Chemistry International 6(3) (2020) 160-167

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Crude oil degradation in loamy soil using Neem root extracts: An experimental study

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ARTICLE INFO

Article type:
Research article
Article history:
Received September 2019
Accepted February 2020
July 2020 Issue
Keywords:
Neem root extracts
Crude oil
Loamy soil
Degradation efficiency
Physicochemical properties
Total petroleum hydrocarbon
Biodegradability

ABSTRACT

The biodegradability of Total Petroleum Hydrocarbon (TPH) in loamy soil amended using neem root extracts was investigated in this study. The neem roots were soaked separately into predetermined volume of water to extract liquids from the roots. The solution of neem-water and extract was then used as treatment to remediate crude oil polluted loamy soil. The loamy soil was used for this study. The physicochemical properties of the loamy soil before pollution, after pollution and at the 84th day of treatment were determined, while analysis for the total bacteria count and TPH were carried out for 84th days at 14 days interval. Density, electrical conductivity (EC), moisture content (MC), total organic carbon (TOC), total nitrogen (TN) and phosphorus (P) in the soils increased after pollution, except for pH, which was the opposite. However, density, EC, MC, TOC, TN and P decreased remarkably with time under treatment, while pH increased from acidity to neutrality after the treatments. The total bacteria count increased across the soils with treatments up to the 56th day of analysis, but declined gradually thereafter due to depletion of the nutrients in the loamy soil. The degradation of TPH in the loamy soil decreased across the process, but higher in the loamy soil with treatment. However, TPH removal was higher with treatment in water solution with performed of neem extract. The TPH degradation percentage at the 84th day was determined and the response thus observed was promising for the degradation of pollutant in soil.

Capsule Summary: The biodegradability of crude oil in loamy soil amended using neem root extracts was investigated and density, electrical conductivity, moisture content, total organic carbon along with other properties was improved of soil.

Cite This Article As: C. P. Ukpaka, S. B. Lezorghia and H. Nwosu. Crude oil degradation in loamy soil using Neem root extracts: An experimental study. Chemistry International 6(3) (2020) 160-167. https://doi.org/10.5281/zenodo.3645478

INTRODUCTION

Crude oil pollution is a known problem across the globe, and in particular, countries where the exploration and exploitation of crude oil is involved. The land, water and air that are receivers of crude oil pollutants suffered greatly, and in extension, everything that inhabits in them. Consequence upon this, efforts has been put in place, such as establishment of legislations that is targeted to managing environmental pollution (Akankali and Nwafili, 2017). Although in Nigeria, this has not yielded the expected results due to poor implementation and weak legislations (Ejiba et al., 2016). This study however, deals with land pollution, but studies on

aquatic pollution by crude oil had been widely reported (Agunobi et al., 2014; Soloviev et al., 2016; Akankali and Nwafili, 2017; Imanian et al., 2017). Most of water pollution by crude oil originates from the land and then migrated into the nearest water body like swamp, river, creek, or even sea. As this happened, several types of contaminants like biological, chemical and particulate matters are carried alongside the crude into the water thereby, rendering the water unsafe for consumption and also, non-conducive for its habitants (Imanian et al., 2017; Ukpaka, 2011).

The contamination of soil by crude oil has been studied with different specific objectives, such as the transport of characteristic of crude oil into soil, effects of crude oil migration in soil, remedial techniques, factors affecting remedial process and many others. Thus, in Amro et al. (2013) the penetration rate and consequences of crude oil migration in soil was reported. This study showed that crude oil transport in soil penetrates through a longer distance in the early stages, but as time progresses, the distance of transport became gradual and slow. This transport behaviour is equally exhibited by different derivatives of crude oil in soil environment, but generally, the concentration of pollutant in soil decreases with increase in depth (Khalilova, 2015 and Ukpaka, 2016).

Soil possesses a distinct feature that purifies itself, therefore some of its pollutants may naturally be removed through this property, while others such as hydrocarbon pollutants may not able (Khalilova, 2015). In Heydataemeh et al. (2017), the self- purifying capacity of soil was investigated using nano-particles soil for removal of green malachite dye from aqueous solutions. This study reported that about 80% of the green malachite dye was removed by the nano-particles soil. However, the contamination of soil by crude oil alters its properties which affect the growth of plants and contamination of groundwater (Adams et al., 2014; Ukpaka, 2017).

Different techniques have been applied for hydrocarbon removal in soils. The use of modified and non-modified adsorbents for removal of crude oil and its derivatives has been widely reported (Elloit, 2016; Bandura et al., 2017; Nnaji, 2017; Olajire et al., 2017). However, biodegradation of hydrocarbon contaminants through application of microorganism or use of materials such as plants, animal wastes, fertilizer etc, to stimulate the population of microorganism necessary to biodegrades contaminant in soil have widely gained attention. This technique used microbial metabolism with sufficient nutrients to breakdown hydrocarbons (Adams et al., 2015; Ukpaka et al., 2016).

According to Das and Chandran (2011), bioremediation technology is cost effective and can achieve complete conversion of organic contaminants into carbon dioxide, water, inorganic compounds, or cell protein. Several studies showed many indigenous microorganisms in contaminated environment has the ability to degrade hydrocarbon contaminants (Obiakalaije et al., 2015; Varjani, 2017). Earlier, Parreira et al. (2011) had studied the biodegradation of benzene, toluene and *o*-xylene (BTX) in soil using different bacterial species, and observed remarkable reduction of BTX in soil. Also, investigation on polycyclic aromatic hydrocarbons degradation by *Pseudomonas* sp. and *Achromobacter xylosoxidans* in soil and water recorded high percentage degradation of polycyclic aromatic hydrocarbons (Esedafe et al., 2015).

The process of obtaining microorganisms can be achieved through biological culturing. Thus, Yuniati (2018) extensively reviewed techniques for microbial culture and its application in bioremediation of contaminated soils. Alternatively, biological and agricultural sources as nutrient stimulants can be applied for soil remediation.

Thus, Ferreira et al. (2012) studied *Yarrowia lipolytica* yeast for degradation of crude oil in soil, with optimum degradation recorded at temperature of 28°C. The use of fertilizer like NPK as microorganism stimulants has been equally been reported with positive results (Adaba (2013), while other researchers had used plant and animal wastes, or combination of NPK fertilizer and plant and animal wastes. In Ofoegbu et al. (2015), formulations of NPK fertilizer, cow dung, palm kernel husk ash and their combinations for bioremediation of arable soil was investigated. The results recorded the highest degradation rate in inorganic fertilizer and cow dung combination. But in Aghalibe et al. (2017), comparison was made between NPK fertilizer, poultry manure and cow dung for biodegradation of petroleum hydrocarbons in contaminated soil, which recorded 73%, to 85% removal of the hydrocarbon pollutant.

Compost and inorganic fertilizers applied as bio-stimulants for microorganisms in hydrocarbon remediation derived from plant and animal wastes are also in practice. Thus, wastes from groundnut shell, beans shell, melon shell, cassava peels, cow dung and pig dung for hydrocarbon biodegradation in soils have been reported (Agarry et al., 2013; Udom and Nuga (2015). Also, comparison of wastes obtained from tea leaf, soy cake and potato skin on the degradation of diesel fuel in silt loam soil showed percentage degradation of 88%, 81% and 75 % diesel fuel in the soil respectively compared to just 35 % for untreated soil (Udom and Nuga, 2015).

Again, in other independent studies, cassava peels was used for the degradation of crude oil in soil, with appreciable degradation rate of petroleum hydrocarbons reported (Akpe et al., 2015; Ogu and Odo, 2015). In Ogu and Odo (2015), the increase in weight of cassava peels reduces the content of crude oil in the soil, while in Akpe et al. (2015), high content of the crude oil at constant treatment weight decreased the degradation rate. Also, the efficacy of mango leave as biodegradation material in soil polluted by crude oil has been documented (Ukpaka and Edwin, 2013).

Animal wastes such as cow dung, poultry droppings and goat manure have equally proven to be effective treatment for soil remediation. This was demonstrated by Obiakalaije et al. (2015), which reported a degradation efficiency of70.7%, 76.6% and87.1%for cow dung, poultry droppings and goat manure in the removal of total petroleum

hydrocarbon in soil. They also reported high growth in microbial bacteria as a result of the treatments.

In another related study, Olu (2017) found that the formulation of cattle dung, goat dung, pig dung and poultry manure for biodegradation of polycyclic aromatic hydrocarbons (PAHs) in polluted soil after 28 days increased the percentage degradation of PAH by 70.8% compared to 10.1% increase for untreated soil sample. The growth rate of hydrocarbon degrading bacteria in soil with the formulation also was greater than the untreated soil. Similar study by Ukpaka and Nkakini (2017) showed that formulations of dried poultry manure, goat dung and fine sawdust are excellent crude oil degradation materials in soil, and they also increased the soil nutrients capable of improving soil fertility. Based on aforementioned facts, present study was performed to check the biodegradability of Total Petroleum Hydrocarbon (TPH) in loamy soil amended using neem root extracts based on soil physico-chemical properties.

MATERIAL AND METHODS

Material, chemical and reagents

The following materials were used in the course of the experiment: Crude oil, soils, plastic vessels (reactors), Neem and Moringa roots, digital weighing balance, stirrer, shovel, sieve, sack bags, measuring cylinders, beakers, conical flask, Kjeldahl flask, pipette, oven, crucible, desiccators, thermometer, pH meter, Gas Chromatography, distilled water, sodium hexa-phosphate(II) $(NaPO_3)_2$ andH_2SO_4.

Sample collection

The soil used in this study is loamy soil. The soil was collected from Kpea Community in Khana Local Government Area of Rivers State. Kpea is one of the communities in Ogoni Ethnic Group in Rivers State. The loamy soil were collected with aid of shovel and then, bagged in sack bags. Thereafter, they were transported to the Department of Chemical/Petrochemical Laboratory, Rivers State University Port Harcourt for laboratory analysis. The Neem roots were collected from the same Wiiyaakara Community in the Khana Local Government Area of Rivers State. They were washed with distilled water to remove debris and then sundried before extraction. Water for extraction was collected from the Laboratory.

Experimental procedure

The roots of Neem plants were separately soaked in water for two weeks to effectively obtain extracts from the roots. The loamy soil sample was weighed into 500g and 600g separately. The loamy soil was divided into two (2) samples. One (1) of the sample weighing 500g was measured into vessels prepared to receive roots extract obtained with water. This was also done for the other one sample weighing 600 g. Upon preparation of the loamy soil samples, they were then contaminated with 50ml of crude oil and mixed properly with the aid of a stirrer, to ensure consistency of the concentration of crude oil in the loamy soil. After stirring for about two minutes, the polluted loamy soil sample was left undisturbed for three days. This was to allow stabilization or settlement of the soils. At the third day, 100mg of the liquid extract obtained with water as solvent, was weighed into the samples as designed. Similarly, the loamy soil sample was prepared as above, but without the root extracts, which were used as control samples. Every 7 day, representative sample of loamy soil was collected for laboratory analysis to determine the physicochemical parameters, Total Petroleum Hydrocarbon (TPH) content and total bacteria count for 42 days (6 weeks).

Sample test analysis

Sample analysis of loamy soil was carried out in the laboratory, and the following parameters determined: pH, electrical conductivity, moisture content, total organic carbon, Total Petroleum Hydrocarbon (TPH) content and Total Bacteria Count (TBC). The soil textural class was also determined. Also, the physiochemical properties of Neem roots were analyzed.

Soil pH

The hydrogen ions concentration of the loamy soil sample was determined using the pH electrical conductivity meter (pH meter). To achieve this, 10g homogenized loamy soil sample (pounded in a soil mortar and sieved down to less than 2mm was weighed and put in a pH cup and the addition of 25ml of deionized water followed suit. This then resulted in soil: water concentration of 1:2.5. The mixture was stirred for 1 hour and reading was taken. The readings were then taken by inserting the probe of the pH/EC meter into solution (soil solution).

Electrical conductivity

The same pH/EC meter used in measuring the soil pH was used to measure electrical conductivity of the soils. Hence, same steps or procedure stated for pH measurement was followed. The EC of the soil samples were measured in micro-Siemens/cm (µS/cm). The probe of the electrode was washed after each reading for accurate results and to avoid cross-contamination.

Moisture content

The moisture content of the samples was determined according to standard method described by Ukpaka and Edwin, (2013). 10g of soil sample was weighed into a crucible and heated in an oven at 105ºC for 24 hours to dry off water content in the soils. After which, the dried soil samples were cooled in desiccator for 30 minutes. On cooling, the samples were reweighed to obtain a constant weight.

Table 1: Physicochemical properties of treatment water extract before application

Parameters	Water	Neem-water extract
pH	6.39	6.25
Chloride (mg/l)	43.70	83.51
Sulphate (mg/l)	6.26	20.80
Total hardness	28.17	47.30
Iron Content (mg/l)	0.030	0.269
Conductivity (µS/cm)	41.04	1.665
TDS (mg/l)	2.80	8.06
TSS (mg/l)	2.80	5.98
Nitrate (mg/l)	0.22	6.20
Calcium (mg/l)	39.88	18.27
Salinity (mg/l)	53.88	89.80
Temperature (ºC)	29.1	29.5
Density (g/cm^3)	1.0007	1.0002

Table 2: Physicochemical properties of loamy soil before and after pollution

Parameters	Before pollution	After pollution
Density (g/cm^3)	1.75	4.80
E.C (µS/cm)	10	15.6
pH	5.85	5.48
MC (%)	9.0	30.5
TOC (%)	2.64	4.77
TN (mg/kg)	2.27	6.46
TP (mg/kg)	1.14	4.68

To calculate the percentage moisture content, Eq. 1 was used.

$$MC(\%) = \frac{w_1 - w_2}{w_1} \times 100\% \quad (1)$$

Where, MC is a moisture contents (%), W_1 is an initial weight of soil sample (g) and W_2 is a weight of dried soil sample (g).

Total organic carbon

Total Organic Carbon (TOC) was determined using a method described by Ukpaka and Edwin, (2013). 1.0g of crushed fine representative soil samples was weighed in duplicate into 250ml beaker. 10ml of potassium dichromate solution was pipette into beakers and then, rotated gently to completely wet the soil sample, and then, followed by addition of 20ml of concentrated H_2SO_4 using an automatic pipette, directing the stream into the suspension. Thereafter, the beaker was gently rotated to obtain a uniform mixture of soil and reagents, and vigorously rotated for the next one minute, for effective and more complete oxidation, before being allowed to stand for 30 minutes on sheet of asbestos. On settling, 100ml of distilled water was added followed by addition of 3-4 drops of 0.5 ml diphenylamine indictor. The solution was titrated with 0.5N ferrous sulfate solution. The end point was noticed as dull green through turbid blue to brilliant green. The process was repeated on distilled water (blank titration), but without soil to standardize the dichromate. The TOC was calculated using Eq. 2. Where, V is volume, W is weight of sample and B is a lank.

$$TOC = B - \{\frac{V \times 0.195}{W}\}100 \quad (2)$$

Total petroleum hydrocarbon (TPH) content

This was determined using ASTM (1999) method D3921. Hydrocarbon content was extracted with dichloromethane in an extractor and treated with 2 ml of activated silica gel. The TPH of the representative samples were then determined with the aid Gas Chromatography – Flame Ionization Detector (GCFID) Model, HP 5890 Series II, U.S.A. The percentage of TPH removed at any given time was determined using Eq. 3.

$$TPH_R(\%) = \frac{TPH_i - TPH_f}{TPH_i} \times 100 \quad (3)$$

Where, TPH_R is the percentage of TPH removed at a given time, TPH_i is the initial concentration of THP and TPH_f is the concentration of TPH at a given time.

Total bacteria count (TBC)

Prepared nutrient agar culture plates were made according to the manufacturer's specification (HIMEDIA) M001-500G, HIMEDIA Laboratories Pvt. LTD Number-400086, India). The culture plates were dried and 0.1ml of the 10^1 diluted soil samples was placed on it and it was spread using a glass rod spreader to dryness on the plate. This was incubated in an incubator at 37ºC for 24hours and the counting of the bacteria was made on the plate after the bacteria have shown growth. The bacteria that did not grow after 24hours were further allowed in the incubator for another 24hours and readings were made on them.

Soil particle size analysis

The particle size of the soils was analyzed using the method of Reddy (2002). 500g of soil was weighed and sieved to 2.0mm particle size. The sieved soil particle size was transferred into a plastic bottle. 50ml of sodium hexameta phosphate $(NaPO_3)_2$ plus 10ml of distilled water was added into the plastic bottle. The mixture was manually shaken for about 3-5 minutes, and then transferred into 100ml measuring cylinder and allowed to settle. The particle sizes that cannot pass through the sieve mesh were weighed, while

Table 3: Physicochemical properties of loamy soil after 84 days of treatment

Parameters	Control	NW-500	NW-600
Density (g/cm^3)	3.94	2.74	2.29
E.C (µS/cm)	14.20	13.73	12.01
pH	5.81	6.74	6.94
MC (%)	25.01	17.39	14.55
TOC (%)	3.72	2.61	2.28
TN (mg/kg)	3.36	2.07	1.79
TP (mg/kg)	2.43	1.50	1.30

Table 4: TPH degradation in loamy soil treated with neem

Time (Week)	Control (mg/kg)	NW-500 (mg/kg)	NW-600 (mg/kg)
0	40232.65	40232.65	37137.30
14	33981.54	28737.57	26462.74
28	30803.88	25793.49	24363.53
42	29333.06	22280.84	20087.57
56	26235.03	18042.31	16342.90
70	23285.27	13730.43	9871.09
84	20961.53	7341.79	3774.64

the different layers of the soils, which had settled in the cylinder, were collected and gently separated. Thereafter, the separated samples were then heated to dryness and weighed to determine the percentage of silt, clay and sand in the respective soil samples. The percentage of silt, clay or sand in the soils was calculated using Eq. 4. Where MBS = mass of bulk sample and MDS = mass of dried sample.

$$Class\,texture(\%) = \frac{MBS - MDS}{MBS} \times 100 \quad (4)$$

From the percentage of soil texture and using the soil texture triangle, the soil textural class was determined.

RESULTS AND DISCUSSION

The biodegradability of Total Petroleum Hydrocarbon under the influence of neem roots solution applied as treatment of crude oil polluted soils has been carried out experimentally, and the results presented in this research work.

Soil physicochemical properties

The physicochemical properties of neem-water solution were determined (Table 1). Also, as a way of determining the extent of variability of loamy soils due to the effect of crude oil pollution and treatment, the physicochemical properties of the loamy soil were evacuated before and after treatment. Table 1 showed that there are changes in the properties of water after soaking neem roots into it. This implied that neem roots were responsible for the change in water property. The composition of parameters studied as presented in Table 1 demonstrates the significance of neem roots in enhancing bioremediation of a polluted soil environment. From Table 2 it is seen that the concentration of the parameters investigated showcase a tremendous change in concentration due to the effect of the neem roots indicating the action of the root in increase the concentration of the functional parameters Table 3 illustrates the comparison of the control sample with the polluted samples as shown in the research work. In Table 3 it is seen that the concentration of the polluted loamy parameters values are increasing and decreasing which reveals the effect of the degree of pollutant on the physicochemical e of the loamy soil studied.

Table 4 demonstrates the TPH degradation in the three environmental conditions of control, NW-500 and NW-600 and the result obtained reveals increase in degradation to have been faster in NW-600 than in NW-500 because of high volume of neem roots extract added in the third container of NW-600. The concentration in the control show slight change and the change can be attributed to the environmental factors. Table 5 showcase the comparison of the percentage degradation of the TPH in each of the bioreactor of NW-500 and NW-600 and the results obtained reveals the container with high volume of neem root extract attained high level of TPH degradation. This process reveals that the higher the volume of the neem root extract the more effective the degradation of TPH only when the concentration of the root extract is high. Table 6 demonstrates the total bacteria count for the container or bioreactor of NW-500 and the result obtained showcase progressive phase from 14days to 56days before sudden decrease in microbial population. Total bacteria count for the container or bioreactor of NW-600 and the result obtained showcase progressive phase from 14 days to 56days before sudden decrease in microbial population in loamy soil environment (Table 7). The biodegradability of crude oil in loamy soil amended using neem root extracts was promising and density, electrical conductivity, moisture content, total organic carbon, total nitrogen and phosphorus in the soils was also improved significantly, hence, the bioremediation of soil contaminated with HCs could possibly be treated by biodegradation technique and previous findings are also in line present investigation (Abbaspour et al., 2020; Ebadi et al., 2018; Hazaimeh et al., 2019; He et al., 2019; Huang et al., 2019; Igun et al., 2019; Kang et al., 2020; Tao et al., 2019; Vasilyeva et al., 2020; Zhao et al., 2019).

CONCLUSIONS

The performance of neem roots soaked in water for bioremediation of crude oil polluted soils has been studied in this work. Physicochemical analysis was carried out and

Table 5: Percentage TPH degradation in loamy soil with treatment-water solution

Time (Week)	NW-500 (%)	NW-600 (%)
0	0	0
14	28.57	28.74
28	35.89	34.40
42	44.62	45.91
56	55.16	55.99
70	65.87	73.42
84	81.75	89.84

Table 6: Total bacteria count for NW-500 treatment

Time (Weeks)	Loamy (cfu/ml)
0	6.85×10^7
14	4.50×10^9
28	5.96×10^9
42	6.35×10^9
56	6.70×10^9
70	6.12×10^9
84	5.64×10^9

Table 7: Total bacteria count for NW-600 treatment

Time (Weeks)	Loamy (cfu/ml)
0	6.85×10^7
14	4.51×10^9
28	6.25×10^9
42	6.47×10^9
56	6.79×10^9
70	6.21×10^9
84	5.96×10^9

results obtained as presented in the research. Thus, there was significant evidence of changes in physicochemical properties of the soils used for this study under the influence of the formulated treatments. The density, electrical conductivity (EC), moisture content (MC), total organic carbon (TOC), total nitrogen (TN) and phosphorus (P) in the loamy soil increased after polluting the loamy soil, except for pH, which its case was the reverse. However, density, electrical conductivity (EC), moisture content (MC), total organic carbon (TOC), total nitrogen (TN) and phosphorus (P) decreased remarkably with time under treatment, while the pH of the loamy soil increased from acidity to neutrality after the treatments.

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