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Influence of sediment chemistry on mangrove-phosphobacterial relationship

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Abstract

The study reveals importance of sediment chemistry in mangrove-phosphobacterial relationship. The various physicochemical parameters of sediment, bacterial distribution, and acidic- alkaline phosphatase activity were studied in *Avicennia marina* mangrove sediments. The sampling sites were four mangrove forests along West India. Rhizospheric, pneumatophoric and bulk/non-rhizospheric sediment were collected. The rhizospheric sediment showed higher bacterial activity. The pH was observed low in the rhizospheric sediment and good abundance of phosphate-solubilizing bacteria (PSB). This was also supported by other physico-chemical parameters. Acid phosphatase activity was found to be higher in rhizospheric sediment indicating acid production at rhizosphere by microbial action and root exudation. It is the first study indicating that alkaline phosphatase activity was observed to be higher in pneumatophoric sediment. This coincides with higher number of phosphatase-producing bacteria (PPB) and high inorganic P in the pneumatophoric sediment, revealing that this region of *A. marina* harbours favourably more PPB with utilization of organic P by bacterial load and conversion to inorganic forms stressing that strong bonding exists between mangrove-sediment-phosphobacterial relationships.

Keywords: Mangrove, Phosphorus (P), Phosphate-solubilizing bacteria (PSB), Phosphatase-producing bacteria (PPB), acid phosphatase activity, alkaline phosphatase activity

Introduction

The tropical and subtropical coastal areas are dominant with mangrove ecosystems, covering nearly 1,40,000 km^2 (Giri *et al.*, 2011) [13] and play a major role in providing excellent feeding and breeding areas for many finfishes, shellfishes, micro-organisms, fungi, plants and animals altogether constituting the mangrove ecosystem (Spalding, 1997) [42]. None of the other aquatic ecosystems have similar productivity and standing crop as that of mangrove forests, which rival the biomass of many tropical rainforests (Saenger, 2002) [40] and this is supported by Kathiresan and Bingham, (2001) [25] that mangrove benefits are 25-fold higher than that of paddy cultivation.

Even though the mangrove ecosystems are organically rich, Nitrogen and Phosphorus are not uniformly distributed (Feller *et al.*, 2002) [10] and Romine and Metzger (1939) [38] observed that it may be low due to strong weathering of the old highly leached soils of the tropics. Phosphorus (P), being one of the essential mineral nutrient, limits the plant growth and not readily available to plants due to its low solubility and high fixation in the soil (McVicker *et al.*, 1963) [31]. Studies shows that mangrove sediments act as a sink for P with high retention capacity (Tam and Wong, 1994, 1995, 1996) [44 45 46] and get easily immobilized in soil but mobilization is a very slow process. A vast group of bacterial genera and functional types exist in mangrove soil and on above ground roots (Kathiresan and Bingham, 2001) [25] and can be helpful in releasing P from organic and inorganic matters through the process of solubilization by PSB and mineralization by PPB altogether constituting the plant growth promoting rhizobacteria (PGPR) in and around the rhizosphere by secretion of organic acids and production of phosphatase enzyme facilitating the conversion of insoluble forms of P to plant-available forms (Kim *et al.*, 1998) [28], through their metabolic activities alter the microenvironment around the roots, releasing nutrients and modifying the redox potential and pH of the soil and in turn, depend on the leakage of dissolved nutrients from the roots as a source of energy (Hyde and Lee, 1995; Holguin *et al.*, 2001) [22, 20].

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Major biological components assuring mangrove productivity are sediment and rhizospheric microorganisms, but at the same time there exist an interaction between physico-chemical and biological components comprising of mangrove-sediment-bacterial relationship which promotes excellent productivity and thus provides basic foundation to understand function and contribution of mangroves to nutrient dynamics in the coastal zone (Alongi, 1988) [2].

Previous studies suggested that physico-chemical characteristics such as pH, available sulphides, alkalinity and redox state also affect dissolution of mineral phosphate (Boto K.G., 1982; Ramanathan *et al.*, 1999) [6, 36] and these factors in turn are often affected by the activity of microbes and larger organisms. Studies also states that there exists correlation between soil characteristics and metabolic processes (Holguin *et al.*, 1999) [19] and the densities of bacterial population may be varying in relation to sediment grain size, organic content, root and infaunal abundance, distribution and to environmental factors, such as temperature (Alongi and Sasekumar 1992) [3]. Gurmeet *et al.*, (2015) [15] suggested that capacity of mangrove sediments to immobilize phosphorus depends on the amount of organic matter, its C: P ratio, and the type and amount of clay minerals present.

So far, there has no attempt made to relate the physicochemical characteristics of the sediment in the mangrove ecosystems with the phosphobacterial load and phosphatase activity in the mangrove rhizospheric, pneumatophoric and bulk sediments. Considering the above facts, the present study was carried out to understand the role of sediment chemistry in mangrove-phosphobacterial relationship.

Materials and Methods

Sampling sites, Sample Collection and Processing

Alibag, Mahul, Versova and Gorai mangrove forests along Mumbai coast, west India were selected as sampling areas during 2013 (Fig 1). The Alibag sampling area extends between latitudes 18° 56’ and 18° 29’ N, and longitudes 72° 50’ and 73° 04’ E. It is one of the coastal areas with less anthropogenic influence, emerging as prominent eco-tourism spot without any industrial units in the vicinity. The Mahul area extends between latitudes 19° 12’ and 19° 71’ N, and longitudes 72° 59’ and 72° 52’ E. Strong industrial influence with several small and large industrial units are located in this area. The Versova sampling unit extends between latitudes 19° 08’ N and longitudes 72° 47’ E. The beach has a lush mangrove system which is able to go through a salt water and freshwater cycle regularly. It features an ocean line and body to the west and a mangrove system within its beach in landward and there is a single outlying break off of a river and contains a vast supply of freshwater. The Gorai area extends between latitudes 19° 14’ and 19° 13’ N, and longitudes 72° 46’ and 72° 47’ E. It is reported that the area has immense development pressure (Anon, 2007) [4]. It is observed that anthropogenic influence is leading to severe destruction of creek and its ecosystem. It was one of the garbage dumping sites in Greater Mumbai region. The creek water gets polluted on account of direct contact with refuse, domestic wastes, etc.

Three sediment types namely bulk sediment (non-rhizosphere), rhizospheric sediment and sediment on the pneumatophores (referred as pneumatophoric sediment) were collected once in triplicates. The coordinates of the sampling points were recorded using GPS (eTrex Venture® HC, Garmin, USA). Bulk sediment was collected in the depth of 0-15 cm. The rhizospheric sediment samples were collected by carefully removing the soil adhering in a 2-3 mm thickness around the individual roots of *A. marina*. The sediment at the base of the pneumatophores was collected. Sediment samples were also collected in triplicates aseptically in sterile Uricol bottles (Hi-Media, India) for the study of phosphatase activity and bacterial enumeration. The samples were kept in ice-box and transported to the laboratory.

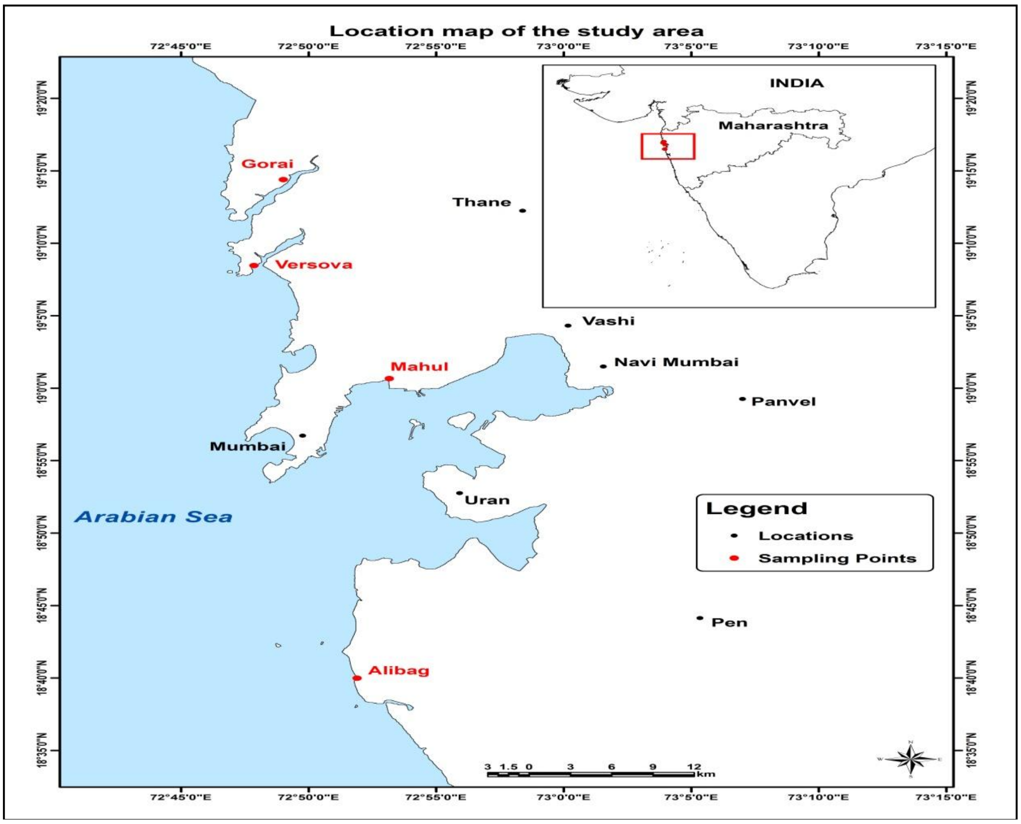


Fig 1: Sampling sites

Physicochemical characteristics of sediment

The samples were air dried for the analysis of physicochemical characteristics of sediment. Texture analysis was carried out using the sieved samples. The sieved sediment was powdered using pestle and mortar and stored for the further analysis. The moisture content was estimated gravimetrically for both wet and air-dried samples for expressing the results on moisture-free basis. The sediment pH and EC was measured using portable pH and EC meters (Eutech Instruments, Malaysia) in sediment and water ratio of 1: 2.5 and the sediment textures was estimated by the international pipette method (Muthuvel and Udayasoorian, 1999) [32]. The organic carbon (C) content of the sediment was estimated by Walkley and Black (1934) [50] method. Organic and inorganic P in sediment was estimated by ignition method. P in ignited sediment provides total P and inorganic phosphorus is determined using the unignited sediment. The difference between the above two gives quantity of organic P (Kuo, 1996) [29]. The available P in sediment was estimated using Olsen's method (Olsen *et al.*, 1954) [33] as the sediment pH was in neutral to alkaline range. The sediment samples were digested for estimation of iron, calcium, magnesium and zinc using microwave based digestion system (Multiwave 3000, Anton Parr, USA). The digested samples was analysed by atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer, USA) using flame atomization.

Acid phosphatase activity of sediment

The activity of fresh sediment was estimated according to Tabatabai *et al.* (1969) [43]. Phosphatase activity is measured in terms of quantity of *p*-nitrophenol released from the substrate p-nitrophenyl phosphate. One gram sediment each was taken in two 50 ml conical flasks. Out of these two, one conical flask was used as control and other as substrate flask. 0.2 ml of toluene and 4 ml of MUB (Modified universal buffer) solution of pH 6.5 was added to both flasks. One ml of p-nitrophenyl phosphate (*p*-NPP) solution was added to substrate conical flasks. After proper swirling, stoppered them and placed in incubator at 37˚C for one hour. After incubation, the stopper was removed and one ml of 0.5 *M* $CaCl_2$ and four ml of 0.5 *M* NaOH was added. One ml of *p*-NPP solution was added to the control. All the suspensions were quickly filtered through Whatman No. 2 filter paper and intensity of the yellow colour (*p*-nitrophenol) in the filtrate was measured at 440 nm using UV-visible spectrophotometer and compared with the standard graph prepared with different concentrations of *p*-nitrophenol (*p*-NP). The results are expressed as µg *p*-NP released g^{-1} hr^{-1}

Alkaline phosphatase activity of sediment

Phosphatase activity of fresh sediment was estimated according to tarafdar *et al.* (1988) [47]. The procedure followed for the measurements of alkaline phosphatase activity of sediment is similar to that of acid phosphatase activity except for pH of the buffer which was adjusted to 11.

Enumeration of Phosphobacteria

Total heterotrophic bacteria were enumerated using ZoBell's marine agar 2216. Pikovskaya's media (PVK) and NBRIP media were used for the enumeration of PSB with prominent halo formation around the colonies and phenolphthalein-phosphate media (PP) was used for the enumeration of PPB by considering the pink coloured colonies on exposure to ammonia vapour. NBRIP medium was prepared in the laboratory and the composition (g/L) is provided as: glucose 10g, tri-calcium phosphate 5g, $MgCl_2$ $6H_2O$ 5g, $MgSO_4$ $7H_2O$ 0.25g, KCl 0.2g, $NH4)_2SO_4$ 0.1g and agar 15g.

Statistical Analysis

All statistical analysis was carried out using SPSS 16.0 (SPSS Inc., Chicago, llinois, USA). The two-way ANOVA was carried out for the factors, sediment type and site. Duncan homogenous grouping of means was carried out at P=0.05 for the significant effects. Pearson correlation matrix was prepared for the physicochemical characteristics and phosphatase activity of sediments.

Results and Discussions

A significant relationship was seen between the different physico-chemical characteristics of the sediment and bacterial load (Table1). PSB load in PVK medium had positive correlation with acid phosphatase activity, organic P, heterotrophic bacterial load in ZoBell's marine agar, PSB load in NBRIP, PPB load in PP agar and zinc, and negative correlation with pH, EC and inorganic P. PSB load in NBRIP medium had highly positive correlation with acid phosphatase activity, heterotrophic bacterial load, PSB load in PVK medium and PPB load, and negative correlation with pH, inorganic P and zinc. PPB in PP agar medium had positive correlation with alkaline and acid phosphatase activity, heterotrophic bacteria, PSB load in PVK and NBRIP, zinc and negative correlation with pH, EC, total P and organic P. Total P had positive correlation with organic C, acid phosphatase activity, inorganic P, organic P, clay, iron and zinc, and negative correlation with pH, EC, PPB load, calcium and magnesium. Organic P had positive correlation with Olsen P, acid phosphatase activity, total P, clay, PSB in PVK medium and zinc, and negative correlation with pH, EC, alkaline phosphatase activity, PPB load and magnesium. Inorganic P had positive correlation with pH, organic C, alkaline phosphatase activity, total P, clay and iron, and significant negative correlation with total heterotrophic load, PSB in PVK and NBRIP medium, calcium and magnesium. Olsen P had highly positive correlation with acid phosphatase activity, organic P, clay and zinc, and negative correlation with pH, alkaline phosphatase activity and iron. Alkaline phosphatase activity in sediment had significant positive correlation with pH, inorganic P, PPB, magnesium and negative correlation with Olsen P, acid phosphatase activity, organic P, calcium, iron and magnesium. Acid phosphatase activity had positive correlation with Olsen P, total P, organic P, clay, heterotrophic bacterial load, PSB, PPB, zinc and a negative correlation with pH, EC, alkaline phosphatase activity and magnesium.

Physicochemical characteristics of sediments

The sediment pH in all the four sites was neutral to slightly alkaline. The lowest value of 7.3 at Versova (Fig 1b) and highest at Alibag (Fig 1b &1c) was observed. In some regions, it has been recorded that pH in mangrove swamps is generally alkaline (Dwivedi *et al.*, 1975) [9]. Alkaline sediment pH in mangrove areas of West Indian coast may be due to the characteristics of the basaltic parent material. During the non-monsoon season the building up of the salinity due to increased incursion of saline water could be the causative factor for maintaining alkaline condition during post and pre-monsoon season. Varshney (1988) [48] documented the gradual increase of sediment pH from inshore to offshore regions in the coastal waters of Versova. pH in the rhizospheric sediment were low compared to bulk and Pneumatophoric sediment. A positive correlation with EC, alkaline phosphatase activity, inorganic P, magnesium, and negative correlation with Olsen P, acid phosphatase activity, total P, organic P, clay, heterotrophic bacterial load, PSB, PPB and zinc was observed (Table 1). This clearly indicates the secretion of organic acid in the rhizospheric region. Gahoonia and Nielsen (1992) [11]; Hinsinger (2001) [18] suggested that pH of the rhizosphere soil may be changed by imbalance uptake of cations and anions by plants, which can affect the P availability in the soil. PSB and PPB in and around the rhizosphere by secretion of organic acids and production of phosphatase enzyme facilitate the conversion of insoluble forms of P to plant-available forms (Kim *et al.*, 1998) [28]. Clay content of the study area ranged from 4.09 % (Fig 1b) to 64.91% (Fig 1b). A positive correlation of clay with organic C, Olsen P, acid phosphatase activity and all the forms of P, and negative correlation with pH, EC, magnesium and calcium was observed (Table 1). Alibag rhizospheric sediment showed low clay content, and this could be due to the lesser riverine strength, which is responsible for clay settlement and more the tidal strength from seaward that could be the reason for the greater percentage of sand. Mahul had higher clay content. It was also observed that the higher clay content in Mahul favoured higher bacterial load. Clayey sediments contain higher phosphobacterial population than the sandy sediments (Ayyakannu and Chandramohan, 1971) [5]. Sediment texture analysis showed different types between samples of same site could be due to seasonal variations, proximity to the sea, magnitude of mixing and activity at the adjoining coasts but also on terrigenous and anthropogenic input. Organic C in the sediment ranged from 0.53% (Fig 1b) in Alibag rhizospheric sediment to 3.38% (Fig 1c) at Mahul bulk sediment, and had positive correlation with EC, inorganic P, total P, clay and iron (Table1). Organic C in sediment is basically derived from within the ecosystem and also by transportation of leaf and eroded materials. As Alibag is a coastal area, the continuous strong tidal action and due to existence of vigorous oxidation activity in the sediment may be a reason for low organic carbon. A higher organic carbon than three percent might be due to the industrial and domestic discharges in Mahul. Sasamal *et al.* (1986) [41] reported sediment organic C varying between 0.59 and 4.12% in Orissa coast.

In the present study, the total P ranged from 466.19 (Fig 2b) to 1524.5 mg kg^{-1} (Fig 2c) and had positive correlation with organic C, acid phosphatase activity, inorganic P, clay and iron (Table1). Hesse (1961; 1963) [16, 17] reported the total P concentration varying between 800 and 1600 µg g^{-1} in Sierra Leone mangroves (West Africa). The result of the present study is comparable to that reported by Vazquez *et al.* (2000) [49] in mangroves of Laguna de Balandra, Mexico and had reported total P as 1092±95 and 1317±70 µg g^{-1} in mangrove rhizospheric sediment and bulk sediment, respectively. Alibag sediments had low quantity of total P when compared to other sites. It was reported that P concentration in sandy coastal zone is comparatively low with respect to clayey and silty deep water zones (Lukawska- Matuszewska and Bolaek, 2008) [30]. The low clay and high sand content explains the low total P in Alibag sediment.

Organic form of P ranged from 58.2 (Fig 2b) to 799.12 mg kg^{-1} (Fig 2a). It may constitute 30–50% of the total P in most soils, although it may range from as low as 5% to as high as 95% (Paul *et al.*, 1988) [34]. It was noted that pneumatophoric sediment had low level of organic P when compared to bulk and rhizospheric sediment. Organic P content is reported as 112±40 µg g^{-1} Laguna de Balandra mangrove sediment (Vazquez *et al.*, 2000) [49]. Organic P had a positive correlation with Olsen P, acid phosphatase activity, total P, clay, iron and zinc (Table1). Moreover, high density of PPB was observed in the pneumatophoric sediment, and hence the conversion of organic P to inorganic form by the microbial action. It has been suggested that higher phosphatase in rhizosphere, compared to the bulk soil, can induce significant depletion of organic P in the rhizosphere (Tarafdar and Jungk, 1988; Chen *et al.*, 2002; Radersma and Grierson, 2004) [47, 7, 35].

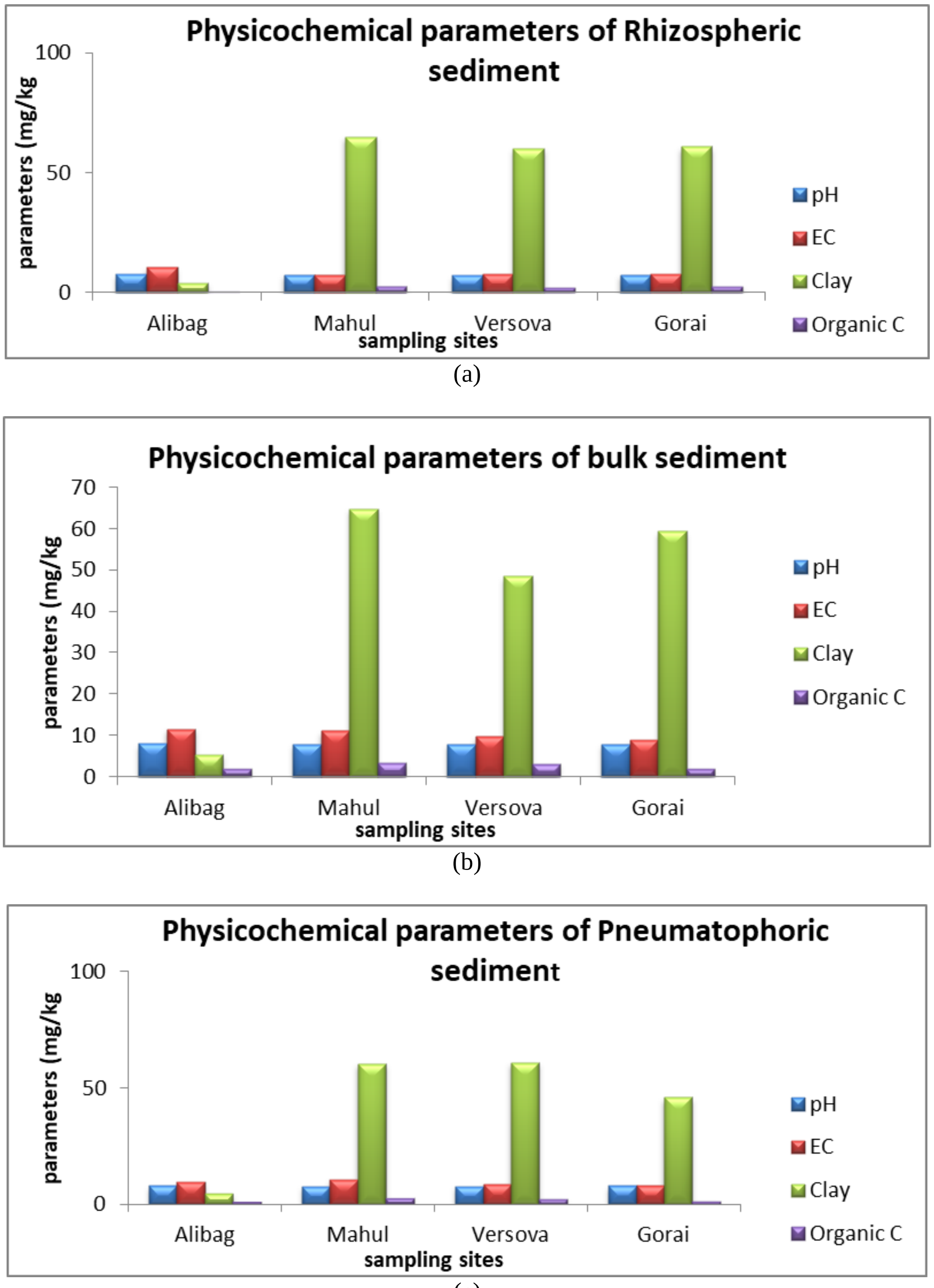
The inorganic P was between 385.32 (Fig 2a) and 1575.82 mg kg^{-1} (Fig 2c) and had positive correlation with pH, organic C, alkaline phosphatase activity, total P, clay and iron (Table1). The rhizospheric sediments showed low inorganic P than bulk and pneumatophoric sediments. This might be due to the microbial utilization or solubilization of insoluble inorganic form of P. Inorganic P in mangrove sediment of Laguna de Balandra, Mexico is reported as 1047±31 µg g^{-1} in rhizospheric sediment and 1317±70 µg g^{-1} in bulk sediment (Vazquez *et al.*, 2000) [49]. Alexander (1978) [1] reported that microbes involved in mineralizing of organic P into orthophosphates, solubilize the inorganic P compounds converting the inorganic available anion into cell protoplasm and bringing about an oxidation or reduction of inorganic P compounds. Further, it is noted that the pneumatophoric sediment had significantly higher iron content than the other sediment type. The precipitation of phosphate as iron phosphate might justify the significantly higher inorganic P in pneumatophoric sediment.

Olsen P in the study sediment was from 3.98 (Fig 2b) to 14.04 mg kg^{-1} (Fig 2c). It was found to be higher in rhizospheric sediments than in bulk and pneumatophoric sediment. This may be due to the solubilization activity of inorganic P to plant available form. It is reported that 19.2 to 26.2 µg g^{-1} of Olsen P in mangrove sediment in Hong Kong (Tam and Wong, 1995) [45]. The higher Olsen P might be due to nutrient enrichment.

Iron in the sediments was from 49410 (Fig 3c) to 126060 mg kg^{-1} (Fig 3b) and had positive correlation with organic C, alkaline phosphatase, inorganic and total P (Table1). The higher levels of iron are justifiable, when considered with the highest abundance of iron in planet earth and the lithosphere (Johnson *et al.*, 1997) [24]. Calcium was 3378 (Fig 3c) to 10222.5 mg kg^{-1} (Fig 3b). Magnesium ranged from 4035.5(Fig 3a) to 10460 mg kg^{-1} (Fig 3c) and had positive correlation with pH, EC, alkaline phosphatase and calcium (Table1). Zinc ranged from 224 (Fig 3b) to 376.5 mg kg^{-1} (Fig 3a) and had positive correlation with Olsen P, acid phosphatase activity, total P, organic P, clay and bacterial load in different media (Table1). Metal content in all sediment types was noted to be higher which might be due to anthropogenic input and also the natural deposition of metals in earth crust. Inorganic P content of Alibag was lower than that of other sites. Rhizospheric sediment had lower inorganic P, pH, iron content, and higher calcium and magnesium than those of bulk and pneumatophoric sediment. The lower inorganic P coincides with lower iron content and higher calcium and magnesium content in Alibag sediment and rhizospheric sediment than that of other sites and sediment types. Probably the low iron content would have resulted in low inorganic P in Alibag sediment and rhizospheric sediment. Fractionation study by Coelho *et al.* (2004) [8] in an estuary revealed that with increasing pH and salinity leads to shifting of iron bound P fraction to $CaCO_3$ bound P and calcite formation through precipitation process.

Acidic and Alkaline Phosphatase Activity of Sediments

Sediment acid phosphatase activity ranged from 0.34 to 15.21 µg *p*-NP released g^{-1} h^{-1}. Alkaline phosphatase activity ranged from 2.33 to 20.97 µg *p*-NP released g^{-1} h^{-1} (Table 2). Gorai sediments showed significantly higher acid and alkaline phosphatase activity. It might be due to higher clayey texture. Alibag showed low acid and alkaline activity due to less clay content and higher salinity. Acid phosphatase was found to be more in rhizospheric sediment compared to bulk and pneumatophore. It might be due to acid production in the rhizospheric region by the action of microbes and root excretion. Alkaline phosphatase was observed to be higher in pneumatophoric sediment. The presence of significantly higher number of PPB in pneumatophoric region when compared to other samples justifies the higher alkaline phosphatase activity. Huang (2005) [21] found sediment acid phosphatase to be 12.6±2.9 and 21.2±0.3 µmol *p*-NP released g^{-1} h^{-1} for the oligohaline and mesohaline habitats, and proposed that phosphatase activity tend to decrease with increasing salinity. A decrease in clay content would, for example, afford less protection for the enzyme due to complex formation between the clay and phosphatase.

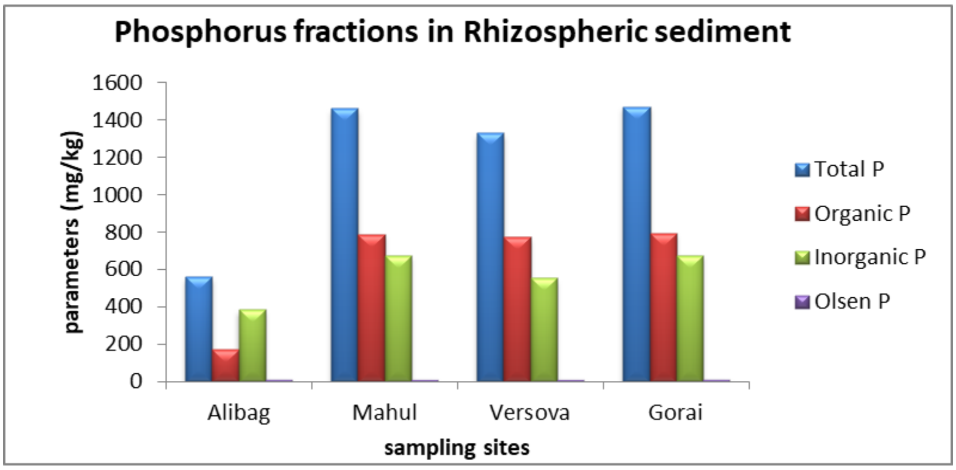


(a)

(b)

(c)

Fig 3: Physicochemical Properties of a) Rhizospheric sediment b) Pneumatophoric sediment c) Bulk sediment



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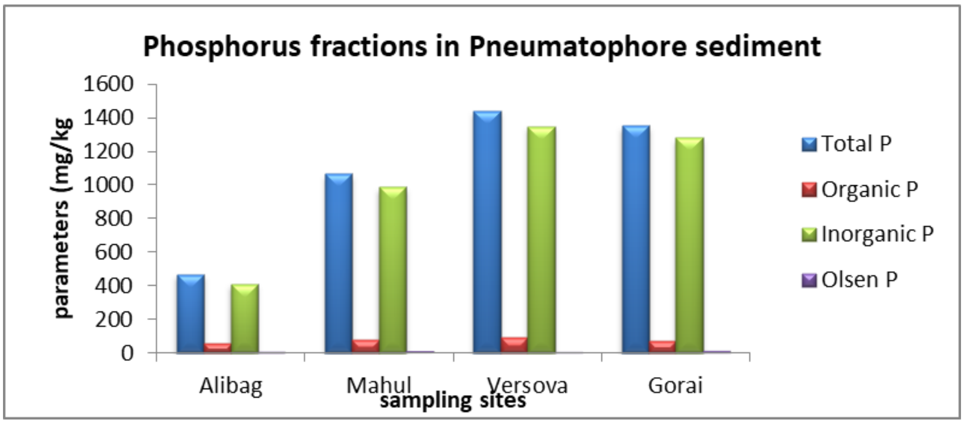

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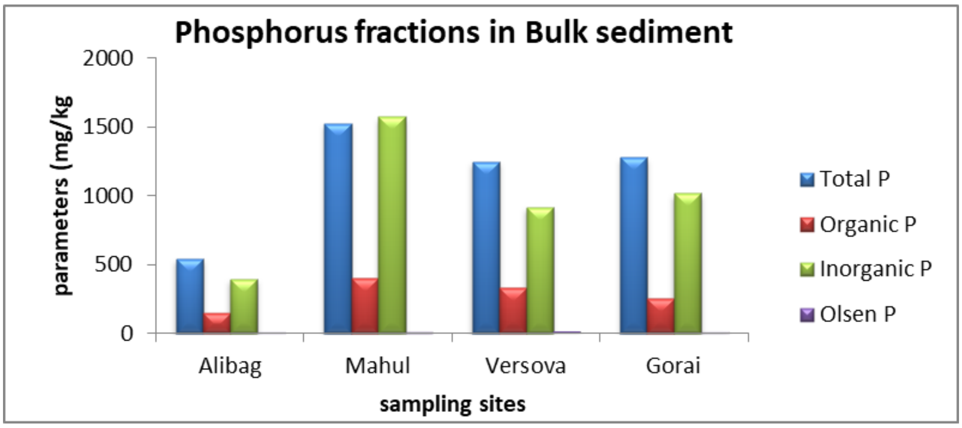
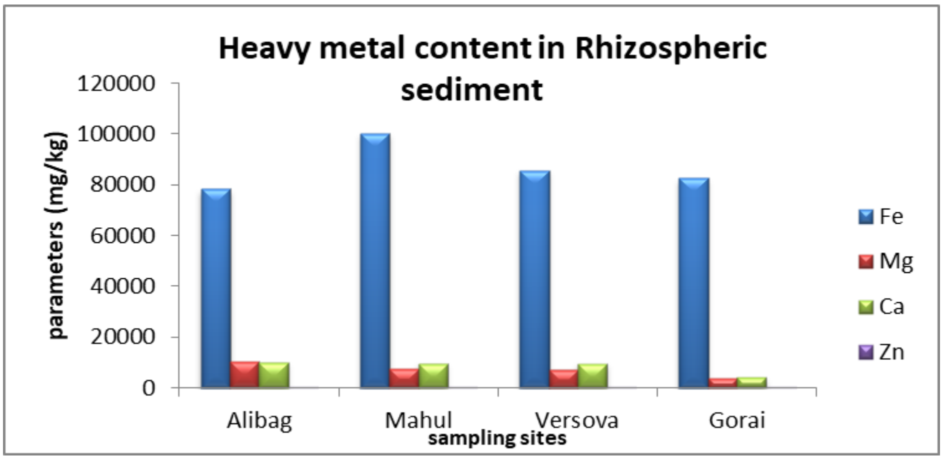


(c)

Fig 2: Phosphorus Fractions in, a) Rhizospheric sediment, b) Pneumatophoric sediment, c) Bulk sediment



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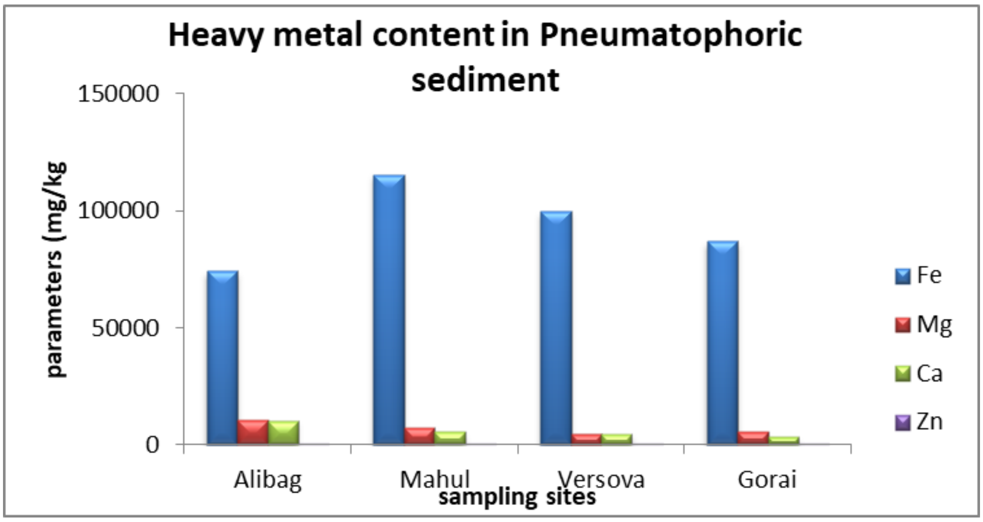

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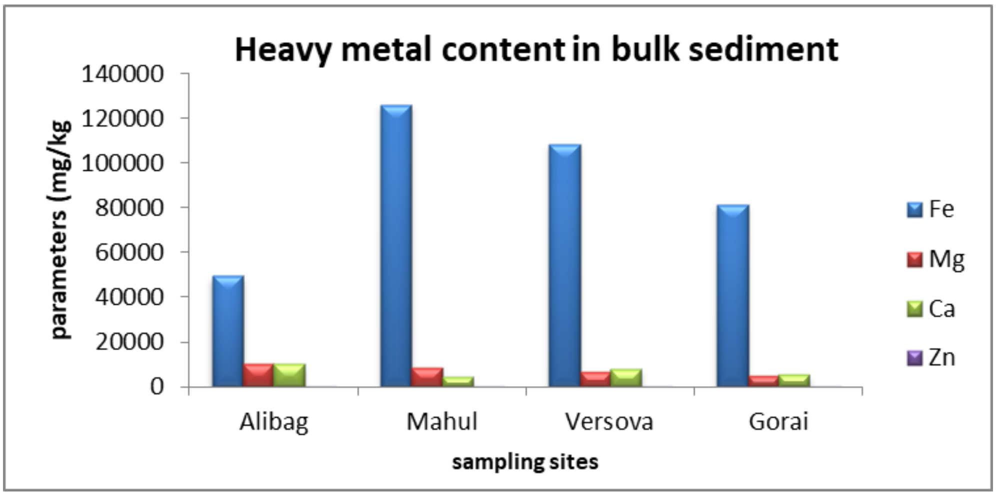

(c)

Fig 3: Heavy metal content in a) Rhizospheric sediment b) Pneumatophoric sediment, c) Bulk sediment

Table 1: Pearson correlation matrix for sediment physicochemical characteristics, phosphatase activity and P- mobilizing bacteria in the study area (n = 108)

	pH	**EC**	**Org C**	**Olsen P**	**Alk PP**	**Acid PP**	**Inorg P**	**Total P**	**Org P**	**Clay**	**M Agar**	**PVK**	**NBRIP**	**PP Agar**	**Fe**	**Ca**	**Mg**	**Zn**
EC	.436**	1																
Org C	.067	.272**	1															
Olsen P	-.350**	-.160	-.040	1														
Alk PP	.257**	-.097	-.143	-.530**	1													
Acid PP	-.709**	-.606**	-.131	.470**	-.260**	1												
Inorg P	.191*	.063	.201*	-.164	.411**	-.158	1											
Total P	-.300**	-.237*	.253**	.149	-.049	.357**	.769**	1										
Org P	-.722**	-.450**	.114	.453**	-.637**	.764**	-.185	.487**	1									
Clay	-.334**	-.320**	.226*	.223*	.060	.363**	.584**	.818**	.460**	1								
M Agar	-.387**	-.360**	-.039	.127	.152	.450**	-.303**	-.187	.126	-.148	1							
PVK	-.474**	-.451**	-.050	.155	.088	.566**	-.233*	-.002	.315**	.043	.652**	1						
NBRIP	-.286**	-.109	-.074	-.096	.107	.397**	-.213*	-.132	.087	-.046	.389**	.354**	1					
PP Agar	-.190*	-.324**	-.059	-.040	.552**	.199*	-.110	-.223*	-.192*	-.095	.725**	.497**	.288**	1				
Fe	.019	-.034	.208*	-.225*	.218*	-.051	.276**	.212*	-.051	.147	-.038	.032	-.118	.027	1			
Ca	-.020	.113	-.070	.058	-.492**	-.167	-.820**	-.662**	.102	-.587**	.074	-.006	-.099	-.092	-.193*	1		
Mg	.275**	.462**	.036	.017	-.410**	-.426**	-.520**	-.620**	-.241*	-.703**	-.004	-.176	-.143	-.065	-.166	.760**	1	
Zn	-.421**	-.308**	.061	.509**	-.149	.459**	.162	.402**	.396**	.290**	.491**	.390**	-.191*	.232*	.060	-.155	-.142	1

Table 2: Alkaline and acid phosphatase activity (*p*-NP released g^{-1} hr^{-1}; in range) of sediment in the study sites

Parameter	Sediment type	Versova	Mahul	Alibag	Gorai
Alkaline phosphatase	Rhizosphere	2.43-4.65	4.10-5.78	5.45-6.27	4.10-5.88
	Pneumatophore	17.38-20.97	12.14-14.45	9.66-12.19	13.84-15.44
	Bulk	3.36-5.36	2.33-4.32	4.10-4.83	9.76-11.34
Acid phosphatase	Rhizosphere	7.02-8.77	9.38-11.07	4.22-4.76	11.95-15.21
	Pneumatophore	2.07-2.78	1.25-2.41	0.34-0.79	6.26-8.80
	Bulk	1.06-1.91	1.37-2.83	1.06-1.72	2.00-2.88

Enumeration of Bacteria in the Sediments

The total heterotrophic count of the sediment enumerated using ZoBell's marine agar ranged from $114.21 \pm 12.83 \times 10^{6}$ cfu g^{-1} to $532.58 \pm 126.50 \times 10^{6}$ cfu g^{-1} (Table 3), and had positive correlation with PSB and PPB. The phosphate-solubilizers ranged from $0.20\text{-}6.07 \times 10^{3}$ cfu g^{-1} in Pikovskaya media (Table 4) and $0.61\text{-}11.45 \times 10^{3}$ cfu g^{-1} in NBRIP medium (Table 5), respectively. The rhizospheric sediment showed higher biological activity. The physicochemical and biological characteristics of rhizospheric sediment are significantly differing from bulk sediment (Gregory and Hinsinger, 1999) [14]. Ryan *et al.* (2001) [39] had proposed that organic anions secreted from plant roots increases the P availability compounds by desorbing inorganic P from a mineral surface and chelating or complexing cations such as iron and Calcium which gets bound to P. Ravikumar *et al.* (2002) [37] isolated three species of phosphobacteria, belonging to the same genus (*Bacillus*) in Pichavaram mangrove. The species diversity was found maximum in the roots and rhizospheric soil of mangrove plant species. This is due to the secretions of carbohydrates and amino acids from root exudates of plants that enhance the growth and multiplication of bacterial species (Kathiresan and Ravikumar, 1995; 1993) [26, 27].

The PPB in PP agar ranged from 0.98×10^{3} to 31.73×10^{3} cfu g^{-1} (Table 6). PPB showed higher activity in pneumatophoric sediment. The higher bacterial load, alkaline phosphatase activity, inorganic P and low organic P in the pneumatophoric sediment indicates the possibility of harbouring favourably the PPB in pneumatophore than rhizosphere or bulk sediments. PPB has capability to produce extracellular enzymes such as phosphatase (George *et al.*, 2002) [12]. This enzyme is able to mineralize organic phosphates into inorganic phosphates that provide high P for plant. Soil phosphatases play a major role in the mineralization processes (dephosphorylation) of organic P substrates. A study on endophytic bacteria colonizing pneumatophores reported that this kind of relationship enhances growth of the entire plant, increasing productivity and the yield of reproductive organs (Janarthine *et al.*, 2011) [23].

Table 3: Distribution of total heterotrophic bacteria (cfu × 10^{6} g^{-1}) in ZoBell's marine agar medium (2216)

Site	Sediment type			Mean
	Bulk	Pneumatophore	Rhizosphere	
Alibag	136.29±40.16	414.56±137.10	532.58±126.50	361.14±199.66^{b}
Mahul	148.62±21.33	343.57±182.41	473.78±27.33	321.99±170.73^{b}
Versova	114.21±12.83	334.74±46.11	239.60±37.95	229.52±98.07^{a}
Gorai	155.05±76.74	348.75±88.58	486.36±96.72	330.05±162.12^{b}
Mean	138.54±45.89^{a}	360.40±123.34^{b}	433.08±140.08^{c}	310.68±167.11

Table 4: Distribution of phosphate-solubilizing bacteria (cfu × 10^{3} g^{-1}) in Pikovskaya's agar

Site	Sediment type			Mean
	Bulk	Pneumatophore	Rhizosphere	
Alibag	0.89	3.56	3.72	2.72^{ab}
Versova	0.47	2.88	6.07	3.14^{bc}
Gorai	0.20	2.97	2.80	1.99^{a}
Mahul	1.54	3.69	5.94	3.73^{c}
Mean	0.78^{a}	3.28^{b}	4.63^{c}	2.90

Table 5: Distribution of phosphate-solubilizing bacteria (cfu × 10^{3} g^{-1}) in NBRIP medium

Site	Sediment type			Mean
	Bulk	Pneumatophore	Rhizosphere	
Alibag	10.5	0.93	6.30	5.91^{b}
Mahul	0.75	8.33	6.22	5.10^{ab}
Gorai	0.61	6.68	3.92	3.74^{a}
Versova	1.02	6.89	11.45	6.45^{b}
Mean	3.22^{a}	5.71^{b}	6.97^{c}	5.30

Table 6: Distribution of phosphatase-producing bacteria (cfu × 10^{3} g^{-1}) in phenolphthalein-phosphate agar medium

Site	Sediment type			Mean
	Bulk	Pneumatophore	Rhizosphere	
Alibag	1.62	31.73	21.35	18.23^{b}
Versova	2.98	30.87	19.63	17.83^{b}
Gorai	0.98	22.65	16.70	13.44^{a}
Mahul	6.59	28.26	19.39	18.08^{b}
Mean	3.04^{a}	28.38^{c}	19.27^{b}	16.89

Note: Values with different letters in superscript in all tables differ significantly at $p = 0.05$ within sediment type

Conclusion

The mangrove ecosystem is very complex in nature and favours a positive relationship between the mangrove-microbial-sediments. It is seen that higher abundance of PSB was found in the rhizospheric sediment with clear indication of low pH in the rhizospheric sediment. Low inorganic P was also found in rhizospheric sediment indicating the solubilization of P. Higher abundance of PPB was found in the pneumatophoric sediment with clear indication of low organic P in the pneumatophoric sediment than that of rhizospheric and bulk sediment. Low organic P in pneumatophoric sediment implies the utilization of organic P by the bacterial load and conversion to inorganic forms. Alkaline phosphatase activity was higher in the pneumatophoric sediment and acid phosphatase activity was higher in the rhizospheric sediments. It shows that the all physico-chemical parameters of sediment correlated significantly with bacterial populations and phosphatase activity indicating influence of sediment chemistry on mangrove-bacterial interactions. The mangroves act as a link between the terrestrial and aquatic life. And hence, the survival within a harsh, waterlogged and anaerobic environment compels them to depend on a close relation among mangroves, microbes and soil parameters.

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References

1. Alexander. Phosphorus-containing pesticide breakdown products: quantitative utilization as phosphorus sources by bacteria. Appl. Environ. Microbiol. 1978; 36(5):668-672.
2. Alongi DM. Bacterial productivity and microbial biomass in tropical mangrove sediments. Microb Ecol. 1988; 5:59-79.
3. Alongi DM, Sasekumar A. Benthic communities. In: Robertson AL and Alongi DM (ed) Tropical mangrove Ecosystem, Am. Geophys. Union, Washington D.C, 1992, 137-171.
4. Anon. State of Forest Report. Forest survey of India, Dehradun, 2000.
5. Ayyakkannu K, Chandramohan D. On the occurrence and distribution of phosphobacteria in the marine environment of Porto Novo. Curr. Sci. 1970; 39:398-399.
6. Boto KG. Nutrient and organic fluxes in mangroves. In: Clough BF (ed) Mangrove Ecosystems in Australia, Australian National University Press, Canberra, 1982, 239-257.
7. Chen YP, Rekha PD, Arun AB, Shen FT, Lai WA, Young CC. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. Appl. Soil Ecol. 2006; 34:33-41.
8. Coelho JP, Flindt MR, Jensen HS, Lillebø AI, Pardal MA. Phosphorus speciation and availability in intertidal sediments of a temperate estuary: relation to eutrophication and annual P-fluxes. Estuarine Coastal Shelf Sci. 2004; 61(4):583-590.
9. Dwivedi SN, Parulekar AH, Goswami SC, Untawale AG. Ecology of mangrove swamps of the Mandovi Estuary, Goa, India. Proc. Int. Symp. Biol. Management of Mangroves. 1975; 1:115-125.
10. Feller IC, Mckee KL, Whigham DF, John P. Nitrogen vs. Phosphorus limitation across an ecotonal gradient in a mangrove forest. Biogeochemistry. 2002; 62:145-175.
11. Gahoonia TS, Nielson NE. The effects of root-induced pH changes on the depletion of inorganic and organic phosphorus in the rhizosphere. Plant Soil. 1992; 143(2):185-191.
12. George TS, Gregory PJ, Wood M, Read D, Buresh RJ. Phosphatase activity and organic acids in the rhizosphere of potential agroforestry species and maize. Soil Biol Biochem. 2002; 34:1487-1494.
13. Giri C, Ochieng E, Tieszen LL, Zhu Z, Singh A, Loveland T, *et al.* Status and distribution of mangrove forests of the world using earth observation satellite data. Glob. Ecol. Biogeogr. 2011; 20:154-159.
14. Gregory PJ, Hinsinger P. New approaches to studying chemical and physical changes in the rhizosphere: an overview. Plant Soil. 1999; 211:1-9
15. Gurmeet Singh, Rita Chauhan, Rajesh Kumar Ranjan, Balakrishna Prasad M, Ramanathanan AL. Current Science. 2015; 108:10.
16. Hesse PR. Some differences between the soils of Rhizophora and Avicennia mangrove swamps in Sierra Leone. Plant Soil. 1961; 14:335-346.
17. Hesse PR. Phosphorus relationships in a mangrove swamp mud with particular reference to aluminium toxicity. Plant Soil. 1963; 19:205-218.
18. Hinsinger P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. Plant Soil. 2001; 237(2):173-195.
19. Holguin G, Bashan Y, Mendoza-Salgado RA, Amador E, Toledo G, Vazquez P, *et al.* La Microbiologia de los manglares. Bosques en la frontera entre el mar y la tierrra. Ciencia Desarrollo. 1999; 144:26-35.
20. Holguin G, Vazquez P, Bashan Y. The role of sediment microorganisms in the productivity, conservation and rehabilitation of mangrove ecosystems: an overview. Biol. Fert. Soils. 2001; 33:265-278.
21. Huang X, Morris JT. Distribution of phosphatase activity in marsh sediments along an estuarine salinity gradient. Mar Ecol Prog Ser. 2005; 292:75-83.
22. Hyde KD, Lee SY. Ecology of mangrove fungi and their role in nutrient cycling: what gaps occur in our knowledge? Hydrobiologia. 1995; 295:107-118.
23. Janarthine SR, Eganathan SP, Balasubramanian T, Vijayalakshmi S. Endophytic bacteria isolated from the pneumatophores of Avicennia marina. Afr. J Microbiol. Res. 2011; 5(26):4455-4466.
24. Johnson TL, Fish W, Gorby YA, Tratnyek PG. Degradation of carbon tetrachloride by iron metal: complexation effects on oxide surface. J Contam. Hydrol. 1997; 29(4):379-398.
25. Kathiresan K, Bingham BL. Biology of mangroves and mangrove ecosystems. Adv. Mar. Biol. 2001; 40:81- 251.
26. Kathiresan K, Ravikumar S. Influence of tannius, aminoacids and sugars on fungi of marine halophytes. Mahasagar: Bull. Natl. Inst. Oceanogr. 1993; 26(1):21-25.
27. Kathiresan K, Ravikumar S. Influence of tannins, sugars and amino acids on bacterial load of marine halophytes. Environ. Ecol. 1995; 13(1):94-96.
28. Kim KY, Jordan D, McDonald GA. Effect of phosphate-solubilizing bacteria and vesicular-arbuscular mycorrhizae on tomato growth and soil microbial activity. Biol. Fertil. Soils. 1998; 26:79-87.
29. Kuo S. Phosphorus. In: Sparks DL, Page AL, Helmke PA, Loeppert RH, Soltanpour PN, Tabatabai MA, Johnston CT, Sumner ME (Eds.), Methods of Soil Analysis: Part 3, Soil Science Society of America Book Series (3rd ed.), No. 5, Soil Sci. Soc. Am and Am. Soc. Agron, Madison, Wisconsin, USA, 1996, 869-919.
30. Lukawska-Matuszewska K, Bolaek J. Special distribution of phosphorus forms in sediments in the Gulf of Gdansk (southern Baltic sea). Cont. Shelf Res. 2008; 28:977-990.
31. McVicker H, Bridger GL, Nelson LB. Effect of halophilic phosphobacteria on Avicennia officinalis

seedling. Soil Sci. Soc. Am. Madison II, Wiscon, 1963, 155-187.

32. Muthuvel P, Udayasoorian C. Soil, plant, water and agrochemical analysis. Tamilnadu Agriculture Univ., Coimbatore, 1999, 301.
33. Olsen S, Cole C, Watanabe F, Dean L. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular Nr 939, US Gov. Print. Office, Washington, DC, 1954.
34. Paul JH, DeFlaun MF, Jeffrey WH, David AW. Seasonal and diel variability in dissolved DNA and in microbial biomass and activity in a subtropical estuary. Appl. Environ. Microbiol. 1988; 54:718-727.
35. Radersma S, Grierson PF. Phosphorus mobilization in agro forestry: Organic anions, phosphatase activity and phosphorus fractions in the rhizosphere. Plant Soil. 2004; 259(1-2):209-219.
36. Ramanathan AL, Subramanian V, Ramesh R, Chidambaram S, James A. Environmental geochemistry of the Pichavaram mangrove ecosystem (tropical), southeast coast of India. Environ. Geol. 1999; 37:223-233.
37. Ravikumar S, Ramanathan G, Suba N, Jeyaseeli L, Sukumaran M. Quantification of halophilic Azospirillum from mangroves. Ind. J Mar.Sci. 2002; 31(2):157-160.
38. Romine DS, Metzger WH. Phosphorus fixation by horizons of various soil types in relation to dilute acid, extractable iron, and aluminium. Agron. J. 1939; 31:99-108.
39. Ryan PR, Delhaize E, Jones DL. Function and mechanism of organic anion exudation from plant roots. Annu. Rev. Plant Physiol. Plant Mol. Biol. 2001; 52:527-560.
40. Saenger P. Mangrove Ecology,Silviculture and Conservation. Kluwer Academic publishers, Dordrecht, 2002, 360.
41. Sasamal SK, Sahu BK, Panigrahy RC. Monthly variation in some chemical characteristics of near shore waters along the south Orissa coast. Indian J Mar. Sci. 1986; 15:199-200.
42. Spalding M. The global distribution and status of mangrove ecosystems. International News letter of Coastal Management-Interlock Network, Special Edition. 1997; 1:20-21.
43. Tabatabai MA, Bremner JM. Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Boil. Biochem. 1969; 1(4):301-307.
44. Tam NFY, Wong YS. Nutrient and heavy metal retention in mangrove sediment receiving wastewater. Water Sci. Technol. 1994; 29:193-200.
45. Tam NFY, Wong YS. Mangrove soils as sinks for wastewater-borne pollutants. Hydrobiologia. 1995; 295:231-241.
46. Tam NFY, Wong YS. Retention of wastewater-borne nitrogen and phosphorus in mangrove soils. Environ. Technol. 1996; 17:851-859.
47. Tarafdar JC, Jungk A. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fert. Soil. 1988; 3(4):199-204.
48. Varshney PK, Govindan K, Gaikwad UD, Desai BN. Macrobenthos off Versova(Bombay), west coast of India in relation to enviromnental conditions. Indian J mar. Sci. 1988; l7:222-227.
49. Vazquez P, Holguin G, Puente ME, Lopez-Cortes A, Bashan Y. Phosphate-solubilizing microorganisms associated with the rhizosphere of mangroves in a semiarid coastal lagoon. Biol. Fertil. Soils. 2000; 30:460-468.
50. Walkley JA, Black JA. Estimation of organic carbon by the chromic acid titration method. Soil Sci. 1934; 37:29-31.