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Bioremediation of Oil-contaminated Sand

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ABSTRACT

The energy demand is set to grow very rapidly and the potential demand for oil around the world is at its highest level. Apart from indigenous oil sources, crude oil is imported by water transportation to fulfill local demand. The occurrence of oil leakage during drilling and transportation in pipelines is a major concern. As a result of this, soil is getting polluted and its geotechnical properties are altered. In this study, the effect of engine oil and diesel contamination on the geotechnical parameters of sea sand has been studied. Further, to enhance the properties of oil-contaminated sand, the bioremediation method was adopted. Sea sand from Ganagalla Peta beach, Andhra Pradesh state, India was taken up for the study. In the laboratory, 4%, 8%, 12% and 16% of engine oil and diesel were used to artificially contaminate the sea sand and geotechnical parameters; namely, compaction, shear strength and permeability, were studied for oil-contaminated sand and compared with those of virgin sea sand. In the next stage, bioremediation of engine oil-and diesel-contaminated sand was carried out to improve the geotechnical properties. It has been found that the geotechnical properties had improved after three days of bioremediation. Fourier transform infrared spectroscopy (FTIR) analysis showed that the contaminated sand is of a lipopeptide nature and showed the presence of carboxyl groups, whereas the IR absorption pattern of the treated sand matches with N-Methyl-N-Vinyl Acetamide.

KEYWORDS: Engine-oil contamination, Diesel contamination, Bioremediation, *Bacillus subtilis*, Geotechnical properties, Compaction characteristics, Shear-strength characteristics, Permeability, FTIR analysis.

INTRODUCTION

Even under the most recent estimates for the expansion of other energy sources, oil will likely continue to be a crucial source of energy for the globe for many years. Most nations have significantly increased their oil consumption (Abousnina et al., 2015) and according to the International Energy Agency (IEA) (Zhang et al., 2021), oil is anticipated to make up 30% of the global energy mix in 2030. In some developed countries, around two-thirds of all oil is used for transportation. However, in most of the rest of the world, oil is largely used for power generation and space heating (Nazir, 2011). Oil is also a crucial component of the global agriculture business, providing food for more than six billion people (Khanam et al., 2017). The Middle East and African countries have enormous oil wells and because of their geographic location, several countries import oil from Middle Eastern countries to meet their demands. Shipping and pipeline transit are the two most common means of import. Although ocean freight is less expensive, it has a longer transit time. Oil pipelines are the most extensively utilized mode of oil transportation, resulting in a smaller carbon impact. Most pipelines are buried between 3 and 6 feet (0.91 and 1.8 m) into the ground to shield pipes from impact, abrasion and corrosion. Pumping stations along the pipeline keep the oil moving at a pace of 1 to 6 meters per second (3.3 to 20 feet per second). Most oil spills occur accidentally during land or marine transportation

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due to storage-tank leaks or during the oil-drilling process (Saeed et al., 2021). However, oil may be intentionally spilled in some circumstances, as occurred during the Persian Gulf war in 1991. When soil becomes severely polluted by oil spills and leaks, restoration and reclamation of the polluted areas warrant significant duties. Several techniques have been presented by oil-extraction companies to clean up the badly damaged soils after the oil lakes have been drained of their liquid petroleum. Among them was blending oily soil with aggregate or consolidation agents to create road-foundation material or a topping layer for parking lots and highways. Vacuum extraction and separation using centrifuge and screen systems, confinement at big burial sites, burning, biological procedures, absorption techniques, soil cleansing techniques and incineration are among the other approaches. The latter strategy would be appropriate for dealing with the remaining oil sludge in areas covered with standing oil. Several research groups have proposed strategies to rehabilitate the quality of polluted soil after examining the effects of oil contamination on soil geotechnical characteristics (Sakthipriya et al., 2015; Ahmadi et al., 2021). For the examined coastal plain's coastal engineering and environmental rehabilitation efforts, knowledge of these impacts of oil pollution is crucial (Khamehchiyan et al., 2007). Remediation and reclamation are necessary to restore the quality of contaminated soil and make it safe and affordable for constructing buildings on it (Hazirbaba and Mughieda, 2019). Bioremediation is an ecologically friendly, easy, safe and cost-effective process that employs biological agents to efficiently modify harmful qualities in soil. Crude oil is a complex blend of resins, asphaltene, aromatics, alkanes and hydrocarbons. Various species of bacteria have the capacity to oxidize petroleum hydrocarbons by utilizing them as a source of energy and carbon (Bao et al., 2014). The primary role of the bacteria is degradation, which makes the environment safe and habitable (Wu et al., 2014). The wide diversity of microorganisms found in nature with the ability to breakdown complex hydrocarbons and recent advancements in the field of biotechnology and genetic engineering have made biodegradation more simple and effective with high operational safety for environmental compatibility and sustainable development. Furthermore, numerous *in-situ* metabolites produced by bacteria, such as biosurfactants, fatty acids, alcohols and solvents, can improve bioremediation by solubilizing the paraffin fractions and lowering the critical micelle concentration (CMC), surface tension and interfacial tension (Banat, 1995). Biosurfactants can boost the bioavailability of microorganisms by enabling them to grow on hydrophobic (hydrocarbon) substrates (Rosenberg, 1984). Various researchers demonstrated effective bioremediation methods and improved the quality of oil-contaminated sea sand using a variety of bacteria (Al-Awadhi et al., 2002). In this study, we have determined the potential bioremediation aspect of *Bacillus subtilis* regarding oil contamination by evaluating and comparing the shear strength, compaction and permeability properties of sea sand and remediated sand after contaminating with engine oil and diesel.

MATERIALS AND METHODS

Soil Sample

The soil sample was collected from Ganagalla Peta beach, Andhra Pradesh state, India, at a latitude and longitude of 18.21° and 83.95°. The soil sample was tested for its basic properties to understand its nature and characteristics. The tests conducted strictly adhered to the Indian standard code of practice. The properties are summarised in Table 1.

Table 1. Properties of virgin soil

S. No.	Properties	Values
1	Specific gravity	2.58
2	Mechanical sieve analysis	
	Gravel	5.01 %
	Sand	92.99 %
	Silt	2.01 %
	Unified soil classification symbol	SP
3	Angle of internal friction	32°

4	Coefficient of permeability	2.5 x 10^{-2} cm/sec
5	Standard proctor test Optimum moisture content Maximum dry density	 7 % 1.65 g/cc

Waste Engine Oil

Engine oil or motor oil is a complex combination of petroleum-based hydrocarbons with various organic compounds, some of which may contain organometallic components (Butler and Mason, 1996). Waste engine oil was collected from the R.B. Auto Garage, Coimbatore. The contaminated samples were prepared by intentionally combining the waste engine oil in varying quantities, such as 4%, 8%, 12% and 16%.

Diesel

Diesel is made by fractional distilling crude oil at atmospheric pressure and temperatures ranging from 200 to 350 degrees Celsius, generating a variety of carbon chains with an average of 9 to 25 carbon atoms per molecule. Diesel was obtained from the Bharat Petroleum fuel station, Coimbatore. Diesel was intentionally blended in ratios, such as 4%, 8%, 12% and 16% to create the contaminated samples.

Bacillus subtilis

Bacillus subtilis surfactants have been proven to improve hydrocarbon biodegradation and enhance microbe bioavailability (Sakthipriya et al., 2015). *Bacillus subtilis* shown in Figure 1, supplied by Tamil Nadu Agricultural University, Coimbatore, was utilized to degrade oil contaminants and treat polluted samples.

Figure (1): *Bacillus subtilis*

Azophos

Azophos, supplied by Tamil Nadu Agricultural University, Coimbatore, depicted in Figure 2, was used to promote and enhance bacterial growth and survival in the contaminated soil sample.

Figure (2): Azophos

Preparation of Contaminated Soil Sample

The virgin soil sample was split into eight parts and dried for 24 hours at 105 °C in an oven. The samples were then pooled with waste engine oil and diesel in various amounts by weight of the dry samples. The contaminants were mixed evenly and carefully. For ageing and equilibrium purposes, the samples were kept in a closed container for three days, allowing for potential interactions between the soil and used motor oil and diesel. In order to assess the consequences of polluted soil, eight mixed and equilibrated soil samples were prepared.

Bioremediation of Contaminated Soil

Bacillus subtilis was used to degrade oil contaminants and Azophos was added to promote bacterial growth. A soil sample of 3 kg was artificially contaminated using waste engine oil and diesel and aged for three days in a covered container. Soil samples were moistened with 300 ml of distilled water. A bacterial solution containing 40g of *Bacillus subtilis* dry powder in 400 ml of distilled water was applied directly to the soil and an amountof 10g of nutrients was supplied periodically. The prepared samples were moistened, maintained at room temperature and sprinkled with nutrients. After three days, the remedied samples were removed to be tested for compaction characteristics and shear-strength characteristics.

FTIR

Fourier transform infrared (FTIR) spectroscopy (Perkin Elmer 1600 FTIR) with an Attenuated Total Reflectance (ATR) Crystal Accessory was used to analyze the chemical composition of the sand polluted with used engine oil and diesel and the sand that had undergone bioremediation. In this experiment an amount of 100 mg of KBr (potassium bromide) was combined with 1 mg of crude biosurfactant before being pressed.

Experimental Programme

Initially, the index and engineering properties of the virgin soil were determined. After that, the artificially contaminated sand samples were prepared by mixing soils with waste engine oil and diesel individually in varying percentages of 4%, 8%, 12% and 16%. The contaminated sand samples were taken out after three days and standard proctor test, direct shear test and permeability test were conducted. The compaction test was conducted as per IS 2720 (Part 7) to determine the compaction characteristics. The direct shear test was conducted as per IS 2720 (Part 13) - 1986 (SP 36-1, (1987) to determine the shear-strength characteristics and the permeability test was conducted as per IS 2720 (Part 17) - 1986 to determine the coefficient of permeability.

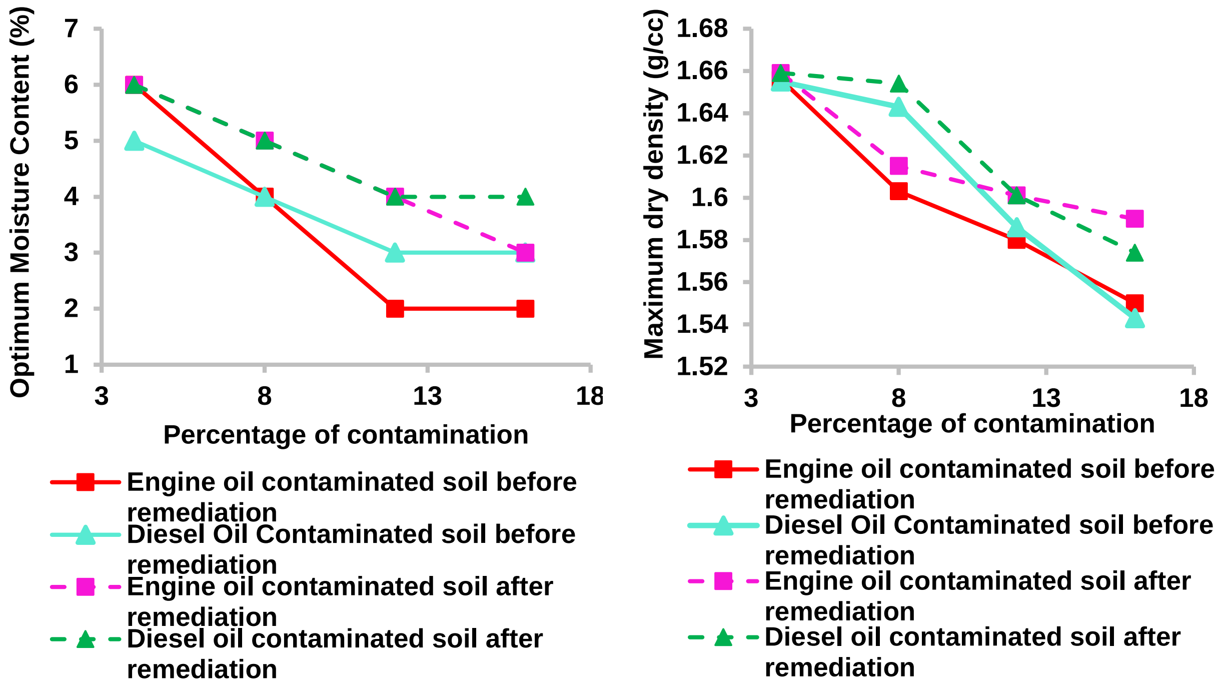
RESULTS AND DISCUSSION

Effect of Contamination and Bioremediation on Compaction Characteristics

The optimum moisture content values and maximum dry density values of the contaminated soil sample and the bioremediated sand sample were compared and are shown in Fig. 3(a) and Fig. 3(b). The maximum dry density and optimum moisture content of sand samples contaminated with waste engine oil and diesel decreased with an increase in the percentage of oil content. Our findings were similar to previously published reports of oil-contaminated sandy soil (Ahmadi et al., 2021). From the test, the optimum moisture content decreased by 14.3%, 42.9%, 71.43% and 71.43% for 4%, 8%, 12% and 16% engine oil-contaminated sand respectively and the maximum dry density decreased by 0.42%, 3.6%, 5% and 6.8% for 4%, 8%, 12% and 16% engine oil-contaminated sand, respectively, when compared to the virgin sand. A similar trend was also observed for diesel oil-contaminated sand. From the data obtained, it is clearly understood that the oil contaminants decrease maximum dry density and optimum moisture content due to the effect of capillary tension. Additionally, compaction test was carried out on remediated sand samples using bacteria to study the consequences of bioremediation on compaction characteristics. The results, summarised in Figure 3, show that the maximum dry density and optimum moisture content of sand samples remediated with bacteria increased. The optimum moisture content increased by 0%, 25%, 100% and 50%, while the maximum dry density increased by 0.18%, 0.75%, 1.33% and 2.58%, for 4%, 8%, 12% and 16% of the bioremediated sand, respectively, compared to the corresponding engine oil-contaminated sand. Also, the diesel-contaminated sand shows a similar trend after bioremediation. This reveals that the bioremediation of soil samples using *Bacillus subtilis* increases the maximum dry density and optimum moisture content, indicating the reduction of oil contamination in the sand samples. The enhancement in the shear strength of sand samples might be due to the improvement in the compaction characteristics of bioremediated sand samples.

Effect of Contamination and Bioremediation on Shear-strength Characteristics

The angle of internal friction values between the contaminated soil sample and bioremediated soil sample is compared and picturized in Figure 4. The angle of internal friction of the poorly graded sand samples contaminated with waste engine oil and diesel decreased with an increase in the percentage of oil content. Various researchers also obtained a similar decreasing trend in the angle of internal friction (Ahmadi et al., 2021; Ostovar et al., 2021). From the direct-shear test results, the angle of internal friction decreased by 62.5%, 65.625%, 75% and 81.25% for 4%, 8%, 12% and 16% engine oil-contaminated sand, respectively, compared to virgin sand. Similarly, the angle of internal friction decreased by 59.4%, 62.5%, 68.8% and 71.9% for 4%, 8%, 12% and 16% diesel-contaminated sand, respectively, compared to virgin sand. Thus, the data shows that the angle of internal friction decreases with an increase in the percentage of oil content. From the trend observed in Figure 4, it could be understood that the waste engine oil and diesel oil contaminants decrease the frictional resistance between the soil particles due to increasing pore fluid viscosity. As the ϕ value decreases, the shear strength of the contaminated poorly-graded sand sample also decreases (Ostovar et al., 2021). From the results of bioremediation of the contaminated soil shown in Fig.4, the angle of internal friction of samples remediated with bacteria increased. The angle of internal friction of the bioremediated 4% engine oil-contaminated sand showed an increase of 158.3% and the bioremediated 16% engine oil-contaminated sand showed an increase of 250% to the respective engine oil-contaminated sand. Furthermore, the angle of internal friction of the bioremediated 4% diesel-oil-contaminated sand showed an increase of 92.31% and the bioremediated 16% diesel-oil-contaminated sand showed an increase of 44.4% to the respective diesel-contaminated sand. Therefore, it is understood that the use of the bacteria *Bacillus subtilis* to bioremediate the sand sample increases the frictional resistance between the soil particles. As the ϕ value increases, the shear-strength characteristics of the remediated poorly-graded sand sample also improve.



(a) (b)

Figure (3): Comparison of (a) Optimum moisture content values; (b) Maximum dry density values of contaminated soil samples and bioremediated sand samples

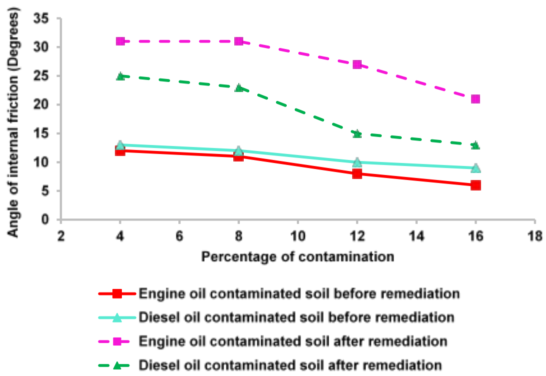


Figure (4): Comparison of angle of internal friction values of contaminated soil samples and bioremediated soil samples

Effect of Contamination and Bioremediation on Permeability Characteristics

Figure 5 shows the variation of the coefficient of permeability values for varying percentages of contamination before and after bioremediation. The coefficient of permeability of sand samples contaminated with waste engine oil and diesel decreased with an increase in the percentage of oil content. However, the coefficient of permeability decreased slightly to 36.8% and 6.4% for 4% engine oil-and diesel-contaminated sand. It decreased further until it reached 57.2% and 40% for 16% engine oil-and diesel-contaminated sand. The decrease in the coefficient of permeability with an increase in the percentage of oil content might be due to the occupancy of highly viscous oil in the voids of sand samples, which blocks the water movement (Ostovar et al., 2021). The value of the coefficient of permeability increased when samples were bioremediated with *Bacillus subtilis,* as represented in Figure 5. The maximum increase in the coefficient of permeability was 14.02% and 16% for 16% bioremediated engine oil-and diesel-contaminated sand, respectively. The bioremediation technique applied to the sand sample ensured the reduction in oil content, thereby increasing the permeability of contaminated sand.

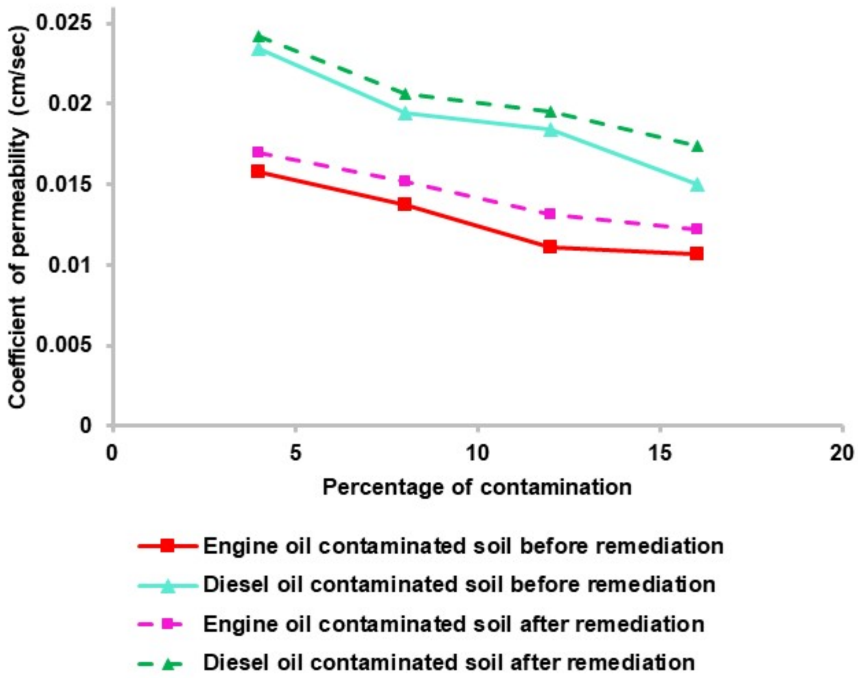


Figure (5): Comparison of coefficient of permeability values of contaminated soil samples and bioremediated soil samples

FTIR Characterization

As shown in Figure 6(a), the FTIR spectrum of the sand contaminated with engine oil showed strong absorbing bands at 2980 cm^{-1} as a result of C=H stretching, which indicates the presence of vinyl groups. Bioremediated sand contaminated with engine oil showed strong bands at 2925 cm^{-1}, indicating the presence of methyl groups. In Figure 6(a), the presence of the nitrites group was indicated by the C=N stretch at 2230 cm^{-1} in both engine oil-contaminated sand and bioremediated sand. The other common bands observed in engine oil-contaminated sand and contaminated sand after bioremediation belong to the phenols group present in the range of 3610-3670 cm^{-1} (O–H stretching) and C–H (benzene) stretching at 800-860 cm^{-1} range, as shown in Figure 6(a). From Table 2, it can be seen that the sand contaminated with engine oil showed a strong absorption band at 1200 cm^{-1} due to the presence of the phenols group. However, the bioremediated sand contaminated with engine oil showed an absorption band in the near similar range of 1380 cm^{-1}, indicating the presence of the methylene groups. The sand contaminated with engine oil showed C=O (carboxylic acid) stretching present at the 1700cm^{-1} range and the presence of the bromo-alkanes groups in the range of 500-600 cm^{-1}, as represented in Table 2. The unique bands found in engine oil-contaminated sand after bioremediation were C-O stretching at 1470 cm^{-1}, indicating alcohol groups, N-O stretching in the range of 1150-1200 cm^{-1}, identifying nitro-compounds and C-H stretching in the range of 500-600 cm^{-1}, showing the presence of monosubstituted benzene (Table 2).

Table 2. Characteristic infrared absorption frequencies present in the tested soil samples contaminated with engine oil

Soil + Engine oil			Soil + Engine oil + Bacteria		
Wavenumber (cm^{-1})	**Intensity**	**Peak Assignment**	**Wavenumber (cm^{-1})**	**Intensity**	**Peak Assignment**
3610-3670	Very weak	O-H stretch (Alcohols, Phenols)	3610-3670	Weak	O-H stretch (Alcohols, Phenols)
2980	Strong	$C=CH_2$	2925	Weak	C-H stretch (Methyl)
2230	Very weak	C=H stretch (Nitrites)	2230	Very weak	C=N stretch (Nitrites)
1700	Very weak	C=O stretch (Carboxylic acid)	1470	Very weak	C-O stretch (Alcohols)
1200	Strong	C-O stretch (Phenols)	1380	Weak	C-H stretch (Methylene)
800-860	Very weak	C-H (Para-disub. Benzene)	1150-1200	Strong	N-O (Nitro-compounds)
500-600	Very weak	C-X stretch (Bromo-Alkanes)	800-860	Very weak	C-H stretch (Para-disub. Benzene)
			500-600	Very weak	C-H stretch (Monosubstituted Benzene)

As represented in Figure 6(b), the FTIR spectrum of the diesel-contaminated sand and diesel-contaminated sand after bioremediation had many common bands, like O-H stretching at 3700 cm^{-1} indicating alcohol groups, C=N stretching at 2230 cm^{-1} showing the presence of nitrites and other compounds, such as alcohols, methylene, nitro-compounds, para disubtituted benzene and monosubstituted benzene. From Table 3, it can be seen that the only unique band found in bioremediated sand contaminated with diesel was C-H stretching at 2960 cm^{-1}, indicating methyl groups. In contrast, the unique bands of sand contaminated with diesel were C-H stretching at 2920 cm^{-1} representing the methylene group and C-O stretching at 1820 cm^{-1} showing the carboxylic acid group. The IR absorption pattern revealed the peptide and carboxyl groups, indicating the lipopeptide nature in the contaminated soil sample (Sivapathasekaran et al., 2010).

Table 3. Characteristic infrared absorption frequencies present in the tested soil samples contaminated with diesel

Soil + Diesel			Soil + Diesel + Bacteria		
Wavenumber (cm^{-1})	**Intensity**	**Peak assignment**	**Wavenumber (cm^{-1})**	**Intensity**	**Peak assignment**
3700	Weak	O-H stretch (Alcohols, Phenols)	3610	Weak	O-H stretch (Alcohols, Phenols)
2920	Weak	C-H stretch (Methylene)	2960	Weak	C-H stretch (Methyl)
2230	Very weak	C=N stretch (Nitrites)	2230	Very weak	C=N stretch (Nitrites)
1820	Strong	C-O stretch (Carboxylic acid)	1160	Very weak	C-O stretch (Alcohols)
1470	Weak	C-H stretch (Methylene)	1470	Weak	C-H stretch (Methylene)
1380	Weak	N-O (Nitro-compounds)	1380	Weak	N-O (Nitro-compounds)
1160	Very weak	C-O stretch (Alcohols)	800-860	Very weak	C-H stretch (Para-disub. Benzene)
800-860	Very weak	C-H stretch (Para-disub. Benzene)	700-750	Very weak	C-H stretch (Monosubstituted Benzene)
700-750	Very weak	C-H stretch (Monosubstituted Benzene)			

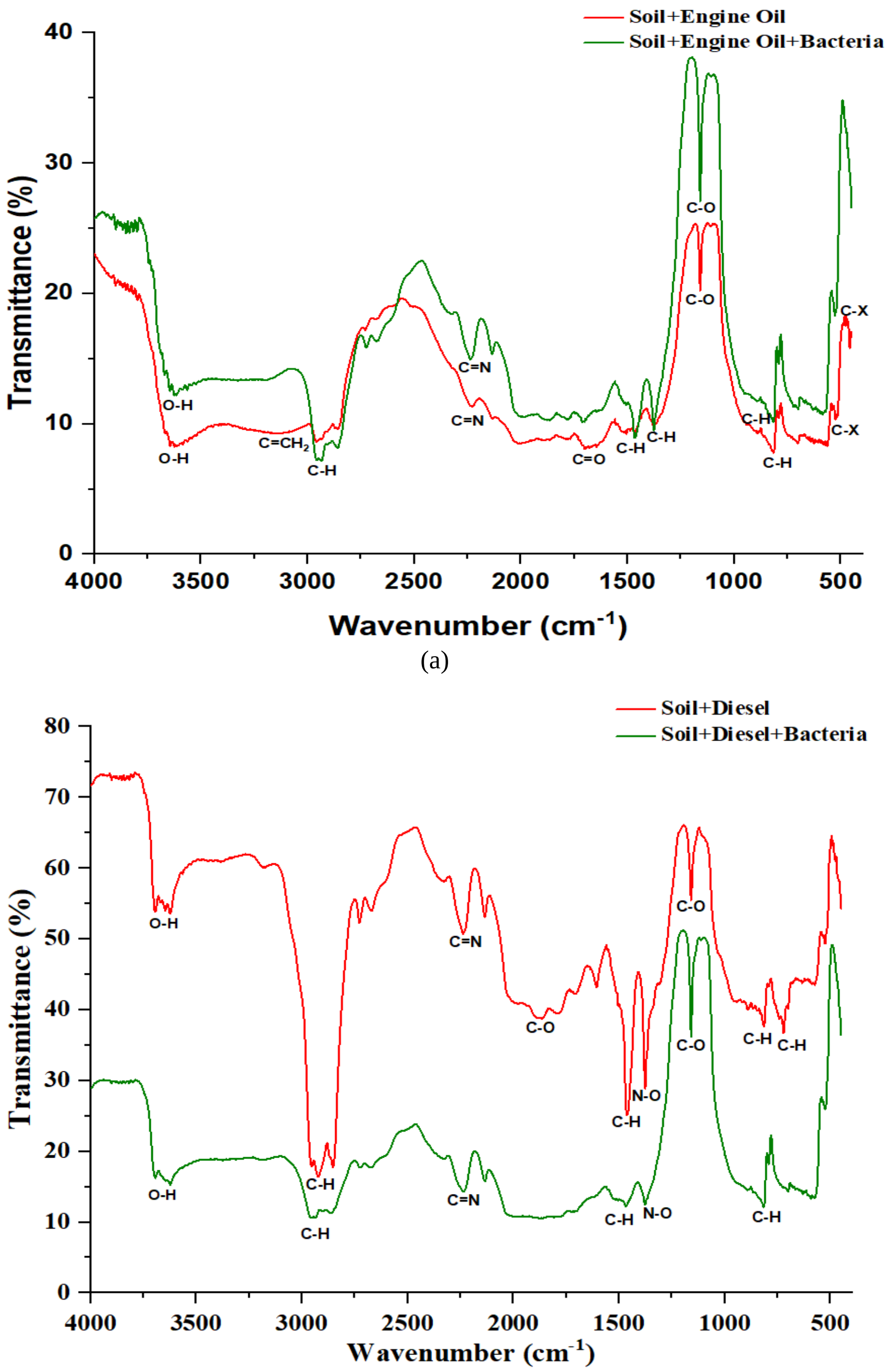


Figure (6): Fourier transform infrared (FTIR) spectra of (a) Soil contaminated with engine oil and the same remediated with *Bacillus subtilis*; (b) Soil contaminated with diesel and the same remediated with *Bacillus subtilis*

Compared to other treatment methods, the bioremediation method using *Bacillus subtillus* and azophos is very cost-effective and the cost of bacteria and nutrients was Rs.200/kg ($2.43/kg).

CONCLUSIONS

An extensive laboratory testing program was conducted to study the effect of waste engine oil and diesel contamination and bioremediation on poorly-graded sand samples. The waste engine oil and diesel were added from an increment of 4% by weight of dry samples to 16%, making the soil sample artificially contaminated. The contaminated soil samples were remediated by adding *Bacillus subtilis*. The following conclusions were drawn from the experimental study carried out.

- Reduced optimum moisture content by 14.3%, 42.9%, 71.43% and 71.43%, for 4%, 8%, 12% and 16% of engine oil-contaminated sand and by 28.58%, 42.86%, 57.14% and 57.14% for 4%, 8%, 12% and 16% of diesel-contaminated sand when compared to virgin sand. This reduction in optimum moisture content was due to the effect of capillary tension.
- Reduced maximum dry density by 0.42%, 3.6%, 5% and 6.8% for 4%, 8%, 12% and 16% of engine oil-contaminated sand and by 0.48%, 1.2%, 4.63% and 7.22% for 4%, 8%, 12% and 16% of diesel-contaminated sand, compared to virgin sand.
- Decreased angle of internal friction by 62.5%, 65.63%, 75% and 81.25% for 4%, 8%, 12% and 16% of engine oil-contaminated sand and by 59.4%, 62.5%, 68.8% and 71.9% for 4%, 8%, 12% and 16% of diesel-contaminated sand when compared to virgin sand. This may be due to the decrease in frictional resistance between the soil particles caused by increasing pore fluid viscosity.
- The coefficient of permeability diminished by 36.8%, 45.2%, 55.6% and 57.2% for 4%, 8%, 12% and 16% of engine oil-contaminated sand and by 6.4%, 22.4%, 26.4% and 40% for 4%, 8%, 12% and 16% of diesel-contaminated sand when compared to virgin sand. This was due to the occupancy of highly viscous oil in the voids of sand samples, which would block the movement of water.
- The bioremediation technique with bacteria showed improved results in the removal of oil contaminants in the soil.
- The optimum moisture content improved after bioremediation using *Bacillus subtilis*. The optimum moisture content increased by 25%, 100% and 50% for 8%, 12% and 16% of engine oil-contaminated sand after bioremediation and by 20%, 25%, 33.3% and 33.3%, for 4%, 8%, 12% and 16% of diesel-contaminated sand after bioremediation when compared to the respective contaminated sand samples.
- The maximum dry density showed an increasing trend with a maximum increase of 2.58% and 2% for 16% engine oil-and diesel-contaminated sand after bioremediation when compared to the respective contaminated sand samples. This increment in dry density was due to the reduction in the effect of oil contamination in the sand samples.
- The angle of internal friction showed an increasing trend after bioremediation with a maximum increase of 250% and 92.31% for 16% and 4% of engine oil-and diesel-contaminated sand after bioremediation, respectively, when compared to the respective contaminated sand samples. This increase in the angle of internal friction was due to the increase of frictional resistance between the soil particles after bioremediation.
- The coefficient of permeability of engine oil and diesel after bioremediation increased by a maximum value of 18.02% and 16% for 12% and 16%, respectively. This increment in the coefficient of permeability was due to a reduction in the oil content present in the sand samples.
- From the FTIR results, the IR absorption pattern revealed the presence of peptide and carboxyl groups, which indicated the lipopeptide nature in the contaminated soil samples. The IR absorption pattern of the contaminated soil after bioremediation matches with N-Methyl-N-Vinyl Acetamide.
- It is concluded that the use of *Bacillus subtillus* and azophos can effectively be used for bioremediation of engine oil-and diesel-contaminated sand to improve the compaction, permeability and shear-strength characteristics of oil-contaminated sand.

Future Scope of Work

It is recommended to conduct similar studies for other site-specific requirements by collecting naturally contaminated samples. Since laboratory trials showed promising results, further research is necessary to study bioremediation of contaminated sand in the field. Moreover, studies may be extended by varying bacteria content, nutrient content and curing.

REFERENCES

Abousnina, Rajab M., Allan Manalo, Jim Shiau, and Weena Lokuge. (2015). "Effects of light crude oil contamination on the physical and mechanical properties of fine sand". Soil and Sediment Contamination, 24 (8), 833-845. https://doi.org/10.1080/15320383.2015.1058338.

Ahmadi, Mahmood, Mohsen Abbaspour, Taghi Ebadi, and Reza Maknoon. (2021). "Effects of crude oil contamination on geotechnical properties of sand-kaolinite mixtures". Engineering Geology, 283, 106021. https://doi.org/10.1016/j.enggeo.2021.106021.

Al-Awadhi, Husain, Redha H. Al-Hasan, and Samir S. Radwan. (2002). "Comparison of the potential of coastal materials loaded with bacteria for bioremediating oily sea water in batch culture". Microbiological Research, 157 (4), 331-336. https://doi.org/10.1078/0944-5013-00168.

Banat, I.M. (1995). "Biosurfactants production and possible uses in microbial enhanced oil recovery and oil-pollution remediation: A review". Bioresource Technology, 51 (1), 1-12. https://doi.org/10.1016/0960-8524(94)00101-6.

Bao, Mutai, Yongrui Pi, Lina Wang, Peiyan Sun, Yiming Li, and Lixin Cao. (2014). "Lipopeptide biosurfactant production bacteria acinetobacter Sp. D3-2 and its biodegradation of crude oil". Environmental Sciences: Processes and Impacts, 16 (4). https://doi.org/10.1039/c3em00600j.

Butler, Clive S., and Jeremy R. Mason. (1996). "Structure-function analysis of the bacterial aromatic ring-hydroxylating dioxygenases". Advances in Microbial Physiology, 38, 47-84. https://doi.org/10.1016/S0065-2911(08)60155-1.

Hazirbaba, K., and Mughieda, O.S (2019). "A comparative study of targeted ground improvement alternatives during site reclamation". Jordan Journal of Civil Engineering 13 (2), 180-196

Khamehchiyan, Mashalah, Amir Hossein Charkhabi, and Majid Tajik. (2007). "Effects of crude oil contamination on geotechnical properties of clayey and sandy soils". Engineering Geology, 89 (3-4), 220-229. https://doi.org/10.1016/j.enggeo.2006.10.009.

Khanam S., Abbas, S., and Abbas, S.M. (2017). "Environmental risk mitigation using optimal mix for road embankments of marble dust and WTP sludge with soil". MOJ Civil Engineering, 2 (5), 151-156. https://doi.org/10.15406/mojce.2017.02.00044.

Nazir, Ashraf K. (2011). "Effect of motor-oil contamination on geotechnical properties of over-consolidated clay." Alexandria Engineering Journal, 50 (4), 331-335. https://doi.org/10.1016/j.aej.2011.05.002

Ostovar, Mojtaba, Reza Ghiassi, Mohammad Javad Mehdizadeh, and Nader Shariatmadari. (2021). "Effects of crude oil on geotechnical specifications of sandy soils". Soil and Sediment Contamination, 30 (1), 58-73. https://doi.org/10.1080/15320383.2020.1792410

Rosenberg, Mel. (1984). "Bacterial adherence to hydrocarbons: A useful technique for studying cell surface hydrophobicity". FEMS Microbiology Letters, 22 (3), 289-295. https://doi.org/10.1111/j.1574-6968.1984.tb00743.x.

Saeed, Maimona, Noshin Ilyas, Muhammad Arshad, Muhammad Sheeraz, Iftikhar Ahmed, and Arghya Bhattacharya. (2021). "Development of a plant microbiome bioremediation system for crude oil contamination". Journal of Environmental Chemical Engineering, 9 (4), 105401. https://doi.org/10.1016/j.jece.2021.105401.

Sakthipriya, N., Mukesh Doble, and Jitendra S. Sangwai. (2015). "Bioremediation of costal and marine pollution due to crude oil using microorganism *Bacillus subtilis*". In: Procedia-Engineering, 116, 213-220. Elsevier, Ltd. https://doi.org/10.1016/j.proeng.2015.08.284

Sivapathasekaran, C., Soumen Mukherjee, and Ramkrishna Sen. (2010). "Matrix-assisted laser desorption ionization-time of flight mass spectral analysis of marine lipopeptides with potential therapeutic implications". International Journal of Peptide Research and Therapeutics, 16, 79-85. https://doi.org/10.1007/s10989-010-9206-z.

SP 36-1. (1987). "Compendium of Indian standards on soil engineering: Part-1: Laboratory testing of soils for civil-engineering purposes".

Standards, Bureau of Indian. (1986). "IS 2720: Part 17: Methods of test for soils: Laboratory determination of permeability". Bureau of Indian Standards, 54 (4).

Wu, Binbin, Tian Lan, Diannan Lu, and Zheng Liu. (2014). "Ecological and enzymatic responses to petroleum contamination". Environmental Sciences: Processes and Impacts, 16, 1501-1509. https://doi.org/10.1039/c3em00731f.

Zhang, Shibin, Rongjian Li, Weishi Bai, and Qiang Yang. (2021). "Effects of oil contamination on the physical-mechanical behaviour of loess and its mechanism analysis". Geofluids. https://doi.org/10.1155/2021/3691549.