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Phosphate Solubilizing Micro-organisms in Water and Sediments of a Tropical Estuary and the Adjacent Coastal Arabian Sea in Relation to their Physico-chemical Properties.

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Phosphate solubililizing bacterial population in both estuarine and coastal environments were much higher than phosphate solubilizing fungal population in these habitats. However, greater proportion of the fungal population was involved in phosphate solubilization than bacteria Solubilization of phosphate in broth was accompanied by a drop in pH. Species of *Bacillus* and *Aspergilius* were responsible for solubilization of phosphate in marine sediment and their solubilization ability varied from 10 to 300μ g P in 10 ml broth with consequent drop in pH ranging from 6 45 to 4.90. Out of eight *Bacillus* three were able to produce phosphatase and out of three *Aspergillus* two isolates possessed phosphatase activity. In estuarine water species of *Bacillus, Aspergillus, Penicillum* and an unidentified fungus were responsible for solubilization of phosphate, whereas, species of *Bacillus, Vibrio, Streptomyces, Aspergillus* and an unidentified fungus were found to solubilize phosphate. Among these isolates solubilization of phosphate ranged from 45 to 1475μ g P in 10 ml broth with consequent drop in pH ranging from 6.0 to 4.15. Among the isolates species of *Bacillus, Aspergillus, Vibrio* and *Streptomyces* showed phosphatase activity.

Availability of reactive phosphate has been found to be the limiting factor for the productivity in lakes and pelagic sea (Redfield, 1934). Micro-organisms are well known to solubilize insoluble phosphate in terestrial soils (Hayman. 1975 ; Banik and Dey, 1981 ; 1982 ; Bhattacharyya et al., 1986). They also play a significant role in solubilization of insolube phosphate in marine and estuarine sediments (ZoBell, 1946 ; Ayyakkannu and Chandra Mohan 1971). However, information on the responsible microflora or on their ability to solubilize insoluble phosphate in marine and estuarine habitat are patchy. So a study was undertaken to charaoterise phosphate solubilizing microflora and determine their ability to solubilize insoluble inorganic phosphate together with a capacity to produce phosphatase enzyme isolated from the waters and sediments of the Cochin backwater—a tropical estuary and the sediments from the adjacent coastal Arabian sea, off Cochin, on the South West coast of India.

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MATERIALS AND METHODS

The study was conducted on water and sediments collected from different locations in the Cochin backwater area viz,. Vypeen island, Ernakulam, Fort Cochin and Pelagic fisheries lab area of C.M.F.R.I. and the adjacent coastal Arabian sea, off Cochin on the South West coast of India

Sediment samples from 10 and 20 m depths, off Cochin during the months of December 1984 and January 1985 and from different locations in the Cochin backwater area at depths ranging between 2 and 5 m in the month of February 1985 were collected by means of a Peterson grab. Water samples were collected in 125 ml. sterilized glass bottles for microbiological analyses, in 500 ml narrow mouthed polythene container for physico-chemical analyses and in 125 ml glass stoppered bottle for the dissolved oxygen content. Sediment samples were transfered to polyethene container immediately after collection. Water and sediment samples were processed immediately for chemical and microbiological analyses.

Temperature of the water samples were immediately noted from the thermometer of the sampler and pH and Eh of the collected water and sediment samples were determined following Cohen (1957). Salinity of water samples were estimated by using Mohr-Knudson argentometry and dissolved oxygen content of water was determined according to the modified Winkler's method (Strickland and Parsons, 1968). Total phosphorus and organic carbon content of sediments were determined from air dry soil following methods described in Jackson (1973). Reactive phosphorus and organic carbon content were determined from water samples following Strickland and Parsons (1968) and Bjorndhal (1975) respectively. Phosphatase enzyme activity of sediment was determined according to Tabatabai and Bremner (1969). Same method was followed for determinig phosphatase activity in water by using 1 ml water in place of 1 g air dry soil.

For determining total heterotrophic bacterial population of sediment and water ZoBell's 2216E agar medium was used whereas for determining total fungal population Czapek—Dox agar medium was employed. For enumeration of phosphate solubilizing microorganisms Pikovskaia's agar medium was employed. The above media were modified by using 75% aged sea water and 25% distilled water for culturing microorganisms from estuarine samples and by using 100% aged sea water for culturing microorganisms from marine sediments. For determining P solubilizing power the respective media were supplemented with 2% and 3% NaCl in addition to usual composition to maintain osmotic balance of estuarine and marine sediment microorganisms respectively. The media were diluted with double distilled water and pH

was adjusted accordingly. and incubated at 28 ± 1°C for 5 to 10 days according to the need to grow maximum number of colonies. Studies were made from fresh sediments but results are expressed in air dry soil basis.

Phosphate solubilizing power of sediment and water was determined in 25 ml Pikovskaia's broth following Banik (1983). The fall of pH in the medium after 7 days incubation at 28 ± 1°C were recorded with the help of a digital pH meter. 1 ml suspension of sediments from 10^1 dilution and 1 ml undiluted water were used as inoculum. 34 colonies forming well defined clear zones were isolated from Pikovskaia's agar plates for identification and further determination of their phosphate solubilizing power and phosphatase producing ability. The isolates were purified by repeated plating followed by microscopic examinations. The bacteria were identified according to Bergey's manual (1974) and fungi according to Gilman (1967). Phosphate solubilizing power of the microorganisms and pH were determined in Pikovskaia's broth afer 7 days and incubated at 28 ± 1°C according to Banik and Dey (1982). For determining phophatase enzyme activity, a loopful of the microorganisms from 24 hrs. cultures were inoculated in medium containing 4 ml Pikovskaia's broth without $Ca_3(PO_4)_2$ and 1 ml standard p-nitrophenyl phosphate solution and incubated at 28 ± 1°C. After 48 hrs. incubation the amount of p-nitrophenol released were estimated colorimetrically following Strickland and Parsons (1968).

RESULTS AND DISCUSSIONS

It is evident from data presented in table 1 that pH values of marine and estuarine waters was in the alkaline range, where as pH of estuarine sediments were slightly acidic in nature and those from sea were alkaline in nature. E_h of estuarine water was more oxidised than sea water, However, dissolved oxygen content in sea water was more in comparison to estuarine water. Organic carbon content in sediments from both the habitats were excepting in samples collected from ernakulam. Organic carbon contents of estuarine water was high. Reactive phosphorus content of sea water was much higher than that of estuarine water although total phosphorus content in both the habitats did not vary much excepting in Ernakulam.

Perusal of ihe results presented in table 1 indicates that the environments were favourable for flourishing aerobic mesophilic heterotrophs. Although the sea sediments were in the reduced E_h range high dissolved oxygen of overlying water must make at least the water sediment interface

Table 1 : Environmentol parameters of water and sediment studied.

Collection area and depth	Dates of Collection	Water temp. (°C)	pH		E_h (mV)		Dissolved oxygen (ml/lit.)	Salinity of water (ppt)	Organic carbon		Phosphorus	
			Water	Sediment	Water	Sedimenl			Water (mg/lit)	Sedi-ment (%)	React ive P of waters (ug/l t)	Total P in sedi- (%)
Arabian sea												
10 met	11.12.84	28 2	8.35	8.35	+70	−150	4.30	31.03	—	4.06	50.22	0.17
20 met		28.5	8.40	8.10	+70	−155	4.41	31.03	—	4.14	18 60	0.21
10 met	19.12.84	28.3	8.20	7.43	+75	− 22	4.16	3 .27	—	3.87	35.34	0.18
20 met		28.4	8.20	7.50	+75	− 28	4.75	31.36	—	4.37	33.48	0.19
10 met	8. 1.85	28.5	8.30	7.40	+105	− 12	3.98	32.38	—	4.02	68.50	0.19
20 met		28.2	8.25	7.80	+110	− 30	4.35	30.62	—	4.29	68.50	0.20
Vypeen	24. 2.85	32.5	8.05	6.35	+267	+ 16	1.94	17.27	124.8	4.67	12.50	0.20
Ernakulam	24. 2.85	33.0	7.90	6.70	+262	+ 26	1.43	16 49	134.2	0.61	0	0.08
Fort Cochin	24. 2.85	31.0	7 95	6.40	+272	+ 16	1.60	17.46	124.8	3.79	6.25	0.17
Pelagic	24. 2.85	32.5	8.25	6.35	+282	+ 21	2.13	16.15	106.0	5.33	12.50	0.20

favourable to aerobic microflora. Organic carbon content both in sediment and water was sufficient to support heterotrophic population. Only exception found in the Ernakulam sediment was perhaps due to its sandy nature. Higher reactive phosphorus content of sea water was due to higher phosphate solubilizing activity in sea water than that of estuary.

Table 2 presents the population density of microorganisms in these environments. Total bacterial population in estuarine sediment were much higher than that in sea sediments and it was so in the cases of total fungi. phosphate solubilizing bacteria and phosphate solubilizing fungi. All the above mentioned microorganisms in estuarine water were densely populated. Microbial population of sediments were approximately 10 times higher than those of water. Population of total fungi were approximately 10 times lesser than those of bacteria. nevertheless, much higher proportion of fungi were involved in phosphate solubilization.

Population of total bacteria, total fungi, phosphate solubilizing bacteria and fungi were high in estuarine water probably due to contamination, by incoming microflora through sewage discharge. It may be thought that microorganisms accumulated in the estuarine sediments in higher concentrations was due to floculation with organic and inorganic colloids (Day 1978). On the other hand the remaining suspended viable fresh water or terrestrial microflora when reached the sea during low tide was exposed to lethal osmotic shock by high salinity of sea water, In addition unfavourable reduced E_h condition prevailing in the sea sediment restricted the growth of aerobic heterotrophs in that habitat, Though fewer in number, fungi played greater role in solubilizing insoluble phosphate probably due to their ability of producing acidity (Banik and Dey, 1982) and this was evidenced by a greater drop in pH in their growth medium.

Phosphate solubilizing power and phos phatase enzyme activity of water and sediments are presented in table 3. On average phosphate solubilizing power of estuarine water was higher than that of sediments. Phosphate solubilizing power of sea sediments was very high. Lowering of pH was recorded from all samples after incubation, No solubilization of phosphate was recorded with water sample collected from Pelagic fisheries lab area and sediment sample from Fort Cochin. Secondary heavy fungal mycellial growth and pH as low as 3.3 and 3 45 were recorded in those culture broths. In general drop in the pH was more in estuarine samples than in sea sediment samples. Phosphatase enzyme activity of estuarine water was more than that of estuarine sediment samples. The enzymatic activity was higher in sea sediments than estuarine sediments.

Table 2 : Enumeration of micro-organisms in water and sediment studied

Collection area and depth	Dates of collection	Number of microorganisms							
		Total bacteria ($\times 10^5$)		Total fungi ($\times 10^4$)		Phosphate solubilizing microorganism			
						bacteria ($\times 10^4$)		fuugi ($\times 10^4$)	
		Water (per ml)	Sediment (per g)	Water (per ml)	Sediment (per g)	Water (per ml)	Sediment (per g)	Water (per ml)	Sediment (per g)
Arabian sea									
10 met.	11.12.84	—	4.2	—	17.0	—	5.67	—	1.38
20 met.		—	3.8	—	12.0	—	5.02	—	1.48
10 met.	19.12.84	—	3.2	—	16.0	—	2.63	—	0.88
20 met.		—	4.7	—	22.0	—	2.50	—	1.08
10 met.	8 1 85	—	3.6	—	17.0	—	4.79	—	3.19
20 met.		—	3 7	—	14 0	—	5.87	—	2.61
Vypeen	24. 2 85	19.0	200.0	16.0	119.0	8.0	70.0	6.0	40.00
Ernakulam	24. 2.85	27.0	190.0	17.0	140.0	6.0	80.0	9.0	40.00
Fort Cochin	24. 2.85	20.0	110.0	18.0	160.0	6.0	120.0	7.0	20.00
Pelagic	24. 2.85	30.0	150.0	13.0	90.0	11.0	190.0	5.0	20.00

Lowering of pH in the culture medium during solubilization of phosphate indicates the mode of solubilization greatly by formation of acids especially organic acids (Banik and Dey, 1981 ; 1982). Although the individual marine isolates could not show higher phosphate solubilizing ability in vitro, microorganisms in mixed culture from raw marine sediments caused higher solubilization probably due to synergistic effect which was not possible in vitro with pure Cultures. However, the same explanation could not be applied to estuarine sediments. So, further intensive study is necessary for finding the actual cause. Virtually no accumulation of soluble phosphate in two sets where pH has reached as low as 2.3 and 3.45 is curious but not surrpising. This phenomena may be thought as an incidence of simultaneously growing phosphate solubilizing and immobilizing microffora especially fungi, the letter acted upon soluble phosphate released by the former and completely consumed it in the secondary stage of growth by virtue of their acid tolerance nature. Drop in pH of growth medium was indeed an indication of growth of organic acid producing phosphate solubilizers especially bacteria in the primary stage. Degree of drop in pH most probably depended on the nature and amount of organic acids liberated by the microorganisms (Johnston 1954). Higher phosphatase activity in estuarine water than in estuarine sediment indicates the thriving of more organic phosphate solubilizers in water than sediment. Nonetheless, this observation is interesting and needs further study for confirmation.

Table 4 presents the genera of microorganisms, their in vitro phosphate solubilizing power. drop in pH of growth medium and the phosphatase enzym activity. Among 11 isolates from the sea sediments eight were bacteria, all belonging to the genus *Bacillus* and three were fungi, all black sporulating *Aspergillus*. Most of the fungi solubilized higher amounts of phosphate and caused a greater drop in pH in growth media than that of bacteria. Among the isolates 3 from bacteria and 2 from fungi were able to produce phosphatase enzyme and bacteria were more efficient than fungi in this respect.

Bacillus and *Aspergillus* were the only two genera involved in solubilization of phosphate in sea sediments indicate clearly that only resistant spore formers dominated in phosphate solubilizing activity in this habitat. However, species of *Bacillus* involved in phosphte solubilization with different capabilities. Majority of the microorganisms were able to solubilize inorganic phosphate only. Which is a characteristics of tropical soil phosphate solubilizing microflora (Banik and Dey 1983). Ability to produce phosphatase enzyme by a few microorganisms in addition to solubilization of

Table 3 Phosphate solubilizing power and phosphatase activity of sediment and water studied

Collection area and depth	Dates of collection	Phosphate solubilizing power in μg P/25 ml medium and pH in broth				Phosphatase activity in μ mole p-nitro phenol released per hr.	
		Water		Sediment		Water	Sediment
		P-sol power	pH	P-sol power	pH	per 100 ml	per g
Arabian sea							
10 met.	11.12.84	—	—	1250.0	4.97	—	1.51
20 met.		—	—	2062.5	4.08	—	1.19
10 met.	19.12.84	—	—	1950.0	3.95	—	1.44
20 met.		—	—	2112.5	4.00	—	1.80
10 met.	8.1.85	—	—	2175.0	3.90	—	1.62
20 met.		—	—	1412.5	4.23	—	2.30
Vypeen	24.2.85	1600.0	3.7	375.0	3.15	1.40	0.46
Ernakulam	24.2.85	400.0	3.0	650.0	3.40	4.20	0.46
Fort Cochin	24.2.85	1200.0	4.0	0.0	3.45	6.20	1.08
Pelagic	24.2.85	0.0	3.3	1512.5	3,55	5.80	1.04

insoluble inorganic phosphate indicates their greater importance in marine ecolog.

23 isolates were taken from the four locations in the estuarine water and sediments of which 11 were bacteria. of the genus *Bacillus* and *Vibrio*, one was *Streptomyces* and the remaining 11 were fungi, of the genus *Aspergillus* and *Penicillium* including two unidentified ones. Five isolates were taken from Vypeen island of which two were bacteria of the genus *Bacillus* and three were fungi of which two belonged to *Aspergillus* and an unidentified one. Among them one species of each *Aspergillus* and *Bacillus* was found to produce phosphatase. Five isolates were collected from Ernakulam samples. They were species of *Bacillus*, *Aspergillus*, *Streptomyces* and an unidentified fungus. Among seven isolates from Fort Cochin three were bacteria and four were fungi Among bacteria *Vibrio* sp, was most officient phosphate solubilizer whereas *Aspergillus* was the most efficient among fungi in performing this function. *Bacillus*. *Vibrio* and *Aspergillus* were able to produce phosphatase. Among six isolates from Pelagic fisheries lab area four were bacterla of which one *Bacillus* sp. were much efficient in solubilizing tricalcium phosphate as well as in producing phosphatase enzyme. Fungal isolates from this location were comparitively less efficient in solubilizing phosphate except *Penicillinm* sp. and none of them could produce phosphatase enzyme. Correlation study revealed that lowering and pH in growth medium was positively correlated to the amount of phosphate solubilization in vitro.

It was evident from in vitro study that the estuarine sediment isolates were more efficient than water isolates both in terms of solubilization of phosphate and production of phosphatase enzvme in general. In particular one species from each genus of *Aspergillus*, *Vibrio* and *Bacillus* was efficient phosphate solubilizer. *Bacillus* sp. (PSB 33) was the most efficient one both in terms of phosphate solubilization as well as phosphatase enzyme production and this isolate may be useful for exploitation in confined brackish water aquaculture system or in saline agricultural soils as phosphatic biofertilizer.

From the foregoing discussion it is evident that bacteria, fungi and actinomycetes are involved in solubilization of phosphate in coastal and estuarine environment and their potentiality of solubilization of phosphate depends on several physicochemical parameters of the ecosystem.

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Table 4 Identification of microorganism and their in vitro power of solubilizing of phosphate and phosphatase activity

Microorganisms		Phosphate solubilizing power in μg P/10 ml broth and the consequent pH		Phosphatase activity in μg of p-nitro phenol released in 48 hr. from 5 ml broth
Coded as	Identified as	P-sol power	pH	
ASB 1	*Bacillus* sp.	140	5.35	0
ASB 2	*Bacillus* sp.	130	5.40	0
ASB 3	*Bacillus* sp.	55	6.25	0
ASB 4	*Bacillus* sp.	25	6.20	200
ASB 5	*Bacillus* sp.	65	5.52	100
ASB 6	*Bacillus* sp.	10	6.20	0
ASF 7	*Aspergillus* sp.	300	4.90	0
ASF 8	*Aspergillus* sp.	240	4.95	25
ASF 9	*Aspergillus* sp.	215	6.20	10
ASB 10	*Bacillus* sp.	25	6 45	0
ASB 11	*Bacillus* sp.	65	6.20	100
VBW 12	*Bacillus* sp.	240	4.50	200
VWF 13	Unidentified fungus	410	4.60	0
VSB 14	*Bacillns* sp.	410	4.74	0
VSF 15	*Aspergillus* sp.	435	4.30	275
VSF 16	*Aspergillus* sp.	1000	4.15	0
EWB 17	*Bacillus* sp.	410	4.40	0
EWB 18	*Bacillus* sp.	200	4.15	200
EWF 19	*Aspergillus* sp.	305	4.75	0
ESB 20	*Streptomyces* sp.	45	5.65	200
ESF 21	Unidentified fungus	585	4.40	0
FWB 22	*Bacillus* sp.	370	4.60	100
FWF 23	*Asperqillus* sp.	565	4.57	200
FWF 24	*Asperqillus* sp.	455	4.70	0
FSB 25	*Bacillus* sp.	200	4.82	0
FSB 26	*Vibrio* sp.	1000	4 60	120
FSB 27	*Asperqillus* sp.	520	4.40	450
FSF 28	*Asperqillus* sp.	695	4.50	0
PWB 29	*Bacillus* sp.	585	6.00	200
PWB 30	*Bacillus* sp.	545	4.45	10
PWF 31	*Penicillium* sp.	610	4.80	0
PSB 32	*Vibrio* sp.	410	4.75	0
PSB 33	*Bacillus* sp.	1475	4.25	550
PSF	*Aspergillus* sp.	455	4.65	0

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