Indian Journal of Marine Sciences
Vol. 5, December 1976, pp. 218-221

Seasonal Occurrence of Phosphate Solubilizing Bacteria in the Vellar Estuary, Porto Novo

T. LINGARAJA, D. CHANDRAMOHAN & R. NATARAJAN
Centre of Advanced Study in Marine Biology, Porto Novo 608502

Received 20 *February* 1976; *revised received* 9 *August* 1976

Relative distribution of limnotolerant (freshwater tolerant) and halotolerant (saline water tolerant) phosphate solubilizing bacteria were estimated from 3 stations in Vellar estuary using different media. In addition, nutrients such as nitrite, nitrate, silicate and phosphate were also analysed. Vertical distribution of phosphate dissolving bacteria at each station showed higher concentration in the sediments than in the corresponding overlying water. The ratio between the marine and freshwater phosphate solubilizing bacteria altered due to variation in salinity. In general, clayey sediment harboured more phosphate solubilizing bacteria than the sandy ones. Influences of abiotic factors like organic carbon, total phosphorus, total nitrogen and salinity on the occurrence and distribution of phosphate solubilizing bacteria were discussed.

PHOSPHORUS economy of estuaries was extensively studied[1-4], but studies on the microbial participation in phosphate regeneration in marine environments were sparse. Insoluble organic and inorganic phosphorus compounds were rendered soluble by the action of enzymes and acids secreted by bacteria[5]. A direct method of isolating these bacteria from marine environments was not available till Ayyakkannu and Chandramohan[6] reported on the definite occurrence and distribution of phosphate dissolving bacteria in marine environment, employing hydroxy apatite medium. They observed these bacteria to be present in interstitial water of open sandy beach, estuarine, and neritic waters and sediments of Bay of Bengal. Further, occurrence of these bacteria was noticed in all water and sediment samples irrespective of the variations in salinity (1-33·4‰)[7]. Subsequently, Harrison *et al.*[8] and Dutka *et al.*[9] reported the occurrence and distribution of phosphate solubilizing bacteria in the sediments of upper Klamath lake and offshore Lake Erie respectively.

So far no detailed investigation on the seasonal occurrence of these bacteria has been carried out either in temperate or in tropical regions. The present report deals, in addition to influences of various physical and chemical factors, with the seasonal distribution of phosphate solubilizing bacteria in Vellar estuary, Porto Novo (11°29'N-79°49'E) (Fig. 1).

Materials and Methods

Three stations were selected for the present study viz., Vellar mouth (I), Biological station (II) and Railway bridge (III). In addition to surface and bottom water samples, sediment samples were also collected.

Water samples were collected with the aid of a ZoBell's bacteriological water sampler and the sediment samples with a Petersen Grab. Salinity and temperature of the samples were measured with a conductivity bridge in the field itself. *p*H of the samples was measured with a Philip's *p*H meter. Light penetration was measured with a Secchi disc and the extinction coefficient was calculated. The depth at the station was recorded on the disc attached to the winch. The depth was not corrected for wire angle as it did not exceed 4° (ref. 10.)

Estimations of dissolved oxygen, phosphate, nitrate, nitrite and silicate were carried out according to Strickland and Parsons[11] methods. Total phosphorus[12], total nitrogen[13], and total organic carbon[14] were also analysed in sediment samples.

For enumerating total and phosphate dissolving bacterial populations, the method of Ayyakkannu and Chandramohan[7] was followed using nutrient medium and special hydroxy apatite medium. For 'halotolerant bacteria' sea water (32‰) medium was used while distilled water medium was employed for 'limnotolerant bacteria'. Total number of fungi and actinomycetes were not taken into account. Various physical, chemical and microbiological factors were analysed over a period of 6 months and totally 12 collections were made at fortnightly intervals.

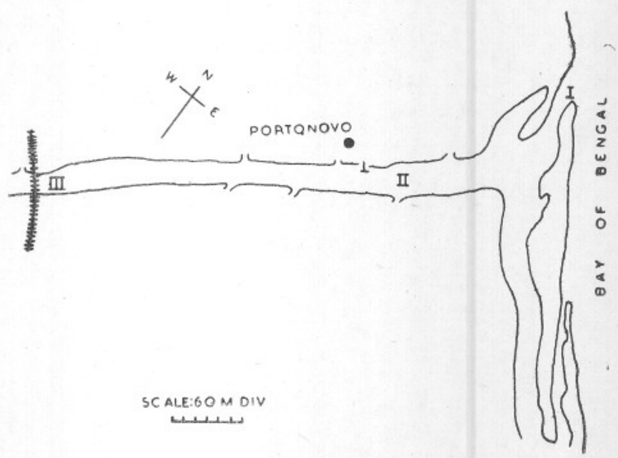


Fig. 1 — Vellar estuary showing sampling stations

Results and Discussion

Since the mouth of the river is always open to the sea, Vellar estuary is considered to be a true estuary subjected not only to variations in salinity but also to various mixing processes resulting from seasonal variation in the amount of freshwater inflow during rainy season and due to tidal rise and fall of water level. At station I high salinities were always recorded (Fig. 2). At stations II and III salinity of surface water was very low (1-8‰) during October since the inflow of fresh water had started. However, during this month bottom samples showed higher salinity. During December (collection No. 9) lowest salinity was recorded at all stations because of the heavy inflow of fresh water. The surface water temperature was always higher (24·0-28·4°C), than the bottom water (23·0-27·4°C). High temperatures were observed both in surface and bottom waters during premonsoon period. Maximum *p*H (8·8) was observed at the mouth (station I) during January and minimum (7·8) during August. At stations II and III maximum *p*H (8·9) was recorded during August and October.

During flood season (December) the water was turbid at all stations, as evidenced by high light extinction coefficient values (24·2-34). Out of the 3 stations, station I was shallow (2·2 m) compared to other 2 stations (3·0 m and 3·92 m). However, during monsoon period the depths fluctuated because of the heavy inflow of fresh water.

Dissolved oxygen of water samples was generally low (2·7-3·72 ml O_2/litre) during premonsoon period in all the stations. However, during December, a sharp rise in the oxygen content (6·58-6·7 ml O_2/litre) was noticed because of the flood. The range of variation of dissolved inorganic phosphate was not high. For surface water it ranged from 1·88 (station I) to 0·02 μg at P/litre (station II) whereas for the bottom water the range was from 1·07 (station I) to 0·02 μg at P/litre (station II). In general, maximum phosphate content for both surface and bottom waters for station I was observed during premonsoon period (August and September), while for stations II and III, high values were observed during monsoon period.

In general, nitrate nitrogen varied from 19 to 34 μg at/litre in surface waters and 17 to 43 μg at/litre in bottom waters. Maximum values of NO_3-N were recorded during November and December. For surface water, a maximum nitrite content of 3·64 μg at/litre was recorded at station II during monsoon period and minimum of 0·01 μg at/litre was observed at station III during August. Nitrite content of bottom water varied from 0·07 to 1·39 μg at/litre at all the stations. The silicate distribution pattern was rather interesting. Maximum values were observed towards the end of monsoon (December-January). Silicate content was always higher at station III than at other 2 stations. Also higher values were recorded in surface water than in bottom water. For surface water the range of variation of silicate was 5 to 233 μg at/litre whereas for the bottom water it ranged from 4·7 to 187 μg at/litre.

Total phosphorus content in the sediment varied considerably during the period of observation. High total phosphate content was observed in samples collected at station III (Table 1). Station I samples were sandy and hence they contained least amounts of total phosphorus than other 2 stations. Total phosohorus content was high in premonsoon period (August) and gradually decreased reaching minimum during December at all the stations. Sediments collected at stations II and III always contained (Table 1) higher amounts of total nitrogen (392 to 709 and 196 to 793 μg NH_3-N/g) than at station I. Total organic carbon content of sediments also fluctuated with the season (Table 1).

Four different types of bacterial populations, viz. total halotolerant, total limnotolerant, halotolerant phosphate solubilizing and limnotolerant phosphate solubilizing, were estimated in water and sediment samples and the results are given in Figs. 3 and 4. In general, higher bacterial populations were recorded only in bottom water. The sediments from stations II and III were clayey in nature and harboured more bacteria than the sediments from station I which was sandy. This further confirms the earlier findings that clay (1 to 5 μm diam. particles) harboured nearly 17 times higher population than sandy sediments (150 to 1000 μm diam. particles)[6,15]. This may possibly be attributed to

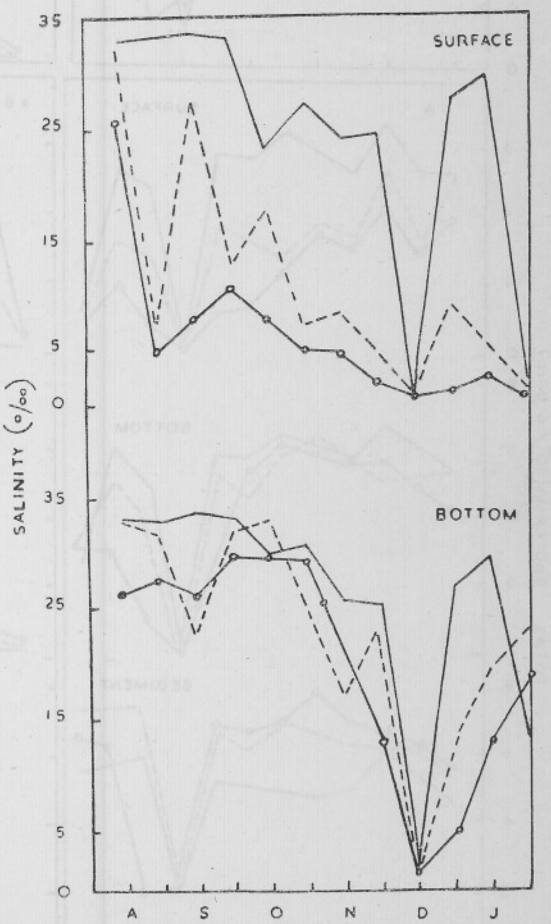
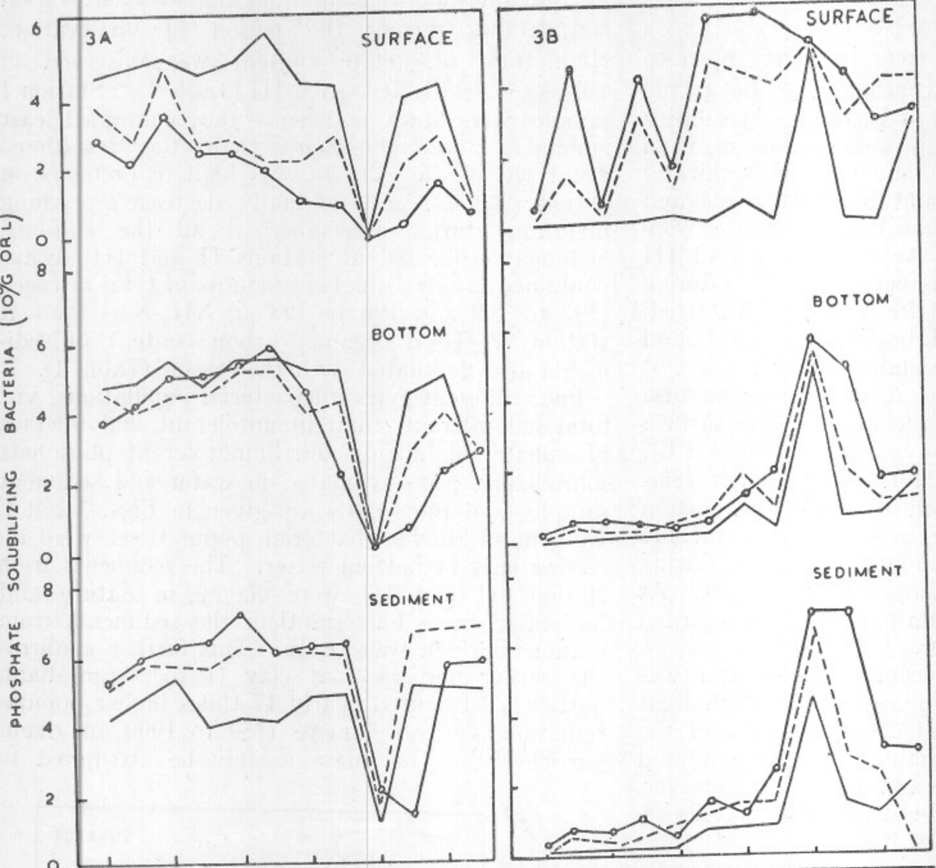


Fig. 2 — Salinity of surface and bottom waters [—, station I; – – –, station II; O—O, station III]



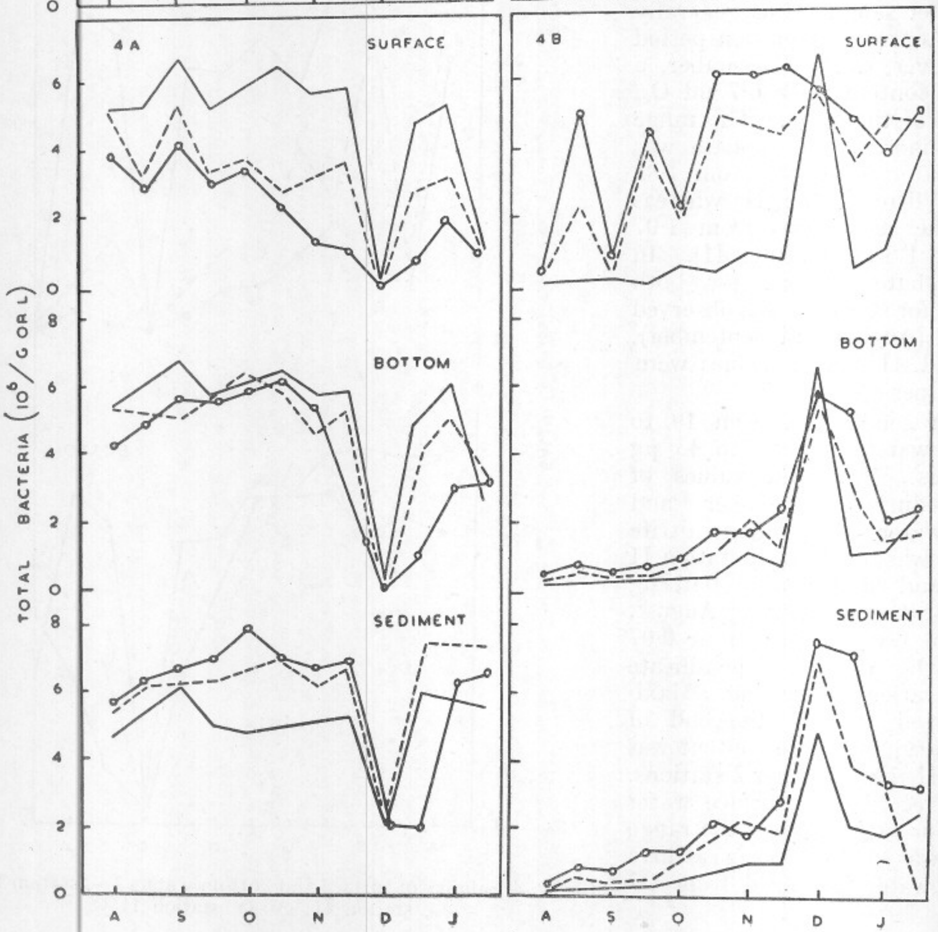


Fig. 3 — Halotolerant (A) and limnotolerant (B) phosphate solubilizing bacterial population [Details of curves same as in Fig. 2]

Fig. 4 — Total halotolerant (A) and limnotolerant (B) bacterial population [Details of curves same as in Fig. 2]

TABLE 1 — TOTAL PHOSPHORUS, NITROGEN AND ORGANIC CARBON CONTENT IN ESTUARINE SEDIMENTS

Station	August		September		October		November		December		January	
						TOTAL PHOPHOSRUS (mg/g)						
I	0·24	0·18	0·2	0·18	0·15	0·12	0·13	0·10	0·10	0·06	0·04	0·11
II	0·32	0·32	0·77	0·31	0·31	0·4	0·48	0·16	0·22	0·2	0·18	0·12
III	0·48	0·6	0·68	0·58	0·41	0·3	0·16	0·21	0·18	0·07	0·1	0·44
						TOTAL NITROGEN ($\mu g\ NH_3$-N/g)						
I	168	178	168	158	177	165	356	308	215	178	149	168
II	513	551	579	569	709	672	513	308	448	541	392	625
III	579	634	728	765	793	644	205	439	178	198	196	579
						TOTAL ORGANIC CARBON (%)						
I	0·056	0·018	0·093	0·025	0·047	0·046	0·057	0·036	0·068	0·041	0·021	0·19
II	0·328	0·546	0·3	0·276	0·552	0·312	0·438	0·396	0·33	0·332	0·528	0·4
III	0·42	0·636	0·492	0·564	0·98	0·428	0·123	0·714	0·032	0·064	0·076	0·54

the rich organic matter content. Rich organic matter may enhance the production of organic acids by the bacteria which are responsible for solubilization of inorganic insoluble phosphates[7].

Differentiation of halotolerant and limnotolerant bacteria during the season gave an interesting relationship between the salinity and the group of bacteria. Whenever the salinity (15-33‰) was high halotolerant group dominated and when the salinity decreased the limnotolerant group predominated. Similar was the case with halotolerant and limnotolerant phosphate dissolving bacteria. Obviously halotolerant phosphate solubilizing bacteria existed in greater numbers in samples collected during premonsoon period when the salinity was high and limnotolerant groups during monsoon period when the salinity was low.

Observations made during this study indicate the possible effect of certain environmental factors on the distribution of phosphobacteria. Station I which is situated at the mouth of the estuary is quite different from the other two. This station being situated in the marine zone is always subjected to the influence of the sea. When surface and bottom water samples were analysed at all the stations, a salt wedge was noticed i.e. bottom water being more saline than the surface. So the sediments at all these stations were constantly exposed to saline water except during December, when heavy inflow of fresh water was noticed because of monsoon rains. Normally, the sediment at station I was sandy while at other 2 stations it was clayey. Because of heavy floods, lot of silt and clay were deposited and the sediments at all the stations became silty clay in January. During the flood season, the water was very turbid as evidenced by high light extinction coefficient values.

Since the nutrient levels in the overlying water are dependent on the nature of sediment and its buffering capacity, the overlying water layers were also analysed. Out of the various factors studied only a few viz., salinity, total phosphate, total nitrogen and total organic carbon, seem to influence the phosphate solubilizing bacterial population.

Compared to bottom water, the surface water showed lower bacterial counts. This may be due to increased temperature which may reduce their number. The increased populations in bottom water may be due to contact with the sediments. The limnotolerant bacterial population was high during the monsoon period because of fresh water flow. During the postmonsoon period the number decreased as the salinity slowly increased. No strict correlation could be made between the number of bacteria in the sediment and in the overlying water. Phosphate solubilizing bacteria were present both in water and sediments irrespective of variations in salinity. This is possible because both the holotolerant and limnotolerant groups of bacteria may adopt themselves to changing salinities. However, the dominance of either the limnotolerant or the holotolerant group of the bacterial population is dependant on the salinity values and the availability of total phosphates.

Acknowledgement

The authors are grateful to the University Grants Commission, New Delhi, for the financial support.

References

1. EMERY, K. O. & STEVENSON, R. E., in *Treatise on marine ecology and paleoecology*, edited by J. W. Hadgepeth: *Geo. Am. Soc. Memoir*, 1957, 67.
2. BRUCE, H. E. & HOOD, D. W., *Pub. Inst. mar. Sci.*, **6** (1960), 133.
3. BALASUBRAHMANIYAN, K., *J. zool. Soc. India*, **13** (1961,) 166.
4. RAJENDRAN, A. & VENUGOPALAN, V. K., *Indian J. mar. Sci.*, **2** (1973), 13.
5. SKUJINS, J. J., in *Soil biochemistry*, edited by A. D. McLaren & G. H. Peterson (Marcel Dekker, New York), 1967, 371.
6. AYYAKKANNU, K. & CHANDRAMOHAN, D., *Curr. Sci.*, **39** (1970), 398.
7. AYYAKKANNU, K. & CHANDRAMOHAN, D., *Mar. Biol.*, **11** (1971), 201.
8. HARRISON, M. J., PACHA, R. E. & MORITA, R. Y., *Limnol. Oceanogr.*, **17** (1972), 50.
9. DUTKA, B. J., BELL, J. B. & LIU, D. L. S., *J. fish. Res. Bd Canada*, **31** (1974), 299.
10. SVEDRUP, H. V., JOHNSON, M. W. & FLEMING, R. H., *The oceans — their physics, chemistry and general biology*, (Printice-Hall, New York), 1942.
11. STRICKLAND, J. D. H. & PARSONS, T. R., *A practical handbook of sea water analysis.*, Bull. No. 167 (Fish. Res. Bd, Canada) 1968.
12. MURPHY, J. & RILEY, J. P., *Anal. Chim. Acta*, **27** (1962), 31.
13. BARNES, B., *Apparatus and methods of oceanography*: *Part I — Chemical* (Allen & Unwin, London) 1959, 341.
14. WAKEEL, S. K. & RILEY, J. D., *J. Du. Conseil*, **22** (1956), 180.
15. ZOBELL, C. E., *J. Sedim. Petrol.*, **8** (1938), 10.