

Influence of anthropogenic activities on the spatial distribution of heterotrophic, indicator and pathogenic bacteria around Andaman and Nicobar Islands

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Abstract

To examine the influence of anthropogenic activities on the marine ecosystem around the Andaman and Nicobar Islands, a study was conducted to investigate the abundance of heterotrophic, indicator and pathogenic bacteria during the monsoon season. We noticed the higher abundance of heterotrophic, indicator (*Escherichia coli* and *Enterococcus faecalis*) and pathogenic bacterial counts (*Aeromonas hydrophila*, *Enterobacter aerogens*, *Escherichia coli* strain O157:H7, *Shigella dysenteriae* and *Vibrio parahaemolyticus*) near the 10° channel, which is the principal route to reach Andaman mainland. Most of the stations are offshore (chosen to cover shipping tracks) at a distance ranging from 41 to 266 km from the coast. The nearest station to the coast was at 21 km away. Apart from those mentioned above, several other pathogenic bacteria were also detected such as *Klebsiella pneumoniae*, *Salmonella paratyphi*, *Vibrio cholera* and *Vibrio vulnificus* but they are sparsely detected at few stations only.

Keywords: Andaman and Nicobar islands; indicator bacteria; pathogenic bacteria; heterotrophic bacteria; marine waters pollution.

1. Introduction

A great percentage (25%) of the human population lives in countries that surround the Bay of Bengal (BoB), such as Bangladesh, India, Indonesia, Malaysia, Myanmar, Sri Lanka, Sumatra, and Thailand. These countries depend on the BoB for maritime activities (Sunil, 2013). Conventionally, the BoB has been a highway of maritime transportation to do business between different countries and hosts significant transport routes within the Indian Ocean. Several anthropogenic pressures such as discharges from domestic and industries from the coastal cities and also non-coastal cities through river discharges, air pollution coming from the Indo-Gangetic plain, mineral pollution through ports, ship traffic, ship-related discharges, fishing activities, recreational activities in the beach waters, oil and natural gas explorations etc. influence the region (Verma and Schulz, 2011; Sarma et al., 2020; Khan et al., 2017; Clark et al., 2003; Green Shipping, 21/07/18). Considerable maritime transport is taking place in the region. Coastal areas are continually influenced by natural and a variety of anthropogenic actions which can alter the value of coastal water rendering it unsafe for recreational activities (Prasad et al., 2015; Simeonov et al., 2003; Sudharani et al., 2018).

The Andaman and Nicobar (A & N) Islands are located in the eastern part of BoB, and separated by other countries such as Indonesia, Malaysia, Myanmar, and Thailand by the Andaman Sea (AS) (Fig. 1). The A & N Islands are sparsely populated, and the region is thought to be least influenced by anthropogenic activities. In the recent decade, the Indian government is giving more importance to the A & N islands as Indian strategic forces are located here to safeguard the waters and to improve the tourism industry which increases the vacation tourism to the islands through cruise ships. Recent studies have shown that the coastal waters of these islands are contaminated with indicator and pathogenic bacteria (Nallathambi et al., 2002; Swarnakumar et al., 2008; Gobalakrishnan et al., 2013). Nallathambi et al. (2002) study in the Port Blair bay (South Andaman) showed high counts of coliforms and indicator bacteria. During the peak monsoon season, the entire bay is flooded with rainwater coming from the island (due to hilly topology of the Port Blair town) and contaminates the bay. They noticed the presence of *Escherichia coli*, *Enterococcus faecalis* (previously known as *Streptococcus faecalis*), *Vibrio cholerae*, and *Vibrio parahaemolyticus* like bacteria. In a different study near Little Andaman Island by Swarnakumar et al. (2008) the authors showed the presence of heterotrophic, indicator (*Escherichia coli* and *Enterococcus faecalis*) and several pathogenic bacteria. A similar study around the coastal waters of Havelock Island by Gobalakrishnan et al. (2013) showed the same results as Swarnakumar et al. (2008). These studies showed the pollution level near the coastal region in this area.

Approximately half of the world's population live within 200 km of a coastline (Scialabba, 1998). Hence the pollution of marine ecosystems with bacterial pathogens is recognised as a notable hazard to both, the ecosystem and human health. Microbial contamination of sea waters takes place through several routes such as by bathers themselves, discharge of domestic waste directly to coastal regions, fishermen activities, river discharges (polluted from anthropogenic activities), sewage effluents and seabird faeces. Ships (especially cruise and passenger ships) also discharge human waste into marine waters, generally after sewage treatment. Which is classified as black water includes waste from toilets and health provisions in the ship, and is rich in indicator and possible chances of pathogenic bacteria which may adversely affect the marine ecosystem. Cruise ships can carry nearly 10,000 people (including passengers and crew) and can discharge around 210,000 gallons of human sewage into the marine waters during a week's voyage (Marine insight, 07.10.2019).

It is necessary to understand the spatial circulation pattern of indicator, and pathogenic bacteria either from natural (e.g. riverine) or anthropogenic (especially fishermen activities and ship-born) discharges in the marine ecosystem to assess their potential hazard to human and marine fauna. However, insufficient data is available regarding the influence of river discharge and anthropogenic activities such as ship born discharges and their spatial distribution in the offshore regions around the A & N Islands. The objective of the present work is to study the heterotrophic, indicator bacteria such as *Escherichia coli* (EC) and *Enterococcus faecalis* (EF) and human pathogenic bacteria such as *Aeromonas hydrophila* (AH), *Enterobacter aerogenes* (EA), *Escherichia coli* strain O157:H7 (EC O157), *Klebsiella pneumoniae* (KP), *Salmonella paratyphi* (SP), *Shigella dysenteriae* (SD), total *Vibrio* (TV), *Vibrio cholera* (VC), *Vibrio parahaemolyticus* (VP) and *Vibrio vulnificus* (VV) abundance and distribution around the A & N Islands during monsoon season to notice the influence of anthropogenic pressures on the marine ecosystem. We assume that the anthropogenic activities offshore the A & N islands are a source of indicator and pathogenic bacteria to the marine waters. To confirm our hypothesis, marine waters were sampled onboard ORV Sindhu Sadhana during August 2014, representing the monsoon period. The present study area was divided into the eastern BoB and central AS (Fig. 1).

2. Materials and methods

2.1. Study site

A large area of Indian exclusive economic zone (EEZ) (covering 30%) around the A & N Islands is more advantageous, rich in resources and economically profiting (Pradeep et al., 2014).

The territory is 220 km north of Aceh (Indonesia) and separated from Myanmar and Thailand by the AS (Fig. 1). A & N Islands are separated by the 10° channel, the Andaman Islands located at the north and the Nicobar Islands at the south of the channel. The A & N archipelago is one of the recognised biodiversity hotspot of the globe with 572 islands (Sawhney and Pravin, 2019). Coastal regions of the A & N Islands are affected by natural pressures such as the discharge of different rivers (Ganges and Irrawaddy) and heavy precipitation during the SW monsoon period. The A & N region is also influenced by the anthropogenic pressures such as massive traffic of cruise (recreational and passenger transport vessels) and cargo ships (Fig. 1c). These pass through either the 6 or 10° channel to reach coastal cities of the A & N Islands, Myanmar, Thailand & Indonesia. The major port in the A & N Islands is in Port Blair. This port handles large number of vessels and imports. During 2014-2015 11,626 vessels called by this port. It handled 804316 MT of Bulk cargo through 37,002 containers. A total of 18.53 Lakhs passengers transit through this port and these include inlanders (native people of the A & N Islands who visited main land, India) and also the tourists. A total of 5 Cruise ships and 38 Yachts visited this port during this period. Most of them pass through the 10° channel to reach this port (<http://andssw1.and.nic.in/ecostat/EconomicReview2017/11.Transport.pdf>).

2.2. Sample collection and analysis

The sampling was performed in the coastal and offshore (EEZ) regions of the A & N islands at a total of 23 stations (15 at the AS and 8 at the BoB) (Fig. 1b). Most of the sampling locations are away from the coast. The distance from the nearest coast of the A & N islands of each station varies from 21 (Station 14) to 266 km (Station 12) (Table 1). The distance of stations from the Ganges and Irrawaddy are also given in Table 1. Salinity, temperature and pressure were observed using a portable conductivity, temperature and depth (CTD) profiler (SBE 19 plus; Sea-Bird Electronics, USA) connected to the rosette sampler. Water samples were collected from surface using a 10 L Niskin bottle attached to the rosette sampler. Bubble-free water samples were initially collected for dissolved oxygen (DO) measurements subsequent the Winkler titration method of Carritt and Carpenter (1966). The analytical accuracy, articulated as standard deviation, was 0.07 % for oxygen. The concentrations of nutrients were calculated following standard spectrophotometric procedures using an autoanalyser (Technicon; Traacs, Australia) (Grasshoff et al., 1983). The precision (SD) for nitrate, ammonium and phosphate were ± 0.05 , ± 0.05 and ± 0.02 μM respectively. A 2-L surface water sample was filtered through a GF/F filter (0.5 μm pore size; Whatman) for chlorophyll determination. Chlorophyll-*a* (Chl-*a*) on the filter was first extracted with N, N-dimethylformamide

(DMF), at 4° C in the dark for 12 h and then spectrofluorometrically analysed (Varian Eclipse fluorescence photometer) following Suzuki and Ishimaru (1990). The analytical precision for Chl-*a* analysis was ± 4 %. Total Suspended Matter (TSM) concentration in the water sample was analysed by filtering about 1L of surface water sample through the pre-weighed 0.22 µm filter (Millipore), dried at 40 °C and reweighed to quantify TSM (mg L⁻¹) retained on the filter.

About 100 ml of surface water sample was taken into a pre-sterilised bottle for bacterial analysis. All samples were collected with the precautions required for microbiological analysis. They were analysed onboard ship microbiology laboratory. Enumeration of heterotrophic, indicator and pathogenic bacteria were done following Sudharani et al. (2018), and Prasad et al. (2015). Different selective media were used for specific bacterial groups. Heterotrophic bacterial (HB) counts were determined on R2A agar. Enumeration of indicator bacteria such as *Escherichia coli* and *Enterococcus faecalis* were determined on McConkey agar and Rapid HiEnterococci (RHE) agar respectively. Enumeration of pathogenic bacteria such as *Aeromonas* on *Aeromonas* Isolation agar, *Escherichia coli* O157:H7 on HiCrome EC O157:H7 agar, *Salmonella* spp. and *Shigella* spp. on Xylose-Lysine-Deoxycholate (XLD) agar. *Vibrio* spp. were determined on Thiosulphate citrate bile salts sucrose (TCBS) agar. Serially diluted (1/10) water samples of 100 µl were spread plated on respective agar plates and incubated at 37 °C for 3-5 days. The specific colonies (unique to the organism of interest) grown on the respective agar media were quantified, and the results were expressed in colony-forming units (CFU ml⁻¹) according to Nagvenkar and Ramaiah (2009), Prasad et al. (2015) and Sudharani et al. (2018). Total microbial (TM) count was determined using SYBR Green I fluorescent dye under an upright fluorescent microscope (Nikon, Japan) according to the method by Noble and Fuhrman (1998).

2.3. Statistical analyses of data

The nonparametric Wilcoxon tests (Wilcoxon, 1945) were performed using Wilcoxon Signed-Ranks Test Calculator to determine significant difference in the data from stations of Bay of Bengal and Andaman Sea. We calculated the nonparametric Spearman's r_s correlation coefficient for each pair of response variables. Significance was considered for $p < 0.05$. To determine the effect of environmental variables on biological parameters principal component analysis (PCA) was performed. Prior to analysis all values were transformed according to the following formula to normalize scale of different parameters. New value (X^*) = $X - \text{Mean} / \text{Standard deviation}$. Analyses were performed with Past version 4.02.

3. Results

3.1. Physico-chemical characteristics of surface waters around the A & N islands

Physical and biogeochemical characteristics demonstrated east-west variations around the A & N Islands in the BoB and AS. The surface water temperature ranged between 28.25 °C and 28.75 °C (Fig. 2a). Salinity of surface water differed between 31.74 and 33.76 practical salinity unit (PSU) around the A & N Islands. Comparatively, lesser salinities were noticed in the northern part than the southern area (Fig. 2b). The Chl-*a* varied from 0.02 to 0.66 µg/L, with relatively higher concentrations in stations 14, 19, 24 and 26 which are near the 10° channel in both the AS and the BoB (Fig. 2c). DO varied from 165 and 191 µmol and concentration was higher in the BoB (Fig. 2d). The TSM varied from 0.5 to 2.7 mg/L (Fig. 3a) with higher values near the 10° channel. Similarly, nutrients, nitrite+nitrate, phosphate and silicate concentrations ranged from 0.01 to 0.27 µM, 0.01 to 0.54 µM and 1.1 to 2.9 µM, respectively, with high levels of silicate and phosphate in the northern area and moderately high concentration of phosphate near the 10° channel (Figs. 3b-d). Wilcoxon test showed that significant difference was observed for silicate concentrations ($Z = -2.0896$; p value: 0.03662) $p < 0.05$) among the BoB and the AS regions.

3.2. Bacterial counts around the A & N islands

The TM counts varied between 1.7 to 9.0 × 10⁶ cells/ml (Fig. 4a). The highest count was observed in station 13. Moderate abundance was detected in stations 15, 23, 24 and 25 which are distributed around the AS and the BoB (near the 10° channel). The HB counts ranged from 0.06 to 8.0 × 10³ CFU/ml around the A & N Islands (Fig. 4b). The highest counts were observed in stations 16 and 17. Moderate abundances were detected in stations 8, 13-21 which are in AS. The HB counts were higher in the AS compared to the BoB and the highest counts were observed near the 10° channel. Wilcoxon test showed that significant variation was observed in case HB counts ($Z = -2.7308$; p value: 0.00634) $p < 0.01$ among the AS and the BoB. The indicator bacteria such as EC and EF counts ranged from 0 to 2.0 × 10³ and 0 to 1.5 × 10² CFU/ml respectively around the A & N Islands (Figs. 5a, b). Apart from being found in higher numbers, EC were found in most of the sampling points, whereas EF was only detected in some stations. Wilcoxon test showed that significant variation was observed in case EC counts ($Z = -2.5508$; p value: 0.01078) $p < 0.01$ among the BoB and the AS. The pathogenic bacteria such as AH, EA, EC 0157, KP, SD, SP, TV, VC, VV and VP counts ranged from 0 to 2.0 × 10³ CFU/ml around the A & N islands (Figs. 6, 7, 8). Each bacterial abundance (CFU/ml) range and percentage of occurrence across the sampling stations is given in Table 2. Interestingly AH, EA, EC 0157, TV and VV were detected in several stations (30 to 78% of

them; Table 2) and AH was the one more detected. Wilcoxon test showed that significant variation was observed in case AH ($Z = -1.9879$; p value: 0.0466) $p < 0.05$ and ECO157 counts ($Z = -2.2014$; p value: < 0.05) $p < 0.01$ among the BoB and the AS. KP and SD were detected in very few stations, mostly detected in AS except KP that was detected in a single station in BoB. SP, and VC were detected in only single stations of the northern part of the area of study (7 and 6) located in the AS and the BoB respectively, while VP was found in station 18, in the 10° channel, were most of the other bacteria were also found. In the case of pathogenic bacteria, there is no specific trend observed in their abundance. PCA analysis of physico-chemical and biological parameters in the AS and BoB were done and the first two vectors in the PCA explained 42.3% of variance in the data. The Principle component analysis (PCA) based on physico-chemical and microbiological variables indicates that the BoB and the AS stations clustered separately. TSM, total Chl-*a*, silicate, KP, EF and VV were grouped closely and fall near the BoB region. HB, EC, AH, EA, ECO157, SD and TV were grouped closely and fall near the AS region. DO and phosphate grouped closely and temperature, nitrate+nitrite, salinity, total microbial counts were grouped closely but not falling in between the BoB and the AS (Fig. 9).

4. Discussion

Spatial variations were noticed with respect to different physico-chemical parameters like temperature, salinity, Chl-*a*, DO, TSM, and nutrients between the AS and the BoB due to influence of discharge from different rivers (Fig. 1a, Table 1). The discharge influence of the Irrawaddy River is present in the north-eastern side of the islands. The stations there located show lower salinity and higher silicate concentration (which may be due to massive influx from the Ganges and Irrawaddy Rivers), but towards the south, the salinity increases and silicate concentration decreases, which is a typical picture of the riverine influence. Salinity inversely correlated with silicate ($r^2 = -0.64$, $p < 0.01$) (Table 3) indicates that as salinity increases silicate concentration decreases and as freshwater discharge increases (comprises higher silicate concentration), which decrease the salinity of surface water. Silicate concentration showed significant variations among the AS and the BoB and higher in the AS which is statistically significant according to the Wilcoxon test and PCA analysis (Fig. 9), which showed that silicate was nearer to the BoB. Although high phosphate and silicate observed in northern stations the phytoplankton growth is limited because of very low concentrations of nitrite + nitrate (Fig. 2c, d and 3b) in that region. Salinity correlated positively with nitrate + nitrite ($r^2 = 0.62$, $p < 0.01$) (Table 3). Similar correlations of salinity with silicate and nitrite + nitrate were observed by Sudharani et al. (2018). The higher DO concentrations in the BoB stations correlates with higher

concentrations of nitrite + nitrate that may have triggered phytoplankton growth (Fig. 2c, d and 3b). DO correlated positively with nitrite + nitrate ($r^2 = -0.63$, $p < 0.01$) (Table 3) indicates that higher concentration of later increase the phytoplankton growth which in turn enhance the DO in that region. This is also explained with PCA (Fig. 9), which showed that nitrite + nitrate and total chlorophyll-*a* were nearer to the BoB. Very high TSM value was found near to the 10° channel stations showed the possible anthropogenic input from ship related discharges.

Relatively lower total microbial counts were observed at stations 7, 10 and 11, which are located far away from the mainland of Andaman coast; whereas the highest was found at station 13 in the 10° channel (Fig. 4a). The distribution of HB load around the A & N Islands illustrated that relatively low HB counts were observed along the stations except at stations 16, 17 and 18. These are located in the 10° channel, which may be due to the high shipping activity, as it is a significant transportation route to the AS, and fishermen activities (Fig 1b and Fig. 4b). Though discharge of the Ganges and the Irrawaddy river water enters the BOB and AS the influence of these waters on the heterotrophic count is not seen as both are far away, especially the mouth of the Ganges (Fig. 1, Table 1). HB counts vary with respect to the habitat, during phytoplankton bloom or due to seasonal changes which is evident from the previous studies along coastal waters in the BoB (Venkateswaran and Natarajan, 1987; Nallathambi et al., 2002; Clark et al., 2003; Swarnakumar et al., 2008; Patra et al., 2009; Gobalakrishnan et al., 2013; Prasad et al., 2015; Sudharani et al., 2018). Nevertheless, our HB counts are lower than those found in previous studies in the BoB, but the sampling stations in the previous studies are closer to the coast as compared to our study where land driven influence is higher and direct. Lower counts in the BOB stations are attributed to the lesser human intervention in this region. Surface water TSM inversely correlated with HB ($r^2 = -0.51$, $p < 0.05$) (Table 3), as higher amounts of the former reduce light penetration and effect photosynthesis resulting in low *in-situ* derived DOC. This is also explained with PCA (Fig. 9), which showed that TSM was closer towards the BoB and HB was closer to the AS. Similar correlation was observed by Prasad et al. (2015) and Sudharani et al. (2018). Heterotrophic bacterial counts showed significant variations among the AS and the BoB and higher in the AS which is statistically significant according to the Wilcoxon test and PCA analysis.

Indicator bacteria are conventionally used to notice the occurrence of pathogenic bacteria in the aquatic ecosystems (Berg, 1978). *Escherichia coli* and *Enterococcus faecalis* are commonly used as indicator bacteria, and their presence in the water suggests the possible occurrence of pathogens (Ashbolt et al., 2001). EC counts were detected in most of the stations in the study region (Fig. 5a).

The presence of EC counts in marine habitats indicates the release of untreated sewage from the coastal cities (Swarnakumar et al., 2008; Nallathambi et al., 2002; Gobalakrishnan et al., 2013; Clark et al., 2003; Patra et al., 2009). The occurrence of the EC in higher abundance in the sampled area designates the possible existence of pathogenic bacteria (Rozen and Belkin, 2001; Piccolomini et al., 1987). Like HB, EC counts are also higher near the 10° channel, which may be due to the high shipping and fishermen activities. Nitrite + nitrate concentrations inversely correlated with EC ($r^2 = -0.72$, $p < 0.001$) (Table 3) showed that these were not from the natural marine water as the former increase the phytoplankton growth and in turn increase the usable *in-situ* DOC as a result autochthonous marine bacteria proliferate. As EC is not the autochthonous it is abundant wherever the anthropogenic activity is more. This is also explained with PCA (Fig. 9), which showed that nitrite + nitrate were closer towards the BoB and EC was closer to the AS. EC counts showed significant variations among the AS and the BoB and higher in the AS which is statistically significant according to the Wilcoxon test and PCA analysis. EF counts were detected only at a few stations in the study region (Fig. 5b). The presence of EF counts may indicate untreated sewage comprised of human faeces was entering into the marine waters in different ways like from fishermen and shipping activities. No counts in the BOB stations are attributed to the lesser human intervention in this region. Nitrite + nitrate concentrations inversely correlated with EF ($r^2 = -0.56$, $p < 0.05$) (Table 3) indicates that these are also allochthonous bacteria enter marine habitat due the anthropogenic intervention. Although we got correlation similar to EC but EF is observed in fewer stations and in PCA also which is in between the BoB and the AS (Fig. 9).

AH and EA counts were detected at several stations in the study region (Fig. 6a, b). Relatively low counts were detected in all stations except at stations 17 and 18 (for AH) and 18 (for EA). These stations are located in the 10° channel; the high number in this region may be due to the high shipping activity and fishery activities (Fig. 4a). TSM inversely correlated with AH ($r^2 = 0.49$, $p < 0.05$) showed that this bacterium is autochthonous and cause diseases in marine fish and will be negatively influenced by the high TSM. ECO157 is enterohemorrhagic serotype transmitted with fouled marine food or water which might cause hemorrhagic diarrhoea and finally kidney failure. ECO157 counts were detected only at a few stations (Fig. 6c). The presence of this pathogenic strain in such a high number in station 17 is alarming (Fig. 6c). AH and ECO157 counts showed significant variations among the AS and the BoB and higher in the AS which is statistically significant according to the Wilcoxon test. This is also explained with PCA (Fig. 9), which showed that AH and ECO157 were closer towards the AS. KP counts were only detected in the stations 19, 20 and 27, with overall low counts but in station 19 (Fig. 6d). KP counts were noticed in lesser number in

marine waters (Gobalakrishnan et al., 2013; Swarnakumar et al., 2008). SP counts were only detected in station 7 (Fig. 7a). SD counts were detected in 4 stations, and higher counts were observed in stations 17 and 18 which are located in the 10° channel (Fig. 7b). However, their occurrence has been noticed in several studies carried in the BoB coastal regions (Clark et al., 2003; Gobalakrishnan et al., 2013; Patra et al., 2009; Swarnakumar et al., 2008; Venkatswaran et al., 1989). AH correlated positively with SD ($r^2 = 0.50$, $p < 0.05$) (Table 3) as high abundance of AH might show the possible presence of SD. This is also shown in the PCA analysis where both AH and SD clustered together (Fig. 9). TV counts were detected in 10 stations, and higher counts were observed in stations 17 and 18 located in the 10° channel (Fig.8a). VV was more abundant over VC, and VP (Figs. 8b-d) in this work and their abundance is lower compared with previous studies in the BoB (Nair et al., 1980; Gobalakrishnan et al., 2013; Nallathambi et al., 2002; Swarnakumar et al., 2008). HB correlated positively with TV ($r^2 = 0.48$, $p < 0.05$) showed that the higher abundance of *Vibrio* species present where higher heterotrophic bacteria as both are autochthonous bacteria which is also evident from the PCA analysis (Fig. 9). EA correlated positively with VP ($r^2 = 0.42$, $p < 0.05$) (Table 3) as high abundance of the former might showed possible presence of VP. TV and EF correlated positively with VV ($r^2 = 0.70$ and 0.42 , $p < 0.001$ and 0.05) (Table 3) as high abundance of the former might showed possible presence of VV.

Most of the bacterial counts such as HB, EC, AH, EA, ECO157, SD and TV were significantly higher in AS, what indicates the impact of anthropogenic activities in this region. This is confirmed by the Wilcoxon test and PCA analysis.

The distance between the nearest sampling station (station 7) from Irrawaddy River is around 264 km, and the distance between the Ganges River and nearest sampling station (station 6) is approximately 942 km (Table 1). So the transportation of bacterial load (indicator and pathogenic bacteria) to such long distance is very low in the surface water. In a study by Prasad et al., (2015) in the coastal to offshore waters of Bay of Bengal from off Krishna river (south) to off Mahanadi river (north), showed that the indicator and some pathogenic bacterial counts were observed from coast to 50 and 100 km (south to north) but their abundance decreased with respect to the distance from the coast. In the present study, the sampling stations distance from the A & N islands varied from 21 to 266 km (Table 1). The sewage discharge from the islands would not reach that much long distance as there are no major rivers in the islands, and also the population density is less compared to the mainland. Hence we hypothesised that the presence of these bacteria has its origin in offshore anthropogenic activities such as passenger or cruise ships and fisheries. High heterotrophic, indicator

and pathogenic counts in the stations near the 10° channel may be due to the high shipping activity as it is a significant shipping channel to AS (<http://andssw1.and.nic.in/ecostat/EconomicReview2017/11.Transport.pdf>). Lower counts in the BoB stations are attributed to the lesser human intervention in this region. The presence of pathogenic bacterial counts in the offshore areas may be because of intense fishermen activity and shipping traffic, which may release the untreated sewage. Untreated sewage discharge (black water) from ships and direct release of human faecal matter by fishermen activities not only intimidate life in marine habitats but also put human health in danger. Consumption of seafood from marine waters contaminated with the pathogenic bacteria cause health issues (Shuval, 1999).

5. Conclusions

HB count illustrates noteworthy spatial distinction around the A & N Islands due to variable anthropogenic pressures. Comparatively elevated heterotrophic bacterial abundance was noticed in the AS stations, especially near the 10° channel, compared to the BOB stations (where shipping traffic is less intense) which is evident from Wilcoxon test and PCA analyses. Coliforms, and possible pathogenic bacteria, were noticed in elevated abundance in the AS area. The occurrence of *Aeromonas hydrophila*, *Klebsiella pneumonia*, *Escherichia coli* O157:H7, *Enterococcus faecalis*, *Enterococcus aerogens*, *Salmonella paratyphi*, *Shigella dysenteriae*, *Vibrio cholera*, *Vibrio parahaemolyticus* and *Vibrio vulnificus* around the A & N island waters, especially in the AS region, is disturbing. As most of the sampling stations were far away from the mainland of the A & N Islands (open ocean region) and also away from riverine discharge, we are anticipating untreated domestic release from land is minimal. So the major cause might be due to anthropogenic offshore activities such as ship related discharges and/or fishermen activities. Marine water contaminated with indicator bacteria deteriorates the quality of the marine ecosystem that increase health risk and subsequent loss of the economy due to the associated pathogens.

CRedit authorship contribution statement:

Srinivas T. N. R.: Conceptualization, Project administration, Methodology, Writing - original draft, Writing - Review & Editing. Sudharani, P., Swarnaprava Behera, Dutta, S.: Supervision, Methodology, Writing - review & editing. Sathibabu, Y., Sampath Kumar, G., Geethika, G.: Formal analysis, Visualization, Software. Swagata, C. and Pratim Kandar, P.: Investigation, Data curation.

Declaration of competing interest:

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Figure Legends:

Fig. 1. Map showing the study area in the north-eastern Indian Ocean (Arrows shows mouth of the Ganges and Irrawaddy Rivers) (a), sampling locations in the Bay of Bengal (BB; station Nos. 6, 22-28) & Andaman Sea (AS; station Nos. 7-21) (Arrow shows mouth of the Irrawaddy River) (b) and shipping traffic on August 2014 (c) in the present study region. Numbers in the map 1a shows the corresponding country. 1. India; 2. Sri Lanka; 3. Bangladesh; 4. Myanmar; 5. Indonesia; 6. Malaysia; 7. Thailand.

Fig. 2. Spatial variation of temperature (a), salinity (b), Chlorophyll-*a* (c) and dissolved oxygen (d) in the surface waters around the A & N Islands.

Fig. 3. Spatial variation of total suspended matter (a), nitrite + nitrate (b), phosphate (c) and silicate (d) concentrations in the surface waters around the A & N Islands.

Fig. 4. Spatial variation of total microbial count (a) and total heterotrophic count (b) in the surface waters around the A & N Islands.

Fig. 5. Spatial variation of *Escherichia coli* (a) and *Enterococcus faecalis* (b) counts in the surface waters around the A & N Islands.

Fig. 6. Spatial variation of *Aeromonas hydrophila* (a), *Enterobacter aerogenes* (b), *Escherichia coli* strain O157:H7 (c) and *Klebsiella pneumoniae* (d) counts in the surface waters around the A & N Islands.

Fig. 7. Spatial variation of *Salmonella paratyphi* (a) and *Shigella dysenteriae* (b) counts in the surface waters around the A & N Islands.

Fig. 8. Spatial variation of total *Vibrio* (a), *Vibrio cholera* (b), *Vibrio vulnificus* (c) and *Vibrio parahaemolyticus* (d) counts in the surface waters around the A & N Islands.

Fig. 9. Principal component analysis (PCA) indicating characteristics of different sampling stations with respect to different water quality parameters in the surface waters around the A & N Islands. Red squares indicate BOB stations and black dots AS stations. Temp, temperature; Sal, salinity; TChl-*a*, total chlorophyll-*a*; DO, dissolved oxygen; TSM, total suspended matter; PO₄³⁻, phosphate; SiO₄⁴⁻, silicate; NO₂⁻+NO₃⁻, nitrite + nitrate; TM, total microbial; HB, heterotrophic bacteria; EC, *Escherichia coli*; EF, *Enterococcus faecalis*; AH, *Aeromonas hydrophila*; EA, *Enterobacter aerogenes*; ECO157, *Escherichia coli* O157:H7; KP, *Klebsiella pneumoniae*; SD, *Shigella dysenteriae*; TV, Total *Vibrio*; VV, *Vibrio vulnificus*; AS, Andaman Sea; BoB, Bay of Bengal; CFU, colony forming unit.

Table 1. Station locations and distance from nearest coast, mouth of Ganges and Irrawady Rivers.

Station Number	Latitude	Longitude	*Distance from coast (Km)	Distance from mouth of Ganges (Km)	Distance from mouth of Irrawaddy (Km)
St.6	13.919	92.503	64	942	348
St.7	13.599	94.164	117	1122	264
St.8	13.069	95.101	223	1235	304
St.9	13.001	93.389	41	1140	359
St.10	12.504	94.141	129	1224	380
St.11	11.638	94.555	198	1330	467
St.12	11.01	95.074	266	1416	531
St.13	10.371	94.005	161	1447	613
St.14	9.287	92.99	21	1498	756
St.15	9.989	92.994	80	1454	682
St.16	10.413	93.278	79	1443	628
St.17	9.795	93.701	116	1520	681
St.18	8.995	94.005	130	1610	761
St.19	8.512	95.000	163	1705	808
St.20	7.49	94.99	127	1785	920
St.21	6.407	94.079	47	1833	1046
St.22	6.095	93.52	82	1841	1088
St.23	8.747	92.301	64	1521	838
St.24	9.165	92.003	81	1466	806
St.25	9.927	91.819	92	1379	794
St.26	10.299	91.504	101	1330	761
St.27	12.003	91.504	113	1151	582
St.28	13.502	91.7	126	1006	447

*, Distance from coast refers to the distance between a given station and its closest coastal location.

Table 2. Hydrographic characteristics of the surface water samples collected from the Bay of Bengal and the Andaman Sea.

Bacteria	Range	Highest count in station	Area	Occurrence (%)
TM (cells/ml)	1.7 to 9.0 x 10 ⁶	13	AS	100
HB (CFU/ml)	0.06 to 8.0 × 10 ³	17	AS	100
EC (CFU/ml)	0 to 2.0 × 10 ³	18	AS	82
EF (CFU/ml)	0 to 1.5 × 10 ²	14	AS	34
AH (CFU/ml)	0 to 8.3 × 10 ²	18	AS	78
EA (CFU/ml)	0 to 2.0 × 10 ³	18	AS	34
EC157 (CFU/ml)	0 to 5.9 × 10 ²	17	AS	30
KP (CFU/ml)	0 to 3.0 × 10 ¹	19	AS	13
SD (CFU/ml)	0 to 5.4 × 10 ²	18	AS	17
SP (CFU/ml)	0 to 1.0 × 10 ¹	7	AS	4
TV (CFU/ml)	0 to 8.1 × 10 ²	17	AS	43
VC (CFU/ml)	0 to 1.0 × 10 ¹	6	BoB	4
VP (CFU/ml)	0 to 3.0 × 10 ¹	18	AS	4
VV (CFU/ml)	0 to 3.0 × 10 ¹	8, 24	AS, BoB	34

TM, total microbial; HB, heterotrophic bacteria; EC, *Escherichia coli*; EF, *Enterococcus faecalis*; AH, *Aeromonas hydrophila*; EA, *Enterobacter aerogenes*; EC157, *Escherichia coli* O157:H7; KP, *Klebsiella pneumoniae*; SD, *Shigella dysenteriae*; SP, *Salmonella paratyphi*; TV, Total *Vibrio*; VC, *Vibrio cholerae*; VP, *Vibrio parahaemolyticus*; VV, *Vibrio vulnificus*; AS, Andaman Sea; BoB, Bay of Bengal; CFU, colony forming unit.

Table 3. Rank correlation matrix (Spearman's rs) of biogeochemical parameters measured in the surface waters around the A & N Islands

	Temp	Sal	DO	TSM	PO ₄ ³⁻	SiO ₄ ⁴⁻	NO ₂ ⁻ +NO ₃ ⁻	Chl- <i>a</i>	TM	HB	EC	EF	AH	EA	ECO157	KP	SD	SP	TV	VC	VP	VV	
Temp	1.00																						
Sal	-0.01	1.00																					
DO	-0.39	0.24	1.00																				
TSM	0.26	0.10	0.08	1.00																			
PO ₄ ³⁻	-0.01	0.39	0.11	-0.01	1.00																		
SiO ₄ ⁴⁻	-0.13	<i>-0.64</i>	0.05	-0.13	0.01	1.00																	
NO ₂ ⁻ +NO ₃ ⁻	-0.04	<i>0.62</i>	<i>0.63</i>	0.32	0.25	-0.31	1.00																
Chl- <i>a</i>	<u>-0.55</u>	0.44	0.45	-0.09	-0.04	-0.31	0.25	1.00															
TM	0.03	0.47	-0.08	0.08	0.16	-0.38	0.33	-0.06	1.00														
HB	-0.44	0.22	0.15	<u>-0.51</u>	0.36	-0.15	-0.11	0.32	0.02	1.00													
EC	-0.15	-0.05	-0.47	-0.21	-0.21	-0.20	-0.72	0.00	-0.03	0.28	1.00												
EF	-0.06	-0.08	-0.44	-0.18	-0.04	-0.26	<u>-0.56</u>	0.01	0.07	0.15	0.30	1.00											
AH	0.04	-0.31	0.23	<u>-0.49</u>	0.10	0.27	-0.17	-0.14	-0.30	0.33	0.30	-0.10	1.00										
EA	<u>0.50</u>	0.39	-0.10	0.30	0.39	-0.42	0.25	-0.20	0.01	0.05	0.17	-0.22	0.16	1.00									
ECO157	0.10	0.21	-0.45	-0.13	-0.30	-0.31	-0.10	0.38	-0.20	0.17	0.31	0.34	-0.11	0.01	1.00								
KP	0.08	-0.20	0.25	0.29	-0.20	0.03	0.10	0.06	-0.30	-0.03	-0.24	-0.27	0.01	0.26	0.00	1.00							
SD	0.25	-0.31	-0.31	-0.37	-0.31	0.08	-0.31	-0.21	-0.32	0.35	0.41	-0.13	<u>0.50</u>	0.02	0.27	-0.18	1.00						
SP	-0.37	-0.42	0.08	0.26	-0.08	0.36	-0.20	0.19	-0.35	-0.29	0.11	-0.15	-0.16	-0.15	-0.14	-0.08	-0.10	1.00					
TV	-0.31	-0.18	0.17	<i>-0.55</i>	-0.04	-0.02	-0.43	0.22	0.11	<u>0.48</u>	<i>0.59</i>	0.22	0.33	-0.16	0.05	-0.32	0.26	0.12	1.00				
VC	0.37	-0.14	-0.20	0.16	-0.14	-0.08	-0.36	-0.13	0.19	-0.23	0.26	0.38	0.08	-0.15	-0.14	-0.08	-0.10	-0.05	0.23	1.00			
VP	0.00	0.00	0.00	-0.22	0.00	0.00	0.00	-0.35	0.10	0.26	0.36	-0.15	0.36	<u>0.42</u>	-0.14	-0.08	<i>0.53</i>	-0.05	0.36	-0.05	1.00		
VV	-0.39	-0.17	0.21	-0.33	-0.04	-0.01	-0.40	0.40	0.17	0.20	0.25	<u>0.42</u>	-0.05	-0.34	-0.12	-0.28	-0.33	0.23	0.70	0.23	-0.15	1.00	

Temp, temperature; Sal, salinity; DO, dissolved oxygen; TSM, total suspended mater; PO₄³⁻, phosphate; SiO₄⁴⁻, silicate; NO₂⁻+NO₃⁻, nitrite + nitrate; Chl-*a*, Chlorophyll-*a*; TM, total microbial; HB, heterotrophic bacteria; EC, *Escherichia coli*; EF, *Enterococcus faecalis*; AH, *Aeromonas hydrophila*; EA, *Enterobacter aerogenes*; ECO157, *Escherichia coli* O157:H7; KP, *Klebsiella pneumoniae*; SP, *Salmonella paratyphi*; SD, *Shigella dysenteriae*; TV, Total *Vibrio*; VC, *Vibrio cholerae*; VP, *Vibrio parahaemolyticus*; VV, *Vibrio vulnificus*.
 p < 0.001 (bold); p < 0.01 (italic); p < 0.05 (underline).

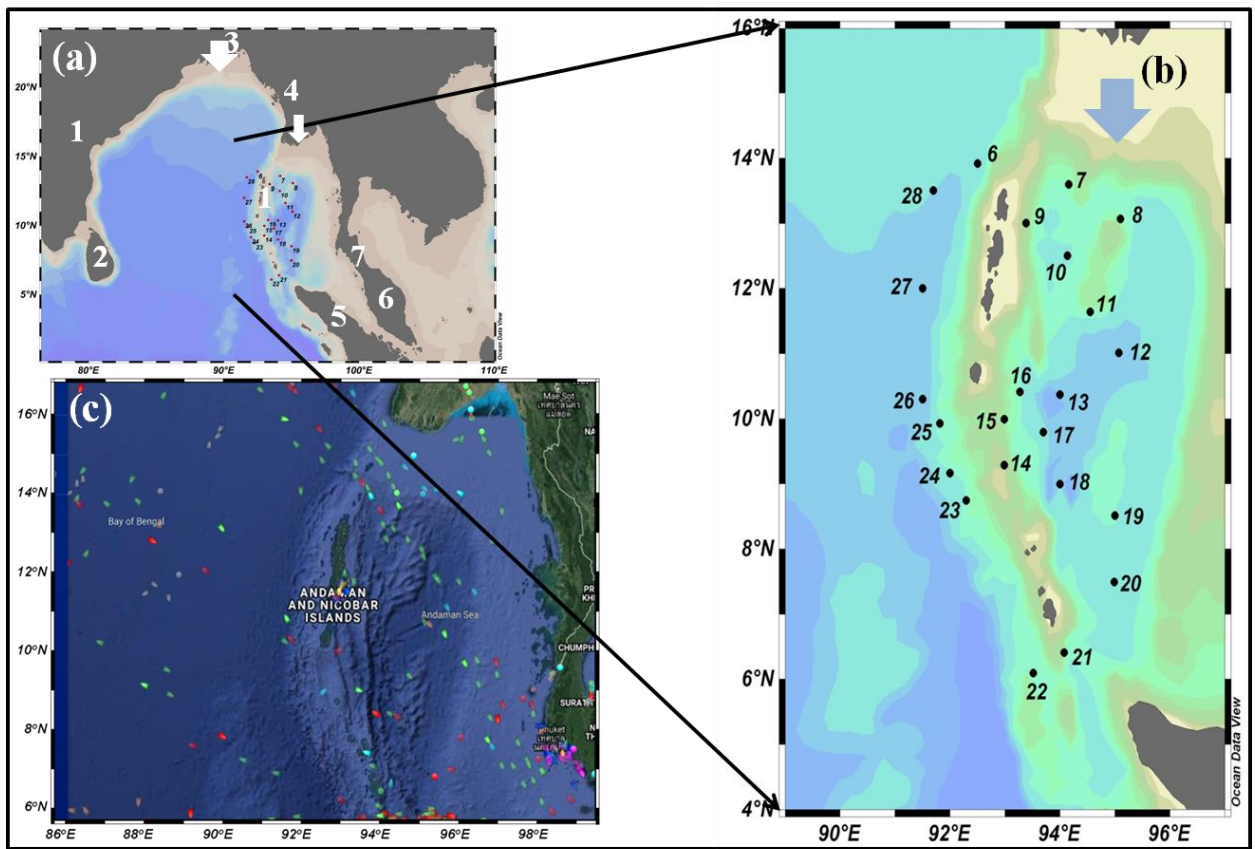


Fig. 1

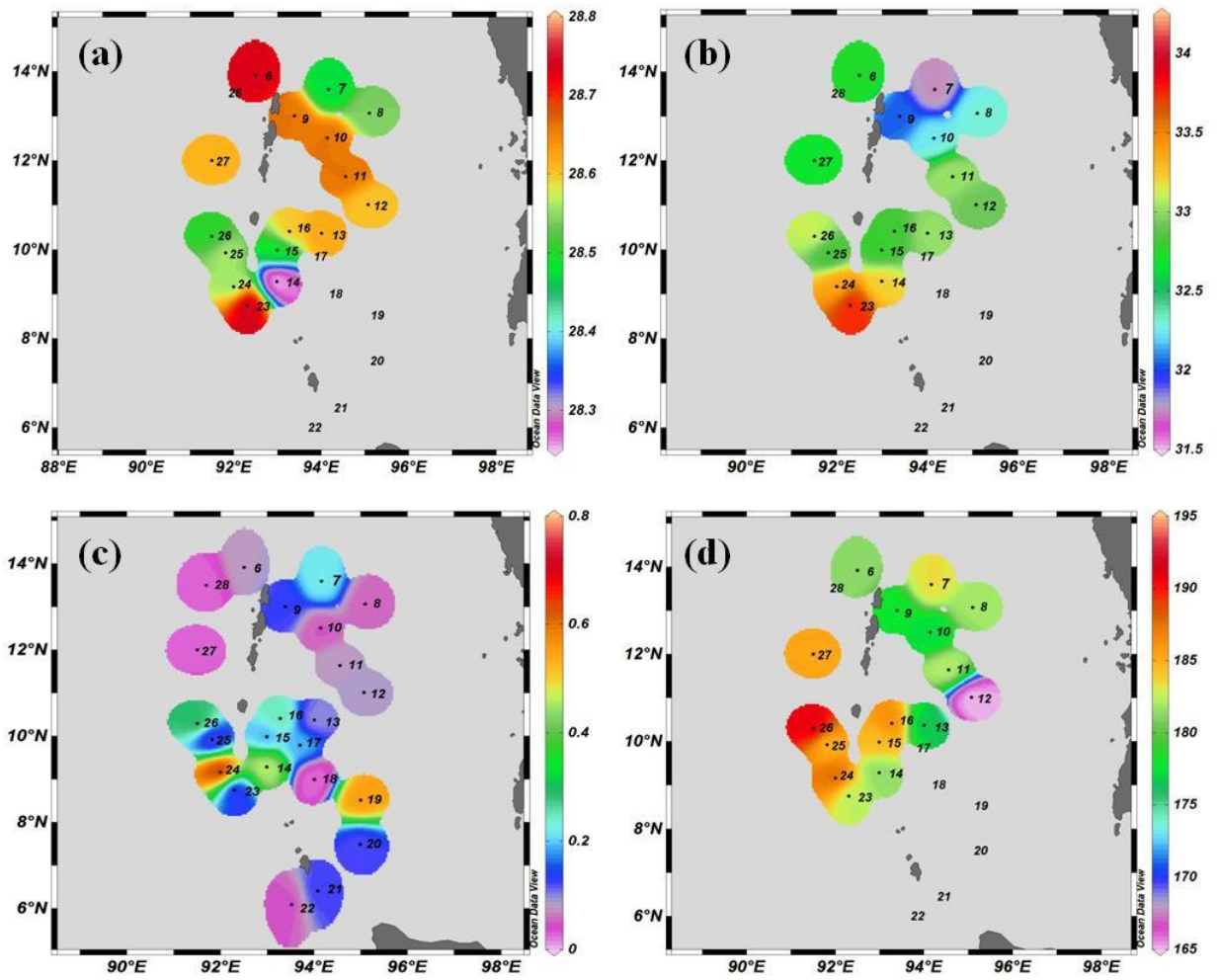


Fig. 2

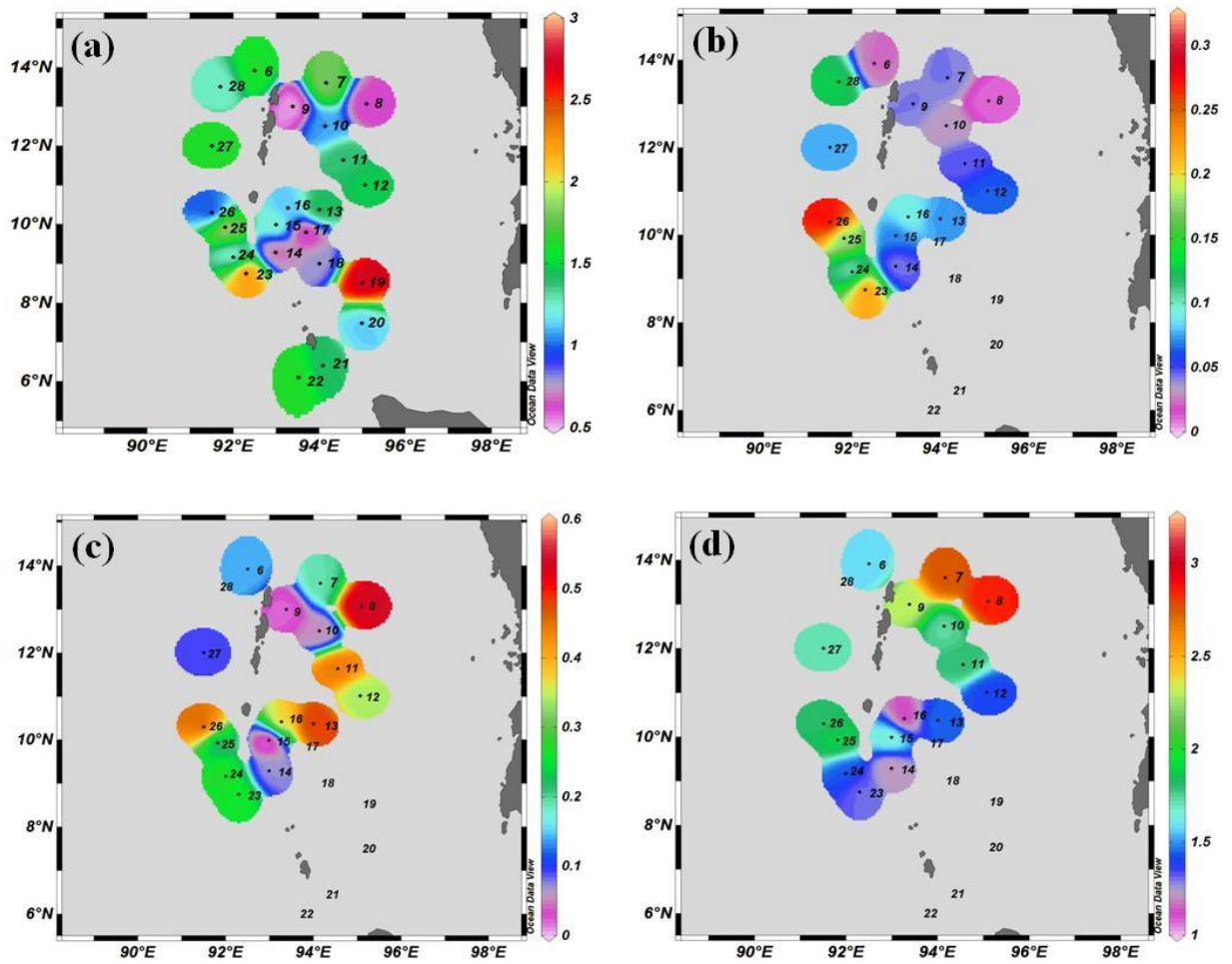


Fig. 3

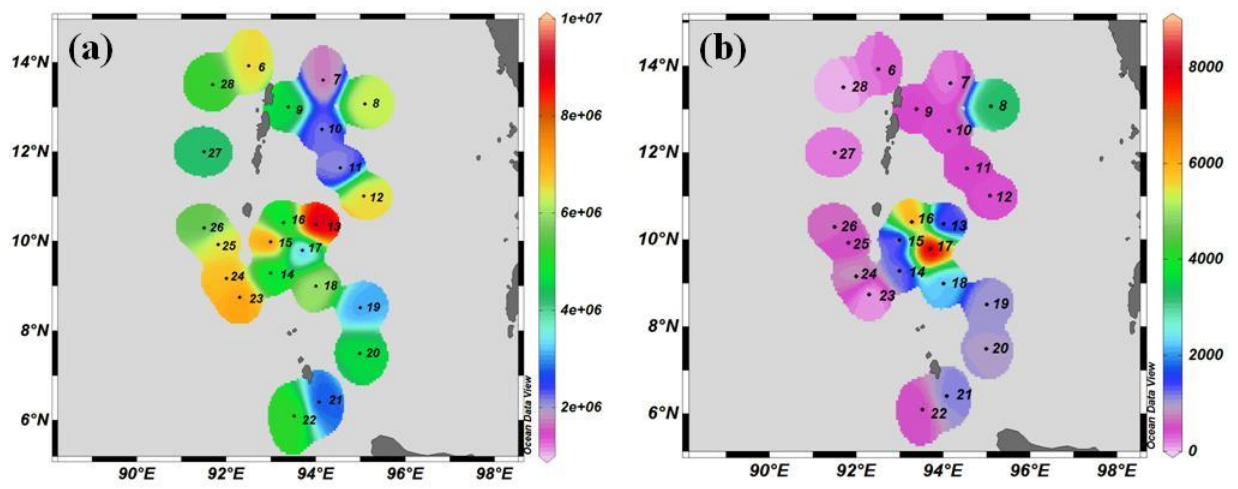


Fig. 4

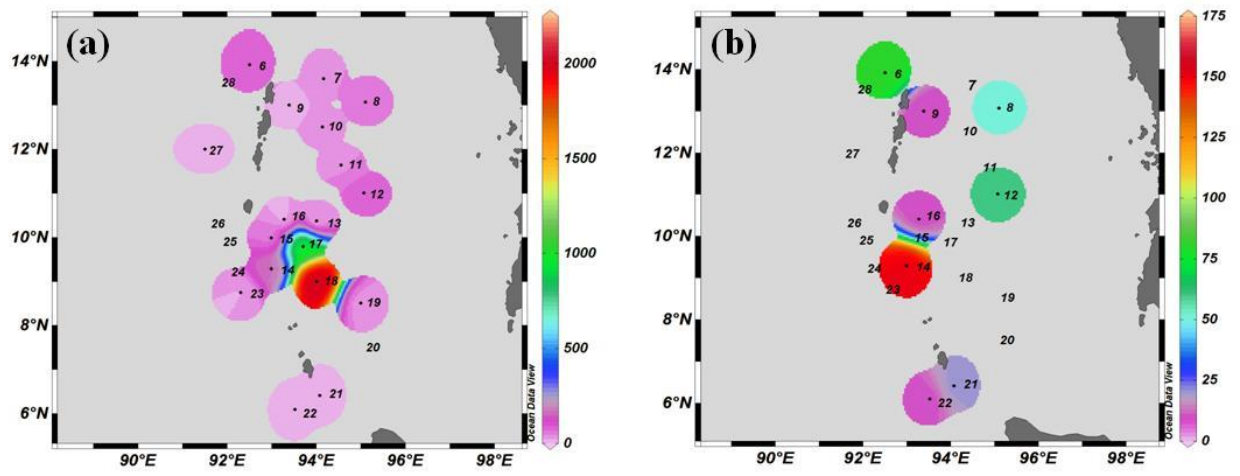


Fig. 5

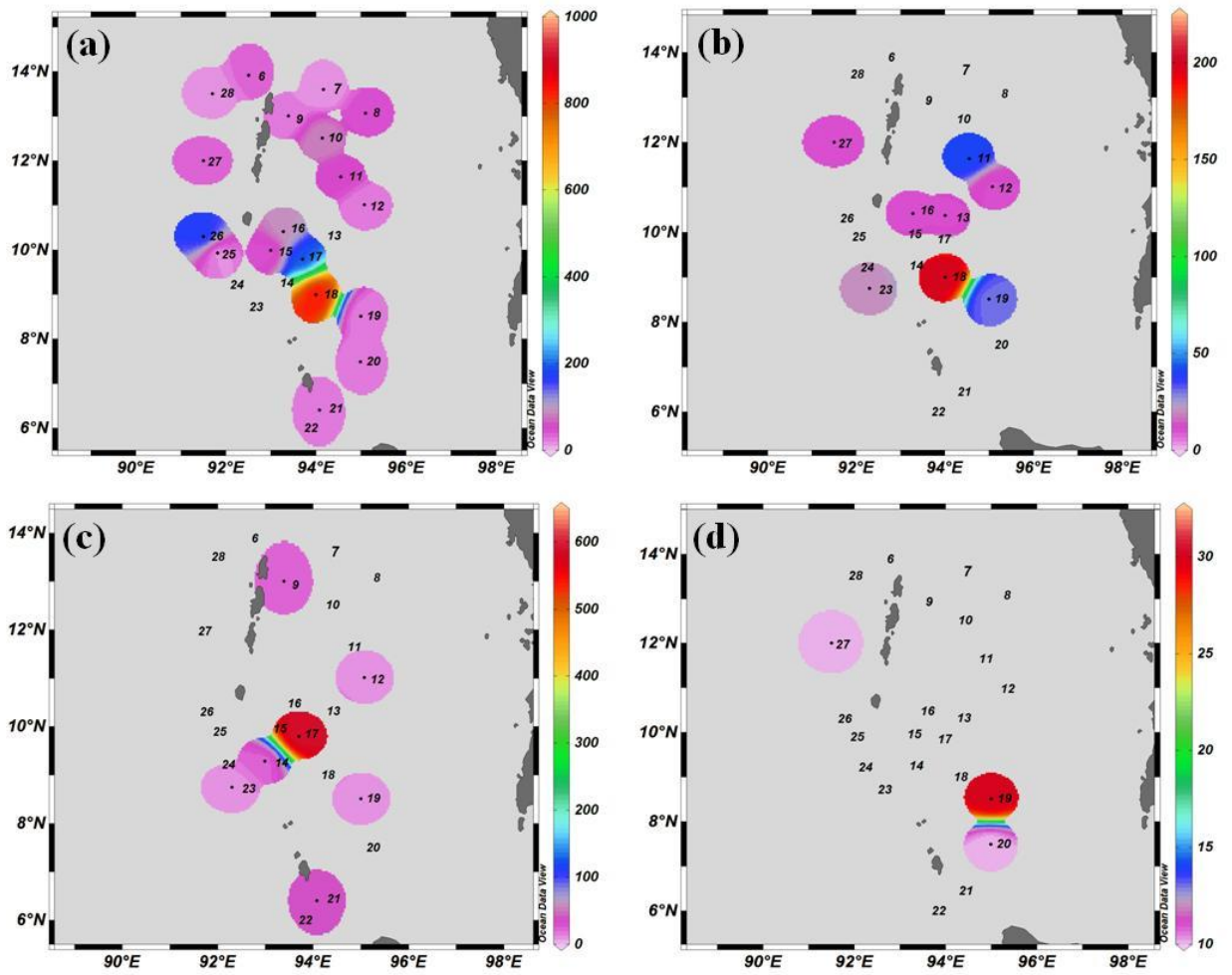


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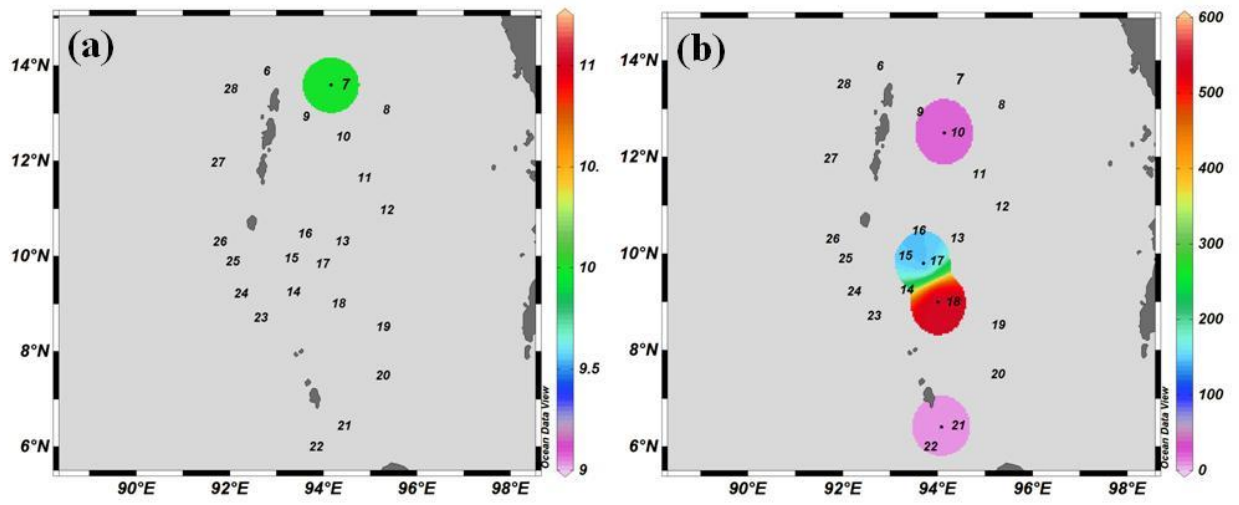


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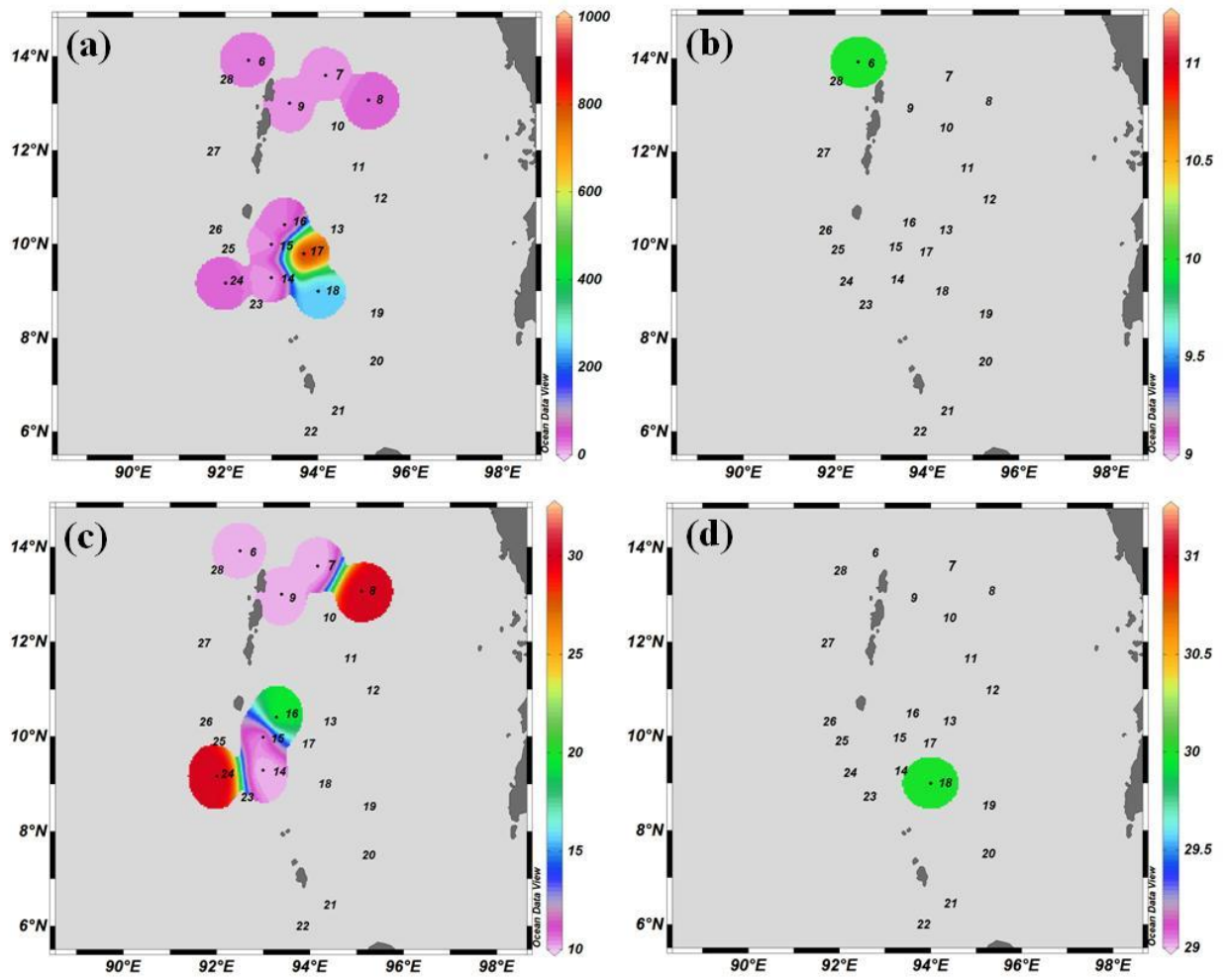
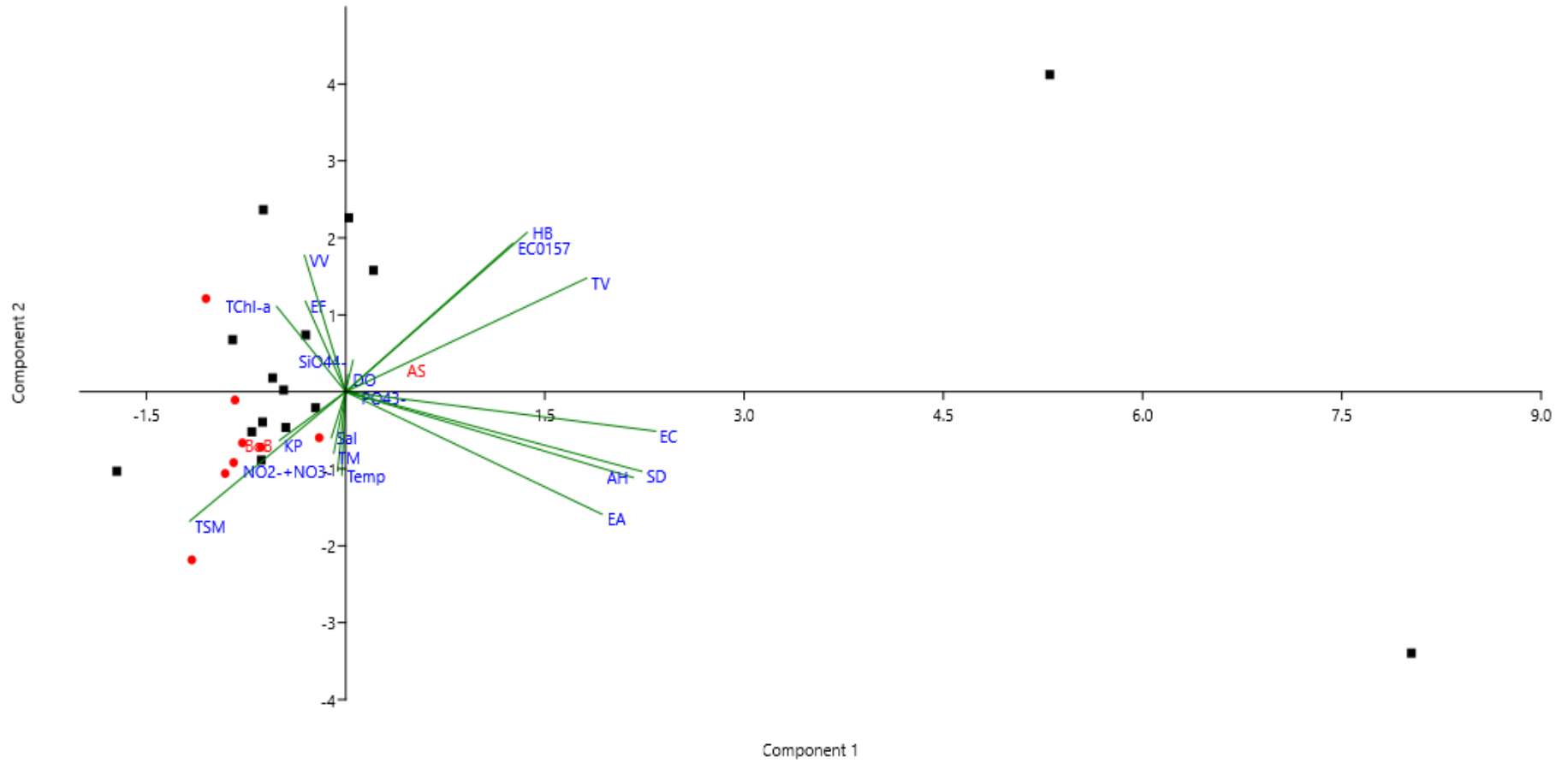


Fig. 8

1



2
3

Fig. 9