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Microbial Diversity in the Surface Sediments and its Interaction with Nutrients of Mangroves of Gulf of Kachchh, Gujarat, India

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Abstract

The microbial population and nutrients status of the surface water indicates the nutrient dynamics in the study area. Nutrients in surface water directly provide the available source of nutrients to the microbes. Nutrients in the water such as NO_3^- *(2.77-46.08mg/L),* PO_4^{3-}*(0.32-2.81 mg/L),* SO_4^{2-}*(1892-3839.98 mg/L) and dissolved silica* (H_4SiO_4) *(3.32-16.98 mg/L) has been reported. Population of nitrate forming bacteria with higher population count at Old Bedi Port (M2), Narara (M5) and Mundra (M8) was reported while Phosphate solubilizing bacteria with higher count at Mundra (M8) and Jodiya (M10). The cellulose degrading bacteria was reported exceptionally high at Mundra, Narara and Old Bedi Port. The higher population count in the microbes synchronized with the nutrients availability in the surface water. Lack of significant correlations among the nutrients indicate the influx of anthropogenic inputs and waste discharges containing nutrients from river runoff into these environments.*

Keywords: Mangroves, sediment, microbes, nutrients.

Introduction

Mangrove ecosystems are unique environment, harboring diverse groups of microorganisms which perform an important role in nutrients cycling in the ecosystem[1,2]. Microbes play a vital role in the biogeochemical cycles of any ecosystem. Mangrove ecosystem is being very rich in organic matter, the presence of microbes especially bacteria are active participants in the ecosystem. Various groups of bacteria and fungi such as nitrogen fixers, phosphate solubilizers, cellulose decomposers, etc are prevalent in this ecosystem[3]. Different groups of bacteria that get nourished by detritus and in turn, support the mangrove ecosystem in various ways[4]. Distribution of bacteria depends on various physico-chemical parameters like water temperature, salinity, pH, available nutrients etc[5]. Halophilic bacteria are believed to be predominant in the mangrove ecosystem as having high salinity[6]. In one hand, microbes help in biogeochemical cycling of the nutrients in the ecosystem and on the other hand act as important source of food for a variety of marine organisms and maintain pristine nature of the environment. Mangroves are one of the most productive ecosystems contributing to the food chains of coastal oceanic areas and form a barrier against the waves and tides with their long spreading root system. Now a day, mangroves are among the most threatened habitats in the world disappearing at an accelerating rate, yet with little public notice. Biological diversity is a key issue of conservation and controlling the continuous degradation and destruction of mangroves, there is a critical need to understand them better. The microbial diversity and its distribution in a mangrove would improve our understanding of microbial functionality and their interactions found in that ecosystem[7-9]. In the present study microbial population and its interaction with nutrients have been investigated.

Material and Methods

Study Area: The Gulf of Kachchh, Gujarat, India lies approximately between latitudes 22° to 23°N and between longitudes 68° to 70° 30° E with an area of approximately 7300 Km^2. The climate is semi- arid and the maximum rainfall is of the order of 50 cm yr^{-1}. There is no major river flow into it and hence little runoff has been observed. The sampling sites at various locations were chosen to get the broader picture of the interaction of various components (viz. anthropogenic input, natural impact etc.) and the sites were fixed by using GPS (figure 1). The major source of this sediment is considered to be the shore material and the load transported by the Indus River[10]. It is now recognized that the damming of the Indus River has drastically reduced the sediment delivery to the Arabian Sea[11, 12]. The reduction in sediment supply of the Indus River results changes in the Indus delta. The process of sediment supply to the Gulf of Kachchh via tidal erosion of the abandoned delta is still active. Many industries like metal smelting, cement, salt, textile, Ship dismantle and petrochemical refineries are situated nearby of towns like Jamnagar, Kandla, Mundra, Mandvi, Sikka and Jam-Salaya which are the potential source of pollution in the estuary of Gulf of Kachchh. The climate is semi-arid which influence the precipitation of air borne pollutants of the nearby cities/towns.

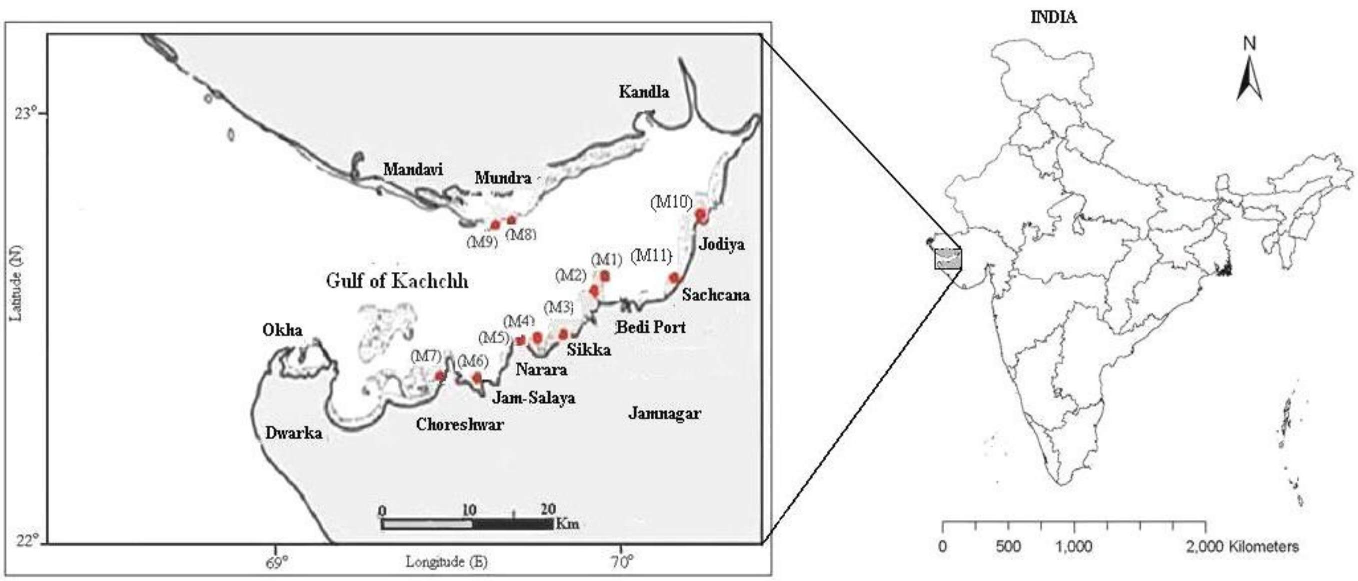

Figure-1
Showing study area in the Gulf of Kachchh, Gujarat, India

Sample collection and preservation: The surface sediments from eleven different locations (upto10 cm depth) were collected from the inter-tidal regions of different mangroves at Mundra, Jodiya, Sachana, New Bedi Bandar, Old Bedi Bandar, Sikka, Jam-Salaya, Narara and Choreshwar during November 2008, taking consideration of anthropogenic input, mixing zones, natural weathering input zones, etc (figure 1). Since major part of the study area is under Marine National Park, conservation of mangroves was carried out at large scale by various governmental and non-governmental bodies. Sediment samples were collected in the pre-cleaned sterilized polythene bags and stored in the ice chest and transported to laboratory.

Water samples were collected in pre-washed polythene bottles separately for nutrient analysis. The sediment samples were collected aseptically by removing off upper layer of the sediment. The samples were kept frozen in ice chest till the transfer in the laboratory where they were stored at 4^{o} C.

Nutrient analysis in surface water: The pH, temperature and total dissolved solids (TDS) in water were measured onsite using the Thermo-Orion water analysis kit (Model Beverly, MA, 01915). Salinity of the water samples were measured using refractometer. Nitrate (NO_3^-), sulphate (SO_4^{2-}), bicarbonate (HCO_3^-), phosphate (PO_4^{3-}) and inorganic silica ($HSiO_3^-$) were analyzed by using standard methods[13].

Microbial analysis: Sediments samples were processed for isolation of microbes. Sterilized Milli-Q water and lab wares were used during entire process of microbial analysis. One gram sediment sample was used for each inoculum. Serially diluted inoculums (10^{-8}) were used for inoculums in pore-plating techniques for isolation of microbes[14]. The modified isolation media for bacteria contained Beef extract (3.0g), bacteriological Peptone (5.0g), $NaNO_3$ (3.0g), KH_2PO_4 (1.0g), $MgSO_4.7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4.7H_2O$ (0.01g), agar (15.0g), distilled water (1.0L), pH (6.8-7.0).

Free living nitrogen fixer has been isolated in a selected medium, comprising mannitol (15.0g), K_2HPO_4 (0.5g), $MgSO_4.7H_2O$ (0.2g), $CuSO_4$ (0.1g), NaCl (0.2g), $CaCO_3$ (5.0g), agar (15.0g), distilled water (1.0L), pH maintained at 8.3.

Phosphate solubilizing bacteria are isolated in the Pikovskayas as media, containing glucose (10.0g), Ca_3 $(PO_4)_2$ (5.0 g), $(NH_4)_2SO_4$ (0.5g), KCl (0.2g), agar (20.0g), distilled water (1.0L), pH maintained at (6.8-7.0).

Cellulose decomposers has been isolated in selective media containing K_2HPO_4 (1.0g), $CaCl_2$ (0.1g), $MgSO_4.7H_2O$ (0.2g), $CaSO_4$ (0.1g), NaCl (0.2g), $NaNO_3$ (2.0g), Agar (12.0g), precipited cellulose (4.0g), distilled water (1.0L).

Fungi isolated in the Czapedox agar media, which contained $NaNO_3$(3.0g), KH_3PO_4 (1.0g), $MgSO_4.7H_2O$ (0.5g), KCl (0.5g), $FeSO_4.7H_2O$ (0.01g), sucrose (30g), agar (15.0g), $ZnSO_4.7H_2O$ (0.05g), distilled water (1.0L). Isolated colonies were measured in colony formation unit (CFU per gram).

CFU/g in original sample = No. of colonies counted/ {(dilution factor) x (volume plated, in ml)}.

The total fungal load was calculated in terms of percentage occurrence, which may be expressed by the formula:

Percentage occurrence = (No. of samples on which particular fungus is recorded x 100)/Total number of samples examined.

Table-1
Physical and chemical parameters of surface water and microbial load in the surface sediments

	Total bact. load	Total fungal load	Temp. (^oC)	pH	Salinity	TDS	HCO_3^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	H_4SiO_4
Range	35-61	7-20	21-24	5.5-8.35	32-37	29.9-33.7	90-301.1	2.77-46.08	0.32-2.81	1892-3839.98	3.32-16.98
Mean± SD	47.7± 9.14	13± 4.02	22.8± 1.03	7.6± 0.77	33.9± 1.33	56.9± 81.63	173.2± 72.59	9.8± 14.44	1.3± 0.88	2635.6± 531.94	8.3± 4.88

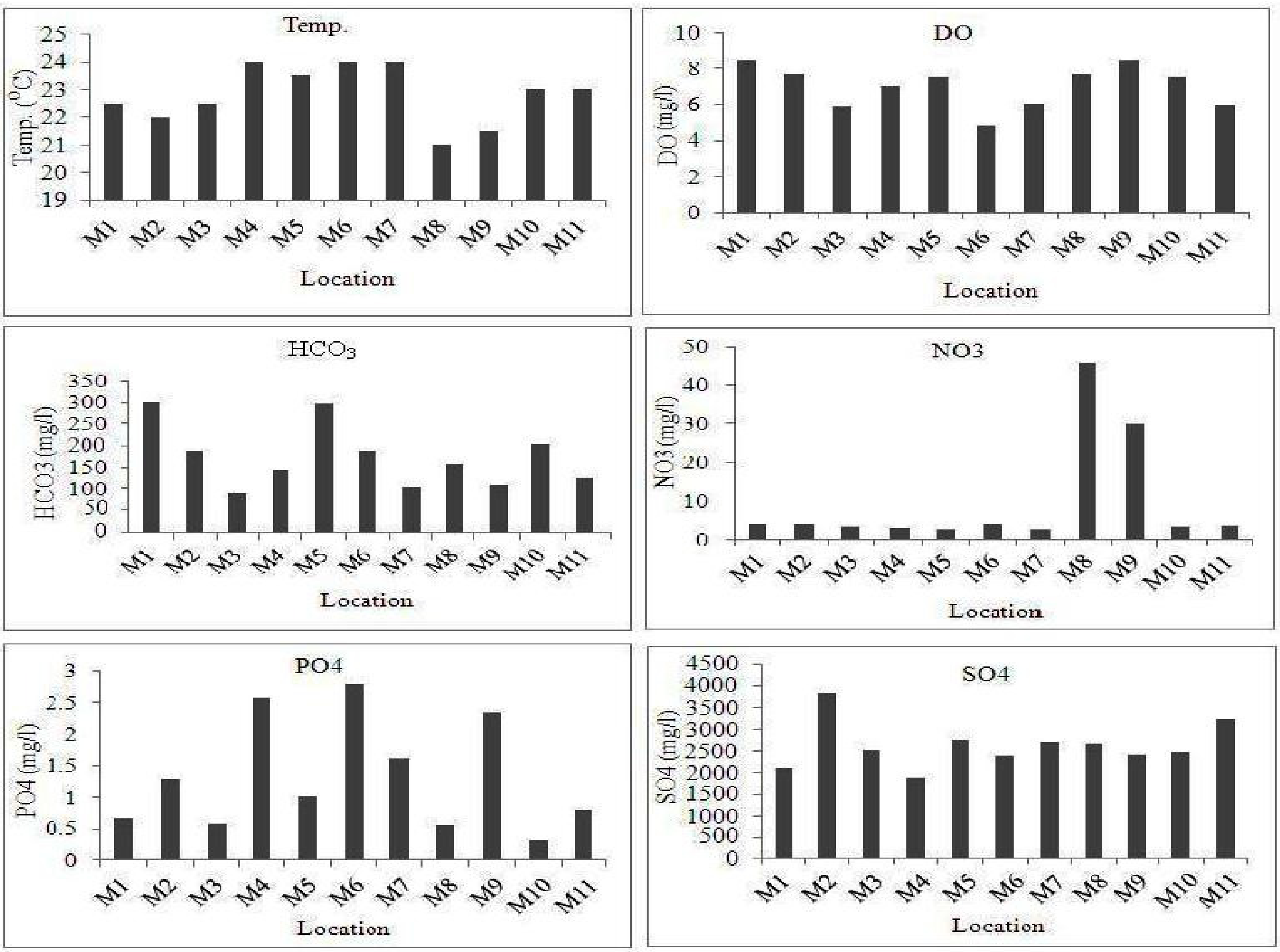

Figure-2
Showing various parameters of surface water

Results and Discussion

Geochemical analysis of surface water: The environmental parameters showed wide variations at different locations in the study area depending upon the various factors such as topography, fresh water influx etc. The highest value of salinity was observed at Narara (35 ppt) and lowest at Mundra (32 ppt). Generally, estuarine mangrove waters have relatively low stocks of dissolved inorganic phosphorus and nitrogen[15,16]. High NO_3^- (46.08 mg/L) concentration was observed at Mundra (M8) indicates the impact of terrestrial runoff. The mean value and range of physico-chemical and microbial parameters of the study area are given in table-1. Water temperature ranged from 21^0C to 24^0C. Surface water temperature is influenced by the intensity of solar radiation, evaporation, fresh water influx and cooling and mixing up with ebb and flow from adjoining neritic waters[17,18]. This higher value of surface water temperature may be contributed by the low water level at Narara (M4), Jama-Salaya (M6) and Choreshwar (M7).The pH value of the surface water varied from 7.01 to 8.35 with maximum at Jam-Salaya (M6). Generally, variation in the pH value is attributed by factors like removal of CO_2 by photosynthesis, sea water dilution by fresh water influx, reduction in salinity and temperature and decomposition of organic matter[19]. The maximum value of bicarbonate (HCO_3^-) was reported at New Bedi Port (301 mg/L). This high value indicates the higher mixing of sea water (figure-2).

Microbial population estimation: Population of the free living Nitrogen fixing, Phosphate solubilizing, Cellulose degrading bacteria have been analyzed in the surface sediments of various location (figure 1). Nitrogen fixing bacteria are known for improving nutrient status in sediments and hence to the mangrove plants[20]. Population of nitrate forming bacteria was reported highest at the locations like Old Bediport (M2), Narara (M4) and Mundra (M9) (4.0x103 cfu/g soil), indicate dominance of nitrogen fixing process which may be the reason for higher concentration of NO_3^- in the overlying water. Phosphate solubilizing bacteria may release phosphate ions from sparingly soluble inorganic and organic Phosphate compounds in the soil, which contribute with an increased phosphorus pool[21]. The Phosphate solubilizing bacteria varied from 3-17 x 10^3 cfu/g soil with highest at M6, M8 and M10 (17.0 x 10^3 cfu/g soil). The cellulose degrading bacterial population was also reported, which varied from 5-13 x10^3 cfu/g soils (figure-3). Besides assessment of microbial population dynamics, an attempt has also been made to identify four selected groups of bacteria such as nitrate forming, free living N2-fixing, phosphate solubilizing, and cellulose degrading. The microbial population in the sediment reflects the status of nutrient dynamics in the study area.

Statistical analysis: Correlation analysis is a bivariate method commonly used to measure and establish the relationship between two variables and generally used to measure the degree of dependency of one variable to other. Strong correlation was observed between NO_3^- and total fungal load (r^2=0.76) and between total bacterial load and PO_4^{3-} (r^2=0.79) indicates these factors significantly influence the microbial population in the sediments (table 2). Lack of significant correlations among the nutrients indicate the influx of anthropogenic input and waste discharge containing nitrogen and phosphorous compounds from river runoff into these environments. Factor analysis is an important statistical method used to explain observed relationship among numerous variables. The mode of factor analysis is R-mode or Q-mode. Factor analysis is termed R-mode when the concern is interrelationships among the variables and Q-mode when concern is the interrelationships between samples[22]. R-mode factor analysis was used to identify major factors controlling the hydrochemistry of surface water of Gulf of Kachchh. Eigen value is greater than 1 reflects significant contribution of corresponding factor. Four factors with an Eigen value >1 were identified. These four factors explain about 86.73 % of the total variance. Factor 1 accounts for 26% variance in the dataset and shows high loading of PO_4^{3-} and H_4SiO_4 among nutrients and negative loading of SO_4^{2-}. This factor explains contribution from silicate weathering, sulphate mineral dissolution, sulphide oxidation, phosphate mineral weathering or anthropogenic inputs. SO_4^{2-} might have derived from sulphate mineral dissolution, hence showing negative loading in comparison to PO_4^{3-} and H_4SiO_4. Factor 2 accounts for 22.4% variance in the dataset and shows strong loading of pH, HCO_3^- and H_4SiO_4, which describes contribution from weathering of carbonate silicate minerals. Factor 3 accounts for 21.4% variance in dataset and shows high loading of NO_3^- among nutrients. This factor represents contribution from runoff and waste water discharge into the Gulf of Kachchh. Factor 4 contributes 16.7% variance in database and shows high loading of salinity and TDS (table 3).

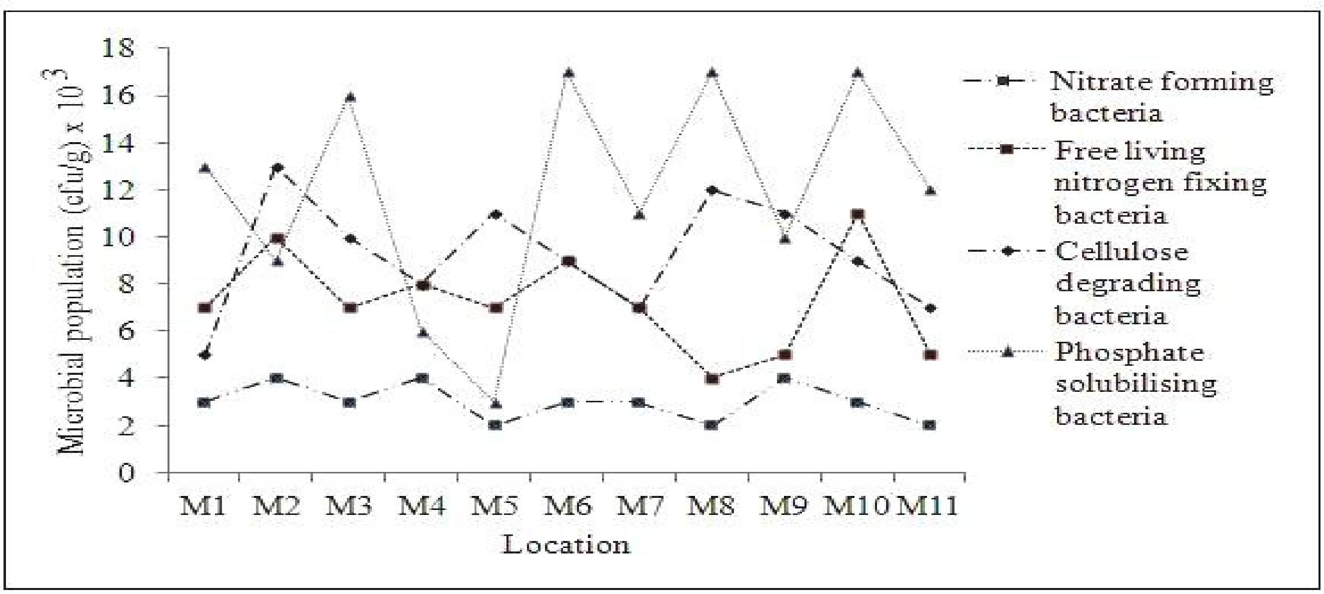

Figure-3
Microbial populations in the surface sediments of mangrove of Gulf of Kachchh, Gujarat

Table-2
Pearsons' correlation matrix for total microbial load, nutrients in surface water and organic carbon content in the surface sediments

	Temp. (°C)	pH	Salinity	TDS	HCO_3^-	NO_3^-	PO_4^{3-}	SO_4^{2-}	H_4SiO_4	Total bact. load	Total fungal load
Temp. (°C)	1.00										
pH	-0.01	1.00									
Salinity	0.50	0.10	1.00								
TDS	0.08	0.09	-0.39	1.00							
HCO_3^-	0.09	-0.73	-0.16	-0.06	1.00						
NO_3^-	-0.76	0.11	-0.46	-0.11	-0.22	1.00					
PO_4^{3-}	0.39	0.38	-0.11	0.28	-0.23	-0.04	1.00				
SO_4^{2-}	-0.27	-0.11	0.11	0.08	-0.08	-0.06	-0.29	1.00			
H_4SiO_4	0.63	-0.49	0.11	0.05	0.70	-0.47	0.25	-0.49	1.00		
Total bact. load	0.52	0.21	-0.08	0.11	-0.20	-0.04	0.79	-0.50	0.26	1.00	
Total fungal load	-0.49	0.22	-0.33	-0.20	-0.13	0.76	0.27	-0.49	-0.06	0.14	1

Table-3
Principal and varimax rotated R-mode factor loading matrix

Variables	Factor 1	Factor 2	Factor 3	Factor 4	Communities
Temp °C	0.626	-0.199	-0.671	0.276	0.958
pH	0.298	0.810	-	-	0.759
Salinity	-	-	-0.286	0.937	0.963
TDS	-0.236	0.213	-	0.871	0.867
HCO_3	-0.115	-0.909	-	-0.157	0.872
NO_3	-0.164	0.234	0.868	-0.221	0.883
PO_4	0.818	0.277	-	-0.186	0.783
SO_4	-0.724	0.301	-0.384	-	0.763
H_4SiO_4	0.510	-0.809	-0.221	-	0.964
Total bacterial load	0.867	0.159	-	-	0.785
Total fungal load	0.278	-	0.923	-0.114	0.944
Eigen value	3.196	2.868	2.428	1.048	
% of variance	26.005	22.465	21.490	16.772	
% of cumulative variance	26.005	48.471	69.961	86.733	

Conclusion

The source of nutrients in the mangroves of Gulf of Kachchh includes anthropogenic input and waste water discharge. A strong correlation between NO_3^- and total fungal load (r^2=0.76) and between total bacterial load and PO_4^{3-} (r^2=0.79) indicates these factors significantly influence the microbial population in the sediments. R-mode factor analysis indicates the anthropogenic input and various minerals dissolution contributed availability of nutrients in the study area. The available nutrients in the surface water directly influence the microbial status and nutrient dynamics in the sediment.

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