Impact of Soybean Waste and Pigeon Dropping in the Total Hydrocarbon Content of Crude-Oil Polluted Soil

ABSTRACT

The contamination of the soil by crude oil is known to cause detrimental effects and advances using organic and inorganic nutrients to clean it up are being exploited. The impact of soybean waste (SBW) and pigeon dropping (PD) in the bioremediation of crude oil polluted soil was investigated. Uncontaminated soil sample was collected from the Rivers State University Research FarmLand. Furthermore, soil sample was contaminated with 5% bonny-light crude oil. SBW and PD were used as the organic supplements. The experimental set-up consisted of 500g of contaminated soil supplemented with SBW and PD separately and in consortium (both SBW and PD) with a control (uncontaminated soil). Bacterial and fungal isolates were determined using standard microbiological methods while the physicochemical parameters were determined using the standard method. The total heterotrophic bacterial load of the soil ranged from 9.5×10^4 to 1.2×10^7 CFU/g. The hydrocarbon utilising bacterial load ranged from 7.1×10^4 to 2.5×10^5 CFU/g. Fungal load ranged from 7.5×10^3 to 1.4×10^5 SFU/g while the hydrocarbon utilising fungal load ranged from 1.3×10^4 to 5.6×10^4 SFU/g. *Bacillus, Serratia, Pseudomonas* and *Mycobacterium* sp were the hydrocarbon utilising bacteria while *Mucor, Aspergillus, Penicillium* sp and *Saccharomyces* sp represented the hydrocarbon utilising fungi. The physicochemical properties of the soil samples before bioremediation showed that the pH ranged from 6.14 to 7.75. The nitrate ranged from 0.813 to 1.216mg/kg; the phosphate ranged from 0.196 to 0.857mg/kg; the total organic carbon (TOC) ranged from 0.88 to 4.89mg/kg and the total hydrocarbon content (THC) ranged from 1419 to 5320mg/kg. The ranges of the physicochemical properties after bioremediation showed that the pH was 6.11 to 7.10, nitrate content 1.903 to 3.016mg/kg, phosphate 0.011 to 0.03 mg/kg, TOC 1.38 to 2.56mg/kg and THC 920 to 4631 mg/kg. The percentage reduction of the THC showed that the highest percentage reduction 52.26% was observed in the crude oil polluted soil amended with SBW followed by the crude oil polluted soil amended with the consortium (50.56%) and PD amended soil had 50.38%, respectively. The % reduction of THC of the unamended crude oil polluted soil was 12.95%). There was no significant difference ($P>0.05$) in the reduction of THC amongst the treatment. Thus, the use of soybean waste and pigeon droppings for bioremediation is recommended.

Keywords: soybean waste, pigeon droppings, bioremediation, crude oil

Introduction

Anthropogenic activities have led to an increase in environmental contamination in recent decades (Sharma, 2019). Unintentional leaks from reservoirs, refineries, and transportation pipes can contaminate soil with petroleum hydrocarbons (Manli, 2016). One of the several catastrophes that humans have produced is environmental deterioration brought on by oil spills during extraction, processing, transportation, and pipeline corrosion or destruction. According to Adams et al. (2014), oil contamination decreases the soil's capacity to sustain plant development, contaminates ground water through seepage, and raises the concentration of heavy metals, which can bioaccumulate and biomagnify and have detrimental health impacts. The physical, chemical, and ecological characteristics of soil are impacted by the presence of petroleum hydrocarbons (Ramadass *et al.*, 2015). Since less proven technology will be needed to access new oil deposits in the environment due to increased economic pressure, the chance of an unintentional oil spill into the environment will continue to be significant for the foreseeable future (Mason et al., 2012).

Strategies for responding to oil spills and evaluating the environmental effects of oil pollution have not kept pace with the rapid advancements in oil drilling technologies in recent decades. Since then, there have been attempts to use physiochemical techniques to clean up hydrocarbons on

contaminated sites, but their use has been discouraged because of they are not eco-friendly (Malhotra et al., 2015). Over time, petroleum hydrocarbon-polluted sites have been treated with synthetic fertilizers, leading to adverse effects like atmospheric pollution (Geddes et al., 2015) and eutrophication, a process marked by an increase in the productivity of aquatic systems brought on by progressive enrichment with minerals like nitrogen and phosphorus (Aleksandrov et al., 2006). Furthermore, due to their high demand as a necessary agricultural input, synthetic fertilizers are extremely costly in developing nations like Nigeria (Danjuma et al., 2012). These issues, along with others, have led to calls for sustainable substitutes for synthetic fertilizers (Dados et al., 2015). The present study therefore seeks to evaluate the impact of soybean waste and pigeon droppings in the reduction of THC of soil polluted with crude oil

Materials and Method

Description of Study Area

The study was undertaken at the Rivers State University, Nkpolu-Oroworukwo, Mile 3, Port Harcourt Local Government Area, Rivers State at latitude 4.8062 N and longitude 6.9864 E.

Sample Collection

Using a sterile spatula, soil samples were taken from the Rivers State University Research FarmLand at a tillage depth of 10 cm. The samples were then homogenized to create composite soil samples. After that, the soil was gathered and moved to the Rivers State University Greenhouse in airtight bags. The Bonny light crude oil was obtained from Nigeria Liquefied Natural Gas (NLNG) Limited, Bonny Island, Nigeria. Soybeans were obtained from the Mile 3 Market, Port Harcourt, Rivers State, Nigeria and processed to obtain the soybean wastes (SBW). After three days of sun drying, the soybean wastes were ground into a fine powder that could fit through a 2 mm mesh filter and kept in a polythene bag until needed. The pigeon dropping was obtained from Big Birds poultry store, Ada-George, Rumuepirikom, Port Harcourt, Rivers State, Nigeria. The pigeon droppings (PD) were sundried for 3 days and then pulverized and stored in a polythene bag until required.

Bioremediation Experimental Setup

The experimental set-up is presented in Table 1. Five hundred grams (500g) each of the prepared soil samples were put into five equal sized pots and then 50mL (3%) of crude oil was used to pollute four of the experimental pots allowing the unpolluted pot, to serve as positive control. The experiment was divided into four treatments and one control. Treatment 1 covers crude oil polluted soil containing 500g soil and 50 mL crude oil. Treatment 2 is similar treatment 1 with 50g of SBW. Treatment 3 is also similar to treatment 1 with addition of 50g PD. However, in treatment 4, additional 25g each of SBW and PD were added along with500g soil and 50 mL crude oil.

Table 1 Experimental Setup

Sample Container	Treatment	Volume (mL) of crude oil (3%)	Concentration of supplement (g)	Final volume/ weight

Control (unpolluted soil)	500g soil	0	Nil	500g
Crude oil polluted soil	500g soil	50mL	Nil	550g
Crude-oil polluted soil +SBW	500g soil	50mL	50g SBW	600g
Crude-oil polluted soil +PD	500g soil	50mL	50g PD	600g
Crude-oil polluted soil +PD+SBW	500g soil	50mL	25gSBW+25gPD	600g

Keys: Soybean waste (SBW), Pigeon dropping (PD)

Preparation of Soil Samples for Inoculation

One gram of the soil samples from each experimental set-up was transferred into 10mL sterile normal saline to form the stock of each sample. One millilitre was withdrawn from the stock using sterile 1mL pipette and transferred into a test tube containing sterile 9mL normal saline. Further Ten-fold serial dilution was carried out to achieve a dilution of 10^{-6} with the help of normal saline

Enumeration and Isolation of Total Heterotrophic Bacteria (THB)

The total heterotrophic bacterial count was enumerated by inoculating an aliquot (0.1mL) of the 10^{-4} dilution into freshly prepared nutrient agar plate. The plate was swabbed using sterile bent glass rod and incubated in an inverted position in the incubator at 37°C for 24 hours. After incubation, colonies on the plates were counted and discrete colonies identified based on colour, shape, elevation and texture were subctured aseptically into freshly prepared nutrient agar plates. These were incubated at similar temperature and observed for pure isolates. The pure isolates were further identified using biochemical methods (Prescott *et al.,* 2011). The colony forming unit (CFU) per millilitre was calculated using the formula below;

$$\text{CFU/g} = \frac{\text{No of Colonies}}{\text{Dilution x Volume Plated}}$$

Enumeration and Isolation of Total Fungi

The fungal counts were determined by spread plate technique on sabouraud dextrose agar (SDA) (Prescott *et al.,* 2011). The aliquot (0.1mL) of the 10^{-2} dilution of the samples were inoculated in duplicate on the surface of dried tetracycline-supplemented SDA plate and spread evenly with a flame sterilized bent glass rod. The plates were incubated in an inverted position at 25°C for 3-5days. After the incubation, the resulting colonies were enumerated, while discrete colonies based on the macroscopic characteristics were subcultured on freshly prepared tetracycline-supplemented SDA plates for isolation of pure fungal isolates.

Enumeration and Isolation of Hydrocarbon Utilising Bacteria and Fungi

The hydrocarbon utilising bacteria and fungi were enumerated by inoculating an aliquot (0.1mL) of the 10^{-2} dilution into freshly prepared Bushnell-Hass agar plate supplemented separately with miconazole (to inhibit fungal growth) and tetracycline (to inhibit bacterial growth while allowing the proliferation of fungal growth). The plate was swabbed using sterile bent glass rod and incubated in an inverted position in the incubator at 25°C and 37°C for 7 days for isolation of fungi and bacteria, respectively. After incubation, colonies on the plates were counted and discrete colonies identified based on colour, shape, elevation and texture were subctured aseptically into freshly prepared nutrient agar plates for bacteria and SDA plates for fungi (Prescott *et al.*, 2011).

Identification of Bacterial Isolate

Various bacteria isolate in discrete colonies were identified based on colonial, morphological (gram stain) and biochemical characteristics. The biochemical characteristics: catalase, oxidase, sugar fermentations, citrate utilisation, Voges Proskauer, Methyl red test, indole and starch hydrolysis were adopted (Prescott *et al.*, 2011). The response to these tests were inputted in the data base of the Online Advanced Bacterial Identification Software (ABIS) to confirm the identities of the isolates.

Identification of Fungal Isolates

Pure cultures of each fungal isolates were used for identification. The identification of the isolated fungi was done macroscopically and microscopically. A mass of fungal mycelium was scraped from each SDA plate and examined based on difference in colour, shape, septate and size of spores. For each isolate, a portion of the fungal mycelium was taken from the margin of a 3 days old culture using a sterile inoculating pin. The slide preparation was stained with lactophenol cotton blue (LPCB) stain and covered with a cover slip. The mounted slide was observed under the microscope with x10 and x40 magnification. The identities were confirmed by comparing the observed characteristics with those in the fungal atlas (Sarah *et al.*, 2016).

Determination of pH

The pH of soil sample was determined by the American Public Health Association (APHA) Standard Methods (APHA, 2012). The pH meter was switched on and allowed for some time. It was then calibrated with buffer solutions of high pH range between 8 and 9 as well as a lower pH range between 1 and 6 by dipping the electrode into the buffer solutions. Sample (10g) was weighed into 100mL beaker; 50mL of distilled water was then added to allow immersion of the electrode, mixing was carried out by stirring frequently for few minutes. Then beaker was allowed to stand for 15 minutes. The electrode was immersed into the sample. The pH value for each sample was recorded accordingly.

Nitrate

Nitrate was determined by Phenol Di-Sulphonic acid method by the methods of Jackson (1973) and Trivedy and Goel (1984). Fifty millilitres (50 mL) of the water sample was taken and evaporated over a hot plate till residues were formed, which was dissolved in three milliliters (3mL) of phenol Di-Sulphonic acid. The reaction was allowed to stand for 10 minutes and then fifteen milliliters (15mL) of distilled water was added. Seven milliliters (7mL) of ammonia solution were added and the final volume was made to be 50mL. The intensity of yellow colour

transmission percentage was measured at 410 nm. The Values of NO_3-N as mg/L was obtained in reference to the calibration curve and value was computed in the following formula: -

$$Nitrate\ N = \frac{mg\ of\ Nitrate\ N}{ml\ of\ Sample} \quad \text{Equation 4}$$

Determination of Phosphate (PO_4^{3-})

The phosphate levels for the samples were determined using an ultraviolet (UV) spectrophotometer. 25mL of 2.5% Acetic acid was added to 1g of soil sample and shaken for 30minutes. The suspension was filtered through a Whatman filter paper 1. Ten (10mL) of the extract was transferred into 50mL volumetric flask. Extract was diluted with distilled water until the flask is about 2/3 full. 2mL of Ammonium Molybdate reagent was added and mixed with extract. 2mL of stannous chloride was also added and mixed; the solution was diluted to 50mL mark with distilled water. The flask was allowed to stand for 30minutes, and the absorbance was measured at wavelength of 690nm (APHA, 2012).

Determination of TOC

About 0.2g of soil sample was measured into a 500mL conical flask. 10mL of 0.5M $K_2Cr_2O_7$ was added and swirled gently. 20mL of concentration H_2SO_4 was added rapidly and directly into suspension but with care to avoid splashing. Immediately swirl gently until the reagents are mixed for 1 minute. Flask was allowed to stand for 30 minutes. 200mL of distilled water and 10mL concentration H_3PO_4 was added cautiously to avoid splashing and mixture was cooled, 3 drops of Ferroin Indicator Solution was added. Solution was titrated to a deep green end-point with 0.25M Ferroin Ammonium Sulphate (FAS) solution (APHA, 2012).

Determination of THC

This was done using a spectrophotometer. During the setup process for spectrophotometric analysis, 10g of soil sample were weighed from each of the setup rubbers containing 1500g of soil sample into sterile beaker and 20mL of xylene was added and shaken properly to extract the oil from the soil and this was allowed to digest for 30minutes and the extracted oil were sieved with Whatman No 1 filter paper to a test tube that was transferred into colorimeter cuvette and placed in a chamber known as infrared spectrophotometer analyzer. The Total Hydrocarbon Content (THC) value was determined by comparing to a calibration curve constructed from dilution of stock solution of a 1:1 bonny light crude and oil soil dispersant. Total Hydrocarbon Content (THC) was analyzed at 420nm wavelength (APHA, 2012).

Statistical Analysis

The microbial counts were expressed as mean ± standard deviation of two replicates. Analyses of variance (ANOVA) was carried out using SPSS (version 27.0) to check for significant difference and mean values were separated using the Ducan multiple range test (DMRT) at $P \leq 0.05$.

Results

The total heterotrophic bacterial load of the various treatments in Table 2 for Days 1, 15 and 30 ranged from 0.95±0.07 to 12.05±0.07×10^6 CFU/g, 0.65±0.02 to 1.84±0.01×10^7 and 2.00±0.00 to

$3.02\pm0.02\times10^7$ CFU/g, respectively. Results further significant differences ($P<0.05$) in the total heterotrophic bacterial load across the treatments. More so, the crude-oil polluted soil amended with the consortium of pigeon dropping and soybean waste had the highest heterotrophic bacterial load across the period of remediation while the contaminated soil with crude oil only in Day 1 had the least count.

Results of the total hydrocarbon utilising bacterial load in Table 3 showed that the hydrocarbon utilising bacterial load in Days 1, 15 and 30 ranged from 0.71 ± 0.01 to $2.5\pm0.7\times10^6$ CFU/g, 0.92 ± 0.02 to $2.59\pm0.02\times10^6$ CFU/g and 1.34 ± 0.06 to $3.16\pm0.02\times10^6$ CFU/g, respectively. The unpolluted soil had the least hydrocarbon utilising bacterial count across the period of the bioremediation while the crude oil contaminated soil amended with pigeon droppings and soybean wastes displayed higher counts of hydrocarbon utilising bacteria. There was significant differences ($P<0.05$) in the counts for Day 1 unlike the Days 15 and 30 which had no significant differences ($P>0.05$) despite the fluctuation in counts across the samples.

Results of the total heterotrophic fungal load of the samples ranged from 0.075 ± 0.00 to $1.4\pm0.00\times10^5$, 3.25 ± 0.21 to $19.7\pm0.01\times10^5$ and 0.77 ± 0.02 to $2.44\pm0.05\times10^5$ CFU/g for Days 1, 15 and 30, respectively. There was significant difference ($P<0.05$) in the fungal counts across the samples in the respective days (Table 4).

The results of the hydrocarbon utilising fungal load ranged from 1.3 ± 0.00 to $5.6\pm0.14\times10^4$, 1.40 ± 0.00 to $5.95\pm0.07\times10^4$ and 1.45 ± 0.21 to $8.50\pm0.42\times10^5$ CFU/g for Days 1, 15 and 30, respectively. There was significant difference ($P<0.05$) in the hydrocarbon utilising fungal counts across the samples in the respective days (Table 4).

The total hydrocarbon utilizing fungal load in Table 5 showed that the mean fungal load for Day 1, 15 and 30 ranged from 1.3 ± 0.00 to $5.6\pm0.14\times10^4$, 1.40 ± 0.00 to $5.95\pm0.07\times10^4$ and 1.45 ± 0.21 to $8.50\pm0.42\times10^4$ SFU/g. Results further showed that there was significant difference ($P<0.05$) in the various samples for the respective period of bioremediation.

Table 2 Total Heterotrophic Bacterial Load of the Soil during the Bioremediation Period

Treatment	Day 1 ($\times10^6$)	Day 15 ($\times10^7$)	Day 30 ($\times10^7$)
Control (unpolluted soil)	2.15 ± 0.07^{c}	0.65 ± 0.02^{a}	2.00 ± 0.00^{a}
Crude oil polluted soil	0.95 ± 0.07^{a}	1.27 ± 0.02^{ab}	2.41 ± 0.02^{a}

Crude-oil polluted soil +SBW	9.05±0.07[b]	1.36±0.02 [b]	2.16±0.02 [a]
Crude-oil polluted soil +PD	5.65±0.07[d]	1.78±0.01 [c]	2.81±0.02 [a]
Crude-oil polluted soil +PD+SBW	12.05±0.07[e]	1.84±0.01 [c]	3.02±0.02 [a]
P-value	0.00	0.00	0.0921

Keys: SBW = soybean waste; PD = pigeon dropping.

Table 3 Total Hydrocarbon Utilising Bacterial Load of the Soil During the Bioremediation Period

Treatment	**Day 1 (×10^6)**	**Day 15 (×10^6)**	**Day 30 (×10^6)**
Control (unpolluted soil)	0.71±0.01[a]	0.92±0.02 [a]	1.34±0.06 [a]
Crude oil polluted soil	0.93±0.00[ab]	1.16±0.00 [ab]	2.15±0.04 [a]
Crude-oil polluted soil +SBW	2.2±0.00 [ab]	2.59±0.02 [b]	3.16±0.02 [a]
Crude-oil polluted soil +PD	2.2±0.7 [ab]	2.32±0.02 [b]	3.10±0.00 [a]
Crude-oil polluted soil +PD+SBW	2.5±0.7[b]	2.27±0.01 [b]	1.69±0.02 [a]
P-value	0.029	0.031	0.914

Keys: SBW = soybean waste; PD = pigeon dropping.

Table 4 Total Heterotrophic Fungal Load of the Soil During the Bioremediation Period

Treatment	**Day 1 (×10^5)**	**Day 15 (×10^5)**	**Day 30 (×10^5)**
Control (unpolluted soil)	0.71±0.01 [b]	7.45±0.07 [b]	0.77±0.02 [a]
Crude oil polluted soil	1.2±0.00 [bc]	13.0±0.14 [c]	1.44±0.00 [ab]

Crude-oil polluted soil +SBW	0.075±0.00[a]	8.75±0.21 [b]	2.06±0.04 [b]
Crude-oil polluted soil +PD	1.4±0.00 [c]	19.7±0.01 [d]	2.44±0.05 [b]
Crude-oil polluted soil +PD+SBW	0.16±0.00 [ab]	3.25±0.21 [a]	0.81±0.03 [a]
P-value	0.00	0.00	0.00

Keys: SBW = soybean waste; PD = pigeon dropping.

Table 5 Total Hydrocarbon Utilising Fungal Load (CFU/g) of the Soil During the Bioremediation Period

Treatment	**Day 1 (×10^4)**	**Day 15 (×10^4)**	**Day 30 (×10^4)**
Control (unpolluted soil)	5.6±0.14[e]	5.95±0.07 [c]	6.45±0.21 [b]
Crude oil polluted soil	4.7±0.00[d]	3.30±0.14 [b]	1.60±0.14 [a]
Crude-oil polluted soil +SBW	1.9±0.00[b]	5.05±0.21 [c]	8.50±0.42 [c]
Crude-oil polluted soil +PD	2.3±0.00[c]	1.80±0.14[a]	1.55±0.21 [a]
Crude-oil polluted soil +PD+SBW	1.3±0.00[a]	1.40±0.00[a]	1.45±0.21 [a]
P-value	0.00	0.00	0.00

Keys: SBW = soybean waste; PD = pigeon dropping.

The total heterotrophic bacterial isolates included *Bacillus* sp, *Staphylococcus* sp, *Serratia* sp, *Escherichia coli, Proteus* sp, *Streptomyces* sp and *Pseudomonas* sp. The hydrocarbon utilising bacterial isolates were *Bacillus* sp, *Mycobacteria* sp, *Serratia* sp and *Pseudomonas* sp.

The fungal isolates included *Aspergillus* sp, *Mucor* sp, *Saccharomyces* sp, *Candida* sp and *Penicillium* sp., while the hydrocarbon utilising fungi were *Aspergillus* sp, *Mucor* sp, *Rhodotorula* sp and *Penicillium* sp.

The physicochemical properties of the soil samples is presented in Figs. 1-5. Before bioremediation the pH ranged from 6.14 to 7.75 (Fig.1). The nitrate ranged from 0.813 to 1.216mg/kg (Fig. 2); the phosphate ranged from 0.196 to 0.857mg/kg (Fig. 3); the TOC ranged from 0.88 to 4.89mg/kg (Fig. 4) and the THC ranged from 1419 to 3602mg/kg (Fig. 5). The physicochemical properties of the soil samples after bioremediation showed that the pH ranged from 6.11 to 7.10, the nitrate content ranged from 1.903 to 3.016mg/kg, phosphate content ranged from 0.011 to 0.03 mg/kg, the TOC ranged from 1.38 to 2.56mg/kg and THC ranged from 920 to 4631mg/kg.

Results of the percentage reduction of the THC is presented in Fig. 6. Results showed that the highest percentage reduction 52.26% was observed in the crude oil polluted soil amended with soybean waste followed by the crude oil polluted soil amended with the consortium (50.56) and pigeon droppings amended soil had 50.38%. The % reduction of THC of the unamended crude oil polluted soil (12.95%).

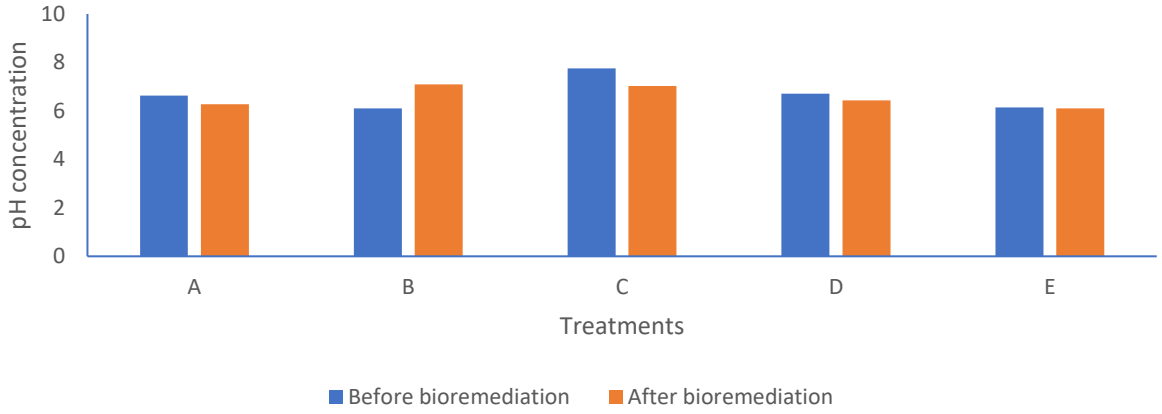

Fig. 1: pH of the soil before and after Bioremediation

Key: A: Unpolluted soil, B: Polluted soil, C: Polluted soil amended with Soybean Waste (SBW); D: Polluted soil amended with Pigeon Dropping (PD), E: Polluted soil amended with Soybean waste Pigeon Dropping (SBW + PD)

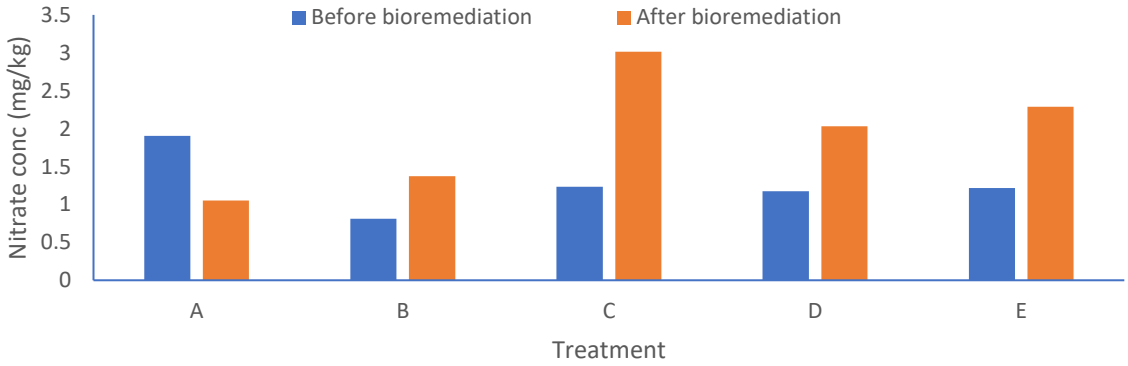

Fig. 2: Nitrate (mg/kg) of the soil before and after Bioremediation

Key: A: Unpolluted soil, B: Polluted soil, C: Polluted soil amended with Soybean Waste (SBW); D: Polluted soil amended with Pigeon Dropping (PD), E: Polluted soil amended with Soybean waste Pigeon Dropping (SBW + PD)

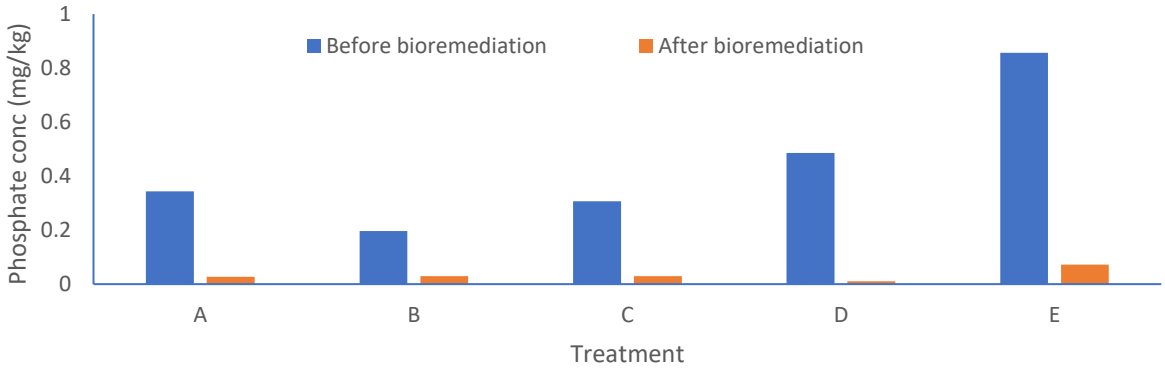

Fig. 3: Phosphate (mg/kg) of the soil before and after Bioremediation

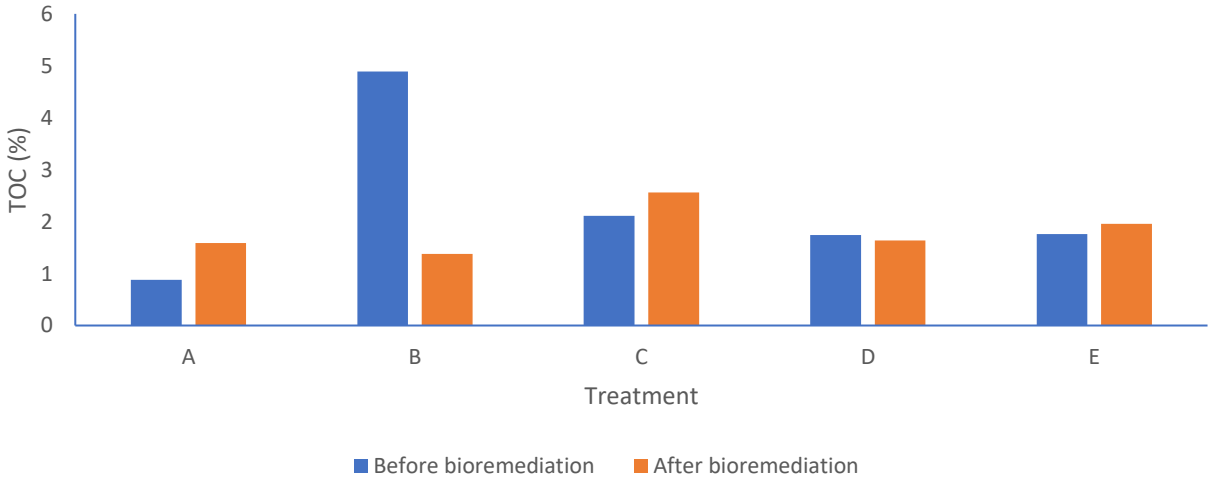

Fig. 4: TOC (%) of the soil before and after Bioremediation

Key: A: Unpolluted soil, B: Polluted soil, C: Polluted soil amended with Soybean Waste (SBW); D: Polluted soil amended with Pigeon Dropping (PD), E: Polluted soil amended with Soybean waste Pigeon Dropping (SBW + PD)

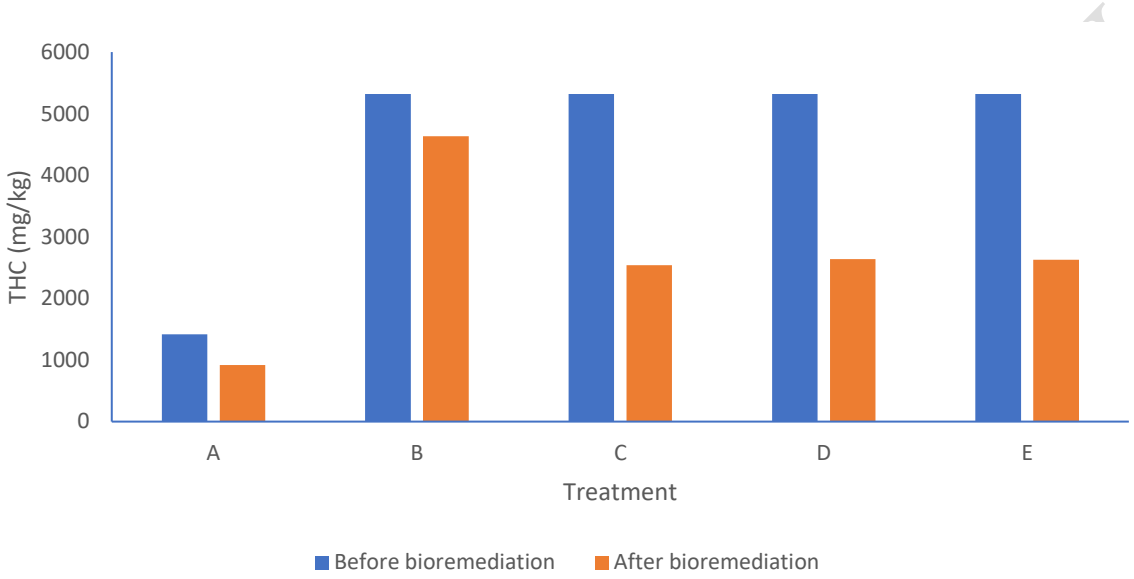

Fig. 5: THC (mg/kg) of the soil before and after Bioremediation

Key: A: Unpolluted soil, B: Polluted soil, C: Polluted soil amended with Soybean Waste (SBW); D: Polluted soil amended with Pigeon Dropping (PD), E: Polluted soil amended with Soybean waste Pigeon Dropping (SBW + PD)

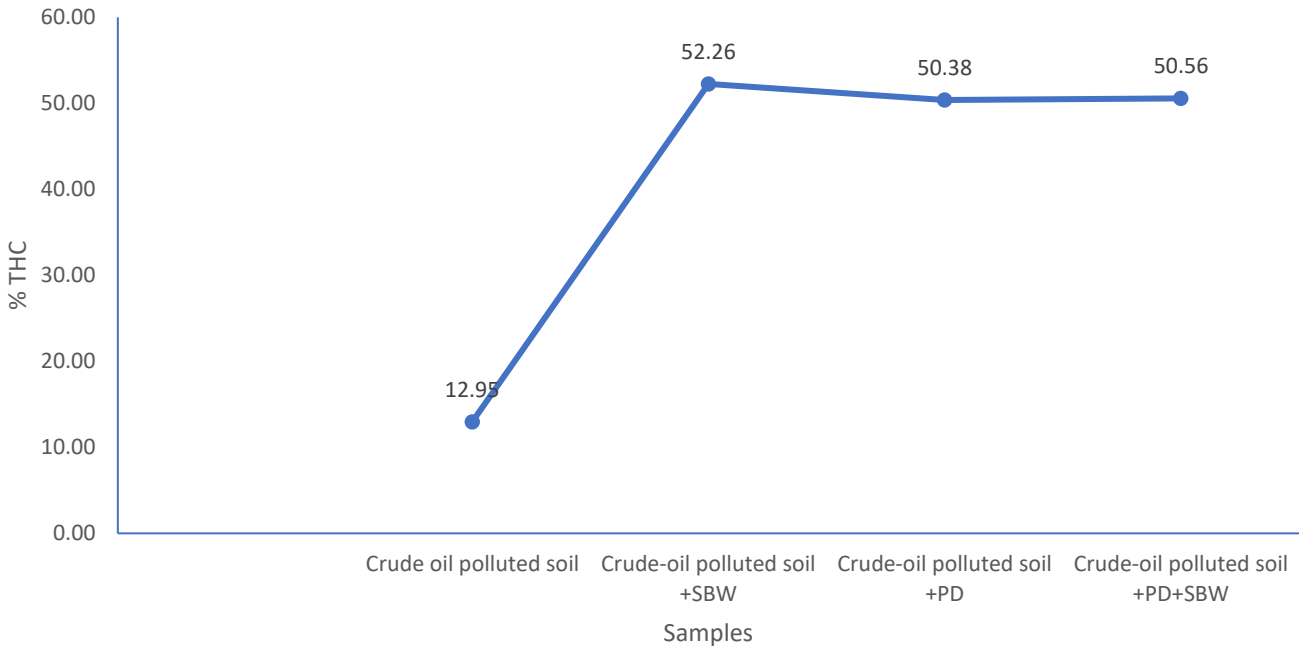

Fig. 6. Percentage reduction of THC after bioremediation

Discussion

The total heterotrophic bacterial counts of the crude-oil polluted soil amended with the consortium of PD and SBW (1.2×10^7) was higher than the total heterotrophic bacterial counts recorded in other samples (9.5×10^5 to 9.0×10^6) for day 1. The crude oil-polluted soil amended with soybean waste which had the second highest (9.05×10^6CFU/g) counts in day 1 and day 30 had a decline in counts in Day 15. For the crude oil-polluted soil amended with pigeon droppings, it was observed that the counts increased exponentially and was higher than the counts recorded for the uncontaminated and contaminated soils without amendment. This observed high counts could be attributed to the nutrients available in the poultry droppings. Similarly, the hydrocarbon utilizing bacterial count fluctuated across the samples and days of bioremediation. The polluted soil with the consortium of pigeon droppings and soybean waste had the highest hydrocarbon utilizing bacterial counts in day 1 while polluted soil amended with pigeon droppings and soybean respectively had similar counts in day 1. Additionally, the hydrocarbon utilizing bacterial counts of polluted soil amended with soybean waste was higher in days 15 and 30 followed by polluted soil amended with pigeon droppings. Williams et al., (2024) reported high bacterial load in crude oil polluted soil amended with pigeon droppings than unamended soil. The heterotrophic fungal and hydrocarbon utilizing fungal counts also increased exponentially especially with the amended soil.

This gradual increase in the microbial population during the period of the bioremediation could be attributed to the presence of nutrients which also enhanced the bioremediation process. This findings is consistent with previous studies (Chikere et al., 2016; Nrior and Mene 2017; Williams

et al., 2024) who reported increased total heterotrophic bacterial and hydrocarbon utilizing bacterial counts. The addition of pigeon droppings and soybean waste boosted the nitrogen and phosphorus content of the polluted soil thereby providing available nutrients for the proliferation of the microorganisms (Ughala & Ogugbue, 2019; Williams et al., 2024). This could also be seen in the gradual depletion of nitrate and phosphate across the samples during the period of the bioremediation. Okafor et al. (2016) reported that the addition of pigeon dropping in a hydrocarbon polluted environment enhances the microbial population thereby resulting in the biodegradation of the crude oil contaminant.

The hydrocarbon utilizing bacteria (*Bacillus* sp, *Mycobacteria* sp, *Serratia* sp and *Pseudomonas* sp) and hydrocarbon utilizing fungi (*Aspergillus* sp, *Mucor* sp, *Rhodotorula* sp and *Penicillium* sp) in the present study have been reported in previous studies to possess the ability to utilize hydrocarbon products thereby aiding in the remediation of the affected environments (Douglas & Ikirikoba, 2018). Ijah et al., (2014) isolated bacteria such as *Pseudomonas, Bacillus, Micrococcus* and Acinetobacter sp; and fungi such as *Aspergillus niger, Mucor mucedo* and *Cephalosporium acremonium*. *Bacillus* sp. and *Aspergillus* sp. were more abundant than other bacteria and fungi. The abundance of *Bacillus* sp could be due to its ability to withstand harsh environmental conditions especially with the presence of spore. *Bacillus* sp and *Pseudomonas* sp was isolated in a previous study from hydrocarbon polluted soil *(*Douglas & Ikirikoba, 2018).

The physicochemical parameters (pH, nitrate, phosphate, TOC and THC) of the amended soil including the controls (unamended soil samples) fluctuated with respect to time of the bioremediation. All the pH of the samples were slightly acidic except the pH of the crude oil polluted soil amended with SBW. The fluctuations in the pH especially on the samples could suggest bioremediation (Edward *et al.,* 2019). Thus, the crude oil pollution in the soil was broken down into less toxic and acidic byproducts, as evidenced by the rise in pH values. pH fluctuations during bioremediation of crude oil polluted environment is well documented (Amenaghawon *et al.,* 2014; Sampson *et al.,* 2016). The TOC in the present study fluctuated before and after the bioremediation. For the unpolluted soil, polluted soil amended with soybean waste and polluted soil amended with the consortium (soybean and pigeon droppings), the TOC increased from 0.88 to 1.59mg/kg, 2.11 to 2.56 and 1.76 to 1.96mg/kg, respectively while for the crude-oil polluted unamended soil and crude oil polluted soil amended with pigeon dropping, the TOC reduced from 4.89 to 1.38mg/kg and 1.74 to 1.64mg/kg, respectively. The TOC is an indicator used in measuring the extent of organic pollution in the environment (Owhonka and Obire, 2019, 2020). The high total heterotrophic bacterial load observed during the bioremediation period may have contributed to the reduction of the TOC especially in the nutrient supplemented crude oil-polluted soil. It is well known that heterotrophic microbes are in charge of using organic carbon, releasing it for use by various food webs (Owhonka and Obire, 2020). The present study contradicts Albert and Anyanwu, (2012) who reported constant TOC in their study.

The nitrate and phosphate content of the samples fluctuated. While the nitrate concentration of the samples excluding the unpolluted soil increased during the 30-days period of the bioremediation, there was a decline in the phosphate concentration in all the samples during the 30 days period of the remediation. The increase in nitrate during the 30 days period of bioremediation could be attributed to the introduction of crude oil in the soil as well as the addition of organic amendments. It could also imply that the nitrate in the soybean waste and pigeon droppings was not readily accessible or used by the microorganisms in the crude oil contaminated soil unlike the

uncontaminated soil which showed decline in the nitrate concentration. Thus, the microorganisms opted for the utilization of the phosphate. Organic manures such as poultry droppings, soybean and pigeon droppings have been reported to be slow release in nature and could be affected by early stage nutrient deficiency phenomena (Ughala & Ogugbue, 2019).

The % reduction in THC of the samples showed that bioremediation took place but was highly enhanced with the application of organic manure (soybean waste and pigeon droppings). The sample supplemented with soybean waste (52.26%) had the highest % reduction of THC while the consortium with % reduction of 50.56 was the second followed by the sample amended with pigeon droppings. the unamended sample despite depicting remediation, had the least 12.95% THC reduction. The reduction in the THC is indicative that the indigenous microorganisms were not only tolerant to the crude oil polluted soil but also possess the ability to degrade the THC. Generally, all the amended soil reduced the THC below the 5000mg/kg DPR limits (EGASPIN, 2018). Additionally, while there was no significant difference in the reduction of THC amongst the amended samples which could be due to presence of utilisable nutrients. There was a significant difference ($P<0.05$) between the polluted soil with nutrient amended to the polluted soil sample without amendment.

Conclusion

The impact of soybean waste and pigeon dropping in the bioremediation of crude oil polluted soil sample was determined in this study. The amended soil varied in their ability to biostimulate the hydrocarbon utilizing bacteria and fungi needed for the reduction of the total hydrocarbon content. Although there was no significant difference in the effect of the soybean waste, pigeon dropping and the consortium in the bioremediation, the most active manure was the soybean waste followed by the consortium. Thus, the use of soybean waste and pigeon dropping as well as the optimization potentials of these manures in bioremediation is recommended.

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