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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 μM P h^{-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.

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 (Benitez-Nelson, 2000; Paytan and McLaughlin, 2007; Thingstad et al., 2005). 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; Zaccone 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; Zaccone 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

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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 μM (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 (Sankaranarayanan et al., 1978; Thomas et al., 2013). 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 (Smitha et al., 2008; Thomas et al., 2013). 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 is 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 (Subina et al., 2012; Thomas et al., 2013).

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 1000 m (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

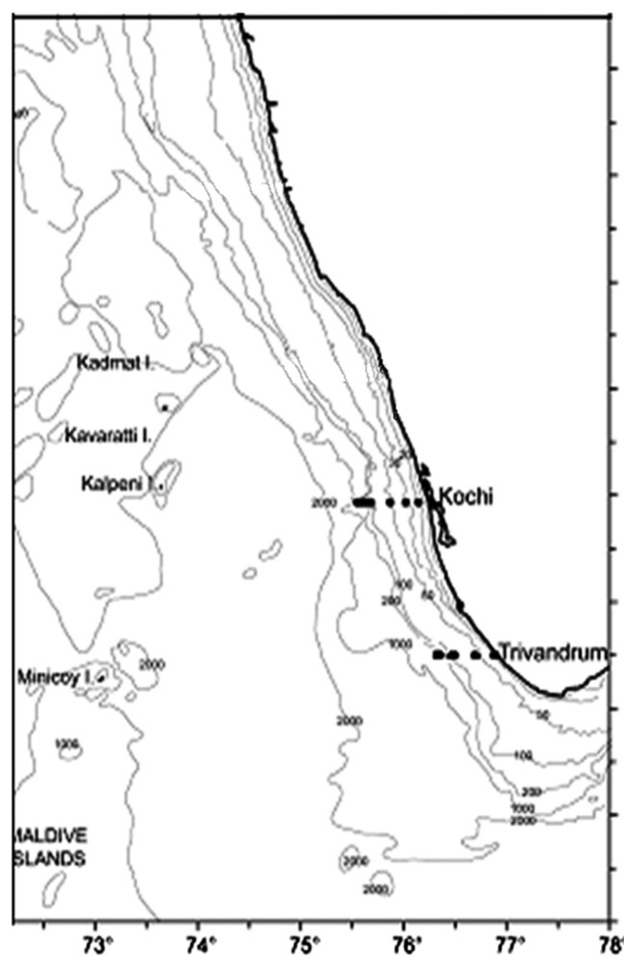

Fig. 1. Map showing sampling locations along the transects off southwest coast of India.