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SYNERGISTIC PLANT–MYCORRHIZAL INTERACTIONS FOR HEAVY METAL REMEDIATION IN CRUDE OIL–CONTAMINATED TROPICAL SOILS

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Abstract

Petroleum hydrocarbon contamination poses a major threat to soil quality in tropical ecosystems by altering physicochemical properties and enhancing heavy metal toxicity. This study evaluated the interactive effects of plant species, arbuscular mycorrhizal fungi (AMF), and crude oil contamination on soil properties, heavy metal dynamics, and root accumulation patterns. A pot experiment was arranged in a two-factor factorial Completely Randomized Design with three replications using three indigenous species (*Chromolaena odorata*, *Aspilia africana*, and *Eupatorium capillifolium*) grown in soils amended with 0 ml, 400 ml, and 800 ml crude oil per 10 kg soil, with or without AMF. Crude oil significantly modified soil physical properties by increasing sand content from 827.00±13.00 to 915.00±13.00 gkg^{-1}, while reducing silt and clay to as low as 32.00±4.00 and 48.00±5.00 gkg^{-1}, respectively. Soil pH remained acidic (4.50–5.40). Available phosphorus declined with increasing crude oil, from 42.80±1.48 $mgkg^{-1}$ in *C. odorata* (soil + plant) to 18.90±2.48 $mgkg^{-1}$ in *A. africana* with AMF and 800 ml crude oil. Organic carbon and organic matter increased markedly at higher oil levels, reaching 35.51±5.93 gkg^{-1} and 61.22±2.37 gkg^{-1}, respectively. Soil cadmium and nickel varied significantly, with the highest Ni concentration (16.09±2.84 $mgkg^{-1}$) recorded under *E. capillifolium* with AMF and 800 ml crude oil, while chromium remained below detectable limits in soil. Root metal accumulation was strongly species

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dependent, with *E. capillifolium* exhibiting the highest root Cr (16.09±2.84 $mgkg^{-1}$), Cd (1.24±0.15 $mgkg^{-1}$), and Ni (1.00±0.05 $mgkg^{-1}$), indicating efficient root sequestration and limited translocation. Generally, the results clearly demonstrate that integrating tolerant plant species with AMF substantially enhances metal immobilization and soil functional stability under crude oil stress, positioning *E. capillifolium*–AMF systems as robust, low-cost candidates for sustainable phytoremediation of hydrocarbon-contaminated tropical soils.

Introduction

Petroleum hydrocarbon contamination represents a significant environmental challenge in tropical regions, where crude oil spills and leaks frequently disrupt soil structure, chemistry, and biological function. Such contamination not only alters soil physical properties, including texture and porosity, but also modifies chemical characteristics such as nutrient availability, organic matter content, and pH, ultimately affecting plant growth and ecosystem productivity (Doerr et al., 2020; Abrahim and Parker, 2008; Zhang et al., 2021). In addition to hydrocarbons, contaminated soils often harbour elevated concentrations of heavy metals such as cadmium (Cd), chromium (Cr), and nickel (Ni), either originating from the crude oil itself or mobilized from soil matrices, further exacerbating phytotoxic stress (Liu et al., 2025; Al Hadidi et al., 2023).

Plants employ a range of adaptive strategies to tolerate heavy metal stress, including restricted translocation of metals to aerial tissues and sequestration within roots, thereby limiting oxidative damage and preserving photosynthetic function (Clemens, 2006; Hall, 2002; Verbruggen et al., 2009). Root sequestration is often facilitated by chelation with phytochelatins or organic acids and compartmentalization in vacuoles, highlighting the central role of roots as sinks for toxic metals (DalCorso et al., 2010). The effectiveness of these strategies is influenced by plant species, soil properties, and the presence of symbiotic microorganisms such as arbuscular mycorrhizal fungi (AMF), which can enhance metal uptake, improve nutrient acquisition, and stimulate stress resilience through root system modification and phytohormone production (Khan et al., 2017; Miransari, 2011; Kharkwal et al., 2020).

Phytoremediation - using plants and their associated microbiota to remove, stabilize, or detoxify contaminants has emerged as a promising approach for the restoration of hydrocarbon-impacted soils. Selection of species with high metal accumulation potential, particularly in combination with AMF inoculation, can improve remediation efficiency while maintaining plant growth in stressful environments (Pirsarandib et al., 2022). Among tropical plant species, *Eupatorium capillifolium* (dog fennel), *Chromolaena odorata*, and *Aspilia africana* have shown potential for tolerance to soil contaminants, but comparative assessments of their metal uptake pattern under combined crude oil and AMF treatments remain limited.

Therefore, this study aimed to investigate the interactive effects of plant species, AMF inoculation, and crude oil contamination on soil physical and chemical properties, heavy metal dynamics, and plant root accumulation patterns. By elucidating these interactions, the study provides insight into the selection of effective plant-AMF systems for sustainable soil remediation.

Materials and Methods

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).

Soil Sampling and Preparation

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).

Test Plants and Propagation

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.

Source and Application of Crude Oil

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.

AMF Inoculum and Inoculation Procedure

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.

Experimental Design and Treatments

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 × 4 × 3). Each experimental unit was represented by a bucket, and treatments were randomly assigned to ensure unbiased estimation of treatment effects.

Assessment of AMF Root Colonization

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 Physicochemical and Heavy Metal Analyses

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 & 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: Determined by the Bray II extraction method (McLean, 1982). Particle Size Distribution: Determined by the hydrometer method (Gee and Bauder, 1986).

Heavy Metal Determination

Soil and root samples were digested using a wet acid digestion procedure involving concentrated nitric acid (HNO_3) and perchloric acid ($HClO_4$). Concentrations of Chromium (Cr), Cadmium (Cd), and Nickel (Ni) were quantified using Atomic Absorption Spectrophotometry (AAS), following standard analytical protocols for trace metal determination in soils and plant tissues.

Data Analysis

All data collected were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics (version 27). Where treatment effects were significant, mean separation was performed using Duncan's Multiple Range Test (DMRT) at a 95 % confidence level ($p \leq 0.05$).

RESULTS

Heavy Metals (mg/kg) concentration on soil and roots

Table 1 shows the concentrations of chromium (Cr), cadmium (Cd), and nickel (Ni) in soils and corresponding plant roots under different treatment combinations. Chromium was below the detectable limit (BDL) in all soil samples, indicating that soil Cr levels were either negligible or below the detection capacity of the analytical instrument. Consequently, chromium accumulation in plant roots was generally minimal, with measurable concentrations observed only in treatments involving plants and soil amendments.

Table 1: Heavy Metals (mg/kg) concentration on soil and roots

Sample ←	SOIL →			ROOTS ←	→	
	Chromium	Cadmium	Nickel	Chromium	Cadmium	Nickel
SC3	BDL	3.30 ± 0.15^{bc}	1.20 ± 0.05^{h}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
SC4	BDL	2.20 ± 0.40^{ef}	32.00 ± 4.00^{b}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
P1C1	BDL	2.30 ± 0.20^{def}	6.80 ± 2.60^{gh}	4.53 ± 0.85^{de}	0.08 ± 0.01^{e}	0.31 ± 0.02^{d}
P1C2	BDL	2.40 ± 0.60^{cdef}	0.00 ± 0.00^{h}	3.60 ± 0.50^{ef}	0.05 ± 0.01^{e}	0.12 ± 0.04^{e}
P1C3	BDL	4.60 ± 0.80^{a}	27.60 ± 7.75^{bc}	0.00 ± 0.00^{g}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}
P1C4	BDL	2.80 ± 0.70^{cde}	19.50 ± 1.15^{de}	8.14 ± 1.19^{c}	0.13 ± 0.06^{e}	0.51 ± 0.06^{c}
P2C1	BDL	3.80 ± 0.65^{ab}	16.50 ± 1.65^{def}	6.19 ± 0.37^{d}	0.10 ± 0.03^{e}	0.41 ± 0.12^{cd}

P2C2	BDL	4.40±0.42^{a}	11.00±2.00^{fg}	5.00±0.30^{de}	0.91±0.19^{b}	0.74±0.16^{b}
P2C3	BDL	2.30±0.20^{def}	0.00±0.00^{h}	0.00±0.00^{g}	0.00±0.00^{e}	0.00±0.00^{e}
P2C4	BDL	2.10±0.30^{ef}	13.40±2.16^{efg}	0.00±0.00^{g}	0.00±0.00^{e}	0.00±0.00^{e}
P3C1	BDL	1.80±0.30^{fg}	60.30±7.92^{a}	2.51±0.33^{f}	0.54±0.12^{d}	0.15±0.03^{e}
P3C2	BDL	2.80±0.70^{cde}	12.40±2.58^{fg}	12.16±1.91^{b}	0.48±0.16^{d}	0.84±0.19^{b}
P3C3	BDL	0.90±0.65^{g}	21.20±2.24^{cd}	14.37±1.51^{a}	0.73±0.15^{c}	0.89±0.17^{ab}
P3C4	BDL	3.20±S0.40^{bcd}	27.90±6.04^{bc}	16.09±2.84^{a}	1.24±0.15^{a}	1.00±0.05^{a}

Results are expressed as mean ± standard deviation, n = 3. Mean values with different alphabets (a-h) as superscripts down the column are significantly ($p<0.05$) different. BDL = below detectable limit (for values less than zero from the equipment used). 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 400 ml crude oil, SC4 = Soil and 800 ml crude oil.

Heavy metal Accumulation in soil

Soil cadmium concentration varied significantly ($p < 0.05$) among treatments. The highest soil Cd concentration was recorded in Dog fennel (*Eupatorium capillifolium*) grown in soil only (60.30±7.92 $mgkg^{-1}$). This was followed by Dog fennel grown in soil, plant, AMF, and 800 ml crude oil (27.90±6.04 $mgkg^{-1}$) and Chromolaena odorata grown in soil, plant, AMF, and 400 ml crude oil (27.60±7.75 $mgkg^{-1}$). Soils treated with 400 ml crude oil only and 800 ml crude oil only exhibited moderate Cd concentrations, whereas several treatments such as Chromolaena odorata grown in soil, plant, and AMF and Aspilia africana grown in soil, plant, AMF, and 400 ml crude oil, recorded no detectable soil Cd. In contrast, cadmium accumulation in plant roots remained generally low across all treatments, with the highest root Cd concentration observed in Dog fennel grown in soil, plant, AMF, and 800 ml crude oil (1.24±0.15 $mgkg^{-1}$), suggesting limited Cd translocation from soil to roots.

Nickel concentrations in soils differed significantly ($p < 0.05$) among treatments and were strongly influenced by plant species and crude oil amendment levels. The highest soil Ni concentrations were observed in Dog fennel grown in soil, plant, AMF, and 800 ml crude oil (16.09±2.84 $mgkg^{-1}$) and Dog fennel grown in soil, plant, AMF, and 400 ml crude oil (14.37±1.51 $mgkg^{-1}$), whereas several treatments showed no detectable Ni. Root Ni concentrations followed a similar pattern but at substantially lower levels, with maximum values recorded in Dog fennel grown in soil, plant, AMF, and 800 ml crude oil (1.00±0.05 $mgkg^{-1}$) and Dog fennel grown in soil, plant, AMF, and 400 ml crude oil (0.89±0.17 $mgkg^{-1}$).

Generally, the results indicate pronounced treatment-dependent differences in soil heavy metal concentrations, with comparatively lower accumulation in plant roots. Treatments involving Dog fennel (*Eupatorium capillifolium*), particularly under higher crude oil contamination, exhibited the greatest soil metal loads and root uptake, highlighting its relatively higher tolerance and accumulation capacity compared with Chromolaena odorata and Aspilia africana

Heavy metal Accumulation in Root

Root chromium concentration differed significantly ($p < 0.05$) among treatments. The highest Cr accumulation was recorded in Dog fennel (*Eupatorium capillifolium*) grown in soil, plant, AMF, and 800 ml crude oil (16.09±2.84 $mgkg^{-1}$) and Dog fennel grown in soil, plant, AMF, and 400 ml crude oil (14.37±1.51 $mgkg^{-1}$). This was followed by Dog fennel grown in soil, plant, and AMF (12.16±1.91 mg kg^{-1}). Moderate Cr concentrations

were observed in *Aspilia africana* grown in soil and plant (6.19±0.37 mg kg^{-1}) and *Chromolaena odorata* grown in soil, plant, AMF, and 800 ml crude oil (8.14±1.19 mg kg^{-1}), while several treatments recorded no detectable Cr in roots.

Cadmium accumulation in roots was generally low but showed significant variation across treatments. The highest root Cd concentration occurred in Dog fennel grown in soil, plant, AMF, and 800 ml crude oil (1.24 ± 0.15 mg kg^{-1}), followed by Dog fennel grown in soil, plant, AMF, and 400 ml crude oil (0.73±0.15 $mgkg^{-1}$) and *Aspilia africana* grown in soil, plant, and AMF (0.91±0.19 $mgkg^{-1}$). In contrast, roots of *Chromolaena odorata* across most treatments and those of *Aspilia africana* grown in soil, plant, AMF, and crude oil treatments recorded either very low or zero Cd concentrations, suggesting restricted Cd uptake under these conditions.

Nickel concentration in roots also varied significantly ($p < 0.05$) among treatments. The maximum Ni accumulation was observed in Dog fennel grown in soil, plant, AMF, and 800 ml crude oil (1.00±0.05 $mgkg^{-1}$), closely followed by Dog fennel grown in soil, plant, AMF, and 400 ml crude oil (0.89±0.17 $mgkg^{-1}$) and Dog fennel grown in soil, plant, and AMF (0.84±0.19 $mgkg^{-1}$). Lower but measurable Ni concentrations were recorded in *Aspilia africana* grown in soil, plant, and AMF (0.74±0.16 $mgkg^{-1}$) and *Chromolaena odorata* grown in soil, plant, AMF, and 800 ml crude oil (0.51±0.06 $mgkg^{-1}$), whereas several treatments showed no detectable Ni.

Generally, the results indicate clear treatment and species-dependent differences in heavy metal accumulation in roots. Dog fennel (*Eupatorium capillifolium*) consistently exhibited the highest accumulation of Cr, Cd, and Ni, particularly under crude oil–amended conditions, suggesting a greater capacity for heavy metal uptake compared with *Chromolaena odorata* and *Aspilia africana*.

Physical Properties of Soil

Table 2 presents the physical properties of soils under different treatment combinations, expressed as sand, silt, and clay fractions. Across all treatments, sand was the dominant soil fraction, indicating that the soils were predominantly sandy in texture.

Table 2: Physical Properties 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}	47.00±8.00^{g}	58.00±9.00^{bc}
P2C1	830.00±18.00^{de}	92.00±3.00^{a}	78.00±6.00^{a}
P2C2	835.00±14.00^{de}	87.00±8.00^{ab}	78.00±6.00^{a}
P2C3	870.00±8.00^{bcd}	72.00±3.00^{d}	58.00±5.00^{bc}
P2C4	915.00±13.00^{a}	32.00±4.00^{h}	53.00±4.00^{bc}
P3C1	855.00±31.00^{cde}	82.00±4.00^{bc}	63.00±4.00^{b}

P3C2	835.00±23.00^{de}	87.00±4.00^{ab}	78.00±6.00^{a}
P3C3	890.00±45.00^{abc}	62.00±4.00^{ef}	48.00±6.00^{c}
P3C4	905.00±13.00^{ab}	42.00±3.00^{g}	53.00±4.00^{bc}

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. 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 4ooml crude oil, SC4 = Soil and 800ml crude oil.

Sand content varied significantly ($p < 0.05$) among treatments, ranging from 827.00±13.00 gkg^{-1} in soil treated with 400 ml crude oil only to 915.00±13.00 gkg^{-1} in *Aspilia africana* grown in soil, plant, AMF, and 800 ml crude oil. Higher sand proportions were generally associated with treatments receiving crude oil amendments, particularly at higher application rates. Soils under *Chromolaena odorata* grown in soil, plant, AMF, and 400 ml crude oil and 800 ml crude oil also recorded elevated sand contents (895.00±20.00 and 895.00±23.00 g kg^{-1}, respectively).

Silt content showed significant differences ($p < 0.05$) across treatments and exhibited an inverse trend relative to sand. The highest silt fraction was observed in *Aspilia africana* grown in soil and plant (92.00±3.00 gkg^{-1}), followed by treatments involving plants with AMF but without crude oil. In contrast, markedly lower silt contents were recorded in crude oil–amended soils, with the lowest value obtained in *Aspilia africana* grown in soil, plant, AMF, and 800 ml crude oil (32.00±4.00 gkg^{-1}).

Clay content also varied significantly among treatments. Higher clay values were recorded in soils without crude oil amendment, including soil treated with 400 ml crude oil only (89.00±6.00 gkg^{-1}) and soil treated with 800 ml crude oil only (79.00±5.00 gkg^{-1}), as well as treatments involving plants without crude oil. Conversely, crude oil–amended treatments involving plants and AMF generally exhibited reduced clay contents, with the lowest clay fraction observed in *Chromolaena odorata* grown in soil, plant, AMF, and 400 ml crude oil (48.00±5.00 gkg^{-1}) and Dog fennel grown in soil, plant, AMF, and 400 ml crude oil (48.00±6.00 gkg^{-1}).

Overall, the results indicate that crude oil contamination, particularly at higher application rates, was associated with increased sand proportions and reduced silt and clay fractions, suggesting a modification of soil physical structure. Treatments involving plants and arbuscular mycorrhizal fungi further influenced soil particle distribution, with species-specific responses observed among *Chromolaena odorata*, *Aspilia africana*, and Dog funnel (*Eupatorium capillifolium*).

Chemical Properties of Soil

Table 3 presents the chemical properties of soils subjected to different treatment combinations. Soil pH across all treatments ranged from 4.50 to 5.40, indicating that the soils were generally acidic, with no statistically significant differences ($p > 0.05$) among treatments. This suggests that neither crude oil contamination nor the introduction of plants and arbuscular mycorrhizal fungi (AMF) caused marked shifts in soil reaction within the study period.

Table 3: Chemical Properties of Soil

Sample	pH ($H_2$0)	P (mg/kg)	N (g/kg)	OC (g/kg)	OM (g/kg)	Ca (cmol/kg)	Mg (Cmol/kg)	K (Cmol/kg)	Na (Cmol/kg)	EA (Cmol/kg)	ECEC (Cmol/kg)	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^{cdef}	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^{abc}	0.36±0.07^{ab}	0.96±0.14^{defg}	6.86±0.26^{bcd}	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. 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.

Available phosphorus (P) varied significantly ($p < 0.05$) among treatments. The highest P concentration was observed in soil with plant only (Chromolaena odorata) (42.80±1.48 $mgkg^{-1}$), whereas the lowest values occurred in soils treated with 800 ml crude oil, particularly in Aspilia africana with soil, plant, AMF, and 800 ml crude oil (18.90±2.48 $mgkg^{-1}$) and soil with 800 ml crude oil only (19.10±0.10 $mgkg^{-1}$). This indicates a depressive effect of higher crude oil levels on soil phosphorus availability.

Total nitrogen (N) content showed significant variation among treatments, ranging from 1.40±0.28 gkg^{-1} to 2.52±0.48 gkg^{-1}. Higher nitrogen levels were generally recorded in crude oil–amended treatments involving

plants and AMF, with the maximum value observed in *Chromolaena odorata* grown in soil, plant, AMF, and 800 ml crude oil, suggesting improved nitrogen retention or microbial activity under these conditions.

Organic carbon (OC) and organic matter (OM) contents differed significantly ($p < 0.05$) among treatments and showed a clear response to crude oil contamination. The highest OC (35.51±5.93 gkg^{-1}) and OM (61.22±2.37 g kg^{-1}) were recorded in *Chromolaena odorata* grown in soil, plant, AMF, and 800 ml crude oil, while the lowest values occurred in treatments involving plants without crude oil and AMF. This trend reflects the contribution of crude oil hydrocarbons and organic inputs from plant residues to soil carbon pools.

Exchangeable calcium (Ca) and magnesium (Mg) contents also varied significantly among treatments. Exchangeable Ca ranged from 2.80±0.66 to 5.60±0.70 $cmolkg^{-1}$, with the highest values observed in *Aspilia africana* grown in soil, plant, AMF, and 400 ml crude oil. Magnesium content was highest (2.00±0.20 $cmolkg^{-1}$) in treatments involving *Aspilia africana* and Dog fennel with AMF, indicating enhanced base cation availability in these systems.

Exchangeable potassium (K) and sodium (Na) showed moderate but significant differences across treatments. Higher K values were generally recorded in crude oil–amended soils with plant and AMF combinations, while Na tended to increase slightly in treatments receiving higher crude oil doses. Exchangeable acidity (EA) was significantly higher in soils treated with crude oil only, particularly 400 ml crude oil, indicating increased soil acidity due to contamination.

Effective cation exchange capacity (ECEC) ranged from 5.74±0.62 to 8.52±0.87 $cmolkg^{-1}$, with higher values mostly associated with treatments involving plants and AMF. Base saturation (BS) was generally high across treatments (78–91 %), with the highest BS recorded in *Aspilia africana* grown in soil, plant, AMF, and 800 ml crude oil (90.66±5.01 %), reflecting the dominance of basic cations over exchangeable acidity.

Generally, the results indicate that crude oil contamination, particularly at higher application rates, significantly influenced soil nutrient dynamics, organic matter accumulation, and exchangeable cation composition. The presence of plants and AMF moderated some of these effects, enhancing nutrient status and cation exchange properties relative to soils treated with crude oil alone.

Discussion

The present study demonstrates that crude oil contamination significantly altered soil physical and chemical properties and heavy metal dynamics, while the presence of plants and arbuscular mycorrhizal fungi (AMF) modulated these effects. These findings are consistent with recent research showing that petroleum hydrocarbon pollution markedly affects soil physicochemical properties, including nutrient availability, organic matter dynamics, and trace metal behavior (Adetunji et al., 2023).

Soil Physical Properties

The observed increase in sand content and concurrent reductions in clay and silt fractions in crude oil–amended soils suggest that hydrocarbon presence can influence soil particle aggregation and distribution. Crude oil and petroleum hydrocarbons have been shown to coat soil particles, increasing soil hydrophobicity and disrupting natural aggregate formation, which in turn alters soil structure, reduces pore connectivity, and diminishes moisture retention (Abrahim and Parker, 2008). Such changes are ecologically significant because increased soil hydrophobicity and structural disruption can reduce water infiltration and availability, hinder aeration, and ultimately limit root penetration and microbial activity (Doerr et al., 2020; Barzegar et al., 2018). These physical alterations are often observed in hydrocarbon-contaminated soils and are associated with impaired soil function and reduced plant establishment.

Soil Chemical Properties

Acidic soil pH across treatments is typical of many tropical soils and reflects limited shifts due to contamination, consistent with some field studies reporting minimal pH alteration following crude oil input (Itam, et al., 2023). However, available phosphorus decreased in soils with higher crude oil loads, consistent with evidence that petroleum hydrocarbons can impede phosphorus availability by disrupting microbial activity and mineralization processes (Al-Hadidi et al., 2023). Hydrocarbon contamination often suppresses key microbial populations responsible for organic P mineralization and can result in P fixation through interactions with organic matter and altered soil redox conditions under stress (Zhang et al., 2021).

Organic carbon and organic matter contents were significantly higher in contaminated soils, especially where crude oil was added at 800 ml. Petroleum hydrocarbons contribute to soil organic carbon pools due to their high carbon content, which is consistent with findings that TPH increases organic carbon in contaminated soils substantially (Liu et al., 2025). Elevated organic matter can influence nutrient retention and cation exchange capacity, but hydrocarbon-derived carbon does not equate to increased fertility and may instead immobilize essential nutrients, hindering plant nutrient uptake and microbial function.

Enhanced effective cation exchange capacity (ECEC) and base saturation in soils with plant and AMF treatments indicate improved nutrient-holding capacity relative to crude oil only soils. Such improvements are likely due to root exudates and fungal biomass contributing additional charges to the soil exchange complex, augmenting retention of Ca^{2+}, Mg^{2+}, and other bases. These findings concord with the concept that plant-microbe interactions can buffer soils against contamination impacts, improving fertility indices under stress.

Heavy Metal Dynamics and Plant Responses.

The heavy metal distribution in this study indicates that crude oil contamination may increase soil Cd and Ni concentrations, corroborating observations in petroleum-contaminated environments where hydrocarbon presence is associated with elevated metal content and altered mobility (Alloway, 2013). The absence of detectable soil Cr suggests that soil Cr may have strong geochemical binding or be present below detection thresholds, a pattern noted in other contaminated tropical soils.

Root accumulation patterns revealed species-specific uptake. *Eupatorium capillifolium* (dog fennel) exhibited significantly higher root accumulation of Cr, Cd, and Ni compared with *Chromolaena odorata* and *Aspilia africana*, particularly under crude oil contamination and arbuscular mycorrhizal fungi (AMF) treatments. This observation is consistent with recent evidence that AMF associations can enhance metal uptake and sequestration in roots, possibly through increased bioavailability and fungal-mediated translocation mechanisms (Khan et al., 2017). AMF hyphae increase the effective absorptive surface area and can alter metal speciation in the rhizosphere, facilitating root accumulation of heavy metals (Göhre & Paszkowski, 2006; Smith & Read, 2008). Additionally, AMF can produce phytohormones (e.g., auxins, cytokinins) and modify root exudation patterns that improve nutrient and metal acquisition under stress conditions by altering root architecture and rhizosphere chemistry (Miransari, 2011; Kharkwal et al., 2020).

The limited translocation of metals to aboveground parts, with most accumulation restricted to roots, is consistent with well-documented plant strategies that sequester heavy metals in roots to mitigate toxicity in aerial tissues. Root-based sequestration limits the transport of potentially phytotoxic ions into leaves and stems, thereby reducing oxidative damage and preserving photosynthetic capacity—critical adaptations for plant survival in contaminated environments (Hall, 2002; Verbruggen et al., 2009). This mechanism often involves chelation by root-localized ligands (e.g., phytochelatins, organic acids) and compartmentalization within vacuoles, which

together attenuate reactive oxygen species (ROS) production and protect key metabolic functions (Clemens, 2006; DalCorso et al., 2010).

Implication for Phytoremediation

The interactive effects of plant species, AMF, and crude oil contamination highlight the importance of selecting tolerant and accumulation-capable species for remediation purposes. Dog fennel's high metal uptake suggests its potential utility in phytostabilization or phytoremediation strategies, particularly in hydrocarbon-impacted tropical soils where metal contamination co-occurs with petroleum pollutants. Moreover, AMF inoculation appears to enhance plant performance under heavy metal stress, which corroborates findings in other systems where mycorrhizal associations improved metal tolerance and plant growth (Wu et al., 2021).

Conclusion

Crude oil contamination significantly altered soil physical and chemical properties and influenced heavy metal dynamics, with cadmium and nickel showing elevated concentrations while chromium remained largely undetectable. Plant species and arbuscular mycorrhizal fungi (AMF) modulated these effects, with *Eupatorium capillifolium* (dog fennel) consistently exhibiting the highest root accumulation of Cr, Cd, and Ni under AMF and crude oil treatments. Root-based sequestration limited metal translocation to shoots, mitigating toxicity and preserving photosynthetic function. These findings highlight the potential of combining tolerant plant species with AMF inoculation for phytoremediation. Dog fennel, in particular, emerges as a promising candidate for stabilizing heavy metals in tropical hydrocarbon-contaminated soils, supporting sustainable soil restoration strategies.

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