

Phytoplankton assemblage and chlorophyll *a* along the salinity gradient in a hypoxic eutrophic tropical estuary-Ulhas Estuary, West Coast of India

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Abstract

Phytoplankton assemblage and chlorophyll *a* along the salinity gradient and oxygen zonation were studied in Ulhas Estuary. Oxic condition exist in euhaline region of the estuary that receives oxygenated coastal waters, while, poly-meso-oligohaline region is hypoxic due to the accumulation of voluminous amount of pollutants from industrial effluents, domestic/sewage and agricultural discharges. Additionally, anthropogenically generated nutrients enhanced phytoplankton biomass (chlorophyll *a*) and their senescence also led to hypoxia. The dominance of diatoms (>70%) was observed in the euhaline-oxic region, while a tremendous decrease in their contribution (18%) was observed in the oligohaline region. Meso to oligohaline region of the estuary is governed by cyanophytes and chlorophytes due to their affinity towards low salinity and high dissolved inorganic nutrients. Carlson's Trophic State Index (TSI) further substantiated the estuary as eutrophic. Additionally, the study could identify the adaptation of cyanophytes and chlorophytes in oxygen deficient water.

Keywords: Eutrophication, hypoxia, salinity gradient, nutrients, phytoplankton.

1. Introduction

Phytoplankton are microscopic autotrophs, which is the major primary producer in any aquatic ecosystem. Their community structure can give more evidence concerning the water quality of the system (Yusuf, 2020) through alteration in their community composition, distribution and proportion of sensitive species (Gharib et al. 2011). To attain a better perspective of the structure and dynamics of the aquatic environment, it is imperative to understand the quantification of phytoplankton biomass and their community composition (Roy et al. 2006). Alteration in abundance and diversity of phytoplankton species is often used as indicators of water quality and hence spatial and temporal variation in the phytoplankton community are also investigated (Gameiro et al. 2007).

In recent decades, anthropogenic interventions such as agro-fertilizer wastes, wastewater discharges from treatment plants and domestic/sewage discharges, degradation of organic nitrogenous matter have exponentially increased nutrient inputs from their original level leading to eutrophication (Bricker et al. 2008; Bouwman et al. 2013; Yu et al. 2020). Nutrient additions cause progressive eutrophic warning signs that often begin with high concentrations of chlorophyll *a* (Ryther and Dunstan, 1971). The measure of phytoplankton biomass (chlorophyll *a*) is commonly employed as an indicator of eutrophication (Bricker et al. 1999) because it provides consistent insights on a stressed area, but it should be monitored together with the phytoplankton assemblage changes (McQuatters-Gollop et al. 2009).

Eutrophication can also lead to high biochemical oxygen demand (BOD) which is the amount of oxygen demand for micro-organisms to oxidize and stabilize dissolved-organic substances in an aquatic system at a certain temperature over a specific time period (Sawyer et al. 2003). BOD is directly proportional to organic load; the greater the BOD, the lower the amount of dissolved oxygen available in the system (Phu 2014). BOD is also used to quantify the strength of pollutant loads (Clark et al. 1977; Boyd, 2000) from industries and domestic/sewage discharges (Mallin, 2000). Elevated BOD concentration caused by the decomposition of phytoplankton blooms and other excessive organic matter are an extensive and growing problem in large open coastal waters, as well as in estuaries, lakes and streams (Boesch et al. 2001). Studies have evidenced that eutrophication propels and increases hypoxia ($\leq 2.0 \text{ mg l}^{-1}$) which is partly allied with the decomposition of organic carbon derived from phytoplankton blooms induced by eutrophication (Rabalais et al. 2014; Wang et al. 2016). BOD is widely recognized geographically and across multi-disciplinary subjects as it provides insights into the dissolved oxygen condition of a given system. It is a standard and easily performed methodology in the quantification of dissolved oxygen concentration in a given ecosystem.

Pioneer studies on the qualitative and quantitative aspects of phytoplankton concerning physico-chemical parameters in spatio-temporal scale have been carried out in Indian waters. Mani et al. 1986 studied about the ecology of phytoplankton blooms in the Vellar Estuary, east coast of India and reported that decreasing salinity and higher nutrient concentration showed increase in phytoplankton population. Devassy and Goes 1988 reported about the phytoplankton community structure in Mandovi-Zuari estuarine complex in the coastal waters of Goa and discussed about the post monsoon period showing maximum cell concentration and that waters with reduced salinity supported greater abundance of phytoplankton in the estuaries. The monsoonal impact on planktonic standing stock and abundance in Cochin backwaters was studied by Madhu et al. 2007 and addressed that the high phytoplankton biomass was related to the enormous input of nutrients into the estuary from various sources such as industrial, agricultural and domestic wastes. Mishra et al. 2009 explained about the seasonal appearance of Chlorophyceae phytoplankton bloom by river discharge off Paradeep at Orissa coast and further established the role of salinity on phytoplankton species composition and seasonal succession. Patil and Anil 2011 reported the variation in phytoplankton community in a monsoon-affected Zuari Estuary, Goa and elucidated that the phytoplankton community was influenced by the change in salinity and nutrient gradients of the estuary. Fine-scale responses of phytoplankton to freshwater influx in Mandovi Estuary was studied by Pednekar et al. 2011 and observed phytoplankton shift according to the salinity changes. Work done by Sahu et al. 2012 on phytoplankton dynamics in the coastal waters of Kalpakkam demonstrated a considerable increase (3 fold) in phytoplankton species composition (185 species) as compared to earlier findings (60 species) and found that salinity was the prime important environmental variable influencing the phytoplankton species composition, standing stock and succession in the study area. Chowdhury et al. 2017 studied the nutrient stoichiometry and freshwater flow in shaping of phytoplankton population in Kundalika Estuary. Karthik et al. 2020 reported diatom bloom in the Amba River which is a nutrient enriched tropical river fed estuary of Maharashtra with importance to how the allochthonous nutrient inputs governed the phytoplankton distribution and abundance. Gauns et al. 2020 studied the shift in community structure of phytoplankton along the central west coast of India in response to dissolved oxygen and reported on phytoplankton species which thrived in different oxygen zonation (oxic to anoxic) and species that tolerate wide range of oxygen concentrations. A brief description on spatial variability in nutrient-phytoplankton relationship was studied by Niveditha et al. 2022 in a shallow tidal creek-Thane Creek, where elevated levels of nutrients enhanced both the phytoplankton biomass and density.

Considerable studies have been carried out in Ulhas Estuary from 2002 to 2020. Scientific proceedings and technical report of Quadros and Athalye, 2002; Borke et al. 2002 and Athalye et al. 2003 studied the phytoplankton and intertidal macrobenthic fauna of Ulhas Estuary and additionally assessed its pollution stress. Hydrological study of Ulhas Estuary was carried out by Mishra et al. 2007. The hydrodynamic study in Ulhas estuary was well explained by Jubin et al. 2019 which defined that Ulhas Estuary is a mixed semi-diurnal hyposynchronous estuary. Due to this property, a gradual decrease in tidal amplitude was noticed from mouth to upstream leading to tidal asymmetry which in turn changes the duration of flood and ebb in the estuary. Mulik et al. 2020 studied the ecological status of Ulhas Estuary using benthic index, AZTI's Marine Biotic Index (AMBI) and defined the vulnerability of the estuary. It has been observed that studies on phytoplankton along the salinity gradient and different oxygen realms have been vaguely reported in the study region. This is the first attempt carried out to understand the distribution of the phytoplankton community in relation to both salinity regime and oxygen zonation. The study focused on both quantitative and qualitative phytoplankton ecology by considering the influence of hydrographical parameters and nutrients. Additionally, a pollution index was also applied to classify the estuary based on its ecological status. The pollutants from non-point sources entering into the estuary have become a major concern for policymakers who have been commissioned with formulating remedial and effective measures towards restoring the estuarine ecosystem. Hence it is essential to study the impact of anthropogenic interventions on the Ulhas Estuary and postulate its management, in order to protect, preserve and sustain the ecosystem and enhance environmental stewardship.

2. Materials and Methodology

2.1 Description of the sampling site

Ulhas River is a westward flowing river in the state of Maharashtra which originates in the Sahyadri hill ranges of Ulhas valley near Udhewadi. After a westerly course of about 100 km through mountainous terrain, the river meets downstream of the confluence of Kalu and Bhatsai Rivers near Kalyan and becomes part of the Ulhas Estuarine system. From Kalyan onwards, the estuary meanders through vast mudflats and extensive mangrove areas and joins the Arabian Sea near Vasai through the Bassein Creek.

Three prominent seasons are observed in the Ulhas Riverine-Estuary system, with high runoff during June to September (monsoon), the least runoff during February to May (pre monsoon; dry season) and the intermediate phase from October to January (post monsoon). The annual mean rainfall of Ulhas catchment receives 2600-2800 mm during June-September (monsoon period). Ulhas Estuary is

an undulating estuary with a wide mouth and the length is around 65km. For the convenience of the study, the sampling locations were zoned into the upper, mid and lower estuary. Generally, Ulhas Estuary experiences a mixed semi-diurnal tide with considerable diurnal inequality. During the dry season, the tidal excursion which is 5-15 km in the lower estuary decreases to 2-5 km in the upper estuary. During the same period, the estuarine flushing time varies from 73 to 211 tidal cycles leading to the possibility of accumulation of pollutants in the upper estuary. However, during monsoon season the estuary is effectively flushed by voluminous river discharge and occasional floods Mulik et al. 2020. Earlier studies of Ram et al. 2003; Ram et al. 2009 stated that the spatial and temporal variation in salinity were noteworthy. During the spring high tide, salinity is close to the seawater value (35.5ppt) in the lower estuary and decreases to about 25ppt in the mid-estuarine zone and further down to 10ppt in the upper zone during May when the riverine flow is lean.

The estuary is heavily affected by anthropogenic interventions and by the uncontrolled discharge of pollutants from both point and non-point sources, such as untreated domestic sewage, runoff from terrestrial and agricultural fields, dredging activities and industrial effluents. Rivers, streams, land runoff, wastewater inputs and industrial discharges are all pathways by which pollutants enter the estuary (Ram et al. 2003). The accumulation of pollutants in the upper estuary depends upon the estuarine flushing rate (Ram et al. 2009). An examination of available studies on Ulhas Estuary indicated that unfavorable ecological conditions like eutrophication and hypoxia occurred when there is little or no freshwater discharge (Ram et al. 2003; Ram et al. 2009).

2.2. Sampling strategy, estimation of environmental variables, chlorophyll a and phytoplankton community

Sampling was carried out during November 2015 and May 2016 which represented the post monsoon and pre monsoon season respectively. The study mainly focused on two different scenarios that existed in Ulhas Estuary. The post monsoon season is the period that is soon after the complete flushing of the estuary and pre monsoon is the period when the estuary is under stress due to the accumulation of pollutants because of least discharge. Samples were collected from eight locations, constituting the lower (stations U1, U2 &U3), mid (stations U4 &U5) and upper (stations U6, U7 &U8) zone of Ulhas Estuary (Figure 1).

Surface water samples were collected from 1m water depth below the surface using a 5 L capacity Niskin water sampler (Germany) for dissolved oxygen (DO), BOD, dissolved inorganic nutrients (nitrite, nitrate, ammonia and phosphate), chlorophyll *a* and phytoplankton taxonomy.

Water temperature was measured using a hand-held thermometer of accuracy $\pm 0.1^\circ\text{C}$. Salinity was measured using an AUTOSAL salinometer (GUILDLIN Instruments Ltd. Canada). The instrument was standardized with IAPSO standard seawater (OSIL, UK). DO was estimated using the Winkler's method (Grasshoff, 1983) and is expressed in mg l^{-1} . Samples for the determination of BOD were collected in BOD bottles and were incubated for 3 days at 27°C . DO concentration in these samples was determined by Winkler's method (Grasshoff, 1983) after fixing the samples immediately on completion of 3 days incubation period. The difference in the DO concentrations on the 1st and 3rd days yielded the measure of BOD which is expressed in mg l^{-1} . Dissolved inorganic nutrients like nitrite (NO_2^- -N), nitrate (NO_3^- -N), ammonia (NH_4^+ -N), and phosphate (PO_4^{3-} -P) were analyzed within 6 h of collection using standard procedures described by Grasshoff, 1983 and is expressed in μM . In the study, the sum of nitrite, nitrate and ammonia was denoted as Dissolved Inorganic Nitrogen (DIN) and phosphate was denoted as Dissolved Inorganic Phosphate (DIP).

For chlorophyll *a* estimation, 500 ml of seawater sample were filtered through Whatman GF/F filter paper (47 mm diameter; pore size $0.7 \mu\text{m}$) and extracted in 90% acetone at 4°C for 24 h. The chlorophyll *a* was estimated using a Turner Trilogy fluorometer (Model 7200) following the standard procedure of Parson et al. 1984. The concentration of chlorophyll *a* in the sample is reported as mg m^{-3} . For phytoplankton taxonomic identification and density enumeration, 500 ml of seawater sample was fixed with Lugol's iodine. After sedimentation in dark, the samples were concentrated to 10ml. An aliquot of 1 ml from each concentrate was taken on Sedgwick Rafter (United Kingdom) chamber and analyzed under a compound light microscope (400 X magnification). The phytoplankton were identified up to the lowest possible taxonomic level following the standard taxonomic keys (Subrahmanyam, 1946; Tomas, 1997). The enumerated phytoplankton taxa were reported as $\text{no.} \times 10^5 \text{ cells l}^{-1}$.

2.3. Statistical analysis

To examine inter and intra group relationship between phytoplankton assemblage and hydrographical parameters and nutrients, Spearman's correlation matrix (*r*) was computed using SPSS-PASW Statistics 18 and the significant level was considered at $p < 0.05$ & $p < 0.01$. Mann Whitney U test (non-parametric) was performed for the two transects; lower to the mid estuary and from mid to upper estuary during post monsoon and pre monsoon for phytoplankton components (chlorophyll *a* and abundance), hydrographical parameters (temperature, salinity, DO, BOD) and nutrients (DIN and DIP). This test was used to understand the difference between two transects and Graph Pad Prism software (version 5.01) was used to perform the test.

The trophic status of the estuary was estimated using Carlson's Index. Carlson's Trophic State Index (TSI) is a frequently used biomass-related trophic state index. In accordance with the definition of trophic state given above, the TSI (Carlson, 1977) uses algal biomass (chlorophyll *a*- CHL) as the basis for trophic state classification. Based on the formula of Carlson and Simpson, 1996 the trophic status of the estuary was estimated using chlorophyll *a* as a proxy:

$$TSI (CHL) = 9.81 \ln(CHL) + 30.6$$

Based upon the TSI, the estuary can be classified bearing the index 30-40 as oligotrophy, 40-50 as mesotrophy and 50-70 as eutrophy.

3. Results

3.1 *Spatio-temporal variation of hydrographical parameters, nutrients and phytoplankton components*

Hydrographical parameters such as temperature, salinity, DO and BOD was examined for two seasons (post and pre monsoon) along the estuary. Temperature showed seasonality in the study region with a 3-4°C increase during pre monsoon (Figure 2a). Being a monsoonal estuary, salinity gradient was prominent during the study period. In the study region, there was indeed a prominent salinity gradient that classified the estuary into euhaline (>30ppt), polyhaline (30-18ppt), mesohaline (18-5ppt) and oligohaline (5-0.5ppt) conditions. During the study period, salinity ranged between 0.6 and 34.6ppt during post monsoon while in pre monsoon it was 9.8 to 36.7ppt (Figure 2b). Salinity wise the estuary experienced euhaline to polyhaline condition in the lower estuary, poly to mesohaline in the mid estuary and meso to oligohaline in the upper estuary during post monsoon. Ingress of coastal water into the estuary during pre monsoon resulted in the complete exclusion of oligohaline condition from the upper estuary (stations U7&U8). Based upon the DO zonation, the study region exhibited oxic to hypoxic conditions from lower to upper estuary. The lower estuary (stations U1-U3) displayed oxic condition ($DO \geq 2 \text{ mg l}^{-1}$) throughout the study period, while mid to upper estuary (stations U4-U8) acted as hypoxic ($DO < 2 \text{ mg l}^{-1}$), however, station U4 showed seasonality and acted as oxic/hypoxic according to the oscillation of tidal water (Figure 2c). Therefore, the study confirmed that the meso-oligohaline region of the estuary was always hypoxic. The above scenario is due to the excess utilization of DO for the decomposition of organic matter. Spatial variation of BOD was very clear from the study, while seasonal variation was least. Lower estuary exhibited low BOD concentration ($BOD < 2 \text{ mg l}^{-1}$), whereas, BOD was $> 20 \text{ mg l}^{-1}$ from mid to upper estuary (stations U5-U8) and maximum concentration was observed at station U8 ($\geq 55 \text{ mg l}^{-1}$) during both the season (Figure 2d).

Fundamentally, DIN is contributed by nitrite, nitrate and ammonia, however, nitrite is thermodynamically less stable, henceforth, nitrate and ammonia contribute more to DIN. In the present study, the sum of nitrite, nitrate and ammonia is considered as DIN. Therefore, the seasonal and spatial variability of DIN noticed during the study period can be speculated based on nitrate and ammonia concentration. During post monsoon, DIN ranged from 25.1 to 105.4 μM and revealed that the poly to oligohaline-hypoxic region (stations U5-U8) of the estuary remarked a high concentration of DIN (av. 88.5 μM). Whereas, in pre monsoon, the range was between 5.8 and 82.2 μM and relatively less concentration (av. 51.6 μM) was observed in the poly to mesohaline region of the estuary (Figure 2e). DIP during post monsoon ranged between 2.5 and 7.6 μM . The concentration of DIP increased from euhaline to mesohaline region (stations U1-U5) and gradually decreased towards the oligohaline region of the estuary. In pre monsoon, euhaline to mesohaline region of the estuary showed an increase in DIP and ranged between 1.2 and 14.8 μM ; except at U8 (Figure 2f). Spatial variability of DIP was not noteworthy; the Mann Whitney U test also indicated the same (Table 4).

Chlorophyll *a* and phytoplankton abundance demonstrated spatial variation during the study period. Chlorophyll *a* ranged from 0.9 to 38.4 mg m⁻³ and exhibited high concentration from meso to oligohaline region (av.33.1 mg m⁻³) during post monsoon. While in pre monsoon high chlorophyll *a* was observed from polyhaline to mesohaline region (av. 23.3 mg m⁻³) and ranged between 1.0 and 33.1 mg m⁻³ (Figure 2g). The seasonal study of the dissolved form of nutrients in the estuary revealed that poly to oligohaline region of the estuary is highly loaded with dissolved nitrogenous forms during both seasons. Therefore, the chlorophyll *a* peak during both seasons can be linked to the DIN and DIP concentration. Phytoplankton abundance showed spatial variation profusely from meso to oligohaline region of the estuary with a peak value at station U8 (179.2 x 10⁵ cells l⁻¹) in post monsoon. On the other hand, in pre monsoon relatively low abundance (60.9 x 10⁵ cells l⁻¹) was observed at the same station (Figure 2h). A 2 fold decrease in phytoplankton abundance was observed during pre monsoon when compared to post monsoon.

The pollution status of the estuary was studied through Carlson's Trophic State Index (TSI) and took chlorophyll *a* as a proxy. Based upon the TSI, the trophic status is classified bearing the index 30-40 as oligotrophy, 40-50 as mesotrophy and 50-70 as eutrophy. The study indicated an apparent spatial variability in TSI while seasonal variability was feeble. The study region exhibited eutrophic condition from mid to upper estuary (stations U5 to U8) of TSI index ranging from 61 to 66.4 for post monsoon and 54.2 to 64.9 for pre monsoon (Figure 3a&b). Lower to mid estuary (stations U1 to U4) exhibited oligotrophy to mesotrophy during both post monsoon (29.5-41.3) and pre monsoon (31-47.3). From the study, it was understood that the eutrophic condition of the estuary co-existed

with low salinity (<25 ppt) and DO concentration (<2 mg l⁻¹). The study concluded that the meso-oligohaline-hypoxic region of the estuary is eutrophic regardless of the season.

3.2 Phytoplankton community in different salinity and dissolved oxygen (DO) realm

Study on phytoplankton community belonging to seven classes viz., diatoms, dinoflagellates, chlorophytes, chrysophytes, cyanophytes, euglenophytes and cryptophytes showed strict salinity preference (Figure 4). During both seasons the supremacy of diatoms (>70%) was found in euhaline and polyhaline region of the estuary. However, in the mesohaline and oligohaline region of the estuary, the contribution of diatoms decreased to 37% and 18% respectively in post monsoon. Further, during the same season, the occurrence of chlorophytes (33%) and cyanophytes (23%) was found in the mesohaline region of the estuary, whereas, the oligohaline region of the estuary was dominated by cyanophytes (76%). In pre monsoon, diatoms and cyanophytes almost equally contributed to the total community in the mesohaline region of the estuary. Complete absence of chlorophytes, cyanophytes and euglenophytes in the euhaline to polyhaline region of the estuary indicated their brackish to freshwater preference.

The DO zonation of the estuary revealed that the oxic zones were dominated by diatoms in both post monsoon (>78%) and pre monsoon (87%) (Figure 5). Besides diatoms, the oxic zones comprised of cryptophytes (17%) and dinoflagellates (12%) during post and pre monsoon respectively. The hypoxic zone exhibited the dominance of cyanophytes (65%), followed by diatoms (20%) and chlorophytes (11%) during post monsoon. While, in pre monsoon, diatoms (55%) and cyanophytes (44%) equally contributed to the hypoxic zone of the estuary.

3.3 Seasonal and spatial distribution of Phytoplankton assemblage in Ulhas estuary

Phytoplankton assemblage study during post monsoon revealed that the maximum density and diversity was found in the meso-oligohaline region, the hypoxic zone of the estuary. In pre monsoon, the hypoxic zone was between poly and mesohaline region that exhibited high density and diversity. During post monsoon, diatoms like *Thalassiosira* sp., *Cylindrotheca* sp., *Odontella* sp., *Pleurosigma* sp., *Nitzschia* sp., *Rhizosolenia* sp. and *Skeletonema* sp. were found in generous in the hypoxic zone (mid to upper estuary) where the salinity condition was between meso to oligohaline (Table 1). During the same season, chlorophytes like *Scenedesmus* sp., *Dictyosphaerium* sp., *Ankistrodesmus* sp., and cyanophytes like *Merismopedia* sp., *Oscillatoria* sp., *Anabaena* sp., *Spirulina* sp. were found in copious in the meso-oligohaline region. In pre monsoon, *Thalassiosira* sp., *Skeletonema* sp., *Cylindrotheca* sp., *Odontella* sp., *Pleurosigma* sp., *Cyclotella* sp. and *Certaulina* sp. was plentiful from poly to mesohaline-hypoxic water (Table 2). *Merismopedia* sp., (mesohaline) and *Oscillatoria*

sp. (poly-mesohaline) were in abundance during the same season. The study summarizes that oxieuhaline waters were less dense and diverse compared to meso-oligohaline hypoxic region. Major phytoplankton genera along the salinity gradient and in different oxygen realms are given in plate 1.

3.4 Spearman's correlation of chlorophyll a and phytoplankton community with physico chemical parameter

Spearman's correlation of chlorophyll *a* and phytoplankton community with physico-chemical parameters were studied in the Ulhas Estuary (Table 3a&b). During post monsoon phytoplankton community like diatoms ($r=-0.71$, $p<0.05$), chlorophytes ($r=-0.78$, $p<0.05$), cyanophytes ($r=-0.91$, $p<0.01$) showed negative correlation with salinity. Chlorophyll *a* ($r=-0.74$, $p<0.05$) also correlated negatively with salinity during the same period. Dinoflagellates showed a positive correlation ($r=0.90$, $p<0.01$) with DO and negative correlation ($r=-0.81$, $p<0.05$) with BOD. Chlorophytes ($r=0.78$, $p<0.05$) and cyanophytes ($r=0.86$, $p<0.01$) exhibited a positive correlation with BOD. Chlorophyll *a* ($r=0.76$, $p<0.05$), chlorophytes ($r=0.90$, $p<0.01$) and cyanophytes ($r=0.84$, $p<0.01$) were positively correlated to DIN. Cryptophytes showed positive correlation with DIP ($r=0.74$, $p<0.05$) (Table 3a).

In pre monsoon the diatoms ($r=-0.79$, $p<0.05$), chlorophytes ($r=-0.79$, $p<0.05$), cyanophytes ($r=-0.87$, $p<0.01$) and chlorophyll *a* ($r=-0.83$, $p<0.05$) showed negative correlation with salinity. Chlorophyll *a* ($r=-0.83$, $p<0.05$) and diatoms ($r=-0.85$, $p<0.01$) showed a negative correlation with DO. Chlorophyll *a* had positive correlation with DIN ($r=0.79$, $p<0.05$). Diatoms ($r=0.74$, $p<0.05$), chlorophytes ($r=0.79$, $p<0.05$) and cyanophytes ($r=0.79$, $p<0.05$) were positively correlated with DIN (Table 3b).

3.5 Mann-Whitney U test

Mann-Whitney U test inferred that parameters like temperature, salinity, BOD, chlorophyll *a* and phytoplankton abundance showed spatial variation during both seasons. While DO, DIN showed spatial variation during pre monsoon and post monsoon respectively (Table 4). DO did not show spatial variation during post monsoon because except for the two values at stations U1 (5.1 mg l^{-1}) and U2 (4.4 mg l^{-1}) in the remaining stations DO values were more or less the same. As mentioned in the result, the DIN concentration varied according to the concentration of nitrate and ammonia, the Mann-Whitney U test also supported the above statement. (Table 4).

4. Discussion

4.1 Spatio-temporal variation of hydrographic parameters, nutrients and phytoplankton components

Water temperature exhibited seasonality (Figure 2a) with a high temperature in pre monsoon which could be due to the warmest month of the season (May 2016) because of the raising heat (Kothawale et al. 2010; Gadhia et al. 2012). Being a monsoon fed estuary, tide and freshwater played a major role in the classification of the estuary (Jubin et al. 2022). The study inferred that, during post monsoon, the lower estuary behaved as euhaline (>30ppt) to polyhaline (30-18ppt) that received tidal water from the adjacent coastal water. In the mid estuary, the tidal water was mixed with the fresh water and exhibited poly to mesohaline (18-5ppt), further upstream, the estuary was meso to oligohaline (5-0.5ppt). Whereas, in pre monsoon, the ingress of coastal water into the estuary led to the complete exclusion of oligohaline condition (av. 2.5ppt) from the upper estuary (station U7&U8) (Figure 2b). According to the study made by Vijith et al. 2009, the distribution of salinity in an estuary is grouped into two classes, namely, salinity-ingress and salinity-egress. The latter is associated with runoff and the former is related to salinity increase in the estuary due to tidal activity. The present study was in agreement with the above statement as the upstream stations (U6-U8) of the estuary picked up the salinity (9.8-19.0ppt) due to ingress of coastal water and lack of river discharge during pre monsoon.

DO is an important water quality parameter that controls the oxidation/reduction processes in any aquatic system. Ulhas Estuary exhibited oxic to hypoxic condition along the salinity gradient during both seasons. Oxic ($DO > 2 \text{ mg l}^{-1}$) condition existed in the lower estuary that always received oxygenated coastal water (Figure 2c). While mid to upper estuary (shallow and narrow region) exhibited hypoxic ($DO < 2 \text{ mg l}^{-1}$) to severe hypoxic condition ($DO < 0.6 \text{ mg l}^{-1}$) that received industrial and domestic wastewater of 437.29 Million Litres per Day (MLD). The technical report of NIO, 2016 and 2018 mentioned that Ulhas Estuary receives voluminous amount of sewage discharges that contain oxidisable organic matter and certain pollutants from industries that consume DO more than the water body can replenish which led to subsequent hypoxia. Bricker et al. 1999, Mallin et al. 2002, Mallin et al. 2006 stated that both allochthonous and autochthonous inputs are the major cause for hypoxia in watershed estuaries. Conversely, the noticeable feature of the study was that the polyhaline region of the estuary exhibited oxic/hypoxic condition according to salinity variation. This scenario was observed at station U4 (mid estuary) that behaved oxic/hypoxic during the study period which can be accorded to tidal oscillation. During post monsoon station U4 behaved as hypoxic, while in pre monsoon the existing condition was shifted to oxic. The probable reason for

the shift of hypoxic to oxic condition can be attributed to the ingress of coastal water that signified the role of tide in the transportation of hypoxic water. Spearman's correlation also showed a linear relationship between DO and salinity during pre monsoon ($r=0.90$, $p<0.01$) (Table 3b) which indicated that DO increases along the salinity gradient as a result of mixing with oxic coastal water. The above findings can be substantiated by the studies made by Ram et al. 2003 and Jubin et al. 2022. The study of Ram et al. 2003 accounted that during dry season (October-May) the estuarine reach is up to 25km inland and is strongly influenced by tides. According to the study made by Jubin et al. 2022, the Ulhas Estuary experienced the flushing time of 1.5 days in monsoon and 2.57 days during non-monsoon period. Pearce and Schumann, 2003 studied the DO characteristics of the Gamtoos Estuary, South Africa and elucidated that encroachment of coastal water having high DO content (10 mg l^{-1}) can replenish the upstream containing low DO concentration.

High BOD concentration ($>20 \text{ mg l}^{-1}$) from mid to upper estuary (station U5-U8) pointed out the input of organic load from varied sources and consumption of DO as stated above (Figure 2d). Mann-Whitney U test also indicated the spatial variation with a significant difference during post monsoon ($p<0.05$) and pre monsoon ($p<0.05$) between the two transects; lower to mid estuary and mid to upper estuary (Table 4). Mocuba, 2010 quoted that BOD value of 2 mg l^{-1} or less are unpolluted waters, whereas water bodies receiving wastewater may have high BOD values up to 10 mg l^{-1} . The BOD concentration was found to be much higher than the above-referred work especially in the upper estuary of Ulhas during post monsoon (av. 37.3 mg l^{-1}) and pre monsoon (av. 39.4 mg l^{-1}). The high concentration obtained in the present study indicates that this zone of the estuary was severely affected by anthropogenically generated organic load. The BOD concentration depends on the requisite amount of DO which ultimately depends upon the type of bacteria (nitrifiers/denitrifiers) present in the wastewater. In the present study BOD negatively correlated with DO during post monsoon ($r= -0.85$, $p<0.01$) and pre monsoon ($r= -0.83$, $p<0.05$) (Table 3a&b) which also indicated the high utilization of DO.

Anthropogenically derived DIN through point and non-point sources lead to the eutrophic condition of the upper estuary. During post monsoon, DIN in the upper estuary peaked at $105.4\mu\text{M}$ while in pre monsoon the maximum concentration was $82.2\mu\text{M}$ (Figure 2e). Additionally, DIN displayed an inverse relationship with salinity and DO, displaying relatively low DIN towards the euhaline-oxic region of the estuary compared to poly-oligohaline-hypoxic region. The study made in river Periyar, West coast of India (Joy et al. 1990), showed that high DIN is from industries through effluent discharges in the upstreams and low DIN in the mouth of the estuary is due to dilution by seawater. A comparative study made by Hao et al. 2021 in eutrophic (Wenchang Estuary, China) and

oligotrophic (Krka, Croatia) estuaries tracing nutrients and organic matter indicated that the major contributor for DIN was ammonia and nitrate.

Even though, the estuary received anthropogenically generated nutrients in the upper estuary (poly-mesohaline region), a low concentration of DIP was found during post monsoon, while in pre monsoon high concentration was detected (Figure 2f). The main cause of high concentration was anthropogenic contaminants generated around the estuary by upstream development, the release of organic matter and nutrients (Ram et al. 2014). Liu et al. 2009 stated that those waters that receive domestic wastes containing detergents, as well as wastes from agro fields, are rich with phosphate-phosphorous fertilizers and pesticides. Conversely, phosphorus is considered as an important macronutrient in an estuarine system and its high concentration can lead to an algal bloom through phytoplankton uptake (Maher and DeVries, 1994).

Chlorophyll *a* did not show a noteworthy seasonal variation rather spatial variation was evident during the study period (Figure 2g). Mann-Whitney U test also validated the above result showing a significant spatial variation during post monsoon and pre monsoon (Table 4). The enhanced chlorophyll *a* from mid to upper estuary can be attributed to the elevated concentration of nutrients (DIN&DIP). The relationship between DIN-DIP and chlorophyll *a* indicated a high concentration of these parameters from mid to upper estuary. Tao et al. 2021 found that the concentration of dissolved nutrients exhibited a clear decreasing trend from the upstream (freshwater) to downstream (coastal water). The study continued to explain that DIN-DIP sources are riverine inputs and land-based pollutants from urban settlements. Additionally, nutrients (DIN&DIP) positively contributed to chlorophyll *a* by the uptake process, whereas, salinity negatively influenced the chlorophyll *a* which is characterized by the external driving forces (tides, bathymetry). The negative correlation of chlorophyll *a* with salinity in post monsoon ($r=-0.74$, $p<0.05$) and pre monsoon ($r=-0.83$, $p<0.05$) and also with DO ($r=-0.83$, $p<0.05$) substantiates the above finding, that the eutrophic zone of the estuary lies in low saline and oxygen-deficient region (Table 3a&b). Bužančić et al. 2016 stated that the anthropogenic inputs such as agro-fertilizer waste, urban runoff, discharges from wastewater treatment plants, domestic/sewage discharges and degradation of organic nitrogenous matter lead to eutrophication which can hike chlorophyll *a* (phytoplankton biomass). The extreme ramification of eutrophication caused by the oxidation of organic matter is the depletion of dissolved oxygen in the system. The organic matter is either added to the estuary or produced within the estuary as an outcome of excessive nutrient input (Boesch et al. 2001). Spearman's correlation between chlorophyll *a* with nutrients during the study period indicated a positive correlation of chlorophyll *a* with DIN during post monsoon ($r=0.76$, $p<0.05$) and pre monsoon ($r=0.79$, $p<0.05$) which may be

due to the adding up of nutrients in the upper estuary through anthropogenic nutrient input that exceeded phytoplankton uptake. Although a positive correlation between chlorophyll *a* and BOD was seen in post monsoon, a significant positive correlation ($r=0.74$, $p<0.05$) was observed only during pre monsoon which indicated that high BOD is mainly by the decomposition of high phytoplankton biomass (chlorophyll *a*) and partly by allochthonous inputs of organic matter (Mallin et al. 2006; Faragallah et al. 2009).

It was also observed that the eutrophic environment not only enhanced the chlorophyll *a* it also spiked the phytoplankton abundance (Figure 2h). In the upper estuary, a 2-fold increase in abundance was observed during post monsoon (av. 94.8×10^5 cells l^{-1}) than during pre monsoon (av. 42.7×10^5 cells l^{-1}) which can be attributed to the occurrence of opportunistic species that can flourish in the enriched environment. Species like *Merismopedia* sp. and *Scenedesmus* sp. are known to proliferate in relatively high phosphate content. The phosphate loving nature of these species was responsible for the drawdown of phosphate in the upper estuary during post monsoon and in pre monsoon at station U8 (Table 1 and 2). The present study could understand that phosphate concentration could be the dominant factor influencing the success of cyanophytes and chlorophytes. Smith, 1990 suggested that absolute P concentrations rather than N:P ratios could be the dominant factor influencing the success of planktonic cyanobacteria (*Merismopedia* sp.). The experimental study made by Pham and Bui, 2020 reported that *Scenedesmus* sp. can effectively draw down 97% of phosphate from wastewater. Microalgae have high potential in the removal of inorganic nutrients especially phosphate from wastewater, *Scenedesmus* sp. is one among the widely studied microalgae for the above-mentioned purpose (Li et al. 2010). In addition to the phosphate concentration, salinity is also a determining factor for their proliferation. During pre monsoon the upper estuary picked up salinity (av.15.2ppt) due to tidal action thereby resulting in the decrease of *Merismopedia* sp. abundance. The study made by Lueangthuwapranit et al. 2011 explained that as the level of dissolved salts decreases, the densities of freshwater forms like *Merismopedia* sp., *Closterium* spp., *Cosmarium* sp., *Spirogyra* sp., etc increase. The abundance of *Scenedesmus* sp. dramatically came down with an increase in salinity and was eliminated in the lower estuary (Zhu et al. 2015).

Carlson's Trophic State Index (TSI) is a biomass-related trophic state index, therefore it was used to identify the pollution status of the estuary and defined mid to upper estuary as eutrophic. Meanwhile, the lower to mid estuary exhibited oligotrophy to mesotrophy (Figure 3a&b). The mid to upper estuary (Stations U5-U8) received the maximum anthropogenic input compared to lower to mid estuary (Stations U1-U4). The eutrophic zone of the estuary spiked the phytoplankton biomass and the lack of flushing led to their accumulation. Gameiro et al. 2004 reported that low river inputs and

long water residence time allow the accumulation of phytoplankton and the development of blooms. Mourao et al. 2020 studied the water quality and eutrophication in the Curuca Estuary, Brazil and took TSI and TRIX to identify alterations and their possible origin in the waters of Curuca municipality. Their study defined that water bodies receiving high nutrient load through anthropogenic interventions are susceptible to eutrophication due to their hydrologic characteristics and diffuse sources of pollution.

4.2 Spatial distribution of phytoplankton community in different salinity and DO realm

Phytoplankton in terms of quantity, community structure and physiological status is closely related to the salinity gradients in the estuary (Ahel et al. 1996). The dominance of diatoms (>70%) was observed in euhaline (>30ppt) and polyhaline (30-18ppt) region of the estuary during post monsoon and pre monsoon (Figure 4). The predominance of diatoms in an estuarine ecosystem is not only due to its high rate of division but also to its euryhaline ability (Ribeiro et al. 2003). Furthermore, in the mesohaline region, cyanophytes (33%) and chlorophytes (23%) propagated, thereby, the percentage contribution of diatoms was brought down from >89% to 37%. In the oligohaline region (eutrophic region), a tremendous decrease in diatoms contribution (18%) was observed due to the overwhelming effect of cyanophytes (76%). The drastic decrease of diatoms was mainly due to the outnumbering of cyanophytes and also due to the competition for the same nutrient source. Even though diatoms are euryhaline, the probable reason for the drastic decrease could be due to the allelopathic effect of cyanophytes. Allelopathy can influence the competition between different photoautotrophs for the same resource and can bring changes to the species succession in the phytoplankton community. Gross, 2003 study stated that cyanophytes can produce effective allelochemicals interfering with the growth of competing algae. Therefore, the present study is also establishing the fact that cyanophytes can have an allelopathic effect on the diatom community.

Phytoplankton communities in different oxygen realms revealed that the oxic zones exhibited the dominancy of diatoms during both seasons (Figure 5). Diatoms in the oxic zone during post monsoon were represented by 78% and in pre monsoon it was 87%. Similarly, cryptophytes and dinoflagellates are also inhabited in the oxic zone in post monsoon (17%) and pre monsoon (12%). The hypoxic-eutrophic zone of the estuary exhibited the dominance of cyanophytes during post monsoon (65%) and pre monsoon (44%). Funkey et al. 2014 reported that nutrient over-enrichment triggers the occurrence of cyanobacteria to bloom condition in aquatic ecosystems. In the Baltic Sea, cyanobacteria regularly occur in the late summer months and their abundance is attributed to human-induced eutrophication. Radwan et al. 1994 mentioned that cyanophytes can flourish in highly organic polluted water. The study also could uncover the complete absence of chlorophytes,

cyanophytes and euglenophytes in the euhaline to polyhaline region of the estuary and emphasized their brackish to freshwater nature. Cañavate et al. 2019 reported the inverse relationship of cyanobacteria, chlorophytes, and euglenophytes to salinity.

4.3 Seasonal and spatial distribution of Phytoplankton assemblage in Ulhas estuary

Seasonal and spatial distribution of phytoplankton assemblage study exhibited high density and diversity in meso-oligohaline-hypoxic region of the estuary (Plate 1). Phytoplankton assemblages were dominated by diatoms (*Thalassiosira* sp., *Cylindrotheca* sp., *Odontella* sp., *Pleurosigma* sp., *Nitzschia* sp., *Rhizosolenia* sp., *Skeletonema* sp., *Cyclotella* sp.), chlorophytes (*Scenedesmus* sp., *Dictyosphaerium* sp. and *Ankistrodesmus* sp.) and cyanophytes (*Merismopedia* sp., *Oscillatoria* sp., *Anabaena* sp. and *Spirulina* sp.) during the study period (Table 1&2). Salinity status and anthropogenically generated nutrients affected the composition of phytoplankton as individual taxa had diverse requirements. Spearman's correlation was carried out and observed that chlorophytes ($r=0.90$, $p<0.01$) and cyanophytes ($r=0.84$, $p<0.01$) had a significant positive correlation with DIN during post monsoon (Table 3a). While in pre monsoon in addition to chlorophytes ($r=0.79$, $p<0.05$) and cyanophytes ($r=0.79$, $p<0.05$), diatoms ($r=0.74$, $p<0.05$) also showed significant positive correlation with DIN (Table 3b). The studies made by Ramaiah et al. 1998 in Mumbai Harbour-Thane estuarine system and De et al. 1994 in Hooghly Estuary illustrated that sewage as well as industrial wastes, principally sustained diatoms like *Thalassiosira* sp., *Skeletonema* sp., *Nitzschia* sp. and *Rhizosolenia* sp. in high density. Therefore, the outnumbering of *Thalassiosira* sp. in the present study can be related to their ability to withstand organic pollution. Cyanophytes and chlorophytes which are less tolerant to salinity were restricted to the low saline region of the estuary and observed only in the meso-oligohaline zone (Akoma, 2008; Opute, 2000). Additionally, these species were dominant at stations (stations U5-U8) that received high nutrient load, industrial wastewater and domestic/sewage effluents (Wang et al. 2013). Salinity and DO are together impacting the phytoplankton community structure in Ulhas Estuary. The occurrence of phytoplankton species in the low saline region and their ability to thrive in hypoxic-meso-oligohaline region of the estuary together play a role in the high phytoplankton biomass and abundance.

Many authors used phytoplankton species as an indicator of water quality and pollution as well as eutrophication. Ajayan and Ajit 2017 reported a few pollution-indicating phytoplankton in their study which were organic pollution-indicating species like *Oscillatoria* sp., *Euglena* sp., *Lepocinclis* sp., *Nitzschia* sp., *Synedra* sp. and *Ankistrodesmus* sp. Zargar and Ghosh 2006 reported that excessive growth of certain phytoplankton genera like *Scenedesmus* sp., *Anabaena* sp., *Oscillatoria* sp., *Melosira* sp. indicate nutrient enrichment of aquatic bodies. Wang et al. 2013 spoke about the

dominance of diatoms, chlorophytes and cyanophytes in eutrophic waters. Davies and Ugwumba 2013 studied the nutrient-phytoplankton relationship in a tidal-influenced creek, Okpoka Creek, Nigeria, observed few phytoplankton as organic pollution indicators (*Nitzschia* sp., *Cyclotella* sp., *Scenedesmus* sp. *Oscillatoria* sp. and *Anabaena* sp.). The present study also could identify a few organic pollutant indicators like *Nitzschia* sp., *Cyclotella* sp., *Anabaena* sp., *Scenedesmus* sp., *Ankistrodesmus* sp., *Oscillatoria* sp., *Merismopedia* sp.

Conclusion

The spatio-temporal variation of hydrographical parameters, nutrients and phytoplankton components depicted that the study region underwent salinity gradation based on dilution and there existed four different salinity regimes. Dissolved oxygen which is an important water quality parameter exhibited two oxygen realms (oxic and hypoxic) along the estuary. The main cause for the hypoxia was anthropogenic interventions such as agro-fertilizer waste, urban runoff, industrial effluents and discharges from wastewater treatment plants. The study continued to explain that DIN-DIP sources are riverine inputs and land-based pollutants from urban settlements. These allochthonous inputs enhanced chlorophyll *a* (phytoplankton biomass) that led to hypoxia through organic matter degradation which ultimately increased the BOD concentration. Phytoplankton in terms of quantity, community structure and physiological status is closely related to the salinity gradient, DO zonation and nutrient concentration in the estuary. Diatoms contributed more to euhaline to polyhaline region of the estuary while cyanophytes and chlorophytes which are less tolerant to salinity were observed in the meso-oligohaline zone of the estuary. The allelopathic nature of cyanophytes was the reason for the overwhelming decrease in the diatom contribution in the meso-oligohaline region of the estuary. Further studies on the phytoplankton genera and their ability to thrive and proliferate in hypoxic region should be investigated to effectively understand their mechanism. Opportunistic species like *Nitzschia* sp., *Cyclotella* sp., *Anabaena* sp., *Scenedesmus* sp., *Ankistrodesmus* sp., *Oscillatoria* sp., *Merismopedia* sp. acted as organic pollutant indicators. The Trophic Status Index (TSI) study concluded that the upper estuary is eutrophic regardless of the season especially the meso-oligohaline-hypoxic region of the estuary. To maintain the sustainability of the estuary throughout, the discharge of effluents should be regulated or metered by the policymakers and a strict monitoring plan should be implemented. Therefore, seasonal observation of hydrographical parameters, nutrients and phytoplankton biomass along with their abundance will be very vital to know the impact of the anthropogenic interventions.

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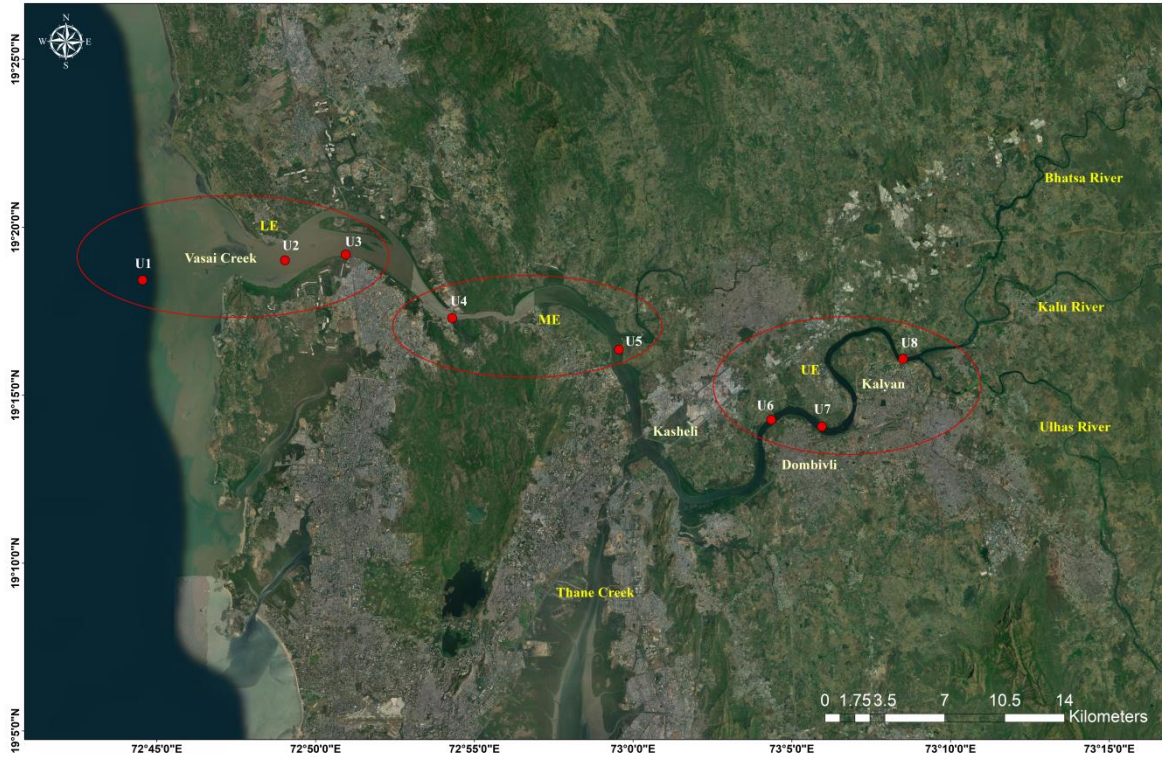


Figure 1. Sampling locations in Ulhas Estuary showing three zones. LE-Lower Estuary, ME=Mid Estuary and UE=Upper Estuary (lower estuary-U1, U2 & U3), (mid estuary-U4& U5) and (upper estuary-U6, U7&U8)

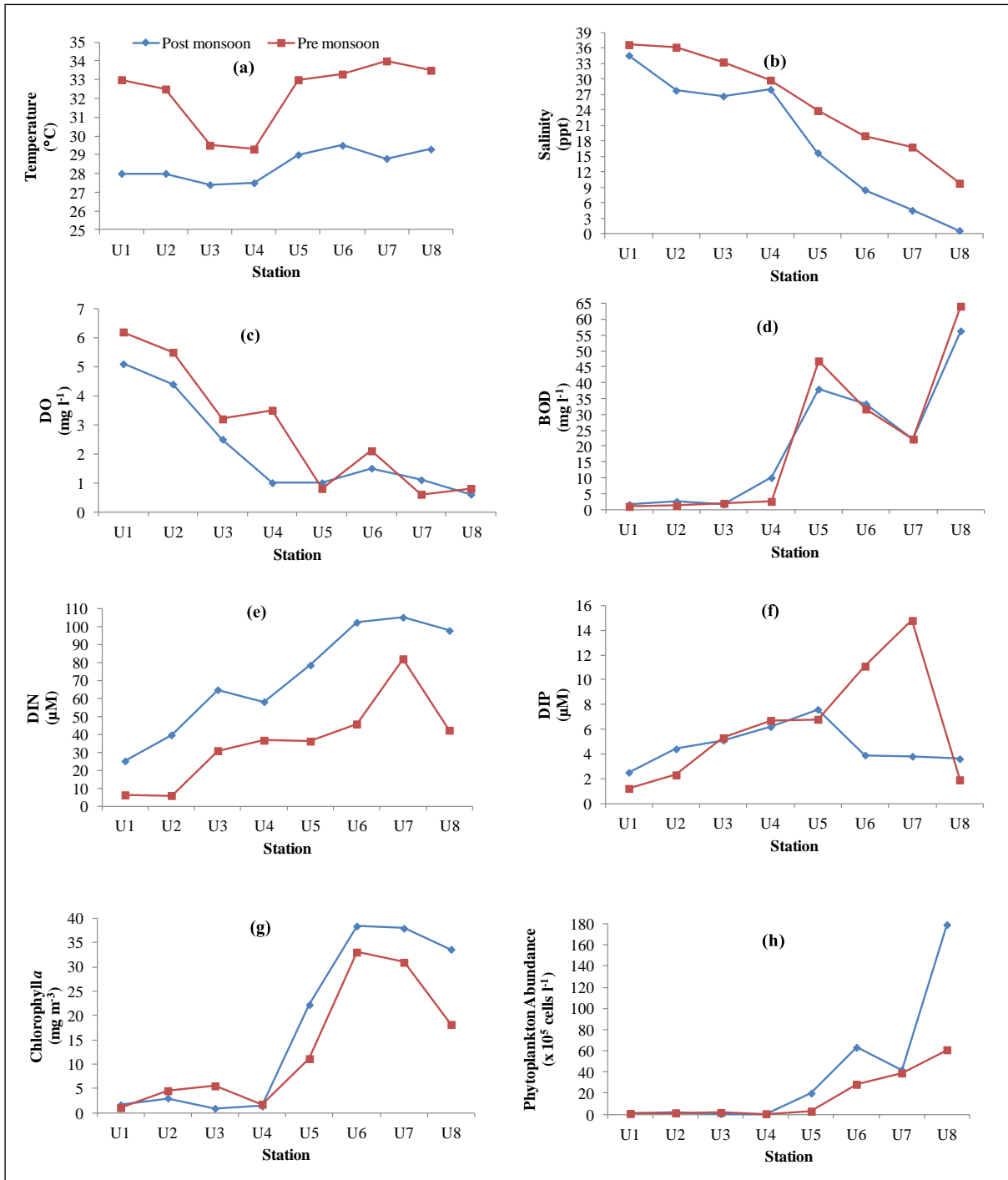


Figure 2. Seasonal and spatial distribution of (a) Temperature, (b) Salinity (c) Dissolved Oxygen (DO), (d) Biochemical Oxygen Demand (BOD), (e) Dissolved Inorganic Nitrogen (DIN), (f) Dissolved Inorganic Phosphate (DIP) (g) Chlorophyll *a*, (h) Phytoplankton Abundance in Ulhas Estuary

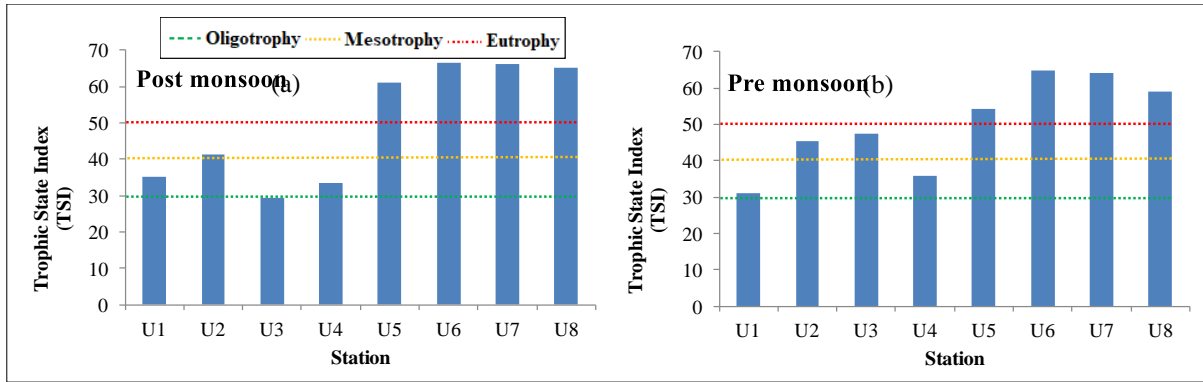


Figure 3. Trophic State Index (TSI) in Ulhas Estuary. (a) Post monsoon and (b) Pre monsoon. The green dash lines represents oligotrophy, yellow dash lines represents transition from oligotrophy to mesotrophy, red dash lines represents transition from mesotrophy to eutrophy.

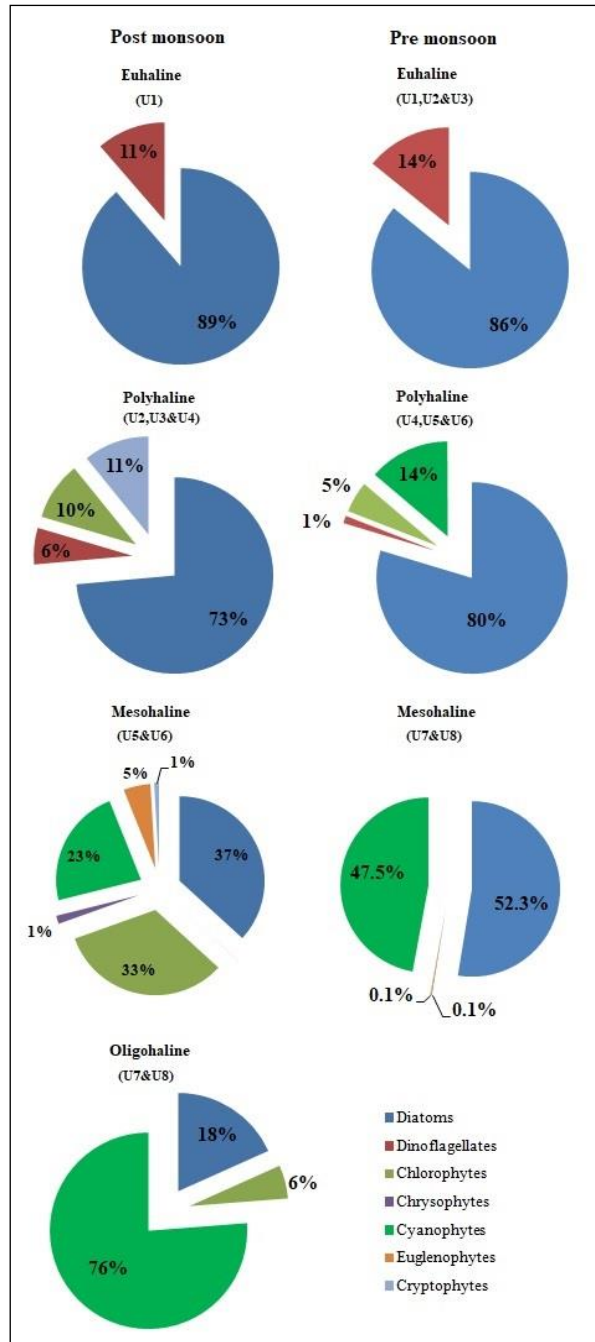


Figure 4. Seasonal distribution of phytoplankton community along the salinity gradient during post monsoon and pre monsoon in Ulhas Estuary

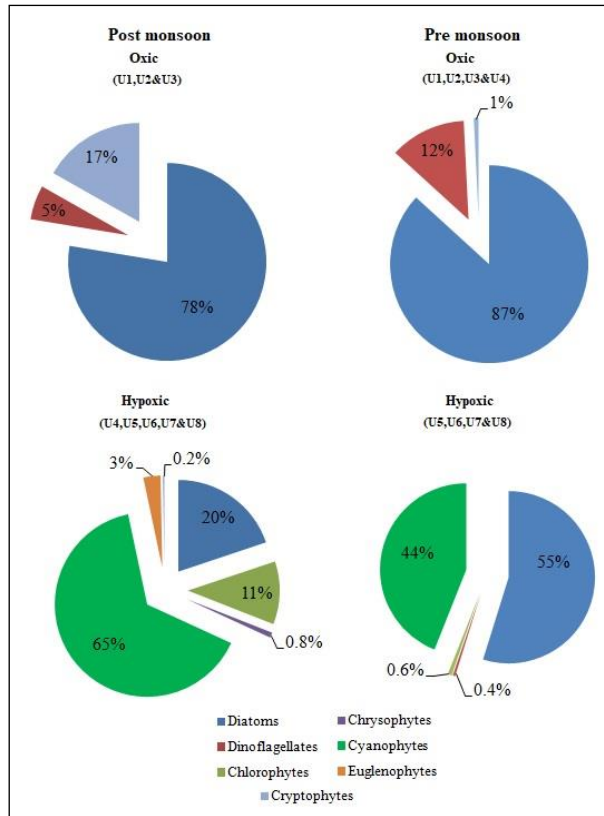


Figure 5. Seasonal distribution of phytoplankton community during post monsoon and pre monsoon against DO in Ulhas Estuary

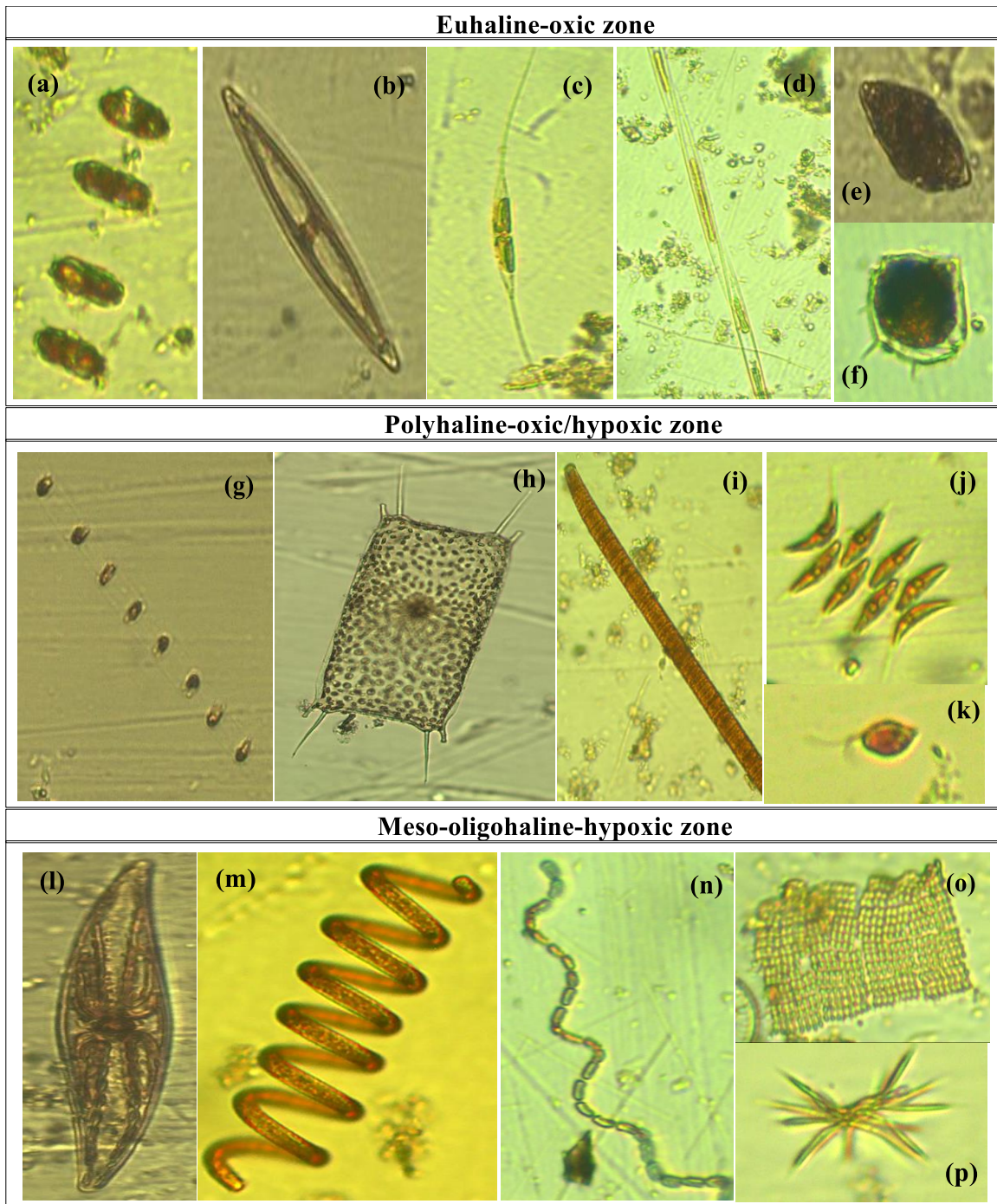


Plate 1: Photographs of phytoplankton genera along the salinity gradient and in different oxygen realm in Ulhas Estuary
 (a) *Thalassiosira* sp. (b) *Navicula* sp. (c) *Cylindrotheca* sp. (d) *Pseudo-nitzschia* sp.
 (e) *Gyrodinium* sp. (f) *Peridinium* sp. (g) *Skeletonema* sp. (h) *Odontella* sp. (i) *Oscillatoria* sp.
 (j) *Scenedesmus* sp. (k) *Plagioselmis* sp. (l) *Pleurosigma* sp. (m) *Spirulina* sp.
 (n) *Anabaena* sp. (o) *Merismopedia* sp. (p) *Ankistodesmus* sp. (Euhaline: a-f;
 Polyhaline: a-c, g-k; Meso-oligohaline: a, c, g, i, j, l-p).

Table 1. Phytoplankton genera ($\times 10^5$ cell l^{-1}) in different salinity-oxygen gradient in Ulhas Estuary during post monsoon

Station	U1	U2	U3	U4	U5	U6	U7	U8
Depth	9 m	8 m	11 m	14 m	14 m	12 m	12 m	5 m
Salinity gradient	Euhaline	Polyhaline			Mesohaline		Oligohaline	
Oxygen gradient	Oxic			Hypoxic				
Diatoms								
<i>Amphiprora</i> sp.	0	0.06	0	0	0	0	0	0
<i>Amphora</i> sp.	0.02	0.004	0.02	0	0	0.24	0	0
<i>Aulacoseira</i> sp.	0	0	0	0	0	0	0	0.44
<i>Chaetoceros</i> sp.	0.04	0.18	0	0	0.04	0	0	0
<i>Coscinodiscus</i> sp.	0.002	0	0	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0.02	0	0.08	0	0	0.002
<i>Cylindrotheca</i> sp.	0.1	0.12	0.02	0.02	0.06	0.64	0.90	0.04
<i>Diploneis</i> sp.	0	0	0	0	0.02	0	0	0
<i>Fragilaria</i> sp.	0	0	0	0	0	0.04	0	0
<i>Guinardia</i> sp.	0.02	0	0	0	0	0.12	0	0
<i>Lithodesmium</i> sp.	0.02	0	0	0	0	0	0	0
<i>Melosira</i> sp.	0	0	0	0	0	0	0.16	0
<i>Navicula</i> sp.	0.10	0.08	0.06	0	0.02	0.14	0	0.12
<i>Nitzschia</i> sp.	0	0	0	0	0	0.44	0.006	0.08
<i>Odontella</i> sp.	0.006	0.02	0	0.002	0.22	0.012	0.46	0
<i>Pleurosigma</i> sp.	0.002	0.02	0	0	0.08	0.30	0.014	0.12
<i>Pseudo-nitzschia</i> sp.	0.04	0	0.08	0.02	0	0	0	0
<i>Rhizosolenia</i> sp.	0	0.04	0.004	0.008	0.30	0.004	0.08	0
<i>Skeletonema</i> sp.	0	0	0	0	0.52	0.24	0.76	0
<i>Surirella</i> sp.	0	0.02	0	0	0	0	0	0
<i>Synedra</i> sp.	0	0.06	0	0	0	0	0	0
<i>Thalassiosira</i> sp.	0.1	0.82	0.1	0	15.04	13.00	28.00	9.20
<i>Thalassiothrix</i> sp.	0.02	0	0.02	0.04	0	0	0	0
Dinoflagellates								
<i>Alexandrium</i> sp.	0	0.02	0.02	0	0	0	0	0
<i>Gymnodinium</i> sp.	0	0	0	0	0	0.006	0	0
<i>Gyrodinium</i> sp.	0.06	0.02	0.02	0	0	0	0	0
<i>Peridinium</i> sp.	0	0	0.02	0	0	0	0	0
Chlorophytes								
<i>Actinastrum</i> sp.	0	0	0	0	0	0	0	0.20
<i>Ankistrodesmus</i> sp.	0	0	0	0	0	10.00	1.60	0
<i>Chlorococcus</i> sp.	0	0	0	0	0	0	0.40	0
<i>Closterium</i> sp.	0	0	0	0	0	0	0.60	0.04
<i>Dictyosphaerium</i> sp.	0	0	0	0	0	8.14	0.56	0
<i>Monoraphidium</i> sp.	0	0	0	0	0	0	0	1.24
<i>Oocystis</i> sp.	0	0	0	0	0	0.16	0.16	0
<i>Scenedesmus</i> sp.	0	0	0	0.08	0.36	9.20	6.10	1.20
<i>Selenastrum</i> sp.	0	0	0	0	0	0.20	0	0
<i>Ulothrix</i> sp.	0	0	0	0	0	0	0	0.04
Chrysophytes								
<i>Dinobryon</i> sp.	0	0	0	0	0.84	0.48	0	0
Cyanophytes								
<i>Anabaena</i> sp.	0	0	0	0	1.94	9.94	1.42	0
<i>Merismopedia</i> sp.	0	0	0	0	0	7.60	0.48	163.20
<i>Oscillatoria</i> sp.	0	0	0	0	0	0.10	0.14	1.08
<i>Spirulina</i> sp.	0	0	0	0	0	0.014	0.06	2.16
<i>Trichodesmium</i> sp.	0	0	0	0	0	0.006	0	0
Euglenophytes								
<i>Eutreptiella</i> sp.	0	0	0	0	0	2.20	0	0
Cryptophytes								
<i>Plagioselmis</i> sp.	0	0.16	0	0	0	0	0	0
<i>Teleaulax</i> sp.	0	0	0	0.02	0.4	0	0	0

Table 2. Phytoplankton genera ($\times 10^5$ cell l^{-1}) in different salinity-oxygen gradient in Ulhas Estuary during pre monsoon

Station	U1	U2	U3	U4	U5	U6	U7	U8
Depth	9 m	8 m	11 m	14 m	14 m	12 m	12 m	5 m
Salinity gradient	Euhaline			Polyhaline			Mesohaline	
Oxygen gradient	Oxic				Hypoxic			
Diatoms								
<i>Aulacoseira</i> sp.	0	0	0	0	0	0	0	0.22
<i>Cerataulina</i> sp.	0	0	0	0	0.008	0.28	1.06	0
<i>Chaetoceros</i> sp.	0	0	0.24	0	0	0	0.18	0
<i>Coscinodiscus</i> sp.	0	0.06	0.014	0	0	0	0	0
<i>Cyclotella</i> sp.	0	0	0	0	0	0.34	0.03	4.00
<i>Cylindrotheca</i> sp.	0.06	0.12	0.34	0.12	0.04	1.68	3.26	2.26
<i>Diploneis</i> sp.	0	0.002	0	0	0	0	0	0
<i>Ditylum</i> sp.	0	0.02	0.006	0	0	0	0	0
<i>Guinardia</i> sp.	0	0.04	0.10	0	0.30	0.22	0.08	0
<i>Gyrosigma</i> sp.	0	0	0	0	0	0	0	0.002
<i>Leptocylindrus</i> sp.	0	0	0	0	0.006	0	0	0
<i>Navicula</i> sp.	0	0.08	0.06	0.08	0.12	0	0.06	0
<i>Nitzschia</i> sp.	0	0.18	0.04	0.08	0	0	0.004	0.006
<i>Odontella</i> sp.	0	0	0.06	0	1.10	2.00	0.24	1.10
<i>Pleurosigma</i> sp.	0	0.01	0	0	0.04	0.14	0.74	8.00
<i>Pseudo-nitzschia</i> sp.	0.06	0.14	0.08	0	0.08	0	0	0
<i>Rhizosolenia</i> sp.	0.04	0.04	0.04	0	0.10	0	0.04	0
<i>Skeletonema</i> sp.	0.20	0	0	0	0.72	4.10	1.48	0
<i>Thalassionema</i> sp.	0	0.04	0.14	0.04	0	0	0	0
<i>Thalassiosira</i> sp.	0.14	0.24	0.36	0.16	0.18	17.00	29.20	0.58
<i>Triceratium</i> sp.	0	0.08	0.02	0	0	0.004	0	0
Dinoflagellates								
<i>Akashiwo</i> sp.	0	0.04	0	0	0	0	0	0
<i>Ceratium</i> sp.	0.08	0.04	0	0	0	0	0	0
<i>Gonyaulax</i> sp.	0	0	0	0	0.004	0	0	0
<i>Gymnodinium</i> sp.	0	0	0.06	0.004	0.06	0	0	0
<i>Peridinium</i> sp.	0.04	0.12	0	0	0.06	0.18	0.08	0
<i>Prorocentrum</i> sp.	0	0.02	0	0	0	0	0	0
<i>Protoperidinium</i> sp.	0.002	0.04	0	0	0.14	0.01	0	0
<i>Warnowia</i> sp.	0	0	0.06	0	0	0	0	0
Chlorophytes								
<i>Ankistrodesmus</i> sp.	0	0	0	0	0	0	0	0.01
<i>Closterium</i> sp.	0	0	0	0	0	0	0.004	0
<i>Scenedesmus</i> sp.	0	0	0	0	0	0	0	0.16
<i>Sphaerocystis</i> sp.	0	0	0	0	0	0.64	0	0
Cyanophytes								
<i>Merismopedia</i> sp.	0	0	0	0	0	0	0.48	41.60
<i>Oscillatoria</i> sp.	0	0	0	0	0	1.66	2.00	3.00
<i>Spirulina</i> sp.	0	0	0	0	0	0	0.002	0
Cryptophytes								
<i>Cryptomonas</i> sp.	0	0	0	0.008	0	0	0	0

Table 3 (a). Summary of Spearman's correlation of chlorophyll *a* and phytoplankton community with physico-chemical parameters during post monsoon in the Ulhas Estuary

	Dia	Dino	Chloro	Chryso	Cyano	Eugl	Crypto	Chl. <i>a</i>	Sal	DO	BOD	DIN	DIP
Dia	1												
Dino	-0.46	1											
Chloro	0.71*	-0.65	1										
Chryso	0.51	-0.24	0.46	1									
Cyano	0.71*	-0.56	0.88**	0.36	1								
Eugl	0.25	0.09	0.59	0.54	0.44	1							
Crypto	0	-0.29	-0.25	0.37	-0.33	-0.28	1						
Chl. <i>a</i>	0.86**	-0.46	0.88**	0.45	0.86**	0.58	-0.19	1					
Sal	-0.71*	0.55	-0.78*	-0.23	-0.91**	-0.25	0.3	-0.74*	1				
DO	-0.26	0.90**	-0.58	-0.23	-0.61	0.08	-0.16	-0.30	0.61	1			
BOD	0.62	-0.81*	0.78*	0.51	0.86**	0.25	0.14	0.69	-0.81*	-0.85**	1		
DIN	0.74*	-0.57	0.90**	0.34	0.84**	0.41	-0.33	0.76*	-0.91**	-0.55	0.71*	1	
DIP	-0.14	-0.24	-0.12	0.44	-0.32	-0.08	0.74*	-0.33	0.17	-0.27	0.12	-0.05	1

*p<0.05; **p<0.01 (Dia=Diatoms; Dino=Dinoflagellates; Chloro=Chlorophytes; Chryso=Chrysoephytes; Cyano=Cyanophytes; Eugl=Euglenophytes; Crypto=Cryptophytes; Chl. *a*=Chlorophyll *a*; Sal=salinity; DO=Dissolved Oxygen; BOD= Biochemical Oxygen Demand; DIN=Dissolved Inorganic Nitrogen; DIP=Dissolved Inorganic Phosphate).

Table 3 (b). Summary of Spearman's correlation of chlorophyll *a* and phytoplankton community with physico-chemical parameters during pre monsoon in the Ulhas Estuary

	Dia	Dino	Chloro	Cyano	Crypto	Chl. <i>a</i>	Sal	DO	BOD	DIN	DIP
Dia	1										
Dino	-0.02	1									
Chloro	0.79*	-0.3	1								
Cyano	0.79*	-0.52	0.91**	1							
Crypto	-0.58	-0.41	-0.28	-0.28	1						
Chl. <i>a</i>	0.95**	-0.02	0.85**	0.76*	-0.41	1					
Sal	-0.79*	0.43	-0.79*	-0.87**	0.08	-0.83*	1				
DO	-0.85**	0.20	-0.58	-0.7	0.25	-0.83*	0.90**	1			
BOD	0.64	-0.19	0.63	0.65	-0.08	0.74*	-0.90**	-0.83*	1		
DIN	0.74*	-0.45	0.79*	0.79*	0.08	0.79*	-0.88**	-0.79*	0.69	1	
DIP	0.59	0.14	0.35	0.24	0.08	0.67	-0.5	-0.65	0.40	0.69	1

*p<0.05; **p<0.01 (Dia=Diatoms; Dino=Dinoflagellates; Chloro=Chlorophytes; Cyano=Cyanophytes; Crypto=Cryptophytes; Chl. *a*=Chlorophyll *a*; Sal=salinity; DO=Dissolved Oxygen; BOD= Biochemical Oxygen Demand; DIN=Dissolved Inorganic Nitrogen; DIP=Dissolved Inorganic Phosphate).

Table 4. Mann-Whitney U Test

Mann-Whitney U Test	Temperature	Salinity	DO	BOD	DIN	DIP	Chl.a	Abundance
Lower to Mid estuary vs Mid estuary to Upper estuary								
Post Monsoon (p value)	0.0294*	0.0286*	0.1465	0.0286*	0.0286*	0.8857	0.0286*	0.0286*
Pre monsoon (p value)	0.0421*	0.0286*	0.0286*	0.0286*	0.0571	0.20	0.0286*	0.0286*

* p<0.05; DO=Dissolved Oxygen; BOD=Biochemical Oxygen Demand; DIN=Dissolved Inorganic Nitrogen;
 DIP=Dissolved Inorganic Phosphate; Chl.a =Chlorophyll *a*