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Pigeon droppings as compositing amendment for enhancement of the biostimulation of soil artificially polluted with diesel oil

Williams Janet Olufunmilayo and Ambrose Favour C

Abstract
Aim: To assess the potential of pigeon droppings as composting amendment for enhancement of the biostimulation of soil artificially polluted with diesel oil.
Study design: This study focused on soil artificially polluted with diesel oil and use of pigeon droppings as amendment to bio remediate the polluted soil. Statistical analysis of data and interpretation was carried out.
Place and Duration of Study: Soil was obtained from an agricultural farmland in the Rivers State University, Nkpolu-Oroworukwo, diesel oil (DO) from Royal Dynasty Filling Station at Ada George Road and Pigeon droppings (PD) were collected into a sterile polythene bag from Mile III Market, Port Harcourt, Nigeria in September, 2022.
Methodology: Surface soil samples used were collected using hand auger between the depths of 0-15cm at different points and mixed thoroughly to form a composite sample. The pigeon droppings were sun dried for 14 days, ground into fine powder and stored in a sterile polythene bag. Treatment of soil samples with pollutant was done whereby 1.5 kg of soil was weighed separately and placed in seven sterile containers and diesel oil was added to the soil in the containers at various concentrations (0, 20, 40 and 80ml) and then mixed thoroughly. (UPS, Soil + 20% DO, Soil + 40% DO, Soil + 80% DO, Soil + 20% DO + PD, Soil + 40% DO + PD, Soil + 80% DO + PD). The polluted and unpolluted soil samples were kept under natural environmental conditions for 14 days before the application of pigeon droppings. During this period, the soil samples were watered within the interval of two days. After 14 days of pollution treatment, pigeon droppings were weighed and added to the soils in the labeled plastic containers. Three (3) grams each of the pigeon droppings was added to the soil with label (Soil + 20% DO + PD, Soil + 40% DO + PD, Soil + 80% DO + PD) while those with the label, Soil + 20% DO, Soil + 40% DO, Soil + 80% DO were used as control for the amendments. Each set was mixed thoroughly with wooden spatulas to obtain homogenized mixtures and enhance aeration. Standard microbiological techniques were used to determine the microbial population. The physicochemical parameters were analyzed using standard methods
Results: The microbiological analysis of the soil revealed that the mean count of the total heterotrophic bacteria in soil polluted with diesel oil and amended with pigeon droppings ranged between 1.6 x 10^8 and 1.8 x 10^8cfu/g. These counts were higher than the counts of the unamended soil which ranged from 4.0 x 10^7 to 1.1 x 10^8 cfu/g. The mean counts of the diesel utilizing bacteria ranged between 1.2 x 10^5 and 1.6 x 10^5 cfu/g. The mean microbial count of fungi in soil polluted with diesel oil and amended with pigeon dropping ranged between 5.4 x 10^4 and 1.2 x 10^5 cfu/g. The diesel utilizing fungi count ranged from 3.3 x10^4 to 7.8 x 10^4 cfu/g. The analysis of variance (ANOVA) showed significant differences among the treatments. The diesel utilizing bacteria identified in soil amended with pigeon droppings were species of *Bacillus, Pseudomonas, Alcaligenes, Micrococcus* and *Serratia.* The diesel utilizing fungi species identified were *Aspergillus, Penicillium, Geotrichum, Fusarium* and *Rhodotorula.* The physiochemical properties of the soil sample before and after contamination with diesel oil were analyzed. Some physiochemical properties such as nitrogen, phosphate, pH and temperature enhanced the growth of these organisms in the polluted environment. The use of pigeon droppings as amendment raised the nitrogen and phosphorus contents of the soil. Soil polluted with diesel oil and amended with pigeon dropping had higher amount of organic carbon than the unamended soil.
Conclusion: The result of this research work showed that pigeon droppings act as good stimulants for enhancing diesel oil biodegradation in the soil, therefore, could be used in remediating diesel oil polluted soil.

Keywords: Pigeon droppings, diesel utilizing bacteria, diesel oil, nitrogen

Introduction

Petroleum hydrocarbons are the lifeline of industrial sectors in any part of the world acting as raw materials in petrochemical industries and refineries for making different products like fuel, synthetic polymers and petrochemicals [1]. According to [2], petroleum is a liquid fossil fuel produced from decaying organic matter such as microalgae and zooplankton. It serves as one of the main sources of energy around the globe including chemical industry that use refined products to manufacture large numbers of consumer products. Oil drilling or oil transportation can cause accidents that lead to contamination of the environment. Spillages resulting from hydrocarbon and its products have posed greater challenges in Nigeria especially in the Niger Delta region known for her endowment of crude resources.

Petroleum is a complex mixture of hydrocarbons and many other organic components. Structurally, the hydrocarbons are classified as alkanes, cycloalkanes, and aromatics. It is composed of about 75% saturated hydrocarbon (primarily paraffin) and 25% aromatic hydrocarbon (including naphthalenes and alkyl benzenes) [3]. These compounds are related to the family of neurotoxic and carcinogenic organic contaminants [4]. Among petroleum products, diesel oil is a complex mixture of alkanes and aromatic compounds that are frequently reported as soil contaminants leaking from storage tanks and pipelines or released in accidental spills posing a serious risk to different life forms on earth [5]. Industrial and municipal discharges as well as natural seeps also cause hydrocarbon pollution of the environment. Oil in soil creates unsatisfactory conditions for plant growth probably due to insufficient aeration in the soil [6]. This condition causes the displacement of air from pore-spaces by oil and an increase in the demand for oxygen brought about by the activities of oil-decomposing microorganisms. Oil occupies macrospores and a coated aggregate reduce oil film thickness around macro aggregates and retards the movement of water in and out of micro aggregates. It has been observed from studies on the effect of oil-based wastes on soil hydraulic properties that drainage is decreased and water retention increases [7].

The use of substances which act as nutrients to stimulate the growth of microbial population especially those with potentials to remediate polluted environments otherwise known as bio stimulation is well documented [8]. A study carried out by [9] using inorganic fertilizer (NPK) in enhancing microbial degradation of petroleum hydrocarbons in soil showed that normal paraffin and isoprenoid (Phitane and Pristane) decreased in the range of 40- 60% in all the treatment categories in less than 10 weeks, thus, improved the CNP ratio of the test setups ultimately and promoting microbial degradation. Due to the challenges posed with inorganic substances, organic substances are being exploited for remediation processes. Based on recent studies, organic nutrients are potentially useful as stimulating nutrients for bioremediation. Contaminated soil containing more than 38,000mg/kg TPH was remediated using sewage sludge and wood chips compost by [10]. Cow dung as organic nutrient source has shown good promises in the bioremediation of crude oil impacted mangrove swamps in the Niger delta part of Nigeria [11]. Pigeon excrement constitutes a highly favorable substrate for microbial growth [12]. Thus, the present study is aimed at investigating the potential of pigeon droppings as a bio stimulant for remediation of diesel oil polluted soil.

Materials and Methods

Description of Study Area

The area of study was the agricultural farm land of Faculty of Agriculture, Rivers State University, Nkpolu-Oroworukwo, and Rivers State with the GPS coordinate of 4.7971ºN, 6.9801ºE. The farm land where the soil samples were collected was a mixed crop farm land with crops: Vegetables, pepper, water leaf, tomato, etc. The farm is close to the Geology department of Faculty of Science, Rivers State University. The soil samples were taken at different points within the same location to give a composite sample. The GPS coordinate of the agricultural farmland was 4.4818ºN, 6.5839ºE.

Collection of Samples

The soil samples used were collected using hand auger at the topsoil between the depths of 0-15cm at different points and mixed thoroughly to form a composite sample. The sample was kept in a sterile perforated polythene bag and transported to the Microbiology Laboratory, Rivers State University for analysis. The diesel oil sample used was obtained from Royal Dynasty Filling Station located at Ada George Road, Port Harcourt, Rivers State, Nigeria. Pigeon droppings were collected into a sterile polythene bag from Mile III Market, Port Harcourt, and Rivers State. The pigeon droppings were sun dried for 14 days, grounded into fine powder and stored in a sterile polythene bag.

Treatment of Soil Samples with Pollutant

The treatment of soil samples with pollutant was carried out as described by [13]. In this method, 1.5 kg of soil sample was weighed separately and placed in seven clean and sterile plastic containers labeled (UPS, Soil + 20% DO, Soil + 40% DO, Soil + 80% DO, Soil + 20% DO + PD, Soil + 40% DO + PD, Soil + 80% DO + PD). Diesel oil was added to the soil in the containers at various concentrations (0, 20, 40 and 80ml) and then mixed thoroughly. The polluted and unpolluted soil samples were kept under natural environmental conditions for 14 days before the application of pigeon droppings. During this period, the soil samples were watered within the interval of two days.

Amendment treatments

After 14 days of pollution treatment, pigeon droppings were weighed and added to the soils in the labeled plastic containers. Three (3) grams each of the pigeon droppings was added to the soil with label (Soil + 20% DO + PD, Soil + 40% DO + PD, Soil + 80% DO + PD) while those with the label (Soil + 20% DO, Soil + 40% DO, Soil + 80% DO) were used as control for the amendments. The experimental design is illustrated in Table 1. The soil labeled UPS was used as the control for both pollution and amendment treatments. The ones labeled Soil + 20% DO, Soil + 40% DO and Soil + 80% DO were used as control for the soil samples with amendments. Each set was mixed thoroughly with wooden spatulas to obtain homogenized mixtures and enhance aeration.

Table 1: Experimental Design

Control	Treatments
UPS	-
1.5kg soil + 20mL Diesel Oil (DO)	1.5kg soil + 20mL DO + 3g Pigeon Dropping
1.5kg soil + 40mL Diesel Oil (DO)	1.5kg soil + 40mL DO + 3g Pigeon Dropping
1.5kg soil + 80mL Diesel Oil (DO)	1.5kg soil + 80mL DO + 3g Pigeon Dropping

Key: UPS = Unpolluted Soil, DO = Diesel Oil, PD = Pigeon Droppings

Determination of physicochemical Parameters

The physicochemical parameters: pH, temperature, total nitrogen, soil organic matter, total organic carbon, phosphate and total hydrocarbon content of polluted and unpolluted soil samples were analyzed using standard methods [14].

Microbiological Analysis

Enumeration of Total Heterotrophic Bacteria: The total heterotrophic bacterial counts of soil samples were determined using the standard plate count method on nutrient agar. A serial ten-fold dilution was prepared using 1g of soil and 0.1ml of 10^5 dilution was inoculated and plated in duplicates. Plates were properly labeled and incubated at 37 ºC for 24 hours [15].

Enumeration of Fungi: The fungal counts were enumerated using the standard plate count method on Sabouraud dextrose agar (SDA) plates fortified with tetracycline antibiotics (to inhibit the growth of bacteria). In this method, aliquot (0.1ml) of 10^{-2} dilution were transferred on prepared Sabouraud Dextrose agar (SDA) plates. The plates were later spread evenly using sterile bent glass rod. Inoculation was done in duplicates and after inoculation, plates were incubated at 28 ºC for 3-5 days. Enumeration of fungal counts was carried out after incubation while distinct fungal colonies were morphologically characterized and sub-cultured on fresh SDA plates for further identification [16].

Enumeration of Diesel Utilizing Bacteria (DUB): The diesel utilizing bacteria (DUB) in the sample was determined using the standard plate count on Bushnell Hass medium. The vapour phase transfer method was adopted with slight modification as reported by (2). In this method, instead of adding 1ml of crude oil on sterile filter papers placed on the lid of Petri dishes, 1ml of diesel (sterilized by heating) was placed on the Whatman filter paper. Ketoconazole antifungal agent was added into the Bushnell-Hass agar after sterilization to inhibit the growth of fungi. Aliquot from 10^{-2} dilution was transferred into the centre of the agar and evenly spread using a sterile bent glass rod. The inoculation was done in duplicates and incubated at 37 ºC for 7days.

Enumeration of Diesel Utilizing Fungi (DUF): DUF was enumerated on Bushnell Hass agar fortified with tetracycline antibiotics (to inhibit the growth of bacteria). Aliquot (0.1 ml) was withdrawn from 10^{-2} dilution using a sterile pipette and plated out in duplicates on Bushnell-Hass agar plates. The plates were evenly spread with bent glass rod and were inverted in the lid of the petri dish containing sterile Whatman filter paper saturated with 1ml diesel oil (vapor phase transfer technique) in an inverted position and incubated at 28 ºC for 7 days.

Characterization and Identification of Bacterial Isolates: The bacterial isolates were characterized by observing them microscopically and subjecting them to series of biochemical tests: Catalase, citrate, oxidase, coagulase, Methyl Red, Motility, indole, starch hydrolysis, Voges Proskauer and sugar fermentation tests. Further confirmation was done by comparing their characteristics with those of known taxa as outlined in Bergey's Manual of Systematic Bacteriology [17]. Fungal isolates were identified using their morphological features such as colony color, shape, texture and size of colony followed by microscopic examination (conidial shape, arrangement of hyphae and type of spore) of their wet mounts prepared with lactophenol cotton blue and reference made to fungal identification manual [18].

Statistical Analysis: The results were expressed as mean ± standard deviation of two replicates. Analyses of variance (ANOVA) were carried out using SPSS (version 25.0) to check for significant difference and mean values were separated using the Ducan multiple range test (DMRT) at $p \leq 0.05$. The percentage occurrence was calculated on Microsoft Excel (Version 2021).

Results

Microbial Population of Samples: The mean microbial counts from the remediation study (Table 2) showed that the total heterotrophic bacterial, fungal, diesel utilizing bacterial and diesel utilizing fungal load were 4.0×10^7 to 1.8×10^8, 4.9×10^4 to 1.19×10^5, 1.2×10^5 to 1.6×10^5 and $3.3\times10^4$to 7.8×10^4 cfu/g, respectively. Results showed that the total heterotrophic bacterial load of 80% diesel oil contaminated soil amended with pigeon droppings was higher than the unpolluted and soils contaminated with diesel oil with and without pigeon droppings amendment. The diesel utilizing bacterial load of the 40% diesel oil contaminated soil with pigeon droppings amendment was higher than the diesel utilizing bacterial load of unpolluted and soils contaminated with diesel oil with and without pigeon droppings amendment. Similarly, the fungal load and the diesel utilizing fungal load of the 80% diesel oil contaminated soil with pigeon dropping amendment were higher than the fungal load and diesel utilizing fungal load of the unpolluted and diesel oil contaminated soil with and without pigeon droppings.

Table 2: Mean Microbial Count of the Remediation

Treatment	THB (10^8)	FC (10^4)	DUB (10^5)	DUF(10^4)
UPS	1.4 ± 0.04^{bc}	7.9 ± 0.05^{ab}	1.2 ± 0.05^{a}	5.8 ± 0.08^{a}
Soil + 20% DO	0.40 ± 0.01^{a}	4.9 ± 0.04^{a}	1.2 ± 0.07^{a}	3.3 ± 0.04^{a}
Soil + 20% DO +PD	1.8 ± 0.05^{c}	5.4 ± 0.04^{a}	1.4 ± 0.07^{a}	4.4 ± 0.06^{a}
Soil + 40% DO	0.53 ± 0.03^{a}	4.9 ± 0.03^{a}	1.3 ± 0.06^{a}	4.8 ± 0.07^{a}
Soil + 40% DO +PD	1.6 ± 0.02^{bc}	6.1 ± 0.05^{a}	1.6 ± 0.04^{a}	5.7 ± 0.08^{a}
Soil + 80% DO	1.1 ± 0.06^{b}	7.0 ± 0.04^{ab}	1.2 ± 0.06^{a}	6.4 ± 0.07^{a}
Soil + 80% DO +PD	1.8 ± 0.06^{c}	11.9 ± 0.7^{b}	1.5 ± 0.06^{a}	7.8 ± 0.08^{a}

*Means with similar alphabet down the group show no significant difference ($p>0.05$)

Results of the phenotypic characterization of the bacterial isolates showed that the bacterial isolates shared similar

characteristics and were identified as *Staphylococcus*, *Klebsiella*, *Serratia*, *Escherichia coli*, *Pseudomonas*, *Shigella*, *Bacillus*, *Micrococcus*, *Alcaligenes*, *Cronobacter*, *Tatumella*, *Cedecea*, *Proteus* and *Providencia* species. All of these isolates were amongst the total heterotrophic bacteria while the diesel utilizing bacterial isolates were *Serratia*, *Pseudomonas*, *Bacillus*, *Micrococcus*, *Alcaligenes* and *Proteus* species. The percentage occurrence of the bacterial isolates (fig. 1) were *Staphylococcus* (4.9%), *Klebsiella* (9.3%), *Serratia* (6.4%), *Escherichia coli* (4.4%), *Pseudomonas* (14.2%), *Shigella* (2.9), *Bacillus* (15.7%), *Micrococcus* (7.4%), *Alcaligenes* (13.7%), *Cronobacter* (6.4%), *Tatumella* (4.9), *Cedecea* (2.5), *Proteus* (5.9%) and *Providencia* (1.4%). Species. Moreso, *Bacillus* and *Shigella* species were the most occurring bacterial isolates followed by *Pseudomonas* sp. while *Providencia* sp. was the least occurring bacterial isolate.

The percentage occurrence of the diesel utilizing bacterial isolates (Fig. 2) were *Serratia* (6.1%), *Pseudomonas* (24.5%), *Bacillus* (32.7%), *Micrococcus* (10.2%), *Alcaligenes* (20.4%) and *Proteus* (6.1%) species. *Bacillus* sp. was the most dominant and occurring DUB isolates followed by *Pseudomonas* sp. while *Serratia* and *Proteus* species which shared similar percentages were the least frequently isolated DUB.

Results for the distribution of bacterial isolates across the samples are presented in Tables 3 and 4. Results showed that the bacterial isolates varied or fluctuated in their distribution across the samples. Some of the bacterial isolates which were isolated from the control were not isolated in the other treatments especially treatments lacking pigeon dropping amendment. Results of the Distribution of bacterial isolates across the soil samples in weeks 1 and 2 showed that only *Klebsiella*, *Bacillus* and *Pseudomonas* species were isolated from all the samples for the first and second weeks despite the diesel oil treatment while *Staphylococcus* sp. having been isolated from all the samples in week 1 was only isolated in the control of week 2 but not in the treated soil samples. There was a variation in the bacterial distribution. Results of the Distribution of bacterial isolates across the soil samples in weeks 3 and 4 are presented in Table 4. Results showed that *Bacillus* and *Pseudomonas* species were isolated from all the samples in the different weeks and were not inhibited by the presence of diesel oil. *Staphylococcus* sp. was only isolated in the unpolluted soil for week 3 but was not isolated in other treatments as well as in unpolluted soil of week 4.

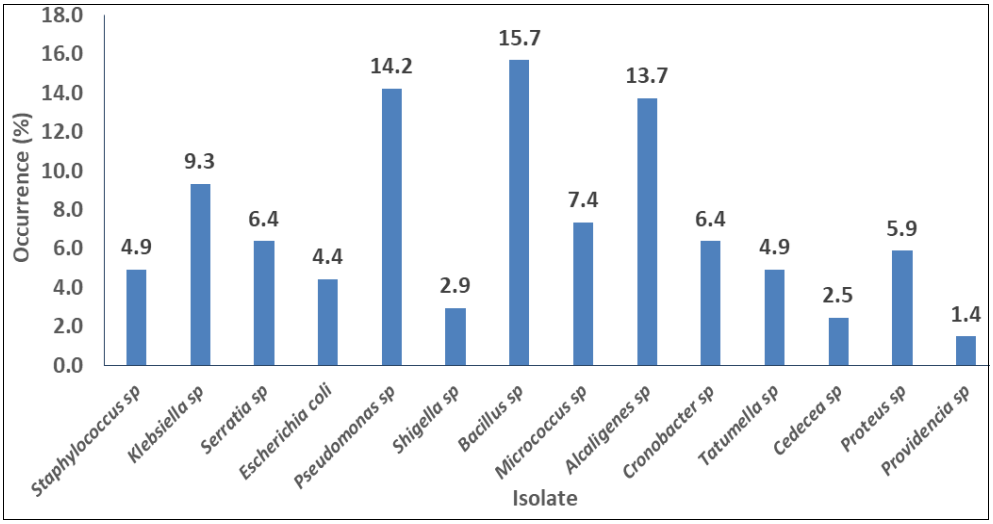

Fig 1: Percentage occurrence of total heterotrophic bacterial isolates

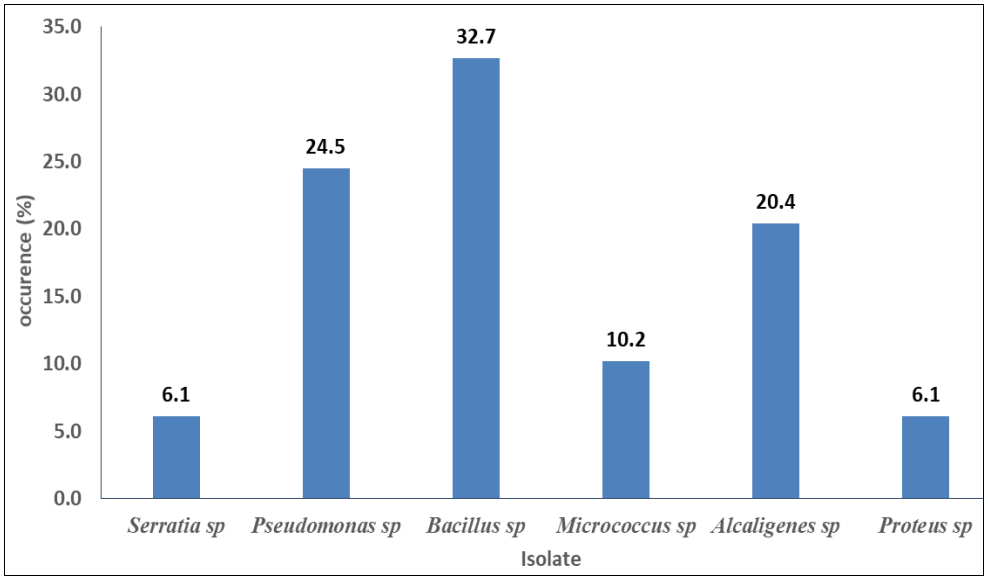

Fig 2: Percentage occurrence of diesel utilizing bacterial isolates

Table 3: Distribution of bacteria across the treatments in weeks 1 and 2

Isolates	Week 1							Week 2						
	UPS	Soil + 20% DO	Soil + 20% DO + PD	Soil + 40% DO	Soil + 40% DO + PD	Soil + 80% DO	Soil + 80% DO +PD	UPS	Soil + 20% DO	Soil + 20% DO + PD	Soil + 40% DO	Soil + 40% DO + PD	Soil + 80% DO	Soil + 80% DO + PD
Staphylococcus sp	+	+	+	+	+	+	+	+	-	-	-	-	-	-
Klebsiella sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Serratia sp	+	+	+	-	-	-	-	+	+	+	-	-	-	-
Escherichia coli	-	-	+	-	+	-	+	-	-	+	-	+	-	+
Pseudomonas sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Shigella sp	-	-	+	-	+	-	+	-	-	+	-	+	-	-
Bacillus sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Micrococcus sp	+	-	-	-	-	-	+	+	+	+	+	-	-	-
Alcaligenes sp	+	+	+	+	+	+	+	-	-	+	-	+	-	+
Cronobacter sp	+	+	+	+	+	+	+	+	-	+	+	-	+	+
Tatumella sp	-	-	-	-	+	+	+	-	-	-	+	+	-	-
Cedecea sp	-	-	-	-	-	-	-	-	-	-	-	-	+	+
Proteus sp	+	-	-	-	-	-	-	+	+	+	-	-	+	+

Key: + = isolated; - = not isolated; DO = Diesel oil; PD = Pigeon Droppings

Table 4: Distribution of Bacteria across the treatments in weeks 3 and 4

Isolates	Week 3							Week 4						
	UPS	Soil + 20% DO	Soil + 20% DO + PD	Soil + 40% DO	Soil + 40% DO + PD	Soil + 80% DO	Soil + 80% DO + PD	UPS	Soil + 20% DO	Soil + 20% DO + PD	Soil + 40% DO	Soil + 40% DO + PD	Soil + 80% DO	Soil + 80% DO + PD
Staphylococcus sp	+	-	-	-	-	-	-	-	-	-	-	-	-	+
Klebsiella sp	+	-	+	-	+	-	+	-	-	+	-	-	-	-
Serratia sp	+	-	+	+	+	+	+	-	-	-	-	-	-	+
Escherichia coli	-	-	+	-	+	-	+	-	-	-	-	-	-	-
Pseudomonas sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Shigella sp	-	-	+	-	-	-	-	-	-	-	-	-	-	-
Bacillus sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Micrococcus sp	+	+	+	+	+	+	+	+	+	-	-	-	-	-
Alcaligenes sp	-	-	-	+	-	+	+	-	-	-	-	-	-	-
Cronobacter sp	-	-	-	-	-	-	-	+	-	-	-	-	-	-
Tatumella sp	-	-	-	-	-	-	-	-	+	+	+	+	+	-
Cedecea sp	-	+	-	-	+	-	+	-	-	-	-	-	-	-
Proteus sp	+	-	+	-	+	-	+	-	-	+	-	+	-	-
Providencia sp	-	-	-	-	-	-	-	-	-	-	+	+	-	+

Key: + = Isolated; - = Not Isolated; DO = Diesel Oil; PD = Pigeon Droppings

Results of the fungal isolates showed that they shared similar characteristics with those presented in fungal atlas and were therefore identified as *Rhizopus, Aspergillus, Rhizopus, Rhodotorula, Gliocladium, Cunninghamella, Candida, Paecilomyces, Blastomyces, Penicillium, Scopulariopsis, Geotrichum, Fusarium, Coccidioides* and *Mucor* species. The percentage occurrence of fungal isolates (fig. 3) were *Scopulariopsis* sp (4.7%), *Candida* sp (12.0%), *Mucor* sp (4.0%), *Aspergillus* sp (19.3%), *Gliocladium* sp (4.7%), *Fusarium* sp (12.0%), *Paecilomyces* sp (4.0%), *Blastomyces* sp (0.7%), *Coccidioides* sp (4.0%), *Penicillium* sp (13.3%), *Rhizopus* sp (7.3%)), *Rhodotorula* sp (7.3%), *Cunninghamella* sp (0.7%) and *Geotrichum* sp (6.0%). The percentage occurrence of diesel utilizing fungal isolates (fig. 4) was as follows: *Fusarium* sp (10%), *Penicillium* sp (20%), *Rhodotorula* sp (15%), *Aspergillus* sp (27.5%), *Geotrichum* sp (12.5%) and *Canida* sp (15%).

Results of the fungal distribution across the samples in the weeks showed high variation in the fungal types across the samples in the respective weeks (Tables 5 and 6). Similar to the changes observed in the bacterial isolates. *Aspergillus* sp was the most dominant isolate and was very prevalent in weeks 1, 2, 3 and 4, while *Candida* sp was prevalent in weeks 3 and 4 despite being the second most prevalent isolate in weeks 1 and 2 but *Penicillium* sp was very predominant in weeks 3 and 4. Results also showed that the amended soil had more bacterial and fungal types than the unamended diesel contaminated soils.

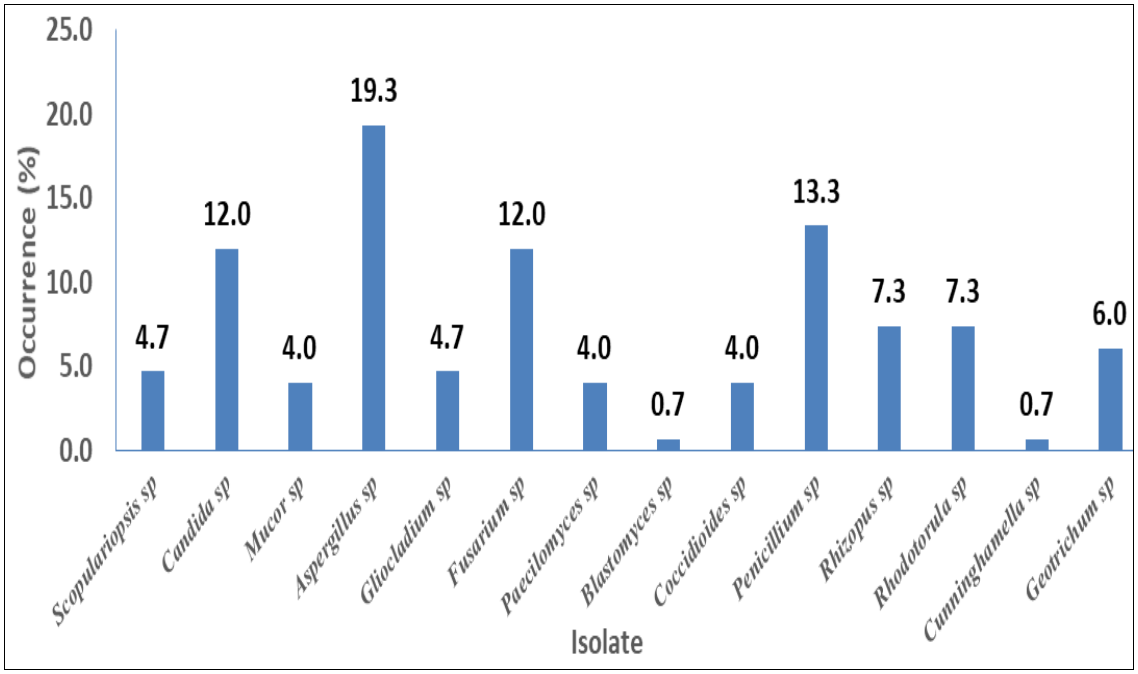

Fig 3: Percentage occurrence of fungal Isolates

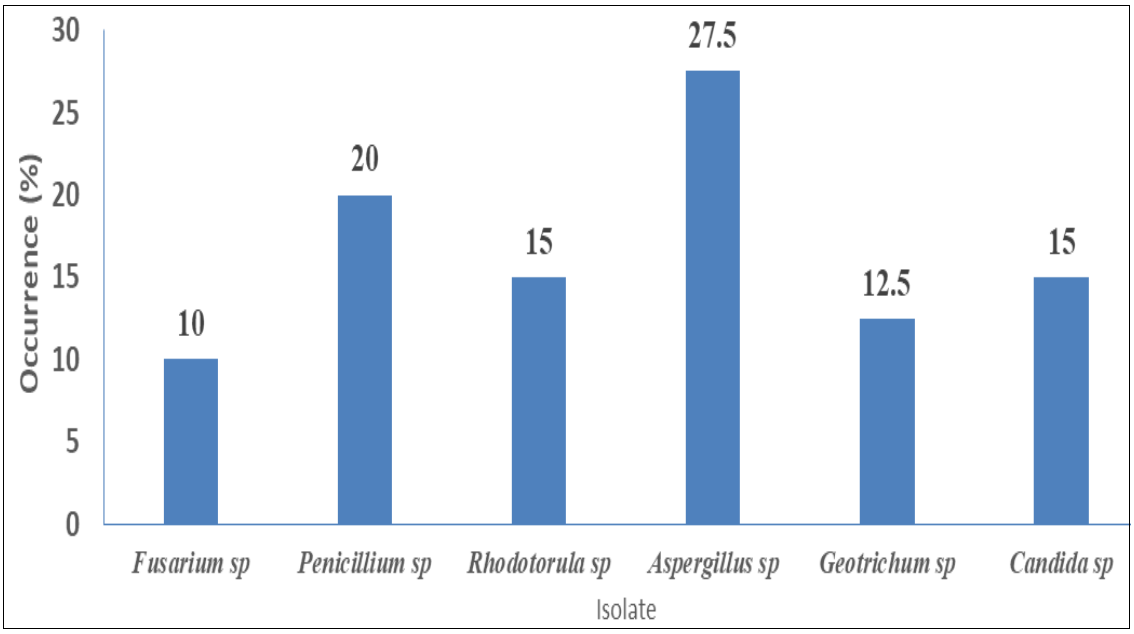

Fig 4: Percentage occurrence of diesel utilizing fungal isolates

Table 5: Distribution of fungal isolates across the treatments in weeks 1 and 2

Isolates	Week 1							Week 2						
	UPS	Soil + 20% DO	Soil + 20% DO + PD	Soil + 40% DO	Soil + 40% DO + PD	Soil + 80% DO	Soil + 80% DO +PD	UPS	Soil + 20% DO	Soil + 20% DO + PD	Soil + 40% DO	Soil + 40% DO + PD	Soil + 80% DO	Soil + 80% DO +PD
Scopulariopsis sp	+	+	-	+	-	-	-	-	-	+	-	-	-	-
Candida sp	+	+	+	-	-	-	+	+	-	+	-	-	-	+
Mucor sp	-	+	+	+	-	-	-	+	-	-	-	+	-	-
Aspergillus sp	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gliocladium sp	-	-	-	-	-	+	+	-	-	-	+	+	-	+
Fusarium sp	+	-	+	+	-	+	+	+	+	+	-	-	+	-
Paecilomyces sp	-	-	-	-	-	-	+	-	-	+	-	-	+	+
Blastomyce ssp	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Coccidioides sp	-	-	+	-	+	-	+	-	-	+	-	+	-	+
Penicillium sp	+	+	+	+	+	+	+	+	-	+	-	-	-	+
Rhizopus sp	+	-	-	-	-	-	-	+	-	+	-	+	-	+
Rhodotorula sp	-	-	-	-	-	-	-	-	-	+	-	+	-	+
Cunninghamella sp	-	-	-	-	-	-	-	-	-	+	-	-	-	-
Geotrichum sp	-	-	-	+	+	+	-	-	-	+	-	-	-	-

Physicochemical Properties

Results of the physicochemical properties of the soil sample before contamination with diesel oil (Baseline study) is presented in Table 7. Results showed that the values for nitrogen, phosphate, pH, temperature, total organic carbon, soil organic matter, total hydrocarbon content and sulphate were 110 mg/kg, 0.093 mg/kg, 6.2, 26.6 °C, 0.26%, 0.45%, 180 mg/kg and 20.5 mg/kg, respectively.

The physicochemical properties of the soil on Day 1 (Table 8) showed that the range values for nitrogen was 28.38 to 110.42mg/kg, phosphate 0.093 to 0.575 mg/kg, pH 6.2 to 7.5, temperature 26.6 to 28.3, total organic carbon 0.25 to 0.4%, soil organic matter 0.45 to 0.69% and total hydrocarbon content 180 to342mg/kg. Result showed that the nitrogen content of the soil was higher in the unpolluted soil and lower in the soil contaminated with 20% diesel oil with pigeon dropping amendment. The phosphate content of the soil contaminated with 40% diesel oil and amended with pigeon dropping was higher than the phosphate content of the unpolluted soil and soils contaminated with diesel oil and those with pigeon droppings and those without pigeon dropping amendment. The pH and temperature of the amended soil with 80% diesel oil was higher than the pH value for other treatments. Similarly, the total organic carbon, soil organic matter and total hydrocarbon content were highest in the soil contaminated with 80% diesel oil.

The physicochemical properties of the soil on Day 28 (Table 9) showed that the values for the soil properties were nitrogen, 32.383 to 110.42 mg/kg, phosphate 0.093 to 0.532 mg/kg, pH 6.2 to 7.5, temperature 26.6 to 28 ºC, total organic carbon 0.105 to 0.26%, soil organic matter 0.181 to 0.45% and total hydrocarbon content 4 to 186mg/kg. Result showed that the nitrogen content in the unpolluted soil was higher than the nitrogen content of diesel oil contaminated soil with and without pigeon droppings. The phosphate, pH and temperature of the soil after contamination were lowest in the unpolluted soil. The phosphate and pH of the soil were higher in the soil contaminated with 40% diesel oil with pigeon dropping amendment. The total organic carbon of the soil was higher in the unpolluted soil and lowest in the soil contaminated with 20% diesel oil without amendment. Similarly, the soil organic matter and total hydrocarbon content was higher in the unpolluted soil and soil contaminated with 80% diesel oil with pigeon dropping amendment, respectively.

Table 6: Physicochemical properties of the soil before contamination (Baseline study)

Set up Code	Nitrogen (mg/kg)	Phosphate (mg/kg)	pH	Temperature	Total Organic Carbon (TOC) (%)	Soil Organic Matter (SOM) (%)	Total Hydrocarbon Content (THC), (mg/kg)	Sulphate (mg/kg)
Unpolluted Soil	110.42	0.093	6.2	26.6	0.26	0.45	180	20.5

Table 7: Physicochemical properties of the soil in Day 1

S/N	Set up Code	Nitrogen (mg/kg)	Phosphate (mg/kg)	pH	Temperature	Total Organic Carbon (TOC) (%)	Soil Organic Matter (SOM), (%)	Total Hydrocarbon Content (THC) (mg/kg)
1	UPS	110.42	0.093	6.2	26.6	0.26	0.45	180
2	Soil + 20%DO	39.72	0.537	7.0	27	0.25	0.431	300
3	Soil + 20%DO +PD	28.38	0.546	7.2	27	0.3	0.517	300
4	Soil + 40%DO	38.39	0.541	6.9	27.5	0.28	0.483	322
5	Soil + 40%DO + PD	39.72	0.575	7.2	28	0.34	0.586	322
6	Soil + 80%DO	45.72	0.55	7.1	28	0.4	0.69	342
7	Soil + 80%DO + PD	48.29	0.58	7.5	28.3	0.37	0.638	330

Table 8: Physicochemical properties of the soil after monitoring (Day 28)

S/N	Set up Code	Nitrogen (mg/kg)	Phosphate (mg/kg)	pH	Temperature	Total Organic Carbon (TOC) (%)	Soil Organic Matter (SOM), (%)	Total Hydrocarbon Content (THC) (mg/kg)
1	UPS	110.42	0.093	6.2	26.6	0.26	0.45	180
2	Soil + 20%DO	38.386	0.526	7.2	28	0.105	0.181	94
3	Soil + 20%DO +PD	39.053	0.517	7.4	28	0.199	0.343	40
4	Soil + 40%DO	36.385	0.532	7	28	0.14	0.241	106
5	Soil + 40%DO + PD	47.057	0.528	7.5	28	0.192	0.329	34
6	Soil + 80%DO	38.386	0.523	7.2	28	0.152	0.262	186
7	Soil + 80%DO + PD	32.383	0.519	7.8	28	0.187	0.322	124

Discussion

Generally, the total heterotrophic bacteria and fungi in the diesel oil contaminated soil with pigeon droppings had higher bacterial and fungal load compared to soil contaminated with diesel oil without pigeon dropping amendment. The reason for this increase in microbial count of the amended soil might be as a result of the addition of pigeon droppings which could contain detectable quantities of nitrogen and phosphorus which are essential nutrients for microbial growth. More so, the high diesel utilizing bacteria and fungi observed in diesel contaminated soil amended with pigeon droppings could also be ascribed to the effect of the pigeon droppings in enhancing growth of the hydrocarbon degraders. Pigeon dropping is known to be an organic stimulant that helps in the stimulation of hydrocarbon in the soil. The addition of this stimulant as nitrogen source helps to increase the microbial population of a contaminated site and also enhances the degradation of the diesel oil in the soil environment [19]. In the study of bioremediation on microbial population in oil sediments, [20] observed that continual ventilation and fertilizer addition had considerable effect on the growth of hydrocarbon degrading bacteria in soil which is consistent with the present study. The low bacterial and fungal counts recorded in diesel contaminated soil without amendment could be ascribed to the detrimental effect being exhibited by the

diesel oil. Diesel oil are environmental pollutants and their availability on soil could hinder abiotic factors such as aeration, water activity, nutrient content and lots more, thereby, affecting microbial growth. According to [21], diesel oil causes reduction in species richness, evenness and diversity when spilled in a soil environment. Lower diesel-utilizing bacterial counts in the unamended sample could be as a result of depletion of limiting nutrients [22]. Furthermore [23], opined in their study that fuels introduced into the soil could stimulate or reduce the number of bacteria depending on the type of contamination and its dose. It was observed that diesel contaminated soil with and without pigeon droppings had higher fungal counts than the unpolluted soil. This observation could be ascribed to the ability of fungi to utilize crude oil products as sources of carbon and energy. In the study carried out by [24] soil contamination with 5% and 10% diesel oil increased fungal counts by 73% and 139%, respectively. This finding demonstrated the capacity of fungi to use diesel hydrocarbons as energy source and their potential ability to biodegrade diesel oil.

Bacterial and Fungal Isolates

The bacterial and fungal types identified in the control for the first week were higher than those recorded for the contaminated soils without amendment. The high bacterial and fungal types observed in amended soils could be ascribed to the presence of pigeon droppings which could harbour microorganisms. Moreso, the bacterial and fungal isolates in this study have been reported in previous studies. [13] Isolated members of various genera such as *Bacillus*, *Lysinibacillus*, and *Enterobacter* from crude oil polluted soil [25]. Isolated *Bacillus* sp, *Pseudomonas* sp, *Acinetobacter* sp, *Micrococcus* sp and *Staphylococcus* which agrees with the present study except for the presence of *Staphylococcus* sp as Diesel utilizing bacteria which does not concur with the present study. The percentage occurrence of diesel utilizing bacteria in the present study is in agreement with theirs which showed that *Bacillus* sp was the most prevalent isolate followed by *Pseudomonas* sp. and *Alcaligenes* sp. was observed to have the highest number of growth on the diesel contaminated soils. This result is in line with [26] who reported that the bacterial isolates from the soil contaminated with petroleum products from the sites showed *Pseudomonas sp*, *Bacillus sp* and *Klebsiella spp.* having highest percentage occurrence of 60%. Similarly, in weeks 3 and 4, *Bacillus sp*. and *Pseudomonas sp* were observed to have the highest occurrence of growth on the diesel contaminated soils. The prevalence of *Bacillus* sp as diesel oil utilizing bacteria could be due to its ability to survive harsh conditions by the formation of spores. Its hydrocarbon degrading enzyme system and ability to emulsify petroleum hydrocarbon is another reason for its high occurrence in Nigerian soils [25]. *Pseudomonas*, *Bacillus* and *Micrococcus* spp. isolated in this study corresponds with the work of [27].

The prevalence of *Aspergillus* sp in the diesel contaminated soil could be attributed to its ability to utilize the diesel oil. This agrees with (13) who reported similar findings. The fungal isolates in this study have been reported in a previous study to be associated with hydrocarbon utilization [28] (Douglas and Tamunonegiyeofori, 2019). In a previous study of soil samples contaminated with crude oil, fourteen fungal genera belonging to *Alternaria sp.*, *Aspergillus sp.*, *Cephalosporium sp.*, *Cladosporium sp.*, *Fusarium sp.*, *Geotrichum sp.*, *Mucor sp.*, *Penicillium sp.*, *Rhizopus sp.* *Trichoderma sp.*, *Candida sp.*, *Rhodotolura sp.*, *Saccharomyces sp*. and *Torulopsis* sp from the control soil, with five hydrocarbon utilizing fungi identified out of the fourteen. In this study, *Cephalosporium* sp was not isolated. The hydrocarbon utilizing bacteria and fungi identified in this study have been shown to have the ability to utilize crude oil as carbon source.

Physicochemical Properties

There was a high fluctuation in the physicochemical parameters of the soil during the study period. Nitrogen, phosphate, organic carbon, and soil organic matter increased throughout the study in amended samples. Unamended samples showed decrease. The increase in these nutrients could be as a result of the addition of pigeon droppings which increased the nutrients in the amended samples. Nitrogenous compounds and other necessary nutrients present in pigeon droppings were reasons for this increase. This result agrees with [25] who observed a similar trend but differs with that of [29] who observed decrease in nitrogen, phosphorus and potassium levels in amended samples for a period of 42 days. The ratio of organic waste to soil used by [29] could have been the reason for the decrease in nutrient content. This study also showed that the pH of all the amended samples increased with time. This is similar to the report of [30] who also observed increase in pH with time after amending soil with organic manure. Bacterial growth and activity are readily affected by pH and in this study, the pH ranged from 6.2 to 7.8. This observation slightly differs from the pH range (6.0 to 8.9) that was reported by [31] as the best pH range for bioremediation of hydrocarbon polluted soils and that these changes in pH level could be due to the release of acidic and alkaline intermediates and final products during biodegradation of hydrocarbons, which has an effect on the pH.

The samples amended with pigeon droppings degraded diesel more than the unamended. This may possibly be due to a higher nutrient level present in pigeon droppings. Poultry droppings like chickens could harbor hydrocarbon-utilizing bacteria [32]. The biodegradation recorded in the unamended soil sample could be due to non-biological factors such as evaporation, photo-degradation [33]; volatilization, adsorption, abiotic factors (temperature and pH) [34]. Reduction of petroleum hydrocarbon in unamended sample has also been reported by previous studies [29, 35].

Conclusion

From the study, it can be concluded that the diesel pollution of soil has adverse effects on soil. More so, reduction of diesel oil in the contaminated soil indicates the presence of diesel degrading microorganisms; and that rate of diesel oil biodegradation in soil could be enhanced by amendment with pigeon droppings that serves as a biostimulation agent. Pigeon dropping is a good organic substrate containing nitrogen and phosphorus which modified the physical, chemical and biological properties of diesel oil polluted soil and improved their nutritional status for enhanced agronomic performances.

Competing interests

Authors have declared that no competing interests exist.

Authors' Contributions

'WJO' designed the study, wrote the protocol and the first draft of the manuscript. 'AF' managed the literature

searches, analyses of the study and performed the statistical analysis. All authors read and approved the final manuscript.

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