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Bioremediation of Petroleum Hydrocarbon-Contaminated Soil Using Mangrove Species

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Abstract

This study aimed to assess the extent of petroleum hydrocarbon contamination in soils from the Zubair oil field and evaluate the efficacy of mangrove-mediated bioremediation. Soil samples were collected from four distinct locations within the field, designated Zubair1, Zubair2, Zubair3, and Zubair4. Two experimental conditions were established for each location: soils amended with NPK fertilizer and unamended control soils according to the fertilizer recommendation. Mangrove seeds were sown in all treatments and cultivated for a period of six months. Following the growth period, 100 g of soil from each treatment was subjected to solvent extraction to quantify the residual hydrocarbon concentration and determine the percentage of biodegradation. The results demonstrated statistically significant differences in biodegradation efficiency between fertilized and non-fertilized soils. The highest biodegradation rate, 77%, was observed in the fertilized soil from Zubair2. Conversely, the lowest rate, 51%, occurred in the unfertilized soil from Zubair4. These findings indicate that NPK fertilization enhances the bioremediation potential of mangroves in petroleum-contaminated soils, with Zubair2 exhibiting the greatest remediation capacity.

Keyword: Contamination oil soil, Biodegradation Mangrove.

Introduction

Industrial and agricultural activities, along with the rapidly increasing global population growth rate, contribute to the rising demand for oil production to obtain energy. (Singha and Pandey, 2021). Bioremediation involves the biological conversion, decomposition, or transformation of organic pollutants by microorganisms such as bacteria, fungi, or plants, or their metabolic byproducts such as enzymes, to remove the toxicity of environmental pollutants (Huang *et al.*, 2019). Also adding items (such as

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fertilizers or other growth stimulants) to contaminated environments to speed up natural biodegradation processes is known as bioremediation Environmental Protection Agency (EUA, 2008).

Due to their ability to store organic carbon, mangrove forests may contribute considerably to carbon emissions when polluted, degraded, or deforested (Hamilton and Friess, 2018). Oil spills can occur anywhere in the oil production chain and cause significant environmental and health damage, making them one of the most catastrophic types of pollution. Therefore, mangrove trees are among the best plants for bioremediation. (Santos *et al*., 2011).

Phytoremediation, which involves the symbiosis between plants and microorganisms in the root zone, is a very common method for rehabilitating contaminated soil. However, its effectiveness depends on the type of pollutant, the type of soil, the types of plants used, and the presence of microorganisms that break down hydrocarbons, thus facilitating the removal of those pollutants. (Afzal *et al*., 2011; Khan *et al*., 2013; Zheng *et al*., 2018). Bioremediation is an effective, affordable and eco-friendly technology that has been gaining favor for the mineralization of organic pollutants in wastewater and soil settings (Aragaw, 2021). Bioremediation of PHC contaminated soil is of special attention for academics since it is ecofriendly (Sayara and Sanchez, 2020).

The microbial organisms change the substance via metabolic or enzymatic activities. The underlying mechanisms are two. Growth and cometabolic. Organic contaminant as the only source of carbon and energy for growth. The process leads to mineralization (degradation) of the organic contaminants. Cometabolism is the metabolism of an organic substance in the presence of a growth substrate, which is the principal source of energy and carbon (Roy *et al*., 2018). Biodegradation process is affected by different microorganisms as bacteria, fungus and yeasts. Reports on the function of algae and protozoa in biodegradation are rare (Fritsche and Hofrichter, 2008). Despite the fact that the processes themselves can vary substantially, the final product of biodegradation processes is frequently carbon dioxide (Das and Chandran, 2011). Organic material may be decomposed either aerobically in the presence of oxygen or anaerobically in the absence of oxygen (Pramila *et al*., 2012). Soil bacterial diversity index and soil total organic carbon, total nitrogen, dissolved organic carbon, ammonium nitrogen, available phosphorus, available potassium content and soil pH value had significant positive correlation. (*Guo et al*., 2010).

A large number of studies have established the effectiveness of bioremediation approaches using bacterial, fungal and rhizosphere microbiome species singly or in combination for the mineralization of PHC-contaminated soils over decades. Moreover, these biological treatments are still under development and need greater understanding. This thorough analysis explores the complicated field of bioremediation as a sustainable and efficient technique for the treatment of PHC polluted soil. The study discusses the importance of microbes in remediation and reviews the ideas and processes that govern the process. The study therefore explores the basic basis of bioremediation, revealing the quiet stars, microbes, whose minute talents underlie ecological restoration, and uncovering the intricate processes involved. This study takes us beyond commonplace analysis to the forefront of scientific innovation and environmental responsibility, demonstrating the changing story of our quest to heal the world.

The process of detoxifying environmental toxins by the biological conversion, disintegration, or transmutation of organic contaminants by microorganisms (fungi and bacteria), plants, or their metabolites (enzymes) is known as bioremediation (Huang *et al.*, 2019). It is the partial or total mineralization of the petroleum-hydrocarbon-contaminated soil. Based on the use on the site, the bioremediation approaches are classified as ex situ and in situ. The most common ex situ bioremediation techniques include landfills, bioreactors, decomposition and land cultivation. In-situ bioremediation methods include natural attenuation, bioventing, phytoremediation, biostimulation (adding of microbial growth stimulating compounds) and bioaugmentation (addition of microbial inoculation) (Chaudhary and kim, 2019). The use of in-situ bioremediation methods is especially ecologically benign and financially efficient. Microbes may survive in extreme situations and enhance the natural conditions of soil, water and detritus (Bala *et al.*, 2022).

Materials And Methods

Sample Collection

Petroleum hydrocarbon-contaminated soil samples were collected using a sterilized spade and placed in plastic containers, which were pre-cleaned with cotton wool saturated with 70% ethanol. Composite (Goutx and Saliot, 2019) samples were prepared by combining soils from four distinct sampling sites post-excavation. The collected material was subsequently transported to the Environmental Microbiology Laboratory, University of Basra, for analysis. All soil samples were obtained from various locations within Basra Governorate, Iraq, specifically from four sites in the Zubair oil field: Zubair 1, Zubair 2, Zubair 3, and Zubair 4. All sites were characterized by soils contaminated with petroleum hydrocarbons.

Extraction of petroleum hydrocarbons from contaminated soil

Petroleum hydrocarbons were extracted from the soil matrix according to the protocol described by. The wet soil samples were initially desiccated in a freeze-drier for 24 hours. The dried samples were then pulverized using a mechanical mortar and pestle and homogenized by sieving through a 62 µm mesh. A 100 g aliquot of the dried, ground, and sieved soil was placed in an extraction thimble. A total of 150 mL of a 1:1 methanol : gasoline mixture was added, and extraction was performed using a Soxhlet apparatus for 24 hours. The extract was subsequently cooled and concentrated to a final volume of 10 mL via rotary evaporation, followed by saponification.

The concentrated extract was transferred to a separating funnel with 50 mL of n-hexane, agitated vigorously, and allowed to separate. Extractable lipids were solubilized in methanol for 2 hours with four additions of an aqueous potassium hydroxide solution, after which 75 mL of a 1:1 methanol: gasoline mixture was added. This resulted in the formation of two distinct phases: a lower saponified layer containing fatty acids, and an upper non-saponified layer containing the petroleum hydrocarbons. The lower phase was discarded, and the upper phase was subjected to chromatographic fractionation.

The fractionation column was packed, from bottom to top, with glass wool, silica gel, alumina, and a layer of anhydrous sodium sulfate. The aliphatic fraction was eluted with 30 mL of n-hexane, and the aromatic fraction was subsequently eluted with 30 mL of benzene. The eluates were dried using a rotary evaporator, and the residue was reconstituted in 5 mL of n-hexane in preparation for Gas Chromatography (GC) analysis.

Preparing soil mixtures for agricultural experiment

A 1000 g subsample of contaminated soil was homogenized with an equivalent mass of uncontaminated soil, and the total concentration of petroleum hydrocarbons was quantified. Subsequently, 1000 g of each contaminated soil sample was weighed and mixed. with 1000 g of uncontaminated agricultural soil at a 1:1 ratio. Fertilizer Recommendation: The recommended fertilizer for mangrove plants is applied at a level of 0.261 gm of urea per plant, dissolved in 350 ml of water (field capacity water). Then, 0.396 gm of superphosphate per plant, dissolved in the field capacity water, is added. Finally, 0.128 gm of potassium sulfate, also dissolved in the field capacity water, is added and mixed together. This quantity of NPK fertilizer is divided into two applications; the first application is applied every three months, followed by the second application.

The soils were homogenized, and a recommended dose of NPK fertilizer was applied to the treatment group. Control replicates were maintained without NPK amendment. The treated soils from the four oil fields were placed in planting vessels, with three replicates established for each treatment. Mangrove seeds were sown, and the soils were irrigated to maintain optimal moisture content, while a set of unamended controls was left untreated. After a six-month growth period, 100 g subsamples were collected from each treatment and analyzed to determine the residual concentrations of petroleum hydrocarbons. The efficacy of bioremediation mediated by mangrove was evaluated by comparison with the untreated control samples.

Statistical Analysis

Data was examined using the statistical analysis software SPSS (2021), with a completely randomized design (CRD). The differences between the treatments were tested for significance at the ($P \leq 0.05$) level using the Least Significant Difference (L.S.D.) test.

Results and Discussion

One hundred grams of dry, petroleum hydrocarbon-contaminated soil were obtained from each of the four sites in the Zubair oil field, Basra. A notable variation in total petroleum hydrocarbon (TPH) concentrations was observed among the sites. The lowest TPH concentration was recorded in the Zubair 2 field at approximately 78 mg/L, followed by the Zubair 3 field at 80 mg/L. The Zubair 1 and Zubair 4 fields exhibited higher concentrations, at 88 mg/L and 86 mg/L, respectively Before the mangrove bioremediation process, as detailed in Table(1)

Table (1): Total concentrations of petroleum hydrocarbons

Location	Z1	Z2	Z3	Z4
Concentration of hydrocarbons	88mg/L	78mg/L	80mg/L	86mg/L

The variability in total crude oil concentrations is attributed to several factors, including the volume of oil extracted, the physicochemical properties of the crude oil, the extent of oil spills, and the duration of extraction activities across the four fields. Regarding soil bio reclamation and the efficacy of biodegradation following a six-month phytoremediation period with mangrove, the highest biodegradation efficiency of 77% was achieved in the Zubair 2 field soil amended with NPK fertilizer. This was followed by the Zubair 3 field with a 72% degradation rate. In contrast, the Zubair 1 and Zubair 4 fields exhibited lower efficiencies of 62% and 56%, respectively, under NPK-amended conditions. In the absence of NPK fertilization, biodegradation rates were consistently lower across all sites: 66% for Zubair 2, 64% for Zubair 3, 52% for Zubair 1, and 51% for Zubair 4. These findings, illustrated in Figures 1 and 2, indicate that NPK biostimulation significantly enhances the phytoremediation potential of mangrove in PHC-contaminated soils.

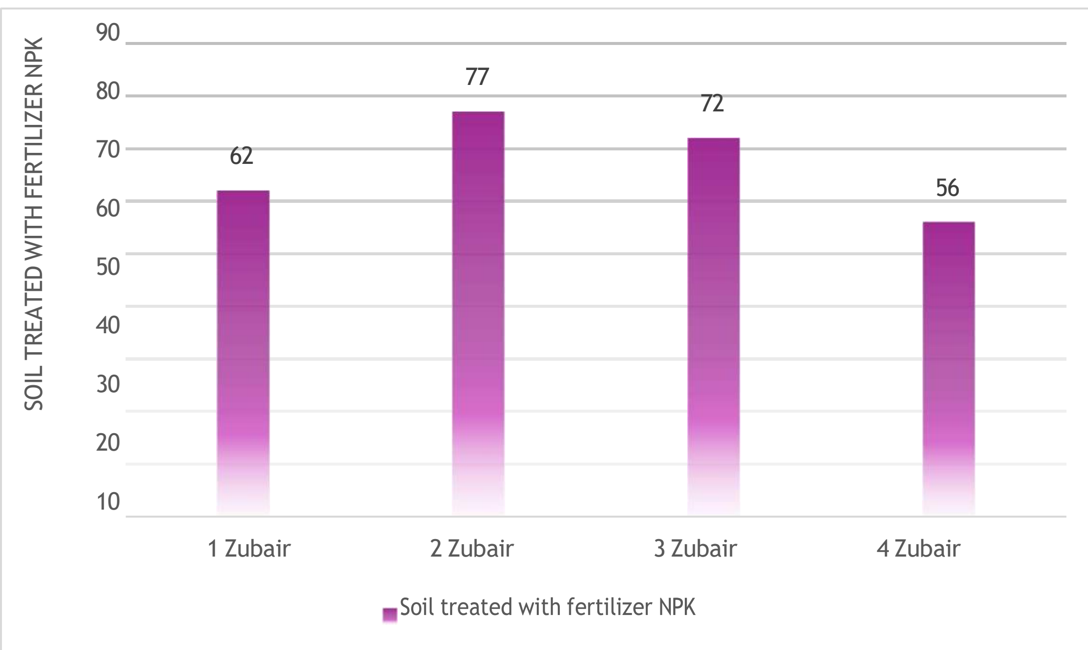

Figure (1): Soil treated with fertilizer NPK.

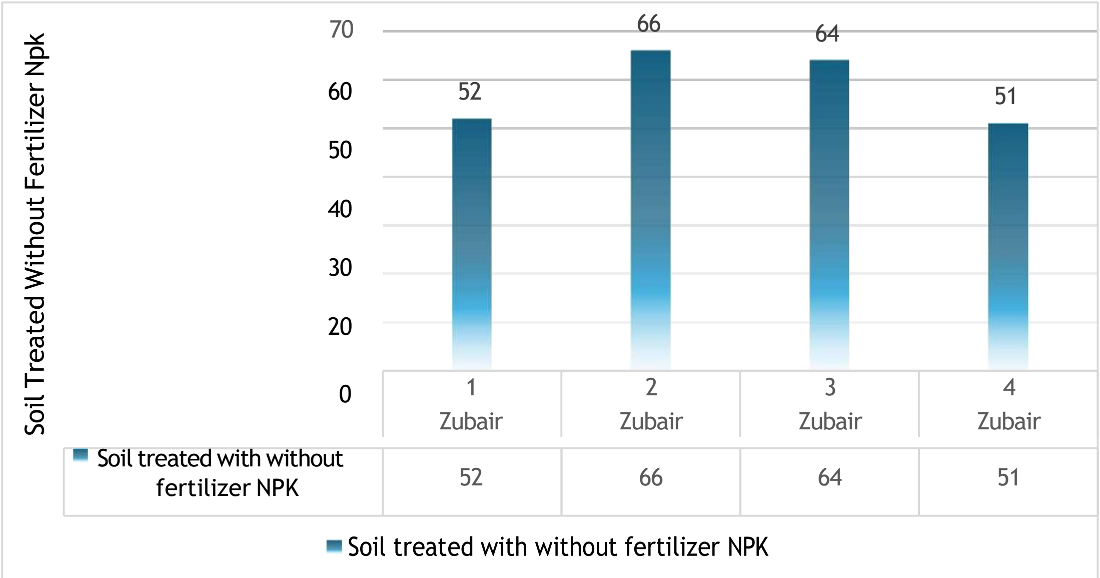

Figure (2): Soil treated without fertilizer NPK.

Fertilizer amendments to improve soil texture, provide the plant with the required nutrients from major elements, and increase the secretion of root hormones, in addition to enzymes secreted by microorganisms. They propagate root microbes that collect organic carbon from oil pollution decomposing into carbon and water. This reveals that phytoremediation helps biodegrade and reclaim crude oil-contaminated soil. The application of fertilizer amendments serves multiple functions: it improves soil physicochemical properties, provides essential macronutrients to the plant, and stimulates the secretion of root exudates and microbial enzymes. These conditions promote the proliferation of rhizosphere microorganisms, which metabolize organic carbon from petroleum contaminants, ultimately mineralizing them to CO_2 and H_2O. These findings substantiate the role of phytoremediation, in conjunction with mangrove, as an effective strategy for the biodegradation and reclamation of crude oil-contaminated soils (Lin *et al.*, 2020; Tsai *et al.*, 2025).

Furthermore, biostimulants introduced into the soil matrix provide essential nutrients that enhance microbial metabolic activity and growth. Organic amendments, such as plant residues, and inorganic nitrogen sources, including ammonium nitrogen, amino acids, and amides, are readily hydrolyzed and can substantially augment soil nutrient availability. Consequently, nutrient limitation such as nitrogen (N), phosphorous (P) and potassium (K) strongly limits the development of microbes and nutrient supply is important to enhance the degradation of petroleum in contaminated soils (Wu, 2016; Deng *et al.*, 2025). Therefore, choosing appropriate plants for petroleum hydrocarbon-contaminated soil restoration is crucial. For example, native plants that are resistant to petroleum hydrocarbons are more economically and ecologically sustainable than alien plants for recovering petroleum hydrocarbon-contaminated lands (Correa *et al.*, 2018; Hoang *et al.*, 2021; Singha and Pandey, 2021).

The results of the statistical analysis in Figure (1) showed significant differences ($p \leq 0.05$) between the different treatments, as the treatment with NPK fertilizer recorded a significant increase in removal of petroleum hydrocarbon concentrations compared to the treatment not treated with NPK fertilizer As shown in Figure 2.

Conclusion

Phytoremediation is a suitable, operationally simple, low-maintenance, environmentally benign, and cost-effective technology for hydrocarbon-contaminated soils. The experimental results demonstrated that mangrove possesses a significant capacity for the biodegradation of petroleum-contaminated soils. Therefore, in-situ treatment using plants and associated microorganisms represents an optimal approach, as it preserves soil structure, minimizes secondary pollution, and yields reclaimed soil that is suitable for agricultural use and enriched with carbon derived from the biodegradation process.

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المعالجة الحيوية للتربة الملوثة بالهيدروكربونات النفطية باستخدام أنواع أشجار المانغروف

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المستخلص

هدفت هذه الدراسة إلى تقييم مدى تلوث التربة بالهيدروكربونات النفطية في حقل الزبير النفطي، وتقييم فعالية المعالجة الحيوية باستخدام أشجار المانغروف. جُمعت عينات التربة من أربعة مواقع مختلفة داخل حقل، زبير 1، زبير 2، زبير 3، وزبير 4. وُضعت لكل موقع تجربة، تربة مُعالجة بسماد NPK، وتربة ضابطة غير مُعالجة. زُرعت بذور المانغروف في جميع المعاملات، لمدة ستة أشهر. بعد انتهاء فترة النمو، اخذ 100 غرام من التربة من كل معاملة لاستخلاص بالمذيبات لتحديد تركيز الهيدروكربونات المتبقية، ونسبة التحلل الحيوي. ظهرت النتائج فروقًا ذات دلالة إحصائية في كفاءة التحلل البيولوجي بين التربة المخصبة وغير المخصبة. سُجِّل أعلى معدل للتحلل البيولوجي، بنسبة 77%، في التربة المخصبة من منطقة الزبير 2. في المقابل، سُجِّل أدنى معدل، بنسبة 51%، في التربة غير المخصبة من منطقة الزبير 4. تشير هذه النتائج إلى أن التسميد بالأسمدة النيتروجينية والفوسفورية والبوتاسيومية يُحسِّن من قدرة أشجار المانغروف على المعالجة البيولوجية للتربة الملوثة بالنفط، حيث أظهرت منطقة الزبير 2 أعلى قدرة على المعالجة.

الكلمات المفتاحية: تلوث التربة بالنفط، التحلل البيولوجي لأشجار المانغروف.