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Prevalence of Autochthonous *Vibrio cholerae* and Role of Abiotic Environmental Factors in their Distribution along the Kerala-Karnataka Coast, India

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Abstract: Occurrence and distribution of *Vibrio cholerae* (VC) with respect to different abiotic environmental factors were studied for a period of three year from 2003-2005 and interpreted using Principal component analysis and Pearson correlation. Study reveals the serious dimensions of increase in VC population (2.67% in 2003, 5.33% in 2004 and 92% in 2005 in Mangalore) over the years. Among all stations, Kochi and Mangalore seems to be highly polluted. The PCA extracted four significant main components explains more than 75% of the variance. Of them the most contributing descriptors in the first PC (24.29%) were total nitrogen, silicate, temperature and *V.cholerae*. On the other hand *V. cholerae* showed significant positive correlation against temperature (0.01 levels) and also with total nitrogen and silicate (0.05 levels). Component plot showed that variables have tendency to accumulate into three distinct groups. *V. cholerae* and temperature belongs to one group and nutrients on the other group, which indicate that temperature and nutrients are the major factor governing the distribution of *V. cholerae*. The result of the study provides insight into the ecology of this aquatic species and is potentially important to the understanding of the epidemiology of cholerae on a global scale.

Key words: *Vibrio cholerae*, pearson correlation, principal component analysis, abiotic environmental factors

INTRODUCTION

Cholera has historically occurred in periodic epidemics, with the most severe epidemics limited to few countries, namely India, Bangladesh and countries in Africa and South America (World Health Organization, 2000) and was suggested to be an autochthonous member of the aquatic environment (Thompson *et al*., 2004). Nearly 200 *V. cholerae* serogroups have been identified to date (Yamai *et al*., 1997), but only two serogroups, serogroups 01 and 0139, are associated with epidemic cholera (Keimer *et al*., 2007). Although, majority of the environmental isolates of *Vibrio cholerae* are members of non-01, non-139 serogroups which is considered to be non pathogenic, recent studies have confirmed that natural population of *Vibrio cholerae* including *Vibrio cholerae* non-01, non-139 isolates can serve as a precursor for new pathogenic or epidemic strains (Faruque *et al*., 2002; Bik *et al*., 1995).

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Because of this inherent risk, it is relevant to understand the mechanisms that affect the natural population of *Vibrio cholerae* in the environment. Colwell (1996) reported that the presence of cholerae in the Indian subcontinent and the re-emergence of cholera in other continents may highly dependent on environmental factors. So, a better understanding of the relation to climate would allow better planning for epidemics (Rodo *et al*., 2000). In the present study, our first objective was to investigate the occurrence of *V. cholerae* with respect to different abiotic environmental factors (salinity, nutrients, temperature etc.) in order to understand how changing environmental conditions might affect the ecology of *V. cholerae* in the environment. Secondly we aimed to investigate the spatial variability and annual distributional status of *V. cholerae* along the Kerala-Kernataka coast.

MATERIALS AND METHODS

Data Acquisition

The study area comprising five different stations, Veli, Neendakara, Kochi, Mangalore and Karwar (Fig. 1), each with 5 sampling locations nearshore (NS), 1, 3, 5 and 10 km from shore. Samples were collected during the cruises of Sagar Purvi and Sagar Paschimi, the coastal research vessel of DOD (Department of Ocean Development, Govt. of India) for a period of 3 years (2003-2005). Water samples from surface, mid and bottom region were collected using Niskin water sampler and sediment samples by Van-Veen grab. Analysis of several parameters of water samples like pH, temperature, salinity, DO (dissolved oxygen), BOD (bio-chemical oxygen demand), TSS (total suspended solids), turbidity and nutrients (NO_2-N, NO_3-N, PO_4-P) have been carried out using standard procedures (Grasshoff, 1983). Spread plate technique using 0.1 to 0.5 mL sample, was adopted for the

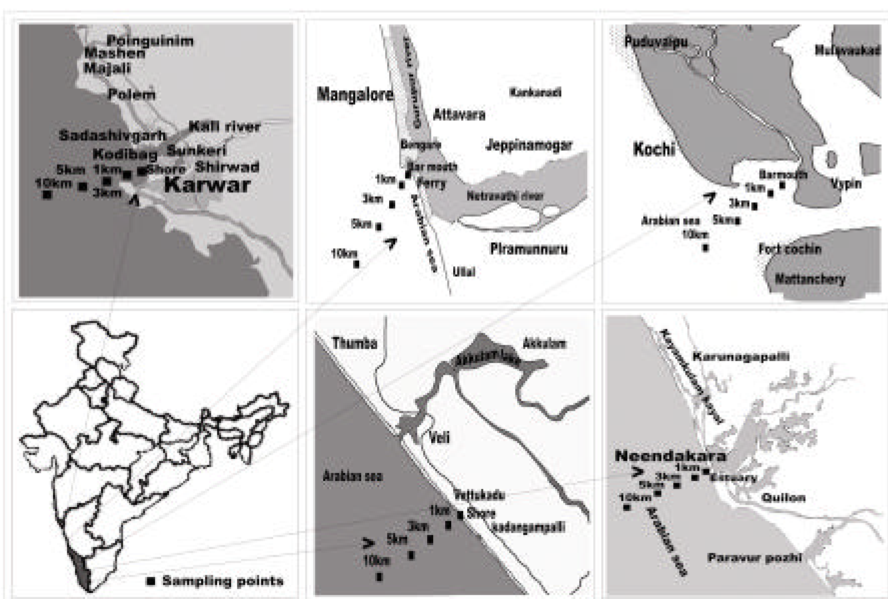

Fig. 1: Study area

Vibrio cholerae and the results were reported in Colony Forming Units (CFU mL^{-1}). One gram of the sediment was dissolved in 100 mL sterile water was used for the isolation of *Vibrio cholerae* from the sediment samples and the results were expressed in CFU g^{-1}. All the samples were plated onto TCBS (thiosulphate citrate bile salts sucrose) agar, a medium that inhibits most other normal faecal flora but supports the growth of the vibrios and were incubated for 18-24 h. *Vibrio cholerae* colonies appear as smooth yellow colonies with slightly raised centres (AOAC, 1995).

Data Analysis

Each water quality data of a transect represent the over all mean of three different seasons, i.e., pre-monsoon, monsoon and post-monsoon, including their average values of surface, mid and bottom and it can mitigate the seasonal influence. A correlation matrix was generated for understanding the degree of mutually shared variability between different chemical water quality parameters and *Vibrio cholerae*. Eigenvalues and Factor loadings for the correlation matrix also were estimated. An Eigenvalue gives a measure of significance of the factor. The Eigen values > 1 were considered as prominent factors as per the Kaiser criterion (Kaiser, 1960), the factors with the highest Eigenvalues are the most significant. After the correlation and Eigen values were obtained, factor loadings were used to measure both the correlations and regression weights between factors and variables. A rule of thumb frequently used is that the absolute value of the factor loading greater than 0.3 is considered significant, greater than 0.4 is more important and greater than 0.50 is very important (Lawley and Maxwell, 1971).

RESULTS AND DISCUSSION

Distributional Status of *Vibrio cholerae*

Vibrio cholerae population showed wider fluctuation during the entire annual study period (Fig. 2-6). During the study the population level kept low in all stations during 2003 and 2004, but has produced an increase in their population towards 2005. Almost all the stations *V. cholerae* levels were 2 to 5 fold higher in 2005 than the values found during 2003 and 2004. Sediment also produced the same output. Sediments reported to have fairly high bacterial population than water. In general, *V. cholerae* hoards in sediments at very high levels than the overlying water column. The same was also reported by Nandini and Somashekar (1999), who stated that sedimentation and adsorption of the microorganisms to sand and clay particles culminated in the increase in the density of bacteria at the bottom zone. Of the study Mangalore estuary reported with comparatively high bacterial population

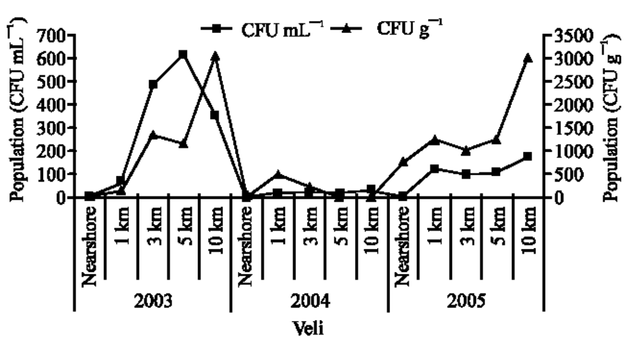

Fig. 2: Distributional status of *Vibrio cholerae* in Veli

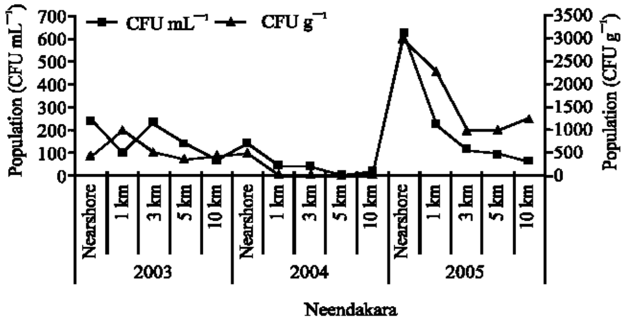

Fig. 3: Distributional status of *Vibrio cholerae* in Neendakara

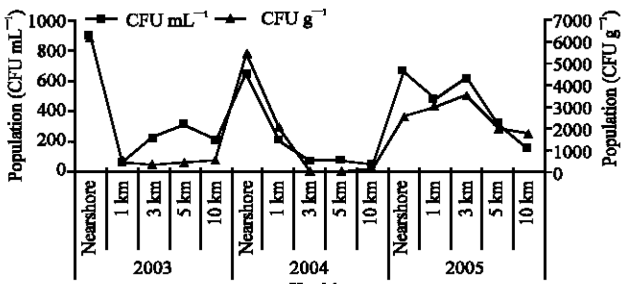

Fig. 4: Distributional status of *Vibrio cholerae* in Kochi

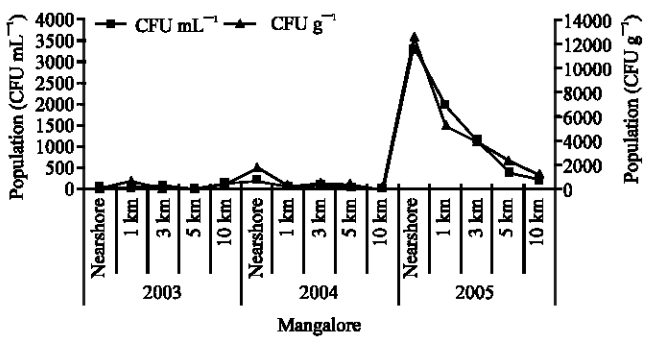

Fig. 5: Distributional status of *Vibrio cholerae* in Mangalore

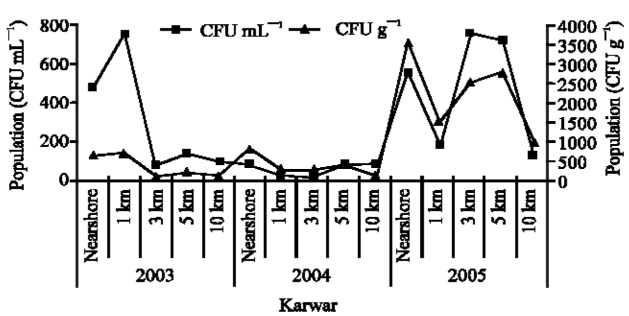

Fig. 6: Distributional status of *Vibrio cholerae* in Karwar

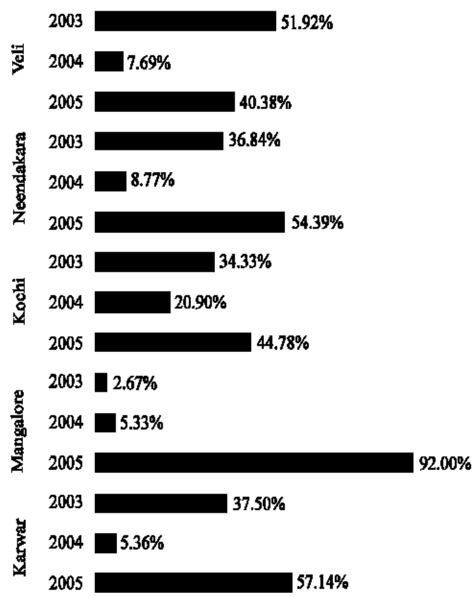

Fig. 7: Progressing annual population of *Vibrio cholerae* in water (%)

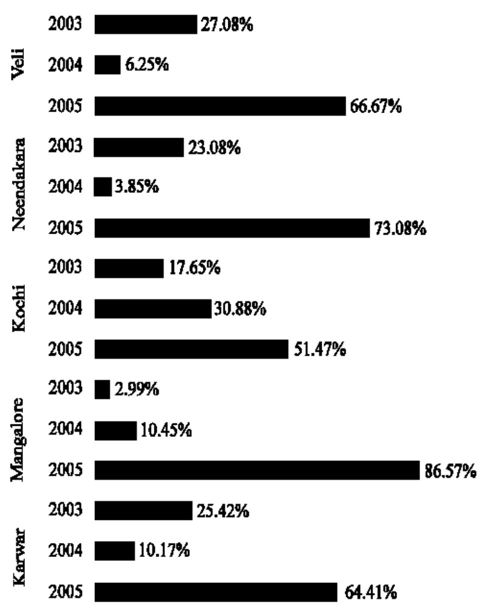

Fig. 8: Progressing annual population of *Vibrio cholerae* in sediment (%)

of 3335 CFU mL^{-1} in water and 12500 CFU g^{-1} in sediment during 2005. In all the stations *V. cholerae* population were more concentrated towards shore region except in Veli where they are more documented towards the offshore region. Ouseph *et al.* (2009) reported that the fairly low microbial population reported in Veli near shore is attributed to the discharge of acidic effluents from the nearby Titanium factory. Progressing *V. cholerae* annual population in both water and sediment are depicted in Fig. 7 and 8, respectively. The maximum percentage population of 92% in water and 86.57% in sediment, both documented from Mangalore station in 2005. In contradiction to the above high value the minimum of 2.67 and 2.99%, respectively in both water and sediment were also reported from Mangalore in 2003, which clearly reveals progressing *V. cholerae* population over the years.

Status of Physico-Chemical Parameters

Water temperature showed variation from 24.0 to 34.0°C (mean of 28.56±2.07°C) during the study period. The minimum and maximum temperature was noticed at nearshore region of Veli (2003) and 5 km of Kochi (2003), respectively. Throughout the study, the temperature of the bottom water was lower than the surface water. Salinity ranged from 15.10 (Kochi-Estuary-2003) to 34.90 psu (Veli-10 km-2005) with an overall mean of 30.60±5.19. The lower salinity in the Cochin estuary may be due to the riverine influence. The pH fluctuated between 2.60 (Veli- NS-2003) and 8.27(Veli-5-2005) with mean of 7.81±0.97 and that of total suspended solids accounted a variation of 2.30 mg L^{-1} (Karwar-NS-2005) to 21.80 mg L^{-1} (Veli-1 km-2003) with mean of 8.17±4.45. The low pH attributed in Veli may due to the impact of acidic effluents from the TTP industry (Ouseph, 1993). The BOD oscillated between 0.52 mg L^{-1} (Veli-Nearshore-2003) to 2.02 mg L^{-1} (Neendakara-1 km-2005) with mean of 1.15±0.35. Dissolved oxygen fluctuated from 3.02 to 6.96 mg L^{-1} with a mean of 4.93± 0.67 mg L^{-1} and the lowest and highest was noticed at nearshore region of Veli (2005) and 5 km of Kochi (2005), respectively. In the over all scenario DO values were comparatively higher in surface water than the bottom region which may be probably due to the atmospheric turbulence. The lower oxygen content at the bottom could also be attributed to oxygen consumption during the decomposition of organic matter (Abowei, 2010).

Nutrient Status

Generally, bottom water showed higher concentrations of nutrients than the surface water. Of the nutrient species, the nitrite-N varied from 0.04 μmol L^{-1} (Veli-10 km-2003) to 1.38 μmol L^{-1} (Veli-10 km-2004) with an over all mean of 0.52±0.30 and that of nitrate-N showed a variation of 0.16 μmol L^{-1} (Veli-5 km-2003) to 11.23 μmol L^{-1} (Kochi-3 km-2003) with a mean of 4.15±2.21. The higher nitrite concentration in Kochi may due to the increased nitrification process (Miranda *et al.*, 2008) prevailing in the environment. The average concentration of silicate during the intact study period was 3.66±1.88 μmol L^{-1}. Inorganic phosphate concentration varied between 0.35 μmol L^{-1} (Mangalore-10 km-2005) to 2.95 μmol L^{-1} (Veli-Nearshore-2005) with a mean of 1.45±0.60 and that of total phosphorous registered a variation of 2.12 μmol L^{-1} (Veli-5 km-2003) to 10.0 μmol L^{-1} (Kochi-3 km-2003) with a mean of 4.15±1.62. The minimum concentration of total nitrogen was 3.92 μmol L^{-1} (Veli-1 km-2005) and the maximum was 33.14 μmol L^{-1} (Cochin-5 km-2003) with an overall mean value of 12.11±4.52. Broadly, Cochin reported with higher concentration of nutrients, may probably due to the release of nutrients from the interstitial sediments owing to prevalent dredging activities (Joseph and Ouseph, 2009; Sudhanandh *et al.*, 2010) and also owing to the unabated domestic waste disposal.

Correlation Analysis

Correlation matrix (Table 1) revealed the existence of a strong relation between *V. cholerae* and temperature at 0.01 level ($p<0.01$), with total nitrogen at 0.05 level ($p<0.05$), and also with silicate at 0.01 level ($p<0.01$). *Vibrio cholerae* also showed a negative correlation with salinity. Association found with *Vibrio cholerae* to temperature were also reported by Siddique *et al.* (1992), Glass *et al.* (1982) and Sack *et al.* (2003) who stated that

Table 1: Correlation matrix showing the interrelationship of different abiotic environmental factors and *Vibrio cholerae*

Parameters	1	2	3	4	5	8	9	12
Temperature	1							
Salinity	-0.064	1						
pH	0.375	-0.097	1					
Total suspended solids	-0.016	-0.354	-0.361	1				
Dissolved oxygen	-0.393	-0.173	0.525*	-0.581*	1			
Biological oxygen demand	0.251	0.356	0.327	-0.209	0.038			
Nitrite	-0.021	-0.468	-0.246	0.663**	-0.518*			
Nitrate	0.155	-0.520*	0.036	-0.072	0.263	1		
Silicate	0.656**	-0.537*	0.127	0.042	0.37	0.495	1	
Inorganic phosphate	-0.237	0.087	-0.429	0.169	-0.325	0.306	-0.215	
Total phosphorous	0.653**	0.014	0.142	0.265	0.081	-0.429	0.158	
Total nitrogen	0.568*	-0.631*	0.311	-0.085	0.553*	0.584*	0.705**	1
Vibrio cholerae	0.701**	-0.112	0.363	-0.509	0.609*	0.11	0.646**	0.605*

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed)

Table 2: Factor loading for different water quality parameters

	Principal components			
Constituent	1	2	3	4
Total nitrogen	0.804	0.405	0.060	0.281
Silicate	0.792	0.419	0.022	-0.302
Temperature	0.780	-0.065	0.434	-0.204
Dissolved oxygen	-0.771	-0.388	0.350	0.148
Vibrio cholerae	0.695	-0.305	-0.130	-0.545
Nitrite	-0.138	0.769	0.423	0.249
Salinity	-0.385	-0.751	-0.012	-0.176
Nitrate	0.392	0.658	-0.492	0.123
Total suspended solids	-0.333	0.617	0.593	-0.179
Total phosphorous	0.307	-0.245	0.772	-0.272
pH	0.525	-0.335	0.041	0.574
Biological oxygen demand	0.066	-0.451	0.515	0.545
Inorganic phosphate	-0.431	0.337	-0.248	-0.098
Eigen vectors	3.95	2.96	2.03	1.38
Percentage variation	24.29	20.70	18.46	12.21
Cumulative variation	24.29	44.99	63.45	75.67

cholera outbreaks occur each year corresponding to the warm seasons before and after the monsoon rains. Quick *et al.* (1995) reported that cholera epidemics are strictly confined to the warm season. Thus temperature seems to be related to the ability of vibrios to grow rapidly in aquatic environment. Furthermore, the positive correlation found between *V. cholerae* to total nitrogen suggest that *Vibrios* are favored in waters rich in organic nutrients such as might be expected in areas heavily impacted by land runoff and wastewater discharges. On the other hand negative correlation of salinity with total nitrogen, nitrate and also with silicate 0.05 level ($p<0.05$) pointed out that sea water was not the source for nutrients. Hence other human anthropogenic activities including the sewage discharge and also the fresh water input which carry industrial and agricultural wastes and other land drainage in the immediate vicinity of the shore region are the major reasons for nutrient enrichment. Joseph *et al.* (2008) observed fresh water run-off, sewage input from the upper reaches of the backwaters and localized disturbance in the harbor as the major sources of nutrients in the Cochin estuary which is in concordance with our study.

Factor Analysis

Principal Component Analysis (PCA) extracted five Principal Components (PCS) from the variance present in the data (Table 2). Of them the first four factors (Eigen value >1) accounted for 75.67% of the observed variation in water quality observations. Additional

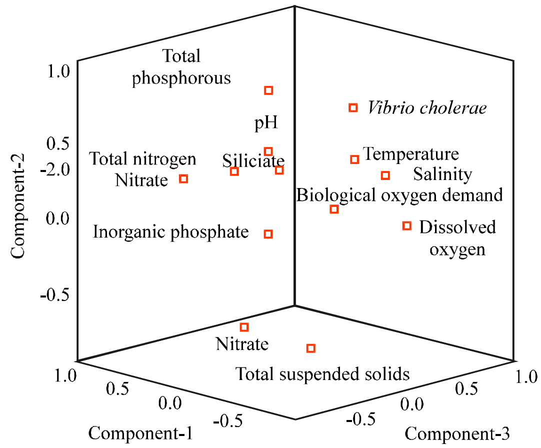

Fig. 9: PCA component plot showing the relativeness of different environmental parameters

factors (5th) provided marginally less explanatory capability and were not examined further. Factor loadings (correlations between the variables and the extracted factors) for the four retained Eigen values are also incorporated with the Table 2. The first principal component has a high positive loading of total nitrogen, silicate, temperature, pH, *Vibrio cholerae* and negative loading of dissolved oxygen and explains 24.29% of the total variance. This factor can be ascribed to the variation of natural condition due to the influx of water which carries heavy nutrient load and also global warming which effect the gases concentration, temperature and influences the bacterial load. So, these components represent the backwater discharge and global warming. Singh and Sarkar (2003) observed an increasing trend of surface water temperature almost through out the year with an increase of 0.03°C $year^{-1}$ in the annual mean due to global warming.

The second PC explained 20.70% of the total variance is found to be strongly associated with NO_2-N NO_3-N and TSS with strong negative loading of salinity and moderately negative loading of DO and pH which strongly indicates that sea water is not the source of pollution. So this component represents anthropogenic pollution sources and can be explained that high levels of dissolved organic matter consume large amount of oxygen which is indicative of the negative loading of DO (Panda *et al*., 2006) and nitrification under oxic condition causes the reduction in water pH values (Kim *et al*., 2003). The third factor is positively loaded with TSS, TP and BOD and explained 18.46% of the total variance. This factor may be correlated with the resuspended sediments through churning action by tidal currents. The fourth factor is loaded with strong positive loading of TP and BOD and strong negative loading of *V. cholerae*. This factor represents industrial effluents especially acidic in nature which reduces the growth potential of *V. choleare*. On the other hand, higher correlations were found with temperature to *Vibrio cholerae* and Total nitrogen to silicate in the correlation matrix (Table 1) and also in the first principal component, explaining themselves as the observed variance and could be considered the most descriminant variables.

The PCA loading plot, representing the scores of the sample (PC1 vs. PC2 vs. PC3) and relationship between the variable is shown in Fig. 9. All the variables were found to be distributed in three groups. *Vibrio cholerae*, temperature, salinity, biological oxygen demand and dissolved oxygen showed one group formed by temperature, attributed to the increased *V. cholerae* population due to global warming, second group constituted by total phosphorous, total nitrogen, silicate, nitrate and inorganic phosphate, might be due to an increase provoked by the nutrient enrichment and the third group constituted by total suspended solids and nitrite due to remobilization of sediments through churning action by tidal currents.

CONCLUSION

Study recapitulated that our coastal waters are stock up with autochthonous *Vibrio cholerae* and make an ample chance for disease out break. It is evident from the study that *V. cholerae* population demonstrates spatiotemporal correspondences with the environmental variations. Varifactors obtained from factor analysis and also significant correlation found with *Vibrio cholerae* to temperature and nutrients suggests that nutrient loading and global warming makes the organism thrive in the environment.

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