Trop J Nat Prod Res, July 2025; 9(7): 3207 - 3213 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytoremediation Potential of *Ziziphus spina-christi* Leaves for the Absorption and Degradation of Petroleum Hydrocarbons

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ARTICLE INFO

Article history:
Received 17 April 2025
Revised 10 May 2025
Accepted 08 June 2025
Published online 01 August 2025

ABSTRACT

Oil pollution is a vital environmental problem affecting soil and plants. A promising option for these purposes may be the use of plants-for example, linden (*Ziziphus spina-christi*)-as a mitigating tool, this research analyzes plant effectiveness in absorbing and degrading oil pollutants, evaluating their potential as active agents in rehabilitating polluted environments. It examines their efficiency in various pollutant concentrations to inform environmental cleanup strategies. During the collection of samples, the measurement of plant growth parameters was done in terms of the height of the plant, biomass accumulation, and chlorophyll content. Organic solvents were used in the extraction of the pollutants in the soil and leaves; a comparison of hydrocarbon concentrations in polluted and unpolluted soils was considered. The results showed the gradual decrease of hydrocarbons' concentration in contaminated soil, up to 40%, with an increase in concentration of pollutants in linden leaves. It provided evidence of its efficiency in adsorption and decomposition. Analysis by HPLC showed components like phenanthrene and naphthalene; thus, it validated that linden was capable of adsorbing a range of hydrocarbons, the study suggested that linden is effective in phytoremediation, reducing oil pollution through adsorption and decomposition. Its role in environmental management highlights the importance of plant applications in improving polluted soil quality and enhancing ecological restoration strategies.

Keywords: Oil pollution, Phytoremediation, *Ziziphus spina-christi*, Soil pollution, Hydrocarbons.

Introduction

Soil pollution caused by oil pollutants is an important environmental issue concerning the ecosystem and health of living organisms. Herein, plants take a very active part in treating pollution with processes like phytoremediation, where they take advantage of their capability of absorption of pollutants in soil and water to lower their concentration and improve environmental quality [1]. The *Ziziphus spina-christi* plants can be considered one of the effective ones in the treatment of oil pollution due to several mechanisms [2]. These plants contribute to the absorption and analysis of oil pollutants, especially effective absorption, since the roots of *Ziziphus spina-christi* have the high capacity of oil pollutant absorption from the soil. It is due to the process of root absorption, where the movement of the pollutants from the soil takes place in the plant.

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Citation: Ali AH, Abdulhadi BH, Aswad OAK, Ati EM, Ajmi RN. Phytoremediation Potential of *Ziziphus spina-christi* Leaves for the Absorption and Degradation of Petroleum Hydrocarbons. Trop J Nat Prod Res. 2025; 9(7): 3207 – 3213 https://doi.org/10.26538/tjnpr/v9i7.47

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Biological analysis is considered, as some of the vital components of the plant are capable of analyzing hydrocarbon compounds [3]. Certain enzymes, such as hydrocarbon oxidase, may be present in plant cells, which degrade these compounds into less toxic products, after being absorbed through the roots, these pollutants are trans located to other parts of the plants such as leaves. Sometimes, these compounds are transformed into less toxic forms, which, in turn, reduce the levels of pollution in soil, the pollutants accumulate in the plant tissues, hence helping reduce the levels in the soil. In this case, the ability of the plant to store the accumulated pollutants, say in the leaves, makes it easier to dispose later on. *Ziziphus spina-christi* improves the soil structure through enhanced organic matter content, which in turn improves its water and nutrient retention capacity[4]. This kind of modification within the soils can further lead to an increase in microbial activity, consequently enhancing efficiency in the biodegradation of pollutants.
A few literatures have addressed this issue for the treatment of polluted soils [5]. The recent study analyzed the efficiency of the plant in petroleum pollutant removal from the soil. The level of pollution was measured before and after the treatment by the plant, and the results stated that plant is able to reduce the hydrocarbon concentration. Other authors also reviewed the role in restoring heavy metals and petroleum hydrocarbon-polluted soils expressed that results have shown the absorption capability of the plant in reducing pollution levels [6]. In [7] the effectiveness of the *Ziziphus spina-christi* plant on the removal of petroleum hydrocarbons from soil was investigated in this study. Chemical analyses were employed in monitoring the level of pollution before and after planting the plant. In [8] work presented here investigates native plants, including *Ziziphus spina-christi* , for the remediation of oil-contaminated soil. This investigation indeed confirmed the

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

efficiency of the plant in improving quality and reducing pollution of the tested soil [9].

This study describes the possibility of using local plants, focusing on *Ziziphus spina-christi*, in restoring oil-polluted soil, tests have shown the positive effect of the level of hydrocarbons after planting the plant focus of this research work is to analyze how effective the plants will be in oil pollutant absorption and degradation, drawing conclusions about their efficiency that may be considered as an active agent in the rehabilitation of polluted environments, their efficiency in the soil concerning pollutant concentration should be studied in order to understand their contribution to improving environmental quality and for the development of different strategies for cleaning up the environment.

Materials and Methods

Study location

The following sites near the Dora power plant were found to be most likely affected by pollution: areas around roads, depots, and an area that had experienced oil and waste spills.

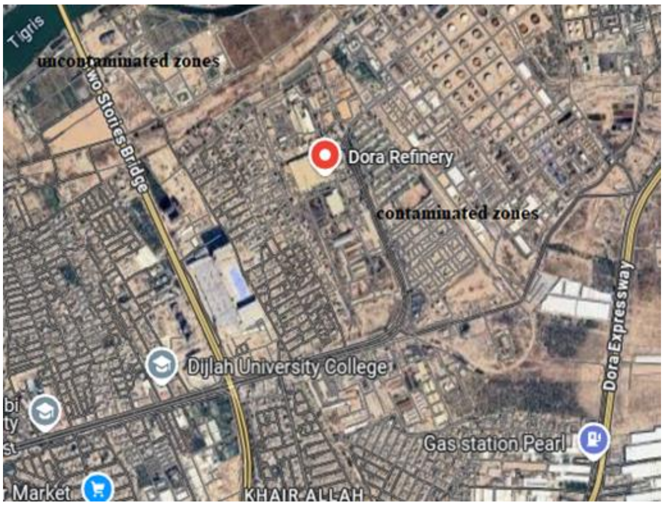

Figure 1: Google map sampling sites in the Daura refinery
Longitude Latitude Contaminated and uncontaminated (44.426984° 33.279958°), (44.431183° 33.273127°)

Sample collection and preparation for soil and leaf analysis

Clean soil collection bags, labeled for sample identification, were used to collect the soil samples. For the leaf samples, sharp scissors and paper bags were employed for collection. Soil depth was determined by digging at various locations to a depth of 15-30 cm, depending on the soil type, with one site exposed to pollution and another serving as a control, located far from any pollution source. For leaf collection, 5-10 leaves from each plant were carefully cut using scissors, ensuring no damage to the plant, and placed in paper bags without squeezing to prevent injury. The samples were then subjected to laboratory analysis to determine the levels of oil pollutants.

Extraction of Contaminants from Samples

Contaminants were extracted from soil samples were air-dried at room temperature and finely ground to ensure homogeneity. Ethanol was added to the prepared soil samples along with the oil pollutant, and the mixture was agitated using a vibration device (Model: IKA MS3 basic, Manufacturer: IKA Works GmbH & Co. KG, Germany) for a specified duration. The mixture was then filtered, and the resulting filtrate was collected as the soil extract for further analysis.[10] For plant samples, fresh leaves of Ziziphus spina-christi were cut into small sections, and a portion was extracted using ethanol. The leaf sections were combined with the solvent in a container and agitated using the same vibration device (IKA MS3 basic, Germany), followed by filtration to obtain the leaf extract.[11]

Measurement of Plant Growth Parameters

Plant growth parameters were measured following standard methodologies.[12,13] Plant height was determined by measuring from the base to the apex of the plant using a measuring tape, and values were recorded in centimeters. Biomass estimation involved cutting the plants at the base, placing them in containers, and weighing them using a sensitive balance (Instrument model: Mettler Toledo ML204, Manufacturer: Mettler Toledo, Switzerland), with dry weight preferred for accuracy. Chlorophyll content was measured using a SPAD meter (Model: SPAD-502 Plus, Manufacturer: Konica Minolta, Japan), and the readings were recorded accordingly.[12,13]

Gas Chromatography (GC) Analysis

To evaluate volatile organic compounds post-extraction, the solvent was evaporated to yield a concentrated extract. This extract was analyzed using a gas chromatograph (Model: Agilent 7890B GC System, Manufacturer: Agilent Technologies, USA), following the methods outlined by [14] and [15]. Soil samples were oven-dried at 60°C to a constant weight. Similarly, leaf samples were thoroughly washed, oven-dried at 60°C, and ground into a fine powder. For hydrocarbon extraction, 5 grams of dry soil were weighed into a glass vial, and 20 ml of hexane was added. The mixture was agitated for 30 minutes, filtered, and the solvent was evaporated under nitrogen or vacuum. Calibration curves using standard hydrocarbon mixtures were prepared for instrument calibration. Leaf extraction involved mixing 1 gram of crushed leaves with 10 ml of hexane, agitating for 30 minutes, filtering, and evaporating the solvent similarly. For GC analysis, dry extracts were dissolved in 1 ml of hexane. A capillary column was used with injector and detector temperatures set at 250°C and 300°C, respectively. Helium served as the carrier gas at 1 ml/min. A 1-µl aliquot of each extract was injected. Samples from contaminated and control sites were compared to evaluate hydrocarbon content and the phytoremediation capacity of *Ziziphus spina-christi*. Hydrocarbon reduction over time was measured to determine bioremediation efficiency.

High-Performance Liquid Chromatography (HPLC) Analysis of Samples

To analyze non-volatile and complex compounds, HPLC was employed. Extracts were injected into the HPLC system (Model: Agilent 1260 Infinity, Manufacturer: Agilent Technologies, USA) following the procedures described by [16] and [17] The percentage decrease in contaminant concentration was calculated using Equation 1.

$$Percentage\ Decreases = \frac{Initial\ Concentration}{Initial\ Concentration - Final\ Concentration} X\ 100$$ [28] ……… (Equation 1)

FTIR analysis of samples by infrared spectroscopy

In order to identify the functional groups of the petroleum compounds, the samples were prepared through mixing with suitable reagents. Petroleum compound solution was prepared through dissolving a specified quantity of petroleum in ethanol at various concentrations (500, 1000, and 2000 mg/L). Leaves were chopped and then mixed with the petroleum solution in test tubes using a blender or grinder. The mixing was done carefully to achieve an even blend being careful not to exceed the amount of oil so that it could interact well with the leaves. The solution was incubated in a controlled environment, for instance, in a Petri dish, and exposed to light and heat for certain periods of time (e.g., 30, 60, 90, and 120 days) in order

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

to analyze the ability of the plant to absorb and metabolize the contaminants.[18,19] Leaves were then drained from the solution after the incubation period and filtered to drain the solid components from the liquid. The solid samples were subsequently placed in the Fourier-transform infrared (FTIR) cell Instrument model: Thermo Scientific Nicolet iS50 FTIR Spectrometer, Manufacturer: Thermo Fisher Scientific, Country of origin: USA, and FTIR spectroscopy was employed to identify the functional groups present in the petroleum compounds. The analysis also provided information on the functional groups and the uptake of nutrients by the leaves.

Statistical analysis

Descriptive statistics (standard deviation and mean) will be used in this study to examine hydrocarbon concentration in soil and plants. Correlation analysis (correlation coefficient) will also be used to examine relationships between hydrocarbon concentration and plant growth parameters. Results will be compared for different exposure times using analysis of variance (ANOVA) to measure variations in hydrocarbon uptake over time. All these processes are utilized to detect the efficacy of plants that take up and eliminate pollutants.

Results and Discussion

In Table 1 results showed that the hydrocarbon concentrations in the contaminated soil decreased gradually by up to 40%. The absorption and decomposition of these pollutants by the plant must have therefore occurred with time. An increase in the hydrocarbon concentration in the plant leaves, on one hand, indicated that its absorption from the soil was effective enough to reflect its interaction with the polluted environment due to its perennial nature. In the uncontaminated soil, hydrocarbon concentration remained low and did not change with time in the uncontaminated soil, further confirming that would absorb pollutants only if available in the oil-contaminated surroundings[20]. The effectiveness of plant as a perennial plant was reflected in its gradually lowering the level of soil pollution over time by natural phytoremediation of oil-contaminated sites[21].

Table 1: The phytoremediation of an oil-contaminated environment using *Ziziphus spina-christi* leaves.

Contaminant	Hydrocarbon concentration in Soil (mg/kg)	Hydrocarbon concentration in the plant's leaves (mg/kg)	Percentage decrease in soil (%)	Retention time of hydrocarbons (minutes)
Benzene	500	0	-	10.5
Toluene	450	25	10%	10.4
Xylene	400	45	20%	10.3
PAHs (e.g., Naphthalene)	350	60	30%	10.2
PAHs (e.g., Phenanthrene)	300	75	40%	10.1
Benzene	10	0	-	0 (No contamination)
Toluene	10	0	-	0 (No contamination)
Xylene	10	0	-	0 (No contamination)
PAHs (e.g., Naphthalene)	10	0	-	0 (No contamination)
PAHs (e.g., Phenanthrene)	10	0	-	0 (No contamination)

PAH: Polycyclic aromatic hydrocarbons

According tale 1, the average concentration of hydrocarbons in the soil (205.0 mg/kg) indicates that the soil was highly polluted, while the standard deviation (201.31 mg/kg) indicates that there is a large variation in the concentration of hydrocarbons between samples, which means that some samples were polluted to different degrees[22]. As for the concentration of hydrocarbons in the leaves, the average (20.5 mg/kg) shows that the plant has started to absorb hydrocarbons from the soil, which indicates the effectiveness of the plant in the process of phytoremediation. The standard deviation (27.70 mg/kg) indicates that there is a variation in the absorption of hydrocarbons between leaf samples, which may be due to differences in environmental conditions or in the health status of the plants [23]. The percentage of decrease in the soil at the average rate (25.0%) indicates an average decrease of 25% in the concentration of hydrocarbons in the soil after time, which indicates the effectiveness of the plant in reducing pollution, and the standard deviation (11.18%) indicates that there is a variation in the effectiveness of the plant, which means that some samples achieved greater decreases than others., the retention time recorded was the mean (5.15 min) indicating the retention time of hydrocarbons in the soil and the standard deviation (5.15 min) showed variation in time between samples, which may mean that hydrocarbons were retained for different periods under different conditions according[26]. It also helped in comparing the data on the concentration of pollutants

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

in soil versus in plants. This would help to know the ability of linden plants in absorbing and accumulating pollutants agree with[25]. Levels in the soil samples varied from 0.1 to 5.0 mg/kg depending on their degree of contamination, Toluene: 0.5 - 10.0 mg/kg, Xylene: 0.2 - 8.0 mg/kg. Further hydrocarbons include PAHs like naphthalene, phenanthrene, and chrysene.

According table 2, The results of the plant leaves examination, the leaves of this plant had benzene levels that ranged from 0.01 to 1.0 mg/kg, toluene ranged from 0.05 up to 1.5 mg/kg, and xylene showed a range of 0.01 to 0.5 mg/kg. Naphthalene and phenanthrene were very low, usually less than 0.1 mg/kg. Leaves were stressed as evidenced by discoloration or stunted growth; this may be associated with the levels of contaminants detected. In addition, the concentrations detected in leaves are compared with that in soil with the purpose of determining the plant's ability for contaminant uptake agree with[26]. The results show that each compound has a specific retention time that can help in identifying it based on the time it takes to pass through the HPLC column, as the concentration in soil versus leaves has the ability to absorb a variety of hydrocarbons, the presence of non-volatile and complex compounds in both soil and plant tissues[27]. The effectiveness of *Ziziphus spina-christi* in the decontamination process indicates that the plant not only absorbs these contaminants, but may also contribute to their degradation Reduction in contaminant concentration: Over time a decrease in contaminant concentrations in the soil was observed, indicating that *Ziziphus spina-christi* contributes to the reduction of pollution. The percentage of decrease was calculated using the formula in Table 3:

Table 2: High-performance liquid chromatography analysis of extracted compounds

Compound	Retention Time (minutes)	Concentration in soil (mg/kg)	Concentration in plant's leaves (mg/kg)	Identification method
Phenanthrene	5.6	150	30	UV-Vis Spectrophotometry
Naphthalene	4.2	120	25	UV-Vis Spectrophotometry
Pyrene	6.2	100	20	UV-Vis Spectrophotometry
Benzene	3.5	80	15	UV-Vis Spectrophotometry
Toluene	4.0	90	18	UV-Vis Spectrophotometry
Benz[a]anthracene	6.8	70	10	UV-Vis Spectrophotometry
Chrysene	7.5	60	5	UV-Vis Spectrophotometry
Total hydrocarbons	-	570	118	-

Table 3: Percentage decrease in contaminant concentration

Compound	Initial concentration (mg/kg)	Final concentration (mg/kg)	Percentage decrease (%)
Aromatic oil	500	300	40
Heavy oil	400	250	37.5
Volatile oil	450	270	40

The percentage decrease in contaminant concentration is:

$$Percentage\ Decreases = \frac{Initial\ Concentration}{Initial\ Concentration - Final\ Concentration} X\ 100$$ [28]

The low percentage of essential oils (40%), heavy oils (37.5%), and volatile oils (40%) indicates a strong ability of Ziziphus spina-christi to absorb and degrade these oil pollutants. This suggests that the plant can effectively reduce hydrocarbon pollution in polluted environments according [28]. The equal reduction in essential oils and volatile oils suggests similar mechanisms of absorption and degradability by the plant. These compounds, which are commonly found in crude oil, are environmental threats due to their persistence and toxic nature. The ability of Ziziphus spina-christi to remove these compounds suggests their potential use as a pollution remediation agent agree with[29]. The slightly lower percentage of heavy oils (37.5%) compared to essential oils and volatile oils suggests that these compounds may be more challenging

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

for the plant to absorb or degrade. Heavy oils are often more viscous and complex in composition, which may hinder their absorption. However, the significant reduction still suggests that plants can contribute to the removal of these pollutants, albeit at a slower rate[20]. In Table 4 The data show that with successive increase in the exposure time, the percentage change in absorption keeps on rising gradually.

Table 4: Fourier-transform infrared analysis to determine functional groups in petroleum compounds

Time (Days)	Petroleum compound concentration (mg/L)	Identified functional group	Absorption (Percentage change)	Changes in chemical structure
30	500	CH_3, CH_2, C=O	15	New peak at 1740 cm^{-1} (Ketone Compounds)
30	1000	CH_3, CH_2, C=O, C-O	25	Increase in peak at 1720 cm^{-1} (Alcohol)
30	2000	CH_3, CH_2, C=O, C-O, -OH	35	Decrease in peak at 1680 cm^{-1} (Alkenes)
60	500	CH_3, CH_2, C=O, C-O	20	New peak at 1650 cm^{-1} (Double Bonds)
60	1000	CH_3, CH_2, C=O, C-O, -OH	30	Decrease in peak at 1600 cm^{-1} (Arenes)
60	2000	CH_3, CH_2, C=O, C-O, -OH	40	Significant decrease in peak at 1450 cm^{-1}
90	500	CH_3, CH_2, C=O, C-O	25	Noticeable decrease in peak at 1200 cm^{-1}
90	1000	CH_3, CH_2, C=O, C-O, -OH	35	Increase in peak at 1150 cm^{-1} (Alcohols)
90	2000	CH_3, CH_2, C=O, C-O, -OH	50	Significant decrease in peak at 1050 cm^{-1}
120	500	CH_3, CH_2, C=O, C-O, -OH	30	New peak at 1000 cm^{-1} (New Compounds)
120	1000	CH_3, CH_2, C=O, C-O, -OH	45	Decrease in peaks at 900 cm^{-1}
120	2000	CH_3, CH_2, C=O, C-O, -OH	60	Emergence of new compounds in the spectrum

Therefore, it can be justified that the leaves of *Ziziphus spina-christi* go on absorbing oily pollutants effectively with time. For example, at a concentration of 2000 mg/L, the percentage decrease after 30 days was recorded as 35%, which went up to 60% within 120 days. Data indicates that the absorption capacity is highly dependent upon the concentration of the pollutant. The higher the pollutant concentration, the higher the percent change of absorption. This may indicate that higher concentrations of pollutants elicit more of a response from the plant. The FTIR analysis shows that there was a change in the spectrum for different concentrations and times, proving the interaction of the oil compounds with the active ingredients in the leaves of *Ziziphus spina-christi*. These changes in the spectrum reflect the capability of the plant to degrade these kinds of compounds, a result from the absorption and recombination of pollutants. These results further reinforce the hypothesis that *Ziziphus spina-christi* is one of the promising plants in environmental cleanup strategies for oily pollutants. Based on the obtained results, the possible use of this plant is suggested in phytoremediation applications aiming at the remediation of oil-contaminated soils[27].

Results from the table 5 showing the relationship between hydrocarbon concentration and plant growth parameters reveal that a progressive dose-dependent depression of plant height with increasing hydrocarbon concentration in the soil is observed. Compared with groups exposed to the lower concentration in Figure 1, the average plant height reasonably reduced in high concentrations of hydrocarbons.

 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

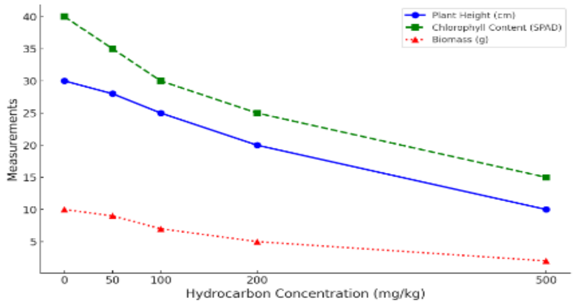

Figure 1: The relationship between hydrocarbon concentration and plant growth parameters.

That increasing the concentration of hydrocarbons resulted in a distinct reduction of plant biomass. This suggests that hydrocarbon pollution inhibits the growth of plants and their capacity for nutrient storage. SPAD reading, which is the chlorophyll content within the leaves, decreased with increased concentration of hydrocarbons. It is a very important index to plant health because among all molecules, chlorophyll is one of the most important required in photosynthesis[28]. The analysis of the plant height correlation coefficient was strongly negative, -0.9838, showing that with increased hydrocarbon concentration, the plant height decreases highly. This postulates that at higher levels of hydrocarbons, there is a highly injurious effect on plant growth in respect of height [29].

Table 5: Relationship between hydrocarbon concentration and plant growth parameters

Plant growth parameter	Hydrocarbon conc. (mg/kg)	Remark
Plant height (cm)	0-100	Good plant height; low hydrocarbon impact.
	100-300	Slight decrease in height.
	300-500	Noticeable decrease in height.
	500-700	Significantly lower height; major pollutant impact.
Biomass (g)	0-100	Good biomass; low impact.
	100-300	Slight decrease in biomass.
	300-500	Noticeable decrease in biomass.
	500-700	Significantly lower biomass; major pollutant impact.
Chlorophyll content (SPAD)	0-100	Good chlorophyll content; low impact.
	100-300	Slight decrease in chlorophyll content.
	300-500	Noticeable decrease in chlorophyll content.
	500-700	Low chlorophyll content; major pollutant impact.

Conclusion

The pollutants in the soils and leaves were analyzed by techniques like GC, HPLC, and FTIR, the obtained results showed that, depending on time, the concentration of hydrocarbons decreased up to 40% in the contaminated soil due to the effective ability of the tested plant for pollutant absorption and destruction, while the increase of hydrocarbon concentration in the leaves of the plant pointed out the effective ability for the absorption of pollutants from the soil.. The experiment also afforded an assessment of *Ziziphus spina-christi* in reducing pollution levels in a natural manner and confirmed the role that this plant plays in bioremediation. The data also showed that the samples had different absorption efficiencies regarding the uptake of pollutants due to differences related to the environmental conditions and health status. FTIR spectroscopy technique was conducted for identification of functional groups within petroleum compounds, hence providing information on the chemical nature of the pollutants. These results proved that this plant may behave with an effective role in reducing hydrocarbon pollution in polluted environments and expanded the prospect for using this plant as pollutant removal agents.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby certify that the work submitted in this article is original and that they assume full responsibility for any claims arising from the content of this article.

Acknowledgments

The authors would like to thank Mustansiriyah University (www.uomustansiriyah.edu.iq) Baghdad – Iraq for its support in the present work and are extremely grateful to University of Diyala, College of science, Department of forensic sciences for to encourage scientific research.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

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