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EAAA, EHLA, and QYMA conceptualised the study; AAMR, MAMH, AMYA, SMSA, IWAM, and LAMA participated in data collection and the conduct of the experiments. EAAA, QYMA, AAMR, MAMH, AMYA, SMSA, IWAM, and LAMA contributed to writing the manuscript. All authors read and approved the final version of the manuscript.

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Efficient Bioremediation of Petroleum Hydrocarbon Wastes by Some Bacterial Strains Isolated from Soil in Yemen

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Abstract:

Petroleum pollution is a well-known problem identified both locally and internationally, which can cause multiple environmental damages and may lead to significant disturbances in the biotic and abiotic components of ecosystems. This study aims to determine certain physical properties of one type of Yemeni crude oil, isolate and identify bacteria that degrade petroleum hydrocarbons, and screen the identified bacterial isolates for their ability to degrade petroleum hydrocarbon wastes. Twenty soil samples (contaminated and non-contaminated with petroleum derivatives) were collected from 4 different governorates (Capital Secretariat, Sana'a, Al-Hudaydah, and Marib). The serial dilution method was used from 10^{-2} dilution on Bushnell and Haas agar medium containing 1% v/v of crude oil by using the spreading method. Colorimetric assay by 2,6-Dichlorophenol indophenol (2,6-DCPIP indicator) was done to screen identified bacterial isolates for their ability to degrade petroleum hydrocarbon wastes. The results showed that crude oil, which was used as a sole carbon source and other additives, was identical to local and international standards according to the Yemeni petroleum company databases. All soil samples that contained bacteria were able to grow on BHA medium. From 40 bacterial isolates, only 26 were identified to genus / species and selected for screening of petroleum hydrocarbon waste degradation. Most common bacterial isolates were *Pseudomonas* spp. (23.07%), followed by *Bacillus* spp., and *Streptococcus* spp. (19.23%) each, *Enterococcus* spp. (11.53%), *Staphylococcus aureus* and *Enterobacter* spp. (7.69%) each and the least common were *Morganella morganii*, *Providencia stuartii*, and *Proteus vulgaris* each represents 3.84%. Our results showed that 13 identified bacterial isolates represented 50% of the total identified isolates showed the highest ability to degrade petroleum hydrocarbon wastes at the shortest time (3- 12 hrs.), 9 identified bacterial isolates represented 34.61% showed moderate ability of degradation at 13-24 hrs. Therefore, this study concluded that bioremediation using bacteria is sustainable and cost-effective. More attention to the management and cleanup efforts of these pollutants based on their type.

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INTRODUCTION

Crude oil is a liquid form of petroleum composed of thousands of hydrocarbon compounds. It serves as a primary source of energy for both industry and daily life, which is why it is often referred to as 'Black Gold' (Aicha *et al.*, 2013; Balba *et al.*, 2013; Shakya *et al.*, 2021; Varjani, 2017). With the rise in population and advancements in industrialization, global energy demand has significantly increased, leading to the excessive consumption of fossil fuels. This, in turn, has resulted in the emission of greenhouse gases (GHGs), particularly carbon dioxide (CO_2), which plays a major role in global warming and climate change (Akın *et al.*, 2023). On the other hand, oil can also lead to organic contamination of water and soil, whether through accidental incidents or human activities. It may enter rivers and marine environments through various pathways, such as accidental spills or the discharge of refinery waste into water bodies. Soil contamination by oil is now considered a major global environmental concern, as it can result in delayed plant growth, reduced soil fertility, and alterations in the soil's physicochemical and microbiological characteristics. Furthermore, it may lead to groundwater contamination, posing serious risks to human health (Abdul-Ameer Ali, 2019; Shakya *et al.*, 2021). It has been known to the family of carcinogens and neurotoxic organic pollutants (Das and Chandran, 2011).

Effective control and treatment strategies are essential to mitigate the harmful impacts of petroleum hydrocarbons (Bidoia *et al.*, 2010). A range of chemical, physical, and biological methods have been developed for the remediation of contaminated soil (Alamri, 2009; Maliji *et al.*, 2013; Mashreghi and Marialigeti, 2005). Biological treatment methods for organic pollutants offer effective, long-term, and environmentally safe solutions. Compared to chemical and physical approaches, they are generally more reliable, cost-effective, and simpler to implement, while also contributing to improved environmental quality (Sonawdekar, 2012). Where certain microbes show an increase, typically in polluted sites due to the use of petroleum hydrocarbons as nutrients (Lee and Levy, 1986; Westlake *et al.*, 1974). Such species are commonly used for remediation of contaminated sites (Lotfinasabasl *et al.*, 2012).

Recent developments in microbial and environmental biotechnology in Yemen and the broader Middle East highlight the increasing significance of biological approaches in pollution management. Bioactive compounds derived from native plants such as *Boswellia sacra* have shown promising antimicrobial properties that can aid microbial remediation (Abdullah *et al.*, 2025b). Additionally, locally sourced nanomaterials, such as silica nanoparticles extracted from bamboo ash, present sustainable and effective tools to improve the efficiency of biodegradation processes (Saleh *et al.*, 2025). Moreover, microbiological studies in Yemen have identified substantial contamination in food products (Abdullah *et al.*, 2025a), emphasizing the urgent need to explore beneficial bacteria with potential environmental applications. Regional research on pathogens like *Campylobacter jejuni* also reveals deficiencies in microbial monitoring systems (Al-Bana *et al.*, 2025), underscoring the importance of adopting integrated biotechnological strategies. In this regard, phytochemicals with broad-spectrum antimicrobial activity remain promising candidates for tackling both microbial and environmental challenges (Al-Arnoot *et al.*, 2025).

Therefore, this study seeks to contribute to the field of environmental bioremediation by evaluating the physicochemical characteristics of a selected Yemeni crude oil sample, isolate and identify petroleum hydrocarbon-waste-degrading bacteria, and screening identified bacterial isolates for their ability to degrade petroleum hydrocarbon wastes. This integrated approach aims to provide scientific insight into the local microbial resources that can be harnessed for the remediation of petroleum hydrocarbon wastes in Yemen.

MATERIALS AND METHODS

Crude oil sample collection and analysis

One crude oil sample (about five liters) was provided from the oil drilling site of Safer Oil Company, Marib province, Yemen. Crude oil was collected in a clean sterile container to avoid contamination, stored at low temperature (4 °C), and to avoid light exposure, which can cause photodegradation of certain compounds in the crude oil (Wang *et al.*, 2024). Some physicochemical properties of the crude oil were studied at the Yemeni Petroleum Company (YPC).

Collection of soil samples

About 20 soil samples (50g for each one) were collected from 4 Yemeni governorates (Capital secretariat, Sana'a, Al-Hudaydah, Marib) at different locations. Thirteen sample of contaminated soil with petroleum product were collected from petrol, diesel stations and mechanic work shop, seven soil samples of uncontaminated soil were collected from agricultural and nonagricultural sites. A scoop was used to remove debris of the organic partials from the soil surface. Soil samples were taken from a depth of 10 cm below the surface. Then the soil samples were transferred to an appropriate, labeled and sterile sample container with a sterile laboratory spatula. Samples were stored in a sterile plastic container at room temperature (28±2 C°) in the laboratory until bacteriological studies (Afuwale and Modi, 2012; Obire and Nwaubeta, 2001).

Isolation of petroleum waste-degrading bacteria

Soil bacteria were isolated by using the serial dilution method as described previously (Khalid *et al.*, 2016; Koch, 2010; Mohammad *et al.*, 2021) with some modifications, and the Bushnell and Haas agar media (BHA) was used as a selective medium for isolating petroleum-degrading bacteria (Bushnell and Haas, 1941). BHA medium was cooled to 45-50 °C, and 1%(v/v) of crude oil was added under aseptic conditions, then it was mixed thoroughly (Ekpo and Udofia, 2008).

Identification of bacterial isolates

Morphologically different colonies of bacteria were purified on nutrient agar medium (Ebah *et al.*, 2023; Saleem *et al.*, 2020). Pure cultures of bacteria were transferred to nutrient agar slants and kept in a refrigerator at 4°C for further studies.

The isolated bacteria, which had the ability to degrade petroleum waste, were identified based on cell morphology, staining and biochemical parameters (Bergey, 1994).

Screening of bacterial isolates for their ability to degrade petroleum waste

All identified bacterial isolates were screened for their ability to degrade petroleum waste by using 2,6-Dichlorophenol Indophenol (2,6-DCPIP indicator) colorimetric assay described previously (Bidoia *et al.*, 2010; Hanson *et al.*, 1993) with simple modifications.

All identified bacterial isolates were streaked onto petri dishes containing Nutrient Agar (NA), then incubated at 37 °C for 24 hours (Ebah *et al.*, 2024; Iqbal *et al.*, 2024). Then each isolate was inoculated in a test tube containing 10 ml Nutrient Broth (NB), and incubated at 37 °C for 24 hours. 50µl of each culture was added to assay tubes (duplicated) with 4 ml Bushnell and Haas Broth (BHB) medium, 200µl DCPIP indicator, and 25µl crude oil.

Control assay (C1-3) was used to evaluate interactions between components and the DCPIP indicator. C1 contains DCPIP, BHB medium, and isolated bacteria to determine the influence of DCPIP over time. C2 contains DCPIP indicator and BHB medium. C3 contains DCPIP indicator, BHB medium, and crude oil. All tubes were incubated at 30±1°C for 48 hours. Each tube was observed for DCPIP color change at intervals of 1 hour till 48 hours. The change of DCPIP color from blue (not degraded isolate) to colorless (degraded isolates) over time indicated the ability of identified bacterial

isolates to degrade petroleum hydrocarbon waste.

RESULTS AND DISCUSSION

Crude oil sample with a dark black color and high viscosity in texture showed density (0.8062 g/mL) and flash point temperature (95 °C). In this study, 40 bacterial isolates were isolated from 20 soil samples taken from four different Yemeni governorates using serial dilution techniques on BHA medium containing 1% crude oil as the sole carbon source. In a similar study, 36 bacterial isolates were found to utilize oil as a carbon source (Afuwale and Modi, 2012), and 42 bacterial isolates were found to be capable of utilizing hydrocarbons (Varjani and Upasani, 2013). This may be due to these microorganisms possessing a specific enzyme system that enables them to degrade hydrocarbons and use them as a source of carbon and energy (Panda *et al.*, 2013).

After the isolation of bacteria from study samples, only 26 bacterial isolates were selected for identification to the genus and species level. Our results indicate that 26 bacterial species belonging to 9 genera were identified. *Pseudomonas* spp. was the most common genus, representing 23.07% of identified bacterial isolates. *Bacillus* spp., *Streptococcus* spp., and *Enterococcus* spp. were the next genus common, representing (19.23%), (19.23%), (11.53%) of the identified bacterial isolates, respectively. While the less common species were *Staphylococcus aureus* and *Enterobacter* species, each represents 7.69%, and *Morganella morganii*, *Providencia stuartii*, and *Proteus vulgaris*, each represents 3.84%, for each one of the identified bacterial isolates (Figure 1). A previous study reported that *Bacillus* and *Pseudomonas aeruginosa* were the most prevalent genera (Selvakumar *et al.*, 2014). Another investigation showed that *Bacillus*, *P. aeruginosa*, *Micrococcus*, *Proteus mirabilis*, *P. vulgaris*, and *Enterobacter* species are capable of utilizing petroleum as a carbon source (Balogun *et al.*, 2015). The prevalence of *the Pseudomonas* genus in this study can be explained by the fact that *Pseudomonas* spp. is one of the best crude oil degraders, capable of utilizing hydrocarbons as carbon and energy sources and producing biosurfactants (Adeline *et al.*, 2009; Jahangeer and Kumar, 2013; Mays *et al.*, 2013). *Bacillus* species were widely distributed due to their diversity and ability to grow on different substrates. This genus has the ability to degrade various hydrocarbons by producing surfactants, which facilitate the degradation process. They also produce a variety of enzymes that are involved in biodegradation, enhancing the efficiency of this process (Gopinathan *et al.*, 2012).

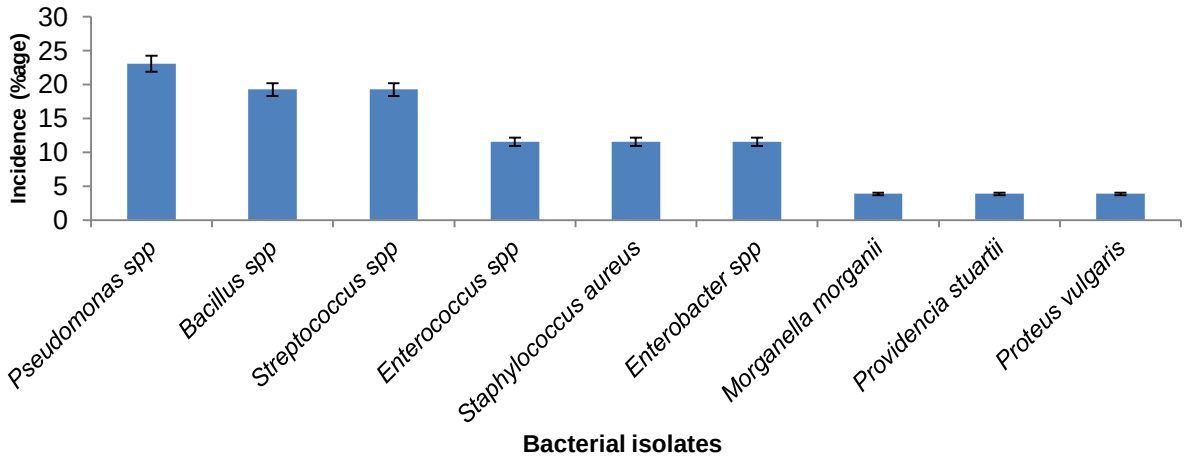

Fig. 1. Percentage of the identified bacterial genera and species during the study.

Table (1) showed the decolorization time of DCPIP in hours by soil-identified bacterial isolates. Results show that all of the bacterial isolates were able to decolorize DCPIP completely in 35 hours of incubation at 30±1 °C. This indicated the ability of these isolates to degrade petroleum waste. Where, 13 (50%) identified bacterial isolates were able to decolorize DCPIP during 3-12 hrs, which had highest ability to degradation, followed by 9 (34.61%) identified bacterial isolates had the moderate ability to degrade petroleum waste 13-24 hrs. and 4 (15.38%) identified bacterial isolates had the lowest ability to degrade hydrocarbons during 24-35 hrs (Figure 2).

Table 1. The decolorization time of DCPIP in hours by soil-identified bacterial isolates.

Bacterial genera \species	Time in hrs.
Enterobacter aerogenes	35
Providencia stuartii	13
Morganella morganii	10
Proteus vulgaris	24
Pseudomonas spp.	13
Enterococcus spp.	8
Pseudomonas spp.	10
Streptococcus spp.	33
Pseudomonas spp.	8
Streptococcus spp.	6
Pseudomonas spp.	9
Pseudomonas spp.	17
Bacillus spp.	14
Bacillus spp.	20
Streptococcus spp.	5
Bacillus spp.	14
Enterococcus spp.	21
Staphylococcus aureus	15
Streptococcus spp.	26
Streptococcus spp.	4
Staphylococcus aureus	11
Pseudomonas spp.	6
Enterobacter cloacae	7
Bacillus spp.	3
Enterococcus spp.	26
Bacillus spp.	7

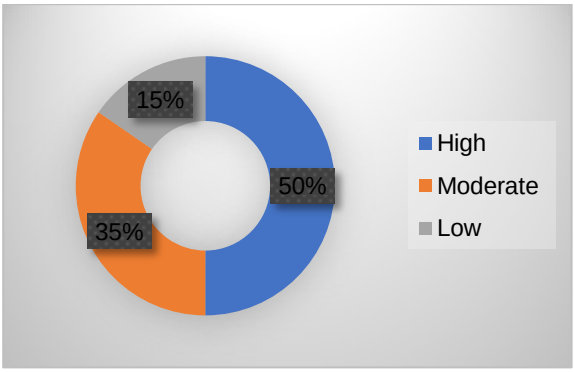

Fig. 2. Percentage of the identified bacterial isolates' ability to degrade petroleum hydrocarbon wastes.

A similar study found that 2 out of 7 isolates completely decolorized DCPIP in about 8 hrs., while 5 other isolates took 10 hrs (Afuwale and Modi, 2012). Another study demonstrated that among 103 bacterial isolates examined for their ability to degrade petroleum waste hydrocarbons using DCPIP, 32 bacterial isolates had the highest ability to degrade petroleum waste hydrocarbons within 5-12 hrs., 5 bacterial isolates had the lowest ability to degrade petroleum waste hydrocarbons (37-48 hrs.), and the rest were in between (Al-Ariki, 2015). The bacterium invades the crude oil compound, which is a rich source of organic compounds suitable for the growth of this bacterium, which also produces biosurfactants that are considered the first stage of the hydrocarbon clastic process (Darsa and Thatheyus, 2014; Gopinathan *et al.*, 2012; Mays *et al.*, 2013).

CONCLUSION

This study concluded that all bacterial genera \species used in this study were able to degrade petroleum hydrocarbon wastes with variable capabilities, especially the genus *Pseudomonas*, *Bacillus,* and *Streptococcus*, which had a high ability to degrade.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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