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Soil physicochemical parameters and bacterial load influences the alkaline phosphatase activity: a study in sediment of brackish water systems along East-coast India

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Key words: Alkaline phosphatase activity, brackish water system, soil physicochemical parameters, total bacterial count.

Abstract

Alkaline phosphatase (E.C.3.1.3.1) is an extracellular enzyme which plays an important role in releasing of phosphorus from sediment which is an essential nutrient for sustaining primary and secondary productivity. It also acts as an indicator of environment pollution. In this present research work which is the first of its kinds, the relationship between alkaline phosphatase activity of the sediment with the various soil physicochemical parameters along with total bacterial count was worked out. For these a total of 20 bulk sediment samples were collected randomly from different sites of the brackish water systems of the east India and processed to determine alkaline phosphatase activity, total bacterial count and other soil physicochemical parameters. Descriptive and inferential statistics were used to analyze the data. It can be inferred from this study that the alkaline phosphatase activity in brackish water systems is influenced by the different soil physicochemical parameters and bacterial population.

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Introduction

Phosphorus (P) is an essential nutrient for sustaining primary and secondary productivity, biological synthesis, energy transfer and global biogeochemical cycle (Liu *et al.*, 2004; Mudryk, 2004). Sediments are the reservoir of P accumulation because it receives high concentration of organic matter due to shallow water depths and river inputs. But it is released from the sediment when the water quality changes from oxic to anoxic condition (Loyer *et al.*, 2008). Most of the P in sediment exists in mineral or organic form (Pettersson *et al.*, 1988). The enzyme phosphatase has received considerable attention on this regard due to its ability to hydrolyze phosphate esters and transformation of phosphate from one organic form to another for providing food to microbial forms like algae, bacteria etc. (Kuenzler *et al.*, 1965; Nausch, 2000). This enzyme is categorized into three types depending upon its pH i.e. acid phosphates, alkaline phosphatase and neutral phosphatase (Sayler *et al.*, 1979). In brackish water system the level of salinity is in between fresh water and sea water. So in such circumferences there is more possibility to get alkaline phosphatase. It is also reported that in water bodies, the activity of alkaline phosphatase is higher than that of acid phosphatase (Mudryk, 2004). Alkaline phosphatase is an extracellular enzyme which is reported from all forms of life ranging from bacteria, algae to higher plants and animals (Orhanovic and Pavela-Vrancic, 2000). It is a dimeric molecule having molecular weight 160 kDa (Oh *et al.,* 2005). The activity of alkaline phosphatase is affected by P loading (Nausch, 2000). Alkaline phosphatase activity (APA) is well correlated with various physicochemical and biological parameters (Kizilkaya *et al.*, 2007). Different reports are available mainly for agricultural soil where it was noted that various soil physicochemical parameters such as soil pH, available P, organic carbon, total nitrogen, microbial biomass etc., influence the release of phosphatase enzymes (Herbien and Neal, 1990; Marinari *et al.*, 2000; Nannipieria *et al.*, 1983; Pagliai and De Nobili, 1993). But there are no such reports available for alkaline phosphatase activity of the brackish water sediment and its possible relationship with soil parameters. So, this study is mainly focused on the alkaline phosphatase activity of the sediment and its relationship with various soil physicochemical parameters and total bacterial count.

Materials and methods

Sample collection

A total of 20 bulk sediment samples were collected randomly from different sites of the brackish water systems of coastal area of the east India (Figure 1) during December 2007 to February 2008. Samples were collected from the upper 5 cm layer of the sediment using hand-operated 5 cm diameter core sampler, as this layer is more reactive due to predominant chemical and microbial changes (Boyd *et al.*, 1999). The sediment samples were placed in sealable plastic bags, transferred to laboratory for analysis and stored in the dark at 4^{o}C till further analysis but for longer storage the samples were placed at -20^{o} C and allowed to thaw at 4^{o}C about 2 days before analysis (Kandeller, 2007). Previous studies show that storage of soil samples for six to eight weeks under these conditions (4^{o}C) has no significant effect on phosphatase activity (Speir and Ross, 1978)

Analyses of sediment samples for physico-chemical properties:

A portion of sediment samples was hand crushed and analyzed for moisture content by gravimetric method (Piper, 1966), pH was measured using pH meter with glass electrode (1:2.5; soil:water suspension) and electrical conductivity by Elico conductivity bridge (1:2.5; soil:water suspension) respectively (Jackson, 2005). The rest portion of the sediment samples was air dried at room temperature (Jackson, 2005), homogenized and gently crushed using an agate mortar and pestle and sieved through a standard sieve of 2mm mesh size (Gelderman and Mallarino, 1998). The total organic carbon (TOC) was determined by chromic acid wet digestion method (Walkley and Black, 1934). Total Nitrogen (TN) was determined by Macro-Kjeldahl method (Piper, 1966). Soil available phosphorus (Soil P) was determined by Sodium bicarbonate extraction method (Olsen *et al.*, 1954) and Exchangeable sodium (Na), Exchangeable potassium (K), Exchangeable Calcium (Ca) & Exchangeable Magnesium (Mg) was analyzed by Flame photometer method (Piper, 1966).

Table 1. Selected soil physicochemical parameters of the soil samples.

Sample no	APA ($\mu g\ g^{-1} min^{-1}$)	MC (%)	Soil P ($\mu g\ g^{-1}$ of P)	TOC (%)	TOM (%)	TN (%)	pH	EC ($mS\ cm^{-1}$)	Na ($cmol_c\ kg^{-1}$)	K ($cmol_c\ kg^{-1}$)	Ca ($cmol_c\ kg^{-1}$)	Mg ($cmol_c\ kg^{-1}$)	TBC (cfu x 10^3 g^{-1} of soil)
S1	12.225	22.75	0.76	10.9	18.79	2	9.02	2.68	10.43	1.74	43.91	4.36	12
S2	13.797	23.73	0.66	10.9	18.79	1.9	8.65	2.33	8.70	1.13	32.04	3.95	11
S3	58.594	21.47	0.62	8.7	15.00	2	8.24	2.78	12.17	0.95	44.91	5.76	129
S4	40.122	23.43	0.75	10.4	17.93	3.1	8.71	2.19	13.04	1.53	44.31	4.03	23
S5	16.909	21.73	0.95	10.7	18.45	3.1	8.82	2.34	12.17	1.71	47.70	5.68	10
S6	39.35	22.97	1.08	9.7	16.72	2.8	9.63	2.87	13.91	1.43	26.75	6.25	38
S7	32.288	22.53	1.15	9.7	16.72	3.5	9.4	3.57	12.17	1.76	36.53	5.43	13
S8	157.09	21.14	1.48	15.4	26.55	1.5	8.76	1.1	15.65	0.87	28.24	5.68	178
S9	126.26	22.28	1.39	12.3	21.21	1.3	9.14	1.86	14.78	1.13	22.85	4.69	295
S10	75.483	24.12	1.44	8.9	15.34	2.7	8.15	1.98	15.65	1.10	42.86	6.50	35
S11	30.236	24.07	1.21	10.1	17.41	3.2	8.04	1.75	14.78	0.90	35.98	6.01	44
S12	56.374	22.60	1.16	9.7	16.72	1.1	7.5	1.28	12.17	0.95	29.04	5.68	27
S13	25.212	23.32	1.27	10.8	18.62	1.3	7.51	1.22	12.17	1.94	22.90	4.61	27
S14	99.483	21.10	1.13	8.6	14.83	1.6	7.55	1.52	11.30	1.20	28.89	4.85	61
S15	51.278	21.98	0.97	9.6	16.55	2.9	8.55	1.4	12.17	1.07	29.39	4.85	58
S16	16.381	23.94	1.17	10.4	17.93	1.1	8.79	2.24	13.91	0.74	42.71	6.17	13
S17	70.037	22.45	1.12	8.8	15.17	3.2	7.91	1.31	9.57	1.23	39.17	5.68	126
S18	96.247	22.37	1.13	9.5	16.38	3.1	8.05	1.38	8.70	1.20	29.39	7.32	329
S19	77.928	21.81	1.14	9.2	15.86	3.2	7.92	1.08	10.43	0.90	44.56	6.50	254
S20	92.593	21.76	1.21	8.5	14.65	3.6	7.93	1.71	10.43	0.90	44.71	6.91	203

(APA, Alkaline phosphatase activity; MC, Moisture content; P, Soil Phosphorus; TOC, Total organic carbon; TOM, Total organic matter; TN, Total nitrogen; pH, pH of soil; EC, Electrical conductivity; Na, Exchangeable sodium ion concentration; K, Exchangeable potassium ion concentration; Ca, Exchangeable calcium ion concentration; Mg, Exchangeable magnesium ion concentration; TBC, Total bacterial count)

Table 2. Pearson's correlation matrix of the selected soil physicochemical parameters of the soil samples.

Variables	APA	MC	Soil P	TOC	TOM	TN	pH	EC	Na	K	Ca	Mg	TBC
APA	1	-0.574	0.580	0.304	0.304	-0.104	-0.182	-0.517	0.203	-0.447	-0.323	0.295	0.748
MC	-0.574	1	-0.008	-0.077	-0.077	-0.025	0.102	0.215	0.210	0.089	0.047	-0.106	-0.460
Soil P	0.580	-0.008	1	0.272	0.272	-0.117	-0.165	-0.459	0.513	-0.223	-0.384	0.432	0.331
TOC	0.304	-0.077	0.272	1	1.000	-0.442	0.390	-0.127	0.426	0.026	-0.352	-0.315	0.092
TOM	0.304	-0.077	0.272	1.000	1	-0.442	0.390	-0.127	0.426	0.026	-0.352	-0.315	0.092
TN	-0.104	-0.025	-0.117	-0.442	-0.442	1	0.107	0.174	-0.249	0.136	0.455	0.376	0.123
pH	-0.182	0.102	-0.165	0.390	0.390	0.107	1	0.712	0.293	0.269	0.031	-0.193	-0.153
EC	-0.517	0.215	-0.459	-0.127	-0.127	0.174	0.712	1	0.076	0.393	0.313	-0.166	-0.441
Na	0.203	0.210	0.513	0.426	0.426	-0.249	0.293	0.076	1	-0.163	-0.131	0.037	-0.175
K	-0.447	0.089	-0.223	0.026	0.026	0.136	0.269	0.393	-0.163	1	-0.059	-0.438	-0.387
Ca	-0.323	0.047	-0.384	-0.352	-0.352	0.455	0.031	0.313	-0.131	-0.059	1	0.215	-0.165
Mg	0.295	-0.106	0.432	-0.315	-0.315	0.376	-0.193	-0.166	0.037	-0.438	0.215	1	0.454
TBC	0.748	-0.460	0.331	0.092	0.092	0.123	-0.153	-0.441	-0.175	-0.387	-0.165	0.454	1

(APA, Alkaline phosphatase activity; MC, Moisture content; P, Soil Phosphorus; TOC, Total organic carbon; TOM, Total organic matter; TN, Total nitrogen; pH, pH of soil; EC, Electrical conductivity; Na, Exchangeable sodium ion concentration; K, Exchangeable potassium ion concentration; Ca, Exchangeable calcium ion concentration; Mg, Exchangeable magnesium ion concentration; TBC, Total bacterial count).

Table 3. Results of test of significance (t-test) between correlation coefficient of alkaline phosphatase activity (APA) and other selected soil physicochemical parameters of the soil samples on study.

Correlation between	r	SE of r	t-test	Significant or Non-significant ($p<0.05$)
APA & MC	-0.574	0.04548742	-12.62143638	Non Significant
APA & P	0.580	0.045270296	12.80420906	Significant
APA & TOC	0.304	0.052934136	5.734547741	Significant
APA & TOM	0.304	0.052934136	5.734547741	Significant
APA & TN	-0.104	0.055253403	-1.885010288	Non Significant
APA & pH	-0.182	0.054627027	-3.332875574	Non significant
APA & EC	-0.517	0.047556456	-10.87021643	Non significant
APA & Na	0.203	0.054393881	3.739910645	Significant
APA & K	-0.447	0.049707098	-8.984872209	Non significant
APA & Ca	-0.323	0.052570172	-6.151735595	Non significant
APA & Mg	0.295	0.053084496	5.555733291	significant
APA & TBC	0.748	0.036848984	20.30910324	significant

(APA, Alkaline phosphatase activity; MC, Moisture content; P, Soil Phosphorus; TOC, Total organic carbon; TOM, Total organic matter; TN, Total nitrogen; pH, pH of soil; EC, Electrical conductivity; Na, Exchangeable sodium ion concentration; K, Exchangeable potassium ion concentration; Ca, Exchangeable calcium ion concentration; Mg, Exchangeable magnesium ion concentration; TBC, Total bacterial count)

Table 4. Variable Importance in the Projection (VIP) statistics of different selected soil physicochemical parameters in relation with alkaline phosphatase activity (APA) which was calculated by Partial Least Squares (PLS) Regression method

Variable	VIP	Standard deviation	Lower bound (95%)	Upper bound (95%)
TBC	1.767	0.311	1.158	2.376
Soil Phosphorus	1.368	0.262	0.854	1.882
Moisture content	1.355	0.391	0.590	2.121
Electrical conductivity	1.220	0.235	0.760	1.680
Potassium ion concentration	1.054	0.372	0.325	1.784
Calcium ion concentration	0.763	0.485	-0.187	1.713
Total organic matter	0.717	0.370	-0.009	1.442
Total organic carbon	0.717	0.370	-0.009	1.442
Magnesium ion concentration	0.696	0.508	-0.299	1.692
Sodium ion concentration	0.480	0.633	-0.761	1.722
Soil pH	0.430	0.610	-0.766	1.625
Total nitrogen	0.246	0.488	-0.711	1.203

Analyzes of Alkaline phosphatase activity of the sediment samples:

The alkaline phosphatase activity of the sediment samples were determined using p-nitrophenylphosphate as a substrate. The released p-nitrophenol was measured by UV-Visible Spectrophotometer (Pharmaspec UV-1700, SHIMADZU, Japan) at 420 nm (Tabatabai and Bremner, 1969).

Enumeration of Total number of bacteria from the sediment samples:

Total bacterial load in the sediment samples were determined by serial dilution and standard plating technique. Serial dilution were made by mixing ten gram of sediment in 95 ml of sterile distilled water which was followed by spread plating of 0.1 ml inoculums on nutrient agar from 10^{-3} dilution. The inoculated Petri plates were incubated at 37°C for 36 hrs. The total number of colonies on nutrient agar was counted using Quebec Colony Counter.

Statistical analysis

Descriptive and inferential statistics were used to analyze the data. Statistical studies have been carried out by calculating correlation coefficients between different pairs of parameters and t-test applied for checking significance. Probabilities less than 0.05 ($p<0.05$) were considered statistically significant. Grouping of the all parameters along with APA was done using the agglomerative hierarchical clustering (AHC) based on the Pearson's correlation coefficient matrix and unweighted pair-group average (UPGA) to visualize the clustering between the all sediment parameters in question. AHC method can display the clustering process clearly. Regression analysis of APA versus soil physicochemical parameters was performed to asses the regression equation model including the multicolinearity analysis. The partial least square (PLS) regression along with variable importance for projection (VIP) statistics were also performed to predict dominant parameters which are important for APA of a brackish water system. Partial least square regression (PLS) is a very useful statistical technique when there are a lot of highly correlated prediction variables (multicollinearity) present. All statistical analyses were done by Microsoft Excel 97 and XLSTAT package from Addinsoft for windows.

Results and discussion

The highest alkaline phosphatase activity of 157.09 µg g-1min^{-1} was observed in sample S8 and lowest of 12.225 µg g-1min^{-1} in sample S1 (Table 1). It was noted that the pH of these sediments samples ranged from 7.5 to 9.63, electrical conductivity from 1.08 to 3.57 mS cm^{-1} and moisture content from 21.0962 % to 24.1153 %. Pearson's correlation coefficient was calculated it was negatively correlated between APA and the above parameters. (Table 2) A similar negative correlation was observed by Kizilkaya *et al.*, (2007) between pH and APA. The reason behind the negative correlation of pH with APA can be explained by the fact that the optimal pH for APA vary with the substrate concentration (Ross *et al.* 1951). As far as chemical properties are concerned TOC ranged from 8.5% to 15.4%, TOM from 14.654% to 26.5496%, TN from 1.1% to 3.6%, available phosphorous from 0.618 to 1.4755 µg g^{-1} soil, exchangeable calcium from 22.85 to 47.70 cmolc per kg, exchangeable magnesium from 3.95 to 7.32 cmolc per kg, exchangeable sodium from 8.70 to 15.65 cmolc per kg, exchangeable potassium from 0.74 to 1.94 cmolc per kg (Table 2). The TOC and available soil P of the soils showed a significant positive correlation with APA ($r=0.304$ and 0.580 respectively), on the other hand TN has negative relationship with APA ($r= -0.104$) which is also supported by the work of Su *et al.* (2005). Total organic matter (TOM) have positive and significant relationship ($r=0.304$) with APA. This observation is supported by Marinari *et al.* (2000). Therefore, changes in soil chemical parameters like TOC, TOM TN and available P can be used as indices for APA in brackish water systems. Exchangeable sodium and magnesium showed a positive correlation with APA, but exchangeable calcium and potassium had a negative correlation with APA. This may be partly explained by the fact that magnesium along with sodium induces the phosphatase activity (Olsen *et* *al.*, 1954). Total bacterial count of the samples ranged from 10 x 10^3 to 329 x 10^3 cfu g^{-1} soil and has a positive correlation with APA (r=0.748, p<0.001), which is supported by the studies of Nannipieria *et al.* (1983) and Jones (1972). (Table 2)

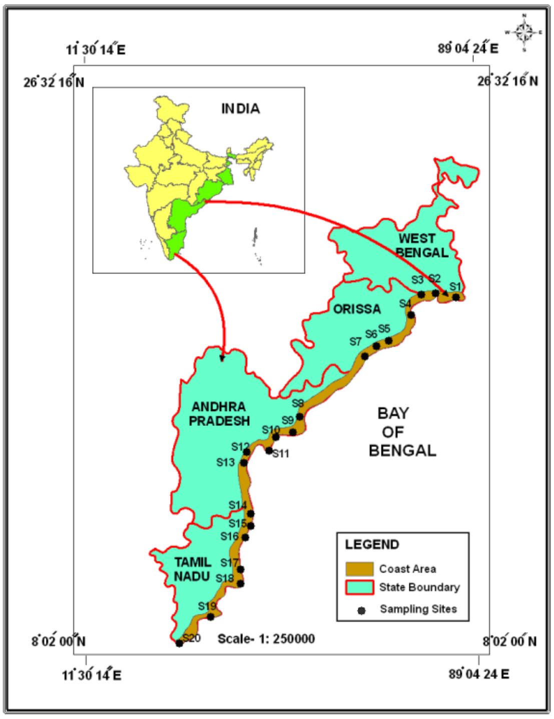

Fig. 1. Geographic locations of East coast of India from where the soil samples were collected. Map was generated using the ArcGIS 9.3 and ERDAS IMAGINE 9.3 with the help of GPS locations of the sampling sites.

The correlation effects between the soil parameters were also studied. It revealed that EC showed a highly significant positive correlation with pH (r=0.712 with p<0.05) while had a significant negative correlation with soil available P (r= -0.459). Likewise, exchangeable Na was highly positively correlated with soil available P (r=0.513, p<0.05). Total bacterial count was positively correlated with exchangeable Mg (r=0.454, p<0.05) and negatively correlated with moisture content (r=0.460, p<0.05). (Table 3, Figure 2) Grouping of the all parameters along with APA was done using the agglomerative hierarchical clustering (AHC) using proximity type Pearson's correlation coefficient (Figure 3). Regression analysis of APA versus soil physicochemical parameters were performed to asses the regression equation model. Due to multicolinearity the TOM (Tolerance = 0.00) was removed from the regression model. Reliability of regression equation was tested with a good F statistics (F = 5.326, p<0.05) and goodness of fit (R^2) 0.880. Depending on the regression analysis a regression model was formulated to reveal the relation between APA and other soil physicochemical parameters.

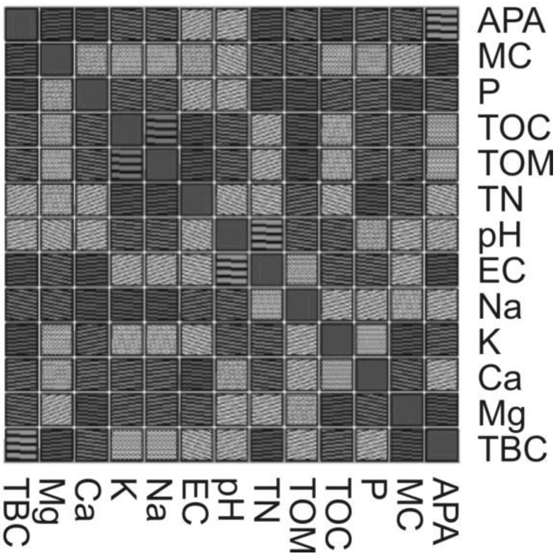

Fig. 2. Correlation map of APA and soil physicochemical parameters based on Pearson's correlation coefficient matrix (p<0.05).

(APA, Alkaline phosphatase activity; MC, Moisture content; P, Soil Phosphorus; TOC, Total organic carbon; TOM, Total organic matter; TN, Total nitrogen; pH, pH of soil; EC, Electrical conductivity; Na, Exchangeable sodium ion concentration; K, Exchangeable potassium ion concentration; Ca, Exchangeable calcium ion concentration; Mg, Exchangeable magnesium ion concentration; TBC, Total bacterial count).

Lastly, partial least square (PLS) regression along with variable importance for projection (VIP) statistics were also performed to predict which soil parameters were the most important for the APA in brackish water systems. From the above statistics (Table 4) it can be inferred that the bacterial population, soil available P, moisture content, exchangeable K and EC are the main contributors with a VIP value more than 0.8 (Wold, 1995). (Fig. 4)

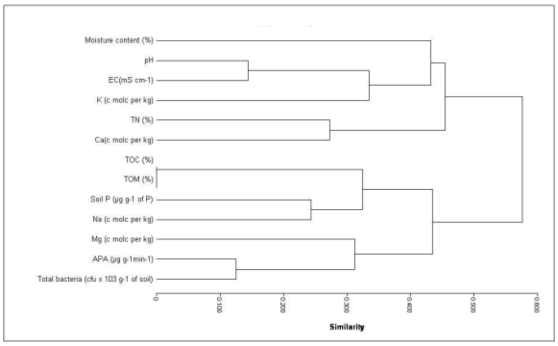

Fig. 3. Grouping of the all the parameters along with Alkaline phosphatase activity (APA). Dendrogram was generated based on agglomerative hierarchical clustering (AHC) using proximity type Pearson's correlation coefficient similarity and unweighted pair-group average (UPGA) matrix. The scale bar represents the Euclidean distance as an index of similarity.

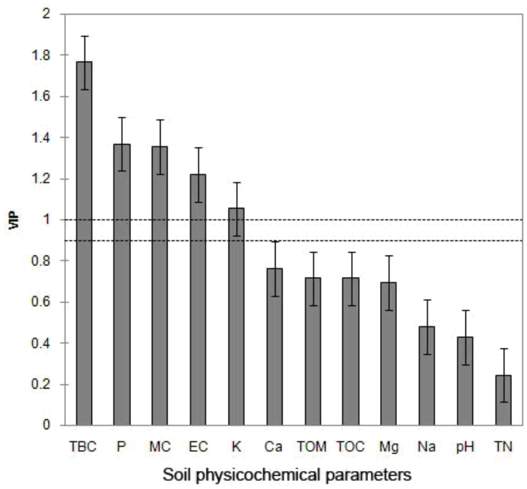

Fig. 4. Graphical representation of the physicochemical parameters of brackish water systems in study based on the variable importance for projection (VIP) value (95% confidence limit). Columns represent VIP values of the parameters; Y-error bars represent the corresponding standard error.

(APA, Alkaline phosphatase activity; MC, Moisture content; P, Soil Phosphorus; TOC, Total organic carbon; TOM, Total organic matter; TN, Total nitrogen; pH, pH of soil; EC, Electrical conductivity; Na, Exchangeable sodium ion concentration; K, Exchangeable potassium ion concentration; Ca, Exchangeable calcium ion concentration; Mg, Exchangeable magnesium ion concentration; TBC, Total bacterial count)

Conclusion

In this present work, the first of it kinds in brackish water systems of east-coast India, the alkaline phosphatase activities of the different sediments of brackish water systems and their relationship with the bacterial population and soil physicochemical parameters reveals that bacteria population is the major factor that determines APA in these sediments; but some other physicochemical properties of soil also influence the APA. Our investigation agrees closely with the fact that APA plays an important role in P cycling in brackish water sediments. These findings were concomitant with strong correlation between APA and other soil parameters TOC, TOM and available phosphorus, indicating that organic pollution and available phosphorus in sediments affects APA remarkably. In a later context, it can be concluded that APA seems to be an indicator of environmental pollution in brackish water systems.

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