BioTechnologia vol. 103 (4) • pp. 385–396 • 2022
Journal of Biotechnology, Computational Biology and Bionanotechnology RESEARCH PAPERS
https://doi.org/10.5114/bta.2022.120707

Microbial effects of manure from poultry droppings and pig dung in diesel-contaminated soil

AYOTUNDE E. COO [1], FAITH E. OVIASOGIE [1], BECKLEY IKHAJIAGBE [1, 2, 3] *

[1] Department of Microbiology, University of Benin, Nigeria
[2] Department of Plant Biology and Biotechnology, University of Benin, Nigeria
[3] Environmnetal Biotechnology and Sustainability Research Group, University of Benin, Nigeria

Abstract

In recent years, an increase in environmental pollution has been observed due to rapid industrialization, unsafe agricultural practices, and increased human activities on energy reservoirs. The wide use of petroleum hydrocarbon products as energy sources has contaminated the soil and the environment, thereby posing serious threats to all life forms, including humans. This study aimed to investigate the role of poultry droppings and pig dung in enhancing the bioremediation of diesel-contaminated soil. Soil samples were collected, processed by air drying and sieving, weighed in experimental bowls (5000 g), and contaminated with 250 ml of diesel. Then, poultry droppings and pig dung were added to the soil samples in different ratios, namely 1 : 1, 1 : 2, and 2 : 1. The diesel-contaminated soil sample without treatment served as the control. Thirty days after exposure to the experimental treatment regimes, the total bacterial count and the hydrocarbon-utilizing bacterial count of the diesel-contaminated soil ranged from 0.4×10^4 to 2.7×10^4 CFU/g and from 0.1×10^4 to 2.1×10^4 CFU/g, respectively. The total fungal count and the hydrocarbon-utilizing fungi count ranged from 0.6×10^3 to 2.1×10^3 SFU/g and from 0.2×10^3 to 1.7×10^3 SFU/g, respectively. *Bacillus subtilis*, *Micrococcus* sp., *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Aspergillus niger*, *Penicillium* sp., and *Mucor* sp were found to be active degraders. A significant reduction in the total aliphatic hydrocarbon (TAH) content of the diesel-contaminated soil was reported, with remediation approaching 95% in 30 days when the poultry droppings – pig dung mixture was added to the soil. The remediation of diesel-contaminated soils is important for the enhancement of the ecosystem. This study has shown that the use of farm waste such as the poultry droppings – pig dung mixture can enhance the remediation of diesel-contaminated soils.

Key words: bioremediation, farm waste, diesel-contaminated soil, petroleum hydrocarbons, hazard quotients, contamination factor

Introduction

The growing population of the world, along with an increasing acceptance of an industrialized lifestyle, has inexorably increased the anthropogenic impact on the biosphere, petroleum exploration and exploitation being the major contributor (Chu and Karr, 2017). Petroleum, like all other fossil fuels, is made up of complex chemical structures known as hydrocarbons. At high dosages, hydrocarbons found in crude oil and petroleum products are extremely toxic to many species, including humans. Due to the global economy's reliance on petroleum products, these toxins are released into populated areas and ecosystems all over the world (Ojumu et al., 2003). The long-term persistence of petroleum contamination depends on the amount and quality of the hydrocarbon mixture, as well as the characteristics of the impacted environment (Ojo, 2005). The petroleum sector has provided economic benefits to many countries but resulted in pollution that has brought environmental and socio-economic difficulties.

Oil-producing areas are frequently in danger because the soil is destroyed and rendered infertile as a result of

* Corresponding author: Department of Microbiology, University of Benin, Nigeria; e-mail: beckley.ikhajiagbe@uniben.edu

oil spills and other concerns related to oil exploration or transportation, preventing crop development. Erhenhi and Ikhajiagbe (2012) have observed that oil has a multitude of phytotoxic effects on plant growth and development, thus resulting in massive agricultural and economic losses in contaminated areas. Other researchers (Vwioko and Fashemi, 2005; Anoliefo et al., 2017; Ikhajiagbe et al., 2017) have reported that petroleum hydrocarbons are hazardous to both plants and mammals. The presence of oil in the soil is a major environmental concern as it creates unfavorable conditions for plant growth, most commonly due to insufficient soil aeration. This could be attributable to oil displacing air from pore spaces and an increase in oxygen demand caused by the activities of oil-decomposing bacteria, which affects normal diffusion processes (Gudin and Syratt, 1975).

Nowadays, physical and chemical technologies are mostly used for cleanup in petroleum hydrocarbon management procedures. The most common cleaning methods are neither straightforward nor environmentally friendly (Ashraf et al., 2014). For example, chemical sorbents and dispersants are considered fail-safe since they introduce more dangerous substances into the environment (Ashraf et al., 2014). Therefore, the bioremediation technology must be relied upon. In bioremediation, biological organisms, especially bacteria, are used in environmental cleanup, such as *B. subtilis*, *Micrococcus varians*, and *P. aeruginosa* (Anoliefo et al., 2006; Ikhajiagbe et al., 2012; Ikhajiagbe and Anoliefo, 2012a, 2012b). These bacteria, as well as many others that use hydrocarbons, help in soil recovery. Bioaugmentation with materials such as animal waste is used to improve the activities of hydrocarbon utilizers and soil properties that are necessary for the intrinsic rehabilitation of hydrocarbon-polluted soils (Ikhajiagbe and Anoliefo, 2012a, 2012b).

Microorganisms have been proven to be cost-effective and ecologically acceptable in the decomposition of petroleum hydrocarbons and their components in the environment (Agarry et al., 2013; Yakubu, 2007).The ability of the soil microbial community to breakdown petroleum pollutants is determined by its structure and diversity. Previous studies have reported that bioaugmentation of contaminated marine and terrestrial environments showed superior treatment efficiency (Tang et al., 2010; Kadali et al., 2012). However, some studies have also reported that bioaugmentation did not result in a significant increase in bioremediation and that the hydrocarbon-degrading bacteria did not show any degradation activity in some cases (Yu et al., 2005). Therefore, the use of microbes and soil amendment materials in bioremediation is becoming increasingly popular (Nwinyi and Akinmulewo, 2019). During the bioremediation process, soil amendments or additions such as sawdust, plant waste materials, plant debris, manure, and fertilizers are used to enhance the activity of microorganisms. Soil amendment improvesthe physical properties of the soil, such as water retention, permeability, water infiltration, drainage, aeration, and structure (Davis and Wilson, 2005). Moreover, most of the animal wastes such as dung contain a diverse spectrum of microorganisms and other expelled components that are crucial for soil amendment. This research aimed to explore how chicken and pig manure alters the microbial composition and bioremediation performance of diesel-contaminated soil.

Materials and methods

Study area

Soil, poultry droppings, and pig dung were obtained from the field, poultry, and pig farm, respectively, in the Nigeria Institute for Oil Palm Research (NIFOR)in Edo State. This research institute (latitude 06°33′N and longitude 05°37′N) is located in the rainforest zone of Nigeria. The rainfall ranges from 1500 to 2135 mm. The mean temperature ranges between 31 and 21°C.

Sample collection

Soil samples were collected at depths of 0–15 cm, and poultry droppings and pig dung were air-driedand sieved with a 2-mm sieve. Diesel was bought from the FAGCoop Filling Station in the University of Benin campus, Nigeria, and transported to NIFOR where the experiment was set up.

Experimental design

First, 1250 ml of diesel was added to 5000 g of soil and mixed thoroughly. The diesel-contaminated soil was subjected to the addition of the poultry droppings – pig dung mixture in three ratios: 1:1, 1:2, and 2:1 (Table 1). The treatments were conducted in five replicates. The soil samples were kept for 4 weeks in plastic containers in a well-ventilated screen house located at the

Table 1. Experimental design for the treatment of diesel-contaminated soil

Treatments	Ratio [pm : pw]	Soil amendments	Replicates
Treatment 1	1 : 1	5000 g soil + 1250 D +100 g pm + 100 g pw	5
Treatment 2	1 : 2	5000 g soil + 1250 D + 50 g pm + 100 g pw	5
Treatment 3	2 : 1	5000 g soil + 1250 D + 100 g pm + 50 g pw	5

pm – poultry manure, pw – pig waste/dung, and D – diesel

Department of Plant Biology and Biotechnology, University of Benin. The setup was constantly wetted with 250 ml of distilled water every other day. Each of the experimental setups had a control, in which diesel was added but not the poultry droppings–pig dung mixture.

Isolation of the total bacteria and fungi

The pour plate method adopted from Adams et al. (2014) and Cheesbrough (2006) was used to isolate bacteria and fungi from the samples (soil, poultry droppings, and pig dung). The samples were air-dried and sieved through a 2-mm mesh to remove debris. Then, they were diluted by transferring 1 g of the samples to 9 ml of sterile distilled water in sterile glass containers as blank. The glass containers were shaken for 5 min, and the samples were taken as 10^{-1} dilution factor; then, 10 ml was transferred from the 10^{-1} dilution into another 9 ml blank to obtain a dilution factor of 10^{-2}, and the same transfer process was repeated twice to obtain a dilution factor of 10^{-4}. For each of the samples, 1 ml of the 10^{-4}serially diluted portion was inoculated onto nutrient agar plates for bacterial count determination and potato dextrose agar plates for fungal count determination. The plates were inoculated at room temperature for 24 and 72 h, respectively, for bacterial and fungal growth. After incubation, colonies were counted, and the colony-forming units per gram (CFU/g) of the soil samples was determined. Nystatin at a final concentration of 0.05 mg/l (Morton Grove Pharmaceuticals, Inc., USA) was added to the nutritional agar to prevent fungal growth during bacterial isolation, whereas 0.05 mg/l of chloramphenicol (Rambaxy Nigeria Limited, Lagos) was added to the potato dextrose agar to prevent bacterial growth during fungal isolation. The Petri dishes were covered, inverted, and incubated for 48 h at 37°C for bacterial isolation and 96 h at 28°C for fungal isolation. Isolation and characterization of bacterial isolates were carried out using the methods of Cowan and Steel (1974) and Cheesebrough (2006).

Fungal isolates were characterized by determining their colonial morphology on plates, texture, and surface appearance. A microscopic examination of the isolates was carried out to determine the nature of the mycelium and the type of the fruiting body. The fungi atlas was used to identify the microorganisms (Adams et al., 2014; Cheesbrough, 2006).

Isolation and identification of hydrocarbon-utilizing bacteria and fungi

For the isolation and identification of hydrocarbon-utilizing bacterial (HUB) and fungal isolates in the diesel-contaminated soil samples, the experiments were set up using 2% (v/v) of diesel as a carbon source in the basal mineral salt medium. The composition of the basal mineral salt medium used in this study was as follows (g/l): $NaNO_3$ (2.0), NaCl (0.8), KCl (0.8), $CaCl_2 \cdot 2H_2O$ (0.1), KH_2PO_4(2.0), $Na_2HPO_4 \cdot 12H_2O$ (2.0), $MgSO_4$(0.2), and $FeSO_4 \cdot 7H_2O$ (0.001).

The initial pH was adjusted to 6.8. The mineral salt agar was sterilized at 121°C for 15 min at 15 psi and allowed to cool to about 47°C. Filter paper moistened with diesel was placed on the lid of each plate. The plates were inverted, and their edges were sealed with masking tape to increase the vapor pressure of the hydrocarbons, which provides carbon as the sole energy source. The viable counts of hydrocarbon-degrading bacteria and fungi in the samples were taken, and their colonies were purified by streaking them on fresh nutrient agar and inoculating them on PDA, respectively. Then, the colonies were stored in slants at 4°C prior to characterization. The developed colonies were counted and expressed as CFU/g for bacteria and spore-forming units per gram (SFU/g) for fungi.

Table 2.Physicochemical characteristics of the contaminated and control soil, poultry droppings, and pig dung

Sample	pH	TN [%]	P [mg/kg]	K [cmol/kg]
Soil	4.8 ± 0.6^{c}	0.12 ± 0.02^{b}	5.33 ± 0.16^{a}	0.11 ± 0.01^{b}
Soil + diesel	5.1 ± 1.1^{bc}	0.15 ± 0.03^{b}	5.17 ± 1.06^{a}	0.12 ± 0.01^{b}
Poultry droppings (dried)	7.1 ± 1.0^{a}	0.42 ± 0.01^{a}	1.14 ± 0.09^{b}	0.60 ± 0.11^{a}
Pig dung (dried)	5.9 ± 0.8^{b}	0.46 ± 0.06^{a}	0.92 ± 0.13^{c}	0.72 ± 0.09^{a}
P-value	0.042	< 0.001	< 0.001	0.003

pH – hydrogen ions, TN – total nitrogen %, P – available phosphorus (mg/kg), K – exchangeable potassium (cmol/kg); mean values in the same column with the same superscriptsare not significantly different from each other ($P < 0.05$)

Table 3. Physicochemical characteristics of the contaminated and treated soil, after 30 days of treatment

Samples	pH	TN [%]	P [mg/kg]	K [cmol/kg]
Soil	4.80 ± 0.10^{a}	0.12 ± 0.02^{a}	28.51 ± 1.33^{a}	0.08 ± 0.02^{a}
Pd soil + Pg diesel	5.77 ± 0.15^{b}	0.18 ± 0.02^{b}	27.31 ± 0.27^{a}	0.05 ± 0.02^{a}
Pd 100 g / Pg 100 g	6.47 ± 0.23^{c}	0.33 ± 0.02^{ab}	36.66 ± 0.58^{b}	0.55 ± 0.05^{b}
Pd 300 g / Pg 300 g	7.27 ± 0.15^{ef}	0.34 ± 0.02^{ab}	76.95 ± 0.84^{c}	0.32 ± 0.07^{af}
Pd 500 g / Pg 500 g	7.37 ± 0.06^{f}	0.42 ± 0.02^{ab}	90.13 ± 0.38^{d}	3.12 ± 0.04^{cf}
Pd 50 g / Pg 100 g	6.57 ± 0.06^{c}	0.24 ± 0.02^{ab}	87.48 ± 0.12^{e}	0.57 ± 0.07^{bf}
Pd 150 g / Pg 300 g	6.73 ± 0.12^{cde}	0.33 ± 0.01^{ab}	88.71 ± 1.14^{de}	1.11 ± 0.25^{d}
Pd 250 g / Pg 500 g	6.77 ± 0.06^{cde}	0.37 ± 0.02^{ab}	90.19 ± 1.73^{d}	2.04 ± 0.04^{e}
Pd 100 g / Pg 50 g	6.67 ± 0.58^{cd}	0.18 ± 0.02^{a}	77.00 ± 1.33^{c}	1.08 ± 0.50^{d}
Pd 300 g / Pg 150 g	6.87 ± 0.78^{cdef}	0.47 ± 0.02^{ab}	88.60 ± 1.01^{de}	0.87 ± 0.38^{bd}
Pd 500 g / Pg 250 g	7.20 ± 0.26^{def}	0.52 ± 0.02^{ab}	89.81 ± 0.02^{d}	2.27 ± 0.23^{e}
LSD (0.05)	0.55	0.54	1.63	0.3695
P-value	0.082	0.128	0.006	0.035

pH – hydrogen ions, TN – total nitrogen (%), P – available phosphorus (mg/kg), K – exchangeable potassium (cmol/kg); mean values in the same column with the same superscripts are not significantly different from each other ($P < 0.05$); LSD – least significant differences; ANOVA – analysis of variance

Table 4. The presence or absence of bacterial isolates in the soil, poultry droppings, and pig dung

Bacterial isolates	Soil	Poultry droppings	Pig dung
Bacillus subtilis	+	+	–
Brucella sp.	+	+	+
Klebsiella sp.	–	+	+
Micrococcus sp.	+	–	–
Proteus vulgaris	–	+	+
Pseudomonas aeruginosa	+	+	+
Salmonella sp.	–	+	+
Shigella sp.	+	+	–
Staphylococcus sp.	–	+	+

(+) – present, (–) – absent

Table 5. Fungal isolates of the soil, poultry droppings, and pig dung

Fungi isolates	Soil	Poultry droppings	Pig dung
Aspergillus niger	+	+	+
Geotrichium sp.	–	+	+
Mucor sp.	–	+	+
Penicillium sp.	+	+	+
Saccharomyces sp.	+	+	+

(+) – present, (–) – absent

Table 6. Bacterial and fungal counts of the contaminated soil, poultry droppings, and pig dung

Samples	TBC	HUB	TFC	HUF
	[$\times 10^4$ CFU/g]	[$\times 10^4$ CFU/g]	[$\times 10^4$ SFU/g]	[$\times 10^4$ SFU/g]
Soil (without diesel)	1.5 ab	0.7 bc	0.08 b	0.05 b
Soil (with diesel)	1.2 b	0.4 c	0.14 c	0.02 b
Pig dung	2.0 a	0.9 b	0.22 a	0.12 a
Poultry droppings	2.2 a	1.2 a	0.17 a	0.09 a
P-value	0.135	0.017	0.046	0.008

TBC – total bacterial count, HUB – hydrocarbon-utilizing bacteria, TFC – total fungal count, HUF – hydrocarbon-utilizing fungi; mean values in the same columns with the same superscripts are not significantly different from each other ($P < 0.05$)

Table 7. Bacterial and fungal counts of the contaminated and treated soil after 30 days of exposure to experimental treatments

Samples poultry droppings/ pig dung	TBC [$\times 10^4$CFU/g]	ΔTBC [%]	HUB [$\times 10^4$CFU/g]	ΔHUB [%]	TFC [$\times 10^3$SFU/g]	ΔTFC [%]	HUF [$\times 10^3$SFU/g]	ΔHUF [%]
Soil	0.7 ± 0.2	−53.33	0.2 ± 0.01	−71.43	0.8 ± 0.1	0	0.6 ± 0.2	20
Soil (with diesel)	0.4 ± 0.1	−73.33	0.1 ± 0.01	−85.71	0.6 ± 0.1	25	0.2 ± 0.1	−60
Pd 100 g/Pg 100 g	1.7 ± 0.2	13.33	1.0 ± 0.1	42.86	0.6 ± 0.2	25	0.4 ± 0.1	−20
Pd 300 g/Pg 300 g	2.1 ± 0.5	40	1.8 ± 0.2	157.14	1.5 ± 0.5	50	1.2 ± 0.2	140
Pd 500 g/Pg 500 g	2.5 ± 0.6	66.66	1.9 ± 0.3	171.14	1.1 ± 0.1	37.5	0.9 ± 0.2	80
Pd 50 g/Pg 100 g	1.2 ± 0.2	−20	1.1 ± 0.1	57.14	1.3 ± 0.2	62.5	1.0 ± 0.5	100
Pd 150 g/Pg 300 g	1.8 ± 0.2	20	1.4 ± 0.1	100	1.4 ± 0.2	75	1.3 ± 0.5	160
Pd 250 g/Pg 500 g	1.9 ± 0.4	26.16	1.5 ± 0.2	114.29	1.7 ± 0.4	112.5	1.3 ± 0.2	160
Pd 100 g/Pg 50 g	2.7 ± 0.5	80	2.1 ± 0.6	200	0.9 ± 0.2	12.5	0.6 ± 0.3	20
Pd 300 g/Pg 150 g	2.1 ± 0.8	40	1.3 ± 0.2	85.7	2.1 ± 0.5	162.5	1.7 ± 0.4	240
Pd 500 g/Pg 250 g	1.2 ± 0.4	−20	0.9 ± 0.1	28.57	1.2 ± 0.2	50	0.7 ± 0.2	40
LSD (0.05)	0.63	N/A	1.54	N/A	1.96	N/A	1.35	N/A
P-value	0.007		0.043		0.019		0.003	

Pd – poultry droppings, Pg – pig dung, Δ%–percentage change to background level, $\Delta TBC_{background}$ – 1.5, TBC – total bacterialcount, −Δ indicates reduction compared with the background, $\Delta HUB_{background}$ – 0.7, HUB – hydrocarbon-utilizing bacteria, +Δ indicates an increase compared with the background, $\Delta TFC_{background}$ – 0.4, TFC – total fungi count, $\Delta HUF_{background}$ – 0.5, HUF – hydrocarbon-utilizing fungi

Physicochemical analysis

The following physicochemical characteristics of the contaminated soil and the treated soil samples were evaluated: pH, total nitrogen, total available phosphorus, and exchangeable potassium. The pH was measured using a pH meter (Mettler Toledo Seven Compact™ pH meter S210). The exchangeable potassium content was determined following the procedure of Udo and Ogunwale (1986). The total nitrogen content was determined using the Kjeldahl technique (Bremner, 1965). The total available phosphorus content was determined using the Bray 1 method (Bray and Kurtz, 1945).

Data analysis

The experiments were performed in triplicate, data were recorded, and statistical analyses were carried out using SPSS, version 16, at the 95% confidence level. The results are presented as means and standard error of means. One-factor ANOVA was conducted upon the assumption of homogeneity of the experimental plot.

Calculation of hazard quotient and contamination factor (CF) of the TAH content in the remediated medium

The hazards quotient (HQ) expresses the probability of a contaminant being an ecological risk factor or a con-

Table 8. Bacterial isolates in the contaminated and treated soil after 30 daysof exposure to the experimental treatments

Sample	*Proteus vulgaris**	*Klebsiella* sp.*	*Staphylococcus* sp.	*Pseudomonas aeruginosa**	*Shigella* sp.	*Micrococcus* sp.*	*Bacillus subtilis**	*Brucella* sp.	*Salmonella* sp.
Soil	–	–	+	+	–	+	+	–	–
Soil (with diesel)	+	+	–	+	–	+	+	+	–
Pd 100 g/Pg 100 g	+	+	–	+	+	+	+	+	–
Pd 300 g/Pg 300 g	+	+	–	+	+	+	+	–	+
Pd 500 g/Pg 500 g	+	+	+	+	+	+	+	–	+
Pd 50 g/Pg 100 g	+	–	–	+	–	+	+	+	–
Pd 150 g/Pg 300 g	+	–	–	+	–	+	+	–	–
Pd 250 g/Pg 500 g	+	–	+	+	+	+	+	–	+
Pd 100 g/Pg 50 g	+	+	–	+	–	+	+	+	–
Pd 300 g/Pg 150 g	+	+	+	+	+	+	+	–	+
Pd 500 g/Pg 250 g	+	+	+	+	+	+	+	–	+
Percentage of occurrence %	90.9	63.6	45.5	100	54.54	100	100	36.4	45.5

(–) – absent, (+) – present, (*) – hydrocarbon utilizers, Pd – poultry droppings, Pg – pig dung

Table 9. Fungal isolates in the contaminated and treated soil after 30 days of exposure to the experimental treatments

Sample	*Aspergillus niger**	*Penicillium notatum**	*Mucor* sp.*	*Saccharomyces* sp.	*Geotrichium* sp.
Soil	+	+	–	–	–
Soil + diesel	+	+	+	–	–
Pd 100 g/Pg 100 g	+	+	+	–	–
Pd 300 g/Pg 300 g	+	+	+	–	+
Pd 500 g/Pg 500 g	+	+	+	+	+
Pd 50 g/Pg 100 g	+	+	+	–	–
Pd 150 g/Pg 300 g	+	+	–	–	–
Pd 250 g/Pg 500 g	+	–	+	+	–
Pd 100 g/Pg 50 g	+	+	+	–	–
Pd 300 g/Pg 150 g	+	+	+	–	+
Pd 500 g/Pg 250 g	+	+	+	+	+
Percentage of occurrence %	100	90.9	81.8	27.3	27.3

(–) – absent, (+) – present, (*) – hydrocarbon utilizers, Pd – poultry droppings, Pg – pig dung

taminant of potential ecological concern, whereas the CF expresses the ratio between the eventual concentrations of a pollutant and its preindustrial concentration (Ikhajiagbe and Anoliefo, 2012a):

$$CF = \frac{\text{Concentration of pollutant}}{\text{Background/preindustrial concentration}}$$

$$HQ = \frac{\text{Measured concentration}}{\text{Toxicity reference value or selected screening benchmark}}$$

When HQ > 1, the contaminant can cause harm. When HQ = 1, the contaminant alone is not likely to cause ecological risk. When HQ < 1, harmful effects are not likely.

Results

Physicochemical characteristics of the remediated oil-polluted substrate

Altogether, the pH of the soil, poultry droppings, and pig dung ranged from 4.8 to 7.4, with the poultry droppings showing the highest pH of 7.4 (Table 2). The total nitrogen content ranged from 0.12 to 0.46%, with the pig dung showing the highest value of 0.46% and the poultry droppings showing a value of 0.42%. Thirty days after the polluted soils were subjected to soil amendments, increases in the total nitrogen, total available phosphorus, and exchangeable potassium contents were observed (Table 3). The pH of the treated soils ranged from 4.80 to 7.27. The pH of the soil with the 1 : 1 treatment ratio was around 7. The 1 : 2 treatment ratio resulted in a slightly acidic pH of 6.57–6.77, whereas the 2 : 1 treatment ratio resulted in a more acidic pH. The total nitrogen content in the soil ranged from 0.12 to 0.52%, and the 2 : 1 treatment ratio resulted in the highest nitrogen content (0.52%).The total available phosphorus content ranged from 27.31 to 90.19 mg/kg, and the 1 : 2 treatment ratio showed the highest value of 90.19 mg/kg.

Bacterial and fungal composition of the polluted medium

A total of nine bacterial isolates were isolated from the soil, poultry droppings, and pig dung altogether (Table 4). *Pseudomonas aeruginosa* and *Brucella* sp. were isolated from the soil, poultry droppings, and pig dung. *Staphylococcus* sp., *Salmonella* sp., *P. vulgaris*, and *Klebsiella* sp. were isolated from poultry droppings and pig dung. Among the five fungal isolates, *Mucor* sp. and *Geotrichium* sp. were not observed in the soil, but present in poultry droppings and pig dung (Table 4).

The bacterial counts were higher than the fungal counts (Table 6). The total bacterial count (TBC) ranged from 1.2 to 2.2×10^4CFU/g. In the diesel-contaminated soil, the TBC decreased from 1.5×10^4 to 1.2×10^4CFU/g (Table 6). The pig-dung-based soil amendment showed

Table 10. Total aliphatic hydrocarbon content of amended and unamended diesel-contaminated soil 30 days after exposure to the experimental treatments

TAH	Control	Combination ratios								
		Pd 300 g/ Pg 300 g	Pd 250 g/ Pg 500 g	Pd 100 g/ Pg 100 g	Pd 500 g/ Pg 500 g	Pd 500 g/ Pg 250 g	Pd 150 g/ Pg 300 g	Pd 100 g/ Pg 50 g	Pd 300 g/ Pg150 g	Pd 50 g/ Pg 100 g
n-Octane	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
n-Nonane	0.533	0.496	0.418	0.098	0.206	0.151	0.189	0.082	0.155	0.152
n-Decane	1.290	0.855	0.623	0.179	0.346	1.285	0.701	0.235	0.001	0.297
n-Undecane	2.641	0.791	0.460	0.060	0.636	2.886	0.161	0.047	0.049	0.146
n-Dodecane	26.608	5.951	6.679	1.107	2.710	3.807	6.812	1.793	1.714	2.097
n-Tridecane	11.277	2.053	2.074	0.104	0.209	0.685	0.344	0.273	0.425	0.255
n-Tetradecane	45.868	14.892	2.715	2.320	2.633	2.625	1.849	2.691	2.732	3.045
n-Pentadecane	18.144	13.243	2.502	7.900	3.608	156.883	10.972	1.757	7.415	1.312
n-Hexadecane	88.102	8.539	9.038	7.648	0.472	30.990	5.778	1.697	3.867	6.997
n-Heptadecane	14.268	18.498	6.516	28.617	13.231	2.923	1.239	12.969	16.455	2.201
Pristane	240.745	7.628	8.053	7.709	2.794	81.275	13.700	1.896	5.112	45.670
n-Octadecane	34.133	12.772	15.743	1.310	9.958	1.033	5.313	8.840	3.175	1.247
Phytane	171.647	22.545	2.781	0.928	0.824	95.037	13.216	0.467	10.114	30.724
o-Nonadecane	53.782	5.535	3.854	0.204	0.031	1.025	0.309	0.288	0.704	0.413
n-Eicosane	25.360	2.126	5.063	0.339	0.109	33.889	0.074	0.569	0.377	3.085
n-Heneicosane	12.998	7.558	9.139	0.092	0.165	18.675	0.967	0.317	1.154	0.319
n-Docosane	4.626	5.754	1.674	0.068	0.059	11.746	0.070	0.277	0.058	0.499
n-Tricosane	3.130	7.262	2.204	0.035	0.180	6.028	0.127	0.096	0.097	0.032
n-Tetracosane	6.620	0.786	4.163	0.012	0.091	0.010	0.263	0.333	0.124	0.110
n-Pentacosane	0.303	0.151	0.094	0.002	0.003	0.000	0.003	0.002	0.007	0.000
n-Hexacosane	0.090	0.030	0.151	0.001	0.002	0.001	0.001	0.010	0.002	0.002
n-Heptacosane	0.304	1.002	1.385	0.002	0.004	0.009	0.006	0.003	0.006	0.003
n-Octacosane	0.118	0.359	0.490	0.275	0.254	0.671	0.066	0.657	0.253	0.188
n-Nonacosane	0.133	0.120	0.576	0.007	0.007	0.007	0.007	0.008	0.007	0.007
n-Triacontane	0.085	0.132	0.131	0.002	0.206	0.001	0.088	0.834	0.331	0.265
n-Hentriacontane	0.204	0.123	0.105	0.058	0.127	0.257	0.030	0.799	0.332	0.271
n-Doctriacontane	0.399	0.377	0.646	9.955	11.879	0.628	0.496	1.465	10.801	0.746
n-Tritriacontane	ND	ND	ND	0.013	0.045	0.024	0.013	0.037	0.047	0.045
Total	763.408	139.578	87.277	69.044	50.789	452.549	62.793	38.445	65.515	100.131

ND – below detection limit, TAH – total aliphatic hydrocarbon, Pd – poultry droppings, Pg – pig dung

Table 11. Evaluation of the progress of remediation after exposure to the experimental treatments

Sample	TAH [mg/kg]	Remediation indices		
		BRE [%]	ΔCF	ΔHQ
At day 0				
soil (no diesel)	11.2	–	–	0.01
soil + diesel	4028 ± 62.1	0	182.86	1.02
At day 30				
soil (without diesel)	< 0.001	NA	NA	NA
soil (with diesel)	763.03 ± 2.87	62.74	68.13	0.38
poultry 100 g/Pig 100 g	69.03 ± 0.02	96.63	6.16	0.03
poultry 300 g/Pig 300 g	139.55 ± 0.97	93.19	12.46	0.07
poultry 500 g/Pig 500 g	50.10 ± 1.39	97.55	4.47	0.03
poultry 50 g/Pig 100 g	100.05 ± 2.01	95.11	8.93	0.05
poultry 150 g/Pig 300 g	61.44 ± 1.40	97	5.49	0.03
poultry 250 g/Pig 500 g	86.50 ± 1.30	95.78	7.72	0.04
poultry 100 g/Pig 50 g	38.40 ± 0.03	98.13	3.43	0.02
poultry 300 g/Pig 150 g	64.00 ± 1.51	96.88	5.71	0.03
poultry 500 g/Pig 250 g	449.51 ± 3.98	78.05	40.13	0.22
LSD(0.05)	14.86	–	–	–
P-value	< 0.001	–	–	–

BRE – bioremediation efficiency (%); ΔCF – contamination factor; the higher the CF, the more likely it is still contaminated compared with the background value; ΔHQ – hazard quotient; when HQ > 1, harmful effects are likely due to the contaminant in question; when HQ = 1, the contaminant alone is not likely to cause ecological risk; when HQ < 1, harmful effects are not likely; TAH – total aliphatic hydrocarbon (limits for TAH 2000 mg/kg); NA – not applicable

a TBC of 2.0×10^4CFU/g and an HUB content of $0.9 \times \times 10^4$CFU/g. Pig manure showed a total hydrocarbon-utilizing fungi (HUF) content of 0.12×10^4CFU/g. At 30 days after the treatment, significant increases in the TBC were observed in all diesel-contaminated soil samples ($P < 0.05$) (Table 7). When the soil was amended with the pig dung–poultry droppings mixture (pg/pd), the TBC increased from 0.4×10^4CFU/g to $1.2–2.7 \times \times 10^4$CFU/g. In soils treated with 500 g of poultry dropping and 500 g of pig dung (abbreviated as Pd 500 g/Pg 500 g), the TBC and the BUB content increased by 66.66% and 171.14%, respectively. The highest HUF content was observed in the Pd 300 g/Pg 150 g treatment, which showed a 240% increase compared with the unamended diesel-contaminated soil. Moreover, increases in the HUF content were affected by soil amendments as well.

Five of the nine bacterial isolates showed the capacity to utilize hydrocarbons present in the soil, namely *P. aeruginosa*, *P. vulgaris*, *Klebsiella* sp., *Micrococcus* sp., and *B. subtilis*. *P. aeruginosa, Micrococcus* sp., and *B. subtilis* were present in all three samples – the soil, pig dung, and poultry droppings, implying that they were present in all of soil, pig dung and poultry dropping samples. *Klebsiella* sp. and *P. vulgaris* showed a percentage of occurrence higher than 60%. In addition, *A. niger* was detected in all experimental treatments. *Saccharomyces* sp. and *Geotrichium* sp. showed the lowest occurrence rates among the fungal species (Table 9).

The list of aliphatic hydrocarbons present in both control and amended soils is provided in Table 10. Some of the hydrocarbons present were *n*-pentacosane, *n*-tetracosane, *n*-hexacosane, *n*-octacosane, and *n*-nonacosane. The control showed a TAH content of 763.40 mg/kg. However, the 2 : 1 treatment ratio of the poultry droppings and pig dung showed a TAH content as low as 38.44 mg/kg, which implied significant remediation. Bioremediation indices were computed to evaluate the

bioremediation efficiency of the pig-dung-amended and poultry-dropping-amended diesel-polluted soil (Table 11). Chromatographic representations of the aliphatic hydrocarbons present in the diesel-contaminated soil and treated with poultry droppings and pig dung are shown in Figures 1–11 in Supplementary materials.

At day 0, the TAH content of the clean soil prior to contamination was 11.2 mg/kg. This was considered the background concentration in this study. Upon contamination with diesel (also at day 0), the TAH content increased to 4028 mg/kg, thus indicating a CF of 182.86 and an HQ of 1.02. According to Ikhajiagbe and Anoliefo (2012a), a contaminant with an HQ > 1 is said to be of environmental concern. The TAH was 763.03 mg/kg after 30 days in the contaminated soil without any modification, implying a CF of 68.13 and an HQ of 0.38. With an HQ value of less than one, the contaminant (diesel) was below the limit, i.e., it was not harmful to the environment. The remediation rate was 62.74%. However, when the contaminated soils were treated with the pig dung – poultry droppings mixture, the remediation rate increased to 98.13%. The CF and the HQ were drastically decreased to 3.43 and 0.02, respectively, at that point. These results emphasize the importance of soil amendments as hydrocarbon remediation facilitators.

Discussion

Petroleum hydrocarbons enter the environment through a variety of channels, such as storage tank leaks, faulty transfer lines, and product transportation from one location to another, andtheir environmental impact can be disastrous; thus, deliberate attempts to eradicate them from the ecosystem are essential (Chikere and Ekwuabu, 2014). Reports have suggested that remediation of hydrocarbon-polluted soils can be enhanced by introducing nutrients and bacteria into them (Ikhajiagbe and Anoliefo, 2012a, 2012b; Agarry et al., 2013). The use of nutritional supplements such as animal manure (Agarry et al., 2013) and other materials like inorganic fertilizers (Margesin et al., 2001) in thebiostimulatation of degraders in contaminated environments has been extensively investigated as well.

The significant reduction in hydrocarbons in the polluted soil observedin this study wasmost likely due to the increased microbial activity in the soil due to the poultry droppings and pig dung amendment. The following microorganisms capable of metabolizing petroleum hydrocarbons were isolated from pig dung and chicken manure: *Shigella* sp., *B. subtilis*, *Micrococcus* sp., *P. vulgaris*, *Klebsiella* sp., *Salmonella* sp., *Brucella* sp., and *P. aeruginosa*, all of which had previously been documented (Williams et al., 1999; Ojumu et al., 2004; Chikere and Azubike, 2014). *Pseudomonas* are well-known biosurfactant-producing bacteria that can use hydrocarbons as a carbon and energy source (Rahman et al., 2003). They have been extensively investigated in the production of glycolipid biosurfactants. Biosurfactants help bacteria consume more hydrocarbons by increasing the surface area of the oil (Nikolopoulou and Kalogerakis, 2009).

Das and Chandran (2010) reported that establishing adequate nutrient concentrations is one of the most crucial factors in guaranteeing optimal microorganism growth rates for petroleum hydrocarbon biodegradation. In the present study, the amended soils showed considerable increases in soil nutrients. This was most likely due to the improvement in remediation capacity. Azubuike et al. (2016) emphasized the relevance of nutrients, especially nitrogen and phosphorus, in the biodegradation of hydrocarbons. Barrett (2008) reported that chicken droppings have considerable levels of nitrogen, phosphate, and potassium. Okolo et al. (2005) used poultry manure as an organic fertilizer in contaminated soil and found that biodegradation was improved by more than 90%in the presence of poultry manure.

Das and Chandran (2010) reported that the pH of a remediating compound should be between 6 and 9, which is similar to that of the present study. Although remediation of the TAH content of the soil was observed, the remediation rate and efficiency were enhanced when the soil was amended with either pig dung or poultry droppings.

Conclusions

The relevance of using organic manure in bioremediation to assist ecosystem processes in polluted areas cannot be overstated. Bioremediation efficiency is critical when the number and types of microbes in polluted soils or other media are increased. Organic amendments such as poultry droppings and pig dung provide a lot of benefits, especially enhancing soil parameters and microbial efficiency. Supplementing contaminated soils with the

poultry droppings – pig dung mixture considerably accelerated the remediation of the diesel-contaminated soil.

Conflicts of interests

The authors declare no conflicts of interest.

References

Adams G.O., Tawari-Fufeyin P., Ehinomen I. (2014) *Bioremediation of spent oil contaminated soils using poultry litter*. Res. J. Eng. Appl. Sci. 3(2): 124–130.

Agarry S.E. (2018) *Evaluation of the effects of organic fertilizers, activated carbon on bioremediation of soil contaminated with weathered crude oil*. J. Appl. Sci. Environ. Manag. 22(4): 587–595.

Agarry S.E., Owabor C.N., Yusuf R.O. (2010) *Bioremediation of soil artificially contaminated with petroleum hydrocarbons oil mixtures: evaluation of the use of animal manure, chemical fertilizers*. Bioremed. J. 14(4): 189–195.

Anoliefo G.O., Ikhajiagbe B., Okonokhua B.O., Diafe F.V. (2006) *Ecotaxonomic distribution of plant species around auto mechanic workshops in Asaba, Benin City: identification of oil tolerant species*. Afr. J. Biotech. 5(19): 1757–1762.

Ashraf M.A., Maah M.J., Yusoff I. (2014) *Soil contamination, risk assessment and remediation*. [in:] *Environmental risk assessment of soil contamination*. IntechOpen. https://doi.org/10.5772/57287

Azubuike C.C., Chikere C.B., Okpokwasili G.C. (2016) *Bioremediation techniques classification based on site of application: principles, advantages, limitations, prospects*. World J. Microbiol. Biotech. 32: 180.

Barrett J. (2008) *FCS Soil Science L3*. FET college series. Pearson Education South Africa: 7.

Bray R.H., Kurtz L.T. (1945) *Determination of total organic, available forms of phosphorus in soils*. Soil Sci. 59: 225–229.

Cheesbrough M. (2006) *Laboratory manual. District laboratory practice in tropical countries*. Cambridge University Press, United Kingdom: 145–157.

Chikere C.B., Ekwuabu C.B. (2014) *Culture-dependent characterization of hydrocarbon utilizing bacteria in selected crude oil–impacted site in Bodo, Ogoniland, Nigeria*. Afr. J. Environ. Sci. Technol. 8(6): 401–406.

Chikere C.B., Azubike C.C. (2014) *Characterization of hydrocarbon-utilizing fungi from hydrocarbon-polluted sediments, water*. Niger. J. Biotech. 27: 49–54.

Chu E.W., Karr J.R. (2017) *Environmental impact: concept, consequences, measurement*. Reference module in life sciences. 2017: B978-0-12-809633-8.02380-3. https://doi.org/10.1016/B978-0-12-809633-8.02380-3. Epub 2016 Oct 31. PMCID: PMC7157458

Collin P.H. (2001) *Dictionary of ecology, the environment*. 4th ed. Peter Collin Publishing, London.

Cowan S.T., Steele K.J. (1974) *Manual for identification of medical bacteria*. 2nd ed. Cambridge University Press, Cambridge, UK: 216.

Das N., Chandran P. (2010) *Microbial degradation of petroleum hydrocarbon contaminants: an overview*. Biotech. Res. Int. Article ID 941810. https://doi.org/10.4061/2011/941810

Davis J.G., Wilson C.R. (2005) *Choosing a soil amendment*. Colorado State Univ. Cooper. Ext. Horticult. 7: 235.

Erhenhi H.A., Ikhajiagbe B. (2012) *Growth responses of fluted pumpkin (Telfairia occidentalis, Hook. F.S) in crude oil polluted soil*. South Asian J. Biol. Sci. 2(1): 01–11.

Anoliefo G.O, Ikhajiagbe B., Omoregie E.S., Aimiebenomor R.A., Okoye P.C. (2017) *Plant growth response, nitrate reductase activities of roots of Chromolaena odorata in a model spent lubricating oil-polluted soil*. Studia Univ. Babeş – Bolyai: Biologia. LXII(2): 97–106. https://doi.org/10.24193/subbbiol.2017.2.09

Gudin C., Syratt W.J. (1975) *Biological aspects of land rehabilitation following hydrocarbon contamination*. Environ. Pollut. 8: 107–112.

IITA (1979) *Selected methods for soil, plant analysis*. IITA Manual Series No 1. Ibadan, Nigeria: IITA.

Ikhajiagbe B., Anoliefo G.O. (2012a) *Phytoassessment of a 5-month old waste engine oil polluted soil after augmentation with Pleurotus tuberregium*. Curr. Res. J. Biol. Sci. 4(1): 10–16.

Ikhajiagbe B., Anoliefo G.O. (2012b) *Substrate bioaugmentation of waste engine oil polluted soil*. Res. J. Environ. Earth Sci. 4(1): 60–67.

Ikhajiagbe B., Anoliefo G.O., Erhenhi H.A., Ogweigor U. Hope (2012) *Post impact assessment of a petroleum effluent dump site located in Midwestern Nigeria*. Arch. Appl. Sci. Res. 4(5): 1923–1931.

Ikhajiagbe B., Omoregie G.O., Ekhator P.O., Mshelbulla P.B. (2017) *Growth, yield responses of okra (Abelmoschus esculentus) in a petroleum hydrocarbon-polluted soil after substrate amendment*. Ethiop. J. Environ. Stud. Manag. 10(7): 866–876.

Kadali K.K., Simons K.L., Sheppard P.J. et al. (2012) *Mineralisation of weathered crude oil by a hydrocarbonoclastic consortia in marine mesocosms*. Water Air Soil Poll. 223: 4283–4295.

Margesin R., Schinner F. (2001) *Bioremediation (natural attenuation, biostimulation) of diesel oil contaminated soil in an Alpine Glacier Skiing area*. Appl. Environ. Microbiol. 67: 3127–3133.

Nikolopoulou M., Kalogerakis N. (2009) *Biostimulation strategies for fresh, chronically polluted marine environments with petroleum hydrocarbons*. J. Chem. Tech. Biotech. 84(6): 802–807.

Nwinyi O.C., Akinmulewo B.A. (2019) *Remediation of soil polluted with spent oil using cow dung*. IOP Conf. Ser.: Earth Environ. Sci. 331 (2019): 012058. https://doi.org/10.1088/1755-1315/331/1/012058

Ojo O.A. (2005) *Petroleum – hydrocarbon utilization by nature bacterial population from a wastewater canal Southwest Nigeria*. Afr. J. Biotech. 5: 333–337.

Ojumu T.V., Bello O.O., Sonibare J.A, Solomon B.O. (2004) *Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria*. Afr. J. Biotech. 4: 31–35.

Ojumu T.V., Solomon B.O., Betiku E., Layokun S.K., Amigun B. (2003) *Cellulase production by Aspergillus flavus Linn Isolate NSPR 101 fermented in sawdust, bagasse, corncob*. Afr. J. Biotech. 2: 150–152.

Okolo J.C., Amadi E.N., Odu C.T.I. (2005) *Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil*. Appl. Ecol. Environ. Res. 3(1): 47–53.

Rahman K.S.M., Rahman T.J., Kourkoutas Y., Petsas I., Marchant R., Banat I.M. (2003) *Enhanced bioremediation of n-alkane in petroleum sludge using bacterial consortium amended with rhamnolipid, micronutrients*. Bioresour. Technol. 90(2): 159–168.

Tang J., Wang R., Niu X. et al. (2010) *Enhancement of soil petroleum remediation by using a combination of ryegrass (Lolium perenne), different microorganisms*. Soil Till. Res. 110: 87–93.

Udo E.J., Ogunwale J.A. (1986) *Laboratory manual for the analysis of soil, plant, water samples*. London. University Press: 174.

Yakubu M.J. (2007) *Biodegradation of lagoma crude oil using pig dung*. Afr. J. Biotech. 6(24): 2821–2825.

Yu K., Wong A., Yau K. et al. (2010) *Natural attenuation, biostimulation, bioaugmentation on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments*. Mar. Pollut. Bull. 51: 1071–1077.