sciendo DOI: 10.2478/ats-2022-0014 AGRICULTURA TROPICA ET SUBTROPICA, 55 OV, 119–132, 2022

Original Research Article

Effect of mycorrhizal inoculation and organic fertiliser on bioremediation of spent engine oil contaminated soil

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Abstract

Hydrocarbon-related environmental pollution is a major environmental hazard due to its toxicity and widespread presence in the environment, resulting in stunted growth of soil microorganisms, plants, and animals. This study was therefore conducted to evaluate the effect of mycorrhizal inoculation and compost made from Cocoa Pod Husk (CPH) and cattle dung in the bioremediation of Spent Engine Oil (SEO)-contaminated soil. About 2.5 kg of sterilised soil was contaminated with SEO at different concentrations: 0, 100, and 150 mL/pot. Compost was then added after two weeks of contamination at the rate of 10 g/pot. Inoculation for treatments containing *Glomus mossaea* (consisting of 20 g of root soil-fungal mixture) was blended into the soil samples as well. It was a 2 × 2 × 3 factorial experiment that was laid out in a completely randomised design and replicated three times. The incubation was allowed to last for twelve (12) weeks before the termination of the experiment. Data were collected on the Total Petroleum Hydrocarbon (TPH), bacterial and fungal biomass of the SEO-contaminated soil. Results obtained indicate that combined application of mycorrhiza with 100 mL/pot SEO resulted in significantly ($p < 0.05$) lower residual TPH content (54.50% degradation) of the contaminated soil compared to the other treatment combinations whereas significantly higher residual TPH content (20.43% degradation) of the contaminated soil was obtained from the interaction between 150 mL/pot SEO and without mycorrhizal inoculation. Interaction between mycorrhiza and 10 g/pot compost had a significantly higher bacterial colony (6.58 CFU/g) compared to other treatment combinations. Mycorrhizal inoculation resulted in a significantly higher fungal colony (5.844 CFU/g) compared with non-mycorrhizal inoculation (3.222 CFU/g). Therefore, it can be concluded that mycorrhizal inoculation and compost were effective in the bioremediation of SEO-impacted soil.

Keywords: *Glomus mossaea*; degradation; compost; Total Petroleum Hydrocarbon; biostimulation; bioremediation

INTRODUCTION

Environmental contamination from a significant volume of spent engine oil has been caused by industrialisation and urbanisation, which have led to an increase in the consumption of petroleum-based goods like engine oil. According to Ogunjobi and Ekanem (2016), used engine oil contamination of soil via leaks and unintentional spills has become a severe issue on a global scale due to the effects it has on the ecology and people's health. Onuoha et al. (2011), stated that soil contamination by spent engine oil occurs frequently in oil-producing and industrialised nations, but the issue is more serious in developing nations like Nigeria where there are no strong environmental regulating laws. Polycyclic Aromatic Hydrocarbons (PAHs), performance-enhancing additives, breakdown products, and heavy metals like lead, vanadium, nickel, arsenic, mercury, and many more that are released by worn-out engine parts are all constituents of spent engine oil (Agarry and Oladipupo, 2012). According to Klamerus-Iwan et al. (2015), Rodríguez-Rodríguez et al. (2016), contamination of soils due to the influence of hydrocarbons disrupts their biological, chemical, and physical characteristics, as well as their wettability. Hydrophobicity of hydrocarbons can alter the wettability of the particles on the soil surface and consequently result in soil water repellency in the course of coating soil particles (Roy and Gill, 2000). It has been reported by Doerr et al. (2000), that repellency of soil water upsets its hydrological and ecological functions with the resultant effect of declining water infiltration, speeding up surface runoff and erosion, and ultimately hampering the growth of plants. Water repellency of soils confines its ability to sorb water and results in irregular moisture distribution, creating preferential water flow in the soil profile (Szatyłowicz et al., 2007; Hewelke et al., 2016).

Biological treatment of polluted environments involves the conversion of harmful pollutants into less harmful ones by employing a single microorganism or a consortium of microorganisms (Zhao et al., 2018; Uzoma et al., 2021). In as much as there is multiplicity in cradles and chemical complications in organic pollutants, there is perhaps more multiplicity in the microbial world as proficiencies to degrade organic compounds are ever evolving (Ramakrishnan et al., 2010). The ecological restoration and reclamation of hydrocarbon-impacted environments can be based on microbial degradation of the pollutants therein (Boonchan et al., 2000). Biologically and biochemically mediated processes in soils are of the utmost importance to ecosystem function. Soil microbes are the driving force behind many soil processes including the transformation of organic matter, nutrient release, and degradation of petroleum hydrocarbons (Zabaloy et al., 2008).

Bioremediation is a technology that adopts the use of microorganisms and suitable nutrients to break down organic and inorganic pollutants into forms that may be less toxic or not toxic (Adeleye et al., 2018; Nkereuwem et al., 2020a). These authors further reiterated that this technology has shown encouraging results over the years as it is economical and environmentally friendly. According to Adeleye et al. (2019), bioremediation technology can degrade or detoxify pollutants documented to be deleterious to humans through the employment of biostimulation technology (supplementation of hydrocarbonoclastic microorganisms with nutrients) or bioaugmentation technology (seeding of hydrocarbon impacted environments with known hydrocarbonoclastic microorganisms). The objectives of this study were to 1) assess the effect of Mycorrhizal inoculation in the degradation of Total Petroleum Hydrocarbon (TPH) content in Spent Engine Oil (SEO) contaminated soil and 2) assess the effect of compost made of Cocoa Pod Husk (CPH) and cattle dung in the degradation of TPH content in SEO contaminated soil and 3) determine the combinatorial effect of Mycorrhizal inoculation and compost made of CPH and cattle dung in the degradation of TPH content in SEO impacted soil.

MATERIALS AND METHODS

Description of the study area

The study was conducted at the Department of Soil Science Teaching and Research Farm, Federal University Dutse, Jigawa State. The area is located on latitude 11.706 and longitude 9.380 with altitude 457.36 in the derived Sudan Savannah of the Northwest agroecological zone which is characterised by two distinct seasons (dry and wet). The climate is tropical wet-dry climate classification and there is a slightly cool period around November–February. The mean annual temperature is 26 °C but the mean monthly values range between 21 °C in the coldest month (December/February) and 38 °C in the hottest months (April/May). The area has an average annual rainfall of 681 mm that falls between the months of May and September/October (Olofin, 1987).

Experimental materials

Soil samples, mycorrhiza (*Glomus mossaea*), polyethylene bags, compost (made from the composting of cocoa pod husk and cattle dung), and Spent Engine Oil (SEO) were used in this study. Mycorrhiza inoculum was sourced from the Soil Microbiology Laboratory, University of Ibadan, Oyo State. Spent engine oil was sourced from the Mechanic village, Dutse while the compost (made from cocoa pod husk and cattle dung) was sourced from the Department of Soil Science, Federal University Dutse, Jigawa State.

Experimental design

A 2 × 2 × 3 factorial experiment using Completely Randomised Design (CRD) with three (3) replicates was adopted in this study. There was a total of twelve (12) treatment combinations giving a total of thirty-six (36) experimental units. The factors are depicted below:

Mycorrhiza inoculum at two levels

With *Glomus mossaea*
Without *Glomus mossaea*

Organic amendment (Compost-C) at two levels

0 g/kg soil
0.01 g/kg soil

SEO at three levels (O)

0 % (w/w) (0 mL/pot)
10% (w/w) (100 mL/pot)
15% (w/w) (150 mL/pot)

Treatment combinations

$GM^+C_1O_1$	$GM^-C_1O_1$
$GM^+C_1O_2$	$GM^-C_1O_2$
$GM^+C_1O_3$	$GM^-C_1O_3$
$GM^+C_2O_1$	$GM^-C_2O_1$
$GM^+C_2O_2$	$GM^-C_2O_2$
$GM^+C_2O_3$	$GM^-C_2O_3$

Soil sample collection and preparation

The experimental soil was randomly collected from the Teaching and Research Farm, Federal University Dutse at the depth of 0–15 cm using a shovel. Subsequently, it was exposed to air so as to form a compound sample as done by Adeleye et al. (2021). The bulked soil was later sieved using a 2.0-mm mesh size (Soretire et al., 2017). Thereafter, SEO (0, 100, and 150 mL/pot) was mixed thoroughly with the soil (2.5 kg/pot) and allowed to stand for two weeks for effective volatilization to take place (Adeleye et al., 2019). Compost was then added after two weeks at the rate of 10 g/pot. This was exhaustively mixed with the soil for equilibration. Inoculation for treatments containing *Glomus mossaea* consisting of 20 g of root soil-fungal mixture was blended into the soil samples as well.

Incubation assay

About 2.5 kg of the sterilised soil was potted. A digital weighing scale was used to measure the two (2) levels of mycorrhiza (0 and 20 g/pot), and two (2) levels of compost (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 in-between and 1 m between the replications. The incubation was allowed to last for twelve (12) weeks in the laboratory before the termination of the experiment.

Laboratory analyses

Spent engine oil-contaminated soil samples were collected and taken to the laboratory whereby its physicochemical indicators, Total Petroleum Hydrocarbon (TPH), and bacterial and fungal biomass were subjected to analyses when the experiment was terminated. Soil sample devoid of SEO contamination was also equally collected for analyses at the start of the study. Particle size analysis of the soil was determined through the hydrometer method (Bouyoucos, 1951). Organic carbon was determined through the Walkey-Black method whereas available phosphorus was determined through the Olsen method. The exchangeable bases ranging between Ca, K, Mg, and Na were determined through flame photometry (Jenway model). Electrical Conductivity (EC) was determined using a method described by (Bower and Wilcox, 1965). The soil pH was determined through Hanna's digital pH meter. The moisture content of the samples was determined using the gravimetric method.

Counting of spent engine oil-utilising bacteria and fungi

Spent engine oil-utilising bacteria and fungi were enumerated through the plate count method (Ochei and Kolhatkar, 2008). Ten grams of 2 mm sieved soil was dispensed into 90 mL of sterile distilled water and was consequently mixed vigorously for 30 minutes. Up to 10^{-6} serial dilutions were made. Surface plating of 1 mL of the serial dilution onto sterile nutrient agar produced total viable counts of aerobic heterotrophic bacteria, while surface plating of 1 mL of the serial dilution onto Potato Dextrose Agar (PDA) produced fungal total viable counts. Plates containing nutrient agar were incubated for 48 hours at ambient temperature (28 ± 2 °C) while plates containing PDA were left on the bench for seven days. After incubation, plates having between 30–300 colonies were selected, and the microbial counts were multiplied by the dilution factor and subsequently expressed as CFU/g of soil.

Measurement of Total Petroleum Hydrocarbons

Total Petroleum Hydrocarbon (TPH) from the SEO contaminated soil was determined using the USEPA 1850C method (USEPA, 2003). The extraction was carried out following USEPA method 3550C (USEPA, 2017). TPH was analysed at Analytical Concept Limited, Port Harcourt, Rivers State, Nigeria. The percentage of TPH degraded in the SEO contaminated soil was calculated using the formula below:

$$\%Degradation = \frac{TPHi - TPHr}{TPHi} \times 100$$

where:
TPHi = initial TPH concentration
TPHr = residual TPH concentration

Table 1. Physical and chemical characteristics of the soil used for the experiment

Variables	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

Table 2. Effect of mycorrhizal inoculation, compost, and spent engine oil concentration on total petroleum hydrocarbon content of contaminated soil

Treatments	Total petroleum hydrocarbon (mg/kg)	Percent degradation
Mycorrhizal inoculation		
With *Glomus moussaea*	33000	67.0
Without *Glomus moussaea*	42812	57.2
LSD (0.05)	449.3	
SE (±)	153.2	
Compost (g/pot)		
Compost at 0 g	43311	56.7
Compost at 10 g	32502	67.5
LSD (0.05)	449.3	
SE (±)	153.2	
SEO (mL/pot)		
SEO at 0 mL	BDL	
SEO at 100 mL	47182	52.8
SEO at 150 mL	66537	33.5
LSD (0.05)	550.3	
M X C	s	
M X SEO	s	
C X SEO	s	
M X C X SEO	s	

SEO = spent engine oil, LSD = least significant difference, BDL = below detection limit, M = Mycorrhizal inoculation, C = Compost. S = significant

Data analysis

Data collected from this study were subjected to Analysis of Variance (ANOVA) using PROC. GLM of GENSTAT (17th edition) and significant means derived were separated using appropriate post-hoc tools.

RESULTS

Table 1 shows the physical and chemical features of the soil used for the experiment with a pH of 8, Total Nitrogen of 0.7%, and Available Phosphorus of 0.9 mg/kg. The soil textural class is sandy loam.

Table 3. Interaction of mycorrhizal inoculation with compost and total petroleum hydrocarbon content of contaminated soil

Mycorrhizal inoculation	Compost (g/pot)	Total petroleum hydrocarbon (mg/kg)	Percent degradation
With *Glomus mossaea*	Compost at 0 g	35658	64.3
Without *Glomus mossaea*	Compost at 10 g	30343	69.7
With *Glomus mossaea*	Compost at 0 g	50964	49.0
Without *Glomus mossaea*	Compost at 10 g	34661	65.3
	LSD (0.05)	635.4	
	SE (±)	216.6	

LSD = least significant difference, SE = standard error

Table 4. Interaction of mycorrhizal inoculation with spent engine oil concentration and total petroleum hydrocarbon content of contaminated soil

Mycorrhizal inoculation	SEO (mL/pot)	Total petroleum hydrocarbon (mg/kg)	Percent degradation
With *Glomus mossaea*	SEO at 0 mL	BDL	
With *Glomus mossaea*	SEO at 100 mL	45503d	54.5
With *Glomus mossaea*	SEO at 150 mL	53498b	46.5
Without *Glomus mossaea*	SEO at 0 mL	BDL	
Without *Glomus mossaea*	SEO at 100 mL	48861c	51.1
Without *Glomus mossaea*	SEO at 150 mL	79576a	20.4

SEO = spent engine oil, BDL = below detection limit. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

Effect of mycorrhizal inoculation, compost, and spent engine oil concentration on TPH content of contaminated soil

Mycorrhiza application resulted in significantly lower TPH content (33000 mg/kg) compared to that with no mycorrhizal application (42812 mg/kg) (Table 2). Experimental pots amended with compost resulted in significantly lower TPH content (32502 mg/kg) compared to those without compost amendment (43311 mg/kg) (Table 2). Spent engine oil application at 100 mL/pot resulted in significantly lower TPH content (47182 mg/kg) compared to that of 150 mL/pot SEO application (66537 mg/kg) (Table 2). However, SEO application at 0 mL/pot was Below Detection Limit (BDL).

Interaction of mycorrhizal inoculation with compost and TPH content of contaminated soil

Experimental pots with mycorrhizal inoculation and 10 g of compost had significantly lower (30343 mg/kg) TPH content compared to others (34661 mg/kg, 35658 mg/kg, and 50964 mg/kg). Similarly, those with 0 g of compost without mycorrhiza had significantly higher (50964 mg/kg) TPH content (Table 3).

Interaction of mycorrhizal inoculation with spent engine oil concentration and TPH content of contaminated soil

Combined application of mycorrhiza with 100 mL/pot SEO resulted in significantly lower residual TPH content (45503 mg/kg) of the contaminated soil compared to the other treatment combinations (Table 4) while significantly higher residual TPH content (79567 mg/kg) of the contaminated soil was obtained from the interaction between 150 mL/pot SEO and without mycorrhizal inoculation. Furthermore, the interactions between mycorrhizal inoculation with 150 mL/pot SEO and 100 mL/pot SEO and without mycorrhizal inoculation were not significantly different (Table 4). However, the interaction between with or without mycorrhiza with 0 mL/pot SEO was Below Detection Limit (BDL) (Table 4).

Interaction of compost with spent engine oil concentration and TPH content of contaminated soil

Interaction between compost at 10 g/pot and SEO at 100 mL/pot resulted in significantly lower TPH content (40619 mg/kg) of the contaminated soil compared to

Table 5. Interaction of compost with spent engine oil concentration and total petroleum hydrocarbon content of contaminated soil

Compost (g/pot)	SEO (mL/pot)	Total petroleum hydrocarbon (mg/kg)	Percent degradation
Compost at 0 g	SEO at 0 mL	BDL	
Compost at 0 g	SEO at 100 mL	53744c	46.3
Compost at 0 g	SEO at 150 mL	76188a	23.8
Compost at 10 g	SEO at 0 mL	BDL	
Compost at 10 g	SEO at 100 mL	40619d	59.4
Compost at 10 g	SEO at 150 mL	56886b	43.1

SEO = spent engine oil, BDL = below detection limit. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

Table 6. Interaction of mycorrhizal inoculation, compost, and spent engine oil concentration and total petroleum hydrocarbon content of contaminated soil

Mycorrhizal Inoculation	Compost (g/pot)	SEO (mL/pot)	Total petroleum hydrocarbon (mg/kg)	Percent degradation
With *Glomus mossaea*	Compost at 0 g	SEO at 0 mL	BDL	
With *Glomus mossaea*	Compost at 0 g	SEO at 100 mL	52001d	48
With *Glomus mossaea*	Compost at 0 g	SEO at 150 mL	54973c	45.0
With *Glomus mossaea*	Compost at 10 g	SEO at 0 mL	BDL	
With *Glomus mossaea*	Compost at 10 g	SEO at 100 mL	39005f	61
With *Glomus mossaea*	Compost at 10 g	SEO at 150 mL	52023d	47.9
Without *Glomus mossaea*	Compost at 0 g	SEO at 0 mL	BDL	
Without *Glomus mossaea*	Compost at 0 g	SEO at 100 mL	55488c	44.5
Without *Glomus mossaea*	Compost at 0 g	SEO at 150 mL	97404a	2.6
Without *Glomus mossaea*	Compost at 10 g	SEO at 0 mL	BDL	
Without# *Glomus mossaea*	Compost at 10 g	SEO at 100 mL	42233e	57.8
Without *Glomus mossaea*	Compost at 10 g	SEO at 150 mL	61749b	38.3

SEO = spent engine oil, BDL = below detection limit. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

other treatment combinations (Table 5). Significantly higher residual TPH content (76188 mg/kg) of the contaminated soil was obtained with the combined use of 0 g/pot compost with 150 mL/pot SEO compared to the combined application of 10 g/pot compost with 150 mL/pot SEO. Combined application of SEO at 0 mL/pot with or without compost was, however, Below Detection Limit (BDL).

Interaction of mycorrhizal inoculation, compost, and spent engine oil concentration and TPH content of contaminated soil

Significant ($p < 0.05$) interaction existed between the parameters measured. Combined application of mycorrhiza, 10 g/pot compost, and 100 mL/pot SEO resulted in significantly lower TPH content (39005 mg/kg) of the contaminated soil compared to other combinations (Table 6). Significantly higher TPH content (61749 mg/kg) was obtained from the combined use of 0 g/pot compost, 150 mL/pot SEO, and without mycorrhiza inoculation compared to the combined application of 10 g/pot compost, 150 mL/pot SEO, and without mycorrhiza inoculation (Table 6).

Effect of mycorrhizal inoculation, compost, and spent engine oil concentration on the bacterial colony of contaminated soil

Mycorrhizal inoculation resulted in a significantly higher (5.38 CFU/g) bacterial colony compared to non-mycorrhizal inoculation (3.17 CFU/g) (Table 7). With reference to compost, amendment with 10 g/pot compost resulted in a significantly higher (5.28 CFU/g) bacterial colony compared to compost at 0 g/pot amendment (Table 7). SEO application at 100 mL/pot

Table 7. Effect of mycorrhizal inoculation, compost, and spent engine oil concentration on bacterial colony of contaminated soil

Treatments	Bacterial colony (CFU/g)
Mycorrhizal inoculation	
With *Glomus mossaea*	5.38
Without *Glomus mossaea*	3.17
LSD (0.05)	0.636
SE (±)	0.217
Compost (g/pot)	
Compost at 0 g	3.27
Compost at 10 g	5.28
LSD (0.05)	0.636
SE (±)	0.217
SEO (mL/pot)	
SEO at 0 mL	4.89
SEO at 100 mL	5.95
SEO at 150 mL	1.98
LSD (0.05)	0.778
M X C	s
M X SEO	s
C X SEO	s
M X C X SEO	s

SEO = spent engine oil, LSD = least significant difference.
s = significant

resulted in a significantly higher (5.95 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 (4.89 CFU/g) (Table 7).

Interaction of mycorrhizal inoculation with compost and the bacterial colony in spent engine oil-contaminated soil

Interaction between mycorrhiza and 10 g/pot compost had a significantly higher (6.58 CFU/g) bacterial colony compared to other treatment combinations (Table 8) whereas a significantly lower (2.34 CFU/g) bacterial colony count was obtained from the combined application of 0 g/pot compost and without mycorrhiza inoculation (Table 8).

Interaction of mycorrhizal inoculation with spent engine oil concentration and the bacterial colony in contaminated soil

Combined application of mycorrhiza and 100 mL/pot SEO resulted in a significantly higher (7.733 CFU/g) bacterial colony count compared to other treatment combinations (Table 9). However, combined applications of mycorrhiza and 100 mL/pot SEO and mycorrhiza with 0 mL/pot SEO were not significantly different (Table 9). Significantly lower (1.483 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 9).

Interaction of compost with spent engine oil concentration and the bacterial colony in contaminated soil

Interaction between 10 g/pot compost and 100 mL/pot SEO resulted in significantly a higher (7.150 CFU/g) bacterial colony count compared to other treatment

Table 8. Interaction of mycorrhizal inoculation with compost and bacterial colony in contaminated soil

Mycorrhizal inoculation	Compost (g/pot)	Bacteria colony (CFU/g)
With *Glomus mossaea*	Compost at 0 g	4.19
With *Glomus mossaea*	Compost at 10 g	6.58
Without *Glomus mossaea*	Compost at 0 g	2.34
Without *Glomus mossaea*	Compost at 10 g	3.99
	LSD (0.05)	0.899
	SE (±)	0.306

LSD = least significant difference, SE = standard error.

Table 9. Interaction of mycorrhizal inoculation with spent engine oil concentration and bacterial colony in contaminated soil

Mycorrhizal inoculation	SEO (mL/pot)	Bacterial colony (CFU/g)
With *Glomus mossaea*	SEO at 0 mL	5.933b
With *Glomus mossaea*	SEO at 100 mL	7.733a
With *Glomus mossaea*	SEO at 150 mL	2.483d
Without *Glomus mossaea*	SEO at 0 mL	3.850c
Without *Glomus mossaea*	SEO at 100 mL	4.167c
Without *Glomus mossaea*	SEO at 150 mL	1.483d

SEO = spent engine oil. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

Table 10. Interaction of compost with spent engine oil concentration and bacterial colony in contaminated soil

Compost (g/pot)	SEO (mL/pot)	Bacterial colony (CFU/g)
Compost at 0 g	SEO at 0 mL	3.350c
Compost at 0 g	SEO at 100 mL	4.750b
Compost at 0 g	SEO at 150 mL	1.700d
Compost at 10 g	SEO at 0 mL	6.433a
Compost at 10 g	SEO at 100 mL	7.150a
Compost at 10 g	SEO at 150 mL	2.267cd

SEO = spent engine oil. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

Table 11. Interaction of mycorrhizal inoculation, compost, and spent engine oil concentration and bacterial colony in contaminated soil

Mycorrhizal inoculation	Compost (g/pot)	SEO (mL/pot)	Bacterial colony (CFU/g)
With *Glomus mossaea*	Compost at 0 g	SEO at 0 mL	3.933cd
With *Glomus mossaea*	Compost at 0 g	SEO at 100 mL	6.333b
With *Glomus mossaea*	Compost at 0 g	SEO at 150 mL	2.300def
With *Glomus mossaea*	Compost at 10 g	SEO at 0 mL	7.933a
With *Glomus mossaea*	Compost at 10 g	SEO at 100 mL	9.133a
With *Glomus mossaea*	Compost at 10 g	SEO at 150 mL	2.667def
Without *Glomus mossaea*	Compost at 0 g	SEO at 0 mL	2.767def
Without *Glomus mossaea*	Compost at 0 g	SEO at 100 mL	3.167de
Without *Glomus mossaea*	Compost at 0 g	SEO at 150 mL	1.100f
Without *Glomus mossaea*	Compost at 10 g	SEO at 0 mL	4.933bc
Without *Glomus mossaea*	Compost at 10 g	SEO at 100 mL	5.167bc
Without *Glomus mossaea*	Compost at 10 g	SEO at 150 mL	1.867ef

SEO = spent engine oil. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

combinations (Table 10), although the combined applications of 10 g/pot compost with 0 mL/pot SEO and 10 g/pot compost with 100 mL/pot SEO were not significantly different. Combined application of compost at 0/g/pot with 150 mL/pot SEO had the lowest (1.700 CFU/g) bacterial colony count and this was significantly different from the combined use of 10 g/pot compost with 150 mL/pot SEO (Table 10). Furthermore, the combined use of 0 g/pot compost with 0 mL/pot SEO and 10 g/pot compost with 150 mL/pot SEO were not significantly different.

Interaction of mycorrhizal inoculation, compost and spent engine oil concentration and the bacterial colony in contaminated soil

Significant ($p < 0.05$) interaction existed between the parameters measured. Combined application of mycorrhiza, 10 g/pot compost, and 100 mL/pot SEO yielded significantly higher (9.133 CFU/g) bacterial colonies compared to other treatments (Table 11). Significantly lower (1.100 CFU/g) bacterial count was obtained from the combined application of 0 g/pot compost with 150 mL/pot SEO and without mycorrhiza inoculation compared to the combined application of mycorrhiza, 0 g/pot compost, and 0 mL/pot SEO.

Effect of mycorrhizal inoculation, compost and spent engine oil concentration on the fungal colony in contaminated soil

Mycorrhizal inoculated treatment resulted in a significantly higher (5.844 CFU/g) fungal colony compared to treatment without mycorrhizal inoculation (3.222 CFU/g) (Table 12). With reference to compost, amendment with 10 g/pot compost resulted in a significantly higher (6.222 CFU/g) fungal colony compared to compost at 0 g/pot amendment (Table 12). SEO application at 100 mL/pot resulted in significantly higher (5.058 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 (5.808 CFU/g) (Table 12).

Interaction of mycorrhizal inoculation with compost and the fungal colony in contaminated soil

Interaction between mycorrhiza inoculation and 10 g/pot compost had a significantly higher (7.978 CFU/g) fungal colony compared to other

Table 12. Effect of mycorrhizal inoculation, compost, and spent engine oil concentration on the fungal colony in contaminated soil

Treatments	Fungal colony (CFU/g)
Mycorrhizal inoculation	
With *Glomus mossaea*	5.844
Without *Glomus mossaea*	3.222
LSD (0.05)	0.3983
SE (±)	0.1358
Compost (g/pot)	
Compost at 0 g	2.844
Compost at 10 g	6.222
LSD (0.05)	0.3983
SE (±)	0.1358
SEO (mL/pot)	
SEO at 0 mL	5.808
SEO at 100 mL	5.058
SEO at 150 mL	2.733
LSD (0.05)	0.4879
SE (±)	0.1663
M X C	s
M X SEO	s
C X SEO	s
M X C SEO	s

SEO = spent engine oil, LSD = least significant difference, SE = standard error, M = mycorrhizal inoculation, C = compost and s = significant

treatment combinations (Table 13) whereas a significantly lower (1.978 CFU/g) fungal colony count was obtained from the combined application of 0 g/pot compost and without mycorrhiza inoculation.

Interaction of mycorrhizal inoculation with spent engine oil concentration and the fungal colony in contaminated soil

Combined application of mycorrhiza and 0 mL/pot SEO resulted in a significantly higher (7.500 CFU/g) fungal colony count compared to other treatment combinations (Table 14). However, combined applications of mycorrhiza and 100 mL/pot SEO and mycorrhiza with 0 mL/pot SEO were not significantly different (Table 14). Significantly lower (1.950 CFU/g) fungal colony count was obtained from the combination of 150 mL/pot SEO and without mycorrhiza inoculation compared to the combined application of mycorrhiza and 150 mL/pot SEO (Table 14).

Interaction of compost with spent engine oil concentration and fungal colony in contaminated soil

Interaction between 10 g/pot compost and 0 mL/pot SEO resulted in a significantly higher (7.900 CFU/g) fungal colony count compared to other treatments (Table 15), although the combined applications of 10 g/pot compost with 0 mL/pot SEO and 10 g/pot compost with 100 mL/pot SEO were not significantly different. Combined application of compost at 0/g/pot with 150 mL/pot SEO had the lowest (2.083 CFU/g)

Table 13. Interaction of mycorrhizal inoculation with compost and fungal colony in contaminated soil

Mycorrhizal inoculation	Compost (g/pot)	Fungal colony (CFU/g)
With *Glomus mossaea*	Compost at 0 g	3.711
Without *Glomus mossaea*	Compost at 10 g	7.978
With *Glomus mossaea*	Compost at 0 g	1.978
Without *Glomus mossaea*	Compost at 10 g	4.467
	LSD (0.05)	0.5633
	SE (±)	0.1921

LSD = least significant difference, SE = standard error.

Table 14. Interaction of mycorrhizal inoculation with spent engine oil concentration and fungal colony in contaminated soil

Mycorrhizal inoculation	SEO (mL/pot)	Fungal colony (CFU/g)
With *Glomus mossaea*	SEO at 0 mL	7.500a
With *Glomus mossaea*	SEO at 100 mL	6.517a
With *Glomus mossaea*	SEO at 150 mL	3.517b
Without *Glomus mossaea*	SEO at 0 mL	4.117b
Without *Glomus mossaea*	SEO at 100 mL	3.600b
Without *Glomus mossaea*	SEO at 150 mL	1.950c

SEO = spent engine oil. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

Table 15. Interaction of compost with spent engine oil concentration and fungal colony in contaminated soil

Compost (g/pot)	SEO (mL/pot)	Fungal colony (CFU/g)
Compost at 0 g	SEO at 0 mL	3.717b
Compost at 0 g	SEO at 100 mL	2.733cd
Compost at 0 g	SEO at 150 mL	2.083d
Compost at 10 g	SEO at 0 mL	7.900a
Compost at 10 g	SEO at 100 mL	7.383a
Compost at 10 g	SEO at 150 mL	3.383bc

SEO = spent engine oil. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

Table 16. Interaction of mycorrhizal inoculation, compost, and spent engine oil concentration and fungal colony in contaminated soil

Mycorrhizal Inoculation	Compost (g/pot)	SEO (mL/pot)	Fungal colony (CFU/g)
With *Glomus mossaea*	Compost at 0 g	SEO at 0 mL	5.133bc
With *Glomus mossaea*	Compost at 0 g	SEO at 100 mL	3.300d
With *Glomus mossaea*	Compost at 0 g	SEO at 150 mL	2.700de
With *Glomus mossaea*	Compost at 10 g	SEO at 0 mL	9.867a
With *Glomus mossaea*	Compost at 10 g	SEO at 100 mL	9.733a
With *Glomus mossaea*	Compost at 10 g	SEO at 150 mL	4.333c
Without *Glomus mossaea*	Compost at 0 g	SEO at 0 mL	2.300def
Without *Glomus mossaea*	Compost at 0 g	SEO at 100 mL	2.167ef
Without *Glomus mossaea*	Compost at 0 g	SEO at 150 mL	1.467f
Without *Glomus mossaea*	Compost at 10 g	SEO at 0 mL	5.933b
Without *Glomus mossaea*	Compost at 10 g	SEO at 100 mL	5.033bc
Without *Glomus mossaea*	Compost at 10 g	SEO at 150 mL	2.433def

SEO = spent engine oil. Means with the same letter(s) are not significantly different at $p < 0.05$ using Duncan Multiple Range Test (DMRT)

fungal colony count and this was significantly different from the combined use of 10 g/pot compost with 150 mL/pot SEO. Furthermore, the combined use of 0 g/pot compost with 0 mL/pot SEO and 10 g/pot compost with 150 mL/pot SEO were not significantly different.

Interaction of mycorrhizal inoculation, compost, and spent engine oil concentration and the fungal colony in contaminated soil

Significant ($p < 0.05$) interaction existed between the parameters measured. Combined application of mycorrhiza, 10 g/pot compost, and 0 mL/pot SEO resulted in significantly higher (9.867 CFU/g) fungal colony compared to other treatment combinations (Table 16). Similarly, a significantly lower (1.467 CFU/g) fungal count was obtained from the combined application of 0 g/pot compost with 150 mL/pot SEO and without mycorrhiza inoculation compared to the combined application of mycorrhiza, 0 g/pot compost, and 0 mL/pot SEO.

DISCUSSION

The results obtained from this study show enhanced TPH degradation due to compost application to the SEO-contaminated soil. The TPH reduction orchestrated through the bio-stimulatory influence of compost in this study corroborates previous findings by Agbor et al. (2012); Agbor et al. (2015); Adeleye et al. (2020); Adeleye et al. (2021), with reference to its capacity for such achievement. This further corroborates the findings of Okoh (2006), who reported that organic wastes bind rapidly to the soil particle, and this facilitates the movement of the pollutants through the soil when natural events like rain occur.

The application of compost made from CPH and cattle dung in this study resulted in a higher bacterial and fungal population compared to treatments devoid of such application. This result is in agreement with the findings of Ijah and Antai (2003), who observed the bacterial count of hydrocarbon degraders in oil-polluted soil to be $\times 10^6$ CFU/g and that of Antai and Mgbomo (1989), who observed the bacterial count of Hydrocarbon Utilising Bacteria (HUB) in hydrocarbon-contaminated soil to be ×10^8 CFU/g. The reason for higher counts of bacteria in amended soil may be due to the presence of appreciable quantities of nitrogen and phosphorus in the organic wastes. This result also corresponds with the findings of Tiquia and Tam (2002), which stated that the colonies of fungi and bacteria increased in their study because of high concentrations of ammonia in the organic wastes utilised.

The results obtained from soil treatments contaminated with SEO show that the microbial population of the soil was affected when compared with uncontaminated soil. The microbial population was reduced probably as a result of the nutrient imbalance created by SEO pollution. This result is also in agreement with Okolo et al. (2005), who used poultry manure to enhance crude oil degradation in sandy loam soil and concluded that poultry and cattle manure improved soil chemical properties irrespective of soil texture.

The addition of compost in this study resulted in enhanced TPH degradation in the SEO-contaminated soil. A similar result was also obtained by Adeleye et al. (2021), where they observed a reduction in TPH and Polycyclic Aromatic Hydrocarbons (PAHs) content of SEO contaminated soil. The reduction in TPH content in this study could be due to the fact that mycorrhizal fungi notable active bio-fertilization interacted with some other beneficial soil organisms in order to achieve complete clean-up of polluted soils (Chibuike, 2013). These results equally corroborate the previous report by Harrier and Watson (2004), that mycorrhizal fungi favour the activities of soil microorganisms and that the amount of pollutants bio-augmented with mycorrhiza fungi is increased due to the activities of these microorganisms. It is also evident in the work of Gao et al. (2010), where they observed remediation of fluorine and phenanthrene with Arbuscular Mycorrhiza (AM) inoculation compared to those treatments that were not inoculated with mycorrhiza.

In this study, mycorrhizal inoculation increased the counts of bacteria and fungi colonies. This result could be attributed to the fact that mycorrhizal fungi favour the activities of soil microorganisms. This equally corroborates the findings of Nkereuwem et al. (2020b), who reported an increase in fungal colony count (CFU) of Bonny light crude oil contaminated soil due to mycorrhiza inoculation compared to non-mycorrhizal inoculation. Mycorrhizal inoculation adopted in this study resulted in enhanced bacterial and fungal colony counts. This result is also in agreement with the findings of Yuniati (2018), who reported 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, thereby resulting in their increased populations. Mycorrhiza fungi also release glomalin in the soil environment which results in better soil structure and high organic matter content coupled with the fact that they also play a major role in the soil aggregation process and stimulation of microbial activity (Miller and Jastrow, 2000).

Increase in SEO concentration levels resulted in higher TPH contents of the contaminated soil and this in turn affects some of the soil properties (e.g., pH). The result of this study is in agreement with the findings of Nkereuwem et al. (2010) on the effect of spent oil on soil properties. Furthermore, Bizecki Robson et al. (2004) reported higher pH values in hydrocarbon oil-contaminated soil. A significant determinant of the elements in the soil that are available for plant absorption is the pH of the soil (Marschner, 1995). The retention of metals to soil organic matter is also weaker at low pH, resulting in more available metal in the soil solution for root absorption (McBride, 1994). Many metal cations are more soluble and available in the soil solution at low pH (below 5.5), including Cd, Cu, Hg, Ni, Pb, and Zn (McBride, 1994). (Ndukwu, et al., 2015; Uchendu and Ogwo, 2014).

CONCLUSION AND RECOMMENDATION

It can be concluded that mycorrhizal inoculation and compost were effective in the bioremediation of SEO-impacted soil. Furthermore, the combined application of mycorrhiza and compost performed best in TPH degradation compared to using mycorrhiza or compost alone. Microbial growth was found to increase significantly due to mycorrhizal inoculation and compost application compared to treatments without mycorrhiza or compost application. Mycorrhiza and cocoa-based compost should be employed in the bioremediation of SEO and other petroleum hydrocarbon related-pollution due to their promising exploits. However, further studies should be conducted with higher concentrations of SEO with a view to ascertaining the combinatorial efficacy of mycorrhiza and cocoa-based compost in the remediation of such a hydrocarbon-impacted environment.

ACKNOWLEDGEMENTS

The current study was independently funded by the authors and they are therefore acknowledged.

CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to research, authorship, and publication of this article.

ETHICAL COMPLIANCE

The authors have followed ethical standards in conducting the research and preparing the manuscript.

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Received: February 11, 2022
Accepted after revisions: September 20, 2022