

**SPATIO-TEMPORAL DISTRIBUTION AND BIOCHEMICAL COMPOSITION OF
PHYTOPLANKTON ALONG THE COASTAL WATERS OFF DAKSHIN
KANNADA DISTRICT, KARNATAKA**

Comment [1]: OF

I. ABSTRACT

The phytoplankton distribution and biochemical composition concerning water quality parameters were studied in the coastal waters off Mangalore, Monthly sampling was carried out from October 2020 to May 2022 at selected stations of Netravathi-Gurupura estuary, latitude 12°50'38.56" N, longitude 74°50'0.23° to coastal waters off Padubidri latitude 13° 8'0. 57" N longitude 74°42'9.47° with an average depth of 8 meters. Spatiotemporally the phytoplankton, chlorophyll-*a* and phytoplankton biochemical composition showed a significant difference for the entire duration. During the present investigation, 35 different phytoplankton genera belonging to 3 groups were observed with complete domination from diatoms with 27 genera followed by Dinoflagellates 7 and blue-green algae. distribution and abundance of the phytoplankton were mainly *Chaetoceros sp.*, *Ceratium sp.*, *Thalassiosira sp.*, *Biddulphia sp.*, *Coscinodiscus sp.*, *Rhizosolenia sp.*, *Ditylum sp.*, *Triceratium sp.*, *Nitzschia sp.* and blue-green algae under Chrysophyta, Bryophyta, Chlorophyta, Cyanophyta were observed, while in biochemical composition of phytoplankton, the carbon contributed to the highest percentage and hydrogen constitute to the lowest percentage. Crude protein and Lipid contents are maximum during post-monsoon and carbohydrates showed their highest content during pre-monsoon for phytoplankton. There is a direct relationship between the biochemical composition and the physicochemical and environmental conditions including nutrients, especially nitrates and phosphates.

Comment [2]: The abstract is the summary of everything. You do not need to include geographical coordinates. What the reader is interested in is what was done, how it was done, the key results and inferences drawn from the research findings. I strongly believe that this abstract can be a lot better than its present form.

Comment [3]: I strongly recommend that you recast these words. Too long sentences. Not easily understood by the reader.

Comment [4]: Statements seem disjointed with no flow. Why not present in the following manner: Results of the biochemical analysis revealed seasonality in variation as the crude protein and lipid contents of phytoplankton were highest during post-monsoon but highest for carbohydrate during pre-monsoon. The biochemical composition of phytoplankton exhibited positive correlation with the physicochemical and environmental conditions of the water and especially with nitrates and phosphates. This study concluded...

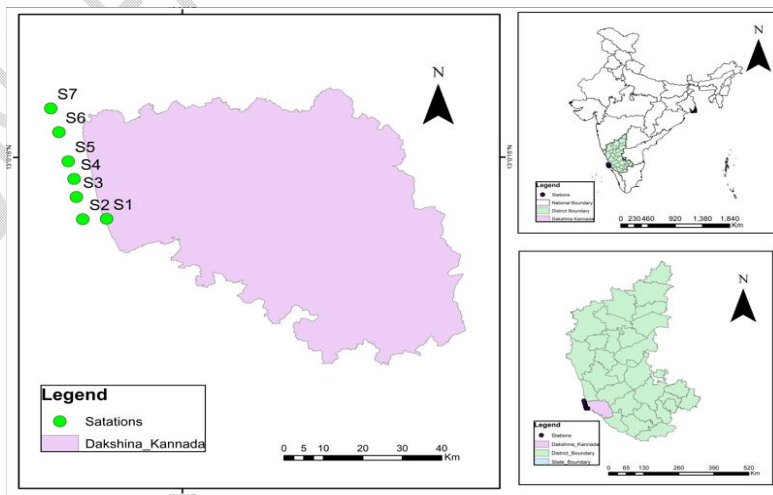
Keywords: Phytoplankton, Chlorophyll-*a*, Crude protein, Lipid, Nutrients, Mangaluru

II. INTRODUCTION

Plankton is considered a good bioindicator in coastal waters. Plankton is a diverse group of organisms that live in the water column and cannot swim against the current. They reflect the effect of environmental changes in a short time. The spatial and temporal variation in the community structure of phytoplankton is influenced by the alterations in the physical and Hydrobiological variables in the marine environment (Rajkumar *et al.*, 2009, Acharya *et al.*, 2010. Gopalakrishnan *et al.*, 2020). Research in the recent past from the present study domain shows the strong biophysical coupling between hydrographical parameters and the kind of plankton existing in diverse environments (Arunpandi *et al.*, 2017, 2021; Karnan *et al.*, 2021). The major features of phytoplankton are its shape and size which controls biochemical Functions such as plankton growth and nutrient intake. Based on the phytoplankton size structure, the total chlorophyll concentration is classified into three functional classes (A. Mohan *et al.*, 2020).

III. MATERIALS AND METHODS

The sampling stations were selected along the coastal waters off Dakshina Kannada, South West coast of India. A total of five stations were selected with an average depth of 8 m. Seven stations were selected in coastal waters off Dakshina Kannada district and designated as Gurupura-Netravathi Estuary S₁, Barmouth region of Netravathi S₂, Coastal waters off Thannirbhavi S₃, Coastal waters off Panambur S₄, Coastal waters off Suratkal S₅, Coastal waters off Mulki S₆ and Coastal waters off Padubidri S₇.



Comment [5]: Which reference backs this? Provide it.

Comment [6]: The word "Plankton" is plural so should be Plankton are a diverse...

Comment [7]: Which reference backs this? Provide it.

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Comment [9]: What are the three functional classes? Do not leave your readers hanging here.

Comment [10]: I strongly recommend you beef up your introduction section. It is too short in its present form. Add some more literature.

Comment [11]: This section seems incomplete. Information on the statistical analysis carried out and the statistical packages used is missing.

Comment [12]: Information on the specific physicochemical and environmental parameters monitored in this study is missing in this section. The methods used in analysing them is missing as well. If you have this information in another paper that is offshoot of the same research, you can simply say so.

Comment [13]: You first said a total of 5 stations were chosen. Then you say seven were selected. This is confusing. How many were selected and sampled in this study?

Comment [14]: What is the criterion for choosing these sampling sites? Are any of the stations pollution prone or judged to be relatively undisturbed? What activities are being carried out at each of the stations?

Map . 1 ArcGIS map showing the selected stations of the study area

Monthly sampling was carried out at selected seven stations from the Barmouth region of Netravathi estuary, latitude 12°50'38.56" N, longitude 74°50'0.23° to coastal waters off Padubidri latitude 13° 8'0. 57" N longitude 74°42'9.47° with an average depth of 8 meters. Phytoplankton samples were collected using a Heron-Tranter net from all the stations. The Heron-Tranter net was hauled horizontally in each station leaving a two-meter safe depth from the bottom. The net is square-shaped with a mouth area of 0.25 m² and has a filtering cone of 1.2 m length with a 60 µ pore size. The net towed for one minute. Phytoplankton samples were then preserved in 4 % seawater formalin on board the vessel for further analysis. Samples were then briefly rinsed with distilled water while still under vacuum, wrapped with aluminum foil, placed into sealed plastic bags, and stored in a freezer. In the laboratory, frozen samples were weighed and then freeze-dried to obtain dry mass. The water content in the samples was calculated from the difference between wet mass and dry mass and expressed as a percentage of wet mass.

The samples of phytoplankton were brought to be identified and classified by a microscope at a magnification of 400x and identification with appropriate tools (Verlecar, X.N. and Desai, S.R., 2004). The Phytoplankton abundance is the number of individuals or cells per unit volume was calculated using the following equation. $N = n \times V_r / V_o \times 1 / V_s$, N= phytoplankton abundance (cell/l), n=number of observed phytoplankton, V_r=volume of filtered water (30 ml), V_o=concentrate volume of Sedgwick rafter Counting Cell (ml), V_s=volume of filtered water sample (100 lit.). Chlorophyll-a was counted using the following equation (APHA, 2005) Chlorophyll-a (mg/m³) = {(11, 85(E664-1.54(E647-0.08(E630) XVe/Vs)}.

Comment [15]: This should be written as Verlecar and Desai, 2004

Comment [16]: Chlorophyll-a cannot be counted. You quantify it through analysis.

IV. RESULTS AND DISCUSSION

The dynamics of phytoplankton populations are controlled by multiple factors including physiological and evolutionary adaptations, environmental and biological processes. The evaluation of physicochemical characteristics in India's southwest coastal waters serves as a visible tool for investigating water quality (Dayala *et al.*, 2014). The interactions between the different factors are important in understanding the response of organisms to these variables. The air temperature of the study area varied between 27.43 °C and 32.59 °C with the highest in the pre-monsoon season and the lowest in the monsoon season. The annual Precipitation averaged 3479 mm during the study period (Agril. Research

Station, Mangaluru). The surface water temperature of the coastal waters ranged from 28.01 °C to 31.65 °C. The maximum mean surface water temperature was observed in the pre-monsoon season (29.39 ±1.0 °C) and the minimum in the wet season (28.01 °C) showed in Table 1. Surface water temperature demonstrated a significant positive correlation with air temperature, and salinity and a negative correlation with chlorophyll-*a*, silicate and rainfall showed in Table 2. Andrade *et al.* (2011) examined maximum and minimum surface water temperatures in Mangalore coastal water during the summer (34.75°C) and winter (28°C) seasons and discovered that this is related to increased solar radiation, evaporation and entry of fresh water. Vajravelu *et al.* (2018) found temperature fluctuations in the Parangipetta coastal waters, Bay of Bengal, South East Coast of India, during the monsoon and summer seasons, due to substantial rainfall throughout the monsoon season, low temperatures were reported. The overall pH values during the study period ranged from 7.23 to 8.62 with its maximum value in the pre-monsoon season. There revealed a significant positive correlation between pH, and salinity. According to Kumar *et al.* (2013), various factors such as rainfall, CO₂ removal by photosynthesis and dilution of seawater by freshwater intake, salinity decrease and temperature control seasonal variations in water pH. Tenjing *et al.* (2017) found that the pH of seawater in Panambur and Padukere along the Karnataka coast ranged from 7.92 in September to 8.26 in May. According to Krishnankutty *et al.* (2019) seasonal pH changes in coastal waters off Cochin, in the south-eastern Arabian Sea, revealed that the monsoon has low pH levels. Salinity ranged from 26.07 to 35.28 PSU for the whole study period.

Mean salinity was found in the dry season (30.83 ± 2.48 PSU) Salinity also showed a positive correlation with temperature and pH and a negative correlation with dissolved oxygen chlorophyll *a* and productivity dissolved oxygen ranged from 3.41 to 7.07 mg L⁻¹ during the study period Bharathi *et al.* (2017) have revealed that salinity levels were higher in the summer and lowest in the monsoon seasons, which could be owing to higher evaporation during the summer season and the lowest salinity in the monsoon season due to heavy rainfall in the Puducherry coastline waters. Madhavi *et al.* (2015) reported a low value of dissolved oxygen during a post-monsoon season in the coastal waters of the Dakshina Kannada and Udupi districts. However, the mean value of dissolved oxygen was observed in the dry season (4.67 ± 1.25 mg L⁻¹). Dissolved oxygen revealed a positive correlation with phytoplankton abundance. The Ammonia, Nitrate, Nitrite and phosphate value in the coastal waters ranged from 0.17 µg/l to 17.20 µg/l, 0.0 µg/l to 5.40 µg/l, 0.15 µg/l to 4.25 µg/l and 0.03 µg/l to 7.58 µg/l respectively recorded for the whole study period. Meanwhile, the

Comment [17]: Which kind of correlation analysis was run? Please signify in your methodology section. Was it Pearson Correlation or Spearman Correlation or CCA?

Comment [18]: What is the implication of these values to the aquatic ecosystem? Is it within acceptable standard or exceeds the standard?

highest mean of silicate was observed in the dry season (0.70.58 to 43.70). In 2010 and 2011, off the coast of Mangalore in the Arabian Sea, Sulochananet *al.* (2014) discovered substantial relationships between rainfall, silicate, and phosphate. Krishnan and Tharavathy (2016) observed that nutrients such as nitrate, nitrite, inorganic phosphate and reactive silica were high during the monsoon in coastal waters at Kushalnagar Beach, Kasaragod, Kerala. The mean value of Organic carbon was observed (12.97%). The maximum mean of chlorophyll a was found in the post-monsoon season (2.02) and the minimum in the monsoon season (0.28). Significant seasonal variations were observed among some hydrological parameters such as surface water temperature. The mean value of phytoplankton demonstrated higher concentration in the dry (1.61 mm/m³) season followed by the intermediate (9.25 mm/m³) season. Phytoplankton demonstrated positive correlation with chlorophyll a and Net primary productivity and negative correlation with water temperature and salinity (Table 2). Silicate (SiO₂) mean found to be in the 14.76. Similarly Madhavi. K. *et al.*, 2014 observed the correlation among the hydrographical and biological parameters showed spatial variations and it could be due to different hydrology of different stations.

Comment [19]: What could be the reason for this result? Has any other researcher reported this?

Comment [20]: Before you report what other people have done, you first report what you did. Then, you report if the work of other researchers corroborates/agrees with what you did or your results are not in line. Then you include a reason for this.

Comment [21]: What is the significance of these results? Does this signify that there was seasonality in variation?

Comment [22]: Which statistical analysis was used to determine the variation? The tool used ought to be included in your methodology. This study was for 19 months. More robust analyses are expected. You could use Duncan's multiple range test to determine if there was significant difference among the stations, seasons and throughout the study period.

Table: 1 mean, minimum and maximum values of different physicochemical parameters in different months during the study period in selected stations.

Parameters	Mean	Minimum	Maximum	Median	Std. Error

UNDER PREPARATION

Air temperature °c	30.08	27.43	32.59	30.06	0.13
Water temperature °c	29.39	28.01	31.65	29.46	0.06
pH	8.04	7.23	8.62	8.03	0.02
Salinity (PSU)	30.83	26.07	35.28	30.93	0.21
Dissolved oxygen (mg/l)	4.67	3.41	7.07	4.54	0.10
BOD (mg/l)	1.90	0.36	6.93	1.63	0.11
Ammonia-Nitrogen (µg/l)	5.51	0.17	17.20	4.15	0.41
Nitrate-Nitrogen (µg/l)	1.13	0.00	5.40	0.65	0.11
Nitrite-Nitrogen (µg/l)	1.40	0.15	4.25	1.21	0.09
Phosphate-phosphorus (µg/l)	2.61	0.03	7.58	2.14	0.20
Silicate-silicon (µg/l)	14.76	0.10	43.70	13.39	0.83
Organic carbon%	12.97	0.10	43.70	12.19	0.81
Chlorophyll- <i>a</i> (mg/m ³)	0.43	0.15	2.02	0.28	0.03
Phytoplankton(mm/m ³)	2.81	1.61	9.29	2.14	0.14
Phyto. abundance(cells/m ³)	3276793.88	111220.00	10798000.0	2369000.0	277955.07
	28.50	8.82	0	0	1.92

IV. 1. Quantitative Phytoplankton analysis

The qualitative analysis of phytoplankton revealed the presence of a large number of phytoplankton cells belonging to different groups such as diatoms, dinoflagellates and blue-green algae (BGA). Spatially shallower water showed a maximum number compared to deeper water with the maximum being recorded at 8m depth. Throughout the study period, Diatoms are the dominant group of phytoplankton contributing to the total phytoplankton production. Greater numbers of diatoms were reported at shallower depths with a variation of 7650 to 17917000 cells/m³ at 8m depth in stn. S₃. Dinoflagellates were reported variation of stn. S₇ 67500 to stn. S₃ 480000 cells/m³ at stn. S₃ Spatially shallower waters reported greater numbers with a positive response to increased sea surface water temperature. Temporal variation exhibited a bimodal variation over time.

IV.1.1. Diatoms

The greater numbers of diatoms were reported in December 2021 at stn. S₃ with a maximum of 17917000 cells/m³. 27 species of diatoms were reported during the investigation period and the species which occurred more than 78 percent in the entire duration. Spatially shallower depths exhibited a higher number of *Biddulphia sp.* with an average monthly range of 1179 to at stn. S₅, 195000 cells/m³. The maximum numbers of *Biddulphia sp.* were

Comment [23]: Change to "were" because this study is in the past.

Comment [24]: I suggest you remove the acronyms and write in full. Station S3 and so on.

recorded in October 2021 and the minimum was recorded in the Feb, March and April 2022.

During the entire study duration. Temporarily an increasing trend was observed with a narrow range of differences in seasons during the study period with the pulsating trend with many minor peaks in the entire study duration. *Chaetoceros sp.* was the most dominant diatom reported during the entire study duration with a range of 835 to 12525000 cells/m³ at station S₆, in November 2021. The monthly average data did not exhibit spatial variation even though greater numbers were reported in the shallower depth contour. Temporarily an increasing trend of *Chaetoceros sp.* was observed over time and the variation was much narrow during the last three months of the study period. Two peaks were exhibited starting from October (post-monsoon) to February (pre-monsoon) then the decreasing trend continued till September 2021 with a second peak later on. The present observation was supported by Lathika *et al.* (2013). They reported the dominance of *Chaetoceros sp.* in the upwelling system of the south-eastern Arabian sea, with a contribution of 60-90% of the total cell density and revealed that these species have high spore production and reseeded rates, which favors their occurrence in the upwelled waters *Cosinodiscus sp.* distribution exhibited a variation of 0 to 1050000 cells/m³ for the entire study duration. The Monthly average data showed no difference in the stations as the variation between the various depths is narrow but even then greater numbers were recorded at station 5 with a range of 8514 to 1050000 cells/m³. Temporal variation of *Cosinodiscus sp.* did not exhibit any noticeable trend for the entire 16 months with maximum peak in post-monsoon February, March and April, with an average of 75000 cells/m³. No spatial variation was reported in the distribution of *Ditylum sp.* in the first 4 Months. For the next 12 Months, a pulsating trend with a maximum peak in the month of S₆ April 2021 was reported and then the variation in different stations was very much narrow. Temporal variation exhibited a maximum peak in S₅ with 110000 cells/m³ of *Ditylum sp.* numbers and a total of 2 peaks were reported in S₆ and S₇. The absence of the *Ditylum sp.* distribution in S₂ and S₃ post-monsoon season became a setback in clear-cut temporal trends. Spatial distribution exhibited at S₂, S₄ and S₇ reported maximum numbers of *Nitzschia sp.* with a variation of 500 to 85000 cells/m³ throughout the investigation. Temporal variation exhibited the highest peak in the year 2021 months of October, November and March at S₄ and S₇ with an average of 8500 12500, and 21250 cells/m³ respectively, Several small peaks in the early stage and then a slightly increasing trend was observed till the pre-monsoon season later decreased till December in S₃ and S₅. The dominance of *Nitzschia sp.* among pennales in the upwelling system of the south-eastern Arabian Sea was also observed by Shruthi. G *et al.* (2013). Overall the temporal variation of *Nitzschia sp.*

Comment [25]: I suggest you include comparisons of your results with those of other researchers that carried out similar studies whose work did or did not tally with your findings.

exhibited two major peaks in S₄ and S₇. The spatial distribution of *Planktoniella sp.* was found to be more at stations S₆ and S₇ with a variation of 835 to 42500 cells/m³. The deeper waters recorded more values only during October, and November at S₆ and S₇ with a variation of 10002 to 42500 cells/m³. Temporal variation exhibited an increasing trend with the highest peak during October and November 2021 with 100 to 27500 cells/m³ and over the period, totally a trimodal oscillation was exhibited. For the entire duration of the study, the *Planktoniella sp.* numbers varied from 0 to 42500 cells/m³. The spatial distribution of *Pleurosigma sp.* at various depths was not clear due to narrow variation with greater numbers at deeper waters. The *Pleurosigma sp.* numbers at 4m, 8m 12m and 15m varied from 89 to 99717, 747 to 490885, 49 to 80400 and 665 to 1065286 cells/m³ respectively. The maximum number was noticed at 15m depth with 1065286 cells/m³. Temporal variation exhibited an increasing trend without much clear variation except with a few minor peaks from 1998 to 2006. Overall a slight increase in the *Pleurosigma sp.* numbers was observed with the highest numbers (1065286 cells/m³) being recorded in 2011. Estuarine waters recorded maximum numbers of *Rhizosolenia sp.* with a variation of 7500 to 975000 cells/m³ at S₁ and S₅. Second dominance was found at S₃ and S₄ with a variation of 1000 to 54000 cells/m³, *Rhizosolenia sp.* numbers were abundantly present at shallower waters compared to deeper waters which were supported by the decreased salinity. For the entire period of investigation. Temporal variation showed bimodal oscillation with two clear peaks during October, November 2021 and 2022. An increasing trend till early pre-monsoon was observed with a maximum number of 54000 to 97500 cells/m³ and later decreased till March and April with the lowest numbers of 0 to 250 cells/m³. Overall a bimodal oscillation was exhibited by the *Rhizosolenia sp.* distribution with a slight increase in the number over the period. Similar observations were also made by Jiyalalram *et al.* (1989), Naik *et al.* (1990), Ramesh (1992), De'souza (2001), Katti *et al.* (2002), Raveesha (2007), Saravanakumar (2008), Karolina *et al.* (2009), Kadam *et al.* (2011), Sushanth and Rajashekhar (2012) and Madhavi (2014) along the west coast of India. Similar reports were also observed along the east coast of India by Gouda and Panigrahi (1996), Sarojini *et al.* (2001), Prabhakar *et al.* (2011), Smitha *et al.* (2010), Muruganatham *et al.* (2012) and Babu *et al.* (2013).

IV.1.2. Dinoflagellates

Dinoflagellates are the second most dominant group after diatoms contributing to the total phytoplankton. Shallower waters (4m depth) recorded more numbers of dinoflagellates with a variation of 800 to 480000 cells/m³. At S₃ 1800 to 480000 population varied and in S₁ 1800 to 69723 cells/m³, Spatially coastal waters reported greater numbers with a positive

Comment [26]: Change to "were" because this study is in the past.

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response to increased sea surface water temperature and decreased sea surface salinity. Temporal variation did not exhibit any significant variation over time. The greater numbers of dinoflagellates were reported in the pre-monsoon season with a maximum of 3507 to 480000 cells/m³. Three main species occurred more than 80 percent in the observation duration which is found to be *Ceratium sp.*, *Dinophysis sp.* and *Peridiniumsp.* The most dominant dinoflagellate with the maximum number found to be at S₅ and S₇ water with a variation of 2500 to 115000 to 1700 to 67500 cells/m³ respectively, for the entire study duration shallower waters dominated in the *Ceratium sp.* distribution numbers except in few pre-monsoon months. Greater numbers were reported from October to November which is in agreement with the decreased salinity during the same duration of the study. Temporal variation exhibited a pulsating trend with a noticeable peak from October to January and a decreasing trend was observed in pre-monsoon season and then an increasing trend reappeared to form a second peak in September 2021. *Dinophysis*sp. is the third most dominant dinoflagellate with a variation of 0 to 10000 cells/m³ at S₃, 0 to 300000 cells/m³ at S₄, 0 to 22000 cells/m³ at S₇ and 0 to 12500 cells/m³. Deeper waters reported greater numbers of *Dinophysis sp.*, compared to shallow waters throughout the study period. The total Monthly variation of *Dinophysis sp.*, showed a variation from 0 to 300000 cells/m³. Temporal variation exhibited three peaks with a maximum peak from 1996 to 1998. A secondary peak was observed to be in the year pre-monsoon and then it decreased to the lowest numbers in the year during the post-monsoon. The third peak was observed in November, December and January at S₃. *Peridiniumsp.* was found to be the second most dominant dinoflagellate with a variation of 0 to 15500 cells/m³ throughout the study period. Spatially, shallower water recorded higher values from September to November at S₁ and S₃ and then it was reversed from December to April in S₂ and S₃. Temporal analysis showed an increasing trend from pre-monsoon to post-monsoon and later a decreased pulsating trend with few minor peaks. The maximum number of *Peridinium sp.* was recorded in December S₃ with 15500 cells/m³. The overall no clear-cut temporal trend was exhibited for the entire study duration.

IV.1.3. Blue green algae

Blue-green algae showed clear spatial variation from S₁ and S₅ due to the Fresh water in flow. As the different genera among the Blue-green algae by numbers and abundance, hence all the genera were pooled together to form a single Blue-green algae group which accounted for several chains of each species rather than several cells individually. Blue-green algae varied from 0 to 341000 cells/m³ at S₄, 100 to 65000 cells/m³ at S₁, 0 to 70056 cells/m³ at S₅ and 1000 to 341000 cells/m³ at S₄. Temporally a decreasing trend is exhibited

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with a maximum peak in January and February with 110000 cells/m³. From January maximum peaks were observed and then a decreasing trend to indicate over time there is a decreasing trend which is in agreement with the decreasing salinity and increasing surface water temperature. Rath, A.R., *et al.*, 2018 Karati, K.K., 2021 Padmakumar, K.B., 2018 reported Overall 54 phytoplankton species (35 genera) were observed in Mangaluru coastal waters according to Rath, A.R., 2018 Diatoms were the dominant group (surface: 80-99.9%, NBW: 97.1-100%), followed by dinoflagellates (surface: 0.1-20%, NBW: 0.1-3.7%) and silicoflagellates (surface: 0.1-0.2%, NBW: 0.1-0.2%) (Fig. 3). Phytoplankton abundance ranged from 1.54 x 10⁴ (MON; outer zone) to 3.73 x 10⁵ cells L⁻¹ (PRM; channel zone) in surface waters and 1.03 x 10⁴ (MON; outer zone) to 4.85 x 10⁵ cells L⁻¹ (PRM; outer zone) in the NBW.

IV.2. Biochemical analysis

IV.2.1. Crude protein

Protein was the principal organic constituent in all species. It represented at least 40 % of the dry weight of cells in all species of diatoms. Seasonal variations in crude protein content in percentage of (dry weight) at selected stations are graphically represented in Fig.1. In the present study protein contributed to the maximum when compared to the other biochemical compounds. Crude protein content was observed highest during post-monsoon when compared with pre-monsoon season. The nutrients, especially the nitrates (NO₃) and phosphate (PO₄) indicated the fertility of the water for the increase and enrichment of the biochemical contents. The average nitrate and phosphate contents were high during post-monsoon when compared with pre-monsoon with the values of 7.44 µg-at/l and 1.19 µg-at/l.

Comment [30]: Include likely reasons for this.

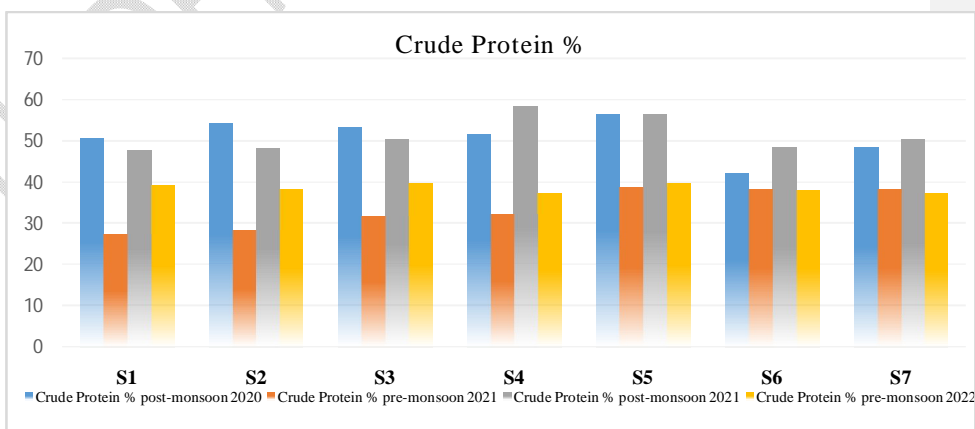


Fig.1. Seasonal variations in Protein content in percentage (dry weight) at selected stations

The maximum protein content was obtained in post-monsoon, 2021 58.54 ± 0.2 % at station S₄ and a minimum of 27.31 ± 1.2 % at station S₁ Pre-monsoon 2021 and followed by maximum content of 56.34 ± 0.1 % at station S₅ Post-monsoon 2022 and minimum content was noticed during post-monsoon, 2022 (37.19 ± 1.0 %) at station S₄ during Pre-monsoon 2022 the study period. Parsons & Strickland (1961). Hedges *et al.* (2002) also observed protein percentages ranging from 51 – 73 %. Protein generally reflects physiologically healthy phytoplankton with high relative growth rates, and high protein contents suggesting that the phytoplankton had no nitrogen limitation in water mass. Mahmoud (2016) reported that the nutrients, especially the nitrates (NO₃) and phosphate (PO₄) indicated the fertility of the water to increase and enrichment of the biochemical contents of phytoplankton. The various Physicochemical parameters viz., temperature, salinity, pH, dissolved oxygen and nutrients are the factors that mainly influence the production and successful propagation of planktonic life in the coastal biotopes (Santhosh Kumar *et al.*, 2012).

Comment [31]: Has any other researcher reported similar or different findings? Include those.

IV.2.2. Total lipid

Algal lipids are composed of glycerol, saturated and unsaturated fatty acids, and some fatty acids of ω₃ & ω₆ families are pharmaceutically important. Seasonal variations in total lipid content in percentage of (dry weight) at selected stations is graphically represented in Fig.2. During the present study, the total lipid content of phytoplankton reached the maximum in post-monsoon compared with pre-monsoon, but there is no significant difference between the two seasons. The maximum lipid content (5.76 ± 1.10 %) was found at station S₇ post-monsoon, 2021 and the minimum (2.43 ± 0.77 %) at station S₂ during the post-monsoon 2022 and followed by the second highest percentage (5.65 ± 0.10 %) was noticed at station S₆ and second lowest percentage (2.65 ± 1.14 %) at station S₁ during the pre-monsoon period.

Comment [32]: What was your finding? You first report your finding before stating what some other researcher did and how it corroborates the results of your own research.

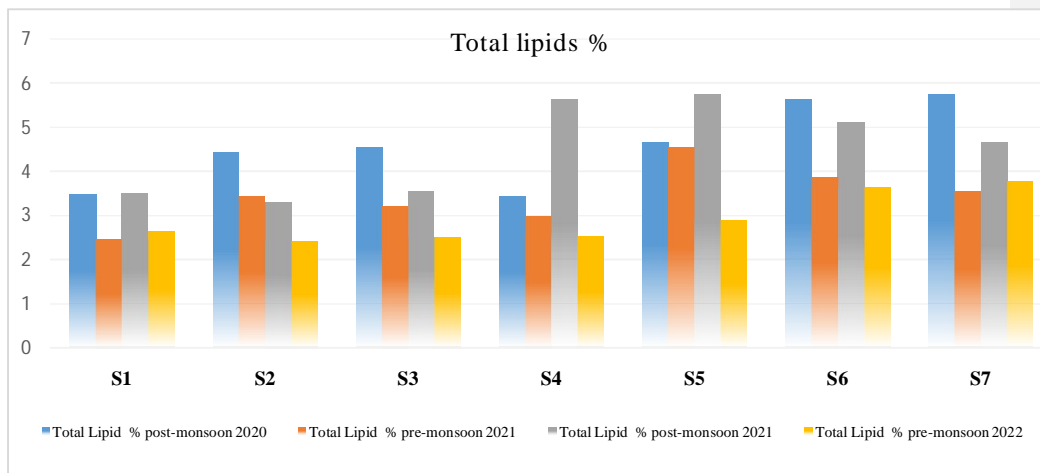


Fig.2. Seasonal variations in lipid content in percentage (dry weight) at selected stations

Hussian *et al.* (2016) reported that N starvation increased carbohydrate percentage and decreased protein percentage, while lipid percentage remained relatively constant. Abd El-Hady *et al.* (2012) reported that protein constitutes the major part of the biochemical contents of the phytoplankton of Ismailia Canal while lipid constitutes the minor one. According to Mahmoud (2016) the highest values of crude protein and total lipid obtained in autumn 2014 52.76 mg/L, at station 9 & 1.76 mg/L, at station 8 respectively while the lowest value of crude protein and total lipid were in summer 2015 was 1.23 mg/L and 0.08 mg/L, respectively.

IV.2.3. Carbohydrate

In the present study, the carbohydrate content of phytoplankton reached a maximum in pre-monsoon compared with post-monsoon. Seasonal variations in carbohydrate content in percentage of (dry weight) at selected stations are graphically represented in Fig.3. The maximum carbohydrate content ($45.54 \pm 0.12\%$) was found at station S₂ pre-monsoon, 2021 and minimum ($10.97 \pm 0.08\%$) at station S₁ during the post-monsoon and second highest percentage ($44.54 \pm 0.10\%$) was noticed at station S₆ pre-monsoon 2022 and lowest percentage ($12.43 \pm 0.18\%$) at station S₇ during the post-monsoon 2021 period. Carbohydrates and protein serve both as structural and storage components and represent the major form of photo-chemically assimilated carbon in the biosphere and are powerful tools in the pathways of biologically important organic materials in nature (Mahmoud, 2016).

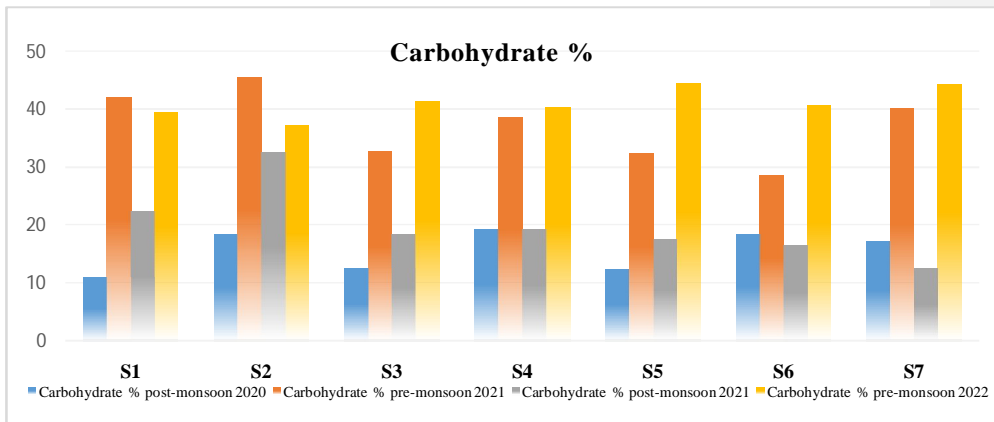


Fig.3. Seasonal variations in carbohydrate content in percentage at selected stations

Carbohydrates in microalgae are found in the form of starch, glucose, sugars and other polysaccharides (Becker, 1994). Abd El-Hady (2012) reported maximum level of total carbohydrate concentration was found in summer, while the minimum level was detected in autumn. Brown and Jeffrey (1992) reported CHO levels ranging from 5.9 – 16.7 % in 10 species of Chlorophyceae & Prasinophyceae. CHO levels ranged from 8 – 56 % dry weight depending on the growth stage and species. An increase in CHO, variability in lipid content, and decrease in protein with advancing growth stage was reported by (Brown and Jeffrey 1992; Wikfors *et al.* 1992), although as already mentioned, growth medium and growth stage will affect biochemical composition (Leonardo's and Lucas, 2000). Thomas *et al.* (1985) also found that N deficiency increased carbohydrate yield in mass cultures of *Isochrysis*.

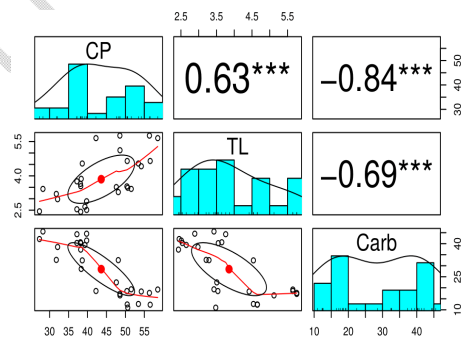


Fig.4 Correlation plot between the crude protein, total lipid and carbohydrates in phytoplankton

The analysis of frequency distributions of Physico-chemical parameters and chlorophyll-*a* revealed a similar pattern with a marginal difference in the trend between 16 months. Therefore, the data were averaged by month and further analysed for spatiotemporal trends. In the west coast environment, the monsoon plays a critical role in the distribution of Physico-chemical parameters that can directly influence the distribution of chlorophyll-*a* (Pednekar *et al.* 2012). The analysis of mean precipitation over the study area showed a similar trend as seen in 10 years of average distribution. Monthly chlorophyll-*a* showed a generally bi-modal distribution, with peaks occurring during pre-monsoon (March) and later stages of monsoon (September). During the pre-monsoon period, blooms of diatoms and dinoflagellate have been reported in past studies S. K. Baliarsingh *et al.* (2016). Indeed, elevated chlorophyll-*a* associated with phytoplankton blooms was observed during pre-monsoon in March. The later stage of monsoon, during September, also experiences higher chlorophyll-*a* in surface waters. This could be attributed to the influx of nutrients due to river runoff after monsoonal precipitation. This elevated chlorophyll-*a* subsequently breaks into patches due to localized effects and moves apart. Confirming the study by Baliarsingh *et al.* (2013) also reported that the maximum spatial variability in chlorophyll-*a* concentrations during March–April was associated with a linear distribution with NO₂, NO₃ and SiO₄. According to Mohanty *et al.* (2007). During this period, river influx was much less, as evident from salinity distribution, restricting the advection of the bloom offshore.

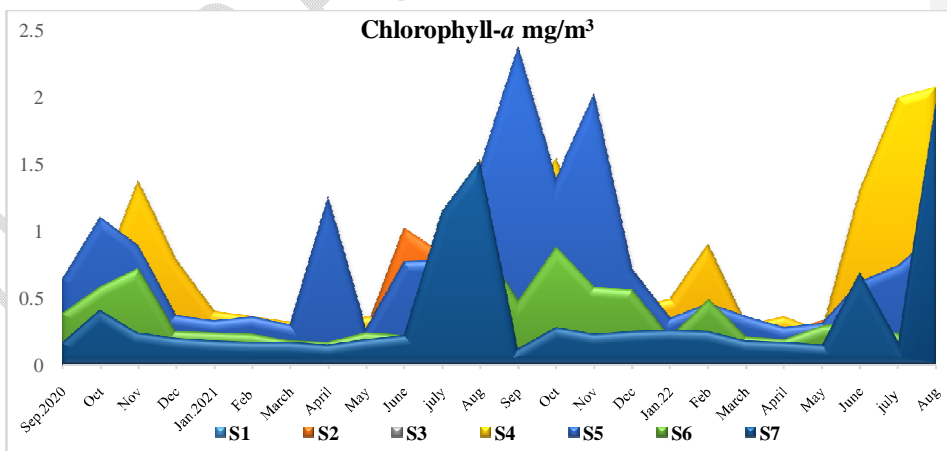


Fig. 5. Seasonal variations in Chlorophyll-*a* mg/m³ at selected stations

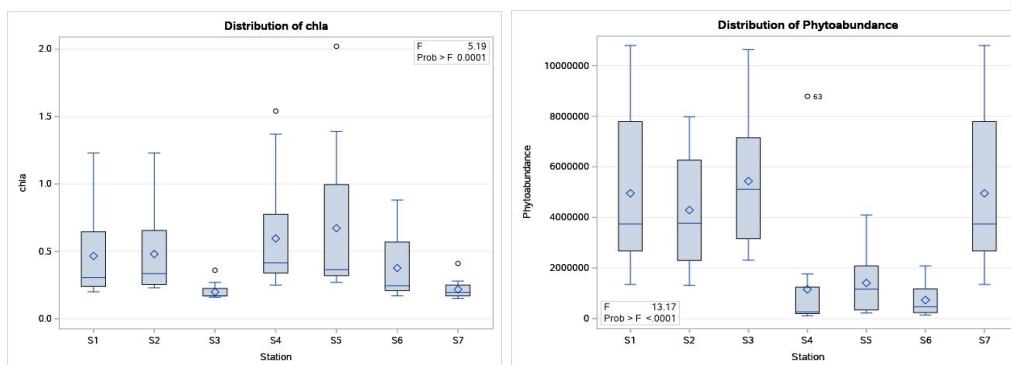


Fig. 6 variations in the distribution of chlorophyll-*a* and phytoplankton abundance in selected stations

Table 2. Correlation coefficient between and Physico-chemical and biological parameters

	<i>Airtemp</i>	<i>watertemp</i>	<i>pH</i>	<i>Salinity</i>	<i>DO</i>	<i>BOD</i>	<i>Ammonia</i>	<i>Nitrate</i>	<i>Nitrite</i>	<i>Phosphate</i>	<i>Silicate</i>	<i>OC</i>	<i>chl-a</i>	<i>phyto</i>	<i>Phytoabundance</i>	<i>NPPV</i>
<i>Airtemp</i>		0.546***	0.188*	0.476***	-0.035	0.028	-0.037	-0.162	-0.121	0.014	-0.216*	-0.119	-0.366***	-0.368***	0.030	-0.258**
<i>watertemp</i>	0.546***		0.286**	0.260**	-0.137	0.098	-0.085	-0.085	-0.167	0.115	-0.213*	-0.081	-0.282**	-0.350***	-0.052	-0.310***
<i>pH</i>	0.188*	0.286**		0.304**	-0.170	0.109	-0.080	-0.236*	-0.034	-0.146	-0.223*	-0.136	-0.093	-0.100	-0.241*	-0.088
<i>Salinity</i>	0.476***	0.260**	0.304**		-0.218*	0.001	-0.192*	-0.384***	0.089	-0.250**	-0.380***	-0.150	-0.340***	-0.358***	0.097	-0.269**
<i>DO</i>	-0.035	-0.137	-0.170	-0.218*		-0.089	0.397***	0.343***	0.045	0.178	0.024	-0.088	-0.021	-0.005	0.216*	0.074
<i>BOD</i>	0.028	0.098	0.109	0.001	-0.089		-0.179	-0.080	-0.089	-0.023	0.028	0.064	0.071	0.081	-0.049	0.048
<i>Ammonia</i>	-0.037	-0.085	-0.080	-0.192*	0.397***	-0.179		0.473***	0.093	0.171	0.056	-0.127	0.011	0.058	0.154	0.058
<i>Nitrate</i>	-0.162	-0.085	-0.236*	-0.384***	0.343***	-0.080	0.473***		-0.057	0.240*	0.309***	0.113	-0.031	-0.008	0.098	0.003
<i>Nitrite</i>	-0.121	-0.167	-0.034	0.089	0.045	-0.089	0.093	-0.057		0.036	0.050	0.034	0.016	-0.000	0.020	0.075
<i>Phosphate</i>	0.014	0.115	-0.146	-0.250**	0.178	-0.023	0.171	0.240*	0.036		-0.015	-0.157	0.079	0.110	0.008	0.002
<i>Silicate</i>	-0.216*	-0.213*	-0.223*	-0.380***	0.024	0.028	0.056	0.309***	0.050	-0.015		0.831***	0.165	0.134	0.081	0.199*
<i>OC</i>	-0.119	-0.081	-0.136	-0.150	-0.088	0.064	-0.127	0.113	0.034	-0.157	0.831***		0.116	0.071	0.086	0.132
<i>chl-a</i>	-0.366***	-0.282**	-0.093	-0.340***	-0.021	0.071	0.011	-0.031	0.016	0.079	0.165	0.116		0.939***	-0.351***	0.789***
<i>phyto</i>	-0.368***	-0.350***	-0.100	-0.358***	-0.005	0.081	0.058	-0.008	-0.000	0.110	0.134	0.071	0.939***		-0.281**	0.821***
<i>Phytoabundance</i>	0.030	-0.052	-0.241*	0.097	0.216*	-0.049	0.154	0.098	0.020	0.008	0.081	0.086	-0.351***	-0.281**		-0.169
<i>NPPV</i>	-0.258**	-0.310***	-0.088	-0.269**	0.074	0.048	0.058	0.003	0.075	0.002	0.199*	0.132	0.789***	0.821***	-0.169	

Computed correlation used pearson-method with listwise-deletion.

V. CONCLUSION

The results indicated that the phytoplankton species in the coastal waters of Mangaluru and Udupi were dominated by *Chaetoceros sp. sp.*, *Ceratium sp.*, *Thalassiocera sp.*, *Biddulphia sp. sp.*, *Coscinodiscus sp.*, *Rhizosolenia sp. sp.*, *Ditylum sp. sp.*, *Triceratium sp.*, *Nitzschia sp. sp.* and blue-green algae under *chrysophyta*, *pyrophyta*, *chlorophyta*, *cyanophyta*. Diatoms were found to be most contributory to the total phytoplankton, remaining dominant species responsible for the high abundance of phytoplankton were *Pleurosigma sp. sp.*, *Coscinodiscus sp.*, *Nitzschia sp. sp.* and *Dinophysissp.* composition and

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diversity of phytoplankton were found relatively high in post and pre-monsoon season, this could be due to water quality, favourable hydro-biological condition. The species composition and abundance of phytoplankton in all the stations was high S₁ and S₅ shows in the post-monsoon season, which could be due to the nutrient and freshwater inflow to the selected stations. Other than seasonality, climatic, and hydrobiological parameters like rainfall, ammonium, and silica also demonstrated the influence on the composition and abundance of phytoplankton in the study area. The phytoplankton biochemical compositions of Mangaluru coastal waters are dominated by protein during post-monsoon when compared with pre-monsoon which showed a positive correlation with nutrient concentrations especially nitrates and phosphates in all the selected stations. Lipid contributed to the minor fraction and showed a non-significant difference between all stations. Carbohydrate content is high in pre-monsoon when compared to post-monsoon also the present study provides the importance of the phytoplankton biochemical composition as a biomarker of the ecosystem of different sites in Mangaluru coastal waters. For future study, detailed biochemical compositions besides macromolecular compositions of phytoplankton provide better clues for environmental change effects on physiological conditions of phytoplankton.

Comment [34]: Recast i.e. rewrite to a more readable format.

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