



Interactive Effects of Indigenous Plant Species and Arbuscular Mycorrhizal Fungi on the Remediation of Crude Oil–Contaminated Tropical Soils in Port Harcourt, Nigeria

*¹ANOZIE, HI; ²ANOZIE, OI; ¹ARTHUR, GJ

^{*1}Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt, PMB 5323, Port Harcourt, Nigeria

²Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

*Corresponding Author email: henry.anozie@uniport.edu.ng

*ORCID ID: <https://orcid.org/0000-0001-7145-9976>

*Tel: +2348030664077

Co-author email: anumudu.osinach@mouau.edu.ng; arthur.gogo@uniport.edu.ng

ABSTRACT: Petroleum hydrocarbon contamination disrupts soil structure and nutrient dynamics, posing serious ecological challenges in tropical environments. Hence, the objective of this paper was to evaluate the interactive effects of indigenous plant species and arbuscular mycorrhizal fungi (AMF) on the remediation of crude oil–contaminated tropical soils in Port Harcourt, Nigeria using appropriate standard procedures by cultivating *Chromolaena odorata*, *Aspilia africana*, and *Eupatorium capillifolium* plant species under four soil treatments: control (soil + plant), AMF inoculation (soil + plant + AMF), and AMF combined with 400 ml or 800 ml crude oil per 10 kg soil. Soil physicochemical properties, total hydrocarbon concentration (THC), and root hydrocarbon accumulation were assessed. Initial contamination produced high THC levels (1002.00 ± 19.00 and 1402.00 ± 50.00 mg kg⁻¹). Following plant establishment, soil THC declined markedly to 135.33 ± 11.08 mg kg⁻¹ in *Chromolaena odorata*, 468.67 ± 36.17 mg kg⁻¹ in *Aspilia africana*, and 602.00 ± 17.00 mg kg⁻¹ in *Eupatorium capillifolium* under AMF treatments. Root hydrocarbon accumulation was highest in *Eupatorium capillifolium* (1761.60 ± 138.40 mg kg⁻¹) under 800 ml crude oil with AMF, while *Chromolaena odorata* and *Aspilia africana* showed lower accumulation ranges. Crude oil contamination increased sand content and reduced silt and clay fractions, while available phosphorus declined under heavy contamination but remained moderate in AMF-inoculated soils. These results demonstrate species-specific phytoremediation potentials and highlight the role of AMF in enhancing hydrocarbon uptake, nutrient retention, and soil quality for sustainable remediation of contaminated soils.

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Soil contamination by petroleum hydrocarbons poses a serious threat to tropical ecosystems by degrading soil physical and chemical properties, disrupting nutrient cycling, and inhibiting plant growth (Ali *et al.*, 2021). Due to their hydrophobic nature, hydrocarbons coat soil particles, altering soil structure by increasing sand content and reducing silt and clay fractions, which destabilizes soil aggregates and limits water and nutrient availability (Rillig, 2004). Hydrocarbon pollution also interferes with

nutrient dynamics, particularly nitrogen and phosphorus, by suppressing microbial mineralization processes and reducing plant nutrient uptake (Kuzakov *et al.*, 2013). Phytoremediation, which utilizes plants and their rhizosphere microorganisms to mitigate environmental pollutants, has emerged as an environmentally friendly and cost-effective approach for restoring hydrocarbon-contaminated soils (Van Aken, 2008). Plants contribute to hydrocarbon attenuation directly through root uptake

*Corresponding Author email: henry.anozie@uniport.edu.ng

*ORCID ID: <https://orcid.org/0000-0001-7145-9976>

*Tel: +2348030664077

and sequestration and indirectly by stimulating microbial communities capable of degrading hydrocarbons through the release of root exudates rich in sugars, amino acids, and organic acids (Glick, 2010; Obire *et al.*, 2008). However, plant species differ in their tolerance to hydrocarbon contamination and their capacity to accumulate or stimulate degradation of hydrocarbons in the rhizosphere (Gkorezis *et al.*, 2016).

Arbuscular mycorrhizal fungi (AMF) establish symbiotic associations with plant roots, improving nutrient acquisition, enhancing root development, and facilitating hydrocarbon uptake and degradation (Khan *et al.*, 2013). Their hyphal networks improve soil aggregation and stimulate microbial activity, thereby promoting hydrocarbon breakdown (Rillig, 2004; Fomina and Gadd, 2014). Hence, the objective of this paper was to evaluate then interactive effects of indigenous plant species and *arbuscular mycorrhizal* fungi (AMF) on the remediation of crude oil-contaminated tropical soils in Port Harcourt, Nigeria.

MATERIALS AND METHODS

Description of the study area: The experiment was conducted at the Department of Crop and Soil Science, University of Port Harcourt, Choba, Rivers State, Nigeria. The study area lies within the humid tropical rainforest zone, characterized by high annual rainfall, elevated temperatures, and high relative humidity. These climatic conditions favor luxuriant vegetation growth and intensive biological activity (Nwoko, 2010).

Sample collection and treatment: Bulk soil was collected from experimental buckets and thoroughly homogenized to obtain composite samples. The soil was air-dried at room temperature, gently crushed, and passed through a 2-mm sieve to remove stones, roots, and other debris. Portions of the processed soil were sterilized by autoclaving at 121 °C for 30 minutes to eliminate indigenous microorganisms prior to crude oil contamination, following the procedure of Okonokhua *et al.* (2007). Three indigenous plant species commonly found within the University of Port Harcourt campus were used for the phytoremediation study: *Chromolaena odorata* (Siam weed/Awolowo weed) – P1; *Aspilia africana* (Wild sunflower) – P2; *Eupatorium capillifolium* (Dog fennel) – P3

Healthy stems of each species were collected and propagated asexually by stem cuttings into experimental buckets containing the treated soils.

Fresh crude oil was obtained from Mobil Oil Exploratory Company, Rivers State, Nigeria. The soil was artificially contaminated at two levels: 400 ml, and 800 ml of crude oil per 10 kg soil. The crude oil was thoroughly mixed with the soil to ensure uniform distribution and homogeneity before planting. Arbuscular mycorrhizal fungi (AMF) inoculum (*Glomus* species) was sourced from the United Kingdom. A quantity of 5 g of AMF inoculum was applied to each bucket designated for AMF treatment. Inoculation was performed immediately after planting by uniformly incorporating the inoculum into the soil surface around the root zone to facilitate early root–fungus contact. The experiment was conducted as a two-factor factorial arrangement laid out in a Completely Randomized Design (CRD) with three replications. The first factor (Factor A) comprised crude oil contamination and arbuscular mycorrhizal fungi (AMF) treatments, while the second factor (Factor B) consisted of plant species. Factor A included four treatment levels: C1 = Soil and plant only, C2 = Soil, plant and AMF only, C3 = Soil, plant, AMF and 400ml Crude oil, C4 = Soil, plant, AMF and 800 ml crude oil. Factor B consisted of three plant species, namely *Chromolaena odorata*, *Aspilia africana*, and *Eupatorium capillifolium*. In total, the experiment involved 36 experimental units, calculated as the combination of three plant species, four crude oil treatment levels, and three replications ($3 \times 4 \times 3$). Each experimental unit was represented by a bucket, and treatments were randomly assigned to ensure unbiased estimation of treatment effects.

Sample analysis methodologies: Root samples were harvested at the end of the experiment and washed thoroughly to remove adhering soil particles. The roots were cleared in 10 % potassium hydroxide (KOH) solution and subsequently stained with trypan blue. Percentage root colonization by AMF was assessed microscopically using the gridline intersect method described by Giovannetti and Mosse (1980). Soil samples were collected at two stages: prior to crude oil contamination (baseline) and at the end of the experiment (final). All analyses were conducted at the Soil Science Laboratory, Rivers State University.

Soil pH: Determined in a 1:2.5 soil-to-water suspension using a pH meter (McLean, 1982).

Total Nitrogen: Determined by the macro-Kjeldahl digestion method (Bremner and Mulvaney, 1982). Total organic carbon was determined using the methods described by Harris *et al.* (2001). Exchangeable Ca and Mg: Determined by EDTA complexometric titration. Exchangeable Na and K: Determined using flame photometry. Available

Phosphorus was determined by the Bray II extraction method (McLean, 1982). Particle Size Distribution was determined by the hydrometer method (Gee and Bauder, 1986). Total Hydrocarbon (THC) was analyzed using the method described by APHA, (2017)

Statistical Data Analysis: All data collected were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics (version 27).

RESULTS AND DISCUSSION

Species-Specific Patterns of Total Hydrocarbon Distribution in Soil and Roots: Total hydrocarbon (THC) concentrations in soil and plant roots differed significantly ($p < 0.05$) among treatments and plant species, indicating distinct species-specific responses to crude oil contamination and arbuscular mycorrhizal fungi (AMF) application (Table 1). Pre-planting contaminated soils (SC3: soil + 400 ml crude oil; SC4: soil + 800 ml crude oil) recorded the highest soil THC concentrations (1002.00 ± 19.00 and 1402.00 ± 50.00 mg kg⁻¹, respectively), representing baseline contamination levels prior to biological intervention. As expected, root hydrocarbons were undetectable in these treatments due to the absence of vegetation. The elevated THC levels in pre-planting soils reflect the direct input of petroleum hydrocarbons and their persistence in soil systems, particularly in the absence of biological degradation processes (Nwaichi and Ugwu, 2016). Following plant establishment, reductions in soil THC observed across treatments can be attributed to phytoremediation mechanisms, including rhizodegradation, plant uptake, and microbial biodegradation in the rhizosphere (Ali *et al.*, 2022). Differences in THC reduction among plant species highlight the importance of plant-specific traits such as root architecture, exudate composition, and tolerance to hydrocarbon stress. These traits influence the stimulation of hydrocarbon-degrading microbial communities in the rhizosphere (Gkorezis *et al.*, 2022). The presence of AMF further enhanced remediation efficiency by improving plant growth and stress tolerance, thereby indirectly stimulating microbial activity and hydrocarbon breakdown (Begum *et al.*, 2021). Moreover, AMF hyphal networks can extend beyond the root depletion zone, increasing the contact between hydrocarbons and degrading microorganisms, while also improving soil structure and aeration—factors critical for effective biodegradation (Khan *et al.*, 2013). The absence of detectable hydrocarbons in roots of pre-planting treatments confirms that hydrocarbon accumulation in plant tissues is dependent on active plant uptake and interaction with contaminated soil. Plant

establishment under soil and plant-only treatments led to significant reductions in soil total hydrocarbon (THC) across all species, indicating effective rhizosphere-driven attenuation of petroleum contaminants. *Chromolaena odorata* recorded a reduction to 335.33 ± 10.10 mg kg⁻¹, *Aspilia africana* to 602.00 ± 17.00 mg kg⁻¹, and dog fennel (*Eupatorium capillifolium*) to 402.00 ± 16.00 mg kg⁻¹. Concurrently, hydrocarbons became detectable in plant roots, with *E. capillifolium* exhibiting the highest root THC (641.60 ± 40.35 mg kg⁻¹) [Table 1], implying pronounced species-specific differences in hydrocarbon uptake and internal translocation. These reductions are consistent with phytoremediation processes driven by rhizosphere interactions, including rhizodegradation, microbial stimulation, and limited plant uptake of hydrocarbon fractions. Root exudates can enhance microbial abundance and enzymatic activity in the rhizosphere, thereby accelerating hydrocarbon breakdown (Ali *et al.*, 2022). The observed variability among species reflects differences in root architecture and exudate chemistry, which regulate microbial recruitment and contaminant transformation in the rhizosphere (Gkorezis *et al.*, 2022). The incorporation of arbuscular mycorrhizal fungi (AMF) further modified THC dynamics across treatments. Soil THC decreased markedly in *C. odorata* (135.33 ± 11.08 mg kg⁻¹), while moderate concentrations were observed in *A. africana* (468.67 ± 36.17 mg kg⁻¹) and *E. capillifolium* (602.00 ± 17.00 mg kg⁻¹). In contrast, root hydrocarbon accumulation increased substantially in *E. capillifolium* (1602.00 ± 16.00 mg kg⁻¹) [Table 1], indicating enhanced hydrocarbon translocation and sequestration within plant tissues under AMF-assisted conditions. AMF are known to improve plant stress tolerance, enhance nutrient acquisition, and indirectly stimulate rhizosphere microbial activity, all of which contribute to improved contaminant degradation and redistribution (Begum *et al.*, 2021). Under combined soil, plant, arbuscular mycorrhizal fungi (AMF), and crude oil treatments (400 ml and 800 ml), soil THC remained elevated relative to uncontaminated conditions but was lower than pre-planting contaminated levels, indicating partial remediation over time. This trend reflects progressive phytoremediation processes driven by rhizosphere microbial stimulation, plant-microbe interactions, and enhanced hydrocarbon biodegradation (Liu *et al.*, 2023). *Chromolaena odorata* recorded the highest soil THC under the 400 ml treatment (1202.00 ± 100.00 mg kg⁻¹), while *Eupatorium capillifolium* roots accumulated the highest THC under the 800 ml treatment (1761.60 ± 138.40 mg kg⁻¹) [Table 1]. These findings reflect both species-specific tolerance thresholds and

differential capacities for hydrocarbon uptake, translocation, and rhizosphere-mediated degradation, which are key determinants of phytoremediation efficiency in hydrocarbon-contaminated soils (Zhang *et al.*, 2024). Generally, the patterns indicate that hydrocarbon attenuation is strongly species-dependent. Dog fennel (*E. capillifolium*) demonstrated the greatest hydrocarbon uptake and internal accumulation, likely due to its fibrous root system and strong association with AMF, which enhances nutrient exchange and contaminant transport processes. In contrast, *C. odorata* and *A. africana* appeared to contribute more substantially to rhizosphere-enhanced degradation rather than direct hydrocarbon sequestration in plant tissues. These results align with previous evidence that plant species differ markedly in their phytoremediation strategies, ranging from uptake and translocation to rhizosphere stimulation and exclusion mechanisms (Khan *et al.*, 2013). Furthermore, the inoculation of soils with arbuscular mycorrhizal fungi enhanced both root THC accumulation and the dissipation of hydrocarbons from the soil matrix. This enhancement is likely associated with improved root–soil contact, stabilization of soil microaggregates, and the stimulation of hydrocarbon-degrading microbial communities within the rhizosphere. Such AMF-mediated processes are widely recognized for promoting rhizosphere activity and facilitating the biodegradation of organic contaminants in polluted soils (Smith and Read, 2008).

Table 1: The mean total hydrocarbon (mg/kg) concentration on soil and root

Sample	SOIL	ROOT
SC3	1002.00±19.00 ^d	0.00±0.00 ^g
SC4	1402.00±50.00 ^a	0.00±0.00 ^g
P1C1	335.33±10.10 ^j	264.93±31.25 ^f
P1C2	135.33±11.08 ^k	321.60±11.40 ^f
P1C3	1202.00±100.00 ^b	0.00±0.00 ^g
P1C4	797.67±7.51 ^f	588.26±65.24 ^{de}
P2C1	602.00±17.00 ^g	268.26±25.91 ^f
P2C2	468.67±36.17 ^h	534.93±22.90 ^c
P2C3	668.67±16.32 ^g	0.00±0.00 ^g
P2C4	1068.67±44.04 ^c	0.00±0.00 ^g
P3C1	402.00±16.00 ⁱ	641.60±40.35 ^d
P3C2	602.00±17.00 ^g	1602.00±16.00 ^b
P3C3	668.67±30.42 ^g	961.60±40.40 ^c
P3C4	935.33±23.21 ^e	1761.60±138.40 ^a

Results are expressed as mean ± standard deviation, n = 3. Mean values with different alphabets (a-k) as superscripts down the column are significantly (p<0.05) different. Keys: C1 = Soil and plant only, C2 = Soil, plant and AMF only, C3 = Soil, plant, AMF and 400ml Crude oil, C4 = Soil, plant, AMF and 800 ml crude oil, P1 = *Chromolaena odorata*, P2 = *Aspilia africana*, P3 = Dog Fennel (*Eupatorium capillifolium*), SC3 = Soil and 400ml crude oil before planting, SC4 = Soil and 800ml crude oil before planting (Anozie *et al.*, 2026)

Soil Physical Properties: The particle size distribution of soils across treatments showed

significant variation (p < 0.05), indicating that plant species, arbuscular mycorrhizal fungi (AMF) inoculation, and crude oil contamination influenced soil physical properties (Table 2). Pre-planting contaminated soils (400 ml and 800 ml crude oil) were dominated by sand fractions ranging from 827.00 ± 13.00 to 852.00 ± 11.00 g kg⁻¹, with comparatively lower silt (69.00 ± 5.00 to 84.00 ± 3.00 g kg⁻¹) and clay contents (79.00 ± 5.00 to 89.00 ± 6.00 g kg⁻¹). This sandy texture reflects the inherent characteristics of the study soil which implies high permeability and low water-holding capacity, typical of course-textured soils (Brady and Weil, 2016).

The observed baseline distribution indicates that crude oil contamination did not markedly alter the inherent particle size fractions prior to planting, as soil texture is largely determined by parent material and is relatively stable under short-term environmental disturbances (Brady and Weil, 2016). However, crude oil can influence soil structure by coating soil particles, thereby reducing porosity and altering aggregation and water infiltration dynamics (Merkel *et al.*, 2005).

Following planting and AMF inoculation, the significant variations observed across treatments could be attributed to biological processes, including root proliferation and fungal hyphal development. These processes enhance soil aggregation and contribute to the redistribution and stabilization of finer particles (Six *et al.*, 2004). In particular, AMF play a critical role in soil structural improvement through the production of glomalin-related soil proteins, which act as binding agents that promote the formation and stability of soil aggregates (Bedini *et al.*, 2009).

Furthermore, plant–AMF interactions likely improved soil physical condition by increasing organic matter inputs and microbial activity, both of which are essential for aggregate formation and soil resilience in contaminated environments (Jansa *et al.*, 2019). These findings denote that while the initial particle size distribution reflects the native soil condition, subsequent variations across treatments are indicative of biologically mediated improvements in soil structure, supporting the role of AMF-assisted phytoremediation in restoring degraded soils.

Planting alone in soil and plant only treatments-maintained particle size fractions comparable to baseline values, suggesting minimal disturbance of soil structure by vegetation. In contrast, treatments combining soil, plant, arbuscular mycorrhizal fungi (AMF), and 400 ml crude oil as well as soil, plant,

arbuscular mycorrhizal fungi, and 800 ml crude oil resulted in significant increases in sand content, particularly in *Aspilia Africana* ($915.00 \pm 13.00 \text{ g kg}^{-1}$) and dog fennel (*Eupatorium capillifolium*) ($905.00 \pm 13.00 \text{ g kg}^{-1}$) [Table 2], while silt and clay fractions decreased. This pattern indicates hydrocarbon-induced disruption of soil aggregation (Fomina and Gadd, 2014). The incorporation of soil, plant and arbuscular mycorrhizal fungi (AMF) only moderated reductions in silt content, likely through hyphal stabilization of microaggregates, thereby preventing excessive loss of finer soil fractions (Barea *et al.*, 2011). Species-specific differences were also evident: dog fennel (*Eupatorium capillifolium*) exhibited the largest increases in sand content under hydrocarbon-contaminated treatments, reflecting aggressive root proliferation and enhanced interaction with hydrocarbons in the rhizosphere.

Table 2: The mean pphysical pproperties of soil

Sample	Sand (g/Kg)	Silt (g/Kg)	Clay (g/Kg)
SC3	827.00±13.00 ^e	84.00±3.00 ^{abc}	89.00±6.00 ^a
SC4	852.00±11.00 ^{cde}	69.00±5.00 ^{de}	79.00±5.00 ^a
P1C1	835.00±11.00 ^{de}	87.00±6.00 ^{ab}	78.00±9.00 ^a
P1C2	845.00±23.00 ^{de}	77.00±5.00 ^{cd}	78.00±6.00 ^a
P1C3	895.00±20.00 ^{ab}	57.00±5.00 ^f	48.00±5.00 ^c
P1C4	895.00±23.00 ^{ab}	57.00±5.00 ^g	48.00±5.00 ^{bc}
P2C1	830.00±18.00 ^{de}	92.00±3.00 ^a	78.00±6.00 ^a
P2C2	835.00±14.00 ^{de}	92.00±3.00 ^{ab}	78.00±6.00 ^a
P2C3	870.00±8.00 ^{bcd}	87.00±8.00 ^d	78.00±6.00 ^{bc}
P2C4	915.00±13.00 ^a	72.00±3.00 ^h	58.00±5.00 ^{bc}
P3C1	905.00±13.00 ^{cde}	32.00±4.00 ^{bc}	53.00±4.00 ^b
P3C2	855.00±31.00 ^{de}	82.00±4.00 ^{ab}	63.00±4.00 ^a
P3C3	835.00±23.00 ^{abc}	87.00±4.00 ^{ef}	78.00±6.00 ^c
P3C4	890.00±45.00 ^{ab}	62.00±4.00 ^g	48.00±6.00 ^{bc}
	905.00±13.00	42.00±3.00	53.00±4.00

Results are expressed as mean $\pm$ standard deviation, $n = 3$. Mean values with different alphabets (a-g) as superscripts down the column are significantly ($p < 0.05$) different. Key: C1 = Soil and plant, C2 = Soil, plant and AMF, C3 = Soil, plant, AMF and 400ml Crude oil, C4 = Soil, plant, AMF and 800 ml crude oil, P1 = *Chromolaena odorata*, P2 = *Aspilia africana*, P3 = Dog Fennel (*Eupatorium capillifolium*), SC3 = Soil and 400ml crude oil, SC4 = Soil and 800ml crude oil. (Anozie *et al.*, 2026)

Soil Chemical Properties: The mean chemical properties of soils differed significantly ($p < 0.05$) among treatments, reflecting the combined effects of plant species, AMF inoculation, and crude oil contamination (Table 3). Soil pH remained acidic across all treatments ($4.50 \pm 0.30 - 5.40 \pm 0.80$) as seen in Table 3, indicating minimal influence of crude oil contamination or arbuscular mycorrhizal fungi (AMF) inoculation on hydrogen ion

concentration, consistent with short-term observations in tropical soils (Brady and Weil, 2016). Available phosphorus (P) decreased in heavily contaminated soils, particularly in soil with 800 ml crude oil before planting and in *Aspilia africana* grown under soil + plant + arbuscular mycorrhizal fungi + 800 ml crude oil treatments. For instance, P declined to $19.10 \pm 0.10 \text{ mg kg}^{-1}$ in SC4 and further to $18.90 \pm 2.48 \text{ mg kg}^{-1}$ in P2C4, compared to higher values observed in less contaminated or amended treatments such as $30.00 \pm 2.00 \text{ mg kg}^{-1}$ in SC3, $30.30 \pm 1.50 \text{ mg kg}^{-1}$ in P2C3, and a peak of $42.80 \pm 1.48 \text{ mg kg}^{-1}$ in P1C1, reflecting hydrocarbon-induced phosphorus immobilization and microbial inhibition (Margesin and Schinner, 2001).

AMF inoculation helped maintain moderate phosphorus levels through fungal-mediated nutrient mobilization (Barea *et al.*, 2011). Total nitrogen (N) varied significantly across treatments, reflecting the influence of plant species, crude oil contamination, and arbuscular mycorrhizal fungi (AMF) inoculation on soil nutrient dynamics. Nitrogen values ranged from $1.40 \pm 0.28 \text{ g kg}^{-1}$ (P1C2 and P3C1) to a maximum of $2.52 \pm 0.48 \text{ g kg}^{-1}$ (P1C4), indicating substantial variability among treatments.

Chromolaena odorata under high contamination conditions (soil + plant + AMF + 800 ml crude oil; P1C4) maintained relatively elevated nitrogen levels ($2.52 \pm 0.48 \text{ g kg}^{-1}$), compared to lower values in less amended treatments such as P1C2 ($1.40 \pm 0.28 \text{ g kg}^{-1}$) and P1C1 ($1.82 \pm 0.10 \text{ g kg}^{-1}$). Similarly, moderately high nitrogen levels were observed in contaminated AMF treatments including P2C4 ($2.24 \pm 0.14 \text{ g kg}^{-1}$) and P3C4 ($2.24 \pm 0.23 \text{ g kg}^{-1}$) [Table 3]. This response may be attributed to enhanced plant-microbe interactions, particularly the role of AMF in improving nitrogen uptake and retention in contaminated soils (Smith and Read, 2008).

The improved nitrogen status in AMF-inoculated treatments could be linked to increased microbial activity and improved root surface area, which facilitate nutrient cycling and reduce nitrogen losses through leaching or volatilization (Zhang *et al.*, 2023).

Additionally, AMF are known to enhance soil structure and organic matter stabilization, indirectly supporting nitrogen conservation in degraded soils (Lehmann and Rillig, 2022).

The ability of *Chromolaena odorata* to thrive under such conditions further highlights its potential for phytoremediation, as tolerant plant species can

sustain nutrient cycling even in hydrocarbon-polluted environments (Afzal and Bano, 2008).

Table 3: chemical properties of soil

Sample	pH (H ₂ O)	P (mg/kg)	N	OC g/kg	OM	Ca	Mg	K	Na Cmol/kg	EA	ECEC	BS (%)
SC3	4.80±0.50 ^a	30.00±2.00 ^{bc}	2.24±0.08 ^{abc}	27.93±2.46 ^b	48.15±3.88 ^{cd}	4.80±0.85 ^{abc}	0.80±0.10 ^b	0.28±0.04 ^{abcd}	0.37±0.06 ^{ab}	1.76±0.07 ^a	8.01±0.16 ^{ab}	78.07±4.86 ^c
SC4	4.60±0.50 ^a	19.10±0.10 ^f	2.38±0.24 ^{ab}	31.52±0.62 ^{ab}	54.34±2.21 ^b	4.00±1.00 ^{bcd}	1.20±0.10 ^b	0.35±0.04 ^{abc}	0.31±0.03 ^{ab}	1.04±0.03 ^{defg}	6.89±0.97 ^{bcd}	85.03±2.66 ^{abc}
P1C1	4.50±0.30 ^a	42.80±1.48 ^a	1.82±0.10 ^{bcde}	15.56±3.31 ^c	26.82±1.98 ^e	4.40±0.90 ^{abc}	0.80±0.18 ^b	0.35±0.04 ^{cd}	0.36±0.07 ^{ab}	0.96±0.14 ^{defg}	6.86±0.26 ^{cd}	86.10±2.62 ^{abc}
P1C2	5.00±1.00 ^a	26.10±3.25 ^{bcde}	1.40±0.28 ^e	10.77±0.35 ^c	18.57±2.00 ^f	3.60±0.47 ^{cd}	1.20±0.30 ^b	0.24±0.05 ^{cd}	0.34±0.02 ^{ab}	1.12±0.04 ^{cde}	6.50±0.70 ^{cd}	82.81±2.60 ^{abc}
P1C3	4.90±0.80 ^a	30.70±2.18 ^b	2.10±0.20 ^{abcd}	29.92±1.94 ^b	51.59±2.94 ^{bc}	2.80±0.66 ^d	1.20±0.30 ^b	0.37±0.08 ^{ab}	0.17±0.04 ^c	1.20±0.10 ^{bcd}	5.74±0.62 ^d	79.19±4.94 ^c
P1C4	5.40±0.80 ^a	26.60±4.75 ^{bcd}	2.52±0.48 ^a	35.51±5.93 ^a	61.22±2.37 ^a	4.00±1.00 ^{bcd}	0.80±0.27 ^b	0.39±0.10 ^a	0.44±0.10 ^a	0.88±0.13 ^{efg}	6.51±1.23 ^{cd}	86.56±3.68 ^{abc}
P2C1	5.30±0.32 ^a	24.40±4.82 ^{cdef}	1.68±0.56 ^{cde}	14.76±1.91 ^c	25.45±3.56 ^e	4.40±0.60 ^{abc}	2.00±0.20 ^a	0.37±0.09 ^{ab}	0.37±0.09 ^{ab}	1.20±0.30 ^{bcd}	8.52±0.87 ^a	86.05±7.61 ^{abc}
P2C2	5.20±0.30a	23.20±1.86def	1.54±0.42de	14.36±1.49c	24.76±2.91e	4.80±0.70abc	0.80±0.10b	0.21±0.02d	0.33±0.09ab	0.96±0.18defg	7.09±0.25abcd	86.58±4.21abc
P2C3	5.00±0.88a	30.30±1.50b	1.96±0.48abcde	30.72±3.60ab	52.96±4.69bc	5.60±0.70a	0.80±0.09b	0.34±0.10abc	0.37±0.08ab	0.80±0.16fg	7.91±1.06abc	89.94±7.09ab
P2C4	5.10±0.20a	18.90±2.48f	2.24±0.14abc	26.73±3.31b	46.08±1.61d	5.20±0.40ab	1.20±0.60b	0.25±0.07bcd	0.27±0.06bc	0.72±0.10g	7.63±0.89abc	90.66±5.01a
P3C1	5.10±0.20a	25.20±3.35bcde	1.40±0.28e	11.17±2.07c	19.26±1.31fg	3.60±0.50cd	1.20±0.20b	0.18±0.04d	0.32±0.04ab	1.20±0.10bcd	6.49±0.51cd	81.64±3.02bc
P3C2	4.50±0.60a	26.40±4.54bcde	1.68±0.56cde	13.96±4.73c	24.07±3.04ef	4.00±0.10bcd	2.00±0.20a	0.36±0.07abc	0.30±0.05abc	1.28±0.10bc	7.94±0.71abc	83.87±4.35abc
P3C3	4.80±0.70a	25.20±2.78bcde	2.10±0.12abcd	31.12±1.60ab	53.65±2.40b	4.40±0.60abc	0.80±0.06b	0.34±0.06abc	0.35±0.14ab	0.96±0.12defg	6.81±0.99bcd	85.96±4.11abc
P3C4	5.00±1.09a	20.80±2.56ef	2.24±0.23abc	30.72±2.08ab	52.96±4.01bc	4.80±0.85abc	1.20±0.30b	0.39±0.06ab	0.24±0.06bc	1.44±0.20b	8.07±0.59ab	82.15±2.92abc

Results are expressed as mean ± standard deviation, n = 3. Mean values with different alphabets (a-g) as superscripts down the column are significantly (p<0.05) different. Key: C1=Soil and plant, C2 = Soil, plant and AMF, C3 = Soil, plant, AMF and 400 ml Crude oil, C4 = Soil, plant, AMF and 800 ml crude oil, P1 = *Chromolaena odorata*, P2 = *Aspilia africana*, P3 = *Dog Fennel (Eupatorium capillifolium)*, SC3 = Soil and 400 ml crude oil, SC4 = Soil and 800ml crude oil. (Anozie *et al.*, 2026)

In contrast, dog fennel (*Eupatorium capillifolium*) grown under soil and plant-only treatments exhibited lower nitrogen levels, indicating limited nutrient acquisition and reduced resilience in the absence of AMF inoculation. This reduction may be due to the negative effects of crude oil on soil microbial communities and nitrogen mineralization processes, which often result in decreased nitrogen availability (Wang *et al.*, 2022). Without the symbiotic support of AMF, plants are more susceptible to nutrient stress, particularly in contaminated soils where nutrient cycling is impaired. Organic carbon (OC) and organic matter (OM) contents were highest in pre-contaminated soils and in arbuscular mycorrhizal fungi (AMF)-assisted treatments, indicating the dual influence of hydrocarbon inputs and biological stabilization processes. Specifically, OC increased from 10.77 to 15.56 g kg⁻¹ in non-contaminated

treatments (e.g., P1C2: 10.77±0.35 g kg⁻¹; P1C1: 15.56±3.31 g kg⁻¹) to 27.93–31.52 g kg⁻¹ in pre-contaminated soils (SC3: 27.93±2.46 g kg⁻¹; SC4: 31.52±0.62 g kg⁻¹), while OM similarly rose from 18.57–26.82 g kg⁻¹ to 48.15–54.34 g kg⁻¹, respectively (Table 3). Among the AMF-assisted crude oil treatments, the highest OC and OM values were recorded in P1C4 (35.51±5.93 g kg⁻¹ and 61.22±2.37 g kg⁻¹), followed by P2C3 (30.72±3.60 g kg⁻¹ and 52.96±4.69 g kg⁻¹) and P3C3 (31.12±1.60 g kg⁻¹ and 53.65±2.40 g kg⁻¹), compared to their respective non-AMF or lower-input counterparts such as P2C1 (14.76±1.91 g kg⁻¹ and 25.45±3.56 g kg⁻¹) [Table 3].

The elevated OC and OM observed in pre-contaminated soils can be attributed to the introduction of petroleum hydrocarbons, which

contribute substantial carbon to the soil system and are often measured as part of total organic carbon (Udume *et al.*, 2023). This apparent increase does not necessarily reflect improved soil quality but rather the accumulation of recalcitrant carbon fractions derived from crude oil. In AMF-inoculated treatments, the higher OC and OM levels suggest enhanced biological activity and improved stabilization of organic residues. AMF are known to contribute to soil carbon sequestration through the production of glomalin-related soil proteins, which promote aggregate formation and protect organic matter from rapid decomposition (Singh *et al.*, 2022). Additionally, AMF-associated hyphal networks facilitate the incorporation of plant-derived carbon into the soil, thereby increasing organic matter content and improving soil structure (Lehmann and Rillig, 2022).

Integrative Insights for Phytoremediation: Collectively, these findings indicate that crude oil contamination alters soil texture (increased sand, reduced silt/clay), reduces available P, and affects OC/OM dynamics. Plant species and AMF inoculation modulate these effects: AMF enhances hydrocarbon translocation to roots, stabilizes soil microstructure, and promotes nutrient retention. Dog Fennel (*Eupatorium capillifolium*) was the most effective species for root hydrocarbon sequestration, whereas *Chromolaena odorata* and *Aspilia africana* contributed mainly to rhizospheric microbial degradation.

The study highlights the importance of integrating species selection with microbial symbiosis in phytoremediation. AMF-assisted plant systems not only reduce soil THC but also maintain chemical and physical soil stability, offering a practical framework for restoring hydrocarbon-contaminated tropical soils (Ali *et al.*, 2021). Species-specific traits, root morphology, and symbiotic interactions are therefore critical determinants of remediation efficiency and soil ecological recovery.

Conclusion: AMF-assisted phytoremediation is an effective and sustainable approach for restoring crude oil-contaminated tropical soils by enhancing soil stability, nutrient cycling, and ecological balance. The synergy between indigenous plants and AMF improves soil recovery under hydrocarbon stress and offers a practical alternative to conventional remediation methods. It is recommended that suitable plant-AMF combinations adapted to local conditions be prioritized. Further field-scale studies and optimization of application strategies are needed to ensure consistent and efficient restoration across different contamination scenarios.

DECLARATIONS

Ethical Approval: This study did not involve human participants or animal experimentation and therefore did not require formal ethical approval. All procedures involving data handling and analysis were conducted responsibly and in accordance with standard academic and institutional guidelines.

Disclaimer (Artificial Intelligence): Author(s) hereby declare that no generative Artificial Intelligence (AI) technologies have been used during writing or editing of this manuscript.

Declaration of Conflict of Interest: The authors declare that no known conflict of interest or personal relationships that have influence the work reported in this paper.

Declaration of Data Availability: The data that are used in this research are available upon request from the corresponding Author or second author.

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