https://dx.doi.org/10.4314/dujopas.v9i4b.23
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 9 No. 4b December 2023*

Microbial Proficiency in Spent Engine Oil Degradation of Polluted Soil from Auto-repair Facilities and Industrial Sites: Isolation and Molecular Characterization of Hydrocarbonoclastic Microorganisms

[1]Nwagu, K.E, [2]Akumah, O.E, *[2]Ibeh, A.G, [3]Enwere, E.N, [4]Azumini, P; [5]Agu, F.C and [6]Abel, C.F.

[1,2,3,4,5,6]Department of Biotechnology,
Alex Ekwueme Federal University
Ndufu Alike Ikwo,
Ebonyi State.
Nigeria

Email: ibeh.gift@funai.edu.ng

Abstract

The disposal of spent engine oil presents a significant environmental challenge due to its toxic and persistent nature. This study aims to isolate and molecularly characterize microbes capable of degrading spent engine oil, with the ultimate goal of developing eco-friendly bioremediation strategies. Soil and water samples from auto-repair facilities and industrial sites were collected, and Mineral Salt Agar (MSA) medium was used to screen for spent engine oil degrading microorganisms. Isolated strains were screened for their ability to utilize spent engine oil through growth experiments and biochemical assays. Other parameters monitored were temperature and pH. The 16S rRNA gene sequencing was employed to identify the isolated microbes at the molecular level. BLAST program in National Centre for Biotechnology Information (NCBI) Genebank database Phylogenetic analysis revealed a diverse array of seven (5) isolates including bacterial (3) and fungal (2) taxa, suggesting a rich microbial diversity involved in spent engine oil degradation. The isolates were identified as bacteria (SA1-Bacillus thuringiensis, SA6-Bacillus cereus, and SB5-Alcanivorax borkumensis) and fungi (SA5-Aspergillus niger and SC3-Aspergillus flavus). The percentage of oil degradation rate was SA5 (43.80%) > SA1 (29.17%) > SB5 (28.82%) > SC3 (6.07%). The fungal isolate, SA5-Aspergillus niger showed a significant ($p < 0.05$) spent engine oil degradation rate compared to the bacteria. The study demonstrates the potential of not only bacteria but indigenous fungal communities in mitigating the environmental impact of spent engine oil. It also provides a foundation for future studies focused on optimizing the biodegradation of complex hydrocarbon pollutants.

Keywords: Bioremediation, Enzyme Activity, Hydrocarbon-Degrading Bacteria, Hydrocarbon-Degrading Fungi

INTRODUCTION

Hydrocarbons, consisting primarily of carbon and hydrogen, are the essential constituents of crude oil, a complex mixture that contains oxygen, sulphur, nitrogen as well as trace metals. Post-refining, petroleum products acquire altered physicochemical properties that enhance complexity and may impede their biodegradation (Logeshwaran *et al*., 2018). Sludge from the oil industry contains pollutants, such as hydrocarbons, sulfides and ammonia, in the soil

*Author for Correspondence

environment. It's expensive and environmentally destructive to use traditional physiochemistry methods like incineration or extraction from solvents (Liu *et al*., 2019). Petroleum hydrocarbons continue to be present in ecosystems, which are affected by activities such as oil spills and drilling with a negative impact on the ecosystem's fish life and health (Poorsoleiman *et al.*, 2021; Radhakrishnan *et al*., 2023).

The disposal of spent engine oil, a hazardous waste generated from automotive and industrial activities, poses significant environmental concerns due to its high toxicity and potential for soil and water contamination (Luo *et al*., 2019). Spent engine oil contains a complex mixture of hydrocarbons, heavy metals, and other contaminants, posing a substantial threat to ecosystems and public health (Deng *et al*., 2014; Wang *et al*., 2016). Traditional methods of spent engine oil disposal, such as landfilling or incineration, are associated with adverse environmental impacts and are progressively being replaced by more sustainable approaches, notably bioremediation (Sathishkumar *et al*., 2018). In contrast, the application of microbial biodegradation presents a promising and eco-friendly alternative for the effective removal of spent engine oil contaminants. With preference to aerobic, anaerobic or facultative anaerobic pathways the metabolic paths involved in hydrocarbon degradation may be significantly different between microbial groups and individual species. Due to changes in availability of hydrocarbon contaminants, microbial populations are able to adapt and evolve; this allows for shifts in the predominant degradation pathways (Alaidaroos, 2023). The complex nature of biodegradation processes due to the variety of compounds that can be reduced by various microbial groups or through distinct pathways, is compounded by hydrocarbon mixtures in contaminated site.

Bacteria and fungi involved in bioremediation transform contaminants into benign substances (Alaidaroos, 2023), with enzymes, biosurfactants, and metabolic products facilitating hydrocarbon degradation (Ossai *et al.,* 2020). Their significance lies in the decomposition of organic pollutants (Fernández *et al*., 2020). Hydrocarbon-oxidizing bacteria attach to spent oil droplets, facilitating their degradation (Pandolfo *et al*., 2023). The bacteria are vital in shifting microbial communities toward hydrocarbonoclastic species capable of using hydrocarbons as source of energy. These bacteria adapt to hydrocarbon exposure through various mechanisms, including the production of biosurfactants that enhance hydrocarbon bioavailability (Ruiz *et al*., 2021). Various hydrocarbon-degrading bacteria, including species from *Bacillus, Cycloclasticus, Stenotrophomonas, Rhodococcus, Mycobacterium, Pseudomonas, Sphingomonas* and *Burkholderia* have been identified (Gupta *et al*., 2019). Recently, fungi have gained prominence in the realm of hydrocarbon degradation research, surpassing bacteria in this aspect. These fungi show effective break down of PAHs, using their enzymes such as laccase and dioxygenase (Al Farraj, *et* al., 2020). Various fungal species such as *Penicillium, Aspergillus, Cladosporium, Phlebiella, Mycoaciella* and *Peniophora* have been reported to exhibit notable proficiency in hydrocarbon degradation (Aranda *et al*., 2017). The purpose of this comprehensive study is to provide a detailed examination of the microbial communities involved in spent engine oil degradation, encompassing their taxonomic diversity. By elucidating the intricate interplay between microorganisms and spent engine oil pollutants, this study aims to not only deepen our understanding of microbial biodegradation processes but also to underscore the potential for harnessing these natural capabilities in developing sustainable and cost-effective bioremediation strategies. Various studies have been carried out on degradation of hydrocarbons by bacterial community but limited reports are available on the exploration and exploitation of fungal species in spent engine oil degradation. Tropical environment like Nigeria could harbour diverse fungal hydrocarbonoclastic microbes that have great potentials in bioremediation of spent engine oil contaminated environments.

MATERIALS AND METHODS

Study Area

The study was carried out within Ikwo Local Government Area of Ebonyi State in South Eastern region of Nigeria, West Africa. The study area is geographically located between Latitude 06°4′28″N and Longitude 8°06′ 02″ E. Ikwo is the largest local government area in Ebonyi State.

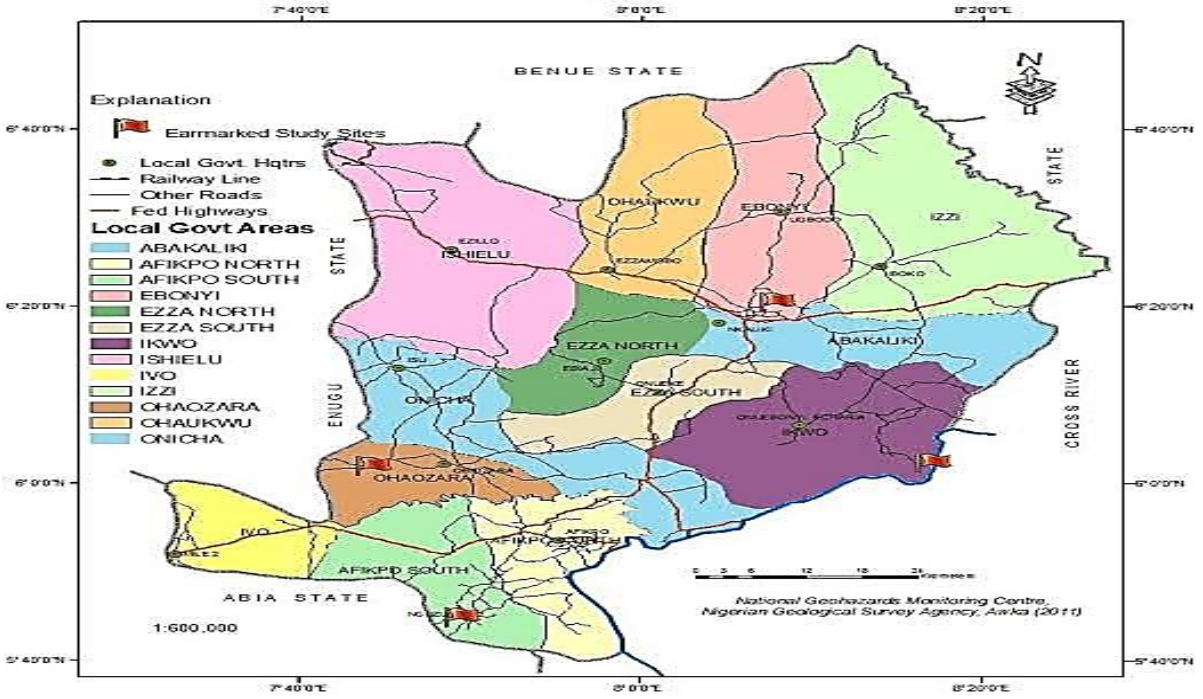

Fig 1: Map of Ebonyi showing Ikwo Local Government Area (Shaded in Dark purple colour)

Sample collection

Soil samples were collected from oil-spilled automobile repair shops using sterile plastic containers at different locations at Ndufu Echara in Ikwo Local Government Area, Ebonyi State. The samples were transported to the lab for further analysis.

Microbial Isolation and Cultivation

Mineral Salt Agar (MSA) and Potato Dextrose Agar (PDA) media were used for the isolation of bacteria and fungi, respectively. The screening media for both bacteria and fungi isolates was Mineral Salt Agar. The screening media supplemented with 2% (v/v) of spent engine oil was used as a natural substrate found in soil samples where the microorganisms were isolated. The screening media was used to confirm the ability of microorganisms to utilize/ degrade spent engine oil.

Isolation of Spent Engine Oil Degrading Microorganisms

The spread plate method of serial dilution (1:10) was used to isolate microorganisms from spent engine oil-rich soils. Aliquots of 1ml were inoculated on MSA and PDA that was previously sterilized by autoclaving at 121 °C for 15min. MSA plates were incubated at 37 °C in an inverted position for 24 h and PDA for 48 h. After regular intervals, sub-culturing of enriched cultures was performed to obtain pure isolates. For future usage, pure bacterial and fungal cultures were maintained on solidified MSA agar slants supplemented with spent engine oil and kept at 4 °C according to Varjani *et al*., (2017).

Spent oil Degradation Activity of Isolates

To check for ability of bacteria and fungi isolates to utilize/degrade spent engine oil, one (1) ml of the isolates inoculum transferred into 100 ml BH (Bushnell Haas) medium supplemented with 2% used engine oil (v/v) in 250 ml Erlenmeyer flasks incubated at 30°C and 100 rpm in shaker incubator. A control devoid of the isolate was prepared for each set of experiments. All experiments were performed in duplicate. Cell growth was measured as optical density at 600 nm (OD600) and was used as a parameter for screening the used engine oil degrading potential of the isolates using a spectrophotometer (Antai *et al.,* 2014; Unimke *et al.,* 2017). Other parameters monitored were temperature and pH.

Spent Engine Oil Degradation Assay

After two weeks of biodegradation, the mixture was separated by pouring it into a separating funnel and allowed to stand undisturbed for about 20 minutes. The oil was separated from the medium; chloroform was used to rinse-off the remaining oil on the body of the separating funnel and allowed to evaporate. After the complete evaporation, the oil residue obtained was weighed and taken as the gravimetric value for further calculation. The level of spent engine oil degradation was determined using the gravimetric analysis. (Chang, 1998; Marquez-Rocha *et al.,* 2001). The percentage of spent engine oil degraded was determined from the following formula:

$$\text{Percentage of oil degradation} = \frac{\text{Amount of spent engine oil degraded}}{\text{Amount of spent engine oil added in the media}} \times 100$$

Amount of spent engine oil degraded = (Weight of spent engine oil added in the media) – (Weight of residual engine oil).

Molecular Characterization of Isolates

In order to extract bacterial and fungal DNA, Zymo Research Kit procedures were followed. To amplify bacterial DNA, the following primers were used: (27F: AGAGTTTGATCCTGGCTCAG) and universal reverse primer (1429R: GGTTACCTTGTTACGACTT) (Inqaba biotech). For fungal DNA amplification, (ITS1: TCCGTAGGTGAACCTGCGG) and universal reverse primer (ITS4: TCCTCCGCTTATTGATATGC) (Inqaba biotech) was used. The 25 µl reaction mixture contained: 12.5µL of Taq 2X Master Mix {New England Biolabs (M0270)}, 2µL of DNA template, 8.5µL Nuclease free water (Inqaba biotech), and 1µL each of the forward and reverse primer for both bacterial and fungal DNA amplification. The thermocycling cycle was carried out with an Initial denaturation at 94˚C for 5 minutes, followed by 36 cycles of denaturation at 94˚C for 30sec, annealing at 56˚C for 30 secs and elongation at 72˚C for 45sec. Followed by a final elongation step at 72˚C for 7 minutes and hold temperature at 10 ˚C forever. Standard electrophoresis techniques were used to electrophorese and observe aliquots (10 µl)) of PCR products in a 2% agarose gel. The BigDye Terminator v3.1 cycle sequencing kit was used for sequencing the amplified fragments on an Applied Biosystems Genetic Analyzer 3130xl sequencer per the manufacturer's instructions. The entire genomic study was performed using MEGA 6 and Bio-Edit software. Using the BLASTN tool, the acquired 16S rRNA gene sequence was compared to those from GenBank. The organisms with highest percentage similarity in the GenBank when compared with our obtained sequences were referenced as the true identity of the isolates.

RESULTS AND DISCUSSION

Isolation and screening of isolates for Spent Engine Oil Degradation

A total of twelve (12) pure isolates were obtained after culturing the soil samples on MSA and PDA. Five (5) isolates that showed significant spent oil degradation potential were selected after screening with Bushnell-Haas medium. The isolates include 3 bacteria (SA1, SA6, and SB5) and 2 fungi (SA5 and SC3). As shown in Table 1, the isolates were identified as bacteria - (SA1- *Bacillus thuringiensis*, SA6-*Bacillus cereus*, and SB5-*Alcanivorax borkumensis*) and fungi (SA5-*Aspergillus niger* and SC3-*Aspergillus flavus*). This result is in correlation with the work reported by Khan *et al*. (2018) and Wu *et al*. (2017) who were able to isolate *Pseudomonas, Bacillus,* and other bacterial strains from engine oil contaminated soil. *Pseudomonas, Bacillus*, and *Rhodococcus* were also isolated from engine oil contaminated soil as reported by Wu *et al*. (2017). Some of the fungal isolates have earlier been reported as hydrocarbon utilizers by Luo *et al*. (2019).

Table 1: Molecular Identification of Fungal and Bacterial Isolates.

Isolate Code	Closest related Organism in the Gene bank	Similarity (%)	NCBI accession number
SA1	*Bacillus thuringiensis*	86.2%	KX057525.1
SA6	*Bacillus cereus*	82.6 %	KC999982.1
SB5	*Alcanivorax borkumensis*	96.5%	OQ834576.1
SA5	*Aspergillus niger*	97.2%	MG659638.1
SC3	*Aspergillus flavus*	97.2%	MG647857.1

Percentage of Spent engine oil degradation.

After carrying out gravimetric analysis using the 5 isolates, the amount of spent engine oil degraded and the percentage of spent engine oil degraded was determined. The organisms degraded the spent engine oil at different rates. The percentage of spent oil degradation is represented in Figure 2 below.

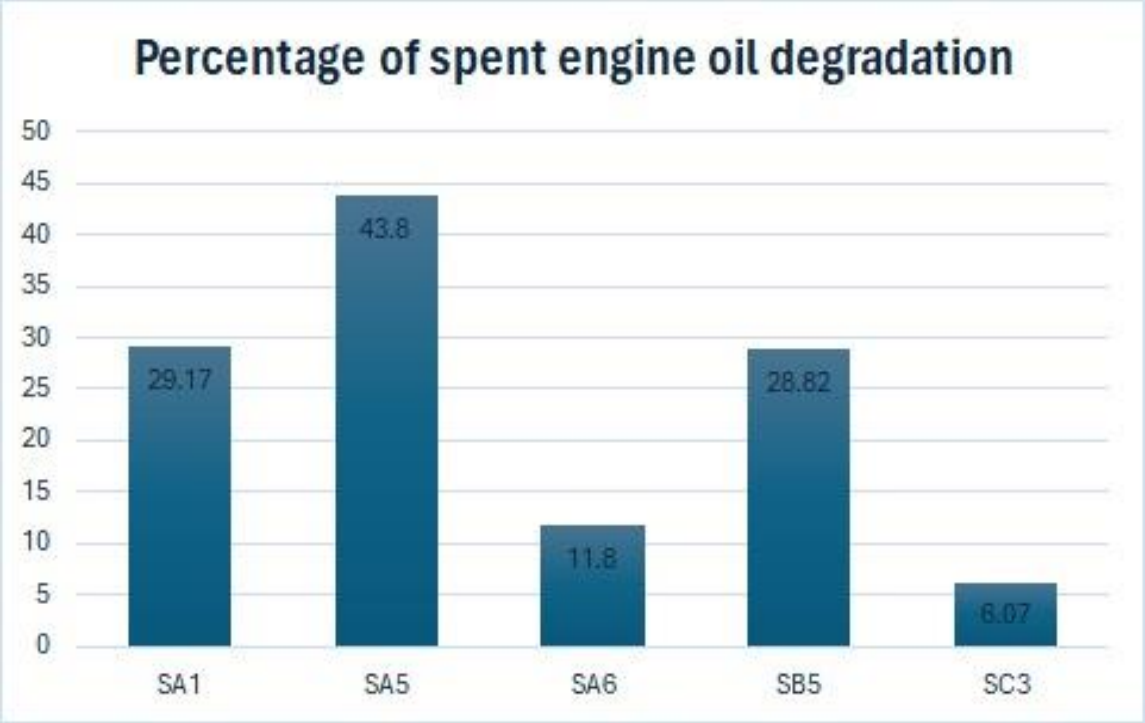

Figure 2: Chart showing the percentage of spent engine oil degraded.

The isolate, SA5 (*Aspergillus niger*) exhibited the highest degradation rate at 43.80% while SA1 (*Bacillus thuringiensis*) demonstrated a moderately high degradation rate at 29.17%. Also, SB5 (*Alcanivorax borkumensis*) exhibited a comparable degradation rate to SA1 at 28.82% while SA6 (*Bacillus cereus*) and SC3 (*Aspergillus flavus*) displayed the lowest degradation rate observed in this study at 11.8% and 6.07%. This result indicates that *Aspergillus niger* (SA5) demonstrated the highest efficiency in degrading the spent oil, followed by the *Bacillus thuringiensis* (SA1), *Alcanivorax borkumensis* (SB5), *Bacillus cereus* (SA6) and finally *Aspergillus flavus* (SC3), which exhibited the lowest degradation rate. The lower efficiency of the *B. cereus* and *A. flavus* specie when compared to the fellow genus of *Bacillus thuringiensis* and *A. niger* may be attributed to physiological and metabolic characteristics of the specie.

This finding is consistent with previous studies that have reported the effectiveness of *Aspergillus niger* in hydrocarbon degradation (Atlas, 2016; Burghal *et al.,* 2016). Additionally, *Alcanivorax borkumensis* has been previously identified as a hydrocarbon-degrading bacterium. The lower degradation rate observed for *Aspergillus flavus* may be attributed to its potentially lower affinity for spent oil components.

The variation in degradation rates among these microorganisms could be attributed to their specific enzymatic capabilities, metabolic pathways, and adaptation mechanisms to utilize hydrocarbons as a carbon source. Furthermore, environmental factors such as temperature, pH, and nutrient availability can also influence the degradation rates of these organisms (Yadav, 2018).

CONCLUSION

The results suggest that *Aspergillus niger* (SA5) is the most effective microorganism for degrading spent oil, followed by *Alcanivorax borkumensis* (SA1), *Alcanivorax borkumensis* (SB5), and *Aspergillus flavus* (SC3). This study underscores the importance of microbial diversity and strain selection in the development of effective bioremediation strategies for spent oil. Further

research into the molecular mechanisms underlying the observed degradation rates will be crucial for optimizing and harnessing the potential of these bacteria and fungi.

CONFLICT OF INTEREST

The authors declare no actual or potential conflict of interest.

ACKNOWLEDGEMENTS

We appreciate the working effort of the Technologist, Mr. Akindele Kayode, in the department of Microbiology, Alex Ekwueme Federal University Ndufu Alike ikwo Ebonyi state, Nigeria and great role in the microbial isolation.

REFERENCES

Alaidaroos, B.A. (2023). Advancing Eco-Sustainable Bioremediation for Hydrocarbon Contaminants: Challenges and Solutions. *Processes*, *11*, 3036. https://doi.org/10.3390/pr11103036.

Al Farraj, D.A., Hadibarata, T., Elshikh, M.S., Al Khulaifi, M.M., & Kristanti, R.A. (2020). Biotransformation and degradation pathway of pyrene by filamentous soil fungus Trichoderma sp. F03. *Water, Air, Soil Pollution*, 231, 1–9.

Antai, S.P., Unimke, A.A., & Agbor, R.B. (2014). Assessment of the heterotrophic and crude oil utilizing microorganisms of Imo River Estuary of the Niger Delta mangrove ecosystem. *American International Journal of Biology*. 2(1),2942.

Aranda, E., Godoy, P., Reina, R., Badia-Fabregat, M., Rosell, M., *et al*. (2017). Isolation of Ascomycota fungi with capability to transform PAHs: Insights into the biodegradation mechanisms of Penicillium oxalicum. *Int. Biodeterior. Biodegrad*, 122, 141–150.

Atlas, R.M. (2016). Biological Degradation of Petroleum Hydrocarbons: *An Environmental Perspective Microbiology Revised* 45, pp.180-209.

Burghal,A. A., Abu-Mejdad, N. M.J., & Al-Tamimi, W. H. (2016). Mycodegradation of crude oil by fungal species isolated from petroleum contaminated soil. *Int.J. Innov.Rese.Sci.Engin.Technol.* 5:1517-1524

Chang, R. (1998). Chemistry (6th edition). McGraw–Hill Companies, Incorporated, 24, 962–963.

Deng, M.C., Li, J., Liang, F. R., Yi, M., Xu, X.M., *et al*. (2014) Isolation and characterization of a novel hydrocarbon-degrading bacterium *Achromobacter* sp HZ01 from the crude oil-contaminated seawater at the Daya Bay, southern China.*Marine Pollution Bulletin,* 83(1),79–86.

Fernández, N.V., Marchelli, P., Tenreiro, R., Chaves, S., & Fontenla, S.B. (2020). Are the rhizosphere fungal communities of Nothofagus alpina established in two different environments influenced by plant genetic diversity? For. *Ecol. Manag*, 473, 118269.

Gupta, G., Kumar, V., & Pal, A. (2019). Microbial degradation of high molecular weight polycyclic aromatic hydrocarbons with emphasis on pyrene. *Polycycl. Aromat. Compd*, 39, 124–138

Khan, M.A.I., Biswas, B., Smith, E., Naidu, R., & Megharaj, M. (2018). Toxicity assessment of fresh and weathered petroleum hydrocarbons in contaminated soil. *A Review Journal on Chemosphere* 212, 755–767.

Liu, S.C., Sun, S.J., Cui, P., & Ding, Y.F. (2019). Molecular modification of fluoroquinolone-biodegrading enzymes based on molecular docking and homology modelling. *Int. J. Environ. Res. Public Health*, 16, 3407.

Logeshwaran, P., Megharaj, M., Chadalavada, S., Bowman, M., & Naidu, R. (2018). Petroleum hydrocarbons (PH) in groundwater aquifers: An overview of environmental fate,

toxicity, microbial degradation and risk-based remediation approaches. *Environ. Technol. Innov.* 12,34.

Luo, C., Tao, L., Zhang, Y., & Lin, X. (2019). Microbial community structure and diversity in soils remediated by a two-phase pilot-scale constructed wetland system. *Ecological Engineering*, 130, 58-66.

Marquez–Rocha, F. J., Hernandez–Rodriguez, V., & Lamela, M. T. (2001). Biodegradation of engine and diesel oil in soil by a microbial consortium. *Water, Air and Soil Pollution*, 128, 313–320.

Ossai, I.C., Ahmed, A., Hassan, A., & Hamid, F.S. (2020). Remediation of soil and water contaminated with petroleum hydrocarbon: A review. *Environ. Technol. Innov*, 17, 100526.

Pandolfo, E., Barra Caracciolo, A., & Rolando, L. (2023). Recent advances in bacterial degradation of hydrocarbons. *Water,* 15, 375

Poorsoleiman, M.S., Hosseini, S.A., Etminan, A., & Abtahi, H.K. (2021). A. Bioremediation of Petroleum Hydrocarbons by using a two-step inoculation composting process scaled-up from a mineral-based medium: Effect of biostimulation of an indigenous bacterial strain. *Waste Biomass Valorization*, 12, 2089–2096.

Radhakrishnan, A., Balaganesh, P., Vasudevan, M., Natarajan, N., Chauhan, A., *et al*. (2023). Bioremediation of Hydrocarbon Pollutants: Recent Promising Sustainable Approaches, Scope, and Challenges. *Sustainability*, 15, 5847.

Ruiz, O.N., Radwan, O.& Striebich, R.C. (2021). GC–MS hydrocarbon degradation profile data of Pseudomonas frederiksbergensis SI8, a bacterium capable of degrading aromatics at low temperatures. *Data Brief*, 35, 106864

Sathishkumar, M., Binupriya, A. R., & Yun, S. E. (2018). Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. *Clean Technologies and Environmental Policy*, 20(8), 1787-1798.

Unimke A.A., Bassey, I.U., Mmuoegbulam, O.A., & Nseabasi, N.O. (2017). Seasonal implications on the microbiological and physico-chemical characteristics of sediments. *Imperial Journal of Interdisciplinary Researc,*3(1),156-164.

Varjani, S.J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresource Technology,* 223,277–286.

Wang, J. J., Jiq, C. R., Wong, C. K., & Wong, P. K. (2016). Characterization of polycyclic aromatic hydrocarbons created in lubricating oils. *Water, Air and Soil pollution*, 120, 381-396.

Wu, M., Li, W., Dick, WA., Ye, X., Chen, K., *et al*. (2017). Bioremediation of hydrocarbon degradation in a petroleum-contaminated soil and microbial population and activity determination. *Chemosphere,* 169,124–130.

Yadav, S. K. (2018). Heavy metal toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. *South African Journal of Botany* 76,167-179.