

Alkaline phosphatase activity at the southwest coast of India: a comparison of locations differently affected by upwelling

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Abstract

The realization of the potential importance of phosphorus (P) as a limiting nutrient in marine ecosystem is increasing globally. Hence, the contribution of biotic variables in mobilizing this nutrient would be relevant especially in productive coastal waters. As alkaline phosphatase activity (APA) indicates the status of P for primary production in aquatic environments, we asked the following question: is the level of APA indicative of P sufficiency or deficiency in coastal waters, especially, where upwelling is a regular phenomenon? Therefore, we have examined the total APA, chlorophyll *a* along with phosphatase producing bacteria (PPB) and related environmental parameters from nearshore to offshore in coastal waters off Trivandrum and Kochi regions differently affected by upwelling during the onset of monsoon. Off Trivandrum, APA in the offshore waters of 5-m layer at 2.23 $\mu\text{M P hr}^{-1}$ was >4 times higher than nearshore. Thus, low APA could be indicative of P sufficiency in coastal waters and higher activity suggestive of deficiency in offshore waters off Trivandrum. In contrast, there was less difference in APA between near and offshore surface waters off Kochi. Our results show that the regions differently affected by upwelling respond differently according to ambient P concentration, distance from shore or depth of water. These observations could apparently be applicable to other coastal systems as well, where gradients in upwelling and phosphate runoff have been noticed. Further studies on other transects would throw more light on the extent and direction of the relationship between APA and ambient P concentration. Such studies would help in understanding the level of control of this nutrient on the productivity of coastal waters.

Key Words: phosphorus; alkaline phosphatase; phosphatase producing bacteria; surface waters; chlorophyll; abundance, southwest coast of India.'

1. Introduction

Phosphorus (P) is an essential element to life, being a structural and functional component of all organisms. It is a major constituent of nucleic acids in all living systems essential in the accumulation and release of energy during cellular metabolism (Paytan and McLaughlin, 2007; Rees et al., 2009). Phospholipids and nucleic acids appear to be the primary reservoirs of P within the planktonic cells in the open ocean (Dyhrman et al., 2006; 2007). However, it is locked up in bedrock, soils, sediments and particulate forms in water column and hence not directly available to organisms for utilization. Phosphorus in the marine ecosystem passes through different stages of biogenic cycle beginning with its migration to deeper waters and ending with fixation in bottom sediments and diagenesis (Benitez-Nelson, 2000; Dyhrman et al., 2006).

P in the ocean exists both in dissolved and particulate forms. Each fraction can either be organic or inorganic. The availability of P can impact the rates of primary production in the ocean as well as species distribution and ecosystem structure (Paytan and McLaughlin, 2007; Benitez-Nelson, 2000). In some marine and estuarine environments like Mediterranean Sea water, Monterey Bay, the availability of P often limits the primary production (Mackey et al., 2012; Zacccone et al., 2003). Marine phytoplankton and autotrophic bacteria take up P in the form of orthophosphate (HPO_4^{2-} , PO_4^{3-}) for their metabolic needs and also have the ability to hydrolyze organic P compounds when their P demand is not satisfied by inorganic orthophosphate (Dyhrman et al., 2007; Hoppe, 1993; Zacccone et al., 2003; Zhu et al., 2011). Dissolved organic P (DOP) can be a significant fraction of the total dissolved P (TDP) pool in surface seawater. It may be an important source of P for phytoplankton and bacteria in dissolved inorganic P (DIP) depleted waters. In nature DOP is diverse and complex because of its diverse source. DOP is supplied by the excretion of actively metabolizing microalgae and bacterial cells, sloppy feeding by zooplankton (Kwon et al., 2011; Paytan and McLaughlin, 2007) and by land runoff. Microbes can transform the bound/apatite form into soluble or dissolved inorganic phosphate by a wide spectrum of enzymes released into the environment (Ahmed and Shahab, 2011; Ayyakkannu and Chandramohan, 1970; 1971; Dyhrman et al., 2007; Zhu et al., 2011). Various extracellular enzymes like phosphatase, phytase, phospholipase, nucleotidase are involved in the mineralization of P. Alkaline phosphatase (AP, EC 3.1.3.1) is an enzyme, which removes phosphate groups by dephosphorylation from many types of molecules, including nucleotides, proteins and alkaloids. Phosphatase activity degrades not only particulate P containing matter but also high molecular dissolved organic matter. This enzyme activity has been suggested as one of the possible mechanisms in the marine environments that releases bioavailable P (Cao et al., 2010; Kwon et al., 2011; Perry, 1972; Sebastian et al., 2004). Several reports show that bacteria, fungi, protozoa and algae, have the ability to secrete phosphatases at the cell surface or release them into their environments. In both free and organism-associated forms, AP is considered to be responsible for the generation of inorganic P from organic P in natural environments (Kwon et al., 2011; Liu et al., 2010; Nausch and Nausch, 2006). The APA depends on the availability of inorganic P in surface water, where phytoplankton dominates. Both in laboratory cultures and natural communities of phytoplankton, AP production is associated with low P which is greatly repressed when P is sufficient (Dyhrman and Ruttenberg, 2006; Kobori and Taga, 1979; Liu et al., 2010; Nausch, 1998; Ruttenberg and Dyhrman, 2005). Measurement of AP activity (APA) is done by addition of artificial substrates like para-nitrophenol phosphate (pNPP) to samples. The ester-bonds of this substrate are cleaved by the phosphatase activity in seawater. APA is generally assumed to

indicate a deficiency of bioavailable inorganic P and so cells attempt to utilize organically bound P (Cao et al., 2010; Sebastian et al., 2004). However, Yamaguchi et al. (2006) found APA to be widely prevalent in coastal waters. The activity has been shown to increase when the orthophosphate concentration in the waters decreases to $< 0.2 \mu\text{M}$ (Nausch, 1998).

Increase in phosphate concentration is generally known to accompany upwelling. This phenomenon brings nutrient rich, cold subsurface water into the euphotic zone, which results in high biological productivity in Arabian Sea which is one of the most productive areas in the world (Habeebrehman et al., 2008; Prasanna Kumar et al., 2001; Sankaranarayanan et al., 1978; Wiggert et al., 2005). Coastal upwelling in the Arabian Sea is an annual phenomenon occurring during summer monsoon. Upwelling along the west coast of India appears to start in the southern regions (8°N) with the onset of summer monsoon in May-June and intensifies in July-August. It gradually proceeds north and has been observed up to 15°N (Thomas et al., 2012; Sankaranarayanan et al., 1978). As APA indicates the status of P for primary production in aquatic environments, we asked the following question: is the level of APA indicative of P sufficiency or deficiency in coastal waters where upwelling is a regular phenomenon? In order to answer this question we have examined APA, chlorophyll *a*, phosphatase producing bacteria (PPB) and related environmental parameters, off Trivandrum and Kochi along the southwest coast of India. The phenomenon initiates at the Cape in the south and gradually wanes towards north (Thomas et al., 2012; Smitha et al., 2008). Observations were carried out onboard FORV Sagar Sampada during the monsoon month of June (07-06-2010 to 10-06-2010) when upwelling signatures begin to become more evident off Trivandrum than off Kochi. The paper contrasts the findings of the two transects and discusses the similarities and differences.

2. Materials and methods

2.1 Study area and sampling locations

The study area was off southwest coast of India in the eastern Arabian Sea, off Trivandrum and Kochi at 8.2°N and 10°N , respectively. The sampling was done along these two transects perpendicular to the coast (Fig. 1). The surface water of the eastern edge of Arabian Peninsula and the southwest coast of India are pushed away by the winds from southwest to northeast during the southwest monsoon. The waters off Trivandrum are the most affected by the coastal upwelling during southwest monsoon as the process starts from this region and moves northward (Thomas et al., 2012; Subina et al., 2012).

The water samples were collected onboard FORV Sagar Sampada (cruise no. 276) off Trivandrum and Kochi during June 2010 (07-06-2010 to 10-06-2010 and 16-06-2010 to 19-06-2010, respectively). Each transects included six stations up to a water depth of 1000m (Table 1). The sample collection was from 30, 50, 100, 200 m, water depths nearshore and down to 500 and 1000 m offshore. Table 1 gives the geographical co-ordinates of each station, the sampling depths and grouping of stations into near and offshore, surface and bottom layers.

2.2 Sub-sampling for chemical and biological parameters

Water samples from pre-selected discrete depths were collected using 10 L Niskin bottles (Table 1). Sub-samples were used for the analysis of various parameters like dissolved oxygen (DO), nutrients and chlorophyll *a* (Chl *a*). Seawater sub-samples for microbiological analyses were collected in

sterile containers immediately after retrieval of the Niskin bottles. The sampling bottles were acid washed with 1 M HCl, rinsed thrice with Milli-Q water and completely dried before use. Bottles were also rinsed with sample before collection. Samples for DO were collected in 125 mL stoppered glass bottles avoiding air bubbles and were immediately fixed with Winkler's reagents. Fixed samples were stored in the dark until analysis (Strickland and Parsons, 1965). For nutrients, samples were passed through pre-combusted GF/F filters and were frozen in polycarbonate bottles till analysis.

For pigment analysis, 1L of water sample was immediately filtered using a glass fibre filter (Whatmann GF/F, 0.7 μm pore size). The filters were stored in 20 mL polyethylene vials at $-20\text{ }^{\circ}\text{C}$ until analysis in the shore laboratory.

2.3 Physical parameters

Vertical profiles for temperature and salinity were produced using the Sea Bird CTD connected to the Niskin sampler rosette.

2.4 Chemical Analyses

Dissolved oxygen (DO) was measured onboard by titrimetric method (Strickland and Parsons, 1965). Nutrients such as nitrite, nitrate, phosphate and silicates in the seawater samples were measured onboard by SKALAR auto analyzer as described by Wurl (2009). Ammonia was estimated manually by spectrophotometric method (Grasshoff, 1983).

2.5 Chlorophyll a (Chl a) and phaeophytin (phae)

The stored filters in 20 mL screw-capped tubes were treated with 10 mL 90 % acetone to extract the pigments. After a period of 24 hr in darkness at $-20\text{ }^{\circ}\text{C}$, the extract was measured following UNESCO (1994) protocol. The readings before and after acidification were measured using Turner Design (10AU) fluorometer and then both Chl *a* and phaeophytin (a degradation product of chlorophyll) concentrations were calculated.

2.6 Abundance of phosphatase producing bacteria (PPB)

Phosphatase producing bacteria (PPB) were enumerated by most probable numbers (PPB-MPN) by microtitre plate method using para-nitrophenol phosphate (pNPP) as substrate (1 mM) as detailed in section 2.7. The samples were incubated in the dark at room temperature ($28\pm 2\text{ }^{\circ}\text{C}$) for scoring positive and negative growth/reactions. Three tube method was suitably adopted to incubate dilution of samples. Scoring/calculations were done using McCrady's table (Rodina, 1972).

2.7 Alkaline phosphatase activity (APA)

The alkaline phosphatase activity (APA, EC 3.1.3.1) was estimated using the method of Taga and Kobori (1978) and as elaborated by De Souza et al. (2000). pNPP (1 mM) was prepared in sea water which was pre-sterilized by autoclaving. Incubation was for 24 hrs at room temperature ($28\pm 2\text{ }^{\circ}\text{C}$) and care was taken to shield all the samples from light. The APA was indirectly measured by the amount of phenol released. The results were based on the increase in absorbance at OD 405 nm. One International Unit (IU L^{-1}) is defined as the amount of enzyme that catalyzes the transformation of one micromole of substrate per hour under specified conditions. Measurements were run against a

control containing an equivalent volume of the seawater samples previously autoclaved. The results represent the average of triplicates corrected for controls.

APA was calculated as follows,

$$\text{AP activity } (\mu\text{M P hr}^{-1}) = (C_f - C_0)/t,$$

where, Product P in μM = para-nitro phenol released; one mole of phenol released is = one mole of inorganic phosphate released by APA.

C_f = Final concentration of the product

C_0 = Initial concentration of the product

t = Incubation time in hours

Experimental values were calculated against a standard curve prepared to cover concentrations from 10 to 1000 nM of para-nitro phenol (pNP).

2.8 Statistical Analysis

Bacterial data was log transformed for statistical analysis. Inter-relationships between biological and environmental parameters were examined using Statistica ver. 6.0 and analysis tool pack in Microsoft Excel. Analysis of variance (ANOVA) was carried out to indicate significant differences between sets of parameters.

3. Results and Discussion

1. 3.1 Environmental parameters

Low sea surface temperature (SST) prevailing near the coast could be indicative of upwelling conditions. Such an upwelling event was detectable off Trivandrum characterized by cooler SST ($25.20\text{ }^{\circ}\text{C}$) at nearshore than offshore ($29.08\text{ }^{\circ}\text{C}$) (Fig. 2a). A marked difference in temperature between near ($28.60\text{ }^{\circ}\text{C}$) and offshore ($28.83\text{ }^{\circ}\text{C}$) was less discernible off Kochi (Fig. 2a) than off Trivandrum. The cooler SST off Trivandrum was again confirmed by the satellite data (TMI) (Fig. 2b). Moreover, off Trivandrum and Kochi, DO content in the surface water increased from 1.23 and 4.17 mL L^{-1} nearshore to 4.33 and 4.51 mL L^{-1} offshore, respectively (Fig. 3a).

Off Trivandrum, phosphate and silicate varied from 0.85 to 0.065 μM and 1.56 to 4.75 μM from nearshore to offshore, respectively (Fig. 3b and 3c). Off Kochi, phosphate and silicate concentration showed a definite increase from 0.85 and 0.89 μM nearshore to 2.17 and 6.58 μM offshore, respectively. Though phosphate and silicate concentrations were similar in the surface layer of both transects, nearshore surface waters off Trivandrum have higher concentration of nitrite (2.52 μM), nitrate (2.88 μM) and ammonia (0.42 μM) than off Kochi (Fig. 4a, 4b and 4c). Though the nutrient concentrations were more stratified off Kochi than off Trivandrum, the nearshore to offshore trend off Trivandrum was definite in surface water (Fig. 3b-4c). Higher concentration of nutrients off Kochi may be attributed to higher anthropogenic forcing from industrial effluents. This stretch of the coast is often associated with land runoff and brackish water residues which contain high nutrients due to dense population and agricultural runoff (Kaladharan et al., 2011; Lakshmanan et al., 1987).

The Chl *a* concentration ranged from <0.01 ug L⁻¹ offshore to a high 3.474 ug L⁻¹ nearshore and phaeophytin varied from 0.04 offshore to 2.87 ug L⁻¹ nearshore off Trivandrum. Off Kochi, the chl *a* and phaeophytin content varied from <0.01 and 0.015 ug L⁻¹ offshore to 1.27 and 2.35 ug L⁻¹ nearshore, respectively (Fig. 5a and b). Thus, the average Chl *a* concentration was more off Trivandrum and was restricted to the surface layers, whereas off Kochi the distribution extends to subsurface region. The SST profile and phytoplankton biomass (Chl *a*) suggested that the initiation of upwelling signatures was more evident off Trivandrum and less off Kochi.

3.2 Abundance of phosphatase producing bacteria (PPB-MPN)

Off Trivandrum, the PPB-MPN was also marginally more nearshore (2.54×10^5 cells L⁻¹) than at offshore stations (2.13×10^5 cells L⁻¹) and marginally more at the bottom layers (3.90×10^5 cells L⁻¹) than at the surface layers (3.38×10^5 cells L⁻¹). Off Kochi, though the difference was small between PPB-MPN offshore (1.11×10^5 cells L⁻¹) and nearshore (0.83×10^5 cells L⁻¹), the abundance of PPB-MPN was one order higher at the surface (1.22×10^5 cells L⁻¹) than bottom (4.3×10^4 cells L⁻¹) (Fig. 6a and b). As the total bacterial counts ranged from 10⁸-10⁹ cells L⁻¹ (Subina et al., 2012), the percentage of PPB ranged from 0.01 to 0.001% of total bacterial counts. The monsoonal rain enriches the coastal environment with essential nutrients. Such increase in the abundance of PPB during monsoon (Kannapiran and Ravindran, 2012; Sri Ram Kumar et al., 2011) and in the coastal waters (De Souza et al., 2000) has been observed earlier. However, in the present study, the higher abundance could be a response not only to monsoon but also to upwelling which is more marked off Trivandrum.

3.3 Variation in APA

Off Trivandrum, APA ranged from non-detectable (nd) levels to 3.61 uM P hr⁻¹ (Fig. 7a). Unlike PPB, APA did not show depth dependent variation. However, the average APA recorded higher activity (3.18 uM P hr⁻¹) at the surface than bottom (2.90 uM P hr⁻¹). Though not significant, average APA was higher offshore (1.78 uM P hr⁻¹), than nearshore (1.32 uM P hr⁻¹). Interestingly, when APA at 5-m layer alone was considered, the values were 0.51 uM P hr⁻¹ nearshore and 2.23 uM P hr⁻¹ offshore. Similar results have also been observed by Liu et al. (2010) in the lateral water exchange between shelf margins during upwelling in Kuroshio waters. Off Kochi there was no difference in APA between near and offshore waters both being around 5.2 uM P hr⁻¹ (Fig. 7b). Even at 5-m layer the difference was only marginal, offshore waters being only 0.66 times more than nearshore. Results showed that APA was significantly (3.4 times, $p < 0.001$) higher off Kochi at an average value of 5.2 uM P hr⁻¹ than off Trivandrum. Though the APA was more off Kochi than Trivandrum, the nearshore to offshore trend off Trivandrum was definite at 5-m layer. Higher APA off Kochi may be attributed to higher anthropogenic forcing from industrial effluents (Kaladharan et al., 2011; Lakshmanan et al., 1987; Verma, 2003). This stretch of the coast is often associated with land runoff and brackish water residues which contain high phosphatase activities. It is possible that cumulative runoff over time could have contributed in distributing the concentration of P beyond coastal region. Waste water effluents have already been suggested as a possible source of APA in shallow water (Zou et al., 2004).

Inorganic P is a competitive inhibitor of APA and also P released from sediment may inhibit APA in the overlying water column (Newman and Reddy, 1993). Similar observations where activity

increases from the coastal to the offshore region but decreases with depth have been made by Kobori and Taga (1979) in Tokyo bay. Zaccone et al. (2003) have also found an inverse relation between APA and P concentrations from the surface to deep waters in the Mediterranean Sea. However, in the present study such inverse relationship is restricted only to the surface water off Trivandrum. In general, there is no single threshold of P concentration for APA. It appears to be different in different aquatic systems (Ammerman and Azam, 1991, Liu et al., 2010; Nausch, 1998). Ammerman and Azam (1991) reported that APA was usually low and sometimes undetectable in phosphate-rich waters. Nausch (1998) observed a significant decrease in APA at ambient P concentration $>1 \mu\text{M}$ in the Baltic Sea. At the lower limit, Liu et al. (2010) stated that APA was expressed only when the detection limits of P concentration in sea water was $<0.01 \mu\text{M}$. Hence, it is possible that different waters have different thresholds. In the present study we could not detect any such threshold.

3.4 Relation of APA and PPB-MPN with environmental parameters

3.4.1 Off Trivandrum

When surface and bottom layers were examined for both transects, off Trivandrum (Table 2), PPB-MPN and P related to each other inversely only in surface waters ($r = -0.59$, $p < 0.05$). Opposite trends were also observed between APA and Chl *a* ($r = -0.71$, $p < 0.01$) and phaeophytin ($r = -0.53$, $p < 0.05$). It is suggested that at the time of sampling there was sufficient P for phytoplankton and bacterial metabolism in the surface layers which could be due to the continuous supply of P from bottom water and also from land runoff during monsoon season. These observations are in contrast to the observation made by others, where APA increases with increase in Chl *a* (Kwon et al., 2011). It is also suggested that APA measured may be due to constitutive production of enzyme by bacteria during organic matter degradation for carbon and nitrogen source. This inference is supported by the studies made by Hoppe and Ullrich (1998) where they show that bacteria produce enzyme continuously in the aquatic environment. Though the level of P was low, it is probable that the turnover rate could be high. It is more likely that the phytoplankton in the newly upwelled waters were growing under P-sufficient conditions. Therefore, there was probably less reason to expend energy in the synthesis of APA. Consequently, the measured enzyme activity could have been low. These results are congruent with the earlier published results which show that APA gets repressed in phytoplankton, bacteria and algal cultures under high ambient P concentrations (Mackey et al., 2012; Durham et al., 2006).

Though opposite trend was observed between P and PPB-MPN (36%), at the surface, P is influenced positively by APA (25%) at the bottom layers. APA also increases with P concentration in the bottom layers ($r = 0.49$, $p < 0.05$) suggesting a tighter coupling between the parameters off Trivandrum. De Souza et al. (2000) also demonstrated that in sediment and seawater samples around the Indian Peninsula, APA of bacterial isolates was higher wherever the total P content of the ambient waters/sediment sampled was high. Again, this relationship is quite contradictory to the one observed in offshore surface waters where the activity is triggered in low ambient P concentration. It is therefore inferred, that the control of APA in euphotic surface and darker bottom waters could be different. In surface waters, APA is generally induced and therefore triggered by very low concentration of P. However, in the bottom water such a control is not evident as APA continues to be expressed constitutively even when the ambient P concentration is measurable. Thus, the APA at the euphotic surface waters could be driven more by phytoplankton under P deficiency and at the

bottom the activity is driven by microbes where their continuous activity replenishes P concentration of the overlying waters. Similar observations have also been made by Mackey et al. (2012) in Monterey Bay and Nausch (1998) in Baltic Sea, where inverse relation between P and APA was observed in the surface waters.

3.4.2 Off Kochi

In the surface water off Kochi (Table 2), PPB-MPN decreased with increase in temperature ($r = -0.70$, $p < 0.01$). However, APA increased with nitrite ($r = 0.48$, $p < 0.05$) in surface water suggesting that this relationship could be indirectly linked to ambient nitrite concentration. Similar observations have been made by Nyman and Graneli (1983) in Laholm Bay, South eastern Kattegat waters where APA increased within a few days after the addition of nutrient rich river water with 150 to 200 μM of nitrogen. The high APA measured in diazotrophic cyanobacteria is also attributed to nitrogen fixation under P deficient conditions (Graneli et al. 1990). Likewise, APA in other phytoplankton may be enhanced by nitrogen enrichment (Vargo and Shanley, 1985).

3.5 Phytoplankton abundance and major groups off Trivandrum and Kochi

Some interesting observations were made on the distribution of phytoplankton in nearshore and offshore waters at 5-m layer. Though the number of dominant phytoplankton species (14 spp.) prevailing off Trivandrum and Kochi were similar in nearshore, the abundance of phytoplankton was more off Trivandrum (nearshore- 6×10^4 cells L^{-1} and offshore- 4×10^4 cells L^{-1}) compared to Kochi (nearshore- 2.7×10^4 cells L^{-1} and offshore- 9.7×10^3 cells L^{-1}) (Table3). Though the number of species known for APA production (*Thalassiosira* spp., *Asterionellopsis* spp., *Chaetoceros* spp., *Guinardia* spp., *Protoperidinium* spp. and *Prorocentrum* spp.) prevailing in both localities were same, at the nearshore station off Trivandrum, *Thalassiosira* spp (38%) and *Asterionellopsis* spp (26%) off Kochi were dominant at the 5-m layers. Previous studies by Ou et al. (2006) also showed that *Thalassionema nitzschioides*, *Pseudo-nitzschia pungens* and *Asterionellopsis glacialis* could contribute to high bulk APA in Taiwan Strait seawaters. However, offshore Trivandrum was dominated by *Guinardia* spp (31%) and *Protoperidinium* spp (5.38%), whereas offshore Kochi was dominated by *Thalassiosira* spp (20%) and *Protoperidinium* spp (7.59%). These observations show that the 5-m layer was more dominated by APA producers offshore, suggesting that they would be active under P deplete condition. Such expressions have been commonly observed in other dinoflagellates species of the genera *Protoperidinium* and *Gymnodinium* in Mediterranean water (Rengefors et al., 2003; Dyhrman and Ruttenberg, 2006; Ou et al., 2006). It is possible that in the present study, the contribution of dinoflagellates in offshore APA production could be considerable under P deplete condition. Activity measurement was also in agreement with the pertinent number and type of prominent APA producing phytoplankton distribution. Different types of phytoplankton (diatom versus dinoflagellates) or even different species within the same type may respond to P availability differently with respect to APA (Lin et al., 2011; 2012).

In the present study, phytoplankton biomass (Chl *a*) and bacterial numbers did not display any relationship to APA and P at Kochi surface water nearshore. We therefore infer that the APA could be due to microbes which are known to produce this enzyme constitutively (Hoppe and Ulrich, 1998; Dyhrman et al., 2007; Know et al., 2011).

Offshore observation shows that the surface water off Trivandrum was marked by higher activity than the nearshore. This differential expression of APA nearshore and offshore is more restricted to 5-m layer off Trivandrum. Though there is a general trend in the distribution of Chl *a* from nearshore to offshore Trivandrum, the pattern is more patchy. However, we could discern an increase in activity at station 5 and beyond, synchronizing with the decrease in Chl *a*. Access to DOP data would have clarified these aspects better. This observation made at the regional scale could apparently be applicable to other upwelling/non-upwelling systems as well.

4. Conclusions

The present study suggests that APA could be an indicator of the status of P availability to phytoplankton and microbes. Off Trivandrum, low APA could be suggestive of P sufficiency in the nearshore surface waters especially 5-m layer while higher activity could be indicative of P deficiency in offshore surface waters. Off Kochi it is intriguing that such a relationship was not evident. Further studies on other transects particularly with those affected by less land runoff would throw more light on the extent and direction of the relationship between APA and ambient P concentration. Such studies would help in understanding the extent of control of this nutrient on the productivity of coastal waters.

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Legends to Figures

Fig. 1 Map showing sampling locations along the transects off southwest coast of India

Fig. 2a Vertical profile of temperature off Trivandrum and Kochi

2b Satellite derived sea surface temperature profile off Trivandrum and Kochi. The grey shaded portions at the bottom (2a and b) indicate land mass.

Fig. 3 Distribution of **a.** Dissolved Oxygen **b.** Phosphate **c.** Silicate concentrations off Trivandrum and Kochi. The grey shaded portions at the bottom (3a, b and c) indicate land mass.

Fig. 4 Distribution of **a.** Nitrite **b.** Nitrate and **c.** Ammonia concentrations off and Kochi. The grey shaded portions at the bottom (4a, b and c) indicate land mass.

Fig. 5 Distribution of **a.** Chlorophyll *a* and **b.** phaeophytin off Trivandrum and Kochi The grey shaded portions at the bottom (5a, b and c) indicate land mass.

Fig. 6 Distribution of most probable number of phosphatase bacteria (PPB-MPN) **a.** off Trivandrum and **b.** off Kochi.

Fig. 7 Alkaline Phosphatase Activity (APA) **a.** off Trivandrum and **b.** off Kochi

Table 1. Details of samples collected from different depths – off Trivandrum and Kochi, June 2010

| Stn. no. | Lat (N) | Long (E) | Water depth (m) | Depths (m) | | Distance (km from coast) |
|-------------------|--------------|--------------|-----------------|----------------|-------------------------|--------------------------|
| | | | | SL | BL | |
| Trivandrum | | | | | | |
| 1-ns | 8°28' 808'' | 76°51' 133'' | 39 | 5,15, | 25 | 81.4 |
| 2-ns | 8°30' 45'' | 76°42' 198'' | 53 | 5, 15, | 25,45 | |
| 3-ns | 8°27' 064'' | 76°29' 981'' | 110 | 5, 25, | 50, 75, 100 | |
| 4-ns | 8°26' 903'' | 76°26' 182'' | 205 | 5, 25, 50, | 75, 150, 200 | |
| 5-os | 8°26' 134'' | 76°20' 734'' | 510 | 5, 25, 50, | 75, 150, 250, 420 | |
| 6-os | 8°26' 007'' | 76°06' 909'' | 970 | 5, 25, 50, 75, | 150,250, 500, 750, 950 | 159 |
| Kochi | | | | | | |
| 1-ns | 10°03' 265'' | 76°01' 331'' | 32 | 5,15, | 25 | 69.54 |
| 2-ns | 9°56' 594'' | 75°51' 014'' | 50 | 5, 15, | 25,45 | |
| 3-ns | 9°55' 39'' | 75°38' 100'' | 109 | 5, 25, | 50, 75, 100 | |
| 4-ns | 9°56' 47'' | 75°35' 024'' | 281 | 5, 25, 50, | 75, 150, 250 | |
| 5-os | 9°56' 348'' | 75°32' 748'' | 482 | 5, 25, 50, | 75, 150, 250, 420 | |
| 6-os | 9°56' 062'' | 75°29' 950'' | 893 | 5, 25, 50, 75, | 150, 250, 500, 750, 890 | 121 |

ns-nearshore; os-offshore; SL-surface layers; BL-Bottom layers

Table 2. Relationships of PPB-MPN and alkaline phosphatase activity (APA) to environmental parameters off Trivandrum and Kochi

| Parameters | Trivandrum | | Kochi | |
|-----------------|--------------------|-------------------|--------------------|--------------|
| | SL (n=16) | BL (n=18) | SL (n=16) | BL (n=18) |
| PPB-MPN | | | | |
| PO ₄ | -0.59 ^a | - | - | - |
| Temp | - | - | -0.70 ^b | - |
| APA | | | | |
| NH ₃ | 0.60 ^b | - | - | - |
| NO ₂ | 0.48 ^a | | | |
| PO ₄ | - | 0.49 ^a | - | - |
| Chl. a | -0.71 ^b | - | - | - |
| Phaeo | -0.53 ^a | - | - | - |

SL-surface layers; BL-Bottom layers ; a - p<0.05; b - p<0.01 and c - p<0.001.

PPB-MPN-phosphatase producing bacteria; APA-alkaline phosphatase activity; phaeo-phaeophytin

Table 3. Phytoplankton abundance and major groups off Trivandrum and Kochi

| Parameters | Trivandrum | | Kochi | |
|---------------------------------|---|--|---|---|
| | ns | os | ns | os |
| Abundance cells L ⁻¹ | 60303 | 40450 | 27403 | 9704 |
| Diatoms (%) | 89.60 | 86.90 | 85.50 | 68.60 |
| Dinoflagellate (%) | 10.40 | 13.10 | 14.50 | 31.40 |
| Major groups (Relative %) | <i>Thalassiosira</i> sps (38.52%) <i>Leptocylindrus</i> sps (33.70%) <i>Thalassionema</i> sps (23.46%) <i>Gymnodinium</i> sps (5.50%) <i>Pseudo-Nitzschia</i> sps (5.31%) | <i>Guinardia</i> sps (30.47%) <i>Leptocylindrus</i> sps (19.75%) <i>Bacterosira</i> sps (16.90%) <i>Rhizosolenia</i> sps (10.26%) <i>Proto-peridinium</i> sps (5.38%) | <i>Asteromphalus</i> sps (45.48%) <i>Asterionellopsis</i> sps (26.42%) <i>Bacterosira</i> sps (19.54%) <i>Thalassiosira</i> sps (17.50%) <i>Guinardia</i> sps (16.02%) | <i>Thalassiosira</i> sps (19.82%) <i>Rhizosolenia</i> sps. (16.30%) <i>Guinardia</i> sps (8.55%) <i>Gonyaulax</i> sps (8.33%) <i>Proto-peridinium</i> sps (7.59%) |

ns-nearshore; os-offshore; bold indicates major producers of APA as cited in literature

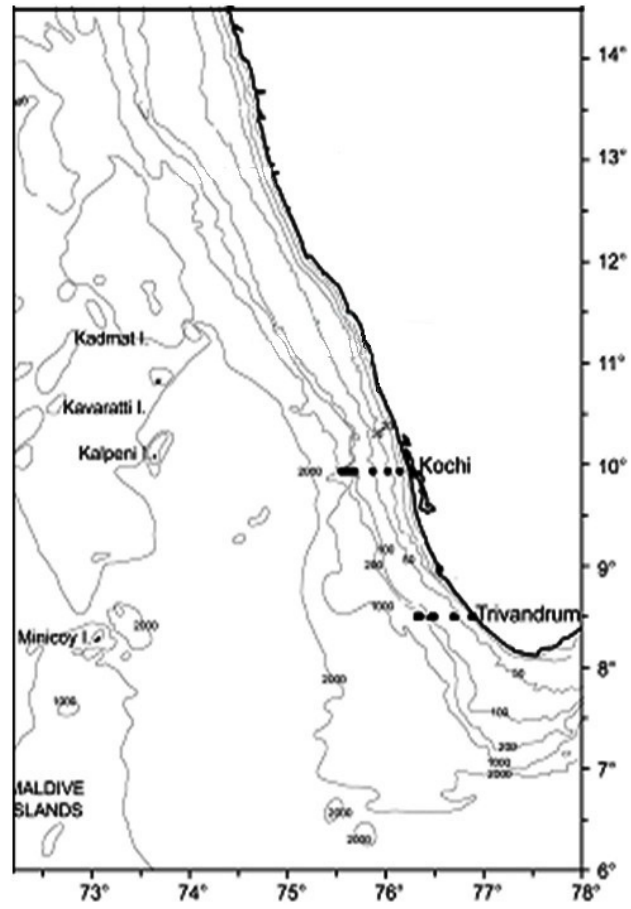


Fig. 1.

Trivandrum

Kochi

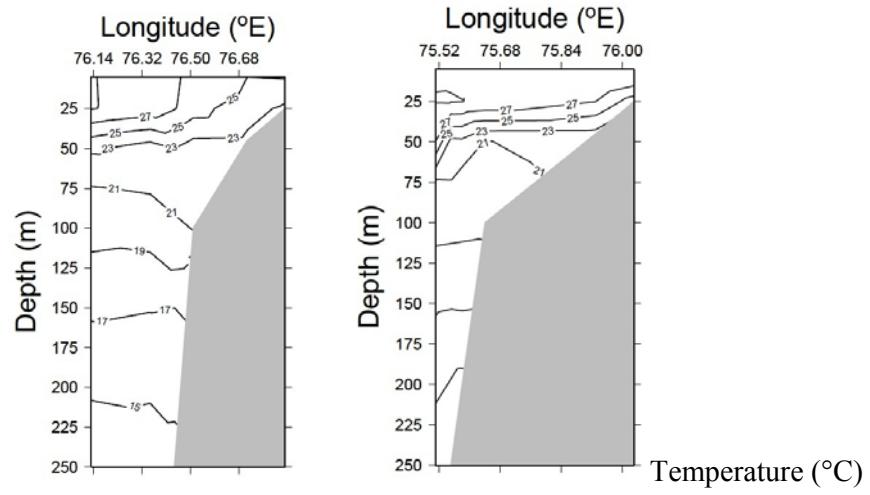


Fig. 2a.

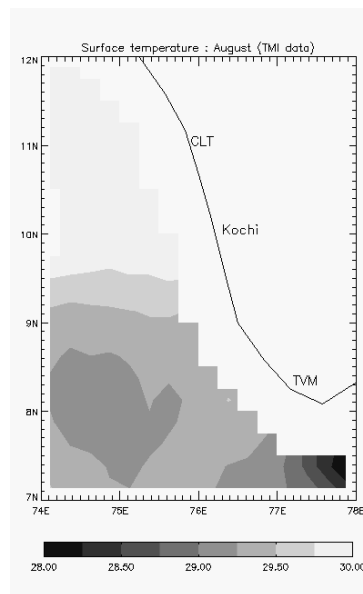


Fig. 2b.

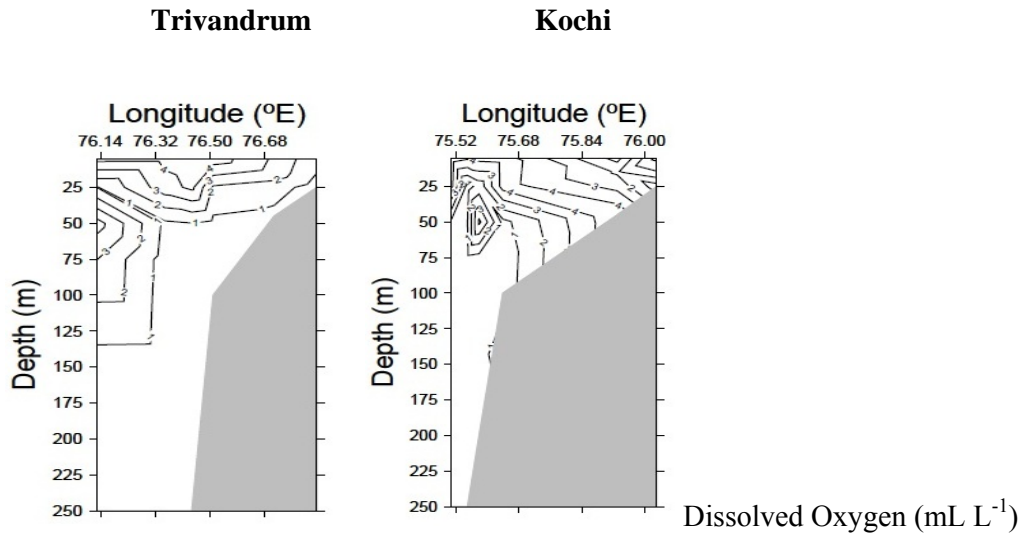


Fig. 3a.

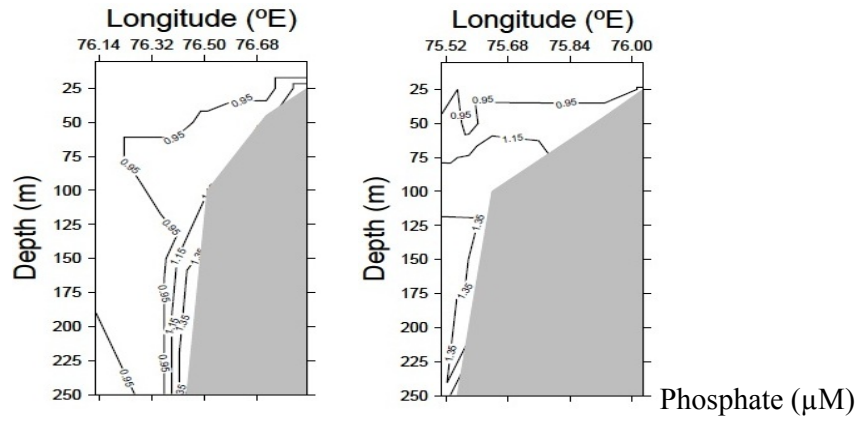


Fig. 3b.

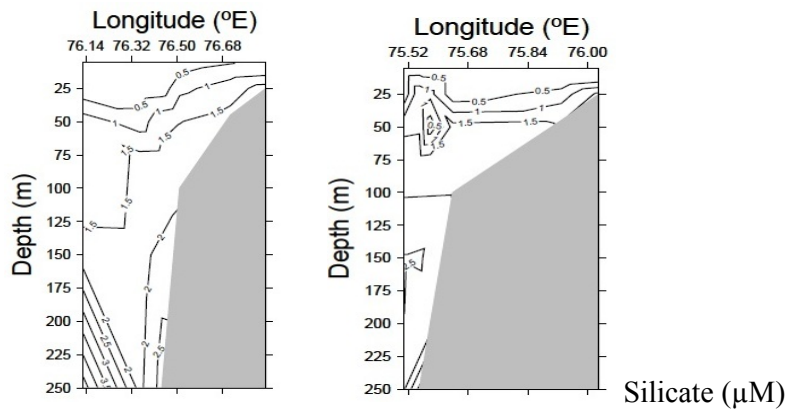


Fig. 3c.

Trivandrum

Kochi

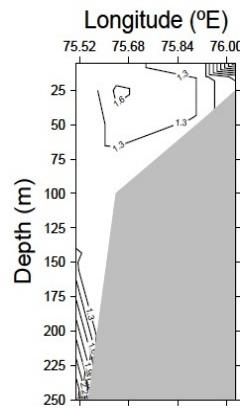
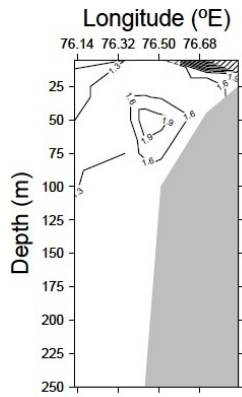


Fig. 4a.

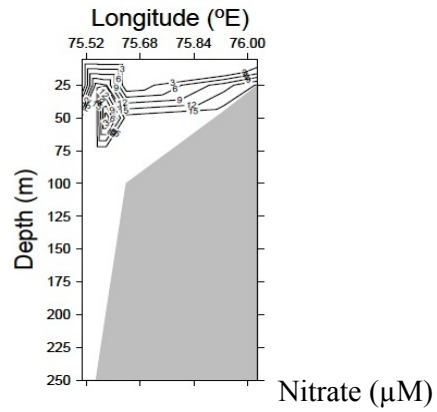
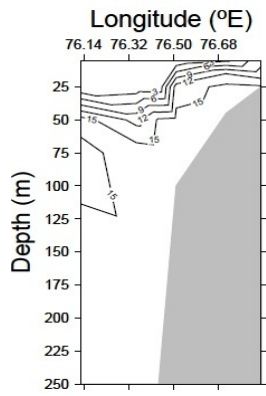


Fig. 4b.

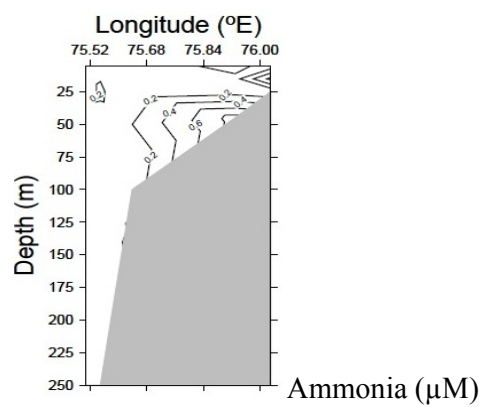
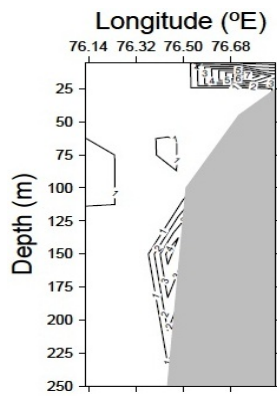


Fig. 4c.

Trivandrum

Kochi

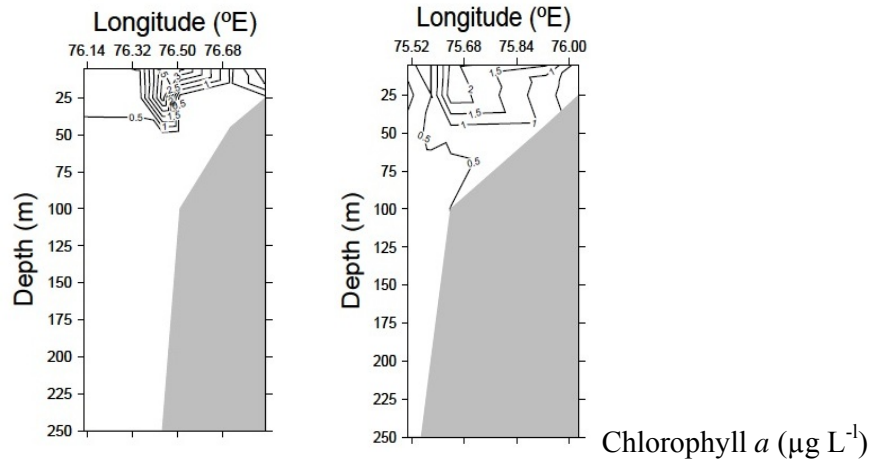


Fig. 5a.

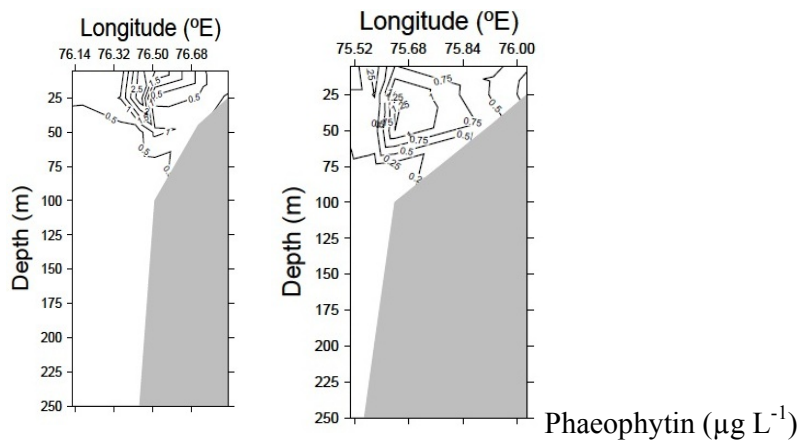


Fig. 5b.

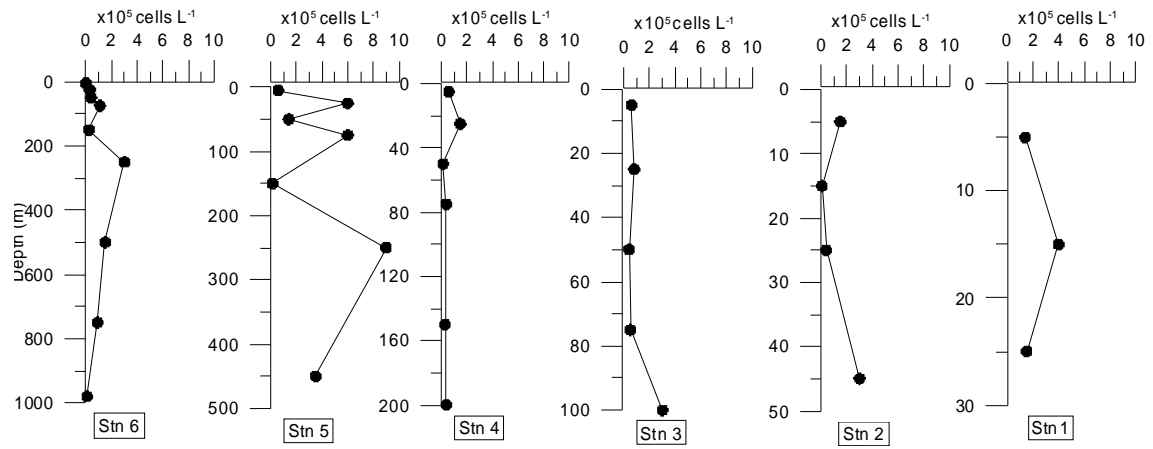


Fig. 6a.

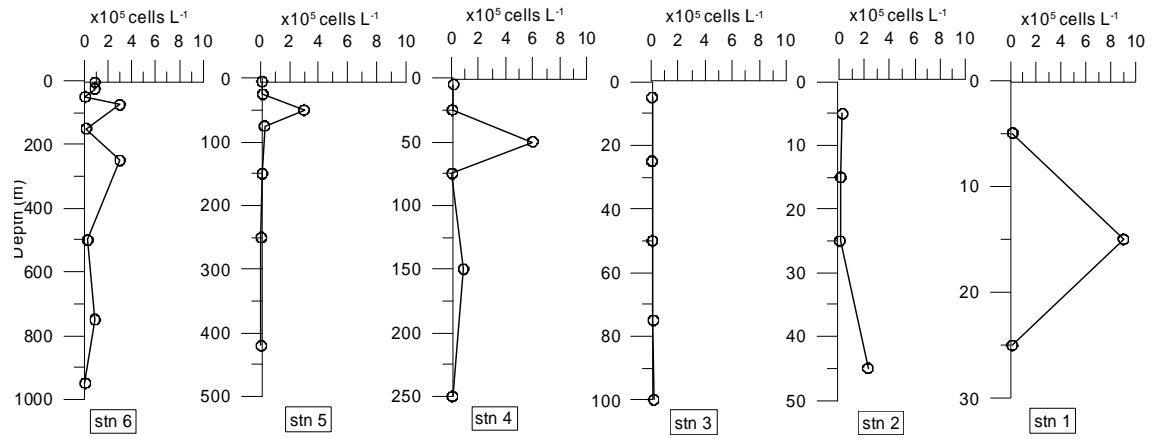


Fig. 6b.

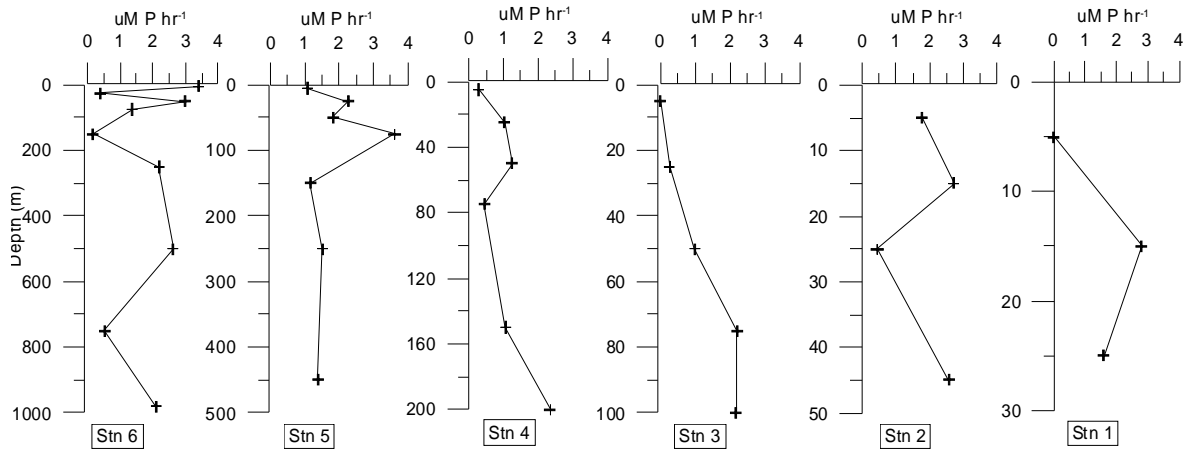
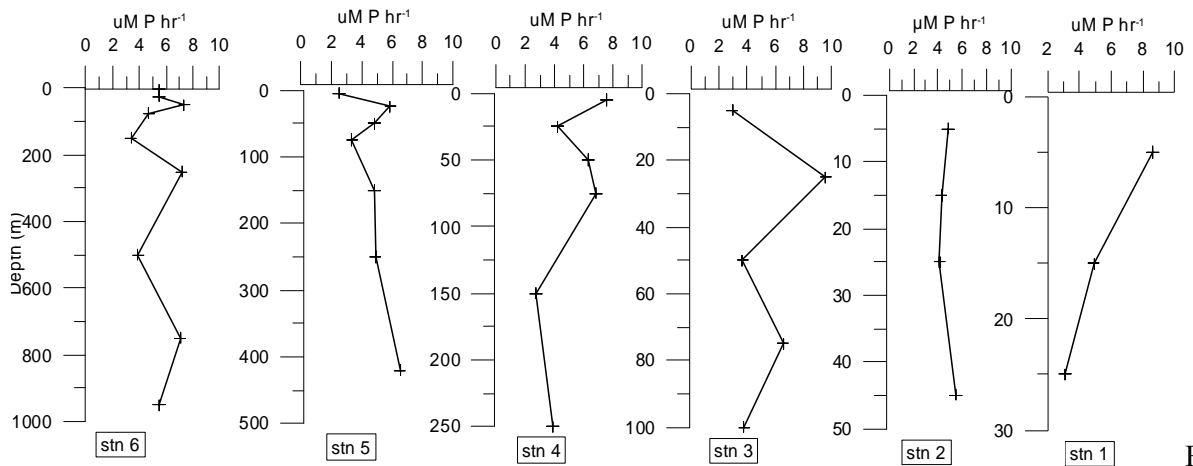


Fig.7a.



7b.

Fig.