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Research Article

Assessment of Total Petroleum Carbon and Heavy Metals of Some Selected Plants Grown in Crude Oil Polluted Soils Using GC-FID

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Abstract

This study was focused on assessing the phytoremediation potentials of some selected plant species on crude oil contaminated soils. Five plant species were cultivated in soils polluted with crude oil at 10, 50, 100, 150 and 200 mg kg^{-1}. The results revealed that there were significant reduction in THC contents of soil ameliorated with the various plants species compared to the control group increases with increasing concentration of crude oil pollutant in soil. THC was 62.24 mg kg^{-1}, 1,120 mg kg^{-1}, 208.67 mg kg^{-1}, 257.87 mg kg^{-1} and 2,762.79 mg kg-1 for *R. simplex*, *P. balfouriana*, *K. pinnata*, *P. fruiticosa* and *T. spathecea* plant species respectively. Results of phyto-extraction of heavy metals by the plant species showed increasing concentration with increasing volume of crude oil pollutant. Nickel concentration ranged from 0.05 mg kg^{-1} in *K. pinnata* to 0.43 mg kg^{-1} in *P. fruiticosa*. Copper concentration varied from 2.28 mg kg^{-1} in *T. spathecea* to 12.64 mg kg^{-1} in *R. simplex*. Iron concentration by the plants varied from 11.36 mg kg^{-1} in *P. balfouriana* to 24 mg kg^{-1} in *K. pinnata*. The concentration of Chromium from the soil by the plant species increased from 0.16 mg kg^{-1} in *R. simplex* to 0.38 mg kg^{-1}. Zinc concentration varied from 2.64 mg l^{-1} *K. pinnata* to 10.26 mg kg^{-1} in *R. simplex*. The uptake of manganese by the plant species increased from 5.56 mg kg^{-1} in *P. balfouriana* to 10.56 mg kg^{-1} in *T. spathecea* while cobalt decreased from 0.08 mg kg^{-1} in *T. spathecea* to 0.001 mg kg^{-1} in *R. simplex*. The concentretion of lead ranged significantly from 0.01 mg kg^{-1} in *K. pinnata* to 0.82 mg kg^{-1} in P. fruiticosa. The study advocated for the use of phytoremediation with *P. balfouriana* and *T. spathecea* plant species.

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Introduction

Over the years since its exploration the petroleum industry has grown to be one of the largest industries in the world and plays a pivotal part in driving a nation's economy. In Nigeria, commercial exploration of petroleum started in Oloibiri Bayelsa State in 1958. Crude oil referred to the populace as "black gold" has gradually grown to be the mainstay of the country's economy. Crude oil is a naturally occurring, unrefined petroleum that is basically composed of hydrocarbon deposits and other organic materials. Crude oil can be processed into more useful products like gasoline, kerosene, jet fuel, diesel, heating oil, and other allied products called petrochemicals by refining process [1]. Exploration of hydrocarbon which has been of immense benefit to Nigeria as the mainstay of the country's economy has also been identified as one of the major environmental pollutants in the Niger – Delta region of the country [2–4]. This is as a result of oil spillage and gas flaring in the region. The resulting effects of these activities over the years has led to environmental degradation, soil depletion, water contamination and atmospheric pollution which have adversely affected the inhabitants and the communities where such activities are carried out [5].

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The effect of oil contamination on the environment, especially on soil quality has been one of the major concerns in Nigeria and regulatory bodies universally. According to reports by Samuel and [1] crude oil spillage and gas flaring causes adverse effects directly or indirectly to the ecosystem. For instance, the quality of soil for agricultural use depends on the concentration of heavy metals in it [6] as soil contaminated with petroleum reduced soil fertility resulting in poor yield. Thus, use of plants for remediation purposes is a possible solution for heavy metal pollution since it includes sustainable remediation technologies to rectify and re-establish the natural condition of the soil to make it fertile. According to Merkl et al. [7] crude oil contamination of soil causes reduction in the germination, growth performance which tends to lead to poor yield of plants. Also, crude oil in soil creates an unsatisfactory condition for plant metabolism due to insufficient aeration brought about by an increase in oxygen demand by oil decomposing micro-organisms.

The accumulation of heavy metals in soil is of concern to environmentalist and agriculturalist due to the adverse effects on food quality, crop growth and environmental health. Heavy metals refer to group name for metals and semimetals that are associated with contamination and potential toxicity or ecotoxicity [8]. The presence of heavy metal content in the soil is largely influenced by crude oil spillage [9]. Heavy metal pollution of the soil is caused by various metals especially copper, nickel, cadmium, zinc, chromium, and lead [10]. Yao et al. [11] documented that pollution caused by heavy metals does not only results in adverse effects on various parameters relating to plant quality and yield but also causes changes in the size, composition and activity of the microbial activities in the soil. Adhikari et al. [12] also added that heavy metals at higher concentrations are toxic in nature to higher life forms because they lead to biomagnifications and their pollution deteriorates the quality of soil and crops produce [13].

Environments that are polluted with crude oil have been known to be detrimental to the health of humans, and the growth and development of plants and animals alike and thus the need to constantly keep the environment free from contamination. Several researches have been carried out by many scholars [12–14] on remediation of polluted soils. However, there is paucity of information on phytoremediation potentials of selected weeds using GC-FID. Hydrocarbon management is a fundamental environmental management tool especially in mining companies that deals with large volumes of hydrocarbons and its hydrocarbon related waste. The need to prevent or ameliorate adverse environmental effect of persistent soil contaminant by crude oil, and to do so at lower cost than existing technologist has brought increase attention to Phytoremediation. These potentials in phytoremediation will address the fundamental mechanism of interactions between plants and crude oil contaminant in soils.

Materials and methods

1) Study location

This study was conducted in the Environmental Biotechnology unit of the Department of Genetics and Biotechnology University of Calabar, Calabar, Cross River State.

2) Experimental laboratories

The total hydrocarbon content (THCs) was carried out in Mifor Consults, Old Ikang Road MCC Calabar. Heavy metal in stem of the plants and the phytochemical analyais were carried out in AKS MST Laboratory Ministry of Science and Technology Uyo, AkwaIbom State.

3) Identification of plant

Five plant species of interest were obtained from different locations within Cross River State and identified by a taxonomist in the herbarium unit of the Department of Plant and Ecological Studies, University of Calabar. Cathedral bells (*Kalachoe pinnata* (Lam). Ming aralia (*Polyscias fruticosa*), Mexican petunia (*Ruellia simplex*) (C. wright), Balfour aralia (*Polyscias balfouriana*) and Boat lily (*Tradescantia spathacea* (Swartz). The five plant species were chosen for the study because they were readily available and locally widespread while being easy and inexpensive to cultivate. The plants were identified via a review of their potential to remove contaminants from soil and air. Also, the plants have been observed to proliferate in the vicinity of petrol stations and crude oil storage facilities within the Niger delta region of Nigeria, and their ability to phytoremediate crude oil has not been well characterized. The plants were grown directly in plastic buckets by stem cutting. All the buckets were watered once daily by spraying to maintain sufficient soil moisture.

4) Collection of soil samples and crude oil

Soil sample were collected from three different points within the University of Calabar, Calabar (Kwa River site, Biological Science and Staff Quarters) however, these are areas with no history of hydrocarbon pollution. The crude oil was purchased from the Nigerian Agip oil company, Port Harcourt River State.

5) Artificial soil pollution

The collected soil samples were bulked to form a composite soil sample, five kilograms (5 kg) each of the composite soil sample was weighed and transferred into labelled plastic buckets with drainage holes at the base.

Seventy-five plastic buckets were used during the experiment; the 90 plastic buckets were divided into 6 experimental groups with 5 plant species. Group A plant (*Kalachoe pinnata*), Group B plant (*Polyscias fruticosa*), Group C plant (*Ruellia simplex*), Group D plant (*Polyscias balfouriana*) and Group E plant (*Tradescantia spathacea*).

Each group was spiked or treated with five different concentrations of crude oil; 0 mL, 50 mL, 100 mL, 150 mL and 200 mL with three replications each. After soil pollution, the soil contained in the various plastic buckets were allowed to stay for the period of two weeks during this period pre remediation was conducted. Thereafter planting was done and allowed for the period of 3–4 months between March-July.

6) Experimental design

The experimental design was carried out in a 5×5 factorial in a completely randomized design (CRD). Factor 1 was the plant species (5 levels) and Factor 2 was the crude oil concentration (5 levels).

7)Extraction methods

7.1) Soil extraction

The collected soil samples were extracted using the procedures by Schramm et al. (2008), with modifications. The soil samples were extracted by pressurized fluid extraction using an accelerated solvent extractor (ASE 200 Dionex). A cellulose filter was inserted into the inner bottom of the extraction cell, then sea sand dried at 550 °C (ca. 1 g) was added. The sample (0.5–5 g) mixed with hydromatrix for drying and dispersing was added into the cell and a filter placed on top. The samples were quantitatively extracted by an accelerated solvent extractor at a temperature of 120 °C and pressure of 120 bar and with n hexane: acetone (75:25) as the extraction solvent mixture. Two static cycles of 10 min were applied for a complete extraction. Another sub-sample of each sample was dried for 24 h at 105 °C and then weighed for moisture and dry weight determination. The extracts were passed over anhydrous sodium sulfate to remove water. The extracts were concentrated using vacuum rotary evaporation to ca. 5 mL then diluted with n hexane to 10 mL and some of them were diluted further by measuring 0.1 mL from that solution and diluting with n-hexane to 10 mL. To remove interferences, the extracts were cleaned-up using silica gel and alumina in glass column (30 cm long with an internal diameter of 2.5 cm) packed, from bottom to top, with 10 g silica gel (grade 60), 5 g alumina with 3% H_2O and 5 g anhydrous sodium sulfate. During clean-up, 50–100 µL from the diluted sample extract were added into the column and spiked with 13C labelled and deuterated internal standards (10 µl of a mixture containing 333–1000 pg μL^{-1} of organochlorine compounds in nonane).

7.2) Plant extraction

2.0 g of various plant, 15 mL of perchloric ($HClO_4$), and trioxonitrate V acid solution were mixed in the ratio of 1:4. After been left overnight, cold digestion was done and heated on hotplate until a transparent solution was observed, but at different temperatures. After cooling, the digested samples were filtered using the What man filter paper No. 42, then diluted up to 100 mL by volume using highly purified deionized water, and stored at room temperature for further analytical procedures.

8) Sample preparation for GC-FID

Samples were collected from the upper 10 cm in each of the plant materials and were wrapped in aluminum foil. Samples were stored in an ice chest before transporting to the laboratory for analysis. In the laboratory samples were stored in refrigerator prior to analysis, so as to preserve its integrity. In the Laboratory samples were air dried for four days after which it was macerated and then sieved through a 1 µm sieve THC determination. 10 g of the homogenized sieved sample was extracted with 100 mL analytical grade dichloromethane (CH_2Cl_2). The dichloromethane extracts were cleaned up by passing through a column packed with cotton wool and 1:1 silica gel and sodium sulphate salt. The resulting extract was concentrated on a rotatory evaporator. Hewlett Packed 5890 series II gas chromato-graphic machine coupled with a flame ionization detector (FID) was used for the analysis. The Hydrocarbon window defining standard was purchased from AccuStandard and the calibration curve was prepared by diluting the 500 ug mL^{-1} standard solution to 300 ug mL^{-1}, 200 ug mL^{-1} and 100 ug mL^{-1} and ran on the GC-FID.

8.1) Determination of THC using gas chromatographic flame ionization detector (GC-FID)

Determination of THC was carried out using Hewlett Packed 5890 series II gas chromatographic machine coupled with a flame ionization detector (FID). Separation of THC in the samples was accomplished with a DB-1 fused-silica capillary column (30 m × 0.32 mm). Helium was used as carrier gas at a flow rate of 0.45 mL min^{-1} and Hydrogen and air was used as the ignition gas, an injection volume of one micro liter. The GC oven temperature was optimized: initial temperature of 50°C was held for 2 min and then ramped at 10°C min^{-1} to 300. It was maintained at this temperature till the end of the run (47 min). The injection port temperature was set at 250°C and the FID detector was set at 300°C. The oven equilibrium time was 1 minute; oven maximum temperature was 350°C and the ambient temperature was 25°C. The hydrogen and air flow rates were 40 mL min^{-1}

and 400 mL min^{-1} respectively. Chromatographs were generated for each sample where the area's count was plotted on the y-axis and the retention time on the x-axis. The area's count explains the area and height of the peaks detected by the GC-FID after matching the sample results with the calibration standard.

The retention time explains the time taken for a particular compound in the sample to elute corresponding retention time in the standard calibration curve. The peaks with the carbon number indicates the compound present in the calibration standard that were detected in the samples analyzed while the peaks without carbon numbers indicates the compounds that were not present in the calibration standard and could not be reported on the chromatogram.

The total run time as imputed in the THC method of GC-FID was 47 min. A calibration equation was generated automatically for each of the compounds in the sample and the formula for calculating the concentration of the compounds was also generated and was used to calculate the various concentrations of the individual compounds in each sample which was summed up to give the total concentration of hydrocarbon present in each sample. The calculated concentrations are in ng ug^{-1}.

9) Heavy metal determination in the plants stem

After digestion heavy metals were determined using computerized atomic absorption spectrophotometer (969 UNICAM thermo elemental AAS). Each heavy metal was determined using their respective lamps and wavelength, the following heavy metals were determined, nickel, iron, copper, manganese, zinc, chromium, cobalt and lead using American Society for testing and material [15].

9.1) Quality assurance and quality control

Separate tools were used to collect different samples from different depths and points. Tools to be reused were thoroughly cleaned with water and soap and rinsed with dichloromethane and acetone. All organic solvents were of picograde quality and were obtained from reputable chemical stores. After use, all glassware and tools were rinsed with a technical mixture of toluene, acetone and hexane, and washed with water and detergent in a washing machine. Thereafter, the glassware was dried in an oven overnight at programmed temperatures up to a maximum temperature of 450°C. Reproducibility and reliability of data were ensured by recalibration of instrument after every ten runs. The validity of analytical protocol was assessed by the use of using spike recovery method. Standard solutions of elements were spiked into already analyzed samples and reanalyzed. High purity multi-cathode lamp (1,000 mg kg^{-1}) from Cambridge CB5 8BZ (UK) was used to obtain the calibration curves for each element. The multi element calibration curves were verified with a multi-element certified material of 1000 mg kg^{-1} (Cambridge CB5 8BZ, UK) with percentages of recovery of 94.5–100% and relative standard deviation RSD between replicate analyses of less than 4%. The limits of detection (LoD) were 0.001 for As, Cd, Hg and V and 0.01 mg kg^{-1} for Pb, while the limits of quantification (LoQ) were 0.0033 for As, Cd, Hg and V and 0.033 mg kg^{-1} for Pb [11].

10) Statistical analysis

Data obtained were analyzed using SPSS in a completely randomized design (CRD), significant means were separated using least significance different (LSD) test.

Results

1) Soil total hydrocarbon content using GC -FID

The five test plants were investigated for their phytoremediation potentials on soils contaminated with crude oil, using the soil THC reduction values as an index for measurement. The reduction value was obtained by subtracting the pre-remediation THC values of the soil for each test plant from the remediation result generated automatically using GC-FID. The samples were analyzed for 38 hydrocarbon components in the calibrated curve and chromatographs were generated along with the results. In the chromatographs the peaks with n-alkanes indicates the presence of hydrocarbons, while the peaks without n-alkanes indicate the absence of hydrocarbons. N-alkane ranged from C2–C38 along with isoprenoid hydrocarbon, Pristine (Pr) and Phytane (Ph). Some n-alkanes were absent at different plant species at different concentrations, this variation in the presence and absence of n-alkanes may probably be due to the variation in the biodegradation processes in the plant species.

From the result analyzed, the higher the mean the lower the n-alkanes in the chromatographs, while the lower the mean the higher the N- alkanes in the chromatographs. The result revealed that there was a significant variation in phytoremediation potential among the five test plants grown on soils of various pollution levels (50 mg, 100 mg, 150 mg and 200 mg), including the control (0 mg). Amongst the five (5) test plants, *T. spathacea* showed significant higher potentials for hydrocarbon remediation on soils treated with 50 mg, 100 mg and 200 mg at $P<0.05$. The mean soil THC reduced by *T. spathacea* was 2762.79±32.63 ng ug^{-1}, 158.62±0.83 ng ug^{-1}, and 1512.07±27.10 ng ug^{-1}, for soils treated with 50 mg, 100 mg and 200 mg of crude oil respectively as shown on Table 1. Also, with a mean total reduction value of 208.67±3.36 ng ug^{-1}, *K. pinnata* showed significant ($P<0.05$) higher hydrocarbon remediation potential on soils treated with 150 mg of crude oil compared to other test

plants. *P. fruticosa*, on the other hand, showed significantly higher potentials for hydrocarbon remediation in the control soils (soils not treated with crude oil) as against other test plants. The mean soil THC reduced by *P. fruticosa* was 126.09±0.54 ng ug^{-1}.

2) Heavy metal content in soil

The results for the various heavy metal contents in the soil is as presented on Table 2.

2.1) Cadmium

The result as presented on Table 2 shows that the Cd in soil grown with *P. fruiticosa* at 50 mL crude oil was the highest, followed by the Cd in polluted soil grown with *P. balfouriana* at 150 ml, *T. spathacea* at 100 mL crude oil and control show no significant difference (P>0.05) in the mean values obtained but significantly higher (P<0.05) than the Cd content in the soil grown with *K. pinnata* (100 mL, 150 mL). The polluted soils with the lowest Cd are the soil grown with *K. pinata* and *P. balfouriana* (200 mL), *P. fruiticosa* (100 mL), *R. simplex* (150 mL, 200 mL), *T. spathacea* (50 mL, 150 mL, 200 mL) of crude oil with no significant difference (P>0.05), while the Cd in soil grown with *K. pinnata* and *T. spathacea* had the lowest mean values.

Table 1 Total Hydrocarbon content (ng ug^{-1}) reduced by test plants in crude oil polluted soils

Plant species	0 mL	50 mL	100 mL	150 mL	200 mL
R. simplex	43.75^{a}±0.25	62.24^{b}±2.89	41.70^{b}±1.13	3.21^{a}±0.04	40.14^{a}±0.96
P. balfouriana	51.39^{a}±1.25	108.46^{c}±12.03	20.49^{a}±0.98	120.60^{e}±9.25	119.78^{b}±2.85
K. pinnata	41.32^{a}±0.93	22.53^{a}±1.10	19.70^{a}±0.36	208.67^{d}±3.36	141.28^{c}±1.04
P. fruticosa	126.09^{c}±0.54	177.20^{d}±4.79	117.79^{c}±2.70	108.38^{b}±2.31	257.87^{d}±2.50
T. spathacea	115.48^{b}±0.9	2762.79^{e}±32.63	158.62^{d}±0.83	185.47^{c}±2.99	1512.07^{e}±27.10

Table 2 Heavy metal concentration in soil of plant species grown in hydrocarbon polluted soil (mg kg^{-1})

Plant species	Conc.	Cadmium (Cd)	Chromium (Cr)	Zinc (Zn)	Copper (Cu)	Iron (Fe)	Manganese (Mn)
K. pinnata	Control	0.40^{b}±0.05	0.022^{c}±0.002	4.89^{d}±0.03	2.46^{i}±0.003	24.09^{c}±0.64	9.19^{b}±0.01
	50 mL	0.001^{d}±0.00	0.03^{c}±0.001	5.26^{d}±0.02	1.48^{j}±0.06	16.5^{d}±1.33	10.6^{b}±0.04
	100 mL	0.24^{c}±0.02	0.154^{b}±0.12	6.96^{c}±0.03	9.47^{a}±0.03	13.72^{d}±0.49	19.88^{a}±0.001
	150 mL	0.16^{c}±0.003	0.26^{b}±0.04	9.68^{a}±0.01	2.67^{i}±0.01	14.49^{d}±0.05	10.26^{b}±0.03
	200 mL	0.001^{d}±0.00	0.024^{c}±0.003	3.54^{e}±0.04	2.48^{i}±0.001	42.98^{a}±0.08	10.68^{b}±0.02
P. balfouriana	50 mL	0.14^{c}±0.003	0.14^{b}±0.003	3.68^{e}±0.023	2.67^{i}±0.002	10.36±0.005	7.74^{b}±0.001
	100 mL	0.22^{c}±0.04	0.32^{a}±0.005	6.28^{c}±0.02	3.42^{h}±0.003	21.42^{c}±0.50	9.86^{b}±0.008
	150 mL	0.40^{b}±0.002	0.18^{b}±0.002	8.48^{b}±0.003	4.82^{f}±0.002	11.45±0.30	7.38^{b}±0.011
	200 mL	0.04^{d}±0.003	0.24^{b}±0.002	4.56^{d}±0.006	8.61^{b}±0.003	43.98^{a}±0.34	8.56^{b}±0.002
P. fruiticosa	50 mL	0.80^{a}±0.03	0.02^{c}±0.04	7.26^{c}±0.03	1.456^{i}±0.01	25.17^{c}±1.1	12.26^{b}±0.03
	100 mL	0.01^{d}±0.00	0.078^{c}±0.002	4.86^{d}±0.01	5.92^{e}±0.04	40.40^{a}±0.67	11.180^{b}±0.006
	150 mL	0.20^{c}±0.05	0.034^{c}±0.003	9.20^{a}±0.02	2.47^{i}±0.004	44.73^{a}±0.15	19.12^{a}±0.07
	200 mL	0.35^{b}±0.02	0.026^{c}±0.01	5.74^{d}±0.01	4.12^{g}±0.003	18.25^{d}±0.55	8.48^{b}±0.023
R. simplex	50 mL	0.17^{c}±0.40	0.034^{c}±0.002	4.98^{d}±0.003	3.62^{h}±0.001	25.17^{c}±0.061	10.58^{b}±0.01
	100 mL	0.26^{c}±0.02	0.018^{c}±0.00	6.60^{c}±0.003	8.12^{c}±0.001	18.58^{d}±0.24	8.34^{b}±0.04
	150 mL	0.001^{d}±0.00	0.240^{b}±0.004	8.46^{b}±0.01	7.62^{d}±0.002	22.46^{c}±0.88	9.76^{b}±0.01
	200 mL	0.01^{d}±0.00	0.022^{c}±0.01	7.64^{c}±0.03	5.84^{e}±0.03	39.38^{a}±0.42	11.68^{b}±0.02
T. spathacea	50 mL	0.001^{d}±0.00	0.0220^{c}±O.04	4.98^{d}±0.003	9.47^{a}±0.00	17.58^{d}±0.42	10.50^{b}±0.003
	100 mL	0.39^{b}±0.01	0.020^{c}±0.002	10.14^{a}±0.02	5.944^{e}±0.01	47.99^{a}±0.07	8.64^{b}±0.01
	150 mL	0.01^{d}±0.000	0.04^{c}±0.05	8.68^{b}±0.04	2.40^{i}±0.17	31.58^{b}±0.89	9.30^{b}±0.05
	200 mL	0.001^{d}±0.00	0.026^{c}±0.01	9.86^{a}±0.06	3.62^{h}±0.01	16.04^{d}±1.13	9.26^{b}±0.03
LSD		0.04	0.03	0.48	0.36	2.70	0.91

Note: Mean with the same superscript along vertical arrays indicates no significant difference (p>0.05).

2.2) Chromium

The Cr in the polluted soils grown with P. *balfouriana* at 100 mL of crude oil was the highest, this was followed by the Cr content in soil grown with P. *balfouriana* (200 mL, 150 mL), of crude oil polluted soils with no significant difference ($P>0.05$) in mean but significantly higher ($P<0.05$) than the crude content in soil grown with *T. spathacea* (50–200 mL), the Cr content in soils grown with T. *spathacea* was the lowest with no difference in mean (Table 3).

2.3) Zinc

The results for Zn shows that the zinc content in soil polluted soils grown with *K. pinata* and *P. fruiticosa* at 150 mL, and *T. spathacea* at 100 ml and 200 ml crude oil polluted soils was the highest with no significant difference ($P>0.05$) in the mean values obtained. The soil grown with *K. pinata* and *P. balfouriana* at 200 mL, 50 mL of crude oil polluted soils had the lowest Zn content in soil. The results obtained shows that the soil grown with *T. spathacea* had the highest Zn content in soil, while the Zn content in soil grown with *P. balfourina* was the lowest in mean.

2.4) Copper

The results for copper shows that the Cu content in soil grown with *K. pinata* and *T. spathacea* at 100 mL, 50 mL of crude oil polluted soil respectively was significantly high ($P<0.05$), this was followed by the Cu in soil grown with *P. balfouriana* at 200 mL crude oil polluted soil. This was significantly higher than the Cu content in soil grown with *R. simplex* at 100 mL and 150 mL of crude oil polluted soils with significant difference among them but also higher than the Cu content in soil grown with *P. fruiticosa*, *R. simplex* and *T. spathacea* at 100 mL, and 200 mL of crude oil polluted soils respectively. The Cu content in soil grown with *K. pinata* at 50 mL of crude oil was the lowest in mean. The results for total heavy metals in soil also shows that the Cu in soil grown with *R. simplex* was the highest, followed by the soil grown with *P. balfouriana* and *T. spathacea* with no significant difference in mean but significantly higher than the Cu in soil grown with *K. pinata* and *P. fruiticosa* that had the lowest mean values.

2.5) Lead

The result for Pb shows that the lead content in soil grown with various concentrations of crude oil had no significantly difference in the mean value obtained. Results for total heavy metal content shows that the Pb content in soil grown with *R. simplex* was significantly higher ($P<0.05$) than in soil grown with P. *balfouriana*, also followed by the Pb content in soil grown with *T. spathacea*, the soil grown with *K. pinata* and *P. fruiticosa* had the lowest Pb values.

2.6) Iron

The Fe content in the soil grown with *K. pinata*, *P. balfouriana*, *R. simplex* at 200 ml, *P. fruiticosa* (100 mL, 150 mL) and *T. spathacea* at 100 mL of crude oil polluted soil was higher with no significant difference ($P>0.05$) in the mean values obtained. Results for total heavy metal shows that the iron content in soil grown with *P. fruiticosa* was the highest, followed by the Fe content in soil grown with *T. spathacea*, while the Fe content in the soil grown with *K. pinata* and *P. balfouriana* had no significant difference ($P>0.05$) in the mean values obtained.

2.7) Manganese

The result shows that the Mn content of the soil grown with *K. pinata*, *P. fruiticosa* at 100 mL and 150 mL of crude oil polluted soils respectively was the highest with no significant difference ($P>0.05$) in the mean values. While the soil grown with *P. balfouriana* had the lowest Mn content.

2.8) Nickel

The Nicontent in soil grown with *K. pinata* (50 mL, 100 mL), *P. balfouriana* (100 mL, 200 mL), *P. fruiticosa* and *R. simplex* (50 mL, 100 mL, 200 mL), and *T. spathacea* at 50 mL of crude oil polluted soil was the highest in no significant change in the mean values. This was followed by the mean values obtained in the soil grown with *P. balfouriana* at 50 mL and 150 mL, *P. fruiticosa* and *R. simplex* at 150 mL with no significant change in mean. It was also followed by the Ni content in soil grown with *K. pinata* and *T. spathacea* at 150 mL crude oil polluted soils and control with no significant variation in mean values. However, significant reduction in the Ni content of the soil was obtained in soil grown with *K. pinata* at 200 mL and *T. spathacea* at 100 mL and 200 mL of crude oil polluted soil with no significant difference ($P>0.05$) in the mean values. Figure 1 shows that the Ni content in soil with *T. spathacea* was the lowest while the Ni content in soils grown with other plants shows no significant difference ($P>0.05$) in mean values obtained.

Table 3 Total Heavy metal contents in soil (mg kg^{-1})

Plants	Cadmium (Cd)	Chromium (Cr)	Zinc (Zn)	Copper (Cu)	Lead (Pb)	Iron (Fe)	Manganese (Mn)	Nickel (Ni)
K. pinata	$0.16^{d}\pm0.04$	$0.05^{c}\pm0.02$	$6.08^{d}\pm0.56$	$3.71^{c}\pm0.78$	$0.02^{d}\pm0.01$	$22.37^{d}\pm2.93$	$12.13^{a}\pm1.04$	$3.73^{a}\pm0.23$
P. balfouriana	$0.24^{b}\pm0.004$	$0.022^{d}\pm0.002$	$5.59^{e}\pm0.4$	$4.39^{b}\pm0.06$	$0.33^{b}\pm0.006$	$22.26^{d}\pm3.24$	$8.54^{d}\pm0.24$	$3.91^{a}\pm0.13$
P. fruiticosa	$0.208^{c}\pm0.42$	$0.360^{a}\pm0.005$	$6.40^{c}\pm0.43$	$3.28^{c}\pm0.42$	$0.015^{d}\pm0.003$	$30.53^{a}\pm2.7$	$12.04^{a}\pm1.01$	$3.64^{a}\pm0.12$
R. simplex	$0.66^{a}\pm0.4$	$0.24^{b}\pm0.002$	$7.58^{b}\pm0.05$	$5.53^{a}\pm0.006$	$0.47^{a}\pm0.10$	$25.9^{c}\pm1.9$	$9.91^{b}\pm0.30$	$3.82^{a}\pm0.02$
T. spathacea	$0.16^{d}\pm0.05$	$0.020^{d}\pm0.01$	$8.27^{a}\pm0.05$	$4.78^{b}\pm0.7$	$0.22^{c}\pm0.00$	$27.4^{b}\pm3.1$	$9.37^{c}\pm0.02$	$3.27^{b}\pm0.002$
LSD	0.03	0.02	0.29	0.40	0.02	0.61	0.21	0.20

Note: Mean with the same superscript along vertical arrays indicates no significant difference ($p>0.05$).

3) Heavy metal in the stem of the plants

The results for the heavy metal contents in the stem of the plants species is as presented on Table 4.

3.1) Nickel

The result of the Ni content in the stem of the plants is as presented on Table 4, the result reveals that the Ni content in the stem of the control plants were significantly higher ($P<0.05$) than the values obtained from the stem of the plants, spike with varying concentrations of crude oil. Among the treated groups with crude oil, the stem of *P. balfouriana* (50 mL, 100 mL, 150 mL, 200 mL), *P. fruiticosa* (50 mL, 100 mL), R. simplex at 100 mL and *T. spathacea* at 200 mL had the lowest level of nickel in the plant. *R. simplex* had significantly high Ni content in the stem as compared to the control.

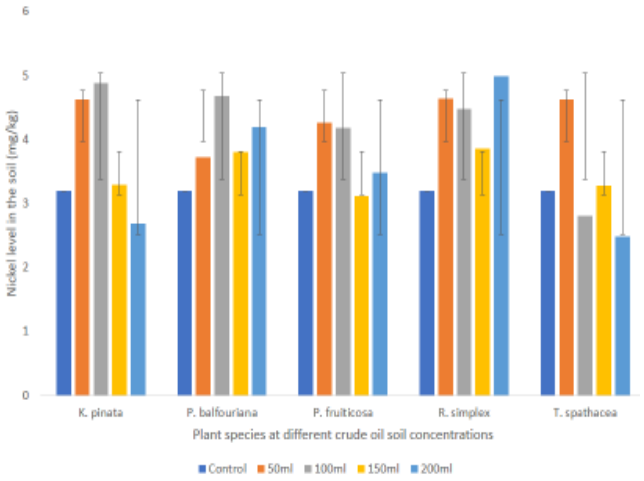

Figure 1 Concentration of Ni in the soil.

Table 4 Heavy metal content in the stem of the plant (mg kg^{-1})

Plant species		Nickel (Ni)	Copper (Cu)	Iron (Fe)	Cobalt (Co)	Chromium (Cr)	Zinc (Zn)	Manganese (Mn)
	Control	$3.86^{a}\pm0.02$	$4.60^{a}\pm0.05$	$20.48^{c}\pm1.05$	$0.140^{b}\pm0.2$	$0.34^{a}\pm0.003$	$17.26^{a}\pm0.10$	$10.28^{a}\pm0.10$
P. balfouriana	50 mL	$0.14^{d}\pm0.03$	$4.87^{a}\pm0.02$	$17.4^{d}\pm0.005$	$0.04^{b}\pm0.00$	$0.22^{a}\pm0.03$	$4.8^{c}\pm0.006$	$9.72^{b}\pm0.30$
	100 mL	$0.14^{d}\pm0.01$	$3.2^{c}\pm0.02$	$19.2^{c}\pm0.60$	$0.42^{b}\pm0.03$	$0.28^{a}\pm0.04$	$9.18^{b}\pm0.4$	$10.48^{a}\pm0.40$
	150 mL	$0.08^{d}\pm0.02$	$4.48^{a}\pm0.03$	$16.32^{d}\pm1.1$	$0.64^{a}\pm0.02$	$0.36^{a}\pm0.1$	$5.7^{c}\pm0.10$	$5.56^{f}\pm0.20$
	200 mL	$0.046^{d}\pm0.01$	$3.12^{c}\pm0.3$	$15.28^{e}\pm0.50$	$0.030^{b}\pm0.01$	$0.16^{a}\pm0.01$	$2.68^{d}\pm0.02$	$10.36^{a}\pm0.10$
P. fruiticosa	50 mL	$0.14^{d}\pm0.30$	$3.88^{b}\pm0.20$	$24.96^{a}\pm0.10$	$0.42^{b}\pm0.10$	$0.180^{a}\pm0.03$	$5.2^{c}\pm0.2$	$9.8^{b}\pm0.06$
	100 mL	$0.064^{d}\pm0.01$	$4.26^{a}\pm0.30$	$22.3^{b}\pm0.04$	$0.20^{b}\pm0.00$	$0.32^{a}\pm0.04$	$6.94^{c}\pm0.04$	$7.3^{e}\pm0.2$
	150 mL	$0.48^{c}\pm0.02$	$3.24^{c}\pm0.20$	$19.74^{c}\pm0.20$	$0.34^{b}\pm0.30$	$0.26^{a}\pm0.30$	$9.68^{b}\pm0.02$	$9.64^{b}\pm0.00$
	200 mL	$0.40^{c}\pm0.01$	$2.89^{c}\pm0.01$	$18.9^{c}\pm0.002$	$0.030^{b}\pm0.00$	$0.20^{a}\pm0.04$	$3.5^{d}\pm0.04$	$8.5^{d}\pm0.10$
R. simplex	50 mL	$0.96^{b}\pm0.00$	$4.60^{a}\pm0.20$	$18.56^{c}\pm0.10$	$0.04^{b}\pm0.04$	$0.20^{a}\pm0.34$	$8.6^{b}\pm0.02$	$8.3^{d}\pm0.50$
	100 mL	$0.14^{d}\pm0.00$	$3.8^{b}\pm0.20$	$16.6^{d}\pm0.07$	$0.040^{b}\pm0.05$	$0.22^{a}\pm0.40$	$9.8^{b}\pm0.1$	$9.2^{c}\pm0.07$
	150 mL	$0.37^{c}\pm0.04$	$2.48^{c}\pm0.40$	$20.4^{c}\pm0.06$	$0.016^{b}\pm0.20$	$0.36^{a}\pm0.20$	$10.26^{b}\pm0.5$	$10.56^{a}\pm0.1$
	200 mL	$0.84^{c}\pm0.10$	$4.2^{a}\pm0.1$	$22.5^{b}\pm0.06$	$0.014^{b}\pm0.00$	$0.16^{a}\pm0.05$	$6.58^{c}\pm0.01$	$7.36^{e}\pm0.2$
T. spathacea	50 mL	$0.36^{c}\pm0.02$	$3.4^{c}\pm0.04$	$16.74^{d}\pm0.08$	$0.014^{b}\pm0.03$	$0.180^{a}\pm0.02$	$6.48^{c}\pm0.04$	$10.56^{a}\pm0.06$
	100 mL	$0.41^{c}\pm0.02$	$3.1^{c}\pm0.30$	$19.06^{a}\pm0.30$	$0.026^{b}\pm0.003$	$0.030^{b}\pm0.00$	$4.5^{c}\pm0.01$	$7.5^{e}\pm0.60$
	150 mL	$0.41^{c}\pm0.02$	$2.28^{d}\pm0.20$	$22.40^{b}\pm0.06$	$0.08^{b}\pm0.00$	$0.32^{a}\pm0.03$	$7.64^{c}\pm0.03$	$8.6^{d}\pm0.10$
	200 mL	$0.084^{d}\pm0.02$	$4.16^{a}\pm0.2$	$14.8^{e}\pm0.04$	$0.042^{b}\pm0.03$	$0.26^{a}\pm0.02$	$3.82^{c}\pm0.04$	$9.2^{c}\pm0.10$
LSD		0.12	0.23	0.74	0.01	0.06	0.84	0.38

Note: Mean with the same superscript along vertical arrays indicates no significant difference ($p>0.05$).

3.2) Copper

The Cu content in the stem of *P. balfouriana* (50 mL, 150 mL), *P. fruiticosa* (100 mL), R. simplex (50 mL, 200 mL) and *T. spathacea* (200 mL) and the control had significantly high (P<0.05) mean values. This was followed by the significant reduction observed in the copper content of the stem of *P. fruiticosa* (50 mL) and *R. simplex* (100 mL) with no significant difference (P>0.05) in mean. The lowest copper content in the stem of the plants was obtained in *T. spathacea* (150 ml) of crude oil (Table 4).

The result for the comparison of the Cu content in the stems among the plants show that the Cu content in the stem of *P. balfouriana* was the highest, followed by *P. fruiticosa* and *R. simplex* while, *T. spathacea* had the lowest Cu content in the stem.

3.3) Iron

The result for Fe shows that the stem of *P. fruiticosa* at 50 mL crude oil pollution had the highest Fe content, followed by the Fe content in the stem of *P. fruiticosa* (100 mL), *R. simplex* (200 mL), and *T. spathacea* (150 mL) of crude oil polluted soils with no significant difference (P>0.05) in the mean while the controls, *P. balfouriana* (100 mL), *P. fruiticosa* (150 mL, 200 mL), R. simplex (50 mL, 150 mL) of the polluted soil show no significant difference (P>0.05) in the mean Fe content in the stem of the plants. With the high value observed in the control, reduction of the Fe content was obtained in *P. balfouriana* (50 mL, 150 mL), *R. simplex* (100 mL) and *T. spathacea* (50 mL) with no significant variation in the mean values. The lowest Fe content was obtained in the stem of *P. balfouriana* and *T. spathacea* at 200 mL crude oil pollution. Table 5 shows that the Fe content in the stem of *P. fruiticosa* was the highest, followed by the Fe content in the stem of *R. simplex* while, *P. balfouriana* and *T. spathacea* had the lowest Fe content in the stem.

3.4) Cobalt, chromium and lead

The Co content in the stem of *P. balfouriana* at 150 mL crude oil polluted soil was the highest while other stems of the plants at varying concentrations and the controls shows no significant difference in the mean values. The Cr content was significantly reduced (P<0.05) in the stem of *T. spathacea* at 100 mL soil pollution while the stem of the control and stems of other plants at varying concentrations had no significant difference (P>0.05) in the mean Cr content of the plants.

The Pb was observed to be high in the stem of *T. spathacea* at 50 mL soil pollution while other groups and controls shows no significant difference (P>0.05) in the mean values (Fig 9). Generally, there was no significant difference (P>0.05) in the mean values of the Co, Cr and Pb content in the stems of the plants.

3.5) Zinc

The result for Zn shows that the control groups had significantly high (P<0.05) Zn content while reduction in the quantity of the Zn value in the stem of *P. balfouriana* (100 mL), *P. fruiticosa* (150 mL), and *R. simplex* (50 mL, 100 mL, 150 mL) was observed. This was followed by the Zn content in the stem of *P. balfouriana* (50 mL, 150 ml), *P. fruiticosa* (50 mL, 100 mL), *R. simplex* (200 mL) and *T. spathacea* (50 mL, 100 mL, 150 mL and 200 mL) with no significant difference (P>0.05) in the mean values obtained. *P. balfouriana* and *P. fruiticosa* at 200 mL soil pollution had the lowest Zn content in the stem of the plants. Table 7 reveals that the Zn in the stem of R. simplex was the highest while *P. balfouriana*, *P. fruiticosa* and *T. spathacea* show no significant difference (P>0.05) in the mean values obtained.

3.6) Manganese

The Mn content in the control, *P. balfouriana* (100 mL, 200 mL), *R. simplex* (150 mL) and *T. spathacea* (50 mL) was significantly high (P<0.05), followed by the Mn content in the stem of *P. balfouriana* (50 ml) and *P. fruiticosa* (50 mL, 150 mL) with no significant difference (P>0.05) in the mean values obtained. This was followed by the manganese content in the stem of *R. simplex* and *T. spathacea* at 100 mL and 200 mL soil pollution respectively. Reduction in the manganese content was also observed in the stem of *P. fruiticosa*, *R. simplex* and *T. spathacea* at 200 mL, 50 mL, and 150 mL crude oil soil pollution respectively, with no significant difference (P>0.05). Total heavy metal content assessment in the stem of the test plants shows that the Mn content in the stem of *P. balfouriana* was the highest followed by the Mn content in the stem of *R. simplex* and *T. spathacea* while P. *fruiticosa* had the lowest manganese content in the stem.

Table 5 Total heavy metal content in plant stem (mg kg^{-1})

Plant species	Nickel (Ni)	Copper (Cu)	Iron (Fe)	Cobalt (Co)	Chromium (Cr)	Lead (Pb)	Zinc (Zn)	Manganese (Mn)
P. balfouriana	$0.21^{b}\pm0.04$	$4.06^{a}\pm0.02$	$17.74^{c}\pm0.006$	$0.063^{a}\pm0.01$	$0.27^{a}\pm0.02$	$0.198^{c}\pm0.08$	$5.93^{b}\pm0.006$	$9.26^{a}\pm0.06$
P. fruiticosa	$0.21^{b}\pm0.04$	$3.48^{b}\pm0.1$	$21.04^{a}\pm0.6$	$0.034^{a}\pm0.00$	$0.240^{a}\pm0.01$	$0.41^{b}\pm0.00$	$5.67^{b}\pm0.007$	$8.50^{c}\pm0.03$
R. simplex	$0.21^{b}\pm0.03$	$3.56^{b}\pm0.2$	$19.08^{b}\pm0.60$	$0.028^{a}\pm0.03$	$0.27^{a}\pm0.02$	$0.63^{a}\pm0.06$	$9.09^{a}\pm0.04$	$8.96^{b}\pm0.03$
T. spathacea	$0.32^{a}\pm0.03$	$3.18^{c}\pm0.02$	$17.36^{c}\pm0.90$	$0.046^{a}\pm0.00$	$0.26^{a}\pm0.01$	$0.033^{d}\pm0.00$	$6.15^{b}\pm0.05$	$8.89^{b}\pm0.03$
LSD	0.02	0.12	0.71	NS	NS	0.09	0.65	0.18

Note: Mean with the same superscript along vertical arrays indicates no significant difference (p>0.05).

Discussions

Crude oil spillage causes the release of large amounts of toxic compounds into the soil. These compounds that include heavy metals, metalloids as well as organic pollutants cause's threats to the environment as they accumulate in soil and are readily passed unto man and animals through the food chain. This lack of control in the contamination of the environment called for the need for provision of appropriate measures to control or curtail the effects of those contaminations. The issue of environmental pollution is a global problem which has drawn a major concern over the years. Consequently, the need to do an environmental audit of some plant species with phytoremediation potential.

From the result obtained in the THC contents in our study, it was observed that *T. spathecea* and *P. balfouriana* which are plants belonging to the grass and legume family demonstrated the greater ability to phytoaccumulate THC. Similar result was also documented by Idris et al. [16]. Ndimele who reported that grasses and legumes have high potential to remediate THC in a crude oil polluted environment [17]. Thus, the results obtained in our study on the THC of plants agrees with the work of Idris et al. who reported that some selected plants bio-accumulate THC. The clear explanation to this could be the presence of extensive fibrous root system with diverse root surface area, which can penetrate deep down into the soil. High value of THC in plants explains the fact that there was high absorption of petroleum hydrocarbon by the plants. The proportion of the concentrations of THC in the plants shows that absorption and accumulation of petroleum hydrocarbon in plants depends on the concentration of the pollutant in the environment.

Glick et al. reported the success of phytoremediation of environmental contaminants with the following plants: Poplar tree (*Populus deltoides*), Willow (*Salix species*), Indian mustard (*Brassica juncea* L.), Indian grass (*Sorghastrum nutans*), and Sunflower (*Helianthus Annuus* L.) [18]. The researchers reported this success to be as a result of their diverse root system that enabled the plant environment for successful growth, and also absorption of large quantities of water. In our results *T. spathecea*, *P. balfouriania*, and *K. picata* were able to grow in the contaminated crude oil soil and bear the phytotoxic nature of the contamination. Hence this research agrees with the work of Anyasi and Atagana who opined that optimal growth of plant in the contaminated soil is significant for phytoremediation to be possible [19]. For plant to grow in a contaminated environment it must have to bear the phytotoxic nature of the contamination, hence such plant should be able to germinate, survive and grow in such harsh condition. The survival of these plants in oil-contaminated soils indicates that they could be used for phytoremediation in the Niger Delta.

The results of heavy metal analysis of the plants showed that the petroleum products had significant effects on heavy metals level of the soil. Here, the contaminated soil had significantly higher values than control soils (p 0.05) with respect to total Cu, Fe, Ni, Mn, and Zn. The Cu level in the soil increased from 2.46 mg kg^{-1} to 9.47 mg kg^{-1}; the Pb content increased from <0.0001 mg kg^{-1} to 0.0328 mg kg^{-1}; while the Fe content increased from 24 mg kg^{-1} to 42.09 mg kg^{-1}. This research relates with that of Scheer who reported that considerable amount of Mn and Fe were found to accumulate in all the plants grown on contaminated soil, while other elements assessed were obtained in trace amount [20]. It is obvious that *T. spathecea* and *P. balfouriana* can thrive in contaminated and uncontaminated soils based on the levels of Fe and Mn, implying that they can be potential materials for phytoremediation. The levels of Fe, Mn and Li were higher than the normal range in plants [21]. Similar result trend was reported by Akpokodje and Uguru, where the Cu content of soil sample increased from 16 mg kg^{-1} to 45.88 mg kg^{-1}; and the Fe content increased from 314 mg kg^{-1} to 432.88 mg kg^{-1} after crude oil contamination [22].

Conclusion

The findings revealed that *T. spathacea* and *P. balfouriana* have phytoremediation potentials compare to others at 50 mL and 200 mL, respectively.

Hence, the research proved that some plants species such as *T. spathecea*, *P. balfouriana* have the ability of taking up heavy metal like Nickel, lead and others at different concentrations of crude oil in the soil compare to others. The present result proved that there is corresponding decrease in phytochemical properties in all selected plant species as the concentration of hydrocarbon in the polluted soil increases except for plants species with phytoremediation potentials that varies significantly.

With the development of industrialization and urbanization the abundance of crude oil pollution in the environment has increase enormously which raise significant concern. In other to tackle this problem plant based technology (phytoremediation) is used to clean contaminated soils. Phytoremdiation has been proven to be a promising technique for re-vegetation of crude oil polluted soil as some plants have shown remediation potentials with a good public acceptance and shows a variety of advantages compared with other physicochemical technique. These methods are efficient, eco-friendly and economical. Nevertheless phytoremediation offers effective alternative. Practically, simple approach is neither possible nor sufficient for effective cleanup of hydrocarbon polluted soil. The combination of different approaches including

genetic engineering, micro-assisted and chelated-assisted approaches is essential for highly effective and exhaustive phytoremediation in the future.

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