

Assessment of enteric bacterial indicators and correlation with physico-chemical parameters in Veraval coast, India

Sunil Borade, Rutuja Dhawde, Aayushi Maloo, Gajbhiye, S.N, Anirudh Ram & Syed G Dastager*¹

CSIR-National Institute of Oceanography, Regional Center, Lokhandwala Road, Mumbai-400053, India

[E-mail: sg.dastager@ncl.res.in]

Received 30 July 2013; revised 7 March 2015

In present study, the prevalence of enteric bacterial indicators like *Escherichia coli*, *Salmonella*, *Shigella*, *Proteus*, *Klebsiella*, *Vibrio cholerae* and *Vibrio parahaemolyticus* like organism in water and sediment of Veraval coast were studied at six different locations in three different seasons. Statistical analysis of data showed that there is a significant increase in count in September (Monsoon) as compared to January (post monsoon). It was also found that the enteric bacterial count was higher in sediment than in surface waters in harbour mouth and during monsoon season. A strong correlation of bacterial counts with physico-chemical parameters was observed. Temperature, pH, salinity, Dissolved oxygen (DO), Nitrite (NO₂⁻) and Nitrate (NO₃⁻) showed negative correlation with the bacterial counts whereas Biological Oxygen demand (BOD), Ammonia (NH₄⁺) and Phosphate (PO₄³⁻) showed a positive correlation with the bacterial counts.

[**Keywords:** enteric bacteria; physicochemical parameters; coastal waters]

Introduction

Marine ecosystem is being threatened by the discharge of untreated sewage and industrial effluents, which ultimately affects the sustainability of the living resources and public health¹. The serious consequence of the increased human settlements near the coastal locations has a negative impact on the water quality². A main effect of such contamination is mainly on human health and the quality of the seafoods. In this regard, water testing is mandatory to enumerate the fecal bacterial count and presence of disease causing organisms³. Some microbial pathogens in the coastal environments were indigenous to the oceans, including *Vibrios*, whereas *Escherichia coli*, *Salmonella* spp, and *Shigella* spp, are allochthonous in nature which are introduced through agriculture, urban surface run off, waste water discharges and from domestic waste⁴⁻⁵. Microbial pathogen levels in the water samples can be estimated by enumerating the indicator organisms⁶. In India, despite several laws and regulations (Water Prevention and Control of Pollution Act 1974) promulgated by the authorities of the Coastal Zone Management, Environmental Protection Agency, and the Pollution Control Boards, sewer outflows, discharges of partially treated /untreated waste

from septic systems and storm water runoffs are still the major sources of pollution of coastal waters⁷.

Veraval lies on the Arabian Sea coast of the Kathiawar Peninsula on the south-west coast of Saurashtra, Gujarat, India. It is a port town with one of the Asia's largest fishing harbour. It also has a healthy boat making industry. Several fish processing factories are located there that export high-quality seafood to the United States, Southeast Asia, Japan, Europe, and the Gulf. Export of these goods provides significant economic source to its residents. One of India's biggest rayon manufacturers, Aditya Birla Nuvo Ltd., is also located in the Port of Veraval. Due to this reason Veraval coast is densely populated which results in sewage pollution as well as industrial pollution of the coastal water. Hence in order to document the microbial pollution, the assessment of enteric bacteria and their seasonal variation during postmonsoon, premonsoon and monsoon was conducted.

Materials and Methods

Veraval coast is located in Gujarat, India (Figure.1) and is a home to a large number of fish processing factories in Gujarat Industrial Development Corporation (G.I.D.C). Veraval

coast was analysed at six stations from the harbour mouth (0km) to offshore (5km). Field collections were carried out in January (postmonsoon), March (Premonsoon) and September (Monsoon) in the year 2011 for microbiological analysis. Water samples in duplicate were collected from six different stations. Details of latitude and longitude of study area shown in Figure.1. Four stations were located near harbour mouth (station-1, 2, 5 and 6) out of which station 1 and station 2 are in inner creek and station 5 and station 6 are in outer creek while two stations (station-3 and 4) were located offshore of the Veraval coast.



Figure.1. Map showing the location (with latitude and longitude) of sampling site in Veraval coast, Gujarat, India.

One litre water sample from each location was collected in to sterile screw capped bottles for bacteriological assessment and the sediment samples were collected by employing an alcohol rinsed and air-dried small Van Veen grab, which were aseptically transferred into new polyethylene bags using a sterile spatula. All samples were collected with precautions required for microbiological analysis, kept in an icebox and transported to the laboratory for further analysis. Following groups of enteric bacteria were quantified. Specific media (Hi-Media) used for enumerating bacterial counts are listed against them.

Sea water nutrient Agar
MacConkey Agar
m-Fc Agar
M7Hrhc Agar
Xylose lysine deoxycholate agar (XLD) Agar

Total viable counts (TVC)
Total coliforms (TC)
Fecal coliforms (FC)
Escherichia coli (EC)
Salmonella (SL)
Shigella (SH)
Proteus, *Klebsiella* (PK)

Thiosulphate citrate bile salts sucrose (TCBS) Agar
Vibrio parahemolyticus (VP), *Vibrio cholerae* (VC)

In this study, to reduce the uncertainties associated with counting the enteric bacteria as 'like organisms' for instance *Escherichia coli* like organism (ECLO), *Salmonella* like organism (SLO), *Shigella* like organism (SHLO), *Proteus Klebsiella* like organism (PKLO), *Vibrio parahemolyticus* like organism (VPLO) and *Vibrio cholerae* like organism (VCLO) were counted to assess the water quality. Different colonies from the specific media were picked out, purified and characterized by biochemical and morphological tests. We found that approximately 80% of ECLO, SLO, SHLO, PKLO, VPLO and VCLO are EC, SL, SH, PK, VP and VC respectively. Using this information, "nearly true" percentages of EC, SL, SH, PK, VP and VC from their 'LO' counts are calculated and presented here. Similar work was done by Mohandas *et al* (2000), Nagvenkar and Ramaiah (2009).

Seawater nutrient agar (SWNA) plates were incubated at room temperature ($26 \pm 0.5^\circ\text{C}$) and all other plates of specific media were incubated at 37°C at least for 24h to 48 h and final counts of colonies were noted. About 1 g wet sediment samples were suitably diluted using saline water blanks to attain 100 or 1000 fold dilutions. Two duplicate aliquots of 0.1 ml and 0.2 ml supernatants from both the dilutions were spread plated respectively on nutrient agar plates (prepared at least three days prior to plating) and selective media and incubated as mentioned above. Total bacterial counts were expressed as colony forming units in water (CFU/mL) and sediments (CFU/g) respectively⁸.

Duplicate water samples were collected by using Niskin water sampler. Atmospheric and surface water temperatures were noted at the sampling point itself using standard mercury filled centigrade thermometer. pH was measured on a microprocessor-based pH analyser, Dissolved oxygen was determined by modified Winkler's method⁹ and Biological Oxygen Demand (BOD) was done by using direct seedling method¹⁰. Analysis procedure for phosphate, nitrate, nitrite and ammonia was adopted from methods as described by Parsons *et al*¹¹. Reagents used for the analysis were AR grade and double distilled water was used for preparation of solutions.

Statistical tests to analyse the data and establish correlations between microbial population and physico-chemical parameters were undertaken

using STATISTICA 7.0 software.

Results and Discussions

On the basis of media manufacturer's guide and on the knowledge of innumerable previous analyses, typical colony morphology characteristics of different bacterial groups were recognized and initial enumeration of enteric bacteria was completed. Typical colony characteristics of each group are listed below:

MacConkeys agar: all pink colored colonies grown on this medium counted as total coliforms. Fecal coliforms were counted using m-Fc agar with rosolic acid as an indicator with blue color colonies. *Escherichia coli* like organisms were enumerated on M7Hrhc agar showing yellow color colonies. Pink with black centered colonies as *Salmonella*, Pink/red colonies were enumerated as *Shigella* and yellow mucoid color colonies as *Proteus Klebsiella* on xylose lysine deoxycholate agar. TCBS Agar: sucrose fermenting, yellow, entire, raised <2mm diameter colonies were counted as *Vibrio cholerae*. Punctate, raised, bluish green, ~1 mm diameter colonies were enumerated as *Vibrio parahemolyticus* like organism.

Microbial counts of total viable count in the water samples varied from 4.8×10^3 to 1.8×10^4 CFU/mL registering minimum and maximum during the premonsoon and monsoon seasons (Table 1). In case of sediment samples, count varies from 48×10^4 to 18.6×10^5 CFU/g during the premonsoon and monsoon seasons. Higher counts of 123 CFU/mL of water sample and 70×10^2 CFU/g of sediment sample were recorded at Station-1 particularly during monsoon season for total coliforms. All bacterial counts were high in sediment samples as compared to water samples. Similar findings were reported by Nagvenkar and Ramaiah¹²; Haller¹³. High bacterial load in sediments may be due to enhanced survival by low or no exposure to stressors, such as sunlight and predation or by increased availability of nutrients as observed and recorded¹⁴⁻¹⁵. Higher TVC counts were observed in monsoon season when compared with postmonsoon and premonsoon. Similar observations were reported at many locations along the south Gujarat coast by Mohandas *et al.*¹⁶. Fecal coliforms recorded maximum count of 98 CFU/mL and 50×10^2 CFU/g at Station-1 in water and sediment samples respectively during monsoon season. Total coliforms and fecal coliforms were not detected in Station-3 and station-4 (offshore located) in water

sample during premonsoon respectively. Maximum counts of *Escherichia coli* 28 CFU/mL and 30×10^2 CFU/g at Station-1 in water and sediment were detected respectively during monsoon season. Water and sediment samples of Station-3 (offshore) in particular does not account for *Escherichia coli* during premonsoon season. Total coliforms, fecal coliforms and *Escherichia coli* counts in Veraval coast was relatively high in monsoon than the postmonsoon and premonsoon, which suggests the role of precipitation on the sources and extent of microbial pollution in the given area. This observation was much correlated with the results reported by Sood *et al.*¹⁷. During the present study, it was observed that, station-1 showed the presence of highest microbial counts as compared to other stations. The coast of Veraval is more densely populated and faces heavy anthropogenic activities near harbour mouth i.e, Station-1. The reason being higher sewage contamination leads to increase in the number of coliforms in natural water bodies.

Hansen and Bech¹⁸ suggest that, in the river environment the proliferation of allochthonous microflora occurs. However, the total coliforms, fecal coliform counts in Veraval coast was lesser than those reported from Mandovi and Zauri river of Goa by Nagvenkar and Ramaiah¹² and Gangetic River by Baghel *et al.*¹⁹. The enteric pathogens studied were *Salmonella*, *Shigella*, *Proteus Klebsiella*, *Vibrio cholerae* and *Vibrio parahemolyticus*. Uncountable pathogenic bacteria constitute the microflora of effluents discharged from the different activities²⁰.

Salmonella in the water samples of Station-6 were as low as 1 CFU/mL during postmonsoon, and as high as 58 CFU/mL at Station-1 during monsoon season. Sediment samples did not show any presence of *Salmonella* in any stations during the premonsoon and postmonsoon seasons. Higher counts of 20×10^2 CFU/g were recorded particularly at Station-1 during monsoon season.

Bacterial counts for enteric bacterium like *Shigella* were minimum during premonsoon, and maximum counts of 48 CFU/mL were observed during monsoon seasons. Sediment samples have not shown counts for *Shigella* during premonsoon whereas maximum counts of 40×10^2 CFU/g were recorded during monsoon seasons. *Proteus Klebsiella* were recorded maximum counts of 70 CFU/mL in water samples during monsoon season whereas in sediment samples counts of 50×10^2 CFU/g were found during monsoon season. Station-1 in particular, for water sample and

Station-2 for sediment samples showed maximum counts of *Proteus Klebsiella* during monsoon season.

Table.1. Average values of enteric bacterial population at different stations during three seasons at Veraval coast in 2011.

Sample site	Enteric Bacterial indicators	Water (CFU/mL)			Sediment (x10 ² CFU/g)		
		Post	Pre	Monsoon	Post	Pre	Monsoon
Station 1	TC	85.0	58.0	123.0	10.0	10.0	70.0
	FC	40.0	46.0	98.0	30.0	10.0	50.0
	E _{CLO}	44.0	18.0	28.0	20.0	10.0	30.0
	SLO	09.0	05.0	58.0	ND	ND	20.0
	SHLO	23.0	13.0	48.0	20.0	ND	40.0
	PKLO	48.0	34.0	70.0	20.0	10.0	20.0
	V _{CLO}	49.0	47.0	58.0	10.0	ND	10.0
Station 2	V _{PLO}	18.0	05.0	67.0	20.0	13.0	26.0
	TC	26.0	18.0	58.0	30.0	40.0	70.0
	FC	44.0	18.0	07.0	30.0	20.0	50.0
	E _{CLO}	07.0	09.0	18.0	ND	10.0	10.0
	SLO	03.0	03.0	16.0	ND	ND	10.0
	SHLO	28.0	04.0	18.0	10.0	ND	10.0
	PKLO	22.0	18.0	36.0	10.0	10.0	50.0
Station 3	V _{CLO}	27.0	13.0	46.0	10.0	ND	10.0
	V _{PLO}	19.0	11.0	58.0	20.0	10.0	40.0
	TC	23.0	00.0	75.0	50.0	ND	10.0
	FC	07.0	00.0	42.0	20.0	ND	10.0
	E _{CLO}	06.0	00.0	23.0	ND	ND	10.0
	SLO	06.0	03.0	13.0	ND	ND	10.0
	SHLO	05.0	03.0	28.0	20.0	ND	20.0
Station 4	PKLO	11.0	05.0	47.0	ND	ND	ND
	V _{CLO}	05.0	00.0	38.0	10.0	ND	60.0
	V _{PLO}	02.0	00.0	05.0	ND	ND	10.0
	TC	25.0	00.0	06.0	30.0	ND	10.0
	FC	17.0	00.0	03.0	10.0	ND	10.0
	E _{CLO}	07.0	03.0	06.0	ND	ND	10.0
	SLO	03.0	03.0	10.0	ND	ND	10.0
Station 5	SHLO	03.0	03.0	08.0	10.0	ND	10.0
	PKLO	03.0	03.0	07.0	ND	ND	ND
	V _{CLO}	05.0	00.0	43.0	13	ND	13.0
	V _{PLO}	07.0	00.0	07.0	10.0	10.0	26.0
	TC	08.0	00.0	22.0	60.0	NS	NS
	FC	18.0	00.0	03.0	10.0	NS	NS
	E _{CLO}	08.0	00.0	06.0	ND	NS	NS
Station 6	SLO	12.0	05.0	03.0	ND	NS	NS
	SHLO	03.0	01.0	08.0	10.0	NS	NS
	PKLO	08.0	06.0	38.0	ND	NS	NS
	V _{CLO}	40.0	00.0	25.0	ND	NS	NS
	V _{PLO}	07.0	00.0	08.0	ND	NS	NS
	TC	07.0	03.0	22.0	NS	NS	NS
	FC	03.0	00.0	03.0	NS	NS	NS
Station 6	E _{CLO}	03.0	00.0	06.0	NS	NS	NS
	SLO	01.0	05.0	08.0	NS	NS	NS
	SHLO	02.0	01.0	13.0	NS	NS	NS
	PKLO	06.0	08.0	10.0	NS	NS	NS
	V _{CLO}	07.0	00.0	28.0	NS	NS	NS
V _{PLO}	12.0	07.0	31.0	NS	NS	NS	

Note: ND, not detected; NS, no sediment sample due to rocky bottom

All reading are average values of three independent microbiological analysis

Bacterial counts for these organisms were recorded low at all stations and such counts are not capable of causing any immediate human health problems in the coastal environments. Counts of *Vibrio cholerae* and *Vibrio parahemolyticus* were not observed in water samples of Station-3, Station-4 and Station-5 in premonsoon season. Higher counts of 58 CFU/mL and 67 CFU/mL respectively were recorded at Station-1 particularly during monsoon season.

Sediment samples did not show presence of *Vibrio cholerae* during premonsoon at any stations, and higher count of 60x10² CFU/g was recorded at Station-3 during monsoon season. *Vibrio parahemolyticus* counts were not observed during postmonsoon and premonsoon seasons and maximum count of 40x10² CFU/g was recorded at Station-2 during monsoon season (Table1). Counts of *Vibrio* spp recorded was lesser than those reported by Swarnakumar *et al.*²¹ and their presence might not always been indicative of the human pathogenic nature. Seasonal variation in the counts of bacteria in water and sediment is represented using Box plots (Figure 2)

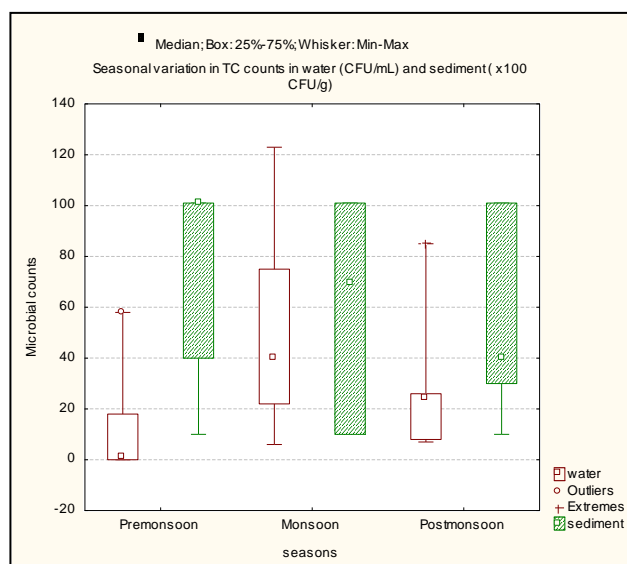


Fig 2(a): Box plot of Total coliforms

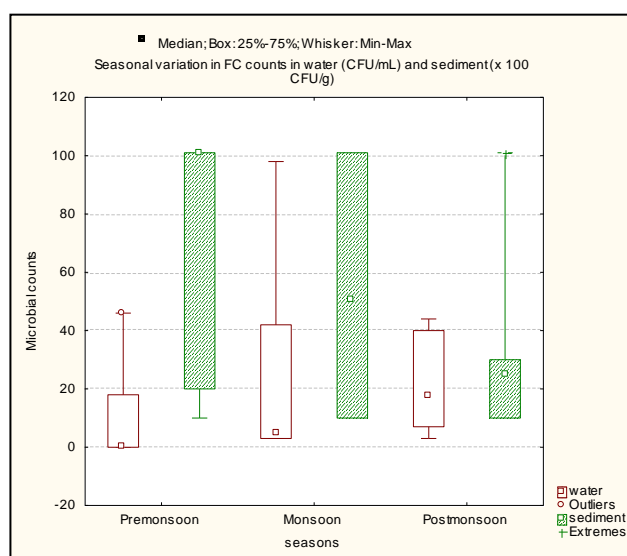


Fig 2(b): Box plot of fecal coliforms

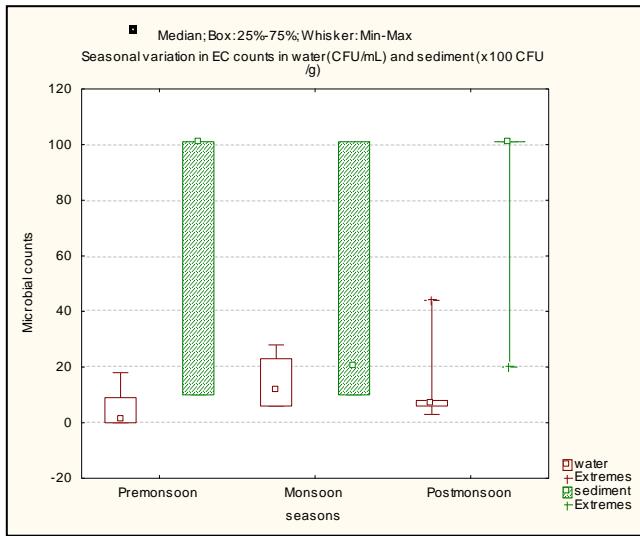


Fig 2(c): Box plot of *Escherichia coli* like organism

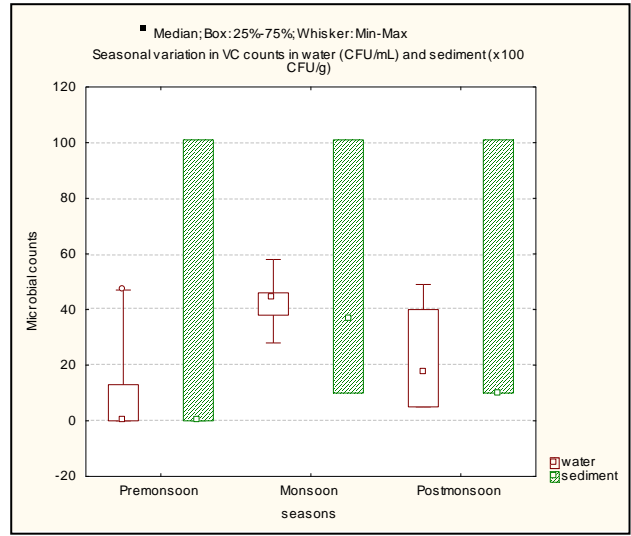


Fig 2(d): Box plot of *Vibrio cholerae* like organism

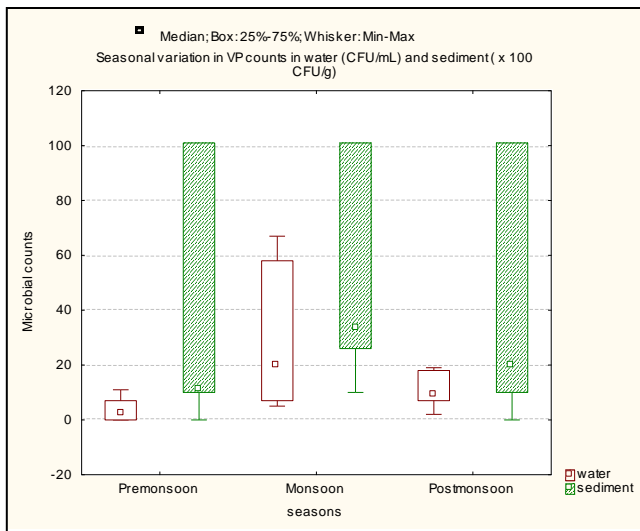


Fig 2(e): Box plot of *Vibrio parahaemolyticus* like organism

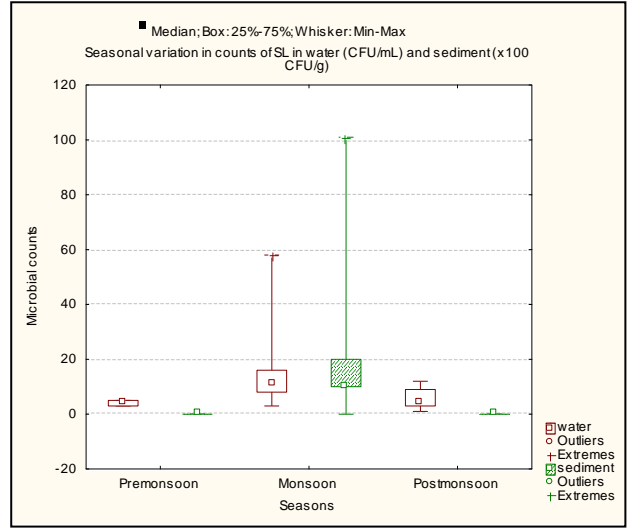


Fig 2(f): Box plot of *Salmonella* like organism

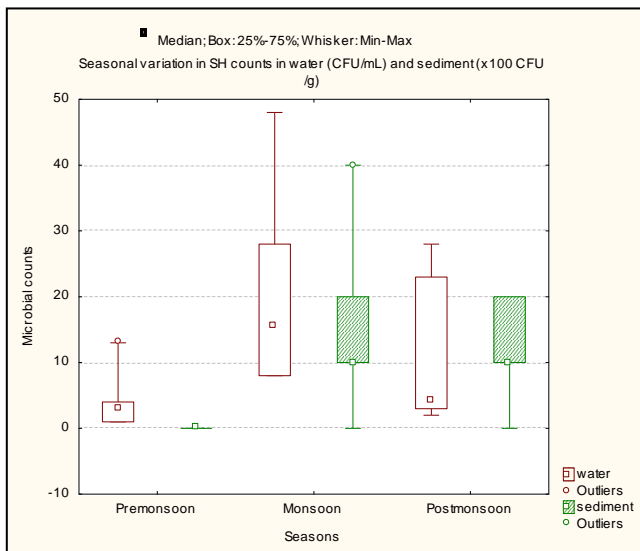


Fig 2(g): Box plot of *Shigella cholerae* like organism

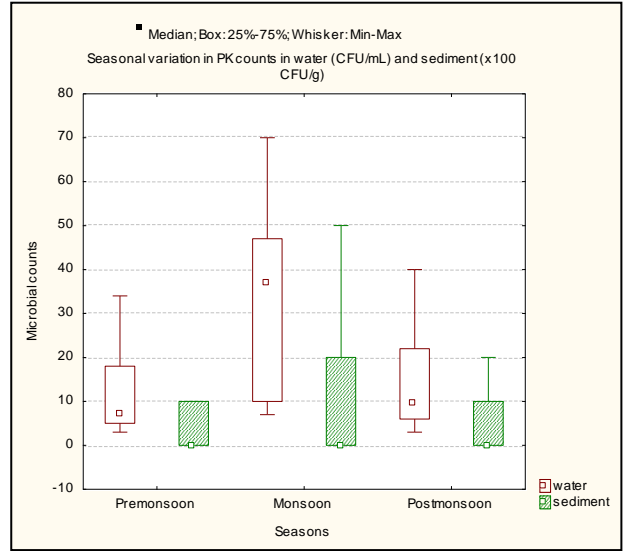


Fig 2(h): Box plot of *Proteus Klebsiella* like organism

Table.2. Correlation matrix showing the significance of environmental condition with bacterial population in different seasons.

	Temp	pH	Salinity	DO	BOD	NH4	NO3	NO2	PO4
TVC	-.9628 ^y	-.4869	-.7401	-.6366	.5197	.3511	-.4063	.0004	.9893 ^z
TC	-.3071	-.9545 ^z	-.8078	-.9450 ^z	.9237^z	.9650 ^z	-.4446	-.1814	.9738^z
FC	-.5515	-.9645 ^z	-.8594	-.9605 ^z	.9553 ^z	.9394^z	-.7897	-.3589	.9449 ^z
ECLO	-.5826	-.9629 ^z	-.9086	-.9659^z	.9808^z	.6611	-.5393	-.2461	.9490 ^y
SLO	-.7204	-.4388	-.5742	-.9245*	.3892	.9463*	-.3098	-.2957	.9843*
SHLO	-.2376	.1090	-.7756	-.7683	.5201	.9880^z	-.7806	-.4972	.9798 ^z
PKLO	-.2845	-.9720 ^z	-.9119	-.9704^z	.9573 ^z	.8911	-.7395	-.5542	.9541^z
VCLO	-.7457	-.9541 ^z	-.9230**	-.9425 ^z	.6760	.9725 ^z	-.8418	-.6165	.9934 ^z
VPLO	-.1894	-.9611*	-.6634	-.7257	.9277*	.8736	-.8620	-.5585	.5891

The application of Mann Whitney test demonstrated significantly higher contamination of Total coliforms, *Vibrio cholerae*, *Vibrio parahemolyticus*, *Salmonella*, *Shigella* and *Proteus Klebsiella* in monsoon as compared to premonsoon and postmonsoon seasons at $p < 0.05$ while fecal coliforms and *Escherichia coli* does not show significant difference in counts with respect to seasons. *Vibrio parahemolyticus*, *Salmonella* and *Shigella* showed significant difference between the counts of water and sediment on application of independent t- test at $p < 0.05$ ($t=2.57, t=2.71, t=2.6$ respectively and p value = 0.02 for all.)

The correlation among the microbial population and the physico-chemical parameters is presented in Table 2. Present results reveal that the biological oxygen demand has a positive correlation with total coliforms, fecal coliforms, *Escherichia coli*, and *Vibrio parahemolyticus* during monsoon season. Ammonia and phosphate concentration also reflected a strong correlation with total coliforms, fecal coliforms, *Shigella*, *Vibrio cholerae* during monsoon season. Temperature, pH and salinity showed negative correlation with the bacterial populations. Overall correlation matrix showed a very good positive relationship with biological oxygen demand, ammonia, phosphate with almost all the microbial populations particularly during monsoon season which indicates the influence of settlements and the mixing of untreated sewage and effluents in the coastal areas. Similar results were reported by Ajithkumar *et al.*²²

Conclusion

Enumeration of enteric bacterial indicators in the Veraval coast revealed anthropogenic activity, sewage and industrial pollution at an alarming rate. Increase in counts of indicator organisms especially in monsoon may be attributed to the change in physico-chemical conditions influence of settlements, addition of terrigenous material and mixing of untreated sewage and industrial effluents in the coastal waters. Albeit being the home for Asia's largest commercial port, Veraval coast has been subjected to heavy pollution from domestic sewage and industrial effluents. Moreover no proper measures has been taken to improve the conditions prevailing there, the present results indicate, there is a need for better designed criteria for construction of proper canals and longer outfall pipes along the coast for discharging sewage at proper location and to allow its better tidal flushing. This may reduce the load and accumulation of enteric and pathogenic bacteria in coastal waters, thereby reducing the chances of threat to humans.

Acknowledgments

Author thanks Director, National Institute of Oceanography for facilities and encouragement. Syed G. Dastager is acknowledging the Council of Scientific and Industrial Research (CSIR), New Delhi, India, supported from the grants under OLP1209 and GAP 2045.

The NIO contribution number is 5724.

References

- Cheung, W.H.S., Chang, K.C.K., and Hung, R.P.S., Health effects of beach water pollution in Hong Kong, *Epidemics Infection.*, **105** (1990) 139-162.
- Ramaiah, N., Coliform and human pathogenic bacteria in tourism affected water bodies in North Goa, *Coastin.*, **7**(2002) 13-16.
- Kumarswamy, P., Vignesh, S., Arthur James, R., Muthukumar, K., and Rajendran, A., Enumeration and Identification of Pathogenic Pollution Indicators in Cauvery Rivers, South India, *Res. J. Of Microbiol.*, **4** (2009) 540-549.
- Fujioka, R., *Microbial Indicators of Marine Recreational Water Quality. In : Manual of Environmental Microbiology*, Hurst, C. J., Crawford, G. Knudsen, M. J. McIneney and L. D. stetzenbach (Eds.), American Society for Microbiology Press, Washington, DC (2002)
- Robin, R.S, Vishnu Vardhan, K., Pradipta, R.M, Srinivasan, M., and Balasubramanian, T., Preponderance of Enteric Pathogens Along the Coastal Waters of Southern Kerala, Southwest Coast of India, *Mar Sci.*, **2** (2012) 6-11.
- WHO., Guidelines for safe recreational –Water Environments, Coastal and Fresh-Waters, World Health Organisation, Geneva, Switzerland (2003)
- Lipp, E.K., Kurz, R., Vincent, R., Rodrigues-Palacios, C., Farah, S.R., and Rose, J.B., The effects of seasonal variability and weather on microbial fecal pollution and enteric pathogens in a subtropical estuary, *Estuaries.*, **24** (2001) 266-276.
- APHA. Standard Methods for Water and Wastewater analysis. 18th edn American Public Health Association, Washington D.C (1985) USA
- Grasshoff, K., Ehrhardt, M., and Kremling, K., Methods of seawater Analysis. (Verlag Chemie Weinheim, Germany) 1999, 159-226.
- APHA, standards methods for the examination of water and waste water. 18th edn, American Public Health Association, Washington, DC (1995) USA
- Parsons, R.P, Maita, Y., and Lalli, C.M., A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford. (1992) 173
- Nagvenkar, G.S., and Ramaiah, N., Abundance of sewage – pollution indicator and human pathogenic bacteria in Mumbai waters , *Curr. Sci.*, **87** (2009) 435-439.
- Haller, L., Pote, J., Loizeau, J.L., and Wildi, W., Distribution and survival of fecal indicator bacteria in the sediment of the Bay of vidy, lake Geneva, Switzerland, *Ecological Indicators.*, **9** (2009) 540-547.
- La Rosa, T., Mrito, S., Marino, A., Alonzo, V., Maugeri, T.L., and Mazola, A., Hetrotrophic bacterial community and pollution indicators of mussels- farm impact in the gulf of Gaeta (Tyrrhenian sea), *Mar. Environ. Res.*, **52** (2001) 301-321.
- Craig, D.L., Fallowfield, H.J., and Cromar, N.J., Enumeration of fecal coliforms from recreational coastal sites: evaluation of techniques for the separation of bacteria from sediments, *J. of Appl. Microbiol.*, **93** (2002) 557-565.
- Mohandas, C., Shanta, N., Achthankutty, C.T., and Loka Bharthi, P.A., Pollution monitoring of coastal and estuarine areas, I- bacterial indicators along the south Gujarat coast, *Ind. J. of Mar. Sci.*, **29** (2000) 43-47
- Sood, A., Singh, K.D., Pandey, P., and Sharma, S., Assessment of bacterial indicators and physicochemical parameters to investigate pollution status of gangetic river system of Uttarkand (India), *Ecological Indicators.*, **8** (2008) 709-717.
- Hansen, B., and Bech, G., Bacteria associated with a marine planktonic copepod in culture. I. Bacterial genera in seawater and body surfaces, intestines and fecal pellets and succession during fecal pellet degradation, *J. of Plankton Res.*, **118** (1996) 257-273.
- Baghel, V.S., Gopal, K., Diwedi, S., and Tripathi, R.D., Bacterial indicators of fecal contamination of the Gangetic river system right at its source, *Ecological Indicators.*, **5** (2005) 49-56.
- Thompson, F.L., Iida, T., and Swings, J., Biodiversity of *vibrios*, *Microbiol. And. Mol. Review.*, **68** (2004) 403-431.
- Swarnakumar, N.S, Maloykumar, S., Sivakumar, K., and Thangardja, T., Assessment of microbial pollution in the coastal environs of the little Andaman island, India. *Ind. J. of Mar. Sci.*, **37** (2008) 146-152.
- Ajithkumar, T.T., Thangaradjou, T., and Kannan, L., Physico-chemical and biological properties of the Muthpettai mangrove in Tamil Nadu, India, *J. of Mar. Bio. Assess.* **48** (2006) 131-138.