



Statistical tools for studying the temporal variations in chlorophyll-*a* concentration along the Southwest Bay of Bengal waters

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Multivariate statistical analysis such as Multiple Linear Regression (MLR) and Principal Component Analysis (PCA) are used to study the effect of physico-chemical parameters on chlorophyll distribution along the southwest Bay of Bengal from January 2012 to June 2014. Physical properties recorded showed clear seasonal patterns in sea surface temperature (26.2 – 32.8 °C), salinity (24 – 36 PSU), pH (7.808 to 8.428), photosynthetic photon flux (522 – 1220.4 $\mu\text{M m}^{-2}\text{s}^{-1}$) with the minimum and maximum values during monsoon and summer seasons, respectively. In contrast, the chemical variables such as nitrite (0.15 to 2.35 μM), nitrate (1.02 to 6.58 μM), ammonia (0.11 – 5.22 μM), total nitrogen (1.04 to 11.58 μM), inorganic phosphate (0.16 – 2.97 μM), total phosphorus (0.55 – 8.60 μM) and reactive silicate (2.00 to 23.95 μM) showed the minimum and maximum concentration during summer and monsoon seasons, respectively. The high and low chlorophyll (0.10 to 6.92 $\mu\text{g l}^{-1}$) and dissolved oxygen (4.07 and 7.884 mg l^{-1}) concentrations are observed during summer and pre-monsoon seasons, respectively. PCA found that nitrogenous nutrients and chlorophyll are positively loaded and Sea Surface Temperature (SST) was negatively loaded in all the seasons except during summer season. Inter-comparison of modeled and *in-situ* chlorophyll-*a* (chl-*a*) concentration showed a significant correlation during monsoon season by 93 % of matchup with a $R^2 = 0.930$, $N = 60$ and $\text{SEE} = \pm 0.369$ compared to other seasons. Regression analysis also predicted the positive influence of nitrate and ammonia and negative influence of SST with chl-*a*.

[**Keywords:** Bay of Bengal, Chlorophyll, Multiple linear regression, Nitrate, Principal component analysis, SST]

Introduction

Marine primary production yields to the world economy > 90 billion kg yr⁻¹ of food that stores 50 times more inorganic carbon than the Earth's atmosphere¹, thereby playing a critical role in the conservation of worldwide carbon budget and climate². Therefore regulating factors of marine production is an important societal interest especially understanding the role of nutrients in determining the growth of phytoplankton that regulates the primary production of the oceans³. Phosphate, along with nitrate and reactive silicate, are the essential micronutrients⁴ capable of determining the species composition and density of phytoplankton in any aquatic environment. Further, changes in the ocean carbon pool are affected by the available nutrients. In addition, temperature, light intensity, dissolved oxygen, pH and salinity can carry seasonal changes in phytoplankton abundance to the global oceans⁵.

Primary productivity is restricted by nutrients availability in tropical watersheds where solar

intensity is not typically a limiting factor⁶, even in overcast conditions, and their bioavailability varies by their remineralization⁷, differential cycling phase of dissolved organic forms and nitrogen fixation⁸. Distribution of individual nutrients varies at time and space and act as the limiting factor in the surface ocean⁹ and nitrogen stands top as limiting factor for primary production in coastal marine ecosystems^{10,11}. There are reports indicating increase in dissolved organic nitrogen inputs in coastal waters and been recognized through the increase in harmful algal blooms (HABs)¹².

Chlorophyll-*a* (chl-*a*) is an important marine phytoplankton photosynthetic pigment that has been used as a proxy to express phytoplankton biomass or ocean productivity¹³. High concentration of chl-*a* would result in high productivity proportional to high phytoplankton biomass. Floristic pattern of chl-*a* is related to the environmental variables and generally interpreted by multivariate procedures such as Multiple Regression Analysis (MLR) and Principal

Component Analysis (PCA) for the reduction, understanding and conclusion of significant data. The relationship between variables is represented by MLR, while PCA categorize the fundamental patterns between all the variables and decreases the number of variables in the cluster. The present study was conducted in the Southwest Bay of Bengal to establish the empirical relationship of environmental variables with chlorophyll concentration.

Materials and Methods

A semi-enclosed coastal basin, the Bay of Bengal, exhibits seasonal changes in circulatory system and climate due to the monsoons. The annual discharge from the tributaries of subcontinent of $1.6 \times 10^{12} \text{ m}^3 \text{ yr}^{-1}$ in the Bay of Bengal is very high compared to $0.3 \times 10^{12} \text{ m}^3 \text{ yr}^{-1}$ for the Arabian Sea¹⁴. Despite this, nitrate which is considered as the region's limiting nutrient, there is also an influx of nutrients such as silicate $\sim 130 \times 10^9 \text{ mol yr}^{-1}$ and phosphorus $\sim 3 \times 10^9 \text{ mol yr}^{-1}$ by Sen Gupta¹⁵ and nitrogen $\sim 10^{10} \text{ mol yr}^{-1}$ by Qasim¹⁶. During summer, the East India Coastal Current (EICC) flows clockwise along the western edge of the Bay of Bengal, while in winter it flow anti-clockwise¹⁷ and plays a vital role in assessing the hydro-biological characteristics of the BoB. The circulation in the Bay of Bengal is strongly affected by the Indian Ocean, monsoon winds and freshwater flow along the northern and central portion of the western Bay of Bengal¹⁸; while the seasonal upwelling and cold core eddy and cyclones are raising the population of phytoplankton in the Bay of Bengal¹⁹.

Analysis of physico-chemical variables

Physico-chemical and biological parameters were measured at monthly intervals in four sampling transects off Chennai (CH), Cuddalore (CU), Parangipettai (PP) and Karaikkal (KL) in southwest coast of BoB from January 2013 to June 2014 (Fig. 1) with the aid of the Global Positioning System (GPS) along the transect perpendicular to the shoreline. The stations were fixed as four sampling stations for longitude and latitude, respectively. Chennai ($80^\circ 16.9' \text{ E} - 13^\circ 3.42' \text{ N}$), Cuddalore ($79^\circ 47.3' \text{ E} - 11^\circ 47.04' \text{ N}$), Parangipettai ($79^\circ 45.5' \text{ E} - 11^\circ 33.8' \text{ N}$) and Karaikal ($79^\circ 50.1' \text{ E} - 10^\circ 54.5' \text{ N}$) were fixed and daily monthly sampling at 0, 1, 2, 3, 4 and 5 km from the shore was carried out. These sampling stations have been developed by taking into account their geographical positions, where domestic discharges,

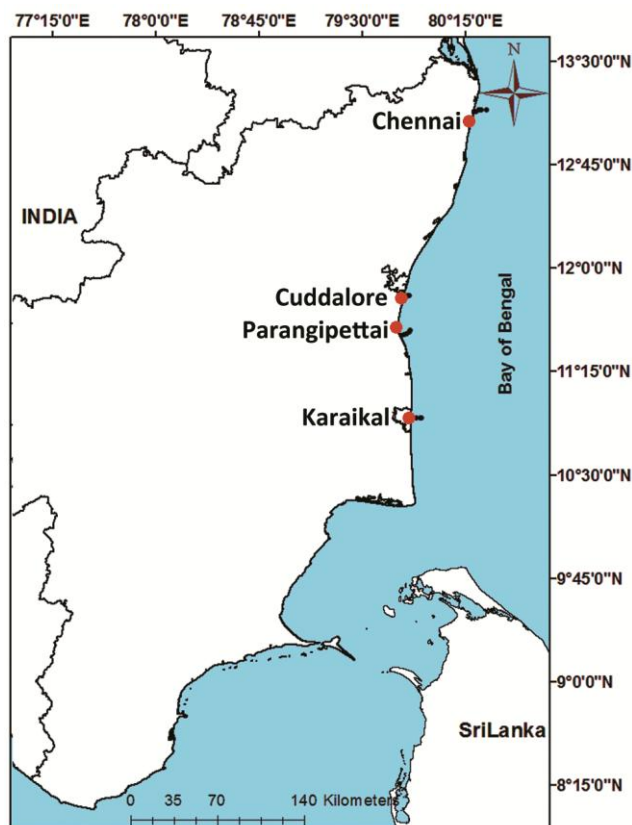


Fig. 1 — A map of the sampling stations

industrial discharges and essential ecosystems, etc., would have no external impact.

Samples of surface water were obtained using Niskin's water samplers. The Sea Surface Temperature (SST) was measured by digital multi sensor of $\pm 0.01 \text{ }^\circ\text{C}$ (Merck Millipore_multi 3420). Salinity was determined using a hand-held refractometer (Atago hand-held refractometer, Japan) and pH was measured with an accuracy of ± 0.001 using a pH meter (EUTECH). Using a digital LUX meter (TES 1332A), Photosynthetic Photon Flux (PPF) was measured and the values were expressed as irradiance using a conversion factor of $\text{LUX} \times 0.018 \mu\text{M m}^{-2} \text{ s}^{-1}$. The 905 Titrandometer (Metrohm) was used to estimate the Dissolved Oxygen (DO) using the modified Winkler method²⁰.

Following the method defined by Strickland & Parsons²⁰, dissolved macronutrients such as nitrite nitrogen (NO_2), Nitrate Nitrogen (NO_3), Total Nitrogen (TN), Ammonia (NH_3), Reactive Silicate (RS), Inorganic Phosphate (IP) and Total Phosphate (TP) were analyzed²⁰. With the exception of TP & TN, water samples were filtered through a 47 mm GF/C filter paper using a millipore filtering system

and UV-VIS spectrophotometer measurements were taken (Shimadzu-UV 2450). The concentration of chlorophyll was calculated using a pre-calibrated spectrophotometer (Shimadzu-UV 2450) using 90 percent acetone and chlorophyll standard (sigma-C6144) using the method defined by UNESCO²¹ (JGOFS).

On the basis of regional climatology, the entire process has been repeated at four wider period representing locations for various seasons, namely Post Monsoon (PM) from January to March, summer (SUM) from April to June and southwest monsoon or Pre-Monsoon (PRM) from July to September, graded on the basis of northeast Monsoon (MON) prevailing in the area from October to December.

Statistical analysis

Of all multivariate statistical techniques, PCA and MLR are the simplest and most analogues to the bivariate techniques commonly used and thus provide the most readily interpretable results. In addition to this, physico-chemical variables were plotted using the box plots for trends. All the statistical analysis was done using SPSS Ver. 16 software for interpretation.

Results

The most important characteristics capable of affecting the growth, abundance and diversity of phytoplankton in the marine environment and showing large temporal and spatial differences were considered to be physico-chemical parameters. Physico-chemical parameters showed consistent seasonal trends typical for the tropical marine environment in the present study (Figs. 2a – m).

The physical properties such as SST (26.2 – 32.8 °C), salinity (24 – 36 PSU), pH (7.808 – 8.428) and PPF (522 – 1220.4 $\mu\text{M m}^{-2} \text{s}^{-1}$) were varied widely (Fig. 2a – d) during different seasons. The chemical properties such as NO_2 (0.15 – 2.35 μM), NO_3 (1.02 – 6.58 μM), NH_3 (0.11 – 5.22 μM), TN (1.04 – 11.58 μM), IP (0.16 – 2.97 μM), TP (0.55 – 8.60 μM), RS (2.00 – 23.95 μM) and DO (4.07 – 7.884 mg l^{-1}) and the chl-*a* concentration (0.10 to 6.92 $\mu\text{g l}^{-1}$) were varied at a narrow width but showed clear seasonal variations. During pre-monsoon 2013 at Cuddalore, higher chlorophyll concentrations were observed and the lowest value was reported at Karaikal during summer 2014. The trend in seasonal mean chlorophyll concentration was in the order of increase from summer (0.72 μM), post monsoon (0.87 μM),

pre-monsoon (1.72 μM) and monsoon (3.00 μM) such trend was noticed in all the sampling stations.

Principal Component Analysis (PCA)

The PCA was performed on a standardized dataset using varimax rotation; only components with eigen values greater than one were considered for interpolation. Four main component (PC) variables were extracted during the post-monsoon season with a cumulative variance of 62.35 percent (Fig. 3a). PC1 explains 21.20 percent of the total variance associated with positive NH_3 , IP, TN and TP loading and negative salinity loading. PC2 accounts for 17.11 percent of the overall variance of SST negative loading and chl-*a* and NO_3 positive loading. With 12.26 percent of total variance, PC3 explains positive loading of PPF and NO_2 , respectively, and positive and negative loading of DO and RS, respectively for PC4 (11.78 %) of total variance.

PCA of summer also extracted four principal components with less (58.81 %) cumulative variance than the other seasons (Fig. 3b). With positive loads of PPF and SST and negative loads of chl-*a* and NO_3 , PC1 accounted for 19.35 % of the overall variance. With the positive loading of NO_2 , RS and TP, PC2 explained 15.74 percent of total variance when salinity was negatively loaded. With a total variance of 13.45 percent, PC3 was positively loaded by NH_3 , IP, TP and pH, while PC4 explained a total variance of 10.27 percent with positive DO and TN loading.

The calculated physico-chemical variables defined 74.57 % of cumulative variance with five component extracts for the pre-monsoon season period (Fig. 3c). With integrated positive loading of NH_3 , chl-*a*, DO and NO_3 and with negative correlation of SST, PC1 accounted for 23.70 percent of total variance. PC2 describes 14.01 percent of the total variance associated with positive RS and TP loading, while PC3 accounted for 12.48 percent of the total variance with positive salinity loading. With a total variance of 12.40 %, PC4 registered positive loading of PPF and pH, while PC5 was positively loaded by IP and NO_2 with a total variance of 11.98 %.

During the monsoon season, the dataset PCA produced four components that explained 71.05 percent of the total variance (Fig. 3d). The first component explains chl-*a*, DO and NO_3 positive loading and PPF and SST negative loading with a cumulative percentage of 24.53 percent. Component 2 with 17.37 % of total variance represented by the positive loading of IP, NO_2 , salinity and negative

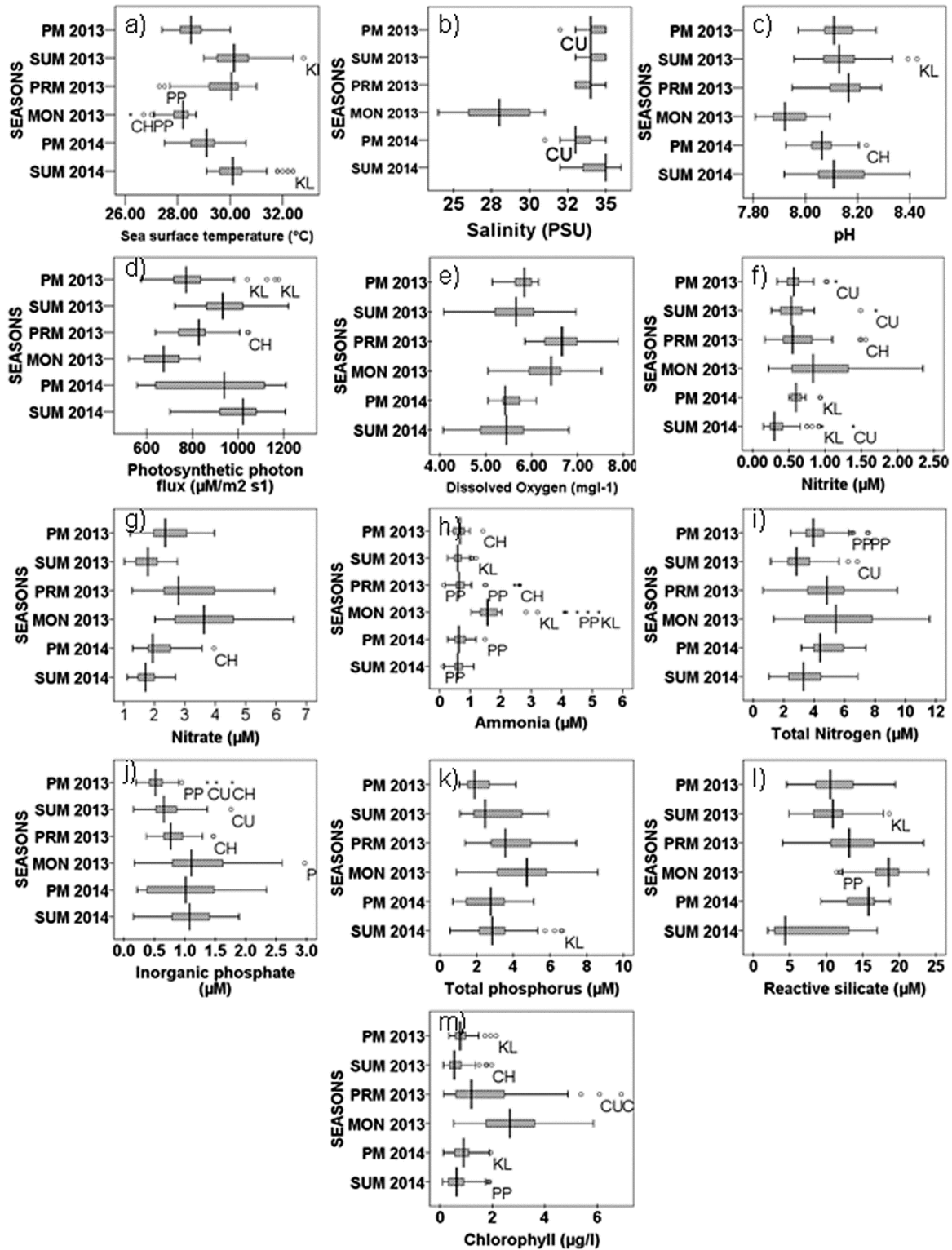


Fig. 2 — Spatial and temporal variations of environmental variables recorded in the southwest BoB

loading of NH_3 although the third component accounted for 15.64 percent of the total variance with NH_3 , RS and TN positive loading; PPF and pH

negative loading. With RS, TN and TP positive loading, the fourth variable (PC4) demonstrated 13.51 percent of the overall variance.

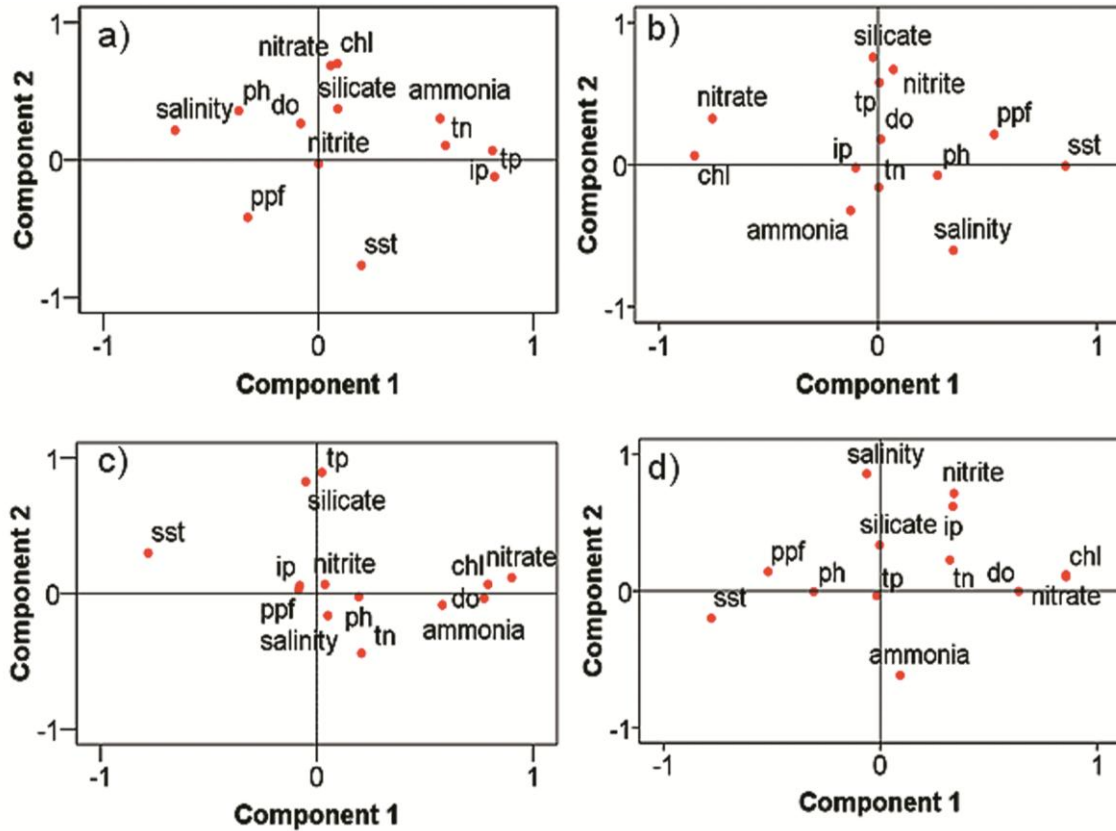


Fig. 3 — The ordination of the physico-chemical variables extracted for PCA during a) PM, b) SUM, c) PRM and d) MON seasons

Multiple Linear Regression (MLR)

MLR were used to assess the effect of physico-chemical variables on the concentration of chlorophyll during all seasons and the findings are summarized in equation 1 to 4. The concentration of chlorophyll was known to be the dependent variable and the remaining environmental variables were treated as independent variables.

$$\begin{aligned} \text{Chl} = & -5.820-0.065(\text{NH}_3)+0.020(\text{DO})+0.128(\text{IP}) \\ & +0.00008162(\text{PPF})+0.200(\text{NO}_3)+0.135(\text{NO}_2) \\ & +0.929(\text{pH})+0.035(\text{salinity}) +0.016(\text{RS}) \\ & -0.099(\text{SST})-0.011(\text{TN})+0.038(\text{TP}) \end{aligned} \dots (1)$$

$$\begin{aligned} \text{Chl} = & 7.056-0.092(\text{NH}_3)+0.028(\text{DO})-0.030(\text{IP}) \\ & +0.00002402(\text{PPF})+0.362(\text{NO}_3)-0.262(\text{NO}_2) \\ & +0.083(\text{pH})+0.016(\text{salinity})+0.002(\text{RS}) \\ & -0.279(\text{SST})+0.017(\text{TN})+0.001(\text{TP}) \end{aligned} \dots (2)$$

$$\begin{aligned} \text{Chl} = & 5.106+0.107(\text{NH}_3)+0.214(\text{DO})+0.652(\text{IP}) \\ & -0.002(\text{PPF})+1.065(\text{NO}_3)+0.182(\text{NO}_2) \\ & -3.463(\text{pH})+0.638(\text{salinity})+0.004(\text{RS}) \\ & -0.005(\text{SST})-0.107(\text{TN})-0.079(\text{TP}) \end{aligned} \dots (3)$$

$$\begin{aligned} \text{Chl} = & 9.983-0.030(\text{NH}_3)+0.075(\text{DO})+0.061(\text{IP}) \\ & +0.000001509(\text{PPF})+0.812(\text{NO}_3)+0.086(\text{NO}_2) \\ & -0.483(\text{pH})-0.090(\text{salinity})+0.071(\text{RS}) \\ & -0.216(\text{SST})+0.053(\text{TN})-0.031(\text{TP}) \end{aligned} \dots (4)$$

The results of regression summary showed the $R^2 = 0.287$ and $N = 106$ with $\text{SEE} = \pm 0.369$, meaning that approximately 30 % of the chl-a concentration was accounted by the variables during post monsoon season among the variables for all stations. Figure 4(a) shows the inter-comparison of modeled and *in-situ* chl-a concentration which indicates underestimation of the *in-situ* values. In case of summer (Fig. 4b), between *in-situ* and modeled chl-a, there was an important association with $R^2 = 0.612$, $N = 128$ and $\text{SEE} = \pm 0.290$. During pre-monsoon (Fig. 4c), around 79 % of the derived modeled chl-a concentration was matched with *in-situ* chlorophyll with $R^2 = 0.79$, $N = 60$ and $\text{SEE} = \pm 0.817$. Modeled chl-a concentration (Fig. 4d) portraits 93 % of matchup with *in-situ* chl-a during the monsoon season with $R^2 = 0.930$, $N = 60$ and $\text{SEE} = \pm 0.369$.

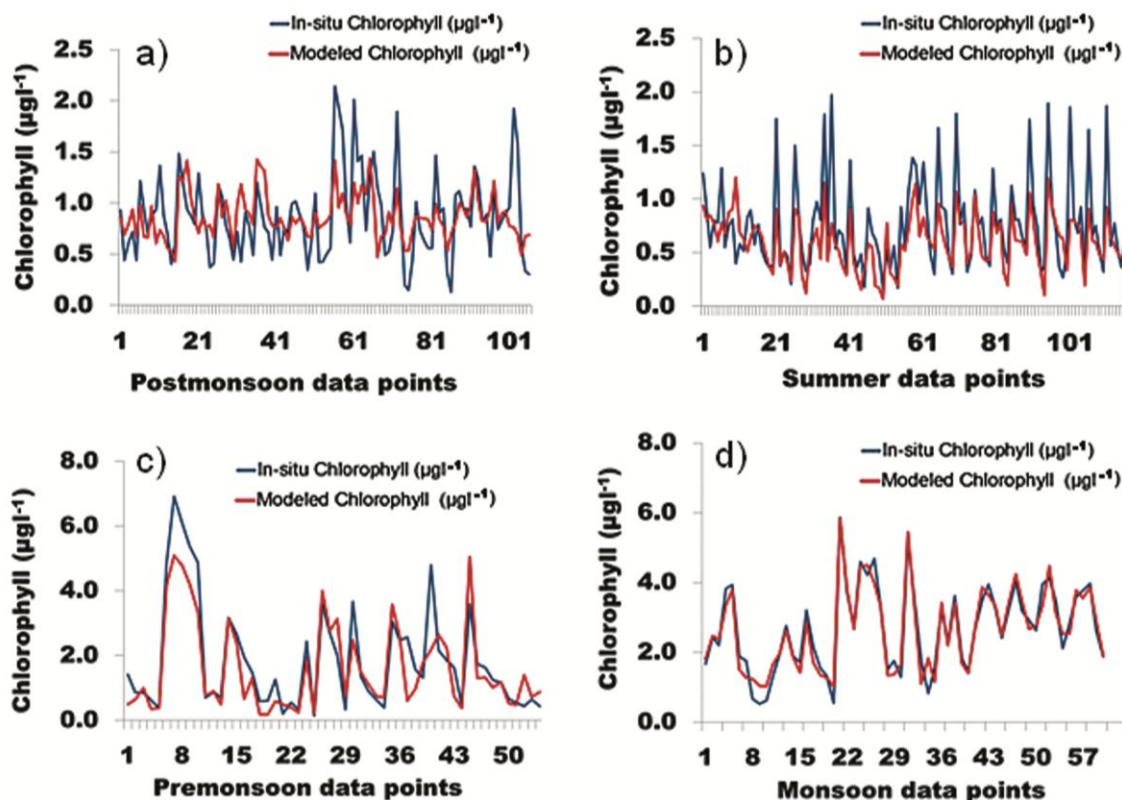


Fig. 4 — Comparison of modeled and *in-situ* concentration of chlorophyll-*a* recorded in the southwest Bay of Bengal for a) PM, b) SUM, c) PRM and d) MON seasons

Discussion

The BoB serves as a major source of nutrients, thereby improving the production; however this is not uniform across the BoB and is mainly limited to the southern sections due to the dominance of seasonal rivers. The distribution of physico-chemical variables and chlorophyll concentration in the BoB is found to be inimitable spatially and temporally. In general, the concentration of chlorophyll is found to be low ($< 1 \text{ mg m}^{-3}$) in the main portion of BoB²².

In addition to the more stagnant state of the surface water, nutrient limitation is the most significant cause of low chlorophyll concentration in the southwest BoB and comparatively narrow continental shelf region with low possibilities of upwelling and hence traditionally seen as less effective compared to its western equivalent, the Arabian Sea²³. Despite these variables, the characteristics of the coastal upwelling and seasonal blooms of primary production²⁴ coincide with post monsoon season when plenty of external nutrient inputs available from the seasonal river inputs.

Temperature is a significant hydrographic parameter that affects almost all the chemical and biological interactions²⁵. SST has generally been

affected by strength of surface winds, solar radiation intensity, evaporation, freshwater invasion and cooling, and by mixing with ebb and flow from adjacent neritic waters²⁶. Due to the weaker winds in the southwest BoB and the increased solar radiation due to the absence of clouds, the higher SST reported during the summer season warms the surface waters²⁷. Although the lowest temperature is experienced in monsoon time due to strong monsoonal winds which makes the surface of the seawater cool. Further, the persistent cloud cover during this season also decreases the solar intensity resulting in decreased sea surface temperature along the southwest BoB. Besides this, the bulk of freshwater influx during this season coinciding the northeast monsoon rains and river inputs also makes the sea surface cooler.

Salinity depicted a clear cyclic pattern with its higher ranges in summer period due to the excess evaporation and less freshwater runoff from the seasonal rivers confluence the Bay, while the low salinity is recorded at Karaikal during the monsoon season. Karaikal is one such region which receives high freshwater inputs from riverlets of Cauvery confluence with BoB at this region. Previous reports

suggest that BoB obtained huge amount of freshwater inflows during the northeast monsoon over the southwest BoB²⁸ from extreme local rain as well as from riverine input. Sea surface salinity in the Bay during this time is around 3 PSU fresher than the average of 36.2 PSU⁶ in the Arabian Sea. In BoB, the freshwater input causes near-surface stratification during the monsoon at the sea surface.

The minimum DO content is observed in summer due to high salinity and SST that are able to reduce oxygen solubility²⁹ by reducing the air sea interaction. Moreover, during this season, the water column of the southwest BoB is highly stratified and the weaker winds prevailing during this season are not capable of breaking this stratification thereby prohibits circulation between water masses leading to low oxygen into the water column. Chlorophyll-*a* concentration is also low during this period which in turn is responsible for release of DO into the water column. The elevated DO content during the pre-monsoon period may have contributed to the photosynthetic release of DO by high phytoplankton density, while the high DO during the monsoon period is directly related to the accumulation of oxygen-rich rainwater from precipitation²⁵.

During the study period, the pH in water bodies always had been alkaline at all stations, with the maximum values during the summer seasons and the lowest values during the monsoon period. Fluctuation at pH levels during different seasons of the year is generally attributed to factors such as the removal of CO₂ by photosynthesis by bicarbonate oxidation, the aliquots of freshwater by seawater, low primary production, reduced salinity and temperature, and degradation of organic materials³⁰. The high pH level could be due to water evaporation and high salt deposition in the Bay during the summer season.

Light is essential for the growth of phytoplankton. On the other hand, too much and too little light can affect the growth rate³¹ by the way of photo inhibition and slowdown the growth respectively. High light intensity also warms the surface of the water column leading to phytoplankton community shifts at different depths controlling the primary production. Southern BoB generally receives minimum light intensity during the northeast monsoon owing to cloudy conditions.

In the ocean, nitrite is primarily derived from NH₃ oxidation and NO₃ reduction, and is thus known to be one of the most volatile sources of inorganic

nitrogen³². In general, the southwest BoB does not entertain any external feedback during most part of the year except during monsoon. During these periods, the water remains highly saline with low nitrate concentration. Increased excretion of phytoplankton, oxidation of NH₃ and decrease of NO₃, and regeneration of nitrogen and bacterial degradation of the planktonic debris found in the environment³³ could lead to higher monsoonal values due to riverine runoff in addition to the terrigenous nitrogen inputs.

In addition to other factors such as nitrogen fixation and seasonal external inputs³, spatial and temporal variation of NO₃ and its decreased organic compounds were mainly affected by biological activity such as phytoplankton utilization and increased bacterial degradation. The reduced NH₃ concentration experienced during the summer season may be due to the rapid assimilation of NH₃ by phytoplankton³⁴, as it is favored to NO₃ by the phytoplankton community under certain marine circumstances³⁵. Higher NH₃ concentration recorded during monsoon is again a clear indication of external inputs which mostly remain at the surface of the water column and is also available only along the coastal zones and often not able to reach up to case 1 water (oceanic). Besides this, death and decay of phytoplankton community also adds on the ammonia concentration in the surface waters³⁶. In addition to localized upwelling and uptake by phytoplankton³², phosphate concentrations in coastal waters are caused by the mixing of freshwater with seawater in the land-sea interaction zone. The minimum summer values might be due to the utilization and absence of external inputs during this period. In contrast maximum IP and TP recorded during the monsoon is due to the external inputs especially, washed away phosphate fertilizers from the agriculture fields reaching the riverlets to estuaries and subsequently to the coastal oceans.

When compared to other nutrients, the RS content is very high in the study period. The seasonal change in RS concentration, however, is largely affected by the external inputs that carry RS materials extracted from land into the ocean through freshwater inputs by rain³. The spatio-temporal variation of reactive silicate³² in the BoB also has a major effect, in addition to the physical mixing of seawater with freshwater, resuspension of sediments due to wave behavior and biological removal of phytoplankton (diatoms and silico-filagellates).

Maximum concentration of chl-*a* experienced during monsoon period is owing to the high freshwater influx which bring additional input of freshwater blue-greens to the water column and nutrients which is capable of increasing the phytoplankton population. The minimum chl-*a* distribution observed during summer season is due to the non-availability of nutrients in the sea surface due to phytoplankton utilization. All these makes the surface water column nutrient limiting as the possibilities of vertical mixing is very low³⁷ due to stratified water and less winds.

Statistical analysis

PCA was used to group the physico-chemical parameters that affect the distribution of chl-*a* by common spatial and temporal changes under certain conditions. Component loading greater than 0.5 can be taken into account in the interpretation³⁸ and the same applies to the present analysis. The first PCA axis is the broad axis (the axis with the greatest variance); the second longest axis perpendicular to the first is the second PCA axis, etc. Component loading, the largest load, either positive or negative, indicates the importance of the dimensions, which measure the degree of closeness between the variables and the PC.

PCA of post monsoon season loaded positively with NH₃, IP, TN and TP and loaded negatively with salinity in PC1 owing to plenty of nutrients in the surface water because of mixing of water during the previous winter season and external inputs due to riverine input. Second component is positively loaded with chl-*a* and nitrate and negative loading of SST. SST is a vital parameter capable of affecting the concentration of chlorophyll, while nitrate is the most essential nutrient and the phytoplankton consumes its final form for its growth and metabolism³⁹. As a result phytoplankton increases drastically with available nutrients and adequate light at the same time increased SST due to clear sky during this season is having a check on the phytoplankton population density by causing photo-bleaching or shifting the plankton community and also composition to the subsurface waters. NO₂ alone loaded in PC3, whereas PC4 accounted negative loading of RS and positive loading of DO. Such inverse relationship between DO and RS as a stand for diatom inhabitants is recorded earlier in the BoB region by Thangaradjou *et al.*²⁴. Diatom highly prefers RS rather than other nutrients so that it may possibly consume silicate and releases dissolved oxygen back.

From the analysis of multiple regressions it is revealed that there is a strong positive association between chlorophyll concentration and DO, IP, PPF, NO₃, NO₂, pH, salinity, RS and TP. It indicates that the above mentioned factors are responsible for chl-*a* largely which is primarily contributed by the population of diatoms, resulting in an increased amount of dissolved oxygen. Comparison of modeled chl-*a* with *in-situ* value did not showed any significant difference.

During summer, PCA extracted four principal components in which SST and PPF are positively loaded in PC1 but NO₃ and chl-*a* loaded negatively. It is well known that the stratification strongly inhibits the nutrient replenishment and impedes growth of phytoplankton⁴⁰, as evidenced by the negative loading of nitrate. Too much of light intensity (direct sunlight) may result in photo-inhibition and is also lethal for many plankton species⁴¹. The inverse relationship between chl-*a* and light is again a clear evidence for the shift of plankton to the subsurface waters. Increased light intensity and high stratification due to increased surface heating and decreased wind stress obstruct the nutrients supply to surface waters during this season, resulting in reduced biological production⁶.

This inverse relationship between the concentration of SST and chlorophyll as a significance of phytoplankton population is already recorded in the BoB region⁴². In PC2, NO₂, RS, TP loaded positively and salinity loaded negatively which indicate that despite the nutrient availability in the surface waters, due to increased light and SST compel the plankton to shift to the subsurface waters. This is also confirmed by the multiple regression analysis, which proved the strong negative and positive correlation of SST and NO₃ with chl-*a*.

Pre-monsoon season PCA plot extracted five components with strong positive loading of NH₃, chl-*a*, DO and NO₃ in PC1; RS and TP in PC2; salinity in PC3; PPF and pH in PC4; and IP and NO₂ in PC5 but SST alone negatively loaded in PC1. Southwest BoB generally experience high wind speed during pre-monsoon season which breaks the stratified summer water and supplement subsurface nutrients to the surface waters resulting in increase of chl-*a* concentration and thereby DO level. Such a positive relationship between chl-*a* and DO and nutrients such as NO₃, NO₂, RS and TN is also predicted by regression analysis. The negative correlation observed between chl-*a* with PPF, SST, pH and TP indicates

the negative role of SST in the distribution of chl-*a* in the region and reduction of carbonic acid pathway due to reduced CO₂ flux. Zepp⁴³ proposed that rapid phytoplankton assimilation and surface run-off enhancement resulted in large-scale spatio-temporal variations of NO₃ in the marine environment. In the pre-monsoon season, a substantial positive relationship of nitrogenous nutrients with chlorophyll concentrations indicates their effect on the distribution of chlorophyll. This is evidenced by the regression analysis derived modeled chl-*a* which matched well with *in-situ* chlorophyll with significant $R^2 = 0.79$ values.

Four components are extracted during the monsoon season with positive relationship of chl-*a*, DO, NO₃ and negative relationship of SST in PC1 clarifies the biological process taking place in phytoplankton and microorganisms. Factor 2 explains IP, NO₂, salinity with positive loadings whereas NH₃ alone loaded negatively indicating utilization of NH₃ by the growing phytoplankton despite the external inputs adding the NH₃ concentration during this period. Moreover the increased organic carbon availability during this season also makes heterotrophic productivity. PC3 explains positive loading of NH₃ during the monsoon season besides TN and RS but PPF and pH loaded negatively; whereas PC4 depicts positive loading of RS, TN and TP. Obviously heavy rainfall induced river runoff lowers light intensity and increases the nutrients (nitrogen, phosphorus and silicate). The dead and degraded organisms are one of the causes for nitrogenous matter. In addition anthropogenic activities also load organic material to the ecosystem, whereas microbial fermentation process occurring on the organic material lowers the pH value⁴⁴.

All the physico-chemical parameters showed clear seasonal pattern representing the tropical marine environment without much spatial variations in the region. As evident from the PCA and MLR analysis, the physico-chemical parameters have a major effect on the distribution of chl-*a* in the southwestern BoB and differ seasonally.

The composition and abundance of phytoplankton is typically significantly different between all seasons, affected by several variables, primarily by water temperature and nutrient availability, and it has been stated that water temperature alone can regulate the seasonal dynamics of chlorophyll concentration. Usually composition and abundance of phytoplankton is typically significantly different between all seasons

influenced by several factors largely by water temperature and nutrient availability and it has reported that water temperature can alone is able to control the seasonal dynamics of chl-*a* concentration⁴⁵. Data from the multivariate study findings also confirms that the most significant factors affecting the seasonal pattern of chlorophyll along the coastal waters of southwest BoB are SST and nitrogen.

Conclusion

The PCA of various seasons clearly explained that phytoplankton (chlorophyll) are highly dependent on nitrogen for its growth and metabolism rather than other nutrients, and it also showed the effect of physical parameters on nutrient distribution and dissolution in southwestern BoB. Nitrogen nutrients (NO₃, NO₂ and NH₃) are positively loaded in all seasons from the results of PCA and regression analysis also predicts positive effects of NO₃ and NH₃ with chlorophyll, which are better evidence of nitrogen nutrients limiting the growth of phytoplankton in the BoB region. Result of PCA further confirmed by the multiple regression analysis, that the chl-*a* is highly correlated with all the nutrients especially with NO₃. An inter-comparison of modeled and *in-situ* chl-*a* concentration shows a significant correlation during monsoon season by 93 % of matchup with a $R^2 = 0.930$, $N = 60$ and $SEE = \pm 0.369$ in the entire regression summary. In this perspective, nutrient availability is seasonally dependent in the surface waters of the southwestern BoB and all nutrients have a significant impact on the distribution of chl-*a*. The present study confirms that the seasonal dynamics of chl-*a* concentration in the BoB is mainly driven by the water temperature and nitrogenous nutrients while RS play a critical role in changing the community structure at seasonal scale.

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Conflict of Interest

Authors declare no competing or conflict of interest.

Author Contributions

DP: Formal analysis, investigation, and original draft writing; RS: Formal analysis and investigation; TT: Conceptualization, review & editing and resources; AS: Supervision and SRK: Funding acquisition.

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