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Comparative Effect of Cattle Rumen Digesta and Poultry Droppings in Bioremediation of Spent Engine Oil-Contaminated Soil

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Abstract. *The study was conducted to compare the bioremediation potentials of two organic wastes (Cattle Rumen Digesta-CRD and Poultry Droppings-PD) in SEO contaminated soil in Dutse, Jigawa State. About 3 kg of soil was contaminated with SEO at 3 levels (0, 100 and 150 mL/pot). After 2 weeks of contamination, CRD and PD were added at 0 and 20 g/pot and thoroughly mixed. It was a 2 x 2 x 3 factorial experiment in completely randomized design replicated thrice. The incubation study lasted for 12 weeks. Data were collected on the Total Petroleum Hydrocarbon (TPH), bacterial and fungal counts of the SEO contaminated soil and analyzed using ANOVA at $p<0.05$. Results obtained shows that CRD and PD application at 20 g/pot yielded significantly ($p<0.05$) lower residual TPH contents (375 mg/kg and 704 mg/kg) of the SEO-impacted soil compared to the control. However, the residual TPH content of the contaminated soil obtained from the combination of 20 g/pot CRD with 100 mL/pot SEO was significantly ($p<0.05$) lower (252 mg/kg) compared to the residual TPH content (641 mg/kg) obtained from the combination of 20 g/pot PD and 100 mL/pot SEO. Interaction of 20 g/pot CRD with 100 mL/pot SEO had higher bacterial and fungal counts (21.03 and 16.80 CFU/g soil) compared to combination of 20 g/pot PD with 100 mL/pot SEO (16.48 and 15.80 CFU/ g soil). Thus, it is concluded that cattle rumen digesta has a higher biodegradation capacity than poultry droppings and was more effective in the bioremediation of SEO contaminated soil.*

Keywords: *Bioremediation, cattle rumen digesta, poultry droppings, spent engine oil, contaminated soil*

1. Introduction

A significant issue brought on by the world's rapid industrialization and urbanization is soil contamination by crude oil and its derivatives (Polyak et al., 2018). Cars, generators and industrial machines usage have skyrocketed in recent years due to the population growth. As a result, during vehicle, generators and industrial machines maintenance and servicing, significant amounts of spent engine oil are produced and dumped indiscriminately into the soil. The dumping of spent engine oil in the soil is hazardous to the ecosystem since it is not recyclable and has no significant additional usage due to the presence of impurities (Bala et al. 2019; Eze et al. 2019; Nna Orji et al., 2018a).

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These contaminants and additives as well as inappropriate disposal techniques, make Spent Engine Oil (SEO) pollution one of the most significant environmental issues in most of the world, particularly in developing countries like Nigeria, where it is even more common than crude oil spills (Abioye et al. 2012). According to Nwite et al. (2016), when spent engine oil enters the environment through soil or waterways, it can have both immediate and long-term effects. In addition to being mutagenic and carcinogenic to humans, spent engine oil pollution and related contaminants alter the nutritional composition available to soil organisms. As a result, soils lose their water holding capacity, their aeration qualities, etc. (Nwite and Alu 2015) thereby impacting the fertility status of the soil negatively manifesting in poor and stunted plant growth and yield (da Silva Correa et al., 2022, Huang et al., 2019). Petroleum hydrocarbon-impacted soil has been treated using a variety of remediation techniques, including solidification and incineration, oil booms, soil washing, and soil vapour extraction. Nevertheless, these conventional physico-chemical methods are costly, time-consuming, and disruptive (Koshlaf and Ball, 2017).

Bioremediation, the use of living organisms (bacteria, fungi and plants) to eliminate, destroy, or transform contaminants into less hazardous substances in the environment or to levels below concentration limits established by regulatory authorities (Abatenh *et al*., 2017), is cost effective, eco-friendly and safer (Tripathi et al., 2021). It can be carried out both in situ and ex situ depending on the cost, soil properties, type of pollutant and concentration levels (Bala et al., 2022). Microorganisms use this naturally occurring degradation process to get energy and nutrition by breaking down complex organic compounds into innocuous substances like water, fatty acids, and carbon dioxide (Osinowo et al., 2020). According to Bala et al. (2022) and Zhang et al. (2020), natural attenuation, biostimulation, Biocharging, biougmentation, and bioventing are various bioremediation techniques. Romantschuk et al. (2023), Curiel-Alegre et al. (2022), and Goswami et al. (2018), stated that biostimulation aims to promote the growth of naturally occurring bacteria for the breakdown of toxins while making sure that any obstacles to this process are eliminated. Biostimulation has been utilized extensively and is very effective, economical, and environmentally friendly (Goswami et al., 2018). There have been reports of the use of organic wastes as stimulants, including cattle dung, saw dust and poultry droppings (Hanson-Akpan et al., 2023), goat droppings (Ogujoifor et al., 2021), fish waste and goat manure (Awari et al., 2020), moss and compost (Mushtaq et al., 2020), water hyacinth compost ((Udume et al., 2023), and compost made from cocoa pod husk and cattle dung (Nkereuwem et al., 2022), in bioremediation of spent engine oil contaminated soil.

The need to reclaim spent engine oil-impacted soil for agricultural and other recreational purposes through bioremediation cannot be over-emphasized. Thus, the study was aimed at the comparative effect of cattle rumen digesta and poultry droppings as biostimulants in bioremediation of spent engine oil contaminated soil.

2. Materials and Methods

2.1. Description of the Study Area

The research was carried out at the Department of Soil Science Teaching and Research Farm, Federal University Dutse, Jigawa State. The area is located on Latitude 11°06'39''N and longitude 9°20'3''E, which is within the derived Sudan Savannah of the Northwest agro-ecological zone. This area experiences two distinct seasons: wet and dry.

The climate is tropically damp and dry, with a cold spell occurring between November and February. The mean monthly temperature ranges from 21° C during the coldest months (November–February) to 38° C during the hottest months (March–May), with an average annual temperature of 26° C (Peel et al., 2007).

2.2. Experimental Materials and Design

The materials used in this study included soil samples, cattle rumen digesta, poultry droppings, polyethylene bags, and spent engine oil. The cattle rumen digesta was obtained from Dutse abattoir, Dutse while the poultry dropping was sourced from the poultry unit, Teaching and Research Farm, Federal University Dutse, Jigawa State. The experiment was laid out in a 2 × 2 × 3 factorial experiment using completely randomized design with 3 replications; giving a total of 12 treatment combinations and 36 experimental units. The factors are listed below:

Poultry droppings (2 levels)

P_0------Without poultry droppings (0 g/pot)

P_{20}------With poultry droppings (20 g/pot)

Cattle rumen digesta (2 levels)

C_0--------Without cattle rumen digesta (0 g/pot)

C_{20}--------With cattle rumen digesta (20 g/pot)

Spent engine oil (3 levels)

S_0---------0 mL/3kg soil

S_{100}---------100 mL/3 kg soil

S_{150}---------150 mL/3 kg soil.

2.3. Soil samples Collection, Preparation and Incubation Study

Soil samples were collected at the study site from 0-20 cm depth using a shovel. The samples were bulked, crushed, air-dried, and passed through a 2 mm sieve. After soil preparation, 3 kg of the soil sample was potted in each polyethylene bag.

Spent engine oil (0, 100 and 150 mL) was mixed thoroughly with the soil and allowed to stand for about 2 weeks. The reason for the two weeks interval is to allow for the early breakdown of some oil components thereby ensuring a more stable soil condition prior to applying amendments. Additionally, the delay lowers possible toxicity and increases the amendment's efficacy by allowing some volatile chemicals to evaporate. Cattle rumen digesta and poultry droppings were applied after 2 weeks at the rate of 0 and 20 g/pot. This was mixed thoroughly with the soil for even distribution. Each treatment was arranged at 0.5 m between treatment and 1 m between replications. The incubation study lasted for twelve (12) weeks after which, soil samples were collected for laboratory analyses.

2.4. Laboratory Analysis

Particle size analysis was done using the hydrometer method (Bouyoucos, 1951) while organic carbon was determined using the Walkley Black Method (1934). Total Nitrogen and Av. P were determined using Kjeldahl method as described by Bremmer (1996) and the Olsen method, respectively. Concentrations of calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) were determined using a flame photometer (Jenway model) to evaluate soil cation-exchange capacity and fertility (Juo et al., 1976), Electrical

conductivity was assessed using the method outlined by FAO (2021) while Hanna's digital pH meter was used to determine the soil pH (McLean, 1982). The plate count approach was used to estimate the number of viable bacteria and fungi as described by Ochei and Kolhatkar (2008). Gram staining was carried out according to Barrow and Feltham (1993) while the procedure established by Ochei and Kolhatkar (2008), was employed in catalase, oxidase and indole test. The methods of Wilson (2012) and Olutiola *et al.* (1991) were adopted for lactose and starch hydrolysis tests, respectively.

2.5. Total Petroleum Hydrocarbon Determination

The Total Petroleum Hydrocarbon (TPH) content from the spent engine oil contaminated soil was determined according to the procedure described in Nkereuwem et al. (2022). The TPH was analyzed at Analytical Concept Limited, Port Harcourt, Rivers State, Nigeria.

2.6. Data Analysis

Data generated were subjected to Analysis of Variance (ANOVA) using PROC GLM of GENSTAT (17th Edition) and significant means were separated using Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT).

3. Results and Discussion

3.1. Physical and Chemical properties of the experimental soil

The soil textural class is sandy loam with a total nitrogen, available phosphorus and Ph of 2.15 g/kg, 6.81 g/kg and 7.1, respectively.

Table 1. Physical and chemical properties of the experimental soil (pre-treatment)

Parameter	Value
Bulk Density (g/cm^3)	1.53
Particle size distribution (%)	
Sand	71
Silt	4
Clay	25
Textural class (USDA)	Sandy loam
Ph	7.1
Electrical conductivity (ds/m)	0.37
Organic carbon (g/kg)	1.93
Total nitrogen (g/kg)	2.15
Available phosphorus (g/kg)	5.31
Exchangeable Bases (cmol/kg)	6.34
Exchangeable Acidity (cmol/kg)	4.16
CEC (cmol/kg)	10.5

Table 2. Chemical properties of the experimental soil (Post-treatment)

Parameter	Value
Ph	6.8
Electrical conductivity (ds/m)	0.23
Organic carbon (g/kg)	3.17
Total nitrogen (g/kg)	3.53
Available phosphorus (g/kg)	9.81
Exchangeable Bases (cmol/kg)	12.26
Exchangeable Acidity (cmol/kg)	2.12
CEC (cmol/kg)	14.38

3.2. Effects of Poultry dropping, Cattle rumen digesta and spent engine oil concentrations on Total petroleum hydrocarbon content of contaminated soil

Poultry droppings application 20 g/pot yielded significantly lower Total Petroleum Hydrocarbon (TPH) content (704 mg/kg) compared to 0 g/pot poultry droppings application (2649 mg/kg) (Table 3). Significantly lower TPH content (375 mg/kg) was obtained from 20 g/pot cattle rumen digesta application as against 0 g/pot cattle rumen digesta (2978 mg/kg) (Table 3). Spent Engine Oil (SEO) at 100 mL/pot and 150 mL/pot resulted in significantly lower and higher TPH contents (1244 mg/kg and 3785 mg/kg), respectively, while 0 mL/pot SEO application was Below Detection Limit (BDL) (Table 3).

Table 3. Effects of poultry dropping, cattle rumen digesta and spent engine oil on total petroleum hydrocarbon content of contaminated soil

Treatment	Total petroleum hydrocarbon (mg/kg)
Poultry dropping (g/pot)	
P_0	2649a
P_{20}	704b
LSD (0.05)	133.8
SE (±)	64.5
Cattle rumen digesta (g/pot)	
C_0	2978a
C_{20}	375b
LSD (0.05)	133.8
SE (±)	64.5
Spent engine oil (mL/pot)	
S_0	BDL
S_{100}	1244b
S_{150}	3785a
LSD (0.05)	163.80
SE (±)	79.0

Means with the same letter (s) are not significantly different at $p>0.05$ using least significant difference. P1= 0 g/pot poultry dropping, P2 = 20 g/pot poultry dropping, C_0 = 0 g/pot cattle rumen digesta C_2 = 20 g/pot cattle rumen digesta, S_0 = 0 mL/pot spent engine oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil, LSD = least significant difference, SE = standard error, BDL = below detection limit

3.3. Interaction of cattle rumen digesta with spent engine oil concentrations on total petroleum hydrocarbon content of contaminated soil

Combined application of 20 g/pot cattle rumen digesta and 100 mL/pot SEO had significantly lower TPH content (252 mg/kg) compared to the other treatment combinations (Table 4) whereas, significantly higher TPH content (6698 mg/kg) was obtained from the combination of 0 g/pot cattle rumen digesta and 150 mL/pot SEO compared to the TPH content obtained from the combination of 0 g/pot cattle rumen digesta and 100 mL/pot SEO (Table 4).

3.4. Interaction of poultry droppings with spent engine oil concentrations on total petroleum hydrocarbon content of contaminated soil

Interaction between 20 g/pot Poultry Droppings (PD) and 100 mL/pot SEO yielded significantly lower TPH content (641 mg/kg) compared with the other interactions (Table 4). Significantly higher TPH content (6100 mg/kg) was obtained from the interaction of 0 g/pot PD and 150 mL/pot SEO compared to the TPH content (1848 mg/kg) obtained from the combined application 0 g/pot PD and 100 mL/pot SEO (Table 4).

Table 4. Interactions of cattle rumen digesta and spent engine oil, Poultry droppings and spent engine oil and Cattle rumen digesta and Poultry droppings on total petroleum hydrocarbon content of contaminated soil

Cattle rumen digesta (g/pot)	Spent engine oil (mL/pot)	Total petroleum hydrocarbon (mg/kg)
C_0	S_0	BDL
C_0	S_{100}	2237b
C_0	S_{150}	6698a
C_{20}	S_0	BDL
C_{20}	S_{100}	252d
C_{20}	S_{150}	872c
Poultry droppings (g/pot)	**Spent engine oil (mL/pot)**	**Total petroleum hydrocarbon (mg/kg)**
P_0	S_0	BDL
P_0	S_{100}	1848b
P_0	S_{150}	6100a
P_{20}	S_0	BDL
P_{20}	S_{100}	641d
P_{20}	S_{150}	1470c
Cattle rumen digesta (g/pot)	**Poultry droppings (g/pot)**	**Total petroleum hydrocarbon (mg/kg)**
C_0	P_0	4983a
C_0	P_{20}	973b
C_{20}	P_0	315c
C_{20}	P_{20}	434c
	LSD (0.05)	189.2
	SE (±)	91.2

Means with the same letter (s) are not significantly different at p>0.05 using Duncan's Multiple Range Test (DMRT). C_0 = 0 g/pot cattle rumen digesta C_{20} = 20 g/pot cattle rumen digesta, P_0 = 0 g/pot poultry dropping, P_{20} = 20 g/pot poultry dropping, S_0 = 0 mL/pot spent engine oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil, BDL = below detection limit, LSD = least significant difference, SE = standard error

3.5. Interaction of cattle rumen digesta and poultry droppings on total petroleum hydrocarbon content of spent engine oil contaminated soil

Combined application of 20 g/pot cattle rumen digesta with 0 g/pot PD resulted in significantly lower TPH content (315 mg/kg) compared to the other combinations (Table 4) while the combination of 0 g/pot cattle rumen digesta and 0 g/pot PD yielded significantly higher TPH content (4983 mg/kg) compared to the TPH obtained from the combination of 0 g/pot cattle rumen digesta and 20 g/pot PD (Table 4).

3.6. Effect of poultry droppings, cattle rumen digesta and spent engine oil concentrations on bacterial colony of contaminated soil

Table 5 shows that cattle rumen digesta application at 20 g/pot resulted in significantly higher bacterial population (17.19 CFU/g soil) compared to 0 g/pot application (13.16 CFU/g soil). The application of 100 mL/pot SEO produced significantly higher bacterial colony (17.73 CFU/g soil) compared to the other concentrations (Table 5) whereas, SEO application at 150 mL/pot yielded significantly lower bacterial colony (13.24 CFU/g soil) compared to SEO application at 0 mL/pot.

Table 5: Effect of poultry droppings, cattle rumen digesta and spent engine oil on bacterial colony in contaminated soil

Treatment	Bacterial colony (CFU/g soil)
Poultry dropping (g/pot)	
P_0	15.00
P_{20}	15.34
LSD (0.05)	0.378
SE (±)	Ns
Cattle rumen digesta (g/pot)	
C_0	13.16b
C_{20}	17.19a
LSD (0.05)	0.378
SE (±)	0.129
Spent engine oil (mL/pot)	
S_0	14.54b
S_{100}	17.73a
S_{150}	13.24c
LSD (0.05)	0.463
SE (±)	0.158

Means with the same letter (s) are not significantly different at p>0.05 using least significant difference. P_0= 0 g/pot poultry dropping, P_{20} = 20 g/pot poultry dropping, C_0 = 0 g/pot cattle rumen digesta C_{20} = 20 g/pot cattle rumen digesta, S_0 = 0 mL/pot spent engine

oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil, LSD = least significant difference, SE = standard error, Ns = not significant.

3.7. Interaction of cattle rumen digesta and spent engine oil concentrations on bacterial colony of contaminated soil

Interaction between 20 g/pot cattle rumen digesta and SEO at 100 mL/pot produced significantly higher bacterial colony (21.03 CFU/g soil) compared to the other treatment interactions (Table 6). Significantly lower bacterial colony (11.55 CFU/g soil) was obtained from the combined application of 0 g/pot cattle rumen digesta and 150 mL/pot SEO compared to the bacterial colony (13.48 CFU/g soil) obtained from the interaction between 0 g/pot cattle rumen digesta and 0 mL/pot SEO (Table 6).

Table 6. Interactions of cattle rumen digesta and spent engine oil, Poultry droppings and spent engine oil and Cattle rumen digesta and Poultry droppings on Bacterial colony of contaminated soil

Cattle rumen digesta (g/pot)	Spent engine oil (mL/pot)	Bacterial colony (CFU/g soil)
C_0	S_0	13.483d
C_0	S_{100}	14.433c
C_0	S_{150}	11.550e
C_{20}	S_0	15.600b
C_{20}	S_{100}	21.033a
C_{20}	S_{150}	14.933c
Poultry droppings (g/pot)	**Spent engine oil (mL/pot)**	**Bacterial colony (CFU/g soil)**
P_0	S_0	13.80d
P_0	S_{100}	18.98a
P_0	S_{150}	12.22e
P_{20}	S_0	15.28c
P_{20}	S_{100}	16.48b
P_{20}	S_{150}	14.27d
Cattle rumen digesta (g/pot)	**Poultry droppings (g/pot)**	**Bacterial colony (CFU/g soil)**
C_0	P_0	11.69d
C_0	P_{20}	14.62c
C_{20}	P_0	18.31a
C_{20}	P_{20}	16.07b
	LSD (0.05)	0.535
	SE (±)	0.183

Means with the same letter (s) are not significantly different at $p>0.05$ using Duncan's Multiple Range Test (DMRT). C_0 = 0 g/pot cattle rumen digesta C_{20} = 20 g/pot cattle rumen digesta, P_0 = 0 g/pot poultry dropping, P_{20} = 20 g/pot poultry dropping, S_0 = 0 mL/pot spent engine oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil, BDL = below detection limit, LSD = least significant difference, SE = standard error

3.8. Interaction of poultry droppings with spent engine oil concentrations on bacterial colony of contaminated soil

Poultry droppings at 0 g/pot combined with SEO at 100 mL/pot recorded significantly higher bacterial colony (18.89 CFU/g soil) compared to the other treatment combinations (Table 6). The interaction between 0 g/pot PD and 150 mL/pot SEO yielded significantly lower bacterial colony (12.22 CFU/g soil) compared to the bacterial colony (13.80 CFU/g soil) obtained from the combination of 0 g/pot PD and 0 mL/pot SEO (Table 6).

3.9. Interaction of cattle rumen digesta and poultry droppings on bacterial colony of contaminated soil

The combined application of 20 g/pot cattle rumen digesta with 0 g/pot PD produced significantly higher bacterial colony (18.31 CFU/g soil) compared to the other combinations (Table 6) whereas, the combination of 0 g/pot cattle rumen digesta with 0 g/pot PD yielded significantly lower bacterial population (11.69 CFU/g soil) compared to the bacterial colony count (14.62 CFU/g soil) obtained from the combined application of 0 g/pot cattle rumen digesta with 20 g/pot PD (Table 6).

3.10. Effect of poultry droppings, cattle rumen digesta and spent engine oil concentrations on fungal colony of contaminated soil

Poultry droppings at 20 g/pot produced significantly higher fungal colony count (12.64 CFU/g soil) compared to treatment devoid of PD (0 g/pot) (Table 7). Cattle rumen digesta application had significantly higher fungal colony (14.48 CFU/g soil) compared to treatment without cattle rumen digesta. Spent engine oil application at 100 mL/pot gave significantly higher fungal population (14.45 CFU/g soil) compared to the other concentrations (Table 7) whereas, significantly lower fungal population (9.68 CFU/g soil) was obtained from 150 mL/pot SEO though not significantly different from the fungal population obtained from 0 mL/pot SEO.

3.11. Interaction of cattle rumen digesta with spent engine oil concentrations on fungal colony of contaminated soil

Significantly higher fungal colony (16.80 CFU/g soil) resulted from the combination of 20 g/pot cattle rumen digesta and 100 mL/pot SEO compared to the other combinations (Table 8). The application of 0 g/pot cattle rumen digesta and 150 mL/pot SEO produced significantly lower fungal colony (6.00 CFU/g soil) compared to that obtained from the combination of 0 g/pot cattle rumen digesta and 0 mL/pot SEO.

3.12. Interaction of poultry droppings with spent engine oil concentrations on fungal colony of contaminated soil

Table 8 shows the interaction between poultry droppings with SEO on fungal population of contaminated soil. Treatment combination involving 20 g/pot PD and 100 mL/pot SEO produced significantly higher fungal colony (15.80 CFU/g soil) compared to the other combinations whilst the combined application of 0 g/pot PD and 150 mL/pot SEO yielded significantly lower fungal colony (9.14 CFU/g soil) compared to that obtained from the combined use of 0 g/pot PD and 0 mL/pot SEO.

Table 7. Effect of poultry droppings, cattle rumen digesta and spent engine oil concentrations on fungal colony of contaminated soil

Treatment	Fungal colony (CFU/g soil)
Poultry dropping (g/pot)	
P_0	10.80b
P_{20}	12.64a
LSD (0.05)	1.342
SE (±)	0.129
Cattle rumen digesta (g/pot)	
C_0	8.96b
C_{20}	14.48a
LSD (0.05)	1.34
SE (±)	0.129
Spent engine oil (mL/pot)	
S_0	11.03b
S_{100}	14.45a
S_{150}	9.68b
LSD (0.05)	1.64
SE (±)	0.158

Means with the same letter (s) are not significantly different at $p>0.05$ using least significant difference. P_0= 0 g/pot poultry dropping, P_{20} = 20 g/pot poultry dropping, C_0 = 0 g/pot cattle rumen digesta C_{20} = 20 g/pot cattle rumen digesta, S_0 = 0 mL/pot spent engine oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil, LSD = least significant difference, SE = standard error.

3.13. Interaction of cattle rumen digesta with poultry droppings on fungal colony of contaminated soil

Cattle rumen digesta application at 20 g/pot combined with 0 g/pot PD produced significantly higher fungal colony (15.03 CFU/g soil) compared to the other combinations (Table 8) however, this was not significantly different from the fungal colony (13.98 CFU/g soil) obtained from the combination of 20 g/pot cattle rumen digesta and 20 g/pot PD. The application of 0 g/pot cattle rumen digesta and 0 g/pot PD yielded significantly lower fungal population (6.57 CFU/g soil) compared to that obtained from the combination of 0 g/pot cattle rumen digesta and 20 g/pot poultry droppings.

Table 8. Interactions of cattle rumen digesta and spent engine oil, Poultry droppings and spent engine oil and Cattle rumen digesta and Poultry droppings on Bacterial colony of contaminated soil

Cattle rumen digesta (g/pot)	Spent engine oil (mL/pot)	Fungal colony (CFU/g soil)
C_0	S_0	8.77c
C_0	S_{100}	12.10b
C_0	S_{150}	6.00d
C_{20}	S_0	13.28b
C_{20}	S_{100}	16.80a
C_{20}	S_{150}	13.37b

Poultry droppings (g/pot)	Spent engine oil (mL/pot)	Fungal colony (CFU/g soil)
P_0	S_0	10.16cd
P_0	S_{100}	13.10b
P_0	S_{150}	9.14d
P_{20}	S_0	11.89bc
P_{20}	S_{100}	15.80a
P_{20}	S_{150}	10.23cd

Cattle rumen digesta (g/pot)	Poultry droppings (g/pot)	Fungal colony (CFU/g soil)
C_0	P_0	6.57c
C_0	P_{20}	11.35b
C_{20}	P_0	15.03a
C_{20}	P_{20}	13.98a
	LSD (0.05)	1.898
	SE (±)	0.2580

Means with the same letter (s) are not significantly different at $p>0.05$ using Duncan's Multiple Range Test (DMRT). C_0 = 0 g/pot cattle rumen digesta C_{20} = 20 g/pot cattle rumen digesta, P_0 = 0 g/pot poultry dropping, P_{20} = 20 g/pot poultry dropping, S_0 = 0 mL/pot spent engine oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil, BDL = below detection limit, LSD = least significant difference, SE = standard error

Table 9. Biochemical Characteristics of Bacteria Isolates in Experimental Soil

Treatment	Gram Rx	Catalase	Oxidase	Lactose	Indole test	Starch hydrolysis	Species identified
$C_0P_0S_0$	+	+	+	+	-	-	*Bacillus cereus*
$C_0P_0S_{100}$	+	+	+	-	-	+	*Proteus mirabilis*
$C_0P_0S_{150}$	-	+	-	-	+	-	*Proteus vulgaris*
$C_0P_{20}S_0$	+	+	+	+	-	+	*Bacillus megaterium*
$C_0P_{20}S_{100}$	-	+	+	-	-	+	*Proteus mirabilis*
$C_0P_{20}S_{150}$	+	+	+	+	-	+	*Bacillus megaterium*
$C_{20}P_0S_0$	-	+	+	+	-	+	*Bacillus licheniformis*
$C_{20}P_0S_{100}$	+	+	-	-	+	-	*Proteus vulgaris*
$C_{20}P_0S_{150}$	-	+	+	-	-	+	*Proteus mirabilis*
$C_{20}P_{20}S_0$	-	+	+	+	-	+	*Bacillus licheniformis*
$C_{20}P_{20}S_{100}$	-	+	-	-	+	-	*Proteus morganii*
$C_{20}P_{20}S_{150}$	-	+	-	+	-	-	*Flavobacterium aquatile*

C_0 = 0 g/pot cattle rumen digesta C_{20} = 20 g/pot cattle rumen digesta, P_0 = 0 g/pot poultry dropping, P_{20} = 20 g/pot poultry dropping, S_0 = 0 mL/pot spent engine oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil, + = positive; - = negative

Table 10. Fungi Species identified in the experimental soil

Treatment	Fungi species identified
$C_0P_0S_0$	*Aspergillus spp. Mucor spp.*
$C_0P_0S_{100}$	*Aspergillus spp. Mucor spp.*
$C_0P_0S_{150}$	*Aspergillus spp. Rhizospus spp.*
$C_0P_{20}S_0$	*Aspergillus spp. Mucor spp.*
$C_0P_{20}S_{100}$	*Rhizospus spp.*
$C_0P_{20}S_{150}$	*Aspergillus spp.*
$C_{20}P_0S_0$	*Aspergillus spp. Mucor spp.*
$C_{20}P_0S_{100}$	*Aspergillus spp. Rhizospus spp. and Penicillium spp.*
$C_{20}P_0S_{150}$	*Aspergillus spp. Mucor spp.*
$C_{20}P_{20}S_0$	*Aspergillus spp.*
$C_{20}P_{20}S_{100}$	*Aspergillus spp.*
$C_{20}P_{20}S_{150}$	*Aspergillus and Rhizospus spp.*

C_0 = 0 g/pot cattle rumen digesta C_{20} = 20 g/pot cattle rumen digesta, P_0 = 0 g/pot poultry dropping, P_{20} = 20 g/pot poultry dropping, S_0 = 0 mL/pot spent engine oil, S_{100} = 100 mL/pot spent engine oil, S_{150} = 150 mL/pot spent engine oil.

The results of this research showed that cattle rumen digesta and poultry droppings effectively enhanced TPH reduction in SEO-impacted soil compared to treatments without the bio-stimulants. The significantly lower residual TPH in the spent engine oil contaminated soil illustrate the bio-stimulatory capacity of these bio-stimulants in bioremediation of oil-impacted soil as also reported by Nunes et al. (2020); Adeleye et al. (2021); Hanson-Akpan et al. (2023); Nkereuwem et al. (2024); Nna Orji (2024). The reduction in TPH may be the consequence of an increase in organic nutrients, which in turn leads to a larger population of microorganisms that use the hydrocarbon as a source of carbon and energy. Osazee et al. (2019) and Abdulkarim et al. (2019) also achieved similar outcomes. Furthermore, the results of this research shows that treatments with cattle rumen digesta resulted in significantly lower residual TPH content in the SEO-contaminated soil compared to poultry droppings amended treatment. This could be attributed to the fact that cattle rumen digesta contain more organic nutrients as a result of the presence of degradative microbes (bacteria, fungi, etc) in the rumen of cattle and it releases more nutrients into the soil (Ekpe et al., 2012b) thereby stimulating microbial activities. This results corroborates the findings of Cherdthong, (2020) who reported that cattle rumen digesta enhances nutrient utilization thus, reducing soil pollution. The outcome of this study also agrees with the findings of Nkereuwem et al. (2024), where they reported significantly lower residual TPH due to cattle rumen digesta application compared to mycorrhizal inoculation. Similar result was also obtained by Hanson-Akpan et al. (2023) who reported superior hydrocarbons degradation capacity of cattle dung in SEO-contaminated soil compared to poultry manure and saw dust.

From the results of this study, cattle rumen digesta and poultry droppings resulted in significantly higher bacterial and fungal populations compared to the control. According

to Ogujoifor et al. (2021) and Benchouk and Chibani (2017), this may be explained by the inherent nutrients, high organic matter content, and microorganisms found in cattle rumen digesta and poultry droppings, which promote the growth of microbes in the soil for the exclusive use of SEO as food, carbon, and energy source. This result is in agreement with the findings of Nkereuwem et al. (2022, 2024), Hanson-Akpan et al. (2023) and Nna Orji (2024), who also reported increase in microbial growth in spent engine oil contaminated soil due to organic amendments.

However, results of this research shows that bacterial and fungal counts were significantly higher in cattle rumen digesta amended treatments compared to poultry droppings amended treatments. This is due to high contents of organic nutrients and microorganisms in the rumen of cattle (Ekpe et al., 2012b), which stimulates microbial growth in the soil for the sole use of hydrocarbons as carbon and energy source. This results corroborates the findings of Nkereuwem et al. (2024), who reported significantly higher bacterial and fungal counts in cattle rumen digesta amended spent engine oil contaminated soil compared to mycorrhizal inoculated treatments. The result of the study reveals that three (3) bacteria genera were isolated and identified. The genera include *Bacillus*, *Proteus* and *Flavobacterium*. This is in agreement with previous findings by Rahman *et al*. (2002) and Nkereuwem et al. (2022), where they isolated crude oil degrading microorganisms belonging to the genera *Micrococcus*, *Corynebacterium*, *Bacillus*, *Enterobacteriaceae*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Moraxella*, *Aeromonas*, *Acinetobacter* and *Vibrio* from crude oil impacted soils.

The fungi species isolated and identified in this study includes: *Mucor spp, Aspergillus spp, Penicillium spp* and *Rhizospus spp*. This is in agreement with Agbor *et al*. (2012), Dawood *et al*. (2015) and Nkereuwem et al. (2020a), where they also identified these fungi species in crude oil contaminated soil. The dominance of *Aspergillus spp* in this study could be attributed to its efficiency in degrading petroleum hydrocarbons. According to Agbor *et al.* (2012), in comparison with other fungi genera, reported that *Aspergillus* and *Penicillium* species were the most efficient metabolizers of hydrocarbons. Conceicao *et al*. (2005), concluded that *Aspergillus* and *Penicillium* possess mechanisms to resist adverse environmental conditions and the ability to degrade oil residues.

Conclusion

The findings of the study shows the bio-stimulatory capacity of cattle rumen digesta and poultry droppings in bioremediation of SEO-contaminated soil. Nevertheless, cattle rumen digesta demonstrated better degradation potential compared to poultry dropping. Additionally, bacterial and fungal counts were higher in cattle rumen digesta amended treatments compared to poultry droppings amended treatments thus, resulting in lower TPH content in the contaminated soil. The study therefore conclude that cattle rumen digesta has a higher biodegradation capacity than poultry droppings and was more effective in the bioremediation of SEO contaminated soil.

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Conflict of interest

The authors declare no conflict of Interest.

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