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Mycoremediation of Hydrocarbon Contaminated Soil-Ftir Based Analysis

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Abstract: Petroleum products composed mainly of hydrocarbons of different types and traces of some potential toxic elements can contaminate soils and leach into ground and surface waters, which are major sources of water for drinking and domestic use and can further get into the food web, becoming accumulated by plants and animals, including man. This can result in ill health effects for the residents of the area**.** Biodegradation of hydrocarbon-contaminated soils, which exploits the ability of microorganisms to degrade and/or detoxify organic contaminants, has been established as an efficient, economic, versatile and environmentally sound treatment**.** The ability of hydrocarbon compounds degrading fungi was tested in soil contaminated with used motor oil spillage. The fungal strains were isolated from the same site from where the contaminated soil has been collected and the degradation potential was evaluated in terms of chemical characterization of the treated and untreated soil with the use of FTIR spectroscopy. The fungal strains isolated seem to have ample potential to remediate the contaminated soil.

Key words: Biodegradation · Fungal strains · Petroleum products · Spillage

INTRODUCTION

Petroleum oil is the life blood of our modern industrial society. It fuels the machines and lubricates the wheels of the world's production, but when this vital resource is mishandled, it can ravage the environment and economy of a whole region. The occurrence of metal and petroleum hydrocarbon contaminants, particularly heavy metals and polycyclic aromatic hydrocarbon compounds have been found in excess of conventional levels and this is a cause for concern. The most commonly marketed refined petroleum products- kerosene, gasoline, diesel and lubricating oils contaminate the environment through spillage accidents involving transportation, overflow leaks in motor vehicles, poor handling, pipeline leakages and vandalisation and leakage of storage tanks [1]. Petroleum products composed mainly of hydrocarbons of different types and traces of some potential toxic elements can contaminate soils and leach into ground and surface waters, which are major sources of water for drinking and domestic use and can further get into the food web, becoming accumulated by plants and animals, including man. This can result in ill health effects for the residents of the area [2]. Over 2 billion tons of petroleum is produced annually worldwide. Spillages of oil have become a common occurrence. Depending on the site location, the level of oil contaminants in the soil may be as high as 10% (w/w) [3]. Large quantities of hazardous substances are carelessly disposed in the environment and are thus creating enormous pollution problems in soils and waters around the world [4].

It is known that the main microorganisms that can consume petroleum hydrocarbons are bacteria and fungi. The potential of microorganisms as degrading agents of several compounds indicates biological treatment as being the most promising alternative for reducing the environmental impact of oil spills [5, 6]. Filamentous fungi play an important role in degrading diesel and kerosene by producing capable enzymes. Because of their aggressive growth, greater biomass production and extensive hyphal growth in soil, fungi offer potential for biodegradation technology [7, 8]. The ability of microbes to degrade organic contaminants into harmless constituents has been explored as a means to biologically treat contaminated environments. Biodegradation of hydrocarbon-contaminated soils, which exploits the

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ability of microorganisms to degrade and/or detoxify organic contaminants, has been established as an efficient, economic, versatile and environmentally sound treatment [9]. The extent of hydrocarbon biodegradation in contaminated soils is critically reliant upon several factors like environmental conditions and the bioavailability of the contaminants to microorganisms [10, 11]. Biostimulation is considered as a most appropriate remediation technique for diesel removal in soil and requires the evaluation of both intrinsic degradation capacities of the autochthonous microflora and the environmental parameters involved in the kinetics of the *in-situ* process [12]. Identification of the key organisms that play a role in pollutant degradation processes is relevant to the development of optimal *in-situ* bioremediation strategies [13, 14]. Protective and preventive measures need to be taken into account to avoid spillage into the environment. Since hydrocarbons are natural products, it is not surprising to find organisms that are able to degrade these energy-rich substrates [15].

With the increase in vehicle use, India has also witnessed a mushroom growth of automobiles and also the service stations where significant amount of used motor oil is erroneously discharged in to the environment. The used motor oil during manual oil changing operation is not recycled but spilled and dumped at station sites, thereby polluting both soil and water [16]. A number of innovative physical and chemical technologies are available to remediate soil contaminated with hydrocarbon pollutants. For example, soil washing, vapour extraction, encapsulation and solidification/stabilization have been successful [17]. These methods, however, are expensive, time consuming and may only be partly effective. In recent years, bioremediation has emerged as an effective technology for treatment of hydrocarbon contaminants in soil. A diverse consortium of micro-organisms is capable of degrading a wide range of hydrocarbon molecules. Trends of hydrocarbon degradation were assessed over time and FTIR was utilized to elucidate the degradation of oil spilled from motorized vehicles in the contaminated soil.

MATERIALS AND METHODS

Study Site: Lucknow (26°5/N latitude, 80°56/E longitude, 128 m above the sea level), the Capital of Uttar Pradesh, spread over an area of 310 sq. km in the central plain of the Indian subcontinent, supporting a population of 36.48 lakhs (2001 Census) has a number of automobile service stations scattering at different sites and also a big railway junction. For the study, following service stations/sampling sites were selected:

- Sunil Auto Centre, SGPGI road- Two wheeler;
- Sonu Workshop, Utrathia- Four wheeler;
- Transport Nagar- Six wheeler and
- Railway service station, Charbagh- Railway track

The top soil was sampled from around the vicinity of the sites so as to know the most contaminated portion of the sampled sites.

Isolation and Inoculation of Fungal Culture: Soil samples contaminated with mobil oil were collected in sterile plastic bags and immediately brought to the laboratory, preserved at 4°C till further analysis. Fungal strains were isolated from the contaminated soil by serial dilution agar plate method. 50g of soil sample from each site was taken and were analyzed using FTIR before inoculation of fungal culture (zero day/untreated/control). After this the samples were inoculated with isolated fungal strains and were also analyzed using FTIR after 10 days and after 20 days (treated) of incubation. Mixing was done manually every fifth day. Moisture was kept at 50%; temperature range was 20-25°C. Water holding capacity was checked before inoculating the soil samples so as to inoculate them with the adequate amount of fungal culture. For the analysis, the samples were grounded with KBr in the ratio of 100:1 (100 mg KBr and 1 mg sample) in an agate motor to make the pellets by using a Hydraullic press (CAP.- 15T) at a pressure of ten tons. Spectra were obtained with a total of 32 scans against a KBr background using FTIR (Fourier-Transform Infrared Spectrometer), Nicolet model- 6700, which works in mid IR range of 4000-400 cm^{-1}. The strains isolated from used motor oil contaminated soil by a selective enrichment culture technique were tentatively identified as *Aspergillus and Pencillium* species following biochemical tests.

RESULTS AND DISCUSSION

The strains isolated from used motor oil contaminated soil by a selective enrichment culture technique were tentatively identified as *Aspergillus and Pencillium* species following biochemical tests. Recently, it was recorded that the genera of fungi such as *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizopus* and *Mucor* are associated with kerosene-polluted soil [18]. All the samples were analyzed using FTIR before and after the inoculation of fungal culture.

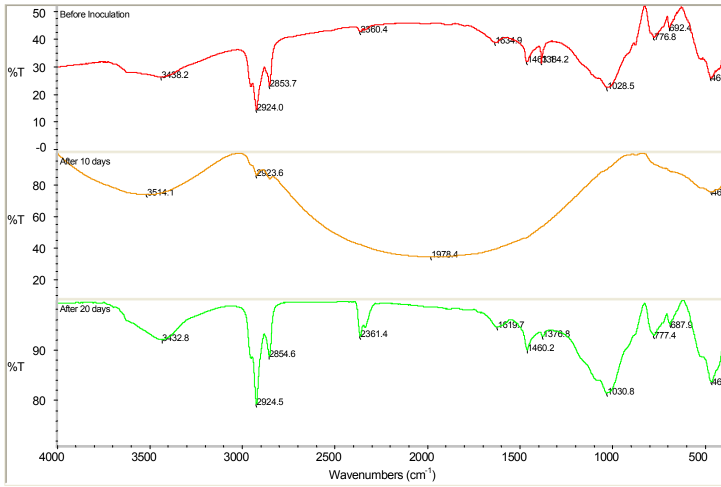

Fig. 1: FTIR Spectra of Two-wheeler Service Centre Soil after different Fungal Treatments

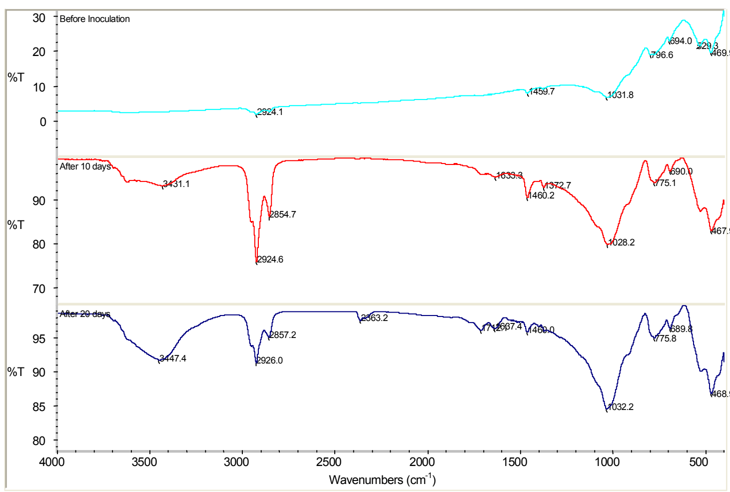

Fig. 2: FTIR Spectra of Four-wheeler Service Centre Soil after different Fungal Treatments

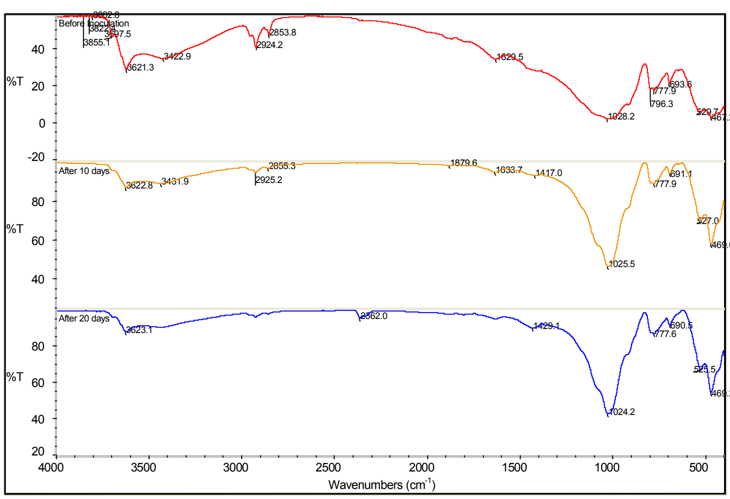

Fig. 3: FTIR Spectra of Six-wheeler Service Centre Soil after different Fungal Treatments

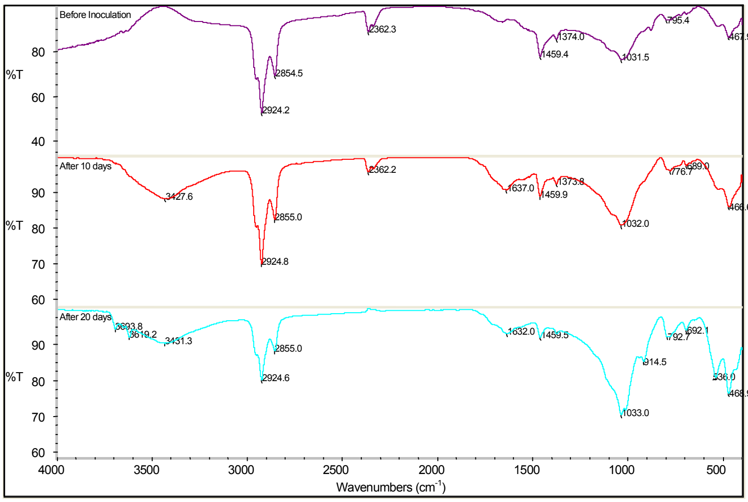

Fig. 4: FTIR Spectra of Railway Track Soil after different Fungal Treatments

Analysis of Two-Wheeler Service Centre Soil: The FTIR spectrum (Fig. 1) of untreated two wheeler service centre soil revealed bands at 3438 cm^{-1} corresponding to hydroxyl stretching of alcohols and phenols; at 2924 cm^{-1} indicating -C-H stretching in aliphatic compounds; at 2360.4 cm^{-1} indicating C-C, C≡N (Nitriles); at 1028.5 cm^{-1} corresponding to stretch of primary alcohols and at 776.8 cm^{-1} indicating the presence of alkyl halides [19]. However, the FTIR spectrum after 10 days of microbial incubation indicated new band at 1978 cm^{-1}, corresponding to C-O stretching [20]. After 20 days the bands were revealed again. These bands could be associated with new carbonyl groups arising from the microbial oxidation of used oil in the rhizosphere. Besides the per cent transmittance increased which shows the high degradation of hydrocarbon compounds by the treatment with the fungal strains.

Analysis of Four-Wheeler Service Centre Soil: The FTIR spectrum (Fig. 2) of untreated four wheeler service centre soil revealed bands at 2924.1cm^{-1} indicating -C-H stretching in aliphatic compounds; at 1031.8cm^{-1} corresponding to stretch of primary alcohol; at 796.6 cm^{-1} indicating the presence of alkyl halides [21]. However, the FTIR spectrum of the used mobil oil contaminated soil (Four Wheeler), recorded after 10 days of microbial incubation indicated new bands at 2854.7 cm^{-1} and at 1633 cm^{-1} indicating C≡N (Nitriles) and C=O stretching respectively [22]. Interestingly after 20 days of microbial incubation, a new band at 2363.2 was observed representing the presence of -C-O stretching for primary alcohols [23], may be due to the microbial oxidation of used oil. Besides it was observed that there is significant change in transmittance which increased after 20 days of treatment with fungi representing degradation of complex mixtures.

Analysis of Six-Wheeler Service Centre Soil: The FTIR spectrum (Fig. 3) of untreated six wheeler soil revealed bands at 3855.1 cm^{-1} indicating hydroxyl stretching in phenols, alcohols; at 2924.2 cm^{-1} signifying -C-H stretching in aliphatic compounds; at 1629.5 cm^{-1} due to the presence of carboxylates; 1028.2 cm^{-1} corresponding to stretch of primary alcohol in; at 777.9 cm^{-1} indicating the presence of alkyl halides [24]. However, the FTIR spectrum after 10 days of microbial incubation indicated new bands at 1879.6 cm^{-1} and 1417.0 cm^{-1}. After 20 days of microbial incubation, maximum bands observed after 10 days disappeared and a new band appeared at 2362.0 cm^{-1} signifying the presence of nitriles [25]. The conversion of -C-H stretching in aliphatic compounds to nitrile groups may be due to degradation by fungal strains. Also the transmittance increased significantly after the treatment with fungal strains.

Analysis of Railway Service Station Soil: The FTIR spectrum (Fig. 4) of untreated railway track soil revealed bands at 2924.2 cm^{-1} indicating -C-H stretching in aliphatic compounds; at 2362.3 cm^{-1} corresponding to C-C, C≡N (Nitriles); at 1031.5cm^{-1} related to stretching of primary alcohols; at 795.4 cm^{-1} signifying the presence of alkyl halides. However, the FTIR spectrum recorded after 10 days of microbial incubation indicated new bands at 1637.0 cm^{-1} and at 689.0 cm^{-1} representing C=O stretching and aryl halides respectively [26]. But after 20 days of microbial incubation, there was not observed much significant change. It was observed that the transmittance increased from zero day to after 20 days of microbial treatment indicating the possible reduction in hydrocarbon compounds used by the micro flora as sole sources of carbon and energy. At given environmental conditions, the degree of hydrocarbon biodegradation is mainly affected by the type of hydrocarbons in the contaminant matrix [27]. Of the various petroleum fractions, n-alkanes and branched alkanes of intermediate length (C10–C20) are the preferred substrates to microorganisms and tend to be most readily degradable. Longer chain alkanes (>C20) are hydrophobic solids and are difficult to degrade due to their inherent recalcitrance and their poor water solubility [28].

CONCLUSION

The FTIR spectra recorded at different time intervals delivered a good indication of hydrocarbon degradability in soil by the isolated fungal strains. The results suggest that the fungal strains prefer C-H aliphatic and aromatic stretches for degradation of long chain alkanes present in used motor oil. It is therefore concluded that by using the isolated fungal strains, the soil which has lost its fertility because of the oil may be remediated and be turned back as fertile soil. Moreover, FTIR spectroscopy can be a very useful tool in performing preliminary tests in order to predict remediation performance so as to select an appropriate approach for clean- up technologies.

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REFERENCES

1. Borneff, J. and H. Kunte, 1965. Carcinogenic substances in water and soil. XVII. About the origin and evaluation of the PAH in water. Arrh. Hyg. Bakt., 149: 226-243.
2. Adebiyi, F.M. and A.F. Adeyemi, 2010. Characterisation of the petroleum hydrocarbons-contaminated soils around a petroleum products depot. Chemistry and Ecol., 26: 137-146.
3. Gogoi, B.K., N.N. Dutta, P. Goswami and T.R.K. Mohan, 2003. A case study of bioremediation of petroleum-hydrocarbon contaminated soil at a crude oil spill site. Adv. Environ. Res., 7: 767-782.
4. Atlas, R.M. and J. Philp, 2005. Bioremediation: applied microbial solutions for real-world environmental cleanup, ASM Press, Washington, D.C: 1-292.
5. Chaineau, C.H., J.L. Morel and J. Oudot, 1995. Microbial degradation in soil microcosms of fuel oil hydrocarbons from drilling cuttings. Environ. Sci. Technol., 29: 1615-1621.
6. Robert, M.G., J.R. Stephen and C.P. Roger, 2003. Biodegradation of fuel oil under laboratory and arctic marine conditions. Spi. Sci. Technol. Bull., 8: 297-302.
7. Delille, D., B. Delille and E. Pelletier, 2002. Effectiveness of bioremediation of crude oil contaminated Subantarctic Intertidal Sediment: the microbial response. Microb. Ecol., 44: 118-126.
8. Saadoun, I., 2002. Isolation and characterization of bacteria from crude petroleum oil contaminated soil and their potential to degrade diesel fuel. J. Basic Microbiol., 42: 422-430.
9. Mehrashi, M.R., B. Haghighi, M. Shariat, S. Naseri and K. Naddafi, 2003. Biodegradation of petroleum hydrocarbons in soil. Iran. J. Public Health., 32: 28-32.
10. El Fantroussi, S. and S.N. Agathos, 2005. Is bioaugmentation a feasible strategy for pollutant removal and site remediation? Curr. Opin. Microbiol., 8: 268-275.
11. Romantschuk, M., I. Sarand, T. Petanen, R. Peltola, M. Jonsson-Vihanne, T. Koivula, K. Yrjala and K. Haahtela, 2000. Means to improving the effect of in situ bioremediation of contaminated soil: an overview of novel approaches. Environ. Pollut., 107: 179-185.
12. Molina-Barahona, L., R. Rodriguez-Vazquez, M. Hernandez-Velasco, C. Vega-Jarquin, O. Zapata Perez, A. Mendoza-Cantu and A. Albores, 2004. Diesel removal from contaminated soils by biostimulation and supplementation with crop residues. Appl. Soil Ecol., 27: 165-175.
13. Abed, M.M.R., N.M.D. Safi, J. Koster, D. DeBeer, Y. El-Nahhal, J. Rullkotter and F. Garcia-Pichel, 2002. Microbial diversity of a heavily polluted microbial mat and its community changes following degradation of petroleum compounds. Appl. Environ. Microbiol., 68: 1674-1683.

14. Watanabe, K., 2002. Linking genetics, physiology and ecology: an interdisciplinary approach for advancing bioremediation. J. Biosci. Bioeng., 94: 557-562.
15. Kenneth, E.H., 1995. Mechanisms for polycyclic aromatic hydrocarbon degradation by ligninolytic fungi. Environ. Health Perspec., 103: 41-43.
16. Bhat, M.M., S. Shankar, Shikha, M. Yunus and R.N. Shukla, 2011. Remediation of Hydrocarbon Contaminated Soil through Microbial Degradation-FTIR based prediction. Advances in Applied Science Res., 2(2): 321-326.
17. Cole, M.G., 1994. Assessment and Remediation of Petroleum Contaminated Sites, Boca Raton, FL: Lewis Publishers: pp: 39-142.
18. Wemedo, S.A., O. Obire and D.A. Dogubo, 2002. Myco-flora of a kerosene-polluted soil in Nigeria. J. Appl. Sci. Environ. Manag., 6: 14-77.
19. Zieba-Paulus, J. and J.P. Koscielniak, 1999. Differentiation of motor oils by infrared spectroscopy and elemental analysis for criminilastic purposes. J. Molecular Structure, 482-483: 533-538.
20. ieira, R.H. and B. Volesky, 2000. Biosorption: a solution to pollution*?* Int. Microbiol., 3: 17-24.
21. Lezcano, J.M., F. González, A. Ballester, M.L. Blázquez, J.A. Muñoz and C. García-Balboa, 2010. Biosorption of Cd(II), Cu(II), Ni(II), Pb(II) and Zn(II) using different residual biomass. Chemistry and Ecol., 26: 1-17.
22. Kadam, A.N. and M.D. Zingde, 1985. Infrared spectroscopic analysis of used crankcase oil. Research and Industry, 30: 382-385.
23. Rashid, H.A., A.A. Alhassan and A. Abdulsalam, 1990. Analysis of used oil by IR-spectroscopic and synthesis of dispersions of a highly basic petroleum calcium sulfonate additive. Fuel Science and Technology International, 8: 899-916.
24. Synytsya, A., J. Copikova, P. Matejka and V. Machovic, 2003. Fourier transform Raman and infrared spectroscopy of pectins. Carbohyd. Polym., 54: 97-106.
25. Elena, D.R. and P. John, 2003. Chemical characterization of fresh, used and weathered motor oil via GC/MS, NMR and FTIR techniques. Proceedings of Indiana Academy of Sci., 112: 109-116.
26. Sheng, P.X., Y.P. Ting and J.P. Chen, 2007. Biosorption of heavy metal ions (Pb, Cu and Cd) from aqueous solutions by the marine alga Sargassum sp. in single- and multiple-metal systems. Ind. Eng. Chem. Res., 46: 2438-2444.
27. Huesemann, M.H., 1995. Predictive model for estimating the extent of petroleum hydrocarbon biodegradation in contaminated soils. Environ. Sci. Technol., 29: 7-18.
28. Facundo, J.M., H. Vanessa and M.L. Teresa, 2001. Biodegradation of diesel oil in soil by a microbial consortium. Wat. Air Soil Pollu., 128: 313-320.