# Investigating the impacts of treated effluent discharge on coastal water health (Visakhapatnam, SW coast of Bay of Bengal, India)

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#### **Abstract**

The present study investigated the impacts of treated effluent discharge on physicochemical and biological properties of coastal waters from three pharmaceuticals situated along the coast of Visakhapatnam (SW Bay of Bengal). Seawater samples were collected (during the months of December' 2013; March'2014 and April' 2014) from different sampling locations (Chippada: CHP; Tikkavanipalem:TKP; and Nakkapalli: NKP) at 0m and 30m depths within 2km radius (0.5km = inner; 1km = middle and 2km = outer sampling circles) from the marine outfall points. Physicochemical and biological parameters, which differed significantly within the stations, were likely to be influenced by strong seasonality rather than local discharge. Dissolved oxygen variability was tightly coupled with both physical and biological processes. Phytoplankton cell density and TChla concentrations were significantly correlated with dissolved inorganic nutrient concentrations. CHP (December) represented a diatom bloom condition where the highest concentrations of diatom cells, TChla, dissolved oxygen coupled with lower zooplankton abundance and low nutrient levels were noticed. The centric diatom, Chaetoceros sp (>50%) dominated the phytoplankton community. TKP (March) represented a post-diatom bloom phase with the dominance Pseudo-nitzschia seriata; zooplankton abundance and nutrient concentrations were minimum. Conversely, NKP (April) represented a warm well stratified heterotrophic period with maximum zooplankton and minimum phytoplankton density. Dinoflagellates abundance increased at this station. Relatively higher water temperature, salinity, inorganic nutrients coupled with very low concentrations of dissolved oxygen, TChla and pH were observed at this station. Copepods dominated the zooplankton communities in all stations and showed their highest abundance in the inner most sampling circles. Treated effluent discharge did not seem to have any significant impact at these discharge points.

**Keywords:** Pharmaceutical effluent, coastal pollution, Visakhapatnam, Bay of Bengal, zooplankton, phytoplankton

#### **Introduction:**

Due to improved medical care, an increasing amount of medicinal products are being manufactured to support the needs of growing population all over the world and consequently, pharmaceutical industry effluent discharge into environment became a major problem (Jørgensen and Halling-Sørensen, 2000; Dębska et al. 2004). The pharmaceuticals and related chemicals are diverse in their therapeutic purposes and mechanisms of action, and may elicit toxic effects in aquatic organisms (Henschel et al. 1997). Some earlier studies reported potential toxicity (mix or individual; Faust et al. 2003) of pharmaceutical products on marine (De Lorenzo and Fleming, 2008), estuarine (Emblidge et al. 2006) and freshwater organisms (Wiegel et al. 2004).

Usually, discharges from pharmaceutical industries as wastewater are channelled to the wastewater treatment plants (WWTP) and then being released as treated effluent in to environment. Nevertheless, many of the compounds released are non-biodegradable and contains several inorganic and organic matters (Dębska et al. 2004) which may not be efficiently removed by conventional (primary, secondary, and tertiary) treatment technologies, leading to an unfavourable accumulation in the aquatic environment (Vieno et al. 2007). Discharging treated effluent from pharmaceuticals may also cause several other impacts to the aquatic systems, like decreasing dissolved oxygen, increasing biochemical oxygen demand, nutrients concentrations and turbidity, etc. These may collectively hinder natural ecosystem processes and essential to monitor.

India has a large coast line and a significant part of its large population live within 40km of the coastlines. Numerous industries have been established along the coastlines of India releasing considerable amount of effluents. India is the largest supplier of bulk drugs to the world market (Larsson, 2008). The state of Andhra Pradesh alone contributes about one-third of the total production of pharmaceuticals in India and one of the largest production centres within India is at Visakhapatnam which is a part of the proposed Special Economic Zone. With the help of 'Andhra Pradesh Industrial Infrastructure Corporation (APIIC)' as a nodal agency several pharmaceutical industries were built for the implementation of pharmaceutical industrial project on a commercial format and the region is often termed as 'Pharma City'. As a consequence, a significant amount of pharmaceutical effluent is being generated within the country and already their treatment, bioremediation and disposal is of great concern (Singh Rana et al. 2014).

Nevertheless, the Ministry of Environment and Forest and Central Pollution Control Board of India set up several criteria for waste water disposal to the adjacent coastal waters. Under this protocol some common physicochemical and biological parameters are routinely monitored to

understand the health of the coastal waters at the discharge points of treated effluent. There are very few studies (Damodhar and Reddy, 2013) reporting the impacts of pharmaceuticals discharge in the natural surface water quality in India and studies from the coastal waters are insufficient. Hence, the present study was conducted at the vicinity of the treated effluent discharge points from three pharmaceutical bulk drug industries along the coast of Visakhapatnam to monitor physicochemical and biological parameters of the coastal waters.

## **Study area and sampling pattern:**

The Bay of Bengal, a semi-enclosed bay forms the eastern arm of the tropical Indian Ocean and is predominantly influenced by the seasonal monsoonal reversal. The bay receives huge amount of fresh water discharge due to the influence of South West Monsoon and a reverse scenario during the dry and cool North East monsoon. Bay of Bengal is characterized by high degree of stratification, warmer sea surface temperature and restricted upwelling (Shenoi et al. 2002) which influences phytoplankton productivity (Prasannakumar et al. 2010). The present study site was at Visakhapatnam, South West coast of Bay of Bengal. Sampling was conducted during December'13, March'14 and April'14 at three different sampling locations, namely, Chippada (CHP; (17°53' - 17°55' N and 83°28' - 83°31' E), Tikkavanipalem (TKP; (17°31' -17°33' N and 83°05' - 83°08' E) and Nakkapalli (NKP; (17°20' -17°21' N and 82°43' - 82°45' E) along the Visakhapatnam coast (Fig. 1).

CHP and TKP are situated 65km apart, whereas NKP is located 45km far from TKP. Two pharmaceutical industries at CHP and NKP are located at a distance of approximately 1.25km away from the high tide line (HTL) of the coast. At TKP, the industry is located 13km far from the coastline. The samples were collected from  $10 \text{km}^2$  area in and around the Marine Out fall Point (MOP/discharge point) from where the effluent is being discharged in to the coastal waters, covering almost 9-12 sampling locations at each station. Furthermore, the sampling stations were divided in to Inner (0.5km), Middle (1km) and Outer (2km) sampling circles considering the distance from the MOP. The aim of such sampling strategy was to understand whether the effluent discharge impacts the coastal water quality and how the effects get diluted spatially with increasing distance from the MOP.

The discharge point to dispose the effluents is positioned at the water depth of approximately at 10 meters, which is approximately 980 meters away from the coast for all three sampling stations. Many channels and creeks especially the rivers like Varaha and Tandava are connected to the sampling site NKP, whereas, river Gosthani is also well connected to the coastal waters at CHP. The

basic physicochemical and biological parameters including salinity, water temperature, dissolved oxygen (DO), pH, total suspended matter (TSM), dissolved inorganic phosphate (DIP), dissolved inorganic silicate (DSi) and dissolved inorganic nitrogen (DIN) including total chlorophyll (TChla), phytoplankton cell density, composition, zooplankton density, community structure and biomass were measured.

#### **Materials and Methods:**

Seawater samples were collected (during the months of December' 2013; March'2014 and April' 2014) from different sampling locations (Chippada: CHP; Tikkavanipalem:TKP; and Nakkapalli: NKP) from both surface (0m) and subsurface ( $\approx$ 30m) within 2km radius (0.5km = inner; 1km = middle and 2km = outer sampling circles) from the MOP. A pre-cleaned 5L Niskin water sampler was used to collect the sample water for the estimation of chemical and biological parameters. Salinity and water temperature were measured by using a Conductivity-Temperature-Depth profiler (CTD; SBE-19 plus, Sea Bird Electronics, USA). DO was estimated by following Winkler's titration method (Grasshoff et al. 1993). Total suspended matter (TSM) was measured based on the weight difference remained on a pre weighed 0.45um polycarbonate filter (Millipore), dried at 40°C and reweighed to quantify TSM (mg L<sup>-1</sup>). Dissolved inorganic nutrients (nitrate, nitrite, ammonia, silicate and phosphate) were analysed following the standard spectrophotometric methods (Grasshoff et al. 1993). Total chlorophyll (TC) was measured following the method of Strickland and Parsons (1972) by soaking in 90% acetone at -20°C overnight for extraction. Measurements were done with the help of Varian CARY eclipse fluorescence spectrophotometer and concentrations were calculated based on the known concentration of standard chlorophyll (Sigma Aldrich, USA). For phytoplankton species composition and cell density was measured by following the methods of Utermohl, 1958 and for enumeration was done following the manuals of Desikachary (1987) and Hasle et al. (1997). Total phytoplankton abundance was calculated in terms of number of phytoplankton cells observed in litre of water sample and is also expressed in terms of relative percentage for each phytoplankton genera identified. Phytoplankton diversity index (H') or Shannonweaver diversity index was calculated according to the following formula:  $H' = -\Sigma(n/N)x \ln(n/N)$ 

Where, n = individual number of species in a community and N = total number of individual in that community.

Zooplankton samples were collected at each station from surface water by horizontal hauling of zooplankton net (mouth area  $0.25m^2$ , mesh size  $200\mu m$ ) for 5 minutes and preserved in 5% formaldehyde solution. A digital flow meter (Hydrobios) was used to determine the volume of water filtered. The collected zooplankton samples were further sub-sampled with the help of Folsom

splitter for quantitative and qualitative analysis in lab. For enumeration and identification standard literatures of Kasturirangan, 1963 and Wimpenny, 1966 were used. Wet biomass of zooplankton was measured as displacement volume (ml 100m<sup>-3</sup>) following the standardized method of Kramer (1972).

#### **Results:**

## Physicochemical parameters:

Minimum, maximum and average values of all physicochemical parameters (temperature, salinity, TSM, dissolved oxygen) are given in Table 1. Seawater temperature (in both surface and subsurface) showed very little variations among the stations and from inner to outer circles. At station CHP, the average surface (25°C) and subsurface (25.3°C) water temperature values were almost identical. The highest temperature (at surface 27.91°C, at subsurface 26.40°C) was observed at TKP among three seasons/stations. Nearly 1°C difference in temperature was noticed between surface and subsurface water masses. At NKP the average temperature showed almost 2°C between surface (26.22°C) and subsurface (24.83°C) waters indicating strong stratification. The maximum salinity values were observed at NKP (> 34psu) followed by TKP (Table 1) and the minimum value was noticed at CHP. There was no significant variation in salinity distribution from inner to outer circles showing less impact of treated effluent discharge on salinity. TSM concentrations were almost 25% and 61% higher at TKP and NKP relative to the value noticed at CHP (22.35±2.49 mg L<sup>-1</sup>). A gradual enhancement (21%) was observed towards the inner circle. Subsurface waters showed higher values of TSM than that of surface for all stations. At NKP, the observed difference between surface and subsurface TSM values was quite high possibly due to stratification.

Surface waters were detected with higher DO concentrations relative to the subsurface waters in all the stations. The highest DO concentrations were observed at CHP (>6.5mg L<sup>-1</sup>) followed by TKP and the lowest concentrations were detected at NKP (< 4 mg L<sup>-1</sup> at the surface and <2 mg L<sup>-1</sup> at the subsurface) (Table 1). The observed DO concentrations at NKP were almost half at the surface and 3.5 times lower at the subsurface. At CHP and TKP, the observed DO values were >6 mg L<sup>-1</sup> at both surface and subsurface waters (Table 1). At CHP the concentrations of DO increased from inner to outer sampling circle in subsurface waters, whereas, a reverse trend was observed in the surface waters. At TKP, there was an increase in DO concentration from the inner to the outer circles in surface waters, whereas, in subsurface waters there was no such trend observed (Table 1). pH values (Table 1) observed at CHP (8.02 -8.23) and TKP (8.15-8.20) were almost similar and comparable with the usual values noticed in coastal waters. There was no significant difference between the values obtained from the surface and subsurface waters. The pH values observed at NKP were

significantly lower (7.43 -8.01) than that of CHP and TKP and showed considerable difference between the surface and subsurface waters indicating a strong stratified condition.

## **Dissolved inorganic nutrients:**

The average concentrations of major dissolved inorganic nutrients are presented in Table 1 (dissolved inorganic nitrogen [DIN] concentrations were the sum of the concentrations of nitrate [NO<sub>3</sub>-1], nitrite [NO<sub>2</sub>-1] and ammonium [NH<sub>4</sub>+1]. The highest DIN concentrations were observed at NKP (6.88-7.51µmolL<sup>-1</sup> and 8.93-9.91 µmolL<sup>-1</sup> in surface and subsurface waters, respectively) and showed an increasing pattern outwards. Similar values of high DIN were observed off Kakinada and Visakhapatnam during April 2014 (unpublished data). CHP (1.52-1.93µmolL<sup>-1</sup> in surface waters and 1.27-2.17 μmolL<sup>-1</sup> in subsurface) and TKP (1.63-2.31μmolL<sup>-1</sup> in surface and 1.42-2.12 μmolL<sup>-1</sup> in subsurface) showed more or less similar DIN values in both surface and subsurface waters and the highest values were observed at the inner circles. The concentrations of DIP (Table 1) showed its highest values at NKP (2.34-3.30 µmolL<sup>-1</sup> in surface and 2.36-2.55 µmolL<sup>-1</sup> in subsurface ). There was a strong increase in DIP concentration from the outer towards the inner circle at NKP. station CHP, the average DIP values were much lower than that of the values observed at station TKP and NKP (0.25-0.31µmolL<sup>-1</sup> in surface and 0.28-0.30 µmolL<sup>-1</sup> in subsurface waters, respectively) (Table 1). At station TKP, the concentrations of DIP in surface were lower (0.39-0.48 μmolL<sup>-1</sup>) than that of the subsurface (0.40-0.52 μmolL<sup>-1</sup>). Dissolved DSi concentrations were >  $15\mu mol L^{-1}$  in both surface and subsurface waters at NKP. At CHP, DSi values were  $> 9~\mu mol L^{-1}$  in both surface and subsurface waters and increased linearly towards the inner circle at the subsurface. The lowest values for DSi were noticed at TKP, both in surface (3.15-5.48 µmolL<sup>-1</sup>) and subsurface waters (3.08-4.54 µmolL<sup>-1</sup>) and an increasing trend was noticed towards the inner circle.

The average DIN:DIP ratios remained well below the classical Redfield ratio (16:1) in all sampling stations (Table 1). At CHP, higher DIN:DIP values observed at the surface in inner circle (6.27±1.95) and increased towards the middle (7.39±2.70) and then decreased towards the outer circles (6.04±4.51). Conversely, in the subsurface waters, the ratio of DIN:DIP increased from 4.48±1.17 to almost 51% at the middle and outer circle. Whereas, the DIN:DIP ratio was 29% lower at the surface and further reduced by 36% in the subsurface waters at TKP than CHP. At NKP, the DIN:DIP ratio was 40% higher than that of TKP at the surface (Table 1), whereas, there was no difference observed in subsurface waters between these two stations. Similarly, DIN:DSi ratios were also lower than the modified Redfield ratio of (1:1). The observed values of DIN:DSi at the subsurface (1:0.58) waters was higher than the surface waters at NKP (1:0.46). A reverse trend was observed at TKP (surface 1:0.53, subsurface 1:0.47). The DIN:DSi ratios of the station CHP (surface 1:0.10, subsurface 1:0.08) were almost 4 times lower than that of TKP and NKP (Table 1).

## Total chlorophyll (TChla), phytoplankton cell density, diversity and community composition:

The maximum TChla value (Table 1) was noticed at CHP (0.43µgL<sup>-1</sup>), followed by TKP (0.32µgL<sup>-1</sup>) and the minimum value was observed at NKP (0.08µgL<sup>-1</sup>) and similar trend was also noticed in phytoplankton cell abundance. Phytoplankton cell density in the surface waters of CHP was 8.78-15.27x10<sup>4</sup> cells L<sup>-1</sup> and relatively lower values were noticed in subsurface waters (4.81-6.60x10<sup>4</sup>cells L<sup>-1</sup>). At TKP phytoplankton cell abundance was higher at the subsurface waters  $(14.08-33.64\times10^4 \text{ cells L}^{-1})$  than that of the surface  $(5.98-7.07\times10^4 \text{ cells L}^{-1})$ . At NKP, cell counts were significantly lower than that of CHP and TKP. There was no trend observed in phytoplankton cell number from the inner to the outer circles in none of the stations. A total of 31 phytoplankton genera were identified during three different study periods, of which 25 were diatom (25, 21 and 20 diatom genera at TKP, CHP and NKP, respectively) and 6 were dinoflagellate (6, 6 and 4 at TKP, CHP and NKP, respectively). Phytoplankton diversity indices ( $\overline{H}$ ) for all the three sampling stations are given in Table 1 and the chain forming centric diatoms showed the highest diversity. The lowest values were noticed at CHP (1.72±0.22 at surface, 1.83±0.17 at subsurface) in the inner circle and reduced towards the middle and outer circle (Table 1). However, this difference was not statistically significant.\_The highest diversity indices were noticed at NKP in both surface  $(2.05\pm0.36)$  and subsurface  $(2.1\pm0.21)$  waters. The higher diversity was found near the inner circles. TKP showed moderate ( $\overline{H}$ ) values relative than that of CHP and NKP.

Phytoplankton species relative % (Table 2) varied considerably among all three sampling stations. At CHP, the phytoplankton community was mainly dominated by the chain forming centric diatom *Chaetoceros* (≈60% relative abundance in the surface and >50% in the subsurface waters). The relative abundance of *Chaetoceros* increased from the inner towards the outer circle in both surface and subsurface waters. The other dominant phytoplankton species observed were *Leptocylindrus danicus*, *Pseudo-nitzschia seriata*, *Thalassionema nitzschioides*, *Dactylosolen fragilissmus* (Table 2). *Nitzschia longissima* contributed only a small fraction (<1%) of the total phytoplankton community at this stations. The dinoflagellate species including *Protoperidinium* sp, *Prorocentrum micans*, *Ceratium furca*, *Dinophysis* sp and *Scrippsiella trochoidea* were common in both surface and subsurface waters (Table 2). Dianoflagellate spores were also noticed.

At TKP (Table 2) the relative abundance of *Chaetoceros* reduced to ≈25% on overage and *Nitzschia longissima* dominated the phytoplankton community in both surface (25.9 - 36.62%) and subsurface waters (27.74-33.34%). *Chaetoceros* were also noticed; however, were relatively lesser than that of CHP. The other abundant phytoplankton species observed at this station were *Pseudonitzschia seriata*, *Skeletonema costatum*, *Leptocylindrus danicus*. *Navicula* sp showed almost equal

distribution from the inner to the outer circle in both surface and subsurface water with very less variability. The dinoflagellate community at TKP were very much similar to CHP in both surface and subsurface waters. The pennate diatom Nitzschia longissima dominated the phytoplankton community at NKP (Table 2). The relative % varied between 12.80 - 34.10% in the surface and 17.70-24% in the subsurface waters. The next dominant species was Thalassionema nitzschioides (8.80 -17.80% and 8.60-12.90% in surface and subsurface waters, respectively). The other dominant phytoplankton species observed were Coscinodiscus radiatus and Asterionella japonica. The relative abundance of dinoflagellate species was considerably higher at the station NKP than the other stations. The dominating dinoflagellate genera were Protoperidinium, Dinophysis, Scrippsiella trochoidea and Prorocentrum micans in surface waters; whereas, Prorocentrum micans and Scrippsiella trochoidea dominated the subsurface waters (Table 2).

## Zooplankton abundance, biomass and community composition:

Zooplankton wet biomass and cell density was measured only for the surface waters. A total of 22 zooplankton genera were identified at the station CHP; whereas, 18 genera at TKP and 14 genera with 1 meroplankton species were identified at the station NKP. The zooplankton number was estimated as cells x10<sup>3</sup>100m<sup>-3</sup> and the highest and lowest zooplankton cell numbers were noticed at NKP and TKP, respectively (Table 1). Zooplankton wet biomass showed a very similar trend. The highest value was noticed at NKP and reduced to almost 42% at CHP and to almost half at TKP (Table 1). Copepods were the most dominant zooplankton group and their relative abundance was consistently high in the inner sampling circles for all three stations (Fig. 2). In all three stations the zooplankton community was dominated by the subclass copepoda. At CHP their % was 68.90 -85.42 followed by rotifers, tintinnids, tunicates and crustacean larvae (Fig. 2a). The relative abundance of the subclass copepoda was highest in the inner circle and reduced almost 24% at the outer circle. Rotifers showed a similar trend with their highest abundance at the inner circle (7.45%) and reduced in the middle (0.28%) and outer circle (1.95%). At TKP copepods relative % ranged between 87.78 -77.91% and a significant reduction was noticed from the inner to the outer circles (Fig. 2b). Unlike CHP, rotifers were not present during the sampling period. Crustacean larvae, cladocerans, polychaete larvae, gastropod larvae were the next abundant zooplankton group. At NKP the relative abundance of copepods were maximum (Fig. 2c) (83.64 -99.23%) and the highest value was noticed in the middle circle (99.33%) which reduced to 83.64% in the outer circle.

## **Correlations between different parameters:**

Considering all values of the measured parameters from three observations, some statistical correlations were done to understand the interactions between physical, chemical and biological

parameters. DO concentrations showed significant linear negative correlation with salinity values ( $R^2 = 0.312$ , p < 0.01, n =70), but insignificant with temperature values ( $R^2 = 0.119$ , p > 0.1). TSM concentrations showed highly significant linear negative correlation with DO concentrations ( $R^2 = 0.519$ , p < 0.001, n =70). pH values revealed a significant positive linear correlation with DO concentrations ( $R^2 = 0.677$ , p < 0.001, n =70). DO values revealed a significant positive correlation with phytoplankton cell counts ( $R^2 = 0.708$ , p < 0.001, n =70) and TChla ( $R^2 = 0.521$ , p < 0.001, n =70). TChla and phytoplankton cell counts, both were exponentially (positive) correlated with DIN ( $R^2 = 0.577$ , p < 0.001, n =70) and DIP ( $R^2 = 0.489$ , p < 0.001, n =70) values. DSi concentrations did not show any significant correlation with TChla; however, cell counts were positively correlated with DSi concentrations ( $R^2 = 0.326$ , p < 0.01, n =70). Phytoplankton cell numbers were negatively correlated with TSM values ( $R^2 = 0.326$ , p < 0.01, n =70). pH values were significantly correlated (logarithmic positive) with both TChla ( $R^2 = 0.427$ , p < 0.001, n =70) and phytoplankton cell density ( $R^2 = 0.555$ , p < 0.001, n =70). Zooplankton abundance and bio-volume showed a logarithmic negative correlation with phytoplankton cell abundance, however, it was not statistically significant (p>0.1).

#### **Discussion:**

The goal of the present study was to understand if treated effluent discharges from the selected pharmaceutical industries are impacting the physicochemical and biological parameters of the coastal waters, but no such trend of was seen. The observed differences in physicochemical and biological parameters between three different coastal stations were mostly due to different sampling seasons which are directly influenced by Indian summer monsoon. Three sampling periods represented distinct physicochemical and biological characteristics; a clear succession of phyto and zooplankton was observed at each station. The overall physicochemical and biological features observed at CHP represented like a diatom bloom scenario with the highest cell and TChla concentrations coupled with the lowest values of diversity index and zooplankton abundance. Occurrences of phytoplankton bloom associated with low salinity and water temperature in this bay during the postmonsoon period (January) has already been reported in our earlier studies (Biswas et al. 2004, Biswas et al. 2010; Shaik et al. 2015). Dominance of larger diatoms like *Chaetoceros* sp Leptocylindrus danicus and Dactylosolen fragilissimus might have inhibited zooplankton active grazing during this time leading to lower zooplankton abundance. Although diatoms have been considered as preferred food items for copepods, since 1993 there are growing evidences showing that diatoms may significantly inhibit copepod egg hatching by producing wound induced aldehydes (Ianora et al. 2004; Ianora and Miralto, 2010). Presence of some anti-miotic compounds in the diatom diet was shown to inhibit cell division during copepod enbryogenesis (Poulet et al. 1994). This can be largely species specific for both diatoms and copepods; nevertheless, some species of *Chaetoceros* sp. has also been reported to show such character (Ianora et al. 1995; Lee et al. 1999). Though we do not have direct evidence to show such activities, the possibility of suppressing zooplankton growth by the dominant diatoms cannot be overruled. However, exceptions have also been reported (Irigoien et al. 2002). Moreover, the increasing TSM concentrations towards the inner circle at CHP could be due to effluent discharge flow which churns up the sediment particle in suspension.

During March at TKP the condition was more like a post-bloom phase with reduced dominance of larger diatom, quite low nutrient concentrations, specially, DIN were relatively lower than DSi (high DIN:DSi values). At this time too zooplankton biomass and abundance became the lowest. Increased diversity index from post to premonsoon was probably due to proliferation of multiple diatom species within the community and has also been reported by D'Costa and Anil (2010) from the west coast of India. According to Margalef (1978) diversity of phytoplankton usually between 1 and 2.5 in coastal waters, 3.5 to 4.5 in the oceanic areas and 5 in open tropical oceanic environments, where phytoplankton productivity will be lower with large number of species. Note that the values reported during this study were within the range of values reported for coastal waters in the respective study areas.

NKP represented a heterotrophic phase when high water temperature, TSM and salinity can largely impede phytoplankton growth. A strong stratification between surface and subsurface water was noticed and was clearly reflected in temperature, DO, DIN and TSM concentration. During the months of March and April (premonsoon) with the weakening of NE monsoon, rise in temperature, flow of saline water from the Indian ocean with no external source of riverine input is a common phenomenon in this bay (Varkey et al. 1996). At this station low TChla values might be due to relatively higher turbidity and significant grazing activity by zooplankton. The observed higher nutrient concentrations at NKP could be attributed to very low consumption by phytoplankton community. Seasonal oxygen depletion is a common phenomena occurring in the coastal shallow waters and mostly controlled by the temperature induced stratification and can be further intensified by enhanced nutrient input. Hence, at NKP high water temperature might have resulted in high heterotrophic activity coupled with significant amount of oxygen consumption due to organic matter degradation. Likewise, a strong positive correlation between thermal stratification and depleted oxygen levels has been observed for a long term period in the coastal waters of Ireland (O'Boyle and Nolan 2010).

The negative correlation with TSM and DO indicated that the presence of higher amount of suspended solids negatively impacted DO concentrations. Particularly, the highest amount of TSM was coupled with the lowest DO concentrations at NKP. The highest TSM levels could be due to churning of sediment influenced by the northerly wind and the northward current prior to the onset of the Southwest monsoon (Varkey et al. 1996; McCreary et al. 1996). Occurrences of turbulent condition in the Indian coastal waters favouring the re-suspension of the bottom sediment due to stirring action (Qasim et al. 1968; Nixon, 1988) is a common phenomenon. Higher TSM concentration absorbs more heat and thus makes the water warmer leading to oxygen loss (Wetzel, 2001). Moreover, TSM can further impede algal photosynthesis by cutting down light and thus may lead to further depletion in DO concentrations. This observation was in well agreement with lower abundance of TChla and phytoplankton abundance at NKP.

Overall, DO values were likely to be influenced by both physical and biological parameters. Salinity showed higher influence on DO distribution relative to temperature. However, autotrophic photosynthesis seemed to influence DO concentration and was seen in its strong positive correlations with TChla and Phytoplankton cell abundance. O'Boyle et al. (2013) conducted an eight-day time series measurement (in every 15 minutes) of DO, pH, salinity, Chla and other parameters during summer in the Lower Slaney estuary, Wexford, and in the Oysterhaven estuary, Ireland. The authors reported that pH and DO were positively correlated. The authors also noticed a tight coupling between TChla and DO values similar to the present study. The significant positive correlation between pH and phytoplankton (both TChla concentrations and cell density) suggested that pH was strongly influenced by biological activities. In general, when any aquatic system is dominated by autotrophy, CO<sub>2</sub> is being removed by the process of photosynthesis and this leads to increase in pH values and was probably the case of CHP and TKP. Conversely, when a system is dominated by heterotrophy, higher amount of CO<sub>2</sub> is being released by microbial respiration relative to photosynthetic carbon uptake and thus reduces pH which was seen at NKP.

Usually, certain physicochemical parameters play a key role in phytoplankton succession and community composition and nutrient availability is one of them (Dugdale, 1967; Ryther and Dunstan, 1971; Smayda, 1980). Consistent lower ratios of DIN:DIP and DIN:DSi suggested that the sampling systems were limited by DIN relative to DIP and DSi. The significant exponential negative correlation between phytoplankton abundance (both cell count and TChla) and DIN/DIP concentrations suggested that phytoplankton growth was mainly influenced by the availability of these nutrients. Low nutrient levels at CHP and TKP were possibly due to active utilization by phytoplankton. A similar observation was also noticed by Bandyopadhyay et al. (2017) in the north-

western coastal Bay of Bengal during the SW monsoon. At NKP the minimum phytoplankton cell density was associated with higher nutrient concentrations indicating restricted consumption of nutrients. However, high temperature and salinity, as well increasing water residence time at NKP can also favour organic matter decomposition, providing a significant amount of nutrient leaching to the water column (Galicka, 1992) coupled with considerable drop in DO and pH level. Biswas et al. (2010) also reported reduced primary production and phytoplankton abundance under high salinity, temperature and higher nutrient levels during a pre-monsoon season in the mangrove waters of Sundarbans (NE Bay of Bengal).

Phytoplankton community of the coastal Bay of Bengal has been reported to be dominated by diatoms (Shaik et al. 2015) and our microscopic analysis also supported this view. Diatom growth in the marine environment can be directly dependent on dissolved silicate concentrations (Sarthou et al. 2005) and there are growing concerns that dissolved silicate plays a key role in coastal water biogeochemical cycling (Conley et al. 1993). However, the high abundance of diatoms (>90%) relative to dinoflagellates could also be attributed to the low DIN:DSi ratios (nitrogen is limited before silicate) and such realms are reported to support diatom dominated systems (Béthoux et al. 2002). The reverse scenario usually supports the proliferation of dinoflagellate growth (Officer and Ryther, 1980) over diatoms. Unlike DIN and DIP, TChla did not show any significant correlation with DSi concentrations, and this suggests that the diatom growth was not limited by silicate availability at these stations.

However, the lowest DSi concentrations were detected at TKP. The demand for DSi by diatoms appears to differ with species (Mochizuki et al. 2002) and such inter-specific differences in silica requirements may be one of the reasons for the observed differences in abundance and distribution of diatom species during three different sampling periods. At CHP during December the large chain forming centric diatom *Chaetoceros* dominated the community and this genera has been reported to uptake dissolved silicate more efficiently at much faster rate relative to the other large diatom like *Rhizosolenia* (Tréguer et al. 1991). *Chaetoceros* grows fast despite its large size and highly competitive for nutrients and inefficiently grazed or even avoided by copepods (Rick & Dürselen, 1995; Roy et al. 1989) which could be a reasonable fact for its higher relative abundance (> 50%) in both surface and subsurface waters of CHP. Biswas et al. (2004) also reported a bloom of *Chaetoceros affinis* during postmonsoon period (February) when water temperature and salinity values were significantly low in a mangrove water in NE Bay of Bengal. On the other hand, pennate diatom *Nitzschia longissima* was the most dominant phytoplankton species observed at both the stations TKP and NKP coupled with higher salinity and water temperature. It has been observed that under fluctuating salinity, *Chaetoceros* dominates, whereas, *Nitzschia longissima* dominated when

the salinity values were high (Zhang et al. 1999). Dinoflagellates being more independent of the nutrient levels, exhibit more diversified trophic behaviour than diatoms. Previous reports indicate that, several species of dinoflagellates can show their prominence at very low nutrient concentrations and consequently a mixo-trophic behaviour can be expected. It is likely that the presence of higher Si:N ratios does not allow any other group than diatom to proliferate in this coastal waters. The higher relative % of dinoflagellates at NKP might have resulted in mixo-trophic behaviour contributing to the net heterotrophy in the study area other than the losses attributed to the zooplankton grazing.

Variability in zooplankton biomass and abundance could be largely influenced by the key parameters like temperature, salinity, and food supply (Lawrence et al. 2004). Warming and freshening of the water column have been found to have significant impacts on zooplankton breeding and hence community and biomass (Greenwood et al. 2001; Rabalais et al. 2002). It is likely that both high water temperature and salinity promoted zooplankton to flourish to a maximum level during the sampling time and there was a significant top-down control on the phytoplankton community at NKP. A positive correlation between mesozooplankton biomass/density and temperature has been reported from the Bay of Bengal during the summer monsoon by Fernandes and Ramaiah, (2009). Dominance of copepods in the zooplankton community at this bay has been previously reported (Nair et al. 1981; Fernandes and Ramaiah, 2009). The abundant zooplankton group copepoda dominated over the other groups possibly due to their continuous breeding behaviour, quick larval development and their well adaptation to the widely changing environmental conditions (Santhanam and Perumal, 2003). Dominance of copepod in the inner sampling circles in all stations was an interesting trend. This could be related to the turbulence created by treated effluence discharge. Turbulent particle motion has significant impact on zooplankton feeding, particularly copepod (Granata and Dicky, 1991). It has been well documented by theoretical and experimental work that under a small scale turbulence copepod feeding can be enhanced by increasing contacts with prey (Schmitt and Seuront, 2008) and by enhanced feeding currents (copepods create a feeding current to entrain the passive particles towards the mouth parts) (Osborn 1996).

Interestingly at CHP the relative abundance of rotifers and TSM values were positively (p<0.01) correlated and increased from the outer to the inner circles. Kirk and Gilbert, (1990) noticed that rotifer abundance can be more under high turbid water. The authors also argued that the rotifers have a special ability with which they can select and identify the food to be ingested through the sensorial bristle, which indeed helps in avoiding the ingestion of inorganic suspended particles;

hence, under high TSM in the water column, rotifers can survive. This was consistent with our observation.

#### **Conclusion:**

Effluent discharge from pharmaceutical industries to the coastal waters can cause significant changes in the physicochemical and biological parameters; however, was not seen during the present study. The observed differences at three sampling stations were mostly due to strong seasonality. The first two sampling locations (sampled in December and March) represented an autotrophic condition which was reflected in all measured physicochemical and biological parameters. The water column was well mixed and no stratification was noticed. In the month of April a strong stratification and heterotrophy was recorded at the third sampling station. This was likely to happen due to thermal stratification during the peak of premonsoon. Diatoms were the dominant phytoplankton group in all three locations with very few dinoflagellates. The heterotrophic condition at NKP probably facilitated relatively higher dinoflagellate abundance at this staion. The presence of excess silicate over nitrate could be the reason for consistent diatom abundance. These observations were consistent with our earlier studies. Copepods, the dominating zooplankton, were more confined to the effluent discharge points with higher turbulent water flow which could be beneficial for prey capture. So the present study concluded that the coastal waters of Visakhapatnam did not show any major indication of pollution due effluent discharge at these marine outfall points. Nevertheless, our study represents just a snapshot of the total system and a continuous monitoring effort is needed in order to understand if there is any variability in the long-term at these locations. Some detailed investigations including some microbiological studies might provide better insight about the ecosystem health at these location.

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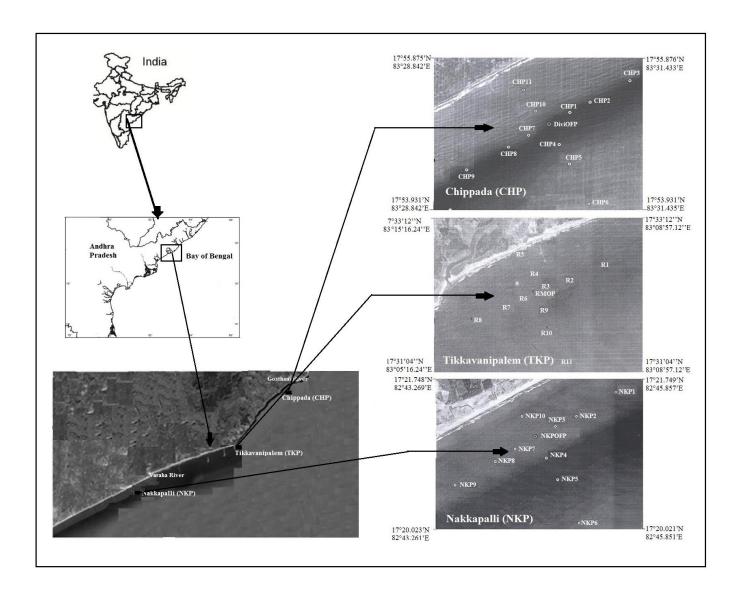
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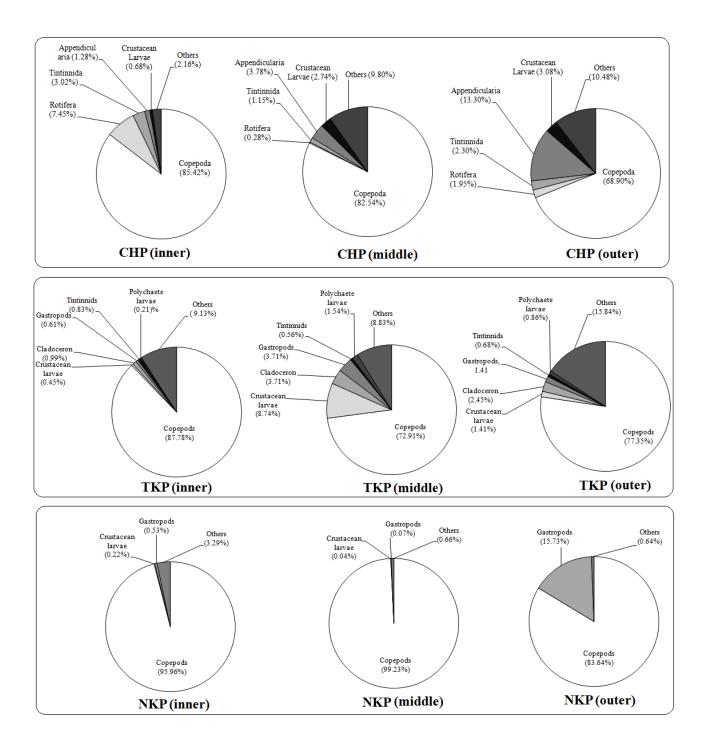
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**Fig. 1**: Map showing the sampling locations along the coast of Visakhapatnam. The inner sampling circle at the station CHP consists of D-OFP, CHP1, CHP2, CHP4; the middle sampling circle includes the stations CHP5, CHP7, CHP8, CHP10 and CHP3, CHP6, CHP9, CHP11 represented the outer sampling circle. For the station TKP, the stations considered for the inner circles are R3, R6, R-MOP; The middle circle was comprised of R2, R4, R7, R9 and R10. The outer sampling circle was consisted of R1, R5, R8, R11. At the station NKP the sampling locations NKP3, NKP4, NKP7, H-OFP represented the inner sampling circle. The middle circle was represented by the locations NKP2, NKP5, NKP8, NKP10. NKP1, NKP6 and NKP9 were considered for the outer circle sampling circle. At CHP the sampling was done during December, 2013; at TKP and NKP the sampling was done during March and April, 2014, respectively.



**Fig. 2**: Relative % of major contributing zooplankton groups in the surface waters at the inner, middle and outer sampling cirles of three sampling stations, namely, CHP (top pannel), TKP (middle pannel) and NKP (lower pannel) during December; 2013, March and April' 2014, respectively.

**Table 1:** Variability in physicochemical and biological parameters in surface (0m) and subsurface waters (30m) of the inner, middle and outer sampling circles at CHP, TKP and NKP (Values given are average  $\pm$  stdev; n =3-5).

		Chippada (CHP)			Tikkavanipalem (TKP)			Nakkapalli (NKP)		
		Inner	Middle	Outer	Inner	Middle	Outer	Inner	Middle	Outer
		Avg±SD	Avg±SD	Avg±SD	Avg±SD	Avg±SD	Avg±SD	Avg±SD	Avg±SD	Avg±SD
Temp (°C)	Sur	24.91±0.29	25.08±0.08	25.19±0.13	27.83±0.21	27.97±0.11	27.90±0.34	26.12±0.12	26.22±0.05	26.36±0.5
	Sub	25.36±0.03	25.35±0.14	25.43±0.09	26.92±0.20	26.18±1.13	26.30±1.45	24.83±0.04	24.81±0.06	24.84±0.12
Salinity (PSU)	Sur	28.29±0.10	27.49±1.49	27.64±1.05	33.47±0.03	33.55±0.03	33.14±0.62	34.18±0.33	34.34±0.04	34.31±0.19
	Sub	28.84±0.20	28.96±0.22	28.92±0.26	33.96±0.05	34.20±0.40	34.19±0.42	34.59±0.04	34.59±0.05	34.64±0.06
TSM (mg L <sup>-1</sup> )	Sur	22.35±2.49	19.80±1.88	18.40±0.37	24.80±3.70	25.64±3.33	25.45±3.17	29.85±9.76	38.20±8.53	29.87±13.60
	Sub	23.30±2.89	22.25±5.73	30.55±21.79	23.80±1.06	25.36±2.17	26.65±1.54	35.90±10.78	41.95±2.66	40.87±2.32
DO (mg L <sup>-1</sup> )	Sur	6.87±0.28	6.71±0.05	6.52±0.25	6.37±0.16	6.53±0.16	6.69±0.13	3.28±0.45	3.11±0.51	2.81±0.43
	Sub	6.23±0.31	6.15±0.35	6.39±0.56	6.47±0.18	6.21±0.19	6.41±0.24	1.69±0.50	1.96±0.51	1.68±0.52
pH (NBS)	Sur	8.18±0.04	8.10±0.09	8.20±0.02	8.18±0.02	8.17±0.02	8.18±0.02	7.84±0.03	7.95±0.10	7.92±0.08
	Sub	8.19±0.03	8.17±0.06	8.15±0.05	8.18±0.07	8.17±0.02	8.15±0.07	7.76±0.25	7.89±0.16	7.87±0.08
$NH_4^+$ (µmol $L^{-1}$ )	Sur	0.80±0.19	1.02±0.54	0.79±0.51	0.33±0.12	0.30±0.12	0.33±0.22	0.14±0.07	0.50±0.47	0.66±0.48
	Sub	0.50±0.44	1.26±0.46	0.94±0.40	0.28±0.06	0.27±0.07	0.45±0.13	0.60±0.47	0.28±0.11	0.69±0.37
$NO_2^-$ (µmol L <sup>-1</sup> )	Sur	0.15±0.03	0.18±0.06	0.18±0.04	0.14±0.03	0.15±0.02	0.13±0.03	0.70±0.12	0.87±0.12	0.75±0.03
	Sub	0.17±0.02	0.19±0.04	0.16±0.03	0.16±0.04	0.16±0.05	0.19±0.06	0.66±0.03	0.72±0.05	0.87±0.36
$NO_3^-$ (µmol L <sup>-1</sup> )	Sur	0.57±0.33	0.73±0.37	0.79±0.62	1.16±0.41	1.36±0.52	1.84±1.62	6.04±0.11	6.04±0.18	6.10±0.21
	Sub	0.60±0.27	0.71±0.28	0.56±0.48	0.98±0.24	1.69±0.80	1.21±0.50	8.14±0.48	7.93±0.48	8.36±1.34
DIN (µmol L-1)	Sur	1.52±0.39	1.93±0.89	1.76±1.05	1.63±0.47	1.81±0.59	2.31±1.83	6.88±0.20	7.40±0.71	7.51±0.57
	Sub	1.27±0.46	2.17±0.72	1.65±0.89	1.42±0.15	2.12±0.84	1.85±0.43	9.40±0.79	8.93±0.44	9.91±1.84
DIP (µmol L-1)	Sur	0.25±0.07	0.26±0.07	0.31±0.09	0.48±0.13	0.45±0.10	0.39±0.04	3.30±0.77	2.83±0.62	2.34±0.56
	Sub	0.28±0.05	0.30±0.09	0.29±0.17	0.40±0.04	0.51±0.14	0.52±0.20	2.55±0.34	2.58±0.83	2.36±0.33
SiO <sub>4</sub> -	Sur	11.61±4.16	9.43±2.93	13.83±5.97	5.48±4.00	3.15±0.51	4.17±1.99	16.15±3.37	16.28±1.25	15.55±1.23
	Sub	13.32±3.26	10.88±2.90	9.36±3.31	3.08±0.68	4.48±1.42	4.54±2.08	16.12±0.58	17.42±5.78	16.58±1.89

DIN:DIP	Sur	6.27±1.95	7.39±2.70	6.09±4.51	3.57±1.39	4.07±1.44	6.17±5.33	2.19±0.65	3.90±1.80	3.34±0.83
	Sub	4.48±1.17	7.48±2.45	6.10±2.51	3.58±0.57	4.05±0.67	3.93±1.53	3.75±0.76	3.90±1.80	4.32±1.30
DIN:Si	Sur	0.14±0.03	0.21±0.07	0.15±0.10	0.43±0.28	0.59±0.26	0.51±0.15	0.44±0.07	0.46±0.07	0.48±0.04
	Sub	0.10±0.05	0.21±0.10	0.20±0.13	0.48±0.13	0.46±0.08	0.47±0.20	0.58±0.03	0.54±0.15	0.67±0.17
Chl a (µg L <sup>-1</sup> )	Sur	0.56±0.31	0.21±0.10	0.52±0.33	0.25±0.12	0.35±0.14	0.22±0.09	0.04±0.02	0.11±0.11	0.11±0.14
	Sub	0.56±0.49	0.33±0.23	0.38±0.19	0.23±0.15	0.37±0.26	0.45±0.25	0.09±0.13	0.05±0.05	0.09±0.13
Phytoplankton cell x	Sur	8.78±4.04	10.45±2.29	15.27±4.03	5.98±4.43	7.07±5.42	6.08±3.42	0.79±0.33	0.93±0.57	0.79±0.48
$10^{5}L^{-1}$										
	Sub	5.20±2.69	4.81±1.33	6.60±1.83	21.09±19.05	14.08±8.44	33.64±5.19	0.64±0.53	0.95±0.58	0.56±0.20
Diversity index (H)	Sur	1.70±0.28	1.38±0.15	1.61±0.27	1.72±0.13	1.76±0.16	1.81±0.19	1.80±0.24	1.55±0.30	1.20±0.13
	Sub	1.83±.016	1.62±0.25	1.70±0.09	1.81±0.06	1.78±0.18	1.81±0.16	1.74±0.15	1.76±0.21	1.54±0.22
Zooplankton density (No 100 <sup>-3</sup> ) x 10 <sup>3</sup>	Sur	6.73±3.72	9.45±7.24	6.04±5.81	1.41±1.35	1.14±0.84	1.41±0.73	7.88±5.37	11.83±13.53	7.21±4.81
Zooplankton biomass (ml 100m <sup>-3</sup> )	Sur	4.38±1.49	5.13±2.02	5.38±3.64	2.27±0.76	2.90±1.79	4.00±1.99	8.40±4.20	6.57±3.11	6.17±2.36

**Table 2:** Relative % of phytoplankton species in the inner, middle and outer circle of Marine Outfall points of Chippada (CHP, December' 13), Tikkavanipalem (TKP, March'14) and Nakkavanipalem (NKP, April'14) in both surface and subsurface waters. Only Phytoplankton genera present >1% has been given here and the rests have been grouped under "others".

		Surface			Sub-surface		
Stations	Name of the Species	Inner	Middle	Outer	Inner	Middle	Outer
CHP							
	Chaetoceros sp.	56.07	61.6	57.66	47.22	52.61	54.22
	Pseudo-nitzschia seriata	7.68	11.43	11.74	12.92	13.04	12.28
	Leptocylindrus danicus	8.98	12.18	11.53	9.99	5.11	8.97
	Dactyliosolen fragilissimus	3.94	0.11	2.64	2.82	2.21	4.25
	Rhizosolenia setigera	2.52	1.48	1.86	2.01	2.82	1.26
	Thalassionema nitzschioides	3.39	2.11	2.06	4.11	3.74	2.38
	Thalassiothrix frauenfeldii	1.64	0.14	0.33	0.11	1.62	0.39
	Dinoflagellate	0.41	1.06	0.28	0.39	0.47	0.78
	Others	15.37	9.89	11.9	20.43	18.38	15.47
TKP							
	Nitzschia longissima	36.62	31.76	25.9	27.74	33.34	28.59
	Chaetoceros sp.	27.67	30.69	19.68	27.27	24.37	21.63
	Pseudo-nitzschia seriata.	8.08	6.47	19.75	13.16	9.7	15.04
	Skeletonema costatum	7.26	3.86	6.65	6.02	2.63	6.33
	Leptocylindrus danicus	2.48	3.79	4.5	5.03	4.22	5.64
	Navicula sp.	2.89	2.85	2.84	3.62	4.05	3.52
	Dactyliosolen fragilissimus	1.53	1.34	1.52	1.69	3.35	2.36
	Rhizosolenia setigera	1.92	0.69	1.08	1.48	2.49	2.67
	Asterionella japonica	1.27	1.13	1.16	1.65	4.18	4.54
	Dinoflagellate	0.67	1.77	3.12	0.34	0.98	0.3
	Others	9.61	15.65	13.8	12	10.69	9.38
NKP							
	Nitzschia longissima	19.1	12.8	34.1	24	17.7	19.5
	Chaetoceros sp.	4.9	3.7	2.4	3.9	11.4	7
	Pseudo-nitzschia seriata		4.8	2.4		1.3	
	Skeletonema costatum	3.9	6.5	3.6	6.6	3.5	
	Navicula sp.	12	13.5	3.2	16.1	13.8	5.2
	Dactyliosolen fragilissimus		2	1.2			
	Synedra sp.	7.6	2.8	3.7	5.5	6.7	11.3
	Asterionella japonica	3.2	5.1	3.3	2.6	4.9	8.5
	Thalassiosira decipiens	2.1	5.4	3.4	7.9	4.6	5.1
	Thalassionema nitzschioides	11.1	8.8	17.8	12.1	8.6	12.9
	Coscinodiscus sp.	3.4	2.5	4.7	8	1.4	10.3
	Dinoflagellate	8.5	8	7.6	2.6	1.4	0.9
	Others	24.2	24.1	12.6	10.7	24.7	19.3