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Bioremediation of Crude Oil-polluted Soil Using Broiler-Chicken Droppings

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Abstract The impact of poultry droppings in the bioremediation of crude oil-polluted soil was evaluated. Different concentrations of the poultry droppings (10%, 30%, and 50%) were also studied. The physicochemical and microbiological properties of the soil were monitored for a period of 6 months. The poultry droppings had total heterotrophic bacterial and fungal counts of 4.2 × 104cfu/g and 1.8 × 104 cfu/g respectively. The total hydrocarbon utilizers increased progressively from month 2 to month 3, after which a decline from month 4 down occuured. The total heterotrophic microbial counts also increased from month 2 to month 4 followed by a decline from month 5 down. The control showed slight increase in microbial growth. The microbial growth rate increased as the concentration of the poultry droppings increased. Statistical analyses showed a significant difference at (P<0.05), level for the amended options and control. The total hydrocarbon content of the oil-polluted soil decreased from 6609.83 to 2951.37ml/g. *Bacillus* spp *Pseudomonas* spp *Flavobacterium* spp *Fusarium* spp, *Aspergillus* spp were isolated. Alkaline pH was observed in the poultry droppings as well as in the amended soils at 50% and lowest at the control. Ecotoxicity assay, measured in terms of germination index was used to evaluate the extent of contaminant removal.. Using seeds of *Viciafaba,* germination index of 95 % was observed in the 50 % amended option only. The study therefore showed that poultry droppings can serve as a good remediation material in the reclamation of a crude oil-polluted lithosphere.

Keywords: *bioremediation, crude oil, polluted soil, chicken droppings, amendment*

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1. Introduction

Bioremediation refers to the use of naturally occurring microorganisms or genetically engineered microorganisms by man to detoxify man-made pollutants. [1]. Since bioremediation is a microbial process, it requires the provision of nutrients among other factors or requirements. The addition of organic waste materials such as poultry litter (PL) and Coir pith (CP) to the soil facilitates aeration through small pores and increases the water holding capacity of the soil, thus enhancing bioremediation [1,2]. It allows natural processes to clean up harmful chemicals in the environment. Microscopic "bugs" or microbes that live in soil and groundwater use certain harmful chemicals such as those found in gasoline and oil spills.

Crude oil is a complex mixture of diverse hydrocarbons including alkanes, aromatics, alicyclics, branched hydrocarbons, and non-hydrocarbon compounds including polar fractions containing hetero-atoms of nitrogen, sulfur and oxygen (NSO fraction), and asphaltens, [3,4,5].

The high demand for petroleum products in the form of cooking gas, aviation fuel, gas oil, engine lubricating oil, asphalt and coal tar means increase in production and this eventually results in oil spills and hydrocarbon contamination of the environment especially through oil well blow out, tanker accidents, accidental rupture of pipelines and routine clean-up operations. These oftenlead to the release of oil into the environment, [6,7]. Current technologies for cleaning hydrocarbon contaminated soil include soil washing, solvent extraction, thermal treatment, composting, chemical oxidation (Fenton's reagent, permanganate, ozone etc) and bioremediation (bioaugmentation, biostimulation and phytoremediation) [8,9].

The most widely used bioremediation procedure is biostimulation of the indigenous microorganisms by the addition of nutrients, as input of large quantities of carbon sources tends to result in rapid depletion of the available pools of major inorganic nutrients, such as nitrogen and phosphorus [10]. Biostimulation is the addition of nutritional amendments to increase microbial metabolism and to encourage bioremediation, [10].

When microbes completely digest these chemicals, they change them into water and harmless gases such as carbon dioxide [11].

Nutrient is one factor that can hinder biodegradation if not handled properly and could limit the rate of hydrocarbon degradation in the terrestrial environment [12,13].

Crude oil pollution adversely affects the soil ecosystem through adsorption to soil particles, provision of an excess carbon that might be available for microbial use and induction of a limitation in soil nitrogen and phosphorus [14]. The above reasons account for a delay in the natural rehabilitation of crude oil polluted soil and various soils treatment have been used in bioremediation strategies to hasten the process.

There are different methods of restoration of oil-polluted soil varying from complete removal of the affected soil to doing nothing at all and allowing nature take its course [13]. According to [15], natural recovery of vegetation of the area affected by light spillages of crude oil hasoccurred without any special treatment. At low levels of contamination of crude oil, cultivation of soil without nutrient amendment is possible since reclamation of the minerals in the soil can take place in a very short time [16,17]. Naturally occurring microbial communities that respond to the presence of contaminating hydrocarbons normally have more than one type of hydrocarbon utilizing microorganisms for seeding oil slicks, therefore mixing of hydrocarbon utilizing microorganism, or genetically engineered microorganisms have been suggested [18].

Oil contamination with petroleum hydrocarbons has caused critical environmental and health defects and increasing attention has been paid for developing and implementing innovative technology for cleaning up this contamination [19].

The effects of oil pollution on the physico-chemical properties of soils have been reported [20] and also the socio-economic impact and health problems that have emerged over the years have been continuously emphasized [21]. Unless properly managed, hydrocarbon polluted environments can remain impacted for long periods of time. However, with active biodegradation process and remediation programme using adapted microbes, significant higher rates of oil removal could be achieved [22].

Biodegradation of oil is undoubtedly the most important process involved in weathering and eventual disappearance of petroleum from the polluted environment. Bacteria, yeasts and molds attack gaseous, liquids and solid hydrocarbons, transforming them into more soluble and usually more reactive compounds that in turn are broken down by microorganisms into simple components and eventually to carbon-dioxide and water. Certain microorganisms can degrade hydrocarbons but cannot utilize the degraded products in their metabolic processes.

Animal waste has over time been used to improve soil fertility [23]. Animal waste includes livestock and poultry manure, bedding and litter. Animal waste should be considered a valuable resource which when managed properly, can reduce the need for commercial fertilizer. Such waste can add organic matter which improves the water holding capacity and improves soil tilth. Animal waste can provide an economical source of nitrogen, phosphorus and potassium as well as other nutrients for plant growth [24,25]. According to [26], the addition of nutrients that can limit biodegradation to the spill site is necessary and those nutrients are not different from fertilizer. Microbial and physiochemical analysis of animal waste is carried out to determine its microbial flora and its mineral contents. [27], reported that chicken droppings habour bacteria and fungi that can utilize crude oil efficiently. Chemical analysis of chicken droppings and cow dung showed that they contain high percentage of nitrogen, phosphorous, potassium and sodium salts [28,29].

Bacteria implicated in contamination of animal wastes include Escherichia coli, Klebsiellaspp, Enterobacter spp, Staphylococcus spp, Pseudomonas spp, and Lactobacilus spp. Fungal isolates identified include Aspergillus spp., Penicilliumspp, and Rhizopus spp. [30,31]. Organic nutrients such as animal dung when added to polluted sites act both as source of nutrients and of microorganisms. Therefore, the aims of this study is to determine the relative concentration of poultry droppings that can effectively remediate a known area of soil polluted with known volume of crude oil. Effectiveness of the use of poultry droppings in remediating crude oil spill as well asecotoxicity in term of germination index using a selected agricultural plant (Viciafaba) was also evaluated.

2. Materials and Methods

2.1. Collection of Sample

Soil samples were collected from four different locations in Aguleri and Nkwelle Ezunaka, both in Anambra State. The soil samples were stored in polythene bags and transported to the laboratory. The soil samples were air dried, sieved through 2mm mesh and stored in polythene bags at room temperature.

The crude oil was collected from Eleme oil field, Eleme L.G.A, Rivers state. The soil amendment material (poultry droppings) was collected from Arroma farms in Awka, and from Anambra Integrated Poultry farms, NkwelleEzunaka, both in Anambra State. The poultry droppings were air dried, ground and stored in the laboratory at room temperature (28±2°C).

2.2. Incorporation of Amendment Material Into The Soil Sample

Three hundred and twenty (320) grams of soil was moistened and kept at room temperature in the Microbiology laboratory for one week. The soil sample was polluted with the crude oil in the ratio of 5:1 i.e. 80g of soil was mixed with 16ml of crude oil and kept for 2 weeks. The poultry dropping was applied at 10%, 30% and 50% respectively to the oil polluted soils. The experimental samples were set up as shown in Table 1 Both the amended soil and the control (polluted soil without amendment) were incubated at room temperature and observed after every two weeks for 24 weeks after pollution and the effect of the amendment on the samples studied.

Table 1. Experimental Design

Microcosm	% chicken dropping	Description
PCI	10	80g of polluted soil + 8g of chicken droppings
PC2	30	80g of polluted soil + 24g of chicken droppings
PC3	50	80g of polluted soil + 40g of chicken droppings
PC4	Control	80g of polluted soil + no amendment

[a]PC Plastic container.

2.3. Bioremediation Study

This was carried out in the field located at Nkwelle Ezunaka, Anambra State. Mounds of the soil samples were made in the field in 4 different places and left sun-dried for 2 weeks (*In situ*). Another portion (*ex situ*) was collected into four different sterile containers and transported into our university microbiology laboratory [32]. 80ml of crude oil was then applied and left for 2 weeks. Appropriate quantities of the poultry droppings were then added to the crude oil polluted soil at the various concentrations of 10%, 30%, 50% and the control was left without amendment [32]. The set up was then left for a period of 12 weeks, while monitoring the physicochemical properties of Ph, calcium, phosphorus, magnesium and nitrogen bi-weekly over a 12 week period. The total petroleum hydrocarbon content was determined using Gas chromatographic methods. After 12 weeks of remediation, the remediated soil was then cultivated with bean seeds and tested for ecotoxicity impact on the agricultural soil.

2.4. Physico-chemical Characterization Of Soil Sample And Soil Amendment Material

The soil pH was determined using pH meter (Jenway 3015 UK). Five grams of air dried soil (passed through 2mmsieve) was weighed into a 20ml beaker and 5ml of distilled water added. This was allowed to stand for 30 minutes and stirred occasionally with a glass rod. The electrodes of the pH meter were then inserted into the partly settled suspension and the pH measured. The pH meter was calibrated at pH 7.0.

Total Nitrogen of the soil samples were determined by the macro kjedahl digestion method, [33]. Five grams of soil sample was ground to pass through 0.5mm sieve and put into a dry 500ml macro kjedahl flask. To this was added one tablet of mercury catalyst. 10grams of K_2SO_4 followed by 30ml of concentrated H_2SO_4. The flask was heated at low heat on the digestion stand until frothing ceased. The heat was then increased until digest cleared after which the mixture was boiled for 5hours. 100ml of distilled water was added to the digest after it has been allowed to cool and then transferred into 750ml macro-kjedahl flask and the sand particles were then washed with 50ml of distilled water 4 times and the aliquot transferred to the 750ml flask each time. 50ml of H_3BO_3 indicator solution was measured into a 500ml Erlenmeyer flask and placed under the condenser of the distillation apparatus. The 750ml macro-kjedahl flask was then attached to the distillation apparatus and 150ml of 10N NaOH poured through the distillation flask by opening the funnel stop cork. Distillation was then carried out at the end of which 150ml of distillate was collected. The ammonium nitrogen in distillate was determined by titrating with 0.01N standard HCL with the endpoint indicated by a colour change from green to pink. The percentage nitrogen content of the sample was obtained by calculation.

Hydrochloric acid (HCL) used x normality of 4 HCL.

Available phosphorus was determined by the Bray No, One method [34] and determined by blue molybdocolometric method [35]. Briefly, 1 g gram of the sample was weighed into a 15ml centrifugation tube and 7ml of the extracting solution of NH_4F and HCL added. This was shaken on a mechanical shaker for 1minute before centrifugation at 2000revolutionperminutes (rpm) for 15minutes, it was then filtered into acid washed container, 2ml of the clear supernant was pipette into a 20ml test tube and 5ml distilled ammonium molybdate (NH_4) $6M_{07}O_{24}$) solution. The content was mixed and 1ml of dilute stannous chloride ($SnCL_2.2H_2O$) was added and mixed again. The absorbance was read at 882nm after 30 minutes [35].

The exchangeable cations were determined by using Atomic Adsorption Spectrophotometer (AAS) [36]. 5g of air dried sample was placed in a 50rnl centrifuge tube. 25ml of 1N ammonium acetate (NH_4OAC) was added. A stopper was placed on the tube and shaken for 30mins; The tube was placed in a centrifuge and spinned at 2000rpm for 10mins. The supernatant was poured into 500ml volumetric flask. This was repeated with an additional 25ml and finally brought up to a volume of 50ml with ammonium acetate (NH_4OAC). The cations (magnesium, phosphorus, calcium, potassium and sodium) were determined using atomic adsorption spectrophotometer (AAS) at wavelength 422.7nm for calcium 285.2nm for magnesium, 703.0nm for potassium.

2.5. Crude Oil Plant Toxicity Assay

Ecotocity is the subject of study of the field of ecotoxicology, which refers to the potential for biological, chemical or physical stressors to affect ecosystem. The remediated soil samples where further subjected to ecotoxicity tests to show the success of the remediation process and to determine the relationship between the growth rate of plants with the remediated soil. A bean seed was first cultivated in the soil sample without pollution to determine its suitability for germination purposes. When this was ascertained, bean seeds were planted in both In-situ and Ex-situ environments on the crude oil polluted soil amended with the poultry droppings added at varying concentrations and the experimental samples were set up.

The equation as given by [37] was used and germination index was determined as follows:

$$\text{Germination Index, GI}(\%) = \frac{(\%\ \text{Seed Germination, SG})\,\text{X}\,(\%\ \text{Growth of root, GR})}{100}$$

Where % Seed Germination,

$$\text{SG} = \frac{(\%\text{Germination on contaminated soil, EG})}{(\ \%\ \text{Germination on control soil, CG})}\text{X100}$$

And % Growth of the root,

$$\text{GR} = \frac{\left(\begin{array}{l}\text{Elongation of root on}\\ \text{contaminated soil, GERm}\end{array}\right)}{\left(\begin{array}{l}\text{Elongation of root on}\\ \text{control soil, GERCm}\end{array}\right)}\text{X100.}$$

2.6. Microbial Enumeration

Serial dilutions were carried out and the isolates were characterized and identified using the taxonomic scheme of [38]. Ten grams of the poultry droppings was measured and introduced into a beaker containing 90ml of distilled water. It was shaken for even distribution. 1ml of the

aliquot was aseptically transferred into sterile test tube containing 9ml of water to give 10^{-1} (tenfold) dilution, further tenfold serial dilution was carried out to factor 10^{-8} dilution factor. This process was also repeated for the polluted soil amended with poultry droppings. After serial dilution process, 1ml of the dilutions from factor 10^{-4} and 10^{-6} were seeded into a sterile petri dish and nutrient agar which was blended with nystatin, was poured into the plate and gently swirled and was allowed to set on the bench for bacterial count. For the fungal count, 1ml from factor 10^{-4} and 10^{-6} were seeded into a sterile petri dish and SDA media, which was blended with chloramphenicol, was poured into the plate and was gently swirled and allowed to set on the bench (10^{-8} for bacteria and 10^{-6} for fungi) by pour plate technique [39] and incubated invertedly at room temperature (28°C) for 24h for bacteria and for 3 to 5 days for fungi. After 24 hours, bacterial colonies that grew were counted using the standard plate counting techniques. The same process was repeated for fungal colonies at the end of 5 days. Discrete colonies from the primary plate was picked with the help of a sterile, wire loop and sub-cultured into a fresh agar plate and incubated. The cultural characteristics of the colonies were observed and pure cultures were sub-cultured on agar slants in Bijou bottles. After 24 hours incubation, the slants were preserved in the refrigerator at 4°C for further sub-culturing for biochemical characterization. The vapour phase transfer method [40] was used. Mineral salts medium was sterilized by autoclaving at 121°C for 15 minutes and dispensed into Petri dishes. The plates were inoculated in duplicates with O.lml aliquots of the 10^{-4} and 10^{-6} ten-fold serially diluted samples using spread plate technique. The plates were inverted over the dish covers containing 9cm Whatman No.1 filter papers earlier impregnated with crude oil. 0.lml aliquot of 10^{-4} and 10^{-6} tenfold serial diluted samples were used for fungal plates and 0.5ml of streptomycin was added to the mineral salt agar to suppress bacterial growth on fungal plate counts and nystatin used on bacterial plates to suppress fungal growth.

The ability of the bacterial isolates to utilize crude oil as the only source of carbon and energy was determined by the method of [41]. 0.1ml of 24 hours old nutrient broth culture was inoculated into each test tube containing 10ml of sterile mineral salt medium (MSM) of [42] and 1% crude oil. Control test tubes were set up containing 10ml of MSM with 1% crude oil but had no added bacteria. The tubes were incubated at 28°C for 10 days. At the end of the incubation period, the growth of the isolates was determined by visual observation of the oil medium for turbidity, as compared to the control tubes [41]. The extent of degradation of the incorporated crude oil by the bacterial isolates was determined by the gravimetric analysis method [27]. The amount of crude oil left after the incubation time was determined by extracting the residual oil with 50ml of toluene from the 100ml culture. The mixture was separated using a separator/ funnel and then filtered off with Whatman No 1 filter paper. The optical density was read on a spectrophotometer at 550nm wavelength. Using a previously prepared standard curve, the weight of the crude oil was determined. The amount of crude oil degraded was calculated by subtracting the weight of residual crude oil from weight of the added (initial) crude oil, divided by the weight of the initial crude oil and then multiplied by 100.

$$\text{Amount degraded} = \frac{\begin{pmatrix}\text{Weight of initial crude oil} \\ -\text{ Weight of residual of crude oil}\end{pmatrix}}{\text{Weight of initial crude oil}} \text{X100.}$$

Crude oil utilization test was carried out for the confirmatory identification of actual petroleum-utilizing fungi using isolates obtained from the oil agar preliminary isolation medium. The vapour phase transfer method was used [43].

Putative petroleum-utilizing fungi isolates were streaked on plates of agar medium (one Isolate per plate). In the inside of the Fein-dish cover was placed a sterile filter paper (Whatman No 1) saturated with filter-sterilized crude oil used in the study. This was aimed at supplying hydrocarbons as sole sources of carbon and energy for the growth of the micro-organisms on the mineral salts agar medium surface through vapour phase transfer. All the plates were inverted and incubated at 28°C for 7 to 14 days [40]. Uninoculatedplates served as control. Colonies which appeared on the mineral salts agar medium plates were picked and purified on plates of potatoes dextrose agar. They were finally transferred onto Sabouraud dextrose agar slants. These were then considered confirmed petroleum-utilizing fungi.

2.7. TPH Estimation

Oil contents of the polluted and remediated soil samples were determined using Gas Chromatographic methods according to the toluene extraction method [32,44] and Sonication water bath methods. Fifteen grams (15g) of each of the sample was weighed into 50ml conical flasks, then 1ml of 60ug/ml of 1-chlorooctadecane surrogate standard was added. Then 30 milliliters of dichloromethane (extraction solvent) was added to extract oil in the soil. After shaking vigorously in water bath for 5hrs, the mixture was allowed to stand for 60 minutes and then filtered through Whatman No.1 filter paper fitted with cotton wool and sodium sulphate into a clean beaker washed with methylene chloride.The residue was then washed with 20ml extracting solvent and then filtered through funnel.The extracted oil was transferred to vial and placed on a GC for analysis. The amount of crude oil degraded was calculated by subtracting the weight of residual crude oil from weight of the initial crude oil, divided by the weight of the initial crude oil and then multiplied by 100 [45].

$$\text{TPH for Soil}(\text{mg / kg}) = \frac{\text{Instrument reading x Total weight of extract}}{\text{Weight of sample}}.$$

2.8. Statistical Analysis

Statistical analysis was carried out using one – way ANOVA and Statistical Package for Social Science (SPSS) version 21.0. The standard deviations (error bars) and statistical differences (5% level of significance) were analyzed by using SPSS and GraphPad Prism 6® software (trial version) (GraphPad Software, CA, USA).

3. Results and Discussion

3.1. Physicochemical Properties of the Poultry Droppings and the Polluted Soil

The physicochemical analysis of the poultry droppings reveals an alkaline pH of 8.1 and a low value of 0.5% magnesium content. The physicochemical properties of the crude oil polluted soil before amendment are shown in Table 2 where a high nitrogen value of 6.08 was obtained and a low potassium value of 0.07 was obtained. The crude polluted soil after 2 weeks of pollution is also represented in Table 2, where a high Nitrogen content of 5.89 and a low potassium value of 0.05. Figure 1- Figure 6 show the changes in the physicochemical properties of the crude oil polluted soil after amendment for the 24 week (6-month) period.

The physicochemical analysis of the poultry droppings as shown in Table 2 reveals an alkaline pH of 8.1 and a low value of 0.5% magnesium content similar to the value reported by [2]. The physicochemical properties of the crude oil polluted soil before amendment as shown in Table 3 showed a high nitrogen value of 6.05 and a low potassium value of 0.07. This value is in agreement with the report of [44], who found out that the crude-oil polluted soil after 2 weeks of pollution before amendment with poultry dropping showed a high Nitrogen content of 5.89 and a low potassium value of 0.05. The pH rose from 5.65 to 5.93 after 12 weeks of pollution as reported by [2]. The physicochemical properties of the crude oil polluted soil after amendment with the poultry dropping for the 24 week(6-month) period illustrated in Figure 1 – Figure 6 showed a rise in pH at the on-set of the remediation process. The pH maintained a steady rise up to the 12th week (3rd month), after which there was a decline from week 14 down. This is also the case with Nitrogen, Calcium, phosphorus, magnesium and potassium which all showed a steady rise in values upto the 12th week, and decline from week 14 down in conformity with the report of [44].

Table 2. Physicochemical analysis

Parameter	Values		
	Chicken dropping	soil before crude oil pollution	Polluted soil after two weeks inoculation
pH	8.10±0.11	5.65±0.10	5.93±0.16
Total Nitrogen (%)	1.80±0.21	6.05±0.40	5.89±0.08
Total PO_4^{2+} (%)	0.90±0.33	1.20±0.12	1.05±0.17
Exchangeable Ca^{2+} (%)	1.80±0.02	2.80±0.34	2.40±0.50
Exchangeable Mg^{2+} (%)	0.50±0.14	1.18±0.00	0.98±0.13
Exchangeable K^{1+} (%)	2.70±0.33	0.07±0.01	0.05±0.22

[a]Values are mean of replicate analysis ± SD.

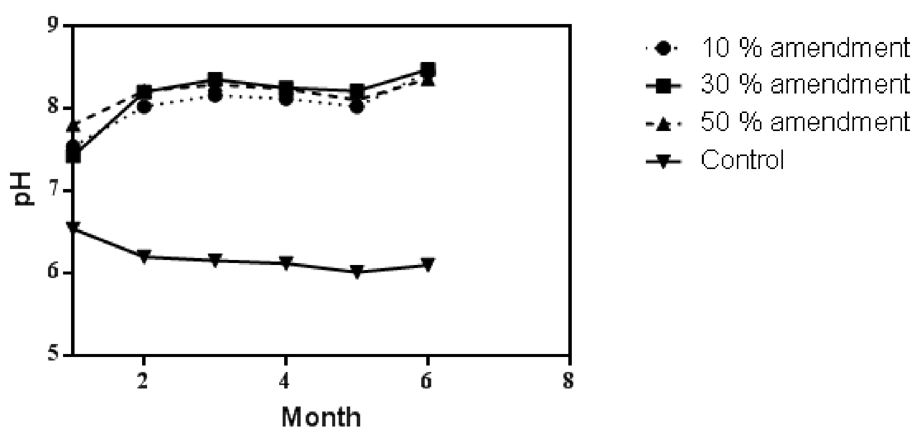

Figure 1. Effect of pH on amended polluted soil

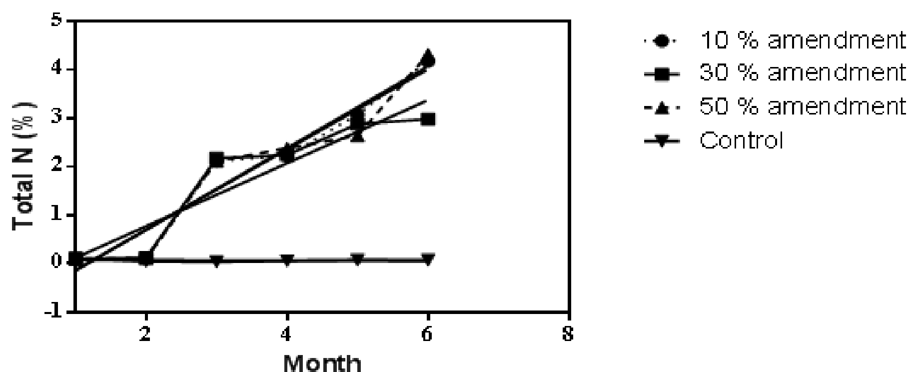

Figure 2. Effect of amendment concentration (chicken dropping) on total nitrogen

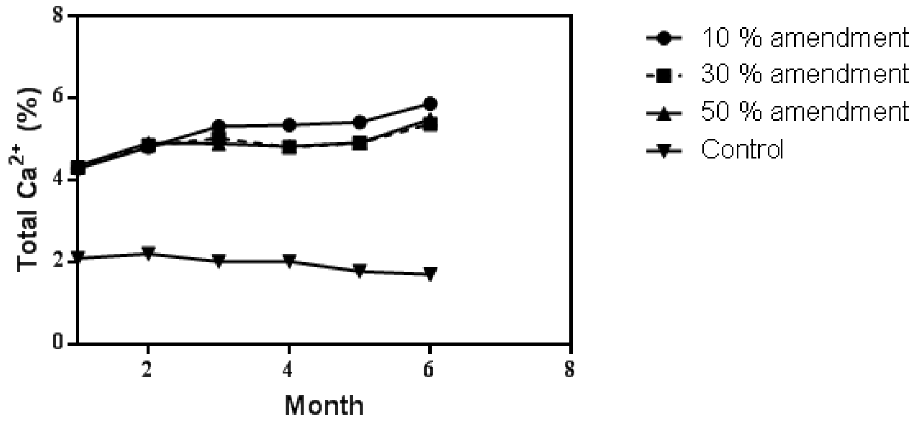

Figure 3. Effect of amendment concentration (chicken dropping) on total calcium

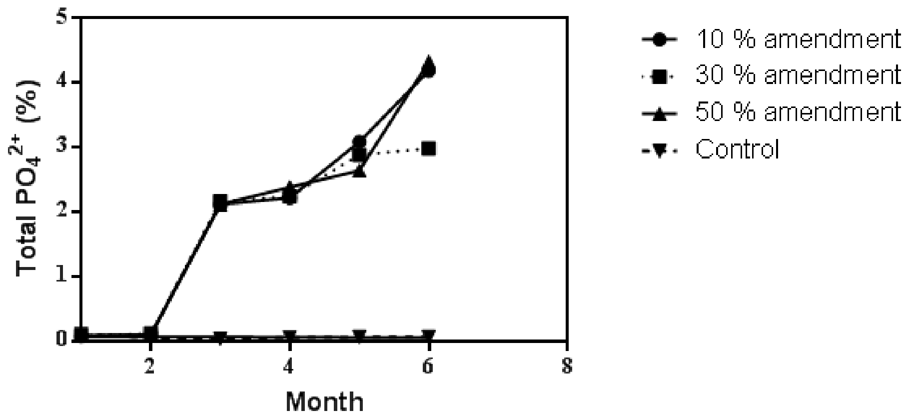

Figure 4. Effect of amendment concentration (chicken dropping) on total phosphorus

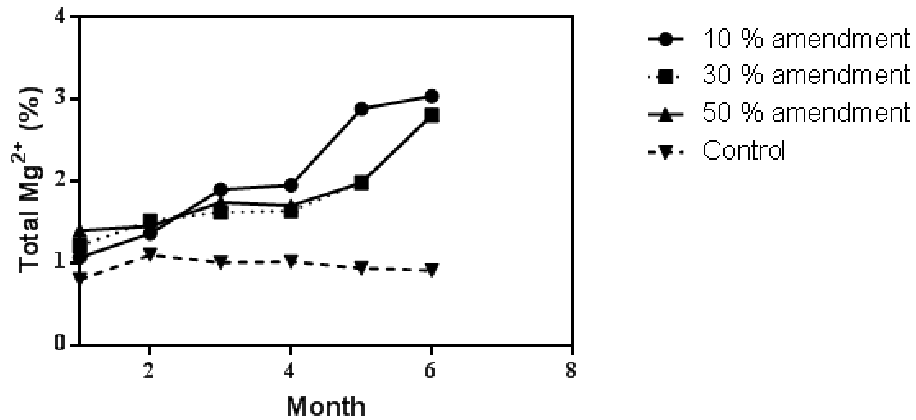

Figure 5. Effect of amendment concentration (chicken dropping) on total magnesium

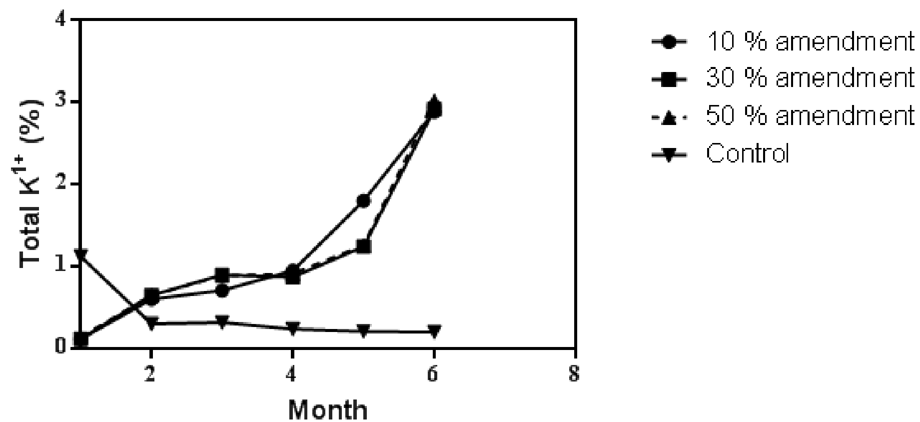

Figure 6. Effect of amendment concentration (chicken dropping) on total potassium

3.2. Microbiological Analysis of the Poultry Droppings and the Polluted Soil

Microbial population in the poutry droppings at the start of the analysis is represented in Table 3. The heterotrophic bacteria isolated included: *Pseudomonas, Serratia, Flavobacterium, Bacillus, Micrococcus and Klebsiella* spp. While that of fungi species included*Candida, Penicillium, Fusarium, Mucor, Cladosporium and Aspergillus*. The mean count of total heterotrophic microorganisms in poultry dropping and the soil before amendment, revealed bacterial count of 3.4 x 10^4 Cfu/g in the poultry dropping and a fungal count of 1.77 x 10^4 Cfu/g. The soil sample showed heterotrophic Bacterial count of 2.58 x 10^4 Cfu/g and Fungal counts of 1.74 x 10^4 Cfu/g. The isolation of diverse genera and species of bacteria and fungi from the poultry droppings in this work was in agreement with earlier report by [46,47]. The microbiological assessment of the poultry droppings in this research revealed that Poultry manure Contains rich organic matter on which many microorganisms thrive including *Arthrobacter. Pseudomonas, Serratia, Flavobacterium, Micrococcus, Bacillus* and *Klebsiella* were isolated and counted. There is a wide variation in the chemical composition of dried poultry waste especially in its crude protein content. This depends on the type of feed being fed, the systems of husbandry and storage conditions as reported by [48,49,50]. The pH of the polluted soil rose from 5.65 to 5.93 after the addition of the poultry droppings. This finding is similar to the report of [51], who noted that chicken manure raised the pH of soil from 6.3 to 7.4, which is optimal for the growth of oil utilizing bacteria such as *Bacillus* species, *Pseudomonas aeruginosa, Proteus, Enterobacter*and *Micrococcus* species. When chicken manure was added to soil contaminated with 10% volume to weight of crude oil to soil, it was reported that 75% of the oil was broken down in soil with the amendment additive after about two weeks; whereas additive-free soil was naturally remediated to just over 50% [51]. The microbial population of the poultry droppings before being used for amendment revealed the mean count of 3.4 x10^4Cfu/g for bacteria and 1.77 x10^4Cfu/g for fungi similar to report by [52]. The heterotrophic bacteria isolated include: *Pseudomonas, Serratia, Flavobacterium, Bacillus, Micrococcus* and *Klebsiella* spp. While that of fungi include *Candida, Penicillium, Fusarium, Mucor, Cladosporium* and *Aspergillus* similar to the findings of [41]. The oil utilizing bacteria isolated from the chicken droppings in this work have previously been implicated in crude oil biodegradation In varying degrees of crude oil degrading capabilities from different sources [27]. This capacity was revealed in this work to be further widespread among the bacteria present in the chicken droppings. Stimulated biodegradation of crude oil is at present being encouraged because it ensures rapid remediation of oil-polluted ecosystems [27].

In this study, seven genera of hydrocarbon utilizing bacteria were identified from the chicken droppings used and they include *Arthrobacter. Pseudomonas, Serratia, Fiavobacterium, Micrococcus, Bacillus* and *Klebsiella*.

Bacillus species was the most predominant bacterial species isolated. Its prevalence could be attributed to the fact that it forms spores, which helps microorganisms to withstand harsh conditions such as sun drying of chicken droppings employed in this work. Isolation of *Bacillus* species from chicken droppings could also be attributed to the ubiquitous nature of the microorganisms. [45] reported the isolation *of Bacillus, Pseudomonas, Flavobacterium,* among other Bacteria from the animal wastes.The hydrocarbon utilizing bacteria and fungi isolated in the course of this research include: *Pseudomonas, Serratia, Flavobacterium, Bacillus, Micrococcus* and *Klebsiella*spp. While that of fungi include *Candida, Penicillium, Fusarium, Mucor, Cladosporium*and*Aspergillus*. This is similar to the report of [41,52]. The mean hydrocarbon utilizing microbial counts of the crude oil polluted soil after 24 weeks of amendment as shown in Table 5 revealed the highest count of hydrocarbon utilizing bacteria at 50% amendment with a value of 1.57 x10^4 Cfu/g, while the least hydrocarbon utilizing bacteria count was obtained in the control sample without amendment with a value of 0.20 x10^4 Cfu/g. The highest count of hydrocarbon utilizing fungi was obtained at 50% amendment with a value of 0.69 x10^4 Cfu/g while the least hydrocarbon utilizing fungi count was obtained in the control sample without amendment with a value of 0.22 x10^4 Cfu/g. This finding was similar to the report of [52].

The rate of growth of the bacterial isolates in the crude oil medium revealed the highest growth in mineral salt medium, was shown by *Bacillus* and *Pseudomonas spp.* while *Serratia* and *Klebsiella spp*. showed least amounts of growth. [40] also reported similar findings.

Table 3. Enumeration of total heterotrophic microorganisms

	Fungi (10^4cfu/g)	Bacteria (10^4cfu/g)
AA	1.17±0.44	3.40±0.12
BB	1.74±0.47	2.58±0.35
CC	0.75±0.27	1.22±0.07
DD	2.10±0.29	3.7±0.18

[a]Values are mean of replicates analysis ± SD
[a]AA Chicken dropping
[a]BBunpolluted soil without amendment
[a]CC polluted soil without amendment
[a]DDPolluted Soil With Amendment (Chicken Droppings).

3.3. Hydrocarbon Degraders

The hydrocarbon utilizing bacteria and fungi isolated in the course of this research include: Pseudomonas, Serratia, Flavobacterium, Bacillus, Micrococcus and Klebsiellaspp. While that of fungi include Candida, Penicillium, Fusarium, Mucor, Cladosporium and Aspergillus. Table 5 shows the mean hydrocarbon utilizing microbial counts of the crude oil polluted soil after 24 weeks of amendment. The highest count of hydrocarbon utilizing bacteria was obtained at 50% amendment with a value of 1.57 x 10^4 Cfu/g, while the least hydrocarbon utilizing bacteria count was obtained in the control sample (sample without chicken droppings amendment) with a value of 0.20 x 10^4 Cfu/g. The highest count of hydrocarbon utilizing fungi was obtained at 50% amendment with a value of 0.69 x 10^4 Cfu/g, while the least hydrocarbon utilizing fungi count was obtained in the control sample without amendment with a value of 0.22 x 10^4 Cfu/g.

3.4. Hydrocarbon Utilizing Screening Test

The ability of the bacterial isolates to grow in the crude oil medium was evaluated. The highest growths in mineral salt medium were observed in *Bacillus* and *Pseudomonas*

spp. while *Serratia* and *Klebsiella* spp. showed least amounts of growth.

Table 4. TotalHydrocarbonoclastic microorganisms in the crude oil polluted soil

Months	Treatments (% chicken dropping)				Treatments (% chicken dropping)			
	10	30	50	Control	10	30	50	Control
	[a]*Bacteria* (10^4*cfu* g^{-1})				[a]*Fungi* (10^4*cfu* g^{-1})			
0	1.20±0.20Aa	1.40±0.20Aa	1.70±0.20Aa	0.80 ±0.20Aa	0.80±0.43XYz	0.50±0.31Wz	0.60±0.30Wz	0.50 ±0.33Wz
1	1.30±0.20Aa	1.70±0.03Ab	1.90±0.02Ab	0.40±0.25Vx	0.80±0.32Wz	0.60±0.34Vy	0.60±0.31Xx	0.20±0.31Xx
2	1.80±0.01Ba	2.00±0.11Ac	1.9±0.31Ab	0.2±0.46Xx	1.20±0.44XYy	0.90±0.31Xx	1.10±0.31Xx	0.30±0.31Xx
3	1.70±0.40Ca	2.30±0.24Aa	2.00±0.28Aa	0.20±0.47YZxy	1.40±0.54XYy	1.20±0.28Xx	1.30±0.31Xx	0.20±0.31Xx
4	1.60±0.20Da	2.00±0.29Bb	1.90±0.20Ba	0.30±0.26Wx	6.26±0.29Zy	6.21±0.20Zy	5.57±0.31Xx	5.57±0.31Xx
5	0.90±0.13Ca	0.70±0.13Aa	0.90±0.19Aa	0.10±0.43Zx	0.30±0.37Zy	0.20±0.46Zy	0.40±0.31Xx	0.10±0.31Xx
6	0.50±0.13Ca	0.50±0.13Ca	0.70±0.19Aa	0.10±0.43Zx	0.10±0.31Xx	0.10±0.31Xx	0.30±0.31Xx	0.10±0.31Xx
Mean	1.290±0.43Ca	1.510±0.03Ab	1.57±0.28Bb	0.20±0.21Aa	0.59±0.31Xx	0.61±0.31Xx	0.69±0.31Xx	0.22±0.31Xx

[a]Values are mean of triplicate analyses ± SD

Same capital letters are not statistically different among treatments by the Tukey test and same lower caps letter are not statistically different among months by the Tukey test ($p < 0.05$). ± Standard error (n = 3).

3.5. Residual TPH

The gas chromatographic technique carried out on the polluted soil amended with poultry droppings revealed that total petroleum hydrocarbon content (TPH) content was greatest in the control sample without amendment with a value of 5327.84 mg/kg, while the least TPH value was obtained in the sample with 50% amendment with a value of 2951.37 mg/kg, showing a significant reduction in the oil content which was expressed statistically. Table 4 shows the changes in total hydrocarbon content (TPH) of the crude oil-polluted soil which was amended with poultry dropping, which showed an initial TPH of 6609.83 ml/g at the start of the remediation and after 24 weeks of remediation with the amendment material, the 50% amendment sample had residual oil content of 2951.37ml/g and showed the highest crude oil removal of 3658.46 ml/g representing 55% oil removal rate, followed by the 30% amendment sample, which had residual oil content of 3144.06 mg/kg and showed a crude oil recovery of 3465.77mg/kg representing 52% oil removal rate. The 10% amendment sample had residual oil content of 3788.03ml/g and showed a crude oil recovery of 2821.80mg/kg representing 42% oil removal rate. The control sample without amendment had a residual oil content of 4192.35mg/kg and showed a crude oil recovery of 2417.48mg/kg representing 36% oil removal rate which happens to be the least. The crude oil recovery rate is obtained by substracting the initial TPH by the residual oil content. The Gas chromatographic technique carried out on the polluted soil amended with poultry droppings as shown in Table 4 revealed that the total petroleum hydrocarbon (TPH) content was greatest in the control sample without amendment with a value of 5327.84ml/g, while the least TPH value was obtained in the sample with 50% amendment with a value of 2951.37ml/g, showing a significant reduction in the oil content which was expressed statistically. [2,55], also reported similar findings.

Table 5. TPH Quantification By Gas Chromatography

Amended samples (%)	Initial TPH (mg/kg)	TPH (mg/kg) after 6 weeks	Residual TPH (mg/kg)	% TPH removal
10	6609.83±1.01	3788.03±1.22	2821.80±1.35	42±1.02
30	6609.83±1.01	3144.06 ±1.07	3465.77±1.71	52±0.08
50	6609.83±0.01	1652.46±1.04	4957.37±1.07	75±0.01
Control	6609.83±1.01	4192.35±1.11	2417.48±1.03	36±0.09

[a]Values are mean of replicates analysis ± SD.

3.6. Ecotoxicity Test

The ecotoxity revealed growth of the seed of *Viciafaba* a (beans seed) after 5 days incubation but only in the 50% amended samples. A 95 % germination index (IG %) was also reported in microcosm with 50 % chicken dropping only. A confirmatory testing with unpolluted soil sample collected from the same site prior to pollution with crude oil produced 100 % germination characteristics in both ex situ and in situ study. There was no growth in the other microcosms (control, 10 %, and 30 % amendment) cultivated. This is possibly due to increased microbial activity and contaminant utilization at a shorter time interval brought about by the 50 % amendment. The ecotoxicity test carried out on the crude oil polluted soil amended with poultry droppings revealed growth of the agricultural seed after 5 days of planting but only in the 50% amendment samples. There was no growth in the other samples cultivated. This is possibly due to the increased number of microorganisms and the great amount of the poultry droppings in the 50% amendment which facilitated higher reduction of oil in the amended soil when compared to other amendment concentrations. This therefore, corroborated work done by [52], which had growths on two out of the three seeds cultivated. This is possibly due to the differences in the rate of crude oil pollution, topography of soil, as well as the differences in the microorganisms carrying out the remediation.

The statistical analysis revealed a significant difference between the crude oil reduction rates of the four polluted soil samples amended with poultry droppings. There was a significant difference between the total petroleum hydrocarbon content in the 50% amended sample and the control sample without amendment. Using a probability level of 0.05, a P-value of 0.033 and a correlation coefficient of 0.967 were obtained showing a significant difference thus, a positive result.

4. Conclusion

The outcome of this study showed that 50% chicken droppings supported high crude oil remediation in the polluted soil. Poultry droppings is a potential source of nutrients for microbial activity and it habours microorganisms capable of utilizing hydrocarbons as source of carbon and energy thus, potentially useful in soil hydrocarbon pollution response action.

In future, more research should be carried out to study and monitor the microbial community before and after application through non-culture based methods like sequencing.

Competing Interests

The authors declare that no conflict of interests exists regarding the publication of this article.

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Author's Contributions

OUC designed the whole study and carried out the experiment and MUO supervised the intellectual content and revised it. The other authors assisted in the research methodology. All the authors approved the final manuscript.

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