Evaluating the Phytoremediation Capacity of *Cupressus Sempervirens* in Crude Oil-contaminated Soils: A Study of Growth Parameters and Hydrocarbon Removal Efficiency

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Manuscript received December 29, 2025; revised February 9, 2026; accepted February 26, 2026; published August 25, 2026

***Abstract*—The experiment conducted in the study aimed at the utilization of the Cypress (*Cupressus sempervirens*) seedlings to phytoremediate soil polluted with crude oil. One- and 2-year-old seedlings were grown in soil polluted with 0.5 and 1.5% weight-to-weight (w/w) crude oil. Plant growth parameters, crude oil degradation, hydrocarbons, soil parameters, nitrogen (N), phosphorus (P), and potassium (K) were measured. The results showed a successful plant growing in crude oil-polluted soil, with no poisonous symptoms or recorded mortality cases. Chlorophyll b, the shoots' biomass, and plant height showed a significant reduction. The pH decreased while both Electrical Conductivity (EC) and Organic Matter (OM) of the soil increased significantly. The pH reduction recorded 0.2 degrees, soil EC, and OM in soil containing 0.5% crude oil marked 811.167 µS/cm and 1.578%; in soil polluted with 1.5% crude oil, the results read differently, marking 889.667 µS/cm and 1.942% respectively. Regarding the Total Petroleum Hydrocarbons (TPH), Gas Chromatography Technique analysis of soil showed that a total of 10 different hydrocarbons (3C–8C and 10C–13C) had vanished. Additionally, Petroleum degradation was found to be in the range of (35.98–37.881%) after 4 months of planting, while reaching (87.333–87.831%) after eight months. N concentration in soil and plant tissues also showed a significant increase. In general, 1- and 2-year-old seedlings showed insignificant results in most of the studied traits, apart from the plant growth parameters. The study provided evidence of this plant's capacity to grow in soil polluted with 0.5% and 1.5% crude oil, and it also exhibited a strong ability to remediate the soil from crude oil.**

Keywords*—phytoremediation, crude oil pollution, hydrocarbons, *Cupressus sempervirens

I. Introduction

Due to over reliance on crude oil as the main source of energy around the world, soil often becomes contaminated with petroleum hydrocarbons. To put this in perspective, the amount of oil spilled worldwide is estimated to hit roughly 600,000 tons [1]. Iraq, including the Kurdistan Regional Government, ranked second in the Organization of the Petroleum Exporting Countries (OPEC) regarding crude oil production. With 145 billion barrels, Iraq has the 5th largest amount of validated, reserved crude oil in the world.

In 2021 alone, Iraq's total primary energy consumption was nearly two quadrillion British Thermal Units (BTU). It ranks fourth in the Middle East with respect to energy consumption [2]. In the Kurdistan Region, oil production began in 2005 in the Tawke fields in the District of Zakho by the Det Norske Oljeselskap (DNO) Company, producing the first barrel of oil in 2007. The Taq Taq and Khurmala fields in Kirkuk joined production next, followed by the remaining fields in the Kurdistan Region.

Crude oil is a flammable and toxic liquid with a black or dark brown color, consisting mostly of alkenes, alkanes, and a mixture of hydrocarbons of different molecular weights, and organic compounds of nitrogen, sulphur, and vanadium [3]. When spilled due to anthropogenic activities, crude oil exposes the environment to hazards that affect agricultural lands, impede plant growth, reduce food quality, and ultimately affect human health [4]. Crude oil pollution is the main cause of major strains to both terrestrial and aquatic ecosystems [5].

The survival of humanity heavily depends on the productivity of soil and the extent to which it supports the growth of healthy and sound plants. Soil is an imperative natural resource for every country, as its quality influences its effectiveness for various intended purposes [6]. Given its importance as a key factor in maintaining the productivity and sustainability of all ecosystems of the Earth, healthy soil is a national resource that must be protected from pollution to maintain its productivity and preserve its sustainability for future generations [7]. There is substantial evidence regarding the effects of crude oil pollution on the chemical and physical qualities of soil [8].

Many countries have recently conducted numerous studies investigating the most effective methods of remediating oil-contaminated soil. Typical methods comprise excavating the soil, followed by both chemical treatment through the use of potent oxidizing agents and physical treatment through heat application [9]. Although effective, these methods are costly and cause significant disruption to the processed area.

The biological method is considered a further economically feasible alternative; the phytoremediation method, for instance, incorporates living green plants in their natural environment to remediate contaminated soil. Furthermore, it holds significant promise for the remediation of petroleum-polluted soil [10]. Through a variety of processes—including degradation, volatilization, sequestration, absorption, and transfer to other compounds—plants play a crucial role in soil remediation by reducing soil contamination levels [11].

Owing to its advantages over chemical techniques, phytoremediation has gained increasing popularity since the 1980s [12]. Due to the fact that plants can directly absorb organic contaminants like Petroleum Hydrocarbons (PHC) and metabolically transform them into other compounds, phytoremediation can be used to remove these toxins from the soil [13]. Plant roots can modify the physical and chemical characteristics of the soil by secreting exudates that enhance microbial metabolism and degrade soil contaminants [14]. Some plant species are preferred for phytoremediation, as they withstand local climate and different soil conditions, making them more likely to grow and propagate in crude oil-contaminated soil [15].

The key factor for an effective phytoremediation practice is the selection of a plant species that tolerates the polluted environment and withstands high contamination levels. However, the majority of research conducted has focused on different plant species, leaving only a limited number of studies that have investigated the effectiveness of trees in remediating crude oil-contaminated soil. Various species belonging to the Fabaceae, Lamiaceae, Euphorbiaceae, Poaceae, Brassicaceae, and Asteraceae families can remediate contaminated soil [16].

In the same vein, Frick *et al* [17] and Kamath *et al* [18] demonstrated the role of Populus sp., Salix sp., Cordia subcordata, Thespesia populnea, Prosopis pallida, and Scaevola serica in tolerating PHC-contaminated soil. Moreover, Ayuba *et al.* [19] investigated the ability of the *Eucalyptus camaldulensis* species to phytoremediate of petroleum hydrocarbon-polluted soil. Whereasa recent study by Abdallah *et al* [20] involved the phytoremediation of crude oil-contaminated soil by Acacia sieberiana Tausch. It is worth noting that several studies have highlighted the significance of a wide range of plant species in the phytoremediation of crude oil-contaminated soil [21–23].

On the other hand, very few studies have examined the use of *Cupressus sempervirens* (*C. sempervirens*) in the remediation of crude oil-contaminated soil, particularly in the Kurdistan Region. The Cypress cultivated in the area has a strong root system and can tolerate extreme weather conditions, including drought and high heat; therefore, it was selected for the current study.

As such, this study aims to evaluate Cypress's potential to remediate crude oil-contaminated soil. This will be realized by (1) examining the response of seedling growth to different levels of crude oil contamination. (2) evaluating the efficiency of these trees in hydrocarbon removal from soil by measuring the decrease in the concentrations of Total Petroleum Hydrocarbons (TPH) over time.

II. Materials and Methods

A. Studied Plant

The current study was conducted between April and December 2023 in the shaded environment of the lath house at the College of Agricultural Engineering Sciences/ University of Duhok in Duhok city. The geographical coordinates for the location are: (36°51′32.39″ N) and (42°52′58.95″ E), with an elevation of 473 meters above sea level.

The selected tree species is the introduced evergreen *C. sempervirens*. The seedlings being evaluated were both one- and two-year-old. These plants were transported from the Malta nursery to the lath house site, where, prior to planting, height measurements were recorded. The seedlings were planted in plastic bags containing 4 kg of soil and irrigated with tap water in the lath house.

Commonly referred to as the Mediterranean Cypress, this type of evergreen coniferous tree can grow to 35–40 meters high, with a trunk diameter that may exceed one meter, though rarely surpassing 2 m. This species, originally from the eastern Mediterranean area, can live for over a century. Yet, they are widely grown as ornamental evergreen trees in places beyond their indigenous range [24]. It flourishes in deep, moist, and well-drained rich soil with a neutral pH level [25]. Notably, this tree has been widely planted in Kurdistan and Iraq for ornamental purposes, including its ability to absorb pollution [26, 27].

B. The Experiment

To investigate the plant species *C. sempervirens*, a Randomized Complete Block Design (RCBD) was employed (due to the experiment's location being close to a building, which created spatial variations in light, the alignment of the blocks was set perpendicular to the light gradient). The study included 2 age groups (one-year-old and two-year-old seedlings) and 3 levels of crude oil concentration (0%, 0.5%, and 1.5%), with 3 replicates and 4 plants per experimental unit. Therefore, the calculation for the total number of experimental units is (1 plant) × (2 age groups) × (3 concentrations) × (3 replicates) = 18 (experimental units) × 4 (plants in each experimental unit) = 72 seedlings.

The soil used in the experiment was collected from the fields of the College of Agricultural Engineering Sciences, soil's physical and chemical properties were determined, air-dried, and thoroughly mixed with crude oil, obtained from local refineries in Duhok city, at concentrations of 0.0%, 0.5%, and 1.5%.

The oil-contaminated soil was placed into pots, 4 kg each, placed at the experimental site, and irrigated with well water for two weeks prior to planting. In April 2023, a single seedling was planted in each pot.

Other pots filled with 0.5% and 1.5% crude oil-contaminated soil without plants were used to evaluate the effect of non-plant factors in oil degradation. The measures were compared to those of the pots planted with *C. sempervirens* to evaluate the trees' effect on the degradation process. Throughout the 8-month study period, no signs of plant toxicity were recorded, such as yellowing, necrosis, or the death of any specific part of the plant. There was only one fatality.

C. Seedlings Rooting Up, Soil and Plant Samples Preparation

At the beginning of November, all Cypress seedlings were gently uprooted from the pots, and the shoot system was separated from the root system for each seedling. The soil stuck to the roots was carefully removed, and both components were washed separately.

Each experimental unit consisted of 4 individual plants (each plant housed in its own pot). Measurements for each unit were derived from all 4 plants and averaged into a single representative value for that unit. For instance, leaves from all four plants were collected and combined to determine chlorophyll levels, while soil samples were taken from all 4 pots and were mixed thoroughly before measuring parameters such as electrical conductivity, pH, organic matter, and removed oil, among others.

The seedlings were then placed on a plastic sheet and left to air-dry for 24 to 48 h. Then, they were subjected to 72 h of oven drying, set at 70°C. Each experimental unit was subsequently placed into an individual paper bag. All samples were cut into small fragments and ground into a powder using a stainless-steel grinder, consistent with the digestion method detailed by Tandon [28]. The samples were then dried in an oven at 60°C overnight.

The soil samples, on the other hand, were first dried at room temperature and then in an oven at 60 °C overnight. After the drying process was completed, the soil samples were sieved using a 2 mm sieve. Furthermore, they were ground into a fine powder and stored in clean jars. Electrical Conductivity (EC), pH, chlorophyll levels, increase in height, biomass, and oil removal were measured at the Research Center of Agricultural Engineering Science College. On the other hand, organic Matter (OM) and Nitrogen, Phosphorus, and Potassium (NPK) content in the samples were evaluated at the College of Agriculture and Forestry, Mosul University.

D. Measurements

Prior to the plant uprooting day, the concentration of chlorophyll pigment was measured. One gram of fresh leaves was extracted using 98% ethanol to determine chlorophyll a and b (Chlo. a and b) in the leaves (mg/g fresh weight). The maximum absorbance (A) of the solution was determined using a spectrophotometer at wavelengths of 645 nm and 665 nm, following the procedure outlined by Wintermans and Mots [29], applying Eqs. (1) and (2).

$$Chlo.a = (13.7)(A665\ nm) - (5.76)\ (A645\ nm) \quad (1)$$

$$Clho.b = (25.8)\ (A645\ nm) - (7.6)\ (A665\ nm) \quad (2)$$

where A665 nm and A645 nm are absorption of the sample at 665 and 645 nm wavelengths respectively.

The height of each seedling was documented at the beginning and end of the study. A tape measure was used to create a straight line from the soil level to the highest point of the seedling. The height increase of the seedlings was calculated by determining the difference between the starting and final height measurements.

The Electrical Conductivity (EC, μs/cm) of the soil was measured using an extract of (1:2.5) soil and water by means of an EC meter model (Microprocessor Conductivity/TDS meter HANNA-HI-9635) as described by Rowell [30]. Moreover, the Organic Matter (OM) content of the soil was evaluated by employing the Walkley and Black method with K2Cr2O7 (1N) in accordance with Allison [31]. The pH level of each soil sample was measured in extracts with a ratio of 1:2.5 using the ESMART Pen Type Water Quality Meter.

Total nitrogen content in soil and shoot samples was determined using the traditional Microkjeldahl method, as outlined by Ryan *et al.* [32]. Total potassium levels in soil and shoot samples were evaluated using a Flame Photometer set to a wavelength of 589 nm [33]. Total phosphorus in root and shoot samples was determined through a colorimetric technique utilizing a 6705 UV/Vis spectrophotometer, in accordance with the procedure described by Ryan *et al.* [32].

Total Petroleum Hydrocarbons (TPHs) were quantified using a Gas Chromatography-Flame Ionization Detection (GC-FID) system, specifically the Agilent 7890A model equipped with an HP-5 column (30 m × 0.32 mm × 0.25 μm). This methodology aligns with recent protocols for TPH determination in environmental samples.

The analysis of Total Petroleum Hydrocarbons (TPH) fractions was performed using an Agilent 7890A Gas Chromatograph equipped with a Flame Ionization Detector (GC FID). The instrument was fitted with an HP-5 capillary column (30 m × 0.32 mm × 0.25 μm), functioning at a detector temperature of 350°C, an inlet temperature of 320°C, and a carrier gas flow rate of 1 mL/min.

Samples were initially kept at 4 °C and subsequently dried with anhydrous sodium sulfate (Na_2SO_4) to eliminate moisture. To extract the Total Petroleum Hydrocarbons, a mixture of hexane and acetone was employed as solvents. Prior to GC FID analysis, the samples were fractionated and purified into aromatic and aliphatic components using silica gel adsorbents. This methodology aligns with modern practices for assessing TPHs in environmental samples [34].

With the aim of measuring the percentage of oil removed from the soil during the phytoremediation period after 4 and 8 months, the level of hydrocarbons in the soil samples was measured using a Soxhlet apparatus, where 10 grams of air-dried soil were extracted with chloroform over a period of 8 h.

The chloroform extract was subsequently evaporated in a pre-weighed dish to facilitate the assessment of Total Petroleum Hydrocarbons (TPHs), and afterward, the percentage loss of TPH was determined [35]. The percentage of petroleum degradation in the studied soil was determined using the weight loss method by Eq. (3).

$$Oil\ degraded = \frac{orig.conc. - fin.conc.}{orig.conc.} \times 100\% \quad (3)$$

where *orig. conc.* is a crude oil concentration in the soil sample before Soxhlet extraction. *fin. conc.* is the crude oil concentration in the soil sample after Soxhlet extraction.

E. Statistical Analysis of Data

All data were analyzed with the Microsoft Statistical Analysis System SAS 2002. The variation between the treatment means and Analysis of Variance (ANOVA) was evaluated using the Duncan Multiple Range Test at a significance level of 0.05 [36].

III. Result and Discussion

A. Soil and Irrigation Water Properties

The pH level of the water was measured at 7.40 (Table 1), which falls within the neutral spectrum. This indicates a balanced level of acidity and alkalinity, making it appropriate for aquatic ecosystems as well as irrigation applications [37]. The Electrical Conductivity (EC) is recorded at 892.7 μs/cm, reflecting a moderate concentration of dissolved ions. This level is considered acceptable for agricultural use, without raising any major salinity issues [38].

The Total Dissolved Solids (TDS) measured at 450.7 mg/L, which is within the allowed range for drinking water (300–600 mg/L) [39]. Total alkalinity was recorded at 386 mg/L, suggesting a strong buffering capacity. This alkalinity helps to mitigate sudden changes in pH levels within water systems [40]. With a total hardness of 480.4 mg/L, the water is classified as very hard. While suitable for agricultural uses, it can lead to scaling problems in both domestic and industrial settings [39].

Table 1. Water parameters used for the irrigation

Water Parameters	Values	Units
pH	7.40	-
Electrical conductivity	892.7	µs/cm
Total dissolved Solids	450.7	mg/L
Total alkalinity	386.0	mg/L
Total hardness	480.4	mg/L

Soil properties in Table 2 show that the soil texture was sandy clay loam, consisting of 41.3% clay, 20.5% silt, and 38.2% sand. This particular soil texture retains sufficient moisture to facilitate plant growth while allowing excess water to drain off. Owing to these characteristics, this soil proves to be well suited for agricultural use. In addition, the incorporation of manure, compost, and other organic materials can improve soil fertility [41].

Table 2. Soil parameters analysis results

Soil parameters	Values	Units
Clay	41.3	%
Silt	20.5	%
Sand	38.2	%
Texture	Sandy clay loam	-
pH	7.88	-
EC	790	µs/cm
Total $CaCO_3$	16.43	%
Total organic matter	1.13	%
Total nitrogen	0.53	%
Total phosphorus	0.081	%
Total potassium	12.34	%

The soil's pH was measured at 7.88, indicating a slightly alkaline environment. This pH level supports the growth of a diverse array of crops; however, it may restrict the availability of specific nutrients like Fe and Zn, which could necessitate targeted amendments [42]. Moreover, the soil's electrical conductivity was recorded at 790 µs/cm, indicating low salinity levels suitable for most crops without posing risks associated with salt stress [38].

The soil was identified as calcareous, with a $CaCO_3$ content of 16.43%, which is classified as moderately calcareous. This affects the availability of certain nutrients, especially phosphorus and other micronutrients, requiring targeted management strategies [43].

Regarding Organic Matter (OM), the total level was found to be 1.13%, which is considered relatively insufficient for promoting healthy soil conditions. The findings highlight the need for organic amendments such as manure or compost to improve soil structure, fertility, and nutrient retention [44]. The soil's total nitrogen concentration was 0.53%, indicating extremely low fertility. Nitrogen is essential for protein synthesis, photosynthesis, and the overall growth of plants [45].

The low level of the soil's phosphorus, measuring 0.081%, combined with a high $CaCO_3$ concentration, makes phosphorus less accessible to plants. These measurements necessitate extra phosphorus fertilization for optimal crop yields [46]. Potassium level in the soil was measured at 12.34%, generally sufficient for various crops. However, higher amounts may be needed for plants requiring more potassium [47].

B. Impact of Crude Oil on Chlorophyll Content

Chlorophyll content (mg/g) serves as an essential indicator of both plant health and photosynthesis efficiency. The ratio of chlorophyll a to chlorophyll b varies across plant species and can be affected by environmental factors [48]. The results indicate that both chlorophyll a and b have recorded greater amounts in the control group relative to the contaminated group, as illustrated in the graph below in Fig. 1.

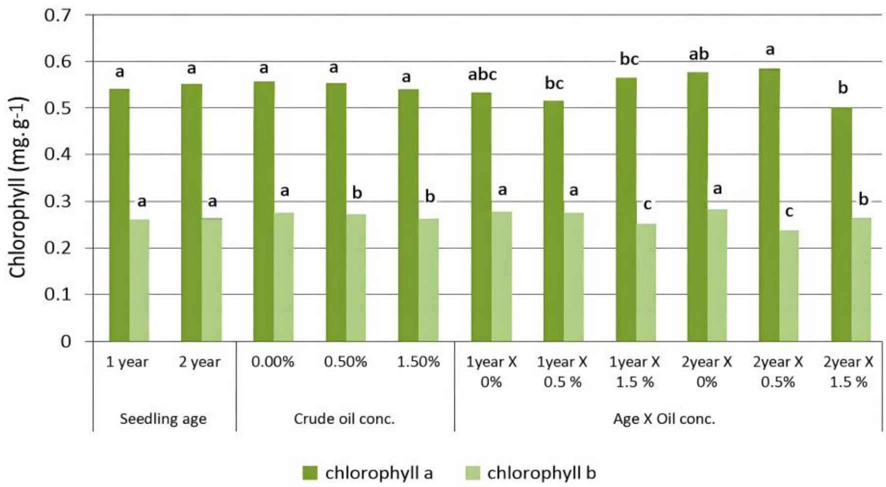

Fig. 1. The Chlorophyll a and b content (mg/g) in *C. sempervirens* seedlings. (For each factor, means that share the same letters do not show significant differences at the 5% level according to Duncan's multiple range test).

Growing plants in crude oil-contaminated soil significantly affects the production of chlorophyll pigments, resulting in a negative impact on the levels of chlorophyll a and b, and the overall chlorophyll content of the leaves [49]. Polycyclic aromatic hydrocarbons generate Reactive Oxygen Species (ROS) and result in symptoms similar to oxidative stress, such as reduction of chlorophyll [50].

The data suggest that older seedlings exhibit more developed root systems and enhanced nutrient absorption capabilities, which in turn facilitate chlorophyll biosynthesis. These findings align with earlier research demonstrating that plant maturity contributes to improved resilience against stress and enhanced photosynthetic performance under optimal conditions [51].

C. Impact of Crude Oil on Seedlings' Dry Weight and Height

It has been well established that mineral availability in soil highly determines plant Dry Weight (DW) and height. Plant growth and biomass are positively affected when minerals are readily available. Contrariwise, insufficient mineral availability impedes nutrient uptake, resulting in reduced dry weight and stunted plant height.

The root dry weight of plants exposed to crude oil showed no significant decrease, indicating that the presence of oil had no impact. However, the DW of shoots varied across treatments and interactions. As shown in Table 3, the maximum dry weight was recorded in 2-year-old seedlings at a crude oil concentration of 0% (12.666 g), followed by those at 0.5% crude oil (10.352 g).

Table 3. Impact of crude oil on the roots' dry weight, shoots' dry weight, and the height of *C. sempervirens* seedlings

Interactions	Treatments	Roots Dry Weight (g)	Shoots Dry Weight (g)	Increased Height (cm)
Seedling age	**1-year**	2.969 a	8.938 b	14.824 a
	2-year	3.133 a	10.219 a	9.891 b
Crude oil conc.	**0%**	3.139 a	11.509 a	16.639 a
	0.50%	2.985 a	9.206 b	9.917 b
	1.50%	3.029 a	8.022 b	10.517 b
Age × Oil conc.	**1-year × 0%**	3.008 a	10.352 b	20.056 a
	1-year × 0.5%	2.905 a	8.70 bcd	11.333 c
	1-year × 1.5%	2.993 a	7.763 d	13.083 b
	2-year × 0%	3.269 a	12.666 a	13.222 b
	2-year × 0.5%	3.065 a	9.713 bc	8.500 d
	2-year × 1.5%	3.064 a	8.280 cd	7.950 d

For each factor, means sharing any same letter (a, b, c, d) are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

On the other hand, the minimum dry weight was noted in 1-year-old seedlings subjected to a 1.5% crude oil concentration (7.763 g). In various crude oil treatments, a concentration of 0% oil consistently yielded higher dry weight (11.509 g) than 0.5% (9.206 g) and 1.5% (8.022 g), indicating the detrimental effects of oil pollution on plant development and enhanced performance of seedlings in conditions of low or no contamination.

Control plants exhibited the greatest increase in height, reaching 16.639 cm, significantly exceeding the height recorded in the 0.5% and 1.5% crude oil treatments. In terms of interaction between age and crude oil, the highest measurement was noted in 1-year-old seedlings with 0% crude oil, reaching 20.056 cm, significantly distinct from all other treatments. The presence of crude oil in the plant-soil microenvironment appears to affect nutrient release and uptake. Besides, it alters soil chemistry and reduces the amount of water available for plants [52].

D. Effect of Crude Oil on the Soil pH, Electrical Conductivity (EC), and Organic Matter (OM)

As shown in Table 4, soil pH is essential for plant vitality, as it affects microbial activity and nutrient availability. Additionally, plants typically thrive when nutrient availability is optimal within a soil pH range of 6.0 to 7.5. Any deviation from this range may result in either toxicities or deficiencies in nutrients, negatively affecting plant development [53]. The data presented in Table 4 show a significant distinction in soil pH values between contaminated soils and the control. These results, however, contradict those of Agbogidi *et al* [54], who reported no notable differences between the 2 groups. The presence of crude oil introduces hydrocarbons that disrupt microbial activity and nutrient cycling, likely resulting in a decrease in soil pH [55].

Table 4. Impact of crude oil on the pH, EC, and OM of the soil

Interactions	Treatments	pH	EC (µs/cm)	OM (%)
Seedling age	1-year	7.813a	858.778 a	1.586 a
	2-year	7.781a	851.111 a	1.647 a
Crude oil conc.	0%	7.904 a	811.167 c	1.328 c
	0.50%	7.768 b	864.000 b	1.578 b
	1.50%	7.718 b	889.667 a	1.942 a
Age × Oil conc.	1-year × 0%	7.967 a	795 e	1.317 d
	1-year × 0.5%	7.735 b	883 ab	1.630 c
	1-year × 1.5%	7.737 b	898.333 a	1.810 b
	2-year × 0%	7.842 ab	827.333 d	1.34 d
	2-year × 0.5%	7.802 b	845 c	1.527 c
	2-year × 1.5%	7.700 b	881 b	2.073 a

For each factor, means sharing any same letter (a, b, c, d) are not significantly different at $p < 0.05$ according to Duncan's multiple range test.

Soil Electrical Conductivity (EC) indicates its ability to transmit electrical current, which is influenced by the concentration of dissolved salts. Crude oil itself has low EC, but when present in the soil, it triggers several biological and geochemical processes that increase soil EC [56]. Consequently, soil EC rises considerably when contaminated with crude oil [57]. Nevertheless, crude oil contamination has been reported in some studies to reduce EC, although the extent of this reduction depends on the properties of the soil and various environmental factors. Seedling age, on the other hand, has no significant effect on EC, as indicated in Table 4. The highest percentage of crude oil, at 1.5%, recorded an EC value of 889.667 (µs/cm), significantly different from both the 0% and 0.5% levels. When considering the interaction of plant age with crude oil concentration, significant differences in EC were observed across all treatments. Higher EC readings, observed in 1.5% crude oil-contaminated soil, suggest an increased concentration of dissolved salts. This can be attributed to the hydrocarbon's breakdown, releasing salts into the soil matrix. Conversely, lower EC values observed in uncontaminated soil (0%) emphasize the stability of nutrient dynamics in unpolluted environments [58].

Organic Matter (OM) is vital for soil fertility and health as it enhances nutrient availability, stimulates microbial activity, and improves water retention. Crude oil-contaminated soil can alter OM dynamics, either increasing OM through hydrocarbon accumulation or decreasing it by impeding microbial decomposition processes [59]. Pots subjected to crude oil treatment exhibited significantly greater OM levels, reaching the highest percentages of 1.942% in 1.5% crude oil-contaminated soil and 1.578% in 0.5% crude oil-contaminated soil.

Regarding the interaction between seedling age and crude oil concentration, 2-year-old seedlings in 1.5% crude oil-contaminated soil exhibited the highest OM value, which was significantly different from all other treatments, whereas 1-year-old seedlings in uncontaminated soil had the lowest OM value at 1.137%, as illustrated in Table 4.

These results align with the research conducted by Polyak *et al.* [59], who reported that crude oil contamination increases soil OM due to the added hydrocarbons while negatively impacting plant growth. This suggests that hydrocarbons can alter the physical, chemical, and biological characteristics of the soil [60].

E. Removed Crude Oil

The contamination levels implemented in this study amount to (0.5% and 1.5% w/w, equivalent to 5000 and 15,000 mg TPH/kg soil, based on typical crude oil density). These levels fall within the (0.4%–2.5%) range, consistent with moderately contaminated field sites. High pollution ranges from (2.5%–10%), while very high pollution ranges between (10%–15%). Contamination levels above 15% TPH are considered extreme or catastrophic pollution [61]. For example, studies on oil spills on land have found TPH concentrations from 40,000 mg/kg (4%) in Iran to over 75,000 mg/kg (7.5%) in Iraq [62].

Numerous studies have shown that Total Petroleum Hydrocarbon (TPH) levels in contaminated soils tend to decline over time, even without any intervention [63, 64]. Conversely, utilizing plants for the phytoremediation of crude oil-contaminated soil accelerates the degradation of oil faster than allowing it to occur naturally, which can be a slow process.

The natural breakdown of TPH is stimulated by microorganisms through a mechanism called bioattenuation, and phytoremediation studies must take this natural background effect into account. It is important to distinguish the effects of phytoremediation from natural microbial degradation, which occurs independently of plant presence.

Accordingly, control pots empty of plants was employed to distinguish the effect of plants and their associated microorganisms from that of microbial activity alone. TPH levels were assessed after both 4- and 8-months post-planting. Consequently, control pots that do not contain plants were employed to distinguish the influence of vegetation and its associated microorganisms from that of microbial activity alone.

TPH levels were measured after 4- and 8-months post-planting (Table 5). After 4 months, 38.03% and 35.98% of the TPH were removed from the planted 0.5% and 1.5% crude oil-contaminated soils, respectively. Conversely, non-planted 0.5% and 1.5% crude oil-contaminated soils saw only 16.85% and 17.58% oil removed, respectively.

Table 5. Percent (%) of removed crude oil during 4 and 8 months in vegetated and non-vegetated pots by *C. sempervirens* polluted with 0.5 and 1.5 % crude oil

Interactions	Treatments	Period of Phytoremediation	
		4 Months (%)	**8 Months (%)**
Seedling age	1-year	36.29	87.45
	2-year	37.72	87.59
Age× Oil conc.	1year × 0.5%	38.19	87.98
	1year × 1.5%	34.38	86.91
	2year × 0.5%	37.86	87.49
	2year × 1.5%	37.57	87.68
Vegetated pots	0.5%	38.03	87.74
	1.5%	35.98	87.30
Non-vegetated pots	0.5%	16.85	45.70
	1.5%	17.58	43.90

After 8 months, the rate of oil degradation in the same treatments climbed to 87.74% and 87.30% in planted soil compared to 45.70% and 43.90% in non-planted soil. The same results in Iran were recorded by Taheri *et al.* [65] on Eucalyptus camaldulensis; 82% oil hydrocarbon reduction was observed in the treatment of 1% oil pollution. These findings indicate that the tested plant may be particularly effective for phytoremediation of soils contaminated with TPH.

The mechanistic supporting evidence of oil phytoremediation effectiveness in planted vs. non-planted soils can be linked to various factors. These include root exudates like sugars, carbohydrates, amino acids, and organic acids, which enhance and boost microbial activity in the rhizosphere and stimulate oil breakdown [66].

Root systems enhance soil porosity, water infiltration, soil aeration, and oxygenation. These conditions favor aerobic degradation of oil by microorganisms [67]. Enzymes like phenol oxidase secreted by plant roots oxidize or detoxify hydrocarbons directly [68].

A comparison between planted and non-planted pots reveals that TPH reduction was significantly greater in the presence of *C. sempervirens*. This supports the plant-mediated remediation mechanism, as opposed to losses occurring solely through non-biological processes.

Cupressus sempervirens exhibited a slower growth rate, particularly during the initial stages, which may be attributed to the growing conditions in pots. These conditions are different from those of farms or natural environments, where roots have more space to access nutrients.

Nonetheless, hydrocarbon stress-induced growth inhibition is a well-recognized problem, even among commonly used phytoremediation species. These plants can establish quickly and thrive at early stages, particularly in dry, rocky soils. However, growth may subsequently decelerate as the trees mature [69].

Notably, even with the reduced biomass, seedlings persisted for the entire 8-month exposure period, maintaining physiological activity, developing roots, and ultimately achieving a considerable reduction in cumulative TPH over time.

These results indicate that *C. sempervirens* has a tolerance for petroleum hydrocarbons. This characteristic is vital for remediation efforts in areas with significant contamination or extreme conditions where rapidly growing crops typically find it difficult to thrive.

F. Composition of Total Petroleum Hydrocarbon (TPH) by Gas Chromatograph

Fig. 2 illustrates the chromatographic profile of total hydrocarbons determined in the crude oil utilized in this study, while Figs. 3 and 4 depict the degradation chromatographic profiles of the TPH fraction degraded throughout the 8-month study period.

It is evident that the phytoremediation process using the *C. sempervirens* plant resulted in the removal of hydrocarbon compounds ranging from Propane to N-Tridecane (N-C3 to N-C13) from the crude oil-contaminated soil. However, the analytical data from the Gas Chromatograph/Flame Ionization Detector GC/FID indicated that Nonane (N-C9) and other hydrocarbons still existed in the soil.

Referring to Table 6, hydrocarbons that were entirely removed from contaminated soil and were no longer detected amounted to approximately 20.42% of the total hydrocarbon ratios measured by the Gas Chromatograph.

This indicates that low molecular weight portions need a considerable period of time to fully volatilize from the soil. This is likely due to the fact that bioremediation of high molecular weight hydrocarbons is difficult [66]. High molecular weight hydrocarbons represent a category of pollutants whose degradation using microorganisms in polluted soil has frequently been unsuccessful [70].

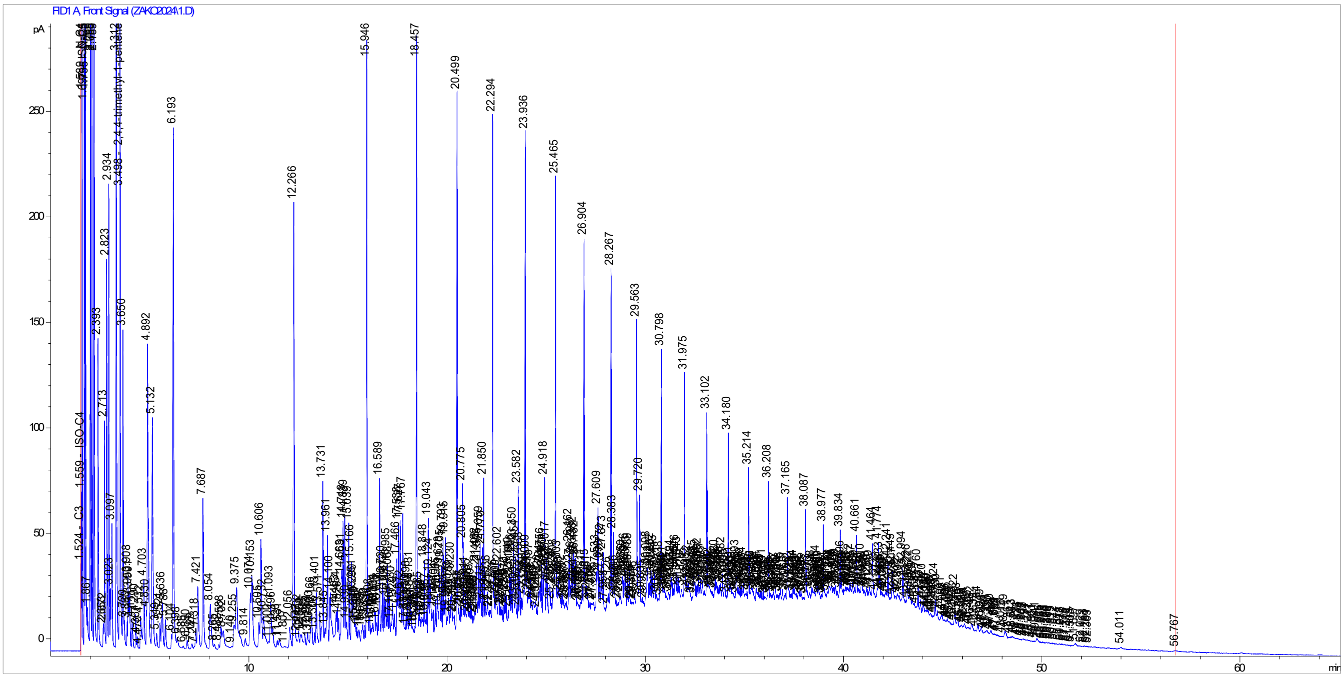

Fig. 2. Chromatographic profile of crude oil used in the experiment.

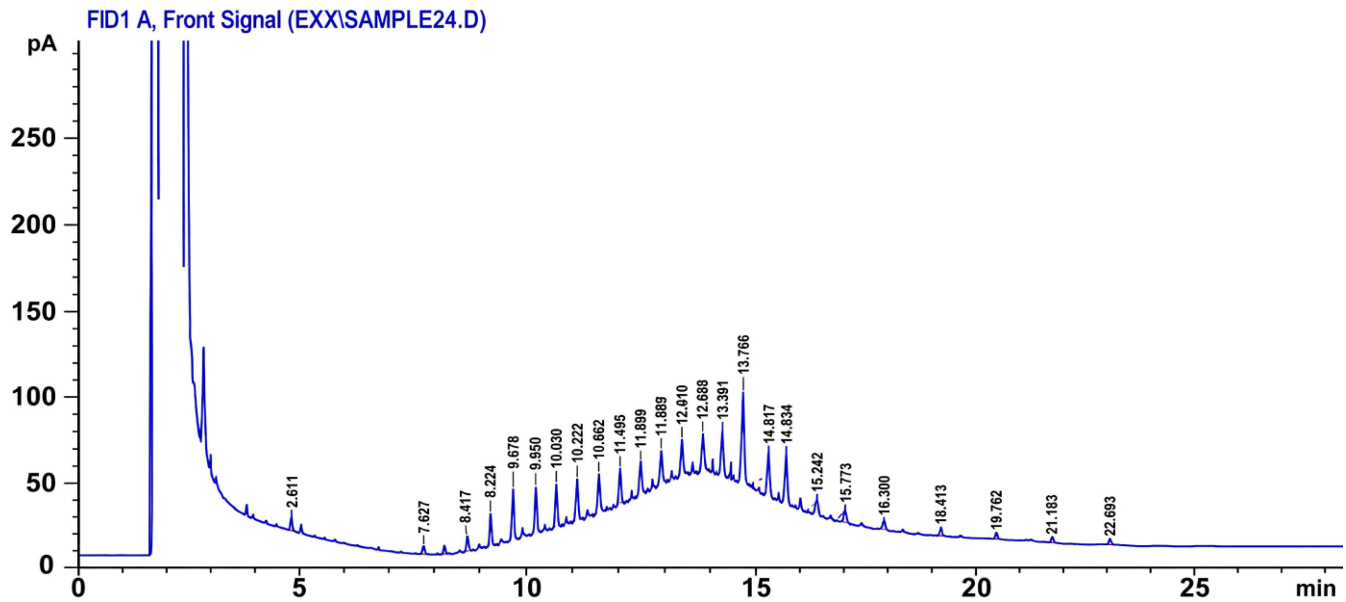

Fig. 3. The chromatographic profile of hydrocarbons in the contaminated soil with 0.5% crude oil after been phytoremediated by *C. sempervirens* plants.

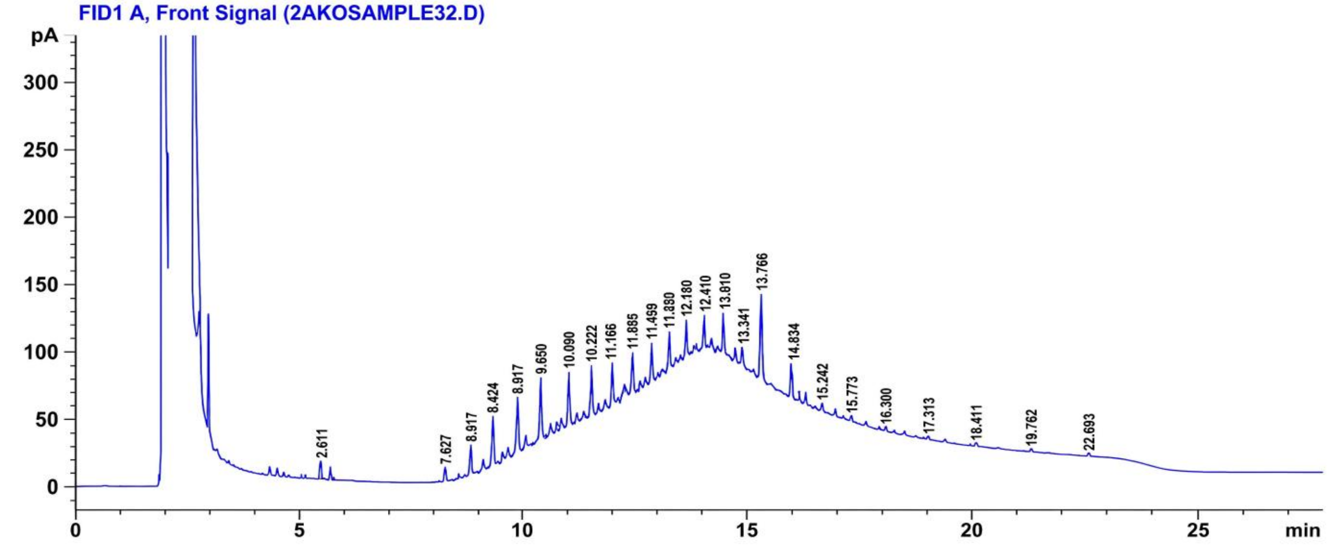

Fig. 4. The chromatographic profile of hydrocarbons in the contaminated soil with 1.5% crude oil after been Phyto remediated by *C. sempervirens* plants.

Table 6. Compositional analysis stabilized oil by gas chromatography technique based on (ASTM D5307)

Component	Hydrocarbon Name	Wt.% in Crude Oil	Presence in Soil at the End of the Study
Methane	N-C1	NP*	NP
Ethane	N-C2	NP	NP
Propane	N-C3	0.041	NP
iso-Butane	N-C4	0.072	NP
n- Butane	N-C4	0.416	NP
iso-Pentane	N-C5	0.596	NP
n-Pentane	N-C5	0.957	NP
Hexanes	N-C6	2.227	NP
Heptanes	N-C7	2.468	NP
Octanes	N-C8	2.818	NP
Nonanes	N-C9	2.69	P**
Decanes	N-C10	3.086	NP
UNPecanes	N-C11	2.796	NP
Dodecanes	N-C12	2.505	NP
Tridecanes	N-C13	2.544	NP
Tetradecanes	N-C14	2.531	P
Pentadecanes	N-C15	2.436	P
Hexadecanes	N-C16	2.248	P
Heptadecanes	N-C17	2.089	P
Octadecanes	N-C18	2.232	P
Nonadecanes	N-C19	1.949	P
Eicosanes	N-C20	1.923	P
Henicosanes	N-C21	1.737	P
Docosanes	N-C22	1.697	P
Tricosanes	N-C23	1.529	P
Tetracosanes	N-C24	1.349	P
Pentacosanes	N-C25	1.274	P
Hexacosanes	N-C26	1.242	P
Heptacosanes	N-C27	1.188	P
Octacosanes	N-C28	1.189	P
Nonacosanes	N-C29	1.226	P
Triacontanes	N-C30	1.257	P
Hentriacontanes	N-C31	0.987	P
Dotriacontanes	N-C32	0.927	P
Tritriacontanes	N-C33	0.847	P
Tetratriacontanes	N-C34	0.634	P
Pentatriacontanes	N-C35	0.486	P
Hexatriacontanes+	N-C36	43.807	P
Total		100	

*NP: not present. **P: present.

Table 6 shows that the hydrocarbon compounds removed from the soil through the phytoreduction process are characterized by short carbon chains. In contrast, hydrocarbon compounds with long carbon chains did not undergo the decomposition process and remained in the soil.

Studies indicate that carbon chain length significantly influences the breakdown of hydrocarbons by plants and the microorganisms present in their rhizosphere [71]. Typically, short-chain hydrocarbons (C10–C20) are more readily degradable due to their higher solubility in water (less hydrophobic), making them easier for plant roots and microorganisms to absorb. They act as a source of carbon and energy for decomposing bacteria (e.g., *Pseudomonas*, *Bacillus*) [72]. Conversely, long-chain hydrocarbons (C20–C40) are more difficult to decompose due to lower solubility and stronger attachment to soil particles (particularly clay). This reduces their ease of transport across the cell membranes of plants or microorganisms, requiring specialized enzymes (such as oxygenases) for long carbon chain cleavage [73].

G. Effect of Crude Oil on N, P, and K Concentration in Soil and Plant

Crude oil contamination has an impact on soil nutrient balance, often resulting in increased sodium (Na) levels and reduced presence of phosphorus (P), nitrogen (N), and potassium (K). This impact is attributed to shifts in soil pH and alterations in microbial activity. Nitrogen is essential for plant growth and chlorophyll production. Phosphorus supports root development and energy transfer, whereas potassium enhances disease resistance and regulates water management. Crude oil presence may result in nutrient deficiencies, reduction in soil fertility, and negative impact on overall plant health [74].

Plants cultivated in crude oil-contaminated soil in Table 7 and Table 8 show higher nitrogen concentration (N) in their shoots in comparison to those cultivated in uncontaminated soil. The lower nitrogen levels indicate that crude oil contamination can reduce the nitrogen available to plants by increasing competition from soil microorganisms that use nitrogen for their growth. The findings indicate that hydrocarbon pollution leads to the immobilization of nitrogen within microbial biomass, which in turn decreases its accessibility for plant absorption [75]. This finding contradicts a previous study [76], which maintains that crude oil pollution reduces plant nitrogen absorption. Nonetheless, the impact of oil contamination on nitrogen absorption and plant growth differs based on the plant species and initial soil properties.

Phosphorus content in plant shoots in Table 7 remained consistent across different treatments, regardless of crude oil concentration, indicating a minimal effect of contamination. The level of phosphorus in soil varied significantly between older and younger seedlings. Two-year-old seedlings planted in 0.5% crude oil-contaminated soil exhibited the highest phosphorus content (0.1%), followed closely by those planted in 1.5% (0.097%) crude oil-contaminated soil. Whereas 1-year-old seedlings planted in crude oil-free soil had the lowest phosphorus content (0.077%).

Table 7. Impact of crude oil on the NPK (%) in soil

Interactions	Treatments	N in Soil	P in Soil	K in Soil
Seedling age	1-year	0.64a	0.078b	12.082b
	2-year	0.63a	0.092a	13.270a
Crude oil conc.	0%	0.48c	0.078b	12.478a
	0.50%	0.67b	0.088a	12.775a
	1.50%	0.74a	0.090a	12.775a
Age × Oil conc.	1-year × 0%	0.49c	0.077bc	11.289b
	1-year× 0.5%	0.68b	0.075c	13.072ab
	1-year× 1.5%	0.75a	0.083b	11.884ab
	2-year × 0%	0.47c	0.079bc	13.666a
	2-year × 0.5%	0.67b	0.100a	12.478ab
	2-year × 1.5%	0.74a	0.097a	13.666a

For each factor, means that share the same letters do not show significant differences at the 5% level according to Duncan's multiple range test.

Table 8. Impact of crude oil on the NPK (%) in shoots

Interactions	Treatments	N in Shoots	P in Shoots	K% in Shoots
Seedling age	1-year	1.537a	0.313a	0.863a
	2-year	1.447b	0.307a	0.662b
Crude oil conc.	0%	1.418c	0.315a	0.750a
	0.50%	1.502b	0.314a	0.751a
	1.50%	1.555a	0.302a	0.787a
Age × Oil conc.	1-year × 0%	1.467b	0.268b	0.857a
	1-year × 0.5%	1.563a	0.358a	0.871a
	1-year× 1.5%	1.580a	0.314ab	0.861a
	2-year × 0%	1.370c	0.361a	0.643c
	2-year × 0.5%	1.440b	0.271b	0.630c
	2-year × 1.5%	1.530a	0.290b	0.714b

For each factor, means that share the same letters do not show significant differences at the 5% level according to Duncan's multiple range test.

The findings indicate that soil phosphorus remains relatively stable regardless of contamination level, with variations likely attributed to seedling age and interactions between plants and soil. The influence of crude oil contamination on soil phosphorus concentration proved to be minimal [77].

Potassium (K) levels in the shoots Table 8 exhibited significant differences based on seedling age. 1-year-old seedlings in 0.5% crude oil-contaminated soil recorded the highest K presence (0.871%) across all treatments, whereas 2-year-old seedlings in the same crude oil concentration had the lowest K content (0.630%) [76]. These results indicate that potassium K accumulation is highly affected by seedling age, with younger seedlings demonstrating higher uptake efficiency. This suggests that age plays a significant role in the patterns of nutrient dynamics between plants and soil [77], as illustrated in Table 7.

H. Translating Results into Practical Applications in the Field

The current study shows the potential of *C. sempervirens* for phytoremediation under lath house conditions. A significant hydrocarbon reduction has been observed over an 80-day period. However, the translation of these findings into an effective field strategy is anticipated to take additional time, a procedure that necessarily addresses important practical constraints. The phytoremediation process may be slowed because of numerous field-condition variables, including fluctuating temperatures (which affect microbial activity and plant metabolism), heterogeneous soil texture and contaminant distribution, and variable moisture regimes [78]. Addressing these variables entails the application of supportive management techniques to set the ground for a successful field implementation. These techniques include irrigation to maintain optimal soil moisture without leaching and initial soil tilling to homogenize the contaminant and incorporate bulking agents for better soil aeration. For an improved degradation consortium, researchers may resort to the application of controlled fertilization and possible inoculation with mycorrhizal fungi or hydrocarbon-degrading rhizobacteria isolated from contaminated sites [79]. In addition, phytostabilization—physical containment by the root system—poses a low risk of upward contaminant transfer into harvestable biomass.

IV. Conclusions

The results of the present study indicate that crude oil contamination reduced soil pH, while both soil EC and organic matter content increased compared with the control. Even though Cypress seedlings exhibited the capacity to grow in 1.5 % crude oil-contaminated soil, chlorophyll b and plant biomass reduced, with no fatality recorded among the tested plants.

By the end of the study, the proportion of phytoremediated crude oil ranged from 87.831% to 87.300%. Short-chain hydrocarbons were removed from the soil as a result of phytoremediation, while long-chain hydrocarbons did not undergo the decomposition process and remained present in the soil. Crude oil was shown to have a notable impact on nitrogen concentration in both soil and plant tissues; however, no significant effects were observed on phosphorus and potassium, except for phosphorus in the plants.

Despite the promising results, there are several limitations that need to be considered. The experiment was conducted in a lath house, which may not fully reflect the complex conditions of a crude oil-contaminated field environment. The focus of the study was mainly growth parameters, hydrocarbon fractions, and total hydrocarbon removal, without thoroughly examining the transformation pathways of hydrocarbons within the soil-plant system. Additionally, the research evaluated only a single phytoremediation cycle, which might not accurately reflect the long-term remediation potential and ecological impacts of *C. sempervirens*.

Future research should prioritize long-term field studies to verify the phytoremediation efficiency of *C. sempervirens* under natural conditions. Further investigations are needed to understand the fate of specific petroleum hydrocarbons within plant tissues and the role of soil microorganisms in improving hydrocarbon breakdown. Furthermore, combining organic amendments such as biochar, compost, and animal manure should be considered to enhance soil quality and hydrocarbon availability.

Conflict of Interest

The author declares no conflict of interest.

Author Contributions

The author performed all aspects of this study, including experimental design, data analysis, manuscript preparation, and approval of the final manuscript.

Acknowledgment

The author would like to sincerely thank the research center of to the College of Agricultural Engineering Sciences for providing the resources and assistance required to finish this study. Special thanks are given to Mrs. Bayan Hazim, Miss Dereen Jaladet, Mr. Hasan Saleem and Mr. Abdulazeez for their invaluable help and support during this research.

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