Spatio-temporal Variations in Bacterial Abundance with an Emphasis on Fecal Indicator Bacteria and *Vibrio* spp. in and around Visakhapatnam Port, East Coast of India

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ABSTRACT The ecological health of port environments, which are dynamic and hotspots of anthropogenic activities, can be analyzed using specific pathogenic bacteria as they provide definite evidence and source of pollution. The influence of seasons and environmental settings on total bacterial count (TBC), distribution of fecal indicators, and *Vibrio* spp. was explored in Visakhapatnam port, located along the east coast of India. South-west monsoon had a significant influence on TBC, fecal indicators, and *Vibrio* spp., and the abundance was influenced by the eutrophic environment in the inner harbor. Fecal indicators were one order higher in sub-surface water when compared with sediment, indicating their inoculation due to turbulent conditions in south-west monsoon. The abundance of *V. cholerae* was influenced by salinity, temperature, and SPM, and was positively correlated to plankton; relating their distribution with disease dynamics and ecosystem functioning is a step ahead. Such an assessment is important from the perspective of human health and marine bioinvasion.

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1. INTRODUCTION

Port environments are hotspots of anthropogenic activity, and the quality of water in such environments is important from the perspective of the health of adjoining water bodies and distant locations where such water is transported through ships' ballast. Microbial communities are significantly influenced in such environments (Halpern et al. 2008). Bacteria are known to influence the food web dynamics in any ecosystem owing to their significant role in biogeochemical cycling (Azam et al. 1983). However, among the multitude of bacteria naturally present within the aquatic environment, including those derived from humans, a small proportion is only pathogenic which are either allochthonous or autochthonous and responsible for causing diseases in marine organisms and human beings (Neogi et al. (2014) and references therein). Ports act as gateways to bioinvasive species due to the extent of ballasting/deballasting activities in these areas. In addition to different macro-bio-invaders, bacteria can survive a long duration in ballast tanks (Desai et al. 2018), and by virtue of their abundance, and potential pathogenicity or toxicity, microorganisms have a greater capacity to invade and cause detrimental effects in new environments (Drake et al. 2007). The bacterial diversity at Paradip port, on the east coast of India, revealed the presence of several phyla and species, which were reportedly influenced by anthropogenic inputs (Pramanik et al. 2016). Thus, microbial communities in port environments are influenced by both anthropogenic and shipping activities, and the associated

risk to aquatic ecosystems and human health can be effectively determined by quantifying the pathogen pool. Natural phenomena, such as tidal influence and riverine discharge (Khandeparker et al. 2017b), also exert pressure on microorganisms and can alter their community structure.

In this study, we explored the influence of environmental characteristics on the abundance and distribution of pathogenic bacteria in the Visakhapatnam port environment, which is located along the east coast of India. Apart from the regular port activities, this port also receives the discharge of industrial effluents and untreated domestic sewage waste (Raman 1995). The spatiotemporal variations in the total bacterial count (TBC), allochthonous fecal indicators (*Streptococcus faecalis* and *Escherichia coli*) and autochthonous *Vibrio* spp. (*Vibrio cholerae*, *V. parahaemolyticus*, and *V. alginolyticus*) were studied in the sub-surface and near-bottom water column, sediment, and those associated with the plankton in the Visakhapatnam port environment. These observations were carried out as part of the Ballast Water Management Program, India. The introduction of pathogens non-native to any environment can have a detrimental impact on human health and economy, for example by affecting the fisheries and diversity of the ocean flora and fauna in general. This is of high importance as a large number of ships exchange or discharge their ballast into different regions of the harbor; it is important to understand the ecological status of the port area. Also, an increase in the coastal urbanization and shipping activity-related environmental disturbances and pollution demands a detailed coastal assessment of port ecosystems in terms

of microbial load, as this provides a reliable consequence of such pollution on marine biota as well as human health, directly or indirectly.

2. MATERIALS AND METHODS

2.1 Study area

Visakhapatnam port is a commercial sea port, located in the state of Andhra Pradesh on the east coast of India and is one of the major ports. It is located at a latitude of 17°41' North and longitude of 83°18' East (Figure 1). It is a landlocked, semi-enclosed water body with four radiating arms and a turner's basin. It receives outflow from sewage treatment plants and untreated sewage from other sources (Clark et al. 2003). The port consists of an outer harbor, an inner harbor, and a fishing harbor. The outer harbor, with a water spread of 200 hectares, has six berths, two breakwaters, and has an access to the open sea (Bay of Bengal). The inner harbor consists of a naturally protected entrance channel, a turning basin, and three navigable arms, and it has a water spread of 100 hectares with 18 berths. Major industries including petroleum, steel, power, and fertilizer, among other manufacturing industries, are setup near the port.

The study area experiences north-east and south-west monsoons and, being close to the sea, the humidity is high. The tidal flushing is inadequate, and stagnant conditions prevail in the Visakhapatnam harbor (Raman 1995).

2.2 Sampling

The present study was a part of the port biological baseline survey carried out for the Ballast Water Management Program, India. The sampling was carried out on three occasions in November–December 2007 (north-east monsoon: NEM), April 2008 (pre-monsoon: PrM) and August 2008 (south-west monsoon: SWM). The data on the microbial pollution in an enclosed area like that of the port ecosystem can be compared any time with the updated results. Thus, the data presented in this study will be useful to any future research related to microbes, either in Visakhapatnam port or other coastal waters.

The sampling stations were selected based on the activities of the port area. Twelve stations were selected for sampling. Out of the twelve stations, WQB-1, WQB-4, EQB-9, TB, HSY-2, WRF, and DCJT (1–7) are the inner harbor (IH) stations, while GCBS, FJT, TRCL, and ORB-2 (8–11) are the

outer harbor (OH) stations. OFSR (12) is the offshore station. All twelve stations were analyzed for water (sub-surface and near-bottom) and sediment, except at TRCL where no sediment was found, owing to high dredging activity. Six stations were analyzed for bacteria associated with plankton: WQB-4 (2), TB (4), WRF (6), DCJT (7), FJT (9), and TRCL (10). During the study period, observations were also carried out for dinoflagellate cysts (D'Silva et al. 2013), macrobenthic communities (Musale et al. 2015), and composition of phospholipid fatty acids (PLFAs; Harji et al. 2010) in the sediments of Visakhapatnam harbor.

2.3 Collection of samples

Niskin sampler (5L) was used for the collection of water samples. The water samples were collected from the sub-surface and near-bottom depths and analyzed for temperature, pH, salinity, nutrients, suspended particulate matter (SPM), dissolved oxygen (DO), and bacterial pathogens. The sediment samples were collected using Van-Veen grab with a grabbing area of 0.04 m², and the plankton samples were collected by taking horizontal hauls using a Heron Tranter (HT) plankton net of 100 µm mesh with a calibrated flow meter attached to it. All the samples were transported on ice for analysis to the laboratory. The samples (water, sediment, and plankton) to be analyzed for TBC were fixed with formaldehyde (final concentration 1 to 2%; v/v).

2.4 Physicochemical parameters

The pH, temperature, and salinity were measured using a multiparameter portable instrument (Merck 340i). The transparency of water was measured using a Secchi Disc 15 cm in diameter. For estimating SPM, a known quantity of seawater was filtered through a pre-weighed Millipore membrane filter (Filter type, HA, 0.4 µm) and reweighed after drying at 60°C. The DO content of the samples was assessed using the Wrinkler method, as described in Parsons et al. (1984). The BOD was assessed by incubating the water samples in amber-colored DO bottles for five days in the dark at room temperature, followed by estimating the unutilized oxygen. The levels of oxygen utilized in the bottles were estimated from the difference between the initial and balance values (Parsons et al. 1984). The nutrients—nitrite (NO₂-N), nitrate (NO₃-N), phosphate (PO₄-P), and silicate (SiO₃-Si)—were estimated using the standard procedures (Parsons et al. 1984).

2.5 Enumeration of culturable pathogenic bacteria from the water column, sediment, and plankton

The culturable pathogenic bacteria were quantified following the methods previously described by Khandeparker et al. (2015, 2017a). Briefly, water (sub-surface and near-bottom), sediment and plankton samples were diluted as required and spread plated (0.1 ml) on Zobell Marine Agar 2216 (Hi-media) plates. Quantification of culturable pathogenic bacteria was achieved using specific media following the manufacturer's instructions (Hi-media). The Thiosulphate-Citrate-Bile Salts (TCBS) Agar (Hi-media) containing sucrose as the carbon source was used for the quantification of *Vibrio* species, which differentiated *Vibrio cholerae*, *V. alginolyticus*, and *V. parahaemolyticus* (Pfeffer and Oliver 2003). The Mac Conkey Agar was used for *Escherichia coli*, and Enterococcus Confirmatory Agar was used for detecting *Streptococcus faecalis* (typically blue, <2 mm dia). All of the plates of specific media were incubated at 37°C for 24 h, and colonies were counted. The verification of dif-

FIGURE 1. Location of stations in Visakhapatnam harbor, east coast of India.

ferent pathogenic bacterial species isolated on different media was achieved using the MALDI TOF MS biotyping (MTB) method (Khandeparker et al. 2015). The culturable pathogenic bacteria in the water samples are expressed as Colony Forming Units (CFU ml⁻¹), whereas, those associated with the sediment and plankton are expressed as Colony Forming Units (CFU g⁻¹).

2.6 Enumeration of total bacterial count (TBC)

In the laboratory, the samples fixed for TBC were quantified using acridine orange and epifluorescence microscopy following the method described by Daley and Hobbie (1975). The offshore station was not sampled during south-west monsoon for water (surface and bottom) and sediment samples. The TBC is expressed as cells ml⁻¹ for water samples and cells g⁻¹ for sediment and plankton samples.

2.7 Data analysis

The spatiotemporal variations in bacterial abundances are presented as SURFER contours. The relationship between the environmental variables and bacterial abundance/bacterial species was investigated using canonical correspondence analysis (CCA), using the CANOCO program version 4.5 (ter Braak and Smilauer 1998). The data on bacterial abundance and bacterial species were log (x + 1) transformed. A forward selection was done on the set of environmental variables, and statistical significance of each variable was tested with a Monte Carlo permutation test using a reduced model with 999 numbers of permutations. Data of three samplings (north-east monsoon, pre-monsoon, and south-west monsoon) were selected for CCA analysis.

3. RESULTS AND DISCUSSION

Spatial and seasonal variations in the total bacterial count (TBC) in the sub-surface water was evident. The average TBC was comparatively higher in the south-west monsoon (Table 1), especially high numbers were evident at WQB-1 ($1.7 \times 10^7 \pm 9.0 \times 10^5$ cells ml⁻¹) WQB-4 ($1.8 \times 10^7 \pm 4.7 \times 10^5$ cells ml⁻¹) and EQB-9 ($1.2 \times 10^7 \pm 7.5 \times 10^5$ cells ml⁻¹), which are the stations located in the inner harbor. The north-east monsoon and pre-monsoon also showed high bacterial numbers in the inner harbor stations (Figure 2). The only outer harbor station showing high TBC irrespective of the seasons was GCBS, whereas during pre-monsoon and south-west monsoon, TBC was high at FJT. The near-bottom water harbored high TBC at most of the stations in the pre-monsoon, however, TBC was maximum during south-west monsoon at the inner harbor station, WQB-1 ($1.9 \times 10^7 \pm 1.71 \times 10^5$ cells ml⁻¹) and the outer harbor station, FJT ($1.23 \times 10^7 \pm 2.8 \times 10^5$ cells ml⁻¹; Figure 3). In the sediment, the TBC was low when compared with sub-surface and near-bottom water (Table 1), and it was low during the north-east monsoon (Figure 4). Out of the six stations that

were analyzed for bacteria associated with the plankton, the TBC was high at FJT during pre-monsoon ($1.7 \times 10^7 \pm 1.52 \times 10^6$ cells g⁻¹), an outer harbor station and at TB ($1.0 \times 10^7 \pm 8.6 \times 10^5$ cells g⁻¹) during north-east monsoon, which is an inner harbor station.

The salinity was high during south-west monsoon and low during north-east monsoon, the pH ranged from 7 to 8.6, and was higher during south-west monsoon. The concentration of DO was lower (0.6–2.9 mg L⁻¹), especially in the inner harbor stations WQB-4 and EQB-9 during the three sampling periods, whereas the BOD ranged between 0 and 20.8 mg L⁻¹ and was high in the inner harbor stations. The CCA analysis used to relate the bacterial abundance to environmental variables indicated that the TBC was influenced by pH in the sub-surface water (Figure 5), whereas it was influenced by NO₃, PO₄, and pH in the near-bottom water (Figure 6). The extent of eutrophication in the inner and outer stations of Visakhapatnam har-

FIGURE 2. Spatial and temporal variation in the TBC (cells ml⁻¹) in the sub-surface water of Visakhapatnam harbor, east coast of India, during different seasons. NEM: north-east monsoon, PrM: pre-monsoon, SWM: south-west monsoon, IH: inner harbor, OH: outer harbor. The scale bar represents log (x + 1) values.

TABLE 1. Seasonal variation in the average total bacterial count (TBC) in the water column (sub-surface, near-bottom), sediment, and plankton during different seasons (NEM, PrM, and SWM).

TBC	NEM	PrM	SWM
Sub-surface water	$6.0 \times 10^6 \pm 4.8 \times 10^5$	$4.6 \times 10^6 \pm 1.9 \times 10^5$	$1.0 \times 10^7 \pm 8.3 \times 10^5$
Near-bottom water	$2.1 \times 10^6 \pm 1.6 \times 10^5$	$4.6 \times 10^6 \pm 1.6 \times 10^5$	$6.1 \times 10^6 \pm 2.2 \times 10^5$
Sediment	$1.5 \times 10^5 \pm 2.3 \times 10^4$	$9.3 \times 10^5 \pm 6.5 \times 10^4$	$4.5 \times 10^5 \pm 4.5 \times 10^4$
Plankton	$4.6 \times 10^6 \pm 3.7 \times 10^5$	$5.3 \times 10^6 \pm 3.0 \times 10^5$	$3.1 \times 10^6 \pm 1.0 \times 10^5$

bor during the study period was evaluated using the nutrient index (I) by D'Silva et al. (2013) and indicated that the water quality of the harbor was mesotrophic except at the northern arm (west and east quay berths; WQB-1, WQB-4, EQB-9, and WRF) of the inner harbor, which was highly eutrophic. A decrease in the DO and high BOD at WQB-4 and EQB-9 in this study can be linked to high bacterial abundance suggesting its active utilization. During south-west monsoon, the concentrations of phosphate, and silicate were higher in the inner harbor stations (Figure 7). Conversely, chlorophyll *a* concentrations were lower during south-west monsoon (D'Silva et al. 2013). Thus, it seems that the microbial loop was predominant during south-west monsoon as the bacterial abundance was high. Visakhapatnam port is a land-locked port and has limitations of natural flushing processes, resulting in the exposure of plant and animal communities to stress-related environmental pollution (Rathod et al. 1995; Tripathy et al. 2005). Periodic outbursts of phytoplankton blooms and fish mortality have been observed in this harbor and have been related to organic pollution (Raman 1995). Recently, D'Silva et al. (2013) reported the distribution and abundance of dinoflagellate cysts in the sediments of Visakhapatnam harbor, which can serve as proxies for anthropogenic eutrophication. The impact of anthropogenic pressures was reflected in the distribution of dinoflagellate cysts in the sediments of Visakhapatnam harbor. The cyst production of *Protoceratium reticulatum*, a potentially harmful dinoflag-

ellate was high in the inner harbor stations, with higher nutrient concentrations. Harji et al. (2010) indicated that the tran-monounsaturated phospholipid fatty acid (PLFA), C18:1n9t, was one of the major PLFAs in the sediments of Visakhapatnam harbor. This PLFA seems to be produced by bacteria from the corresponding cis-isomer in response to stress (Findlay et al. 1990). The harbor receives municipal sewage, factory discharge, sulfur, and petroleum products (Kadam and Bhargale 1993), the presence of which creates anoxic conditions in the harbor which may put stress on benthic microbial communities. The existence of trans-monounsaturated PLFAs in the harbor sediments point out the stress-induced response of the bacterial communities. Je et al. (2004), while studying the changes in benthic communities along a pollution gradient in Vancouver Harbor, also showed the inner harbor to be dominated by pollution-tolerant species. (Harji et al. 2010) also assessed the bulk $\delta^{13}\text{C}_{\text{oc}}$ sediment signatures in Visakhapatnam harbor sediments collected during the sampling time of this study and suggested that the organic matter was of mixed type and from marine and terrestrial sources with a relative abundance of the latter at most of the stations (Harji et al. 2010). Another study by Musale et al. (2015) in Visakhapatnam port on the benthic communities, the occurrence of which provides information on the health of the ecosystem revealed an increase in the number of polychaete species observed over the last 20 years from this region. The abundance of macrobenthic forms varied with the inner and outer harbor

FIGURE 3. Spatial and temporal variation in the TBC (cells ml^{-1}) in the near-bottom water of Visakhapatnam harbor, east coast of India, during different seasons. PrM: pre-monsoon, SWM: south-west monsoon, NEM: north-east monsoon, IH: inner harbor, OH: outer harbor. The scale bar represents $\log(x+1)$ values.

FIGURE 4. Spatial and temporal variation in the TBC (cells g^{-1}) in the sediment of Visakhapatnam harbor, east coast of India, during different seasons. PrM: pre-monsoon, SWM: south-west monsoon, NEM: north-east monsoon, IH: inner harbor, OH: outer harbor. The scale bar represents $\log(x+1)$ values.

FIGURE 5. Canonical correspondence analysis (CCA) for the bacterial abundances in the sub-surface water and the environmental variables during PrM, SWM, and NEM in Visakhapatnam harbor, east coast of India. (a) Station biplot where different seasons are indicated by symbol, (b) sub-surface water bacterial species biplot.

regions, higher species diversity was observed in the outer harbor, suggesting that the outer harbor is semi-polluted or less stressed.

Nutrients and SPM variability are important factors in determining the bacterial abundance in a coastal environment (Khandeparker et al. 2017a), whereas silicate has been used as a proxy for diatoms, which forms a major portion of the microphytoplankton, and can further influence bacterial abundance and composition (Khandeparker et al. 2014). Musale et al. (2015) indicated that the monsoon season showed higher organic carbon in the sediment when compared to other seasons in the inner harbor. The coastal waters are subjected to natural as well as various forms of anthropogenic activities which can change their quality. The different sources of water pollution and the river runoff during the monsoon brings pathogenic bacteria into the coastal and estuarine waters (Pandey et al. 2014). Most of these pathogens are capable of causing deadly diseases in humans, such as typhoid, gastroenteritis, diarrhea, dysentery, cholera, infection, and food poisoning. Coliform pathogens from fecal contamination have been the most important indicators for monitoring the sanitary value of drinking and recreational waters, and their assessment has a vital role in marine pollution studies that have received public and scientific attentions in the recent years (Li et al. 2014). They are introduced into the aquatic habitats either through direct defecation, surface runoff, untreated

FIGURE 6. Canonical correspondence analysis (CCA) for the bacterial abundances in the near-bottom water and the environmental variables during PrM, SWM, and NEM in Visakhapatnam harbor, east coast of India. (a) Station biplot where different seasons are indicated by symbol, (b) near-bottom water bacterial species biplot.

sewage, or wastewater discharge. In the sub-surface water of Visakhapatnam harbor, fecal indicator *E. coli* was dominant at EQB-9 (6.7×10^3 CFU ml⁻¹) during pre-monsoon and at WQB-4 ($6.7 \times 10^3 \pm 5.6 \times 10^3$ CFU ml⁻¹) during south-west monsoon and north-east monsoon ($1.5 \times 10^4 \pm 4.1 \times 10^3$ CFU ml⁻¹), respectively, and both of these stations are located in the inner harbor. The average numbers of *E. coli* were low in the sediment during south-west monsoon, whereas they were high in numbers in the sub-surface water during this time (Table 2). The same was true in the case of *S. faecalis*, as well. Typically, bacteria in aquatic environments are strongly associated with particulate matter. The association of bacteria is stronger in finer sediments and is strongly influenced by the quantity and type of clay minerals and organic matter (Hassard et al. 2016; Gardade and Khandeparker 2017). The attachment of fecal coliforms to the fine particles increases their viability and transport to the sediment bed (Fries et al. 2006). The re-suspension of sediment can inoculate these bacteria back into the overlying water column. A large proportion of the pathogenic organisms present in water are associated with the sediment, which can be subjected to re-suspension. The decrease in bacterial abundance in the sediment with a subsequent increase in the water column suggests the impact of turbulent monsoon conditions on their re-suspension. Evaluating the survival and persistence of such pathogens under different environmental settings has implications in micro-

bial ecology and is of concern to human health. The abundance of *E. coli* and *S. faecalis* was significantly correlated with PO_4 , SPM, NO_3 , NH_4 , and Si in the inner harbor during north-east monsoon and south-west monsoon (Figure 5). In the estuarine and coastal environments, the survival of fecal indicator bacteria (mainly *E. coli*) has been positively correlated with the concentration of the SPM in the water column (Solo-Gabriele et al. 2000).

In the near-bottom water, *E. coli* numbers were also high during south-west monsoon in the inner harbor station, WRF ($7.7 \times 10^2 \pm 5.4 \times 10^2$ CFU ml^{-1}). However, an outer harbor station, TRCL, showed maximum numbers of *S. faecalis* compared with the other stations ($1.6 \times 10^3 \pm 6.6 \times 10^2$ CFU ml^{-1}). During SWM, WQB-4 showed the highest abundance of *E. coli* ($3.0 \times 10^5 \pm 6.0 \times 10^4$ CFU g^{-1}), associated with the zooplankton, whereas at TRCL during all three seasons high numbers of *E. coli* were observed. During the south-west monsoon, abundance of *S. faecalis* ($6.9 \times 10^5 \pm 3.5 \times 10^5$ CFU g^{-1}) was also high at WQB-4, which is an inner harbor station. The rivers bring a significant amount of viable bacteria to the coastal Bay of Bengal, and their abundance is proportional to the magnitude of discharge (Prasad et al. 2015). The influence of domestic or industrial pollution on viable bacterial counts dominated river-derived sources, as indicated by higher counts in the seawater off Visakhapatnam city (Prasad et al. 2015). It seems that in the present study, during the high runoff period of the monsoon, other than the terrestrial organic matter and nutrients, the plankton-associated bacteria were also high, leading to a higher abundance of indicator pathogens in the port. A previous study by Khandeparker and Anil (2013) demonstrated that zooplankton contains a high number of pathogenic bacteria, such as *V. cholerae*, *E. coli*, and *S. faecalis*.

Vibrio spp. are members of Gammaproteobacteria, which are generally found in marine and brackish environments and are serious human as well as seafood pathogens (Thompson et al. 2004; Mookerjee et al. 2014). Among the autochthonous bacteria, *V. cholerae* has been the most extensively studied bacterium in ballast water and is responsible for the life-threatening diarrheal disease, cholera, which causes rapid dehydration and even death of infected persons (Finkelstein 1996). *Vibrio cholerae* dominated the sub-surface water at DCJT (81%) during south-west monsoon and at WQB-4 (97%) in the north-east monsoon in the inner harbor. The near-bottom water also showed maximum abundance during south-west monsoon at DCJT ($1.6 \times 10^4 \pm 2.1 \times 10^4$ CFU ml^{-1}) and at WRF ($2.3 \times 10^4 \pm 2.4 \times$

10^4 CFU ml^{-1}), which is also an inner harbor station, during the pre-monsoon season, respectively. The abundance of *V. cholerae* and *V. alginolyticus* in the sub-surface water was influenced by temperature during south-west monsoon and nitrite (NO_2) in the pre-monsoon. In contrast, in the near-bottom water all *Vibrio* spp. were influenced by temperature, salinity, and SPM during south-west monsoon. Khandeparker et al. (2017a) have also reported that the *V. cholerae* abundance in Zuari estuary, located at the west coast of India, was strongly related to salinity and nitrite, and related to a possible reduction in nitrate by nitrate reducing *Vibrios*. A similar observation was also reported by Lara et al. (2011) at the eastern Sundarbans mangrove forest, Bangladesh. A recent study by Khandeparker et al. (2015) reported a high abundance of *Vibrio* spp. towards the high saline area of the Zuari estuary, indicating that their growth is favored by saline conditions. A study carried out by Neogi et al. (2012) along the tropical Karna-phuli estuary, located at the southern part of Bangladesh also indicated high *Vibrio* spp. abundance in saline water. *Vibrio cholerae* associated with the plankton showed matt growth and were high in numbers in almost all of the stations during south-west monsoon. During this time, fish-ery jetty (FJT) showed the highest abundance of *V. cholerae* in the sediment ($6.8 \times 10^5 \pm 1.4 \times 10^4$ CFU g^{-1}). Thus, as suggested by Khandeparker et al. (2015), the changes in plankton abundance, which are also governed by environmental factors seem to influence the autochthonous bacterial populations, especially that of *Vibrio* spp. The response of the bacterial populations to the changing environmental conditions is rapid, and an assessment of the health of such an ecosystem has a societal relevance. Although more than 200 O-antigen serogroups of *V. cholerae* are identified so far, only two serogroups, O1 and O139, are toxigenic and responsible for the cholera epidemics and pandemics (Sack et al. 2003). Kanungo et al. (2010) reported that high numbers of cholera outbreaks in India were found in West Bengal, Orissa, Maharashtra, and Kerala in 1997–2006. Cholera epidemics in India are seasonal, crop up in the Gangetic delta (Colwell 1996), located at the east coast of India, and usually relapse twice a year (Alam et al. 2007). In general, virulent pathogenic *Vibrio* species are expected to appear more frequently in tropical marine environments, owing to an increase in the virulence gene expression at elevated temperatures (Mahoney et al. 2010). Thus, relating their distribution and abundance with the disease dynamics and ecosystem functioning is a step ahead.

FIGURE 7. Variations in the environmental parameters (nitrate, nitrite, phosphate, and silicate) in sub-surface and near-bottom water at different stations in and around Visakhapatnam port during different seasons.

TABLE 2. Average abundances of bacterial populations at different stations of Visakhapatnam port from sub-surface (SW) and near-bottom (BW) water, sediment (S), and plankton (P) during different seasons. The values in the case of water are expressed as CFU ml⁻¹, and in the case of sediment and plankton as CFU g⁻¹.

Bacterial population	Source	NEM	PrM	SWM
<i>Escherichia coli</i>	SW	$1.4 \times 10^3 \pm 4.2 \times 10^2$	$7.5 \times 10^2 \pm 9.7 \times 10^1$	$1.6 \times 10^3 \pm 1.0 \times 10^3$
	BW	$5.1 \times 10^1 \pm 3.2 \times 10^1$	$1.3 \times 10^2 \pm 6.0 \times 10^1$	$1.4 \times 10^2 \pm 8.4 \times 10^1$
	S	$2.7 \times 10^3 \pm 2.0 \times 10^3$	$4.2 \times 10^3 \pm 2.5 \times 10^3$	$1.2 \times 10^2 \pm 2.8 \times 10^1$
	P	$4.8 \times 10^4 \pm 3.1 \times 10^4$	$3.3 \times 10^4 \pm 9.1 \times 10^3$	$1.5 \times 10^5 \pm 4.9 \times 10^4$
<i>Streptococcus faecalis</i>	SW	$2.9 \times 10^3 \pm 3.0 \times 10^3$	$1.3 \times 10^2 \pm 1.1 \times 10^2$	$4.5 \times 10^3 \pm 1.6 \times 10^3$
	BW	$1.0 \times 10^0 \pm 2.3 \times 10^0$	$1.4 \times 10^2 \pm 8.2 \times 10^1$	$1.5 \times 10^2 \pm 6.3 \times 10^1$
	S	$2.3 \times 10^4 \pm 2.3 \times 10^4$	$7.5 \times 10^3 \pm 8.3 \times 10^3$	$8.0 \times 10^2 \pm 3.5 \times 10^2$
	P	$4.4 \times 10^3 \pm 2.3 \times 10^3$	$9.2 \times 10^3 \pm 4.2 \times 10^3$	$1.5 \times 10^5 \pm 9.0 \times 10^4$
<i>Vibrio cholerae</i>	SW	$1.1 \times 10^3 \pm 8.9 \times 10^2$	$7.7 \times 10^2 \pm 4.4 \times 10^2$	$7.2 \times 10^4 \pm 9.3 \times 10^4$
	BW	$3.2 \times 10^1 \pm 2.9 \times 10^1$	$3.7 \times 10^3 \pm 3.6 \times 10^3$	$3.4 \times 10^3 \pm 2.7 \times 10^3$
	S	$1.8 \times 10^4 \pm 1.9 \times 10^4$	$7.3 \times 10^4 \pm 4.0 \times 10^4$	$1.4 \times 10^5 \pm 7.4 \times 10^4$
	P	$1.7 \times 10^5 \pm 1.1 \times 10^5$	$1.9 \times 10^5 \pm 1.6 \times 10^5$	$1.0 \times 10^7 \pm 1.3 \times 10^6$
<i>Vibrio parahaemolyticus</i>	SW	$5.7 \times 10^2 \pm 2.1 \times 10^2$	$1.7 \times 10^2 \pm 8.4 \times 10^1$	$1.0 \times 10^3 \pm 1.0 \times 10^3$
	BW	$1.6 \times 10^1 \pm 7.0 \times 10^0$	$3.5 \times 10^2 \pm 1.9 \times 10^2$	$2.7 \times 10^2 \pm 3.0 \times 10^2$
	S	$4.7 \times 10^3 \pm 2.8 \times 10^3$	$1.0 \times 10^4 \pm 4.4 \times 10^3$	$1.2 \times 10^4 \pm 4.8 \times 10^3$
	P	$7.4 \times 10^4 \pm 2.8 \times 10^4$	$1.7 \times 10^4 \pm 5.5 \times 10^3$	$6.4 \times 10^2 \pm 5.8 \times 10^2$
<i>Vibrio alginolyticus</i>	SW	$1.1 \times 10^3 \pm 8.9 \times 10^2$	$9.1 \times 10^2 \pm 3.4 \times 10^2$	$6.0 \times 10^2 \pm 2.6 \times 10^2$
	BW	$4.5 \times 10^1 \pm 1.5 \times 10^1$	$6.7 \times 10^2 \pm 2.9 \times 10^2$	$1.2 \times 10^3 \pm 4.6 \times 10^2$
	S	$9.3 \times 10^3 \pm 6.6 \times 10^3$	$1.1 \times 10^4 \pm 7.0 \times 10^3$	$7.7 \times 10^3 \pm 3.8 \times 10^3$
	P	$2.0 \times 10^5 \pm 1.0 \times 10^5$	$2.7 \times 10^4 \pm 2.0 \times 10^4$	$1.1 \times 10^4 \pm 7.3 \times 10^3$

Since the conventional plating method used for different culturable bacteriological analysis do not account for the non-culturable bacteria which may be metabolically active and infectious, the use of modern molecular tools to characterize the bacterial populations is the need of the hour. Recently, flow cytometry (FCM), an advanced analytical tool capable of quantifying both culturable and non-culturable bacteria, is being extensively used for counting microorganisms (Khandeparker et al. 2017a). The Fluorescence In-Situ Hybridization (FISH) allows the molecular phylogenetic discrimination and identification of specific bacteria within environmental samples. The coupling of FISH and FCM techniques can detect specific pathogens in the environmental samples and have been used recently for detecting and quantifying *Vibrio cholerae* populations from different geographic regions (Khandeparker et al. 2018). The genetics of uncultured microorganisms, metagenomics, is also emerging as a powerful tool (Khandeparker et al. 2017b). Therefore, future studies should integrate both molecular and automated analytical methods allowing quick and specific identification of target microorganisms. These methodological challenges can then unravel risk assessment due to the pathogenic load, their dynamics, and fate in the water column, which is poorly understood.

4. CONCLUSIONS

This study comprehensively elucidated the influence of seasons and environmental settings on bacterial abundance and distribution of pathogenic bacteria with an emphasis on allochthonous fecal indicators, and autochthonous *Vibrio* spp. in the Visakhapatnam port environment adjoining the Bay of Bengal, which is located along the east coast of India. This is important as a large number of vessels exchange or discharge their ballast into this region, so it is essential to understand the ecology and status of the port area. As such, a detailed assessment of the port ecosystem serves

as baseline data, which can be used as an initial snapshot to track progress and compare future or recent updates.

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AUTHORS' CONTRIBUTIONS

Conceptualization: LK, DD, SS, ACA; Sample collection: LK, DD, SS, VK; Sample analysis and data processing: LK, DD, VK; Writing, review & editing: LK, DD, SS, ACA; Funding acquisition: ACA. All authors have read and agreed to the published version of the manuscript.

COMPETING INTERESTS

The authors declare that they have no conflict of interest.

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