Ogoanah SO *et al.* (2020)
Notulae Scientia Biologicae 12(2):387-398
DOI:10.15835/nsb12210617
Research Article

Enhancement of the soil quality of an oil-polluted ultisol using livestock wastes

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Abstract

The study investigated the enhancement of soil quality of an oil-polluted ultisol using livestock wastes. Top soil (0 - 10 cm) was obtained as a pooled sample and polluted with spent lubricating oil at 10% w/w. The soil was subsequently amended with sun-dried goat (GT), rabbit (RB), and poultry (PG) dung at 10% w/w on dry weight basis both in singles, double-mixed, and triple-mixed combinations. Twelve weeks after treatment application, results showed that there was a 93.9% decrease ($p<0.05$) in bacterial colony count in the oil-polluted soil compared to the control. *Penicillium notatum* and *Aspergillus niger* as well as *Bacillus* sp. and *Proteus* sp. were the prominent fungal and bacterial species identified respectively. The most abundant plant in the soil seed bank was *Panicum maximum* with 10.4% abundance and this showed possible involvement of the plant in remediation of oil-pollution. The total hydrocarbon content of the oil-polluted soil was 9984.0 mg/kg, compared to 3170.6 mg/kg when amended with RB+GT, implying 76.77% remediation efficiency. Among several trials employed in this study, the combination of rabbit and goat wastes proved to be more effective in reducing the total hydrocarbon content of oil-polluted soil and therefore, is recommended as a potential candidate for application in the bioremediation of such soil.

Keywords: bioremediation; pollution; remediation efficiency; soil amendment; ultisol; waste engine oil

Introduction

For several decades, the environment has been exposed to degradation at monumental levels, following the globalized industrial revolution. The resulting distortion of balance in the natural ecosystem is deemed a great obstacle to sustainable development, especially in third world countries (Adams *et al.*, 2014). This industrial menace, particularly that of oil pollution has persisted in the environment as one of the major issues that causes enormous havoc to the global economy, renders useful lands for agriculture useless, causes an imbalance in the natural equilibrium of the ecosystem, destroys aquatic life, and poses health challenges.

Received: 02 Dec 2019. Received in revised form: 13 Jun 2020. Accepted: 25 Jun 2020. Published online: 29 Jun 2020.

Furthermore, plants that are cultivated in such soils usually have lowered germination percentage (Ekundayo *et al.*, 2001), induced stress (Achuba, 2006; Ohanmu *et al.*, 2014), low yield (Al-Qahtani, 2011) and productivity (Ohanmu and Bako, 2017). This will adversely affect food production, reduce income for the farmers, and cause them to abandon their farm lands due to low crop yield.

The productivity of soils polluted with oil is marked with significant reduction in its nutrients content, microbial diversity, and a hydrophobic layer, which in turn, affects plant growth and development. Abosede (2013) reported adverse effects of oil pollution on the soil's physical properties, while the drastic reduction in nitrogen and organic carbon components of the soil were recorded (Agbogidi *et al.*, 2007; Wyszkowski and Ziolkowska, 2008). In addition, pore spaces might be clogged, and this could reduce soil aeration and water infiltration as well as increase bulk density, subsequently affecting plant growth. In spite of the stated hazardous effects that oil portends to the environment, most plants in Benin are cultivated in an utisol thereby worsening an already precarious condition. Ultisols (red soils) are marginals soil due to the relative low mineral content, fertility and a high pH value (Anikwe *et al.*, 2016).

Restoration of oil-polluted marginal soils could enhance their suitability for agriculture and food production (Ohanmu and Ikhajiagbe, 2018). Various methods have been proposed as ways to remediate oil-polluted soils, namely; physical, chemical and biological methods. The use of livestock waste, a biological method has been reported to enhance the performance of plants in oil-polluted soils (Amadi and UeBari, 1992; Ogboghodo *et al.*, 2004; Ewulo, 2005; Akinde and Obire, 2008; Ikuesan *et al.*, 2016), and enhance the nutrient status of soil for oil degradation by natural microbial population (Powel *et al.*, 1998; Boopathy, 2001; Bidwell *et al.*, 2002; Ikpe and Powel, 2002; Ogboghodo *et al.*, 2004). Also, Gupta and Baummer, (1996) reported that the chemical, microbiological, and physical characteristics of poultry litter are suitable substrates and nutrient sources for potential applications in the soil bioremediation industry. Moreover, poultry litter increases the rate of atrazine biodegradation and may be suitable for the remediation of gasoline-contaminated soil (Gupta and Tao, 1996; Anikwe, 2011). However, none of these studies reported the effect of animal waste, singly or in combinations, on an oil-polluted ultisols which is widespread in Benin.

Since Benin soils are known ultisols characterized by high pH and low productivity, it was necessitated to introduce a variety of animal waste as an ameliorant in a bid to reclaim and improve the vitality and productivity of the soil. To bridge this gap, the main objective of this research work was to determine the capacity of animal waste in enhancing the soil quality of an oil-polluted ultisol in the Benin area of Edo, Southern Nigeria.

Materials and Methods

Preparation of soil

The field experiment started on the 3rd day of March, 2018 and spanned for 12 weeks, was carried out beside the Plant Biology and Biotechnology (PBB) botanic garden on a marked plot measuring 5 m by 3 m. The plot was manually cleared to ground level and the debris were carefully removed from the site. Plastic bowls of 5 L by volume and 0.5 m circumference were procured from a nearby market and set in randomized block design (RBD). The top soil (0-10 cm) was gathered randomly from 10 locations in the garden using a hand auger, and pooled together to obtain a composite sample. Before proceeding with the experiment, a sample of the pooled soil was sent to the laboratory for physiochemical analysis in order to obtain a baseline data. Five (5) kg of the sun-dried soil was measured into 27 bowls arranged already in blocks.

Procurement of livestock and mixture of spent engine oil

Goat, rabbit, and poultry dungs were obtained from the University's animal farm and sun-dried to constant weight. The dried waste materials were crushed manually into powdery form for easy application as

well as to increase the surface area. The water holding capacity of the sun-dried soil was estimated according to method of Anoliefo *et al.* (2016) and the set up was moistened with 514 ml of water. The soil (5 kg) was contaminated with spent engine oil (SEO) by thoroughly mixing 500 g of SEO with the soil to obtain 10% w/w concentration (Ikhajiagbe and Anoliefo, 2011). A sample of the contaminated soil was collected and analyzed for total hydrocarbon content (Paiga *et al.*, 2012), whereas fungal and bacterial composition of soil was determined following standard procedure (Cowan and Steel, 1965; Cheesebrough, 2004; Saroj and Keerti, 2013). The set up was left for one (1) week so the contaminated soil would undergo natural attenuation before the introduction of livestock wastes as amendment.

Amendment of spent engine oil-polluted soil

The livestock dung (goat, rabbit, and poultry) was manually broken and weighed. Fifty grams (50 g) of the dung was used as amendment in the present study. The amendment was such that each of goat, rabbit, and poultry dung was applied, singly and then combined in group of two, and finally, as a pool of the three dungs as follows: goat dung (GD), rabbit dung (RD), poultry dung (PD), poultry + goat dung (POGT), rabbit + goat dung (RBGT), rabbit + goat dung (RBGT), and rabbit + poultry + goat dung (RBPOGT) respectively. Care was taken to ensure that each combination had the same weight (50 g). The total application per 5 kg bowl was 50 g, amounting to 10% w/w. For double mix, 25 g each of both wastes was added; whereas in the triple mix, 15.6 g of each waste was used. The study was observed for 12 weeks following which total hydrocarbon content as well as soil bacterial and fungal compositions were determined.

Data analysis

The data collected from the experiment was subjected to analysis of variance (ANOVA) for randomized complete block design and the differences between treatment means were separated using Fisher's least significant difference at $P=0.05$. The IBM SPSS software version 20®, was used to run the analysis.

Results and Discussion

Soil physicochemical properties of the soil and waste engine oil

The physicochemical properties of the soil and waste engine oil before soil contamination is presented on Table 1. The soil was ferruginous, with a total soil nitrogen content of 0.17%. The total hydrocarbon (THC) contents of both the soil and waste engine oil (WEO) were 7.20 mg/kg and 530101.67 mg/kg respectively. Both the soil and WEO were acidic (pH=5.27 and 5.03 respectively).

Culturable bacterial counts and isolates

The culturable bacterial counts and isolates of treatment substrates before and after exposure to oil contamination is reported (Table 2). Before soil pollution with WEO, the bacterial count was 1.79×10^5 $cfug^{-1}$, but reduced to 0.11×10^5 $cfug^{-1}$ after pollution. Although the control soil was not polluted with oil, its bacterial count reduced to 1.03×10^5 $cfug^{-1}$ over the 12-week period. There was a significant reduction ($P<0.05$) in bacterial count upon soil contamination with oil. However, WEO had no significant changes in bacterial count of soils amended singly (PS+GD, PS+PO, PS+RD), compared to the significant improvement in the amended soil of PS+RBPO respectively. Prominent bacterial isolates were *Bacillus* sp. and *Proteus* sp. The reduction in bacterial count observed in the present study could be as a result of immediate change in the microenvironment of the bacteria. Waste engine oil contains some toxic fractions that can kill or inhibit the growth of microorganisms (Obire and Anyawau, 2009). As oil is immiscible with water, it tends to cause water unavailability forming a film (Ohanmu *et al.*, 2014), that prevents microorganisms from accessing the available water. One of the reasons behind the decrease in the bacterial count is the absence of oxygen. The WEO covered

the air pores through which obligate aerobes receive oxygen. It could be suggested that proliferation of obligate aerobes was halted following the exogenous introduction of WEO (Farhana *et al.*, 2010). Sutton *et al.* (2013) reported that oil initiates anaerobic environment and asphyxiate aerobic microorganisms in the soil by disorganizing soil particles and blocking air diffusion in the soil pores.

Table 1. Physicochemical properties of soil and waste engine oil before soil pollution

Parameters	Soil	Waste engine oil (WEO)
pH	5.27	5.03
Electrical conductivity (µs/cm)	301.34	ND
Total organic carbon (%)	0.41	ND
Total Nitrogen (%)	0.17	ND
Exchangeable acidity (meq/100 g soil)	0.29	ND
Na (meq/100 g soil)	9.94	ND
K (meq/100 g soil)	1.52	ND
Ca (meq/100 g soil)	12.92	ND
Mg (meq/100 g soil)	11.94	ND
NO^{-3} (mg/kg)	28.49	ND
Clay (%)	5.13	ND
Silt (%)	12.06	ND
Sand (%)	82.81	ND
Fe (mg/kg)	1011.92	ND
THC	7.20	530101.67

Table 2. Culturable bacterial counts and isolates from treatment substrates before and after exposure to oil pollution

Treatments	Bacterial count ($\times 10^5$ cfug^{-1})			Isolates
	1WAP	12WAP	p-value	
Control (No oil)	1.79	1.03	*0.328*	*Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Streptococcus* sp.
PS only	0.11	0.16	*0.311*	*Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Streptococcus* sp.
PS+GD	0.37	0.40	*0.303*	*Bacillus* sp., *Enterobacter* sp., *Streptococcus* sp.
PS+PD	0.22	0.27	*0.527*	*Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp.
PS+RD	0.40	0.37	*0.460*	*Bacillus* sp., *Proteus* sp.
PS+RBPO	0.39	8.50	*<0.001*	*Bacillus* sp., *Proteus* sp., *Staphylococcus* sp., *Pseudomonas* sp.
PS+RBGT	3.50	4.10	*0.068*	*Bacillus* sp., *Enterobacter* sp., *Streptococcus* sp., *Proteus* sp.
PS+RBPOGT	1.40	1.10	*0.141*	*Bacillus* sp., *Enterobacter* sp., *Streptococcus* sp., *Proteus* sp., *Staphylococcus* sp., *Pseudomonas* sp.
PS+POGT	1.70	1.68	*0.742*	*Bacillus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Enterobacter* sp., *Streptococcus* sp.
p-value	*0.022*	*<0.001*		-

*1WAP – One week after pollution; 12WAP – 12 weeks after pollution. PS-polluted soil, GD- Goat dung, PD- Poultry dung, RD- Rabbit dung, RBGT- Rabbit + Goat dung, RBPO- Rabbit + Poultry dung, POGT- Poultry + Goat dung, and RBPOGT- Rabbit + Poultry + Goat dung

Changes in percentage culturable bacterial count

The percentage changes in culturable bacterial count in the treated soil are presented (Table 3). There was a 93.9% decrease in bacterial colony count in the oil-polluted soil compared to the unpolluted soil at the first week after soil pollution (1 WAP). However, at the 12th week there was a slight improvement in colony count. The reduction in bacterial colony attributed to oil pollution reduced from -93.9% to -84.5%. Similarly, reduction in bacterial colony attributed to oil pollution also reduced from -77.7% to -64.1% in the rabbit dung amended soil. Significantly, there was over 700% increase in bacterial colony at 12 WAP compared to what obtained at the first week, when oil resulted in 78.2% reduction in colony count. Significant reduction in bacterial count was not observed in oil-polluted soils amended with goat dung (GD), poultry dung (PD), and rabbit dung (RD). Generally, microorganisms require nutrients for their growth and development (Mishra *et al*., 2004) and their proliferation drops as nutrients are used up. The decrease observed in unpolluted soil overtime could be due to progressive drop in nutrient composition of the soil under experimental conditions.

In the case of oil-polluted soil, bacterial community faced a cascade of unfavourable environment ranging from anoxia to presence of toxic chemicals; dehydration to decrease in nutrient composition (Ekpo and Nwaankpa, 2005; Akinde and Obire, 2008). However, when oil-polluted soil was augmented with GD, PD, and RD, there was a significant difference in bacterial count. The present investigation revealed that addition of GD, PD, and RD replenished the basic nutrients required for the growth and development of heterotrophic bacteria and it resulted in improved bacterial count over the 12-week period. This observation agrees with the findings of Obiakalaije *et al.* (2015) who reported an increase in bacterial population in a crude polluted soil after amendment with animal waste. Enhanced microbial counts for hydrocarbon utilizing bacteria, total heterotrophic bacteria, total heterotrophic fungi and hydrocarbon consuming fungi in crude oil-polluted soils amended with organic and inorganic nutrient sources have been investigated by other researchers (Eziuzor and Okpokwasili, 2009; Chikere *et al*., 2012; Orji *et al*., 2012). The following bacterial genera, *Acinetobacter, Alcaligenes, Arthrobacter, Staphylococcus, Bacillus, Flavobacterium, Norcardia* and *Pseudomonas* have been described by Leahy and Colwell (1990) as the renowned hydrocarbon degraders in marine sediments. In the present study, *Bacillus sp* and *Proteus sp* were the prominent isolates.

Table 3. Percentage changes in culturable bacterial count

Treatments	**Δcfu (1WAP) (%)**	**Δcfu (12WAP) (%)**
PS only	-93.9	-84.5
PS+GD	-79.3	-61.2
PS+PD	-87.7	-73.8
PS+RD	-77.7	-64.1
PS+RBPO	-78.2	+725.3
PS+RBGT	+96.1	+298.4
PS+RBPOGT	-21.8	+7.2
PS+POGT	-5.0	+65.2
p-value	<0.001	<0.001

Δcfu changes in colony count; 1WAP – One week after pollution; 12WAP – 12 weeks after pollution. Values represent percentage reduction (-ve) or increase (+ve) in total bacterial count compared to original soil used in the study. PS-polluted soil, GD- Goat dung, PD- Poultry dung, RD- Rabbit dung, RBGT- Rabbit + Goat dung, RBPO- Rabbit + Poultry dung, POGT- Poultry + Goat dung, and RBPOGT- Rabbit + Poultry + Goat dung

Cultural fungal count and isolates

Results of the study in Table 4 show that there was reduction (*P>0.05*) in fungal count from 0.83× 10^5 cfug^{-1} to 0.68× 10^5 cfug^{-1} with time in the control soil. However, soil amended with RBPO, RBGT, POGT and RBPOGT resulted to significant increase in fungal count after 12 weeks. *Penicillium notatum*, *Aspergillus niger, A. flavus, Mucor mucedo,* and *Rhizopus* sp. were the fungal isolates obtained in the control soil prior to oil

pollution. *A. niger* was present in all treatments irrespective of amendments. Comparatively, the present study reported a minimal reduction ($p>0.05$) in fungal count in control soil within a space of 12 WAP compared to that of bacterial count. This result is based on the fact that fungal species have higher capacity to thrive under unfavorable condition than bacterial species under the same experimental condition. Although some bacteria species can produce spores, most fungi produce spores that can thrive under unfavorable condition. *Bacillus sp* was the only bacterial isolate in the present study that are capable of producing spores while all the fungi isolates produce spores. Thus, fungal count reduced minimally compared to bacterial count.

The fungal species isolated in this study were *Penicillium notatum*, *Aspergillus niger, A. flavus, Mucor mucedo,* and *Rhizopus* sp. These are hydrocarbon-degrading fungi and their bioremediation capacity has been examined in previous studies (Mukala *et al.*, 1975; Obire and Anyawu, 2009). However, reduction in fungal count was reported for 12 WAP due to effects of waste engine oil pollution. In this study, the reported significant increase in fungal count upon the application of soil amendments, either singly, or in combinations is not different from previous investigations (Ekpo and Ebeagwu, 2009; Nwogu *et al.*, 2015; Obiakalaije *et al.*, 2015). Of all fungi species isolated and identified in the present study, *Penicillium notatum* and *Aspergillus niger* were the most successful at thriving under the influence of petroleum hydrocarbon pollution and the application of amendments further improved their proliferation. These fungal species have the capacity to enhance bioremediation of waste engine pollution.

Table 4. Culturable fungal counts and isolates from treatment substrates before and after exposure to oil contamination

Treatments	Fungal count ($\times 10^5$ cfug^{-1})		p-value (1WAP vs 12WAP)	Isolates
	1WAP	12WAP		
Soil (no oil)	0.83	0.68	***0.153***	*Penicillium notatum*, *Aspergillus niger, A. flavus, Mucor mucedo, Rhizopus* sp.
PS only	0.06	0.14	***0.062***	*P. notatum, A. niger, A. flavus, M. mucedo, Rhizopus* sp.
PS+GD	0.06	0.32	***0.059***	*A. niger, A. flavus, M. mucedo*
PS+PD	0.08	0.11	***0.094***	*A. niger, P. notatum*
PS+RD	0.10	0.17	***0.152***	*M. mucedo, P. notatum, A. niger*
PS+RBPO	0.50	1.10	***0.048***	*M. mucedo, P. notatum, A. niger*
PS+RBGT	0.70	1.40	***0.043***	*M. mucedo, P. notatum, A. niger, A. flavus*
PS+RBPOGT	0.60	1.00	***0.052***	*M. mucedo, P. notatum, A. niger, A. flavus*
PS+POGT	0.60	1.17	***0.049***	*P. notatum, A. niger, A. flavus, M. mucedo*
p-value	***0.031***	***0.015***		-

1WAP – One week after pollution; 12WAP – 12 weeks after pollution. PS-polluted soil, GD- Goat dung, PD- Poultry dung, RD- Rabbit dung, RBGT- Rabbit + Goat dung, RBPO- Rabbit + Poultry dung, POGT- Poultry + Goat dung, and RBPOGT- Rabbit + Poultry + Goat dung

Changes in percentage culturable fungal count

The percentage changes in culturable fungal count in the treatment amended soils are show in Table 5. Generally, when oil-polluted soils were amended with dung in the duos or trios' combinations, fungal colonies increased. Take for instance, PS+RBGT significantly increased when compared to PS+RD or PS+GD 12 WAP. It could be inferred that addition of rabbit and goat wastes enhanced the growth and development of the fungal species through nutrient replenishment. This could be ascribed to the presence of appreciable quantities of nitrogen and phosphorous in animal waste, two necessary nutrients for bacterial biodegradation activities (Adesodun and Mbagwu, 2008). Also, the presence of indigenous microorganisms in the animal waste

could be responsible for the higher total heterotrophic and hydrocarbon utilizing fungal counts in amended samples.

Nwogu *et al.* (2015) observed that the total heterotrophic fungi and total hydrocarbon utilizing fungi increased with time during their study. This resulted in a corresponding removal of hydrocarbon with time particularly in the nutrient amended samples thus agreeing with the inference drawn from the present findings. It is needful to highlight the fact that rabbit dung was prominent in all amendment combinations that resulted in increased percentage culturable fungi colonies. The observed synergistic action of heterotrophic fungi, hydrocarbon utilizing fungi, heterotrophic bacteria, and hydrocarbon utilizing bacteria was hugely responsible for the reduction in total hydrocarbon content (THC) of the waste engine oil-polluted soil. The decrease in THC indicated that the microorganisms have metabolized the carbon content of the toxicant and probably converted the harmful polycyclic aromatic hydrocarbons into their non-toxic and environmental-friendly forms.

Table 5. Percentage changes in culturable fungal count

Treatments	Δcfu (1WAP) (%)	Δcfu (12WAP) (%)
PS only	-92.77	-79.41
PS+GD	-92.77	-52.941
PS+PD	-90.36	-83.82
PS+RD	-87.95	-75.01
PS+RBPO	-39.76	+61.76
PS+RBGT	-15.66	+105.9
PS+RBPOGT	-27.71	+47.06
PS+POGT	-27.71	+61.76
p-value	0.035	<0.001

Δcfu changes in colony count; 1WAP – One week after pollution; 12WAP – 12 weeks after pollution. Values represent percentage reduction (-ve) or increase (+ve) in total fungal count compared to original soil used in the study. PS-polluted soil, GD- Goat dung, PD- Poultry dung, RD- Rabbit dung, RBGT- Rabbit + Goat dung, RBPO- Rabbit + Poultry dung, POGT- Poultry + Goat dung, and RBPOGT- Rabbit + Poultry + Goat dung

The remediation efficacies of the poultry waste amendments

The remediation efficacies of the various tested soil amendments were ascertained by using total hydrocarbon contents of soil after application of soil amendments (Table 6). The results showed significant decreases in THC upon amendment with livestock dung. THC of the oil-polluted soil was 9984.0 mg/kg, compared to 3170.6 mg/kg in RBGT. This amounted to a 76.77% remediation efficiency of the combined animal wastes. Contamination factor at the 12th week (CF_{final}) was 1385.01 in the oil-polluted soil compared to initial CF of 1893.36; indicating a 26.83% reduction in CF. The highest percentage reduction in CF was obtained with oil-polluted soil amended with RBGT (76.8%). The decline in THC was probably because heterotrophic fungi, hydrocarbon utilizing fungi, heterotrophic bacteria, and hydrocarbon utilizing bacteria initiated the utilization of the carbon compounds in the soil. Previous studies have revealed that different microorganisms have the capacity to metabolize organic compounds of different carbon chains (Ijah and Antai, 2003; Shabir *et al.*, 2008; Lladó *et al.*, 2012). The livestock dung introduced in the oil-polluted soil acted as biostimulant. The indigenous microbiota utilized the mineral content (N, P, and K) of the dung and then turned to the petroleum hydrocarbon in the soil as source of carbon. Okolo *et al.* (2005), and Okieimen and Okieimen (2005) worked independently and shared similar reports on biodegradation of petroleum hydrocarbon polluted soil using animal wastes as biostimulants. The researchers recounted that degradation of crude oil was enhanced when animal manures were used as biostimulants. This confirms the findings of the present study in which THC of oil-polluted soil decreased with time when amendments were added compared to oil-polluted soil without such amendments.

Interestingly, one of the significant observations made in the present study was the fact that remediation efficiency was highest (76.77 %) in oil-polluted soil that was amended with a mixture of rabbit and goat dung (RBGT) compared to what was obtained in other amendments; needless to mention unamended soil. Comparable observations were recorded in the research carried out by Nwogu *et al.* (2015) although these investigators worked with only goat dung unlike the present study in which a mixture of dungs were employed. Contamination factor was computed at 12 weeks after amendment and results showed that oil-polluted soil without amendment had the highest contamination factor whereas the oil-polluted soil in which RBGT was used as biostimulant had the lowest contamination factor.

Table 6. Concentration of THC in the soil at 12 weeks after amendment

Treatment	Concentration of THC (mg/kg)	$CF_{initial}$ (%)	CF_{final} (%)	ΔCF (%)	Remediation efficiency (%)
PS only	9984.902^{a}	1893.36	1385.01	26.8	26.83
PS+GD	7097.147^{b}	1893.36	984.17	48.0	47.99
PS+RD	4890.207^{cd}	1893.36	677.82	64.2	64.17
PS+PD	7129.800^{b}	1893.36	988.70	47.8	47.76
PS+RBGT	3170.614^{d}	1893.36	439.12	76.8	76.77
PS+RBPO	5619.595^{c}	1893.36	779.07	58.9	58.82
PS+POGT	6400.003^{bc}	1893.36	887.40	53.1	53.11
PS+RBPOGT	5007.554^{cd}	1893.36	694.11	63.3	63.31
p-value	0.024	-	-	-	-

Values for THC on the same column having similar alphabetic superscripts don not differ from each other ($p>0.05$). CF – contamination factor, ΔCF (%) – Percentage reduction in CF over remediation period. PS-polluted soil, GD- Goat dung, RD- Rabbit dung, PD- Poultry dung, RBGT- Rabbit + Goat dung, RBPO- Rabbit + Poultry dung, POGT- Poultry + Goat dung, and RBPOGT- Rabbit + Poultry + Goat dung

Bioreclamation of the oil-polluted ultisol

One of the parameters for assessing bioreclamation of the oil-polluted soil after amendment with livestock wastes was by assessing plant recovery potential (Table 7). In the present study, this was determined by comparing percentage of plant stands that recovered after exposure to experimental conditions when compared to unpolluted soil. There was a 32.6% recovery for plants in oil-polluted soil amended with RBGT and 30.2% for POGT-amended soils. Percentage growth suppression by oil pollution was also used for assessing bioreclamation of the oil-polluted soil after amendment. Statistically, there were reductions in plant suppression percentage when soils where amended with combinations of animal wastes than when applied in singles.

Table 7. Bioreclamation of the oil-polluted soil after amendment with livestock wastes

Treatment	*Plant stands count (>3cm ht.)	Phytorecovery efficiency (%)	Plant suppression (1-recovery) (%)
Soil (unpolluted)	86^{a}	Nil	Nil
PS only	9^{e}	10.5	89.5
PS+GD	13^{de}	15.1	84.9
PS+RD	15^{cde}	17.4	82.6
PS+PO	23^{bcd}	26.7	73.3
PS+RBGT	28^{b}	32.6	67.4
PS+RBPO	18^{bcde}	20.9	79.1
PS+POGT	26^{bc}	30.2	69.8
PS+RBPOGT	16^{cde}	18.6	81.4
p-value	<0.001	-	-

*Values have been presented to the nearest whole number. Means on the same column having similar alphabetic superscripts do not differ from each other ($p>0.05$). PS-polluted soil, GD- Goat dung, RD- Rabbit dung, PD- Poultry dung, RBGT- Rabbit + Goat dung, RBPO- Rabbit + Poultry dung, POGT- Poultry + Goat dung, and RBPOGT- Rabbit + Poultry + Goat dung

The plant recovery potential was established by comparing the percentage of plants stands that recovered after exposure to oil-polluted soils when compared to the unpolluted soil. This assessment was necessitated because having been reclaimed or biodegraded, the previously polluted soil would be used mainly for plant production and must support plant growth and development. Here, 32.6% recovery was recorded for plants in oil-polluted soil amended with RBGT, while those amended with POGT had 30.2%. These findings, as recounted in the present investigation, hold out RBGT as potential biostimulant combination that can successfully be employed in bioreclamation of oil-polluted soils. Also, plant suppression percentage was utilized to ascertain the best practice in the usage of biostimulants in bioremediation. It was discovered that there were reductions in plant suppression percentage when soils were amended with combinations of animal wastes than when applied in singles. Bonilla *et al.* (2012) opined that organic amendments have suppressive effects on plant health through the associated microbiota. This can be estimated by using several parameters including number of plants and plant dry weight (Tuyen *et al.*, 2018). In the present study, plant suppression percentage was estimated using the number of plants standing.

Conclusions

The enhancement of the soil quality of an oil-polluted ultisol by livestock wastes has been investigated. Inferences made during the study showed that waste products from farm animals can be used as biostimulants in restoring an arable land that has been polluted with petroleum hydrocarbon. Several fungal and bacterial species were isolated and characterized in the course of the investigation in which rabbit, goat, and poultry wastes were specifically used. *Penicllium notatum* and *Aspergillus niger* as well as *Bacillus sp* and *Proteus sp* were the prominent fungal and bacterial species identified respectively. This work demonstrated that upon application of the animal wastes in oil-polluted soil, these microorganisms proliferated and then utilized the carbon content of the oil-polluted soil thereby orchestrating the biodegradation of the pollutant. Among several trials employed in this study, the combinations of rabbit and goat wastes proved to be more effective in reducing the total hydrocarbon content of oil-polluted soil and therefore could be recommended as a potential candidate for implementation in bioremediation of petroleum hydrocarbon polluted ultisol.

Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors. The researchers are grateful to members in the Department for their assistance during this study.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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