AJMAS
https://journal.utripoli.edu.ly/index.php/Alqalam *eISSN 2707-7179*

Original article

Bioremediation of Crude Oil Residues in Libyan Agricultural Lands

Mona Allafe[1]*, Entesar Omar[2], Abdullah Abdullah[3], Fayiz Mohammed[1]

[1]*Department of Environmental Sciences, Faculty of Natural Resources and Environmental Sciences, Tobruk University, Libya*
[2]*Department of Botany, Faculty of Science, Tobruk University, Libya*
[3]*Department of Natural Resources, Tobruk University, Libya*

ARTICLE INFO
Corresponding Email. *mona.allafa@tu.edu.ly*
Received: *23-09-2022* ***Accepted:*** *09-10-2022* ***Published:*** *13-10-2022*
Keywords: *Bioremediation, Hydrocarbon, Crude Oil, Soil Contamination, Paenibacillus Macerans*

ABSTRACT

Background and aims. *Libya like other most of the oil-producing countries in the world suffers from many environmental problems that negatively affect the balance of the ecosystem. Petroleum hydrocarbons are the most important of these problems and the main pollutants that increase the damage to the ecosystem, whether the pollution of waters seas, and oceans, which affected biodiversity, which also reached large areas of agricultural land, which led to the deterioration of biodiversity. The microbial treatment of these petroleum hydrocarbons is one of the economic and environmental methods trends currently used, where the bioremediation technology using bacteria has proven to be a safe, effective, and environmentally friendly method compared to other chemical methods. Thus, it can be considered one of the best techniques for dealing with soil contaminated with oil and replanting it again with crops.* ***Methods***. *This experiment was conducted in wooden boxes to evaluate the role of Paenibacillus macerans in the disinfection of agricultural land affected by petroleum hydrocarbons. Barley seeds were grown in contaminated soil with a concentration of 10% of crude oil, and bacteria were added at sowing, and 20 days pre-sowing, compared to control (uncontaminated soil). The germination and growth characteristics of barley seedlings were studied as one of the indicators of the ability of Paenibacillus macerans bacteria to disinfect biodegrade crude oil pollutants.* ***Results.*** *The results showed the positive effect of the bacterial strain and the ability to biodegrade petroleum hydrocarbons and the success of barley cultivation in contaminated soil up to a concentration of 10% of these petroleum residues.* ***Conclusion.*** *Bacteria Paenibacillus macerans has ability to decompose petroleum pollutants resulting from the extraction and transportation of petroleum oil and its hydrocarbon derivatives. This allow us to successfully cultivate contaminated farmland again.*

Cite this article: *Allafe M, Omar E, Abdullah A, Mohammed F. Bioremediation of Crude Oil Residues in Libyan Agricultural Lands. Alq J Med App Sci. 2022;5(2):498-502.* https://doi.org/10.5281/zenodo.7191167

INTRODUCTION

Most of the oil-producing countries in the world suffer from many environmental problems that negatively affect the balance of the ecosystem. Petroleum hydrocarbons are the most important of these problems and the main pollutants that increase the damage to the ecosystem, whether the pollution of waters seas and oceans, which affected biodiversity, which also reached large areas of agricultural land, which led to the deterioration of biodiversity [1,2]. Libya, like the rest of the world's oil-producing countries, has been affected by it, which is evident in the increase in the areas of agricultural land and pastures, in which plant life has been completely lost. Thus, in order to develop these lands again, it was necessary to develop alternative and environmentally friendly techniques to remove these pollutants from the soil, and the microbial treatment of these petroleum hydrocarbons is one of the economic and environmental methods trends currently used, as it was found that the main components of crude oil are degradable through the use of microbes (Some types of bacteria, algae, and fungi) where the bioremediation technology using bacteria has proven to be a safe, effective and environmentally friendly method compared to other chemical methods. Thus, it can be considered as one of the best techniques for dealing with soil contaminated with oil and replanting it again with crops.

Bioremediation is one of the methods used to treat pollution from hydrocarbons in the soil resulting from leakage from oil production pipelines and increased human activities, which are the main components of crudest oils and are characterized

by their susceptibility to decomposition. Many different types of microbes have been used, such as bacteria, yeast and fungi. Where the performance and efficiency of the biological analysis of these microorganisms varied, we find that it ranged between 6% to 82% when using fungi, while the efficiency of marine bacteria reached 100% [3-5]. As it is well known that there are many microorganisms that have a high ability to use hydrocarbons as a source of carbon and energy [6,7].
The microbe used varies according to the composition of the hydrocarbons found in the polluted soil. There are 4 main types of hydrocarbons: alkanes, alkenes, alkynes and aromatic hydrocarbons, all of which differ in their bonding properties [8,9]. The aromatic hydrocarbons (naphthalene, phenanthrene, anthracene, pyrene, dibenzoanthracene, benzopyrene) are the most important and dangerous pollutants in soil and water environments because they contain many cytotoxic, mutagenic and possibly carcinogenic compounds [10,11].
But the biodegradation of crude oil is a complex process that mostly depends on the nature and quantities of hydrocarbons present in the soil, of all the microbes used, bacteria are the most active and successful agents in the biodegradation of petroleum. Despite this, the most important limiting factor for completing this process in the environment is the limited availability of microorganisms, as the efficiency of performing that microbial inoculation in soil and sediments for biological treatment and pollution control is affected by some factors such as the concentration of active inoculants, the interaction between inoculating microorganisms, indigenous populations, the nutrient supply of the target microorganisms, and the type of hydrocarbon targeted and present in the soil [12,13].
The microbial world is the largest source of biodiversity which exists in diverse ecologies, which play a great promise because the role of microbes in nutrient cycling, environmental detoxification [14,15]. *Paenibacillus* which formerly known as *Bacillus* has attracted considerable interest because of its great usage in sustainable agriculture.
In 1991, according to Ash C., a more accurate representation relationship among these bacteria was attained when the 16S rRNA gene was sequenced for standard strains from 51 species and then identified as *Bacillus*.
The *Paenibacillus* is almost a *Bacillus* Comparative 16S rRNA, which contains various species such as *B. macerans, B. alvei, B. gordonae, B. amylolyticus, B. azotofi xans, B. larvae, B. macquariensis, B. pabuli, B. polymyxa, B. pulvifaciens and B. validus* [16]. *Paenibacillus* species can be utilized in the removal environmental contaminants, through bioflocculation or enzymatic activities, which are often used in wastewaters or at sites of environmental spills where they produce biological flocculants consisting of sugars, proteins, or other *macromolecules. P. jamilae, P. macerans*, P. *polymyxa* and *P. validus* strains have been shown to enhance the biomagnification of heavy metal ions or acidic pigments, through the production of various enzymes that metabolize aliphatic and aromatic organic pollutants, including dehydrogenase and ligninlytic enzymes [17,18]. *Paenibacillus* strains can also degrade pollutants derived from extracting, refining, and transporting petroleum including crude oil [4] and the polycyclic aromatic hydrocarbons (PAHs) [19]. So we illustrate the role of microbial treatment of petroleum hydrocarbon residues in agricultural lands, and the use of bioremediation technology using *Paenibacillus macerans* bacteria as one of the safe, effective and environmentally friendly methods for disinfecting oil-contaminated soil and re-cultivating it again with crops.

METHODS

Experiment

Experiments were conducted in Tobruk University, Faculty of Natural Resources and Environmental Sciences, the Department of Environmental Sciences, Libya, to study the effect of bacteria *Paenibacillus macerans* on the disinfection of agricultural lands contaminated with petroleum residues and the extent of their ability to biodegrade these hydrocarbon compounds in the soil and the extent to which they can be grown again, barley was grown as one of the important crops and as an indicator of the effect of bacteria.
The one m^2 wooden boxes were prepared and the height of the soil was 25 cm, the soil was treated as follows: 1). Soil contaminated with crude oil at a concentration of 10%, as a control without addition bacteria. 2). Soil contaminated with crude oil at a concentration of 10%, with the addition of bacteria *Paenibacillus macerans* when sowing. 3). Soil contaminated with crude oil at a concentration of 10%, with the addition of bacteria *Paenibacillus macerans* pre 20 days of sowing. Barley (Mexican variety) was grown using a completely random design in 3 replicates during the 2020-2021 season, each replicate containing 100 grains.

Measurements

Morphological characteristics included Germination (%), feather and Root length according to AOSA [20], leaf area (cm^2) according to Thomas [21], wet weight (gr.).

Statistical analysis

The results were statistically analyzed according to Gomez and Gomez [22], by analysis of variance (ANOVA), using L.S.D at 5% for comparison among different factors by using the SPSS program.

RESULTS

Barley germination

The results showed in table (1) the positive role of bacteria *Paenibacillus macerans* usage in the process of biodegradation of hydrocarbons, which was evident in the success of the germination process of barley in the ponds to which bacteria were added, but on the contrary, the percentage of germination was 0% in the control treatment (without adding bacteria). It was also noted that the addition of bacteria to the soil 20 days before planting led to an increase in the germination rate by 25% over the germination rate of barley which has been sowed with the addition of bacteria, which amounted to 69%.

Furthermore, we had found that adding the bacteria *Paenibacillus macerans* to contaminated soil 20 days pre barley planting also led to faster germination of barley grains than the soil in which bacteria were added when planting, where barley seedlings appeared after 4-7 days in the first treatment, but it took about 12-15 days in the second treatment. This shows that adding bacteria 20 days pre sowing has led to the biodegradation of hydrocarbons and the soil is ready for cultivation.

Table 1. Germination energy (%) and germination force (%) of barley grains under planting in contaminated soil by crude oil

Treatment	**Number of days after planting**												**Germination**
	4	5	6	7	8	9	10	11	12	13	14	15	(%)
Without bacteria (control)	×	×	×	×	×	×	×	×	×	×	×	×	0%
Bacteria *Paenibacillus macerans* when sowing	18	36	31	9	×	×	×	×	×	×	×	×	94%
Bacteria *Paenibacillus macerans* pre 20 days of sowing	×	×	×	×	×	×	×	×	12	27	19	11	69%

Table 2. Barley seedling vigor under planting in contaminated soil by crude oil (petroleum hydrocarbons).

Treatment	**Root length (cm)**	**Feather length (cm)**	**Wet weight (gr)**	**Leaf Area (cm^2/plant)**
Without bacteria (control)	0.0	0.0	0.0	0.0
Bacteria *Paenibacillus macerans when sowing*	6.65	8.04	7.0	1.7
Bacteria *Paenibacillus macerans* pre 20 days of sowing	8.71	9.24	14.4	3.3

DISCUSSION

This study demonstrates the possibility of biodegradation of pollutants derived from petroleum extraction, refining, and transportation, including crude oil, by the *Paenibacillus spp*., which was reported by Almeida [4] and Haggblom [19], that the crude oil and polycyclic aromatic hydrocarbons (PAHs) degrade by strains of the genus *Paenibacillus*. This was evident in the disinfection of oil-contaminated soil when bacteria were added and make suitable for cultivation again, which had a positive effect on the germination of barley grains when planted in this soil.

On the other hand, another studies reported that many Paenibacillus species promote crop growth through nitrogen fixation, phosphate dissolution, and production of Indole Acetic Acid (IAA) and other plant hormones [23,24]. Auxin, in addition to increasing the ability of plants to resist insects and plant pathogens, which positively affected the growth characteristics of barley seedlings.

CONCLUSION

According to what has been achieved through experiments, it is clear the ability of bacteria *Paenibacillus macerans* to decompose petroleum pollutants resulting from the extraction and transportation of petroleum oil and its hydrocarbon derivatives, and the possibility of re-cultivating lands that have been contaminated with such waste.

Acknowledgement

The authors extend their sincere thanks and gratitude to Tobruk University, Libya. Also, we wish to acknowledge the chairman and members of the Environmental Sciences Department, Faculty of Natural Resources and Environmental Sciences, and Botany Department, Faculty of Science.

Disclaimer

The article has not been previously presented or published, and is not part of a thesis project.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

REFERENCES

1. Adeola, AO, Akingboye A S, Ore OT, Oluwajana OA, Adewole AH, Olawade, DB, Ogunyele AC. Crude oil exploration in Africa: socio-economic implications, environmental impacts, and mitigation strategies. Environment Systems and Decisions. 2021;1-25.
2. Kuch S, Bavumiragira JP. Impacts of crude oil exploration and production on environment and its implications on human health: South Sudan Review. International Journal of Scientific and Research Publications (IJSRP). 2019; 9(4):247-256.
3. Abha S, Singh CS. Hydrocarbon pollution: effects on living organisms, remediation of contaminated environments, and effects of heavy metals co-contamination on bioremediation. Introduction to enhanced oil recovery (EOR) processes and bioremediation of oil-contaminated sites. 2012; 186-206.
4. Almeida PFD, Moreira R, Almeida RCDC, Guimaraes A, Carvalho A, Quintella C. Selection and application of microorganisms to improve oil recovery. Engineering in life sciences. 2004;4(4):319-325.
5. Bento FM, Camargo FA, Okeke BC, Frankenberger WT. Comparative bioremediation of soils contaminated with diesel oil by natural attenuation, biostimulation and bioaugmentation. Bioresource technology. 2005;96(9):1049-1055.
6. Jyothi K, Babu KS, Clara N, Kashyap A. Identification and isolation of hydrocarbon degrading bacteria by molecular characterization. Helix. 2012;2(1):105-111.
7. Pornsunthorntawee O, Wongpanit P, Chavadej S, Abe M, Rujiravanit R. Structural and physicochemical characterization of crude biosurfactant produced by Pseudomonas aeruginosa SP4 isolated from petroleum—contaminated soil. Bioresource Technology. 2008;99(6):1589–1595.
8. Chen Q, Li J, Liu M, Sun H, Bao M. Study on the biodegradation of crude oil by free and immobilized bacterial consortium in marine environment. PloS one. 2017;12 (3):e0174445.
9. Atlas RM, Bartha R. Hydrocarbon biodegradation and oil spill bioremediation. Advances in microbial ecology: Springer; 1992. 287-338.
10. Lim H, Sadiktsis I, de Oliveira Galvão MF, Westerholm R, Dreij K. Polycyclic aromatic compounds in particulate matter and indoor dust at preschools in Stockholm, Sweden: Occurrence, sources and genotoxic potential in vitro. Science of the Total Environment. 2021;755, 142709.
11. Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnol Res Int. 2011;2011:941810.
12. Bharathi B, Gayathiri E, Natarajan S, Selvadhas S, Kalaikandhan R. Biodegradation of crude oil by bacteria isolated from crude oil contaminated soil–a review. International journal of development research. 2017;7 (12):17392-17397.

13. Tsai P-J, Shieh H-Y, Lee W-J, Lai S-O. Health-risk assessment for workers exposed to polycyclic aromatic hydrocarbons (PAHs) in a carbon black manufacturing industry. Science of the total environment. 2001;278(1-3):137-150
14. Ash C, Priest FG, Collins MD. Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Antonie van leeuwenhoek. 1993; 64(3):253-260.
15. Lal S, Tabacchioni S. Ecology and biotechnological potential of Paenibacillus polymyxa: a minireview. Indian Journal of Microbiology. 2009; 49(1):2-10.
16. Ash C, Farrow J, Wallbanks S, Collins M. Phylogenetic heterogeneity of the genus Bacillus revealed by comparative analysis of small‐subunit‐ribosomal RNA sequences. Letters in Applied Microbiology. 1991;13(4):202-206.
17. Abbasian F, Lockington R, Mallavarapu M, Naidu R. A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. Applied biochemistry and biotechnology. 2015;176 (3):670-699.
18. Haritash A, Kaushik C. Biodegradation aspects of polycyclic aromatic hydrocarbons (PAHs): a review. Journal of hazardous materials. 2009;169(1-3):1-15.
19. Hilyard EJ, Jones-Meehan JM, Spargo BJ, Hill RT. Enrichment, isolation, and phylogenetic identification of polycyclic aromatic hydrocarbon-degrading bacteria from Elizabeth River sediments. Appl Environ Microbiol. 2008 Feb;74(4):1176-82.
20. AOSA. Contribution No. 32 to the Handbook on Seed Testing. AOSA Springfield, IL, 1983.
21. Thomas H. The growth response of weather of simulated vegetative swords of single genotype of lolium perenne. J Agric Sci Camb. 1975; 84:333-343.
22. Gomez KA, Arturo AG. Statistical Procedures for Agricultural Research. John wiley & sons, 1984.
23. Sharma SB, Sayyed RZ, Trivedi MH, Gobi TA. Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. Springer Plus. 2013;2(1):1-14.
24. Grady EN, MacDonald J, Liu L, Richman A, Yuan ZC. Current knowledge and perspectives of Paenibacillus: a review. Microbial cell factories. 2016;15(1):1-8.