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Phytoremediation potential of *Hevea brasiliensis* saplings for heavy metal in crude oil contaminated soil

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Abstract
Crude oil contamination remains a major environmental challenge in oil-producing regions, causing profound alterations in soil quality and threatening the productivity of economically important crops such as *Hevea brasiliensis*. This study evaluated the effects of varying crude oil contamination levels (0, 1, 3, and 5% w/w) on soil physicochemical properties, elemental composition, hydrocarbon accumulation, and the biochemical characteristics of rubber saplings cultivated for 26 weeks. Soil physicochemical parameters, inductively coupled plasma–optical emission spectrometry (ICP–OES), Fourier transform infrared spectroscopy (FTIR), and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDS) were employed to characterize contamination-induced changes. Increasing crude oil concentration significantly ($p < 0.05$) acidified the soil (pH 6.85–5.51), elevated total petroleum hydrocarbons (45.2–6,920 mg/kg) and electrical conductivity (182.4–402.8 µS/cm), while reducing nitrogen, available phosphorus, moisture content, cation exchange capacity, and essential exchangeable cations. ICP–OES revealed progressive accumulation of Fe, Zn, Cu, Pb, Cd, Cr, Ni, V, As, and Na in both soils and plant tissues, whereas Ca, Mg, K, and Mn declined significantly with increasing contamination. FTIR spectra demonstrated progressive enrichment of aliphatic hydrocarbon functional groups and shifts in hydroxyl- and carbohydrate-associated bands, indicating biochemical modification of plant tissues. SEM–EDS analysis further confirmed contamination-induced structural deterioration and alterations in elemental distribution within rubber saplings exposed to higher crude oil concentrations. Collectively, the findings demonstrate that crude oil contamination substantially degrades soil quality, promotes uptake of potentially toxic elements, and induces marked biochemical and structural changes in *H. brasiliensis*. These results provide important insights into the mechanisms of crude oil toxicity in rubber cultivation and offer a scientific basis for developing sustainable remediation strategies for contaminated plantation soils.

Keywords: *Hevea brasiliensis*, crude oil contamination, soil physicochemical properties, ICP–OES, FTIR, phytotoxicity

Introduction

Crude oil contamination is one of the most persistent environmental problems affecting terrestrial ecosystems in petroleum-producing regions worldwide. The exploration, production, transportation, and refining of petroleum frequently result in accidental spills that introduce complex mixtures of hydrocarbons and associated heavy metals into agricultural soils (Adeyemi, & Akpomedaye, 2026) [1]. These contaminants profoundly alter soil physicochemical characteristics, disrupt microbial activity, reduce nutrient availability, and impair plant growth, ultimately threatening agricultural productivity, ecosystem stability, and food security. In oil-producing regions such as the Niger Delta of Nigeria, repeated crude oil spills have resulted in long-term degradation of cultivated lands, including plantations of economically important perennial crops such as *Hevea brasiliensis* (Bahar *et al*., 2024; Okoye *et al*., 2024[9, 37].

Although petroleum hydrocarbons are often regarded as the primary toxic constituents of crude oil, increasing evidence indicates that petroleum-associated heavy metals constitute an equally important source of environmental concern. Crude oil contains appreciable concentrations of potentially toxic elements including iron (Fe), lead (Pb), cadmium (Cd), chromium (Cr), nickel (Ni), vanadium (V), arsenic (As), copper (Cu), and zinc (Zn), many of which persist in soils long after hydrocarbon degradation has occurred. Unlike petroleum hydrocarbons, which may gradually undergo microbial degradation, heavy metals are non-biodegradable and may accumulate within soils, enter plant tissues, and subsequently transfer through terrestrial food webs. Their persistence not only prolongs ecological degradation but also increases the risks of phytotoxicity, biodiversity loss, groundwater contamination, and human exposure through agricultural products (Nwawuike *et al*., 2023; Oghenerukevwe *et al*., 2026) [34, 35].

Heavy metal contamination adversely affects virtually every aspect of plant physiology. Elevated concentrations interfere with nutrient uptake, disturb cellular ion homeostasis, inhibit photosynthesis, impair enzymatic activities, stimulate excessive production of reactive oxygen species (ROS), and induce oxidative damage to proteins, lipids, nucleic acids, and cellular membranes. These biochemical disturbances ultimately manifest as reduced chlorophyll synthesis, impaired vegetative growth, decreased biomass accumulation, altered water relations, and disruption of normal metabolic processes. Consequently, remediation strategies for crude oil-contaminated soils should address not only hydrocarbon degradation but also the immobilization, extraction, or detoxification of associated heavy metals ((Adeyemi & Otugboyega, 2025) [3].

Conventional remediation technologies, including soil excavation, thermal treatment, chemical oxidation, and soil washing, have demonstrated effectiveness in removing contaminants but are frequently constrained by high operational costs, disturbance of soil structure, generation of secondary pollutants, and limited applicability over large contaminated landscapes (Sut-Lohmann *et al*., 2023). These limitations have stimulated increasing interest in phytoremediation as an environmentally sustainable and cost-effective alternative for restoring contaminated ecosystems. Phytoremediation exploits the natural capacity of plants and their associated rhizosphere microorganisms to extract, accumulate, stabilize, transform, or degrade environmental contaminants while simultaneously improving soil quality and ecological function. Depending on plant species and contaminant characteristics, phytoremediation may occur through phytoextraction, phytostabilization, phytodegradation, phytovolatilization, or rhizodegradation (Javeed *et al*., 2024) [24].

Successful phytoremediation depends largely on the selection of plant species capable of tolerating contaminant stress while maintaining sufficient biomass production and contaminant uptake. Plants possessing extensive root systems, high adaptability to adverse environmental conditions, rapid growth, and efficient metal accumulation are generally considered promising candidates for remediation programs. In addition to contaminant removal, such species contribute to soil stabilization, enhancement of microbial activity, and gradual restoration of ecosystem functions (Anjitha *et al*., 2025) [8].

Hevea brasiliensis (rubber tree) is one of the world's most economically valuable plantation crops because of its production of natural rubber. The species is extensively cultivated throughout tropical regions, including the Niger Delta, where commercial rubber plantations frequently coexist with petroleum exploration and production activities. This geographic overlap exposes young rubber plantations to repeated episodes of crude oil contamination, potentially compromising plantation establishment and long-term productivity. Despite its economic importance, relatively little attention has been devoted to evaluating the capacity of *H. brasiliensis* to remediate petroleum-associated heavy metals while simultaneously tolerating contaminated soils. Most previous investigations have focused primarily on growth inhibition, hydrocarbon toxicity, or general physiological responses, whereas information concerning heavy metal uptake and phytoremediation potential remains limited (Golomb *et al*., 2021; Zhang *et al*., 2023) [17, 51].

The present study was designed to address this knowledge gap by comprehensively evaluating the phytoremediation potential of *Hevea brasiliensis* saplings grown in crude oil-contaminated soil. Specifically, the study investigated changes in soil physicochemical properties, heavy metal concentrations in soil and plant tissues, elemental redistribution, and associated biochemical and structural responses of the saplings following exposure to graded crude oil contamination. The underlying study demonstrated that crude oil contamination altered soil properties, promoted the accumulation of petroleum-associated elements in roots, stems, and leaves, and activated physiological defense mechanisms that indicate measurable tolerance to moderate contamination, supporting the potential application of *H. brasiliensis* in phytoremediation of contaminated soils.

By integrating soil chemistry, elemental analysis, plant biochemistry, and structural characterization, this study provides new insights into the role of *Hevea brasiliensis* as a candidate species for heavy metal phytoremediation in crude oil-contaminated soils. The findings contribute to the growing body of knowledge on sustainable remediation technologies and provide a scientific basis for the restoration and management of petroleum-impacted plantation ecosystems.

Materials and Methods

Study Design

A laboratory-controlled pot experiment was conducted to evaluate the phytoremediation potential of *Hevea brasiliensis* saplings grown in crude oil-contaminated soil. The experiment lasted 26 weeks under controlled environmental conditions.

Sample Collection and Preparation

Bonny Light crude oil was obtained from the Warri Petrochemical Refinery, Effurun, Delta State, Nigeria. Healthy *Hevea brasiliensis* saplings (improved clone) were procured from the Nursery Unit of the Rubber Research Institute of Nigeria (RRIN), Benin City, Edo State, Nigeria.

Experimental Treatments

Four treatment groups were established based on crude oil concentration in soil:

- **T1 (Control):** Uncontaminated soil (0% crude oil)
- **T2:** Soil contaminated with 1% (w/w) crude oil
- **T3:** Soil contaminated with 3% (w/w) crude oil
- **T4:** Soil contaminated with 5% (w/w) crude oil

Each treatment was replicated three times to ensure statistical reliability.

Sampling Schedule

Baseline soil and sapling samples were collected before contamination to establish initial conditions. Periodic measurements were conducted during the experimental period, while comprehensive biochemical, structural, and elemental analyses were performed at the end of the experiment.

Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) was performed on plant tissues to identify functional groups and evaluate chemical alterations associated with crude oil exposure.

Heavy Metal Determination

Heavy metal concentrations in both soil and plant samples were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP–OES). The analysis was conducted to quantify elemental concentrations and assess heavy metal uptake and bioaccumulation by *Hevea brasiliensis* saplings.

Scanning Electron Microscopy

Structural changes induced by crude oil contamination were examined using Scanning Electron Microscopy (SEM). Root and leaf samples were prepared for microscopic examination to evaluate morphological alterations resulting from contaminant exposure.

Soil Physicochemical Analysis

Soil physicochemical properties, including pH, organic matter content, and nutrient status, were determined.

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) to determine significant differences among treatment groups. Where significant differences were observed, Tukey's Honestly Significant Difference (HSD) test was applied for multiple comparisons. Statistical significance was accepted at $p < 0.05$.

Results

Physicochemical Characteristics of Soil

The physicochemical properties of soil following crude oil contamination are presented in Table 1. Soil pH decreased progressively with increasing crude oil concentration, declining significantly ($p < 0.05$) from 6.85 ± 0.08 in the control (T1) to 5.51 ± 0.05 in the 5% crude oil treatment (T4). Conversely, electrical conductivity increased significantly across the treatments, rising from 182.4 ± 5.6 µS/cm in the control to 402.8 ± 8.3 µS/cm at the highest contamination level.

Organic carbon content exhibited a significant concentration-dependent increase, with values ranging from 1.82 ± 0.09% in the control to 6.21 ± 0.18% in T4. In contrast, total nitrogen and available phosphorus decreased significantly with increasing contamination. Total nitrogen declined from 0.15 ± 0.01% in the control to 0.06 ± 0.01% in T4, while available phosphorus decreased from 18.6 ± 0.9 mg/kg to 7.2 ± 0.5 mg/kg.

Total petroleum hydrocarbon (TPH) concentrations increased markedly with contamination level, from 45.2 ± 6.5 mg/kg in uncontaminated soil to 6,920 ± 210 mg/kg in the 5% crude oil treatment. Moisture content also declined significantly from 12.4 ± 0.6% in the control to 7.6 ± 0.3% in T4.

Bulk density increased progressively from 1.32 ± 0.03 g/cm³ in the control to 1.52 ± 0.04 g/cm³ under the highest crude oil concentration. Similarly, cation exchange capacity decreased significantly from 12.8 ± 0.7 cmol/kg in the control to 8.1 ± 0.4 cmol/kg in T4.

Exchangeable calcium, magnesium, and potassium all declined significantly with increasing crude oil concentration. Exchangeable calcium decreased from 4.85 ± 0.21 cmol/kg to 2.41 ± 0.14 cmol/kg, magnesium from 2.14 ± 0.12 cmol/kg to 1.09 ± 0.07 cmol/kg, and potassium from 0.42 ± 0.03 cmol/kg to 0.21 ± 0.01 cmol/kg. In contrast, exchangeable sodium increased significantly from 0.31 ± 0.02 cmol/kg in the control to 0.72 ± 0.05 cmol/kg in the highest contamination treatment. Different superscript letters within each row indicate significant differences among treatments ($p < 0.05$).

Table 1: Physicochemical Characteristics of Soil Samples (Mean ± SEM)

Parameter	T1 (Control, 0%)	T2 (1% w/w)	T3 (3% w/w)	T4 (5% w/w)
pH	6.85 ± 0.08^a	6.42 ± 0.06^b	5.98 ± 0.07^c	5.51 ± 0.05^d
Electrical Conductivity (µS/cm)	182.4 ± 5.6^a	245.7 ± 6.2^b	318.5 ± 7.1^c	402.8 ± 8.3^d
Organic Carbon (%)	1.82 ± 0.09^d	2.95 ± 0.11^c	4.63 ± 0.15^b	6.21 ± 0.18^a
Total Nitrogen (%)	0.15 ± 0.01^a	0.12 ± 0.01^b	0.09 ± 0.01^c	0.06 ± 0.01^d
Available Phosphorus (mg/kg)	18.6 ± 0.9^a	14.3 ± 0.7^b	10.8 ± 0.6^c	7.2 ± 0.5^d
Total Petroleum Hydrocarbon (mg/kg)	45.2 ± 6.5^d	1,250 ± 85^c	3,480 ± 120^b	6,920 ± 210^a
Moisture Content (%)	12.4 ± 0.6^a	10.8 ± 0.5^b	9.1 ± 0.4^c	7.6 ± 0.3^d
Bulk Density (g/cm³)	1.32 ± 0.03^d	1.38 ± 0.04^c	1.45 ± 0.05^b	1.52 ± 0.04^a
Cation Exchange Capacity (cmol/kg)	12.8 ± 0.7^a	11.2 ± 0.6^b	9.5 ± 0.5^c	8.1 ± 0.4^d
Exchangeable Ca (cmol/kg)	4.85 ± 0.21^a	3.92 ± 0.18^b	3.05 ± 0.16^c	2.41 ± 0.14^d
Exchangeable Mg (cmol/kg)	2.14 ± 0.12^a	1.78 ± 0.10^b	1.42 ± 0.08^c	1.09 ± 0.07^d
Exchangeable K (cmol/kg)	0.42 ± 0.03^a	0.35 ± 0.02^b	0.28 ± 0.02^c	0.21 ± 0.01^d
Exchangeable Na (cmol/kg)	0.31 ± 0.02^d	0.44 ± 0.03^c	0.58 ± 0.04^b	0.72 ± 0.05^a

ICP–OES Determined Elemental Composition of Soil

The elemental composition of soils following crude oil contamination is presented in Table 2. The concentrations of several potentially toxic elements increased significantly ($p < 0.05$) with increasing crude oil contamination. Iron (Fe) increased from 2,145 ± 85 mg/kg in the control to 3,520 ± 125 mg/kg in T4. Similar concentration-dependent increases were observed for zinc (42.6 ± 2.1–95.4 ± 3.9 mg/kg), copper (18.2 ± 1.0–46.8 ± 2.0 mg/kg), lead (6.5 ± 0.4–33.2 ± 1.5 mg/kg), cadmium (0.42 ± 0.03–2.18 ± 0.09 mg/kg), chromium (9.8 ± 0.6–31.5 ± 1.3 mg/kg), nickel (7.3 ± 0.5–29.6 ± 1.2 mg/kg), vanadium (3.2 ± 0.2–17.9 ± 0.9 mg/kg), arsenic (1.8 ± 0.1–8.6 ± 0.4 mg/kg), and sodium (145 ± 8–275 ± 14 mg/kg).

Conversely, essential nutrient elements generally decreased with increasing crude oil concentration. Manganese declined significantly from 112.5 ± 5.4 mg/kg in the control to 86.3 ± 3.9 mg/kg in T4. Calcium decreased from 1,285 ± 60 mg/kg to 780 ± 41 mg/kg, magnesium from 485 ± 22 mg/kg to 298 ± 15 mg/kg, and potassium from 365 ± 18 mg/kg to 198 ± 12 mg/kg across the treatment groups.

Table 2: ICP–OES Determined Elemental Composition of Soil Samples (mg/kg, Mean ± SEM)

Element	T1 (Control, 0%)	T2 (1% w/w)	T3 (3% w/w)	T4 (5% w/w)
Fe	2,145 ± 85^d	2,480 ± 92^c	2,965 ± 110^b	3,520 ± 125^a
Zn	42.6 ± 2.1^d	58.3 ± 2.8^c	76.5 ± 3.2^b	95.4 ± 3.9^a
Cu	18.2 ± 1.0^d	25.7 ± 1.3^c	34.9 ± 1.6^b	46.8 ± 2.0^a
Pb	6.5 ± 0.4^d	12.8 ± 0.7^c	21.6 ± 1.1^b	33.2 ± 1.5^a
Cd	0.42 ± 0.03^d	0.88 ± 0.05^c	1.46 ± 0.07^b	2.18 ± 0.09^a
Cr	9.8 ± 0.6^d	15.4 ± 0.8^c	22.7 ± 1.0^b	31.5 ± 1.3^a
Ni	7.3 ± 0.5^d	13.2 ± 0.7^c	20.8 ± 0.9^b	29.6 ± 1.2^a

Mn	112.5 ± 5.4[a]	104.2 ± 4.8[b]	95.6 ± 4.2[c]	86.3 ± 3.9[d]
Ca	1,285 ± 60[a]	1,120 ± 52[b]	945 ± 48[c]	780 ± 41[d]
Mg	485 ± 22[a]	420 ± 20[b]	360 ± 18[c]	298 ± 15[d]
K	365 ± 18[a]	310 ± 16[b]	255 ± 14[c]	198 ± 12[d]
Na	145 ± 8[d]	185 ± 10[c]	228 ± 12[b]	275 ± 14[a]
V	3.2 ± 0.2[d]	6.8 ± 0.4[c]	11.5 ± 0.6[b]	17.9 ± 0.9[a]
As	1.8 ± 0.1[d]	3.5 ± 0.2[c]	5.9 ± 0.3[b]	8.6 ± 0.4[a]

ICP–OES Determined Elemental Concentrations in Hevea brasiliensis Tissues

The elemental concentrations in *Hevea brasiliensis* tissues after 26 weeks of exposure are presented in Table 3. Accumulation of several heavy metals increased significantly ($p < 0.05$) with increasing crude oil contamination. Iron concentrations increased from 185.4 ± 7.8 mg/kg in the control to 402.3 ± 13.8 mg/kg in T4. Similar trends were observed for zinc (28.6 ± 1.4–61.5 ± 2.6 mg/kg), copper (9.8 ± 0.5–25.4 ± 1.2 mg/kg), lead (1.25 ± 0.08–12.4 ± 0.52 mg/kg), cadmium (0.18 ± 0.01–1.35 ± 0.06 mg/kg), chromium (2.95 ± 0.14–14.2 ± 0.58 mg/kg), nickel (2.14 ± 0.11–12.7 ± 0.51 mg/kg), vanadium (0.85 ± 0.04–6.12 ± 0.25 mg/kg), arsenic (0.62 ± 0.03–4.75 ± 0.19 mg/kg), and sodium (95.4 ± 4.6–198.7 ± 7.9 mg/kg).

In contrast, the concentrations of several essential nutrients declined significantly with increasing crude oil concentration. Manganese decreased from 65.3 ± 2.9 mg/kg in the control to 44.2 ± 2.0 mg/kg in T4. Calcium declined from 1,125 ± 48 mg/kg to 710 ± 35 mg/kg, magnesium from 365 ± 18 mg/kg to 235 ± 12 mg/kg, and potassium from 825 ± 36 mg/kg to 592 ± 27 mg/kg.

The pattern of elemental accumulation in plant tissues closely reflected the corresponding changes observed in contaminated soils. Increasing crude oil concentration was associated with progressive accumulation of potentially toxic elements and sodium in *H. brasiliensis* tissues, while concentrations of essential nutrient elements decreased significantly. All measured parameters exhibited statistically significant differences among treatments as indicated by the differing superscript letters ($p < 0.05$).

Table 3: ICP–OES Determined Elemental Concentrations in Rubber Plant Tissues after 26 Weeks (mg/kg dry weight, Mean ± SEM)

Element	T1 (Control, 0%)	T2 (1% w/w)	T3 (3% w/w)	T4 (5% w/w)
Fe	185.4 ± 7.8[d]	245.7 ± 9.2[c]	318.6 ± 11.5[b]	402.3 ± 13.8[a]
Zn	28.6 ± 1.4[d]	36.9 ± 1.8[c]	48.7 ± 2.1[b]	61.5 ± 2.6[a]
Cu	9.8 ± 0.5[d]	13.6 ± 0.7[c]	18.9 ± 0.9[b]	25.4 ± 1.2[a]
Pb	1.25 ± 0.08[d]	3.85 ± 0.18[c]	7.62 ± 0.31[b]	12.4 ± 0.52[a]
Cd	0.18 ± 0.01[d]	0.42 ± 0.02[c]	0.86 ± 0.04[b]	1.35 ± 0.06[a]
Cr	2.95 ± 0.14[d]	5.72 ± 0.26[c]	9.48 ± 0.39[b]	14.2 ± 0.58[a]
Ni	2.14 ± 0.11[d]	4.86 ± 0.21[c]	8.15 ± 0.34[b]	12.7 ± 0.51[a]
Mn	65.3 ± 2.9[a]	58.7 ± 2.6[b]	51.4 ± 2.3[c]	44.2 ± 2.0[d]
Ca	1,125 ± 48[a]	980 ± 42[b]	845 ± 39[c]	710 ± 35[d]
Mg	365 ± 18[a]	318 ± 16[b]	276 ± 14[c]	235 ± 12[d]
K	825 ± 36[a]	745 ± 32[b]	668 ± 29[c]	592 ± 27[d]
Na	95.4 ± 4.6[d]	128.6 ± 5.8[c]	162.3 ± 6.7[b]	198.7 ± 7.9[a]
V	0.85 ± 0.04[d]	1.96 ± 0.09[c]	3.84 ± 0.17[b]	6.12 ± 0.25[a]
As	0.62 ± 0.03[d]	1.48 ± 0.07[c]	2.96 ± 0.12[b]	4.75 ± 0.19[a]

SEM–EDS Elemental Composition of Dried Hevea brasiliensis Saplings

The elemental composition of dried Hevea brasiliensis saplings determined by scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDS) is presented in Table 4. Variations in elemental composition were observed among the treatment groups following 26 weeks of cultivation in crude oil-contaminated soils.

Carbon was the predominant element detected in all treatments. Its weight percentage increased from 53.15% in the control (T1) to 76.70% in the 1% crude oil treatment (T2), remained relatively high at 72.03% in the 3% treatment (T3), and reached 76.67% in the 5% crude oil treatment (T4).

Potassium showed a marked reduction with crude oil contamination, decreasing from 18.52% in the control to 5.10%, 6.26%, and 3.63% in T2, T3, and T4, respectively. Calcium also declined from 10.11% in the control to between 6.11% and 6.84% across the contaminated treatments. Similarly, phosphorus decreased substantially from 7.54% in the control to 0.82% in T2, followed by slight increases to 1.41% and 2.73% in T3 and T4, respectively.

Iron was detected at 6.51% in the control, decreased to 2.62% in T2, increased to 5.28% in T3, and was not detected in the highest contamination treatment (T4). Silicon remained detectable across all treatments, varying from 2.93% in the control to 4.46%, 3.88%, and 3.57% in T2, T3, and T4, respectively.

Chlorine was detected only in the control treatment, with a weight percentage of 1.23%, and was not detected in any of the contaminated treatments. Titanium and aluminium were absent in the control but were detected in all crude oil-treated saplings. Titanium occurred at 1.64%, 0.71%, and 0.91% in T2, T3, and T4, respectively, while aluminium was present at 1.54%, 1.48%, and 0.98% across the corresponding treatments.

Sulfur was not detected in the control but appeared in all contaminated treatments, with values of 0.67% in both T2 and T3 and 1.09% in T4. Magnesium was detected at low concentrations in contaminated samples, occurring at 0.35% in T2 and increasing to 1.12% in T4, but was not detected in the control or T3.

Vanadium was absent in both the control and the 1% crude oil treatment but was detected at 1.99% and 2.18% in T3 and T4, respectively.

Sodium was detected only in the highest contamination treatment at 0.27%, whereas manganese was not detected in any of the treatment groups.

Table 4: SEM–EDS Elemental Composition of Dried Rubber Saplings Cultivated in Crude Oil-Contaminated Soils for 26 Weeks (Weight %)

Element	Symbol	T1 (Control)	T2 (1% Crude Oil)	T3 (3% Crude Oil)	T4 (5% Crude Oil)
Carbon	C	53.15	76.70	72.03	76.67
Potassium	K	18.52	5.10	6.26	3.63
Calcium	Ca	10.11	6.11	6.31	6.84
Phosphorus	P	7.54	0.82	1.41	2.73
Iron	Fe	6.51	2.62	5.28	ND
Silicon	Si	2.93	4.46	3.88	3.57
Chlorine	Cl	1.23	ND	ND	ND
Titanium	Ti	ND	1.64	0.71	0.91
Aluminium	Al	ND	1.54	1.48	0.98
Sulfur	S	ND	0.67	0.67	1.09
Magnesium	Mg	ND	0.35	ND	1.12
Vanadium	V	ND	ND	1.99	2.18
Sodium	Na	ND	ND	ND	0.27
Manganese	Mn	ND	ND	ND	ND

ND = Not Detected.

Tables 3 and 4 provide complementary information on elemental accumulation in *Hevea brasiliensis* but are derived from different analytical techniques. Table 3 presents quantitative elemental concentrations determined by ICP–OES (mg/kg dry weight), whereas Table 7 reports surface elemental composition obtained by SEM–EDS as weight percentages (%). Although the units differ and direct numerical comparison is not appropriate, both datasets exhibit consistent trends in elemental changes following crude oil contamination.

Carbon showed the greatest increase in SEM–EDS analysis, rising from 53.15% in the control to approximately 77% in contaminated treatments. This trend corresponds with the increased petroleum-derived carbon associated with crude oil exposure but was not measured by ICP–OES because carbon is not routinely quantified by this technique.

Both datasets demonstrated a progressive enrichment of several heavy metals with increasing crude oil concentration. ICP–OES showed concentration-dependent increases in zinc, copper, lead, cadmium, chromium, nickel, vanadium, arsenic, and sodium within plant tissues. SEM–EDS similarly detected the appearance or increased surface abundance of vanadium and sodium in contaminated saplings, with vanadium absent in the control but present at 1.99% and 2.18% in the 3% and 5% treatments, respectively, while sodium was detected only in the highest contamination treatment.

The two techniques also consistently demonstrated depletion of essential plant nutrients following crude oil exposure. ICP–OES revealed progressive reductions in calcium (1,125–710 mg/kg), magnesium (365–235 mg/kg), potassium (825–592 mg/kg), and manganese (65.3–44.2 mg/kg). Likewise, SEM–EDS showed marked decreases in potassium (18.52–3.63 wt%), calcium (10.11–6.84 wt%), and phosphorus (7.54–2.73 wt%) across contaminated treatments, indicating deterioration of the mineral nutritional status of the saplings.

Some differences between the datasets reflect the analytical principles of the two techniques rather than contradictory findings. Iron concentrations determined by ICP–OES increased substantially with contamination, from 185.4 to 402.3 mg/kg, indicating greater total accumulation within plant tissues. In contrast, SEM–EDS showed lower surface abundance of iron, declining from 6.51 wt% in the control to non-detectable levels in the highest contamination treatment. This disparity is expected because ICP–OES measures total elemental content after complete sample digestion, whereas SEM–EDS analyzes only the elemental composition of the sample surface.

Similarly, magnesium was consistently quantified by ICP–OES in all treatments but was detected by SEM–EDS only in the contaminated samples and at relatively low surface percentages. Manganese exhibited decreasing concentrations in the ICP–OES analysis but was not detected by SEM–EDS, suggesting that its surface abundance was below the detection limit of the SEM–EDS instrument.

SEM–EDS additionally identified elements such as silicon, aluminium, titanium, sulfur, and chlorine that were either not included or not quantified in the ICP–OES dataset. Chlorine was detected only in the control sample, whereas aluminium, titanium, sulfur, and vanadium appeared only after crude oil contamination, indicating contamination-induced changes in surface elemental composition.

Fourier Transform Infrared Spectral Characteristics of Hevea brasiliensis Saplings

The Fourier Transform Infrared (FTIR) spectral characteristics of dried *Hevea brasiliensis* saplings cultivated in crude oil-contaminated soils for 26 weeks are presented in Table 5. Distinct absorption bands corresponding to hydroxyl, aliphatic hydrocarbon, aromatic, carbohydrate, and inorganic functional groups were observed across all treatments, with noticeable variations in peak positions and spectral composition as crude oil contamination increased.

The control treatment (T1) was characterized by a broad hydroxyl (O–H) stretching band at 3554.69 cm^{-1}, together with prominent absorption peaks at 1638.54 cm^{-1} assigned to aromatic C=C stretching or absorbed water bending, 1416.55 cm^{-1} corresponding to C–H bending, 1099.32 cm^{-1} attributed to C–O stretching of polysaccharides, and 561.23 cm^{-1} associated with C–Cl or metal–oxygen stretching vibrations.

Exposure to 1% crude oil contamination (T2) resulted in the appearance of additional absorption bands. A free hydroxyl stretching vibration was observed at 3842.84 cm^{-1}, accompanied by another O–H stretching peak at 3571.50 cm^{-1}. New aliphatic hydrocarbon bands appeared at 2926.58 cm^{-1}, while an absorption band at 2024[24].87 cm^{-1} corresponding to C≡C stretching or aromatic overtones was detected. The aromatic C=C stretching band shifted slightly to 1634.36 cm^{-1}, whereas carbohydrate-associated C–O stretching was recorded at 1104.43 cm^{-1}. The low-frequency region (694.39–404.84 cm^{-1}) exhibited multiple absorption bands attributed to C–Cl, C–Br, and metal–oxygen stretching vibrations.

In saplings grown under 3% crude oil contamination (T3), the hydroxyl stretching vibration occurred at 3606.38 cm^{-1}, while the petroleum-associated overtone at 2024[23].29 cm^{-1} remained evident. Aromatic C=C stretching was observed at 1638.45 cm^{-1}, accompanied by a distinct CH_2 bending vibration at 1476.67 cm^{-1}, indicative of aliphatic hydrocarbon chains. The C–O stretching vibration associated with polysaccharides occurred at 1092.45 cm^{-1}, while absorption bands between 639.08 and 406.93 cm^{-1} were assigned to halogenated compounds and metal–oxygen interactions.

The highest contamination treatment (T4) exhibited a hydroxyl stretching band at 3507.49 cm^{-1} together with two prominent aliphatic C–H stretching vibrations at 2926.71 cm^{-1} and 2854.51 cm^{-1}, corresponding to asymmetric and symmetric stretching of long-chain hydrocarbons, respectively. Aromatic C=C stretching was observed at 1647.75 cm^{-1}, while C–H bending and C–O stretching bands occurred at 1417.31 cm^{-1} and 1099.41 cm^{-1}, respectively. The lower spectral region (617.55–409.09 cm^{-1}) contained absorption bands assigned to C–Cl, C–Br, and metal–oxygen stretching vibrations.

Table 5: FTIR Spectral Characteristics of Dried Rubber Saplings Cultivated in Crude Oil-Contaminated Soils for 26 Weeks

Treatment	Peak Position (cm^{-1})	Assigned Functional Group	Vibrational Mode	Possible Biochemical Constituents
T1 (Control Soil)	3554.69	O–H	Stretching	Phenolics, alcohols, carbohydrates
	1638.54	C=C / H–O–H	Aromatic stretching / water bending	Lignin, absorbed moisture
	1416.55	C–H	Bending	Cellulose, hemicellulose
	1099.32	C–O	Stretching	Polysaccharides, cellulose
	561.23	C–Cl / Metal–O	Stretching	Mineral residues, inorganic components
T2 (1% Crude Oil Contamination)	3842.84	Free O–H	Stretching	Alcoholic and phenolic compounds
	3571.50	O–H	Stretching	Hydroxyl-containing metabolites
	2926.58	C–H	Asymmetric stretching	Aliphatic hydrocarbons, lipids
	2024[24].87	C≡C / Aromatic overtone	Stretching	Hydrocarbon-derived compounds
	1634.36	C=C	Stretching	Aromatic structures, lignin
	1104.43	C–O	Stretching	Carbohydrates
	694.39–404.84	C–Cl, C–Br, Metal–O	Stretching	Inorganic constituents and petroleum residues
T3 (3% Crude Oil Contamination)	3606.38	O–H	Stretching	Phenolics and alcohols
	2024[24].29	C≡C / Aromatic overtone	Stretching	Petroleum hydrocarbon derivatives
	1638.45	C=C	Stretching	Aromatic compounds
	1476.67	CH_2	Bending	Lipids and hydrocarbon chains
	1092.45	C–O	Stretching	Polysaccharides
	639.08–406.93	C–Cl, C–Br, Metal–O	Stretching	Petroleum-associated residues
T4 (5% Crude Oil Contamination)	3507.49	O–H	Stretching	Phenolic compounds and alcohols
	2926.71	C–H	Asymmetric stretching	Aliphatic hydrocarbons
	2854.51	C–H	Symmetric stretching	Long-chain alkanes, lipids
	1647.75	C=C	Stretching	Aromatic compounds
	1417.31	C–H	Bending	Cell wall polysaccharides
	1099.41	C–O	Stretching	Cellulose and carbohydrates
	617.55–409.09	C–Cl, C–Br, Metal–O	Stretching	Petroleum-derived and inorganic residues

Scanning Electron Microscopy Morphological Characteristics of Hevea brasiliensis Saplings

The scanning electron microscopy (SEM) observations of dried Hevea brasiliensis saplings cultivated in crude oil-contaminated soils for 26 weeks are presented in Table 6. Progressive morphological alterations were observed with increasing crude oil concentration across all magnifications.

In the control treatment (T1), examination at 500× magnification revealed compactly aggregated plant tissues with preserved cellular architecture and an intact fibrous matrix. The tissue surface appeared relatively smooth with limited pores and few particulate deposits. At 1000× magnification, a dense cellulose-rich fibrillar network composed of closely packed tissue fragments was observed, exhibiting slight surface roughness and minor natural voids. Examination at 2000× magnification showed well-organized fibrous bundles embedded within a compact matrix characterized by low porosity and the absence of visible erosion or fractures.

Saplings exposed to 1% crude oil contamination (T2) exhibited noticeable morphological modifications. At 500× magnification, fragmented fibrous structures with increased particulate attachment were evident, accompanied by moderate surface roughness and pore formation. At 1000× magnification, fibre bundles coated with granular deposits and fragmented cell wall residues produced a more irregular surface with increased porosity. At 2000× magnification, extensive particulate accumulation on fibres and exposed vascular elements became apparent, while the tissue surface displayed heterogeneous roughness and numerous microvoids.

More pronounced structural alterations were observed in saplings grown under 3% crude oil contamination (T3). At 500× magnification, extensive fibre exposure and fragmented vascular tissues were associated with roughened surfaces containing conspicuous cracks and particulate deposits. Examination at 1000× magnification revealed disruption of the fibrillar arrangement together with partial collapse of the tissue matrix, resulting in increased fissures and surface irregularities. At 2000× magnification, fibre surfaces appeared severely eroded with disruption of cellular organization, accompanied by marked surface roughness and localized collapse of structural components.

The highest crude oil concentration (T4) produced the greatest degree of morphological alteration. At 500× magnification, tissues exhibited extensive fragmentation of fibrous structures with loss of compact organization and the development of highly irregular, porous surfaces. At 1000× magnification, vascular bundles appeared disintegrated with numerous broken fibre fragments, extensive cracking, and pronounced structural disorganization. Examination at 2000× magnification revealed a highly disrupted tissue matrix characterized by collapsed fibres, enlarged voids, extensive porosity, increased surface roughness, and widespread fragmentation throughout the observed microstructure.

Table 6: SEM Morphological Characteristics of Dried Rubber Saplings Cultivated in Crude Oil-Contaminated Soils for 26 Weeks

Treatment	Magnification	Morphological Features Observed	Surface Characteristics	Interpretation
T1 (Control Soil)	500×	Compact aggregation of plant tissues with preserved cellular architecture and intact fibrous matrix	Relatively smooth surface with limited pores and few particulate deposits	Indicates healthy tissue integrity and normal lignocellulosic organization with minimal structural degradation
	1000×	Dense cellulose-rich fibrillar network with closely packed tissue fragments	Slightly rough surface; minor natural voids observed	Suggests preserved cell wall structure and stable biomass composition
	2000×	Well-organized fibrous bundles and compact matrix	Low porosity and absence of visible erosion or fractures	Reflects normal physiological development and structural stability
T2 (1% Crude Oil Contamination)	500×	Fragmented fibrous structures with increased particulate attachment	Moderate surface roughness and pore formation	Indicates initial crude oil-induced modification of tissue morphology and deposition of hydrocarbon-associated particles
	1000×	Fibre bundles coated with granular deposits and fragmented cell wall residues	Increased porosity and irregular texture	Suggests adsorption of petroleum constituents and onset of structural alteration
	2000×	Extensive particulate accumulation on fibres and exposed vascular elements	Rough heterogeneous surface with microvoids	Evidence of early stress response and disruption of cell wall integrity
T3 (3% Crude Oil Contamination)	500×	Pronounced fibre exposure with fragmented vascular tissues	Roughened surface with conspicuous cracks and particulate deposits	Indicates moderate structural degradation resulting from prolonged hydrocarbon exposure
	1000×	Disrupted fibrillar arrangement and partial collapse of tissue matrix	Increased fissures and surface irregularities	Suggests deterioration of cellulose–lignin network and enhanced tissue stress
	2000×	Severely eroded fibre surfaces with disrupted cellular organization	High roughness and localized collapse of structural components	Reflects significant damage to plant microstructure under moderate contamination
T4 (5% Crude Oil Contamination)	500×	Extensive fragmentation of fibrous tissues with loss of compact organization	Highly irregular and porous surface	Indicates severe crude oil-induced deterioration of plant tissues
	1000×	Disintegrated vascular bundles and broken fibre fragments	Extensive cracking and structural disorganization	Suggests marked degradation of lignocellulosic components
	2000×	Highly disrupted matrix with collapsed fibres and enlarged voids	Maximum roughness, porosity, and fragmentation among treatments	Demonstrates severe morphological damage associated with prolonged exposure to high crude oil concentration

Discussion

Crude oil contamination caused concentration-dependent deterioration of soil physicochemical properties, resulting in progressive acidification, increased electrical conductivity, elevated total petroleum hydrocarbons (TPH), reduced moisture content, increased bulk density, lower cation exchange capacity, depletion of essential nutrients, and sodium enrichment. These changes collectively indicate declining soil fertility and the development of unfavorable conditions for the growth of *Hevea brasiliensis* saplings. The observed reductions in nitrogen, phosphorus, calcium, magnesium, and potassium, together with increased sodium, further suggest impaired nutrient availability and ionic imbalance associated with petroleum contamination. These findings agree with previous reports describing petroleum-induced degradation of soil quality and fertility (Bahar *et al*., 2024; Okoye *et al*., 2024; Omenna *et al*., 2024; Okebalama *et al*., 2024; Owordi *et al*., 2025; Mekonnen *et al*., 2024; Ma *et al*., 2026; Wang *et al*., 2024; Mohanta *et al*., 2023; Cheng *et al*., 2024; Wu *et al*., 2025; Hashmat *et al*., 2024; Mbadra *et al*., 2024; Osibemhe *et al*., 2025; Rana *et al*., 2025; Jabbarov *et al*., 2024; Tavassoli *et al*., 2024; Hosseini *et al*., 2024; Tehrani & Besalatpour, 2024; Baio *et al*., 2026; Mathur *et al*., 2026; Huang *et al*., 2025; Muhammad *et al*., 2025) [9, 10, 12, 21, 22, 27, 28, 30, 31, 32, 36, 37, 38, 40, 41, 42, 46, 47].

ICP–OES analysis revealed substantial enrichment of heavy metals, including Fe, Zn, Cu, Pb, Cd, Cr, Ni, V, As, and Na, alongside depletion of Mn, Ca, Mg, and K in contaminated soils. These changes demonstrate that crude oil pollution altered the elemental balance of the soil, increasing potentially toxic elements while reducing essential nutrients required for plant growth. Similar elemental enrichment and nutrient depletion have been reported in crude oil-contaminated soils from petroleum-producing regions (Ebong *et al*., 2022; Nwawuike *et al*., 2023; Oghenerukevwe *et al*., 2026; Adeyemo *et al*., 2025; Cho *et al*., 2026; Anasrullah *et al*., 2023; Rodrigues *et al*., 2023; Bajahmoum & Almaghamsi, 2025; Gürbüz *et al*., 2025) [5, 7, 11, 13, 14, 34, 35, 44].

The elemental composition of *H. brasiliensis* tissues closely reflected the soil contamination gradient, with progressive accumulation of Fe, Zn, Cu, Pb, Cd, Cr, Ni, Na, V, and As, and concurrent reductions in Ca, Mg, K, and Mn. These findings indicate efficient uptake of petroleum-associated metals by the saplings while demonstrating impaired acquisition of essential nutrients. The observed elemental imbalance is consistent with reports of heavy metal accumulation and nutrient deficiency in plants exposed to petroleum-contaminated soils (Adeyemi & Otugboyega, 2025; Nemati *et al*., 2024; Adeyemi & Akpobaro, 2026; Adeyemi & Opia, 2025b; Adeyemi & Akpomedaye, 2026[1, 2, 3, 21]; Sut-Lohmann *et al*., 2023; Javeed *et al*., 2024) [24].

FTIR analysis demonstrated progressive biochemical modifications in rubber saplings following crude oil exposure. Contaminated plants exhibited shifts in hydroxyl, aromatic, and carbohydrate-associated functional groups together with the appearance and increased intensity of aliphatic hydrocarbon bands, indicating incorporation of petroleum-derived compounds and alterations in cellular biomolecules. Despite these changes, cellulose- and lignin-associated functional groups remained detectable throughout the treatments, suggesting persistence of structural components with modified molecular organization. These observations are consistent with previous FTIR studies of petroleum-stressed plants (Adeyemi & Okwadje, 2025; Holden *et al*., 2024; Ren *et al*., 2025; Adeyemi & Opia, 2025b; Aliyeva, 2024; Eze & Amuji, 2024; Golomb *et al*., 2021; Zhang *et al*., 2023; Anjitha *et al*., 2025; Jalal *et al*., 2024) [2, 4, 6, 8, 15, 17, 20, 50, 51].

SEM observations revealed progressive deterioration of tissue morphology with increasing crude oil concentration. Healthy, compact cellular organization in the control was replaced by increasing surface roughness, fissures, pore deformation, tissue fragmentation, and collapse of cellular structures in contaminated treatments, indicating extensive disruption of plant microstructure. Comparable structural alterations have been documented in plants exposed to petroleum hydrocarbons and related environmental contaminants (Fitrisyaah *et al*., 2025) [8].

SEM–EDS analysis complemented the ICP–OES and SEM findings by demonstrating concurrent changes in elemental composition and tissue morphology. Contaminated saplings showed enrichment of petroleum-associated elements alongside depletion of calcium, magnesium, and potassium, while regions of greater elemental accumulation corresponded with more pronounced structural deformation. Together, these findings indicate that crude oil contamination altered elemental distribution, disrupted nutrient balance, and contributed to progressive structural injury in *Hevea brasiliensis* saplings (Vieira *et al*., 2025) [48].

Conclusion

This study demonstrated that crude oil contamination progressively altered the physicochemical and elemental properties of soil, resulting in conditions that adversely affected the nutritional status and structural integrity of *Hevea brasiliensis* saplings. Increasing crude oil concentrations caused soil acidification, elevated electrical conductivity, accumulation of total petroleum hydrocarbons, and depletion of essential nutrients, reduced cation exchange capacity, and increased bulk density, collectively indicating progressive deterioration of soil quality.

Elemental analyses further showed that crude oil contamination promoted the accumulation of petroleum-associated heavy metals in both soil and plant tissues while simultaneously reducing the availability and uptake of essential macro-elements such as calcium, magnesium, potassium, and manganese. The increasing concentrations of lead, cadmium, chromium, nickel, vanadium, and arsenic in the saplings confirmed the capacity of *H. brasiliensis* to absorb and accumulate potentially toxic elements from contaminated soils.

Spectroscopic and microscopic analyses supported these findings by demonstrating concentration-dependent biochemical and structural modifications. FTIR revealed alterations in hydroxyl-, aromatic-, and hydrocarbon-associated functional groups, indicating biochemical responses to petroleum exposure, whereas SEM observations showed progressive disruption of tissue organization, including surface roughening, fibre fragmentation, cellular collapse, and increased porosity. SEM–EDS further confirmed changes in tissue elemental composition that paralleled the accumulation of heavy metals and depletion of essential nutrients.

Collectively, these findings demonstrate that *Hevea brasiliensis* exhibits measurable uptake and accumulation of petroleum-associated heavy metals while maintaining survival under crude oil stress, indicating its potential as a candidate species for phytoremediation of contaminated soils. Although increasing contamination progressively compromised soil quality and plant structural integrity, the ability of the saplings to accumulate heavy metals suggests that *H. brasiliensis* could contribute to the remediation and ecological restoration of crude oil-contaminated environments. Further field-based investigations are, however, required to evaluate its long-term phytoremediation efficiency under natural environmental conditions.

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