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MICROBIAL FLORA ISOLATED FROM POLLUTED AND NON-POLLUTED COASTAL WATERS OF KARACHI

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Abstract

Marine pollution has now become worldwide environmental concern. Continuous discharge of untreated industrial effluent, municipal and power plant's contaminated wastewater has been a serious threat to marine habitat, aesthetic values and interest of visitors to coastal areas. Karachi is the largest city of Pakistan and industrial hub of the Arabian Sea. In this investigation, samples of sediment, water, flora and fauna were taken from nine selected stations on Karachi coast included three stations represented major creeks i.e. Korangi, Gizri and Chinna Creek. These samples were taken during north-east and south-west monsoonal period in 2014. Bacterial flora isolated and identified from samples collected from these sites by conventional method. Among isolated and identified bacteria e.g. *Vibrio alginolyticus, Escherichia coli* and *Streptococuss anginosus* were the most dominant species contributing 21.43, 19.64 and 15.18 percent of total assemblage respectively. Among selected sample stations, Korangi creek station was found to be most polluted with coliform and other pathogenic bacteria. These results clearly indicate that threats from these pathogens are not only to marine life but also to the large number of visitors coming to beaches and residents of surrounding area. Moreover; immediate action should be taken to restrict the growth of these pathogens by taking measures to treat the municipal and industrial effluent to avoid outbreak of any disease in future.

Key words: Industrial effluent, Northeast monsoon, Southwest monsoon, Marine bacteria, Marine pollution

Introduction

Marine pollution has now become a worldwide environmental concern (Kennish, 1997). Anthropogenic activities play a major role in disturbing the ecosystem. Continuous discharge of untreated industrial effluent, municipal and power plant's contaminants to an alarming level is a major threat not only to marine life but deteriorating the aesthetic values of the coastal areas and resulting in loss of interest by national and international tourist thus indirectly effecting the Gross Domestic Product (GDP) of the country (Rabalais & Nixon, 2002; Jilani & Khan, 2013). This environmental crisis can only be solved by monitoring of marine coastal environment and identifying the root cause of origin, distribution, fate and behavior of marine pollutants, this can help to formulate an effective management strategy (De Wolf *et al*., 2005).

Pakistan coastal area is 990 km long starting from Southeast (Run of Katch) to Northwest (Gwader) along the Arabian Sea, divided into two geographical zones i.e. Balochistan coastal belt (745 Km) and Sindh coastal belt (245 Km). The Balochistan coastal belt is relatively pollution free. Sindh coastal belt includes Indus Delta and Karachi Coast. Metropolitan Karachi suffers very serious environmental problems, especially Manora Channel, is heavily polluted due to untreated industrial wastewater and Metropolitan municipal sewage which are indiscriminately discharged into coastal waters through Layari and Malir rivers. According to a report (Anon., 2007), only 20 percent of total annual wastewater produced in Metropolitan Karachi is treated and rest is discharged directly into coastal waters. Oil spills from cargo ships and oil tankers further enhancing the coastal water pollution. The dredging of channel round the year also contributes to coastal pollution in terms of suspended sediments load.

Researcher like Ali & Jilani, (1995); Saleem & Kazi, (1998) investigated the pollution load of Karachi coastal water but they only use physico-chemical and hydrological techniques. Mashiatullah *et al.* (2009; 2010); Nawaz & Ahmed (2011) reported marine bacteria but only *Escherichia coli* were observed in former study and antibacterial activity of marine bacteria were discussed in later.

Distribution, diversity and activities of marine bacteria in marine environment have been controlled by many hydro biogeochemical and physicochemical factors present in marine environment that have been well studied (Azam *et al.,* 1983). Marine environment provides favorable habitat for marine bacteria in respect of space and nutrients for which they have to compete. Such stressed condition push marine bacteria to produce many bioactive products having much medical, industrial importance (Armstrong *et al*., 2001). Marine bacteria received attention in aquaculture especially in processing and preservation of fish (Okazaki *et al*., 1975).

Large fraction of the total microbial community is undetectable by simple conventional methods. Therefore, most of the research on microbial diversity conducted by various researchers on sediment samples was based on molecular techniques (Polymenakou *et al.,* 2009; Schauer *et al.,* 2010). However, Physiological and phylogenic characteristics of marine microorganisms present in sediments and marine environments can be studied in better way by conventional methods, also giving the advantage to identify the new species that may have other biotechnological interest (Zengler *et al*., 2002; Pettit, 2011). Research conducted by Gärtner *et al*. (2011) in Mediterranean Sea revealed that most of the bacteria isolated and identified belong to the two phylogenetic groups Firmicutes and Actinobacteria.

Many techniques are available to monitor the marine coastal pollution (Costanzo *et al*., 2001). However, choice of the technique/s is governed by several factors, including the nature of pollutants and pollution problems, viability of the monitoring techniques and their cost effectiveness. In the present work, we have isolated and identified marine bacteria by conventional method from sea water, mud, animal and plant samples collected from different coastal stations of Karachi. It is aimed to monitor the biodiversity among bacterial flora, seasonal variation and spatial distribution of pathogenic and non-pathogenic bacterial flora along Karachi coast. This study will also provide a precise and comprehensive database of marine bacteria for future studies.

Materials and Methods

Study area: Karachi coast is located at latitude 24° 48´ N and longitude 66° 59´ E of the Arabian Sea. Total nine stations fwere selected on Karachi coast for this study as shown in Fig. 1 namely Karachi Fish Harbor, Chinna Creek, Mac Donald Point, Sea View, Village Wall Point, Sahil Avenue 2, Do Darya, Gizri Creek and Korangi Creek. Samples were collected in southeast and northwest monsoonal period of year-2014.

Sample collection: At time of sample collection, date, time and tide type were noted. Water quality parameters such as pH (HANNA Instruments, Romania), salinity (Refractometer ATAGO S/Mill-E, Japan) were measured, air temperature and sediment temperature were also noted using standard mercury filled centigrade thermometer. For bacteriological analysis sea water and mud sample were collected from surface (0 meter) in 50 ml Non-toxic, sterile, screw capped polyethylene falcon tubes while surface swab samples of rocks, flora and fauna present on coast were taken with TRANSWAB (M40 Compliant, medical wire, UK) having amies medium for transportation of aerobes and anaerobes. All samples were stored in ice box and transported to the laboratory within 2 hours.

Culture media: Marine Agar 2216, Nutrient Agar, Brain Heart Infusion Agar were used for propagation of marine bacterial cultures from samples. All three media were prepared in filtered, autoclaved aged sea water in order to give same conditions. Other selective media, Thiosulfate citrate bile salts sucrose agar (TCBS), Eosin methylene blue (EMB), Blood Agar, MacConkey's Agar, were also prepared in filtered, autoclaved aged sea water.

Bacteriological analysis: One ml of sea water sample and 1 gmof mud was dissolved in 9 ml filtered, autoclaved aged sea water (10-1dilution) and shaken vigorously. 100 µL of each diluted solution were inoculated in to sterile Petri plates of Marine Agar 2216, nutrient agar and Brain Heart Infusion Agar. Swab samples of fauna, flora and rocks were streaked on to the three main media. After inoculation plates were inverted and incubated at 28±2°C. After three days, different well isolated colonies were circled on the back of the plate and numbered. Cells of selected colonies were restreaked onto a nutrient agar plate, incubated at 28±2°C for 24hrs. Each colony was then identified on the basis of colonial, gram staining, morphological and biochemical testing. All of isolated bacterial strains were then preserved and stocked in nutrient broth containing 20% (vol/vol) glycerol and store at freezing temperature.

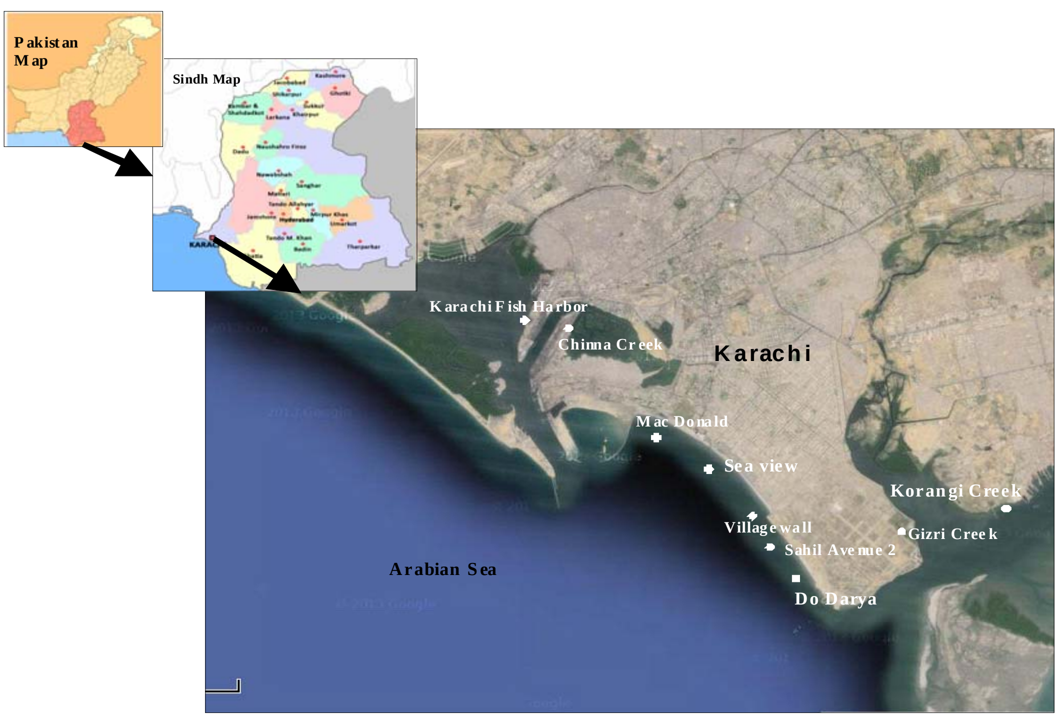

Fig. 1. Sampling stations of coastal waters of Karachi (Google Earth Map).

Results and Discussion

Total 112 bacterial strains were isolated from different samples taken in northeast and southwest monsoon season during 2014 from nine different selected stations on Karachi Coast. Bacteria were identified by culture plate method as per bergey's Manual of Determinative Bacteriology (Holt *et al.,* 1994). In identified marine bacteria, gram negative bacteria were in abundance (69.23%) as compare to gram positive bacteria (30.77%). Table 1 showing the Physical parameters observed at time of sampling during northeast (NE) and southwest (SW) monsoon period in year-2014. Tide type selected for sampling was high ranging from 1.89-2.43 mm length in NE whereas 2.2-2.92 mm length in SW. Temperature of atmosphere was ranging from 26-31^{o} C in NE where as 35-40^{o} C in SW. Whereas Temperature of Sea water was ranging from 25-30^{o} C in NE and 33-39^{o} C in SW. pH were in range of 7.4-9.2 in NE while 7.0-7.3 in SW. Salinity of sea water were measured in range of 35-38‰ in NE where as 35-37‰ in SW.

Some microbial pathogens in coastal environments are indigenous to the sea including Vibrios while others e.g. *Escherichia coli, salmonella* sp., and *shigella* sp., are introduced through urban surface run off, agricultural, waste water discharge and domestic and wild animals (Mahalakshmi *et al.,* 2011). Thirteen different marine bacterial species were isolated from samples collected during this study comprising gram positive bacteria which include *Bacillus subtilis, Staphylococuss aureus, Streptococuss anginosus* and *Streptococuss constellatus* and gram negative bacteria which include *Enterococuss faecalis*, *Escherichia coli*, *Proteus mirabilis*, *Psuedomonas aeroginosa, Salmonella typhimurium, Shigella boydii, Shigella sonnei, Vibrio alginolyticus* and *Vibrio parahaemolyticus.* Table 2 indicates a noticeable difference in percentage composition and types of each bacterial strain isolated from specific stations in northeast & southwest monsoon.

By the enumeration of bacteriological status on each station, public health significance and alarming indicator bacteria *Vibrio alginolyticus* and *Escherichia coli* (fecal origin) were in highest abundance with 21.43 & 19.64 percent of total assemblage respectively from all station's samples as represented in Fig. 3 Presence of high level of pathogenic bacteria in coastal waters e.g. (*Vibrio alginolyticus* and *Escherichia coli* with other pathogens) indicating high load of organic pollutants, heavy metals with domestic sewages(Nergis *et al.,* 2012). Presence of contaminant and their accumulation in microorganisms through sediments can cause biomagnifications via food chain as they increase biological oxygen demand, heavy metals, chemical oxygen demands, total salts, total suspended solids, fecal coliform and then eventually harm the human being. Unlike sewage, pH level of industrial effluents is beyond 6-9 (Nergis *et al.,* 2012) and once they mix with seawater, will eventually change the scenario of aquatic life.

Vibrio alginolyticus is gram negative, curved bacillus, single polar flagellum and facultative anaerobic organism that naturally inhabitant of sea water and distributed worldwide (Nichols *et al.,* 2000). Baker *et al.*(2010) suggested that vibrio species along with other pathogenic bacteria mostly appears in warm surface seawater and it is also attributed to climate change. *Vibrio alginolyticus* was dominantly found in present study along with *Vibrio parahaemolyticus*. Whereas, other researchers reported 35 species of *Vibrio* from marine environment, genus is able to multiply in salty water (Halophilic) at high temperature (Schets *et al.,* 2006) as in present study, seasonally highest positive isolations of marine bacteria were observed in southwest monsoon season followed by northeast monsoon season in period of 2014 shown in Fig. 2. It also known to cause diseases like wound infection, ear infection, gastroenteritis and septicemia, food intoxication, hemorrhaging and dark skin ulcer in human beings (Van Hooijdonk *et al*., 2006). Main pathogen that causes disease in marine animals such as marine fish, shrimp and shellfish is *Vibrio alginolyticus (*Selmin Ozer *et al.,* 2008). It was also noticed that clinical virulent potential of *Vibrio alginolyticus* were spread to human via consumption of raw or under cooked shell fish in form of gastroenteritis and invasive septicemia and contact to sea water in form of wound infection (Gomathi *et al.,* 2013). Whereas *Escherichia coli,* the second highest isolated stain, is gram negative, rod shaped, non spore forming bacteria that belongs to the family enerobacteriaceae and generally known as Coliform. It is mostly present in soil, water and digestive systems of warm blooded animals. These are present in large numbers in the feces and intestinal tracts of human that is way they are called as Coliform (Sattar & Ramia, 1981). Contact with contaminated water can lead to ear or skin infections, and inhalation can cause respiratory diseases (Mashiatullah *et al.,* 2010).

On the basis of coastal condition with season, noticeable difference was also observed in percentage composition of pathogenic bacterial population during both northeast & southwest monsoon in 2014. Creek areas of Karachi coast appear to be more polluted than other coastal areas. Korangi creek samples gave higher count for pathogenic bacteria (25%) in northeast monsoon where as village wall point samples gave higher count (19.44%) in southwest monsoon indicating overall unhygienic condition of coast around both monsoon as summarized in Fig. 4. Comparatively, Highest positive isolation of pathogenic bacteria were observed in Korangi creek samples followed by Gizri creek, Village wall point and Chinna creek in descending order 22.3, 14.3, 14.3 and 13.4 percent respectively as shown in Fig. 5. As there are more than 6300 industries are located at four main sites of Karachi coast e.g., Korangi Industrial area (KIA), Sindh Industrial Trading Centre (SITE), Landhi Industrial Area (LIA) and West Walf Industries (WWI) and one at Hub near to Karachi. All of them have no pre and post treatment facility in these industries and drain all of their effluent and municipal sewage to sea (Jilani *et al.,* 2013). Present study results also described the sources of bacterial contamination. Industrial effluent and municipal untreated sewage are the main sources that made the creeks area specially Korangi and Gizri creeks the most polluted coast with pathogenic bacteria as also mentioned in earlier publication, about 26.5% effluent reaches the coastal waters through Gizri-Korangi Creeks via Malir river and about 73.5% reaches through Karachi Harbour via Lyari river (Saleemi, 1993).

Table 1. Physical parameters observed during NE and SW monsoon in year-2014.

Physical parameters	Sampling stations: Karachi Fish Harbour		Chinna Creek		Mac Donald Point		Sea View		Village Wall Point		Sahil Avenue 2		Do Darya		Gizri Creek		Korangi Creek	
	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW
Date (DD/MM)	23/3	29/5	26/2	29/5	23/3	29/5	25/1	29/5	25/1	29/5	23/3	29/5	23/3	29/5	23/3	29/5	26/2	29/5
Time (24 Hrs)	17:30	14:00	7:00	13:30	17:00	13:00	6:30	12:30	6:00	12:00	16:45	11:30	16:30	11:00	16:00	10:20	8:30	10:00
Tide type	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High	High
Tide length mm)	2.16	2.2	2.42	2.39	2.28	2.61	1.89	2.74	2.1	2.92	2.31	2.91	2.35	2.92	2.43	2.75	2.24	2.59
Temperature (air), °C	29 ± 0	40 ± 0	27 ± 0	40 ± 0	29 ± 0.4	39 ± 0.4	26 ± 0	37 ± 0	26 ± 0	36 ± 0.5	30 ± 0	36 ± 0	30 ± 0	36 ± 0	31 ± 0	35 ± 0	28 ± 0	35 ± 0
Temperature (water), °C	29 ± 0	39 ± 0.2	26 ± 0.1	39 ± 0.1	29 ± 0	38 ± 0	25 ± 0	36 ± 0	25 ± 0	35 ± 0	29 ± 0	35 ± 0.1	30 ± 0	34 ± 0.9	29 ± 0	33 ± 0.9	27 ± 0	33 ± 0.8
pH	7.4	7.2	8.26	7.25	7.32	7.35	7.45	7.37	7.21	7.38	7.38	7.36	7.46	7.36	7.7	7.07	7.84	7.08
Salinity (‰)	36 ± 0.2	35 ± 0	35 ± 0	35 ± 0	3 6± 0.2	36 ± 0	38 ± 0	36 ± 0	36 ± 0	36 ± 0	36 ± 0	36 ± 0	36 ± 0	36 ± 0	35 ± 0.5	35 ± 0	36 ± 0	37 ± 0.7

Note: NE = Northeast monsoon & SW = Southwest monsoon

Table 2. Isolation of Marine bacteria from samples of Karachi coastal waters during NE and SW Monsoon in year-2014.

Bacterial species	Sampling stations: Karachi Fish Harbour		Chinna Creek		Mac Donald Point		Sea view		Village wall Point		Sahil Avenue 2		Do Darya		Gizri Creek		Korangi Creek	
	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW	NE	SW
Bacillus subtilus	-	-	+	-	-	+	+	+	+++	+	+	+	+	-	+++	+	+++	-
Enterococuss faecalis	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	-	+	++++	++	-	-	++	-	+	++	+	-	-	-	-	++	++++++	+
Proteus mirabillis	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+
Psuedomonas aeroginosa	-	-	-	-	++	-	-	-	-	+	-	+	-	-	-	-	+	-
Salmonella typhimurium	-	-	-	-	-	-	+	-	-	-	+	-	-	+	-	-	-	-
Shigella boydii	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+++	-	-	-
Shigella sonnei	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	++	-	+
Staphylococuss aureus	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptococuss anginosus	-	+	++	-	+	-	+	-	++++	-	++	-	+	-	+	-	+++	+
Streptococuss constellatus	-	-	-	-	-	-	++	-	-	-	-	-	-	-	-	-	-	-
Vibrio alginolyticus	-	-	++	++	-	+	+	-	-	+++	++++	++	-	-	-	+	++++++	++
Vibrio parahaemolyticus	-	-	+	-	-	-	++	-	+	-	-	-	-	-	+++	-	-	-

Note: + = Indicating number of strains isolated from individual sample, - = Indicating no isolation of bacterial strain, NE = Northeast monsoon & SW = Southwest monsoon

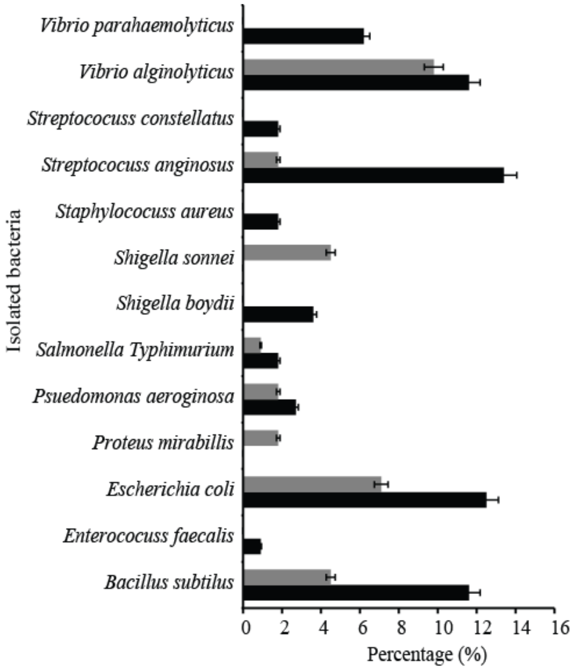

Fig. 2. Percentage composition of bacterial strains isolated from in northeast (NE) and southwest (SW) monsoon.

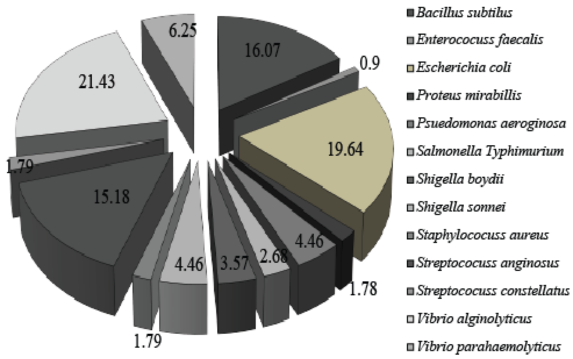

Fig. 3. Comparative abundance (in percentage) of bacterial strains isolated from coastal waters of Karachi.

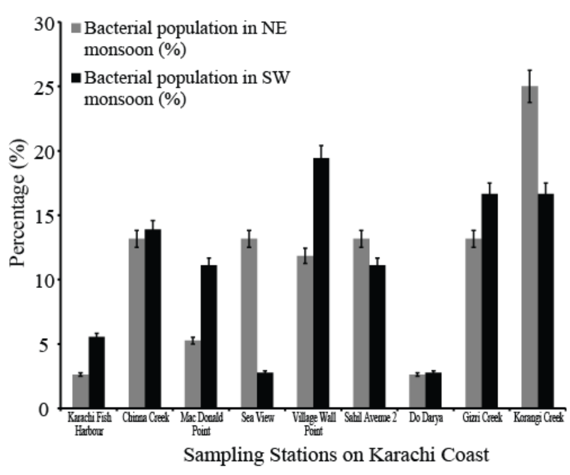

Fig. 4. Percentage (%) contamination of Karachi coast with bacterial population.

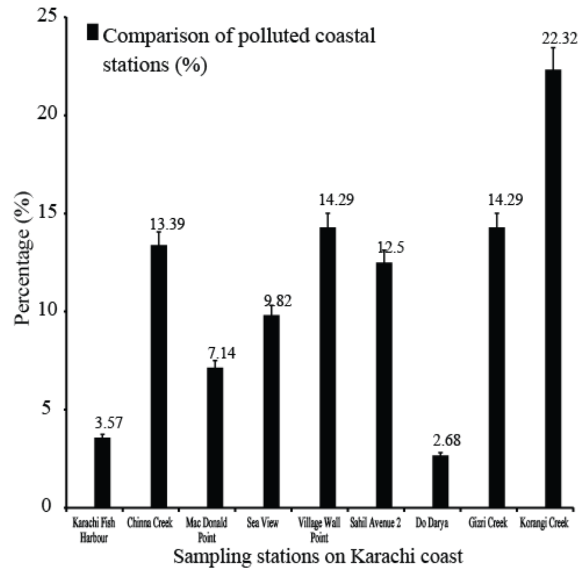

Fig. 5. Comparative polluted coastal waters of Karachi with pathogenic bacteria (%).

The mixing pattern of polluted rivers water with sea water appears to be dependent on high/low tide and distance of the sampling sites from the confluence points of polluted water bodies with the sea. Moreover; due to very close location of restaurant on village wall point sampling may also become the cause of mixing of untreated municipal sewage to the sea at this sampling site. High tide conditions retard the mixing of polluted water with seawater and low tide environment facilitates the mixing of pollutants. Due to semi enclosed nature, Layari river pollution is not completely flashed out during high tide condition, which results in increasing pollution levels in this area (Qadir *et al.,* 2011). It is also evident that pollution load of Malir indicates the large amount of domestic waste in the sea (Zaigham, 2004).

It is worth mentioning that in this study not only abundance of pathogenic marine bacteria were recorded in southwest monsoon period but isolation of same pathogenic bacteria form most polluted coast (Korangi creek) were also recorded during southwest monsoon period in 2014. Distribution of nutrients in sea water is mainly effected by movement of currents. During northeast monsoon (winter monsoon) currents in seawater move away from the coast and cause greater dilution to the pollutants in coastal waters where as during southwest monsoon (summer monsoon) currents in seawater sets in reverse. These changes in the current and wind patterns influence the nutrient circulation in seawater accordingly. Moreover; Due to which contamination of marine microorganisms with pathogenic bacteria from untreated municipal and industrial effluents flowing in Karachi coastal waters.

Conclusion

The present study may change total scenario of Karachi coastal waters condition. Those coastal waters which were considered to be as non pollutant (Mac Donald Point, Sea view, Village wall Point, Sahil Avenue 2 and Do Darya), this study clearly indicated that these non polluted coastal waters are also contaminated with domestic and industrial effluents and we can now not consider them safe for public usage. This situation urgently demands the community participation to make the protocol having effective procedures for improvement of quality of coastal waters. In industrialized countries, many types of water born diseases have been effectively controlled through the general introduction of innovative methods of water treatment and improved means of sewerage collection, treatment and disposal methods. So this situation in our coastal waters can also be controlled by enforcement of national standards, licensing and policing of discharges, strictly following the policy for treatments of discharges by industries near to coast, continuous check and balance of all these industries for waste management treatments plants.

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