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Counts of Indicator Bacterial Assemblages and Concentration of Trace Metal and Physicochemical analysis of oil polluted water and sediment samples from Automobile Garage in Tiruchirappalli city, India

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Abstract

Oil pollution is a common environmental problem in worldwide and was affected the surrounding ecosystems at large extent, and it is an unsatisfactory for use by the general public has become a health problem. The present study was conducted to examine the effects of physiochemical, trace metals and microbiological concentrations in waters and sediment (6) samples of three selected locations in Tiruchirappalli city, Southern India which is receiving several pollutants from various sources. The ranges of pH, TDS, EC, DO, BOD, TA, TH, Ca, Mg, Na, K, HCO_3, CO_3, Cl, SO_4, N-NO_2, O-PO_4 and oil/ grease in sewage water sample of summer season were 8.11-8.64, 800.8-1070.6, 1271.1-1699.3 (µS/cm), 0-1, 3.1-4.6, 61.2-70.1, 141.0-202.0, 148.0-174.8, 68.9-83.5, 79.1-91.3, 93.4-120.5, 54.7-71.6, 136.4-185.6, 4.6-16.4, 247.8-400.8, 60.5-81.3, 6.4-14.5, 11.4-25.9 and 9.4-13.4 mg/L, respectively. Metal contents of oil polluted water samples such as Cd, Cr, Cu, Fe, Ne Pb and Zn concentrations are between 2.12-4.1, 0.57-1.12, 2.78-4.23, 7.45-10.56, 0.31-0.52, 1.87-2.64 and 4.12-5.98 mg L^{-1}, respectively. In oil polluted water sample, counts of TVC, TC, TS, FC, FS, VLO, SC and PC were in the range of 41.0-81.0 x [10^3] CFU/mL, 5.8-16.4 x [10^2] CFU/mL, 1.4-4.2 x [10^2] CFU/mL, 1.2-6.5 x [10^2] CFU/mL, 0.4-1.4 x [10^2] CFU/mL, 3.5-8.6 x [10^2] CFU/mL, 0.7-1.4 x [10^2] CFU/mL and 5.7-10.3 x [10^3] CFU/mL, respectively. The sediment samples got higher concentrations of all the parameters than the water samples due to the flocculation process. The general distribution pattern suggests that the nature of the water and sediment has a significant role in the retention of indicator bacteria and the physiochemical and trace metals were helped their growth.

Keywords: Oil pollution, trace metals, indicator bacteria, physiochemical parameters.

Introduction

Anthropic activities strongly influence the natural status of almost all aquatic ecosystems. The input of heavy amounts of foods, pollutants and urban wastewater discharges can alter the character of the aquatic ecosystems. From an ecological point of opinion, it is well known that aquatic microbial communities are useful indicators of ecological status because microorganisms are able to rapidly react to environmental changes by modifying their community structure and constitution. Urban sewage often represents the primary origin of microbial contamination of waters, which often defines a big limitation for human health in recreational activities and the use of economic resources. Several factors, including light, temperature, inorganic nutrient availability, and river flow, may influence the estuarine microbial distribution, biomass and growth. From the sanitary standpoint, the presence of pathogenic microorganisms in the aquatic environment is related to their ability to resist and survive to different natural factors of stress, such as low temperature, low or high pH, high salinity, and scarce organic matter[1]. At any one time, 1.5 billion people worldwide are estimated to suffer from waterborne diseases, and 3.4 million people die from directly or indirectly consuming water contaminated with bacteria, eukaryotic parasitic, or viral pathogens[2]. Indexes of the presence of pathogens, for exemplar, are utilized in large part because of the intractability of measuring all microbial pathogens that might be present in environmental samples in a timely and cost-effective manner[3].

In parallel to increase in types of use, the need of drinking and usage waters, river waters are significantly contaminated, especially with metals due to improper irrigation in agriculture, increasing usage of fertilizers and increasing number of industrial installations. Metals are very important for humans, plants and the environment. Some of these factors are indispensable for humans and plants while some are toxic. In addition, concentrations of beneficial elements above a certain level may also create toxic effect. Metals can move with the food chain. Therefore, in order to define concentrations of most metals in waters and soil; some regulations are applied[4]. The determination of trace metal contents in river sediments is one of the important indicators for the appraisal of environmental pollution[5].

The pollutants mainly constitute heavy metals, petroleum hydrocarbons and bacterial contaminants[6]. Among these contaminants, heavy metals and petroleum hydrocarbons are thought as the most serious pollutants due to their toxicity and persistence. Oil products are carcinogens and affect a sort of biological processes and potent cell mutagens. The majority of this rapidly associates with hydrophobic organic matter -and suspended particulates, while the volatile portion evaporates. The non-volatile (emulsion or mousse) part of these petroleum products eventually sinks to the bed and goes down onto the sediment[7]. The worst impacts of litter and suspended fractions of the spilled petroleum hydrocarbons are on larvae, low motility organisms and the drastic changes in feeding or reproductive cycles that ultimately affect population size and fertility. On the other hand, the petroleum hydrocarbons settled in the sediments affect the filter feeders and benthic organisms with the bioaccumulation of toxic compounds in tissues, genetic mutations and cell atrophy[8]. Heavy metals once entered in water system are accumulated by marine invertebrates, associated with particulates and subsequently removed to the maximum extent by adsorption onto the sediments leaving partially in waters in a suspended or soluble complex form leading to serious bioaccumulation atrophy[9].

Material and Methods

Sampling site: The Tiruchirappalli is the fourth largest municipal corporation and the biggest urban agglomeration in the country. Tiruchirappalli sits almost at the physiographies center of the state. Densely populated industrial and residential areas have recently been established in all portions of the city and close to 1 million people are surviving in this metropolis. According to the 2011 Indian census, Tiruchirappalli had a population of 847,387, 9.4% of whom were below the age of six, living in 214,529 families within the municipal corporation limits. The Cauvery Delta begins to form 16 kilometers (9.9 mile) west of the city where the river separates into two streams the Cauvery and the Kollidam to form the island of Srirangam. As Tiruchirappalli is on the Deccan Plateau the days are exceedingly tender and dry; evenings are cooler because of frigid winds that blast from the southeast. From June to September, the city receives a moderate climate tempered by hard rain and thundershowers. Rain is heaviest between October and December because of the northeast monsoon winds, and from December to February the climate is cool and moist.

Sampling: The three water and sediment samples from three different sources in Tiruchirappalli city were collected during summer 2015 for physiochemical, trace metal and bacteriological analysis. The water and sediment samples collected from automobile garage located in Woraiyur, K.K.Nagar, and Tennur. The water samples were marked as W1, W2, W3 and sediment samples marked as S1, S2 and S3 respectively. The water samples were collected from 0 to 20 cm below the surface. The 2000 mL of water samples were collected with a 2500 mL sterile container in each location. The sediment samples were collected by sterile spatula and stored in sterile plastic bags[10,11].

Physiochemical analysis: The physiochemical parameters, i.e., pH, electrical conductivity (EC) and total dissolved solids (TDS) were measured using field kit (Thermo Orion 5-Star pH Multi-Meter) on the site and the concentrations of soluble cations and anions (Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, CO_3^{-}, HCO_3^{-}, Cl^{-} and SO_4^{2-}) were determined according to the standard methods[12-14]. All samples were collected with precautions required for microbiological analysis, held on iceboxes and processed within 12 h of collection.

Trace metal analysis: For heavy metal analysis, the one liter of oil polluted water was acidified immediately with concentrated nitric acid (HNO_3). For trace metal study, acidified test water samples were filtered by Whatman No.1 filter paper and processed (APDC + MIBK) for metal analysis. The sediment samples were air-dried and smaller than (>) 63 µm in size were kept back in pre-cleaned properly. Thenceforth, the dried sediment samples were crushed by agate mortar and pestle. Both the samples were processed with an aqua - regia mixture (i.e. HCl: HNO_3= 3:1) in Teflon bomb and were incubated at 140 °C for 2-3 days after dried and sieved samples. After incubation, the reaction mixture was filtered with Whatman No.1 filter paper. The trace metals in the sea water, sea sediment and crab samples were determined by the atomic absorption spectrophotometry (GBC SensAA - AAS, Australia) in flame mode[15].

Bacteriological analysis: All the specific/selective media were prepared with the addition of double distilled water and autoclaved properly. The bacterial populations in different samples were estimated by pure culture technique (spread plating method) on selective medium plates with 100 µL of suitable dilutions[16]. All the media plates were incubated at 37°C ± 1°C for 24–48 h, except M-FC agar plates. The M-FC agar plates were incubated at 44.5°C ± 1°C for 24–48 h. After incubation, the final counts of colonies were noted and all trials were performed in triplicate. On the basis of the media manufacturer's guide, typical colony morphology characteristics of different bacterial groups were recognized and initial enumeration of pathogenic pollution indicator bacteria was completed.

Since recommended selective media were used in all organisms, specific biochemical tests were performed for identification and they are therefore referred to as like organisms (LO). For confirmation of the pathogens, typical colonies were inoculated into Rapid Microbial Limit Test kits recommended for diagnostic microbiology supplied by Hi-media Laboratories Limited. Typical colony characteristics of each bacterial group and specific media used for enumerating them are listed in table-1.

Results and Discussion

Rapid urban/industrial changes and increased pressure of people on ecosystems have caused global environmental changes that encompass global warming, stratospheric ozone depletion, resource depletion, loss of biodiversity, urbanization and widespread environmental contamination. Water can be obtained from a number of sources, among which are streams, lakes, rivers, ponds, rain, springs, wells and oceans. Unfortunately, clean, complete and safe water only exists briefly in nature and is immediately polluted by prevailing environmental factors and human actions. Pollution of surface and coastal waters may arise from various sources, such as the discharge of sewage and industrial waste from many outfalls, the dumping of wastes at aquatic ecosystems, the discharge of sewage and rubbish from the oil industry, petrol / oil cost, ships, the handling of cargo, the exploration and exploitation of the surface and sea bed and aquatic floor, accidental pollution by oil and other substance of pollutants from the land by air and other routes.

The increasing rate of crude oil pollution on farmlands caused by oil product leakage from tanks and pipes, trucks during the distribution operation as easily as by car and railway transport and gas station is turning. The sack of used oil from vehicles or motorcycles is a major source of petroleum pollution in mechanic workshop and its environs[17]. In the present work, the results of Physicochemical, trace metal and microbiological parameter analysis are presented in figures-1 to 6. The ranges of pH, TDS, EC, DO, BOD, TA, TH, Ca, Mg, Na, K, HCO_3, CO_3, Cl, SO_4, N-NO_2, O-PO_4 and oil/ grease in sewage water sample of summer season were 8.11-8.64, 800.8-1070.6, 1271.1-1699.3 (µS/cm), 0-1, 3.1-4.6, 61.2-70.1, 141.0-202.0, 148.0-174.8, 68.9-83.5, 79.1-91.3, 93.4-120.5, 54.7-71.6, 136.4-185.6, 4.6-16.4, 247.8-400.8, 60.5-81.3, 6.4-14.5, 11.4-25.9 and 9.4-13.4 mg/L, respectively. But the range of pH, TDS, EC, DO, BOD, TA, TH, Ca, Mg, Na, K, HCO_3, CO_3, Cl, SO_4, N-NO_2, O-PO_4 and oil/ grease in sewage sediment sample were 7.98-8.64, 1054.1-1255.8, 1673.1-1993.3 (µS/cm), 0-1, 1.8-2.8, 89.4-105.7, 171.5-224.8, 222.7-276.4, 101.4-124.8, 121.3-151.6, 108.9-129.5, 64.8-76.8, 160.1-203.4, 11.4-21.4, 334.5-402.6, 76.5-84.7, 10.5-18.9, 12.4-29.8, 14.5-17.6 mg/kg, respectively (table-1). Runoff from different sources typically has elevated levels of enteric organisms and metals[18]. Here, we may concluded that runoff from sewage/industrial/shipping activities/agricultural/storm drains are significant source of trace metals, indicator organisms and associated pathogens in study area.

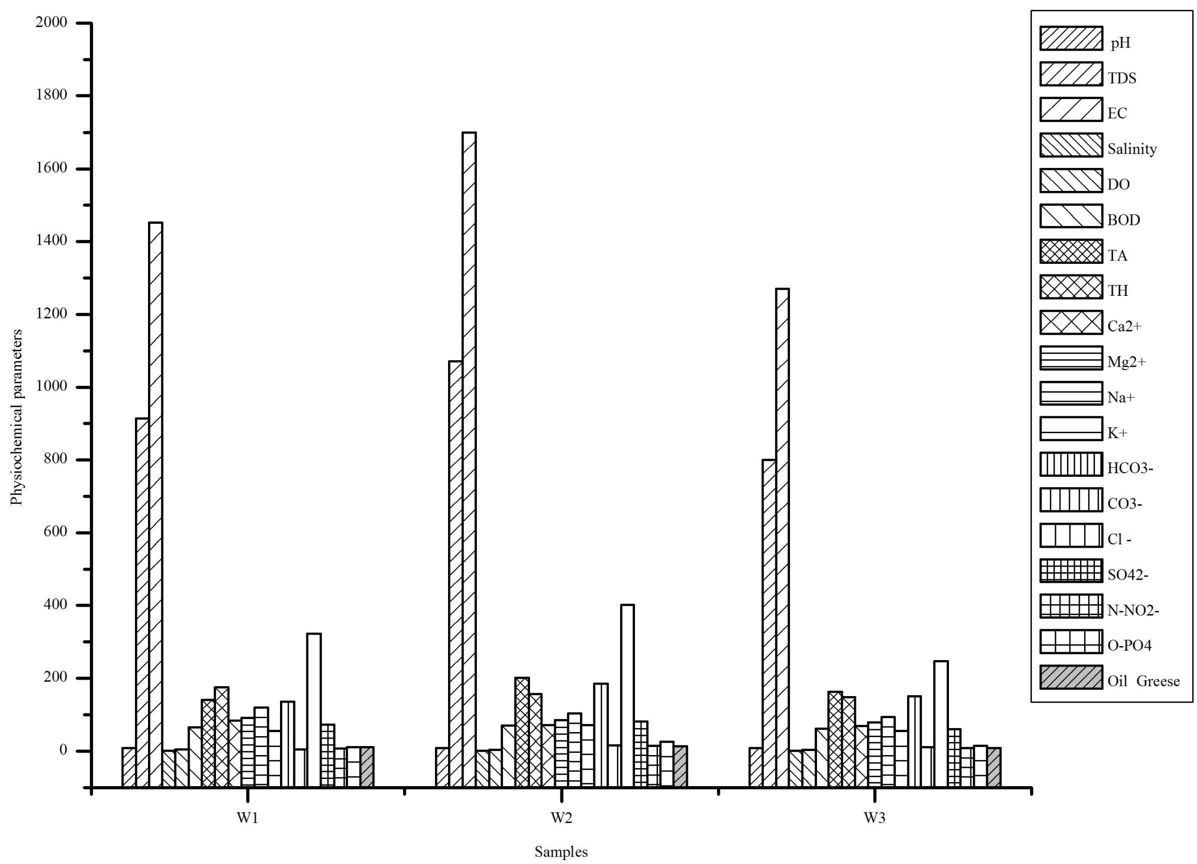


Figure-1
Comparative Graph between three different oil polluted water samples such as W1, W2, W3 against physicochemical parameters

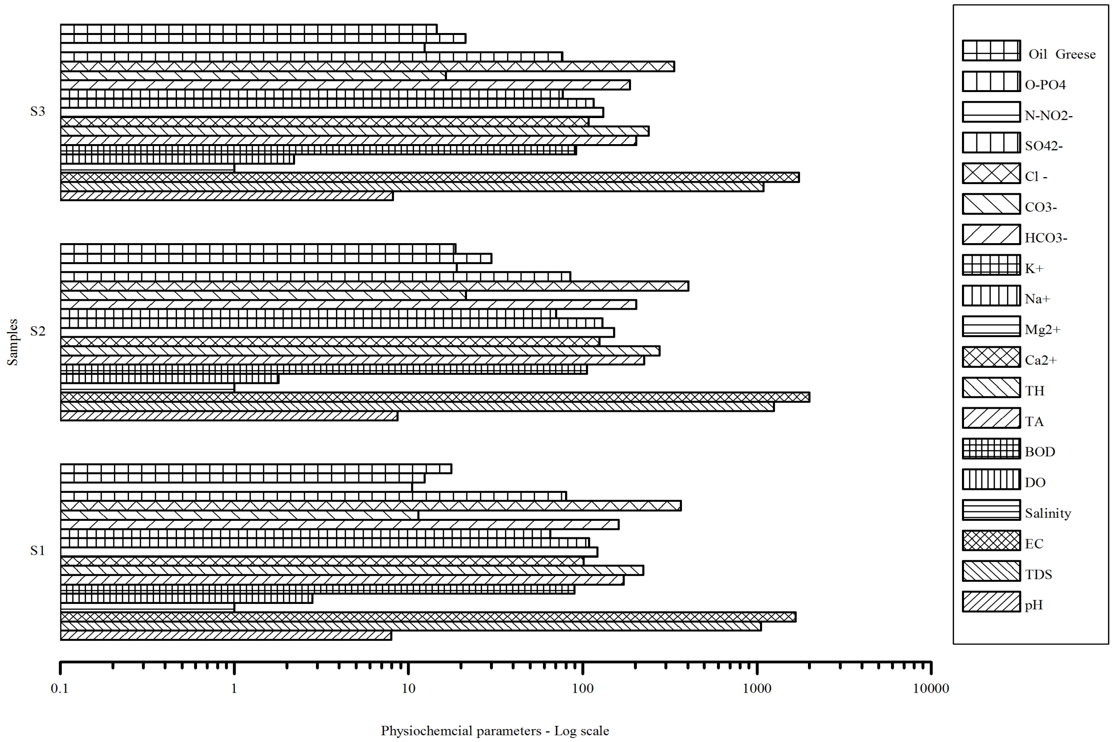


Figure-2
Comparative Graph between three different oil polluted sediment samples such as S1, S2, S3 against physicochemical parameters

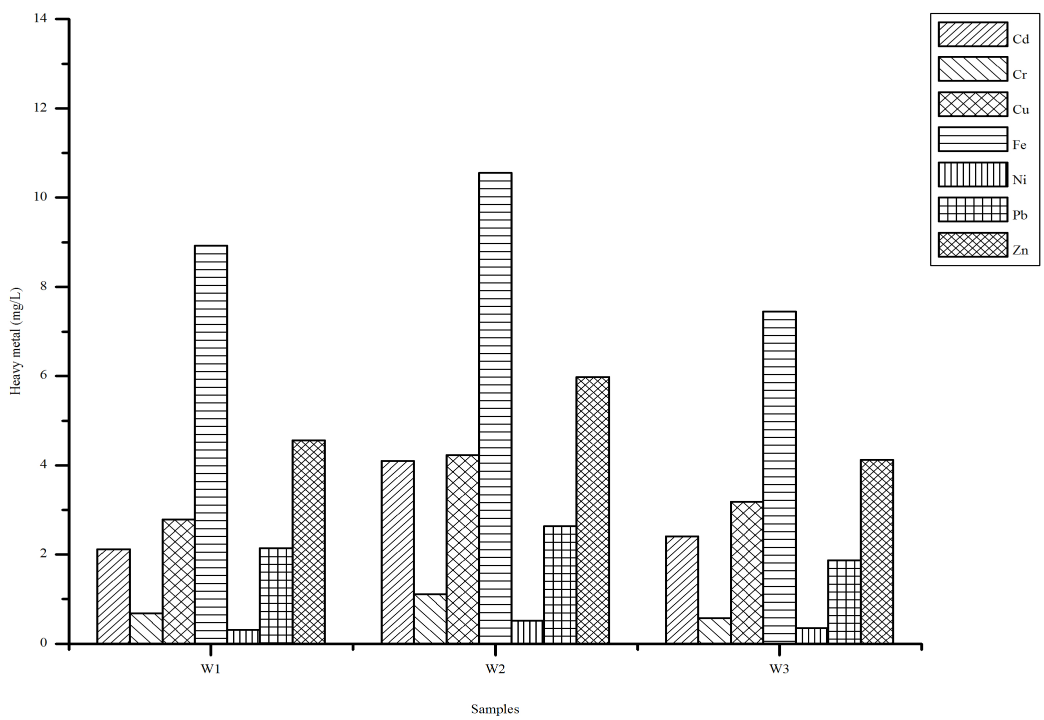


Figure-3
Comparative Graph between three different oil polluted water samples such as W1, W2, W3 against trace metal content

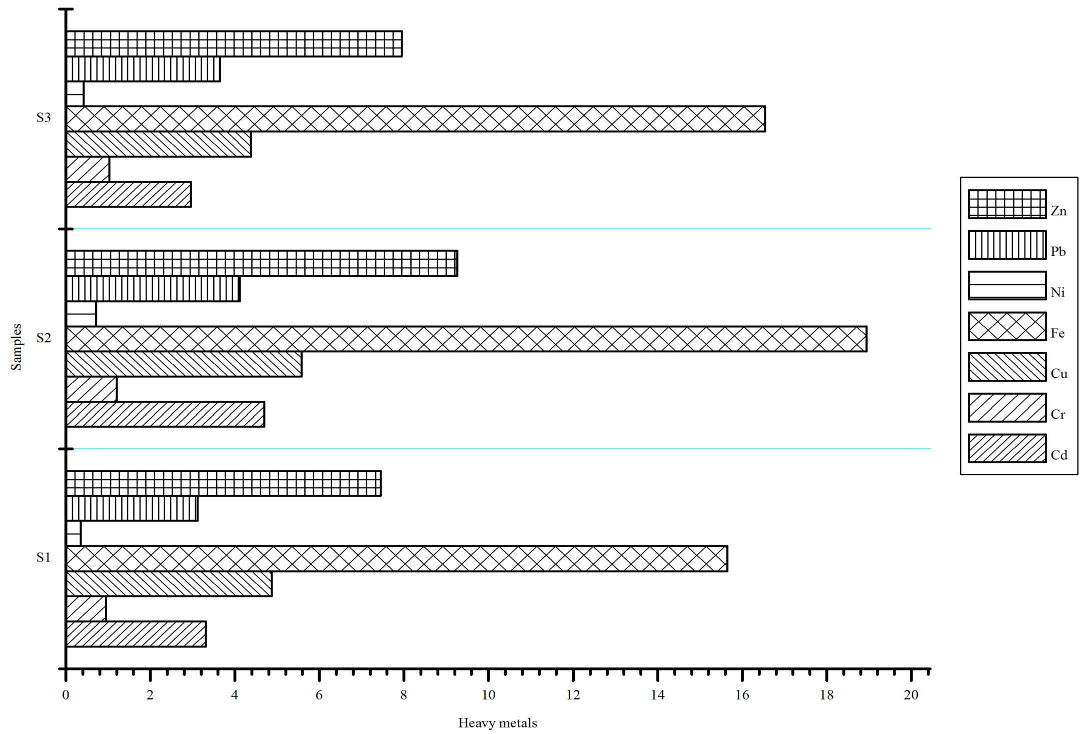


Figure-4
Comparative Graph between three different oil polluted sediment samples such as S1, S2, S3 against trace metal content

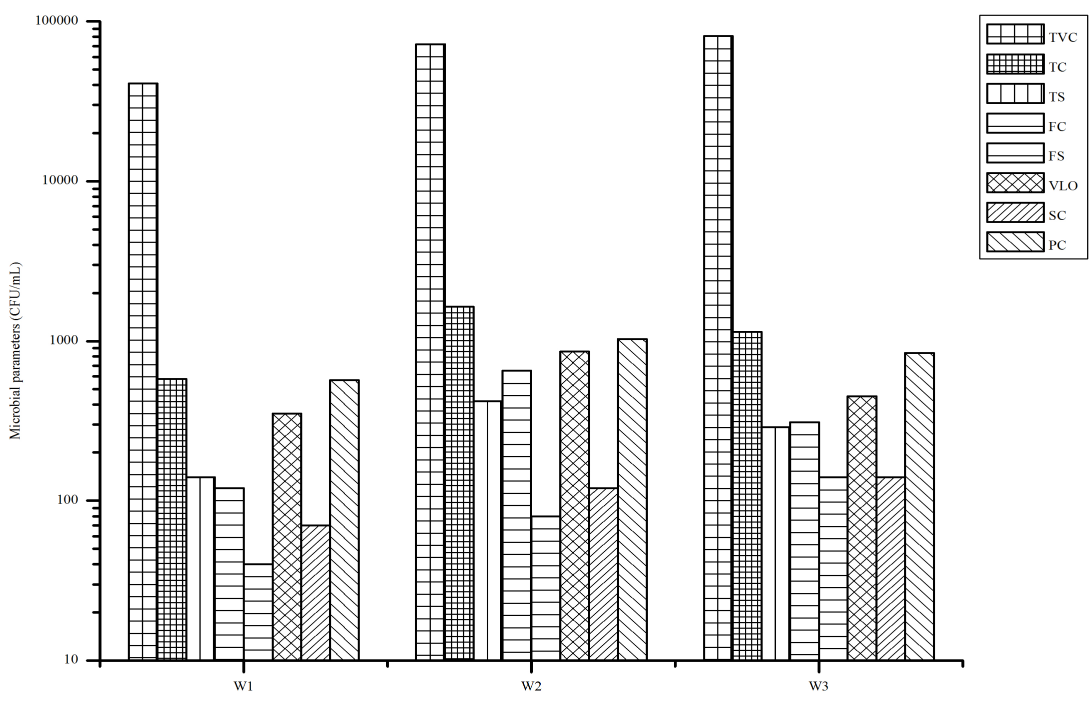
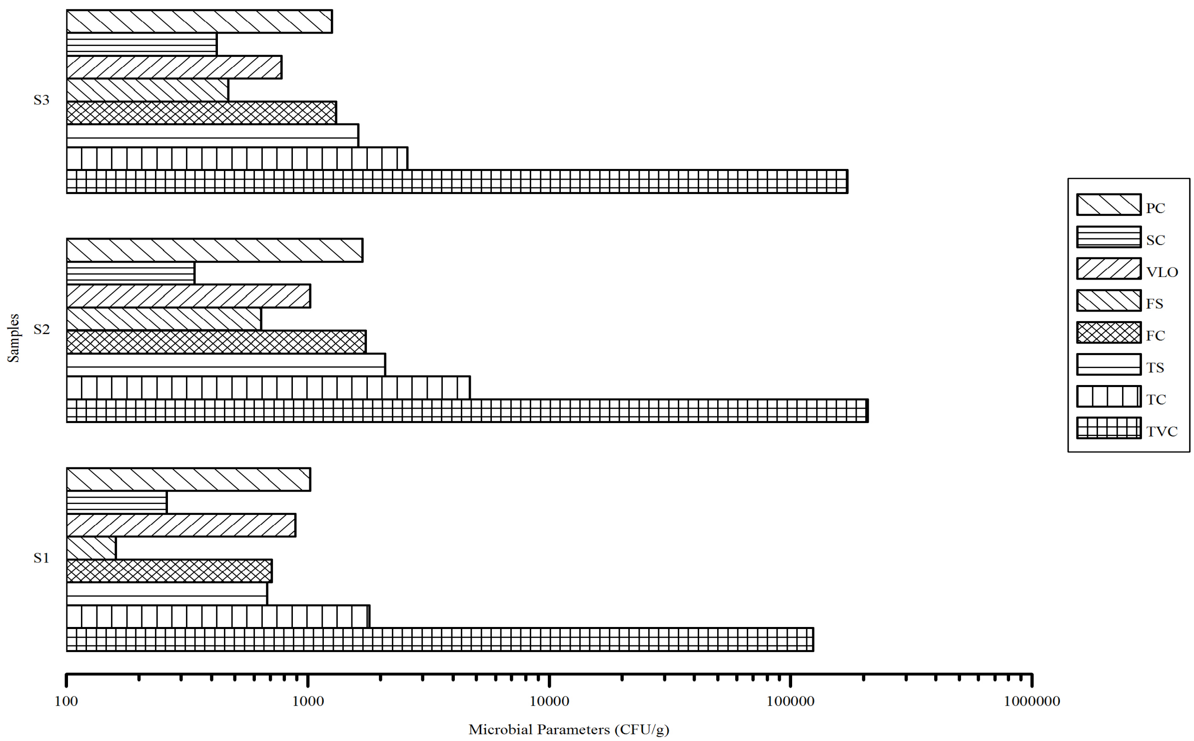


Figure-5
Comparative Graph between three different oil polluted water samples such as W1, W2, W3 against Total Microbial Counts



Figue-6
Comparative Graph between three different oil polluted sediment samples such as S1, S2, S3 against Total Microbial Counts

Contamination of the natural environment by heavy metals is a worldwide problem as these metals are durable and have toxic effects on living things when they pass a certain concentration limit[19]. Heavy metals in the environment mostly come from lithogenic and anthropogenic origins. Metal contents of oil polluted water samples such as Cd, Cr, Cu, Fe, Ne Pb and Zn concentrations are between 2.12-4.1, 0.57-1.12, 2.78-4.23, 7.45-10.56, 0.31-0.52, 1.87-2.64 and 4.12-5.98 mg L^{-1}, respectively. But in the sediment sample, the ranges of Cd, Cr, Cu, Fe, Ne Pb and Zn were 2.96-4.69, 0.95-1.21, 4.38-5.58, 15.65-18.94, 0.35-0.72, 3.12-4.12 and 7.45-9.26 mg kg^{-1}, respectively (table-2). Through the natural process of biomagnifications, minute amounts of metals become part of the diverse food chains and concentrations become elevated to levels which can turn out to be toxic to both human and other living organisms[20].

Water and sediment quality problems stemming from contamination by sewage and, runoff containing pathogenic organisms increase the incidence of illnesses among users[21] potentially leading to extensive water closures. In oil polluted water sample, counts of TVC, TC, TS, FC, FS, VLO, SC and PC were in the range of 41.0-81.0 x $[10^3]$ CFU/mL, 5.8-16.4 x $[10^2]$ CFU/mL, 1.4-4.2 x $[10^2]$ CFU/mL, 1.2-6.5 x $[10^2]$ CFU/mL, 0.4-1.4 x $[10^2]$ CFU/mL, 3.5-8.6 x $[10^2]$ CFU/mL, 0.7-1.4 x $[10^2]$ CFU/mL and 5.7-10.3 x $[10^3]$ CFU/mL, respectively. In sediment sample, the TVC, TC, TS, FC, FS, VLO, SC and PC ranges were 124.0-172.0 x $[10^3]$ CFU/g, 18.0-47.0 x $[10^2]$ CFU/g, 6.8-20.9 x $[10^2]$ CFU/g, 7.1-17.4 x $[10^2]$ CFU/g, 1.6-6.4 x $[10^2]$ CFU/g, 7.8-10.2 x $[10^2]$ CFU/g, 2.6-4.2 x $[10^2]$ CFU/g and 10.2-16.8 x $[10^2]$ CFU/g, respectively (table-3). The role of bacteria as water and sediment quality indicators specify fecal contamination and therefore can be utilized as a signal to determine why such contamination is present, how severe it is and what steps can be engaged to get rid of it. The higher the level of indicator bacteria, the higher the degree of fecal pollution and the larger will be the risks of water borne diseases[22]. In this study, the indicator bacterial levels were crossed the permissible/ standard limits[23,24]. The sediments generally contain higher concentrations of bacteria than the water column[25]. In increase, urban canals receiving domestic sewages and several pollutants like oil pollutants from petrol bunks, mechanic shops, cement/ paint waste, etc. may contribute to the establishment of dissemination routes by microorganism carrying antimicrobial resistance genes[26].

Conclusion

For understanding the existing and possible effects on aquatic environment and human life from toxic metal pollutants, indicator pathogens and phytochemical parameters, so, it is necessary to look into the current concentrations and spatial distribution features of metal pollutants in the survey region. The microbiological and trace metal analysis of the survey indicated that the sites were polluted with high pathogens and metal concentrations which were cutting through the permissible bounds of the BIS[23] and WHO[24]. The pollutant levels in sediment samples were 2-10 folds higher than the water samples. Established on the study, among the three sampling sites, site 2 is highly contaminated/ vulnerable region than site 1 and 2. Hence, through impoundment needed to maintain the sanitation.

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References

1. Ramaiah N., Kenkre V.D. and Verlecar X.N., Marine environmental pollution stress detection through direct viable counts of bacteria, *Water Research,* **36,** 2383–2393 **(2002)**

2. WHO (World Health Organization), Health-based monitoring of recreational waters: the feasibility of a new approach (the ‘‘Annapolis Protocol’’), Geneva, **(1999)**

3. Nagvenkar G.S. and Ramaiah N., Abundance of sewage-pollution indicator and human pathogenic bacteria in a tropical estuarine complex, *Environmental Monitoring Assessment,* **155,** 245–256 **(2009)**

4. Yalcin MG, Narin I and Soylak M, Heavy metal contents of the Karasu creek sediments, Nidge, Turkey, *Environ Monit Assess,* **128,** 351-357 **(2007)**

5. Soylak M, Narin I, Elci L and Dogan M, Investigation of some trace element pollution in Karasu, Sarmisakli Cayi and Kizilirmak Rivers, Kayseri-Turkey, *Fresenius Environ Bullet*, **8,** 014–017 **(1999)**

6. Srinivasa Reddy M., Basha Shaik, Sravan Kumar V.G., Joshi H.V. and Ghosh P.K., Quantification and classification of ship scrapping waste at Alang–Sosiya, India, *Marine Pollution Bulletin,* **46,** 1609–1614 **(2003)**

7. Capone D.G. and Bauer J.E., Environmental microbiology, Clarendon Press, Oxford, **(1992)**

8. Peters E.C., Gassman N.J., Firman J.C., Richmonds R.H. and Power E.A., Ecotoxicology of tropical marine ecosystems, *Environmental Toxicology and Chemistry,* **16,** 12–40 **(1997)**

9. Rainbow P.S., Heavy metals in marine invertebrates, In: Furness, R.W., Rainbow, P.S. (Eds.), Heavy metals in the marine environment, CRC Press, Boca Raton, *FL,* 67–80 **(1990)**

10. Vignesh S, Human impacts on coastal environment in southeast coast of India: A microbial approach. Ph.D. Thesis, Bharathidasan University, Tiruchirappalli, Tamil Nadu, 620 024, India **(2012)**

11. Vignesh S, Muthukumar K, Santhosh Gokul M and Arthur James R, Microbial pollution indicators in Cauvery river, southern India. In Mu. Ramkumar (Ed.), On a Sustainable Future of the Earth’s Natural Resources, *Springer earth system sciences,* 363–376. doi 10.1007/978-3-642-32917-3-20 **(2013)**

12. APHA (American Public Health Association), Standard methods for the examination of water and wastewater. 19th edn, Washington, DC, **(1998)**

13. Vignesh S, Dahms HU, Emmanuel KV, Gokul MS, Muthukumar K, Kim BR and James RA, Physicochemical parameters aid microbial community? A case study from marine recreational beaches, Southern India, *Environmental Monitoring and Assessment,* **186(3),** 1875–1887 **(2014)**

14. Vignesh S, Hans-Uwe Dahms, Kumarasamy P, Rajendran A, Hyoung-Joo Jeon and Arthur James R., Microbial effects on geochemical parameters in a tropical perennial river basin, *Environmental Processes,* **2,** 125-144 **(2015)**

15. Vignesh S, Muthukumar K and James RA, Antibiotic resistant pathogens versus human impacts: A study from three eco-regions of the Chennai coast, southern India, *Marine Pollution Bulletin,* **64,** 790–800 **(2012)**

16. Kumarasamy P, Vignesh S, Arthur James R, Muthukumar K and Rajendran A, Enumeration and identification of pathogenic pollution indicators in Cauvery River, South India, *Research Journal of Microbiology,* **4,** 540–549 **(2009)**

17. Rameshwari R. and Meenakshisundaram M., Microbiological and Physicochemical Analysis of petroleum and diesel oil contaminated samples from motor mechanic workshop in Trichy city, Tamilnadu, India, *Journal of Environmental Science, Computer Science and Engineering and Technology,* **4(3),** 834-842 **(2015)**

18. Clark A, Turner T, Dorothy KP, Goutham J, Kalavati C and Rajanna B., Health hazards due to pollution of waters along the coast of Visakhapatnam, east coast of India, *Ecotoxicology and Environmental Safety,* **56,** 390–397 **(2003)**

19. Macfarlane G.R. and Burchett M.D., Cellular distribution of Cu, Pb and Zn in the greymangrove Avicennia marina (Frorsk.) Vierh, *Aquatic Botany,* **68,** 45–59 **(200)**

20. Bryan E.W., The effects of heavy metals (other than mercury) on marine and estuarine organisms, *Journal of London,* **177,** 389–394 **(1971)**

21. Haile R.W., Witte J.S., Gold M., Cressey R., McGee

C.D. and Millikan R.C., The health effect of ocean water contaminated by storm drain runoff, *Epidemiology,* **10,** 355–363 **(1999)**

22. Pipes W.O., Bacterial indicators of pollution. CRC Press Inc., Boca Raton, FL, 242 **(1981)**

23. BIS, Indian standards drinking water specification, Bureau of Indian Standard, Indian Standard (second revision) -10500, **(2009)**

24. WHO, Guidelines for Safe Recreational Water Environments: Coastal and Fresh Waters Vol. 1, Geneva, **(2003)**

25. Irvensen A., Kühn I., Rahman M., Franklin A., Burman L.G. and Olsson-Lijequist T., Evidence for a transmission between humans and the environment of nosocomial strain of *Enterococcus faecium*. *Environmental Microbiology,* **6,** 55–59 **(2004)**

26. Meirelles-Pereira F., Pereira A.M.S., Silva M.C.G., Gonc-alves V.D., Brum P.R., Castro E.A.R., Pereira A.A., Esteves F.A. and Pereira J.A.A., Ecological aspects of the antimicrobial resistance in bacteria of importance to human infections, *Brazil Journal of Microbiology,* **33(4),** 287–293 (**2002**)