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Research Article

POTENTIAL OF MYCORRHIZAL INOCULATION AND CATTLE RUMEN DIGESTA IN THE BIOREMEDIATION OF SPENT ENGINE OIL CONTAMINATED SOIL

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ABSTRACT

Soil pollution by crude oil contamination has become a major constraint on agricultural productivity. Physicochemical techniques are often expensive. However, bioremediation of petroleum hydrocarbon polluted soil is cost-effective. Therefore, the study was carried out to analyze the influence of mycorrhiza and cattle rumen digesta on bioremediation of Spent Engine Oil (SEO) contaminated soil in Dutse, Jigawa state. Soil samples were randomly collected from the University Research and Teaching Farm. About 2.5 kg of sterilized topsoil (0–15 cm) was filled into pots and arranged in a 2×2×3 factorial experiment in completely randomized design with three replications. Mycorrhiza and cattle rumen digesta were at two levels, while SEO was at three levels. Data were collected on the total petroleum hydrocarbon (TPH) content, bacterial and fungal colony count. Data were analyzed using ANOVA at α 0.05. Results obtained from the study show that mycorrhiza and cattle rumen digesta increased the colonies of fungi and bacteria resulting in significantly enhanced TPH degradation in the contaminated soil. However, cattle rumen digesta significantly ($p<0.05$) enhanced TPH degradation, bacterial and fungal population the most compared to mycorrhiza alone. Combined cattle rumen digesta and mycorrhiza application resulted in significantly ($p<0.05$) lower residual TPH content in the contaminated soil compared to using cattle rumen digesta or mycorrhiza alone. Thus, cattle rumen digesta and mycorrhiza should be used in bioremediation of petroleum hydrocarbon impacted soils.

Keywords: Mycorrhizal inoculation, Cattle rumen digesta, Bioremediation, Spent engine oil, Contaminated soil, Total petroleum hydrocarbon.

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INTRODUCTION

Soil pollution is a consequence of land degradation which is caused by the presence of synthetic chemicals or other changes to the natural soil in our surroundings (Richmond, 2015). Numerous activities can lead to pollution, such as careless garbage dumping, industrial procedures, and agricultural chemicals. The most often discovered contaminants that have been connected to soil pollution are pesticides, heavy metals, solvents, and petroleum hydrocarbons. Petroleum hydrocarbon-related soil pollution, according to Umoren *et al*. (2019), can linger for a number of years and alter the physicochemical quality of the soil. The rate of industrialization in any civilization and the quantity of chemicals discharged into the environment have a major impact on the amount of pollution that can accumulate (Akanksha, 2020). Crude oil contamination of agricultural soil often puts severe pressure on soil health and productivity (Odukoya *et al*., 2019).

Studies have shown that crude oil spillage on arable land has been on the increase since the 20th century when global production doubled (Onosode, 2003). Spent engine oil (SEO) is typically referred to as used motor oil that has been collected from mechanical workshops, garages, and industrial sources such as hydraulic oil, turbine oils, process oil, and metalworking fluids (Olufemi and Oladeji, 2008). Disposal of SEO into gutters, watercourses, open vacant plots, and farmland are common practice among auto machine operators (Adeleye *et al*., 2020). This practice practically increases the incidence of oil pollution of agricultural soils and the built environment.

Rumen digesta, a by-product of the abattoir, is mostly derived from the partially digested feed consumed by ruminants. Crude protein (13.5–46.1%) and a few necessary elements (microbial cells, amino acids, minerals, and volatile fatty acids) were found in the rumen digesta of cattle (Linn *et al*., 2021). Cattle rumen digesta is recommended for use in bioremediation because it enhances soil nutrient utilization and reduces pollution (Cherdthong, 2020). It is possible to both remediate the contaminated soil *in situ* and revegetate the region at the same time if the right mycorrhizal fungus is introduced into the soil (Tangahu *et al*., 2011; Nkereuwem *et al*., 2022). Over time, bioremediation technology has demonstrated positive results in eliminating hydrocarbon levels from affected ecosystems (Nkereuwem *et al*., 2022; Nkereuwem *et al*., 2020a; Adeleye *et al*., 2021). These bioremediation techniques are economically and ecologically attractive. It is based on this background information that this study was staged with a view to assessing the potential of remediating SEO-contaminated soil with cattle rumen digesta and mycorrhizal fungus.

METHODS

Description of the study area

The study was conducted at the Teaching and Research Farm, Department of Soil Science, Federal University Dutse, Jigawa State. Dutse is on latitude 11°42 '04"N, 9°20'31"E and longitude 11°.70'11"N, 9.3°41'94"E. There is little rainfall throughout the year, average precipitation of 743 mm falls annually with an average temperature of 26.5°C (Peel *et al*., 2007).

Experimental materials and design

The materials used in carrying out the experiment included soil sample, mycorrhizal inoculum (*Glomus mossaea*), polyethylene bags, cattle rumen digesta (dried), and SEO. Mycorrhiza inoculum was sourced from the Soil Microbiology Laboratory, Department of Soil Resources Management, University of Ibadan, Ibadan, Oyo State while the SEO was sourced from the Mechanic village, Dutse, Jigawa State. The experimental design was Completely Randomized Design (CRD). It

was a 2×2×3 factorial experiment with three (3) replicates. There were twelve treatment combinations and a total of 36 experimental units. The factors are listed below:

Mycorrhizal inoculum (G. mossaea) at two levels

- *G. mossaea* at 20 g/pot (GM^+)
- *G. mossaea* at 0 g/pot (GM^-)

Cattle rumen digesta at two levels

- Cattle rumen digesta at 0 g/pot (D_1)
- Cattle rumen digesta at 10 g/pot (D_2)

SEO at three levels

- SEO at 0 mL/pot (O_1)
- SEO at100 mL/pot (O_2)
- SEO at150 mL/pot (O_3)

Treatment combinations

- $GM^+D_1O_1$ $GM^-D_1O_1$
- $GM^+D_1O_2$ $GM^-D_1O_2$
- $GM^+D_1O_3$ $GM^-D_1O_3$
- $GM^+D_2O_1$ $GM^-D_2O_1$
- $GM^+D_2O_2$ $GM^-D_2O_2$
- $GM^+D_2O_3$ $GM^-D_2O_3$

Soil sample collection and preparation

The experimental soil was randomly collected from the Teaching and Research Farm, Federal University Dutse at a depth of 0–15 cm using a shovel. Afterward, the soil samples collected were bulked, air-dried, and crushed. The bulked soil was later sieved using 2-mm mesh size. Thereafter, SEO (100 and 150 mL/pot) was mixed thoroughly with the soil (2.5 kg/pot) and allowed to stand for 2 weeks for effective volatilization to occur. Cattle rumen digesta was added after 2 weeks at the rate of 10 g/pot. This was mixed thoroughly with the soil for even distribution. Inoculation for treatments containing *G. mossaea* consisted of 20 g of root soil-fungal mixture blended into the soil samples.

Incubation studies

The experimental soil used was sterilized in a drying oven at 85°C for 20 min and about 2.5 kg of the sterilized soil was then potted. A digital weighing scale was used to measure the two levels of mycorrhiza (0 and 20 g/pot), and two levels of cattle rumen digesta (0 and 10 g/pot), while a measuring cylinder was used to measure the SEO (0, 100 and 150 mL/pot). The pots were arranged at a spacing of 0.5 m between the pots and 1 m between replications. Mycorrhiza and cattle rumen digesta were added to the samples after 2 weeks. Treatments with mycorrhizal inoculation were thoroughly mixed with the soil. A similar technique was employed in the application of cattle rumen digesta. The incubation lasted for 12 weeks in the laboratory before the experiment was terminated.

Laboratory analyses

SEO contaminated soil samples were collected and taken to the laboratory whereby its physicochemical parameters, total petroleum hydrocarbon (TPH), and bacterial and fungal biomass were analyzed at the end of the experiment. Soil sample without SEO contamination was also collected for analysis before the commencement of the study. Particle size analysis of the soil was determined via the hydrometer method (Bouyoucos, 1951). Organic carbon was determined by adopting Walkley–Black method while available phosphorus was determined using the Olsen method. The exchangeable bases (Ca, K, Mg, and Na) were determined using a flame photometer (Jenway model). Electrical conductivity was determined using a method described by FAO (2021). The soil pH was determined using Hanna's digital pH meter.

Enumeration of SEO utilizing bacteria and fungi

The plate count approach was used to estimate the number of SEO utilizing viable bacteria and fungi as described by Ochei and Kolhatkar (2008). This was done by dispensing 10 g of 2 mm sieved soil into 90 mL of sterile distilled water and was subsequently agitated for 30 min. Up to 10^{-6} serial dilutions were made. Surface plating 1 mL of the serial dilution onto sterile Nutrient Agar (NA) yielded total viable counts of culturable aerobic heterotrophic bacteria, while surface plating of 1 mL of the serial dilution on potato dextrose agar (PDA) yielded total viable counts of fungi. Plates containing NA were incubated for 48 h at ambient temperature (28±2°C) whereas plates containing PDA were left on the bench for 7 days. After incubation, plates with counts of 30–300 colonies were selected, and the counts were multiplied by the dilution factor and expressed as colony forming unit (CFU)/g of soil.

Determination of TPHs

TPH from the SEO-impacted soil was determined using the USEPA 1850C method (USEPA, 2003). The extraction was done according to U.S EPA method 3550C outlined in USEPA (2017). A method known as United States Environmental Protection Agency 8015C described by USEPA (2017) was adopted in the estimation of TPH contents of the SEO-contaminated soil sampled. TPH was analyzed at Analytical Concept Limited, Port Harcourt, Rivers State, Nigeria.

Data analysis

All data collected from this research were subjected to Analysis of Variance using PROC. GLM of GENSTAT (17th edition) and significant means obtained were separated using appropriate *post hoc* tools.

RESULTS

The physical and chemical properties of the soil used for the experiment is presented in Table 1.

Effect of mycorrhiza, cattle rumen digesta, and SEO on TPH content of contaminated soil

Mycorrhiza application resulted in significantly lower (32473 mg/kg) TPH content compared to treatments with no mycorrhiza application (37912 mg/kg) (Table 2). With reference to cattle rumen digesta, pots amended with cattle rumen digesta resulted in significantly lower (34128 mg/kg) TPH content compared to those without cattle rumen digesta amendment (36257 mg/kg) (Table 2). SEO application at 100 mL/pot resulted in significantly lower (48770 mg/kg) TPH content compared to that of 150 mL/pot SEO application (56808 mg/kg) (Table 2). However, SEO application at 0 mL/pot was Below Detection Limit (BDL).

Table 1: Physical and chemical parameters of the soil used for the experiment

Parameters	Values
pH	7.9
Organic Carbon (%)	0.18
Total Nitrogen (%)	0.7
Available Phosphorus (mg/kg)	0.9
Exchangeable Bases (meq/100 g)	
K	0.022
Na	0.055
Mg	0.044
Ca	0.019
Exchangeable acidity	0.504
CEC	0.644
Particle size (%)	
Sand	74.50
Silt	16.85
Clay	8.65
Textural class (USDA)	Sandy loam

Source: Nkereuwem *et al*. (2022)

Table 2: Effect of mycorrhizal inoculation, cattle rumen digesta, and spent engine oil on total petroleum hydrocarbon content of contaminated soil

Treatments	Total petroleum hydrocarbon (mg/kg)
Mycorrhizal inoculation	
M^+	32473
M^-	37912
LSD (0.05)	440.3
SE (±)	212.3
Cattle Rumen Digesta (g/pot)	
D_1	36257
D_2	34128
LSD (0.05)	440.3
SE (±)	212.3
SEO (mL/pot)	
O_1	BDL
O_2	48770
O_3	56808
LSD (0.05)	539.3
SE (±)	260.0

M^+: With *Glomus mossaea*, M^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot, LSD: Least significant difference, SE: Standard error, BDL: Below detection limit

Interaction of mycorrhiza inoculation with cattle rumen digesta and SEO on TPH content of contaminated soil

Pots with mycorrhizal inoculation and 10 g of cattle rumen digesta had significantly lower (31426 mg/kg) TPH content compared to others (33521, 38994, and 36831 mg/kg) (Table 3). Similarly, those with 0 g of cattle rumen digesta without mycorrhiza had significantly higher (38994 mg/kg) TPH content.

Combined application of mycorrhiza with 100 mL/pot SEO resulted in significantly lower (4716 mg/kg) residual TPH content of the contaminated soil compared to the other treatment combinations (Table 3) while significantly higher (6336 mg/kg) residual TPH content of the contaminated soil was obtained from interaction between 150 mL/pot SEO and without mycorrhizal inoculation. Furthermore, interactions between mycorrhizal inoculation with 150 mL/pot SEO and 100 mL/pot SEO and without mycorrhizal inoculation were not significantly different (Table 3). However, interactions between with or without mycorrhiza with 0 mL/pot SEO were BDL (Table 3).

Interaction of cattle rumen digesta with SEO on TPH content of contaminated soil

Interaction between cattle rumen digesta at 10 g/pot and SEO at 100 mL/pot resulted in significantly lower (47126 mg/kg) TPH content of the contaminated soil compared to other treatment combinations (Table 4). Significantly higher (58357 mg/kg) residual TPH content of the contaminated soil was obtained with a combined use of 0 g/pot cattle rumen digesta with 150 mL/pot SEO compared to combined application of 10 g/pot cattle rumen digesta with 150 mL/pot SEO. Combined application of SEO at 0 mL/pot with or without cattle rumen digesta was, however, BDL.

Interactions of mycorrhizal inoculation, cattle rumen digesta, and SEO on TPH content of contaminated soil

Significant ($p<0.05$) interaction existed between the parameters measured. Combined application of mycorrhiza, 10 g/pot cattle rumen digesta, and 100 mL/pot SEO resulted in significantly lower (45501 mg/kg) TPH content of the contaminated soil compared to other combinations (Table 5). Significantly higher (64989 mg/kg) TPH content was obtained from the combined use of 0 g/pot cattle rumen digesta, 150 mL/pot SEO, and without mycorrhiza compared to the combined application of 10 g/pot cattle rumen digesta, 150 mL/pot SEO and without mycorrhiza (Table 5).

Table 3: Interactions of mycorrhizal inoculation with cattle rumen digesta and spent engine Oil on total petroleum hydrocarbon content of contaminated soil

Mycorrhizal inoculation	Cattle rumen digesta (g/pot)	Total petroleum hydrocarbon (mg/kg)
M^+	D_1	33521
M^+	D_2	31426
M^-	D_1	38994
M^-	D_2	36831
	LSD (0.05)	622.7
	SE (±)	212.3
Mycorrhizal inoculation	**SEO (mL/pot)**	**Total petroleum hydrocarbon (mg/kg)**
M^+	O_1	BDL
M^+	O_2	47169^c
M^+	O_3	50250^b
M^-	O_1	BDL
M^-	O_2	50372^b
M^-	O_3	63365^a

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test, M^+: With *Glomus mossaea*, M^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, LSD: Least significant difference, SE: Standard error, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot

Table 4: Interaction of cattle rumen digesta with SEO on total petroleum hydrocarbon content of contaminated soil

Cattle rumen digesta (g/pot)	SEO (mL/pot)	Total petroleum hydrocarbon (mg/kg)
D_1	O_1	BDL
D_1	O_2	50415^c
D_1	O_3	58357^a
D_2	O_1	BDL
D_2	O_2	47126^d
D_2	O_3	55258^b

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot, BDL: Below detection limit

Effect of mycorrhizal inoculation, cattle rumen digesta and SEO on a bacterial colony of contaminated soil

Mycorrhizal inoculation resulted in significantly higher (5.73 CFU/g) bacteria colony compared to non-mycorrhizal inoculation (3.73 CFU/g) (Table 6). With reference to cattle rumen digesta, amendment with 10 g/pot cattle rumen digesta resulted in significantly higher (5.93 CFU/g) bacterial colony compared to cattle rumen digesta at 0 g/pot amendment (Table 6). SEO application at 100 mL/pot resulted in significantly higher (6.22 CFU/g) bacterial colony compared to SEO application at 150 mL/pot although this was not significantly different from SEO application at 0 mL/pot SEO (5.79 CFU/g) (Table 6).

Interaction of mycorrhizal inoculation with cattle rumen digesta and SEO on the bacterial colony of contaminated soil

Interaction between mycorrhiza and 10 g/pot cattle rumen digesta had significantly higher (6.87 CFU/g) bacterial colony compared to other treatment combinations (Table 7) while significantly lower (2.86 CFU/g) bacterial colony count was obtained from the combined application of 0 g/pot cattle rumen digesta and without mycorrhiza (Table 7).

Combined application of mycorrhiza and 100 mL/pot SEO resulted in significantly higher (7.30 CFU/g) bacterial colony count compared to other treatment combinations (Table 7). However, the combined application of mycorrhiza and 100 mL/pot SEO and mycorrhiza with

Table 5: Interaction of mycorrhizal inoculation, cattle rumen digesta, and SEO on total petroleum hydrocarbon content of contaminated soil

Mycorrhizal inoculation	Cattle rumen digesta (g/pot)	SEO (mL/pot)	Total petroleum hydrocarbon (mg/kg)
M^+	D_1	O_1	BDL
M^+	D_1	O_2	48837[d]
M^+	D_1	O_3	51725[c]
M^+	D_2	O_1	BDL
M^+	D_2	O_2	45501[e]
M^+	D_2	O_3	48776[d]
M^-	D_1	O_1	BDL
M^-	D_1	O_2	51992[c]
M^-	D_1	O_3	64989[a]
M^-	D_2	O_1	BDL
M^-	D_2	O_2	48751[d]
M^-	D_2	O_3	61741[b]

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. M^+: With *Glomus mossaea*, M^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot, BDL: Below detection limit

Table 6: Effect of mycorrhizal inoculation, cattle rumen digesta, and SEO on bacterial colony of contaminated soil

Treatments	Bacterial colony (CFU/g soil)
Mycorrhizal inoculation	
GM^+	5.73
GM^-	3.93
LSD (0.05)	0.451
SE (±)	0.154
Cattle Rumen Digesta (g/pot)	
D_1	3.73
D_2	5.93
LSD (0.05)	0.451
SE (±)	0.154
SEO (mL/pot)	
O_1	5.79
O_2	6.22
O_3	2.48
LSD (0.05)	0.552
SE (±)	0.188

M^+: With *Glomus mossaea*, M^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot, LSD: Least significant difference, SE: Standard error

0 mL/pot SEO were not significantly different (Table 8). Significantly lower (1.97 CFU/g) bacterial colony count was obtained from the combination of 150 mL/pot SEO and without mycorrhiza compared to the combined application of mycorrhiza and 150 mL/pot SEO (Table 7).

Interaction of cattle rumen digesta with SEO on bacterial colony of contaminated soil

Interaction between 10 g/pot cattle rumen digesta and 0 mL/pot SEO resulted in significantly higher (7.58 CFU/g) bacterial colony count compared to other treatment combinations (Table 8), although the combined applications of 10 g/pot cattle rumen digesta with 0 mL/pot SEO and 10 g/pot cattle rumen digesta with 100 mL/pot SEO were not significantly different. Combined application of cattle rumen digesta at 0/g/pot with 150 mL/pot SEO had the lowest (1.73 CFU/g) bacterial colony count and this was significantly different from the combined use of 10 g/pot cattle rumen digesta with 150 mL/pot SEO (Table 8). Furthermore, the combined use of 0 g/pot cattle rumen digesta with 0 mL/pot SEO and 10 g/pot cattle rumen digesta with 150 mL/pot SEO were not significantly different.

Table 7: Interactions of mycorrhiza inoculation with cattle rumen digesta and SEO on bacterial colony of contaminated soil

Mycorrhizal inoculation	Cattle rumen digesta (g/pot)	Bacterial colony (CFU/g soil)
GM^+	D_1	4.60
GM^+	D_2	6.87
GM^-	D_1	2.86
GM^-	D_2	5.00
	LSD (0.05)	0.638
	SE (±)	0.217
Mycorrhizal inoculation	**SEO (mL/pot)**	**Bacterial colony (CFU/g soil)**
GM^+	O_1	6.90[a]
GM^+	O_2	7.30[a]
GM^+	O_3	3.00[c]
GM^-	O_1	4.68[b]
GM^-	O_2	5.13[b]
GM^-	O_3	1.97[d]

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. M^+: With *Glomus mossaea*, M^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, LSD: Least significant difference, SE: Standard error, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot

Table 8: Interaction of cattle rumen digesta with SEO on bacterial colony of contaminated soil

Cattle rumen digesta (g/pot)	SEO (mL/pot)	Bacterial colony (CFU/g soil)
D_1	O_1	4.00[c]
D_1	O_2	5.45[b]
D_1	O_3	1.73[d]
D_2	O_1	7.58[a]
D_2	O_2	6.98[a]
D_2	O_3	3.23[c]

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. D_1: Without cattle rumen digesta, D_2: cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot

Interaction of mycorrhizal inoculation, cattle rumen digesta and SEO on bacterial colony of contaminated soil

Significant ($p<0.05$) interactions existed between the parameters measured. Combined application of mycorrhiza, 10 g/pot cattle rumen digesta and 0 mL/pot SEO resulted in significantly higher (9.20 CFU/g) bacterial colony compared to other treatment combinations (Table 9). Similarly, significantly lower (1.10 CFU/g) bacterial count was obtained from the combined application of 0 g/pot cattle rumen digestawith150 mL/pot SEO and without mycorrhiza inoculation compared to the combined application of mycorrhiza, 0 g/pot cattle rumen digesta and 0 mL/pot SEO.

Effect of mycorrhizal inoculation, cattle rumen digesta and SEO on fungal colonies of contaminated soil

Mycorrhizal inoculation resulted in significantly higher (5.961 CFU/g) fungal colony compared with non-mycorrhizal inoculation (4.022 CFU/g) (Table 10). With reference to cattle rumen digesta, amendment with 10 g/pot CRD resulted in significantly higher (6.689 CFU/g) fungal colony compared to cattle rumen digesta at 0 g/pot amendment (Table 10). SEO application at 100 mL/pot resulted in significantly higher (5.480 CFU/g) fungal colony compared to SEO application at 150 mL/pot although this was not significantly different from SEO application at 0 ml/pot SEO (6.250 CFU/g) (Table 10).

Table 9: Interaction of mycorrhizal inoculation, cattle rumen digesta and SEO on bacterial colony of contaminated soil

Mycorrhizal inoculation	Cattle rumen digesta (g/pot)	SEO (mL/pot)	Bacterial colony (CFU/g soil)
GM^+	D_1	O_1	4.60^{d}
GM^+	D_1	O_2	$6.83^{b,c}$
GM^+	D_1	O_3	2.37^{g}
GM^+	D_2	O_1	9.20^{a}
GM^+	D_2	O_2	7.77^{b}
GM^+	D_2	O_3	$3.63^{d,e,f}$
GM^-	D_1	O_1	$3.40^{e,f,g}$
GM^-	D_1	O_2	$4.07^{d,e}$
GM^-	D_1	O_3	1.10^{h}
GM^-	D_2	O_1	5.97^{c}
GM^-	D_2	O_2	6.20^{c}
GM^-	D_2	O_3	$2.83^{f,g}$

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. M^+: With *Glomus mossaea*, M^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot

Table 10: Effect of mycorrhizal inoculation, cattle rumen digesta and SEO on fungal colony of contaminated soil

Treatments	Fungal colony (CFU/g soil)
Mycorrhizal inoculation	
GM^+	5.961
GM^-	4.022
LSD (0.05)	0.3127
SE (±)	0.1066
Cattle Rumen Digesta (g/pot)	
D_1	3.294
D_2	6.689
LSD (0.05)	0.3127
SE (±)	0.1066
SEO (mL/pot)	
O_1	6.250
O_2	5.408
O_3	3.317
LSD (0.05)	0.3830
SE (±)	0.1306

GM^+: With *Glomus mossaea*, GM^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot, LSD: Least significant difference, SE: Standard error

Interaction of mycorrhizal inoculation with cattle rumen digesta and SEO on the fungal colony of contaminated soil

Interaction between mycorrhiza and 10 g/pot cattle rumen digesta had significantly higher (7.989 CFU/g) fungal colony compared to other treatment combinations (Table 11) while significantly lower (2.656 CFU/g) fungal colony count was obtained from the combined application of 0 g/pot cattle rumen digesta and without mycorrhiza (Table 11).

Combined application of mycorrhiza and 100 mL/pot SEO resulted in significantly higher (6.250 CFU/g) fungal colony count compared to other treatment combinations (Table 11). However, combined applications of mycorrhiza and 100 mL/pot SEO and mycorrhiza with 0 mL/pot SEO were not significantly different (Table 11). Significantly lower (2.500 s CFU/g) fungal colony count was obtained from the combination of 150 mL/pot SEO and without mycorrhiza compared to the combined application of mycorrhiza and 150 mL/pot SEO (Table 11).

Interaction of cattle rumen digesta with SEO on fungal colony of contaminated soil

Interaction between 10 g/pot cattle rumen digests and 0 mL/pot SEO resulted in significantly higher (8.333 CFU/g) fungal colony count

Table 11: Interactions of mycorrhizal inoculation with cattle rumen digesta and SEO on fungal colony of contaminated soil

Mycorrhizal inoculation	Cattle rumen digesta (g/pot)	Fungal colony (CFU/g soil)
GM^+	D_1	3.933
GM^+	D_2	7.989
GM^-	D_1	2.656
GM^-	D_2	5.389
	LSD (0.05)	0.4422
	SE (±)	0.1508
Mycorrhizal inoculation	**SEO (mL/pot)**	**Fungal colony (CFU/g soil)**
GM^+	O_1	7.500^{a}
GM^+	O_2	6.250^{b}
GM^+	O_3	4.133^{d}
GM^-	O_1	5.000^{c}
GM^-	O_2	$4.567^{c,d}$
GM^-	O_3	2.500^{e}

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. GM^+: With *Glomus mossaea*, GM^-: Without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, LSD: Least significant difference, SE: Standard error, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot

compared to other treatment combinations (Table 12), although the combined applications of 10 g/pot cattle rumen digesta with 0 mL/pot SEO and 10 g/pot cattle rumen digesta with 100 mL/pot SEO were not significantly different. Combined application of cattle rumen digesta at 0/g/pot with 150 mL/pot SEO had the lowest (2.283 CFU/g) fungal colony count and this was significantly different from the combined use of 10 g/pot cattle rumen digesta with 150 mL/pot SEO (Table 12). Furthermore, the combined use of 0 g/pot cattle rumen digesta with 0 mL/pot SEO and 10 g/pot cattle rumen digesta with 150 mL/pot SEO were not significantly different.

Interactions of mycorrhizal inoculation, cattle rumen digesta, and SEO on the fungal colony of contaminated soil

Significant ($p<0.05$) interaction existed between the parameters measured. Combined application of mycorrhiza, 10 g/pot cattle rumen digesta, and 0 mL/pot SEO resulted in significantly higher (9.933 CFU/g) fungal colony compared to other treatment combinations (Table 13). Similarly, significantly lower (1.600 CFU/g) fungal count was obtained from the combined application of 0 g/pot cattle rumen digesta with150 mL/pot SEO and without mycorrhiza inoculation compared to the combined application of mycorrhiza, 0 g/pot cattle rumen digesta and 0 mL/pot SEO.

DISCUSSION

The results obtained from treatments amended with cattle rumen digesta show lower TPH content compared to those without cattle rumen digesta amendment. These results corroborate the findings of Okoh (2006), who reported that organic wastes bind rapidly to the soil particle, and that this facilitates the movement of the pollutants through soil when natural events such as rainfall occurs. The results are also in agreement with previous studies on enhanced bioremediation of impacted media (Ibiene *et al*., 2011; Agary and Ogunleye, 2012; Abioye *et al*., 2012; Chukwudozie, 2013; Omoni *et al*., 2015). Cattle rumen digesta application also resulted in higher colonies of bacteria and fungi compared to treatments without cattle rumen digesta amendment. The increase in the colonies count may be attributed to increased water holding capacity and cation exchange capacity of substrates, providing a slow release of nutrient source, complex toxic metals thus, boosting microbial activity (Tordoff *et al*., 2000). The results obtained in this study are also in agreement with previous report by Tirguia and Tam (Tiquia and Tam, 2002), where they reported increase in bacterial and fungal colonies due to high concentrations of ammonia in organic wastes.

Table 12: Interaction of cattle rumen digesta with SEO on fungal colony of contaminated soil

Cattle Rumen Digesta	SEO (mL/pot)	Fungal colony (CFU/g soil)
D_1	O_1	4.167^{c}
D_1	O_2	3.433^{d}
D_1	O_3	2.283^{e}
D_2	O_1	8.333^{a}
D_2	O_2	7.383^{b}
D_2	O_3	4.350^{c}

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. D_1: Without cattle rumen digesta, D_2: Cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot

Table 13: Interactions of mycorrhizal inoculation, cattle rumen digesta and SEO on fungal colony of contaminated soil

Mycorrhizal inoculation	Cattle rumen digesta (g/pot)	SEO (mL/pot)	Fungal colony (CFU/g soil)
GM^+	D_1	O_1	5.067^{e}
GM^+	D_1	O_2	3.767^{f}
GM^+	D_1	O_3	2.967^{f}
GM^+	D_2	O_1	9.933^{a}
GM^+	D_2	O_2	8.733^{b}
GM^+	D_2	O_3	$5.300^{d,e}$
GM^-	D_1	O_1	3.267^{f}
GM^-	D_1	O_2	3.100^{f}
GM^-	D_1	O_3	1.600^{g}
GM^-	D_2	O_1	6.733^{c}
GM^-	D_2	O_2	$6.033^{c,d}$
GM^-	D_2	O_3	3.400^{f}

Means with the same letter (s) are not significantly different from each other at $P<0.05$ using Duncan's multiple range test. GM^+: With *Glomus mossaea*, M^-: without *Glomus mossaea*, D_1: Without cattle rumen digesta, D_2: cattle rumen digesta at 10 g/pot, SEO: Spent engine oil, O_1: SEO at 0 mL/pot, O_2: SEO at 100 mL/pot, O_3: SEO at 150 mL/pot

The results of this study show that the microbial population of soil was affected by SEO contamination compared to uncontaminated soil. The microbial population of the polluted soils compared to the unpolluted soil was reduced probably due to nutrient imbalance created by SEO contamination. This result corroborates the findings of Okolo *et al.* (2005), who used poultry manure to enhance crude oil degradation in a sandy loamy soil and concluded that poultry and cattle manure improved soil chemical properties irrespective of soil texture. Mycorrhizal application resulted in enhanced TPH degradation in the SEO contaminated soil. A similar result was also obtained by Nkereuwem *et al.* (2022, 2020a) where they observed reductions in TPH content of crude oil-contaminated soil. The reduction in TPH content could be due to the fact that mycorrhizal fungi interact with some other beneficial soil organisms in order to achieve complete clean-up of polluted soils (Chibuike, 2013). The results of this study corroborate previous report by Harrier and Watson (Harrier and Watson, 2004), that mycorrhizal fungi favour the activities of soil microorganisms and that the amount of pollutants remediated with mycorrhiza-assisted remediation is increased due to the activities of these microorganisms. It was also evident in the work of Gao *et al*. (2010), where the remediation of fluorine and phenanthrene with arbuscular mycorrhiza was compared.

The results obtained from the study equally show that mycorrhiza increases the total fungal colony count compared to treatments that had no mycorrhizal inoculation. This is in agreement with Nkereuwem *et al.* (2020b), who reported that the total fungal colony count in their work ranged from 4×10^4 to 8×10^4 CFU/g soil, whereby the treatments inoculated with mycorrhiza had the highest total fungal colony count. Likewise in this study, mycorrhizal inoculation significantly ($p<0.05$) enhanced bacterial and fungal colony count. This result equally corroborates the report of Yuniati (2018), who postulated that mycorrhiza can assess water and nutrient in the smallest pores in the soil, thus making the environment favorable for the growth of bacteria and fungi, resulting in their population getting increased. Mycorrhiza also releases glomalin in the soil environment which results in better soil structure and high organic matter content, they also play a major role in the soil aggregation process and stimulate microbial activity (Miller and Jastrow, 2000).

CONCLUSION

Mycorrhizal inoculation greatly increased the microbial population resulting in enhanced TPH degradation in SEO-impacted soil compared to treatments that were devoid of mycorrhizal inoculation. Cattle rumen digesta also resulted in higher colonies of bacteria and fungi resulting in enhanced TPH degradation in SEO-impacted soil. The use of cattle rumen digesta in bioremediation of petroleum hydrocarbon-impacted soil should be encouraged as it is effective, readily available, and environmentally friendly. Mycorrhizal technology should be used in bioremediation of related petroleum hydrocarbon-impacted soils.

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AUTHOR CONTRIBUTIONS

MN conceptualized the research, design the experiment, and analyzed the data. AA, FK, LS, and CI drafted the manuscript. MN, MB, and AL monitored the research and collected data. MN and AA finalized the manuscript. All the authors approved this research and take responsibility for its integrity.

CONFLICT OF INTEREST

The authors declare that they have no financial or non-financial conflict of interest with regard to the current manuscript.

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