

## QUANTITATIVE ANALYSES OF POLLUTION-INDICATOR AND PATHOGENIC BACTERIA IN MUMBAI WATERS FROM BALLAST WATER EXCHANGE PERSPECTIVE

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**Abstract.** As large numbers of ships either take in ballast from or discharge their ballast into the Mumbai Harbor region, it is pertinent to quantify microorganisms of health concerns and suggest on the suitability of water for ballasting purposes. To meet with this main aim, a selected set of general and pathogenic bacterial groups and their seasonal variations were studied from the Mumbai Harbor area. Sampling was carried out during postmonsoon (November 2001), pre-monsoon (April 2002) and monsoon (October 2002) periods. We quantified total coliforms and eight other groups of known human pathogenic bacteria from water, sediment, marine plant and animal samples collected in and around Mumbai Harbor. When compared with similar studies from other parts/harbors of the world, the Mumbai Harbor area has over 100-times higher levels of coliforms. The serotypes of *Escherichia coli* O157 and *Shigella-Alkaligens* Dispar group were abundant throughout the year. Even *Vibrio cholerae*, *V. parahaemolyticus*, *Salmonella* spp., campylobacters and aeromonads were present in large numbers. This comprehensive study, conducted with a view to quantify these bacterial groups and to evaluate the suitability of these waters for ballasting purposes, clearly suggests that both ballasting and deballasting be avoided in this region and alternative procedures should be developed for treating/handling ballast water.

**Keywords:** human pathogenic bacteria, ballast water management, Indian Ocean, Mumbai Post, serotyping, culturable bacterial populations

### 1. Introduction

From the annual translocation of ca. 12 billion tonnes of ballast water by bulk cargo ships, increasing numbers of non-indigenous organisms have become established in new locations around the world (Rigby, 2001). Environmental surveillance for documenting prevalence, persistence and transmittal, in particular of pollution indicator bacteria and those of human health concern, are the essential components of ballast water management. Their vast species diversity, wide-physiological adaptability and invidious capacities of a few of them to cause diseases in plants and animals including human beings call for careful investigations for an understanding of the deleterious effects that can be brought about through ballast movements. In addition to a variety of macro-organisms documented as bio-invaders (APHA, 1980; Carlton, 1989; Hallegraeff and Bolch, 1992; Hallegraeff, 1998), many bacteria can

survive long durations of 1–3 weeks in the ballast (McCarthy and Khambaty, 1994). When released in either coastal or oceanic zones, species/strains capable of competing with their indigenous counterparts finally become established. However, direct studies on translocation of bacteria through ballast are rare. From the sparse literature, it is explicit that several alien species/strains might have already been established in many new locations. Thus, even before the realization of – and scrutiny for – bio-invasion, the situations are apparently complicated to ascertain as to which bacterium is local, which one an invader.

Recently, Rigby (2001) reviewed the progress of a 10-year plan of mitigating the risks of translocating ballast water organisms. Many maritime countries developed or proposed several management and treatment options in the ensuing period. However, the exchange of ballast water at sea or in a designated area, continues to be the primary treatment option identified by the International Maritime Organization and all countries adopting some form of voluntary or mandatory control. Replacements of approximately 95% to close to 100% of the original water have been said to be possible through exchange of ballast water. As aptly suggested by this author, the efficiency of ‘removal’ of organisms is quite complex. Even after ballast water exchange, large numbers of harmful organisms may still be present in the water discharged into the receiving port. Concerns on locations where exchange takes place and the possible effects on the receiving port of alien organisms taken in during exchanges including the open ocean ones need scientific evaluation.

Mumbai Port is the busiest of the 11 major ports along the Indian coasts. Over 5000 ships of trade, defense, tourism, research and recreation interest visit this port annually and probably exchange ca. 50 million tons of ballast. Ships with bulk exporting and oil importing operations might exchange their ballast in the vicinity of this Port. Instances of the fouling bivalve, *Mytilopsis sallei* outwitting the local organisms around this port have been well documented (Anil *et al.*, 2002). Already, innumerable microbial species might have been transported or, have been brought into the port in the past. However, it is important to document the current levels of pollution indicators as well as pathogenic bacteria in any harbor to realize the levels of microbial hazards and provide baseline information useful for ballast water management. Besides the seasonal differences, this study has thus quantified a variety of bacterial groups.

## 2. Materials and Methods

Various sampling locations sampled in and around the Mumbai Harbor are shown in Figure 1. Sampling was carried out during November 2001, April and October 2002 representing post-monsoon (November–February), pre-monsoon (March–May) and monsoon (June–October) seasons, respectively. As ballasting and deballasting are usually within the docks and adjacent anchorage owing to stability concerns of the vessels, samples were collected from many docks. They included the

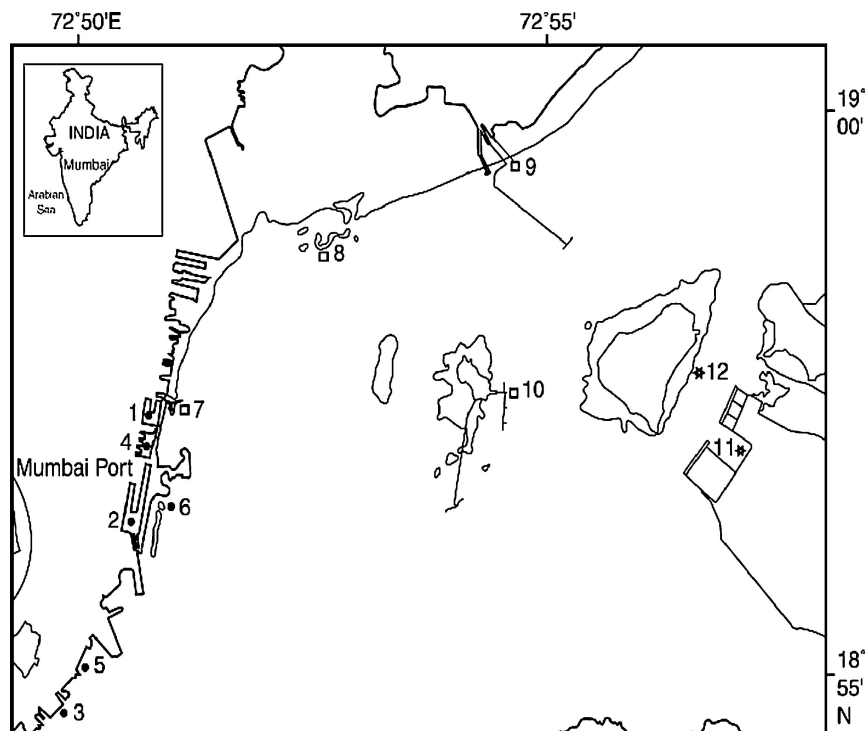


Figure 1. Sampling Locations in Mumbai Harbor area. Locations in most polluted zone (indicated by circles) are: (1) Princess Dock; (2) Indira Dock; (3) Sassoon Dock; (4) Victoria Dock; (5) Apollo Bunder; (6) Berth 20. Locations in moderately polluted zone (indicated by squares) are: (7) Ferry Wharf; (8) Mahul Creek; (9) New Pirpau Point and (10) Jawahar Dweep. Locations in less polluted zone (indicated by stars) are: (11) Jawaharlal Nehru Port Trust (JNPT) and (12) near Elephanta island. A control site (not shown in the map), at  $18^{\circ} 57' N$  and  $72^{\circ} 46' E$  located ca 25 km southwest of Princess Dock was sampled during all three collections for this study.

Princess Dock (PD), Indira Dock (ID), Sassoon Dock (SD) and Victoria Dock (VD). In the anchorage and other areas samples were taken from Apollo Bunder (AB), Berth 20 (B-20), Ferry Wharf (FW), Mahul Creek (MC), New Pirpau Point (NPP), Jawahar Dweep (JDB), Jawaharlal Nehru Port Trust (JNPT) and near Elephanta island (ELE). For comparison, samples were collected during all three seasons from a control site (CONT) about 25 km seaward from the PD. Sampling was so planned to represent most-polluted (PD, ID, SD, VD, AB and FW), moderately polluted (MC, B-20, NPP and JNPT) and less-polluted (JDB and ELE) zones in the Mumbai harbor – Mahul creek – JNPT areas. The sampling locations were chosen to generally represent a seaward gradient in sewage and other industrial effluents. Depths at sampling locations ranged from 9 to 17 m. Temperatures varied from 24.5 to 25.7 °C during November 2001, 28.5–30 °C during April and 26–26.5 °C during October 2002. Salinity is generally lower during October/November ranging from

29 to 34 PSU and quite high (32 to 36 PSU) during the premonsoon month of April. Water samples from ca. 1 m below the surface were collected by using a Niskin (5 L capacity, General Oceanics, FL) sampler. Five hundred milliliters of sample from each location was collected in to pre sterilized bottles. Approximately 100 g of sediment samples collected by a van Veen grab at different locations were removed aseptically into polythene bags. Up to 5 mL samples of zooplankton collected using a Heron Tranter net (200  $\mu$ m mesh sized bolting silk) were taken out for microbiological analyses. About 10–15 sessile barnacles attached to hard substrates were collected during each season. All samples were collected with precautions required for microbiological analyses, held on ice in an icebox and transported to the shore laboratory in Mumbai Port for all analyses.

During all the three collections, water samples were taken from PD, ID, SD, VD, AB, B-20, FW, MC, NPP, JDB, JNPT, ELE and CONT. While, sediment samples were collected from PD, ID, VD, B-20, FW, NPP, JNPT and CONT. Seaweed (mostly *Chaetomorpha* from Custom Jetty near FW: Figure 1), barnacles from PD and zooplankton samples collected from B-20 were also examined for different groups of bacteria.

The following groups of bacteria were quantified and specific media (Hi-Media, Mumbai, India) used for enumerating them are listed below.

Nutrient agar	– Total viable counts (TVC)
McConkey agar	– Total coliforms (TC)
Thiosulphate citrate bile salts sucrose (TCBS) Agar	– Total vibrios (VLO) <i>Vibrio cholerae</i> (VC) <i>Vibrio parahaemolyticus</i> (VP)
Xylose Lactose Deoxycholate (XLD) Agar	– <i>Salmonella</i> spp. (SA) <i>Shigella</i> spp. (SH)
<i>E. coli</i> O157 agar	– <i>E. coli</i> O157
Hi-Crome <i>Salmonella</i> agar	– <i>Salmonella</i> spp. (SA)
M <i>Enterococcus</i> agar	– Total Streptococci (TS)
<i>Aeromonas</i> isolation agar	– <i>Aeromonas</i> spp. (AA)
Blood-free <i>Campylobacter</i> selectivity agar base (BFCSAB)	– <i>Campylobacter</i> spp. (CB)
Bile esculin azide agar	– <i>Streptococcus faecalis</i> (SF)

All media except the TCBS (prepared using deionized water) were prepared with the addition of aged seawater to attain a minimum of 15 PSU salinity and autoclaved/steam sterilized as required. All plates were prepared 5–7 days prior to sampling. Samples were serially diluted using autoclaved 50% seawater blanks (1:1::aged seawater:deionized water) and aliquots of 0.2 mL and 0.3 mL of 10- or 100-times diluted water samples were spread plated in duplicates on nutrient agar plates prepared at least 5 days prior to plating. These volumes spread on to the medium in 90 mm dia plates were easily absorbed without any trace of liquid. For all selective media, 1–5 mL sample was filtered through 0.22  $\mu$ m membrane

filters (Millipore, Bangalore) and placed on respective selective media plates listed above. All plates were incubated at 37 °C at least for 24 h and final counts of colonies were noted. When very high counts of any specific group of bacteria were observed in any media ca. 24 h incubation, water samples held in a refrigerator were diluted 10- or 100-fold and reexamined as above by both spread plating and membrane filtration technique. In addition to these plate counts, total direct counts (TDC) of bacteria were enumerated from all the water samples following Hobbie *et al.* (1977) using acridine orange as the fluorochrome.

About 12–15 g wet sediment samples were suitably diluted using 50% seawater blanks to attain 100- or 1000-fold dilutions. Two replicate aliquots of 0.1 and 0.4 mL supernatants from both the dilutions were spread plated respectively on nutrient agar plates (prepared at least 5 days prior to plating) and selective media and incubated at 37 °C for 24 h. About 10–12 g wet animal and plant samples were homogenized in a tissue blender with appropriate volumes of seawater blanks to attain 100 or 1000 fold dilutions and replicate aliquots of 0.1 and 0.4 mL supernatants plated as done for sediments. Dry weights of all sediment, plant and animal samples used for enumeration of bacterial groups were determined and colony counts expressed on dry weight basis.

Typical colonies of *E. coli* O157 and *Shigella* spp. were serotyped using the antisera (Difco) by following the manufacturer's protocol. The specific antisera used were *Shigella-Alkaligenes* Dispar group and *E. coli* O antiserum O157 (Difco, MI)

About 100 typical *E. coli* O157 colonies from *E. coli* agar and *Shigella* like colonies from XLD agar were picked out carefully and checked for their agglutination.

### 3. Results

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 pollution indicator and pathogenic bacteria was completed. Typical colony characteristics of each group are listed below:

McConkey's agar all colonies grown on this medium counted as total coliforms. MEnterococcus Agar: all colonies counted as streptococci. TCBS Agar: all colonies grown on this medium counted as *Vibrio*-like organisms. Sucrose fermenting, yellow, entire, raised <2 mm dia colonies counted as *Vibrio cholerae*-like organisms. Punctate, raised, bluish green, ~1 mm dia colonies as *Vibrio parahaemolyticus*-like organisms. XLD Agar: reddish, small, round, convex colonies with black centers counted as *Salmonella* spp. (SA) and reddish, small colonies without black centers as *Shigella* spp. *E. coli* O157 Agar: bluish, entire, raised, glistening colonies counted as those of *E. coli* O157. *Aeromonas* Isolation Agar: yellow, entire 2–3 mm dia, glistening, convex colonies counted *Aeromonas* spp. Blood-free *Campylobacter*

TABLE I

Log values of mean  $\pm$  S.D. of different fractions of bacteria in water samples (no. mL<sup>-1</sup>) collected during different seasons from Mumbai Harbor area. These values represent the mean of 13 samples collected during each season

Bacterial types	Seasons		
	Premonsoon	Monsoon	Postmonsoon
Total direct counts	6.7938 $\pm$ 3.4595	6.7205 $\pm$ 4.1078	6.5261 $\pm$ 3.3312
Total viable counts	5.5711 $\pm$ 2.3867	5.9320 $\pm$ 2.6075	4.0190 $\pm$ 1.7497
Total coliforms	4.6687 $\pm$ 3.0318	4.1941 $\pm$ 0.4441	3.5784 $\pm$ 0.7952
<i>Vibrio</i> -like organisms	4.1232 $\pm$ 1.4549	3.7530 $\pm$ 0.6571	3.7692 $\pm$ 0.5539
Total streptococci	3.7012 $\pm$ 0.2577	3.9133 $\pm$ 1.3738	3.9621 $\pm$ 1.4180

Selectivity Agar Base (BFCSAB): translucent, whitish colonies counted as *Campylobacter* spp. Bile esculin azide Agar: lemon-yellow, entire, 2 mm dia colonies counted as *Streptococcus faecalis* (SF).

### 3.1. TOTAL AND INDICATOR BACTERIAL POPULATIONS

Although the sampling area covered for this study was quite large, on comparing the abundance between the sampling sites within the docks and in the anchorage area, differences in the abundance were not apparent. Thus, we present here the average abundances in water samples for each season (Table I) and collective averages for other samples (Table II).

Total direct counts (TDC) for water samples were the highest during premonsoon and the least during monsoon. The mean TDC ranged from 3.22–8.88  $\times 10^6$  mL<sup>-1</sup> during premonsoon, 0.30–1.51  $\times 10^6$  mL<sup>-1</sup> during monsoon and 2.40–3.85  $\times 10^6$  mL<sup>-1</sup> during post monsoon. Variations in total viable counts

TABLE II

Log values of mean  $\pm$  S.D. of different fractions of bacteria in sediment and animal (log numbers g<sup>-1</sup> dried wt.) samples collected during different seasons from Mumbai Harbor. In all, data from 24 sediment (eight samples during each season) and three (one per season) each of other sample types are presented here

Bacterial type	Sample type			
	Sediment	Barnacle	Zooplankton	<i>Chaetomorpha</i>
Total viable counts	8.3504 $\pm$ 6.9243	8.1547 $\pm$ 6.3850	11.3197 $\pm$ 9.5579	10.6497 $\pm$ 8.8784
Total coliforms	5.2506 $\pm$ 4.5690	7.05459 $\pm$ 5.1757	8.1362 $\pm$ 6.2411	8.5353 $\pm$ 6.7739
<i>Vibrio</i> -like organisms	7.3191 $\pm$ 3.5837	7.4506 $\pm$ 5.5684	8.9220 $\pm$ 7.1130	8.5831 $\pm$ 6.7410
Total streptococci	5.7437 $\pm$ 4.4343	5.0788 $\pm$ 3.2422	6.8303 $\pm$ 4.8431	4.2636 $\pm$ 2.4770

(TVC) were large; they were more during post monsoon and low during monsoon. In locations with stagnant waters such as ID, PD, SD, AB and B-20, the TVC were usually high. Among the fecal pollution indicator bacteria, such as TC, EC and TS, the counts of TC were the highest during premonsoon and the least during postmonsoon. They ranged from  $0.31\text{--}12.66 \times 10^4 \text{ mL}^{-1}$  during the premonsoon to  $0.11\text{--}3.69 \times 10^4 \text{ mL}^{-1}$  during monsoon and lower during postmonsoon. In the premonsoon month of April 2002, counts of *Vibrio* like organisms were very high ( $0.49\text{--}2.46 \times 10^4 \text{ mL}^{-1}$ ). TS did not show any location-wise trend. The mean densities of TS were higher during the postmonsoon and lower during premonsoon.

There were no seasonal differences in the counts either of TVC, TC, VLO or TS. Hence, their mean abundance and ranges in the sediment ( $n = 24$ ; 8 samples in each season), *Chaetomorpha* ( $n = 3$ ), zooplankton ( $n = 3$ ) and barnacle, *Balanus* sp. ( $n = 3$  but a minimum of three animals were homogenized together) samples are furnished in Table II.

### 3.2. SEROTYPING

Sixty-seven of the 102 (>65%) colonies counted as “*Shigella*-like organisms” were found to be serotypes of *Shigella-Alkaligens* group. Similarly, 85% (85/97) of *E. coli* O157 colonies agglutinated with *E. coli* O157 antiserum. From a previous fatty acid finger-print analysis (unpublished results) it was found that nearly 65% (8 of the 12 tested) isolates of *Vibrio parahaemolyticus*-like organisms from TCBS medium proved to be strains of *Vibrio parahaemolyticus*. Using these inferences we assumed that at least 50% colonies counted as like organisms actually belong to species we looked for. Therefore, the counts of all the following pathogenic bacteria are 50% of those counted as like organisms.

### 3.3. PATHOGENIC BACTERIAL ABUNDANCE

The abundance (log numbers) of eight pathogenic bacteria in the water samples during premonsoon (March–May; sampled in April), monsoon (June–October; October) or postmonsoon (November–February; November) are represented in Figure 2. Since we sampled over 13 sites each season, the data collected are numerous and therefore we have taken the mean values for all three seasons and represented them in this figure.

Briefly, during April and October, counts of human pathogenic *E. coli* O157 were generally high at several locations. On a seasonal basis, their counts were lower during premonsoon. Counts of *Vibrio cholerae* and *Vibrio parahaemolyticus* showed a similar trend as was seen with many other pathogens, both VC and VP were more abundant during premonsoon.

The mean abundance of *Salmonella* and *Shigella* spp. were higher during the premonsoon. Further, counts of *Salmonella* spp. (SA) and *E. coli* (EC) on Hi-Crome Salmonella Agar were in ranges comparable to those counted on XLD

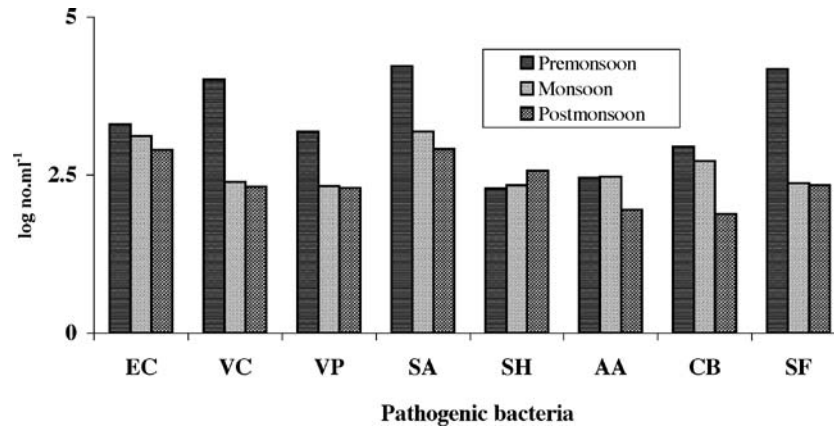


Figure 2. Log numbers of different pathogenic bacteria in water samples collected during different seasons. EC denotes *Escherichia coli O157*, VC: *Vibrio cholerae*, VP: *Vibrio parahaemolyticus*, SH: *Shigella* sp.; SA: *Salmonella* spp.; AA: *Aeromonas* spp., CB: *Campylobacter* spp. and SF: *Streptococcus faecalis*.

agar. *Streptococcus faecalis* (SF) varied widely between locations and seasons. Similar to most other pathogenic groups, their counts were very high during the premonsoon month of April 2002.

Colony forming units of *Aeromonas* spp. were recorded from most locations, in particular at all dock locations during all the seasons. Densities of *Campylobacter* spp. were also high during the premonsoon.

In the samples of sediment, *Chaetomorpha*, zooplankton and barnacle, though highly abundant (Figure 3), there were large variations among the pathogenic bacterial types; without any season- or location-wise trend.

#### 4. Discussion

Fecal pollution indicator bacteria such as TC, EC, TS are the ones routinely examined for an understanding of the preponderance of human pathogenic bacteria (APHA, 1980; Bordner and Winter, 1978). As is universally accepted, higher the counts of these organisms higher would be the incidence of human pathogenic bacterial species. It is adequate to realize the presence of pathogenic bacteria through indicator bacterial quantification. However, an understanding of the incidence of as many individual groups as possible would be necessary in ballast water management arena owing to the involvement of different geographic locations, shipping types, routes and other navigational characteristics.

Distribution of fecal coliforms, total coliforms and heterotrophic bacteria in the coastal waters of Hong Kong, one of the regions with most shipping related activities, was investigated by Ni and Lin (1986) with a view to establish a baseline



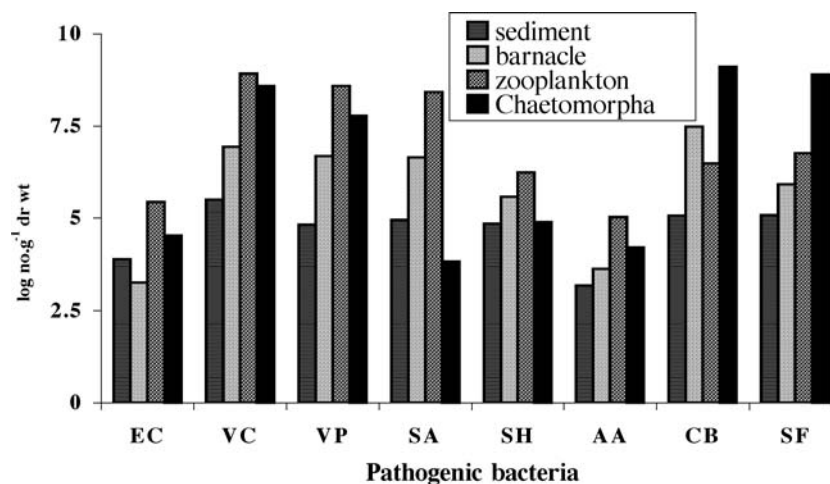


Figure 3. Log numbers of different pathogenic bacteria in sediment and other samples collected during different seasons. EC denotes *Escherichia coli* O157, VC: *Vibrio cholerae*, VP: *Vibrio parahaemolyticus*, SH: *Shigella* spp.; SA: *Salmonella* spp.; AA: *Aeromonas* spp., CB: *Campylobacter* spp. and SF: *Streptococcus faecalis*.

information. The numbers of fecal coliforms ranged from  $10^2$ – $10^5$  cells  $100\text{ mL}^{-1}$ , but were about  $10^4$  cells  $100\text{ mL}^{-1}$  for most stations. The numbers of total coliforms and heterotrophic bacteria ranged from  $10^4$ – $10^6$  and  $10^3$ – $10^7$  cells  $100\text{ mL}^{-1}$ , respectively. In comparison with the Hong Kong Harbor waters, total coliform counts are 2–3-orders higher in Mumbai Harbor. In addition, counts of coliforms observed during different seasons from Mumbai Port area are at least 2 orders of magnitude higher than those observed by George *et al.* (2001) in the Seine River and its estuary. In general, the Mumbai Harbor – Thana creek – Bassein Creek consisting of over 75 km stretch forming the eastern edge of the Mumbai Metropolis is understood to have high microbial activity, abundance (Neelam *et al.*, 1995; Ramaiah, 1994) and ecologically versatile bacterial types (Ramaiah and De, 2003). Though our sampling locations were chosen to represent pollution gradient in the area, we did not find significant reduction or seasonal differences of both indicator and pathogenic bacterial numbers from high- to low-pollution zones. Our observations during different seasons are helpful to understand the annual variations of both indicator (total coliforms) and, many human pathogenic bacteria. These observations are useful to suggest the non-suitability of waters from the Mumbai Harbor area for ballasting throughout the year.

*Vibrio cholerae* (causing cholera in humans), *V. parahaemolyticus* (gastroenteritis), *Salmonella* spp. (food poisoning), *Shigella* spp. (dysentery), *Streptococcus* spp. (meningitis and skin infections), *Campylobacter* spp. (campylobacteriosis) and aeromonads (septicaemic conditions) are potent human pathogens. As mentioned above, these pathogens were abundant in most locations despite pollution gradient

within the precincts of Mumbai Harbor. As is well known, biochemical characterization of different pathogens we enumerated would be laborious, time taking and often ending with large uncertainties in speciation. Thus, we adapted a combination of using highly reliable specific media to count the “like organisms” and results from serotyping of two groups of pathogens to arrive at most conservative estimates of the pathogenic bacterial species we looked for. We suggest that all those pathogenic groups with  $\geq 50$  cells  $\text{mL}^{-1}$  as of high intensity. This criterion of  $\geq 50$  cells of each specific pathogenic bacterium  $\text{mL}^{-1}$  was decided to be of high intensity for two reasons. Serotyping of a few groups was helpful to realize that there are large numbers of specific pathogens in the study area. Even when we are reporting only 50% of the “like organisms” as actual ones, their numbers far exceed 50 000 cells  $\text{L}^{-1}$  in many water samples. Secondly, with their survival being long enough (see below), any pathogenic bacterium with over 50 cells  $\text{mL}^{-1}$  is likely to survive long voyages and establish in newer locations under favorable environmental conditions. Though our list of different pathogenic bacterial groups examined is not exhaustive and – arguably – their identity yet putative, from the abundance of these bacterial types the implicit health hazard in these waters is easily discernible. In addition, continued daily effluent discharges of several million liters into this Harbor area from the Mumbai Metropolis bring in fresh stocks of coliforms and a plethora of other pathogenic bacteria, most of which can proliferate substantially in the adjoining creeks and dock waters.

Further, this first time comprehensive study on different pathogenic bacterial groups has yielded useful insights from the ballast water management perspective. In that, despite seasonal differences in the abundance of various groups of pathogenic bacteria studied, their counts are far more than those reported from Chesapeake Bay (Ruiz *et al.*, 2000). For example, the colony forming units of *Vibrio cholerae* were far more during the postmonsoon month of November 2001. Campylobacters were more during premonsoon (April 2002). Pathogenic bacteria formed quite a fraction of the total plate (viable) counts in sediment samples during different sampling months (Table III). Despite the apparently low percentages, most examined pathogens were quite numerous in all marine plant (*Chaetomorpha*) and animal (zooplankton and barnacle) samples (Figure 3). In the water samples, many pathogens accounted for 20–80% of plate counts.

In ballast tanks, pathogenic bacteria associate with living amoebae, many stages of shellfish larvae (Thom *et al.*, 1992) and dinoflagellate cysts. Their attachment to exoskeletons of chitin-bearing copepods, other plankton and seaweeds as well as their ability to survive unattached in ballast water contribute significantly to their survival (for several days to months) and distribution in the marine environment (Colwell *et al.*, 1977; Xu *et al.*, 1982; McCarthy, 1996). We have examined four pathogenic strains in seawater microcosms without any added nutrients (see Figure 4 as an example, and Vrushali-Kolhe and Ramaiah, unpublished results) had high numbers of direct viable counts (DVC, examined by the procedure of Kogure *et al.*, 1979) even after 10 weeks. Such observations are suggestive that many

TABLE III

Percent of indicator and pathogenic bacteria in total counts of colony forming units on nutrient agar from various samples collected from Mumbai Harbor. Percent of specific pathogens from EC O157 to CB were calculated as 50% of like organisms counted on their respective isolation media and after the serotyping of representative pathogens [see text]

Bacterial types	Water			Sediment	Plant and animal samples		
	Premonsoon	Monsoon	Postmonsoon		Barnacles	Zooplankton	<i>Chaetomorpha</i>
TC	12.52	1.83	36.25	0.08	7.04	0.07	0.096
VLO	3.56	0.66	56.25	9.30	19.77	0.40	0.858
TS	1.35	0.96	87.70	0.25	0.08	0.0032	0.000041
EC O157	1.59	0.45	22.73	8.56	0.0004	0.0395	0.0224
VC	8.21	0.08	5.77	3.35	17.98	0.12	2.57
VP	1.21	0.07	5.56	0.708	10.35	0.56	0.401
SA	13.34	0.53	22.88	0.947	9.375	0.38	0.000046
SH	0.15	0.075	10.43	0.757	0.797	0.0027	0.00051
FS	12.28	0.08	6.23	1.27	1.70	0.0084	5.39
AA	0.23	0.10	2.49	0.017	0.009	0.00015	0.000107
CB	0.71	0.18	2.15	1.245	64.45	0.0044	8.67

EC O157 denotes *Escherichia coli* O157, VC: *Vibrio cholerae*, VP: *Vibrio parahaemolyticus*, SA: *Salmonella* spp.; SH: *Shigella* sp. FS: *Streptococcus faecalis*; AA: *Aeromonas* spp. and CB: *Campylobacter* spp.

pathogenic strains can survive prolonged starvation or low nutrient situations. As ships' stability is the most major concern, vessels cannot be moved out of the ports for ballasting purposes. Thus, it is possible that most of the oil tankers and cargo vessels take in the ballast from the harbor area itself. Several investigators have examined distribution of indicator and human pathogenic bacteria as well as certain viruses in coastal waters with a view to quantify and understand their relationship with relevant environmental factors (Colwell *et al.*, 1977; Marchand, 1986; Patti *et al.*, 1987; Piccolomini *et al.*, 1987; Ruiz *et al.*, 2000). The mortality and survival rates of the fecal contamination indicator *Escherichia coli* in the marine regimes have also been studied (Darakas, 2001; Thom *et al.*, 1992). Their results affirm persistence of allochthonous microflora in the marine environment. As noted for different species of bacteria (Colwell *et al.*, 1981; Darakas, 2001; Xu *et al.*, 1982; Huq *et al.*, 1984; McCarthy *et al.*, 1992; McCarthy and Khambaty, 1994; Ramaiah *et al.*, 2002; Wait and Sobsey, 2000), at least small numbers of all pathogens we examined survive in seawater for one to several weeks. When taken in with ballast water and on deballasting, they disperse to other regions.

From the high counts of pathogens and their percentage fractions of TVC (Table III) as well as the total coliform loads in the study area we suggest that Mumbai waters are not suitable for ballast intake. A note of caution however, is that ships deballasting into these already poor quality waters might have been adding

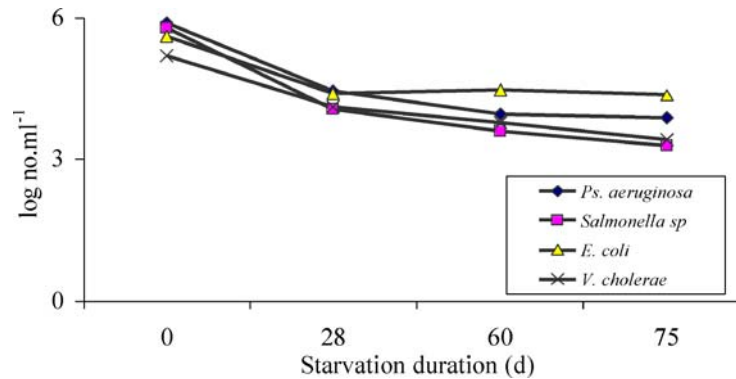


Figure 4. Persistence of direct viable counts (DVC) of bacteria in 2.5 L microcosms containing natural, filtered seawater without added carbon and energy sources. All four strains of pathogenic bacteria examined in this study were isolated from Mumbai Port waters and identified by serotyping/biochemically (in the case of *Pseudomonas aeruginosa*). Samples were drawn from the microcosms at definite intervals and DVC determined following Kogure *et al.* (1979).

the pathogens from other regions of the world. Even though there are demonstrable instances of bio-invasion of microbial species, the immediate and visible benefits from trade are overwhelming. Thus the deleterious, long-term consequences of invisible microbial invasions are subjected to “inattention” or are never investigated. Though it was not the purpose of this study at any stage to look at which organism(s)/species came from which part of the world, our results amply demonstrate that the hazards of transporting numerous pathogens out of this contamination hot-spot are imminent. Similarly, their incoming from the foreign ballast is also implicit. Molecular and genetic comparisons of bacterial pathogens by ascertaining their first-reported-locations are required to specifically pinpoint the possible origin of the pathogens.

Although significant quantities of pathogenic bacteria may be taken on in ballast water from such contamination hot-spots, their survival, persistence and reaching the human environment are dictated by environmental conditions (such as organic load, temperature, sediment characteristics) in the newer-deballasting-locations. Probably, microfaunal (dinoflagellates, some protozoans, microzooplankton) and macrofaunal (bivalves, comb-jellies, fouling ones) invasions are of more imminent threats to the natural ecosystems than those of pathogenic bacteria. It may be added here that though it is not desirable to ballast when high loads of bacteria are present. But, this may not generally be possible as the ship must ballast to maintain safety. Such concerns i.e., high counts of pathogenic bacteria and ships’ stability have to be addressed through concerted research efforts and developments related to alternative ballast treatment/management procedures. As Rigby (2001) suggests, the ongoing world-wide research and lab-scale demonstrations of alternative treatment options that would improve removal or elimination of non-indigenous organisms

contained in ballast water, compared with that available with various exchange options will be essential to reduce the long-term risk of new introductions.

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