

Seasonal variations in the water quality and antibiotic resistance of microbial pollution indicators in the Mandovi and Zuari estuaries, Goa, India.

**Ashwini D. Toraskar, Cathrine S. Manohar, Charmaine L. Fernandes, Durbar Ray*,
Abigayle D. Gomes, Ajit Antony**

CSIR- National Institute of Oceanography, Dona Paula. Goa- 403004.

*Corresponding Author: Email: dray@nio.org ; ORCID: 0000-0002-6681-5857

Abstract

The two adjacent estuaries of the rivers Mandovi and Zuari, along the Goa coast in the central west coast of India, are a large complex aquatic system, hosting diverse natural habitats. The water quality in these habitats is affected by various anthropogenic activities as they are extensively used for transportation, fisheries and various recreational activities. In the present study, changes in the water quality and levels of microbial pollution during the pre-monsoon, monsoon and post-monsoon seasons were determined. The water quality index was estimated based on the parameters; temperature, salinity, pH, dissolved oxygen, biochemical oxygen demand and nutrients. The seasonal changes in the microbial pollution load were also assessed based on the abundance of pollution indicator organisms and their resistivity towards multiple antibiotics. Results show that the water quality index status was 'poor' in the pre-monsoon and post-monsoon season and it was 'good' only in the monsoon period. Levels of pollution indicator organisms determined show that the counts were the highest in the pre-monsoon season, which reduced in the monsoon and further declined during the post-monsoon season. However, the estimated Multiple Antibiotic Resistance (MAR) index, suggests bacterial isolates in monsoonal water and sediment samples have maximum resistance towards antibiotics. This shows that, though the basic water quality improved during the monsoon possibly due to substantial dilution, the increased terrestrial inputs brought harmful pathogens into these estuarine waters, which may be of potential health risk. Understanding the ecological status of the estuarine habitats is important for successful environmental management and sustainable development.

Keywords: Mandovi, Zuari, Estuary, Water Quality Index, Microbial Pollution Indicators, Antibiotic Resistance

Introduction

Estuaries represent productive aquatic habitats and are characterised by dynamic biogeochemical processes. They provide some of the most invaluable ecosystem resources and services. Besides that, estuaries have drawn much attention as they support the livelihood of a large majority of the human population globally and hence are constantly exposed to anthropogenic perturbations (Liu et al., 2016). Such semi-enclosed environments with limited flushing capacity are influenced by seasonal variations, especially due to freshwater influx in the monsoon season and tend to accumulate a variety of anthropogenic wastes (VishnuRadhan et al., 2015). The 7,500 km long Indian coastline is drained out by major rivers; about 14 of them, 44 medium and nearly 162 minor rivers and thus characterised with many complex estuarine habitats (Kumar & Sarma, 2018). Various factors such as the geophysical locations, riverine fluxes and other man-made developments influence the environmental status of Indian estuaries. The estuaries along the west coast of India are largely influenced by intense monsoonal precipitation during the southwest monsoon season and hence experience significant seasonal variations in the physicochemical parameters (Shetye et al., 2007).

Along the central west coast of India, the Mandovi and Zuari estuaries of Goa are two important estuarine habitats (Kessarkar et al., 2010). They receive maximum freshwater runoff (up to $\sim 400 \text{ m}^3 \text{ s}^{-1}$ discharge) in June-September each year during the active phase of the monsoon (Manoj et al., 2009) and with the withdrawal of monsoon, the flow becomes primarily tidal, until the following monsoon season (Sundar & Shetye, 2005). Such seasonal variability along with regular tidal influence brings about large variations in the physicochemical and microbiological parameters of the two estuaries. Due to incessant rains during the monsoon season the water column in these estuaries is stratified, however the waters stay well mixed in the summer before the monsoonal rains and also throughout the post-monsoonal season. Intrusion of saltwater up to the far upstream region makes these estuaries a marine-dominated estuarine complex (Shetye et al., 2007; VishnuRadhan et al., 2015). These semi-enclosed coastal water bodies, which are connected to the open sea and the seawater in these habitats are measurably diluted by freshwater, due to terrestrial drainage. This freshwater influx from rivers helps in maintaining the ecological characteristics of the estuary.

Earlier studies show that due to rapid increase in anthropogenic activities, the level of terrigenous material entering these estuarine environments along the river discharge has been rising at an alarming rate in the Mandovi and Zuari estuaries which are considered to be the lifeline of Goa (Alagarsamy, 2006; Shynu et al., 2015). Studies also show deterioration of environmental quality due to an increase in the levels of various pathogenic microbes such as coliforms, *Vibrio sp.*, *Salmonella sp.*, *Shigella sp.* and *Streptococcus sp.* (Nagvenkar & Ramaiah, 2009; Rodrigues et al., 2011). Adding to this, regular urban effluents bringing in medical wastes, especially antibiotic load, increases the

threat to the ecosystem (Vignesh et al., 2014). This not only introduces antibiotic-resistant microbial strains into the environment but also accelerates the spread of resistance genes to indigenous bacterial flora. As a consequence, the emergence of multidrug resistance is a major threat faced by today's world (De Souza et al., 2006). Considering the current scenario, regular monitoring of the estuarine habitats to determine the health of the coastal water bodies is required. As it can directly impact the surrounding riverine systems, and its deterioration can have a major threat to the human population dependent on them. In this study, we have estimated the impact of monsoon season on the water quality index (WQI) and the multiple antibiotic resistance index (MAR) of microbial pollution indicators in the estuarine environments.

Materials and methods

Study location and sampling details

Sampling for this study was carried out within the estuarine regions of the Mandovi and Zuari rivers along the Goa coast (Fig. 1). These two adjacent tropical estuaries experience strong seasonal changes; very warm and humid pre-monsoon (February - May), intensive precipitation during monsoon (June - October) and relatively dry, post-monsoon (November - January) conditions. To understand the possible impacts of these seasonal variations on the water quality and microbial community structure, sampling was carried out in April 2019 (pre-monsoon), September 2019 (monsoon) and January 2020 (post-monsoon season). The water and sediment samples were collected from 16 sampling locations around this estuarine system (Fig. 1) on a trawl boat. At each sampling location, water samples from the surface (~0.5 m water depth) and close to the bottom (~0.5 m above the sediment) using pre-cleaned Niskin samplers (capacity: 5 L) were collected. Sediment samples were collected using a steel-plated Van Veen grab (capacity: 0.045 sq mt) after water sampling at each location. Water and sediment samples were immediately subsampled for physicochemical and microbiological analysis with necessary precautions and immediately processed or preserved at 4°C following the standard protocols.

Analysis of physicochemical parameters in water samples

To assess the water quality in this estuarine system, the parameters such as temperature, salinity, pH, dissolved oxygen (DO), biochemical oxygen demand (BOD) and essential nutrients were analysed in the water samples from all the sampling stations during each season. During field sampling, the temperature, salinity and depth of the water column at each sampling station was determined using a portable CTD system (Sea Bird Electronics). The pH was measured immediately after returning to the laboratory using a benchtop pH meter (Thermo Fisher Scientific) with an accuracy of ± 0.1 pH unit. The Winkler's titrimetric method was used to determine the DO levels in the water samples and the

values are expressed in mg/l. Similarly, the BOD was estimated with 5 days incubation at 27 °C. The essential nutrients including dissolved nitrate, nitrite, phosphate and silicate were analysed in all the water samples following the photometric methods described by Grasshoff (1983).

Estimation of WQI

The seasonal changes in the WQI of Mandovi and Zuari estuaries were calculated based on the physicochemical parameters. The water quality parameters determined included temperature, salinity, pH, DO, BOD, nitrate and phosphate concentration by using the following equation (Adamou et al., 2020):

$$WQI = \frac{\sum q_n W_n}{\sum W_n}$$

Where quality rating of the nth parameter, q_n was calculated for each parameter as:

$$q_n = \frac{V_n - V_{id}}{S_n - V_{id}} \times 100$$

In the above equation, V_n and V_{id} are the estimated and ideal values of nth parameter respectively, V_{id} value of 0 was used for all the parameters, however for pH the value 7 and for dissolved oxygen the value 12 mg/l, which is the maximum saturation limit of dissolved oxygen in seawater at 0 °C and 25 PSU (Fondriest Environmental, 2013) were used. S_n is the standard permissible value of nth water quality parameter, which was followed for each parameter based on the USEPA, and national standards recommended for water quality (Table 1). W_n represents the relative weight of the nth parameter, computed as:

$$W_n = k/S_n$$

Where k is the proportionality constant calculated from S_n:

$$\frac{1}{\sum \frac{1}{S_{n=1,2...n}}}$$

The WQI values obtained were used to determine the quality in both the estuaries across the three seasons following the standard ranking scale (Table 2).

Microbiological analysis

The microbiological load in the Mandovi and Zuari estuaries was estimated based on the total viable counts (TVC) of bacteria. The microbial pollution in both the estuaries was determined based on the enumeration of Pollution Indicator Organisms (PIOs). The multiple antibiotic resistance (MAR) index of the PIOs was also estimated. Estimates of bacterial TVC in water and sediment samples were carried out using standard microbiological, spread plating method on Zobell Marine Agar (HiMedia). The colony forming units (CFUs), were enumerated after 48 hrs of incubation at 28 ± 0.5°C and are

represented as CFU/ml for water and CFU/g dry weight for sediment samples. The PIOs from the same samples were isolated on selective bacteriological media (Hi-Media, India) according to the manufacturer's guide. The different groups of PIOs such as Total coliforms, Fecal coliforms / *Escherichia coli* like organisms (ECLO) and other major PIOs; *Streptococcus faecalis* (SFLO) *Staphylococcus aureus* (SALO), *Pseudomonas aeruginosa* (PALO), *Salmonella sp.* (SLO), *Shigella sp.* (SHLO), *Klebsiella aerogenes* (KALO), *Proteus vulgaris* (PVLO), and *Vibrio cholerae* (VCLO), *Vibrio parahaemolyticus* (VPLO) and *Vibrio vulnificus* (VVLO) represented together as *Vibrio* like organisms (VLO) were determined based on the colony morphology on specific media. The number of PIOs from the samples was estimated by the spread plate method on selective media and CFUs with specific colony characteristics were enumerated. Samples were diluted in saline to obtain isolated colonies and the CFUs were counted after 24-48 hrs of incubation at $37 \pm 0.5^\circ\text{C}$ for all the PIOs, except thermotolerant ECLO which was grown at 44°C . Statistical analysis to determine the significance in the seasonal variations of physicochemical and microbiological parameters was carried out based on one factor ANOVA using Microsoft Office Excel.

Evaluation of antibiotic resistivity in PIOs: Estimation of MAR index

Representative bacterial morphotypes of the major PIO groups from all three seasons were studied for their antibiotic susceptibility profiles. Altogether 93 morphotypes were tested against a broad spectrum of 16 antibiotics, belonging to different classes of bacteriostatic and bactericidal agents, at recommended concentrations, procured from HiMedia, India. The antibiotics used to determine the MAR index included bacteriostatic agents such as Chloramphenicol, Tetracycline, Azithromycin and Co-trimoxazole and bactericidal agents like Ciprofloxacin, Nalidixic acid, Ofloxacin, Clindamycin, Netillin, Rifampicin, Vancomycin, Ampicillin, Ceftizoxime, Ceftriaxone, Piperacillin and Ertapenem. The disk diffusion method on Mueller Hinton Agar plates was used for Antibiotic susceptibility testing (Bauer, 1966). For this, initially, 200 μl of pure bacterial suspensions of each morphotype was grown to reach an optical density of 0.5 McFarland standard, corresponding to 10^8 CFU/ml. Each bacterial suspension was spread plated using a sterile cotton swab onto the media to develop homogenous growth. Then the antibiotic discs were placed on the agar surface aseptically. Plates were incubated at $37 \pm 0.5^\circ\text{C}$ for 24 hrs and at the end of the incubation period, zones of bacterial growth inhibition formed surrounding the antibiotic discs were measured. Necessary controls were also maintained as per the manufacturer's instructions. To evaluate the relative susceptibility, the Multiple Antibiotic Resistance (MAR) index of each isolate was estimated based on the total number of antibiotics tested and the number of antibiotics against which it exhibits resistance (Chitanand et al., 2010; Maloo et al., 2017).

Results and discussion

Seasonal variations of physicochemical parameters in the estuarine waters

Estuarine environments are governed by complex physicochemical and biological processes. Hydrographical parameters; temperature, salinity, pH, DO, BOD and nutrient levels can largely influence the water quality (Moss et al., 2006). Hence, to evaluate the environmental conditions in the Mandovi and Zuari estuaries, these physicochemical parameters were analysed in the surface and bottom water samples at all 16 sampling locations, in all three sampling seasons. The results obtained for each parameter analysed, are presented as the mean of eight individual sampling locations from each of the estuaries (Fig. 2 and 3).

The seasonal monitoring of the Mandovi and Zuari estuarine system based on water column parameters at 16 sampling stations showed that the average water temperature and salinity ranged between 28 - 31 °C and 24 - 36 PSU respectively. The average water temperature and salinity show distinct seasonal changes in both the estuaries, however, the pH was within a narrow range of 8 ± 0.5 in all three sampling seasons (Fig. 2). Temperature is a principal component affecting the solubility of oxygen and the rates of chemical and biological processes. The seasonal changes of temperature in the surface and bottom waters of Mandovi and Zuari estuarine system measured showed that the surface waters in the pre-monsoon and monsoon season were approximately 1 °C higher than the bottom waters. However, in the post-monsoon season, the surface and bottom waters were uniform and the temperature was 28 ± 1 °C in the water column (Fig. 2) Studies have recommended that the standard temperature should not exceed 30 °C in the coastal waters (Gupta et al., 2003). The higher temperature values in the surface waters of pre-monsoon and monsoon season could be due to the high intensity of solar radiation combined with sluggish mixing of waters (Qasim & Gupta, 1981). Similar patterns were seen in both Mandovi and Zuari estuaries. The temperature in the water column was within the permissible range of 30 °C in both the estuaries in all three seasons, except in Mandovi and Zuari surface waters in the pre-monsoon season.

Salinity is the defining parameter of the estuarine habitats and it shows wide variations with lower values during monsoon season due to the river runoff and increased values during the pre-monsoon or summer season due to reduced inflow from rivers. Our observations in the Mandovi and Zuari estuaries during the pre-monsoon show that the surface water values were 33.6 and 34.2 PSU respectively and the bottom waters were close to 35 PSU (Fig. 2). In monsoon season the lowest salinity values of 25 ± 2 PSU were recorded in the surface waters and the bottom waters were 30 ± 2 PSU. Distinct stratification with a salt wedge in the bottom layer was observed only during the monsoon season. However, in the post-monsoon season, the waters were well mixed and the water

column salinity was close to 34 PSU. Though both the estuaries have salinity values close to seawater, with the onset of the monsoon, a sharp decrease in salinity with distinct stratification in the water column is seen due to the heavy freshwater runoff during the southwest monsoon (Vijith et al., 2009). The warm and lower saline, relatively less dense surface waters float over the dense bottom waters; thus restricting the vertical mixing within the water column (Fig. 2). Due to the strong buffering capacity of seawater, the pH range in the estuarine habitats is generally between 6.5 and 8.5. The pH values measured were within a narrow range of 8 ± 0.5 in all the three sampling seasons in the Mandovi and Zuari estuaries (Fig. 2).

Dissolved oxygen is the prime parameter that determines the water quality in any aquatic habitat. DO level of > 5 mg/L is considered as the optimum value for a healthy water body (Alam et al., 2007; Avannavar & Shrihari, 2008; Şener et al., 2017). Values lower than this are indicative of waste discharges high in organic load and nutrients in the sampling region with increased microbial degradation. The DO levels in both Mandovi and Zuari estuary during all three seasons were above the recommended limits except in the pre-monsoon season in Zuari estuary, the average DO value was 4.8 mg/L (Fig. 2). In both the estuaries, relatively higher DO values were found at the surface than in the bottom waters. Such differences were more prominent during the monsoon and pre-monsoon (Fig. 2). The DO levels greatly improved in the monsoon season and this could be attributed to water turbulence during increased runoff. According to Lin et al. (2006), temperature also has a direct effect on DO levels and accordingly in the pre-monsoon season, when the temperature was high, DO levels were also low in the estuaries.

The amount of oxygen required to biochemically oxidize organic material present in water is quantified as Biochemical Oxygen Demand (BOD) and it is directly dependent on the existing DO levels (Hocking, 2005). According to USEPA (1986) recommendations, the BOD levels should be < 3 mg/L (Table 1). In this study, BOD values were > 3 mg/L in both the estuaries before and after the rains in the pre and post-monsoon season (Fig. 2). However, during the rains when the DO levels were good, BOD values reduced to < 3 mg/L. Values less than 3 mg/L show that during the monsoon season there was increased river runoff which ventilated the water. The seasonal variations in the different physical parameters was supported with very good statistical significance for temperature, DO and BOD (Table 3).

Coastal ecosystems, especially the estuaries, receive inputs through riverine runoff from the terrestrial regions and are rich in organic matter and nutrients (Ram et al., 2003). These runoffs from the terrestrial regions influence the concentration of major nutrients like nitrates, phosphates and silica in the estuarine system. These help in enhancing the numerous biological activities in the ecosystem like re-suspension of benthic diatom propagules in response to nutrient availability as reported by

Khandeparker et al., (2015). However, excessive supply of nutrients can also have a negative impact in many instances. Increased nutrients can lead to deleterious effects such as hypoxia and anoxia from eutrophication and an increase in algal growth (Chakraborty, 2017; Şener et al., 2017). Total nitrogen comprising of nitrate, nitrite and ammonia and total phosphates are the key elements controlling primary production in the coastal habitats. Studies report that if the total nitrogen levels are $\sim 300 - 500 \mu\text{g /L}$ and the total phosphate level around $20 \mu\text{g /L}$ then the water body can be considered eutrophic (Yang et al., 2008).

Eutrophication in estuaries can occur due to increased nutrient concentration and lead to harmful consequences which can destabilise the delicate ecosystem processes. The water quality guidelines for coastal habitats recommend that the total nitrate and nitrite levels should be $< 2\mu\text{M}$ respectively (Jha et al., 2015). The water samples analysed during this study show that the average nitrate levels exceeded the limit in both the estuaries in all three seasons. Values were $\sim 4 \mu\text{M}$ during the summer, before the rains; $\sim 3 \mu\text{M}$ during the monsoonal rains, which was the lowest and $\sim 5.5 \mu\text{M}$ in the post-monsoon season (Fig. 3). The nutrient levels dropped significantly in the monsoon season which is evident that it was due to the dilution resulting from the incoming water flow. The increased nitrate levels could be attributed to improper agricultural practices such as increased usage of inorganic fertilizers and manure which leaches into the natural habitats (Bhavya et al., 2016). The nitrite levels, on the other hand, showed relatively lower concentrations than nitrates (Fig. 3). All the samples analysed were within the recommended standard limits. The values across the seasons were almost negligible to $1.2 \mu\text{M}$ concentration. The absence of nitrite levels shows that the ecosystem was not anaerobic as nitrite is one of the major indicators of denitrification activity (Jayakumar et al., 2004). The levels of total nitrate and nitrite in the Mandovi and Zuari region was close to $4 \mu\text{M}$ especially in the pre-monsoon and post-monsoon season which shows that the ecosystem is moderately eutrophic. The monsoonal flushing seems to be playing an important role in reducing the levels of nitrates and preventing the system from becoming eutrophic.

Phosphates are found in extremely low concentrations in waters as it is actively taken up by the primary producers. The maximum permissible limit for total phosphates in coastal environments is determined as $1.2 \mu\text{M}$ (USEPA, 1986). However, studies report that the ecosystem can become eutrophic even at $\sim 0.2 \mu\text{M}$ concentration (Yang et al., 2008). The phosphate levels in the Mandovi and Zuari waters were within the permissible limit values (Fig. 3). The highest concentrations of $0.78 \pm 0.25 \mu\text{M}$ phosphate were observed during the pre-monsoon season in both estuaries. Levels declined in monsoon season to $0.29 \pm 0.14 \mu\text{mol/L}$ and again increased in the post-monsoon season up to $0.56 \pm 0.25 \mu\text{M}$. Residual detergents, in the domestic waste waters, industrial effluents and fertilizer run-off are

the major contributors of phosphates. Furthermore, all estuaries are known to have phosphate load due to constant adsorption and dissolution at the sediment-water interface and even a small increase in the concentration can impair the water quality level of the habitat, making the system eutrophic. Also, due to continuous adsorption and dissolution at the sediment-water interface, almost all estuaries are known to have phosphate load and even a slight increase in the concentration can reduce the water quality level of the habitat, making the system eutrophic (Sharpley et al., 2001; Manoj et al., 2009).

Nutrients like silica are known to significantly influence the diversity and distribution of phytoplankton and some dominant species are also known to alter the community structure. The highest level of silica was recorded in the pre-monsoon season which was $\sim 13 \pm 4 \mu\text{M}$ and the level declined in the monsoon season to $\sim 10 \pm 6 \mu\text{M}$. The values in the post-monsoon season were $\sim 8 \pm 4 \mu\text{M}$ in both the estuaries. There are no standard limits for silicate concentrations; however, the levels of nitrates and phosphates together with increased silicate levels are very crucial for determining the water quality of the estuarine habitat (Carey & Fulweiler, 2014). Levels of the chemical parameters determined for the Mandovi and Zuari estuaries show that the quality of the waters is close to eutrophicated waters in the pre-monsoon season with increased nitrate and phosphate levels. Seasonal variations of the different chemical parameters between the three seasons was supported with very good significance in both the Mandovi and Zuari estuary based on ANOVA analysis (Table 3). The runoff and increased flushing cause a reduction in the nutrient load during the monsoon season thereby impacting the water quality of this monsoonal estuary. Earlier studies have also indicated that the mixing of wastewaters draining out from local urban areas is the major source of excess nutrients in these estuarine waters (Maya et al., 2011).

Seasonal variation of estuarine water quality: Evaluation of WQI

To evaluate the composite impact of the physicochemical parameters on the estuarine waters, the Water Quality Index (WQI) was estimated for the Mandovi and Zuari estuaries during the three sampling seasons. The WQI is used to evaluate the changes in water quality of the habitats based on the levels of these parameters and their combined effect (Kannel et al., 2007). A total of seven parameters; temperature, salinity, pH, DO, BOD, nitrate and phosphate were considered to evaluate the WQI. Each of these parameters plays a crucial role in determining the ecosystem health and permissible limits are provided by international and national environmental agencies (Table 1).

The WQI was evaluated by comparing the average values of each of the parameters with the standard limits. A cumulative index was estimated based on the deviation of these parameters from the set limits which was used to derive the WQI for the study area across the three seasons (Adamou et al., 2020). WQI can range from 0 to 300 and the habitat studied is assessed into different categories

such as Excellent, Good, Poor, Very poor and unfit for usage (Table 2). The quality of the water in aquatic ecosystems is considered to be excellent if the WQI was less than 50, and both the estuaries were not found to be in this category in any of the seasons (Fig. 4). During the pre-monsoon season, the WQI ranged from 100 to 130 (Fig. 4), indicating poor water quality as the standard limit of WQI from 100 to 200 is categorised as “Poor”. Though most of the physicochemical parameters in the pre-monsoon season were within limits, a wide difference in major parameters like BOD and nitrate was observed. The values of temperature, salinity and phosphate were also very close to the permissible limits and clearly contributed to the reduction in WQI in the estuaries during the pre-monsoon season. The WQI was greatly improved in the monsoon season as all the physical parameters analysed were within the permissible range and all the values showed improved water quality. The WQI values between, 50 to 100 which is considered to be “Good” waters was observed only during the monsoon season (Fig. 4). This could be due to the increased river runoff and flushing. Earlier studies also showed that increased freshwater runoff during monsoon season dilutes the concentration of the contaminants and remove them out of the estuarine system (VishnuRadhan et al., 2015).

The WQI improved during the monsoon period, however, in the post-monsoon season that followed, water quality status deteriorated in both the estuaries. The WQI during the post-monsoon season was categorised as “Poor” as the values were 130 and 120 in the Mandovi and Zuari estuaries respectively. This decline can be linked to the leaching of domestic waste discharge and agricultural fertilizers into the area which is evident due to the high nitrate and BOD values during this season and the further drop in the WQI in the pre-monsoon season in both, Mandovi and Zuari estuaries. This can be associated with the dumping of agricultural wastes as reflected in the elevated levels of essential nutrients like nitrate and phosphate. The abundant nutrient supply encourages further growth of microbial flora accompanied by increased oxygen demands. Another possible factor contributing to this, is extensive tourism activities along the coastal area, especially during November to February. WQI serves to improve the understanding of water quality issues, by generating a score describing water quality status and aids in evaluating the water quality trends (Cude, 2001; Hoorman et al., 2008).

Assessment of microbial pollution: Seasonal variations in TVC and PIOs

Estuarine waters have wide variations in the physical and chemical parameters, especially the nutrient concentration and the organic matter composition can influence the microbial load and its community composition. Apart from it, the inflow of waters from different terrestrial habitats brings in various microbes of allochthonous origin. They also have an influence on the composition of the natural bacterial community and changes in the native bacterial load can be seen (Khandeparker et al., 2015). If the natural balance is not established, there are instances when the terrestrial population can replace

the estuarine community and alter the delicate balance which can lead to an outbreak of pathogenic bacterial communities. The occurrence of PIOs in the samples reflects a higher incidence of sewage indicator bacteria in any environment (Fujioka & Yoneyama, 2002). Assessing the levels of pollution indicators are essential to understand the microbial pollutants present in the Mandovi and Zuari estuaries.

The total bacterial counts were determined based on the TVC and the highest counts were seen during the pre-monsoon and monsoon season with $\sim 10^4$ to 10^6 CFU/ml in the water and sediment samples. However, the microbial load was only $\sim 10^2$ to 10^3 CFU/ml during the post-monsoon season in both the estuaries (Fig 5). The levels of PIOs: TC, ECLO, SFLO, SALO, PALO, SLO, SHLO, KALO, PVLO and VLO were estimated in the water and sediment samples (Table 4). TC, SFLO and SALO with maximum numbers of $\sim 10^2$ CFUs were present only during monsoon and the numbers were below detectable limits in the pre and post-monsoon season. The PIO counts in the water and sediment samples relate to an increase in fecal contamination around the sampling site due to river runoff. Maximum PALO numbers of 10^4 - 10^6 CFU/ml were recorded in the pre-monsoon period and the numbers during the rains and in the following post-monsoon season numbers were less than 100 CFU/ml. SHLO were also found to be maximum with 10^2 - 10^4 CFU/ml in the pre-monsoon season and the numbers were 5 to 300 CFU/ml in the monsoon and after the rains in post-monsoon. The highest VLO counts were also recorded in the pre-monsoon period, with 10^3 – 10^5 CFUs and in the other two seasons, it was ~ 1 to 120 CFUs. This could be due to anthropogenic contamination which is increased during the pre-monsoon season due to increased evaporation and reduced runoff (Table 4). PIOs such as ECLO, SLO, PVLO and KALO were below detectable levels in all three seasons. Seasonal changes in the levels of TC, SALO, PALO, SHLO and VLO was supported with statistical significance (Table 3).

The occurrence of PIOs in the samples reflects the presence of microbial pollution in the ecosystem, which directly reflects the presence of pathogenic bacteria that can affect the people who are exposed to these habitats (Fujioka & Yoneyama, 2002). Earlier studies on the counts of pollution indicator bacteria have also concluded that urban sewage outfalls impact the water quality in these regions due to the mixing of waters from various point and non-point sources into the Mandovi and Zuari estuarine waters (Rodrigues et al., 2011). Periodic investigations on the levels of PIOs and their distribution are required for efficient management of the effluent outfalls into these delicate estuarine systems. Protecting the ecological health of these habitats can be achieved only based on these scientific studies for developing stringent guidelines and appropriate policies. These can provide appropriate methods for reducing the detrimental impact of microbial pollution on human health (Nagvenkar & Ramaiah, 2009).

Characterisation of antibiotic resistivity in PIOs: Estimation of MAR index

Approximately, 90 representative isolates of major pollution indicator bacterial groups from all three seasons, were tested for their susceptibility towards 16 different antibiotics belonging to bacteriostatic and bactericidal classes. Among the 35 isolates tested from the pre-monsoon season, 30 isolates showed resistance to Clindamycin; while all of them were susceptible to Ofloxacin and Tetracycline (Supplementary Table S1). About 86 % of the isolates tested showed a MAR index of < 0.2 (Table 5) which can be categorised as low risk PIOs. Isolates with a MAR index of > 0.4 accounted for about 5.6 % which included isolate PR13 and PR27 belonging to *Vibrio* and *Shigella* group of PIOs (Table 6). Enumerating the MAR Index, proves to be a useful method for health risk assessment and isolates having values >0.2 suggest it as possible means of contamination which could be categorised as 'high risk' (Davis and Brown, 2016). Similar evidence of partial or complete susceptibility for multiple drugs in pathogens has also been reported earlier in other coastal waters and in most cases, contamination was caused by urban wastes (Dang et al., 2008; Henriques et al., 2008; Austin et al., 2009).

During the monsoon sampling, a total of 33 isolates were screened and a coliform isolate from Mandovi estuary, (M7) showed resistance to all the antibiotics tested except Clindamycin, which appeared with a very small inhibition zone of 8 mm only (Table 6). Approximately 75.8 % of the isolates exhibited a MAR index values < 0.2 and 9.1 % had the MAR values of > 0.4 (Table 5). This includes isolates M2, M7 and M20 exhibiting resistance to 9, 15 and 7 antibiotics and MAR index of 0.56, 0.94 and 0.44 respectively. The intensity of resistance, shown by these isolates during this season, is alarmingly high. Antibiotic resistance in the post-monsoon was fairly low with only one isolate (PT13) exhibiting maximum resistance to 9 antibiotics with a MAR index of 0.56 (Table 5 and 6). Isolates with MAR indices > 0.4 pose a great threat as a majority of the local population depends on the estuaries for food and recreational activities.

Such studies on antibiotic resistance in indigenous waters are therefore important, as they indicate the extent of human alteration of water ecosystems (Baquero et al., 2008). An increase in the load of pathogenic bacteria leads to an alteration of the natural microbiota and the strains resistant to commonly used antibiotics can be a major threat to those involved in fishing and recreational activities in these regions (Ramaiah et al., 2004). Most of the isolates showed resistance to the β lactam, Lincosamide, Glycopeptide and Quinolone classes of antibiotics. Therefore, resistance strategies that prevent the inhibition of cell wall biosynthesis, preventing DNA replication and inhibition of transcription are likely the major processes by which these isolates developed resistance to these antibiotics. Understanding the mechanism by which the bacteria acquire resistance can provide better treatment options. Such studies might also help detect radical changes in terms of antibiotic resistance

of emergent pathogens of the marine system in order to alert the government, public and social institutions, of their implications. Calculation of the MAR index provides a better understanding of the virulence of PIOs and serves as an excellent tool for health risk assessment (Chitanand et al., 2010). Though in this study the presence of antibiotic residues was not carried out, its influence is clearly evident based on the natural resistance observed in bacterial communities from these regions. Studies have also shown that the increased density of resistant bacteria and the presence of favourable growth conditions for their proliferation can lead to higher health risks (Diwan et al., 2010).

Conclusion

In this study, the seasonal changes of the physicochemical parameters and microbial load were determined in the Mandovi and Zuari estuaries. The water quality index was computed to understand the current environmental status of the estuaries. Results show that the overall water quality conditions remain considerably good due to the annual southwest monsoon. It has a positive impact on the major physicochemical parameters such as temperature, salinity and dissolved oxygen levels. Studies on the microbial load and the levels of PIOs especially based on CFUs also suggest that the load of bacteria is increased in the pre and post-monsoon period and the numbers reduced during the monsoon period. However, the MAR indices determined for the different PIOs suggest that the water quality is not completely safe during the monsoon season due to increased terrestrial load, which could bring in PIOs with a very high MAR index. Bacteria with > 0.2 MAR index are considered to be highly detrimental to the health of the individuals exposed to these microbes. In this study, we have observed that during the monsoon season an isolate with the highest MAR index of 0.94 was obtained. This shows that the seasonal changes along with the increased terrestrial load from different sources can alter the water quality in the estuarine region and the virulence of the microbes in these habitats.

Mandovi and Zuari estuaries are well ventilated by the annual monsoonal rains and the water quality status is maintained by the increased river runoff each year. However, this study emphasizes the need to study not only the physicochemical parameters and the microbial numbers, but also the pathogenicity, virulence and antibiotic sensitivity of the PIOs present in the habitat. Any reduction in the monsoonal rains and an increase in the number of virulent PIOs entering the estuarine habitat can alter the microbial community structure. Hence, adequate treatment of urban runoff and terrestrial inputs into the estuarine regions has to be undertaken. Studies on the expression of virulence genes by pathogens in the estuary need to be taken up to ensure the long-term safety of those dependent on the estuarine habitat for their livelihood. Such studies can play an important role to frame policies for sustainable development and conservation of these ecologically important coastal zones.

Supplementary Information

Supplementary Table S1. Multiple Antibiotic Resistance (MAR) index of pollution indicator organisms isolated from pre-monsoon, monsoon and post-monsoon season, associated with this article is provided in the file “Supplementary Material.xlsx”.

Acknowledgements

Authors are thankful to Director, CSIR-National Institute of Oceanography, Goa for extending all the support and required facilities to accomplish this work. Research funding to study the Seawater Quality in the Mandovi and Zuari estuarine system from Ministry of Earth Sciences, Govt. of India is gratefully acknowledged. Thanks to all other Project Assistants who participated in seasonal field work and helped in sample collection. This is CSIR-NIO contribution No. XXXX.

Declarations

Funding

Ministry of Earth Sciences, Government of India, under the research program, MoES/ICM AM-PD/Suppl. Order/81/2017.

Conflict of Interest/Competing interests

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Availability of data and material

Not applicable

Code availability

Not applicable

References

- Adamou, H., Ibrahim, B., Salack, S., Adamou, R., Sanfo, S., Liersch, S. (2020). Physico-chemical and bacteriological quality of groundwater in a rural area of Western Niger: a case study of Bonkougou. *Journal of Water and Health*, 18(1), 77-90. <https://doi.org/10.2166/wh.2020.082>
- Alagarsamy, R. (2006). Distribution and seasonal variation of trace metals in surface sediments of the Mandovi estuary, west coast of India. *Estuarine, Coastal and Shelf Science*, 67(1-2), 333-339. <https://doi.org/10.1016/j.ecss.2005.11.023>
- Alam, M. J., Islam, M. R., Muyen, Z., Mamun, M., Islam, S. (2007). Water quality parameters along rivers. *International Journal of Environmental Science and Technology*, 4(1), 159-167.
- Austin, C. B., McArthur, J. V., Lindell, A. H., Wright, M. S., Tuckfield, R. C., Gooch, J., Warner, L., Oliver, J., Stepanauskas R. (2009). Multi-site Analysis Reveals Widespread Antibiotic Resistance in the Marine Pathogen *Vibrio vulnificus*. *Microbial Ecology*, 57, 151-159. <https://doi.org/10.1007/s00248-008-9413-8>

- Avvannavar, S. M., Shrihari, S. (2008). Evaluation of water quality index for drinking purposes for river Netravathi, Mangalore, South India. *Environmental Monitoring and Assessment*, 143(1-3), 279-290. <https://doi.org/10.1007/s10661-007-9977-7>
- Baquero, F., Martínez, J. L., Cantón, R. (2008). Antibiotics and antibiotic resistance in water environments. *Current Opinion in Biotechnology*, 19(3), 260-265. <https://doi.org/10.1016/j.copbio.2008.05.006>
- Bauer, A. W. (1966). Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 45, 149-158. NII Article ID (NAID) 10024190092
- Bhavya, P.S., Kumar S., Gupta G.V.M., Sudheesh V., Sudharma K.V., Varrier D.S., Dhanya K.R., Saravanane N. (2016) Nitrogen uptake dynamics in a tropical eutrophic estuary (Chochin, India) and adjacent coastal waters. *Estuaries and Coasts*. 39, 54-67. <https://doi.org/10.1007/s12237-015-9982-y>
- Carey, J. C., Fulweiler, R. W. (2014). Salt marsh tidal exchange increases residence time of silica in estuaries. *Limnology and Oceanography*. 59(4), 1203-1212. <https://doi.org/10.4319/lo.2014.59.4.1203>
- Chakraborty, P. (2017). Oyster reef restoration in controlling coastal pollution around India: a viewpoint. *Marine Pollution Bulletin*. 115(1-2), 190-193. <https://doi.org/10.1016/j.marpolbul.2016.11.059>
- Chitanand, M. P., Kadam, T. A., Gyananath, G., Totewad, N. D., Balhal, D. K. (2010). Multiple antibiotic resistance indexing of coliforms to identify high risk contamination sites in aquatic environment. *Indian Journal of Microbiology*, 50(2), 216-220. <https://doi.org/10.1007/s12088-010-0042-9>
- Cude, C. G. (2001). Oregon water quality index a tool for evaluating water quality management effectiveness. *JAWRA Journal of the American Water Resources Association*, 37(1), 125-137. <https://doi.org/10.1111/j.1752-1688.2001.tb05480.x>
- Dang, H., Ren, J., Song, L., Sun, S., An, L. (2008). Diverse Tetracycline Resistant Bacteria and Resistance Genes from Coastal Waters of Jiaozhou Bay. *Microbial Ecology*. 55(2), 237-246. <https://doi.org/10.1007/s00248-007-9271-9>
- Davis, R., Brown, P. D. (2016). Multiple antibiotic resistance index, fitness and virulence potential in respiratory *Pseudomonas aeruginosa* from Jamaica. *Journal of Medical Microbiology*, 65(4), 261-271. <https://doi.org/10.1099/jmm.0.000229>
- De Souza, M. J., Nair, S., Bharathi, P. L., Chandramohan, D. (2006). Metal and antibiotic-resistance in psychrotrophic bacteria from Antarctic Marine waters. *Ecotoxicology*, 15(4), 379-384. <https://doi.org/10.1007/s10646-006-0068-2>
- Dheenan, P. S., Jha, D. K., Vinithkumar, N. V., Ponmalar, A. A., Venkateshwaran, P., Kirubagaran, R. (2014). Spatial variation of physicochemical and bacteriological parameters elucidation with GIS in Rangat Bay, Middle Andaman, India. *Journal of Sea Research*. 85, 534-541. <https://doi.org/10.1016/j.seares.2013.09.001>

- Diwan, V., Tamhankar, A. J., Khandal, R. K., Sen, S., Aggarwal, M., Marothi, Y., Iyer, R.V., Sundblad-Tonderski, K., Stålsby-Lundborg, C. (2010). Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. *BMC Public Health*, 10(1), 414. <https://doi.org/10.1186/1471-2458-10-414>
- Fondriest Environmental (2013, November 19). Dissolved Oxygen: Fundamentals of Environmental Measurements. Retrieved December 10, 2020, from <https://www.fondriest.com/environmental-measurements/parameters/water-quality/dissolved-oxygen/>
- Fujioka, R. S., Yoneyama, B. S. (2002). Sunlight inactivation of human enteric viruses and fecal bacteria. *Water Science and Technology*, 46(11-12), 291-295. <https://doi.org/10.2166/wst.2002.0752>
- Grasshoff, K. (1983). Determination of nutrients. *Methods of seawater analysis*, 125-187.
- Gupta, A. K., Gupta, S. K., Patil, R. S. (2003). A comparison of water quality indices for coastal water. *Journal of Environmental Science and Health. Part A*, 38(11), 2711-2725. <https://doi.org/10.1081/ESE-120024458>
- Henriques, I.S., Fonseca, F., Alves, A., Saavedra, M.J., Correia, A. (2008). Tetracycline-resistance genes in Gram-negative isolates from estuarine waters. *Letters in Applied Microbiology*, 47(6), 526-533. <https://doi.org/10.1111/j.1472-765x.2008.02452.x>
- Hocking, M. B. (2005). Water quality measurement. In M. B. Hocking (Eds.), *Handbook of Chemical Technology and Pollution Control* (3rd ed., pp. 105-135). San Diego, California, USD: Elsevier Inc.
- Hoorman, J., Hone, T., Sudman, T., Dirksen, T., Iles, J., Islam, K. R. (2008). Agricultural impacts on lake and stream water quality in Grand Lake St. Marys, Western Ohio. *Water, Air, and Soil Pollution*, 193(1-4), 309-322. <https://doi.org/10.1007/s11270-008-9692-1>
- Jayakumar, D. A., Francis, C. A., Naqvi, S. W. A., Ward, B. B. (2004). Diversity of nitrite reductase genes (nirS) in the denitrifying water column of the coastal Arabian Sea. *Aquatic Microbial Ecology*, 34(1), 69-78. <https://doi.org/doi:10.3354/ame034069>
- Jha, D. K., Devi, M. P., Vidyalakshmi, R., Brindha, B., Vinithkumar, N. V., Kirubakaran, R. (2015). Water quality assessment using water quality index and geographical information system methods in the coastal waters of Andaman Sea, India. *Marine Pollution Bulletin*, 100(1), 555-561. <https://doi.org/10.1016/j.marpolbul.2015.08.032>
- Kannel, P. R., Lee, S., Lee, Y. S., Kanel, S. R., Khan, S. P. (2007). Application of water quality indices and dissolved oxygen as indicators for river water classification and urban impact assessment. *Environmental Monitoring and Assessment*. 132(1-3), 93-110. <https://doi.org/10.1007/s10661-006-9505-1>
- Kessarkar, P. M., Rao, V. P., Shynu, R., Mehra, P., Viegas, B. E. (2010). The nature and distribution of particulate matter in the Mandovi estuary, central west coast of India. *Estuaries and Coasts*. 33(1), 30-44. <https://doi.org/10.1007/s12237-009-9226-0>
- Khandeparker, L., Anil, A. C., Naik, S. D., Gaonkar, C. C. (2015). Daily variations in pathogenic bacterial populations in a monsoon influenced tropical environment. *Marine Pollution Bulletin*. 96(1-2), 337-343. <https://doi.org/10.1016/j.marpolbul.2015.04.051>

- Kumar B.S.K., Sarma V.V.S.S. (2018). Variations in concentrations and sources of bioavailable organic compounds in the Indian estuaries and their fluxes to coastal waters. *Continental Shelf Research*, 166, 22-33. <https://doi.org/10.1016/j.csr.2018.07.001>
- Lin, J., Xie, L., Pietrafesa, L. J., Shen, J., Mallin, M. A., Durako, M. J. (2006). Dissolved oxygen stratification in two micro-tidal partially-mixed estuaries. *Estuarine, Coastal and Shelf Science*, 70(3), 423-437. <https://doi.org/10.1016/j.ecss.2006.06.032>
- Liu, B., Wang, W. L., Han, R. M., Sheng, M., Ye, L. L., Du, X., Wu, X.T., Wang, G. X. (2016). Dynamics of dissolved oxygen and the affecting factors in sediment of polluted urban rivers under aeration treatment. *Water, Air and Soil Pollution*, 227(6), 172. <https://doi.org/10.1007/s11270-016-2869-0>
- Maloo, A., Fulke, A. B., Mulani, N., Sukumaran, S., Ram, A. (2017). Pathogenic multiple antimicrobial resistant Escherichia coli serotypes in recreational waters of Mumbai, India: a potential public health risk. *Environmental Science and Pollution Research*, 24(12), 11504-11517. <https://doi.org/10.1007/s11356-017-8760-8>
- Manoj, N. T., Unnikrishnan, A. S., Sundar, D. (2009). Tidal asymmetry in the Mandovi and Zuari estuaries, the west coast of India. *Journal of Coastal Research*, 1187-1197. <https://doi.org/10.2112/08-1056.1>
- Maya, M. V., Soares M. A., Agnihotri R., Pratihary A. K., Karapurkar S, Naik H., Naqvi S.W.A. (2011). Variations in some environmental characteristics including C and N stable isotopic composition of suspended organic matter in the Mandovi estuary. *Environmental Monitoring and Assessment*, 175, 501–517. <https://doi.org/10.1007/s10661-010-1547-8>
- Moss, J. A., Nocker, A., Lepo, J. E., Snyder, R. A. (2006). Stability and change in estuarine biofilm bacterial community diversity. *Applied and Environmental Microbiology*, 72(9), 5679-5688. <https://doi.org/10.1128/AEM.02773-05>
- Nagvenkar, G. S., Ramaiah, N. (2009). Abundance of sewage-pollution indicator and human pathogenic bacteria in a tropical estuarine complex. *Environmental Monitoring and Assessment*, 155(1-4), 245. <https://doi.org/10.1007/s10661-008-0432-1>
- Qasim, S. Z., Gupta, R. S. (1981). Environmental characteristics of the Mandovi-Zuari estuarine system in Goa. *Estuarine, Coastal and Shelf Science*, 13(5), 557-578. [https://doi.org/10.1016/S0302-3524\(81\)80058-8](https://doi.org/10.1016/S0302-3524(81)80058-8)
- Ram, A. P., Nair, S., Chandramohan, D. (2003). Seasonal shift in net ecosystem production in a tropical estuary. *Limnology and Oceanography*, 48(4), 1601-1607. <https://doi.org/10.4319/lo.2003.48.4.1601>
- Ramaiah, N., Kolhe, V., Sadhasivan, A. (2004). Abundance of pollution indicator and pathogenic bacteria in Mumbai waters. *Current Science*, 87(4), 435-439. <https://www.jstor.org/stable/24109171>
- Rodrigues, V., Ramaiah, N., Kakti, S., Samant, D. (2011). Long-term variations in abundance and distribution of sewage pollution indicator and human pathogenic bacteria along the central west coast of India. *Ecological Indicators*, 11(2), 318-327. <https://doi.org/10.1016/j.ecolind.2010.05.010>

- Şener, Ş., Şener, E., Davraz, A. (2017). Evaluation of water quality using water quality index (WQI) method and GIS in Aksu River (SW-Turkey). *Science of the Total Environment*, 584, 131-144. <https://doi.org/10.1016/j.scitotenv.2017.01.102>
- Sharpley, A. N., McDowell, R. W., Kleinman, P. J. (2001). Phosphorus loss from land to water: integrating agricultural and environmental management. *Plant and Soil*, 237(2), 287-307. <https://doi.org/10.1023/A:1013335814593>
- Shetye, S. R., Shankar, D., Neetu, S., Suprit, K., Michael, G. S., Chandramohan, P. (2007). The environment that conditions the Mandovi and Zuari estuaries. In S. R. Shetye, D. Kumar & D. Shankar (Eds.), *The Mandovi and Zuari Estuaries* (pp. 3-26). National Institute of Oceanography, Goa
- Shynu, R., Rao, V. P., Sarma, V. V. S. S., Kessarkar, P. M., Mani Murali, R. (2015). Sources and fate of organic matter in suspended and bottom sediments of the Mandovi and Zuari estuaries, western India. *Current Science*, 108(2); 226-238. <https://www.jstor.org/stable/24218162>
- Sundar, D., Shetye, S. R. (2005). Tides in the Mandovi and Zuari estuaries, Goa, west coast of India. *Journal of Earth System Science*, 114(5), 493-503. <https://doi.org/10.1007/BF02702025>
- USEPA (1986). Ambient Water Quality Criteria for Bacteria (Washington: U.S. Environmental Protection Agency, EPA440/5-84-002).
- Vignesh, S., Dahms, H. U., Emmanuel, K. V., Gokul, M. S., Muthukumar, K., Kim, B. R., James, R. A. (2014). Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India. *Environmental Monitoring and Assessment*, 186(3), 1875-1887. <https://doi.org/10.1007/s10661-013-3501-z>
- Vijith, V., Sundar, D., Shetye, S. R. (2009). Time-dependence of salinity in monsoonal estuaries. *Estuarine, Coastal and Shelf Science*, 85(4), 601-608. <https://doi.org/10.1016/j.ecss.2009.10.003>
- VishnuRadhan, R., Sagayadoss, J., Seelan, E., Vethamony, P., Shirodkar, P., Zainudin, Z., Shirodkar, S. (2015). Southwest monsoon influences the water quality and waste assimilative capacity in the Mandovi estuary (Goa state, India). *Chemistry and Ecology*, 31(3), 217-234. <https://doi.org/10.1080/02757540.2014.961435>
- Yang, X., Wu, X., Hao, H., He, Z. (2008). Mechanisms and assessment of water eutrophication. *Journal of Zhejiang University Science B*, 9(3), 197–209. <https://doi.org/10.1631/jzus.B0710626>

Figure Captions

Fig.1 Sampling locations in the Mandovi and Zuari estuary off Goa, India

Fig.2 Seasonal variations of temperature, salinity, pH, dissolved oxygen (DO) and biochemical oxygen demand (BOD) in the surface (white bars) and bottom (grey bars) water samples of (a) Mandovi and (b) Zuari estuary, dotted lines indicate permissible limits, error bars represent standard deviation (n=8)

Fig.3 Seasonal variation of nitrate, nitrite, phosphate and silicate in the surface (white bars) and bottom (grey bars) water samples of (a) Mandovi and (b) Zuari estuary, dotted lines indicate permissible limits, error bars represent standard deviation (n=8)

Fig.4 Water Quality Index in Mandovi (white) and Zuari (grey) estuary

Fig.5 Total viable counts in (a) Mandovi and (b) Zuari estuary, error bars represent standard deviation (n=8)

Table 1

List of water quality parameters and their permissible limits

Parameters	Standard values	Reference
DO(mg/L)	>5	1
BOD (mg/L)	<3	1
Temp (°C)	30	2
pH	6.5-8.5	1
Salinity (PSU)	35	2
Nitrate (µM)	<2	3
Phosphate (µM)	<1.2	1

1: USEPA, 1986; 2: Dheenan et al., 2014; 3: Jha et al., 2015

Table 2

Water quality index ranking criteria

WQI range	Category
<50	Excellent
50-100	Good
100-200	Poor
200-300	Very Poor
>300	Unfit for usage

Table 3 ANOVA: one factor to show the significance of seasonal changes between different parameters in the Mandovi and Zuari estuary.

Variables	MANDOVI				ZUARI			
	Df	F value	F critical	p value	Df	F value	F critical	p value
Temperature	2	23.03	3.20	<0.001***	2	1.12	3.20	0.33
Salinity	2	2.67	3.20	0.08	2	0.20	3.20	0.82
pH	2	2.46	3.20	0.10	2	0.31	3.20	0.74
DO	2	0.50	3.20	0.61	2	9.97	3.20	<0.001***
BOD	2	0.96	3.20	0.39	2	5.84	3.20	<0.005**
Nitrite	2	5.25	3.20	<0.008**	2	10.00	3.20	<0.001***
Nitrate	2	1.69	3.20	0.20	2	13.34	3.20	<0.001***
Phosphate	2	13.34	3.20	<0.001***	2	23.72	3.20	<0.001***
Silicate	2	5.62	3.20	0.006**	2	6.48	3.20	0.003**
TVC-water	2	3.91	3.20	0.02*	2	5.87	3.20	0.005**
TVC-sediment	2	0.96	3.47	0.40	2	2.64	3.47	0.09
TC- water	2	3.71	3.20	0.03*	2	2.84	3.20	0.06
TC-sediment	2	1.35	3.47	0.28	2	0.98	3.47	0.39
SFLO- water	2	2.95	3.20	0.06	2	0.77	3.20	0.47
SFLO-sediment	2	2.94	3.47	0.07	2	1.44	3.47	0.26
SALO- water	2	1.97	3.20	0.15	2	5.61	3.20	0.006**
SALO-sediment	2	2.44	3.47	0.11	2	1.04	3.47	0.37
PALO- water	2	1.58	3.20	0.22	2	4.11	3.20	0.02*
PALO-sediment	2	1.01	3.47	0.38	2	7.25	3.47	0.004**
SHLO- water	2	2.59	3.20	0.08	2	4.90	3.20	0.01*
SHLO-sediment	2	18.36	3.47	<0.001***	2	1.45	3.47	0.26
VLO- water	2	6.06	3.20	0.004**	2	15.82	3.20	<0.001***
VLO-sediment	2	3.23	3.47	0.05	2	5.60	3.47	0.01*

Df = degree of freedom, F value greater than F critical value indicates statistical significance, ***significant at 0.1 %, **significant at 1 % and *significant at 5 %. TVC: Total viable counts, TC: Total coliforms, SFLO: *Streptococcus faecalis* like organisms, SALO: *Staphylococcus aureus* like organisms, PALO: *Pseudomonas aeruginosa* like organisms, SHLO: *Shigella* like organisms, and VLO: *Vibrio* like organisms

Table 4 Average \pm standard deviation (n=8) abundance of pollution indicator organisms (CFU ml⁻¹ /g⁻¹) in the Mandovi and Zuari estuary

MANDOVI		TC	SFLO	SALO	PALO	SHLO	VLO
Pre-monsoon season	Surface water	ND	0 \pm 0	0 \pm 0	215 \pm 17 ^a	344 \pm 122	116 \pm 13 ^a
	Bottom water	ND	3 \pm 1	0 \pm 0	39 \pm 9 ^a	873 \pm 84	18 \pm 4 ^a
	Sediment	ND	2 \pm 3	0 \pm 0	4565 \pm 18 ^a	1179 \pm 120	3044 \pm 184 ^a
Monsoon season	Surface water	15 \pm 12	128 \pm 167	1 \pm 0	1 \pm 2	316 \pm 439	3 \pm 2
	Bottom water	11 \pm 2	230 \pm 186	1 \pm 2	4 \pm 5	393 \pm 551	4 \pm 4
	Sediment	5 \pm 4	141 \pm 64	367 \pm 75	1 \pm 1	2 \pm 0	4 \pm 4
Post-monsoon season	Surface water	2 \pm 2	0 \pm 0	1 \pm 1	2 \pm 3	23 \pm 11	6 \pm 5
	Bottom water	1 \pm 1	12 \pm 1	1 \pm 0	84 \pm 113	15 \pm 20	3 \pm 2
	Sediment	1 \pm 1	5 \pm 2	8 \pm 5	4 \pm 6	18 \pm 17	114 \pm 50
ZUARI		TC	SFLO	SALO	PALO	SHLO	VLO
Pre-monsoon season	Surface water	ND	0 \pm 0	0 \pm 0	23 \pm 4 ^a	128 \pm 42	226 \pm 12 ^a
	Bottom water	ND	7 \pm 3	0 \pm 0	45 \pm 15 ^a	59 \pm 3	303 \pm 21 ^a
	Sediment	ND	446 \pm 316	0 \pm 0	18214 \pm 1092 ^a	220 \pm 311	4723 \pm 217 ^a
Monsoon season	Surface water	1 \pm 2	6 \pm 3	4 \pm 3	1 \pm 1	17 \pm 4	5 \pm 6
	Bottom water	1 \pm 2	7 \pm 1	4 \pm 4	24 \pm 34	3 \pm 2	2 \pm 1
	Sediment	177 \pm 251	63 \pm 44	146 \pm 17	1 \pm 2	6 \pm 8	16 \pm 15
Post-monsoon season	Surface water	0 \pm 0	2 \pm 1	1 \pm 1	1 \pm 0	18 \pm 22	2 \pm 2
	Bottom water	0 \pm 0	1 \pm 1	1 \pm 0	36 \pm 25	6 \pm 6	6 \pm 8
	Sediment	3 \pm 1	17 \pm 6	24 \pm 4	3 \pm 1	17 \pm 16	74 \pm 98

^avalues represented are higher by the order of 10²

ND: Not determined, TC: Total coliforms, SFLO: *Streptococcus faecalis* like organisms, SALO: *Staphylococcus aureus* like organisms, PALO: *Pseudomonas aeruginosa* like organisms, SHLO: *Shigella* like organisms, and VLO: *Vibrio* like organisms

Table 5 Percentage frequency of isolates with varying Multiple Antibiotic Resistance (MAR) index

Category	MAR Index	Pre-monsoon	Monsoon	Post-monsoon
Low risk	< 0.2	86.1	75.8	68
High risk	0.2 - 0.4	8.3	15.2	28
Very high risk	> 0.4	5.6	9.1	4

Table 6 Pollution indicator organisms from Mandovi and Zuari estuaries with very high Multiple Antibiotic Resistance (MAR) index

		Pre-monsoon		Monsoon			Post-monsoon
Isolates ^a		PR13 Vibrio	PR27 <i>Shigella</i>	M2 Vibrio	M7 Coliform	M20 Coliform	PT13 Vibrio
Class of Antibiotic	Antibiotic tested (µg)	Diameter of growth inhibition zone (mm)					
Bacteriostatic agents							
Chloramphenicol	Chloramphenicol (30)	0	0	19	0	15	14
Tetracyclines	Tetracycline (30)	21	16	15	0	9	7
Macrolides	Azithromycin (15)	0	29	0	0	0	0
Sulfonamides	Co-trimoxazole (25)	21	28	10	0	10	0
Bactericidal agents							
Quinolones	Ciprofloxacin (5)	0	0	14	0	16	14
	Nalidixic Acid (30)	10	0	0	0	12	0
	Ofloxacin (5)	14	16	11	0	11	0
Lincosamides	Clindamycin (2)	0	0	0	8	7	0
Aminoglycoside	Netillin (30)	17	20	10	0	8	18
Ansamycins	Rifampicin (5)	0	0	0	0	0	0
Glycopeptides	Vancomycin (30)	0	0	0	0	0	0
β Lactam	Ampicillin (10)	0	16	0	0	0	0
	Ceftizoxime (30)	0	22	0	0	0	17
	Ceftriaxone (30)	0	27	0	0	0	21
	Piperacillin (100)	18	0	0	0	0	0
	Ertapenem (10)	15	35	19	0	12	20
% Resistance		56.3	43.8	56.25	93.75	43.75	56.25
MAR index		0.56	0.44	0.56	0.94	0.44	0.56

^a representative isolates with MAR index > 0.4, complete list of pollution indicator organisms tested are provided in the Supplementary Table S1
PR: Pre-monsoon, M: Monsoon, PT: Post-monsoon

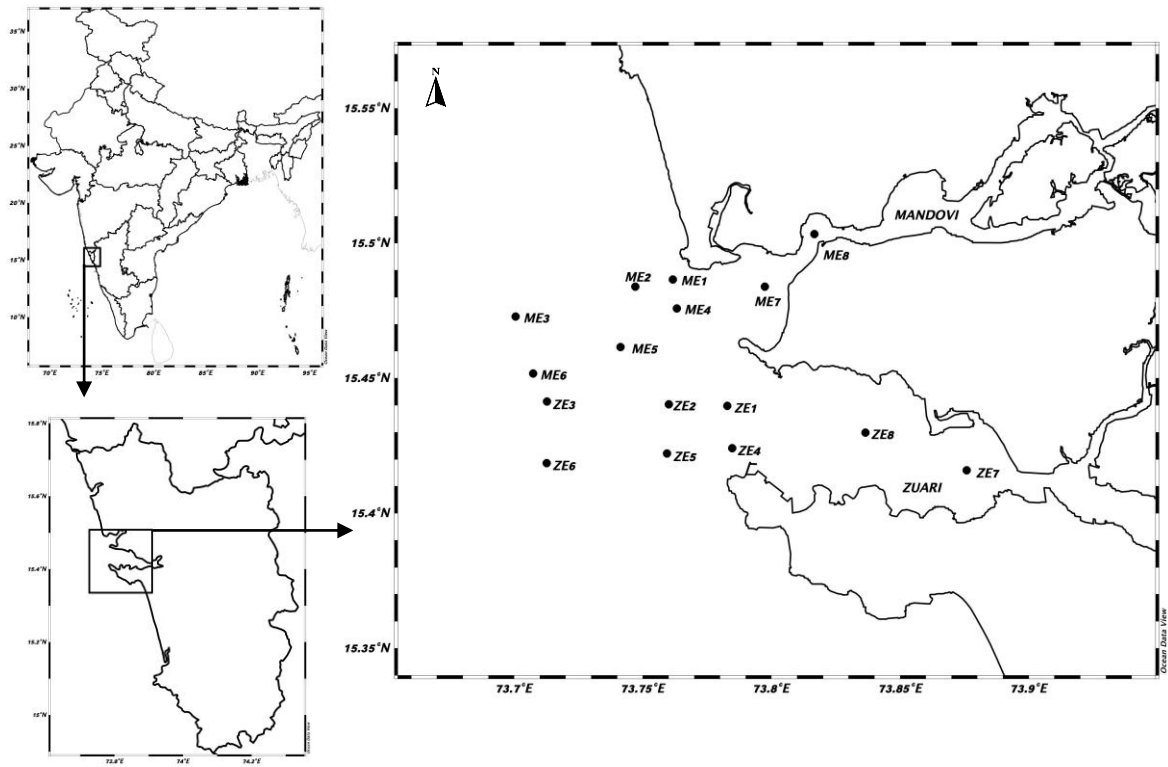


Fig.1 Sampling locations in the Mandovi and Zuari estuary off Goa, India

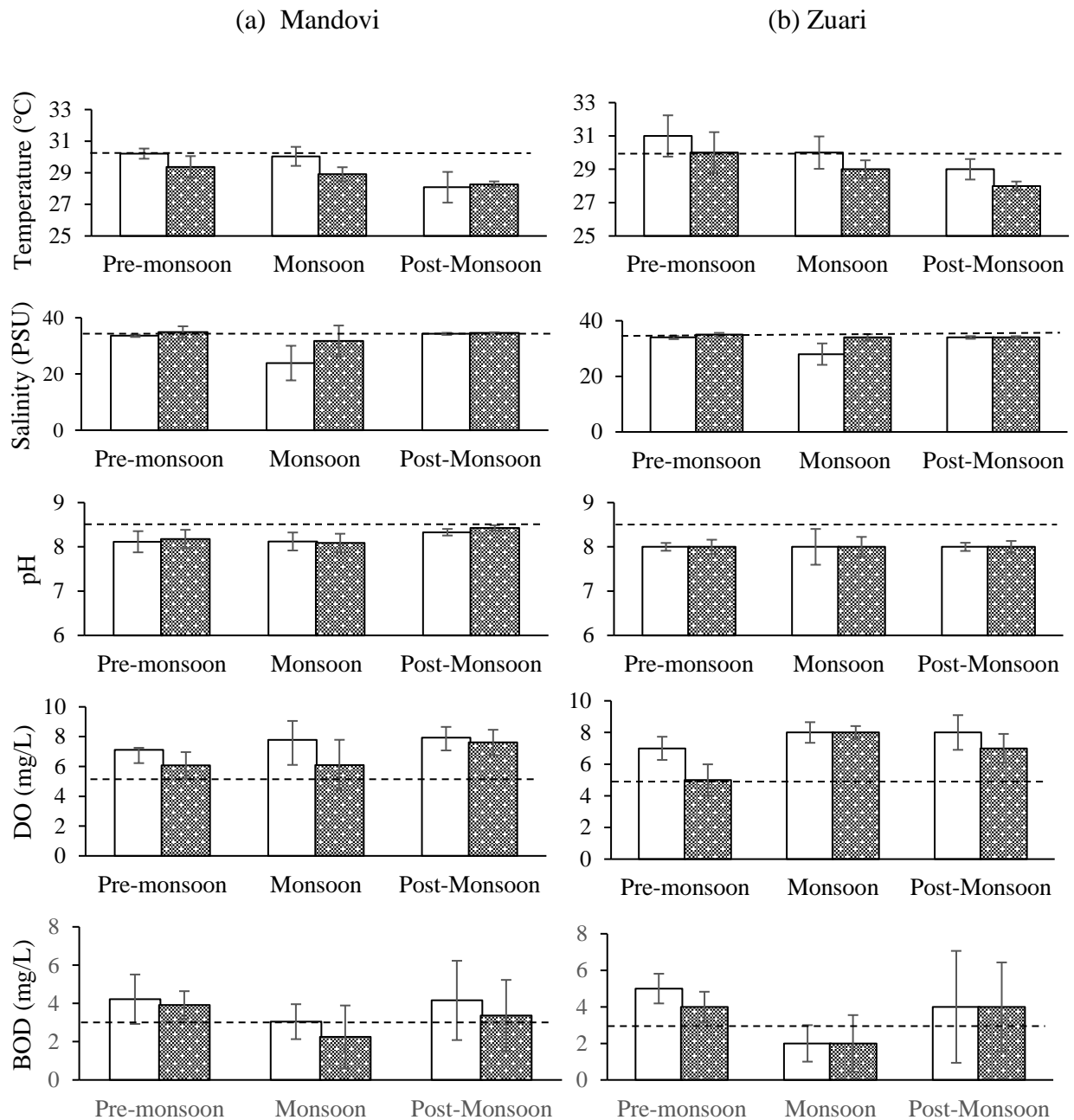


Fig.2 Seasonal variations of temperature, salinity, pH, dissolved oxygen (DO) and biochemical oxygen demand (BOD) in the surface (white bars) and bottom (grey bars) water samples of (a) Mandovi and (b) Zuari estuary, dotted lines indicate permissible limits, error bars represent standard deviation (n=8)

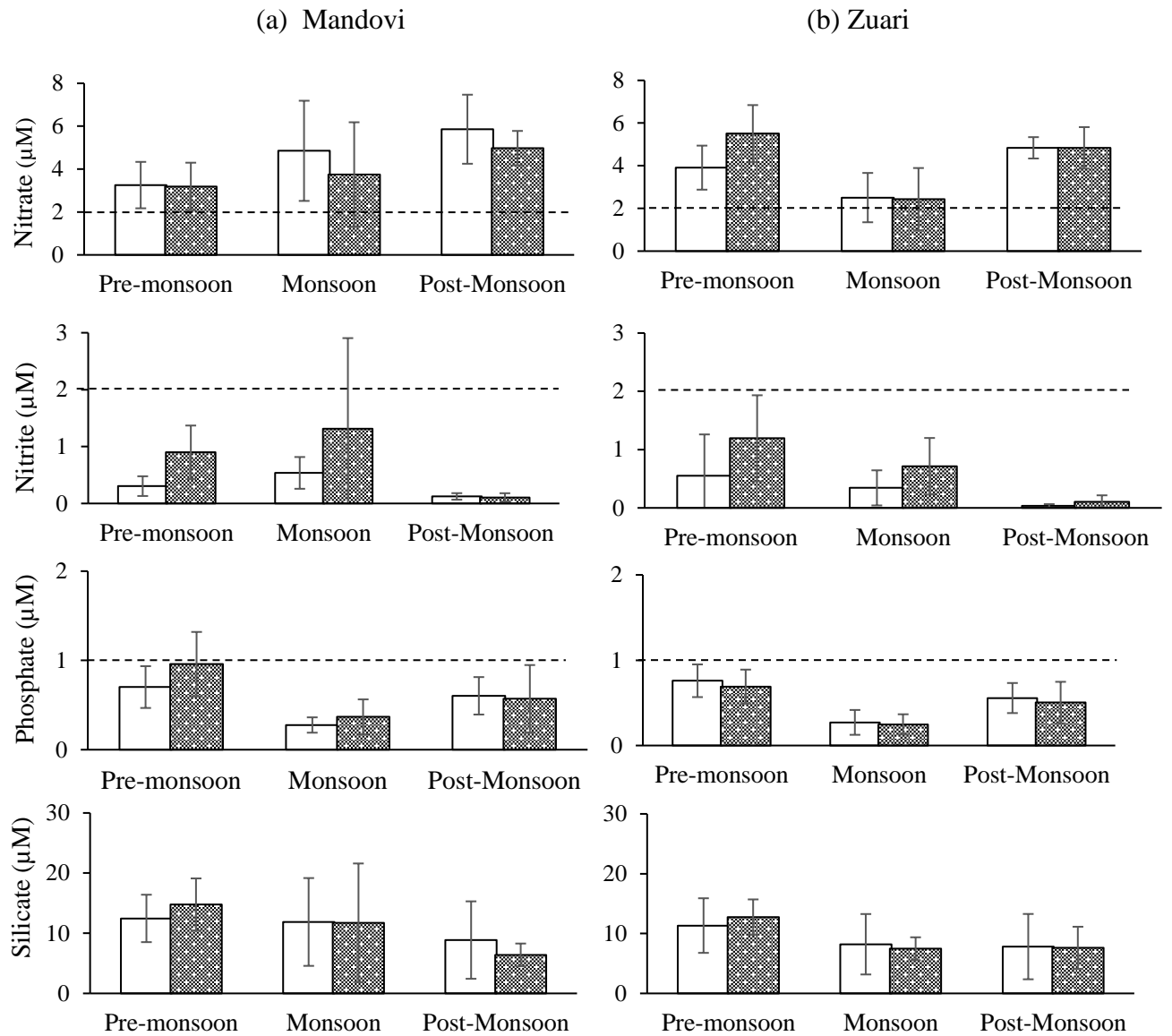


Fig.3 Seasonal variation of nitrate, nitrite, phosphate and silicate in the surface (white bars) and bottom (grey bars) water samples of (a) Mandovi and (b) Zuari estuary, dotted lines indicate permissible limits, error bars represent standard deviation (n=8)

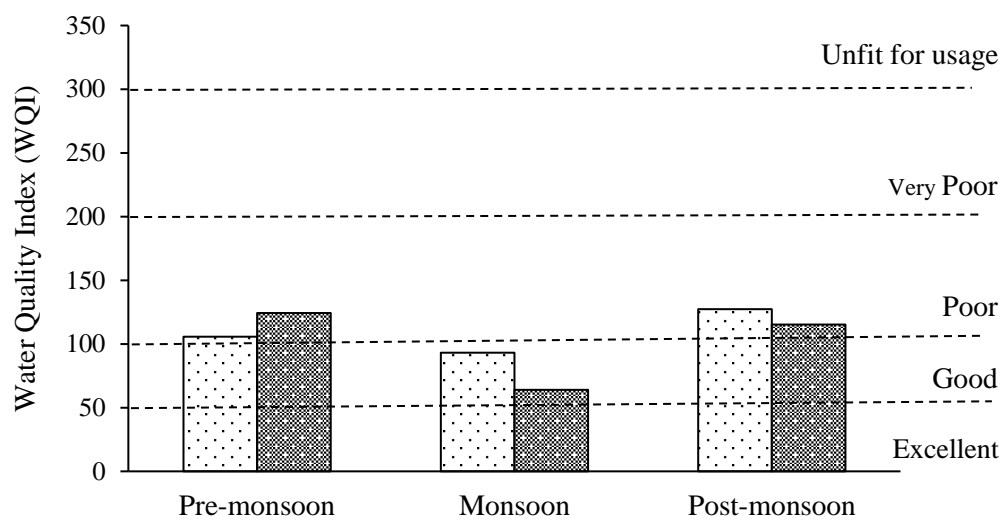


Fig.4 Water Quality Index in the Mandovi (white) and Zuari (grey) estuary

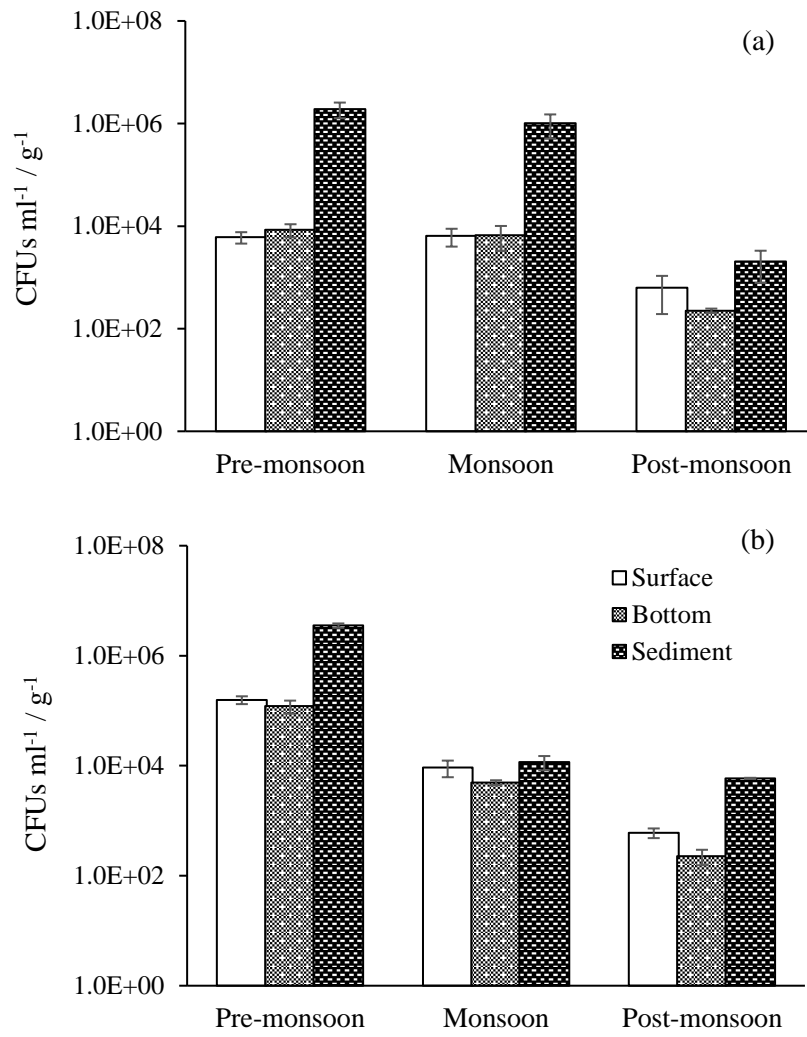


Fig.5 Total viable counts in (a) Mandovi and (b) Zuari estuary, error bars represent standard deviation (n=8).