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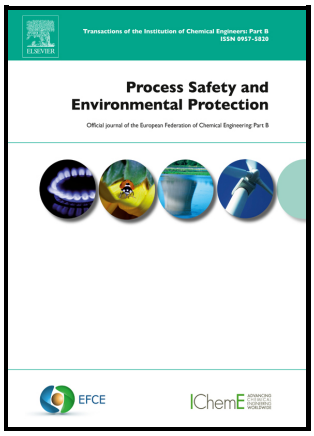

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Field studies on monitoring the marine oil spill bioremediation site in Chennai

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Abstract

Oil spills have become a threat to the ecosystem by releasing the petroleum hydrocarbons and gained substantial public concern in the Chennai coast. This study assesses the effectiveness of bioremediation and its impact on the environment due to remedial operations in the site. Soil and water samples were collected from the bio remediation site at regular intervals of the pit from the topsoil and 20 cm below the ground level from June 2017 to Nov 2019. The average

TPH concentration present in the bottom and top soil of bioremediation pit were vary in the range of 21238.4- 46600 mg/kg and 17577-26910 mg/kg. Central Pollution Control Board (CPCB) allowable limit for TPH concentration present in the soil should be 5000 mg/kg. We have also observed that the mixing was not uniform in the pit and major amount of oil has been penetrated deep inside the soil. Results on gravimetric analysis showed that there was still a large amount of untreated long-chain hydrocarbons are there in the pit. From the results, we can conclude that nC30-nC40 and lower carbon range alkane intermediates have to be treated with additional treatment like thermal smoldering and pyrolysis.

Keywords: Petroleum Hydrocarbons, Bioremediation, Gravimetric Analysis, Microbial growth

1. Introduction

Global industrialization and demand for energy increase the soil and water contamination due to sudden leakage or gradual discharge of crude oil, and other refined products to the environment (Wang and Fingas, 1997). The current increase in petroleum consumption has stimulated exploration and transportation of oil in the marine environment and holds the risk of spillage. Spillage of oil releases a large amount of crude oil into the sea and become a threat to the environment (Margesin et al., 2000). Large quantities of oil released into the atmosphere due to oil tanker collision, oil rig explosion, leakages in oil carrying pipelines, failures in oil storage facilities, etc. (Das and Kumar, 2016; Zabbey and Olsson, 2015; Iturbe et al., 2010). Release of hydrocarbons through spills and leaks from steamers, underground tankers, unplugging of oil wells, etc., causes groundwater, ocean, and soil contamination (Varjani, 2017; Souza et al., 2014). The spilled oil tends to disperse in the aquatic system, produce bulk emulsions, and

creates critical problems to the community if not correctly managed and cleared (Ibrahim et al., 2012).

The first oil spill, which gained more attention, was happened on English Canal on 1969, and after that, numerous spillages had been reported (Atlas, 1995). Oil spills in the Gulf of Mexico, Exxon Valdez spill, Montora, and tanker collision in the Mumbai coast are powerful examples (Sakthipriya et al., 2015). The major disaster that happened to cause an imbalance in the ecosystem in the northern regions of the Chennai coast, Tamilnadu, is the heavy fuel oil spill. Chennai, situated on the southeastern coast of India, and Coromandel Coast along the Bay of Bengal has two main ports, namely,Ennore (Kamarajar) port and Chennai port. BW Maple, anLPG carrier and MT Dawn Kanchipuram, an oil tanker containing petroleum products collided at the Ennore port on January 2017, and released more than 196 MT of fuel bunker oil. Based on the report given in Times of India, a strong wind has blown out the spilled oil upto 34 km till Vettuvankeni in the south and contaminated beaches in Chennai. Figure S1 shows the glimpses of oil spilled at Ennore port.

Many groups have been engaged in the removal of a gigantic oil spill from the coastal area of Chennai, which has raised doubts of severe ecological impairment(Sivagami et al., 2019). Particulars about the actual contamination of the environment by oil are tedious to measure due to the accidental contamination. The oil was collected using 3 super suckers and submersible pumps along with the aid of manual work like scooping with buckets done by local fishermen. Additionally, about 2000 liters of oil spill dispersant was sprayed by the coast guard to disperse the oil into small droplets for biodegradation and also to avoid the oil from reaching the shore.

When responding to oil spills or design a countermeasure, prior knowledge of oil properties is of supreme importance (Reed et al., 1999). Various factors, such as type of oil,

amount of oil spilled, location of the spill, source of contamination,and rate of spillage, determines the severity of oil spill (Ansell et al., 2001, Chikere et al., 2011). Fuel oil is a complex mixture of hydrocarbons and its derivatives with boiling points ranging from a hardly any to several hundred(Kumar et al., 2011; Wang and Fingas, 1997). Once the oil is spilled, it gradually starts decaying under the impact of simultaneous processes collectively known as oil weathering (Hu et al., 2013). Weathering of an oily sludge modifies its behavior and makes it more persistent to marine waters and enduring its lifespan in marine biology (Geraci and Aubin, 1988). In some occasions, the light crude oil spilled on the sea may dissipate naturally due to evaporation and volatilization. This condition was proved when 85,000 tons of light crude oil was dissipated naturally after the spill from cargo on Shetland Isles, the UK, in January 1993 (White and Mollay, 2003). On the other hand, heavy crude oil will remain persistent in the sea.

Various methods, including physicochemical and biological treatment, have been investigated for controlling oil contamination from polluted sites. Any effective remediation procedure should not merely transfer the contaminants to other parts of the environment (Singh et al., 2011). Many of those methods are expensive due to excavation cost and transportation of large amount of contaminants for *ex-situ* treatment (Yuniati, 2018). Bioremediation is the process by which toxic substances are made harmless by degradation using microorganisms, thereby bringing the area contaminated to a harmless state. It is a natural process wherein the microorganisms break the contaminants into non-toxic or less toxic substances by their metabolic activities (Brown, 2010). Bioremediation is performed by the addition of exogenous microbial populations or stimulating indigenous ones, and raise the rates of degradation (Atlas and Barta 1998). It is less expensive and environmentally friendly when compared to other methods of treatment like incineration, oxidation, etc. Also, hydrocarbon utilizing microorganisms are

universally distributed in the aquatic environment and indeed biodegrade various petroleum hydrocarbons following oil spills (Liu et al., 2020). These indigenous microorganisms may

require some nutrients to degrade the contaminants. Alternately, a specific microorganism capable of decomposing the contaminants can be cultured in a laboratory and sprayed over the site (Kumar and Gopal, 2015).

Existing monitoring techniques require the purpose of the disappearance of the pollutants or contaminants and their degradation products to regulatory limits. The assessment of successful bioremediation and the monitoring problems and bioremediation have been successfully have been widely recognized(Hamoudi-Belarbi et al., 2018; Nwogu et al., 2015; Nyman, 1999). Different bioremediation approaches have been effectively applied to degrade or remove contaminants from polluted soils. Researchers have found that the activity of microbes accounts for most of the transformation of contaminants in the soil (Cui et al., 2020; Trindade et al., 2005). Wu et al. (2017) reported that the microbial activity enhances alkane degradation, but no correlation was observed for polycyclic aromatic hydrocarbons (PAH) degradation. Mohn et al. (2001) observed the reduction of total petroleum hydrocarbons (TPH) from 196 to below 10 mg per kg of soil at a site, and from 2,109 to 195 mg per kg of soil at another site after a year of bioremediation. Sasek et al., (2003) established that the successful application of bioremediation of sites affected by oil. He also stated that the time consumption would be more when recalcitrant species, such as high atomic weight hydrocarbons are present. The liquid fraction in soil tends to be extremely toxic due to the presence of PAH and other toxic compounds, and the contaminated soil has to be remediated quickly to avoid a serious threat to the environment (Vidonish et al., 2016; Kuppusamy et al., 2017).

The main objective of the research work is to assess the efficacy of bioremediation and environmental impact due to treatment operations of oil spill sludge at the Ennore Kamarajar Port area. Specific objectives are: Systematic characterization and monitoring of the total petroleum hydrocarbons and n-alkanes present in the Ennore bioremediation pit; Compare and evaluate the TPH degradation efficiencies in the field and lab-scale bioremediation setup; Analysis of microbial population and characterization of microbes present in the soil. Also,the concentration of persistent organic pollutants such as PAHs and heavy metals present in the bio remediated soil was studied after three years time period.

2. Materials and Methods

2.1. Study area

Ennore Port, situated 2.6 km north of the Ennore creek, islocated on Chennai, a city of Tamil Nadu on the Eastern Coastal Plains and Coromandel Coast in the Bay of Bengal. This port experiences minimal variations in seasonal temperatures ranging from a maximum of 38–42 °C in summer to a minimum of 18–20 °C in winter. Oily sludge collected from different locations were transported to Ennore Kamarajar port for storage. An ex-situ bioremediation was carried out by a major oil company in India. The company has proposed the treatment technique of bioremediation to dispose the spilled oil. The spilled oil sludge was transported to the site of bioremediation by trailers and manpower offered another public sector unit. An open and ventilated area was selected for the bioremediation of 180 tons of oil sludge. The pit dimensions were 165 m X 16 m with depth 45 cm. Soil was excavated from the pit and a polythene sheet of sufficient thickness was laid to prevent the oil from infiltrating into the ground and contaminating the ground water. The excavated soil was backfilled for a depth of 3 cm as a

buffer layer over which a polythene sheet was spread. The remaining excavated soil and the oil sludge mixed mechanically in the ratio 9:1 along with a suitable bacterial consortium called oilivorous mixture capable of degrading the oil was spread evenly over the polythene sheet. Water containing dissolved nutrients was also sprayed over the layer to aid the bacterial growth and increase their performance. Tilling and watering was also done weekly to give better aeration to the bacteria and to facilitate bioremediation. Samples from the Ennore bioremediation site and monitoring wells (E1, E2, E3, E4, E5, E6, E7, W1, W2, W3, S1 and N1) have been collected and regularly monitored for TPH and microbial growth.

Bioremediation pit was monitored for the Total Petroleum Hydrocarbon (TPH), Poly Aromatic Hydrocarbons (PAHs) concentration present in the soil over a period of three years. Forty soil samples were collected at regular intervals of the pit from the top surface and 20 cm below the ground level. Ten water samples were collected from bore wells around the bioremediation pit. These water and soil samples were analysed from June 2017 to November 2019 with a regular interval of time. The initial total petroleum hydrocarbon (TPH) concentration present in the soil during the month of February 2017 was around 83,000 mg/kg.

2.2. Bioremediation of oil sludge

The oil spill sludge around 250 tonnes was collected on the shores of Chennai beach and moved to the bioremediation pit dug inside the Ennore port. The pit with an area of 2,000 m^2 was 1.5 ft deep and protected from direct external influences by a polyethylene cover. Layers of earth, sand, sludge, and contaminated soil collected from beaches were laid and bioagents were sprayed in the pit. Soil samples were collected from bioremediation pit at two different depths, namely 10 and 20 cm. Hollow stem auger was used to aid soil sampling. Samples were taken

from south to north direction and left to the right side of pits shown in Figure 1. Twenty samples were collected inside the pit, and one sample has been collected outside the pit to identify the total petroleum hydrocarbon concentration and its transformation as intermediates. The decrease in the TPH content and its fractions with time and the percent biodegradation was calculated from the TPH data of the samples.

2.3 Soil monitoring

Samples were collected according to the guidelines given by the central pollution control board (CPCB) of India. The samples were collected at two different depths (0 to 10 cm and 10 to 20 cm). Around 20 soil samples of 200 g each were collected at different locations using spades and core samplers. As per the CPCB guidelines for the pit of the larger area, the rows and columns to be divided for sample locations should be 10 to 15 m, and for the smaller area the distance should be 3 to 5 m. However, since such specifications led to a large number of sampling points, the number of sample locations was reduced by increasing the distance between the rows and columns above the specified limits, but it was made sure that they were equally spaced. Figure 1 shows the schematic diagram of the sampling points at the bioremediation pit. Total petroleum hydrocarbons (TPH) analysis was carried out using Gas Chromatography-Mass Spectroscopy (GC-MS) and gravimetric method.

2.3.1. Solvent extraction of hydrocarbons for soil samples

The collected soil samples were extracted using Dichloromethane (DCM) (Sakthipriya et al., 2016). About 10 mL of DCM was added to 5 g of soil collected from the bioremediation pit (that is 1:2 ratio of soil to DCM) 50 mL centrifuge tube. The mixture was subjected to vortexing

and sonication so as to make the petroleum hydrocarbons get dissolved in DCM. Vortexing was carried out for 2 min at medium speed and sonication for about half an hour. The mixture was then centrifuged for about 5 to 10 min at 6000 rpm as a method of sedimentation. The supernatant was then separated from the residue, and the solution was concentrated to 1 mL by evaporating the solvent DCM by nitrogen purging in a fume hood. The concentrated solution was then injected into the GC-MS.

2.3.2. Analysis of Total Petroleum Hydrocarbons (TPH) and Poly Aromatic Hydrocarbons (PAHs) using GC- MS

GC-MS identification of n-alkanes in oil sludge was done using GC-MS (Agilent Technologies, USA). A HP-5 MS (30m x 0.25 mm) silica based cross-linked column was used. The injector and detector temperatures were maintained at 300°C. The initial temperature was 50 °C for a min, then ramped to 110 °C at 10 °C/min, held for 2 min, and again ramped up to 250 °C at the rate of 5 °C/min and held for 2 min and to 300°C at the rate of 3 °C/min and maintained for 15 min. An aliquot of 1µL was injected in the splitless mode. Helium was used as a carrier gas at a rate of 1 mL/min. MS detector was scanned from 35 to 550 amu at 1.562 u/s by selecting full scan mode. MS source temperature was 230 °C, and MS quadrupole temperature was maintained at 150°C. Calibration curves were prepared through multiple dilution 20 element multi-elemental total petroleum hydrocarbons (TPH) standard (Supelco, USA). The amount of TPH present in the oil spill sludge was enumerated using Agilent Technologies Mass Hunter software. The calibration standard solution, containing thirty four paraffins from n-heptane to n-tetracontane (nC_7H_{16} to $nC_{40}H_{82}$), was used to build a GC-MS calibration curve with five calibration points at concentration levels of 5, 10, 30, 50 and 100 mg/L. The calibration

standards were made from an original stock solution of 1000 mg/L alkane mixture in n-Hexane (Product code: 49452U; Sigma Aldrich)

For analyzing various PAHs and their respective parent PAHs, SIM (GC/MS) method was used. The 15 element PAHs standard was quantified by employing Agilent Technologies Mass Hunter quantitation software. The PAH standard solution was used to build a GC/MS calibration curve with six calibration points at concentration levels 25, 50, 100, 250, 500, and 750 µg/L. Calibration curves were developed for naphthalene, acenapthylene, acenaphene, anthracene,benz(a) anthracene, Benz(b)fluoranthene, Benz(k)fluoranthene,Benzo (ghi) perylene, Chrysene, fluorene, fluoranthene, pyrene,andphenanthrene,. Average response from three injections were used to compute these calibration points. For quantitation, a minimum of five calibration points were used to obtain a linearity level of 0.97- 0.99. Furthermore, the calibration points were inverse concentration weighted to minimize the bias. PAHs concentrations were measured at the initial GC oven temperature (50 °C) was ramped to 120 °C at 25 °C/min; ramped to 160 °C at 10 °C/min; ramped to 300 °C at 5 °C/min (4 min hold). The target ions monitored during the SIM analysis and standards use were monitored (Rajasekhar et al., 2021). Injector and transfer line temperatures were maintained at 280 °C and 300 °C respectively. MS quad and MS source temperatures were 280 °C and 300 °C respectively

2.3.3. Soil gravimetric analysis

The collected soil samples were extracted using Dichoromethane (DCM). The 10 g of soil was mixed with 20 g of sodium sulphate to remove the moisture content and 15 mL of DCM was added to the mixture. The mixture was subjected to vortexing and sonication so as to make the petroleum hydrocarbons get dissolved in DCM. Vortexing was carried out for 2 min at

medium speed and sonication for an hour. The mixture was then centrifuged for about 5 to 10 min at 6000 rpm as a method of sedimentation. Decant the solvent to a fresh vial (preweighed) and evaporate in a gentle stream of nitrogen. The gain in weight was calculated. The remaining soil in the vial was continuously extracted two more times and the weight gain was noted.

$$\text{Concentration of TPH (mg/kg)} = \frac{(\text{gain in weight of the vial (g)} - \text{Initial weight of the vial (g)}) \times 10^6}{10\,g}$$

2.3.4 Analysis of heavymetals in soil samples

Heavy metals from soil samples were extracted into aqueous phase using the acid digestion process defined in the US EPA 3050B standard method.The concentration of six heavy metals such as Zn, Cu, Cr, Pb, Cd and Fe were determined using inductively coupled plasma mass spectrometry (ICP-MS). The operating conditions of ICP-MS were provided in Table S1 in the supplementary information.

2.4. Monitoring of groundwater system

Groundwater monitoring was done based on the direction of flow of groundwater. Water samples of 500 mLwere collected from the monitoring wells, namely, E1, E2, E3, E4, E5, E6, E7, W1, W2, W3, S1 and N1. The labels E1 to E7 indicate monitoring wells located on the east side of the bioremediation pit. Similarly W1, W2 and W3 represent west side monitoring wells where as S1 and N1 are south and north side moniroing wells, respectively. The positions of all of these monitoring wells were depicted in Figure 1. After the collection of the water sample, it was subjected to TPH analysis and microbial analysis similar to soil samples. However, the extraction procedure for water is slightly different from that of the soil system.

2.4.1. TPH analysis using GC-MS for water samples

DCM was added to the water sample in the ratio 1:3. Accordingly, 10 mL of DCM was added to 30 mL of the water sample. The solution was stirred using a magnetic stirrer for about 30 min at 800 rpm. The solution was then transferred into a separating funnel was shaken for four or five times and left to stand for some time to get a clear separation of the two immiscible liquids (water and DCM containing dissolved petroleum hydrocarbons). Water, having density lower than DCM, will float over DCM. Hence the lower portion, which is DCM is collected in centrifuge tubes, concentrated to 1 mL by nitrogen purging and injected into the GC-MS.

2.4.2. TPH analysis using gravimetric method for water samples

The extraction process of hydrocarbons involved the addition of 50 mL of n-hexane, 10 mL of sulphuric acid (to degrade any biogenic matter if any) and 5 mL of ethanol (to enhance the separation of the two immiscible liquids-water and n-hexane) to 200 mL of the water sample. The solution was transferred to a separating funnel and was shook vigorously to enable the dissolution of the petroleum hydrocarbons in the solvent, n-hexane. Hexane being lighter than water floated over water and was collected in a crucible after disposing of the water. The empty weight of the crucible was found out before collecting hexane. The hexane containing the petroleum hydrocarbons was evaporated to dryness in a hot plate maintained at 100 °C. The final weight of the crucible was noted and the concentration of TPH was calculated from the difference in initial and final weights of the crucible.

2.5. Characterization of microbes and analysis of microbial growth

Colony-forming units (CFU), assessing theviable bacterial cells was calculated by the spread plate procedure described by Tehrani and Herfatmanesh (2015). Soil sample of 10 g was added to 95 mL of deionized water, and the suspension was shaken well. Deionized water of 0.9 mL was added to ten centrifugal tubes, and consequently, 0.1 mL of soil water suspension was added to the first tube and mixed well. Thereafter, 0.1 mL from the first tube was dropped into the second and so on. An aliquot (0.1 mL) from each tube was spread in to the separate Petri dishes containing an agar medium using an 'L' rod. The Petri dishes were placed in the orbital shaker for 24 h and counted for the number of cells (counts). A similar procedure was followed for groundwater samples too. The colonies were isolated and characterized to find the species.

3. Results and Discussion

The average and standard deviation of TPH concentration present in the surface and 20 cm below the soil surface of the bioremediation pit for the month of June 2017 to Nov 2019 were shown in supplementary Tables S2 and S3. The average concentration in the top soil was varied in the range of 17577-26910 mg/kg, and 20 cm below soil has an average concentration of 21,238-46600 mg/kg. The central pollution control board allowable limit for TPH concentration present in the remediated soil should be 5000 mg/kg.

3.1. Degradation of total petroleum hydrocarbons (TPH) in the bioremediation pit

Gravimetric analysis have been used to measure the total petroleum hydrocarbons present in the top and bottom soil of the bioremediation pit. Figure 3 shows the overall TPH observed in the soils collected from the bioremediation pit from June 2017 to September 2019. The amount of TPH degradation was higher in the month of June to December 2017. It was found that the bottom layer has less quantity than top layer in the month of June and July and vice versa in

other cases. High-density compounds had percolated deep into the soil and lower density compounds had been floated and moved from the pit after the northeast monsoon. Comparing the gravimetric studies, we have observed that there was still a maximum amount of hydrocarbon should be treated from the pit (Figure 3). From this, we can observe that the concentrations of hydrocarbons are still higher in the bioremediation pit. This includes linear chain alkanes, branched-chain alkanes, and other polyaromatic hydrocarbons. Initially, in the month of June, July, and August, the concentration of hydrocarbons in the top layer was higher. The maximum TPH has been observed in the soil samples collected from the pit (BR7 and BR7A). The minimum amount of TPH was observed at BR9 on the top layer and BR5A on the bottom layer. During the months progress, the concentration in the bottom layer has increased due to volatilization and photo degradation of low carbon range hydrocarbons and sinking of higher density of nC30-nC40 compounds in the soil. In November 2017, the top layer TPH concentration was measured as 5000 to 320000 mg/kg, and the bottom layer consists of 6000 to 56000 mg/kg with an average value of 22930 mg/kg. Soil was completely mixed with the oil. We observed the oil deposit when it was drilled deep inside the bioremediation pit. The various images taken during the sampling during this time period were shown in Figures S1 and S2. We have observed the floating of oil samples over the pit during our visit after the rainfall Figures S3 and S4). It was also observed that a maximum of 40 mg/kg of TPH has been observed in the soil samples collected from the pit (BR5 and BR6A). A minimum amount of TPH was observed at BR4 on the top layer and BR10A on the bottom layer. The floating of free oil samples and flowing of oil-contaminated rainwater from bioremediation pit to the outside area through the opening in was also observed. Floating oil was observed to cross the bioremediation pit due to the aperture on a compound wall in the bioremediation site. This shows the less effectiveness of

the bioremediation process happening in the pit. Higher level of water stagnation in the pit almost reduced the activity of micro organisms present in the remediation site.

The TPH concentration in the month of January and July 2018 in the top and bottom layer were measured as 7000 to 33,000 mg/kg , 5000 to 29,000 mg/kg and the bottom layer consists of 7,000 to 39,000 mg/kg , 10,000 to 38,000 mg/kg of TPH per gram of soil analysed. From the month of January to June 2018, the reduction in TPH concentration was almost very minimum. TPH concentration measured in the month of Nov 2019 in the top and bottom layer was measured as 8035-23340 mg/kg, and 13000-46600 mg/kg. These higher molecular weight alkanes were highly waxy in nature and remain persistent on the surface. Alkanes have relatively low acute toxicity, but alkanes having higher carbon numbers up to C12 have narcotic properties, particularly following inhalation exposure to high concentrations. (Sakthipriya et al., 2015, 2016).

3.2. Concentrations of individual n-alkanes in the bioremediation pit

The saturated alkanes are another important class of petroleum compounds present in oil spill samples (Wang and Fingas, 1997). The n-alkanes chromatogram shown in this Figure 4 provides a higher resolution of the saturated hydrocarbon distribution. Figure 4 shows the n-alkanes concentration measurement of total petroleum hydrocarbons present in the soil collected from the bioremediation pit on July-2017. The top layer showed the presence of C10 to C36, and the bottom layer indicated the presence of C10 to C29. Variation of TPH concentration in the top and bottom layer of the pit reveals the improper mixing of oil in the pit. Maximum quantity of TPH was observed in the soil samples collected from the BR5 and BR9A in the month of August. Lower molecular weight alkanes were not observed in the month of August due to its high volatility. The top layer showed the presence of C11 to C37, and the bottom layer shows the

presence of C10 to C37. Chennai was flooded with rainfall in the period of September to November 2017. GC- MS finger printing of sep 2019 had shown that the concentration of C21-C40 compounds were measured high in the bottom layer and the C14 to C34 were measured in the top layer. The removal of higher molecular weight hydrocarbons present in the bio remdiation pit mainly depends upon intrinsic ability and factors like moisture, nutrients present in the soil (Das and Chandran, 2011, Sihag et al., 2014). Poor operation and maintenance of bioremediation site, excess water level in the monsoon (Sep-Nov 2017) and very less moisture present in the soil , higher ambient temperature (45-50 °C) made the microbial population almost inactive and the TPH concentration becomes almost stable from June 2018 to Nov 2019.

3.3. Concentration of PAHs and heavy metals in treated soil

The concentrations of the US EPA priority pollutants, such as PAHs, were measured in treated samples of bioremediation pit and the results were shown in Table 1. Persistence of the PAHs in environmental matrices is of major concern as they are acutely toxic and carcinogenic to biota (Sarma et al., 2016). One of the main sources of PAHs occurring in the environment is the use of various crude oil refined fuels such as diesel, lubricating oil, bunker oil and furnace oil (Wilson and Jones, 1993). Six PAHs have been identified as fluoranthene (Flu), pyrene (Pyr), benzo[a]anthracene (BaA), benzo[k]fluoranthene (BkF), chrysene (Chr) and benzo[g,h,i] perylene (BghiP) in both the top and bottom soils of the remediation pit. These six PAHs were found to be high molecular weight PAHs containing four to six fused aromatic rings. The total concentration of PAHs (ΣPAHs) in this study ranged from 0 to 554 µg/kg in the topsoil and 6 to 269 µg/kg in the bottom soil. The ΣPAHs concentration in the topsoil was found to be higher than in the bottom soil. The maximum concentrations of individual PAHs were observed to be 12. 20 µg/kg for Flu, 66.30 µg/kg for Pyr, 296.16 µg/kg for BaA, 115.35 µg/kg for BkF, 121.70

µg/kg for Chr, 258.46 µg/kg for BghiP. Of the six detected PAHs, highest distribution was found in the top soil (5 PAHs) relative to the bottom soil (4 PAHs) (Table 1). Among all PAHs, BaA was detected in most of the sampling spots, whereas BghiP, BkF, Chr, Flu and Pyr were less prevalent (Table 1). Lower molecular weight PAHs (2 to 3 aromatic rings) have not been detected in any of the soil samples. The absence of LMW PAHs in treated soils may indicate their complete biodegradation. In addition to biodegradation, natural weathering processes such as volatilization and photo-degradation could significantly reduce the PAHs concentrations in the soil as the bioremediation pit was operated in the ambient atmosphere (Yin et al., 2015). Previous studies also reported that LMW PAHs exhibited relatively higher rates of biodegradation compared to HMW PAHs (Dearyet al., 2016; Lu et al., 2019).

Besides PAHs, the concentrations of heavy metals in the treated samples were also measured. Variations in the concentrations of each heavy metal ranged from 12.2 to 27.7 mg/kg (Zn), 10.8 to 23.2 mg/kg (Cu), 3.2 to 33.0 mg/kg (Cr), 5.5 to 41.2 mg/kg (Pb), 1.0 to 18.6 mg/kg (Cd) and 14.4 to 25.5 mg/kg (Fe). The concentrations of Zn, Cu, Pb and Cr in the treated soils were below the permissible limits of Indian and European standards such as Zn: 300 to 600 mg/kg, Cu: 135 to 270 mg/kg, Pb: 250 to 500 mg/kg and Cr: 200 to 300 mg/kg (Awashthi, 2000; Sharma et al. 2007; Tóth et al., 2016). However, the observed concentrations of Cd were above the permissible range of 3 to 56 mg/kg. Hence the soil must be further treated for the removal of Cd to avoid the leaching into groundwater and mixing with runoff streams during rainy seasons. Although the concentrations of most heavy metals are below the regulatory limits in this study, presence of these metals as co-contaminants inhibits the biodegradation of PAHs in the soil (Deary et al., 2016).

3.4. Water analysis

The total petroleum hydrocarbons in the water samples E3, E6, S1, and W2 were 101.212, 32.876, 26.769, and 111.773 ppm, respectively. The water sample from W2 was found to have higher concentrations of C11, C21, and C23 (33.62, 16.231 and 8.935 ppm). The highest concentrations of C13, C15, C17, and C19 recorded in this group were 16.591, 14.369, 15.198, 10.190 ppm in sample E3 (Table 2). Samples E6 and S1 had relatively low concentrations of alkanes. Initially, in the month of June and July, contamination of TPH was less, and increased further in consecutive months, and the contamination was less than 100 ppm. It may be inferred that the penetration of hydrocarbons in to ground water is increasing over the coursew of time.The water sample from E2, E3, and E6 was found to have higher concentrations of undecane, heneicosane and tricosane (32-40, 28-43, and 18-45 ppm). The highest concentrations of tridecane, pentadecane, heptadecane and nonadecane recorded in this group were 16-25, 14-33, 15-42, 1038 ppm in sample E2,E3, and E6 (Table 2). Samples N1 and S1 had relatively low concentrations of alkanes. The total petroleum hydrocarbons in the water samples E1, E6, S1 and N1 were 101.212, 32.876, 26.769 and 111.773 ppm, respectively. The average and standard deviation of TPH concentration present in the bore well water around the bioremediation pit were in the range of 47.14 - 79.56 mg/L and 11.45-120.71 mg/L. During the course of time volatile lighter alkanes has been reduced and the concentration of heavier ones have been increased.

3.5. Microbial analysis

Ability of the microbes to grow on the bioremediation site has been observed using colony forming units. Figure 6 shows the growth of microorganisms in the bioremediation pit. It is inferred from the figure that the growth of microorganisms has not followed any pattern. It showed largest growth in certain month in one place and lowest in another place. For example in the month of October, the growth of microbes are lower at the position BR7 and higher at BR1. However, the growth of microorganisms at the bottom layer is always higher when compared to the ground level. We may infer that the circumstances to promote the growth is better at the bottom layer. Another point to be noted here is the role of n-alkanes in the growth of microorganisms. It is observed that the growth of microbes is higher at lower concentration of TPH.

4. Conclusion

This study investigated the performance of large-scale bioremediaton of oil spilled sludge at Ennore Kamarajar port in Chennai, India. The concentrations of total petroleum hydrocarbons (TPH), n-alkanes and persistent pollutants such as polycyclic hydrocarbons (PAHs) and heavymetals in the soil were assessed during the bioremediation operation for a period of 3 years. The reduction in TPH concentration and transformation of higher molecular weight hydrocarbons was observed after six months of bioremediation. The average intial concentration of TPH in the soil ranged from 60,000 to 80,000 mg/kg inside the bioremediation pit. The continuous monitoring of soil samples shown that the bioremediation of the TPH concentration was reduced to 50 to 60% from their intial concentrations. It was also found that the TPH concentrations are not uniform across the pit during the entire period of treatment. TPH levels in the bottom soil (20 cm below) were found to be two times higher than that in the top soil (10 cm

below) inside the pit. The average concentration in the top soil was varied in the range of 8035-23340 mg/kg, and 20 cm below soil has an average concentration of 13000-46600 mg/kg, which is higher than the CPCB allowable limit of 5000 mg/kg. PAH compounds like pyrene, benzoanthracene, benzoflouroanthene, benzoperylene and chrysene were identified in the remediated soil. The effective degradation of hydrocarbons mainly depends upon the microbial population, the concentration of TPH, and its bioavailability, moisture, and nutrients present in the soil over a period of time. Groundwater near the bioremediation pit was slghtly impacted by the petroleum hydrocarbons, most probably due to the infiltrarion of oil-laden water into subsurface during rainy seasons.

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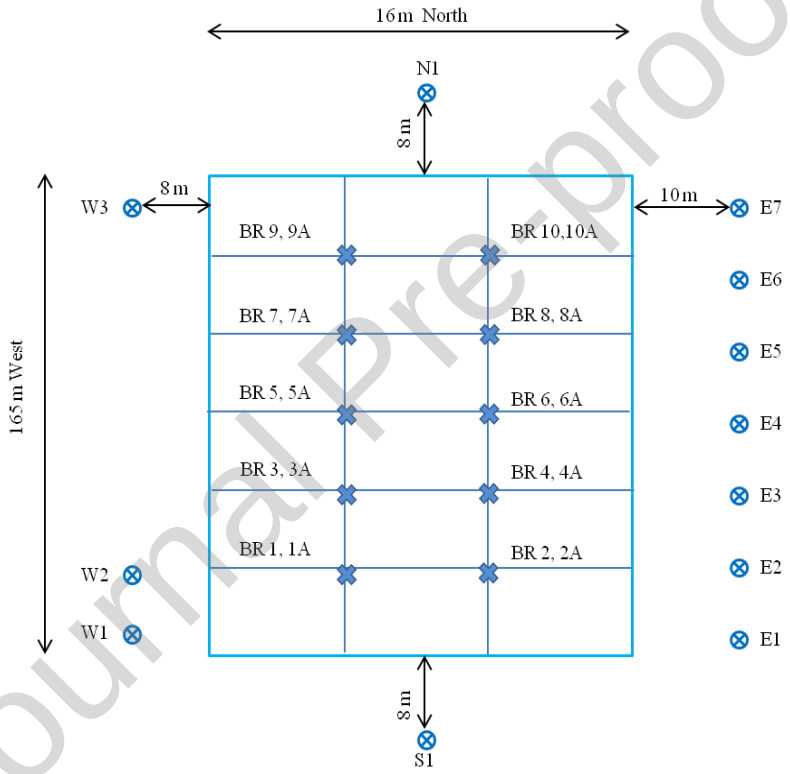


Figure 1. Sampling locations at Ennore oil spill bioremediation site;

BR – 0 to10 cm depth; BR A – 10 to 20 cm depth.

E1 to E7: East side, W1and W2: West side, S1: South side, and N1: North side monitoring wells.

Figure 2. Oil floating on the Ennore bioremediation pit during northeast monsoon (October-2017). Oil deposits in bioremediation pit (January-2018).

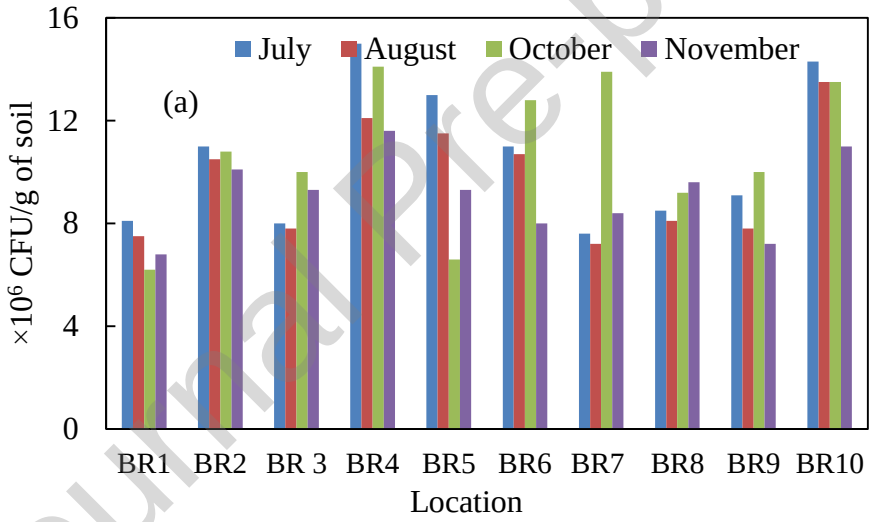
Figure 3 – (a) Gravimetric studies on TPH concentration (mg/kg) present in the bottom (20 cm below the ground) layer of the bio remediation pit. (b) Gravimetric studies on TPH concentration (mg/kg) present in the top soil of the bio remediation pit.

(a)

(b)

Figure 4 – (a) Determination of n-alkanes concentration present in the bottom (20 cm below the ground) layer of the bio remediation pit (b) Determination of n-alkanes concentration present in the top soil of the bio remediation pit (Nov 2019)

Figure 5 – (a) Total petroleum hydrocarbon concentration present in the water samples around the bioremediation pit (June 2017 – Sep 2019) (b) Determination of n-alkanes distribution in the water samples using GC-MS (Sep 2019)



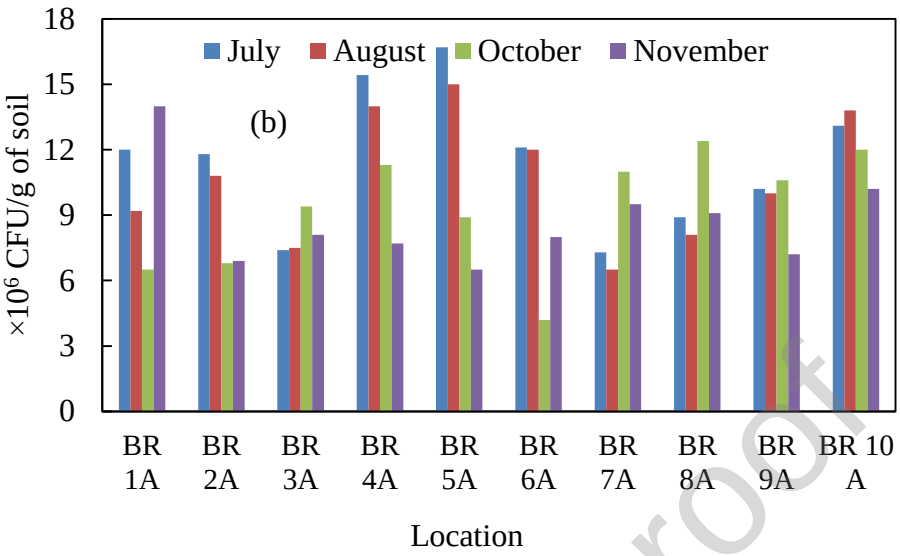


Figure 6. Microbial analysis of soil.

List of Tables

Table 1 Concentrations of PAHs in the treated soil of bio-remediation pit						
Sample location	**Pyrene (µg/kg of soil)**	**Benzo[a] anthracene (µg/kg of soil)**	**Benzo [k] fluoranthene (µg/kg of soil)**	**Benzo [g,h,i] perylene (µg/kg of**	**Fluoranthene (µg/kg of soil)**	**Chrysene (µg/kg of soil)**

				soil)		
Top soil						
BR1	5.562	-	-	-	-	-
BR2	-	13.55	-	-	-	-
BR3	-	-	-	-	-	-
BR4	66.299	133.208	48.973	-	-	-
BR5	-	20.259	-	-	-	-
BR6	-	3.955	-	-	-	-
BR7	-	296.155	-	258.463	-	-
BR8	-	5.967	-	-	-	-
BR9	-	14.147	-	-	-	-
BR10	-	2.577	-	-	-	-
Bottom soil						
BR1A	-	4.937	15.381	-	-	-
BR2A	-	62.075	115.346	-	-	-
BR3A	-	7.429	-	-	-	-
BR4A	13.582	51.521	24.365	-	-	-
BR5A	10.482	31.822	46.053	-	-	-
BR6A	-	135.221	-	-	12.199	121.699
BR7A	-	7.9236	-	-	-	-
BR8A	-	15.324	-	-	-	-

BR9A	-	6.023	-	-	-	-
BR10A	-	22.587	-	-	-	-

Table 2: Concentration of different hydrocarbons in water using GCMS analysis (ppm)

ID	C11	C13	C15	C17	C19	C21	C23	TPH
E3	30.854	16.591	14.369	15.198	10.190	6.678	7.328	101.212
E6	10.552	4.114	9.936	2.854	1.849	1.969	1.599	32.876
S1	3.312	1.377	10.420	1.656	7.616	2.385	ND	26.769
W2	33.629	15.734	13.680	14.678	8.883	16.231	8.935	111.773

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.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Dr.K. Sivagami
Dr.Bokam Rajasekhar
Dr.Indumathi M. Nambi

Graphical Abstract

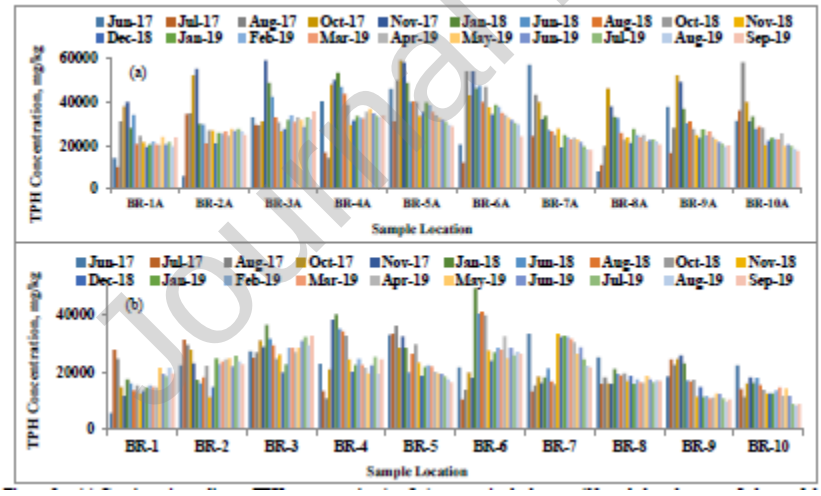

Fig 3

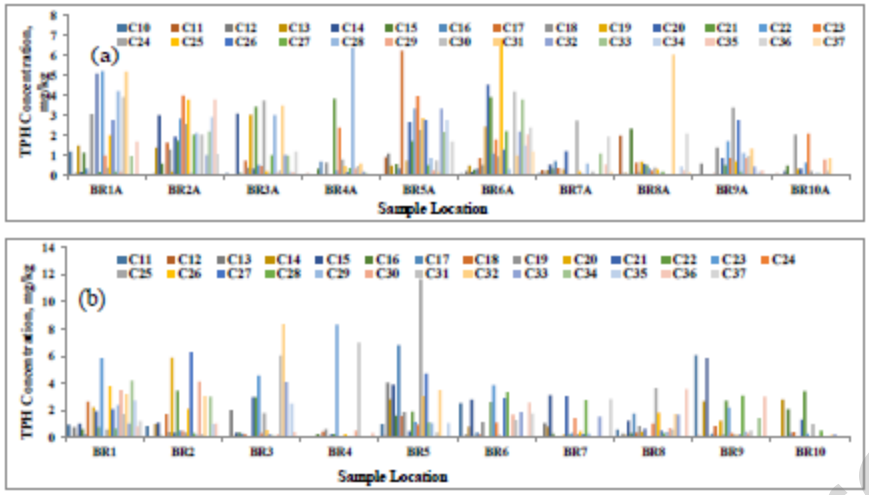

Fig 4

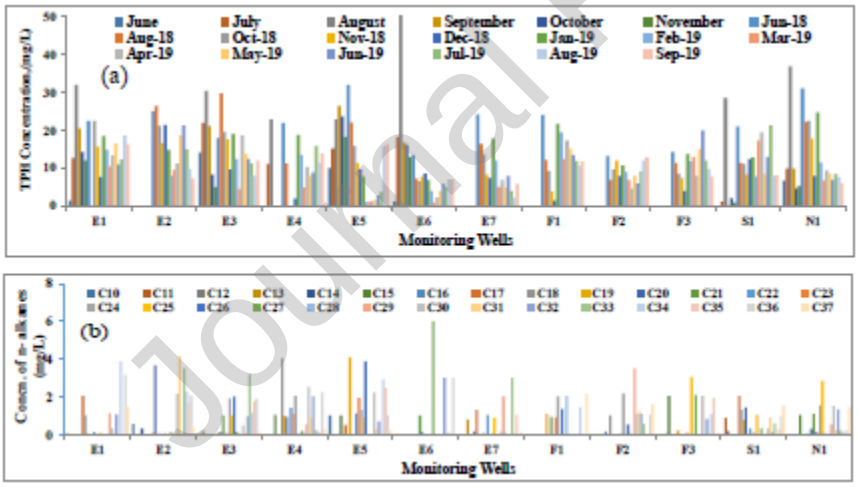

Fig 5