

## CHAPTER - V

## BENTHIC PHOSPHORUS CYCLING IN THE MANDOVI ESTUARY

**5.1. Introduction**

Phosphorus is an essential nutrient required by all organisms for energy transport and growth. It is the eleventh most abundant element in the earth's crust. The importance of P as a nutrient was not realized until the mid-1800s, and it was not until the early 1900s that the role of P in the transfer of energy was realized. The discovery of adenosine triphosphate (ATP) in the 1930s and 1940s revolutionized the understanding of cellular energy metabolism (e.g. Corbridge, 1990). Later the discovery of phosphate esters as key components of cells by 1940s and the identification of the molecular structure of DNA by Watson and Crick in 1953 firmly established the importance of P in the growth and development of all organisms. Over the last few decades, enormous progress in the field of biochemistry has further demonstrated the importance of P in a wide variety of metabolic processes (Bridger and Henderson, 1983; Lehninger *et al.*, 1993). Also marine P has received considerable attention in recent decades, with particular emphasis on source and sink terms in its oceanic budget (Froelich *et al.*, 1982; Meybeck, 1982; Ruttenberg, 1993; Sutula *et al.*, 2004). Phosphorus is the one of the well studied nutrients in aquatic ecosystems. Dissolved phosphate availability in seawater is a dominant factor controlling biological productivity of the oceans (Broecker & Peng, 1982; Garrels & Mackenzie, 1971; Holland, 1978). It is even referred to as the ultimate limiting nutrient in the ocean over geological time scales (Redfield, 1958; Van Cappellen & Ingall, 1994 & 1996; Tyrell, 1999). Phosphorus has particularly drawn attention because of its frequent limitation of primary production in fresh water lakes in contrast to estuaries and coastal seas which are often found to be N - limited (Ryther & Dunstan, 1971; Schindler, 1977; Hecky & Kilham, 1988). Moreover there are areas such as the Mediterranean where phosphorus has been found to limit primary production (Krom *et al.*, 1991 & 1992).

Finally availability of P in oligotrophic (N-depleted) open ocean waters is essential for N<sub>2</sub> fixation (Mills *et al.*, 2004)

#### *P speciation in estuaries and oceans*

The cycling and availability of P in estuaries are largely dependent upon P speciation. Consequently total P (TP) has traditionally been divided into total dissolved P (TDP) and total particulate P (TPP) fractions which can further be divided into dissolved and particulate organic P (DOP and POP) and dissolved and particulate inorganic P (DIP and PIP) pools. The most important P species is DIP which is also often referred to as soluble reactive phosphorus (SRP) since it is the potentially bioavailable. Much of the work to date has focused on SRP which is characterized as the P fraction that forms a blue colored phosphomolybdate complex. The DIP fraction is composed of PO<sub>4</sub><sup>3-</sup>, HPO<sub>4</sub><sup>2-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and H<sub>3</sub>PO<sub>4</sub>. The relative abundance of these species varies with pH in the aquatic systems, making H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and HPO<sub>4</sub><sup>2-</sup> the most abundant species in fresh water and seawater respectively (Morel, 1983). A fraction of DIP comprising polyphosphates and phosphonates is referred to as soluble non-reactive phosphorus (SNP) since it neither forms phosphomolybdic complex nor takes part in biogeochemistry in natural systems.

#### *Sources and Sinks*

Phosphorus enters the oceans predominantly through rivers though there are other sources such as atmospheric input and volcanic eruptions. Continental weathering of crustal materials, which contain on an average 0.1% P<sub>2</sub>O<sub>4</sub> (Jahnke, 2000), is the major source of riverine P. Only about 5-10% of the P eroded from continental rocks is believed to be carried to the oceans in dissolved form, the rest is presumably carried as inert P in fluvial detrital grains. Rivers are the major source of P to the ocean via estuaries, where major chemical and biological transformations of P occur before it is delivered to the ocean (Froelich *et al.*, 1982). Apatite is the most abundant phosphate mineral in the earth's crust, representing more than 95% of all the crustal P. Froelich *et al.* (1982) estimated the input of total P to the ocean as close to 3.3 x 10<sup>11</sup> mol P yr<sup>-1</sup>. However including anthropogenic activities such as deforestation and the use of fertilizers, the total flux increases by over a factor of 3, to 7.4-15.6 x 10<sup>11</sup> mol P yr<sup>-1</sup> (Froelich *et al.*, 1982; Howarth *et al.*, 1995).

Input of P through atmospheric deposition is generally considered to be insignificant in coastal systems since it only represents <10% of the riverine flux of reactive P (Duce *et al.*, 1991; Delaney, 1998). Only in highly oligotrophic systems such as oceanic gyres and eastern Mediterranean (Krom *et al.*, 1991, 1992), can such inputs have a significant effect on primary production. Dry fallout of aeolian P from dust, industry and sea salts on land has been estimated as 1.0 and  $1.05 \times 10^{12}$  g yr<sup>-1</sup> in prehuman and present day P fluxes to the ocean, respectively (Compton *et al.*, 2000).

The removal mechanisms of P from marine systems have been recently reviewed by Delaney (1998) and Follmi (1996). As P has no stable gaseous form, its only removal from the world's ocean is through sedimentary burial. However this process is inefficient as less than 1% of P reaching the sea floor is ultimately trapped in sediments and removed from marine P cycle. There are essentially four processes which have been identified as important removal mechanisms of P from the water column (1) organic matter burial (2) P sorption and precipitation with clays and iron oxyhydroxides (3) phosphorite burial and (4) hydrothermal processes (Froelich *et al.*, 1977; Mach *et al.*, 1987; Howarth *et al.*, 1995; Follmi, 1996; Delaney, 1998). Upon integration over long time scales ( $10^4$ - $10^5$  y), the PO<sub>4</sub><sup>3-</sup> concentration in seawater represents the balance between inputs from rivers and atmosphere and removal to sea floor sediments being associated with these four processes (Froelich *et al.*, 1982).

Many clay minerals have strong tendency of adsorbing DIP. But these adsorptions are reversible in nature *i.e.* P can also get desorbed from the mineral surface. Apart from clay minerals iron oxides, oxyhydroxides, aluminium oxides and manganese oxide particles also adsorb DIP significantly. These adsorptions are favoured at low pH usually encountered in fresh water (Hawke *et al.*, 1989). Suspended particles carried by river usually contain large amount of adsorbed P. After being carried into the estuary, P desorbs from the suspended particles and released to the water owing to increase in pH (Froelich, 1988; Hawke *et al.*, 1989) and salinity. Metal oxides of Fe (III) and Al have variable charge surfaces that generally carry a net positive charge at low pH and a net negative charge at high pH. This facilitates desorption of P from the particle surface in estuary. Moreover, the increase in salinity causes high competition for surface charge sites of the mineral particles. Anions such as SO<sub>4</sub><sup>2-</sup> compete with PO<sub>4</sub><sup>3-</sup> for adsorption

sites. Increase in pH results also results in competition from  $\text{OH}^-$  ions for the adsorption sites (Lijklema, 1977; Bostrom *et al.*, 1982).

Under oxic conditions P remains adsorbed on to amorphous ferric oxides and Mn oxides and also co precipitates with ferric oxyhydroxides (Krom & Berner, 1981; Klump & Martens, 1981; Froelich *et al.*, 1982; Lucotte & D'Anglejan, 1983, 1988; Martens, 1993; Ingall & Jahnke, 1994). But under anoxic condition  $\text{PO}_4^{3-}$  is released from these minerals during their reductive dissolution (Krom & Berner, 1980, 1981; Froelich *et al.*, 1982; Sundby *et al.*, 1992).

$\text{PO}_4^{3-}$  is also incorporated into the lattice of authigenic calcium fluoroapatite (CFAP) during its formation and gets buried in sediment. Phosphorus in sediment thus occurs in six major phases *i.e.* (1) loosely adsorbed P (Lsor-P), (2) iron bound inorganic P (Fe-P) (3) leachable organic P (Lea-OP), (4) authigenic apatite (CFAP), (5) detrital apatite (FAP), and (6) refractory organic P (Ref-OP).

To understand the processes controlling phosphate exchange across sediment-water interface a series of core incubation experiments were carried out in the laboratory.

## **5.2. Methodology**

### **5.2.1. Sediment core collection and pore water analyses**

Sediment cores were collected in different seasons from the site where benthic chamber was deployed. Additional cores were collected from two stations closer to the mouth of the estuary during premonsoon period (May). 30 cm long 54 mm ID transparent acrylic core liners were used for this purpose.

Cores were immediately transferred to the laboratory. There the cores were sectioned into 1 cm thick slices in a transparent acrylic glove box flushed continuously with  $\text{N}_2$ . Sectioned sediment layers were put into 50 ml plastic centrifuge tubes kept inside the glove box and closed. They were then centrifuged at 4000 rpm for 20 minutes. The separated pore water was frozen at  $-20^\circ\text{C}$  until further analysis.

#### *Analysis of DIP and Total Phosphorus (TP)*

Pore water DIP was analyzed by a SKALAR segmented flow Autoanalyzer by using the method described by Koroleff (1983), discussed in detail in chapter-III.

For total P determination a portion of pore water was first mixed with a solution of  $\text{H}_2\text{SO}_4$  and potassium peroxodisulfate ( $\text{K}_2\text{S}_2\text{O}_8$ ) in a screw capped oxidation flask. It was then autoclaved for 30 minutes.  $\text{K}_2\text{S}_2\text{O}_8$  is a very strong oxidant and under high temperature and pressure it oxidizes all organically bound P to  $\text{PO}_4$ . After cooling the samples were analyzed for DIP as described before. Corresponding DIP values were subtracted from TP values to get DOP.

### 5.2.2. Laboratory Incubation experiments

#### Experiment-1

A fresh sediment core was collected in a 64 mm ID acrylic liner. Freshly collected estuarine bottom water was artificially enriched with  $\text{PO}_4^{3-}$ , and slowly introduced over the sediment core ( $\text{PO}_4^{3-}$  concentration of the estuarine water before enrichment was  $0.09 \mu\text{M}$ ). The volume of the overlying water was 630 ml at the start of the experiment. The overlying water was kept aerated throughout the experiment which lasted for 50 hours. Sampling was done at a regular time interval of 6 hours.

This experiment was repeated 4 times by varying  $\text{PO}_4^{3-}$  concentration of the overlying water. In five experiments *i.e.* exp-1a, 1b, 1c, 1d & 1e, overlying  $\text{PO}_4^{3-}$  concentrations at T0 was 6.45, 7.14, 0.71, 0.74 and  $1.02 \mu\text{M}$  respectively. Experiments 1a & 1b and 1c & 1d were actually replicates although with slightly different initial  $\text{PO}_4^{3-}$  content. The fifth experiment (expt.1e) where initial  $\text{PO}_4^{3-}$  was  $\sim 1 \mu\text{M}$  did not have any duplicate.

#### Experiment-2

(i) Two sediment cores collected in two 30cm long, 64mm ID acrylic liners from the estuary were overlain by estuarine bottom water. The overlying water was gently stirred at 60 rpm not to cause suspension of surficial sediments. One of these cores *i.e.* core-A was kept air-tight and overlying water in another (core-B) was deoxygenated completely by purging zero grade helium gas. Both the cores were incubated in dark nearly at *in-situ* temperature. Cores were incubated first for 48 hours in a short-term experiment with regular sampling every 6 hours. Samples were collected for dissolved oxygen and nutrients and replaced by equal volumes of zero grade  $\text{N}_2$  gas through an opening in the lid.

(ii) In a *long term experiment* two separate sediment cores were collected from the same site and overlain by estuarine bottom water. Incubation was carried out in the same way as done in experiment-2 (iii) but this experiment was continued for ~6 days with regular sampling at 6 hourly intervals.

### 5.3. Results & Discussion

#### 5.3.1. Phosphate in Mandovi estuary

The distribution of phosphate in the estuarine water column has been studied by De Souza *et al.* (1981), De Souza (1983) and Qasim & Sen Gupta (1981) and its behavior in sediment has been investigated previously by Rajagopal & Reddy (1984), D'Silva & Bhosle (1990) and Nasolkar *et al.* (1996).

In the premonsoon period water column  $\text{PO}_4^{3-}$  varies from 0.6-1  $\mu\text{M}$  with decreasing trend from the mouth upstream. Low  $\text{PO}_4^{3-}$  concentrations observed in this period may be due to utilization by primary producers which are maximal in number during this season and low supply associated with minimal river runoff. But in the monsoon season owing to heavy river flow  $\text{PO}_4^{3-}$  in the estuary increases significantly ranging between 1 and 2.8  $\mu\text{M}$  with almost same concentration through out the estuarine length. This concentration range is maintained in the estuary during the entire monsoon season which possibly because of two reasons (1) high turbidity and larger cloud cover diminishes primary production and in turn biological  $\text{PO}_4^{3-}$  assimilation and (2) high suspended sediment load (Cherian *et al.*, 1974, Varma *et al.* 1975). High iron content (Kamat & Sankaranarayanan, 1975) coupled with low salinity, low pH and and high oxygen level (Qasim & Sen Gupta, 1981) may favour removal of excess  $\text{PO}_4^{3-}$  through its adsorption to clay particles and ferric oxide minerals and co precipitation with ferric oxyhydroxides (Krom & Berner, 1981). De Souza *et al.* (1981) showed that phosphate was removed from the estuary during the premonsoon and monsoon season while it is added during the post-monsoon. However in the post monsoon period because of reduced river flow and turbidity and increasing tidal penetration enriches the estuary with  $\text{PO}_4^{3-}$  which exceeds P removal by biological uptake, hence there is a net addition of  $\text{PO}_4^{3-}$  to the estuarine water column. Phosphate concentration during the post monsoon typically varies between 0.6

and 2.4  $\mu\text{M}$  with a decreasing trend towards upstream as mentioned by De Souza *et al.* (1981).

The data collected in the present study from July 2004 to June 2005 show that  $\text{PO}_4^{3-}$  varied between 0.2 and 0.4  $\mu\text{M}$  in premonsoon season. But heavy fresh water runoff during the monsoon raised  $\text{PO}_4^{3-}$  level to 1.42  $\mu\text{M}$  in June. Again  $\text{PO}_4^{3-}$  concentration decreased to low values (0.36-0.46  $\mu\text{M}$ ) in the post monsoon season. It indicates that riverine flow remains the major source in Mandovi estuary.

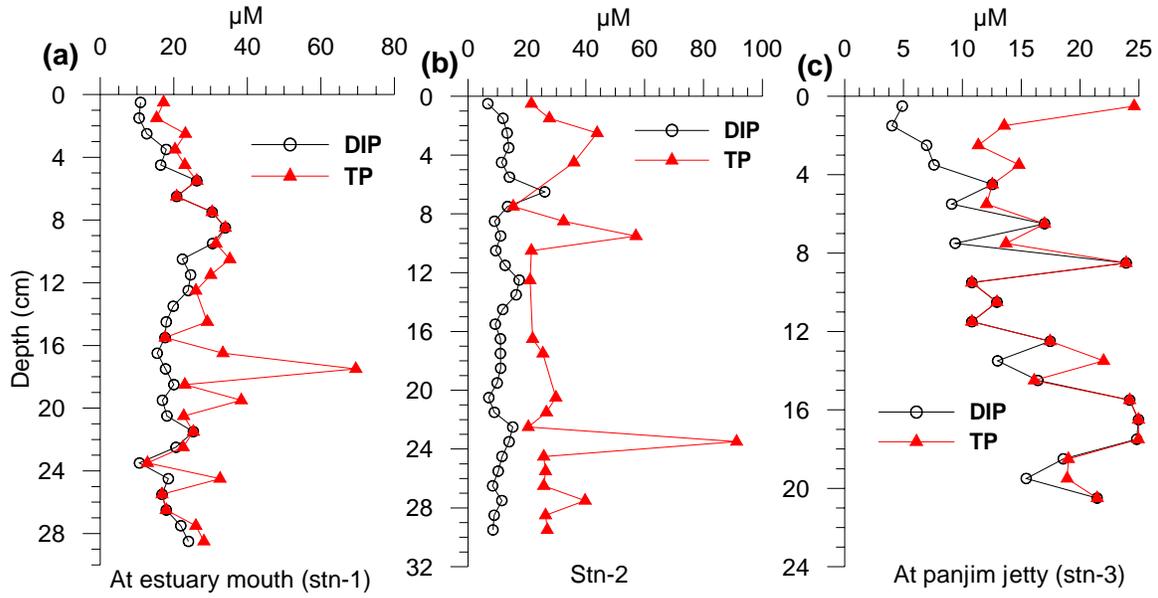
### 5.3.2. Phosphorus in the estuarine sediments

The DIP and total P in the sediment porewaters was measured at three stations, at (1) mouth of the estuary (15° 28' 54.19" N, 73° 46' 52.15" E) (2) regular chamber deployment site (15° 30' 04.94" N, 73° 49' 43.85" E) and (3) at a station located between these sites but closer to the mouth (15° 29' 22.93" N, 73° 48' 05.58" E) (See the blue points in Fig.2.1). Significant spatial variation (ANOVA,  $p < 0.001$ ) in vertical distribution of DIP was observed between Panjim jetty and the estuary mouth whereas spatial variation in total P between these stations was statistically insignificant ( $p > 0.05$ ). DIP of surficial porewater varied between 4.8 and 10.9  $\mu\text{M}$  at these stations with an increasing trend and TP varied between 17.2 and 24.6  $\mu\text{M}$  with a decreasing trend from the jetty to the mouth (Fig. 5.2). At two stations near to the mouth pore water DIP did not show any regular trend with the depth, but maxima and minima were conspicuous (Fig. 5.1). In contrast pore water DIP in jetty sediment showed an overall increase with depth varying between 4.8 and 24.9  $\mu\text{M}$ . This irregularity in the pore water profiles can be attributed to bioturbation and biorrigation (Callender & Hammond, 1982; Davis, 1974; Robbins *et al.*, 1979; Goldhaber *et al.*, 1977) that ventilate the burrows with fresh oxic bottom water, thereby stimulating adsorption and precipitation of DIP with ferric oxides and oxyhydroxides. Due to large adsorption potential of these ferric oxyhydroxides for  $\text{PO}_4^{3-}$  (Krom & Berner, 1980), DIP concentration of interstitial water in contact with oxidized sediment tend to be low (Klump & Martens, 1981; Froelich *et al.*, 1979). Otherwise, anoxic porewater generally contains large concentrations of interstitial phosphate (Theis & McCabe, 1978; Carignan & Flett, 1981). Thus benthic macrofaunal activity seems to be more towards the mouth resulting in an irregular profile of porewater  $\text{PO}_4^{3-}$ . Ansari *et al.* (1986) reported a significant decrease in benthic faunal population with increasing

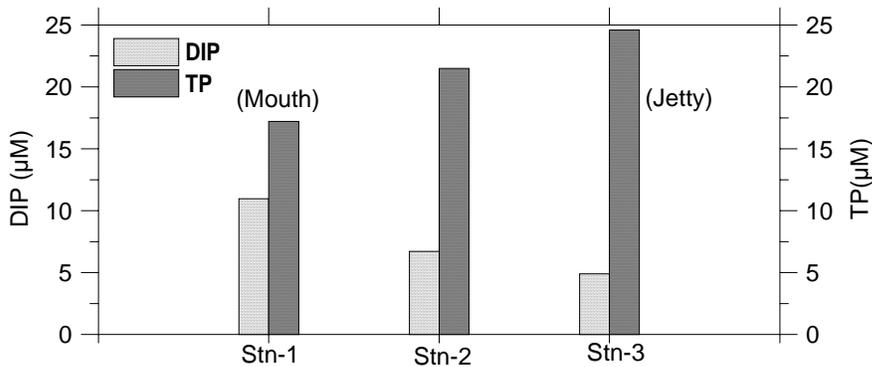
distance from the estuary mouth. Also, they observed significant decrease in faunal population in monsoon which indicates their intolerance to low salinity which dominates the estuary in the monsoon season. Increase in salinity towards the mouth increases the pH. Increase in major ions and  $\text{OH}^-$  with increasing salinity leads to replacement of  $\text{PO}_4^{3-}$  from sorption sites by  $\text{OH}^-$  and other major ions (Lijklema, 1977; Bostrom *et al.*, 1982). Moreover, higher  $\text{SO}_4^{2-}$  reduction in sediment with increasing salinity can decrease the abundance of iron oxides (Carignan & Tessier, 1988) leading to decreased P-sorption (Schindler, 1985; Caraco *et al.*, 1989). Furthermore, increasing pore water DIP with depth can result in an upward migration of DIP along the concentration gradient, and the observed lower DIP in bottom waters of the stations ( $0.09 \mu\text{M}$  at stn-1 and  $0.5 \mu\text{M}$  at stn-2&3) as compared to higher pore water DIP in the surficial sediment would create a concentration gradient, ultimately causing benthic flux of  $\text{PO}_4^{3-}$  to the overlying water. Though benthic flux measurements were not done at station-1 and 2, taking comparatively higher pore water DIP and higher benthic activity at station-1 and 2 into account, it can be hypothesized that benthic release of DIP at these stations may be higher as compared to station-3 (chamber deployment site).

Dissolved phosphorus in pore water represents a small portion (>1%) of total phosphorus in sediment, but it is an important sediment phosphorus fraction since it is directly exchangeable with the overlying water. The pore water phosphate concentration is largely determined by solubility product of  $\text{Ca}_3(\text{PO}_4)_2$  and adsorption/desorption equilibria which will be discussed afterwards. It is likely that pore water phosphate provides a fairly good reflection of the phosphorus saturation level in the sediments.

Nasolkar *et al.* (1996) showed that sedimentary P (phosphate in porewater + sediment) varies from  $0.46$  to  $6.74 \text{mgg}^{-1}$  in the estuary. Rajagopal and Reddy (1984) reported that the phosphate retention capacity of the estuarine sediment is pH dependent. They observed maximum retention of  $\text{PO}_4^{3-}$  (64-95%) of estuarine sediment at pH 7. However TP values indicates that sediment at stn-1 and stn-2 seem to retain less P than that at stn-3 which may be because higher P immobilization at lower salinity (Caraco *et al.*, 1989).



**Fig.5.1:** Vertical profile of porewater DIP and total P at (a) estuary mouth, (c) panjim jetty and (b) at a station in between them (stn-2).



**Fig.5.2:** spatial variability of surficial porewater DIP and TP in the estuarine sediment.

### 5.3.3. Phosphate adsorption and buffering capacity of estuarine sediment

In estuarine, coastal and oceanic sciences, the phosphate buffer mechanism has come to mean the influence of sediments, whether benthic or suspended, in controlling the dissolved reactive  $\text{PO}_4^{3-}$  concentration in the water at some near constant value regardless of biological removal and allochthonous input (Carritt and Goodgal, 1954; Pomeroy, 1965). Similar to pH buffering,  $\text{PO}_4^{3-}$  has been observed to be taken up by sediment particles when its concentration is high and to be released from the sediment, when its

concentration is low (Pomeroy, 1965; Gessner, 1960; Carritt & Goodgal, 1954; Sundby *et al.*, 1992). Conceptually a large pool of  $\text{PO}_4^{3-}$  on inorganic particles is available for release or desorption to the water. Evidence of the process has been derived primarily from two types of observations. First, phosphate concentrations in some estuarine waters are higher than that can be explained by mixing surface sea water with river water. The additional  $\text{PO}_4^{3-}$  must either be released from bottom sediments or from suspended particles. Second, sediment and suspended particulates from estuaries and rivers has been found to release or take up  $\text{PO}_4^{3-}$  to or from solution rapidly in laboratory experiments. Stephenson (1949) suspended estuarine sediments in water and filtered them through 3  $\mu$  pore size filters. He found a significant change in  $\text{PO}_4^{3-}$  in the water. Gessner (1960) added  $\text{PO}_4^{3-}$  to suspensions of sediment and found that the sediment adsorbed additional  $\text{PO}_4^{3-}$  as the concentration in the water increased. This complex behavior of phosphate-particle interaction (Barrow, 1983b) involves the uptake and release of dissolved phosphate via a two step process: rapid surface adsorption/desorption of  $\text{PO}_4^{3-}$  onto/off of reactive surfaces followed by a slow penetration (or release) by solid-state diffusion of this surface phosphate into/out of subsurface zone within the particles. The first step, adsorption-desorption on surfaces has fast kinetics *i.e.* minutes to hours, but the solid state diffusion of adsorbed  $\text{PO}_4^{3-}$  from surface into the interior of particles as well as out of them has slow kinetics *i.e.* days to months or perhaps years (Carritt & Goodgal, 1954; White & Taylor, 1977b; Novak & Petschauer, 1979; Barrow, 1983). Desorption of  $\text{PO}_4^{3-}$  to the water when its  $\text{PO}_4^{3-}$  concentration becomes low, seems to take place via molecular diffusion that occurs because of  $\text{PO}_4^{3-}$  concentration gradient between the outer surface of particles/sediment and surrounding/overlying water. At equilibrium between sorbed and dissolved P, the rates of the forward reaction (sorption) and reverse reaction (desorption) are equal. If the concentration of  $\text{PO}_4^{3-}$  in water increases by some external process, then more  $\text{PO}_4^{3-}$  would sorb onto surfaces to re-establish the equilibrium. If the concentration were to fall by removal from the solution, then  $\text{PO}_4^{3-}$  would be released from the solids. In the oxidizing surface sediment, a major portion of sedimenting organic P is mineralized and the released  $\text{PO}_4^{3-}$  is partitioned between the porewater and surface adsorption sites. Surface adsorbed  $\text{PO}_4^{3-}$  is released to the porewater as needed to

maintain the equilibrium concentration and replace the  $\text{PO}_4^{3-}$  that fluxes to the overlying water. More phosphate is released deeper in the sediment column from iron oxides undergoing reduction. This phosphate migrates upwards and participates in exchange reactions with the remaining adsorption sites in the oxidized surficial sediment. If all the adsorption sites are occupied, it comes out of sediment and is released to the overlying water. Sedimentation, and macrofaunal mixing transport adsorbed, sequestered and organic P downward into the deeper reducing region of sediment column. There again  $\text{PO}_4^{3-}$  is released to the porewater via anoxic decomposition and reductive dissolution of iron oxyhydroxides (Krom & Berner, 1981; Sundby *et al.*, 1992). Molecular diffusion and bioturbation and gas ebullition again transport the mobilized  $\text{PO}_4^{3-}$  to the oxidized sediment surface where it can get re-adsorbed. Hence, pore water  $\text{PO}_4^{3-}$  can be recycled several times across the redox boundary for Fe before escaping the sediment.

Experiment -1, which was carried out to evaluate the phosphate adsorbing capacity of the sediment, revealed the existence of an equilibrium  $\text{PO}_4^{3-}$  concentration in the overlying water which seems to be maintained by sorption on the underlying sediment surface. In this experiment where overlying water contained  $>6 \mu\text{M}$   $\text{PO}_4^{3-}$ , showed significant decrease in  $\text{PO}_4^{3-}$  concentration in the overlying water with time. In experiment-1a,  $\text{PO}_4^{3-}$  decreased from  $6.45 \mu\text{M}$  to  $0.91 \mu\text{M}$  in 50 hours after which it increased slightly, implies an equilibrium  $\text{PO}_4^{3-}$  concentration  $0.91 \mu\text{M}$  (Fig. 5.3a). In exp-1b where  $t_0$  phosphate was  $7.1 \mu\text{M}$ , the equilibrium  $\text{PO}_4^{3-}$  concentration of similar magnitude was reached little earlier (in 36 hours) (Fig. 5.3b). Exp-1c where initial  $\text{PO}_4^{3-}$  was kept at  $0.74$ , revealed a slow decrease in  $\text{PO}_4^{3-}$  to an equilibrium concentration  $0.7 \mu\text{M}$  after 18 hours followed by further increase (Fig. 5.3c) but in case of exp-1d, equilibrium concentration  $0.62 \mu\text{M}$  was attained after 24 hours from a  $t_0$  value  $0.71 \mu\text{M}$ , which was maintained for 12 hours followed by a sharp increase to  $1.09 \mu\text{M}$  (Fig. 5.3d). In Exp-1e an equilibrium value of  $0.73 \mu\text{M}$  was attained after 30 hours from a  $t_0$  value  $1.02 \mu\text{M}$  and after which  $\text{PO}_4^{3-}$  increased slowly.

A buffer diagram (Fig. 5.4a) was constructed by plotting the change in  $\text{PO}_4^{3-}$  concentration ( $\Delta P = P_{\text{init}} - P_{\text{final}}$ ) as a function of the initial  $\text{PO}_4^{3-}$  concentration ( $P_{\text{init}}$ ) (Froelich, 1988; Sundby *et al.*, 1992). The value of  $P_{\text{init}}$  at  $\Delta P = 0$  was found to be  $0.75$

$\mu\text{M}$ , which is referred as the “*zero equilibrium phosphate concentration*” ( $\text{EPC}_0$ ) by Froelich (1988). It represents the concentration at which the sediment displays the maximal buffering capacity. Within a limited range of  $\text{EPC}_0$ , equilibrium  $\text{PO}_4^{3-}$  concentration is re-attained rapidly, if varied slightly. Thus, the equilibrium concentration of  $\text{PO}_4^{3-}$  in Mandovi estuary seems to be in the range 0.7-0.91  $\mu\text{M}$ . Pomeroy *et al.* (1965) found Doboy Sound sediments to be in equilibrium with water having a  $\text{PO}_4^{3-}$  content exactly in the same range. Chase and Sayles (1980) experimented with Amazon river sediments and observed a rapid release of P to the solution that reached quasi-equilibrium values of 0.7-1.0  $\mu\text{M}$  within 24 hours but after 8 days they observed a final  $\text{PO}_4^{3-}$  concentration between 1.3 and 1.4  $\mu\text{M}$ . Fox *et al.* (1986) suspended Amazon estuarine sediments and observed a final  $\text{PO}_4^{3-}$  equilibrium concentration of 2.0-2.5  $\mu\text{M}$  after 2-4 days. Numerous other studies in a variety of different estuaries have also observed the capability of estuarine and freshwater sediments to “buffer”  $\text{PO}_4^{3-}$  concentration in the overlying water (Liss, 1976; Stirling & Wormald, 1977; Van Benekom *et al.*, 1978; Boynton *et al.*, 1980; Sharp *et al.*, 1982; Fox *et al.*, 1985; Edmond *et al.*, 1985; Pilson, 1985).

The slope of  $\Delta\text{P}$  vs.  $\text{P}_{\text{init}}$  can be interpreted as the *buffer intensity* of the sediment. Froelich (1988) suggested that a perfect buffer would be represented by a slope equal to one. In the present study buffer diagram the slope value is 0.95 (Fig. 5.3a), which indicates that estuarine sediments of the Mandovi appear to behave as a nearly perfect buffer for phosphate.

The slope of the linear regression line is also called the *adsorption coefficient* ( $K$ ) and is defined as the number of moles of phosphate added to (or removed from) the system to change the  $\text{PO}_4^{3-}$  concentration in water by 1 mole/litre near  $\text{EPC}_0$ . The quantity of phosphate adsorbed on solid at equilibrium is termed as *equilibrium sorbed phosphate* ( $\text{ESP}=\text{Ps}-\text{EPC}_0$ ) and is a measure of the mass of reactive P per gram of solid that is available for release into a solution of infinite volume devoid of  $\text{PO}_4^{3-}$  (*e.g.* the surface ocean). The average ESP value from exp-1a and 1b was found to be 5.1  $\mu\text{M}$ .

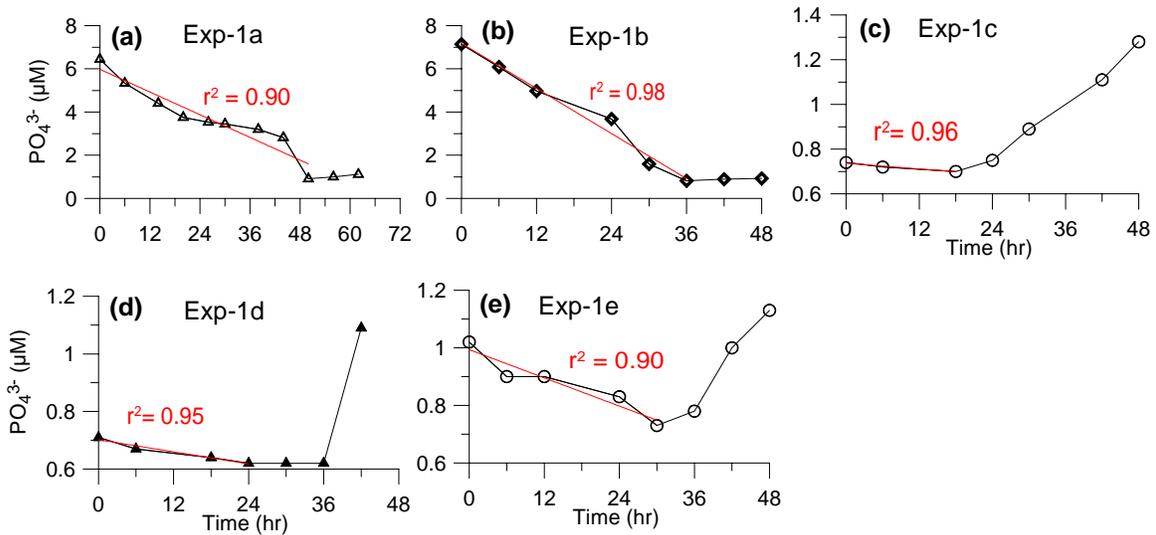
The amount of  $\text{PO}_4^{3-}$  adsorbed appears to be dependent on the concentration of  $\text{PO}_4^{3-}$  present initially. The  $\text{PO}_4^{3-}$  adsorption with time appears to take place almost in a linear

fashion ( $r^2=0.9$ ) (Fig. 5.3). The rate of adsorption was observed to increase with increase in initial  $\text{PO}_4^{3-}$  concentration (Fig. 5.4b) with a very high correlation ( $r^2 = 0.99$ ) between two. However the percentage of adsorbed phosphate increased logarithmically with initial  $\text{PO}_4^{3-}$  concentration in the solution (Fig. 5.4c). The release of  $\text{PO}_4^{3-}$  from the sediment is determined by interplay between benthic efflux of  $\text{PO}_4^{3-}$  occurring mainly because of molecular diffusion and adsorption of upward migrating  $\text{PO}_4^{3-}$  along the concentration gradient, by oxic surficial sediment. The observed increase in  $\text{PO}_4^{3-}$  concentration in the overlying water, after equilibrium concentration (EPC) is attained is probably because of comparatively higher rate of release of  $\text{PO}_4^{3-}$  from organic matter mineralization and consequently higher diffusion rate, which surpasses the rate of  $\text{PO}_4^{3-}$  adsorption by the oxic surficial sediment.

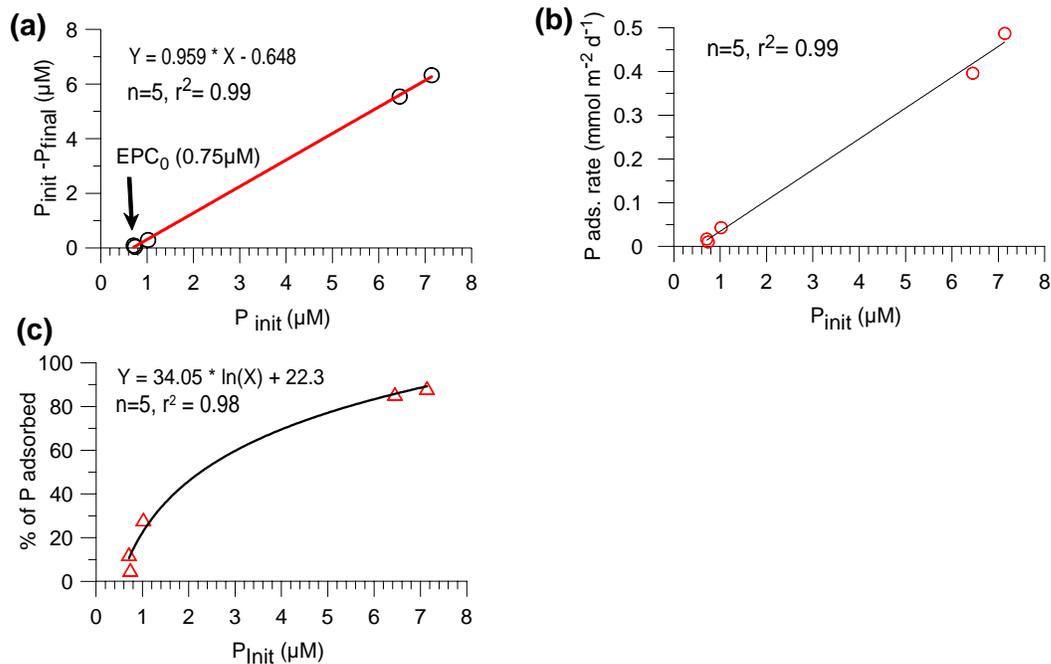
#### *Effect of pH and salinity on P adsorption*

Sorption experiments carried out by Carritt & Goodgal (1954) and White & Taylor (1977a) showed that maximal sorption occurs in the pH range of 4.8-6 where  $\text{H}_2\text{PO}_4^-$  is the dominant species in solution and this has been interpreted as evidence for  $\text{H}_2\text{PO}_4^-$  being the species getting adsorbed on to surfaces. Estuarine waters often exhibit significant change in salinity seasonally or with tidal cycle. Increasing salinity increases the pH the concentration of  $\text{SO}_4^{2-}$ ,  $\text{F}^-$ ,  $\text{B}(\text{OH})_4^-$  and  $\text{OH}^-$  which compete with  $\text{PO}_4^{3-}$  for surface adsorption sites (Rajan & Watkinson, 1976), thereby decreasing the sorption of  $\text{PO}_4^{3-}$ . Lijklema (1977) and Bostrom *et al.* (1982) also concluded that increase in pH in the water in contact with the sediment decreases the P-binding capacity of iron and aluminium oxides, primarily due to ligand exchange reactions in which  $\text{OH}^-$  ions replace  $\text{PO}_4^{3-}$  from the sorption sites.

However the effect of pH on P sorption in calcareous sediments may be different.  $\text{CaCO}_3$  precipitation is induced by high pH level and P may be co-precipitated with  $\text{CaCO}_3$  or adsorbed onto the precipitate (Otsuki & Wetzel, 1972; Gunatilaka, 1982). Moreover formation of hydroxyapatite on  $\text{CaCO}_3$  crystals is strongly pH-dependent and is favoured by higher pH levels (Stumm & Leckie, 1971).



**Fig.5.3:** Adsorption of water column  $PO_4^{3-}$  by sediment surface. Red line is the linear fit from  $P_{init}$  to EPC.



**Fig. 5.4:** (a) Buffer diagram for phosphate,  $EPC_0$  is the zero equilibrium phosphate concentration, (b) Correlation between phosphate adsorption rate and initial phosphate concentration. (c) Logarithmic increase in phosphate adsorption with increase in initial phosphate concentration.

Caraco *et al.* (1989) suggested three mechanisms of decrease in P sorption with increase in salinity, (1)  $\text{SO}_4^{2-}$  competes with  $\text{PO}_4^{3-}$  for anion sorption sites, which may inhibit P sorption by sediment in brackish and marine systems, (2) sulfides produced from  $\text{SO}_4^{2-}$  reduction in anoxic estuarine and coastal sediments, can bind iron to form insoluble iron sulfides (Carignan & Tessier, 1988) and ferrous phosphate within anoxic sediments, thereby significantly decreasing re-supply of Fe-oxides to surface sediment (Carignan & Tessier, 1988) and decreasing P sorption by surficial sediment and (3)  $\text{SO}_4^{2-}$  reduction in anoxic sediment or bottom waters can increase alkalinity and elevate pH which inhibits P-sorption onto iron oxides in sediments (Hawke *et al.*, 1989).

#### *Effect of solid composition*

The second slow diffusion step is particularly strongly dependent on the composition of solid phases. Sediments containing iron and aluminum oxyhydroxides or surfaces coated with these phases, display much higher “slow” sorption capacity, probably due to the reaction of  $\text{PO}_4^{3-}$  with oxides themselves (chemisorption, exchange with  $\text{OH}^-$  and precipitation of Fe and Al phosphates. The capacity of these oxides to sorb P decreases with their age and apparent crystallinity (McLaughlin *et al.*, 1981).

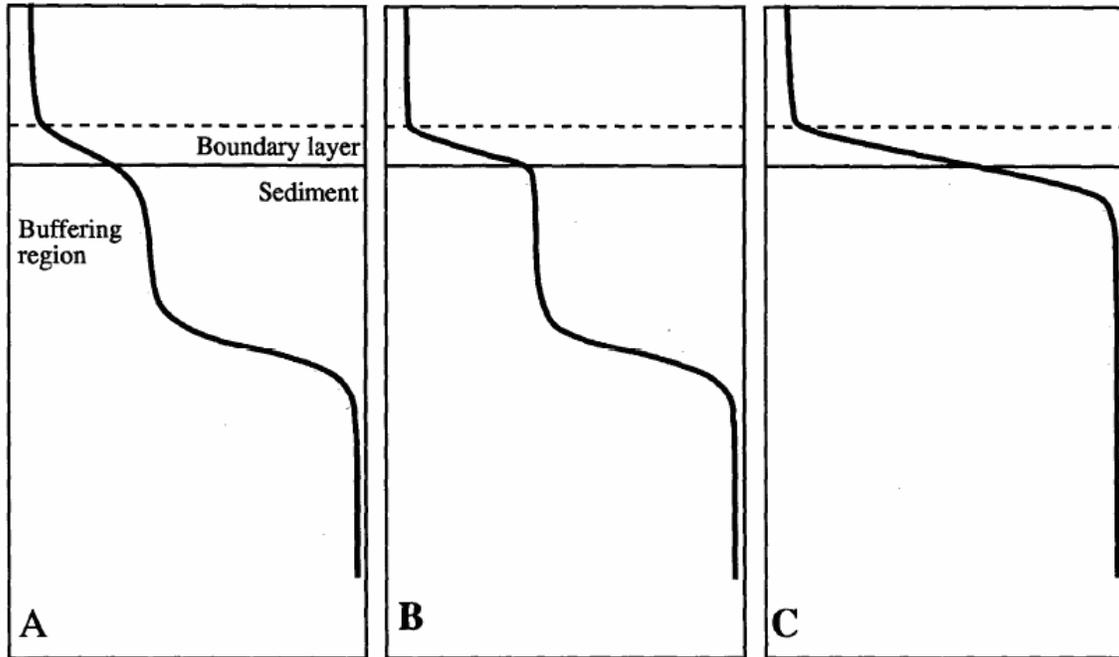
#### *Buffering capacity and pore water profile*

$\text{PO}_4^{3-}$  is primarily produced in pore water via organic matter decomposition. The net phosphate production rate is the difference between  $\text{PO}_4^{3-}$  regeneration rate and  $\text{PO}_4^{3-}$  adsorption and burial rate in sediment. Both total and net  $\text{PO}_4^{3-}$  production rates can be expected to vary seasonally with variation in P input, water temperature and activity of benthic organisms. Sundby *et al.* (1992) suggested that the pore water  $\text{PO}_4^{3-}$  concentration near sediment-water interface depends on the production rate of  $\text{PO}_4^{3-}$ , the buffering capacity of the sediment and the thickness of the diffusive boundary layer in the following manner. For a given environment, there is a rate of net  $\text{PO}_4^{3-}$  production below which the buffering capacity controls the concentration of  $\text{PO}_4^{3-}$  in the pore water. When the production rate rises above this value, the flux of  $\text{PO}_4^{3-}$  across the boundary layer increases until it becomes limited by boundary layer resistance. This results in the addition of excess  $\text{PO}_4^{3-}$  to the reservoir of adsorbed phosphate which leads to a

temporary seasonal storage of  $\text{PO}_4^{3-}$  in the sediment. However, the boundary layer resistance can only constrain the flux out of the sediment as long as the capacity of sediment to maintain a constant pore water  $\text{PO}_4^{3-}$  concentration *i.e.* buffering capacity is not exceeded. When that happens, the concentration will rise, the concentration gradient across the sediment-water interface will steepen and flux will increase to match the net  $\text{PO}_4^{3-}$  production rate again and the flux will be controlled by and equal the net production rate of  $\text{PO}_4^{3-}$ .

The interaction between the net  $\text{PO}_4^{3-}$  production rate, the buffering capacity of the sediment and the thickness of the boundary layer will also control the shape of the pore water profile as the following discussion shows. At low net production rate, as long as buffering capacity is not exceeded, sorption equilibria buffer the concentration of pore water  $\text{PO}_4^{3-}$  and the pore water profile becomes S-shaped (Fig. 5.5A). As the production rate increases, the boundary layer begins to limit the flux which results in the accumulation of more  $\text{PO}_4^{3-}$  in the adsorbed phase (Fig. 5.5B). When the buffering capacity is exceeded, the  $\text{PO}_4^{3-}$  concentration in the pore water and the concentration gradient across the interface increase until the flux again matches the net production rate of  $\text{PO}_4^{3-}$ . This will make the pore water profile attain a concave-down shape (Fig. 5.5C). The pore water profiles of phosphate in the Mandovi estuary show significant seasonal changes in the shape due to the aforementioned interplay between the thickness of diffusive boundary layer, buffering capacity of sediment and production rate of phosphate in the sediment. Pore water profiles in pre-monsoon at Panjim jetty (Fig. 5.6a & b), bear resemblance with the fig. 5C which indicates that phosphate production rate during this season exceeds the buffering capacity. However in March the profile less steep (Fig. 5.6a) than in May (Fig. 5.6b). This can be because of lower regeneration rate of  $\text{PO}_4^{3-}$  in March as compared to that in May since increasing temperature in May can result higher  $\text{PO}_4^{3-}$  regeneration. Moreover profiles at two other sites in the estuary (Fig. 5.6c and d) in May have similar shape as those at Panjim jetty (Fig. 5.6b). The profile near the estuary mouth shows a steep gradient indicating higher  $\text{PO}_4^{3-}$  production rate compared with the other two stations. Heavy precipitation and persistent high cloud cover which decreases solar heating in the monsoon season causes lower the temperature of estuarine

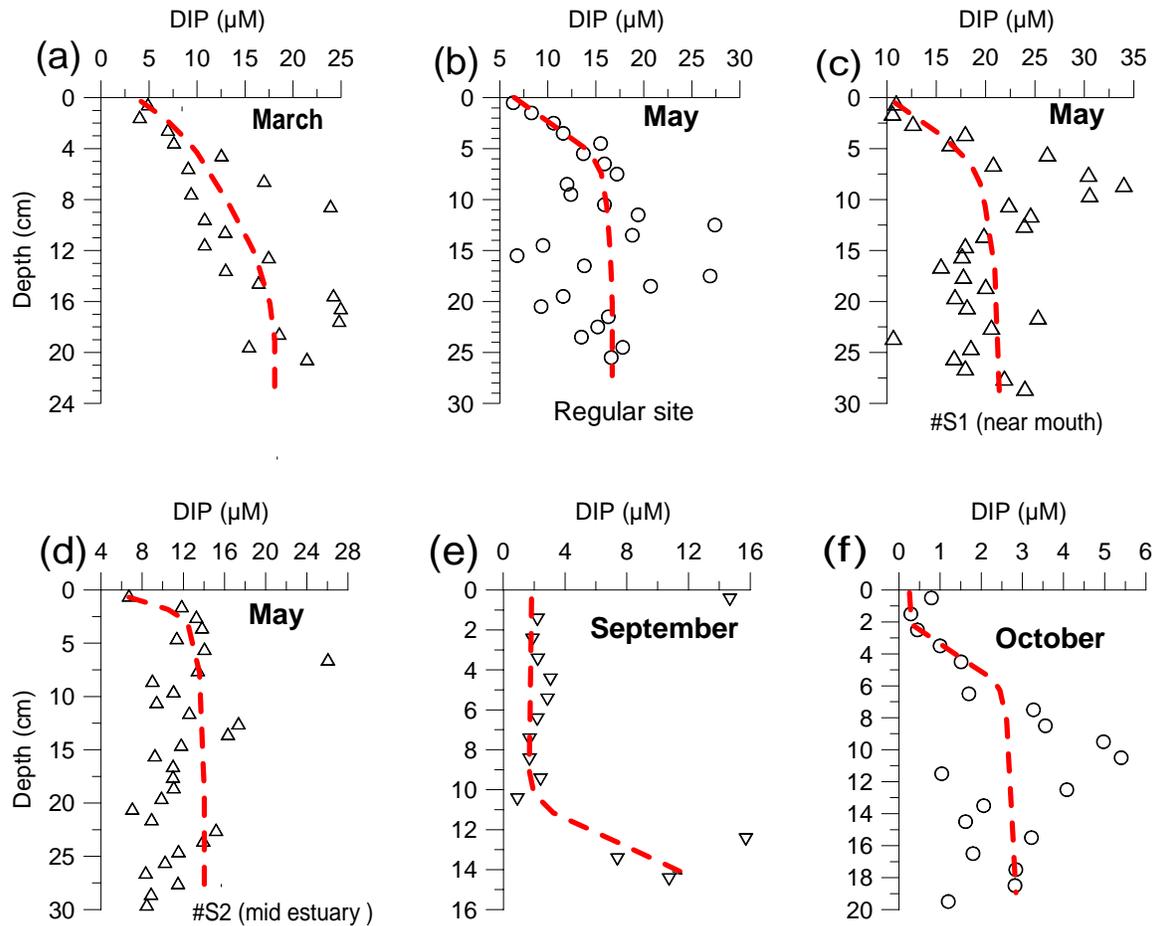
water which may lead to lower bacterial remineralization rate of sedimentary organic matter. This leads to a decrease in phosphate production rate in the sediment that does not exceed the buffering capacity of the sediment. Pore water  $\text{PO}_4^{3-}$  in the upper sediment layer remains well buffered in monsoon as evident in the fig. 5.6e which is more similar to fig-5A, a perfectly S-shaped presumably because the core was not



**Fig. 5.5:** Idealized pore water profile of  $\text{PO}_4^{3-}$  reflecting relative effect of diffusive boundary layer (DBL), Buffering capacity of the sediment and the net production of  $\text{PO}_4^{3-}$  (Sundby *et al.*, 1992).

long enough to contain the lower part where  $\text{PO}_4^{3-}$  concentration is expected to have reached a nearly constant value. Fig. 5.6e shows that  $\text{PO}_4^{3-}$  buffering can occur up to a depth of 10cm which means that the sediment particles have retained some of their capacity to buffer the phosphate concentration even though they are surrounded by an anoxic layer. Krom and Berner (1980) have shown experimentally that anoxic sediments of Long Island Sound adsorb less than 1/25th as much phosphate as oxic sediments from the same site. As temperature increases in the post-monsoon season, it causes an increase in the production rate of  $\text{PO}_4^{3-}$  which may become further dominant over the buffering

capacity, thereby giving a shape similar to Fig. 5C again in October. Buffering of pore water  $\text{PO}_4^{3-}$  in October (Fig. 5.6f) seems to be limited to top 2cm of the sediment column. Thus the above results imply that maximum buffering of pore water



**Fig.5.6:** Seasonal changes in pore water profile of  $\text{PO}_4^{3-}$  in Mandovi estuary which is due to interplay between buffering capacity of sediment, phosphate production rate in sediment and diffusive boundary layer.

phosphate in the estuary occurs during monsoon season and it is minimal during the premonsoon and post-monsoon period in the estuary.

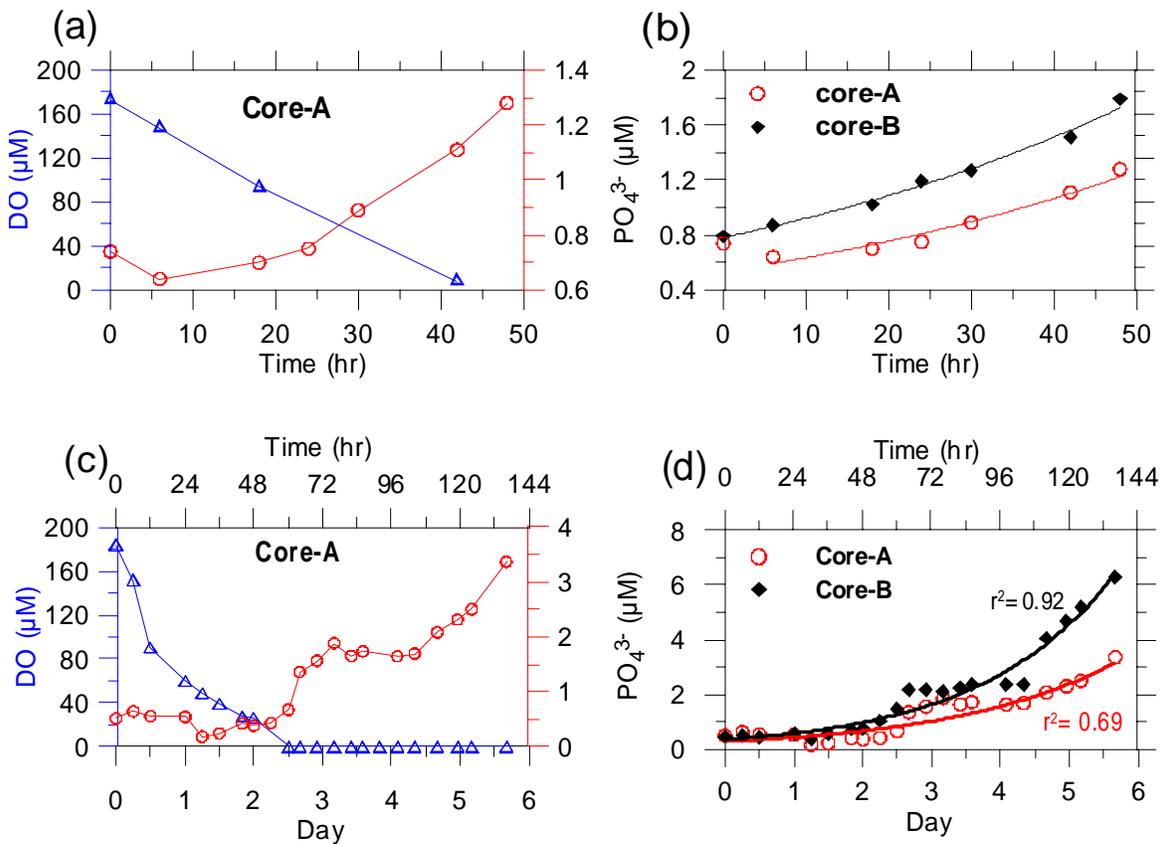
#### 5.3.4. Phosphate release in anoxic condition

**Experiment-2** showed the difference in phosphate release rate between *core-A* in which oxygen in overlying water was allowed to be consumed with time and *core-B* in which overlying water was deoxygenated from the beginning. In *core-A* oxygen depletion in the

overlying was rapid and the concentration reached below Winkler's detection limit after 2 days even though phosphate also did not show any appreciable increase within first 48 hours (Fig. 5.7c). But when the overlying water became completely anoxic afterwards, it exhibited a large increase from 3.8  $\mu\text{M}$  at 48 hours to 3.3  $\mu\text{M}$  at 136 hours. Similarly in core-B phosphate underwent a gradual increase up to 24 hours, but after that its release from the sediment became exponential (Fig. 5.7d). Interestingly  $\text{PO}_4^{3-}$  release rate in core-B was higher than that in core-A, since the overlying water in core-B was already anoxic prior to the start of incubation. In oxygenated systems, a band of poorly crystalline ferric oxyhydroxide occurs to depths of a few millimeters below the water-sediment interface in organic rich lacustrine and estuarine sediments (Williams *et al.*, 1976). Numerous studies have shown that iron oxides and oxyhydroxides are major scavengers of dissolved inorganic phosphate by adsorption and co precipitation under oxic condition (Froelich *et al.*, 1979; Lijklema, 1977, 1980; Golterman, 1976; Parfitt *et al.*, 1975; Krom & Berner, 1980, 1981). But reductive dissolution of above minerals under anoxic condition, serves as a source of reactive phosphorus (Krom & Berner, 1981; Sundby *et al.*, 1992; Jensen *et al.*, 1995). Fe (III) reduction in anoxic condition, which releases adsorbed  $\text{PO}_4^{3-}$  may be partly the result of microbial utilization of Fe (III) as an electron acceptor (Sorensen, 1982; Jansson, 1986). Earlier benthic chamber experiments or core incubation experiments were confined to at most 48 hours and though oxygen in those experiments fell to suboxic or anoxic levels after 48 hours, the effect of prolonged anoxia in overlying water on the benthic phosphate release could not be assessed. In those experiments linear increase  $\text{PO}_4^{3-}$  concentration of overlying water was observed with time like in the present short-term experiment (Fig. 5.7a & b). Froelich (1988) suggested that the adsorption and desorption of  $\text{PO}_4^{3-}$  into the crystal lattice of ferric oxyhydroxides, is a slow process which occurs on the time scale of days to months. This may be reason why exponential release of  $\text{PO}_4^{3-}$  to overlying water was not immediate.

Though  $\text{PO}_4^{3-}$  concentration in both the cores remained almost equal to each other at a given time up to 12 hours, *core-B* exhibited higher rate of increase of  $\text{PO}_4^{3-}$  after that and the exponential increase in  $\text{PO}_4^{3-}$  concentration in this was much more prominent. The abrupt increase in  $\text{PO}_4^{3-}$  in both the cores between 60 hours and 98 hours happened

because of inadvertent power failure in the laboratory. But stirring was continuous after that which accounts for slower phosphate increase. Sundby *et al.* (1986) explained that when stirring stops, the diffusive boundary layer through which oxygen diffuses to sediment surface, thickens which in turn, leads to smaller concentration gradient and a reduced oxygen flux to the sediment. So the anoxic region developed below the boundary layer results in release of Fe and  $\text{PO}_4^{3-}$  to the overlying water. Upon resumption of



**Fig. 5.7:** (a) Oxygen consumption and phosphate release with time in core-A and (b) Comparative exponential release of  $\text{PO}_4^{3-}$  in core-A and core-B in the short term experiment. (c) oxygen consumption and  $\text{PO}_4^{3-}$  release and (d) comparative release of  $\text{PO}_4^{3-}$  in core-A & core-B in the long term experiment.

stirring, the boundary layer thickness reduces significantly and downward diffusion of oxygen again becomes prominent which results in adsorption of excess  $\text{PO}_4^{3-}$  to the sediment. Zwolsman (1994) found the dissolution of ferric oxyhydroxides to be

responsible for remobilization of  $\text{PO}_4^{3-}$  in the Scheldt estuary. Similar  $\text{PO}_4^{3-}$  mobilization has been observed in the anoxic head waters of the Rhode river estuary (Jordan *et al.*, 1991) and the Tiber estuary (Pagnotta *et al.*, 1989). Similar exponential release of  $\text{PO}_4^{3-}$  upon negative redox turn over was also shown by Sundby *et al.* (1986). The critical role of oxygen in controlling the release  $\text{PO}_4^{3-}$  from fluvial, estuarine and marine sediments has been indicated in many other studies as well (Boynton & Kemp, 1985; Sundby *et al.*, 1992; Ishikawa & Nishimura, 1989).

### **5.3.5. Salinity and Phosphate release**

The effect of salinity on benthic phosphate release has already been described in detail in *Chapter-IV*.  $\text{PO}_4^{3-}$  flux showed a positive correlation with salinity and negative correlation with  $\text{PO}_4^{3-}$  concentration in the overlying water. Lijklema (1977) and Bostrom *et al.* (1982) also suggested that increased pH in the water overlying the sediment, decreases the P-binding capacity of Fe and Al oxide minerals, primarily due to ligand exchange reaction in which  $\text{OH}^-$  ions replace  $\text{PO}_4^{3-}$  from adsorption sites. Later Lebo (1991) and Zwolsman (1994) reported that the increase in pH with salinity increase may prevent phosphate adsorption on to ferric oxyhydroxides, by shifting speciation of phosphate from  $\text{H}_2\text{PO}_4^-$  to  $\text{HPO}_4^{2-}$  and changing the surface charge on iron oxyhydroxides. In the Mandovi estuary the benthic flux of  $\text{PO}_4^{3-}$  seems to be controlled by the resultant effect of the interplay between positive effect of salinity and negative effect of water column  $\text{PO}_4^{3-}$  concentration. At negligible variation in  $\text{PO}_4^{3-}$  concentration with changing salinity of the overlying water, increasing salinity enhances benthic  $\text{PO}_4^{3-}$  release which was observed in the core incubation experiment (*Chapter-IV*). In contrast, the effect of increasing salinity is overwhelmed by the negative effect of decreasing concentration gradient between pore water and overlying water when there is considerable increase in water column  $\text{PO}_4^{3-}$  concentration with increasing salinity and that ultimately results in lower  $\text{PO}_4^{3-}$  release from sediment. This effect was evident in the results of benthic chamber experiment. ■

## CHAPTER - VI

## NUTRIENT UPTAKE AND BENTHIC FLUXES

**6.1. Introduction**

It is a well known fact that sediment can supply considerable amounts of nutrients to the overlying water column through benthic regeneration in estuaries and coastal environments. As it has been discussed in *Chapter-IV*, benthic fluxes in the estuarine and coastal systems of Goa have potential to contribute significantly to the pelagic productivity. Such benthic inputs appear to occur continuously even though their magnitude vary seasonally. They make significant contribution to the pelagic nutrient inventories, but the question remains as to whether or not they can be utilized by algal biomass to increase whole system productivity particularly in that season and if so then whether the uptake rate is in balance with the benthic flux rate. In order to address this question, nutrient enrichment experiments were carried out in Mandovi estuary as well as at the mentioned coastal station (G5).

**6.2. Methodology***(i) Experiment in Mandovi estuary*

The first experiment was carried out in February in which surface waters were collected in a Niskin sampler and filtered through 200 $\mu$  mesh to remove macrograzers such as meso-zooplankton and then filled into three transparent polycarbonate Nalgene bottle of 25 litre capacity. The first bottle (bottle-A) was enriched with  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$ , second one (bottle-B) was spiked with  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  and the third one (bottle-C) was kept as control (without any nutrient addition). Ambient  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  were 0.51, 0.09, 2.28, 0.57 and 10.92  $\mu\text{M}$  respectively just before the incubation. The bottles with added nutrients were shaken thoroughly and deployed at 1 meter depth by hanging them from a moored floating raft which minimized their horizontal displacement with the current. The first sampling was done after 16 hours of

incubation and after that it was done at 24 hourly interval. Collected samples were analyzed for nutrients, chlorophyll *a* and phytoplankton. While phytoplankton samples were immediately preserved by adding Lugol's iodine solution, chlorophyll *a* and nutrient samples were immediately frozen till the analyses. Nutrient and chlorophyll samples were analyzed within 24 hours of the collection. Incubation was continued for ~10 days.

The second experiment was done in March at the same location but *bottle A* was not enriched with  $\text{NH}_4^+$  thereby making it as a duplicate of Bottle-B. Bottle-C served as the control. Physico-chemical condition of the estuary was quite similar in February and March and ambient concentrations of  $\text{NO}_3^-$ ,  $\text{NO}_2^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  at the time of second experiment were 0.35, 0.12, 0.46, 0.67 and 8.41  $\mu\text{M}$  respectively. This time the incubation was carried out for ~5 days with sampling at 4 hour interval.

(ii) *Experiment at the coastal station (G5)*

At G5, the enrichment experiment was conducted in a similar manner as in the estuary. Bottles were deployed by hanging them from a floating buoy. The buoy was tied to the anchored vessel CRV *Sagar Sukti* in order to minimise lateral displacement of the bottles. Incubation was continued for ~4 days. Samples were collected for nutrients, chlorophyll and phytoplankton at 6 hourly intervals. Chlorophyll-*a* was analyzed immediately on board the vessel whereas nutrient samples were frozen immediately at  $-20^\circ\text{C}$  and analyzed within 24 hrs.

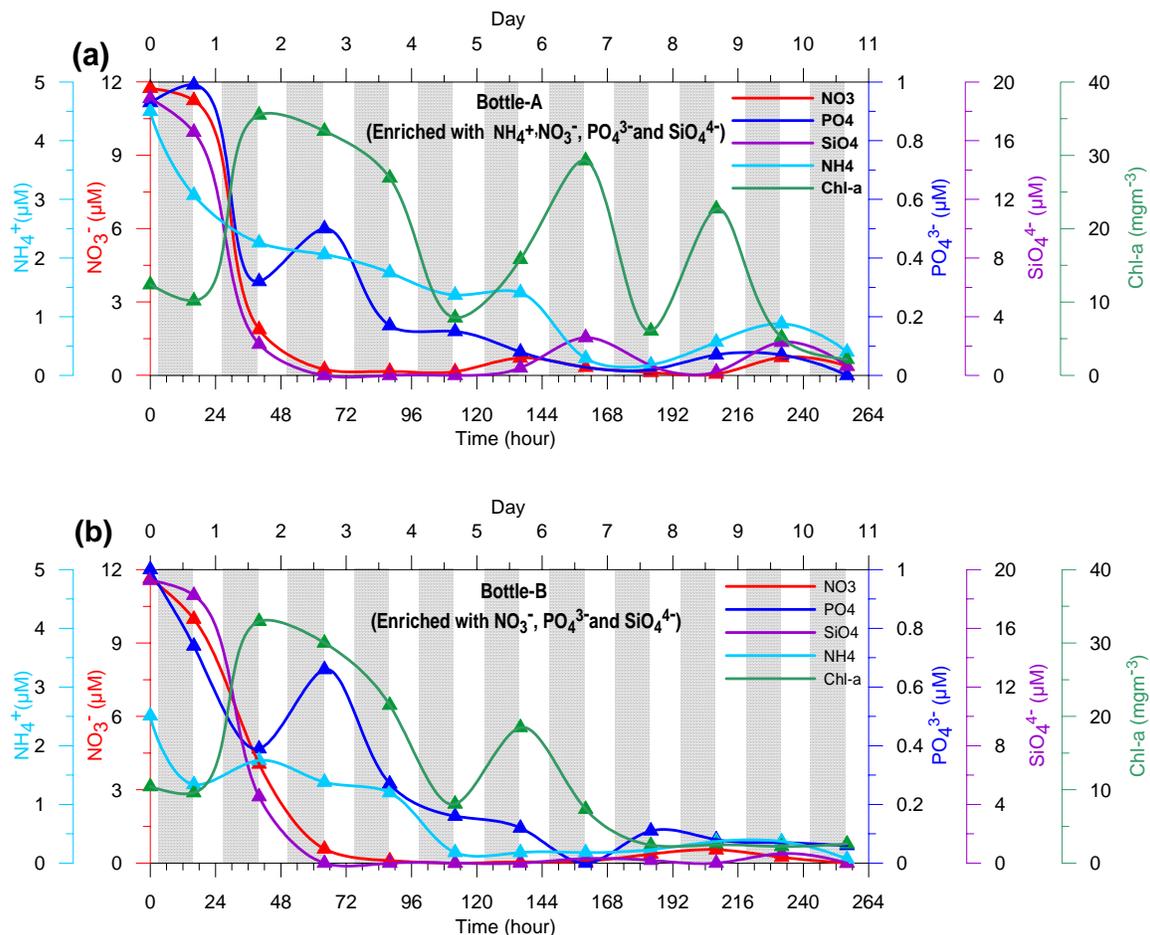
### 6.3. Results & Discussion

#### 6.3.1. Nutrient uptake in the estuary

##### 6.3.1.1. Uptake in February

In the first experiment in the estuary incubation was initiated at around 1600 hours in the afternoon and after  $T_0$  sampling, next sampling was carried out after 16 hrs i.e. next day morning 0800 hours. This was done expecting that photosynthesis could not occur at night, hence no nutrient uptake. Due to delayed incubation on the first day, only three hours of sunlight were available. As a result there was no significant decrease in nutrient or chlorophyll concentrations in the first 16 hours of the incubation. However within next 24 hrs,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  were rapidly taken up in bottles A and B by phytoplankton

(Fig. 6.1). But no significant change in nutrient was observed in the control bottle ( $p > 0.05$ ) (Fig. 6.2). The  $\text{NH}_4^+$  concentrations decreased to  $\sim 50\%$  of its  $T_0$  concentration ( $4.5 \mu\text{M}$ ) after 40 hours at a much slower rate. These decreases in nutrient concentration coincided with sharp increase in chlorophyll-*a* concentration between 16-40 hrs. After 40 hours  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  decreased to 16, 32 and 11% respectively of their initial concentration whereas chlorophyll-*a* increased by a factor of 3. Being a reduced form of nitrogen,  $\text{NH}_4^+$  was expected to be taken up preferentially over  $\text{NO}_3^-$ , the most oxidized form of N. But interestingly, the difference in  $\text{NO}_3^-$  uptake pattern in *bottle-A* and *bottle-B* was found to be statistically insignificant (ANOVA,  $p = 0.9$ ). Though  $\text{NH}_4^+$  was also taken up by



**Fig.6.1:** Uptake of nutrients and increase of chlorophyll with incubation time in (a) bottle-A and (b) bottle-B in February in Mandovi Estuary. Shaded area represents night time.

phytoplankton along with  $\text{NO}_3^-$  and  $\text{NO}_2^-$ , the uptake rate was much lower and it became negligible only after  $\sim 6$  days; much after  $\text{NO}_3^-$  depletion. The water remained oxygenated ( $\text{O}_2 > 4$  ml/L) through out the incubation experiment thereby ruling out the possibility of  $\text{NO}_3^-$  loss due to denitrification or build up of  $\text{NH}_4^+$  through  $\text{SO}_4^{2-}$  reduction process which occur in reducing environment. The uptake pattern of nutrients and biomass growth (Chlorophyll-*a*) appeared to be similar in bottle-A and bottle-B (Fig. 6.2). To compare the uptake pattern of  $\text{NO}_3^- + \text{NO}_2^-$ ,  $\text{PO}_4^{3-}$ ,  $\text{SiO}_4^{4-}$  and chlorophyll-*a* production between *bottle-A* and *bottle-B*, a one way ANOVA was applied between corresponding parameters of both bottles. No significant difference was found between these two data sets ( $p > 0.05$ ). This shows that *bottle-A* and *bottle-B* can be considered almost as duplicates. Chlorophyll-*a* maxima were observed at  $\sim 36$  hrs in *bottle-A* and *bottle-B* whereas in control (*bottle-C*) the variation with time was negligible (Fig. 6.2).

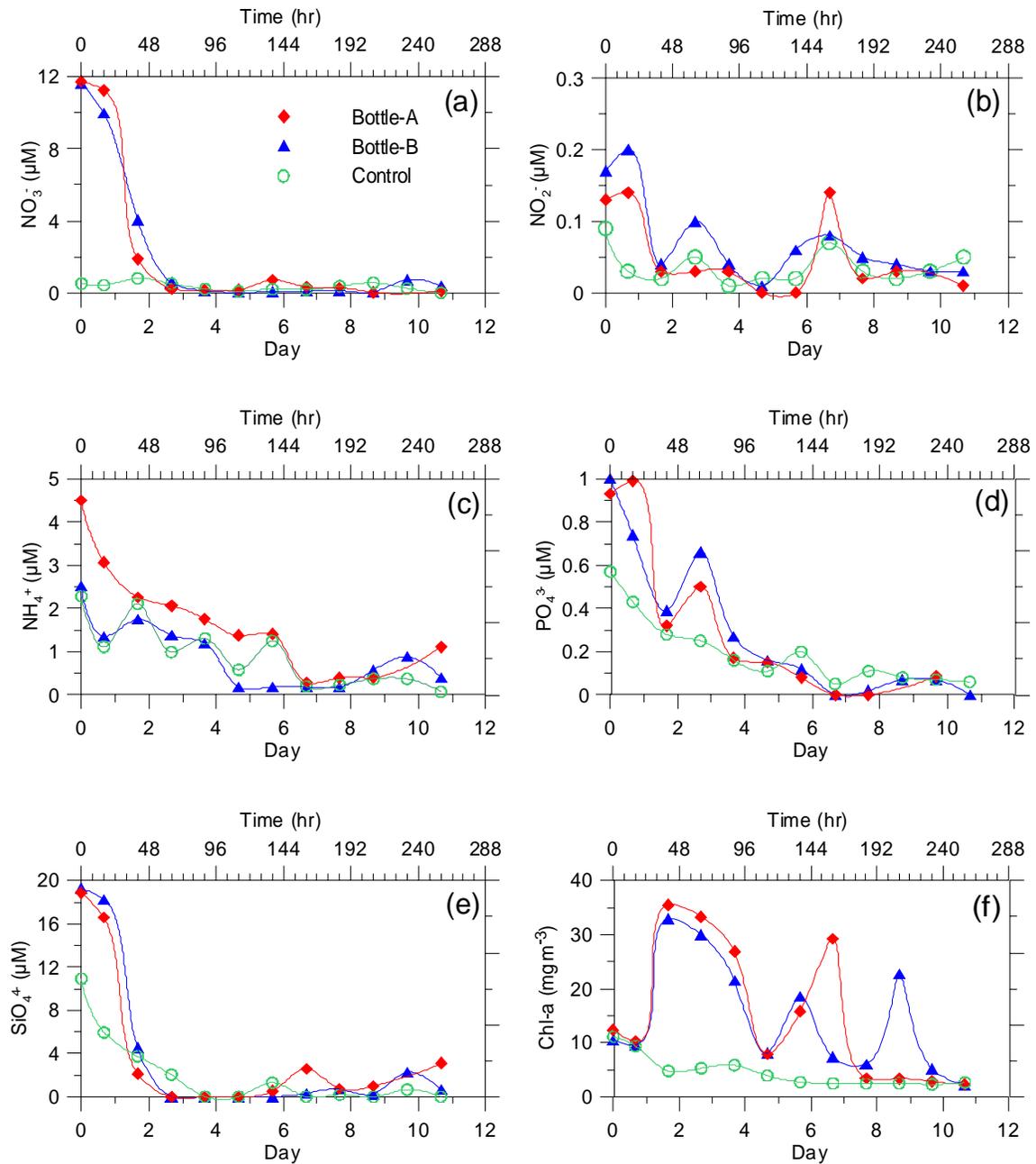
#### 6.3.1.2. Uptake in March

In the February experiment since bottles were exposed to sunlight only for  $\sim 3$  hrs after their deployment, the reason for non-uptake of nutrients during the first 16 hrs *i.e.* whether it was due to non-occurrence of photosynthesis at night or a time lag in phytoplankton response could not be ascertained. In the second experiment, bottles were exposed to sunlight for much longer period (11 hrs).

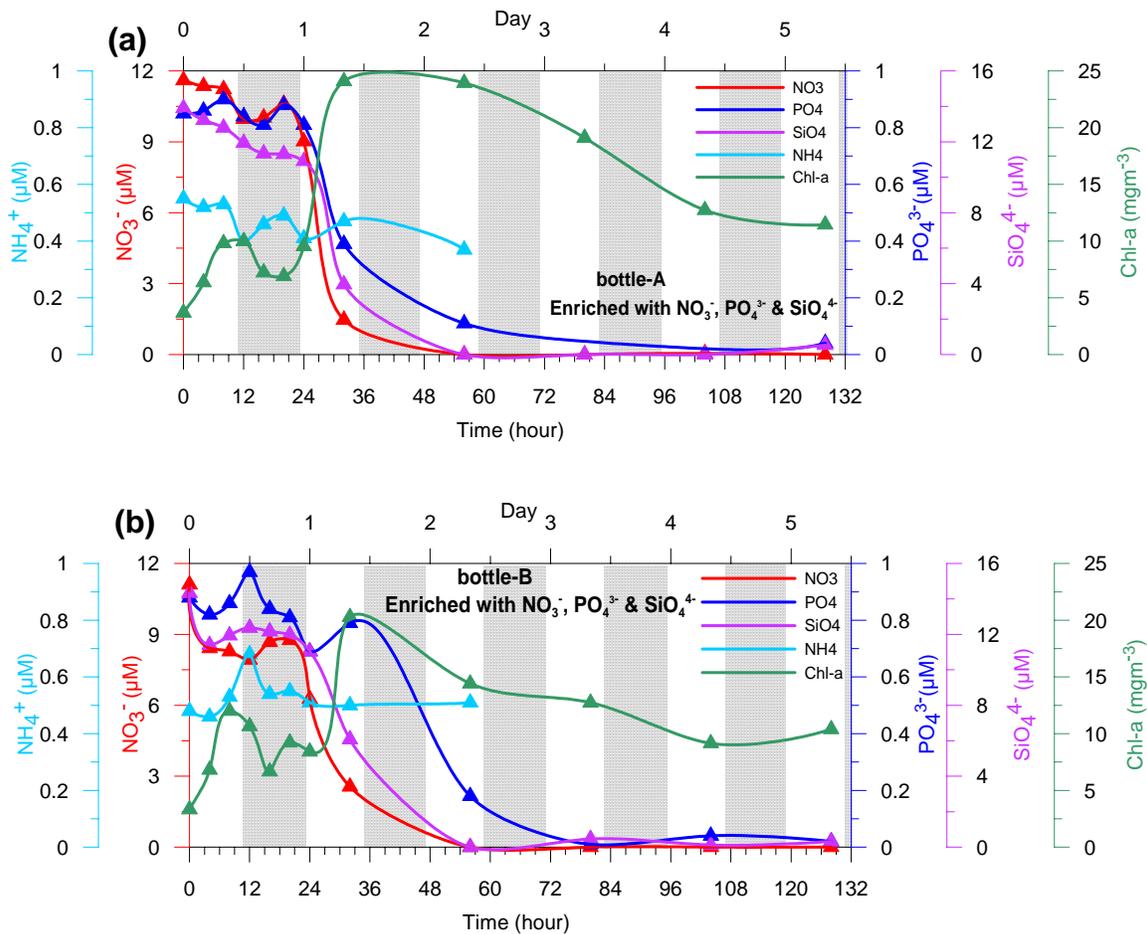
This time bottles were deployed early in the morning and sampling was done at much shorter time interval (4hrs) within first 32 hrs to closely observe the uptake pattern in the first 16 hours. Afterwards sampling was done 24 hourly.

Though this time bottles received sunlight for 11 hours, the observed changes in nutrient and chlorophyll concentrations ( $p > 0.05$ ) were relatively smaller within that period. Apparently nutrients started decreasing within the first day of incubation itself, accompanied by increases in chlorophyll (Fig. 6.3). However considerable changes did not occur in next 12 hrs because of darkness. Sharp decreases in nutrient and increase in chlorophyll-*a* concentration were observed only after 24 hrs of incubation time (*i.e.* on the next day). Nutrients decreased sharply between 24 to 32 hrs of incubation. Chlorophyll peak was attained at 32 hrs. Both in bottle-A and B, similar uptake patterns and chlorophyll increases were observed whereas negligible changes in these parameters

occurred in the control (Fig. 6.4). One way ANOVA revealed the variation in uptake pattern between corresponding parameters of bottles A and B were insignificant ( $p>0.05$ ).



**Fig.6.2:** Comparative uptake of nutrients and increase of Chlorophyll-a in different bottles with incubation time in the February nutrient enrichment experiment in Mandovi estuary. Bottle-A was enriched with  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$ . Bottle-B was enriched with  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$ . Bottle C was kept as control (without nutrient addition).

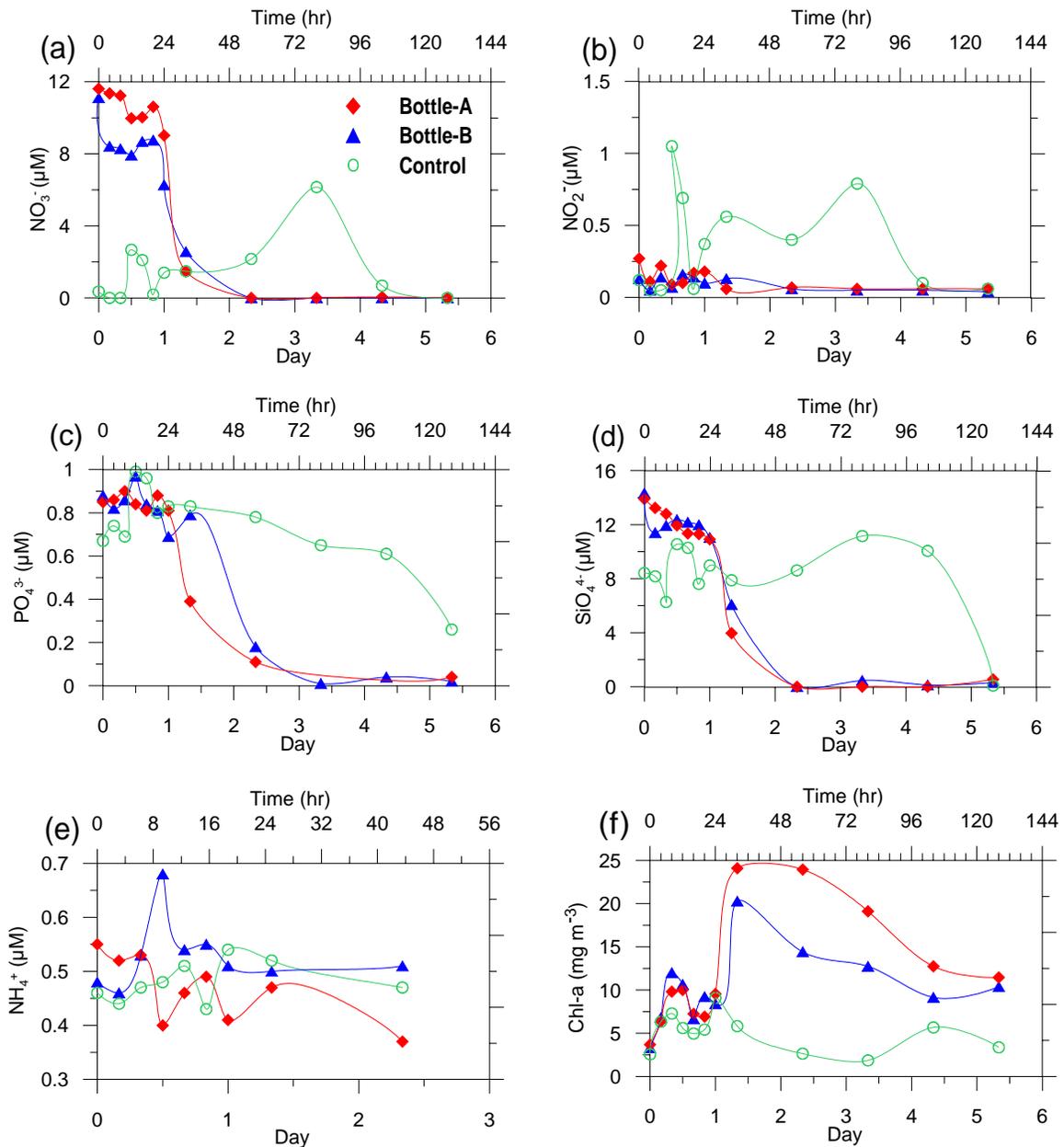


**Fig.6.3:** Uptake of nutrients and increase in chlorophyll with time in (a) bottle-A and (b) bottle-B in Marchin Mandovi Estuary. Shaded area represents night time.

After ~2 days of incubation ambient water entered into the control bottle through a leakage which increased nutrient concentration but once it was remedied, nutrients decreased due to algal uptake which resulted in some increase in chlorophyll (Fig. 6.4f). However the difference between initial and final nutrients and chlorophyll concentrations in the control, was still found to be statistically insignificant (ANOVA,  $p=0.9$ ).

### 6.3.1.3. Response of phytoplankton to nutrients

Nutrients are usually assimilated by phytoplankton during day time in the process of photosynthesis. From above two enrichment experiments, it was observed that the response of estuarine phytoplankton to nutrient enrichment is almost immediate. Though in February negligible changes in nutrient and chlorophyll concentrations occurred, few



**Fig.6.4:** Comparative nutrient uptake and chlorophyll increase with time in different bottles in the March enrichment experiment in Mandovi estuary. Bottle-A and Bottle-B were enriched with  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  and bottle-C was kept as control (without nutrient addition).

hours after enrichment, which was because of short exposure of the bottles to solar radiation (Fig. 6.1 & 6.2), in March, the changes in these parameters would be noticed with just a few hours time lag (Fig. 6.3 & 6.4). This could be because of increase in intensity of solar radiation from February to March. However if night times are excluded

from consideration, two types of uptake appear to exist, *i.e.* the slow uptake which occurred in the first ~8hrs after enrichment and the fast uptake which occurs after that. This was particularly very prominent in March experiment. It indicates that though the response of the estuarine phytoplankton to ambient nutrient enrichment is immediate, they take a while for rapid uptake and this time lag may be due their slow physiological adaptation to eutrophication.

Three groups of phytoplankton *i.e.* diatoms, dinoflagellates and silicoflagellates are found to grow in all bottles in both the experiments. Diatoms always remained the dominant group comprising >99% of the algal biomass leaving a very small percentage for dinoflagellates and silicoflagellates. A total of 34 genera of diatom, 6 genera of dinoflagellate and only one genus of silicoflagellate were found in the samples. Among diatoms, three major species *i.e.* *Thalassionema nitzschoides*, *Asterionella japonica* and *Chaetoceros curvisetus* were found in February whereas *Thalassionema nitzschoides*, *Melosina spp* and *Skeletonema costatum* were major species in March. Among these species *Thalassionema nitzschoides* is a pennate and others are centric diatoms.

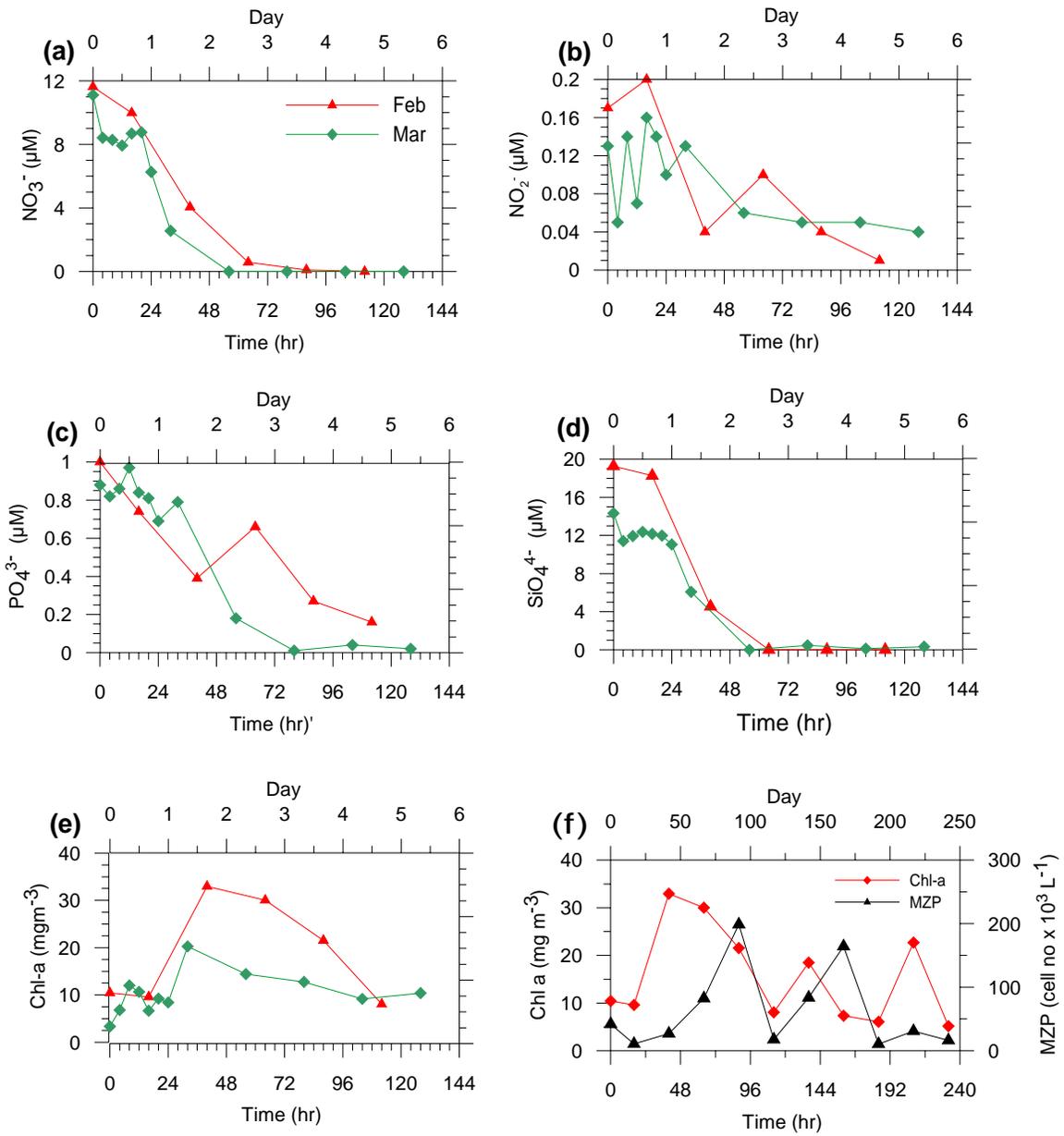
The apparent net growth rate ( $\mu$ ) of the phytoplankton was calculated from changes in the chlorophyll biomass (Pederson & Borum, 1996) *i.e.*

$$\mu = (\ln B_t - \ln B_0) / t$$

Where  $B_0$  and  $B_t$  are initial and final biomass respectively after  $t$  days of incubation.

Net growth rate in February was calculated to be 0.63 and 0.69  $\text{mg m}^{-3} \text{d}^{-1}$  in *bottle-A* and in *bottle- B* respectively, but increased solar radiation in March might have induced higher uptake rate of nutrients (Table 6.1) and hence stimulated higher growth rate as it was found to be 1.40 and 1.35  $\text{mg m}^{-3} \text{d}^{-1}$  in *bottle-A* and in *bottle- B* respectively. This closeness of  $\mu$  in both the bottles in February indicates that presence of  $\text{NH}_4^+$  did not make any significant increase in algal biomass.

From Fig. 6.5, it is apparent that the nutrient enrichment experiments done in February and March gave almost same results which consolidates the fact that the phytoplankton uptake occurs at a rapid rate after about 24 hours in the estuary. Chlorophyll concentration attains maxima only after ~36h of incubation in response to available nutrient concentration. However, the subsequent drastic decrease in chlorophyll possibly occurs due to grazing pressure exerted by micro-grazers ciliates in particular (Fig. 6.5f).

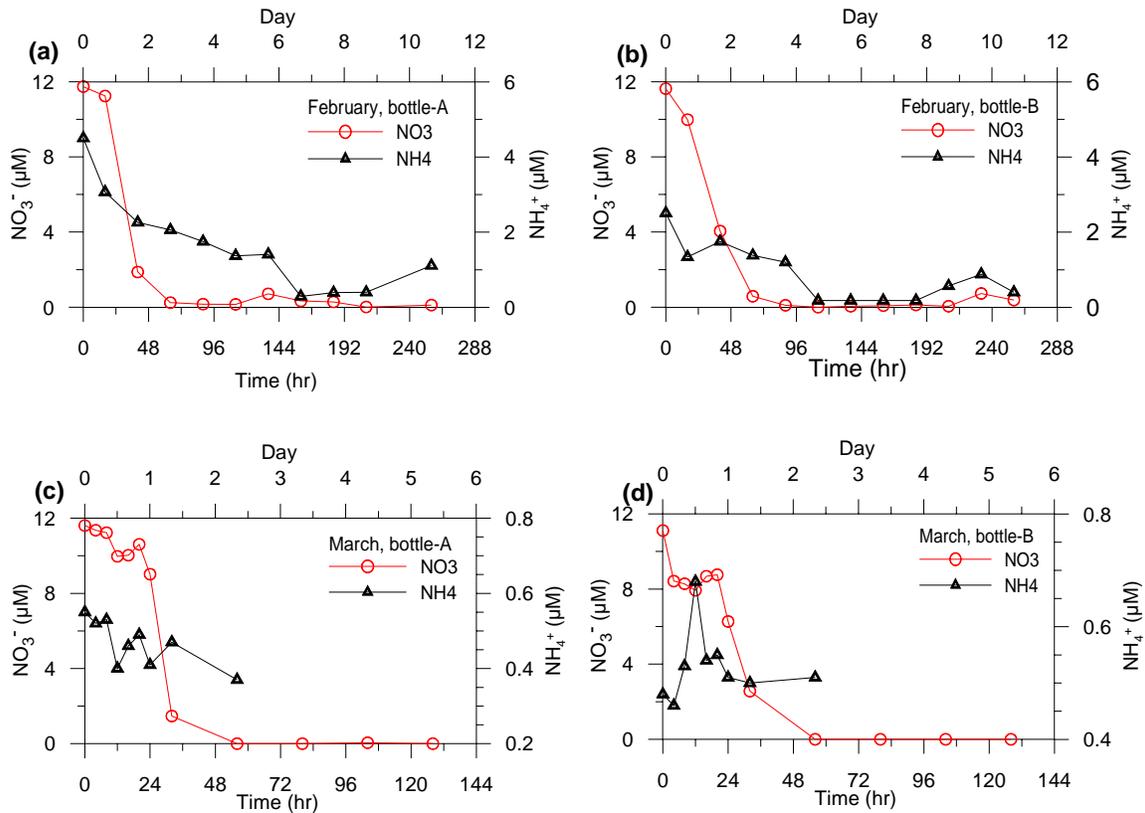


**Fig.6.5:** (a), (b), (c), (d)-Comparative nutrient uptake, (e) chlorophyll production with time in February & March and (f) relative growth of chlorophyll and microzooplankton (MZP) in February with incubation time in Mandovi Estuary.

### 6.3.1.4. Nitrate versus Ammonium uptake

Studies with unialgal laboratory cultures and artificially enriched coastal seawater have shown that marine phytoplankton prefer  $\text{NH}_4^+$  over  $\text{NO}_3^-$  as a nitrogen source (Eppley *et al.*, 1969; Eppley & Rogers, 1970; McCarthy & Eppley, 1972). Several other authors (e.g. Glibert & McCarthy, 1984; Goering *et al.*, 1970; Glibert *et al.*, 1982a; Probyn & Painting, 1985; McCarthy *et al.*, 1977; Dortch & Postel, 1989) have also reported the inhibitory effect of  $\text{NH}_4^+$  on algal uptake of  $\text{NO}_3^-$  or algal preference of  $\text{NH}_4^+$  over  $\text{NO}_3^-$  which severely reduces the rate of  $\text{NO}_3^-$  uptake. Dortch & Conway (1984), McCarthy *et al.*, (1975, 1977), Kuenzler *et al.* (1979), Garside (1981), Paasche & Kristiansen (1982a) found a threshold ammonium concentration of 1  $\mu\text{M}$ , above which  $\text{NO}_3^-$  uptake is largely inhibited despite high concentration of ambient  $\text{NO}_3^-$ . Losado & Guerrero (1979) and Syrett (1981) arrived at a similar conclusion based on theoretical considerations of the relative energy requirement for the utilization of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . However in some cases (e.g. Goering *et al.*, 1970; Harvey & Caperon, 1976; Ronner *et al.*, 1983; Kokkinakis & Wheeler, 1987; Kristiansen & Lund, 1989) no correlation was found between ambient  $\text{NH}_4^+$  concentration and  $\text{NO}_3^-$  uptake. Glibert *et al.* (1982b) observed stimulation of  $\text{NO}_3^-$  uptake by  $\text{NH}_4^+$ . Kuenzler *et al.* (1979), Harrison *et al.* (1982, 1983 & 1985), Price *et al.* (1985) and Collos *et al.* (1989) reported simultaneous uptake of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . Interestingly a preference for  $\text{NO}_3^-$  over  $\text{NH}_4^+$  was observed by Wafar *et al.* (1983) and Harrison *et al.* (1987).

In the experiment  $\text{NO}_3^-$  was taken up before  $\text{NH}_4^+$  was exhausted. In March  $\text{NH}_4^+$  concentration was low (0.5  $\mu\text{M}$ ) but although ambient  $\text{NH}_4^+$  was  $>2\mu\text{M}$  in February, no inhibition was observed in bottles A and B. These results go against the widely held view that phytoplankton prefer  $\text{NH}_4^+$  over  $\text{NO}_3^-$ . Interestingly Malone (1980) and Kokkinakis and Wheeler (1987) found that large sized phytoplankton cells preferentially assimilated  $\text{NO}_3^-$  over  $\text{NH}_4^+$ . Dham *et al.* (2002) also reported comparatively greater uptake of  $\text{NO}_3^-$  than  $\text{NH}_4^+$  in Achara river estuary (central west coast of India).



**Fig.6.6:** Comparative uptake of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  with time in the Mandovi Estuary.

(a) & (b) show uptake in bottle-A and bottle-B respectively in February and (c) & (d) show uptake in bottle-A and bottle-B in March.

**Table.6.1:** Comparative uptake of ammonium and nitrate by estuarine phytoplankton. *Bottle-A* was enriched with  $\text{NO}_3^-$  and  $\text{NH}_4^+$  and *bottle-B* was enriched with  $\text{NO}_3^-$ .

		February				March			
		% N nutrient	% N utilized	uptake rate ( $\mu\text{mol L}^{-1} \text{h}^{-1}$ )	RPI	% N nutrient	% N utilized	uptake rate ( $\mu\text{mol L}^{-1} \text{h}^{-1}$ )	RPI
<i>bottle-A</i>	$\text{NO}_3^-$	71	84	0.39	1.19	93	87	0.78	1.04
	$\text{NH}_4^+$	27	49	0.01	0.19	0.04	14	0.003	0.06
<i>bottle-B</i>	$\text{NO}_3^-$	81	65	0.18	1.16	94	76	0.5	1.07
	$\text{NH}_4^+$	17	30	0.01	0.41	0.04	0.09	0.003	0.05

The relative preference index (RPI), originally introduced by McCarthy *et al.* (1979) was meant to compare the utilization of a particular nitrogen compound relative to its availability and is expressed as:

$$\text{RPI for NH}_4^+ = (\rho_{\text{NH}_4^+} / \rho_{\text{DIN}}) / (\text{NH}_4^+ / \text{DIN}) \text{ ----- (1)}$$

Similarly 
$$\text{RPI for NO}_3^- = (\rho_{\text{NO}_3^-} / \rho_{\text{DIN}}) / (\text{NO}_3^- / \text{DIN}) \text{ ----- (2)}$$

Where  $\rho_{\text{NH}_4^+}$ ,  $\rho_{\text{NO}_3^-}$  and  $\rho_{\text{DIN}}$  are the uptake rate of  $\text{NH}_4^+$ ,  $\text{NO}_3^-$  and DIN ( $\text{NH}_4^+ + \text{NO}_2^- + \text{NO}_3^-$ ) respectively. Subsequently several authors (Glibert *et al.*, 1982; Probyn, 1985; Smith & Nelson, 1990 and Owens *et al.*, 1990) have used RPI to study the preferential N uptake.

If RPI >1 in eq-1 and <1 in eq-2, then  $\text{NH}_4^+$  uptake is preferred and

if RPI <1 in eq-1 and >1 in eq-2, then  $\text{NO}_3^-$  is preferentially taken up over  $\text{NH}_4^+$ .

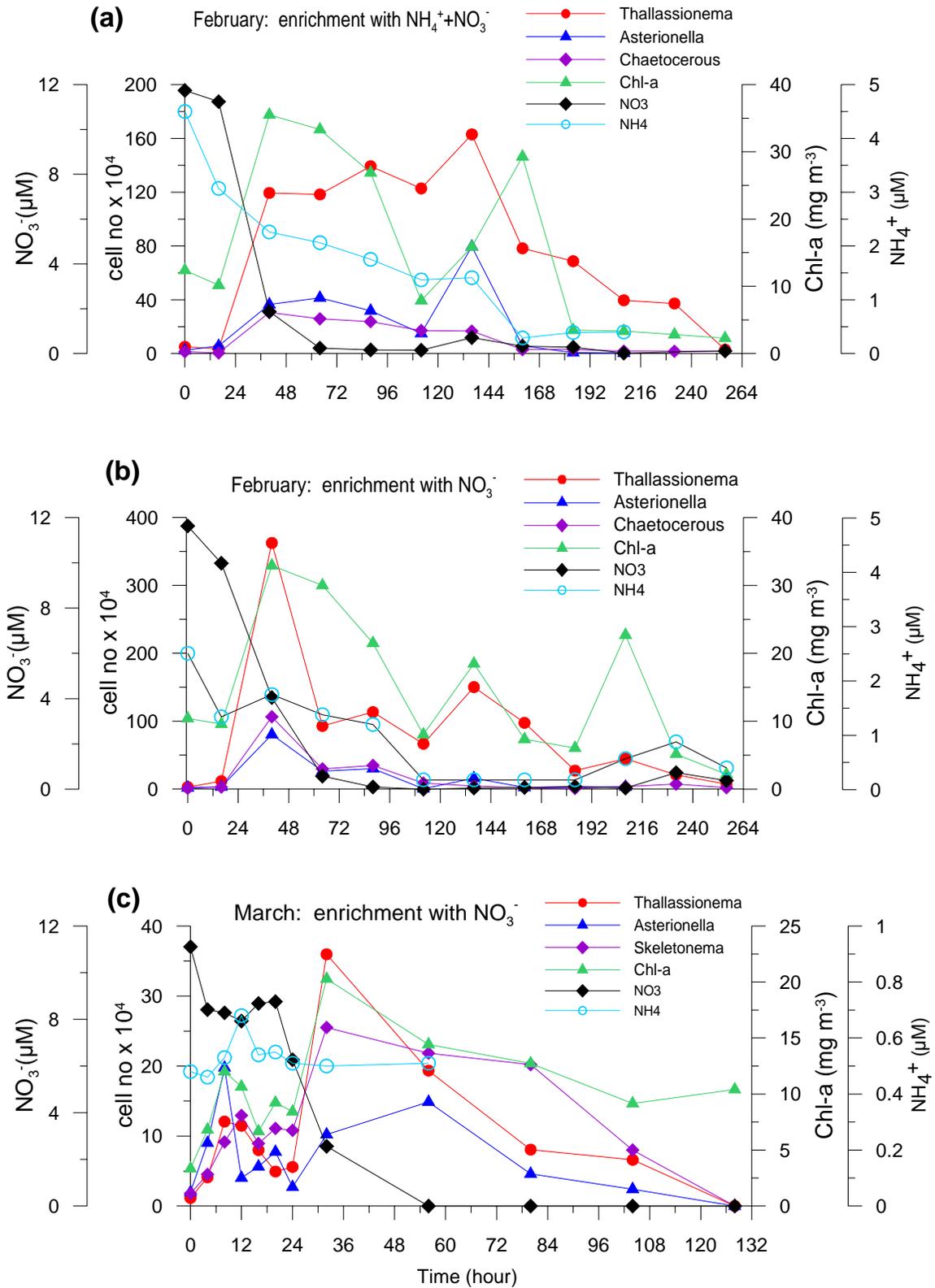
A consistent value of RPI for  $\text{NO}_3^-$  more than 1 in both the bottles in February and March (*see* Table 6.1) indicates that  $\text{NO}_3^-$  is the preferred N substrate for phytoplankton in the estuary irrespective of higher ambient  $\text{NH}_4^+$  concentration. This observation is a contrast to the finding of Dortch *et al.* (1982) who inferred from a laboratory culture experiments that when both  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were supplied to nitrogen deficient phytoplankton, the ability to take up  $\text{NH}_4^+$  increased and ability to take up  $\text{NO}_3^-$  decreased. They summarized that these changes were adaptations to the mechanisms by which  $\text{NO}_3^-$  and  $\text{NH}_4^+$  are added to the euphotic zone as well as their relative availability when N is in short supply. They explained that since phytoplankton growing in areas with low N concentrations are less likely to be exposed sporadically to high  $\text{NO}_3^-$  concentrations than to high  $\text{NH}_4^+$  concentrations, there is no need to develop a rapid uptake mechanism for  $\text{NO}_3^-$ . However DIN in the Mandovi estuary has been observed to remain low in non-monsoon period (October-May) unless there are some episodic inputs of nutrients from sewage discharge. But in *in-situ* enrichment experiment in which estuarine water was spiked with both  $\text{NO}_3^-$  and  $\text{NH}_4^+$  shows rapid uptake of  $\text{NO}_3^-$  irrespective of presence of inhibiting amount of  $\text{NH}_4^+$  (Fig. 6.6). This observation is not consistent with the

hypothesis of Dortch *et al.* (1982). However the presence of  $\text{NO}_3^-$  did not inhibit  $\text{NH}_4^+$  uptake rate as the uptake pattern of  $\text{NH}_4^+$  in *bottle-A* and control in February experiment were found to be similar. As stated above Malone (1980) and Kokkinakis & Wheeler (1987) reported that large sized cells have a distinct preference for  $\text{NO}_3^-$  over  $\text{NH}_4^+$ . Dham *et al.* (2005) found that in the Achara river estuary, the major form N utilized by netplankton (20-200  $\mu\text{m}$  size) was  $\text{NO}_3^-$  and that by nanoplankton (0.8-20  $\mu\text{m}$ ) was  $\text{NH}_4^+$ . Among diatoms they consistently observed  $\text{NO}_3^-$  preference by pennate forms and  $\text{NH}_4^+$  preference by centric forms.

From Fig. 6.7 it is evident that in February a major pennate diatom *i.e.* *Thalassionema nitzschoides* dominates the N uptake scenario irrespective of ambient  $\text{NH}_4^+$  concentration. The growths of other two centric diatoms was found to be comparatively much less. However though in March *Thalassionema* again became dominant among diatoms by assimilating  $\text{NO}_3^-$  preferentially, *Skeletonema* population increased during the later part of the experiment. *Thalassionema* comprised 56% of the diatom community in February but decreased considerably to 29% in March which could be because of increasing temperature. Thus preferential rapid uptake of nitrate in the estuary seems to be due to dominant population of pennate diatoms.

#### **6.3.1.5. Benthic regeneration versus pelagic nutrient uptake**

In experiments carried out both in February and March, nutrient uptake patterns in *bottle-A* ( $\text{NO}_3^- + \text{NH}_4^+ + \text{PO}_4^{3-} + \text{SiO}_4^{4-}$  enriched) and *bottle-B* ( $\text{NO}_3^- + \text{PO}_4^{3-} + \text{SiO}_4^{4-}$  enriched) varied insignificantly ( $p > 0.05$ ) which meant that two systems behaved almost as duplicates. Presence of considerable amount of  $\text{NH}_4^+$  ( $> 2 \mu\text{M}$ ) did not inhibit the rate of  $\text{NO}_3^-$  uptake by phytoplankton. Though in the experiments  $\text{NH}_4^+$  did not seem to contribute directly to the growth of biomass as  $\text{NO}_3^-$  was the most preferred N nutrient, still its conversion to  $\text{NO}_3^-$  via nitrification, can indirectly meet the N requirement of phytoplankton. It also leads to speculation that ammonium can be taken up significantly



**Fig.6.7** : Uptake of nitrate and ammonium by major species of diatoms with time in (a),(b) in February and (c) March in Mandovi estuary.

when ambient  $\text{NO}_3^-$  remains considerably low. This speculation arises from the fact that although  $\text{NO}_3^-$  remains low ( $<1\mu\text{M}$ ) as compared to  $\text{PO}_4^{3-}$  ( $>0.5\mu\text{M}$ ) or  $\text{SiO}_4^{4-}$  ( $>8\mu\text{M}$ ) in the non-monsoon season, maximum primary production is at its maximum in premonsoon (March-April) and post monsoon (October-November) period (Krishnakumari *et al.*, 2002; Pradeep Ram *et al.*, 2003). Moreover  $\text{NH}_4^+$  has been found to be always present at a concentration  $\sim 4\mu\text{M}$  in non-monsoon seasons which may enter the system from nearby mangrove swamp, sewage discharge, resuspension of sediment and benthic regeneration. Further benthic chamber experiments (*Chapter-IV*) have proved that the estuarine sediment is a sink for  $\text{NO}_3^-$  whereas  $\text{NH}_4^+$  remains the dominant N form that was released from sediments in significant quantity in non-monsoon months. Only during the monsoon season (June-September) does the estuarine water get enriched with nutrients particularly  $\text{NO}_3^-$  ( $\sim 8\mu\text{M}$ ) along with  $\text{PO}_4^{3-}$  ( $2.5\mu\text{M}$ ) and  $\text{SiO}_4^{4-}$  ( $\sim 60\mu\text{M}$ ) but even then  $\text{NO}_3^-$  greatly remained unutilized because of cloud cover and turbidity which results in low algal productivity ( $23\text{ mmol C m}^{-2}\text{ d}^{-1}$ , Pradeep Ram *et al.*, 2003). This implies that it is  $\text{NH}_4^+$  rather than  $\text{NO}_3^-$ , probably supports the estuarine productivity in non-monsoon period.

Nutrient uptake rate measured directly from *in-situ* nutrient enrichment experiment and that calculated from primary productivity values measured by Pradeep Ram *et al.* (2003) agree with each other up to a great extent (Table. 6.2) lending support to estimates based on enrichment experiments.

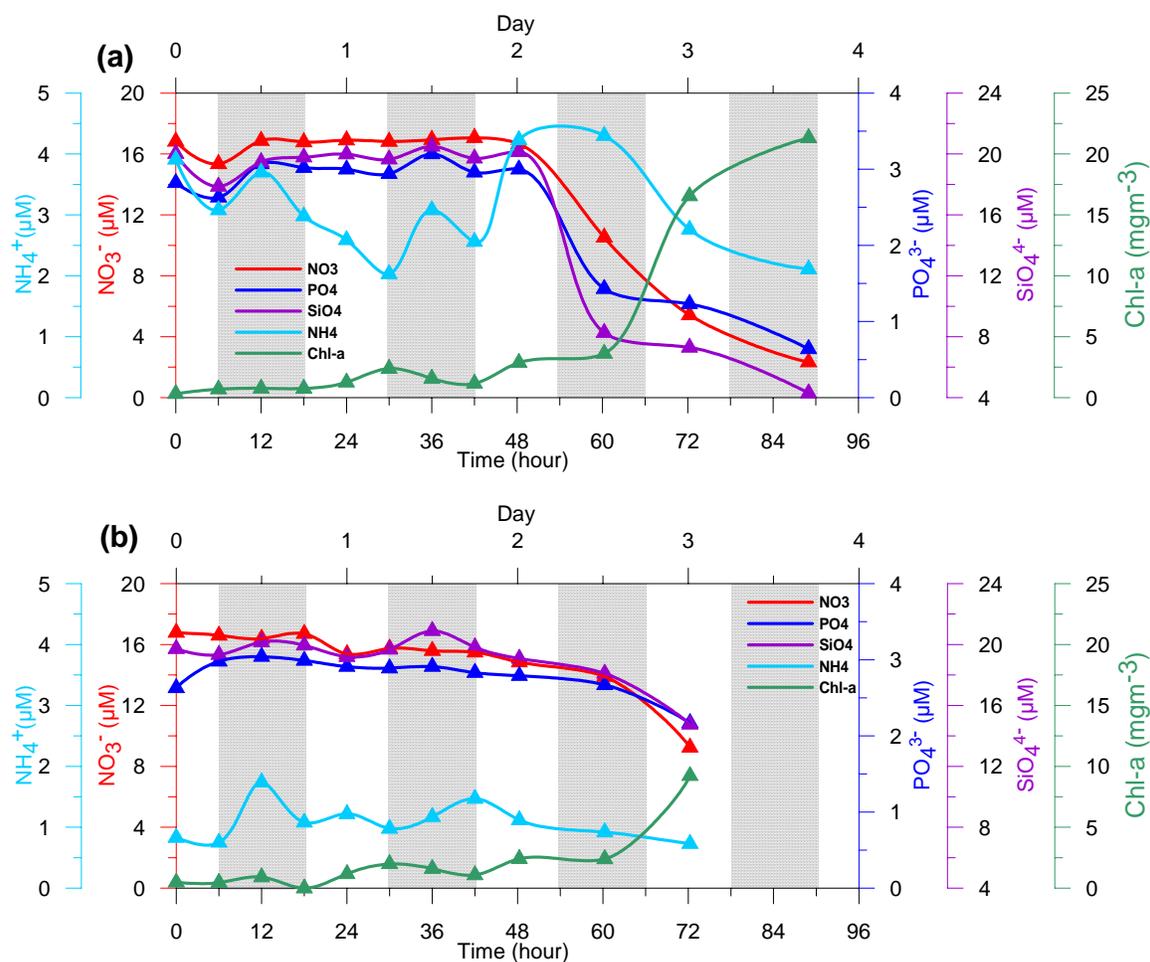
**Table 6.2:** Contribution of benthic flux to Primary productivity; “A” values are calculated from *in-situ* measured uptake rate and “B” values are calculated from primary productivity values.

Month	Nutrient	<i>In-situ</i> measured uptake rate (mmol m <sup>-2</sup> d <sup>-1</sup> )	Calculated uptake rate from GPP (mmol m <sup>-2</sup> d <sup>-1</sup> )	Benthic flux rate (mmol m <sup>-2</sup> d <sup>-1</sup> )	Contribution of Benthic flux to GPP (%)	
					A	B
Feb	DIN	10.55	11.32	3.086	29.39	27.26
	PO <sub>4</sub> <sup>3-</sup>	0.62	0.71	0.128	20.71	18.15
	SiO <sub>4</sub> <sup>4-</sup>	15.97	11.32	4.605	28.96	40.68
Mar	DIN	31.46	28.41	5.096	16.20	17.94
	PO <sub>4</sub> <sup>3-</sup>	1.74	1.78	0.130	7.45	7.30
	SiO <sub>4</sub> <sup>4-</sup>	27.81	28.41	5.780	20.78	20.35

### 6.3.2. Nutrient uptake in coastal waters

Uptake pattern of nutrients at the coastal station was found to be different from that of the estuary. No significant change in nutrient concentration in *bottle-A* (enriched with NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, PO<sub>4</sub><sup>3-</sup> & SiO<sub>4</sub><sup>4-</sup>) and in *bottle-B* (enriched with NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> & SiO<sub>4</sub><sup>4-</sup>) was observed till 48h of the incubation time (Fig. 6.8). Uptake became discernible after 48h in *bottle-A* and after 60h in *bottle-B*. Changes in nutrients and chlorophyll concentrations with time in the control (*bottle-C*) were statistically insignificant (ANOVA,  $p = 0.7$ ). Chlorophyll increased dramatically between 60hrs and 72 hrs in both bottles A and B. However because of logistic problems, experiment was terminated before the chlorophyll could attain its maximum, but its increase from 3.6 to 16.5 mg m<sup>-3</sup> in A and from 2.4 to 9.2 mg m<sup>-3</sup> in B within 12 hrs, accompanied by simultaneous decreases in nutrients clearly indicates a high rate of primary production by phytoplankton.

During the incubation ambient seawater somehow entered to the control bottle through a small leakage which resulted in sudden change in nutrient concentration at 36 h. However the pattern was back to normalcy after the leakage was sealed.



**Fig.6.8:** Uptake of nutrients and increase in chlorophyll with incubation time (a) bottle-A (enriched with  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  &  $\text{SiO}_4^{4-}$ ) and bottle-B (enriched with  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  &  $\text{SiO}_4^{4-}$ ). Shaded area represent night time.

### 6.3.2.1. N preference

Despite the presence of  $\sim 4 \mu\text{M}$   $\text{NH}_4^+$  in the *bottle-A*,  $\text{NO}_3^-$  uptake was not inhibited as its uptake pattern remained almost same as in *bottle-B* where ambient  $\text{NH}_4^+$  was less than  $1 \mu\text{M}$  (Fig. 6.9a). Moreover RPI value for,  $\text{NO}_3^-$  was consistently more than unity (Table 6.3) which shows that irrespective of ambient  $\text{NH}_4^+$  level, coastal phytoplankton like their estuarine counterparts, assimilated  $\text{NO}_3^-$  preferentially over  $\text{NH}_4^+$  (Fig. 6.10). Two major groups e.g. diatoms and dinoflagellates were found in the coastal water of which the former comprised 98% of the algal biomass. Among diatoms three major pennate forms *i.e.* *Navicula spp*, *Pseudonitzschia spp* and *Thalassionema nitzschoides* and three major centric forms *i.e.* *Leptocylindrus minimus*, *Melosira spp* and *Skeletonema costatum* were

abundant. Overall diatom population was dominated by pennate forms (65%) which accounts for the preferential,  $\text{NO}_3^-$  uptake over  $\text{NH}_4^+$ .

#### **6.3.2.2. Benthic inputs and Nutrient uptake**

Uptake rates of DIN,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  measured directly from *in-situ* nutrient enrichment experiment and that calculated from the reported primary productivity value of the coastal region *i.e.*  $90.3 \text{ mmol C m}^{-2} \text{ d}^{-1}$ , Pradeep Ram *et al.*, 2003) agreed to a large extent (Table 6.4) without significant variance ( $p > 0.05$ ) which is reassuring. Though nutrient demand of coastal phytoplankton was less than that of estuarine community, benthic fluxes of nutrients were comparatively higher (*Chapter-IV*). This indicates higher potential contributions of benthic fluxes to primary production in the shelf region. Unlike the experiment where phytoplankton were given a sudden pulse of nutrients, the natural processes nutrify the water column rather slowly through processes such as convective mixing and upwelling in this coastal environment. Thus rather long lag period of 48 hours is required for significant assimilation of nutrients to occur presumably reflecting the slower physiological adaptation of the phytoplankton cells to suddenly enhanced nutrient supply. However rapid uptake of nutrients after 48 hours of no response time indicates that the nutrients of the benthic origin could be utilized within at most a few days.

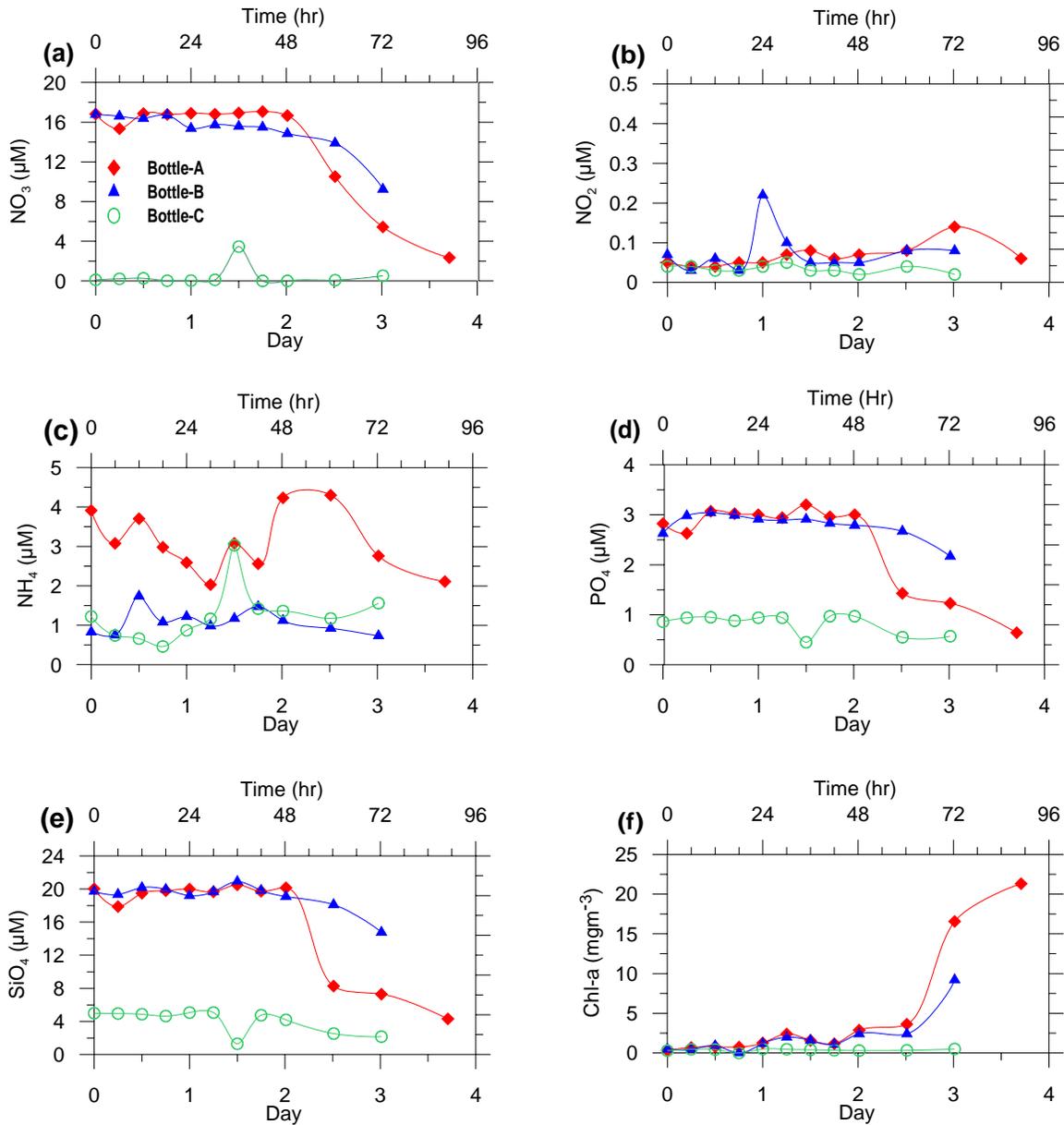


Fig-6.9: Nutrient uptake and chlorophyll increase with incubation time in the nutrient enrichment experiment at G5. Bottle-A was enriched with  $\text{NO}_3^-$ ,  $\text{NH}_4^+$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$ , bottle-B was enriched with  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SiO}_4^{4-}$  and bottle-C was kept as control.

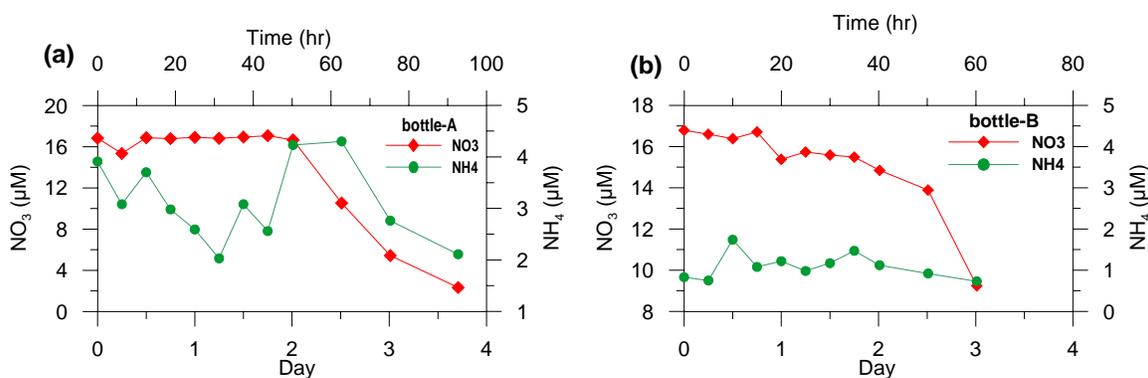


Fig-6.10: Comparative uptake of nitrate and ammonium in the coastal water.

**Table 6.3:** Comparative uptake of ammonium and nitrate by coastal phytoplankton. *Bottle- A* was enriched with NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and SiO<sub>4</sub><sup>4-</sup> and *bottle-B* was enriched NO<sub>3</sub><sup>-</sup>, PO<sub>4</sub><sup>3-</sup> and SiO<sub>4</sub><sup>4-</sup>.

	Nutrient	% N	% N utilized	Uptake rate (μmol L <sup>-1</sup> h <sup>-1</sup> )	RPI
Bottle-A	NO <sub>3</sub> <sup>-</sup>	79	67	0.46	1.11
	NH <sub>4</sub> <sup>+</sup>	20	33	0.061	0.55
Bottle-B	NO <sub>3</sub> <sup>-</sup>	93	37	0.38	1.03
	NH <sub>4</sub> <sup>+</sup>	0.06	34	0.01	0.63

**Table 6.4:** Nutrient uptake rate and contribution of benthic flux to the gross primary productivity in the shelf region. Values under column “A” are calculated from *in-situ* measured uptake rate and the values under column “B” are calculated from uptake rate from Primary Productivity values.

	Nutrient	<i>In-situ</i> measured uptake rate (mmol m <sup>-2</sup> d <sup>-1</sup> )	Calculated uptake rate from GPP (mmol m <sup>-2</sup> d <sup>-1</sup> )	Benthic flux rate (mmol m <sup>-2</sup> d <sup>-1</sup> )	Contribution of benthic flux to PP (%)	
					A	B
May	DIN	19.51	13.63	2.260	11.58	16.58
	PO <sub>4</sub> <sup>3-</sup>	1.29	0.85	0.080	6.20	9.41
	SiO <sub>4</sub> <sup>4-</sup>	15.08	13.63	3.470	23.01	25.46