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Original Research Article

Phytoremediation of Petroleum Hydrocarbon-Contaminated Soil Using Calligonum Comosum

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Abstract: Soil contamination by crude oil is considered one of the major environmental problems because of its harmful effects on plants and soil ecosystems. This study aimed to evaluate the potential of Calligonum comosum for the phytoremediation of crude oil-contaminated soil and to investigate its ability to accumulate petroleum hydrocarbons under different contamination levels. Soil samples (5 Kg each) were contaminated with crude oil obtained from the Shaybah Oil Field at concentrations of 20, 40, 60, and 80 g/Kg (w/w). Each pot was planted with four cuttings of Calligonum comosum (4 cm length). Several soil properties, including pH, electrical conductivity (EC), moisture content, total organic carbon (TOC), total nitrogen (TN), and total petroleum hydrocarbons (TPH), were analysed before and during the experiments at 45, 90, and 120 days. Hydrocarbon accumulation in plant tissue was also evaluated. The results showed a gradual reduction in TPH concentration in contaminated soils over time. Calligonum comosum demonstrated considerable tolerance to crude oil contamination and the ability to accumulate petroleum hydrocarbons across varying contamination levels, indicating its potential for phytoremediation of oil-polluted soil.

Keywords: Soil contamination, Petroleum Hydrocarbon, degradation, Phytoremediation.

INTRODUCTION

Soil is a complex and variable system which has a significant influence on plant growth and development. But, if there is contamination of petroleum hydrocarbons, there are various environmental, agricultural and health dangers like reduction in soil fertility and Inhibition of plant growth. This can affect soil physical characteristics and the capacity of water to penetrate the soil and oxygen to flow through it. Petroleum hydrocarbons can also impact soil microbial communities and hence nutrient cycling processes and soil health. These pollutants can have adverse health effects in humans and ecosystem imbalance if present for a long time.

Numerous remediation methods have been implemented to reduce the petroleum contamination in soils, such as physical, chemical, biological and phytoremediation. The final technology received much attention in relation to its low cost, effectiveness in the degradation of petroleum hydrocarbons, and its enhancement of biological conditions. But there are some factors that affect the efficiency of this method, such as species, soil moisture, nutrients and microbial activity in the rhizosphere. (Singh & Pant, 2023). A number of abiotic processes have a major role in the dissipation of hydrocarbons in soil, such as leaching, volatilisation, sorption and radical and microbial degradation, which are all stimulated by the presence, volume and biomass of plant roots (Tarigholizadeh *et al.*, 2023). In general, owing to their sessile habit, some plants, such as Phragmites australis (Common reed), Cynodon dactylon (Bermuda grass), Medicago sativa (Alfalfa), and Festuca arundinacea (Tall fescue), develop mechanisms to resist various environmental stressors, including the elimination, destruction, or sequestration of hazardous substances, thereby enhancing the remediation of petroleum-contaminated soils (Kongkham *et al.*, 2026). Previous laboratory and pot studies have reported that certain plants can enhance the removal of polycyclic aromatic hydrocarbons (PAHs). For example, Gabriele *et al.*, (2023) observed a clear and significant reduction in the concentrations of petroleum hydrocarbons and/or PAHs in the rhizosphere compared with the unplanted control soil. This reduction was greater in planted soils than in bare soils. On the other hand, Sarfaraz *et al.*, (2025) demonstrated that combining plants and microorganisms accelerates hydrocarbon degradation and reduces contamination within the rhizosphere. The Basrah governorate contains many petroleum and industrial plants that contaminate a large area (Saleem *et al*., 2022).

Calligonum comosum is a perennial woody desert shrub of Polygonaceae, very tolerant of high temperatures, dry soil, and drought conditions (Soliman *et al*., 2018). It is naturally distributed in the arid and sandy areas in the south of Iraq, including the edge of the desert in Basrah Governorate. This plant plays an important ecological role in desert ecosystems, but there is no report on its application in phytoremediation of petroleum hydrocarbon-contaminated Soil. The purpose of the present study is to assess the ability of Calligonum comosum to clean up hydrocarbon-polluted soil.

MATERIALS & METHODS

Experimental Design

The experiments were conducted at the Petroleum Refining and Testing Laboratory, College of Engineering/ University of Basrah/ Iraq during January, February, March and April in 2026. The soil samples were taken from the agricultural fields in the Al- Zubayr city in Basrah governorate. The crude oil (CO) from Shaybah Oil Field (Oil Company) was added to the samples at concentrations of 20, 40, 60 and 80 g/ Kg (w/w) and mixed well. Four (4 cm long) cuttings of Calligonum comosum were planted in each pot. Two sets of control samples were prepared from each pot, one set was treated with oil not planted, and the other set was planted but not exposed to pollutants. The pots were watered daily with tap water. The experiment was conducted for 120 days to allow sufficient time for the establishment, growth, and root development of Calligonum comosum under laboratory conditions. This procedure was repeated three times, and three samples were obtained for chemical analysis and then taken immediately to the laboratory. Plants for root and shoot length measurements were carefully removed from the soil, thoroughly washed and blotted.

Soil's Physical and Chemical Properties

For further examination, soil samples were taken from both affected and uncontaminated locations. They were homogenised separately with a pestle and mortar, dried in air at room temperature, and then filtered via a 1mm mesh screen. Features of the soil, including electrical conductivity (EC), potential of hydrogen (pH), content of moisture, organic matter, total organic carbon (TOC), the amount of nitrogen, and the ratio of carbon to nitrogen were evaluated before and during the experimental period according to the procedures described by Qzar *et al*., (2025).

Determination of Total Petroleum Hydrocarbons

In soil and tissue from plants (roots and shoots), overall total petroleum hydrocarbons (TPHs) were measured at 45, 90, and 120 days using the Soxhlet extraction method. Samples were collected from the beginning of the experiment and analysed based on the process outlined by Goutex & Saliot (1980). The following formula was used to determine the biological concentration factor (BCF) for plants:

BCF is equal to the organism's PH concentration divided by the soil's PH concentration. The formula provided by Al-Mansoory *et al.,* (2017) was used to determine the percentage of hydrocarbons in soil that degraded over time:

$$Degradation\ Percentage\ (\%) = \frac{C_0 - C_t}{C_0}$$

Where;

C_0 is the initial PH concentration, and C_t is the final concentration.

Analysis of Statistics

Three replications of Randomized Complete Block Design (RCBD) were used in the experiment.

The statistical data analysis was performed using SPSS version 20. Bartlett's test verified the homogeneity of variances (P = 0.05). The LSD test was used to evaluate mean differences at the 0.5% likelihood value (Table 1).

Table 1: ANOVA (analysis of variance) for the conditions under study

Source of variation	Degrees of Freedom (df)	EC (Ms cm^{-1})	pH	Moisture (%)	Total N(%)	Organic matter (%)	C/N ratio	TOC (%)
R stratum	1	0.0148	0.143	0.086	0.0106	0.00089	0.0	0.00109
Time	2	28.47	0.94	1.87	0.63	18.71	9.81	0.59
Concentration	4	1.61	0.054	0.73	0.47	2.11	0.37	2.06
Concentration Time	8	1.12	0.032	0.43	0.26	0.86	0.31	0.42

RESULTS & DISCUSSION

Soil's Physical and Chemical Properties

Prior to contamination and before the beginning of the trial, preliminary soil analysis was carried out. The findings showed that the pH of the soil was almost neutral, a moderate amount of organic matter, a moderate electrical conductivity, and no petroleum hydrocarbons (Table 2).

Table 2: Soil's physical and chemical propertiesprior to the experiment

Parameters	pH	EC (Ms cm^{-1})	Moisture (%)	TOC (%)	Total N (%)	Organic matter (%)	C/N ratio	Total petroleum hydrocarbons in soil (µg g^{-1})
Value	7.4	2.8	3.8	2.7	2.1	3.4	1.29	0

Before the experiment, all soil samples were sieved through a 2 mm mesh. Between 45 and 90 days, the soil's physical and chemical properties were assessed. Parameters such as pH, moisture content, and TOC trended to decline with increasing petroleum hydrocarbon concentration and time. Conversely, between 90 and 120 days, PH, total nitrogen, and organic matter increased, whereas the C/N ratio gradually increased with increasing contamination duration across all experimental times (Fig. 1).

During the experimental period, moisture content varied with irrigation depending on the water holding capacity of the soil. Overall, the moisture content in the treated pots was less over time compared to the control pots, indicating that petroleum hydrocarbon contamination can affect the water retention properties of soil. These findings are in agreement with the findings obtained previously by Hewelke & Gozdzowski (2020). Furthermore, the results obtained from a study conducted by Li *et al*., (2024) indicated that petroleum hydrocarbon contamination causes an increase in soil hydrophobicity and alters the pore structure distribution, thereby decreasing water retention and water availability in soil.

In fact, the pH values of the soil are influenced by oil as well as environmental and biological factors (Weil & Brady, 2016). The soil pH of the control pots was higher than that of the soil in the pots modified with different concentrations of hydrocarbons. In addition, the total nitrogen content of contaminated soil was generally greater than that of the control pot, indicating changes in nitrogen dynamics due to petroleum hydrocarbon contamination. The results obtained in this work support the previous findings obtained by Gao *et al*., (2022) and Saikia *et al*., (2023) that referred to the significant changes in soil properties (pH, organic matter, moisture, nutrients, etc.) as a result of the presence of crude oil contamination. In general, the observed changes in soil property values could be caused by interactions between components of crude oil and soil that change the soil physicochemical properties and nutrient dynamics. These changes may also be influenced by irrigation conditions as it affects soil moisture and the distribution of nutrients during the experimental period (Mekkiyah *et al*., 2023).

Total Hydrocarbons in Soil

The amount of all petroleum hydrocarbons in the experimental plants' soil was monitored and analysed throughout a 120-day study period; the results are presented in Fig. 2.

The hydrocarbon degradation percentage decreased progressively with increasing crude oil concentration, as shown in Fig. 3.

The highest degradation efficiency (72%) was obtained at 20 g/kg, while the least (40%) was obtained at 80 g/Kg. Uncultivated control pots were used to determine concentrations of petroleum hydrocarbons related to abiotic losses such as volatilisation, irrigation and environmental exposure. The observed declines in these pots suggest that a fraction of this hydrocarbon loss was non-plant related, and should be taken into account when evaluating degradation efficiency. In general, increasing the removal of hydrocarbons led to lowering the contamination level, indicating lower toxicity and better natural attenuation, while the higher contamination levels may have depressed removal, indicating lesser natural attenuation. The results support the report by Fang *et al*., (2025) that demonstrated the high levels of petroleum hydrocarbons in soil result in great toxic stress, which would negatively impact plant growth, root activity, and remediation efficiency. Phytotoxic effects, related to the high concentration of petroleum hydrocarbons in soil, may affect plant growth and physiological activity and decrease the efficiency of phytoremediation. Plants grown under high hydrocarbon concentrations have reduced degradation and accumulation abilities compared to other plants that grow under low hydrocarbon concentrations (Nemati *et al*., 2024). Petroleum hydrocarbons were significantly lower in the phytoremediation treatments compared to the control treatments ($P < 0.05$) as determined by statistical analysis. The chemical composition and physicochemical properties determine how petroleum hydrocarbons degrade in soil, while the environment (including temperature and moisture) collectively determines their fate and persistence in soil ecosystems (Mekonnen *et al*., 2024).

Various processes contribute to hydrocarbon loss, including volatilisation from the soil surface into the atmosphere, photooxidation induced by sunlight, and microbial degradation that transforms hydrocarbons into simpler, less toxic compounds. These combined processes play a crucial role in reducing petroleum contamination in soil systems (Mekonnen *et al*., 2024).

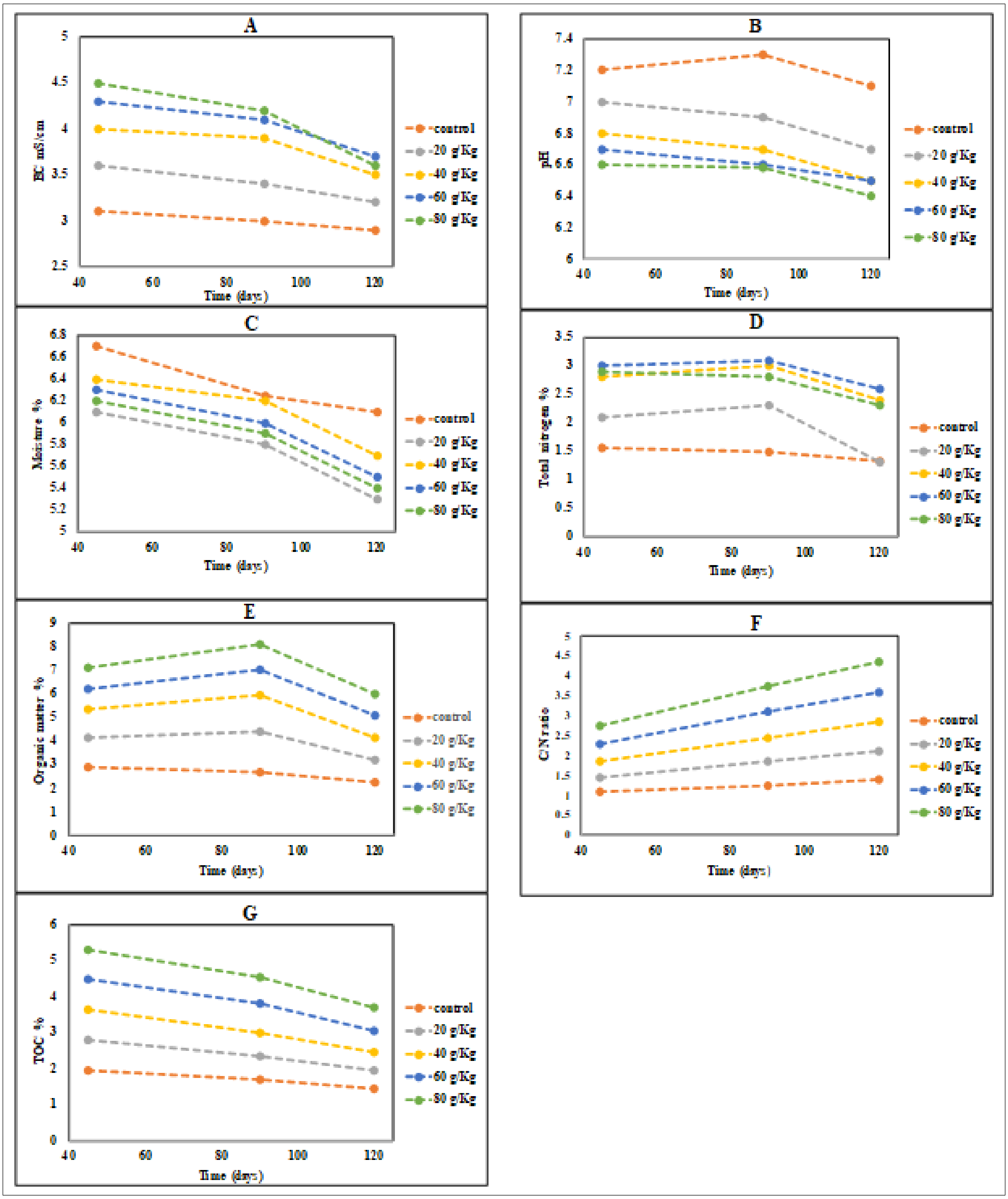

Fig. 1: Soil physical and chemical properties over the 120-day experimental period. (A) mScm¯1, or electrical conductivity. (B) pH level. (C) Moisture percentage. (D) Total nitrogen percentage. (E) Organic matter percentage. (F) Ratio of carbon to nitrogen. (G) The percentage of total organic carbon

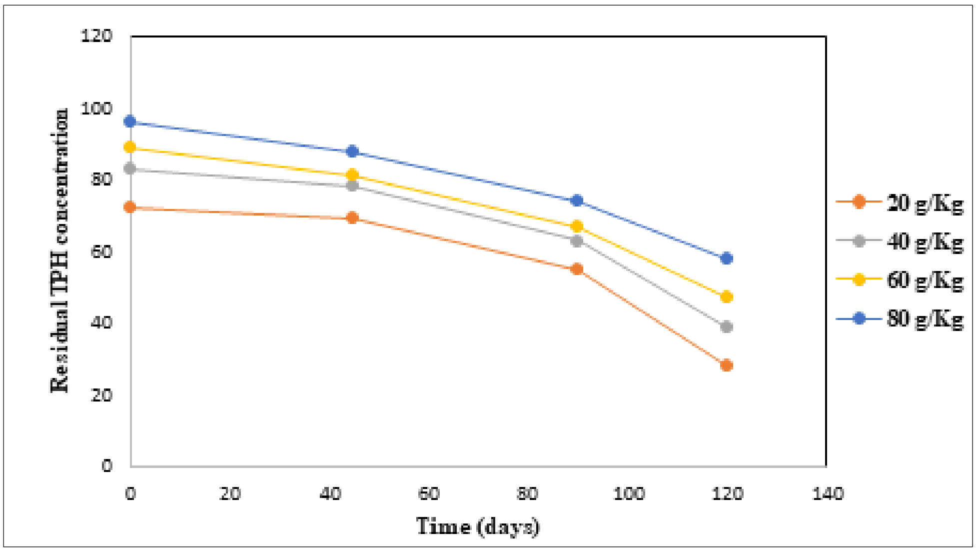

Fig. 2: Residual TPH concentration (g/Kg) in soil during the Experimental Period

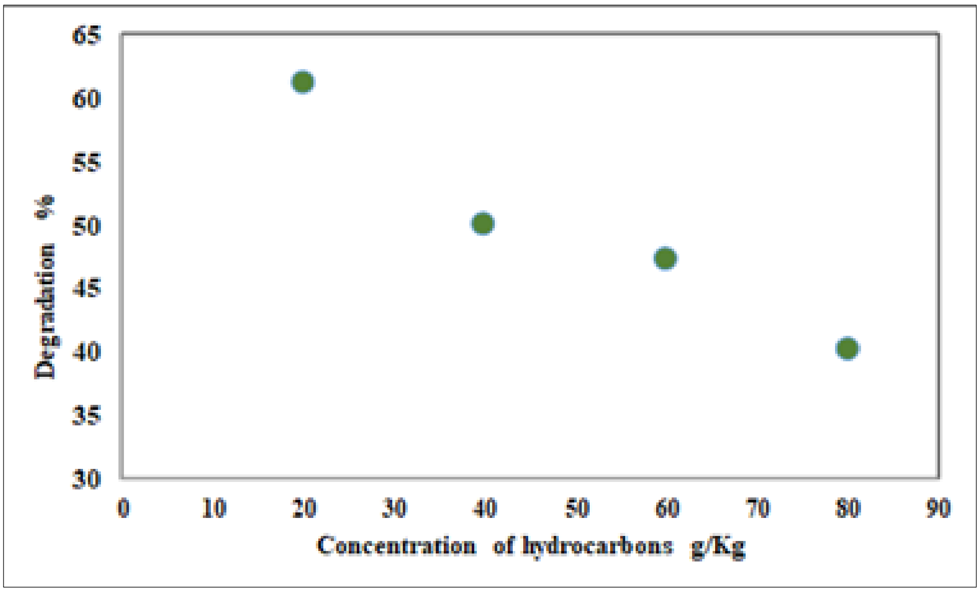

Fig. 3: Hydrocarbon degradation in pots throughout the study period

Total Hydrocarbons in the Plant

Various desert plant species have been considered for soil restoration in arid environments due to their high tolerance to harsh environmental conditions. The observed performance of Calligonum comosum under contaminated conditions may be attributed to its high adaptability to arid, nutrient-limited environments, which may have facilitated plant establishment and indirectly enhanced hydrocarbon dissipation through rhizosphere-associated processes.

The impact of oil contamination on plant growth was clearly evident. Plants grown in soils with lower petroleum hydrocarbon concentrations showed superior growth performance compared with those exposed to higher contamination levels. In contrast, plants subjected to high oil contamination exhibited clear toxic symptoms, including stunted growth, reduced leaf number, and a lack of flowering. Meanwhile, control pots showed normal, healthy plant development. Hydrocarbon levels were also assessed in unpolluted control pots to account for potential background contamination or incidental pollution (Table 3).

The concentration of petroleum hydrocarbons in plant tissues increased initially with exposure time (Fig. 4), reaching its peak after 90 days, then slightly declining after 120 days, which may be attributed to enhanced microbial degradation and reduced hydrocarbon bioavailability in the soil.

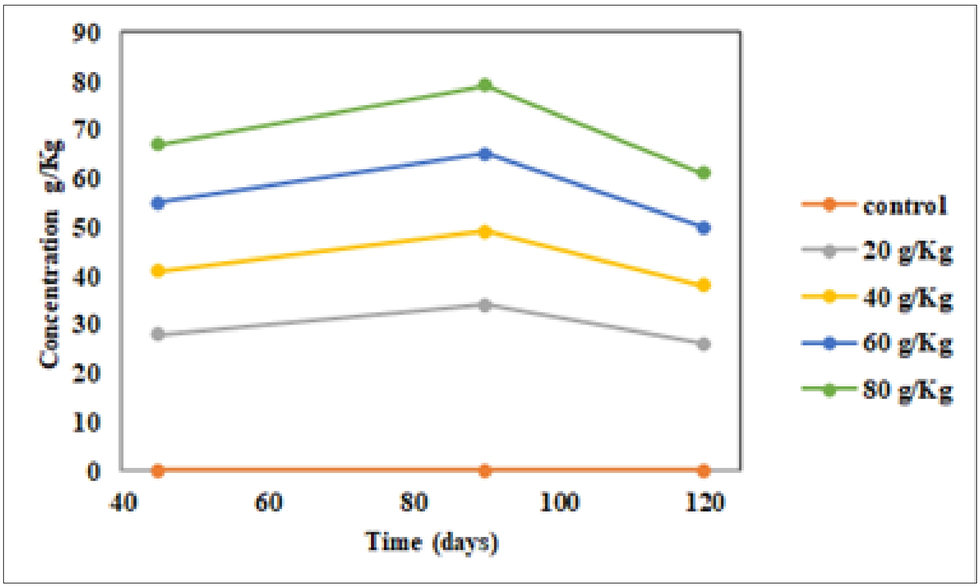

Fig. 4: Level of total hydrocarbons (g/kg) in plants throughout the experiment

The observed biphasic pattern of hydrocarbon accumulation in Calligonum comosum, characterised by an initial increase followed by a decline, is consistent with the findings obtained by Hunt *et al*., (2019). Moreover, recent studies on rhizosphere-mediated phytoremediation reported that the time-dependent reductions in hydrocarbon bioavailability can lead to enhanced microbial degradation and rhizosphere activity (Lü al., 2024), (Zhang *et al*., 2023).

Bioconcentration factors (BCF) calculated at 90 days were around 0.16, 0.73, 0.89 and 0.90 for 20, 40, 60 and 80 G/Kg treatments, respectively. The results show better uptake and accumulation of petroleum hydrocarbons into the plant tissues during the vegetative growth phase. The higher accumulations seen during this time frame could be related to soil root activity and/or more available hydrocarbons during this time frame. However, when exposed for a longer time (120 days), slight variations in BSF values were observed maybe because of the reduction in residual hydrocarbons in soil and possible enhancement of microbial degradation processes which reduced the availability of hydrocarbons for plant uptake. These results suggest that the plants are not very capable of accumulating hydrocarbons in plant tissues, and therefore the plant could mainly contribute to the remediation of petroleum hydrocarbons in the soil through the uptake-assisted degradation mechanisms (Ismail *et al*., 2024).

Lengths of Roots and Shoots

As seen in Table 3, the root and stem lengths of the plants in contaminated soil were reduced compared to the control. The decrease was more significant as the concentration of crude oil increased from 20 g/Kg to 80 g/Kg. The root length and stem length of the control treatment were consistently the highest as compared to other treatments after 45, 90 and 120 days. After 45, 90 and 120 days the root length and stem length of the control treatment were consistently the highest as compared to others. For instance, root and stem length for control after 120 days were 10 cm and 24 cm, respectively while the same reduced to 4 cm and 11 cm respectively for 80 g/Kg treatment. The results show that plants grown in the crude oil contaminated soil were under stress from crude oil and this was reflected in a negative growth response in both the root and stem system. Statistical analysis revealed a strong negative correlation between crude oil concentration and plant growth parameters ($r = -0.95$, $p < 0.05$), indicating that increasing crude oil contamination significantly suppressed root and stem development. These findings are in agreement with those reported by Merkl *et al*., (2004), who found that petroleum-contaminated soils significantly reduced root and shoot growth in plants.

Table 3: Effect of different hydrocarbon concentrations on plant development in potted soil

Time of treatment	**control**		**20 g/Kg**		**40 g/Kg**		**60 g/Kg**		**80 g/Kg**	
	(Root length)	**(Stem length)**	**(Root length)**	**(Stem length)**	**(Root length)**	**(Stem length)**	**(Root length)**	**(Stem length)**	**(Root length)**	**(Stem length)**
After 45 days	4	9	3.5	8	3	7	2.5	6	2	5
After 90 days	7	16	6	14	5	12	4	10	3	8
After 120 days	10	24	8	21	7	18	5	14	4	11

CONCLUSION

In this study, the adverse effect of crude oil contamination on root and stem growth of Calligonum comosum was found, and as the concentration of crude oil increased, the growth of the roots and stems decreased significantly compared to the control treatment. The inhibitory effects were increased with increasing contamination level, especially at 80 g/Kg suggesting the sensitivity of the plant to stress caused by petroleum hydrocarbons. The plant survived under contaminated conditions during the experimental period with reduced growth parameters, indicating some tolerance to crude oil. The results showed that Calligonum comosum is a potential plant in phytoremediation and rehabilitation of crude oil polluted soil in arid environments.

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Conflicts of Interest: No potential conflicts of interest are disclosed by the authors.

Ethical Approval

Environment: Throughout the investigation, all safety precautions were taken, and all experimental material was disposed of in compliance with accepted safety procedures.

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