Почвознание агрохимия и екология, 59, 1/2025
Bulgarian Journal of Soil Science Agrochemisty and Ecology, 59, 1/2025

DOI: https://doi.org/10.61308/AHBZ7374

Bioremediation of heavy metals in spent engine oil-contaminated soil using mycorrhiza and cattle rumen digesta

Michael Nkereuwem[1]*, Adeniyi Adeleye[2], Christiana Ijah[3], Paul Tersoo[2], Mustapha Muhammad[1], Asimya Lemuel[4], Godiya Saleh[1]

[1]*Department of Soil Science, Federal University Dutse, Jigawa, Nigeria*
[2]*Department of Environmental Sciences, Federal University Dutse, Jigawa, Nigeria*
[3]*Department of Soil Science, Akwa Ibom State University, Akwa Ibom, Nigeria*
[4]*Department of Pharmacognosy, Bingham University, Karu, Nasarawa State, Nigeri*a
Corresponding author*: mikenkereuwem@yahoo.com

Citation: Nkereuwem, M., Adeleye, A., Ijah, C., Tersoo. P., Muhammad, M., Lemuel, A., & Saleh, G. (2025). Bioremediation of heavy metals in spent engine oil-contaminated soil using mycorrhiza and cattle rumen digesta. *Bulgarian Journal of Soil Science Agrochemisty and Ecology, 59*(1), 40-53.

Abstract

Spent engine oil (SEO) is a complex mixture of petroleum hydrocarbons, heavy metals, and chemical additives and its contamination of agricultural soils poses severe challenges to soil health and fertility. The traditional remediation techniques have cost implications. Nevertheless, bioremediation is a cost-effective and sustainable remediation strategy. The aim of this study was to determine the efficacy of mycorrhizae and cattle rumen digesta in remediating heavy metals (Cr and Cu) in SEO-contaminated soil. Mycorrhiza, cattle rumen digesta and SEO were at two levels each. Data on Cr and Cu concentrations, bacterial and fungal populations were collected and analyzed using ANOVA at α 0.05. The results of the study reveals that mycorrhiza and cattle rumen digesta application yielded significantly higher bacterial and fungal populations leading to significant ($p<0.05$) reductions in Cr and Cu concentrations in SEO-contaminated soil. The combined application of mycorrhiza with 40 g/pot cattle rumen digesta had significantly ($p<0.05$) lower Cr and Cu concentrations compared to single application of either mycorrhiza or cattle rumen digesta. Mycorrhiza and cattle rumen digesta are therefore recommended for use in bioremediation of Cr and Cu contaminated soil.

Key words: mycorrhiza, cattle rumen digesta, heavy metals, bioremediation, spent engine oil, contaminated soil

Introduction

Serious environmental issues can arise from crude oil and its derivatives, such as Spent Engine Oil (SEO), particularly in regions where oil is widely extracted and used (Amadi et al., 2020; Pinheiro et al., 2020). Due to the inappropriate disposal of spent engine oil by auto specialists and associated craftspeople in Nigeria, contaminants have accumulated in soils, affecting soil health and agricultural productivity (Oghenerobor et al., 2021). According to Omotayo et al. (2022), the complex blend of petroleum hydrocarbons, heavy metals, and chemical additives found in SEO can linger in the environment thereby resulting into long-term ecological consequences. Moses et al. (2023) reported that elevated levels of heavy metals such as chromium, copper, and nickel in spent engine oil can harm human health by causing anemia, neurological disorders, and compromised immune systems, all of which can result in mortality and population-level impacts. Additionally, according to Mitra et al. (2022), some heavy metals are harmful or carcinogenic, damaging the central nervous system (manganese, mercury, lead, arsenic), the skin, bones, or teeth (nickel, cadmium, copper, chromium), or the kidneys or liver (mercury, lead, cadmium, copper). Chromium, heavy metal found in spent engine oil, is a priority pollutant, according to studies. At high concentrations, particularly hexavalent chromium, it is carcinogenic, teratogenic, and toxic (Abdelhafeez et al., 2024). The environment and human health are at risk from trace amounts of heavy metal poisoning because the metals accumulate in the ecosystem over time after seeping into the surrounding agricultural soil. As a result, there is a lot of concern about potential health risks and issues with food safety that arise when the general public eats potentially contaminated vegetables (Gebeyehu and Bayissa 2020; Zhao et al. 2022). Conventional techniques of cleaning up contaminated soils can be costly and lead to more environmental disruptions. Cost-effective and environmentally friendly remediation techniques, including bioremediation, which employs living organisms to cleanse contaminated environments, are therefore becoming more and more popular (Abdulwahab et al., 2021). Bioremediation uses bacteria, fungi, and plants to restore soils that have been contaminated with organic pollutants. These environmentally friendly and cost-effective methods have demonstrated promising outcomes in the recovery of polluted soils, particularly by petroleum hydrocarbons (Graj et al., 2013; Nkereuwem et al., 2024). Arbuscular mycorrhizal (AM) fungi has gained attention for its potential to enhance the phytoremediation capabilities of plants in contaminated soils (Nkereuwem et al., 2020a, Ezugwu et al., 2022; Nkereuwem et al., 2024). Arbuscular mycorrhizal fungi use glomalin to immobilize metals in the soil and metal adsorption on their surface to directly detoxify heavy metal contamination (Vilela and Barbosa, 2019). According to Olalekan and Oyedepo (2023), AM fungi also develop symbiotic interactions with plant roots, which enhance phosphorus uptake and boost plant resistance to environmental stressors such as heavy metals. This symbiosis can help clean-up by improving plant survival and growth in contaminated soils (Ayodele et al., 2023).

Cattle rumen digesta is a by-product of the abattoir generally derived from the incompletely digested feed consumed by ruminants. Investigation shows the possible use of organic waste to improve the productivity and fertility of soils (Asadu and Igboka; 2014). Cattle rumen digesta is recommended for use in bioremediation because it enhances soil nutrient utilization and reduces pollution (Cherdthong, 2020). Study by Nkereuwem et al. (2024) evaluated the potential of cattle rumen digesta in the bioremediation total petroleum hydrocarbon (TPH) in spent engine oil contaminated soil and they reported significantly lower TPH in spent engine oil contaminated soil due to cattle rumen digesta application compared to treatment without cattle rumen digesta. These techniques are economically and eco-friendly.

The aim of this study was to determine the efficacy of mycorrhizae and cattle rumen digesta in remediating heavy metals (Cr and Cu) in SEO-contaminated soil.

Materials and methods

Study area description

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 (Olaniyi et al., 2022).

Experimental materials and design

The materials utilized in this research included soil samples, mycorrhizal inoculum, polyethylene bags, and spent engine oil. The mycorrhiza was obtained from the Soil Microbiology Laboratory at the Department of Soil Resource Management, University of Ibadan, Oyo State while the cattle rumen digesta was obtained from Dutse abattoir. Using a Completely Randomized Design (CRD), the research was designed as a 2×2×2 factorial experiment with three replicates. There were 8 different treatment combinations, resulting in 24 experimental units. The factors are detailed below:

Mycorrhiza inoculum at 2 levels

With Glomus deserticola- M^+ (30 g/pot)

Without Glomus deserticola- M^- (0 g/pot)

Cattle rumen digesta at 2 levels

With cattle rumen digesta- C_1 (40 g/pot)

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

Spent engine oil at 2 levels

With spent engine oil-S_{200} (200 ml/pot)

Without spent engine oil- S_0 (0 ml/pot)

Treatment combinations

$M^+C_1S_{200}$	$M^-C_1S_{200}$
$M^+C_1S_0$	$M^-C_1S_0$
$M^+C_0S_{200}$	$M^-C_0S_{200}$
$M^+C_0S_0$	$M^-C_0S_0$

Soil samples collection, preparation and incubation study

Soil samples were gathered from the experimental location at 0-20 cm depth using a shovel. The samples were crushed, air-dried, and passed through a 2 mm sieve. The soils were then sterilized in an electric oven at 105° C for about 1 hour and each polyethylene bag contained 10 kg of the sterilized soil sample.

Spent engine oil (0 and 200 ml) was mixed thoroughly with the soil and allowed to stand for 2 weeks. Cattle rumen digesta was applied after 2 weeks at the rate of 0 and 40 g/pot. This was mixed thoroughly with the soil for even distribution. Inoculation in treatment containing mycorrhizae inoculum consisted of 30 g of Glomus deserticola. The incubation study lasted for six (6) weeks after which, soil samples were sampled for laboratory analyses.

Laboratory analysis

Particle size analysis was done using the hydrometer method (Bouyoucos, 1951) while organic carbon was determined using the Walkley Black Method (Walkley and Black, 1934). Total nitrogen and available phosphorus were determined using Kjeldahl method as described by Bremmer (1996) and the Olsen (Olsen et al. 1954) 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 (Jackson, 1973), 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).

Heavy metal determination

The standard flame atomic absorption method as described by Aluko et al. (2018) was used to determine the chromium and copper contents in the soil.

Data analysis

The collected data were subjected to Analysis of Variance (ANOVA) using GENSTAT version 17 and significant means were separated using Least Significant Difference (LSD) and Duncan's Multiple Range Test (DMRT) at a 5% probability level.

Results

Physical and chemical properties of the soil used

The results obtained from the soil analysis are presented in the table 1 below. The soil analysis revealed that the study area is predominantly sandy loam in texture with a pH of 7.1. The Organic carbon and total nitrogen contents were 1.93 g/kg and 2.15 g/kg while the Available phosphorus and bulk density were 6.8 g/kg and 1.53 g/cm³, respectively.

Effects of mycorrhiza, cattle rumen digesta and spent engine oil on chromium and copper contents in contaminated soil

Mycorrhiza inoculation resulted in significantly lower (6.14 mg/kg) chromium content compared to treatment without mycorrhiza (table 2). Copper content was also significantly lower (10.59 mg/kg) due to mycorrhiza inoculation compared to non-mycorrhiza inoculated treatment.

With respect to cattle rumen digesta application, there were no significant differences in chromium and copper contents in the soil.

Spent engine oil application resulted in significantly higher (8.57 and 12.88 mg/kg) chromium and copper contents in the soil respectively, compared to treatment without spent engine oil application (table 2).

Interaction of mycorrhiza and cattle rumen digesta on chromium and copper contents in oil contaminated soil

The interaction between mycorrhiza and cattle rumen digesta yielded significantly lower (6.13 and 10.44 mg/kg) chromium and copper contents in the soil compared with other treatment combinations (table 3) while significantly higher (7.85 and 12.02 mg/kg) chromium and copper contents in the soil were obtained from the combined application of 0 g/pot cattle rumen digesta and without mycorrhiza, respectively; compared to the combined application of 40 g/pot Cattle Rumen Digesta (CRD) and without mycorrhiza inoculation.

Interaction of mycorrhiza and spent engine oil on chromium and copper contents in oil contaminated soil

Significantly lower (4.32 and 8.40 mg/kg) chromium and copper contents in the soil were obtained from the combination of mycorrhiza and 0 ml/pot SEO (table 4) compared to other combinations. The combined application 200 ml/pot SEO and without mycorrhiza inoculation resulted in significantly higher (9.190 and 12.98 mg/kg) chromium and copper contents in the soil respectively, compared to the combined application of mycorrhiza and 200 ml/pot SEO (table 4).

Interaction of cattle rumen digesta and spent engine oil on chromium and copper contents in oil contaminated soil

The combined application of 40 g/pot CRD and 0 ml/pot SEO had significantly lower (4.96 and 8.27 mg/kg) chromium and copper contents in the soil compared to the other treatments (table 5). Significantly higher (8.64 and 14.19 mg/kg) chromium and copper contents in the soil resulted from the combined use of 0 g/pot CRD and 200 ml/pot SEO compared to the chromium and copper contents obtained from the combination of 0 g/pot CRD and 0 ml/pot SEO.

Interaction of mycorrhiza, cattle rumen digesta and spent engine oil on chromium and copper contents in contaminated soil

Significant ($p<0.05$) interactions existed between the factors. The combined application of mycorrhiza, 40 g/pot Cattle Rumen Digesta (CRD) and 0 ml/pot SEO resulted in significantly lower (4.29 and 7.95 mg/kg) chromium and copper contents in the soil respectively, compared to the other treatment combinations (table 6) although the chromium and copper contents obtained from the

Table 1. Physical and chemical properties of the experimental soil

Parameter	Value
Bulk Density (g/cm3)	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)	6.81
Exchangeable Bases (cmol/kg)	6.34
Exchangeable Acidity (cmol/kg)	4.16
CEC (cmol/kg)	10.5

Table 2. Effects of mycorrhiza, cattle rumen digesta and spent engine oil on chromium and copper contents in contaminated soil

Treatment	Chromium (mg/kg)	Copper (mg/kg)
Mycorrhiza		
M^+	6.14b	10.59b
M^-	7.66a	11.76a
SE (±)	0.12	0.11
Cattle Rumen Digesta (g/pot)		
C_0	6.99	11.23
C_1	6.80	11.11
	Ns	Ns
Spent Engine Oil (ml/pot)		
S_0	5.22b	9.47b
S_1	8.57a	12.88a
SE (±)	0.12	0.11

Means with the same letter (S) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), M^+ = with mycorrhiza, M- = without mycorrhiza, CRD = cattle rumen digesta, C0 = without CRD, C_1 = CRD at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot, SE = standard Error, Ns = Not significant

Table 3. Interaction of mycorrhiza and cattle rumen digesta on chromium and copper contents in oil contaminated soil

Mycorrhiza	Cattle rumen digesta (g/pot)	Chromium (mg/kg)	Copper (mg/kg)
M^+	C_0	6.145b	10.73b
M^+	C_1	6.132b	10.44b
M^-	C_0	7.853a	12.02a
M^-	C_1	7.460a	11.49a
	SE (±)	0.1628	0.1545

Means with the same letter (S) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), M^+ = with mycorrhiza, M^- = without mycorrhiza, CRD = cattle rumen digesta, C_0 = without CRD, C_1 = CRD at 40 g/pot, SE = standard error

Table 4. Interaction of mycorrhiza and spent engine oil on chromium and copper contents in oil contaminated soil

Mycorrhiza	SEO (ml/pot)	Chromium (mg/kg)	Copper (mg/kg)
M^+	S_0	4.325d	8.40c
M^+	S_1	6.123c	10.53b
M^-	S_0	7.952b	12.76a
M^-	S_1	9.190a	12.98a
	SE (±)	0.1628	0.1545

Means with the same letter (S) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), M^+ = with mycorrhiza, M^- = without mycorrhiza, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot, SE = standard Error

Table 5. Interaction of cattle rumen digesta and spent engine oil on chromium and copper contents in oil contaminated soil

Cattle rumen digesta (g/pot)	SEO (ml/pot)	Chromium (mg/kg)	Copper (mg/kg)
C_0	S_0	8.502a	11.55b
C_0	S_1	8.640a	14.19a
C_1	S_0	4.965c	8.27d
C_1	S_1	5.483b	10.66c
	SE (±)	0.1628	0.1545

Means with the same letter (s) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), CRD = cattle rumen digesta, C_0 = without CRD, C_1 = CRD at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot, SE = standard Error

Table 6. Interaction of mycorrhiza, cattle rumen digesta and spent engine oil on chromium and copper contents in contaminated soil

Mycorrhiza	Cattle rumen digesta (g/pot)	SEO (ml/pot)	Chromium (mg/kg)	Copper (mg/kg)
M^+	C_0	S_0	5.570d	8.59d
M^+	C_0	S_1	7.930b	10.51c
M^+	C_1	S_0	4.290e	7.95e
M^+	C_1	S_1	4.360e	8.86d
M^-	C_0	S_0	7.973b	12.93b
M^-	C_0	S_1	9.350a	15.46a
M^-	C_1	S_0	6.677c	12.47b
M^-	C_1	S_1	9.030a	12.60b

Means with the same letter (S) are not significantly different from each other at p>0.05 using Duncan's Multiple Range Test (DMRT), M^+ = with mycorrhiza, M^- = without mycorrhiza, C_0 = without cattle rumen digesta, C_1 = cattle rumen digesta at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot

combined use of mycorrhiza, 40 g/pot CRD and 0 ml/pot SEO and mycorrhiza, 40 g/pot CRD and 200 ml/pot SEO were not significantly different. The combination of 0 g/pot CRD, 200 ml/pot SEO and without mycorrhiza yielded significantly higher (9.35 and 15.46 mg/kg) chromium and copper contents in the soil compared to chromium and copper contents obtained from the combination of 40 g/pot CRD, 200 ml/pot SEO and without mycorrhiza inoculation.

Effects of mycorrhiza, cattle rumen digesta and spent engine oil on bacterial colony in contaminated soil

Mycorrhiza inoculation resulted in significantly higher (58.08 x 10^4 CFU/g soil) bacterial colony count compared to treatment without mycorrhiza inoculation (table 7).

Cattle rumen digest at 40 g/pot yielded significantly higher (60.81 x 10^4 CFU/g soil) bacterial colony count compared to 0 g/pot CRD (table 7).

Application of 200 ml/pot SEO had significantly higher (55.99 x 10^4 CFU/g soil) bacterial population compared to 0 ml/pot SEO (table 7).

Interaction of mycorrhiza and cattle rumen digesta on bacterial colony in contaminated soil

Significantly higher (64.21 x 10^4 CFU/g soil) bacterial colony in the soil was obtained from the interaction of mycorrhiza and 40 g/pot CRD compared to the other treatment combinations (table 8) while the combined application of 0 g/pot CRD and without mycorrhiza yielded significantly lower (49.29 x 10^4 CFU/g soil) bacterial colony count in the soil compared to the bacterial colony obtained from the interaction between mycorrhiza and 0 g/pot CRD.

Interaction of mycorrhiza, cattle rumen digesta and spent engine oil on bacterial colony in contaminated soil

The combined application of mycorrhiza, 40 g/pot CRD and 200 ml/pot SEO had significantly higher (65.23 x 10^4 CFU/g soil) bacterial colony count in the soil compared to the other interactions (table 9). The interaction of 0 g/pot CRD, 0 ml/pot SEO and without mycorrhiza produced significantly lower (42 x 10^4 CFU/g soil) lower bacterial population compared to the bacterial population obtained from the combined use of 0 g/pot CRD, 200 ml/pot SEO and without mycorrhiza inoculation.

Table 7. Effects of mycorrhiza, cattle rumen digesta and spent engine oil on bacterial colony in contaminated soil

Treatment	Bacterial colony (CFU x 10^4/g soil)
Mycorrhiza	
M^+	580846a
M^-	533442b
SE (±)	1241.5
Cattle Rumen Digesta (g/pot)	
C_0	506233b
C_1	608054a
SE (±)	1241.5
Spent Engine Oil (ml/pot)	
S_0	554329b
S_1	559958a
SE (±)	1241.5

Means with the same letter (S) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), M^+ = with mycorrhiza, M- = without mycorrhiza, CRD = cattle rumen digesta, C_0 = without CRD, C_1 = CRD at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot, SE = standard Error, Ns = Not significant

Table 8. Interaction of mycorrhiza and cattle rumen digesta on bacterial colony in contaminated soil

Mycorrhiza	Cattle Rumen Digesta (g/pot)	Bacterial colony (CFU x10^4/g soil)
M^+	C_0	519583c
M^+	C_1	642108a
M^-	C_0	492883d
M^-	C_1	574000b
	SE (±)	1755.8

Means with the same letter (S) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), M^+ = with mycorrhiza, M^- = without mycorrhiza, CRD = cattle rumen digesta, C_0 = without CRD, C_1 = CRD at 40 g/pot, SE = standard error

Effects of mycorrhiza, cattle rumen digesta and spent engine oil on fungal colony in contaminated soil

Mycorrhiza inoculation produced significantly higher (98.46 x 10^3 CFU/g soil) fungal population in the soil compared to treatment without mycorrhiza inoculation (table 10).

Cattle rumen digesta at 40 g/pot yielded significantly higher (83.75 x 10^3 CFU g/soil) fungal colony in the soil compared to 0 g/pot CRD application (table 10).

Treatment with 0 ml/pot SEO had significantly higher (84.21 x 10^3 CFU/g soil) fungal population compared to 200 ml/pot SEO (table 10).

Interaction of cattle rumen digesta and spent engine oil on fungal population in contaminated soil

The combined application of 40 g/pot CRD

Table 9. Interaction of mycorrhiza, cattle rumen digesta and spent engine oil on bacterial colony in contaminated soil

Mycorrhiza	Cattle rumen digesta (g/pot)	SEO (ml/pot)	Bacterial population (CFU x104/g soil)
M^+	C_0	S_0	532500e
M^+	C_0	S_1	615500c
M^+	C_1	S_0	631883b
M^+	C_1	S_1	652333a
M^-	C_0	S_0	420000g
M^-	C_0	S_1	435833f
M^-	C_1	S_0	549933d
M^-	C_1	S_1	619167c

Means with the same letter (S) are not significantly different from each other at p>0.05 using Duncan's Multiple Range Test (DMRT), M^+ = with mycorrhiza, M^- = without mycorrhiza, C_0 = without cattle rumen digesta, C_1 = cattle rumen digesta at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot

Table 10. Effects of mycorrhiza, cattle rumen digesta and spent engine oil on fungal population in contaminated soil

Treatment	Fungal colony (CFU x 10^3/g soil)
Mycorrhiza	
M^+	98458a
M^-	69042b
SE (±)	884.9
Cattle Rumen Digesta (g/pot)	
C_0	61450b
C_1	83750a
SE (±)	884.9
Spent Engine Oil (ml/pot)	
S_0	84208a
S_1	83292b
SE (±)	884.9

Means with the same letter (S) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), M^+ = with mycorrhiza, M^- = without mycorrhiza, C_0 = without cattle rumen digesta, C_1 = cattle rumen digesta at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot, SE = standard error

Table 11. Interaction of cattle rumen digesta and spent engine oil on fungal population in contaminated soil

Cattle rumen digesta (g/pot)	SEO (ml/pot)	Fungal population (CFU x 10^3/g soil)
C_0	S_0	82000bc
C_0	S_1	81083c
C_1	S_0	86417a
C_1	S_1	85500ab
	SE (±)	1251.4

Means with the same letter (S) are not significantly different from each other at p>0.05 using Least Significant Difference (LSD), C_0 = without cattle rumen digesta, C_1 = cattle rumen digesta at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot, SE = standard error

Table 12. Interaction of mycorrhiza, cattle rumen digesta and spent engine oil on fungal population in contaminated soil

Mycorrhiza	Cattle rumen digesta (g/pot)	SEO (ml/pot)	Fungal population (CFU/g soil)
M^+	C_0	S_0	69.67 x 103c
M^+	C_0	S_1	94.17 x 103b
M^+	C_1	S_0	10.48 x 104a
M^+	C_1	S_1	10.05 x 104a
M^-	C_0	S_0	68 x 103c
M^-	C_0	S_1	68 x 103c
M^-	C_1	S_0	70.5 x 103c
M^-	C_1	S_1	94.33 x 103b

Means with the same letter (S) are not significantly different from each other at p>0.05 using Duncan's Multiple Range Test (DMRT), M^+ = with mycorrhiza, M^- = without mycorrhiza, C_0 = without cattle rumen digesta, C_1 = cattle rumen digesta at 40 g/pot, SEO = spent engine oil, S_0 = SEO at 0 ml/pot, S_1 = SEO at 200 ml/pot

and 0 g/pot SEO resulted in significantly higher (86.42 x 103 CFU g/soil) fungal population compared to the other combinations (table 11) although the fungal population obtained from combinations 40 g/pot CRD with 0 g/pot SEO and 40 g/pot CRD with 200 ml/pot SEO were not significantly different. The interaction of 0 g/pot CRD and 200 ml/pot SEO yielded significantly lower (81.08 x 10^3 CFU/g soil) fungal population in the soil compared to the fungal population obtained from the interaction of 40 g/pot CRD and 200 ml/pot SEO.

Interaction of mycorrhiza, cattle rumen digesta and spent engine oil on fungal population in contaminated soil

Significantly higher (10.48 x 10^4 CFU/g soil) fungal population was obtained from the of mycorrhiza, 40 g/pot CRD and 0 ml/pot SEO compared to the other interactions (table 12) however, the fungal population obtained from the combinations of mycorrhiza, 40 g/pot CRD and 0 ml/pot SEO and mycorrhiza, 40 g/pot CRD and 200 ml/pot SEO were not significantly different. The combined application of 0 g/pot CRD, 0 and 200 ml/pot SEO and without mycorrhiza inoculation resulted in significantly lower (68 x 10^3 CFU/g soil) fungal population compared to the fungal populations obtained from the interactions of mycorrhiza, 0 g/pot CRD and 200 ml/pot SEO and 40 g/pot CRD, 200 ml/pot SEO and without mycorrhiza.

Discussion

The result of the soil analysis shows that the experimental soil is consistent with soil of northern Nigeria's semi-arid and arid regions which is characterized by sandy soils (Ogunwole and Ogunleye, 2005). The soil pH aligns with the typical pH range for Sudan Savannah soils as reported by Nkereuwem et al. (2022b). Organic carbon content was low, which corroborates the findings of Amanze et al. (2016) for northern Nigerian soils and follows the rating by Akinrinde and Obigbesan, (2000). Total nitrogen was categorized as low according to Schoeneberger et al. (2002). This low nitrogen level is a common feature of tropical semi-arid soils, primarily caused by nutrient leaching and erosion due to intense tropical rainfall. Available phosphorus and exchangeable bases, according to Bray1 standards (<8.0 mg/kg) and the criteria established by Usman et al. (2005) were low, respectively.

The utilization of Cattle Rumen Digesta (CRD) in this study has demonstrated significant potential in the remediation of heavy metals in Spent Engine Oil (SEO) contaminated soil. Significant reductions in Cr and Cu concentrations in the soil due to cattle rumen digesta application were obtained. The reason for the reduction might be that cattle rumen digesta, being an organic amendment, enhances soil properties by increasing organic matter, improving microbial activity, and promoting heavy metal immobilization through adsorption and complexation (Nkereuwem et al., 2020a; Pinheiro et al., 2020). The results of this study is in agreement with the findings of Adeleye et al. (2019; 2023) and Abdelhafeez et al. (2024), where they reported significant reductions in heavy metal concentrations in spent engine oil-impacted soil due to organic amendments.

The results of this study shows that treatment with mycorrhiza (Glomus deserticola) experience significant reduction in Cr and Cu concentrations in the SEO-contaminated soil. The lower Cr and Cu concentrations recorded in this study could be explained by the fact that on the surface of the fungal structure, there are positive charge particles such as cysteine, glutathione, amino acids and thiol groups which adsorb and later reduce heavy metals from one form to another (Joutey et al., 2015). Additionally, by directly engaging metal adsorption on the fungal surface and glomalin-induced soil immobilization, arbuscular mycorrhizal fungi detoxify heavy metal contamination (Vilela and Barbosa, 2019). The result of this study corroborates the findings of Olusola et al. (2017) who reported reduction in heavy metal concentrations in Oil Refinery Effluent polluted soil. The findings of this research agrees with the report of Ibrahim et al. (2023), who also obtained significant reduction of heavy metal concentrations in multi-contaminated soil.

Treatments with Glomus deserticola and 40 g/pot Cattle Rumen Digesta (CRD) showed lower Cr and Cu concentrations compared to treatments with mycorrhiza or cattle rumen digesta alone. This revealed that there was positive and productive interaction between Glomus deserticola and cattle rumen digesta in bioremediation of heavy metals in SEO-contaminated soil. This result corroborates the findings of Olusola et al. (2017) who also reported lower concentrations of heavy metals in Oil Refinery Effluent polluted soil when G. hoi and P. aeruginosa were combined compared to single inoculation of Glomus hoi or Pseudomonas aeruginosa.

The higher bacterial and fungal colonies obtained in this study is in agreement with previous findings by Nkereuwem et al. (2020b and 2024) who also recorded higher bacterial and fungal populations in Crude oil and SEO impacted soil. This could be explain explained by the fact that mycorrhiza employs different techniques in bioremediation of contaminated soil and one of such techniques is Mycorrhiza Assisted Remediation (MAR).

Conclusion

The study investigated the efficacy of mycorrhizae and cattle rumen digesta in remediating heavy metals (Cr and Cu) in SEO-contaminated soil. The results revealed that mycorrhiza inoculation and cattle rumen digesta brought about significant reduction in Cr and Cu concentrations in spent engine oil contaminated soil compared to treatments that were devoid of mycorrhiza and cattle rumen digesta. Greater reductions in Cr and Cu concentrations in the SEO-contaminated soil were obtained from the combined application of mycorrhiza with 40 g/pot cattle rumen digesta as against single application of either mycorrhiza or 40 g/pot cattle rumen digesta. Mycorrhiza should be use used either singly or in combination with other soil amendments in restoring heavy metals impacted soil. The use of cattle rumen digesta is therefore recommended for use in bioremediation of heavy metals contaminated soil as it is effective, affordable and eco-friendly.

Acknowledgements

The authors acknowledge all those who contributed to the success of this research.

Conflict of interest

The authors declare that they do not have any financial or non-financial conflict of interest with regard to this manuscript

Funding source

The authors sponsored the research

Data availability

All the data needed have been provided in the article.

Ethics statement

None

References

Abdelhafeez, I. A., El-Tohamy, S. A., & El-Dars, F. M.(2024). Enhanced phytoaccumulation dynamics of chromium and nickel from spent engine oil-contaminated soil amended with biomass derived bulking agent. *Scientiae Radices, 3*(3), 142-156

Abdulwahab, R., Bello, M., & Adamu, A. (2021). Bioremediation: An eco-friendly approach to soil contamination. *Journal of Environmental Science and Technology, 15*(4), 112-120.

Adeleye, A. O., Ibrahim, A., Nkereuwem, M. E., Shiaka, P.G., & Yerima, M. B. (2023). Bio-stimulatory effects of cattle dung on lead decontamination potential of indigenous fungal population isolated from spent engine oil-polluted soil. *Science Heritage Journal, 7*(1), 18-23.

Adeleye, A. O; Yerima, M. B; Nkereuwem, M. E; Onokebhagbe, V. O; Shiaka, P. G; Amoo, F. K., & Adam, I. K. (2019). Effect of organic amendments on the decontamination potential of heavy metals by Staphylococcus aureus and Bacillus cereus in soil contaminated with spent engine oil. *Novel Research in Microbiology Journal, 3*(5), 471-484.

Akinrinde, E. A., & Obigbesan, G. O. (2000). Evaluation of the fertility status of selected soils for crop production in five ecological zones of Nigeria. *Proceedings of the 26th Annual Conference of the Soil Science Society of Nigeria*, 279–288.

Aluko, T. S., Njoku, K. L., Adesuyi, A. A., & Akinola, M. O. (2018). Health Risk Assessment of Heavy Metals in Soil from the Iron Mines of Itakpe and Agbaja, Kogi State, Nigeria. *Pollution, 4*(3), 527-538.

Amadi, A. N., Uchegbu, L. C., & Nwachukwu, C. (2020). Petroleum hydrocarbon contamination in Nigeria: Sources, impacts, and remediation strategies. *Nigerian Journal of Environmental Sciences, 12*(1), 56-70.

Amanze, C., Amapu, I., Chude, V. O., & Chikwendu, D. (2016). Soil fertility assessment and mapping of some soils in the Sudan savanna agroecological zone of Nigeria. *Journal of Soil Science and Environmental Management, 7*(4), 48-57.

Asadu, C. L. A., & Igboka, C. R. (2014). Effects of Animal Faeces and Their Extracts on Maize Yield in an Ultisol of Eastern Nigeria. *J. Agric. Sustain. 5*(1),1-13

Ayodele, A. E., Akinyemi, O. M., & Odebisi, O. J. (2023). Role of arbuscular mycorrhizal fungi in enhancing plant tolerance to heavy metal stress. *Fungal Ecology Reports, 11*(2), 79-92.

Bouyoucos, G. J. (1951). A recalibration of the hydrometer method for making mechanical analysis of soils. *Agronomy Journal, 43*(9), 434-438.

Bremner, J.M. (1996). Nitrogen total. In: *Methods of soil analysis. Part 3. Chemical Methods. Soil Science* Society of America. Madison, Wis. p. 1085

Cherdthong, A. (2020). Potential use of rumen digesta as ruminant diet-a review. T*ropical Animal Health Production, 52*, 1-6

Ezugwu, J. A., Nweze, C. E., & Amadi, C. R. (2022). Enhancing phytoremediation efficiency through arbuscular mycorrhizal fungi. *Journal of Bioremediation Science, 14*(3), 103-119.

FAO (2021). *Guidelines for soil description (5th ed.). Food* *and Agriculture Organization of the United Nations.*

Gebeyehu, H. R., & Bayissa, L. D. (2020). Levels of heavy metals in soil and vegetables and associated health risks in Mojo area, Ethiopia. *PLoS One. 15*(1), e0227883.

Graj, W. M., Pawlik, B., & Mrozik, A. (2013). Bioremediation of petroleum hydrocarbon-contaminated soil: Microbial and plant roles. *Environmental Biotechnology Journal, 22*(5), 289-297.

Ibrahim, E. A., El Sherbini, M. A. A., & Selim, E. M. (2023). Effects of biochar, zeolite and mycorrhiza inoculation on soil properties, heavy metal availability and cowpea growth in a multi contaminated soil. *Scientific Report. 13*, 6621

Jackson, M. L. (1973). *Soil chemical analysis*. Prentice Hall of India Pvt Ltd.

Joutey, N. T., Sayel, H., Bahafd, W., & El Ghachtouli, N. (2015). Mechanisms of hexavalent chromium resistance and removal by microorganisms. *Rev. Environ. Contam. Toxicol. 233*, 45-69.

McLean, E. O. (1982). Soil pH and lime requirement. In *A. L. Page, R. H. Miller, and D. R. Keeney (Eds.), Methods of soil analysis. Part 2. Chemical and microbiological properties* (pp. 199–224). American Society of Agronomy.

Mitra, S., Chakraborty, A. J., Tareq, A. M., Emran, T. B., Nainu, F., Khusro, A., ... & Simal-Gandara, J.(2022). Impact of Heavy Metals on the Environment and Human Health: Novel Therapeutic Insights to Counter the Toxicity. *J. King Saud. Univ. Sci. 34*(3), 101865.

Moses, K. K., Aliyu, A., Hamza, A., & Mohammed-Dabo, I. A. (2024). Recycling of Waste Lubricating Oil: A Review of the Recycling Technologies with a Focus on Catalytic Cracking, Techno-Economic and Life Cycle Assessments. *J Environ. Chem. Eng., 11*(6), 111273.

Nkereuwem, M. E., Adeleye, A. O., Kamaldeen, F., Sowunmi, L. I., Ijah, C. J., Nzamouhe, M., ... & Lemuel, A. (2024). Potential of Mycorrhizal Inoculation and Cattle Rumen Digesta in the Bioremediation of Spent Engine Oil Contaminated Soil. *Innovare Journal of Sciences 12*, 1-7.

Nkereuwem, M. E., Adeleye, A. O., Karfi, U. A., Bashir, M., & Kamaldeen, F. (2022). Effect of mycorrhizal inoculation and organic fertiliser on bioremediation of spent engine oil contaminated soil. *Agricultura Tropica Et Subtropica, 55*, 119-132.

Nkereuwem, M. E., Fagbola, O., Okon, I. E., Adeleye, A. O., & Nzamouhe, M. (2020a). Bioremediation potential of mycorrhiza fungi in crude oil contaminated soil planted with Costus lucanusianus. *Amazonian Journal of Plant Research, 4*, 441-455.

Nkereuwem, S. O., Akpan, E. T., & Essien, M. C. (2020b). Application of organic amendments in the bioremediation of hydrocarbon-contaminated soils. *Soil Remediation Journal, 6*(4), 89-101.

Ochei, J. O., & Kolhatkar, A. A. (2008). *Medical laboratory science: Theory and practice (7th ed.)* (pp. 820-821). New York: Tata McGraw Publishing Company Limited.

Oghenerobor, E. I., Okonkwo, O. J., & Abah, J. (2021). Spent engine oil pollution in Nigeria: Challenges and remediation strategies. *African Journal of Environmental Studies, 14*(3), 88-95.

Ogunwole, J.O., & Ogunleye, P.O. (2005). Influence of Long Term Application of Organic and Mineral Fertilizers on Quality of Savanna Alfisols. *Journal of Sustainable Agriculture, 26*(3), 5-14

Olalekan, A. A., & Oyedepo, S. J. (2023). Arbuscular mycorrhizal fungi: A biotechnological tool for contaminated soil restoration. *Biotechnology in Agriculture, 15*(1), 55-68.

Olaniyi, O. A., Usman, A. B., & Ibrahim, S. T. (2022). Climatic variability and its impact on agricultural productivity in the Sudan Savannah region of Nigeria. *Journal of Agro-Environmental Research, 15*(2), 75-88.

Olsen, S. R., Cole, C. V., Watanabe, F. S., & Dean, L. A. (1954). *Estimation of available phosphorus in soils by extraction with sodium bicarbonate.* USDA Circular No. 939.

Olusola, S. A., Oladele, O. D., & Orinami, A. P. (2017). Bioremediating Effect of Glomus Hoi and Pseudomonas Aeruginosa on the Organic Content and Heavy Metals of Soil Polluted with Oil Refinery Effluent using Amaranthus Cruentus as a Test Plant. I*nternational Journal of Environment, Agriculture and Biotechnology, 2*(4).

Omotayo, F. O., Adebayo, T. S., & Obanla, A. E. (2022). The persistence of petroleum hydrocarbons in soil ecosystems: A case study of spent engine oil contamination. *International Journal of Environmental Pollution Studies, 19*(6), 133-148.

Pinheiro, A. C., Souza, L. A., & Santos, R. F. (2020). Bioremediation potential of biochar and compost in oil-contaminated soils. *Soil Remediation Journal, 9*(4), 102-118.

Schoeneberger, P. J., Wysocki, D. A., Benham, E. C., and Soil Survey Staff. (2002). *Field book for describing and sampling soils (Version 2.0).* USDA-Natural Resources Conservation Service, National Soil Survey Center.

Usman, A. R. A., Kuzyakov, Y., & Stahr, K. (2005). Effect of clay minerals and organic matter on the immobilization of heavy metals in soils. *Communications in Soil Science and Plant Analysis, 36*(5-6), 601-612.

Vilela, L. A. F., & Barbosa, M. V. (2019). Contribution of arbuscular mycorrhizal fungi in promoting cadmium tolerance in plants. Cadmium *Tolerance in Plants*. Elsevier. 553-586.

Walkley, A., & Black, I. A. (1934). An examination of the Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. *Soil Science, 37*(1), 29-38.

Zhao, H., Lan, X., Yu, F., Li, Z., Yang, J., & Du, L. (2022). Comprehensive assessment of heavy metals in soil-crop system based on PMF and evolutionary game theory. *Science of the Total Environment, 849*, 157549.

Received: 12th February 2025, ***Approved:*** 15th March 2025, ***Published***: March 2025