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Effectiveness of Bioremediation Technique on Oil Contaminated Marine Soil

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Abstract. Oil contamination due to accidental spill, fuel usage on ship and leakages in the pipeline along the coastal regions have created considerable threat to aquatic life. These contaminants are found to alter geotechnical property of soil as well. Bioremediation technique is found to be an effective tool for removing oil contaminants. Soil from coastal areas of Vishakhapatnam was collected. Routine classification tests and shear test were performed. Soil was then artificially contaminated with used engine oil. Shear strength of soil was found to decrease upon oil contamination. To study the effectiveness of bioremediation technique, five different conditions are maintained on the soil samples kept separately on different trays at room temperature. Two kinds Bacillus Thuringiensis species were maintained with two different nutrient supplies which form four test samples on oil contaminated soil. Fifth sample of oil contaminated marine soil without any treatment was also taken for reference. FTIR analyses were performed after one month on all five soil samples. The area under peak indicated that absorbance has decreased with treatment corresponding to certain hydrocarbon groups. Minor new peaks were also witnessed after treatment. The remediation treatments are being extended to study further progress with time.

Keywords: Marine Soil, Oil Contaminants, Bioremediation, Biostimulation, FTIR Analysis.

1 Introduction

Oil spills from offshore facilities or fuel usage can result in contamination of soil. These oil spills cause a threat to aquatic life and the soil depletes its natural properties. Degradation of hydrocarbons differs in soil and the aquatic environment (Andressa D et al., 2017; Bijay T et al., 2012). Oil is a complex mixture of hundreds or thousands of chemical compounds aliphatic, branched and aromatic hydrocarbons which are mostly toxic to living organisms (Brian A et al., 2006). Petroleum hydrocarbons are hazardous to human health, cause pollution of the groundwater. The oil contaminants are released to the environment due to numerous anthropogenic activities such as industrial manufacturing, refining, oil-tanker spills and accidents during transportation of oil

(Sunita V et al., 2020). The major problem with oil contamination is that the oil-producing countries are not major oil consumers. Petroleum products are generally transported through underground pipelines or offshore line tankers and in both the ways there are chances of oil spillage (Ronald M et al., 1991). Oil Pollution persisting in the soil can be effectively remediated by microbes when proper nutrient supply and optimum environmental conditions are available (Sungho Y et al., 2012). This method is a green approach and cost-effective as well (Brown L.D et al., 2016).

Temperature effects the rate of degradation naturally (Janne Fritt-R et al., 2013). The most frequently used bioremediation procedure is Biostimulation of microorganisms by adding of nutrients enhances microbial growth (Gogoi B.K et al., 2003; Margesin R et al., 2001). The present study deals with the suitability of bioremediation technique for the treatment of marine soil which is artificially contaminated using used automobile engine oil.

2 Methodology

2.1 Sampling sites and procedure

Soil was collected from Vishakhapatnam beach at a latitude and longitude of 17.7° and 83.3°. The site location was as mentioned in Fig. 1. The depth of sampling was 0.2 m. Visually no oil contaminants were observed at the site from where soil sample was collected. The soil was 24 hours oven-dried at 100°C and basic geotechnical parameters were determined as per IS: 2720 (Part II) – 1973. Environmental properties of soil such as pH, electrical conductivity and Chlorides are determined. Used Engine oil was collected and mixed to oven-dried soil sample to simulate oil spill condition artificially. The oven-dried sample was artificially contaminated with used 10% w/v of used engine oil and the same testing procedures were repeated to study the impact of oil contamination. After 30 days of treatment, FTIR analysis was performed. The amount of hydrocarbon present in the soil after 30 days with and without treatment was studied.

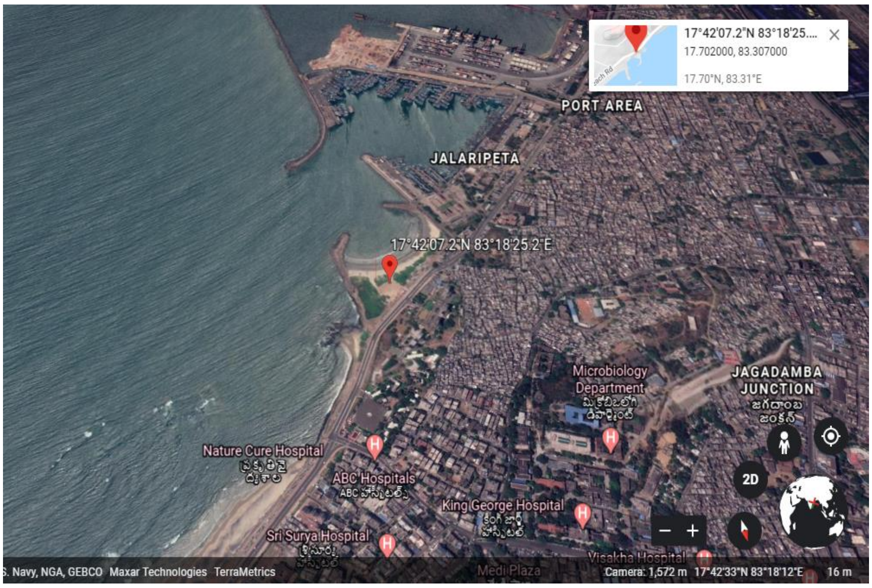

Fig. 1. Satellite image of site location (Source: Google map)

2.2 Bacteria and Nutrients

Endospores of Bacillus Thuringiensis Israelensis (shortly as BTI) and *Bacillus Thuringiensis Kurstaki* (shortly as BTK) supplied by Agri life, Hyderabad. were used for the degradation of oil contaminant. These bacterial species were used in the degradation of the Exxon Valdez oil spill occurred at Alaska (Maczulak. Anne 2017). The present study tried to enhance bacterial growth by adding fertilizers. Two types of nutrients such as NPK (Nitrogen Phosphorus and Potassium) (Morais Eduardo et al.,2009) and Organic Fertilizer were taken. NPK supplements are essentials to support bacterial growth in the marine and oil spill environment (Ogbonna et al., 2019; Das, Nilanjana et al., 2011; Macaulay et al., 2014). Soil sample of 3kg was artificially contaminated using used engine oil (10% w/v) with 4 different combinations for treatment and a reference contaminated sample was maintained without any treatment. Particulars of treatment combination were depicted in Table 1.

300ml (10% w/v) distilled water was used to moisturize soil samples. 40g of Bacterial endospores in the form of powder was diluted in 400 ml of distilled water and was directly added to the soil. 10 grams of nutrients were added periodically. The prepared samples were placed at room temperature and moisturized, nutrients were sprinkled over once in 3 days.

Table 1. Treatment combinations

Combination No.	Content
1	Soil+Oil+BTI+NPK
2	Soil+Oil+BTI+Organic

3	Soil+Oil+BTK+NPK
4	Soil+Oil+BTK+Organic
5	Soil+Oil

3 Results and Discussion

3.1 Preliminary Test Results

Per cent finer and Particle size distribution were determined for untreated soil as per IS 2720 Part 4 (1985) and shown in Fig. 2. Coefficient of uniformity (Cu) and Coefficient of curvature (Cc) of soil sample is =2.57, 1.01. Hence soil was classified as Poorly graded Sand (SP) as per Indian standard.

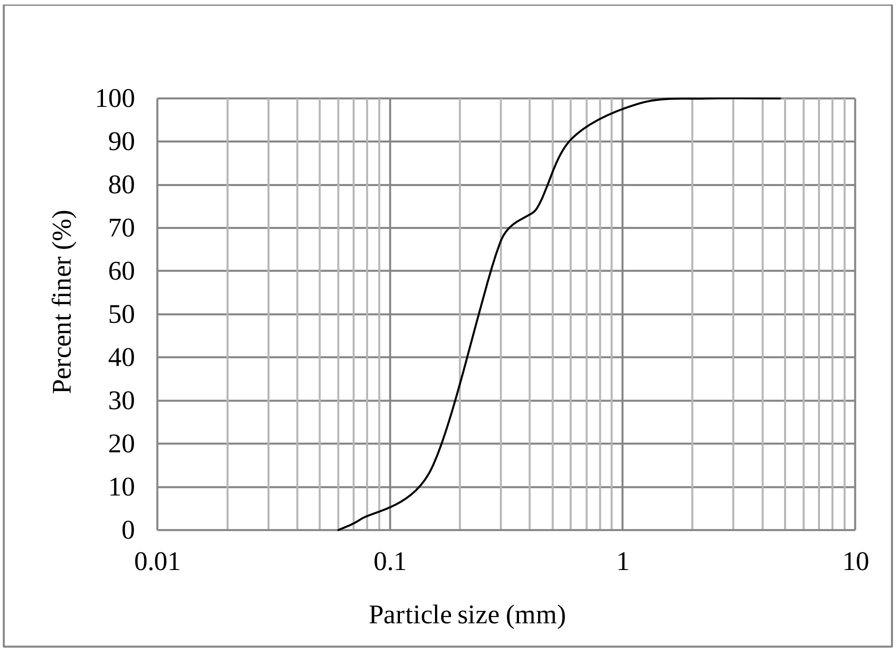

Fig. 2. Grain size distribution curve of uncontaminated soil

Optimum moisture content and maximum dry density were determined for the uncontaminated soil as per IS 2720 Part 7. OMC, MDD were obtained as 8% and 1.64g/cc as represented in Fig. 3. The compaction curve has shown depression initially due to the bulking effect of marine sand.

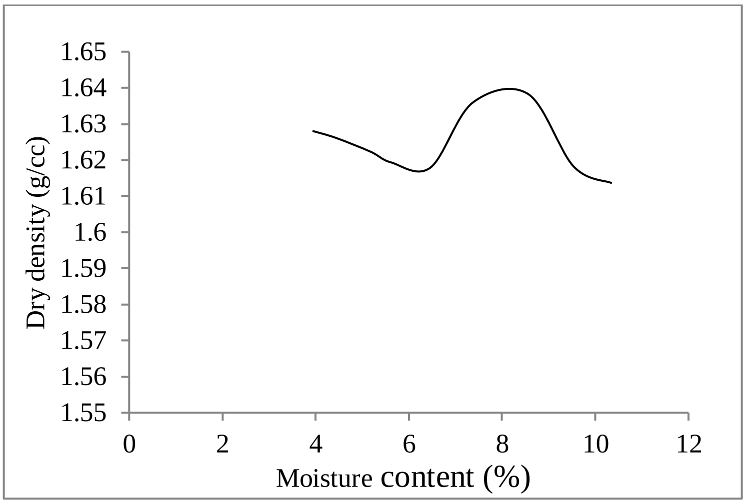

Fig. 3. Compaction curve for uncontaminated soil

Direct shear test as per IS 2720 Part 13 was conducted in the virgin soil at OMC and MDD. Contaminated soil was prepared by adding 10% used engine oil to soil and the same test was repeated. Angle of internal friction and cohesion determined for both uncontaminated soil and soil contaminated with 10% oil are represented in Fig. 4 & 5. Environmental and Shear parameters were tabulated in Table 2. The apparent cohesion reported was due to the presence of water and oil in uncontaminated and contaminated soil respectively. Addition of used engine oil has decreased the angle of internal friction by 29%. This is due to the fact that used engine oil has acted as a lubricant between the particles and because of which the soil particles slipped at the interface.

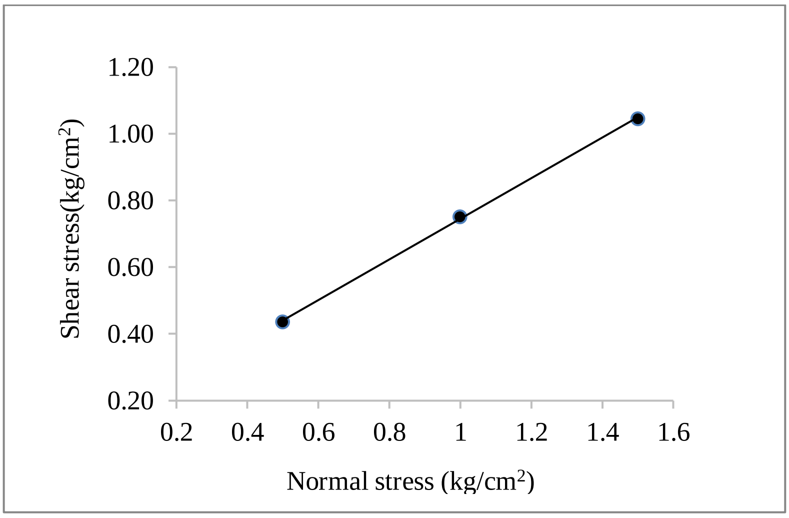

Fig. 4. Shear stress and Normal stress of uncontaminated soil

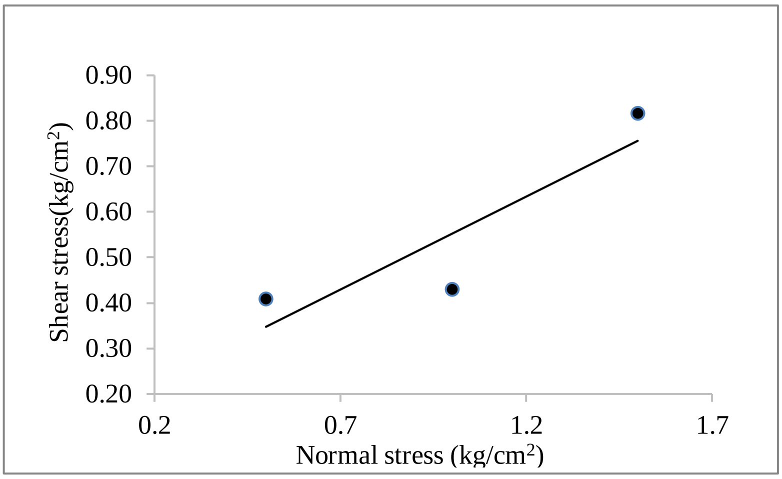

Fig. 5. Shear stress and Normal stress of contaminated soil

Table 2. Properties of uncontaminated and contaminated soil

Properties	Uncontaminated soil	Contaminated soil
Ø	31°	22°
C	0.136 kg/cm^2	0.143 kg/cm^2
pH	7.53	6.45
Electrical Conductivity	412 µS/cm	250 µS/cm
Chlorides	44.2 mg/L	146 mg/L

The specific gravity of uncontaminated soil is 2.61. The pH of the soil was found to decrease upon the addition of the oil. The electrical conductivity of soil was found to decrease in the contaminated soil due to the non-polar nature of oil. The chloride content of the sample was found to increase in contaminated soil due to the presence of chlorinated hydrocarbons that generally exist in engine oils.

3.2 FTIR analysis of Soil samples

Fig. 6 gives the absorbance spectrum obtained through Fourier Transform Infrared (FTIR) Spectroscopy on contaminated soil which was maintained as reference sample for the period of 30 days without subjecting to any treatment.

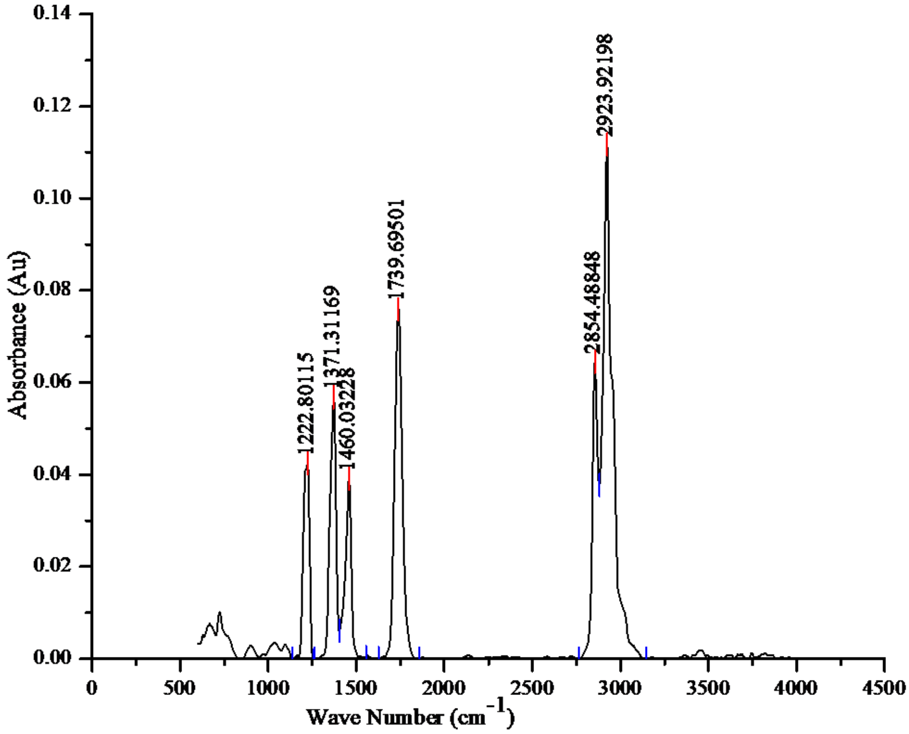

Fig. 6. FTIR spectrum of untreated artificially contaminated soil after 30 days

Enhancement of bacterial growth and oil biodegradation were determined (Deivkumari.M et al., 2020; Margesin R et al.,2000). Fig. 7-10 represents the FTIR spectrum of treated soil sample with two different species and nutrients combinations. The details of the functional group that corresponds to the distinct wave number band observed and the intensity of bonds are given in Table 3. The areas under each peak were calculated and listed in Table 4. The Peak wave numbers observed on treated samples were similar to those obtained for the reference sample. But the area under peak has got reduced after 30 days of treatment. The reduction in area under the peak indicates the reduction in used engine oil concentration added. The overall area under the peak in case of the untreated sample was 18.8 units whereas that of the sample treated with BTK species in NPK nutrient medium has shown a 65% reduction in overall area under the peak and that in organic nutrient medium was around 22%. BTI treated sample supported by NPK fertilizer has 5.3 units which was around 72% decrement. Contaminated soil sample bioremediated by BTI species and bio stimulated with organic fertilizer has also shown reduction in oil contaminant added. But the reduction of overall area under the peaks was around 46%.

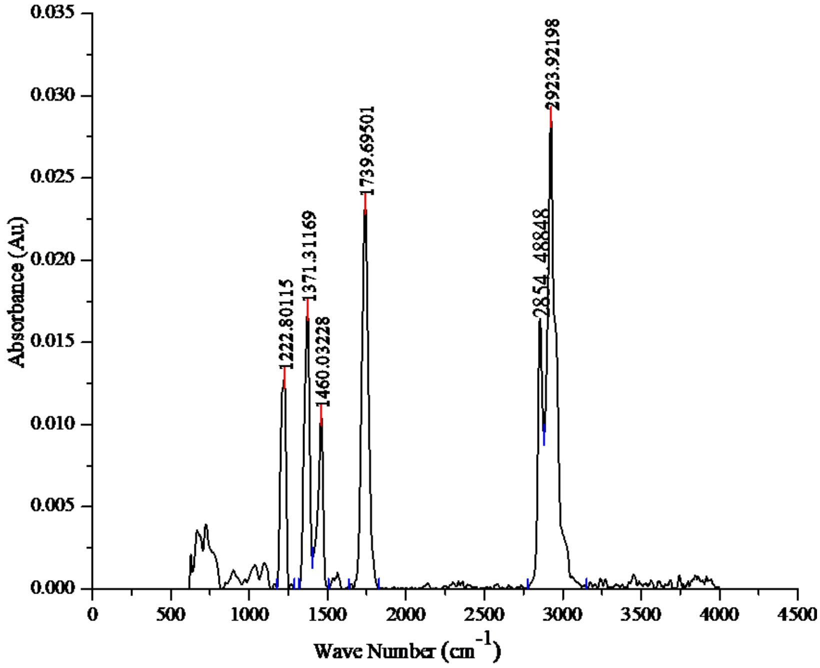

Fig. 7. FTIR spectrum of Contaminated soil treated with BTI and NPK nutrient supply

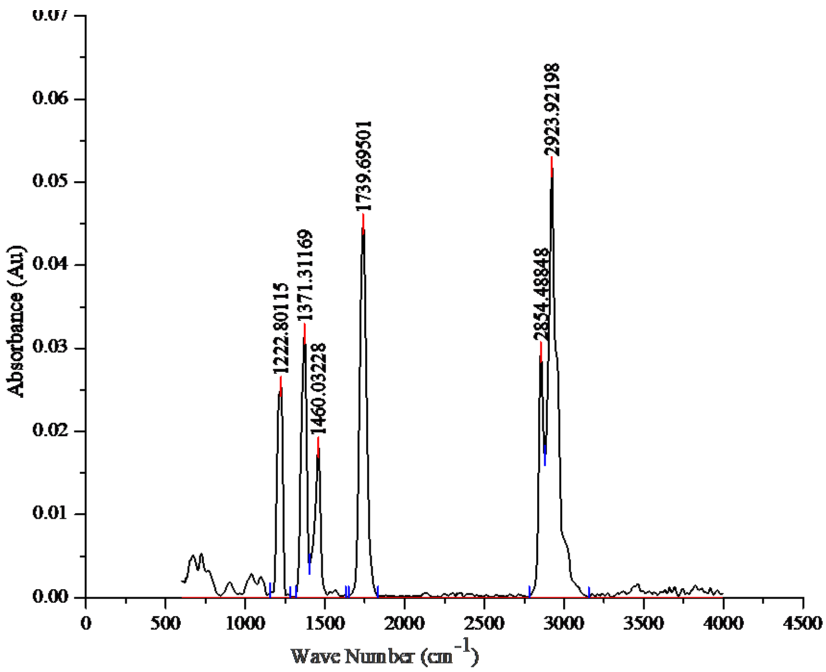

Fig. 8. FTIR spectrum of Contaminated soil treated with BTI and organic nutrient supply

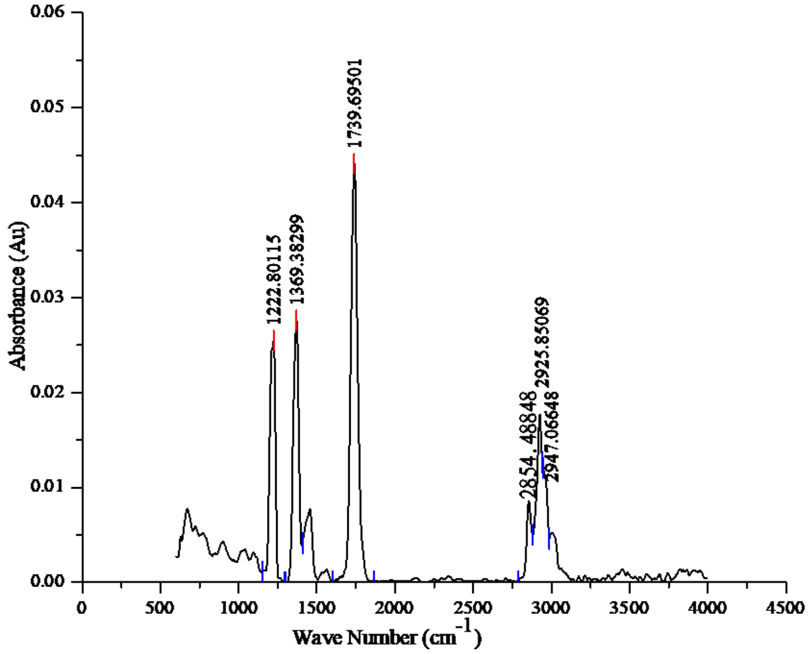

Fig. 9. FTIR spectrum of Contaminated soil treated with BTK and NPK nutrient supply

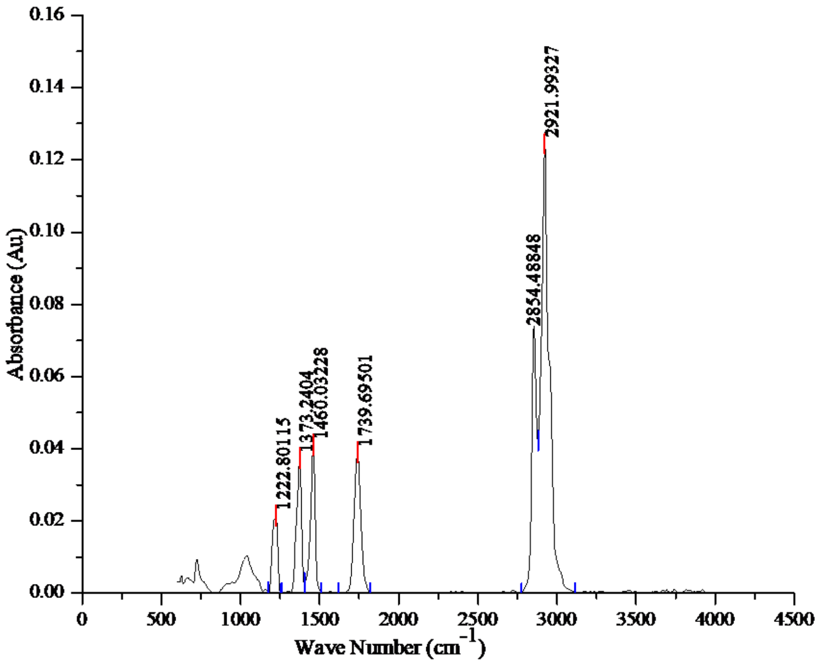

Fig. 10. FTIR spectrum of Contaminated soil treated with BTK and organic nutrient supply

Table 3. Peak wavenumber and functional groups of soil and oil reference sample

Wave number (cm^{-1})	Functional group	Intensity
1225-1200	Vinyl Ether C-O (stretch)	Strong
1372-1335	Sulfonate S=O (stretch)	Strong
1750-1735	δ-lactone C=O (stretch)	Strong
3000-2840	Alkane C-H (stretch)	Medium
3000-2840	Alkane C-H (stretch)	Medium

Table 4. Peak wave number and corresponding area under the peak for five samples after 30 days

Wave number	Area of peak Absorbance				
	Untreated	BTI with NPK	BTI with Organic	BTK with NPK	BTK with Organic
1223	1.7	0.5	1	1	0.8
1371	2.2	0.7	1.3	1.5	1.3
1460	1.5	0.4	0.8	0.2	1.4
1740	3.8	1.2	2.3	2.2	2
2854	2.4	0.6	1.1	1.2	2.7
2924	7.2	1.9	3.5	0.5	6.4
Overall area	18.8	5.3	10	6.6	14.6

FTIR method was efficient in analysing in percent reduction in oil contamination (Dash, Hirak et al., 2014; Agarry et al.,2014). The bacteria utilise hydrocarbon as their carbon source, metabolise and break them into simple compound (Margesin et al., 2007; Avotamuno et al., 2006).

From the overall area under peak comparison, it has been evident that BTI species performed better than BTK species. The bio-stimulation was effective with NPK fertilizer compared to organic fertilizer. Detailed information on percentage reduction of areas under the peak for each sample in comparison to the untreated sample is given in Table 5. As organic fertilizer has also supported a certain amount of degradation, it may be recommended if the delay in degradation is permitted and the problem of oil contamination at the site demands a green approach.

Table 5. Comparision of precentage of reduction of different bonds with respect to reference sample after 30 days

Band width (cm^{-1})	Compound	BTI with NPK	BTI with Organic	BTK with NPK	BTK with Organic
1222	C-O stretching	70	41	41	55
1371	S=O stretching	70	43	30	42
1460	C-H bending	73	50	85	9
1739	C=O stretching	69	40	42	48
2854	C-H stretching	75	55	50	19
2923	C-H stretching	74	51	92	11

Certain new minor peaks were also absorbed as a result of degradation in treated soil samples. Fig.11 and Fig.12 illustrates the effectiveness of nutrient supplies on biodegradation process with BTI and BTK species with respect to the untreated sample. Fig.11 and Fig.12 represents graphs drawn with single bacteria comparing the difference in degradation between NPK and organic fertilizer, formation of new bonds in compounds.

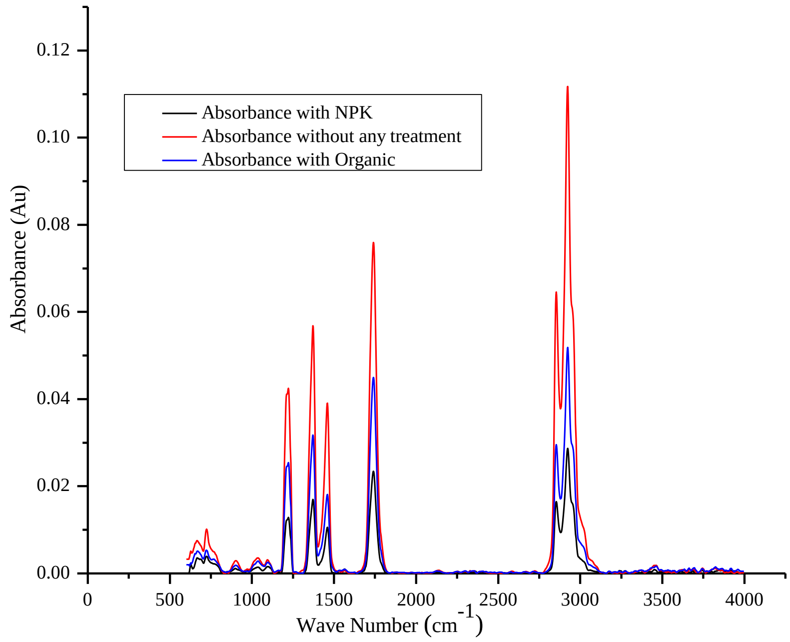

Fig. 11. Comparison of untreated and BTI treated sample supplied with NPK and organic fertilizers

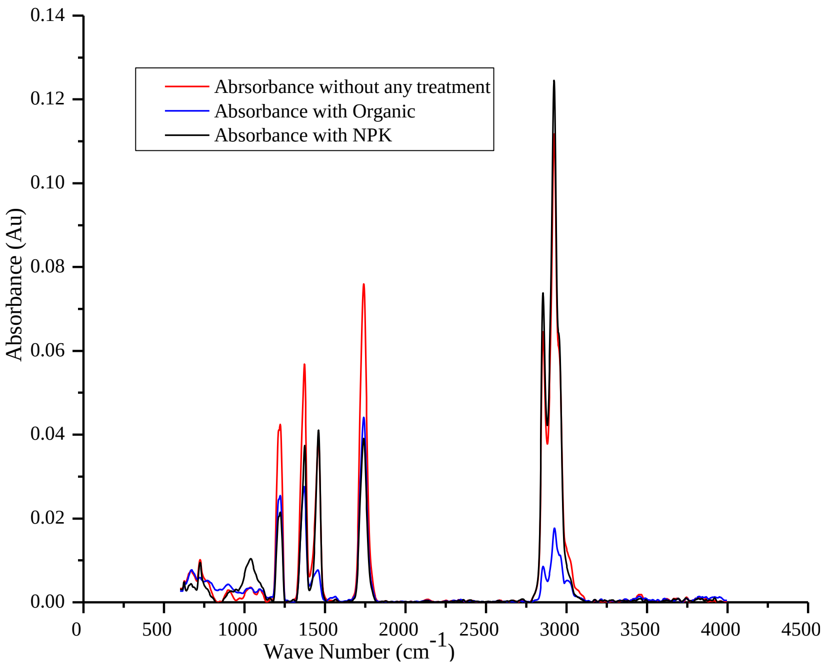

Fig. 12. Comparison of untreated and BTK treated sample supplied with NPK and organic fertilizers

4 Conclusions

Marine soil collected from Vishakapatnam coastal area, artificially contaminated with used engine oil has responded to the bioaugmentation process induced by two different *Bacillus* species at room temperature. FTIR analysis performed after 30 days of treatment showed that compared to BTK (Bacillus Thuringiensis Kurstaki) species BTI (Bacillus Thuringiensis Israelensis) has shown a higher rate of degradation. NPK fertilizer has bio-stimulated oil degradation process better than organic fertilizer. The overall percentage reduction of area under peak reported in case of BTI bio-stimulated by NPK fertilizer in comparison to the reference sample was around 72% after a month of treatment. But organic fertilizer can be used as nutrient if the duration of degradation does not have greater consideration and the oil decontamination requires a greener solution.

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