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Determination of spatio-temporal influences on the distribution of fecal indicator organisms along the north-west coast of India

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Coastal ecosystem is susceptible to discharge of untreated sewage waste. The present study is aimed to determine water quality status prevailing along the north-west coast of India by monitoring spatial and temporal variations associated with fecal indicator organisms (FIOs) (namely, total coliforms, fecal coliforms, *Escherichia coli* and *Streptococcus fecalis*) along the five designated coastal water sites – Veraval, Hazira, Mumbai, Ratnagiri and Malvan for years (2012-14). Spatial waters illustrated that the concentration of FIOs significantly reduced away from the shoreline. Temporal-based sampling elucidates decreasing trend in fecal loads: Monsoon>post-monsoon>pre-monsoon. Based on the resemblance of water quality characteristics applied by hierarchical cluster analysis, these sites were grouped into three categories: Comparatively less polluted, moderately polluted and highly polluted. Regular trends in coastal FIOs variability, collective information about water quality and environmental factors appear useful for monitoring and management towards pollution encumbered at coastal region.

[Keywords: Water quality; Fecal indicator organisms; North-west coast; Hierarchical cluster analysis; Principal component analysis.]

Introduction

Expulsion of wastewater into the coastal areas is not infrequent particularly in the Arabian coastal region^[1]. Unprecedented increase in urbanization and township along the coasts, which provide a source of food, employment, recreation and residence seems to encounter a broad nature of stressors distressing ecosystem and human health via waste disposal practices. Pollution input sowing to alterations in land use and hydrology with massive waste entering on a regular basis may lead to the introduction of an elevated level of nutrients and enteric pathogens combined with flora-faunal changes thereby altering the ecosystem. There exists a fragile balance between the needs of the coastal communities and health of the aquatic ecosystem; therefore, mandates have been implemented to address water quality problems associated with primary point and non-point sources.

Anthropogenic activities alongside land-water interface have a strong likelihood to contribute towards ecological and human health related problems. Over past decades, increasing human activities, growing coastal tourism and disposal of raw sewage into the coastal waters has been a very common practice in the Indian history^[2]. While a wide variety of pathogenic indicators have been

proposed, the most commonly used estimator is the bacterial abundance of fecal indicator organisms (FIOs) including TC, FC, EC and SF. The abundance of these FIOs have been documented to be associated with various respiratory pathogens, as reported in the literature³⁻⁷. FIOs have been presumed to origin due to the anthropogenic activity via point and non-point sources⁸. Untreated domestic sewage discharged through non-point sources is attributed to be the major cause of prevalence of multiple drug-resistant FIOs, specifically *Escherichia coli* in coastal waters of Mumbai, Maharashtra⁹. The proliferation and survival of FIOs will depend on the ability to tolerate wide ranges in physico-chemical parameters, such as salinity, pH, temperature, oxygen saturation, suspended solids, organic content, tide, sunlight penetration etc.^{10,11}. Further, the microbial quality will vary due to the extent of inputs, dispersion of organisms as a result of hydrodynamics, rate of deposition, rate of die-offs, surface runoffs, interflow, ground water flow, outflow as a result of changing environmental conditions such as rainfall, tides etc. which consequently affects the extent of pollution^{12,13}. During recent years, the coastal waters face many constraints that result in weakening and degradation of natural oceanic ecosystems. The universal problem

being monitoring complexities coupled with evaluating large sets of measured variables that may or may not contain valuable information of the water quality. Classification, modeling and interpretation of monitored data are also crucial steps in the assessment of water quality. As a part of national level assessment, efforts were made to study the disturbances in water quality that inexorably lead to deterioration of oceanic ecosystems. Therefore, to endow with a representative and reliable estimation of surface water quality, to understand the hydro-morphological characteristics for an effective, long-term management of coastal waters and to assign the relative risk levels - spatial as well as temporal variations in water quality- the assessment program was carried out for three progressive years. The complex data obtained was subjected to different multivariate statistical techniques such as HCA and PCA to build up a better understanding of water quality and ecological status, to spot the similarities and dissimilarities between sampling sites/sources/factors that influence water systems, to study the influence of spatial and temporal variations and other hidden factors so as to provide reliable tools for water management in addition to rapid pollution related problems.

Materials and Methods

Sampling sites

The north-west coast of India comprises Gujarat and Maharashtra, which covers a coastline of about 2130 km. With the prompt development in terms of urbanization and tourism, coastal water has endured anthropogenic impact, recipient of agricultural, domestic-industrial sewage and rainwater. For this study, a total of five different study sites were selected along the coast, namely, Veraval, Hazira, Mumbai, Ratnagiri and Malvan. To evaluate anthropogenic and natural influences on the variability of different bacterial populations and to obtain the overall distribution pattern of FIOs along the entire stretch of coastline, the data set for 34 water quality stations was monitored (Fig. 1). The south-west monsoons prevail from June to September and bring humid air from low latitudes, resulting in gentle monsoonal rainfall. By contrast, as the monsoon subsides, cold weather predominates from October to February followed by summers during March to May. Sampling sites selected were monitored seasonally viz., pre-monsoon, monsoon and post-monsoon over

three years from 2012-2014. For transect studies, six locations at each site (0, 0.5, 2 and 5 km away from the shore) were covered to collect near-shore as well as offshore samples.

Sample collection

Surface water samples were collected with the help of hired trawlers at an interval of 3 hrs onboard using 5l Niskin sampler (General Oceanics, FL) at 1 m depth into pre-autoclaved polypropylene bottles for microbiological analysis and deposited in an insulated container with sufficient artificial ice packs to maintain a temperature of 2-8 °C during transportation. Seawater samples for analysis of nutrients were collected in PVC bottles for nutrients (NH₄-N, NO₃-N, TN and TP), total suspended solids (TSS) and DO/BOD bottles; these were tested in accordance with the methods for marine pollution monitoring. All samples collected were preserved, stored and analyzed within approximately 24 h as charted in standard methods for the examination of water and wastewater. Sampling variability was abridged by collecting and analyzing samples in the same manner, at or near tide.

Bacterial enumeration

Diverse bacterial populations were enumerated from these samples, such as total viable count (TVC), total coliforms (TC), fecal coliforms (FC), *Escherichia coli* (EC) and *Streptococcus fecalis* (SF). Spread plate technique was opted for enumeration. The samples after appropriate dilution were aseptically spread on selective media to an incubator at appropriate selective temperature for a suitable time to allow duplication of the indicator organisms. Visually identifiable colonies were counted, and outcome was articulated in a number of 'colony forming units' (CFU) per 100 ml of the original sample. The analysis was carried out following the standard methods for the examination of water and wastewater prepared and published by American Public Health Association (APHA) 21st edition¹⁴. Nutrient agar plates prepared with 50% sea water were used to enumerate TVC. Pink colonies on MacConkey agar were quantified as TC, whereas typical blue colonies on MFC agar were quantified as FC. Yellow colonies with a halo on M7HrFC agar were enumerated as EC. Typical dark pink colonies on M-Enterococcus agar were enumerated as SF. The colonies obtained were cross-confirmed using appropriate biochemical tests and coliforms were

confirmed by growing on HiCrome Agar. All media were procured from Himedia Pvt. Ltd., India.

Environmental parameters

Ecological variables were included in this study because of their putative influence on the abundance of FIOs. The data utilized were depth, tide, season along with other physico-chemical parameters namely water temperature, nutrients ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$, TN and TP), total suspended solids (TSS), salinity, pH, DO and BOD (Table 1). Dissolved oxygen (DO) and biochemical oxygen demand (BOD) were determined by Winkler's titration method. TSS of surface water was determined by gravimetric method upon filtration through $0.45\ \mu\text{m}$ filter membrane at $105\text{-}110\ ^\circ\text{C}$ ¹⁵. pH and salinity were determined using the pH meter and salinometer, respectively. Nutrients in the surface layers were determined spectrophotometrically, in accordance with the methods by Grasshoff et al.¹⁶.

Statistical analysis

Relationships between different microbial determinants (e.g., environmental conditions, sediment and water characteristics) and FIOs were examined using multivariate techniques: Cluster Analysis (CA) and Principal Component Analysis

(PCA). All mathematical and statistical analyses were computed using Statistical 6.0 and Excel 2010.

Cluster analysis

Cluster analysis encompasses a number of different classification algorithms which can be used to assemble objects into clusters based on the characteristics they attain with regard to predetermined selection criterion¹⁷. Each cluster has a different threshold of similarity. Varying levels of similarity will sequentially relate to all parameters in water quality. This way by hierarchical agglomerative clustering (HCA), it is possible to obtain a graphical representation in the form of dendrogram of the evoked potential parameters versus the water quality with a dramatic reduction in dimensionality of the original data. The Euclidean distance outlines the likeness amidst two samples and the distance can be represented by the variance between analytical values from the samples¹⁸. In this study, HCA was performed on the data by means of Ward's method, wherein Euclidean distance quantified similarity by means of linkage distance reported as Dlink/Dmax -multiplied by 100 as a way to normalize the linkage distance¹⁹⁻²². Cluster analysis was adopted to visually summarize intra-relationship amongst variations in parameters that can lead to a better perceptive of governing factors.

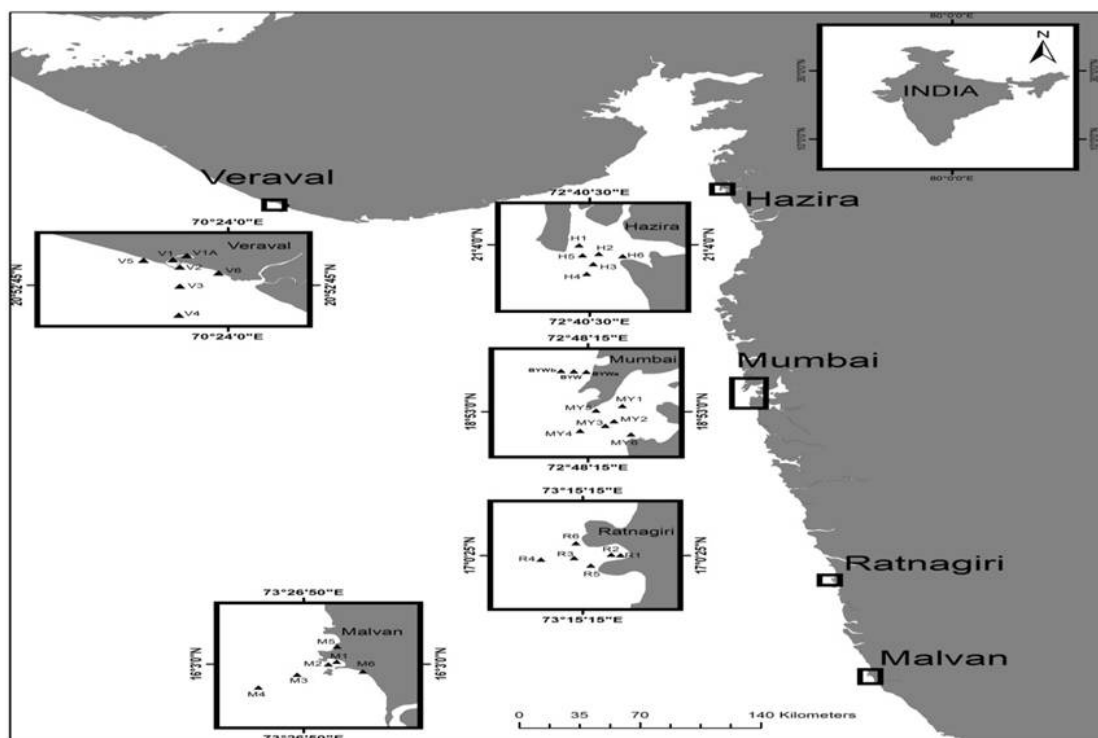


Fig. 1 — Map of study area and water surface quality stations along the north-west coast of India.

Table 1— Water quality parameters, units and analytical methods used during 2012-2014 for evaluating surface water quality across the north-west coast of India

Parameters	Abbreviations	Units	Analytical methods										
• Physicochemical parameters													
Water temperature	WT	°C	Mercury thermometer										
Total suspended solids	TSS	mgL ⁻¹	Gravimetric method										
pH	pH	pH unit	pH meter										
Salinity	Salinity	PSU	Salinometer/Volumetric Argentometric method										
Dissolved oxygen	DO	mgL ⁻¹	Winkler Azide method										
Biochemical oxygen demand	BOD	mgL ⁻¹	Winkler Azide method										
Nitrate nitrogen	NO ₃ ⁻ -N	µmolL ⁻¹	Spectrophotometric method:										
Ammonical ammonium nitrogen	NH ₄ ⁺ -N	µmolL ⁻¹	Spectrophotometric										
Total nitrogen	TN	µmolL ⁻¹	Phenate Spectrophotometric										
Total phosphorus	TP	µmolL ⁻¹	Spectrophotometric										
• Microbiological parameters													
Total viable count	TVC	CFU/mL	Spread plate technique on: Nutrient agar supplemented with 50% sea water										
Total coliform	TC	CFU/mL	MacConkey agar										
Fecal coliform	FC	CFU/mL	MFC agar										
<i>Escherichia coli</i> counts	EC	CFU/mL	M7HrFC agar										
<i>Streptococcus fecalis</i> counts	SF	CFU/mL	M-Enterococcus agar										
Pre-monsoon													
	WT	TSS	pH	Salinity	DO	BOD	NO ₃ ⁻ -N	NH ₄ ⁺ -N	TN	TP	TC	EC	SF
WT	1												
TSS	0.34	1											
pH	0.37	-0.63	1										
Salinity	-0.31	-0.99	0.66	1									
DO	0.86	-0.14	0.77	0.20	1								
BOD	-0.85	-0.27	-0.49	0.17	-0.87	1							
NO ₃ ⁻ -N	-0.12	0.94	-0.68	-0.91	-0.28	-0.21	1						
NH ₄ ⁺ -N	-0.85	-0.23	-0.53	-0.14	-0.89	1.00	-0.17	1					
TN	-0.90	0.09	-0.69	-0.09	-0.94	0.72	0.33	0.75	1				
TP	-0.85	-0.21	-0.55	0.12	-0.90	1.00	-0.15	1.00	0.75	1			
TC	-0.67	-0.84	0.25	0.87	-0.23	0.41	-0.61	0.39	0.40	0.38	1		
EC	-0.48	-0.50	0.05	0.59	-0.14	0.10	-0.22	0.10	0.42	0.10	0.85	1	
SF	-0.29	-0.58	0.20	0.62	-0.04	0.18	-0.44	0.17	0.18	0.17	0.76	0.86	1
Monsoon													
	WT	TSS	pH	Salinity	DO	BOD	NO ₃ ⁻ -N	NH ₄ ⁺ -N	TN	TP	TC	EC	SF
WT	1												
TSS	0.53	1											
pH	0.4	-0.45	1										
Salinity	-0.84	-0.86	-0.05	1									
DO	0.59	-0.20	0.91	-0.23	1								
BOD	-0.28	-0.16	-0.46	0.45	-0.49	1							
NO ₃ ⁻ -N	0.64	0.99	-0.35	-0.91	-0.10	-0.16	1						
NH ₄ ⁺ -N	-0.26	-0.13	-0.47	0.42	-0.49	1.00	-0.13	1					
TN	0.15	-0.01	-0.22	0.13	-0.24	0.91	0.06	0.92	1				
TP	-0.26	-0.10	-0.50	0.40	-0.52	1.00	-0.10	1.00	0.91	1			
TC	-0.12	-0.03	-0.33	0.36	-0.31	0.98	-0.10	0.98	0.95	0.98	1		
EC	-0.16	-0.17	-0.30	0.42	-0.24	0.95	-0.16	0.95	0.89	0.95	0.99	1	
SF	0.10	-0.01	-0.26	0.18	-0.23	0.93	0.05	0.94	0.99	0.94	0.98	0.94	1

(Contd.)

Post-monsoon

	WT	TSS	pH	Salinity	DO	BOD	NO ₃ ⁻ -N	NH ₄ ⁺ -N	TN	TP	TC	EC	SF
WT	1												
TSS	-0.61	1											
pH	0.74	0.04	1										
Salinity	0.94	-0.82	0.51	1									
DO	0.53	0.33	0.80	0.23	1								
BOD	-0.62	-0.24	-0.92	-0.37	-0.95	1							
NO ₃ ⁻ -N	-0.48	0.33	-0.09	-0.40	-0.44	0.21	1						
NH ₄ ⁺ -N	-0.62	-0.19	-0.96	-0.41	-0.83	0.97	-0.03	1					
TN	-0.75	0.24	-0.54	-0.58	-0.77	0.65	0.88	0.47	1				
TP	-0.73	-0.09	-0.94	-0.51	-0.92	0.99	0.27	0.96	0.70	1			
TC	-0.48	-0.32	-0.90	-0.28	-0.78	0.94	-0.17	0.99	0.33	0.91	1		
EC	-0.63	-0.19	-0.95	-0.41	-0.84	0.97	0.01	1.00	0.49	0.97	0.99	1	
SF	-0.51	-0.29	-0.87	-0.31	-0.82	0.96	-0.08	0.98	0.41	0.93	0.99	0.98	1
p<0.01	Highly significant												
p<0.05	Significant												
p>0.05	Not significant												

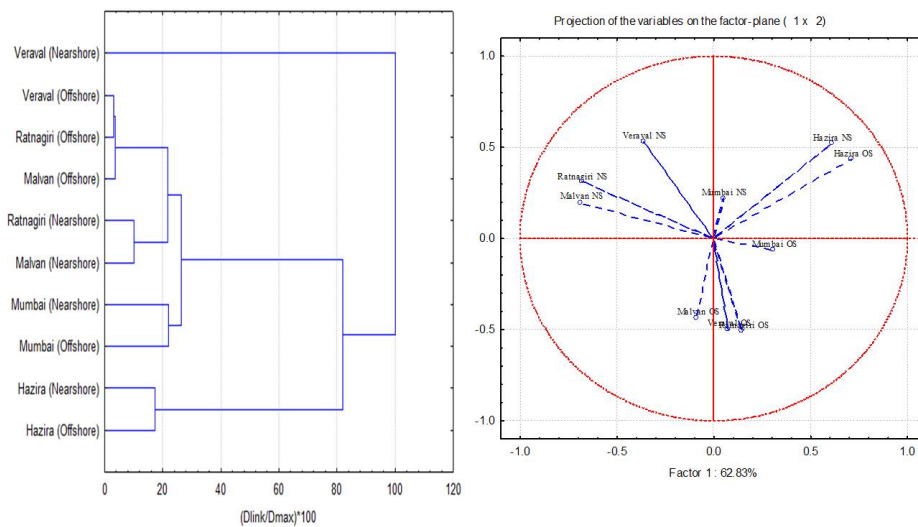


Fig. 2 — Dendrogram and PCA correlation circle showing clustering of sample sites according to surface water quality characteristics along the north-west coast of India.

Principal component analysis

Principal component analysis (PCA), a promissory multivariate technique that attempts to analyze the data in which observations are described by several inter-correlated quantitative dependent variables. It allows quick transformation of valuable information and sets new orthogonal variables called principal components, in response to the pattern of similarity of the observations and of the variables as points in maps. It permits handling of heterogeneous sets of variables that describe an entire data set allowing data reduction with minimum loss of original information²³⁻²⁴. To be used as a management tool, all parameters were standardized and uniformed to enable uniform collection and comparison of data. PCA was opted to verify the potentiality in investigating the influence of different

parameters in the quality of surface waters that contributes to the abundance of FIOs at varying spatial and temporal variations.

Results and Discussion

The selected coastal areas were situated under the appalling environment characterized by industrial and residential set-up alongside. Agglomerative hierarchical clustering analysis was adopted to determine the spatial differences grouping for each monitoring station along the coast. Different geographic locations elicit different response. Based on the normalized data matrix, the dendrogram generated (Fig. 2) grouped all the five sampling sites into three statistically meaningful clusters at (Dlink/Dmax)-100 < 80. The three sampling sites, namely, Malvan, Ratnagiri and offshore stations of

Veraval form a cluster 1 which shows mutual similarity and comprises relatively less polluted sites. These stations receive pollution mostly from sand mining, agricultural and fishing activities. Cluster 2 includes Mumbai offshore and nearshore as these resemble almost one another. Cluster 2 corresponds to moderately polluted sites. Hazira forms cluster 3 and is highly polluted. Veraval near-shore exhibits low mutual similarities as compared to other clusters. Clusters 2 and 3 are areas of industrialization where huge amount of sewage discharge loadings directly into the waters. The results demonstrate HCA as a useful technique in accredited classification of surface waters; hence, the number of sampling sites and respective cost in the future monitoring plans can be reduced. This study and other reports by Kim et al. 2005; Shrestha et al. 2007; and Singh et al. 2004, 2005, implicated the partial attribution of cluster analysis in water quality programs^{15,19,220,23}.

Natural processes and anthropogenic activities that have contributed to the deterioration of surface water quality includes hydrological features, agricultural land use, sewage discharge, precipitation, and climate change¹¹. Spatial-based samplings inferred that the concentrations of bacteria dwindled significantly with distance from the shoreline. Results indicate that this increase may have principally occurred due to increase in anthropogenic activity across the coast. High counts of fecal indicator organisms were attributed to the build-up of ammonical-nitrogen (Spearman's Rank=0.4) and biological oxygen demand for FIO's (Spearman's Rank = 0.8) indicating sewage discharge. A higher magnitude of fecal coliforms and pathogenic bacteria in neritic waters implies threats to environmental and human health. To reduce the dimensionality of multivariate data set containing 13 variables that we have presented for five different zones along the coast, PCA was carried out as outlined by Mardia et al.²⁶. Nearshore data on a large picture contributes to the correlation in PCA as compared to offshore data, wherein BOD is related to ammonia, TP and TN build-up along with micro-parameters suggesting sewage pollution in nearshore waters. However, PCA for offshore data did not give any significant values that infers no fecal contaminations, no organic load and hence no specific correlation of the parameters was observed.

Temporal variation impacts on water quality

The depiction of seasonal changes in surface water quality stands as an essential feature for evaluating temporal variations of coastal pollution due to natural

or anthropogenic inputs of point and non-point sources²⁷. Wu et al., reported the importance of seasonality influence on hydrochemistry²⁸. Temporal-based sampling elucidates decline in the occurrence of FIOs loadings given as monsoon > post-monsoon > pre-monsoon respectively. The levels of fecal coliforms had distinct patterns, peaking at wet season (≤ 14523 CFU/100 ml) and diminishing during dry seasons - Pre-monsoon (≤ 5900 CFU/100 ml) and post-monsoon (≤ 9366 CFU/100 ml) which exhibits unreliable compliance with the imperative Central Pollution Control Board (CPCB) standards (FC ≤ 100 CFU/100 ml). The occurrence of elevated counts in monsoon remains attributed to runoffs and non-point sources. This trend in bacterial load is wholly independent of any recent or long-term climatic variation. Bu et al., specified that the average annual coastal water quality was solely dominated by urban wastewater disposal²⁹. PCA was implemented on 13 variables for five coastal sampling sites across three seasons, with a view to identify seasonally significant water quality parameters. The impact of each factor is computed by eigen values, values of 1.0 or greater resembles the highest level of significance (Shrestha et al. 2007). Factor loading were classified into 'highly significant' values of > 0.75, 'moderate significant'-values of 0.75-0.50 and 'insignificant' values of 0.50-0.30, as prescribed by Liu et al.³⁰. Variable loadings elucidate variance as presented in Table 2 with significant loading values highlighted.

Table 2 — Loadings of experimental variables on significant principal components for different seasons across the coast.

Factor loadings of chemical and microbiological parameters in the study area during pre-monsoon, monsoon and post-monsoon

NS+OS	Pre-monsoon		Monsoon		Post-monsoon	
	PC1	PC2	PC1	PC2	PC1	PC2
WT	0.97	-0.09	0.35	-0.73	0.78	-0.57
TSS	0.27	-0.94	0.16	-0.93	0.02	0.89
pH	0.53	0.74	0.52	0.28	0.94	0.12
Salinity	-0.22	0.97	-0.48	0.88	0.57	-0.7
DO	0.92	0.41	0.56	0.06	0.9	0.14
BOD	-0.93	-0.07	-1	-0.04	-0.98	-0.2
NO ₃ ⁻ -N	0.1	-0.88	0.17	-0.97	-0.3	0.72
NH ₄ ⁺ -N	-0.94	-0.11	-1	-0.07	-0.95	-0.29
TN	-0.9	-0.29	-0.87	-0.29	-0.72	0.53
TP	-0.94	-0.12	-1	-0.09	-1	-0.07
TC	-0.59	0.77	-0.96	-0.11	-0.89	-0.44
EC	-0.42	0.58	-0.93	-0.05	-0.96	-0.28
SF	-0.36	0.65	-0.9	-0.27	-0.91	-0.38
Eigen Value	7.09	4.84	7.47	3.35	9.42	3.06
% Total Variance	50.64	34.53	53.32	23.87	67.27	21.82

The foremost dual factors of PCA accounts for over 85%, 77% and 89% of the variability during pre-monsoon, monsoon and post-monsoon, respectively. Water temperature, nitrate, total suspended solids, ammonical nitrogen, total nitrogen, and BOD appear as the most significant parameters contributing to water quality variations throughout the three seasons along the coast. Similar observation was highlighted by Xu et al., conducted in Yuqiao Reservoir Basin³¹. The temperature with strong loadings is the most significant parameter contributing to water quality variations and it represents the temporal impacts.

Principal component 1 (PC1) showed positive weights for temperature and DO whereas negative weights for BOD, ammonia, TN and TP. Consequently, samples with lowered temperature and DO tend to have higher TN, TP, ammonia and BOD. Irrespective of the season, variation in TC, EC and SF along with BOD and ammonia, within PC1 would indicate fecal contamination. Simultaneously, BOD showed strong positive correlation with ammonia, TP, TN, TC, EC and SF supporting the PCA results. Nitrate and total phosphate with negative loading value serve as the most significant parameters contributing to water quality variations throughout the season, which implies that significant amounts of inorganic nutrients due to an excessive influx of agricultural sewage encompasses almost throughout the year. Kumarasamy et al. revealed that NO₂ and PO₄ affect the seasonal and spatial variations and the involvement of natural weathering processes other than anthropogenic; similar incidence was observed at Hazira³². The biochemical oxygen demand and fecal coliform with strong factor loadings, the most crucial parameters in water quality variations, illustrate the entry of domestic wastewater that causes considerable pollution and extra variations in river water quality most time of the year. Non-significant correlation of other parameters with season signifies contribution of anthropogenic sources in catchment areas.

Authors worldwide such as Toochukwu; Mavukkandy et al.; Vieira et al.; Yang et al. and Pejman et al. have studied local effects caused in waters³³⁻³⁷. Wang et al., directed that TN, WT, and pH are significant variables affecting temporal variation caused by soluble salts (natural), point source pollution of phosphorus and non-point pollution of nitrogen (anthropogenic) similar to this study³⁸. Along the north-west coast of India, the main sources of pollution were identified as agricultural activities,

natural pollution, industrial waste, and domestic wastewater. Results illustrate that each site had unique physical and chemical characteristic due to its different natural and anthropogenic features and that pollution factors that play important roles in prompting water quality status in one environment may not be important in another³⁹. Observations suggest that the concentrations of TC were pronounced during monsoon. Bacterial concentrations in areas of Mumbai and Hazira are higher than the rest. The incidence of EC and SF in the nearshore stretches of the coast are of serious health concern since these areas are utilized for recreation and fishing purpose. All the above results enable validating large data sets using multivariate statistical techniques.

Conclusion

In the present study, multivariate statistical techniques were adopted to determine and explore possible polluting sources, spatial and temporal variations in surface water quality across the north-west coast of India during 2012–2014 based on seasonal and annual time series observations. Based on the obtained information, hierarchical cluster analysis grouped the sampling areas into three clusters with analogous characteristics. While reduction of data was not achieved by principal component analysis. However, it enabled to identify potential sources and factors that induce deterioration of water quality. Parameters solely responsible for spatial disparity in water quality include wastewater discharges containing fecal coliforms, nutrients and BOD. In case of seasonal variations, PCA yielded data reduction highlighting few indicator parameters (fecal source, oxygen demand, nutrients, temperature) accounting to more than 75-80% of variation in water quality throughout all three seasons. Besides significant correlations between coliform groups at many locations, this study is useful to recognize statistically significant season-wise and annual disparities in a load of coliforms. Thus, this study emphasizes on the usefulness of multivariate techniques to optimize monitoring and cost incurred during water quality assessment programs for active management.

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