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Full Length Research Paper

Water quality effects of harbour activities assessed with integrated ecotoxicological parameters in Kerala, India

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Ecological tools were developed to study the water quality in Cochin harbour, a complex aquatic ecosystems, through the integration of microbiological monitoring (faecal coliforms and *Pseudomonas* species) and heavy metal contamination (lead, cadmium and mercury). One way ANOVA indicates statistically significant differences (P = 0.002) in bacterial population between sites and at the same comparatively higher population in site I, reveals the possible impacts of the harbour activities. Total coliforms (TC), faecal coliorms (FC) and facecal streptococci (FS) reported their mean high values of 789.33 CFU/ml, 535 CFU/ml and 231.67 CFU/ml respectively at site 1 which is designated for the anchoring of the ships. FC/FS ratio also suggests the pollution is more with human faecal origin. Reported higher concentrations of lead (44.81 ppm), cadmium (1.5 ppm) and mercury (1.21 ppm) depicts the industrial origin. The relationship between petroleum hydrocarbons (PHC) and *Pseudomonas aeruginosa* (PA) signify the reliability of PA as a biological marker for PHC contamination.

Key words: Harbour activities, pollution, faecal contamination index, *Pseudomonas aeruginosa*, petroleum hydrocarbons, heavy metals.

INTRODUCTION

Marine harbours are highly modified aquatic ecosystems with a high environmental health risk. Manufacturing and harbour activities including dredging, reclamation, construction and shipping on the estuaries are reported to cause impacts such as high sediment load, re-suspension of particulate heavy metals, organic pollution, etc. (Sin et al., 1991; Goh and Chou, 1997). This brings risks to health of the ecosystem and to the humans when acute perturbing phenomena diffuse into the bathing waters. The risk level is influenced by many components, such as the intensity of the crafts traffic, the nature of the transported materials, as well as those materials used in the harbour area, the spatial dislocation of the harbour, and the harbour's exposure to the wave motions and sea currents (DeDonno et al., 2008).

With the increased harbour activities in Cochin estuary over the last decade, there has been a lot of anthropogenic stress on the aquatic ecosystem. With this background, there is an urgent need to evaluate the pollution status of the aquatic ecosystem so as to protect marine life and to minimize the impact of industrial activities on the surrounding ecosystem. There is also a need for the development of robust and reliable ecotoxicological monitoring tools for the harbor to help prevent the potential damages to the environment. In this regard a set of bio-chemical parameters were studied in Cochin harbour region so as to find out their reliability and also to explore the present pollution status.

Atlas (1995) reported that the most important causes of the pollution in harbour areas are the hydrocarbons; these are responsible for chronic pollution due to their small and continuous introduction to the marine ecosystem. These pollution problems often result in huge disturbances of both the biotic and abiotic components of the ecosystems (Mueller et al., 1992), more so that some hydrocarbon compounds have been known to belong to a family of carcinogenic and neurotoxic organopollutants (Hallier-Soulier et al., 1999). However, utilization of

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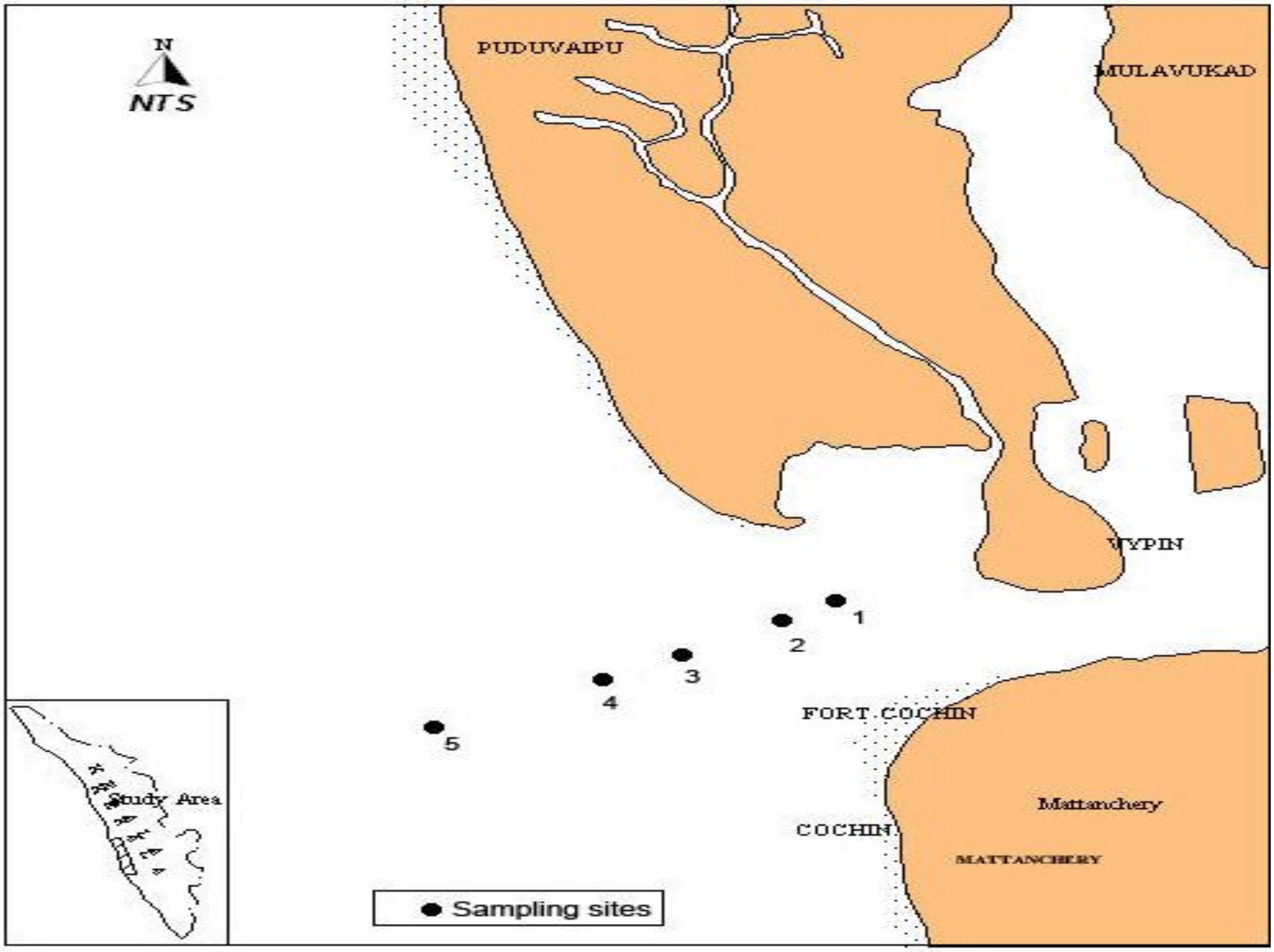

Figure 1. Study area.

petroleum hydrocarbons by *Pseudomonas* sp *(*Leahy and Colwell, 1990; Emtiazi et al., 2005, Ghazali et al., 2004) especially *P. aeruginosa* were well studied (Bhattacharya et al., 2000; Pfaender and Buckley, 1984). They are able to adapt, in the event of oil contamination and specialize their role in decomposing oil molecules, consequently showing higher abundance. Faecal contamination index is another important factor to assess the water quality (Shunder et al., 1974). Lin and Evans (1974) stated that, proportion of faecal coliforms, total coliforms and total viable bacterial population has been used as an index of faecal pollution. Further, the faecal streptococci ratio has also been employed as an indicator of faecal contamination. (Gelgrich and Jenner, 1969). In addition heavy metals like Cd, Hg and Pb, may exhibit extreme toxicity even at low concentrations, thus necessitating regular monitoring of modified aquatic environments such as harbour (Peerzada et al., 1990).

This study has been framed with the following objectives, firstly to evaluate the water quality of Cochin (Kerala, India) through the analysis of the dynamics of some marine autochthon microbial populations (CAHB and hydrocarbon degrading bacteria), correlation of water quality correlated to some risk factors, including urban impact and the impact of harbour activities and estimation impact through the search for the classic faecal contaminants, in sea waters, and through the measuring of the mercury, cadmium and lead and finally the development of a reliable ecotoxicological tool which is sensitive to detect potential damage to environment, especially marine harbours.

MATERIALS AND METHODS

Study area and sample collection

For studying the water quality effects of harbour activities, 5 sites were fixed in relation to horizontal proximity from the harbour region at Cochin (Figure 1 and Table 1). Site 1 is located in the harbour mouth in Cochin estuary. Site 2, 3, 4 and 5 are located 1, 3, 5 and

Table 1. Geographical coordinates of the sampling sites.

Site	Latitude	Longitude
1	9°57'6.9	76°14'29
2	9°56'16	76°13'55
3	9°56'16	76°12'50
4	9°56'14	76°11'48
5	9°56'18	76°09'13

10 km respectively from Site 1. Samples were collected for a period of 1 year (2008 to 2009) during the cruises of Sagar Purvi, the coastal research vessel of MoES (Ministry of Earth Science, Government of India). Water samples of surface and bottom regions were collected using Niskin water sampler and sediment samples by Van-Veen grab, and kept frozen till analysis.

Culturable aerobic heterotrophic bacteria, faecal contamination indexes and hydrocarbon degrading bacteria

Culturable aerobic heterotrophic bacteria (CAHB) were enumerated by employing routine spread plate technique. Nutrient agar with the following composition was used for enumeration: peptone 5.0 g; yeast extract 3.0 g; beef extract 2.0 g; agar 15.0 g; aged sea water 500 ml and deionised water 500 ml and a final pH of 7.2. Various aliquots ranging from 0.2 to 1.0 ml were plated in duplicate, in order not to miss the lower limits. The plates were incubated at room temperature (28 ±2°C). All colonies were counted as CAHB.

Readymade media (Himedia) were used for the isolation of different faecal indicator organisms. Total coliforms were screened using MacConkey agar (M081), and the plates were incubated for 24 h at a temperature of 37°C; the count was performed considering the pink-red colonies. Faecal coliforms were identified using m-FC agar (M1122) and rosolic acid, incubated for 24 h at 44.5±0.2°C, characteristic colonies have blue colour. The medium m-enterococcus agar (M081) was used for the investigation of FS. After the filtration and the incubation of the plates at 37°C for 48 h, the pink–red colonies were considered as positive.

Pseudomonas aeruginosa were taken as hydrocarbon degrading bacteria and were identified using cetrimide agar (MM024). The plates were incubated for 24 to 48 h at 37°C, colonies of *P. aeruginosa* were colorless. Characterization of each group was determined by APHA (1992) method and all indicator bacterial groups after identification expressed as colony forming unit/milliliter (CFU/ml).

Petroleum hydrocarbons

Petroleum hydrocarbons (PHC) in water samples were extracted with n-hexane immediately after sampling. The extract was evaporated in dryness in a rotary evaporator at 30°C under reduced pressure. The concentrate was taken up in n-hexane and fluorescence measured using fluorescence spectrophotometer at 360 nm with excitation at 310 nm.

Heavy metal

For cadmium (Cd) and lead (Pb) sediments were finely powdered, dried (at 70°C) and digested in a mixture of HF-$HClO_4$-HNO_3 using the microwave digester until a clear solution was obtained and made up to 25 ml using Milli Q water. Samples were analyzed on a flame AAS (AAanalyst 100 Perkin Elmer) after calibration with suitable E Merck elemental standards. Cold vapour techniques were adopted for mercury (Hg) analysis. In this method, Hg is reduced to elemental Hg from a digested solid sample by the addition of stannous chloride. From this, the amount of Hg present at trace level in solid samples can be determined.

$$Sn^{2+} + Hg^{2+} \rightarrow Sn^{4+} + Hg^{0}$$

All determinations were performed in duplicate for single samples and the results for each sampling sites were expressed as their mean.

RESULTS AND DISCUSSION

Culturable aerobic heterotrophic bacteria

The data of the analysis were presented in box- and –whisker diagram and are shown in Figure 2. The diagram depicts the distribution in quartiles for the results obtained in each site, and for every parameter it also shows the maximum, the minimum and the median.

Site 1 showed substantially higher counts of CAHB where organic inputs due to human activities (bathing, fishing activities, dredging canal, ships berth) were overriding. The total counts of CAHB in the study area vary from 2400 CFU/ml (surface-site 5) to 17600 CFU/ml (surface-site 1). The lowest mean of 4583.30 CFU/ml was registered at site 5 and the highest mean was found at site 1 (11933 CFU/ml). The one way ANOVA test indicates statistically significant differences (P=0.002) between site 5 and site 1. In all the sites, CAHB showed an overall mean of 8153.33 CFU/ml. The higher CAHB population especially towards the shore region is explained by the fact that inputs of organic substances into the marine coastal environment caused an increase in the growing rates and of the biomass of the heterotrophic bacterial community, accelerating the mineralization of the nutrients.

Faecal contamination indexes

Faecal contamination index also showed the same distributional status as that of CAHB in which they were more concentrated towards site 1 where the nutrient input was comparatively high. For the parameter TC (Figure 3), the maximum value was found in the surface water

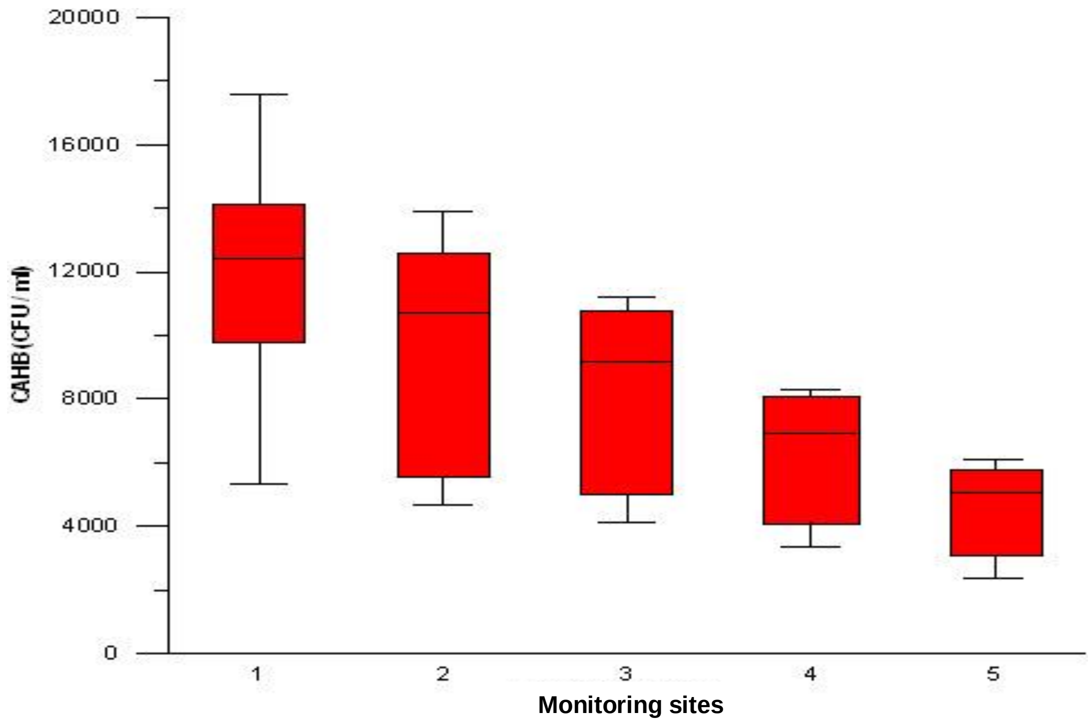

Figure 2. Distributional status of culturable aerobic heterotrophic bacteria in each monitoring sites.

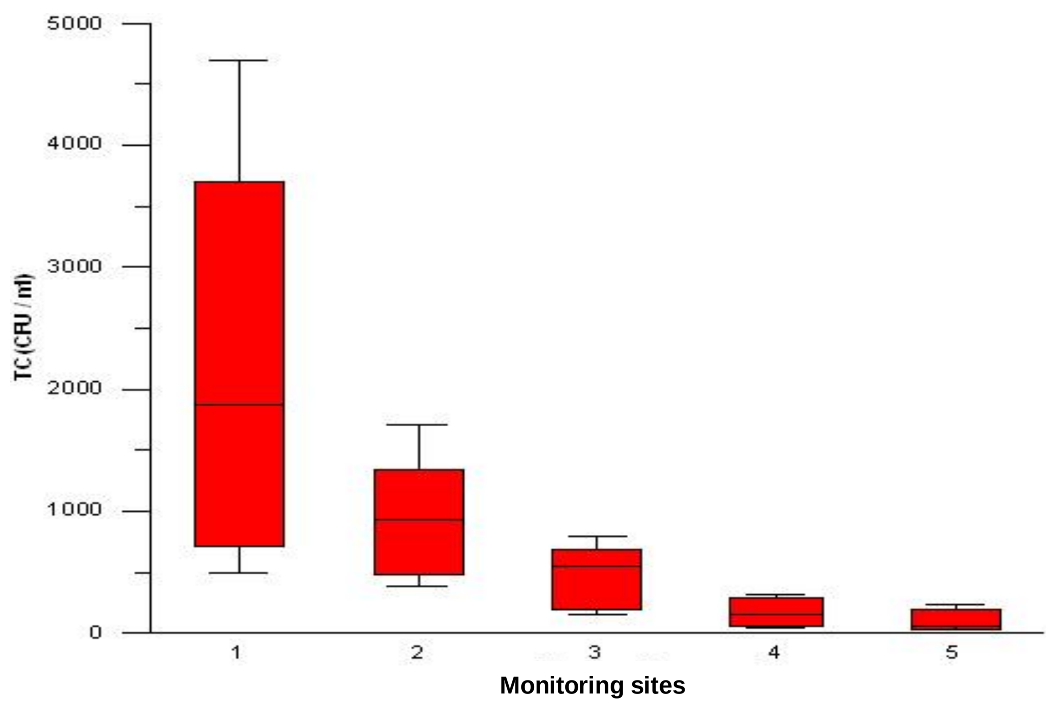

Figure 3. Distributional status of total coliforms in each monitoring sites.

samples, taken from site 1 (4700 CFU/ml), highest medium value was also reported from the same site. TC showed a mean variation of 101.67 CFU/ml (10 km) to 961.67 CFU/ml (1 km) with an overall mean of 789.33 CFU/ml through out the study. Faecal coliforms (Figure 4) fluctuated from 0 (10 km, surface and bottom) to 920 CFU/ml (site 1, surface), while FS (Figure 5) ranged between 0 (5 and 10 km, surface and bottom) and

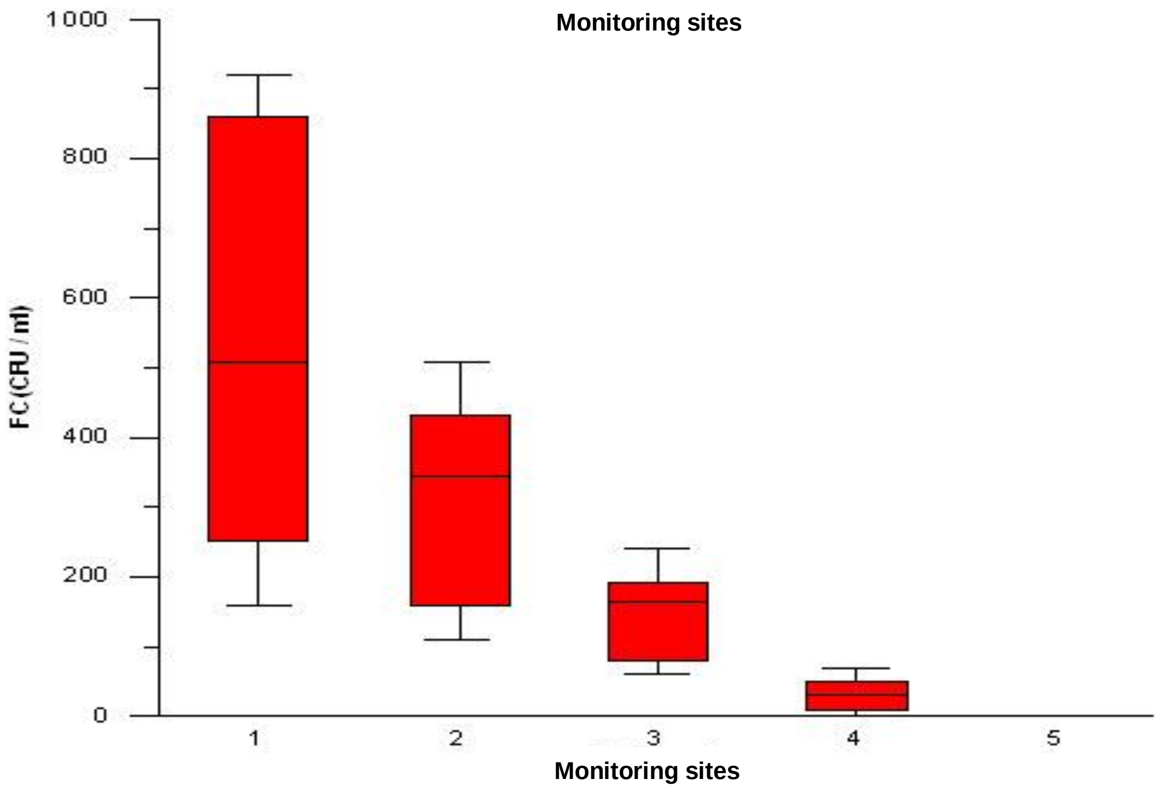

Figure 4. Distributional status of faecal coliforms in each monitoring sites.

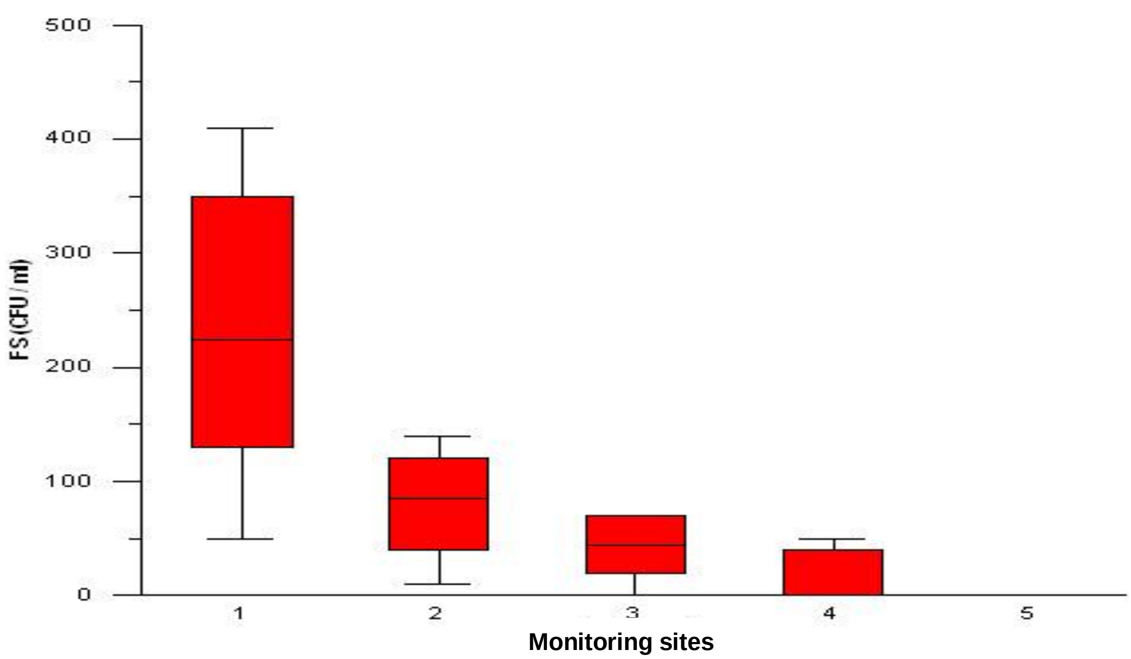

Figure 5. Distributional status of faecal streptococci in each monitoring sites.

410 CFU/ml (site 1, surface). Both showed their mean high values also at site 1 of 535 and 231.67 CFU/ml respectively. Counts were significantly higher for both indicators across sites located close to the shoreline, which are known to receive faecal pollution from variable point sources including in and around harbor sites.

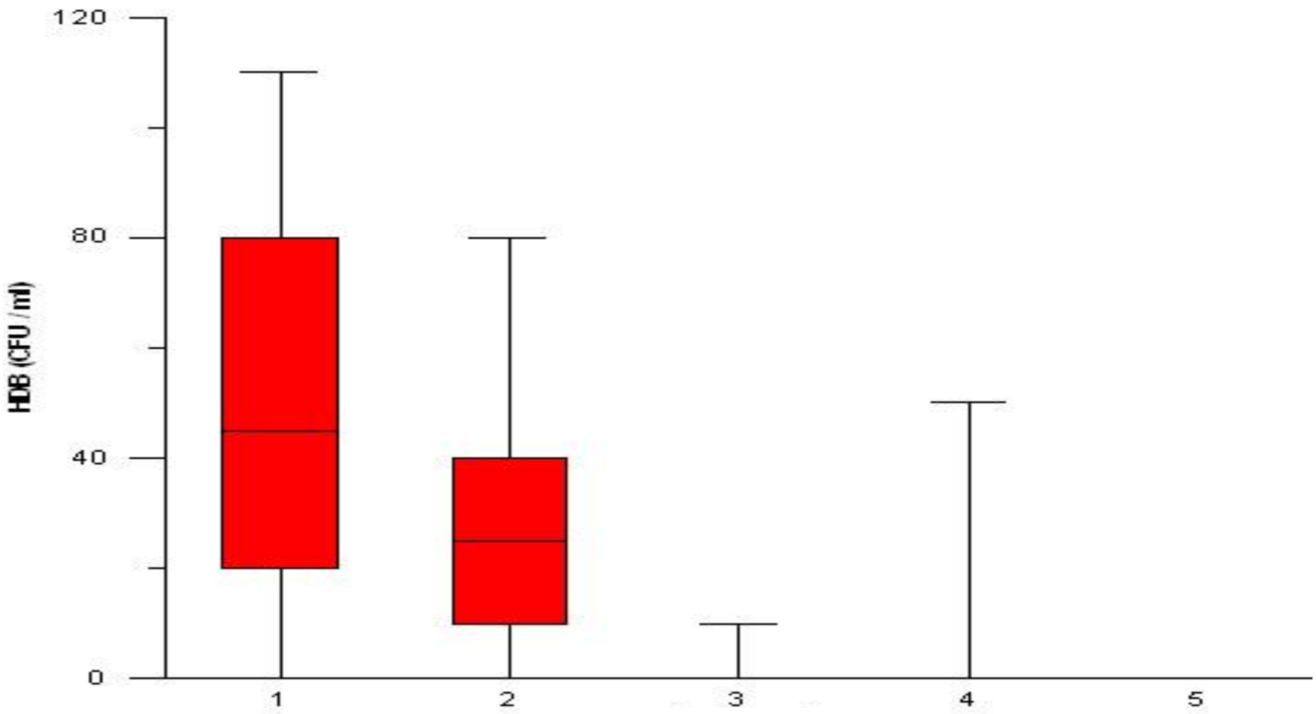

Figure 6. Distributional status of hydrocarbon degrading bacteria in each monitoring sites.

The one-way ANOVA tests suggest significant differences between site 1 and site 5 and 4 (P=0.002) for FC and between site 1 and site 5 (P=0.002), site 4 (P=0.002), site 3(P=0.009) and site 2 (0.041) for FS.

The available results suggest that FC/FS ratio in excess of 2 probably represents primarily human faecal pollution, while ratio of 1 or less may be due to the non-human sources. Intermediate value cannot be interpreted reliably. In accordance with this FC/FS ratio, the faecal pollution in Kochi may be principally of possible human sources. The same was also supported by the fact that site 1 is subjected to severe human interferences (bathing, ships berth). The higher occurrences of faecal indicator organisms also suggest that they are beyond the limit of acceptance.

Hydrocarbon degrading bacteria

Results of the hydrocarbon degrading bacteria are illustrated in Figure 6. HDB counts indicate a discontinuous, but a low concentration. The higher percentages were found mostly in the sites placed near the area used for mooring and transit of crafts and merchant ships. In addition to the high median value, the maximum value of 110 CFU/ml was also reported from site 1. The lowest mean was registered at site 5 (0 CFU/ml), and the highest mean was reported at site 1 (50 CFU/ml) with an overall mean of 18 CFU/ml in the entire study area. The correlation between the density and the relative abundance of HDB and the level of contamination by hydrocarbons has already been proven by the scientific community: the research carried out by Delille et al. (1998) in the Antarctic waters has shown a high abundance of various bacteria species adapted to oil degradation in chronically oil-polluted sites. Atlas (1995) reported that the fraction of HDB was 10% higher than the total heterotrophic component in the marine environments contaminated by hydrocarbons. However similar trend was not observed in our study, in such a way that total heterotrophic component remains the dominant group through out the study. Through out the study surface water reported to have comparatively high HDB population than bottom suggesting that hydrocarbons were more available on the upper strata of the water body. The one-way ANOVA test indicates statically significant differences between site 1 and site 5 (P= 0.015), site 4 (P= 0.041) and also with site 3 (P=0.015), which also indicate that the HDB population probably arose from hydrocarbon pollution due to the harbour activities. The same was also supported by the significant positive correlation (0.05 levels) observed between petroleum hydrocarbons and HDB population.

Petroleum hydrocarbons

Data for petroleum hydrocarbons are summarized in Figure 7. PHC showed wide fluctuations, vary from a

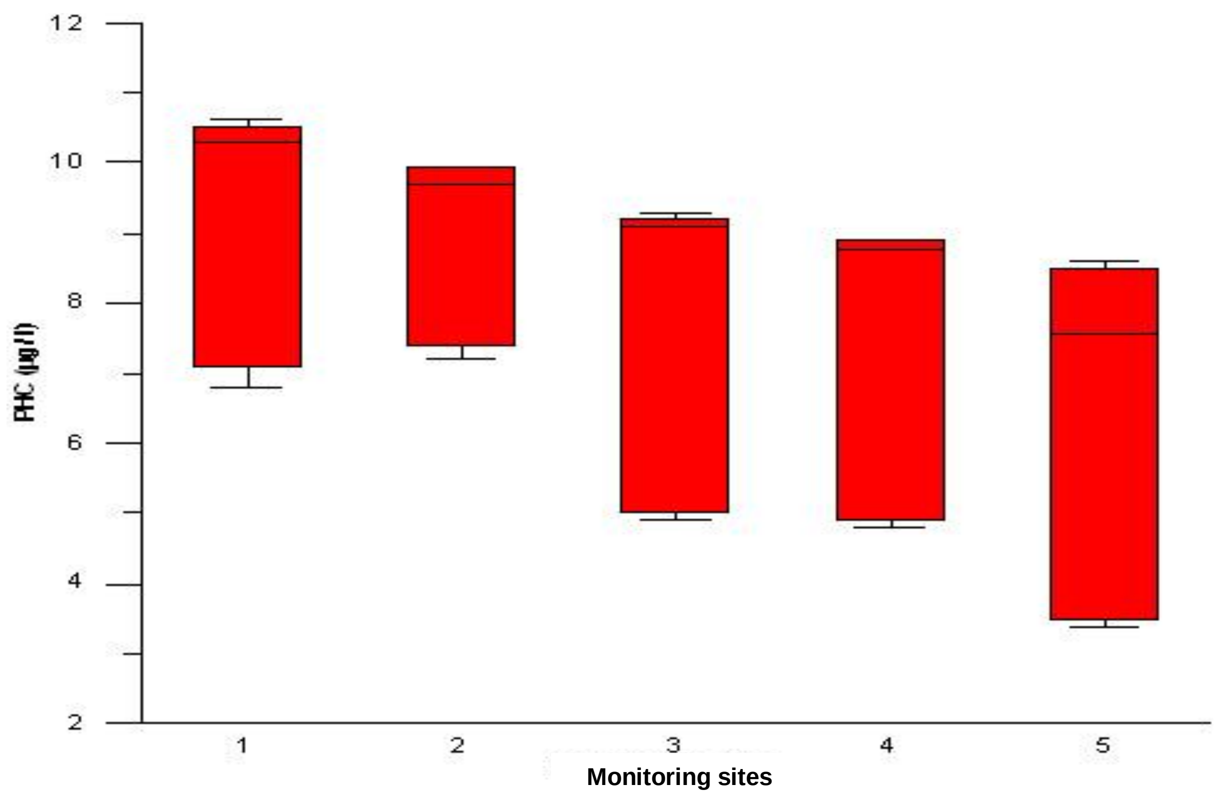

Figure 7. Distributional status of petroleum hydrocarbons in each monitoring sites.

maximum of 10.50 µg/l at site 1 to a minimum of 3.40 µg/l at site 5, suggests that concentration of hydrocarbons tends to increase near the area used for mooring and /or traffic of crafts. Site wise study registered a mean variation of 6.5 µg/l at site 5 to 9.23 µg/L at site 1. However it showed an overall mean of 8.01±2.18 through out the study area. Shipping activities are suspected to be responsible for their mean high concentration. Oily wastes generated from ships such as bilge water, ballast water, washing water, lubricant oil and other residues in machinery space comes under this category.

Heavy metals

The results of the evaluation of heavy metals are shown in Figures 8 to 10. Accumulation trend of all the studied heavy metals were quite different from the previous results. Lead (Figure 8), cadmium (Figure 9) and mercury (Figure 10) recorded their maximum values at site 4 respectively of 44.81 ppm, 1.5 ppm and 1.21 ppm. They showed an overall mean of 33.68 ± 8.07 ppm, 0.79 ± 0.29 ppm and 0.585 ± 0.30 ppm. Comparatively higher accumulation monitored at site 4 may be due to the dislocation of sediments influenced by the dredging activities and tidal current. Site wise study also reported the same output. Site 4 showed mean high concentrations of lead, cadmium and mercury respectively of 40.25 ± 3.79, 1.08 ± 0.34 and 0.79 ± 0.32. Ouseph (1992) reported that the base line concentration of Hg, Cd and Pb in Cochin harbour region was found to be 0.08 ppm, 0.15 ppm and 40 ppm respectively. The observed values are higher than the base line values for Hg and Cd. Data emphasizes, concerning the cadmium, concentrations up to 10 orders of magnitude higher than the base line values. This shows that there is an increased anthropogenic input of these metals into the region. The baseline concentration for Pb is more or less the same. However comparatively higher concentration of all the heavy metals through out the study area is drastic because of their environmental persistence, toxicity and ability to be incorporated into food chains (Kishe and Machiwa, 2003). The strong positive correlation between these heavy metals suggests that all are originating from the same source. The movement of ships, ship repair activities, barges, fishing and passenger boat may be responsible for this increase.

Conclusion

Summarizing the findings from this study, it was apparent that an area that extended for more than 3 km seaward from the harbour mouth at least had been severely polluted as a result of the harbour activities. Study also reveals that arrays of bio-chemical parameters, used in

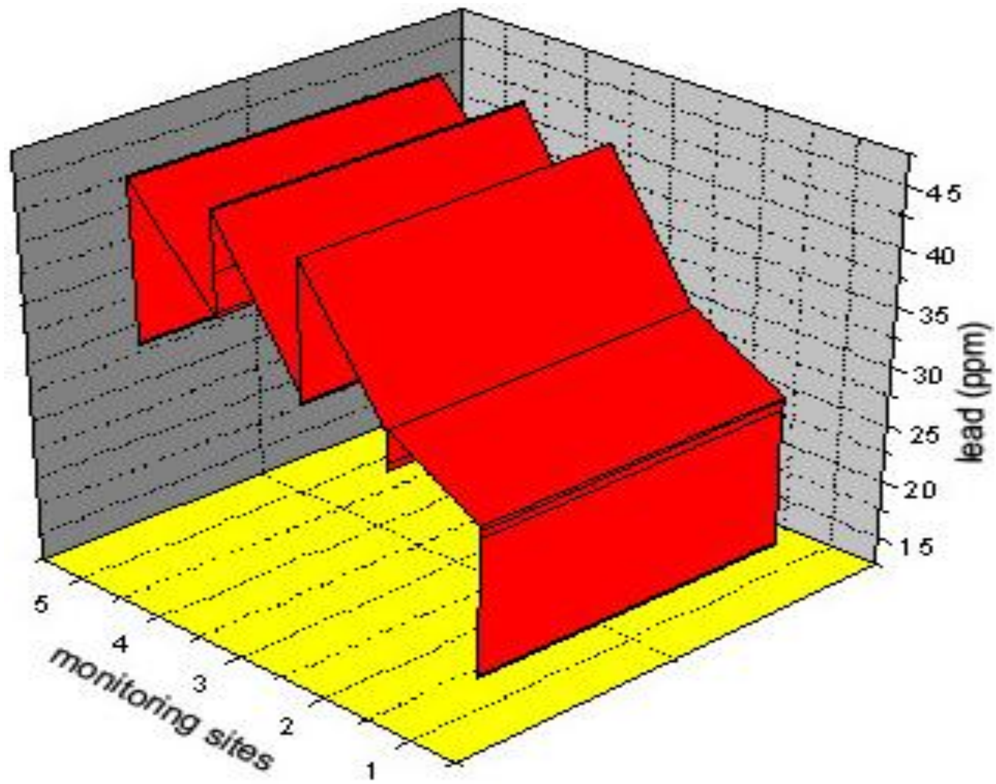

Figure 8. Distributional status of lead in each monitoring sites.

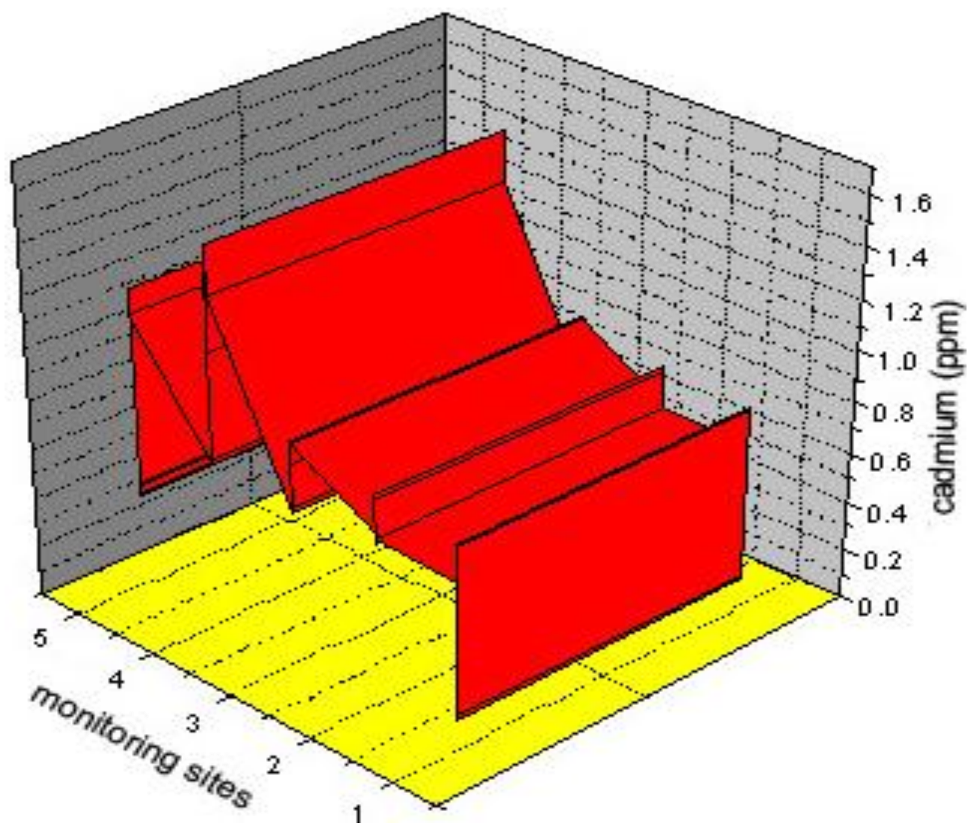

Figure 9. Distributional status of cadmium in each monitoring sites.

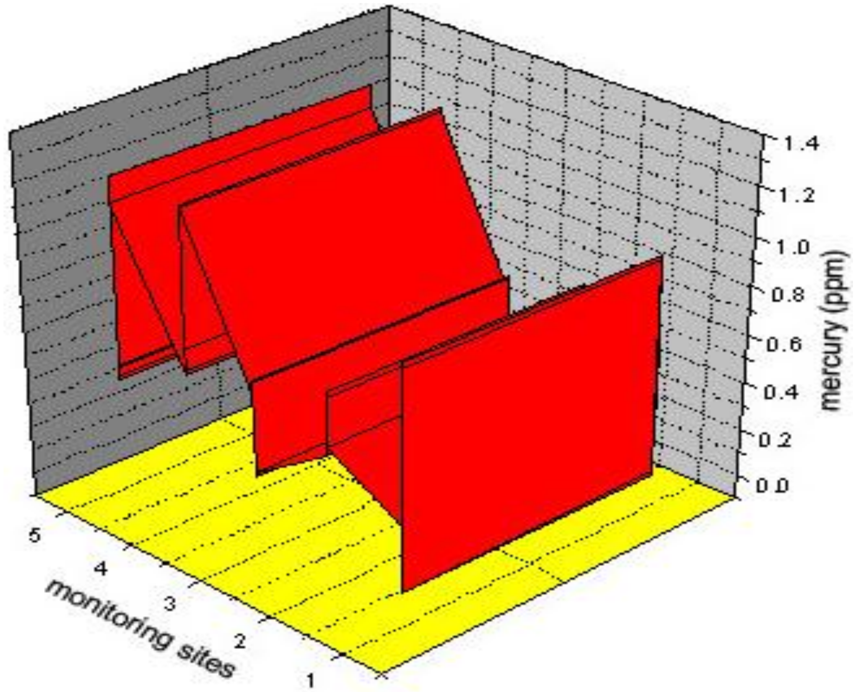

Figure 10. Distributional status of mercury in each monitoring sites.

this study, are useful to explore the water quality status of complex aquatic ecosystem and should be used as an ecotoxocological tool for further monitoring studies. However it is not impossible to establish clean water harbours by adapting technological advances, strict monitoring and compliance of the upkeep of environment in its near nascence.

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