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BIOSTIMULATION POTENTIALS OF CHICKEN DROPPING ON SPENT OIL CONTAMINATED SOIL FROM AUTOMOBILE WORKSHORPS IN KEFFI, NASARAWA STATE NIGERIA

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Abstract

Research into the use of organic waste for the biostimulation and bioremediation of hydrocarbon contaminated soils has expanded rapidly over the years. In this study, the possibility of chicken dropping to stimulate the growth of hydrocarbon utilizing bacteria and optimize total hydrocarbon biodegradation of oil contaminated soil from mechanic workshop was investigated. Soil samples from five (5) different automobile workshops within Keffi, Nassarawa State Nigeria were mixed with poultry droppings at concentrations of 0% (control), 10% and 30%. The set up was then left for a period of 4 weeks. The chicken droppings had total bacteria and fungi counts of $2.51 \cdot 10^7$ cfu g^{-1} and $1.98 \cdot 10^6$ cfu g^{-1} respectively, while soil samples had the bacterial count in the range of $3.92 \cdot 10^6$–$4.87 \cdot 10^6$ cfu g^{-1}. Chicken dropping had mean pH, nitrogen, phosphorus, carbon and moisture contents of 7.06±0.78, 0.88±0.07%, 36.90±3.11 mg kg^{-1}, 3.08±0.11% and 44.78±1.90% respectively. The nitrogen and carbon content in all experimental soil decreases with

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increase in bioremediation time. All the bio-remediated soil had significantly ($p < 0.05$) higher hydrocarbon utilizing bacteria (HUB) than the control (un-remediated). In all the soil samples remediated, 30% remediation had significantly ($p < 0.05$) highest HUB in the range of $16.87 \cdot 10^6$–$19.87 \cdot 10^6$ when compared with those remediated with 10% chicken droppings ($14.78 \cdot 10^6$–$16.89 \cdot 10^6$). This study demonstrated that chicken droppings are good organic substrate containing nitrogen, phosphorus, and carbon, with great potentials for biostimulation of hydrocarbon utilizing bacteria in oil contaminated soil.

Introduction

Petroleum-based products are the major source of energy for industry and daily life. The amount of natural crude oil seepage was estimated to be 600,000 metric tons with a range of uncertainty of 200,000 metric tons per year (Al-Mutairi et al. 2008). In Nigeria, oil pollution problems have been prevalent since the commencement of oil exploration and development of the petroleum industry (Okolo et al. 2005). During the exploration and production of crude oil, numerous oil fields, tank farms, flow stations, pipelines, tankers and loading jetties constantly provide potential sources of oil pollution (Ijah and Antai 2003). However, common forms of pollution come from household wastes, agricultural wastes, gas flaring, oil spills and spent lubricating oil (Ofoegbu et al. 2014).

Spent engine oil, usually obtained after servicing and subsequently draining used oil from automobiles and generator engines, is indiscriminately disposed into gutters, water drains, open vacant plots and farms in Nigeria by auto mechanics and allied artisans with workshops (Nwankwegu et al. 2016). Soils polluted with petroleum hydrocarbons (PHCs) or spent engine oil differ from unpolluted soils and are not able to support adequate crop growth and development (Al-Mutairi et al. 2018). There is need to treat these soils so as to satisfy the food requirement of the ever-increasing world population.

Bioremediation is a cost-effective method of soil remediation which uses organisms for the treatment of polluted soils. It has been used across the globe for the treatment of a wide range of organic soil pollutants (Cai et al. 2010). However, nutrient limitation in hydrocarbon-contaminated soils presents a challenge to bioremediation; nonetheless, addition of nutrients generally benefits soil hydrocarbon utilizing bacteria via biostimultion resulting in enhanced bioremediation of hydrocarbon polluted environment (Liu et al. 2014).

Poultry droppings is a mixture of poultry excreta, spilled feed, feathers, and material used as bedding in poultry operations (Ofoefule and Uzodinma 2006). Poultry manure is rich in organic manure since solid and liquid excreta are excreted together resulting in no urine loss. Poultry manure is used as a source of N, P and K and some micronutrients (Mullins et al. 2002). These nutrients could be very useful in providing adequate environmental medium for efficient growth and reproduction of the hydrocarbon utilizing bacteria. The present study aimed at evaluating the biostimulation effect of chicken dropping on hydrocarbon utilizing bacteria in soil sample collected from mechanic workshop.

Materials and Method

Description of study area and sample collection

The soil sample used in this study was collected from Keffi, Nassarawa State, Nigeria. The main occupations of the people are land farming and subsistent agriculture, of which there is no history of crude oil pollution in this environment. Oil contaminated soil was collected from 5 different automechanic workshops at Keffi garage, Opposite New Keffi hotel, Angwan kwara, High court, and Angwan Tanko, all in Keffi, Nasarawa State Nigeria. At each sampling point, two samples were collected at a depth of 10 cm and bulk samples were collected in polythene bags and immediately transported to the laboratory for analysis. The soil amendment material (chicken droppings) was collected from Lawal Poultry Farms, Pyanku, Nasarawa State, Nigeria.

Isolation and identiftcation of isolates from the spent oil contaminated soil

Total microbial analysis was carried out on the soil by weighing 10 g of soil sample, serially diluted and inoculated unto nutrient agar and potato dextrose agar to culture bacteria and fungi. The culture plates were incubated for 24 and 78 hours respectively. On completion of the culture, microbial strains were identified. Each isolate was examined for its size, shape, margin, consistency, elevation, pigmentation, Gram reaction and cell morphology. The isolates were characterized as described by holt et al. (1999). Biochemical tests which were carried out included production of catalase, indole and oxidase enzymes. Spore production and oxidation/fermentation of sugars were also examined.

Isolation and identiftcation of hydrocarbon utilizing bacteria (HUB)

HUB counts in the soil was determined by plating an aliquot of 0.1 ml of the serially diluted 1 g of the soil on oil agar (OA) [1.8 g K_2HPO_4, 4.0 g NH_4Cl, 0.2 g $MgSO_4.7H_2O$, 1.2 g KH_2PO_4, 0.01 g $FeSO_4.7H_2O$, 0.1g

NaCl, 20 g agar, 1% used engine oil in 1000 ml distilled water, pH 7.4], and incubated at 30^0C for 72 hours . Discrete colonies that developed were counted and expressed in cfu g^{-1} (Adam et al. 2014).

Physicochemical analyses of soil sample and amendment material

Physicochemical parameters including moisture, pH, total nitrogen, phosphorus and carbon contents were carried out on both the chicken droppings before amendment and on the soil sample after amendment with chicken droppings. The analysis was carried out on a weekly basis throughout the study period. The pH was determined by the according to the modified method of McLean (1982), the total organic carbon was determined by the modified (Nelson and Sommers 1982) wet combustion method (Walkey and Black 1934) available nitrogen was ascertained using semi-micro Kjeldhal method (Bremner and Mulvaney 1982), the available phosphorus by Brays No.1 method (Olsen and Sommers 1982).

Bioremediation study

This was carried out ex situ in the Microbiology laboratory at Nasarawa State University Keffi, Nasarawa State. Eighty grams (80 g) of the soil samples was mixed with chicken droppings at various concentrations of 10% and 30% (Table 1). The control was left without amendment. This remediation exercise was carried out in a well perforated 1.5 litres plastic container with an estimated depth of 13 cm. This research work was conducted for four weeks, during which samples from each group were taken to the laboratory for analysis of total hydrocarbon content, once in every 3 days. Hydrocarbon-Utilizing Bacterial (HUB) content was analysed using standard methods (APHA 1992).

Table 1

Experimental design for bioremediation

	Experimental Groups	Description
T1	chicken dropping added at 10%	80 g of polluted soil + 8 g of chicken droppings
T2	chicken dropping added at 30%	80 g of polluted soil + 24 g of chicken droppings
T3	control	80 g of polluted soil only (control)

Results

Bacteria and fungi species isolated from auto-mobile polluted soil

The morphological and biochemical characteristic of bacteria species isolated from the contaminated soil are shown in Table 2. Data confirmed the identity of the organism to be *Bacillus subtilis*, *Micrococcus luteus* and *Pseudomonas aeruginosa* strain. Fungal species including *Aspergillus niger, Aspergillus fumigatus* and *Mucor* species were isolated (Table 3).

Table 2

Morphological biochemical characteristic of bacterial isolate

Test	*Micrococcus species*	*Bacillus species*	*Pseudomonas species*
Shape	Cocci	Rod	Rod
Gram Stain	*G+*	*G-*	*G-*
Catalase	+	+	+
Methyl Red	+	-	-
Oxidase	+		
Indole	-	+	+
Glucose	A G	AG	AG
Fructose	*A*	*G*	AG
sucrose	AG	AG	AG
Lactose	AG	AG	AG

Key: **(+)** – positive; (-) – negative; *G+* – gram positive; *G-* – gram negative; *A* – acid; AG acid and gas production

Table 3

Morphological characteristics of fungal isolated

Test	*Aspergillus niger*	*Aspergillus fumigatus*	*Mucor* species
Macroscopy	black and powdery like	gray – green fluggy colonies	whitish/light cotton like
Microscopy	conidiophores smooth walled and non septate	long erect non septate conidiophores	round, conidia non – septate

Total bacteria and fungi count

The total bacteria and fungi counts in the soil contaminated samples and the chicken droppings used for bioremediation are shown in Table 4. The chicken droppings had total bacteria and fungi counts of $2.51 \cdot 10^7$ cfu g^{-1} and $1.98 \cdot 10^6$ cfu g^{-1} respectively. The soil samples had the bacterial count in the range of $3.78 \cdot 10^6$–$4.87 \cdot 10^6$ cfu g^{-1} and fungal count in the range $2.89 \cdot 10^5$–$3.11 \cdot 10^5$ cfu g^{-1}.

Table 4

Bacteria and fungi count in the soil contaminated samples and the chicken droppins

	Bacteria count	Fungi count
Keffi garage	$4.87 \cdot 10^6$	$3.06 \cdot 10^5$
Opposite New Keffi hotel	$3.78 \cdot 10^6$	$2.89 \cdot 10^5$
Angwan kwara	$4.56 \cdot 10^6$	$2.91 \cdot 10^5$
High court	$4.08 \cdot 10^6$	$3.11 \cdot 10^5$
Anguwan Tanko	$3.92 \cdot 10^6$	$2.81 \cdot 10^5$
Chicken droppings	$2.51 \cdot 10^7$	$1.98 \cdot 10^6$

Physicochemical properties of chicken droppings

Physicochemical properties of chicken droppings used for bioremediation are presented in Table 5. Chicken dropping had mean pH of 7.06±0.78, nitrogen content of 0.88±0.07%, phosphorus contents of 36.90±3.11 mg kg^{-1} while the organic carbon and moisture content were 3.08±0.11% and 44.78±1.90 % respectively.

Table 5: Physicochemical properties of chicken droppings used for bioremediation

Parameters	Chicken droppings
pH	7.06±0.78
Nitrogen [%]	0.88±0.07
Phosphorus [mg kg^{-1}]	36.90±3.11
Organic C [%]	3.08±0.11
Moisture [%]	44.78±1.90

Values are mean ± standard error of mean (SEM) of 3 determinations

Nutrients Compositions of oil contaminated soil sample for remediation

The physicochemical properties of engine oil contaminated soil are shown in Table 6. There were no significant differences ($p > 0.05$) in the pH, nitrogen, organic carbon and moisture contents of the soil samples from the five (5) locations. However, the phosphorus content was significantly higher ($p < 0.05$) in soil sample collected from "anguwan kwara workshop' when compared with soil collected from other sample location. pH of the soil ranged between 6.22±0.29 and 7.90±1.02, nitrogen contents ranged between 11.74±0.43% and 1.85±0.12%, phosphorus ranged between 15.07±0.95 mg kg^{-1} and 19.78±0.45 mg kg^{-1}, organic carbon ranged between 2.30±0.11% and 2.94±0.09 % while moisture contents ranged between 8.67±0.33% and 9.56±0.56%.

Table 6

Physicochemical properties of engine oil contaminated soil

	Soil sample location				
Parameters	Keffi garage	Opposite New Keffi hotel	Angwan kwara	High court	Angwan Tanko
pH	6.23±0.06^{a}	6.55±0.10^{a}	7.90±1.02^{b}	6.22±0.29^{a}	6.41±0.31^{a}
Nitrogen [%]	1.78±0.10^{a}	1.81±0.11^{a}	1.74±0.43^{a}	1.80±0.09^{a}	1.85±0.12^{a}
Phosphorus [mg kg^{-1}]	15.98±0.67^{a}	16.89±1.32^{a}	19.78±0.45^{b}	16.90±1.89^{a}	15.07±0.95^{a}
Organic C [%]	2.56±0.12^{a}	2.89±0.15^{a}	2.94±0.09^{a}	2.30±0.11^{a}	2.42±0.54^{a}
Moisture [%]	9.56±0.45^{a}	8.74±0.28^{a}	8.67±0.33^{a}	8.75±0.45^{a}	9.56±0.56^{a}

Values are mean ± standard error of mean (SEM) of 3 determinations. Values along the same column with different superscripts are significantly different ($p < 0.05$).

Effect of remediation on nutrient composition of the contaminated soil sample

Nitrogen content

The nitrogen content in all experimental soil decreases with increase in bioremediation time (Fig. 1). However, the un-remediated control soil had the highest nitrogen content. The decrease in nitrogen content was dependent on concentration of chicken droppings except for sample collected from Anguwan tanko and high court automobile workshop.

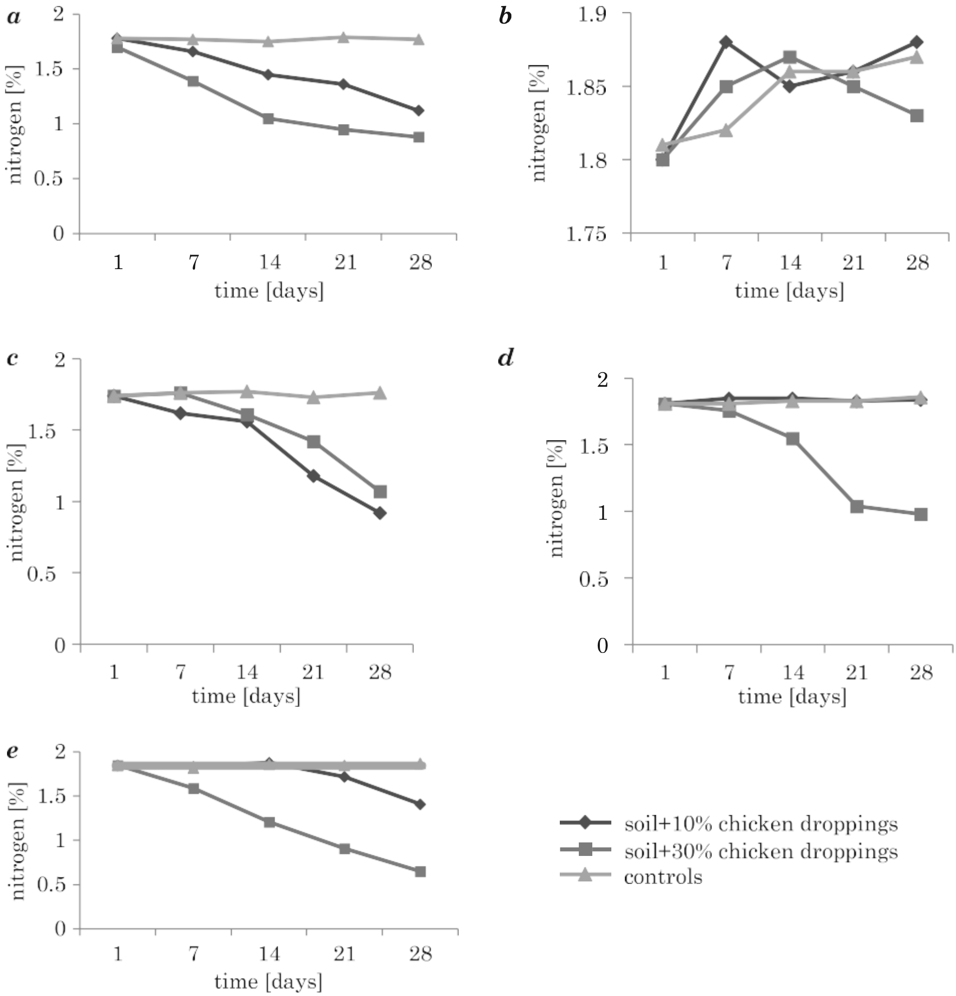

Fig. 1. Effect of remediation time on nitrogen contents of contaminated soil sample: *a* – Keffi garage automobile workshop; *b* – high court automobile workshop; *c* – Anguwan kwara automobile workshop; *d* – New Keffi hotel automobile workshop; *e* – Anguwan tanko automobile workshop

Carbon content

The carbon content in all experimental soil decreases with increase in bioremediation time (Fig. 2). However, the un-remediated soil had the highest carbon content. The decrease in carbon content was dependent on concentration of chicken droppings for all the soil sample except for high court automobile contaminated soil.

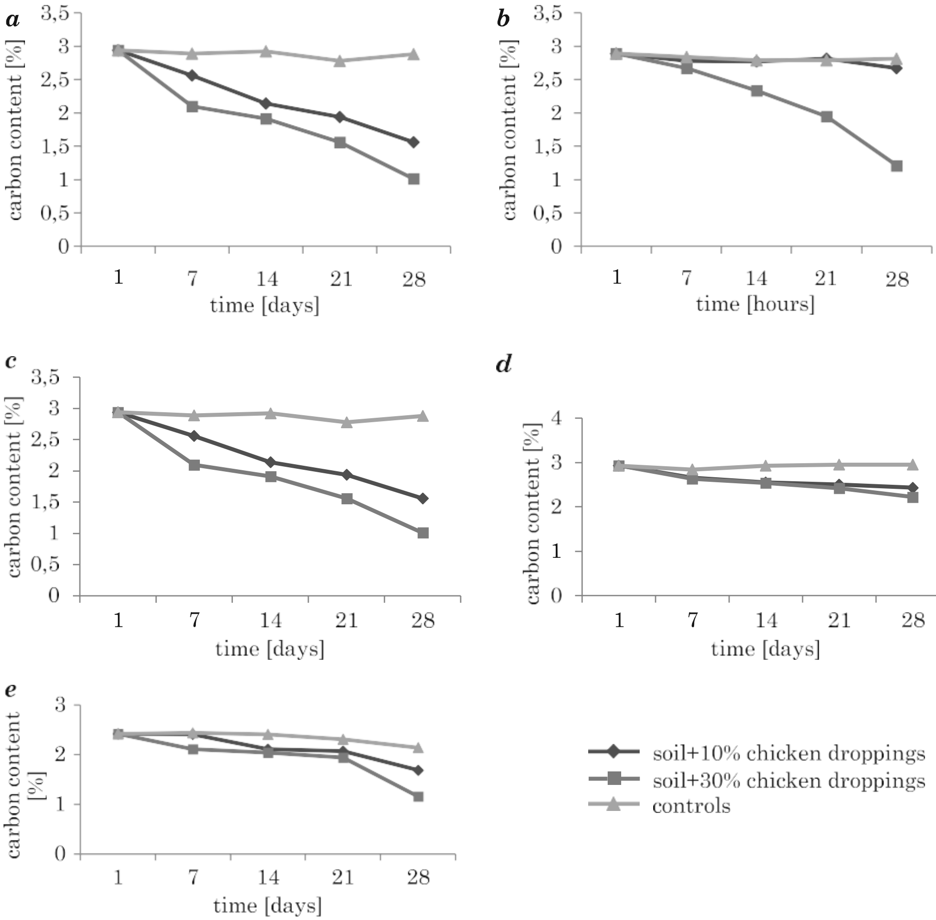

Fig. 2. Effect of remediation time on carbon contents of contaminated soil sample: *a* – Keffi garage automobile workshop; *b* – high court automobile workshop; *c* – Anguwan kwara automobile workshop; *d* – New Keffi hotel automobile workshop; *e* – Anguwan tanko automobile workshop

Hydrocarbon-utilizing bacterial (HUB)

The hydrocarbon-utilizing bacterial (HUB) count in all experimental soil after four (4) weeks of bioremediation with chicken droppings are presented in Figure 3. All the bio-remediated soil had significantly ($p < 0.05$) higher HUB than the control (un-remediated) soil. In all the soil samples remediated, 30% remediation had significantly ($p < 0.05$) the highest HUB in the range of $16.87 \cdot 10^6$–$19.87 \cdot 10^6$ cfu g^{-1} when compared with those remediated with 10% chicken droppings ($14.78 \cdot 10^6$– $16.89 \cdot 10^6$ cfu g^{-1}). The non-remediated control had the least HUB count range of $4.87 \cdot 10^6$–$7.32 \cdot 10^6$ cfu g^{-1}.

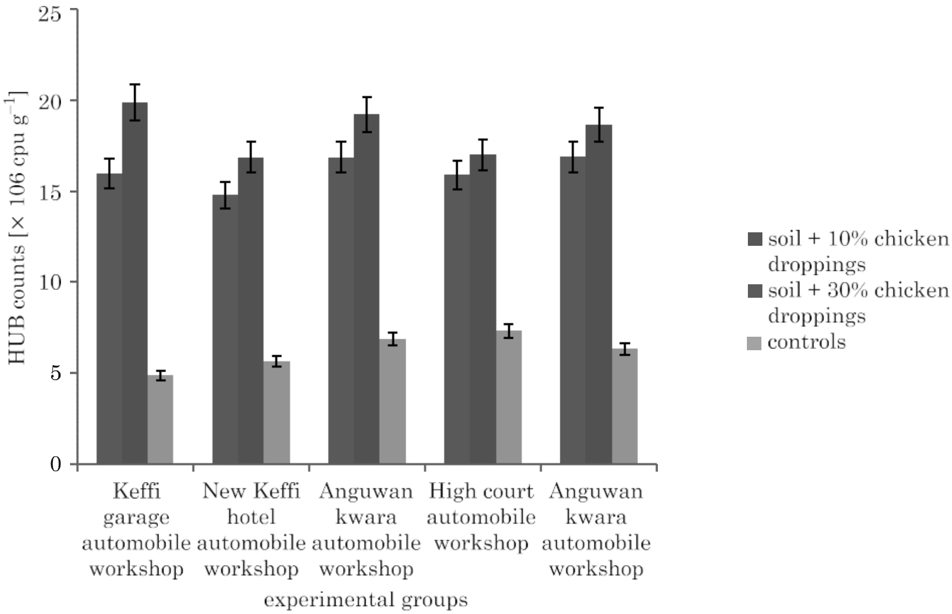

Fig. 3. Counts of Hydrocarbon-Utilizing Bacterial (HUB) population in polluted soil after 4 weeks of bioremediation

Discussion

Biodegradation is increasingly being considered as a less expensive alternative to physical, mechanical and chemical means of disposing hydrocarbon pollutants (Umar et al. 2013). Studies have reported that microbial growth in soil is controlled not by the total amount of resource available but by the scarcest resources (limiting factor), which are, in this case, N, P and C (Gorban et al. 2011).

Morphological and biochemical identification of organism in the contaminated soil confirmed the identity of *Bacillus subtilis*, *Micrococcus luteus* and *Pseudomonas aeruginosa* strain *Aspergillus niger, Aspergillus fumigatus* and *Mucor* species in the soil sample. Previous studies have implicated *Acinetobacter, Micrococcus, Pseudomonas, Nocardia,* and *Bacillus* as the major hydrocarbon utilizing bacteria in oil contaminated soil (Das and Mukherjee 2007). This is also in consistence with the findings of Okafor et al. (2016) who identified *Bacillus* spp., *Pseudomonas* spp., *Flavobacterium* spp., *Fusarium* spp. and *Aspergillus* spp. in crude oil contaminated soil from Anambara State. The ability to isolate high numbers of certain oil degrading microorganisms from an environment is commonly taken as evidence that those organisms are the active degraders of the constituents of that environment. Therefore, the presence of these organisms in crude oil contaminated soil is an indication that these organisms are indigenous hydrocarbon utilizing organism for carbon and energy source (Okolo et al. 2005). It has been reported that some organism cannot survive high diesel contamination level of up to 11.11% (Stephen et al. 2013). However, the hydrocarbon (HC) degrading activities of the surviving microbes could be enhanced by the addition of nutrients required for optimum activities and reproduction of the organism. Although microorganisms are present in contaminated soil, their numbers might not be sufficient to initiate remediation of contaminated sites. The microbial (Fungi and bacteria) population of chicken droppings in this study are higher than $3.4 \cdot 10^4$ cfu g^{-1} and $1.77 \cdot 10^4$ cfu g^{-1} for bacteria and fungi count reported for chicken droppings use for bioremediation in Anambara State (Okafor et al. 2016). These discrepancies could be attributed to the differences in the nutritional quality of feed been fed to the chicken which results to differences in nutrient contents of the droppings and consequently effect bacterial growth. Similarly, differences in environmental factors such as temperature, pH, humidity could affect the optimum activities and reproduction of the organism. The high microbial count reported in this study would therefore favour hydrocarbon degradation when used in bioremediation.

The contaminated soil sample from the automobile workshop in this study had the bacterial count in the range of $3.92 \cdot 10^6$–$4.87 \cdot 10^6$ cfu g^{-1} and fungal count in the range $2.91 \cdot 105$– $3.11 \cdot 10^5$ cfu g^{-1}. In agreement with this study, Daniel et al. (2017), reported bacteria count in the range of 2.94×10^6 cfu g^{-1} and $2.39 \cdot 10^6$ CFU g^{-1} from automobile workshops in Benue State. However, Stephen et al. (2015), reported a low bacteria count in range of $8.0 \cdot 10^3$ to $9.8 \cdot 10^4$ cfu g^{-1} in mechanic workshop soil from Kogi State.

During the bioremediation, it was observed that C and N decrease as the remediation time increases. This observation is in agreement with previous studies that reported enhanced degradation of crude oil using other animal manures i.e goat manure (Nwogu et al. 2015), chicken-drop (Ijah and Antai 2003), poultry manure (Adesodun and Mbagwu, 2008). The decrease in total N and C is due to their utilization for microbial growth. The counts of HUB in all the soil remediated with chicken droppings were higher compared to that of non-remediated control soil; these counts are comparable to those of Ibiene et al. (2011) who reported that the total culturable hydrocarbon utilizing bacterial count in crude oil contaminated soil ranged between $\cdot 10^3$ cfu g^{-1} to $\cdot 10^6$ cfu g^{-1} , also Ijah and Antai (2003), who observed counts of hydrocarbon degraders in oil polluted soil to be $\cdot 10^6$ cfu g^{-1} but lower than those obtained by Antai and Mgbomo (1989) whose counts of HUB in hydrocarbon-contaminated soil was · 10^8 CFU g^{-1} ; this may be due to differences in microbial ecology of the soil or characteristics of the experimental soils. The reason for higher counts of bacteria in bio-remediated soil may be the result of the presence of appreciable quantities of nitrogen and phosphorus in chicken droppings, which are necessary nutrients for bacterial biodegradative activities (AdesOdun and MbAgwu 2008).

Hydrocarbon-polluted soil is toxic for plants, possibly due to direct inhibiting effects of hydrocarbons or their metabolites as well as to changed soil conditions (MOrelli et al. 2005). Apart from this, oil pollution may lead to changes of the microbial community structure, favoring the dominance of phytotoxin-producing species (lAbud et al. 2007, steligA et al. 2012). Fortunately, the decreases in nutrient composition of the chicken dropping amended soil lead to corresponding increase in Hydrocarbon utilizing bacteria, this will consequently lead to increased oil biodegradation in the soil. By implications, the biostimulation technique proposed here for soils polluted with crude oil could be suitable in field, because of its low costs and its low environmental risk associated with volatile hydrocarbon losses.

Conclusion

This study demonstrated that chicken droppings are good organic substrate containing nitrogen, phosphorus, and carbon, which have great potentials for enhanced bioremediation of diesel contaminated soil. The wide availability of chicken droppings in commercial quantity in Nigeria makes these waste products a potential and viable biostimulant for enhanced bioremediation of crude oil polluted environments.

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Conflict of interest

The authors declared no conflict of interest exist.

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References

Adams G.O, Tawari-Fufeyin P., Ehinomen I. 2014. *Bioremediation of spent oil contaminated soils using poultry litter*. Research Journal in Engineering and Applied Sciences, 3(2): 118–124.

Al-Mutairi N., Bufarsan A., Al-Rukaibi F. 2008. *Ecorisk evaluation & treatability potential of soils contaminated with petroleum hydrocarbon-based fuels*. Chemosphere, 74: 142–148.

Antai S.P., Mgbomo E. 1989. *Distribution of hydrocarbon utilizing bacteria in oil spill areas*. Microbiology Letter, 40: 137.

APHA, AWWA, WPCF 1992. *Standard methods for the examination of water and wastewater*. 18th ed. American Public Health Association, Washington DC, USA.

Bremner J.M., Mulvaney C.S. 1982. *Total nitrogen determination*. In: *Method of soil analysis*. Eds. A.L. Page, Miller R.H., D.R. Keeney. American Society of Agronomy, Madison, Wisconsin, USA.

Cai Z., Zhou, Q. Peng, S. Li K. 2010. *Promoted biodegradation & microbiological effects of petroleum hydrocarbons by Impatiens balsamina* L. *with strong endurance*. Journal of Hazard Materials, 183(1): 731–737.

Daniel I.O., Osazee O.J., Nwaeze I.O., Osamudiamen J. 2017. *Isolation and characterization of hydrocarbon-degrading bacteria in top and subsoil of selected mechanic workshops in Benin City Metropolis, Nigeria*. Journal of Applied Science and Environmental Management, 21(4): 641–645.

Das K., Mukherjee A.K. 2007. *Crude petroleum-oil biodegradation efficiency of Bacillus subtitles and Pseudomonas aerations strains isolated from a petroleum-oil contaminated soil from North-East India*. Bioresearch Technology, 98(7): 1339–1345

Gorban I.I., Pokidysheva E.V., Smirnova E.V., Tyukina T.A. 2011. *Law of the minimum paradoxes*. Bulletin of Math Biology, 73(9): 2013–2044.

Holt J.G., Krieg N.R., Sneath P.H.A, Staley J.T., Williams S.T. 1999. *Bergey's manual of determinative bacteriology*. 9th ed. Williams and Wilkins, pp. 71–561.

Ijah U.J.J., Antai S.P. 2003. *The potential use of chicken-drop micro-organisms for oil spill remediation*. Environmentalist, 23(1): 89–95.

Labud V., Garcia C., Hernandez T. 2007. *Effect of hydrocarbon pollution on the microbial properties of a sandy & a clay soil*. Chemosphere, 66: 1863–1871.

Liu H., Yao J., Yuan Z., Shang Y., Chen H., Wang F., Choi M.M. 2014. *Isolation & characterization of crude-oil-degrading bacteria from oil-water mixture in Dagang oilfield, China*. Journal of Hazard Material, 87: 52–59.

McLean E.O. 1982. *Soil pH and lime requirement*. In: *Methods in soil analysis. chemical and microbiological properties*. Part II. Ed. C.A. Black. American Society of Agronomy, Madison, Wisconsin, USA.

Morelli I.S., del Panno M.T., de Antoni G.L., Painceira M.T. 2005. *Laboratory study on the bioremediation of petrochemi – calsludge – contaminated soil*. International Biodeterioration & Biodegradation, 55: 271–278.

Nelson D.W., Sommers L.E. 1982. *Determination of organic carbon*. In: *Methods of soil analysis*, vol. 2. Eds. A.L. Page, R.H. Miller, D.R. Keeney. American Society of Agronomy, Madison, Wisconsin, USA. p. 539.

Nwankwegu A.S., Onwosi C.O., Orji M.U., Anaukwu C.G., Okafor U.C., Azi F., Martins P.E.J. 2016. *Effect of chicken droppings ammendment on bioremediation of crude oil polluted soil*. Environmental Management, 172: 136–142.

Nwogu T. P. Azubuike C.C., Ogugbue C.J. 2015. *Enhanced bioremediation of soil artificially contaminated with petroleum hydrocarbons after amendment with Capra aegagrus hircus (Goat) Manure*. Biotechnology Research International, https://doi.org/10.1155/2015/657349.

Ofoefule A.U., Uzodinma E.O. 2006. *Optimization of the qualitative and quantitative biogas yield from poultry waste*. Proceedings of World Renewable Energy Congress IX, August 19–25, 2006. University of Florence, Italy Elsevier.

Ofoegbu R.U., Momoh Y.O.L., Nwaogazie I.L. 2014. *Bioremediation of crude oil contaminated soil using organic and inorganic fertilizers*. Journal of Petroleum Environmental and Biotechnology, 6: 198. doi:10.4172/2157-7463.1000198

Okafor U.C., Orji M.U., Nwankwegu M.S., Anaukwu C.G., Onuorah S.C., Kingsley C.A. 2016. *Effect of chicken droppings amendment on bioremediation of crude oilpolluted soil*. European Journal of Experimental Biology, 6(4): 62–68.

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*. Applied Ecology & Environmental Research, 3(1): 47–53.

Olsen S.R., Sommers L.E. 1982. *Determination of available phosphorus*. In: *Methods of soil analysis*. Eds. A.L. Page, R.H. Miller, D.R. Keeney. American Society of Agronomy, Madison, Wisconsin, USA.

Steliga T., Jakubowicz P., Kapusta P. 2012. *Changes in toxicity during in situ bioremediation of weathered drill wastes contaminated with petroleum hydrocarbons*. Bioresource Technology, 125: 1–10.

Stephen E., Okwute L.O., Okai A.I. 2015. *Bioremediation of mechanic workshop polluted soil amended with poultry litter*. Bioscience Research in Today's World, 1: 77–83.

Stephen E., Usman A.S., Okolo M.O., Akogu E.A. Abioye O.P. 2013. *Microbial and physicochemical properties of diesel simulated soil*. FUTA Journal Research Science, 4(1): 82–86.

Umar H., Umar A., Ujah U.J.J., Hauwa B., Sumayya B.I., Shuaibu M. Yakubu M.S. 2013. *Biodegradation of waste lubricating oil by bacteria isolated from the soil*. Journal of Environmental Science Toxicology and Food Technology, 3(6): 62–67.

Walkey A., Black A.I. 1934. *An examination of the Degtjareff methods for determining soil organic matter and proposed modification of the chromic acid titration method*. Soil Science 37: 29–38.