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Phytoremediation of crude oil-polluted soil from Egbema Nigeria using *Hibiscus cannabinus*

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Abstract

Crude oil pollution is an environmental problem affecting different areas around the world. The continual use of crude oil and its combustion products impact our environment and human health negatively. Phytoremediation is a process with recorded success in remediation activities. Physicochemical properties of unpolluted agricultural soil (control) crude oil-polluted soil and Egbema polluted soils were analyzed using standard methods prior to and after 90 days planting of *Hibiscus cannabinus*. Total petroleum hydrocarbon (TPH) content of soil samples were analyzed using gas chromatography-flame ionization detector (GC-FID). Results show that sample A3 had the highest agronomic properties (number of leaves, fresh weight and total plant height) which corresponds to the highest percent remediation (46.01%) obtained in the study. This shows a correlation between biomass production and crude oil removal in the phytoremediation plant *Hibiscus cannabinus*. In addition, soil pH and moisture content increased from mean range 4.80-7.30 and 2.50-15.50% to mean range of 5.10-8.20 and 5.00-27.90% respectively showing remediation activity of polluted soils. *Hibiscus cannabinus* therefore offer great potential in crude oil remediation from Egbema polluted sites.

Keywords: Crude oil pollution; Hydrocarbon remediation; Plant biomass; Organic amendment; Plant-growth experiment

1. Introduction

Pollution of the environment as a result of anthropogenic activities is of major concern globally. Crude oil pollution is one of the major problems devastating even the remote areas of the world. Crude oil or petroleum is an admixture of natural hydrocarbon and polar chemical substances obtained from within the earth's crust, usually under the sea [1]. The growing dependence on petroleum and its products has resulted in its over-exploitation, exploration and downstream processing, leading to devastating effects on the environment [2]. Often, the use of petroleum products, such as petrol, diesel and lubricating oils, has resulted in the release of various harmful chemicals, including polycyclic aromatic hydrocarbons (PAHs) and heavy metals, into the environment [3, 2]. Contaminants, such as PAHs, are not readily degradable by microorganisms within the environment due to high levels discharged or their recalcitrance.

The toxic effects of oil spills on soil are of great concern. This issue comprises the focus of several research groups [4, 5, 6]. Tang et al. [7] reported the harmful effects on earthworms, bacteria and plants at 10.57% soil petroleum contamination. At 2% crude oil contamination, the mortality rate of earthworms was reported to be 90% after 7 days, while no earthworm survived at 3% and above crude oil contamination. Likewise, 1% crude oil concentration inhibited

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approximately 100% bacteria. Growth inhibition was recorded in maize and wheat to be 51.3% and 48.4% respectively, on exposure to 3% crude oil concentration. Similarly, high concentrations of oil were reported to inhibit root growth [7]. An increase in crude oil content from 31 mg/kg to 1000mg/kg greatly reduced the survival rate of earthworms after 14 days from 80% to 33% [8]. Additionally, the study by Ramadass et al. [9] reported that used lubricating oil resulted in total mortality of earthworms above 3.88 g/kg soil contamination. Therefore, oil spillage on soil greatly impacts the surrounding environment. This emphasizes the vital need for efficient removal of crude oil contaminants from soil.

Plants are used for the removal of diverse chemical pollutants from the environment [10]. This clean-up method is environmentally friendly, affordable and a natural process of remediation. Plants absorb, degrade and compartmentalize chemical pollutants through their extensive root structure [11]. This cost-effective approach is less invasive and generally accepted than other methods of remediation. The choice of Egbema for this study was based on the fact that recent reports have shown that soil in the Egbema axis has high acidity, low nitrogen, exchangeable bases, phosphorus and organic matter content [12]. The need to enhance soil fertility and quality for improved health conditions, sustainable agricultural output and food security was reported as necessary in Egbema [13, 12]. The study was aimed at assessing the physical and chemical properties of crude oil-polluted soil and Egbema-polluted soil samples before and after the plant growth experiment using *H. cannabinus* and therefore determine the phytoremediation activities of the plant on crude oil-polluted soil.

2. Material and methods

2.1. Collection and preservation of soil samples

Crude oil-polluted soil samples were collected from surface soil using a sterilized soil auger 0-25cm deep, from an oil-polluted site behind the Nigerian Petroleum Development Company Limited reservoir (Lat 5º55'56''N, Long 6º76'34''E) at Ukwugba Obiakpu, in Egbema/Ohaji Local Government Area, Imo State. Unpolluted agricultural soil was obtained from FUTO farms, Federal University of Technology Owerri. Soil samples were placed in pre-labeled sterile polyethylene bags and stored in an ice chest. Subsequently, samples were transported to the laboratory for analysis within 2 h of collection. Soil samples for the plant growth experiment were air dried at room temperature for 7 days and sieved through a 2 mm mesh size net. The fine earth was used for plant growth while the coarse particles were discarded. Bonny light crude oil was obtained from Ukwugba Obiakpu in Egbema/Ohaji Local Government Area, Imo State. The crude oil sample was placed in a properly labeled plastic container, wrapped in black plastic bags and transported to the laboratory within 2 h of collection. The crude oil obtained was filter-sterilized using Whatman #1 filter paper and stored in pre-labeled plastic containers before use. Appropriate safety measures were applied while using petroleum products.

2.2. Collection and preservation of organic amendments

Fresh banana peels were sun-dried for two weeks, ground to powder using a clean mortar and pestle and stored in pre-labeled air-tight containers. Brewery spent grains was obtained from Intafact Beverages Limited, Onitsha, Anambra State. Brewery spent grains was placed in sterile polyethylene bags, properly labeled and transported to the laboratory. Spent grains were sun-dried for seven days and stored in pre-labeled air-tight containers.

2.3. Raising of seedlings

Seedlings were raised from viable *H. cannabinus* seeds on a nursery bed (1×3 m^2) containing sandy-loamy soil. Nursery beds were kept moist by sprinkling 200 ml of tap water onto the nursery beds daily. Seedlings were raised for 2 weeks in the nursery. Thereafter, seedlings of similar heights were transplanted and subjected to treatment.

2.4. Preparation of crude oil amended soil and treatment

Five kilograms of air-dried soil samples were introduced into pots. Test samples were amended with varying concentrations of crude oil (Table 1). The second batch of samples was treated with soil from Egbema, an oil seepage and polluted site (Table 2). Control was set up with no contaminant. All soil samples were amended with dried and ground banana peels and brewery spent grains except the negative control test samples. *H. cannabinus* about 20 cm in height, from the nursery, were planted in control as well as treated pots. The plants were grown for 90 days and watered with 200 mL of tap water daily. Each treatment was conducted in triplicate.

Table 1 Experimental design I

Groups	Number of plants	Composite amendment	Exposure concentration of crude oil (mL/Kg)
A1 (+ve control)	3	Amended	5 Kg unpolluted soil
A2 (-ve control)	3	Not-amended	5 Kg unpolluted soil
A3	3	Amended	5 ml/5Kg
A4	3	Amended	10 ml/5Kg
A5	3	Amended	25 ml/5Kg
A6	3	Amended	50 ml/5Kg

Table 2 Experimental design II

Groups	Number of plants	Composite amendment	Exposure concentration of crude oil
B1 (+ve control)	3	Amended	5 Kg polluted soil
B2 (-ve control)	3	Not-amended	5 Kg polluted soil
B3	3	Amended	1 Kg polluted soil + 4 Kg agricultural soil)
B4	3	Amended	2.5 Kg polluted soil + 2.5 Kg agricultural soil

2.5. Exposure of *H. cannabinus* to different concentrations of crude oil

Plants were allowed to grow for 90 days according to the method described by Tiwari et al. [11]. Three (3) plants, healthy and uniform in height of *H. cannabinus* (Table 1), were transplanted in each pot (25 cm x 20 cm x 20 cm, l x b x h). Treatments included: A1) Control pots with no contaminant amended with banana peels and brewery spent grains (positive control) A2) Control pots with no contaminant and no amendment (negative control) A3) Planted pots amended with composite with 5 mL/5Kg crude oil A4) Planted pots amended with composite with 10 mL/5Kg crude oil A5) Planted pots amended with composite with 25 mL/5Kg crude oil A6) Planted pots amended with composite with 50 mL/Kg crude oil. All pots were covered with aluminium foil to avoid possible photodegradation and evaporation of volatile constituents of crude oil from the soil. Each treatment was carried out in triplicate. Planting was done in a greenhouse with constant aeration. Water temperature of 30 °C and natural photoperiod (12 hr light: 12 hr dark) were maintained daily.

2.6. Determination of Physico-chemical Properties of Crude oil Polluted and Unpolluted Soil samples

Soil samples were analyzed according to standard methods. All chemicals and reagents were of analytical grade. Moisture content was determined using the gravimetric method outlined in [14]. Soil samples were weighed prior to and after oven-drying at 105 ºC for a minimum of 12 hours [14]. The core sampling method was used for the determination of bulk density and particle density [15].

Soil-water suspension of ratio 1:2 (1:5 for only electrical conductivity) was prepared and shaken for one hour on a shaker. It was then filtered through Whatman #1 filter paper and the filtrate was used for analysis. The pH of soil samples was determined using a calibrated pH meter. The electrical conductivity (EC) was measured using a temperature-compensated electronic switchgear meter in µS/cm at 25 ºC [14]. Calcium and magnesium were estimated by versenate titration method [16]. Sodium and potassium in soil-water samples were determined by the flame photometric method [16]. The flame photometer was calibrated with standard solutions of sodium and potassium (10, 25, 50 and 75 ppm). The total organic matter (OM) and organic carbon (OC) of soil samples were determined using the titrimetric method of Walkley and Black [17]. The distillation method was used to determine the available nitrogen [16]. Soil phosphorus was determined by spectrophotometry at an absorbance of 880 nm [18].

2.7. Determination of growth parameters of *Hibiscus cannabinus* during phytoremediation

Growth parameters of *H. cannabinus* including number of leaves, leaf width and plant height were recorded at 0, 15, 30, 45, 60 and 90 days after planting (DAF). The height of plants was calculated by the addition of below- and above-ground parts of test and control plants and recorded immediately after harvesting of plants. The length of the roots (below-ground part) was measured from the tip of the longest root to 2 cm above ground level. The length of the shoot (above-ground part) was determined by measuring plant height from the tip of the highest flower or blade of the highest leaf down to 2 cm above ground level. Fresh weight of below-ground and above-ground (2 cm above ground level) parts of test and control plants were recorded immediately after harvesting of plants.

2.8. Statistical Analyses

Data collected for all parameters were subjected to statistical analysis using Statistical Package for Social Science (SPSS) version 20. One-way analysis of variance (ANOVA) was used to test for differences between samples.

3. Results and discussion

3.1. Physico-chemical Assessment of Crude oil-polluted and Unpolluted Soil Samples before and after Plant Growth

Moisture content observed in the study was within the range of 2.50% to 15.50% before plant growth (Table 3). After 90 days of plant growth, moisture content increased to a mean range of 5.0% to 27.9% showing soil remediation. Similar to the findings in this study, Edori and Iyama [19], observed moisture to be in the range of 16.66 to 21.07% in their study. Additionally, Oliveira et al. [20] reported moisture content within the range of 17.74% to 40.10% in their study. The moisture content of soil shows their water retention characteristics. This serves as an indication of soil type, soil texture and soil health. Sandy soils have low moisture retention capacity, clay soils have very high moisture retention capacity while loamy and humus soils have average moisture content. Soil samples in the study were mainly acidic ranging from 4.80 to 7.30 with the polluted soil from Egbema the most acidic (4.80-6.60) prior to the plant growth experiment (PGE). However, after PGE, an increase in pH was observed in the range 5.10-8.40 (Table 4, Table 5). The pH results obtained from the study were similar to those reported by Okorie et al. [12] in their study of soil samples from Egbema, with a range of 4.8 to 5.4. Additionally, Edori and Iyama [19] reported acidic pH values (4.59-4.99) in their study. Microbial activity, nutrient solubility and availability depend on soil pH. In highly acidic soils, micronutrients are not readily available to plants than in neutral soils. Hence the poor growth of test plants in Egbema soil (Sample B1 and B2).

Table 3 Physical parameters of soil samples prior to and after plant growth

Soil Sample Code/ Parameters	Unpolluted soil		Crude oil polluted soil				Egbema polluted soil			
	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4
MC1(%)	2.50± 0.05	2.50± 0.02	5.00± 0.20	5.00± 0.15	5.00± 0.50	5.00± 0.10	10.00± 0.20	10.00± 0.30	12.50± 0.10	15.50± 0.10
MC2(%)	5.00± 0.00	5.00± 0.00	10.00± 0.00	10.00± 0.00	10.00± 0.00	10.00± 0.00	25.40± 0.20	27.90± 0.10	15.30± 0.20	20.60± 0.10
BD1(g/cm3)	1.10± 0.20	1.10± 0.10	1.30± 0.10	1.28± 0.02	1.40± 0.01	1.42± 0.04	1.70± 0.02	1.70± 0.01	1.35± 0.02	1.55± 0.03
BD2(g/cm3)	1.25± 0.01	1.10± 0.00	1.38± 0.00	1.49± 0.00	1.52± 0.00	1.57± 0.00	1.75± 0.02	1.82± 0.01	1.20± 0.02	1.40± 0.03
PD1(g/cm3)	2.68± 0.02	2.68± 0.04	2.46± 0.02	2.38± 0.01	2.52± 0.04	2.71± 0.01	2.80± 0.01	2.80± 0.02	2.42± 0.01	2.65± 0.02
PD2(g/cm3)	2.61±0. 00	2.05±0. 00	2.53±0. 00	2.54±0. 00	2.68±0. 00	2.58±0. 00	2.77±0. 03	2.85±0. 01	2.15±0. 02	2.34±0. 01

Key: A1 = 5 Kg unpolluted agricultural soil + organic amendment; A2 = 5 Kg unpolluted agricultural soil –organic amendment; A3, A4, A5 and A6 = 5 ml/5Kg, 10ml/5Kg, 25ml/5Kg and 50 ml/5Kg crude-oil polluted soil samples + organic amendment respectively; B1 = 5 Kg polluted soil + organic amendment; B2 = 5 Kg polluted soil –organic amendment; B3 = 1 Kg polluted soil + 4 Kg unpolluted soil + organic amendment; B4 = 2.5 Kg polluted + 2.5 Kg unpolluted soil + organic amendment respectively. MC_1 and MC_2; BD_1 and BD_2; PD_1 and PD_2 = Moisture Content, Bulk Density and Particle Density before and after plant growth experiment respectively.± Standard deviation

Table 4 Chemical parameters of crude oil-polluted and unpolluted soil samples before and after plant growth

Soil Sample Code /Parameters	Unpolluted soil		Crude oil polluted soil				Standards	
	A1	A2	A3	A4	A5	A6	FMEnv Std	WHO Std
pH1	7.30±0.10	7.30±0.10	7.10±0.10	6.80±0.10	6.80±0.10	6.70±0.10	6.5-8.5	6.5-8.0
pH2	8.20±0.10	7.60±0.10	8.00±0.10	7.80±0.10	7.80±0.10	7.60±0.10	6.5-8.5	6.5-8.0
EC_1(µS/cm)	30.00±2.00	30.00±2.00	67.00±1.00	44.00±1.00	52.00±1.00	34.00±1.00	1000	--
EC_2(µS/cm)	23.40±0.10	18.50±0.20	27.40±0.10	29.80±0.10	25.60±0.10	20.10±0.10	1000	--
$Ca^{2+}{}_1$(mg/Kg)	11.54±0.01	11.54±0.01	25.38±0.01	16.92±0.01	20.00±0.00	13.70±0.01	--	100-300
$Ca^{2+}{}_2$(mg/Kg)	9.23±0.02	6.30±0.02	11.43±0.02	14.94±0.01	13.15±0.01	7.61±0.02	--	100-300
$Mg^{2+}{}_1$(mg/Kg)	7.85±0.01	7.85±0.01	17.28±0.02	11.52±0.02	13.61±0.01	8.90±0.02	20	--
$Mg^{2+}{}_2$(mg/Kg)	6.09±0.02	5.37±0.01	7.08±0.01	7.95±0.01	6.23±0.02	4.61±0.02	20	--
$Na^{+}{}_1$(mg/Kg)	14.08±0.01	14.08±0.01	30.99±0.01	20.66±0.01	24.41±0.01	15.96±0.02	200	200
$Na^{+}{}_2$(mg/Kg)	11.35±0.02	9.84±0.02	13.66±0.02	15.46±0.02	16.59±0.02	10.24±0.01	200	200
$K^{+}{}_1$(mg/Kg)	16.30±0.10	16.30±0.10	35.87±0.01	23.91±0.02	28.26±0.02	18.47±0.01	--	8.0
$K^{+}{}_2$(mg/Kg)	12.03±0.02	10.20±0.10	15.79±0.01	16.04±0.02	13.23±0.01	10.11±0.01	--	8.0
OC_1 (%)	0.32±0.01	0.32±0.01	0.32±0.01	0.66±0.02	0.32±0.01	0.64±0.01	--	--
OC_2 (%)	0.22±0.02	0.15±0.01	0.38±0.01	0.32±0.02	0.28±0.01	0.45±0.01	--	--
OM_1 (%)	0.64 ±0.01	0.64 ±0.01	0.69 ±0.01	1.37 ±0.02	0.65 ±0.01	1.28 ±0.02	--	--
OM_2 (%)	0.53±0.02	0.50±0.01	0.55 ±0.02	0.89 ±0.02	0.51 ±0.01	0.95 ±0.02	--	--
N_1 (%)	0.077±0.001	0.077±0.001	0.063±0.002	0.053±0.001	0.063 ±0.002	0.060±0.001	--	--
N_2 (%)	0.047±0.001	0.042±0.001	0.051 ±0.002	0.051 ±0.001	0.049 ±0.001	0.032 ±0.001	--	--
P_1(mg/Kg)	0.400±0.000	0.400±0.000	0.470±0.010	0.430±0.020	0.390 ±0.010	0.400±0.010	--	0.01-0.19
P_2(mg/Kg)	0.300±0.020	0.150±0.010	0.370 ±0.020	0.310 ±0.010	0.380 ±0.020	0.360 ±0.010	--	0.01-0.19

Key:A1 = 5 Kg unpolluted agricultural soil + organic amendment; A2 = 5 Kg unpolluted agricultural soil –organic amendment; A3, A4, A5 and A6 = 5 ml/5Kg, 10ml/5Kg, 25ml/5Kg and 50 ml/5Kg crude-oil polluted soil samples + organic amendment respectively. pH1 and pH2 = pH; EC_1 and EC_2 = Electrical Conductivity; $Ca^{2+}{}_1$ and $Ca^{2+}{}_2$= Calcium; $Mg^{2+}{}_1$ and $Mg^{2+}{}_2$= Magnesium; $Na^{+}{}_1$ and $Na^{+}{}_2$= Sodium; $K^{+}{}_1$ and $K^{+}{}_2$= Potassium; OC_1 and OC_2 = Organic carbon; OM_1 and OM_2 = Organic matter; N_1 and N_2 = Nitrogen andP_1 and P_2 = Phosphorus before and after plant growth experiment respectively.± Standard deviation

Electrical conductivity (EC) in this study had mean range values of 30µS/cm to 180µS/cm before plant growth and 18.50µS/cm to 130.0µS/cm after plant growth. Okafor [21] reported electrical conductivity of 80.79-84.32µS/cm and 93.95-108.25µS/cm before and after three months of phytoremediation of crude oil-polluted soil using *Phaseolus vulgaris* (beans). The results obtained in our study were higher than the values of 592µS/cm reported by Apakama et al. [22]. However, EC values obtained in this study were within the Federal Ministry of Environment (FMEnv) acceptable limit of 1000 µS/cm. Soil calcium content ranged from 3.45-25.38 mg/Kg prior to PGE and 1.81-14.94 mg/Kg after PGE. Apakama et al. [22] reported lower results of calcium (0.892 ppm) than those recorded in the study. The calcium content of soil samples was lower than the WHO stipulated standard (100-300 mg/Kg) showing the unavailability of calcium for plant growth. This could be as a result of nutrient depletion or the high acidic levels of the soil which alter nutrient availability. Values obtained for magnesium in this study ranged from 7.85-17.28 mg/Kg for crude oil-polluted soil and 2.56-4.53 mg/Kg for Egbema-polluted soil before plant growth. However, a reduction after plant growth was observed with values obtained for magnesium ranging from 1.22 mg/Kg to 7.95 mg/Kg. Similar to the findings of our study,

Apakama et al. [22] reported the magnesium content of Egbema-polluted soil in their study as 1.56-4.77 ppm. Additionally, values recorded in the study were within the FMEnv standard of 20 mg/Kg.

Table 5 Chemical parameters of Egbema polluted Soil before and after plant growth

Soil Sample Code /Parameters	Egbema polluted soil				Standards	
	B1	B2	B3	B4	FMEnv Std	WHO Std
pH1	4.80 ±0.10	4.80 ±0.10	6.60 ±0.20	5.90 ±0.10	6.5-8.5	6.5-8.0
pH2	5.40 ±0.10	5.10 ±0.10	5.80 ±0.20	6.10 ±0.10	6.5-8.5	6.5-8.0
EC_1(µS/cm)	180.00±2.00	180.00±2.00	85.00 ±1.00	126.00±2.00	1000	--
EC_2(µS/cm)	130.00±2.00	105.00 ±1.00	74.00 ±1.00	88.00 ±2.00	1000	--
$Ca^{2+}{}_1$(mg/Kg)	3.45 ±0.01	3.46 ±0.01	5.32 ±0.01	7.83 ±0.01	--	100-300
$Ca^{2+}{}_2$(mg/Kg)	2.13 ±0.01	1.81 ±0.01	2.91 ±0.01	3.38 ±0.02	--	100-300
$Mg^{2+}{}_1$(mg/Kg)	2.56 ±0.02	2.56 ±0.01	4.53 ±0.01	3.96 ±0.02	20	--
$Mg^{2+}{}_2$(mg/Kg)	1.67 ±0.02	1.22 ±0.02	2.50 ±0.01	2.06 ±0.01	20	--
$Na^{+}{}_1$(mg/Kg)	7.15 ±0.02	7.15 ±0.02	7.88 ±0.02	9.31 ±0.01	200	200
$Na^{+}{}_2$(mg/Kg)	4.25 ±0.01	4.04 ±0.01	3.45 ±0.01	3.82 ±0.01	200	200
$K^{+}{}_1$(mg/Kg)	3.78 ±0.02	3.78 ±0.02	4.11 ±0.01	5.54 ±0.01	--	8.0
$K^{+}{}_2$(mg/Kg)	3.50 ±0.10	3.16 ±0.10	2.78 ±0.01	3.59 ±0.10	--	8.0
OC_1 (%)	2.45 ±0.02	2.45 ±0.02	0.86 ±0.01	1.79 ±0.01	--	--
OC_2 (%)	1.58 ±0.02	2.12 ±0.02	0.45 ±0.01	0.67 ±0.02	--	--
OM_1 (%)	3.88 ±0.01	3.88 ±0.01	1.54 ±0.02	2.62 ±0.01	--	--
OM_2 (%)	3.11 ±0.01	3.51 ±0.01	0.64 ±0.02	1.95 ±0.01	--	--
N_1 (%)	0.081 ±0.001	0.081 ±0.001	0.078 ±0.001	0.085 ±0.001	--	--
N_2 (%)	0.069 ±0.001	0.074 ±0.001	0.063 ±0.002	0.056 ±0.001	--	--
P_1(mg/Kg)	0.250 ±0.010	0.250 ±0.010	0.290 ±0.010	0.320 ±0.010	--	0.01-0.19
P_2(mg/Kg)	0.210 ±0.020	0.230 ±0.010	0.260 ±0.020	0.280 ±0.020	--	0.01-0.19

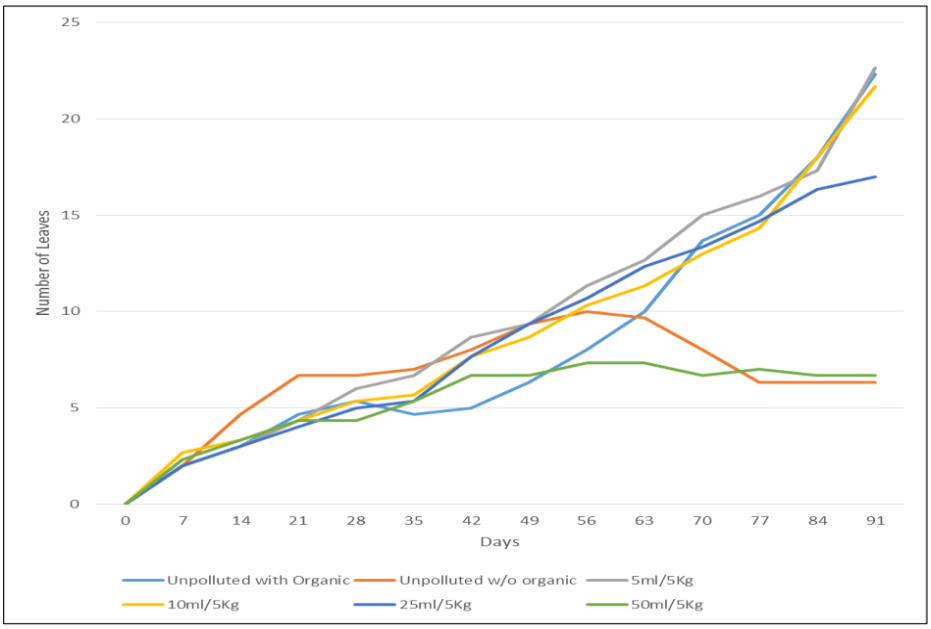
Key: B1 = 5 Kg polluted soil + organic amendment; B2 = 5 Kg polluted soil –organic amendment; B3 = 1 Kg polluted soil + 4 Kg unpolluted soil + organic amendment; B4 = 2.5 Kg polluted + 2.5 Kg unpolluted soil + organic amendment. pH1 and pH2 = pH; EC_1 and EC_2 = Electrical Conductivity; $Ca^{2+}{}_1$ and $Ca^{2+}{}_2$= Calcium; $Mg^{2+}{}_1$ and $Mg^{2+}{}_2$= Magnesium; $Na^{+}{}_1$ and $Na^{+}{}_2$= Sodium; $K^{+}{}_1$ and $K^{+}{}_2$= Potassium; OC_1 and OC_2 = Organic carbon; OM_1 and OM_2 = Organic matter; N_1 and N_2 = Nitrogen andP_1 and P_2 = Phosphorus before and after plant growth experiment respectively.; ± Standard deviation

The sodium content of the soil was higher in polluted soil samples (14.08-30.99 mg/Kg) compared with samples from Egbema (7.15 mg/Kg). In contrast to the findings of our study, Apakama et al. [22] reported lower values of sodium (0.75 ppm) in soil samples studied. Low values obtained in the Egbema soil could result in a low growth rate of *H. cannabinus* (62 cm) as sodium serves as an important macronutrient necessary for plant growth. Potassium in soils is not readily available for plant uptake due to its ability to form complexes. Potassium levels obtained in this study had mean range values of 16.30-35.81 mg/Kg and 10.11-16.04 mg/Kg for crude oil-polluted soil before and after PGE respectively. For soil samples from Egbema, potassium levels were within the range of 3.78-5.54 mg/Kg and 2.78-3.59 mg/Kg before and after plant growth respectively. Similar findings (3.02 ppm) were reported by Apakama et al. [22] in their study of Egbema soils. However, the results obtained in this study were higher than the values (0.02mg/Kg) reported by Ezeji and Chukwudi [23] in their phytoremediation study of used motor oil using cowpea. Higher potassium levels obtained in the study could be a result of the agricultural soil with no history of pollution. Hence nutrient availability was greater in crude oil-polluted soil samples compared to soil samples from Egbema.

The soil organic carbon (OC) is the amount of carbon in the given soil sample. Organic carbon in the study ranged from 0.32 to 2.45% prior to PGE and 0.15-2.12% after PGE. Similar to the range obtained in this study, Ezeji and Chukwudi

[23] reported organic carbon in their study as 0.72%. However, Okorie et al. [12] reported higher findings of organic carbon 3.0-24.9% (average 11.74%) in their study on Egbema soils. Differences observed could be as a result of sampling location and seasonal variations. The organic matter content of soil samples in the study ranged from 0.64% to 3.88% prior to PGE. After PGE, organic matter was slightly reduced to a mean range of 0.50-3.51%. Similarly, Apakama et al. [22] reported organic matter of 3.61% from their study. Alternatively, the study by Edori and Iyama [19] recorded higher organic matter of range 22.33-29.58% than those observed in this study. The differences in the values could be a result of study location as abattoirs are characterized by dumping of animal wastes and animal blood which are potential sources of soil organic matter. Total nitrogen observed in the study before the growth of the plant ranged from 0.053-0.085% and reduced to 0.032-0.074% after plant growth. Higher nitrogen values (4.34%) were reported by Ezeji and Chukwudi [23] in their study of motor oil-contaminated soil. Additionally, Okafor [21] reported high nitrogen levels before (4.14-5.01%) and after (4.84-5.94%) 3 months of growth of *Phaseolus vulgaris*. Phosphorus levels observed in the study prior to PGE had mean range values of 0.25-0.47 mg/Kg and reduced to mean range values of 0.20-0.37mg/Kg after plant growth. Ezeji and Chukwudi [23] reported similar results (0.02mg/Kg) in their study. Alternatively, Okafor [21] reported higher values of 3.81-4.78 mg/Kg in their study. In addition, values obtained were slightly higher than the WHO standard of 0.01-0.19mg/Kg.

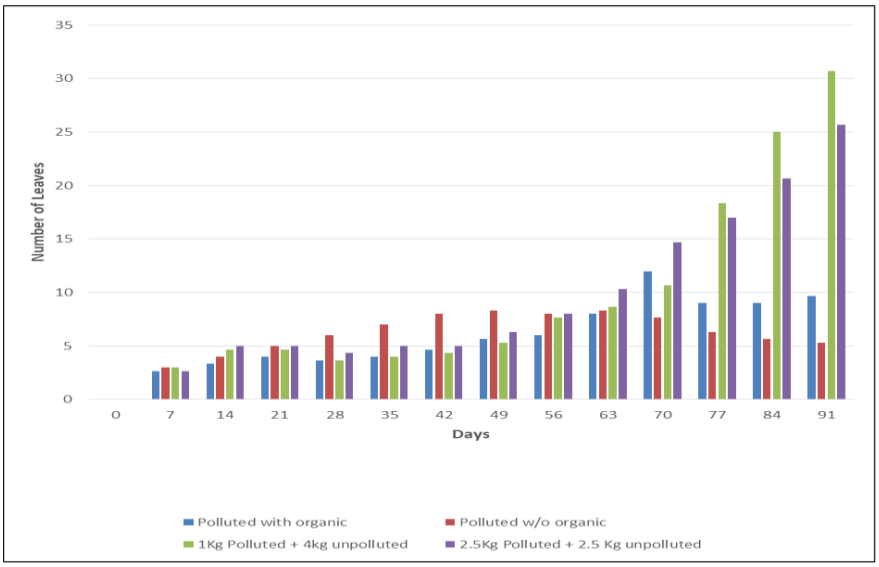
3.2. Number of leaves of *H. cannabinus* during phytoremediation



Key: A1 (Light blue) = 5 Kg unpolluted agricultural soil + organic amendment; A2 (Orange) = 5 Kg unpolluted agricultural soil –organic amendment; A3 (Gray), A4 (Yellow), A5 (Dark blue) and A6 (Green) = 5 ml/5Kg, 10ml/5Kg, 25ml/5Kg and 50 ml/5Kg crude-oil polluted soil samples + organic amendment respectively.

Figure 1a Number of leaves of *H. cannabinus* during plant-growth I

The total number of leaves recorded during plant growth I (PGI) is shown in Figure 1a. After 90-day plant growth, Group A3 had the highest observed number of leaves (23 leaves), closely followed by Group A1 (22 leaves), Group A4 (21 leaves) and Group A5 (17 leaves). The least number of leaves was observed in Group A2 followed by Group A6 having 6 and 7 leaves respectively. The results obtained in our study show the ability of *H. cannabinus* to adapt and utilize petroleum hydrocarbon at concentrations of 5ml/5kg to 25ml/5kg which offered higher biomass production than unpolluted agricultural soil without organic amendment. The results for the total number of leaves observed during PGII (Egbema polluted soil; Figure 1b) show that Group B3 had the highest number of leaves (31 leaves) recorded followed by B4 (26 leaves). Group B1 recorded 10 leaves while Group B2 had the lowest leaves number (6 leaves) after 90 days of plant growth. The findings of our study show the higher remediation potential of introducing unpolluted soil to an oil-polluted site at a ratio of 1:4 during the remediation of oil-polluted sites. This could be a result of the decrease in pollutant concentration as well as the introduction of additional microorganisms from the unpolluted soil which served as bioaugmentation agents that aided the native microorganisms in the polluted site in their remediation activities.



Key: B1 (Blue) = 5 Kg polluted soil + organic amendment; B2 (Orange) = 5 Kg polluted soil–organic amendment; B3 (Gray) = 1 Kg polluted soil + 4 Kg unpolluted soil + organic amendment; B4 (Yellow) = 2.5 Kg polluted + 2.5 Kg unpolluted soil + organic amendment.

Figure 1b Number of leaves of *H. cannabinus* during plant-growth II

3.3. Effect of crude oil pollution on plant fresh weights

Fresh weights of unpolluted, crude oil polluted and Egbema-polluted soil are shown in Table 6. The highest reduction in total fresh weight 94.05% was observed in sample A6 having the highest crude oil contamination (50 ml/5Kg) as compared to the control (A1) for both above- and below-ground parts. Mean fresh weight was reduced by 81.92% and 95.97% for roots and shoots respectively compared to the control. After 90 days of plant growth (PGII) on Egbema-polluted soil samples, the highest total mean fresh weight (101.99 g) was observed in sample B3. Moreover, total mean fresh weights of 69.27 g, 27.12 g and 7.59 g were recorded for samples B4, B1 and B2 for both root and shoot fresh weights respectively. For Egbema polluted soil, the highest reduction in total fresh weight 92.57% was observed in B2 compared to A1. The total mean fresh weight of B3 (101.99 g) was approximately equal to control A1 (102.20 g) indicating the positive effects of bioaugmentation and biostimulation in remediation activities. On average, shoot fresh weight was more reduced than root fresh weight in crude oil polluted soil while root fresh weight was more reduced than shoot fresh weight in Egbema polluted soil.

Petroleum hydrocarbon in soil results in decreased uptake of water and nutrients, root growth, plant growth and subsequently biomass yield [24, 25, 26]. Root and shoot dry weight of *Festuca arundinacea* decreased by 29.70% and 53.50% when grown on soil contaminated with pyrene and phenanthrene respectively [27]. The study by Liste and Felgentreu [28] revealed that in soil contaminated with petroleum hydrocarbon (1517 mg/kg TPH), the shoot and root of ryegrass decreased by 38.90 and 52.60% after a 95-day plant growth respectively. Nutrient availability in crude oil-contaminated soil is relatively low [29], therefore the addition of organic fertilizer serves to improve the growth of plants in these soils [30]. In our study, Fresh weights of A2 (control without organic amendment) and B2 (Egbema polluted soil without organic amendment) were reduced (81.18% and 92.57% respectively) indicating additional nutrient content in organic amendments and their effect on plant yield compared to control. Wang et al. [31] revealed that the root and shoot dry weight of alfalfa and ryegrass increased significantly in compost-amended soil compared to un-amended soil contaminated with pyrene after 90 days. Additionally, the report by Amadi et al. [32] revealed the growth increase in maize grown on poultry manure-amended soils contaminated with crude oil compared to contaminated soil without manure amendment. Sample A3 had the highest shoot, root and total mean fresh weight across all treatments (Table 6). Barati et al. [33] revealed that greater root biomass was related to more elaborate root exploration of soil resulting in higher microbial population and activity necessary for hydrocarbon degradation. Therefore, while plant height and shoot weight are strong indicators of plant health, higher shoot weight does not often imply a more efficient remediation process, rather, higher root weight is associated with greater petroleum hydrocarbon remediation [34]. Results obtained in our study revealed that the plant with the highest root fresh weight (25.19 g) offered the greatest remediation (46.01%).

Table 6 Fresh weight of above- and below-ground parts of *H. cannabinus* after 90-day plant growth

Soil Sample Code /Weight	Unpolluted soil		Crude oil polluted soil				Egbema polluted soil			
	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4
Total Fresh Weight (g)	102.20 ±0.03	19.23 ±0.04	119.33 ±0.02	60.93 ±0.02	106.28 ±0.01	6.08 ±0.00	27.12 ±0.03	7.59 ±0.01	101.99 ±0.02	69.27 ±0.02
Fresh Weight of Roots (g)	13.94 ±0.02	10.02 ±0.02	25.19 ±0.01	8.77 ±0.01	23.43 ±0.02	2.52 ±0.01	6.36 ±0.02	2.71 ±0.02	16.96 ±0.01	16.55 ±0.02
Fresh Weight of Shoots (g)	88.26 ±0.01	9.21 ±0.02	94.14 ±0.01	52.16 ±0.01	82.85 ±0.03	3.56 ±0.01	20.76 ±0.01	4.88 ±0.01	85.03 ±0.02	52.72 ±0.00

Key:A1 = 5 Kg unpolluted agricultural soil + organic amendment; A2 = 5 Kg unpolluted agricultural soil –organic amendment; A3, A4, A5 and A6 = 5 ml/5Kg, 10ml/5Kg, 25ml/5Kg and 50 ml/5Kg crude-oil polluted soil samples + organic amendment respectively; B1 = 5 Kg polluted soil + organic amendment; B2 = 5 Kg polluted soil –organic amendment; B3 = 1 Kg polluted soil + 4 Kg unpolluted soil + organic amendment; B4 = 2.5 Kg polluted + 2.5 Kg unpolluted soil + organic amendment respectively.; ± Standard deviation

3.4. Effect of crude oil pollution on plant heights

Sample A3 had the highest total plant height 137 cm after phytoremediation of crude oil polluted soil samples (Table 7), closely followed by samples A1 (134.00 cm) and A4 (132.00 cm) respectively. Samples A5 and A6 were observed to have total mean plant heights of 125.00 cm and 86.00 cm respectively while sample A2 had the least mean total plant height (74.53 cm) observed. Our findings correlate with the values recorded for the total number of leaves showing a significant relationship between the total plant height and the total number of leaves in the study. Sample B3 had the greatest total plant height 144.0 cm after 90 days of plant growth on Egbema-polluted soil. Total plant heights of 130.0 cm and 126.0 cm were recorded in samples B4 and B1 respectively while sample B2 had the least recorded total heights of plants (69.0 cm). These results were similar to the values observed for the total number of leaves and total fresh weight of plants showing a significant relationship between the number of leaves, fresh weight of plants and heights of plants (biomass production).

Table 7 Heights of *H .cannabinus* after 90-day plant growth

Soil Sample Code /Heights	Unpolluted soil		Crude oil polluted soil				Egbema polluted soil			
	A1	A2	A3	A4	A5	A6	B1	B2	B3	B4
Total Height of Plant (cm)	134.00± 1.00	74.53± 0.25	137.00± 2.00	132.00± 0.90	125.00± 1.00	86.00± 0.30	126.00± 2.00	69.00± 0.10	144.00± 1.00	130.00± 2.00
Height of above-ground parts (cm)	126.00± 1.20	68.03± 0.15	127.00± 1.80	122.00± 1.00	117.50± 0.10	80.00± 0.10	118.00± 2.10	62.00± 0.10	132.00± 1.00	120.00± 1.00
Length of Roots (cm)	8.00±0. 20	6.50±0. 40	10.00±0 .20	10.00±0 .10	7.50±0. 90	6.00±0. 20	8.00±0. 10	7.00±0. 20	12.00±2 .00	10.00±3 .00

Key:A1 = 5 Kg unpolluted agricultural soil + organic amendment; A2 = 5 Kg unpolluted agricultural soil –organic amendment; A3, A4, A5 and A6 = 5 ml/5Kg, 10ml/5Kg, 25ml/5Kg and 50 ml/5Kg crude-oil polluted soil samples + organic amendment respectively; B1 = 5 Kg polluted soil + organic amendment; B2 = 5 Kg polluted soil –organic amendment; B3 = 1 Kg polluted soil + 4 Kg unpolluted soil + organic amendment; B4 = 2.5 Kg polluted + 2.5 Kg unpolluted soil + organic amendment respectively.± Standard deviation

Results obtained in our study show a 35.82% reduction in total plant height for sample A3 compared to control (A1; Table 7). For the highest exposure concentration (A6), the above-ground parts (shoots) recorded a 36.51% reduction while the below-ground parts (roots) recorded a 25.00% reduction when compared with the control. Other studies have shown the negative effect of crude oil-polluted soils on plant growth [35, 36]. Shanker et al. [17] revealed that plant height reduction was a result of growth reduction of roots and reduced translocation of nutrients to aerial parts of plants which affected shoot cell metabolism. Similar to the findings of our study, Martin et al. [38] recorded a decrease in the shoot height of sunflowers grown on soil contaminated with crude oil. Additionally, Barati et al. [33] reported a 34.75% and 37.25% decrease in the shoot height of barley and oats at 8% soil TPH level. According to Chirakkara and Reddy [39], the addition of fertilizers (organic and inorganic) has a positive effect on plant growth in contaminated soils through biostimulation. Our results show that the addition of organic amendments (banana peel and brewery spent grains) significantly affected the total plant height of A1 (positive control) compared to A2 (negative control) after 90 days of plant growth. The total height of sample B3 was 1.07 times that of the control (A1).

3.5. Total Hydrocarbon Degradation of Soil Samples

In our study, the total hydrocarbon content of soil samples prior to and after plant growth I (Table 8) shows samples A3 and A6 had the highest (46.01%) and least (5.08%) percentage remediation respectively. Group A3 having the highest number of leaves, total fresh weight and total plant height offered the highest percentage of crude oil remediation when compared to other samples. Barati et al. [33] reported 21.76% and 20.36% TPH remediation in their study using barley and oats amended with poultry biochar while Prematuri et al. [40] recorded 38% remediation in 40 g/Kg using plants of the Aster family. Alternatively, Abdallah et al. [41] observed a higher crude oil degradation percentage (79%) using *Acacia siberiana* than those recorded in our study after six months of phytoremediation. Our findings show a significant relationship between plant biomass and the remediation ability of *H. cannabinus*. Sample B3 offered the highest percentage of remediation (39.53%) using PGII while samples B4 and B1 had the respective percentage of remediation of 27.94% and 8.61%. Sample B2 had the lowest percentage of remediation (4.63%) across all samples analyzed. The low values recorded in sample B2 correlate with the low biomass yield obtained for the sample. Our findings show that the high pollution index of Egbema soil negatively impacted plant growth and therefore remediation ability was significantly hindered. However, the high percentage remediation values obtained for samples B3 (39.53%) and B4 (27.94%) show the synergistic effect of augmenting Egbema polluted soil with unpolluted soil, offering higher crude oil remediation potential compared to the un-augmented samples B1 and B2 with percentage remediation of 8.61% and 4.63% respectively.

Table 8 Total Petroleum Hydrocarbon Degradation of Soil samples prior to and after PGE

Soil Sample Code /Heights	**Unpolluted soil**		**Crude oil polluted soil**				**Egbema polluted soil**			
	A1	**A2**	**A3**	**A4**	**A5**	**A6**	**B1**	**B2**	**B3**	**B4**
TPH before PGE(µg/mL)	21.5671 ± 0.015	21.5671 ± 0.015	32.3306 ± 0.025	46.9172 ± 0.208	80.4671 ± 0.031	94.1905 ± 0.015	179.579 9± 0.021	179.579 ± 0.021	77.4659 ±0.025	98.3665 ± 0.031
TPH after PGE(µg/mL)	11.7158 ±0.003	19.0589 ±0.110	17.4560 ±0.020	32.2811 ±0.160	45.6140 ±0.060	89.4031 ±0.350	164.111 2±0.250	171.263 0±0.050	46.8459 ±0.060	70.8826 ±0.120
% Remediation	45.68%	11.63%	46.01%	31.20%	43.13%	5.08%	8.61%	4.63%	39.53%	27.94%

Key:A1 = 5 Kg unpolluted agricultural soil + organic amendment; A2 = 5 Kg unpolluted agricultural soil –organic amendment; A3, A4, A5 and A6 = 5 ml/5Kg, 10ml/5Kg, 25ml/5Kg and 50 ml/5Kg crude-oil polluted soil samples + organic amendment respectively; B1 = 5 Kg polluted soil + organic amendment; B2 = 5 Kg polluted soil –organic amendment; B3 = 1 Kg polluted soil + 4 Kg unpolluted soil + organic amendment; B4 = 2.5 Kg polluted + 2.5 Kg unpolluted soil + organic amendment respectively.; ± Standard deviation

4. Conclusion

To the extent of this study, the acidic pH results imply pollution of soil from Egbema/Ohaji and indicate the need for remediation. After planting *H. cannabinus*, pH values and moisture content increased showing remediation activity of the plant. Morphological changes observed during plant growth showed that group A3 (5 ml/5Kg polluted soil + organic amendment) had the highest number of leaves, total fresh weight and fresh weight of roots, offering the highest crude oil remediation (46.01%) recorded, while group B2 (polluted soil - organic amendment) showed the least remediation (4.63%); indicating a correlation between increase in biomass and percentage remediation. Samples with organic amendment offered additional nutrients resulting in an increase in petroleum degradation in amended soils compared to un-amended soils.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest in the publication.

Author Contribution Statement

All authors contributed equally towards the manuscript.

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