Journal of Coastal Development
Volume 15, Number 1, October 2011 : 9 – 16

ISSN : 1410-5217
Accredited : 83/Kep/Dikti/2009

Original Paper

ABUNDANCE AND OCCURRENCE OF PHOSPHATE SOLUBILIZING BACTERIA AND PHOSPHATASE IN SEDIMENT OF HOOGHLY ESTUARY, NORTH EAST COAST OF BAY OF BENGAL, INDIA

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Received : March, 31, 2011 ; Accepted : August, 15, 2011

ABSTRACT

The abundance and occurrence of a special group of bacteria, capable of solubilizing or mobilizing insoluble phosphates were studied in estuarine environment, especially in sediments. The possible role of various factors of the sediments in maintaining the phosphate availability in the overlying water was described. The phosphatase activity was recorded in all samples irrespective of salinity variations. Total number of bacteria showed higher value in lower salinity. The total phosphate content in soil showed positive correlation with the phosphatase activity (r = 0.890; P-Value = 0.000; n-=15). Clayey sediment contents more phosphate solubilizing bacteria and phosphatase than sandy sediment in a tropical estuarine region of River Hooghly.

Key Wards: Hooghly estuary; sediment; phosphate; phosphatase; phosphate solubilizing bacteria (PSB).

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INTRODUCTION

Phosphorus normally occurs as phosphates in both inorganic and organic compounds. Microorganisms assimilate inorganic phosphate and mineralize organic phosphorus compounds and microbial activities are involved in the solubilization or mobilization of phosphate compounds. The phosphate content in water depends upon various factors, of which the bottom deposits play a major role. Phosphate solubilizing microorganisms refer to a group of soil microorganisms are that act as components of phosphorus cycle, can release it from insoluble sources by different mechanisms (Mehrvarz and Chaichi., 2008). Earlier studies by Moore (1930), Rochford (1950), Seshappa (1953), Balasubramanyan (1961) and Pomeroy *et al.*,; (1965) indicated clearly the nature of phosphate exchange between the sediment and the water, suggesting that the sediment act as a buffer on the phosphate concentration in the overlying water. Apart from being good indicators of soil fertility, phosphatase enzyme plays a key role in the soil system (Dick *et al.*, 2000). For example, when there is a signal indicating phosphate deficiency in the soil, the phosphatase secretion from plant roots is increased to enhance the solubilization and remobilization of phosphate, thus influencing the ability of the plant to cope with phosphate - stressed conditions (Karthikeyan *et al.*, 2002; Mudge *et al.*, 2002; Versaw and Harrison 2002). Microorganismsare involved in a range of processes that affect the transformation of soil phosphorus and are thus an integral part of the soil phosphorus cycle. In particular, soil microorganisms are effective in releasing phosphorus from inorganic and organic pools of total soil phosphorus through solubilization and

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mineralization (Hilda and Fraga, 1999). The ability of phosphate rocks and minerals solubilization by Phosphate solubilizing bacteria was related to the production of citric and gluconic acids or both, respectively (Reyes *et al.*, 2001). It is generally accepted that the mechanism of mineral phosphate solubilization by PSB (Phosphatase solubilizing bacteria) strains is associated with the release of low molecular weight organic acids which through their hydroxyl and carboxyl groups chelate the cations bound to phosphate, thereby converting it into soluble forms (Kpomblekou and Tabatabai, 1994). However, phosphorus solubilization is a complex phenomenon, which depends on many factors such as nutritional, physiological and growth conditions of the culture (Reyes *et al.*, 1999). The mechanism for phosphate solubilization probably involves the production of several organic acids (Vazquez *et al.*, 2000). Seshadri *et al.*, (2002) studied on the variations of heterotrophic and Phosphate solubilizing bacteria from southeast coast of India and more recently Chen *et al.*, (2006) described Phosphate solubilizing bacteria from subtropical soil. Recently Khan *et al.*, (2009) reported on the occurrence, mechanisms and role in crop production of Phosphate solubilizing bacteria and Malboobi *et al.*, (2009) isolated PSB strains capable of hydrolyzing both organic and inorganic P compounds more effectively form alkaline soil. It thus seems probable that the phosphatase activity in marine and estuarine sediments may regulate the phosphate content of the sediments, which in turn, will influence the phosphate level of the overlying water. The present study reflects the nature and activity of phosphatase and phosphate solubilizing bacteria in estuarine sediments of Hooghly estuary, north east coast of Bengal, India.

MATERIALS AND METHODS

Sampling site

The present work was carried out during the month of May, 2010, in the Hooghly Estuary (**Fig. 1**) , North East Coast of Bay of Bengal, West Bengal, India. It is a coastal plain estuary and lies approximately between $21^{0} 32^{'}$ - $22^{0} 40^{'}$ N and. $88^{0} 05^{'}$ - $89^{0}00^{'}$E .The estuarine part of the River Ganges, is one of the most dynamic, complex and vulnerable bioclimatic zones in a typical geographical location in the coastal region of Bay of Bengal. Samples were collected from 15 stations along the River Hooghly (**Table. 1**).

Collection of sediment samples

The locations of the stations were based on the nature of sediments and salinity. In the estuary, samples were collected from the middle of the section of the river and from the surface layer of the sediments with a Peterson grab. The central portions of the samples were aseptically transferred to sterile glass bottle.

Isolation of Phosphate Solubilizing Bacteria

Serial dilution of the samples were made with sterile water of appropriate salinities and plated in sterile petridishes containing Pikovskaya's Agar (glucose – 10 g, $Ca_3(PO_4)_2$ – 5 g, $(NH_4)_2SO_4$ – 0.5 g, KCl – 0.2 g, $MgSO_4$ – 0.1 g, $MnSO_4$ – traces, $FeSO_4$ – traces, Yeast Extract – 0.5 g, Agar – 15 g, water of required salinity – 1 L, pH – 7.0) . Plating was done in triplicate and incubated at 30± 5 ^{o}C for 48–96 h. Phosphate solubilization was indicated by the formation of a solubilization or a clear zone around the bacterial colonies. Single bacterial colonies having clear solubilization zones were isolated separately on to fresh Pikovskaya's agar plates, incubated at 30±5ºC (Ramachandran *et al.*, 2007). Phosphate solubilizing bacteria were identified by the clear solubilization zones around their colonies on the third day of incubation.

Measurement of Phosphatase activity

The remaining portions of the samples were air-dried and the phosphatase activity was estimated by a modified method of Kramer and Erdei (1958). The samples were incubated with phenyldisodium orthophosphate in appropriate salinities and the phosphatase activity was indirectly measured by the amount phenol released. Total and adsorbed phosphate in various sediment samples were estimated by standard procedures of Strickland and Persons (1968). Suitable controls were maintained in all

ISSN : 1410-5217
Accredited : 83/Kep/Dikti/2009

analyses and the results represent the average of three replicates.

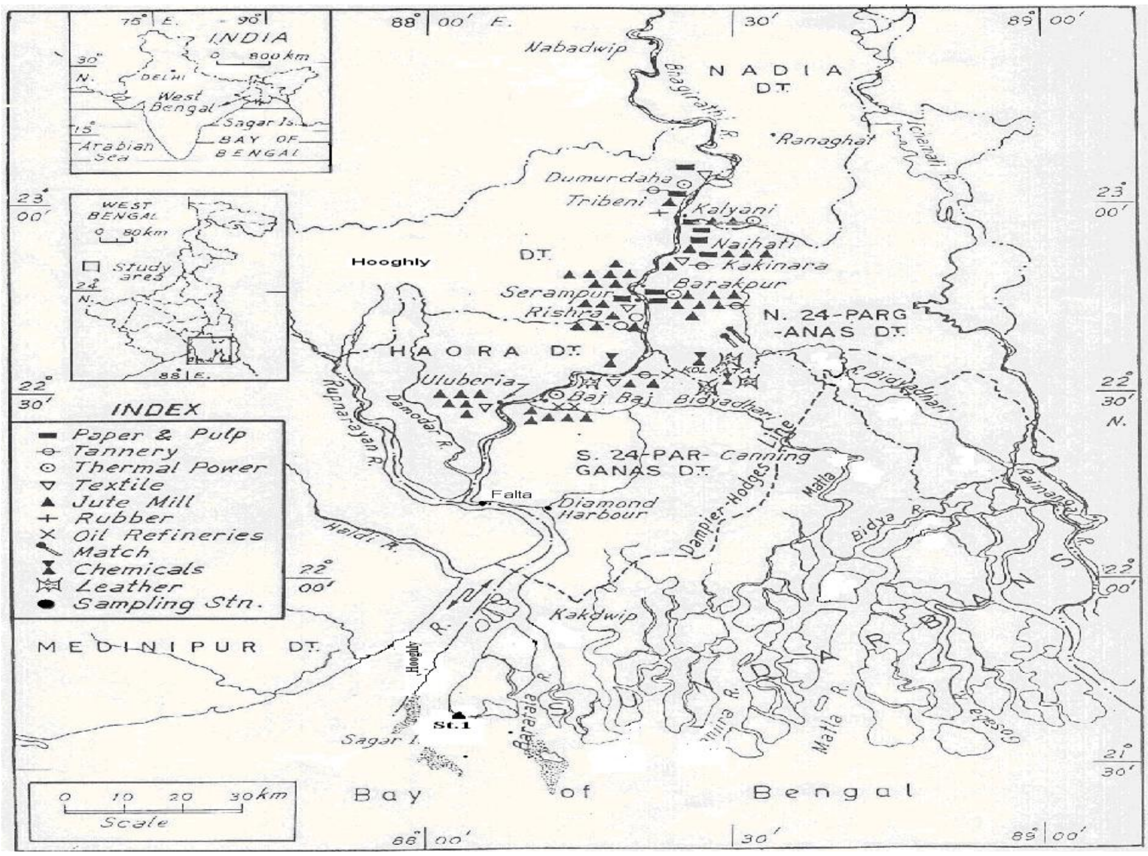

Fig.1. Sampling stations in the Hooghly Estuary, W.B., India

Table 1. Sampling stations in the Hooghly Estuary, West Bengal, India.

Station No	Description	Distance from Station No.1(km)	Nature of sediment	pH of water	Salinity of overlying water(psu).
1	Sagar South	0	Clay	8.32	27.5
2	Beguakhali	3	Clay	8.28	25.5
3	Chemaguri	15	Sand	8.31	24.2
4	Harinbari	18	Sand	8.24	23.5
5	Mandirtala	24	clay	8.21	22.4
6	Kachuberia	30	clay	8.18	20.5
7	Lot No. 8	34	clay	8.15	19.8
8	5 No. Hat	40	Clay	8.05	17.6
9	Kakdwip	45	Clay	8.12	20.2
10	Namkhana	55	Sand	8.09	19.5
11	Nischindipur	60	Sand	7.95	16.3
12	Karanjali	65	Sand	7.92	12.6
13	Kulpi	75	Clay	8.01	6 .3
14	Diamondharbour	87	Clay	7.96	2.1
15	Falta	95	Clay	7.91	0.92

ISSN : 1410-5217
Accredited : 83/Kep/Dikti/2009

RESULTS AND DISCUSSIONS

Table 2. shows the phosphatase activity in all 15 samples, irrespective of salinity variations. In general, clayey substances reveal higher phosphatase activity than sandy substrata. The total phosphate also shows the same trend i.e. higher value for clayey substrata. The total bacterial population and phosphate solubilizing bacteria were detected from all samples, but the clayey samples appear to be much richer than the sandy samples (**Table 2**). Present investigation reveals that phosphatase activity exists in marine, estuarine and freshwater sediments, irrespective of the salinity variations. In aquatic environment, a mixed population of microbes is essential to promote enzymatic degradation of naturally occurring organic phosphorus compounds (Ponmurugan P., Gopi C. 2006). Wherever the total phosphorus content was found to be high, the phosphatase activity was also high (**Fig. 2**) . [The Regressions equation is : Phosphatase = 0.42 + 25.64 Total Phosphate; F=49.78, P=0.00, n=15] i.e. the activity is mostly governed by the availability of total phosphate. Such correlation has also been reported by Ayyakkanu and Chandramohans (1971) at Proto Novo. Pomeroy *et al.,* (1965) stated that the sediments containing a large fraction of silty and clay were rich in phosphate. The present study confirms these results. Generally, the organic matter content in clay is higher than in sand and similarly the total organic phosphate content is higher in clay. Zobel (1938) reported that clay (1 to 5 µ diameter particles) harboured as many as 3.9×10^5 bacteria/g soil, while sand (50 to 1000µ diameter particles) contained only 2.2×10^4 bacteria/g soil. The present study also confirms the earlier finding of Zobel. An inverse relationship is noticeable between the total bacterial population and salinity in different sediments (**Fig. 3**). [The regression equation is: Total bacterial population = 7.86 – 0.14 Salinity; F=2.83, P=0.11, n=15]. In general, freshwater (low saline) river-sediment contained a large number of bacteria than marine sediments. Further, the total bacterial population was higher in clayey sediments than in sandy sediments. This may be attributed to the rich organic matter content of the clay. The phosphate solubilizing bacterial population estimated in different samples seems to be directly related to the total bacterial population, even though a strict correlation could not be made. Phosphate solubilizing bacteria appeared to be less in number in sandy sediments. A direct relationship is noticeable between the number of phosphate solubilizing bacteria and phosphatase activity in different sediments (**Fig. 4**). [The regression equation is: Phosphatase = 2.59 + 1.21 Phosphate solubilizing bacteria; F=29.68, P=0.00, n=15]. Though, microbial extracellular secretion of phosphatase has already been reported (Skujins, 1967), but this may not be the only source for phosphatase in sediments, since the flora and fauna of an area may also contribute to the phosphate pool. Phosphate solubilizing bacteria are the most common in aquatic and terrestrial environments and influence the phosphorous cycling in a number of direct and indirect ways. Significant population of phosphate solubilizing bacteria along with soil phosphatase activity may represent a sustainable ecological condition of the particular sampling site concerned. In the coastal region active participation of phosphate solubilizing bacteria may be considered as a consistent factor with relation to the coastal productivity. Since biological processes in soils are fundamental to their ecological function and to maintain biodiversity, it is important to manage the sensitive coastal soil to maintain the microbial and biochemical activities of the constrained saline environment. However, to manage the salt-affected soil, particularly soils of a coastal region, a prerequisite is to have knowledge on the variables related with salinity sodicity and biological processes regarding coastal productivity (Tripathi *et al.,* 2007). Soil phosphatase activity may be considered to regulate the productivity in coastal regions through contribution of available phosphorous to green plants. The overall results and regression analyses suggest that phosphatase and phosphate solubilizing bacteria may play a major role in increasing the phosphate concentration and consequently the buffering capacities of the sediments.

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Table 2. Phosphatase activity in marine environment in relation to total phosphate and abundance of phosphate solubilizing bacteria. [Results represent the averages of three replicates with Standard deviation (SD)

No.	Phosphatase activity (µg phenol /g/h)	SD (±)	Total phos-phate (mg/g)	SD (±)	Adsorbed phosphate (mg/g)	SD (±)	Total bacteria (10^6/g)	SD (±)	Phosphate solubilizing bacteria (10^6/g)	SD (±)
1	12.92	0.06	0.56	0.012	0.059	0.003	6.96	0.12	5.72	0.12
2	11.32	0.15	0.42	0.009	0.065	0.006	6.33	0.16	5.32	0.21
3	4.92	0.02	0.24	0.017	0.026	0.004	1.64	0.08	1.12	0.08
4	3.12	0.03	0.27	0.013	0.032	0.002	1.72	0.15	0.92	0.06
5	8.12	0.02	0.34	0.012	0.021	0.003	4.98	0.06	3.21	0.12
6	6.92	0.12	0.30	0.008	0.031	0.002	6.72	0.12	5.34	0.15
7	11.52	0.02	0.44	0.012	0.025	0.001	5.92	0.14	5.24	0.07
8	9.32	0.06	0.41	0.007	0.023	0.004	7.62	0.15	6.06	0.06
9	6.12	0.12	0.22	0.006	0.026	0.002	7.96	0.18	5.94	0.02
10	3.21	0.04	0.24	0.009	0.031	0.001	1.82	0.21	0.84	0.13
11	2.69	0.15	0.09	0.012	0.009	0.002	1.52	0.09	0.68	0.05
12	3.24	0.09	0.11	0.011	0.023	0.002	2.62	0.12	1.12	0.02
13	7.59	0.12	0.32	0.009	0.032	0.003	7.93	0.14	6.28	0.08
14	10.96	0.06	0.34	0.012	0.024	0.001	8.37	0.06	6.64	0.16
15	11.22	0.12	0.36	0.014	0.026	0.003	8.96	0.15	6.84	0.22

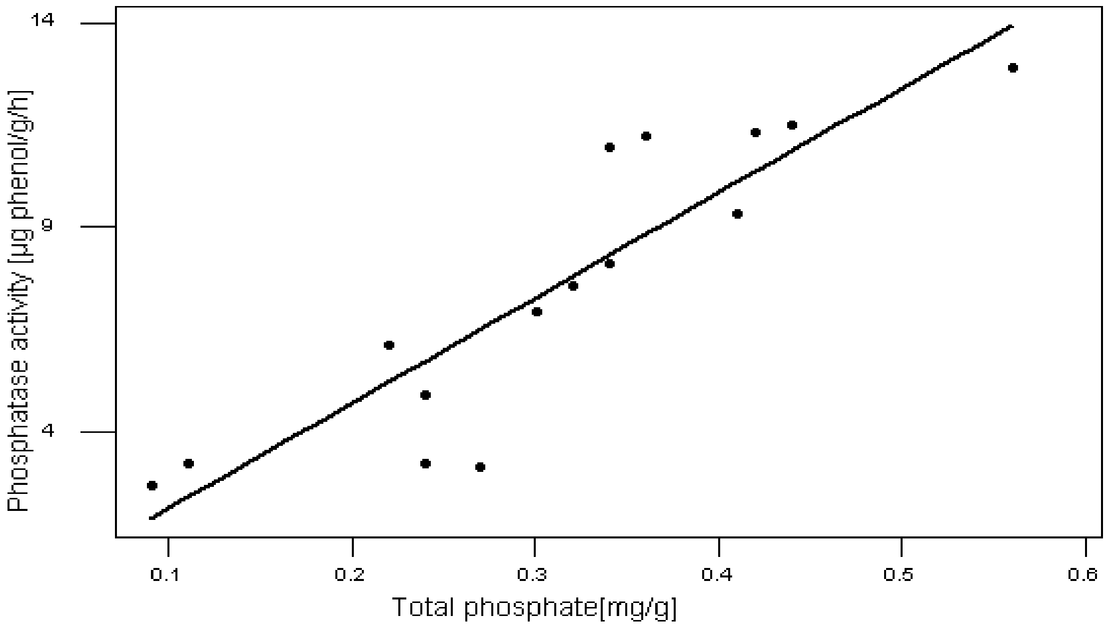

Fig. 2. Relationship between phosphatase activity and total phosphate content in the Hooghly Estuary.

ISSN : 1410-5217
Accredited : 83/Kep/Dikti/2009

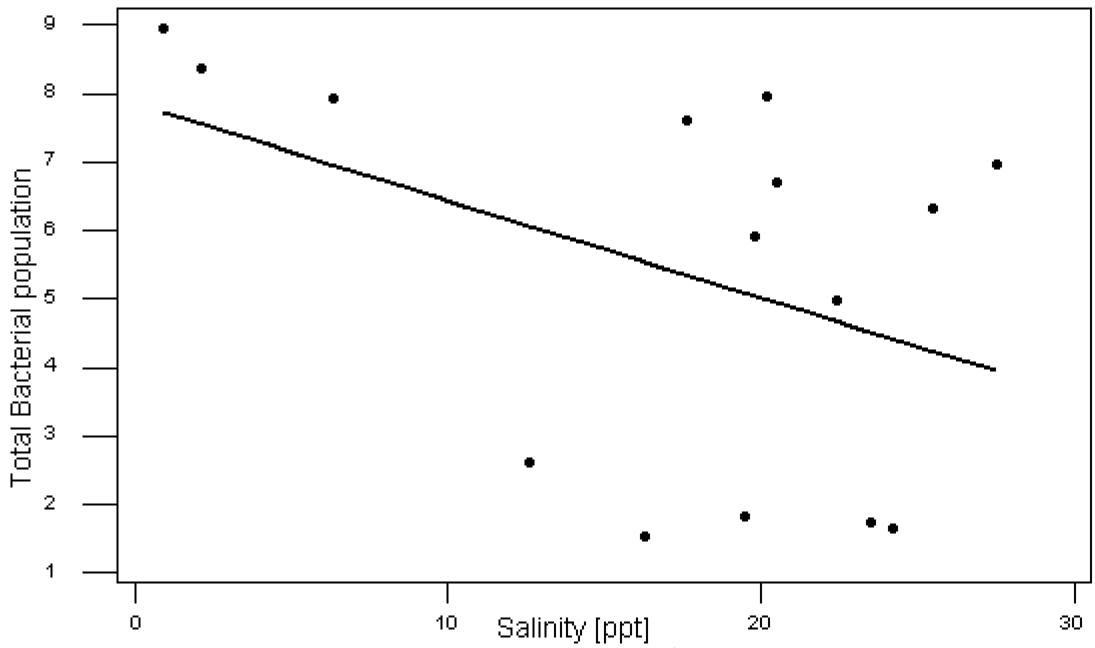

Fig.3. Relationship between Total bacterial populations (x10^6 /g) and salinity in the Hooghly

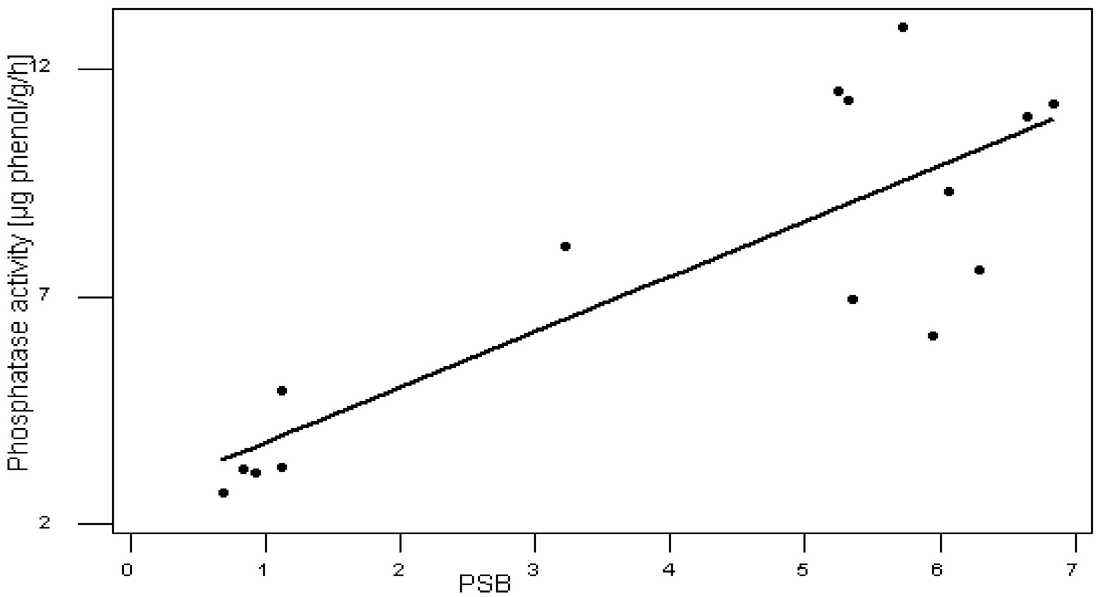

Fig.4. Relationship between phosphatase activity and phosphate solubilizing bacteria population(PSB x 10^6/g) in the Hooghly Estuary.

CONCLUSIONS

Phosphatase and phosphate solubilizing bacteria were present in all 15 samples, irrespective of salinity variations. Generally, clayey sediments contained higher total phosphate and phosphatase activity. The clayey sediments also harboured a greater number of total bacteria and phosphatase solubilizing bacteria than sandy ones. An inverse relationship is noticeable between the total bacterial population and salinity in different sediments but A direct relationship is observed between the number of phosphate solubilizing bacteria and phosphatase activity in various sediment of Hooghly estuary. It is evident from the present investigation that phosphatase and phosphate solubilizing bacteria may play a major role in increasing the phosphate concentration and further studies are in progress relating to the nature of phosphatase and productivity.

ISSN : 1410-5217
Accredited : 83/Kep/Dikti/2009

ACKNOWLEDGEMENT

The financial assistance from DST, New Delhi, Govt. of India and Department of Environment, Govt. of west Bengal, are gratefully acknowledged.

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