplankton in rainy season is due to dilution of area by rain water[16].

It was Palmer who first developed an "Algal index of Pollution" based on the observations of 269 reports by 165 authors who reported algae tolerant to organic pollution (Table.2). The pollution index is based on the relative number of total points scored by each algae[10] (Table. 3). According to Palmer's algal pollution index, Vembanad estuary is represented with pollution tolerant genera like *Oscillattoria*, *Chlorella*, *Navicula*, *Cyclotella*, *Closterium,* *Chlorella*, *Scenedesmus, Navicula, Phacus, Euglena* and *Nitzschia*.

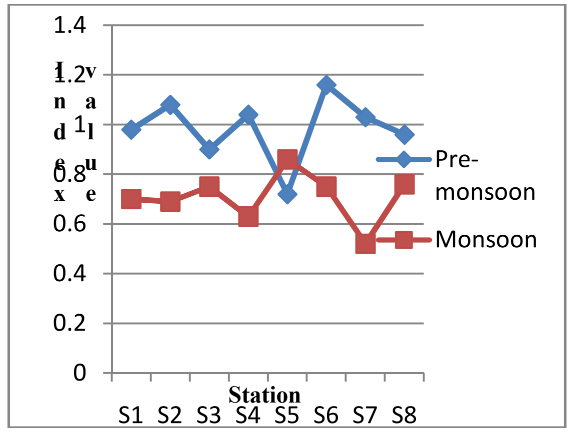

Figure 2: Shannon diversity index of selected locations of Vembanad estuary

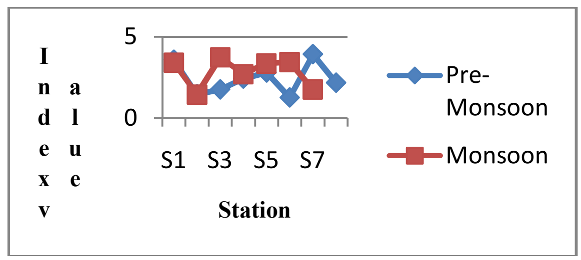

Figure 3: Simpson diversity index of selected locations of Vembanad estuary

The presence of genera like *Euglena, Oscillatoria, Scenedesmus, Nitzchia* and *Navicula* are generally found in organically polluted waters[17]. The average index score values in both the seasons (17.3 and 15.6) suggests probable high organic pollution in Vembanad estuary (Table. 4).

Solid wastes and sewage wastes might be the major source of pollution in Vembanad estuary. The discharge of agricultural fertilizers from the Kuttanad region may also increase the pollution in Vembanad estuary [4].

Microalgae have received a lot of attention in the scientific community and biotechnology industry as they are very good sources of various nutraceutical compounds such as carotenoids and polyunsaturated fatty acids.

Algal Genera	Index Value	Algal Genera	Index Value
Anacystis	1	*Micractinium*	1
Ankistrodesmus	2	*Navicula*	3
Chlamydomonas	4	*Nitzschia*	3
Chlorella	3	*Oscillatoria*	5
Closterium	1	*Pandorina*	1
Cyclotella	1	*Phacus*	2
Euglena	5	*Phormidium*	1
Gomphonema	1	*Scenedesmus*	4
Lepocinclis	1	*Stigeoclonium*	2
Melosira	1	*Synedra*	2

Table 2: Algal Genus Pollution Index Described by Palmer (1969)

0-10	Lack of organic pollution
10-15	Moderate pollution
15-20	Probable high organic pollution
20 and above	High organic pollution

Table 3: Palmer's Algal Pollution Indexscore for phytoplankton based on Palmer (1969)

Station	Pre-Monsoon index value	Monsoon
S1 Thevara	18	15
S2 Kumbalam,	18	16
S3 Kumbalangi	18	15
S4 Ezhupunna,	11	10
S5 Pallippuram,	14	14
S6 Vayalaar	22	21
S7Thanneermukkom South	20	18
S8Thanneermukkom North	18	16
Average index score	17.3	15.6

Table 4: Palmer's Algal Genus Pollution Index of Vembanad Estuary

Nutraceuticals have been defined as food or food products that can provide nutrition and pharmaceutical benefits to the mankind such as prevention and treatment of diseases[18]. The presence of nutraceutically valuable members like *Chlorella ellipsoidea, Chlorella vulgaris, Chaetoceros* sp, *Nitzschia* sp, *Scenedesmus* sp, *Oscillatoria* sp and *Euglena* sp from Vembanad estuary suggests more research in this area for the discovery of value-added products from locally available micro algal strains (Plare. 1). In this context we isolated and inoculated the nutraceutically significant genera in to different artificial culturing media. Further studies on extraction, fractionation and biochemical analyses of nutraceutical compounds are the ongoing investigation in our group so as to gain better understanding of bio-prospecting of microalgae.

Algae	Functional compounds reported	Reference
Chlorella ellipsoidea *C. vulgaris*	Lutein,α-carotene, β-carotene, violaxanthin, antheraxanthin, zeaxanthin, and astaxanthin.	Cha *et al,* 2008[21]
Skeletonema costatum	Ascorbic acid	Brown and Muller. 1992[20]
Chaetoceros sp	Thiamine	Brown *et al.*, 1999
Nitzschia sp	Arachidonic acid Eicosapentaenoic acid	Chu *et a*l., 1994[22]
Scenedesmus sp *Oscillatoria* sp *Euglena* sp	Protein	Becker. 2017[19]

Figure 4: List of nutraceutically valuable taxa reported from Vembanad estuary

***Melosira* sp** **2. *Scenedesmus* sp** **3. *Rhizosolenia* sp**

4. *Asterionella* sp **5. *Skeletonemacostatum*** **6. *Oscillatoria* sp**

7. *Chaetoceros* sp **8. *Nitzschia* sp** **9. *Coscinodiscus marginatus***

Plate 1 : Photomicrographs of major phytoplankton observed in Vembanad estuary

Conclusion

In the present study a total of 42 species of phytoplankton were observed from the central to southern zone of Vembanad estuary, among which seven were found to be potentially valuable for nutraceuticals hence inoculated in artificial culture media for further investigation.

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