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Phytoremediation-Induced Recovery of Crude Oil-Polluted Soil Using *Vetiveria zizanioides* and *Panicum maximum* and Subsequent Enhancement of Okra (*Abelmoschus esculentus* L.)

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ABSTRACT

Crude oil pollution reduces soil fertility, suppresses beneficial microbial activity, and limits crop productivity in many oil-producing agricultural areas. This study evaluated the capacity of Vetiveria zizanioides and Panicum maximum, used singly and with poultry manure amendment, to remediate crude oil-contaminated soil and improve the subsequent performance of okra (Abelmoschus esculentus L.). The phytoremediation phase was laid out in a completely randomized design with six treatments and four replicates over fourteen weeks, after which okra was cultivated on the remediated soils. Total petroleum hydrocarbon (TPH), soil physicochemical properties, microbial population indices, and okra growth and yield traits were determined. Vetiver combined with poultry manure produced the greatest TPH reduction (82.62%), followed by guinea grass with poultry manure (76.21%). The amended phytoremediation treatments improved soil nutrient status, increased hydrocarbon-utilizing bacterial populations, and supported better okra growth and yield than the polluted unremediated control. The findings show that integrating tolerant grasses with organic amendment is a practical, low-cost, and sustainable approach for restoring crude oil-contaminated agricultural soil and improving crop productivity.

Keywords: Crude oil contamination, phytoremediation, *Vetiveria zizanioides*, *Panicum maximum*, poultry manure, total petroleum hydrocarbon, okra productivity.

Authors' Contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

1. Introduction

Agricultural soil contamination by petroleum hydrocarbons remains a serious environmental and food-security problem in oil-producing regions of the tropics and subtropics. Crude oil exploration, transport, storage, and processing can introduce aliphatic hydrocarbons, aromatic fractions, and polycyclic aromatic hydrocarbons (PAHs) into soil systems [23,24]. Because many petroleum compounds are chemically complex and poorly soluble, their natural attenuation may be slow and strongly dependent on soil texture, moisture status, nutrient balance, climate, and the structure of resident microbial communities [4,15].

In cultivated soils, crude oil pollution reduces soil quality by increasing hydrophobicity, lowering aeration, impairing water movement, and limiting nutrient availability. Hydrocarbon films around soil particles can restrict root penetration and microbial activity, while petroleum-derived carbon may accumulate without contributing to effective fertility [1]. The resulting imbalance in carbon, nitrogen, and phosphorus cycling often suppresses seed germination, vegetative growth, and crop productivity [2,3]. These effects are especially important in rural agricultural areas where contaminated soils may remain in use for food production.

Phytoremediation offers a low-cost and environmentally compatible option for restoring hydrocarbon-impacted soils. The process depends not only on plant tolerance but also on the ability of roots to stimulate rhizosphere microorganisms that transform or mineralize petroleum compounds [5,27,31,32]. For petroleum hydrocarbons, rhizodegradation is particularly important because many high-molecular-weight components are not readily translocated into plant tissues, but can be degraded by microorganisms supported by root exudates.

Vetiver grass (*Vetiveria zizanioides* (L.) Nash) is a perennial C4 grass widely cultivated in tropical regions because of its extensive root system, environmental adaptability, and soil stabilization ability. Its deep and fibrous roots create a large rhizosphere capable of supporting microbial populations involved in hydrocarbon degradation [34]. Previous studies have demonstrated the ability of vetiver to enhance petroleum hydrocarbon removal by stimulating hydrocarbon-utilizing microorganisms and improving rhizosphere processes [10,13,16].

Guinea grass (*Panicum maximum* Jacq.) is a fast-growing tropical forage grass with extensive biomass production and a dense fibrous root system. Although less extensively investigated than vetiver, Panicum species have shown potential for petroleum-contaminated soil restoration due to their tolerance to hydrocarbon stress and ability to support rhizosphere microbial activity [21,26].

The application of organic amendments such as poultry manure can improve phytoremediation efficiency by supplying nutrients required for microbial growth and plant establishment. Petroleum-contaminated soils often have nutrient limitations that restrict biodegradation processes; therefore, organic amendments can enhance microbial biomass, enzyme activity, and hydrocarbon degradation rates [6,29]. Poultry manure is particularly suitable in tropical agricultural systems because of its availability and ability to improve soil fertility.

Although several studies have investigated phytoremediation of petroleum-contaminated soils, fewer studies have evaluated soil restoration and subsequent crop productivity within the same experimental system. Therefore, this study aimed to compare the effectiveness of *Vetiveria zizanioides* and *Panicum maximum*, with and without poultry manure amendment, in reducing crude oil

contamination, improving soil properties, enhancing microbial recovery, and restoring the productivity of okra (*Abelmoschus esculentus* L.) cultivated on remediated soils.

2. Materials and Methods

2.1. Study and Experimental Site

The experiment was conducted in the screenhouse of the Department of Crop Science, University of Agriculture and Environmental Sciences, Umuagwo, Nigeria, between April and August 2025. The experimental site is located in southeastern Nigeria at approximately latitude 5°37' N and longitude 7°02' E within the tropical rainforest agroecological zone. The area experiences annual rainfall of approximately 2,000-2,500 mm, mean daily temperatures of 28–32°C, and relative humidity ranging from 70-85%.

2.2. Soil Collection, Contamination, and Baseline Characterization

Topsoil (0–20 cm depth) was collected from an uncontaminated portion of the Crop Science Farm of the University. The soil was air-dried, homogenized, and packed into 24 perforated black polyethylene bags containing 8 kg soil each.

Bonny Light crude oil was applied at 5% (w/w) contamination level, equivalent to 50 g crude oil per 1000 g dry soil. The contaminated soils were allowed to equilibrate for 14 days before planting, while the unpolluted control treatment remained uncontaminated.

Baseline soil characterization was carried out before contamination. Soil pH was determined using a 1:2 soil-to-water suspension with a calibrated glass electrode pH meter [20]. Organic carbon was determined using the Walkley–Black method [35]. Total nitrogen was determined using the Kjeldahl method [12], while available phosphorus was determined using the Bray and Kurtz extraction method [11]. Exchangeable cations were determined using ammonium acetate extraction followed by atomic absorption spectrophotometry [28]. Particle size distribution was determined using the hydrometer method [9].

The experiment consisted of two phases: phytoremediation and okra productivity assessment.

2.3. Phase I: Phytoremediation Experiment

2.3.1. Experimental Design and Treatments

The experiment was arranged in a Completely Randomized Design (CRD) with six treatments and four replicates, resulting in 24 experimental units. Poultry manure was incorporated into Treatments T5 and T6 at a rate equivalent to 5 t ha^{-1} before planting.

Table 1. Treatment structure for Phase I phytoremediation experiment

Treatment	Description
T1	Unpolluted soil without grass or amendment (control)
T2	Crude oil-polluted soil without grass or amendment (polluted control)
T3	Crude oil-polluted soil planted with *Vetiveria zizanioides*
T4	Crude oil-polluted soil planted with *Panicum maximum*

Treatment	Description
T5	Crude oil-polluted soil planted with *Vetiveria zizanioides* + poultry manure
T6	Crude oil-polluted soil planted with *Panicum maximum* + poultry manure

Poultry manure used in Treatments T5 and T6 was obtained from a commercial poultry farm in Umuagwo and incorporated into the soil before planting at an equivalent rate of 5 t ha^{-1}.

2.3.2. Plant Establishment, Soil Sampling, and Growth Assessment

Tillers of *Vetiveria zizanioides* and *Panicum maximum* were obtained from the University Botanic Garden. The planting materials were trimmed to approximately 15 cm shoot length and 10 cm root length before transplanting. The tillers were surface sterilized with 0.1% sodium hypochlorite solution for 5 minutes and rinsed with sterile water before planting.

Three tillers were planted per experimental unit in Treatments T3-T6, while T1 and T2 remained unplanted. All experimental units were irrigated daily to maintain adequate moisture throughout the 14-week remediation period.

Composite soil samples were collected at Week 0, Week 7, and Week 14. Samples were collected from three points within each bag at 0-15 cm depth and mixed to obtain a representative sample for each replicate.

At the end of the remediation period, plants were harvested and evaluated for plant height, number of tillers, root length, fresh biomass, and dry biomass. Dry biomass was determined after oven drying samples at 70°C until constant weight.

2.3.3. Total Petroleum Hydrocarbon (TPH) Determination

Total petroleum hydrocarbon (TPH) concentration was determined using solvent extraction followed by gravimetric analysis and expressed as mg kg^{-1} dry soil.

Percentage reduction in TPH was calculated as:

$$\text{TPH reduction (\%)} = \frac{\text{TPH}_{initial} - \text{TPH}_{final}}{\text{TPH}_{initial}} \times 100$$

2.3.4. Soil Physicochemical Properties

Soil samples were analyzed for pH, organic carbon, total nitrogen, available phosphorus, exchangeable potassium, and cation exchange capacity using standard laboratory procedures described earlier.

2.3.5. Microbial Analysis

Microbial populations were determined using culture-based serial dilution techniques. Ten grams of soil from each experimental replicate were suspended in 90 mL sterile physiological saline (0.85% NaCl) and serially diluted up to 10-^{5}. Total heterotrophic bacteria (THB) were enumerated on nutrient agar and incubated at 28°C for 48 hours.

Fungal populations were determined using potato dextrose agar supplemented with chloramphenicol and incubated at 28°C for 5 days. Hydrocarbon-utilizing bacteria (HUB) were enumerated using Bushnell–Haas mineral salt agar with crude oil as the sole carbon source following the vapour-phase transfer method of Mills *et al.* [22]. Microbial counts were expressed as colony-forming units per gram of dry soil (cfu g^{-1}). Mean values from the four experimental replicates were used for analysis.

2.4. Phase II: Okra Productivity Experiment

2.4.1. Soil Preparation

After completion of the phytoremediation phase, grasses and roots were removed carefully and soils from each treatment were homogenized. The soils were repacked into new perforated polyethylene bags containing 8 kg soil and arranged in a Completely Randomized Design with four replicates. The treatments were:

Table 2. Treatment structure for Phase II okra productivity experiment

Treatment	Description
O1	Soil from T1 (unpolluted control)
O2	Soil from T2 (polluted unremediated soil)
O3	Soil from T3 (Vetiver-remediated soil)
O4	Soil from T4 (Guinea grass-remediated soil)
O5	Soil from T5 (Vetiver + manure-remediated soil)
O6	Soil from T6 (Guinea grass + manure-remediated soil)

2.4.2. Okra Planting

Seeds of okra (*Abelmoschus esculentus* L. Moench) were obtained from the Department of Crop Science seed unit. Seeds were surface sterilized using 1% sodium hypochlorite for 2 minutes, rinsed with sterile distilled water, and soaked for 12 hours before planting. Five seeds were planted per bag at a depth of 2 cm. Seedlings were thinned to two plants per bag at 14 days after sowing (DAS). No fertilizer was applied during the growth period.

2.4.3. Growth and Yield Measurements

Germination percentage was calculated as:

$$\text{GP}\ (\%) = \frac{\text{Number of germinated seeds}}{\text{Total number of seeds planted}} \times 100$$

Emergence rate index was calculated according to Maguire [19].

Plant height, leaf number, stem girth, and chlorophyll content were measured at predetermined growth stages.

Leaf area was estimated using:

$$\text{Leaf area} = \text{Length} \times \text{Width} \times 0.75$$

Leaf area was estimated using the non-destructive method of Bhatt and Chanda [7].

Yield parameters including days to flowering, pods per plant, pod length, pod diameter, fresh pod weight, and total pod yield were recorded from first harvest until final harvest at 10 weeks after sowing.

2.4.4. Final Soil Analysis

After okra harvest, composite soil samples were collected from each experimental unit and analyzed for pH, organic carbon, total nitrogen, available phosphorus, exchangeable potassium, TPH concentration, THB, fungi, and HUB.

2.4.5. Statistical Analysis

All experimental data were subjected to one-way analysis of variance (ANOVA) using SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Treatment means were separated using Duncan's Multiple Range Test (DMRT) at a significance level of $P \leq 0.05$. Values presented in tables represent mean ± standard error of four replicates.

3. Results

3.1. Baseline Soil Physicochemical Properties

The experimental soil was classified as sandy loam, containing 51.2% sand, 13.4% silt, and 15.4% clay (Table 3). The soil was slightly acidic (pH 6.35) with organic carbon of 1.43%, total nitrogen of 0.71%, available phosphorus of 11.45 mg kg^{-1}, exchangeable potassium of 0.65 cmol kg^{-1}, and cation exchange capacity of 15.0 cmol kg^{-1}. Following crude oil contamination, initial TPH concentration increased to 42,200 mg kg^{-1}.

Table 3. Baseline physicochemical properties of experimental soil before pollution induction

Parameter	Value	Unit	Remark
Soil pH (H2O)	6.35	—	Slightly acidic
Organic carbon	1.43	%	Moderate
Total nitrogen	0.71	%	Low-moderate
Available phosphorus	11.45	mg/kg	Moderate
Exchangeable K+	0.65	cmol/kg	—
CEC	15.0	cmol/kg	—
Sand	51.2	%	Sandy loam
Silt	13.4	%	
Clay	15.4	%	
Initial TPH (post-pollution)	42,200	mg/kg	5% w/w applied

CEC = cation exchange capacity; TPH = total petroleum hydrocarbons.

3.2. Total Petroleum Hydrocarbon (TPH) Reduction

Significant differences occurred among treatments after 14 weeks of phytoremediation ($P \leq 0.05$; Table 4). The polluted control (T2) recorded the highest remaining TPH concentration (30,132 mg kg^{-1}), corresponding to only 28.6% reduction. Plant-based treatments significantly improved TPH removal. Vetiver alone (T3) reduced TPH by 68.8%, while Guinea grass alone (T4) achieved 63.8% reduction. The addition of poultry manure enhanced remediation efficiency. Vetiver + manure (T5) produced the highest reduction (82.6%), followed by Guinea grass + manure (T6) with 76.2% reduction. The lowest final TPH concentration was recorded in T5 (7,335 mg kg^{-1}).

Table 4. Total petroleum hydrocarbon (TPH) concentrations and percentage reduction at weeks 0, 7, and 14 (mean ± SE, n = 4)

Treatment	Week 0 (mg/kg)	Week 7 (mg/kg)	Week 14 (mg/kg)	Reduction (%)
T1 – Unpolluted control	336 ± 58.60[b]	365 ± 1.00[f]	352 ± 1.00[f]	—
T2 – Polluted, no grass	42,200 ± 1.00[a]	35,533 ± 404[a]	30,132 ± 231[a]	28.60
T3 – Vetiver only	42,200 ± 1.00[a]	25,599 ± 360[c]	13,165 ± 209[c]	68.80
T4 – Guinea grass only	42,200 ± 1.00[a]	30,199 ± 172[b]	15,245 ± 67.0[b]	63.87
T5 – Vetiver + manure	42,200 ± 1.00[a]	19,135 ± 963[e]	7,335 ± 573[e]	82.62
T6 – Guinea grass + manure	42,200 ± 1.00[a]	21,233 ± 207[d]	10,039 ± 67.2[d]	76.21

3.3. Soil Physicochemical Properties After Phytoremediation

Soil physicochemical properties varied among treatments after the 14-week phytoremediation period (Table 5). The polluted unremediated soil (T2) recorded the lowest soil quality indicators, with pH of 5.12, total nitrogen of 0.08%, available phosphorus of 4.20 mg kg^{-1}, and exchangeable potassium of 0.14 cmol kg^{-1}. Organic carbon was highest in T2 (4.61%), reflecting the accumulation of petroleum-derived carbon following crude oil contamination.

Table 5. Soil physicochemical properties at Week 14 (end of Phase I) across treatments

Treatment	pH	OC (%)	Total N (%)	Av. P (mg/kg)	Exch. K+ (cmol/kg)
T1 – Unpolluted control	6.35	1.43	0.71	11.45	0.65
T2 – Polluted, no grass	5.12	4.61	0.08	4.2	0.14
T3 – Vetiver only	5.98	3.14	0.14	8.9	0.24
T4 – Guinea grass only	5.81	3.42	0.12	7.6	0.21
T5 – Vetiver + manure	6.15	2.72	0.17	11.4	0.25
T6 – Guinea grass + manure	6.12	2.91	0.19	10.2	0.22

3.4. Microbial Population Indices

Microbial populations differed significantly among treatments at Week 14 ($P \leq 0.05$) (Table 6). The polluted unremediated soil (T2) showed a reduction in total heterotrophic bacteria (THB) and fungi compared with the initial values. THB decreased from 8.4×10^6 to 2.1×10^6 cfu g^{-1}, while fungal population decreased from 3.1×10^6 to 0.7×10^6 cfu g^{-1}. In contrast, planted and manure-amended treatments stimulated microbial recovery. The highest microbial populations were recorded in T5 (Vetiver + manure), with THB, fungi, and HUB values of 22.6×10^6, 8.1×10^6, and 28.4×10^6 cfu g^{-1}, respectively. T6 (Guinea grass + manure) also recorded high microbial abundance, with THB of 19.2×10^6 cfu g^{-1} and HUB of 24.1×10^6 cfu g^{-1}.

Table 6. Microbial population indices ($\times 10^6$ cfu/g dry soil) at Week 0 and Week 14 across treatments (mean ± SE, n = 4)

Treatment	THB Wk 0	THB Wk 14	Fungi Wk 0	Fungi Wk 14	HUB Wk 0	HUB Wk 14
T1 – Unpolluted control	8.4±0.4^{a}	8.6±0.3^{a}	3.1±0.2^{a}	3.2±0.2^{a}	0.9±0.1^{a}	0.8±0.1^{a}
T2 – Polluted, no grass	8.4±0.4^{e}	2.1±0.3^{e}	3.1±0.2^{e}	0.7±0.1^{e}	0.9±0.1^{e}	3.4±0.3^{e}
T3 – Vetiver only	8.4±0.4^{c}	14.8±0.6^{c}	3.1±0.2^{c}	5.4±0.4^{c}	0.9±0.1^{c}	18.6±1.2^{c}
T4 – Guinea grass only	8.4±0.4^{d}	12.4±0.5^{d}	3.1±0.2^{d}	4.8±0.3^{d}	0.9±0.1^{d}	15.2±0.9^{d}
T5 – Vetiver + manure	8.4±0.4^{b}	22.6±0.8^{b}	3.1±0.2^{b}	8.1±0.6^{b}	0.9±0.1^{b}	28.4±1.8^{b}
T6 – Guinea grass + manure	8.4±0.4^{b}	19.2±0.7^{b}	3.1±0.2^{b}	6.9±0.5^{b}	0.9±0.1^{b}	24.1±1.5^{b}

THB = total heterotrophic bacteria; HUB = hydrocarbon-utilizing bacteria. All values $\times 10^6$ cfu/g dry soil. DMRT letters apply at $P \leq 0.05$.

3.5. Phytoremediation Plant Growth (Phase I)

Growth parameters of *Vetiveria zizanioides* and *Panicum maximum* differed significantly among treatments after 14 weeks (Table 7). Among the grass treatments, T6 (Guinea grass + manure) recorded the highest shoot growth performance, with plant height of 87.33 cm, 15.00 tillers, fresh biomass of 215.40 g, and dry biomass of 72.10 g. However, T5 (Vetiver + manure) produced the longest roots (98.40 cm), indicating stronger root development. Vetiver without amendment (T3) showed the lowest growth performance among planted treatments, recording plant height of 68.0 cm and dry biomass of 48.2 g.

Table 7. Growth parameters of phytoremediation grasses at Week 14 (mean ± SE, n = 4)

Treatment	Height (cm)	Tillers (n)	Fresh biomass (g)	Dry biomass (g)	Root length (cm)
T3 – Vetiver only	68.0±1.00^{d}	9.43±0.51^{d}	138.07±8.4^{d}	48.2±3.1^{d}	84.6±5.2^{d}
T4 – Guinea grass only	71.63±6.4^{c}	11.20±0.8^{c}	164.3±1.13^{c}	52.4±3.8^{c}	61.2±4.1^{c}

Treatment	Height (cm)	Tillers (n)	Fresh biomass (g)	Dry biomass (g)	Root length (cm)
T5 – Vetiver + manure	83.10±0.85[b]	12.33±0.5[b]	198.33±2.1[b]	68.4±4.4[b]	98.4±6.8[b]
T6 – Guinea grass + manure	87.33±1.53[a]	15.00±0.3[a]	215.40±0.3[a]	72.1±4.8[a]	74.8±5.1[a]

T1 and T2 had no planted grass. DMRT letters apply to dry biomass column ($P \leq 0.05$).

3.6. Okra Germination, Emergence, and Vegetative Growth (Phase II)

Okra performance varied significantly among treatment soils (Table 8). The polluted unremediated soil (O2) recorded the poorest performance, with germination percentage of 42.5%, emergence rate index of 1.94, plant height of 29.6 cm, 5.8 leaves per plant, stem girth of 7.2 mm, and SPAD value of 18.6. The unpolluted control (O1) produced the highest values, with 95.0% germination, plant height of 68.4 cm, and SPAD value of 42.8. Among remediated soils, O5 (Vetiver + manure) showed the best crop response, recording 92.5% germination, 65.8 cm plant height, 11.8 leaves per plant, 14.2 mm stem girth, and SPAD value of 41.2. O6 (Guinea grass + manure) also improved okra performance but remained slightly lower than O5.

Table 8. Okra germination, emergence rate, and vegetative growth parameters across treatment soils (mean ± SE, n = 4)

Treatment	GP (%)	ERI	Height 8WAS (cm)	Leaves 8WAS (n)	Stem girth 8WAS (mm)	SPAD
O1 – Unpolluted control	95.0	4.82	68.4±2.8[a]	12.4±0.6[a]	14.8±0.8[a]	42.8±1.4[a]
O2 – Polluted unremediated	42.5	1.94	29.6±2.1[e]	5.8±0.4[e]	7.2±0.5[e]	18.6±1.2[e]
O3 – Vetiver-remediated	77.5	3.61	54.2±2.4[c]	9.8±0.5[c]	12.1±0.7[c]	36.4±1.2[c]
O4 – GG-remediated	72.5	3.28	49.8±2.2[d]	9.1±0.5[d]	11.4±0.6[d]	33.8±1.1[d]
O5 – Vetiver + manure	92.5	4.64	65.8±2.6[ab]	11.8±0.6[ab]	14.2±0.8[ab]	41.2±1.3[ab]
O6 – GG + manure	87.5	4.31	61.4±2.5[b]	11.1±0.5[b]	13.6±0.7[b]	38.6±1.2[b]

GP = germination percentage; ERI = emergence rate index [19]; WAS = weeks after sowing; SPAD = chlorophyll meter reading at 6 WAS. DMRT letters based on plant parameters at 8 WAS ($P \leq 0.05$).

3.7. Okra Yield Parameters

Yield parameters were significantly affected by soil treatment ($P \leq 0.05$) (Table 9). The polluted unremediated soil (O2) recorded delayed flowering (68.6 days), the lowest pod number (4.2 pods

plant^{-1}), shortest pods (7.8 cm), and lowest total fresh pod yield (22.7 g plant^{-1}). The unpolluted control (O1) produced the highest yield with 18.4 pods plant^{-1} and total yield of 342.2 g plant^{-1}. Among remediated soils, O5 produced the highest recovery, recording 17.8 pods plant^{-1} and total fresh pod yield of 318.6 g plant^{-1}, followed by O6 with 265.7 g plant^{-1}.

Table 9. Okra yield parameters across treatment soils (mean ± SE)

Treatment	Days to flower	Pods/plant	Pod length (cm)	Pod diam. (mm)	Pod wt (g)	Total yield (g/plant)
O1 – Unpolluted control	48.2±1.4^{a}	18.4±0.9^{a}	14.6±0.6^{a}	22.4±0.8^{a}	18.6±0.9^{a}	342.2±14.1^{a}
O2 – Polluted unremediated	68.6±3.2^{e}	4.2±0.4^{e}	7.8±0.5^{e}	12.6±0.6^{e}	5.4±0.4^{e}	22.7±4.2^{e}
O3 – Vetiver-remediated	54.4±1.8^{c}	13.6±0.7^{c}	12.4±0.5^{c}	18.8±0.7^{c}	14.2±0.7^{c}	193.1±10.4^{c}
O4 – GG-remediated	57.8±2.1^{d}	11.8±0.6^{d}	11.8±0.5^{d}	17.6±0.7^{d}	13.1±0.6^{d}	154.6±9.8^{d}
O5 – Vetiver + manure	49.6±1.5^{ab}	17.8±0.8^{ab}	14.2±0.6^{ab}	21.8±0.8^{ab}	17.9±0.8^{ab}	318.6±13.2^{ab}
O6 – GG + manure	51.4±1.6^{b}	16.2±0.8^{b}	13.6±0.6^{b}	20.4±0.8^{b}	16.4±0.8^{b}	265.7±12.1^{b}

3.8. Final Soil Properties After Okra Harvest

Final soil properties after okra cultivation differed among treatments (Table 10). The polluted unremediated soil (O2) maintained poor soil conditions, with pH of 5.04, total nitrogen of 0.06%, available phosphorus of 3.8 mg kg^{-1}, and the highest residual TPH concentration (35,400 mg kg^{-1}). The remediated soils showed improved recovery. The lowest residual TPH among remediated treatments was recorded in O5 (7,240 mg kg^{-1}), followed by O6 (9,610 mg kg^{-1}). The soil treated with Vetiver + manure (O5) also maintained improved fertility characteristics, with pH 6.34, total nitrogen 0.16%, and available phosphorus 11.1 mg kg^{-1}.

Table 10. Final soil physicochemical properties and TPH concentration after okra harvest across treatment soils

Treatment	pH	OC (%)	Total N (%)	Av. P (mg/kg)	Exch. K+ (cmol/kg)	Final TPH (mg/kg)
O1 – Unpolluted control	6.38	1.72	0.17	11.6	0.28	362
O2 – Polluted unremediated	5.04	4.88	0.06	3.8	0.11	35,400
O3 – Vetiver-remediated	6.02	2.88	0.13	9.4	0.22	12,180
O4 – GG-remediated	5.86	3.11	0.11	8.2	0.19	14,860
O5 – Vetiver + manure	6.34	2.44	0.16	11.1	0.27	7,240
O6 – GG + manure	6.22	2.68	0.15	10.4	0.24	9,610

OC = organic carbon; Av. P = available phosphorus; Exch. K+ = exchangeable potassium; TPH = total petroleum hydrocarbons. DMRT letters apply to final TPH column ($P \leq 0.05$).

4. Discussion

4.1. Effect of Crude Oil Contamination on Soil Physicochemical Properties

Crude oil contamination adversely affected soil physicochemical properties, as evidenced by the lower pH, total nitrogen, available phosphorus, and exchangeable potassium recorded in the polluted unremediated treatment (T2) compared with the unpolluted control (T1). The reduction in nutrient availability may be attributed to hydrocarbon-induced disruption of soil microbial processes responsible for nutrient mineralization and cycling. Petroleum hydrocarbons can create hydrophobic conditions that reduce soil aeration and nutrient mobility, thereby limiting nutrient availability to plants and microorganisms [1,2].

The elevated organic carbon content observed in the polluted soil reflects the accumulation of carbon-rich petroleum hydrocarbons. Similar increases in soil organic carbon following crude oil contamination have been reported by Ite and Ibok [14], who noted that petroleum-derived carbon may artificially increase measured soil carbon while simultaneously reducing soil fertility. The decline in soil pH observed in T2 may also be associated with the formation of acidic metabolites during hydrocarbon degradation and changes in soil chemical equilibrium resulting from petroleum contamination [29].

4.2. Phytoremediation Efficiency of *Vetiveria zizanioides* and *Panicum maximum*

Both *Vetiveria zizanioides* and *Panicum maximum* significantly enhanced petroleum hydrocarbon removal compared with the polluted unremediated treatment. After fourteen weeks, *Vetiveria zizanioides* reduced TPH concentration by 68.8%, whereas *Panicum maximum* achieved a reduction of 63.9%. The greater remediation efficiency observed in vetiver-treated soil may be attributed to its extensive root system and larger rhizosphere zone, which provide favourable conditions for microbial colonization and hydrocarbon degradation.

The higher hydrocarbon-utilizing bacterial population recorded in vetiver-treated soil (18.6×10^6 cfu g^{-1}) compared with Guinea grass-treated soil (15.2×10^6 cfu g^{-1}) supports the role of rhizosphere-mediated degradation in petroleum hydrocarbon removal. Vetiver has previously been reported to promote hydrocarbon degradation through root-induced stimulation of microbial activity and improved soil biological conditions [10,13,16].

Although Guinea grass exhibited slightly lower TPH removal efficiency, it demonstrated substantial remediation potential and maintained vigorous growth under petroleum stress conditions. This observation agrees with the findings of Merkl *et al.* [21] and Ologidi *et al.* [26], who reported the suitability of tropical grasses for remediation of hydrocarbon-contaminated soils.

4.3. Influence of Poultry Manure on Remediation Performance

The addition of poultry manure significantly enhanced phytoremediation efficiency in both grass species. *Vetiver* combined with poultry manure (T5) achieved the greatest TPH reduction (82.6%), while Guinea grass combined with poultry manure (T6) achieved 76.2% reduction. These values

were considerably higher than those recorded for the grass-only treatments.

The enhanced performance of manure-amended treatments is likely related to improved nutrient availability for microbial growth and metabolism. Petroleum hydrocarbon degradation is often limited by deficiencies in nitrogen and phosphorus, and the addition of organic amendments helps overcome these constraints by supplying essential nutrients required for microbial proliferation [6,29].

The highest populations of total heterotrophic bacteria, fungi, and hydrocarbon-utilizing bacteria were recorded in T5, indicating that poultry manure stimulated microbial activity and accelerated biodegradation processes. Similar findings have been reported by Oghoje *et al*. [25], who observed improved hydrocarbon degradation and microbial activity following poultry manure application in petroleum-contaminated soils.

4.4. Microbial Dynamics During Remediation

Crude oil contamination altered microbial population dynamics in the soil. The polluted unremediated treatment exhibited substantial reductions in total heterotrophic bacteria and fungi compared with the initial population levels. This decline indicates the toxic effect of petroleum hydrocarbons on sensitive microbial groups and the resulting disruption of normal soil biological functions [17]

In contrast, hydrocarbon-utilizing bacteria increased in contaminated soils because these microorganisms are capable of using petroleum hydrocarbons as carbon and energy sources. The increase in HUB population from 0.9×10^6 cfu g^{-1} at Week 0 to 3.4×10^6 cfu g^{-1} in the polluted control at Week 14 demonstrates the selective enrichment of hydrocarbon-degrading microorganisms under petroleum stress.

The considerably higher microbial populations observed in planted and manure-amended treatments indicate that phytoremediation promoted biological recovery of the contaminated soil. Root exudates produced by *Vetiveria zizanioides* and *Panicum maximum* likely stimulated microbial activity by supplying readily available carbon compounds and creating favourable rhizosphere conditions for microbial growth [18,32,33].

4.5. Growth Performance of Phytoremediation Grasses

Both grass species established successfully in contaminated soils and produced substantial biomass throughout the remediation period. Poultry manure significantly improved plant growth, resulting in increased plant height, tiller production, biomass accumulation, and root development.

Guinea grass combined with poultry manure produced the highest shoot growth and biomass, whereas Vetiver combined with poultry manure developed the longest root system. The extensive root development observed in Vetiver may partly explain its superior hydrocarbon removal efficiency because larger root systems increase rhizosphere volume and enhance plant–microbe interactions involved in hydrocarbon degradation [8,34].

The positive response of both grasses to manure amendment demonstrates that nutrient supplementation not only enhanced microbial activity but also improved plant establishment and growth under petroleum-contaminated conditions.

4.6. Residual Effects of Remediation on Okra Growth and Yield

The poor germination, vegetative growth, and yield recorded in the polluted unremediated soil demonstrate the adverse effects of residual petroleum hydrocarbons on crop productivity. Hydrocarbon contamination reduced germination percentage, chlorophyll content, plant growth, flowering, and pod yield, indicating persistent soil toxicity.

These findings agree with studies of Sagaya *et al.* [30] who reported growth inhibition and yield reduction in crops cultivated on petroleum-contaminated soils. The residual TPH concentration of 35,400 mg kg^{-1} recorded in O2 likely contributed to the poor performance of okra through restricted nutrient uptake, impaired root development, and reduced physiological activity.

In contrast, soils subjected to phytoremediation supported substantially better crop performance. Among the remediated treatments, O5 (Vetiver + manure) produced the highest germination percentage, vegetative growth, and yield, achieving 318.6 g $plant^{-1}$ compared with 342.2 g $plant^{-1}$ in the unpolluted control. This indicates that the integrated remediation strategy restored a large proportion of the soil's productive capacity.

The superior performance of O5 corresponds with its lower residual TPH concentration and improved soil fertility status. The results suggest that successful hydrocarbon removal, combined with nutrient restoration, created favourable conditions for crop establishment and productivity.

4.7. Final Soil Recovery Following Okra Cultivation

The final soil analysis demonstrated continued differences among treatments after okra harvest. The remediated soils maintained higher pH values, improved nutrient status, and lower residual TPH concentrations than the polluted unremediated soil.

The lowest residual TPH concentration among remediated treatments was recorded in O5 (7,240 mg kg^{-1}), indicating sustained remediation benefits beyond the initial phytoremediation phase. Improved nutrient availability and reduced hydrocarbon toxicity likely contributed to the enhanced crop performance observed in these treatments.

Although residual hydrocarbons remained detectable in all previously contaminated soils, the substantial reductions achieved indicate significant progress toward soil recovery. These findings support the use of integrated phytoremediation and organic amendment as a sustainable strategy for the restoration of petroleum-contaminated agricultural soils.

5. Conclusion

This study showed that crude oil contamination negatively affected soil properties, microbial populations, and okra productivity. Phytoremediation using *Vetiveria zizanioides* and *Panicum maximum* significantly reduced TPH levels, with greater efficiency when combined with poultry manure. The treatment improved microbial activity, nutrient recovery, and crop yield performance. The findings demonstrate that grass-based phytoremediation integrated with organic amendment is an effective and sustainable approach for restoring petroleum-contaminated agricultural soils.

Declaration of AI Use

This manuscript was prepared through the combined contributions of all author(s), including contributions to the study design, data, content development, results, interpretation, and related scholarly work. The author(s) acknowledge the use of ChatGPT to assist with grammar checking, language refinement, reference formatting. These AI-assisted tools were not used as authors and did not replace the intellectual contributions or scholarly judgment of the author(s). All AI-assisted outputs, including content, references, and interpretations, were carefully reviewed, revised, verified, and approved by the author(s). The author(s) accept full responsibility for the accuracy, integrity, and final content of the manuscript.

Conflict of Interest

The authors declare that they have no competing interests.

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