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Research article

Bioremediation of crude oil pollutants in the soil by *Pseudomonas aeruginosa* and other soil microorganisms

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ABSTRACT

Soil pollution with oily residues is one of the biggest challenges facing the environment. Microorganisms, especially Pseudomonas aeruginosa play an important role in decomposing oil in soil. The current study aims to highlight the role of *P. aeruginosa* and the synergistic effect of other microorganisms in cleaning the soil from oily residues. Here, nine isolates of *P. aeruginosa* were isolated from soil contaminated with oil. Use biochemical methods and VITIK II technology in identifying the isolates. An oil-contaminated soil model was established in the laboratory, and eight experimental groups were used in the current study; group a, sterilized soil mixed with crude oil and *P. aeruginosa* (OD^{600}: 0.8); group b, soil mixed with crude oil and *P. aeruginosa* (OD^{600}: 0.8); group c, sterilized soil mixed with crude oil; group d, soil mixed with 5 ml of crude oil; group e, sterilized soil mixed with waste cooking oil (WCO) and *P. aeruginosa* (OD^{600}: 0.8); group f, soil mixed with WCO and *P. aeruginosa*; group g, sterilized soil mixed with WCO; group h, soil mixed with WCO. The results showed that the highest percentage of decrease in WCO and crude oil was found in the soil group that was not sterilized in the presence of *P. aeruginosa*. A moderate rate of decomposition was also found in soil that had not been sterilized, and also in sterilized soil in the presence of *P. aeruginosa*. The sterilized soil (only) did not show any significant decrease in the WCO and crude oils. It can be concluded that *P. aeruginosa* is capable of decomposing WCO and crude oils and the presence of other soil microorganisms has increased the rate of decomposition of oil in the soil, which confirms the synergistic role of microorganisms and *P. aeruginosa* in decomposing WCO and crude oils.

Keywords: Bioremidation, Crude oil, Waste cooking oil, *Pseudomonas aeruginosa*.

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1. INTRODUCTION

Crude oil contamination has significant impacts on soil quality and long-term soil health. The presence of crude oil leads to changes in soil physicochemical properties, such as increased acidity, reduced cation exchange capacity, and altered mineralization processes [1]. It also affects the microbial community, reducing microbial diversity and biomass [2]. Nitrifying bacteria which play a crucial role in soil fertility, are particularly sensitive to crude oil pollution, with increased activity and abundance in polluted soils [3]. However, the dominance of certain nitrifier genera may shift in polluted soils [4]. Crude oil contamination also affects soil enzymes, such as lipases and catalase, which are early sensitive bioindicators of soil quality [5]. The long-term

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impacts of crude oil pollution on soil health include reduced plant growth, altered soil pH, and changes in nutrient content. It is important to develop effective remediation methods to mitigate the negative effects of crude oil pollution on soil health.

Pseudomonas aeruginosa is a bacterium that has been found in soil samples contaminated with oil residues [6]. It has also been discovered in the soil surrounding the roots of plants and public gardens [7]. Environmental isolates of *P. aeruginosa* have been examined and found to have various antibiotic resistance profiles [8]. Additionally, *P. aeruginosa* has been studied for its potential in the treatment of synthetic and real textile wastewater. *P. aeruginosa* is an important member of the soil microbial community and plays a significant role in the regulation of the agricultural soil ecosystem [9].

P. aeruginosa is effective in treating oil-contaminated areas. The strain *P. aeruginosa* (DKB1) has been shown to have high hydrocarbon degrading potential and can effectively recover hydrocarbon-polluted environments [10]. Another study found that the combination of *P. aeruginosa*, *Achromobacter* sp., and a bio-surfactant crude product resulted in the remarkable degradation of petroleum-contaminated soil and petroleum hydrocarbons in oil sludge [11]. Additionally, *P. aeruginosa* strains have been isolated and identified as capable of n-Hexadecane biodegradation, making them suitable for bioremediation of hydrocarbon-contaminated soils [6]. The present study aims to identify the potential role of *P. aeruginosa* isolated from the contaminated area with crude oil in the bioremediation of crude oil in the laboratory.

1. MATERIALS and METHODS

2.1. Isolation and identification of *P. aeruginosa*

Seventy samples of soil (one gram) were collected from different depths ranging from 1 to 10 cm from soil contaminated with crude oil residues and from different areas in Iraq. The samples were placed in a sterile plastic container and then transported to the laboratory. The soil samples were placed in sterile test tubes and 10 ml of sterile normal saline was added to each tube. The test tubes were shaken gently. 100 µl of suspension was inoculated onto MacConkey agar and Cetrimide agar. Biochemical tests, as well as morphological characteristics of colonies, were used to identify *P. aeruginosa* isolates. The VITEK® 2 Compact system (*BioMérieux*) was used to further identification of *P. aeruginosa* isolates. The isolates were inoculated onto nutrient agar (37 ºC for 18) and stored at 4 ºC for 2 to 4 weeks.

2.2. Experiment

In this experiment, the effect of *P. aeruginosa* on the crude oil that contaminated the soil was evaluated in the lab. Four experimental groups were prepared in 12.5 cm diameter glasses Petri dishes.

- Group a, 50 grams of autoclaved soil (121 ºC, 15 ps, 15 min) mixed with 5 ml of crude oil and 5 ml of overnight growth *P. aeruginosa* in nutrient broth (optical density at 600 was 0.8).
- Group b, 50 grams of soil mixed with 5 ml of crude oil and 5 ml of overnight growth *P. aeruginosa* in nutrient broth (optical density at 600 was 0.8).
- Group c, 50 grams of autoclaved soil (121 ºC, 15 ps, 15 min) mixed with 5 ml of crude oil (control).
- Group d, 50 grams of soil mixed with 5 ml of crude oil (control).
- Group e, 50 grams of autoclaved soil (121 ºC, 15 ps, 15 min) mixed with 5 ml of waste cooking oil (WCO), and 5 ml of overnight growth *P. aeruginosa* in nutrient broth (optical density at 600 was 0.8);
- group f, 50 grams of soil mixed with 5 ml of WCO and 5 ml of overnight growth P. aeruginosa in nutrient broth (optical density at 600 was 0.8);
- Group g, 50 grams of autoclaved soil (121 C, 15 ps, 15 min) mixed with 5 ml of waste cooking oil (WCO) (control).
- Group h, 50 grams of soil mixed with 5 ml of WCO (control).

The plates were incubated for 4 days at 37 ºC. The amounts of crude oil and WCO were measured before and after incubating to evaluate the changes in the levels of crude oil and WCO.

2.3. Total of crude oil and WCO

The standard method of Jayashreel et al. (2012) was followed to measure the amount of remaining oil in the soil that was either treated with bacteria or untreated with bacteria to check the changes in the amount of oil. Briefly, 50 ml of chloroform was added to each soil sample and mixed well. The samples were centrifuged at 6000 rpm for 30 min. The supernatants were collected and put in clean and dried petri dishes. The dishes were dried at 60 ºC for 60 min to evaporate the chloroform [12]. The remaining oil was weighed. The following equation was followed to calculate the percentages of lost oil.

$$lost\ oil\ (\%) = \frac{Wieght\ of\ oil\ before\ treatemnet - Weight\ of\ oil\ after\ treatemnet}{Wieght\ of\ oil\ before\ treatemnet} x100$$

2.4. Statistical analysis

Student t-test was employed to evaluate the changes in the lost oil before and after treatment. The values were presented in mean ±SD.

3. RESULTS

3.1. Bacterial isolates

In the current study, nine isolates of *P. aeruginosa* (Pa1 – Pa9) were isolated and identified. The ability of these isolates to bio-degrade the WCO and crude oil was measured. It was found that isolate number five (Pa5) showed the highest ability to depredate fats as well as crude oil. Therefore, this isolation was used to estimate the ability of *P. aeruginosa* to bio-degrade the crude oil and WCO in the soil under different conditions.

3.2. Role of *P. aeruginosa* in degrading crude oil

Fig. 1 shows the percentage of loss of crude oil after 96 h of incubation at 37 ºC. The results showed that the highest percentage of loss (decomposition) of crude oil was seen in the soil that had not been previously sterilized with autoclave and to which the *P. aeruginosa* was added (26 %) followed by the percentage decrease in crude oil in soil that was not sterilized with autoclave and in which *P. aeruginosa* was not added, which indicates the role of other microorganisms and their enzymes in the soil on the degrade of crude oil (18%). The soil sterilized with autoclave, to which *P. aeruginosa* was added, gave a percentage of decomposition in crude oil reached to 14%. No significant decrease in crude oil was recorded in the soil that was sterilized by autoclaves, and *P. aeruginosa* was not added, which confirms the role of microorganisms in the decomposition of crude oil.

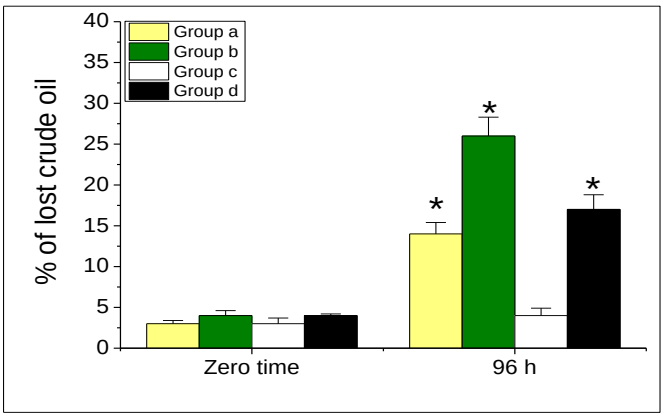

Fig. 1. Percentage of lost crude oil from contaminated soil with crude oil at different time intervals (0 and 96 h). Group a, 50 grams of autoclaved soil (121 °C, 15 ps, 15 min) mixed with 5 ml of crude oil and 5 ml of overnight growth *P. aeruginosa* in nutrient broth (optical density at 600 was 0.8). Group b, 50 grams of soil mixed with 5 ml of crude oil and 5 ml of overnight growth *P. aeruginosa* in nutrient broth (optical density at 600 was 0.8). Group c, 50 grams of autoclaved soil (121 °C, 15 ps, 15 min) mixed with 5 ml of crude oil (control).Group d, 50 grams of soil mixed with 5 ml of crude oil (control). Asterisks indicate a significant difference from the corresponding group at zero time (P<0.05).

3.3. Role of *P. aeruginosa* in degrading WCO

A similar finding was seen in the case of soil that was contaminated (in Lab) with WCO. The results showed that the highest percentage of loss (decomposition) of WCO (40.1 %) was seen in the soil that had not been previously sterilized with autoclave and to which the *P. aeruginosa* was added, followed by the percentage decrease in WCO in soil that was not sterilized with autoclave (no *P. aeruginosa* was not added), which indicates the role of other microorganisms and their enzymes in the soil on the degrade of WCO (20.8%). The soil sterilized with autoclave, to which *P. aeruginosa* was added, gave a percentage of decomposition in WCO reached to 14%. No significant decrease in WCO was seen in the soil that was sterilized by autoclaves (*P. aeruginosa* was not added), which confirms the role of microorganisms in the decomposition of crude oil.

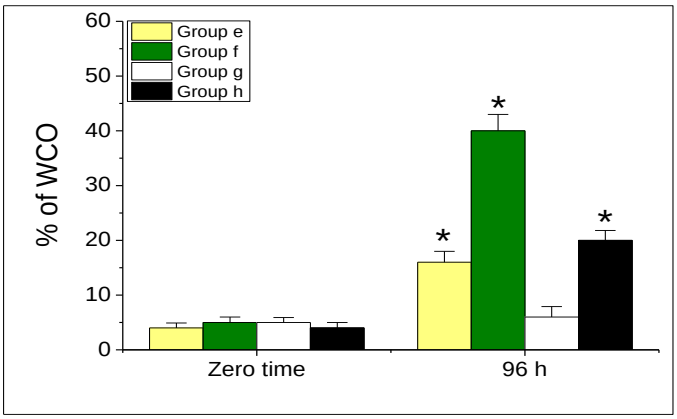

Fig. 2. Percentage of lost waste cooking oil (WCO) from the soil contaminated with WCO at different time intervals (0 and 96 h). Group e, 50 grams of autoclaved soil (121 °C, 15 ps, 15 min) mixed with 5 ml of WCO and 5 ml of overnight growth *P. aeruginosa* in nutrient broth (OD^{600} was 0.8). Group f, 50 grams of soil mixed with 5 ml of WCO and 5 ml of overnight growth *P. aeruginosa* in nutrient broth (OD^{600} was 0.8). Group g, 50 grams of autoclaved soil (121 °C, 15 ps, 15 min) mixed with 5 ml of WCO (control). Group h, 50 grams of soil mixed with 5 ml of WCO (control). Asterisks indicate a significant difference from the corresponding group at zero time (P<0.05).

The results of the current study, as shown in Figs 1 and 2, demonstrated the ability of *P. aeruginosa* bacteria and soil microorganisms to decompose WCO faster and higher than their ability to decompose crude oil.

4. DISCUSSION

There are several microorganisms involved in the decomposition of oil in soil that have been found to have a strong ability to degrade crude oil and its fractions, as well as other hydrocarbon contaminants in soil including bacteria such as *Burkholderia, Mycobacterium, Polaromonas, Pseudomonas, Bacillus anthracis, Bacillus cereus, Achromobacter sp.,* and *P. aeruginosa*, as well as fungi such as *Aspergillus lentulus, Rhizopus arrhizus, Galactomyces geotrichum,* and *Trichoderma virens* [13]. They contribute to the decomposition process by utilizing various metabolic pathways and catabolic genes to break down the hydrocarbons into less harmful compounds. The presence and abundance of these microorganisms in oil-contaminated soil can vary depending on the specific contaminants and their concentrations, but they are capable of effectively removing oil from the environment [14].

In the current study, eight groups of laboratory soil models were used. Sterilized soil by autoclave and non-sterilized soils were contaminated with two types of oils (crude oil and WCO). *P. aeruginosa* was added to some of the soil groups (as shown in the results chapter). The results showed that the *P. aeruginosa* were able to degrade crude oil as well as WCO with good efficiency, but it was noted that the ability of this bacteria to decompose oil was high in non-sterilized soil, which shows the role of microorganisms supporting *P. aeruginosa* in decomposing oils in soil. The results of this study greatly support the possibility of using microorganisms, especially *P. aeruginosa* in cleaning soil from oils.

Microorganisms in soil have specific enzymes and metabolic pathways involved in the decomposition of oil. These enzymes include lipases, dehydrogenases, hydrolases, oxygenases, and isomerases [15]. Lipase-producing microorganisms, such as *P. aeruginosa* have been found to directly degrade waste oil and promote the degradation of oily waste [16]. The metabolic pathways involved in the degradation of oil compounds include the degradation of aliphatics, aromatics, asphaltenes, and waxes. These pathways are utilized by diverse aerobic and anaerobic bacteria, as well as fungi, for the biodegradation of petroleum hydrocarbons. By understanding these enzymes and metabolic pathways, it is possible to develop bacterial consortia for the efficient degradation of oil-contaminated soils.

5. CONCLUSION

The results showed the ability of *P. aeruginosa* bacteria to decompose the crude oil as well as WCO with good efficiency, but it was noted that the ability of this bacteria to decompose oil was high in cooperation with other soil microorganisms, which shows the role of microorganisms supporting *P. aeruginosa* bacteria in decompose the oils in soil. The results of this study greatly support the possibility of using *P. aeruginosa* and other normal soil microorganisms' flora in cleaning soil from oils.

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Conflict of interest

The authors declare that they have no conflict of interests.

Ethical Approval

This review was approved by the Ministry of Oil, Baghdad, Iraq (104, A2023).

Author contributions

Mohammed AJ: Data curation, Investigation; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft. Writing - review & editing.
Kanber SA. Conceptualization, and Formal analysis, Visualization; and Writing - review & editing.
Talib MM: Methodology, Writing - review & editing.
Rasheed HJ: Writing - review & editing.

6. REFERENCES

[1] **Ebulue MM.** (2022) Toxicological Indices of Crude Oil-Polluted Soil Ecosystem. *Saudi J Biomed Res* **7**(6):190-196. https://doi: 10.36348/sjbr.2022.v07i06.001.

[2] **Huang L, Ye J, Jiang K, Wang Y, Li Y.** (2021) Oil contamination drives the transformation of soil microbial communities: Co-occurrence pattern, metabolic enzymes and culturable hydrocarbon-degrading bacteria. *Ecotoxicol Environ Saf* **225**:112740. https://doi.org/10.1016/j.ecoenv.2021.112740.

[3] **Gao Y, Yuan L, Du J, Wang H, Yang X, et al.** (2022) Bacterial community profile of the crude oil-contaminated saline soil in the Yellow River Delta Natural Reserve, China. *Chemosphere* **289**:133207. https://doi.org/10.1016/j.chemosphere.2021.133207.

[4] **Huang L, Ye J, Jiang K, Wang Y, Li Y.** (2021) Oil contamination drives the transformation of soil microbial communities: Co-occurrence pattern, metabolic enzymes and culturable hydrocarbon-degrading bacteria. *Ecotoxicol Environ Saf* **225**: 112740. https://doi.org/10.1016/j.ecoenv.2021.112740

[5] **Ossai IC, Ahmed A, Hassan A, Hamid FS.** (2020) Remediation of soil and water contaminated with petroleum hydrocarbon: A review. *Environ Technol Innov* **17**: 100526. https://doi.org/10.1016/j.eti.2019.100526.

[6] **Deivakumari M, Sanjivkumar M, Suganya AM, Prabakaran JR, Palavesam A, Immanuel G.** (2020) Studies on reclamation of crude oil polluted soil by biosurfactant producing *Pseudomonas aeruginosa* (DKB1). *Biocatal Agric Biotechnol* **29**: 101773. https://doi.org/10.1016/j.bcab.2020.101773.

[7] **Ali AA, Hameed KW, Nadder MI.** (2022) Isolation of Pseudomonas Aeruginosa from soil and production of lipase enzyme. *In IOP Conf. Ser. Earth Environ Sci* **961** (1) 012087. IOP Publishing. https://doi.10.1088/1755-1315/961/1/012087.

[8] **Kaszab E, Radó J, Kriszt B, Pászti J, Lesinszki V, et al**. (2021) Groundwater, soil and compost, as possible sources of virulent and antibiotic-resistant *Pseudomonas aeruginosa*. *Int J Environ Health Res* **31**(7), 848-860. https://doi:10.1080/09603123.2019.1691719.

[9] **Deredjian A, Colinon C, Hien E, Brothier E, Youenou B, et al.** (2014) Low occurrence of Pseudomonas aeruginosa in agricultural soils with and without organic amendment. *Front Cell Infect Microbiol* **4**:53. https://doi: 10.3389/fcimb.2014.00053. PMID: 24809025; PMCID: PMC4010769.

[10] **Jacob EL, Mohan AP, Joseph AV.** (2022) Bioremediation of petroleum-polluted soil using biosurfactant producing bacteria, *Pseudomonas* sp. *J Sci Res* 66(1): 224-231. https://doi:10.37398/JSR.2022.660124

[11] **Ahmad AA, Muhammad I, Shah T, Kalwar Q, Zhang J, et al.** (2020) Remediation methods of crude oil contaminated soil. *World J Agric Soil Sci* **4**(3):1-8. https://doi:10.33552/WJASS.2020.04.000595.

[12] **Jayashree R, Nithya SE, Rajesh PP, Krishnaraju M.** (2012) Biodegradation capability of bacterial species isolated from oil contaminated soil. *J Academia Indust Res* **1**(3), 127-135.

[13] **Liu Q, Tang J, Gao K, Gurav R, Giesy JP.** (2017) Aerobic degradation of crude oil by microorganisms in soils from four geographic regions of China. *Sci Rep* **7**(1): 14856. https://doi.org/10.1038/s41598-017-14032-5

[14] **Ikuesan F.** (2017) Evaluation of crude oil biodegradation potentials of some indigenous soil microorganisms. *J Sci Res Rep* **13**(5): 1-9. https://doi.org/10.9734/JSRR/2017/29151

[15] **Das N, Bhuyan B, Pandey P.** (2022) Correlation of soil microbiome with crude oil contamination drives detection of hydrocarbon degrading genes which are independent to quantity and type of contaminants. *Environ Res* **215**: 114185. https://doi.org/10.1016/j.envres.2022.114185.

[16] **Zouaoui B, Bouziane A.** (2012) Isolation, purification and properties of lipase from Pseudomonas aeruginosa. *Afr J Biotechnol* **11**(60): 12415-12421. https://doi:10.5897/AJB11.3951.

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