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PHYTOREMEDIATION POTENTIAL OF *Catalpa bignonioides* IN CRUDE OIL-CONTAMINATED SOILS: EVIDENCE FROM DUHOK, KURDISTAN REGION

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ABSTRACT:

Phytoremediation is a promising method for cleaning crude oil-contaminated soils. This study aimed to evaluate the potential of *Catalpa bignonioides* seedlings for remediating soil polluted with 1% and 2% (w/w) crude oil. One- and two-year-old seedlings were grown for eight months under contaminated conditions. Plant growth parameters, crude oil degradation percentage, total petroleum hydrocarbons (TPH), soil pH, electrical conductivity (EC), organic matter (OM), and nitrogen (N), phosphorus (P), and potassium (K) levels in both soil and plant shoots were measured. The seedlings successfully grew in contaminated soil, with no plant mortality observed, despite some leaf yellowing and necrosis. Chlorophyll a remained unaffected, while chlorophyll b significantly decreased. Plant height, shoot and root biomass were significantly reduced at 2% oil concentration. Soil pH slightly decreased, while EC and OM increased with contamination. TPH analysis showed complete removal of 10 hydrocarbon fractions (C3–C8, C10–C14), with degradation rates ranging from 70.37% to 84.02%. Crude oil significantly affected soil N and P levels but not K; in plant tissues, only N was significantly altered. Two-year-old seedlings exhibited greater growth and higher N and K content than younger plants. These findings confirm the species' potential for phytoremediation of crude oil-contaminated soils.

KEYWORDS: Crude Oil Pollution, Phytoremediation, Hydrocarbons, Soil Remediation, *Catalpa Bignonioides*

1. INTRODUCTION

Environmental pollution is currently the most significant challenge facing humanity, leading to increased rates of mortality and morbidity. Pollution caused by industrial and human activities leads to the decline in the quality of the natural environment (Landrigan, 2017). Environmental degradation is an inevitable consequence of contemporary economic development that relies on fossil fuel use and industrial output (Haas, 2001). In recent years, the natural environment has undergone changes, becoming more vulnerable, delicate, and exceedingly intricate. Humanity carries a profound responsibility to protect the natural environment (Butnariu, 2018).

Likewise, the genesis of environmental contamination frequently lies in the introduction of harmful materials, such as sewage, industrial discharges, agricultural runoff, and electronic waste into aquatic environments; toxic materials, particulate matter, and gaseous pollutants into the air; along with actions that contribute to soil contamination, including unlawful dumping, mining, deforestation, and landfills (Ukaogo *et a., l*2020).

Soil pollution apart from earthquakes, erosion, and other natural disasters that tend to damage the soil, centuries of anthropogenic activities have given rise to widespread issues related soil pollution around the globe (Koul,2018). The main human caused sources are the chemicals used in or produced as a by-product of industrial, domestic and municipal wastes including crude oil products (Rodríguez *et al.,* 2018).

Fossil fuels from electric-generating plants, petrochemical plants and petroleum refineries also endorse soil pollution (Ukaogo *et al*, 2020). Entry of pollutants either directly or indirectly has been proved to contaminate wide areas of groundwater bodies and soil resources influencing crop production likewise human and animal health through food contamination and the environment in general (Saha *et al*, 2017). Crude Oil contains many different kinds of hydrocarbons, the most prevalent ones are paraffin, naphthene as well as arenes, additionally, a proportion of asphalt compounds. These hydrocarbons have different properties and uses such as lubricants, fuel and chemical feedstock. In order to obtain these products crude oil, undergo process which involve refining, petroleum exploration, and distribution through road transport that consequently led to soil contamination (Ekperi *et al*., 2021). The above-mentioned situation demands prompt attention and resolution. Although cleaning up terrestrial and aquatic ecosystems with conventional techniques considered efficient, however, a high cost with a specialized staff and equipment are needed. Bioremediation and phytoremediation proven to be an eco-friendly and a cost-efficient solution (Hussein *et al*, 2022). Furthermore, phytoremediation is one of the rehabilitation techniques relied on for the sites contaminated with total petroleum hydrocarbons (TPH), by utilizing green plants with the aim of restoring the biological, chemical and physical features of the environment demolished by anthropogenic activities.

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Nowadays, a wide range of plants identified for Phyto-remediating the area polluted with petroleum hydrocarbons (Masu *et al*, 2016) due to their ability to absorb, accumulate and detoxify pollutants from the soil (Ayuba *et al*, 2020). Many studies have been conducted on the cleaning of crude oil polluted soils with phytoremediation using different plant species (Wang *et al*., 2008, Moubasher *et al*., 2015; Xiao *et al*., 2015, Tang & Angela 2019). Nevertheless, limited number of studies have investigated the use of trees for remediation (Ayuba *et al*., 2020; Oyedeji *et al*., 2022; Abdallah *et al*., 2023). In spite the fact that Iraq is one of the largest crude oil producers in the world that transport, produce and refine oil, few studies on trees have been reported (Al-Obaidy *et al*., 2016., Al-Obaidy *et al*., 2018, and Al-Obaidy *et al*., 2019). This study examined to investigate the effect of crude oil contamination on soil properties and plant growth parameters, also to test the effectiveness of *Catalpa bignonioides* tree in phytoremediation of crude oil-contaminated soil, measured by the reduction of total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAH) in soil samples, as part of the broader environmental pollution concerns already highlighted in previous studies in the Duhok region (Khaled *et al*., 2023).

2. MATERIALS AND METHODS

Tree Description :

Catalpa bignonioides is known as Indian Bean Tree, Cigar Tree or Southern Catalpa. It's a deciduous tree of medium-sized, growing 15–18 meters tall, with a trunk reaching 1 meter diameter. The tree characterized with its brown to gray bark, evolving into sturdy plates or ridges as it matures (Keeler, 2005). Additionally, it has a short thick trunk which form a broad and irregular head. While, the roots are fibrous, fleshy and poisonous. The foliage is of vibrant green, large sized with a heart shaped form (Sun *et al*, 2024).

Catalpa trees endure broad range of soil conditions, encompassing acidic to alkaline ranges with wet clay soils, sandy terrain, loamy compositions and more. Good drainage is constantly preferred; however, Catalpa can subsist both flooding and further drought periods (Keeler, 2005).

Study Area:

This study was carried out in the lath house of the College of Agricultural Engineering Sciences within Duhok city area which is situated 430-540m above sea level in the north of Kurdistan region (N 36˚ 52′ 03″ E 42˚ 59′ 34″) (Mohammed, 2013). The ongoing study was conducted during February and persisted further till December of the year 2023.

Crude Oil Used in the Study:

The crude oil used in the experiment was obtained from local oil refineries in Kwashe industrial area in Duhok city, the Compositional Analysis Stabilized Oil by Gas Chromatography Technique Based On ASTM D5307 showed the following hydrocarbon in the crude oil used in the study**;** Propane (N-C3), iso-Butane (N-C4), n- Butane (N-C4), iso-Pentane (N-C5), n-Pentane (N-C5), Hexanes (N-C6), Heptanes (N-C7), Octanes (N-C8), Nonanes (N-C9), Decanes (N-C10), Undecanes (N-C11), Dodecanes (N-C12), Tridecanes (N-C13), Tetradecanes (N-C14), Pentadecanes (N-C15), Hexadecanes (N-C16), Heptadecanes (N-C17), Octadecanes (N-C18), Nonadecanes (N-C19), Eicosanes (N-C20), Henicosanes (N-C21), Docosanes (N-C22), Tricosanes (N-C23), Tetracosanes (N-C24), Pentacosanes (N-C25), Hexacosanes (N-C27), Heptacosanes (N-C27), Octacosanes (N-C28), Nonacosanes (N-C29), Triacontanes (N-C30), Hentriacontanes (N-C31), Dotriacontanes (N-C32), Tritriacontanes (N-C34), Tetratriacontanes(N-C34), Pentatriacontanes(N-C35), and Hexatriacontanes (N-C36)

Soil Preparation :

Soil was collected from the fields of the College of Agricultural Engineering Sciences. Physical and chemical properties of soil and water used in the study are represented in the tables 1 and 2 respectively.

Crude oil was incorporated into the air dried soil at concentrations of 0%, 1%, and 2% (weight: weight), ensuring uniform mixing. Four kilograms of the contaminated soil were placed into pots, which were then set up at the experimental site. The soil was irrigated for two weeks to stabilize before planting. Uniform one- and two-year-old Catalpa seedlings were sourced from the Directorate of Nurseries in Duhok. Careful handling ensured minimal root damage as seedlings were removed from pots. Their roots were rinsed with water to clear soil residues and immediately planted into the experimental pots. Watering was conducted as needed to support seedling establishment.

Seedlings Selection:

Catalpa tree species was chosen for the current study. The seedlings of both ages one and two years old were selected and transferred from Malta plants nursery to the study site. The initial and final height measurements were taken for both before planting. The seedlings were planted in April 2023, in a polyethylene bags (each of 4 kg of soil). Throughout the entire experiment duration, the seedlings were consistently watered with well water.

Design of the Experiment :

The Randomized Completely Block Design (RCBD) was followed during the current study. Catalpa seedlings species of the two different ages (one- and two-years old), with three crude oil concentrations (0%, 1% and 2%), three replicates and four plants within each experimental unit.

Accordingly, the experimental unit's number = 1 plant X 2 ages X 3 concentration X 3 replicates = 18 experimental units X 4 plants in each experimental unit = 72 seedlings

Measurement of Seedling Height Increase :

At the beginning and end of the study, the height of each seedling was measured from the soil surface to the apex using tape. The increase in seedlings' height was computed by subtracting the initial measurement from the final one (Figure 3**).**

Measurement of Chlorophyll A, Chlorophyll B and Total Chlorophyll:

Before plants rooting up Chlorophyll a, b, and total chlorophyll (mg/g fresh weight) were measured by extracting one gram of leaves in 98% ethanol, measuring absorbance at 645 nm and 665 nm, calculating concentrations using formulas from Wintermans and DeMots (1965).

Chlorophyll a:

mg chl. a/ml solution = (13.7) (A 665 nm) – (5.76) (A 645 nm)

Chlorophyll b:

mg chl. b/ml solution = (25.8) (A 645 nm) – (7.6) (A 665 nm)

Total chlorophyll:

Total chlorophyll = (chlorophyll a) + (chlorophyll b)

Root Biomass (Seedling Uprooting)

After 8 months of experimental study exactly in 2022, November all seedlings were rooted up. Each plant was delicately uprooted and the shoot system was separated from the root system. Soil attached to the roots was meticulously cleared, and both shoot and root systems were washed individually. Subsequently, they were left to air dry 24 to 48 hours. After the drying period, the plants were 72 hours oven dried at 70°C. Finally, each experimental unit was individually placed in paper bags.

Analysis of Soil Samples:

Soil samples underwent a natural air-drying process at room temperature. While for those retaining moisture an overnight oven treatment at 60 °C was applied. Subsequently all soil samples were crushed and sieved using a mesh with 2-mm aperture size and further ground into a fine powder which was then preserved in sanitized jars (for digestion). EC, pH, chlorophyll content, shoot and root biomass and removed oil were analyzed at Research Center of Duhok Agricultural Engineering Science College. While, OM and NPK samples were analyzed at College of Agriculture and Forestry, University of Mosul.

Soil Electrical Conductivity (EC, µs/Cm)

The electrical conductivity of the studied soil was measured in soil extracts of (1:2.5) utilizing EC meter model (Microprocessor Conductivity/TDS meter HANNA-HI-9635) as performed by (Rowell, 2014).

Organic Matter Content :

The soil (O.M) content was determined during this research, as documented by Walkley and Black technique using $K2Cr2O7$ (1N) according to (Allison, 1965).

$$O.C\,(\%) = \frac{NFeSO4 \times [VSample - VBlank] \times 1.3 \times 0.003 \times 100}{\text{Weight of soil}} * 100$$

$$\text{Organic matter}\,(\%) = \mathbf{OC}\,(\%) \times \mathbf{1.72}$$

Determination of Ph:

ESMART Pen Type Water Quality Meter was used to measure the pH of all the soil samples. The soil pH of extracts (1:2) was determined, utilizing pH meter model (HI 9023) as described by (Jackson, 1958).

Digestion of Shoots and Soil Samples :

Shoots and Soil samples were digested following the guidelines outlined by Tandon in 1999.

Determination of Total Nitrogen in Shoots and Soil:

The total Nitrogen was determined by classic Microkjelhdahls, following the procedure of (Ryan & Astafan, 2003).

N%=V.HCl×N.HCl×14.00710001005500.5

Determination of Potassium in Shoots and Soil :

By following Flame-phtometer JENWAY PFP 7, and the equation below:

ppm=sample result1.12350100010000.5

P%=ppm/10000

Determination of Total Phosphate in Shoots and Soil:

The Phosphate in shoots and soil samples were detected by colorimetric method, 6705 UV/Vis. Spectrophotometer. Based on the procedure of (Ryan & Astafan, 2003) with using the following formula:

ppm=ABs0.077×50×50510.5

P%=ppm/10000

Determination of Total Nitrogen % in Plant:

Soil Nitrogen determined by Kjeldal apparatus according to the steps previously applied by (AOAC., 2004)

Determination of Phosphorus % in plant:

Phosphate was determined by using colorimetric methods based on the method of (AOAC., 2004)

Determination of Potassium in Plant:

Potassium in leaves was Measured using the flame photometer method at 589 nm wavelength. (AOAC., 2004).

Fraction Analysis of Total Petroleum Hydrocarbon in Soil by: GC-Gas Chromatography

The determination of Total Petroleum Hydrocarbons fraction was conducted using an Agilent 7890A Gas Chromatograph coupled with a Flame Ionization Detector (GC-FID). The system utilized an HP-5 capillary column (30 m × 0.32 mm × 0.25 µm), operating with a detector temperature of 350 °C, an inlet temperature of 320 °C, and a carrier gas flow rate set at 1 mL/min. Samples were initially stored at 4 °C, then dried using anhydrous sodium sulfate (Na_2SO_4) to remove moisture. Extraction of Total Petroleum Hydrocarbons was achieved using a combination of hexane and acetone as solvents. Prior to GC-FID analysis, samples underwent fractionation and cleanup into aromatic and aliphatic components using silica gel adsorbents. This approach is consistent with contemporary methods for analyzing TPHs in environmental matrices (TPHCWG, 1998).

Percentage of Petroleum Degradation :

Percent of petroleum decomposition in the soil was evaluated using an updated weight loss protocol (Li *et al.*, 2021). A 10 g soil sample contaminated with crude oil was extracted with 20 ml dichloromethane (DCM) in a container of known weight. The mixture was gently agitated to enhance oil extraction and then allowed to sit at room temperature for 24 hours to ensure full solvent evaporation. The container's weight with the residual oil was measured, and the percentage of oil degradation was calculated using the formula:

$$\%\text{Oil degraded} = \frac{original\ conc.in\ soil - \text{final conc. in soil}}{original\ conc.in\ soil} * 100$$

Analysis of Data:

The data were analysed by using Microsoft Statistical Analysis System (SAS) 2002. The variations among the treatment means and ANOVA (Analysis of variance) were tested with Duncan Multiple Range Test at 0.05 probability level (Duncan, 1955).

3. RESULTS AND DISCUSSION

Water Parameters:

Irrigation water parameters in table 1; The measured pH of the water is 7.4; the value indicates a slightly alkaline condition which, according to WHO guidelines falls within the optimal range for agricultural use, most freshwater organisms, and human consumption (WHO, 2017). The electrical conductivity (EC) of 892.7 µS/cm indicates moderate salinity suitable for various daily activities. This result aligns with WHO standards (<1000 µS/cm) and poses minimal risk to crops and soil health (Ayers & Westcot, 1985 and WHO, 2017). The total dissolved solids (TDS) value of 450.7 mg/L falls within the acceptable range for necessary daily uses, as recommended by WHO with a maximum limit of 1000 mg/L for palatability (WHO, 2017). The total alkalinity of 386.0 mg/l implies a high buffering capacity, supporting irrigation and aquatic life while stabilizing pH by controlling acidity-related stress (Chapman, 1996). Lastly, the total hardness of 480.4 mg/l reflects a high concentration of magnesium and calcium ions, classifying it as hard water. This hardness positively contributes to plant nutrition by providing essential minerals (Ayers & Westcot, 1985).

Table 1: Irrigation water parameters

Water parameters	Values	Units
pH	7.40	
Electrical conductivity	892.7	(µs.cm^{-1})
Total dissolved Solids	450.7	mg. l^{-1}
Total alkalinity	386.0	mg. l^{-1}
Total hardness	480.4	mg. l^{-1}

Soil Parameters:

As shown in table 2; the particle size distribution was 41.3% clay, 20.5% silt, and 38.2% sand, classifying it as sandy clay loam texture, which corresponds to moderate drainage with good nutrient and water retention capacity. This soil type is suitable for a wide range of agricultural purposes (Ma *et al*, 2022). However, the high clay content may require proper management to prevent compaction and improve aeration (USDA, 1999). The soil pH of 7.88 indicates slight alkalinity. Despite its suitability for many crops, some acid-loving plants may suffer from nutrient deficiencies, requiring adjustments such as sulfur or the addition of organic matter to lower the pH (Brady & Weil, 2008). The soil has an electrical conductivity (EC) of 790 µs.cm^{-1}, indicating low salinity, ideal for most crops (FAO,2024). The total calcium carbonate ($CaCO_3$) content of 16.43% classifies the soil as calcareous, which decreases the availability of phosphorus and micronutrients such as iron and zinc (Lal & Shukla, 2004).

The organic matter content of 1.13% is relatively low and may restrict microbial activity, soil fertility, and water retention (Brady & Weil, 2008). At 0.0053 mg/kg, the soil's total nitrogen content is incredibly low and insufficient for healthy plant growth (Stevenson & Cole, 1999). Testing indicates a total phosphorus level of 0.00081 mg/kg, which is considered a very low availability of phosphorus in the soil. This nutrient level is critical for plant development and energy transfer, particularly under stressful circumstances (Havlin *et al*., 2016). With a potassium level of 12.34 mg/kg, the soil is classified as moderately fertile, which is essential for plant resilience and growth under stress. This baseline level is necessary for evaluating external factor impacts and nutrient dynamics (Marschner, 2012).

Table 2: Physical and chemical properties of soil

Soil parameters	Values	Units
Clay	41.3	%
Silt	20.5	%
Sand	38.2	%
Texture	Sandy clay loam	---
pH	7.88	---
EC	790	µs.cm^{-1}
Total $CaCO_3$	16.43	%
Total organic matter	1.13	%
Total nitrogen	0.0053	mg/kg
Total phosphorus	0.00081	mg/kg
Total potassium	12.34	mg/kg

Effect of Crude Oil on Chlorophyll :

Chlorophyll, essential for photosynthesis, point to plants overall health and efficiency. The crude oil contamination interrupts microbial activity and nutrient availability, eliminating chlorophyll synthesis and photosynthesis (Martins, *et al*., 2023). The total chlorophyll content in Catalpa seedlings was affected by seedling age and the concentration of crude oil, the results of Baruah *et al*. (2014) study align with and support our findings regarding the effect of crude oil on chlorophyll content. Two-year-old seedlings exhibited significantly higher levels (0.734 mg/g). Considering crude oil concentration, the highest chlorophyll content (0.751 mg/g) was observed under 0% crude oil, reflecting optimal growth conditions as evidenced by figure 1. Seedling aged two years maintained more constant chlorophyll levels, suggesting greater tolerance likely due to more advanced root and physiological development (Wilson & Carter, 2017). The crude oil is a mix of organic compounds of high molecular weight, aliphatic and, aromatic which suppress the essential enzymes to synthesize chlorophyll (Baruah, P., *et al.* 2014).

Th study by Pilipović *et al*. (2012) on poplar clones exposed to crude oil-contaminated soil contrast with the results of our study, which revealed the chlorophyll A and B contents in Catalpa seedlings indicate no significant variations across treatments, with values remaining statistically constant regardless of seedling age or crude oil concentration (0%, 1%, 2%). This stability suggests that chlorophyll A and B are less affected by external stresses, including crude oil pollutants, which likely supports photosynthetic function under stress even when total chlorophyll is compromised (Smith & Adams, 2020).

A **B**

For each factor means with same letters are not significantly different at 5% level based on Duncan's multiple range Test.

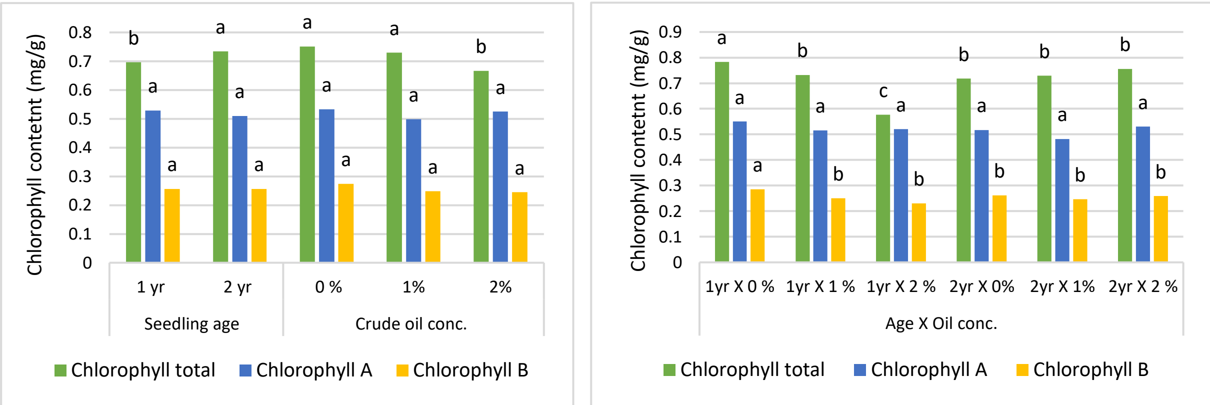


Figure 1 (A): Effect of crude oil contamination on chlorophyll a, b and total in *Catalpa bignonioides.* **(B):** Interaction effect of crude oil contamination and seedling age on chlorophyll a, b and total in *Catalpa bignonioides.*

Shoots and Roots Dry Weight:

Dry weight (DW) and increase in plant height stand as key indicators of mineral readiness in soil and plants. A reduction in mineral availability restricts nutrient uptake, resulting in stunted growth and lower biomass (DW). The analysis of dry weight (DW) in roots revealed that two-year-old seedlings retained higher DW values (9.254 mg/g) consistently compared to their younger counterparts, which were more affected (seedlings under 1% crude oil experienced a reduction to 5.221 mg/g), as outlined in the chart 2 below. The findings imply that older seedlings withstand stress better and accumulate biomass even under crude oil contamination, likely due to more developed root systems and better environmental stress adaptation (Wilson & Carter, 2017 and Smith & Adams, 2020).

A **B**

For each factor means with same letters are not significantly different at 5% level based on Duncan's multiple range Test.

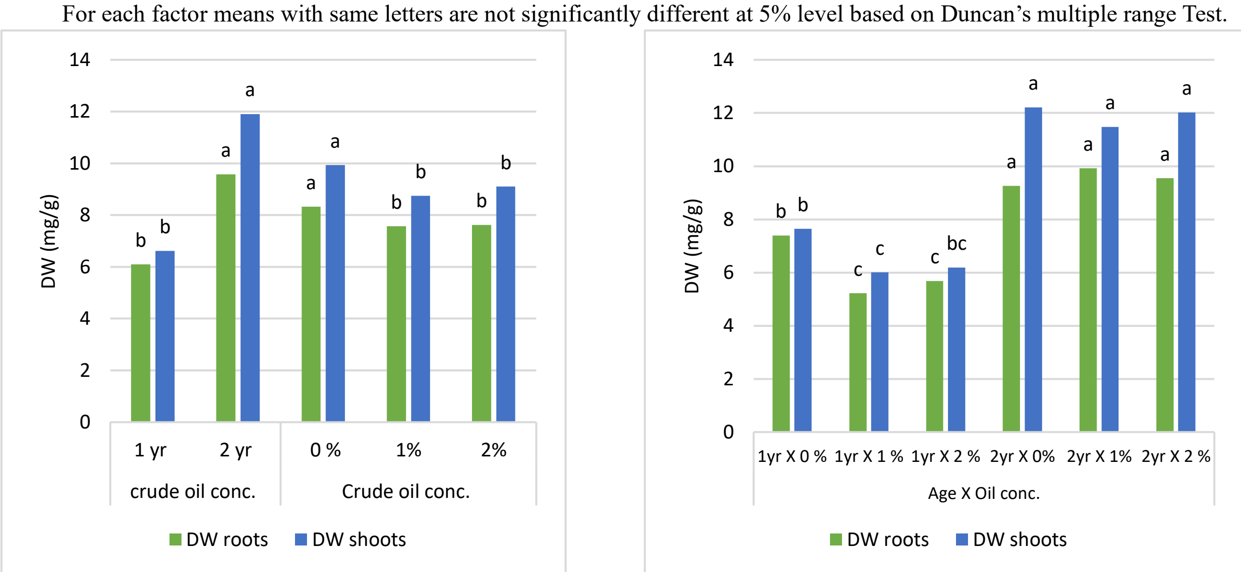
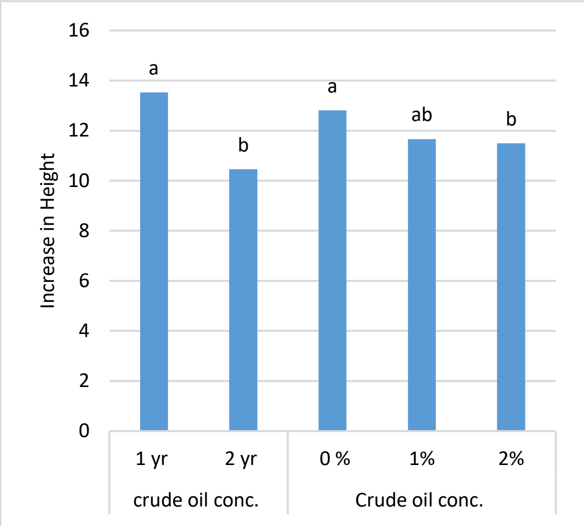
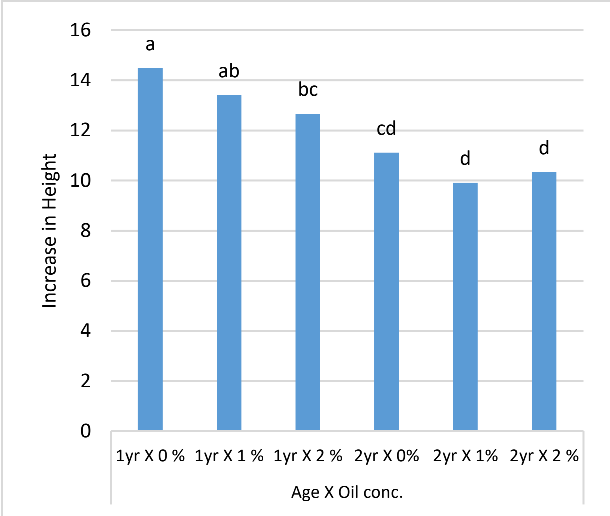


Figure 2 (A): Effect of crude oil contamination on roots and shoots dry weight mg. plant^{-1} of *Catalpa bignonioides.* **(B):** Interaction Effect of crude oil contamination and seedling age on roots and shoots dry weight mg. plant^{-1} of *Catalpa bignonoides.*

Seedlings Height:

Figure 3 indicates that increase in seedling height was at its maximum under 0% crude oil (14.500 cm) and the least increase in height were found under highest concentrations 2% of crude oil (9.917 cm). This reduction suggests that growth is hindered by crude oil stress (Smith & Adams, 2020). The obtained results were in agreement with those obtained by (Adenipekun *et al.*, 2009) that oil in soil above 2% concentration affects the growth severely, according to non-availability of necessary water, which in turn lead to imbalance nutrient uptake and mobility.





A **B**

For each factor means with same letters are not significantly different at 5% level based on Duncan's multiple range Test.

Figure 3 (A): Effect of crude oil contamination on height increase cm. plant^{-1} of *Catalpa bignonioides*. **(B):** Interaction effect of crude oil contamination and seedling age on height increase cm. plant^{-1} of *Catalpa bignonioides.*

Soil Ph, Electrical Conductivity and Organic Matter :

Soil pH is vital for plant health as it controls nutrient availability, microbial activity, and prevents element toxicity, with most nutrients being accessible at a pH range of 6.0–7.5. (Wang *et al*., 2013). From the interaction involving two years old seedlings, soil pH exhibited minimal variation the with the highest value of 7.958 at 0% crude oil and the lowest of 7.722 at 1% oil concentration illustrated in table 3. These minor differences reflect the limited influence of crude oil on soil alkalinity (Brown & Lee, 2020). This study supports evidence from previous observations of Barua *et al*. (2011), who demonstrated that contaminated soils are modestly more acidic as a result of the formation of toxic acids in the oil spill.

Electrical Conductivity (EC) is a measure of a soil's capacity to conduct electrical current, it is directly connected to the soluble salts' concentration in soil. From the interaction regarding EC, higher values demonstrate elevated salinity, which can be due to crude oil contamination (Chukwu, *et al*., 2022). EC values varied across treatments, with the highest EC values (840.667 µS/cm and 836.333 µS/cm) observed under 2% and 1% crude oil treatments, respectively. The lowest EC value (752.000 µS/cm) was found in two-year-old seedlings under 0% crude oil as appeared in table 3. These discrepancies indicate that crude oil contamination exacerbates salinity or ion concentration in the soil environment, particularly affecting younger seedlings (Fu *et al*., 2022). The results of other research has reported reductions in EC under crude oil contamination, suggesting that hydrocarbon coatings might limit ion exchange in certain soils (Saikia *et al*., 2023). These variations indicate that while increasing crude oil levels generally raise EC, the extent of this effect depends on soil type and specific contamination characteristics.

Organic matter (OM) content peaked in two-year-old seedlings (2.123) and was lowest in one-year-old seedlings (1.613). Crude oil contamination appeared to slightly increase OM through the accumulation of decomposed hydrocarbons and organic debris in the topsoil. The more extensive root systems in older seedlings may correlate with higher organic contributions and microbial activity, contributing to elevated OM levels (Fageria and Moreira 2011).

Degradation Percentage of Petroleum Hydrocarbons in Soil:

Petroleum hydrocarbons residues in soil determinate after 8 months, and percentages degradation was calculated as compared with initial crude oil concentration 1 and 2% (Table 3). According to the data, we observe that the phytoremediation process by Catalpa plants degraded most of the crude oil, with a percentage degradation range of 71.131-84.040 %. This range is comparable to what researcher (Abdallah *et al*., 2023) found when he planted seedlings of *Acacia sieberiana* Tausch in soils polluted with 0.5, 1, 1.5, and 2% crude oil; he recorded 49-79% oil degradation after 3 months. Both the current results and those of other researchers highlight the potential of using trees for soil remediation in oil-contaminated areas. Additionally, other plants are used for crude oil polluted soils phytoremediation such as cotton that removed 50.66% of the TPHs from soil polluted with 5% crude oil after 5 months (Al-Obaidy, 2018). Furthermore, *Cyperus laxus* Lam has been utilized in phytoremediation of soil contained with 6 % hydrocarbons and 55 % hydrocarbons removed after 6 months (Escalante-Espinosa *et al*., 2005)

There were no significant differences between one- and two-year-old seedlings in the process of crude oil phytoremediation from the soil. The higher removal rates are possibly linked to increased microbial activity in the rhizosphere, as root exudates enhance hydrocarbon breakdown. This highlights the role of plant-microbe interactions in oil remediation, influenced by localized soil texture, aeration, and nutrient availability around the seedlings (Lee and Park, 2019 and Brown *et al*., 2020).

Table 3: Effects of seedling age, crude oil contamination and their interaction on pH, EC, OM and oil percentage degradation.

Treatments		pH	EC (µS/cm)	OM %	Oil degradation%
Seedling age	1-yr	7.844a	817.222a	1.871111 a	77.956
	2-yr	7.805a	797.444a	1.907778 a	75.350
Crude oil conc.	0 %	7.923a	763.333b	1.748333 c	Why there is not value here
	1%	7.794ab	826.167a	1.865 b	82.175
	2%	7.758b	832.500a	2.055 a	71.131
Age X Oil conc.	1-yr X 0 %	7.888ab	774.667bc	1.753333 c	Why there is not value here
	1-yr X 1 %	7.866ab	836.333a	1.873333 ab	84.02
	1-yr X 2 %	7.778ab	840.667a	1.986667 ab	71.895
	2-yr X 0%	7.958a	752.000c	1.743333 c	Why there is not value here
	2-yr X 1%	7.722b	816.000ab	1.856667 ab	80.333
	2-yr X 2 %	7.737b	824.333a	2.123333 a	70.367

For each factor means with same letters are not significantly different at 5% level based on Duncan's multiple range Test.

Composition of Total Petroleum Hydrocarbon (TPH) By Gas Chromatograph:

Figure 4 represent total hydrocarbons chromatographic profile of crude oil used in the study, while figures 5 and 6 (1 and 2% crude oil polluted soil) are Chromatographic profiles of the TPH fraction degradation during the course of the eight-month experiment. It is evident that the period of phytoremediation by Catalpa plants resulted in the complete disappearance of hydrocarbon compounds (N-C3 to N-C8 and N-C10 to N-C14) from the soil contaminated with 2% crude oil. Hydrocarbon fraction analysis of soil polluted with 2% crude oil showed that hydrocarbons (N-C3 to N-C8 and N-C10 to N-C12) were entirely removed; however, the Gas Chromatograph (GC/FID) analysis revealed that Nonane (N-C9) and other hydrocarbons still remained in the soil. Similar findings were reported by Mehrasbi *et al*. (2003), who noted that the remediation of long-chain hydrocarbons requires more time for complete removal from oil-polluted soils, even with microbial intervention. This indicates that a considerable period of time was required for the low molecular weight portions to fully volatilize from the soil.

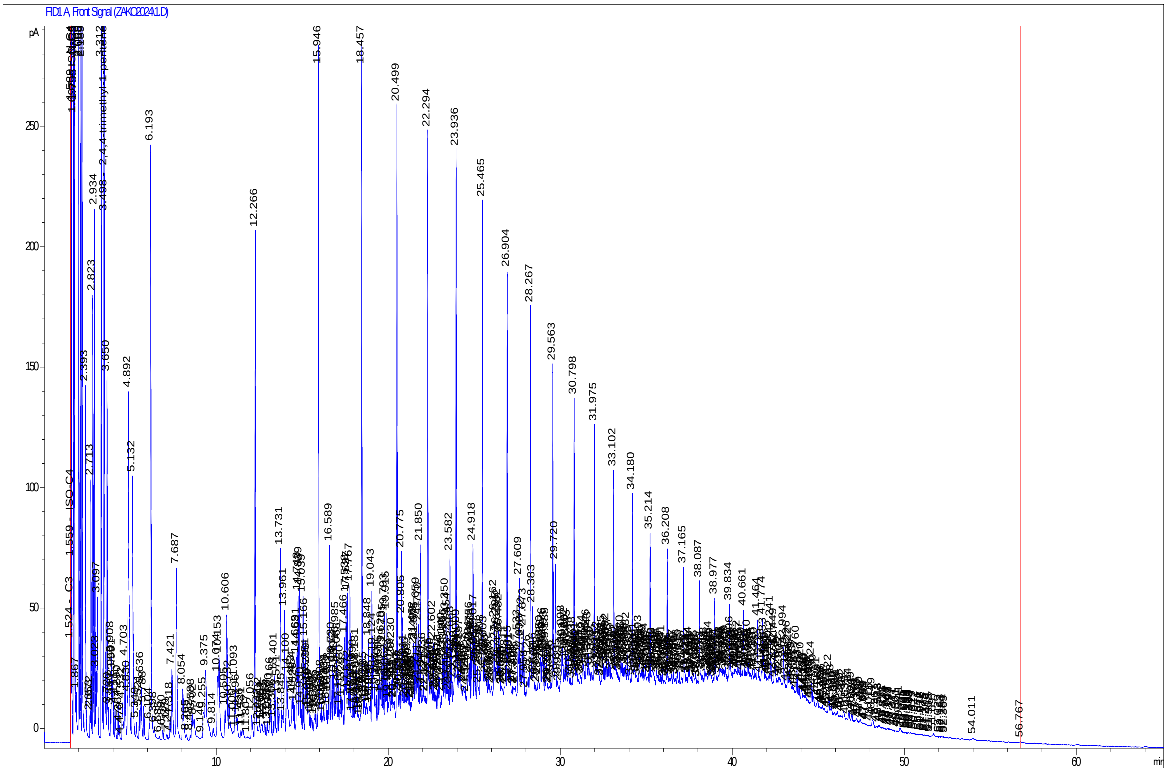


Figure 4: Chromatographic profile of crude oil used in the experiment

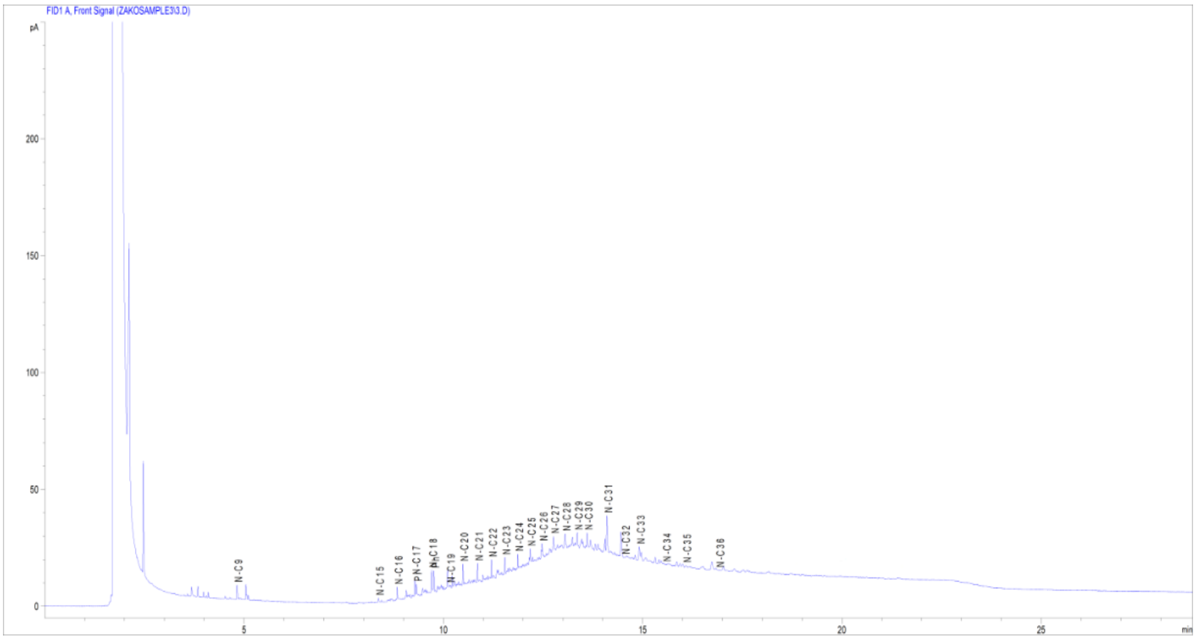


Figure 5: Chromatographic profile of hydrocarbon compounds in the soil polluted soil with 1.0 % crude oil after 8 months of phytoremediation by Catalpa plants

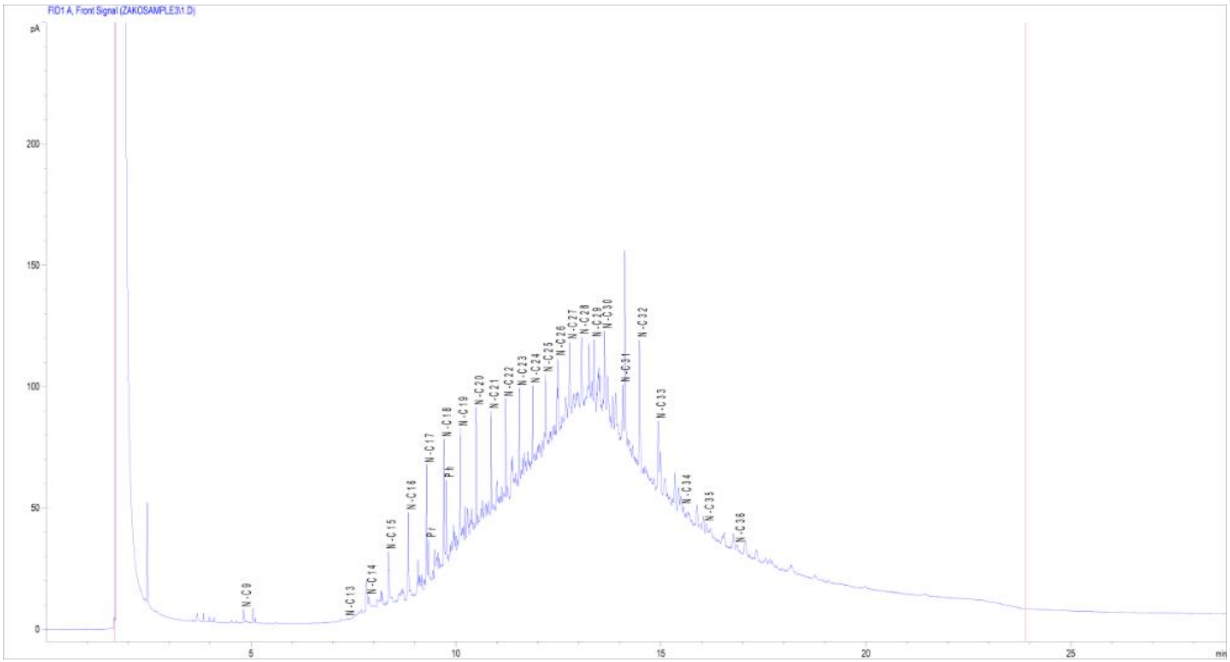


Figure 6: Chromatographic profile of hydrocarbon compounds in the soil polluted soil with 2.0 % crude oil after 8 months of phytoremediation by Catalpa plants

Nitrogen, Phosphorous and Potassium Content:

N, P and K are vital elements for soil fertility and plant development. N is vital for the production of chlorophyll and protein synthesis, P enhance energy flow and root development, and K promote disease tolerance and regulates water balance. These nutrient abundance in the soil can be disrupted by crude oil contamination Agim *et al*., (2021).

The nitrogen content in Catalpa seedlings showed significant differences regarding each of seedling age and the interaction between ages and oil concentrations. Where the two ages, with the highest values found in younger seedlings exposed to 1% and 2% crude oil as clarified in table 4. However, this contrasts with the present findings, which show that soil nitrogen concentrations differ significantly, with the highest value (0.0068) observed in the interaction of 1-year-old seedlings with 2% crude oil. This underscores the role of hydrocarbons in altering nitrogen dynamics. These results suggest that hydrocarbon contamination triggers nitrogen immobilization into microbial biomass, reducing its availability for plant uptake (Agnello *et al*., 2020). John *et al*. (2016) demonstrated that the amount of nitrogen (N) in the shoots of tested plants and the soil was higher in uncontaminated soil compared to soils with varying hydrocarbon content, suggesting that crude oil contamination reduced nitrogen uptake by plants.

Phosphorus levels in the soil ranged from 0.00084 mg/g to 0.00063 mg/g, showing significant variation across treatments. Moreover, the rate increased correlates with the rising percentage of crude oil in the soil. These outcomes differ from Research by Nwakwasi, et . (2019) and Osipova *et al.* (2020) estimate the available phosphorus (P) decreased as the concentration of crude oil increased. As crude oil contamination modifies microbial communities which considered an essential part of phosphorus cycling, resulting in a shortage of available phosphorus in soil (Nwakwasi *et al*., 2019).

The potassium content in shoots was significantly influenced by seedling age but not by crude oil treatments. Values ranged from 0.665% in older seedlings to 0.426% in younger ones, suggesting that age-related physiological factors, such as root development and nutrient uptake efficiency play a more critical role than crude oil contamination (Smith & Adams, 2020). In the soil, potassium levels showed no significant differences across treatments, except for the interaction of 1-year-old seedlings under 2% crude oil (13.666 mg/g) and 1% crude oil (10.695 mg/g).

Table 4: Effect of crude oil contamination on N, P, and K (%) in soil and Catalpa shoots

Treatments		N in shoots	N in soil	P in shoots	P in soil	K in shoots	K in soil
Seedling age	1 yr	1.592a	0.0062a	0.228a	0.00072b	0.609a	12.082a
	2 yr	1.496b	0.0057b	0.221a	0.00079a	0.477b	12.159a
Crude oil conc.	0 %	1.453b	0.0056b	0.223a	0.00072b	0.540a	11.956a
	1%	1.597a	0.0057b	0.221a	0.00074b	0.544a	11.586a
	2%	1.582a	0.0066a	0.229a	0.00079a	0.546a	12.818a
Age X Oil conc.	1yr X 0 %	1.380e	0.0059c	0.204a	0.00063d	0.550c	11.884ab
	1yr X 1 %	1.700a	0.0058c	0.242a	0.00068cd	0.612b	10.695b
	1yr X 2 %	1.697a	0.0068a	0.236a	0.00084a	0.665a	13.666a
	2yr X 0%	1.527b	0.0054d	0.241a	0.00081ab	0.530c	12.029ab
	2yr X 1%	1.493c	0.0055d	0.200a	0.00081ab	0.476d	12.478ab
	2yr X 2 %	1.467d	0.006b	0.221a	0.00074bc	0.426e	11.970ab

For each factor means with same letters are not significantly different at 5% level based on Duncan's multiple range Test.

CONCLUSION

Depending on the results of this study, it was concluded that *Catalpa bignonioides* seedlings can be grown in soil polluted with crude oil and can tolerate oil concentration up to 2%. Additionally, there was 84.02 % loss of TPH at the end of the eighth month. The results revealed that soil acidity decreased with crude oil contaminated, on the other hand, electrical conductivity and organic matter increased. Crude oil treatment of the soil resulted in a significant increase in total nitrogen concentration, while no significant changes were observed for phosphorus (P) and potassium (K). Moreover, two-year-old seedlings showed better growth performance and higher nutrient uptake than one-year-old seedlings. The species also contributed to the complete removal of light hydrocarbon fractions (C3–C14), indicating its efficiency in degrading low-molecular-weight hydrocarbons. Based on the obtained results, it can be concluded that *Catalpa begnonoides* is suitable for phytoremediation as a phytoextraction technique of hydrocarbon-contaminated soil.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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