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Phytoremediation of Petroleum Hydrocarbon Contaminated Soil Using *Senna occidentalis* Seeds

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Abstract

Plants and the associated enzymes have shown great potential to effectively transform and detoxify polluting substances. This work aims at assessing the potential of *Senna occidentalis* and associated rhizospheric enzymes to remediate a hydrocarbon-polluted environment. Viable seeds of *S. occidentalis* were randomly collected from an abandoned farmland at Sabo in the Yaba Local Government Area (LGA) of Lagos. Phytoremediation study using *S occidentalis* were assessed ex-situ in a screen house using two concentrations each of spent engine oil and crude oil for 150 days. Results for physicochemical analysis showed an alkaline soil with pH (9.64±0.00a), Nitrate (53.61±0.15d), organic matter (90.45±0.35e), organic carbon (44.56±0.06c), electrical conductivity (677.5±0.71g), phosphate (92.95±0.21f), and Cation Exchange Capacity of (37.53±0.03b). Morphological characterization of *S. occidentalis* in SEO and crude oil contaminated soil showed a decrease with increasing concentration of the pollutants. The least morphometric was observed in the highest concentration of the combined pollutant. Enzyme activity, however, did not correlate with the concentration of the pollutant. Polyphenol oxidase had the lowest activity (15.07±0.32a) in unpolluted soil and the highest activity (26.89±0.08cg) in mixed pollutants. Mechanism of phytoremediation by *S. occidentalis* is a combination of Phytovolatilization, Phytodegradation, Rhizofiltration, and Rhizodegradation. Degradation of contaminants was seen to decrease with increasing concentration for most of the components of the pollutants. *S. occidentalis* possesses potential for phytoremediation but may be best in conjunction with other organisms.

Keywords: Phytoremediation, *Senna occidentalis,* Enzymes, Hydrocarbon, Spent engine oil

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Introduction

The world population has far exceeded seven billion, with attendant pressure on bio-resources and the environment [1]. The negative effects of anthropogenic disruption of ecosystems through industrialization and urbanization are glaring. Among all heavy metals, cadmium is said to be the most severe, whose health risks to man include anemia, kidney failure, neurological damage, cognitive impairment, cancer, and much more [2]. Crude oil and spent engine oil, due to their toxicity and persistent nature, pose serious threats to the environment. Spent engine oil (SEO), obtained from servicing and drainage of used oil from vehicles and generator engines, contains heavy metals and potentially hazardous polycyclic aromatic hydrocarbons [3]. Contamination of soil by crude and spent engine oil may inhibit or kill soil microorganisms, decrease fertility by making the soil toxic, and inhibit seed germination, thus hindering plant growth. Since the 1950s, petroleum-based (fossil) fuels have been the primary source of energy [4]. The environmental impact of petroleum exploration, production, refining, and transportation is alarming worldwide [5]. Disposing of this SEO is of major environmental concern as it's commonly disposed of into water drainage channels, farmlands, roadsides, or any available open spaces [4].

One of the most promising biological approaches to deal with the problem of hydrocarbon contamination of the environment is phytoremediation. Phytoremediation, recognized as a sustainable and environmentally friendly approach, has shown promise in mitigating the impact of heavy metals and organic compounds on the environment [6]. Phytoremediation uses plants to naturally remove, detoxify, or immobilize contaminants, thereby remediating contaminated environments [7]. Organic pollutants (such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phenols, and pesticides) from industries, urban, and agricultural activities persist in the environment due to their hydrophobic nature and can bioaccumulate, causing toxicity in organisms and disrupting ecosystems [6]. Inorganic pollutants, such as heavy metals, metalloids, radionuclides, phosphates, and nitrates, pose serious health risks to both humans and wildlife, leading to chronic anemia, neurological damage, developmental delays, kidney damage, cognitive impairment, and cancer [8]. Phytoremediation, due to its ecological soundness, cost-effectiveness, and versatility, is the new normal in achieving environmental health. Successful implementation requires understanding of the bioavailability of metals and organic compounds, which guides appropriate plant selection and remediation strategies [9]. The uptake of metals and organic compounds by plants is influenced by the species of plants, soil characteristics, and other prevailing environmental conditions [10]. Phytoremediation processes are used to recover metals, such as Ni, Se, Zn and Fe, by phytomining and biofortification through agronomic practices, plant breeding, and modern biotechnology [11]. Plants produce chelating agents to facilitate metal absorption through diffusion or actively via specialized transporter proteins. Similarly, hydrophobic organic compounds are absorbed passively while actively transporting water-soluble compounds. Detoxification of organic contaminants involves several steps, including chemical modification, functionalization, conjugation, and compartmentalization, with enzymes playing a crucial role in either completely mineralizing pollutants or partially degrading them into stable intermediates [12].

Several plants have shown potential for phytoremediation [13-15]. *Senna occidentalis* L, commonly called "coffee Senna", has several applications in traditional medicines as an analgesic, antibacterial, antifungal, antihepatotoxic, anti-inflammatory, antiseptic, laxative, in the treatment of liver diseases, among others [16]. The potential of *S. occidentalis* to hyperaccumulate heavy metals has been highlighted by Habib *et al.* [17]. This study aims to investigate the phytoremediation potential of *Senna occidentalis* in crude oil and spent engine oil-contaminated soil, determine the effect of rhizosphere enzymes in the remediation, as well as assess the morphological characteristics of *Senna occidentalis* in crude oil and spent engine oil-contaminated soil

Materials and Methods

Sample collection

Matured and dried seeds of *S. occidentalis* was randomly collected from an abandoned farmland at Sabo in Yaba Local Government Area (LGA) of Lagos state (6°30'25.7"N, 3°22'49.3"E). The pods were opened and the dried seeds were pollutant, crude oil was gotten from the Nigeria National Petroleum Cooperation Depot at Apapa Lagos, stored into a 5 Liters keg and labelled (OC). Spent Engine oil (mixture of auto vehicles and other machinery) was obtained from the mechanical workshop at Yaba College of Technology, and stored in a 5-liter keg and labelled (SC). Using a hand trowel, about 50kg of sandy-loam soil was collected at (0-30 cm) depth in Yaba College of Technology Staff Quarters, with no history of any contaminant (6°31'02.3"N, 3°22'41.9"E). The soil was sieved through a 10 mm diameter sieve and left to air-dry for 7 days before use.

Physicochemical analysis of soil, crude oil and spent engine oil

Physico-chemical characteristics of the experimental soil, crude oil and spent engine oil were analyzed. Soil pH was measured in distilled water using soil liquid ratio of 1:1, electrical conductivity was measured using conductivity bridge. Nitrate was determined by the phenol-disulphonic acid, Organic carbon was measured using wet combustion method. Exchangeable cation was determined on atomic absorption spectrophotometer [18-20]. Electrical conductivity (EC) was determined using a glass electrode conductivity meter. Viscosity (V), specific gravity (SG), Flash point (FP), Acid Number of the pollutants, Saponification value (SV), Base Number (BN) were measured according to AOAC [21], and Marinova *et al.* [22].

Phytoremediation study

This Experiment was carried out in a screen house in the Biological Garden, Yaba College of Technology (6'31"N, 3'40"E). Using a weighing balance, 5 kg of the pulverized sandy-loamy soil was weighed into a 10-liter perforated experimental buckets. Spent engine oil and crude oil (50, 100 and 150 mL) were each introduced independently into some of the buckets and mixed thoroughly and allowed to homogenize for 24 h. Randomized complete block design was used to apply 16 treatments including OC_1 = Crude oil concentration 1 (50 ml), OC_2 = Crude oil concentration 2 (100 ml), OC_3 = Crude oil concentration 3 (150 ml), SC_1 = Spent Engine oil concentration 1 (50 ml), SC_2 = Spent Engine oil concentration 2 (100 ml), SC_3 = Spent Engine oil concentration 3 (150 ml), P only = *S. occidentalis* (P) in unpolluted soil, POC_1 = Plant + 50 ml Crude Oil, POC_2 = Plant + 100 ml Crude Oil, POC_3 = Plant + 150 ml Crude Oil, PSC_1 = Plant + 50 ml Spent Engine Oil, PSC_2 = Plant + 100 ml Spent Engine Oil, PSC_3 = Plant + 150 ml Spent Engine Oil, PSC_1OC_1 = Plant + 50 ml Spent Engine Oil + 50 ml Crude Oil, PSC_2OC_2 = Plant + 100 ml Spent Engine Oil + 100 ml Crude Oil. PSC_3OC_3 = Plant + 150 ml Spent Engine Oil + 150 ml Crude Oil. Viable seeds of *S. occidentalis* were planted 24 h after crude oil and SEO introduction at a depth of 3 cm. Five (5) seeds were planted per hole and later thinned to two. Seed viability was confirmed by floatation methods as suggested by Ani *et al.* [23] before planting. Five milliliter (5 ml) bowl was placed under each bucket treated with SEO and crude oil to retain extra water seepage and was re-introduced back into the set up. Each experiment was set in 3 replications.

Data collection

The effect of crude oil and spent engine oil on plant height, internode length, leaf area, number of leaf and circumference of the stem, were assessed at 10 days interval for 120 days. Leaf area (LA) was determined according to Ani *et al.* [23]. For each analysis, a composite sample of each contaminated soil was used.

GC-MS analysis

Phytoremediation potential of *S. occidentalis* in crude oil and SEO was determined using gas chromatography mass spectroscopy (GC-MS) ran at day 0 and at day 150 of the experiment using a composite soil sample while the GC-MS of the plant samples was done at day 150 only.

Enzyme assay

The activities and quantification of Indole Acetic Acid (IAA) from sample was done using Salkowski reagent.

Urease activity was assessed by determining the amount of ammonium released by urease activity when soil is incubated with tris(hydroxymethyl)aminomethane (THAM) buffer, urea solution, and toluene at 37 °C for 2 h [24]. Succinate Dehydrogenase (SD), Alkaline Phosphatase and Polyphenol oxidase (PPO) were determined according to determined according to Abatabai and Bremner [25].

Statistical analysis

Results were analyzed statistically with SPSS version 23 using student general linear model (GLM) which incorporates the univariate analysis of variance (ANOVA) and the pair wise test comparison at ($p<0.05$).

Results and Discussion

Table 1 shows the study soil physico-chemical properties. The soil was alkaline (pH 9.64±0.00). This reduces microbial activities and bioavailability of essential micronutrients such as Iron, and manganese. This is an indication of stress-adapted ability of *S. occidentalis* to sustain metabolic function, and physiological resilience to thrive in alkaline environments [26]. The Nitrate levels was moderately high (53.61±0.15 mg/L) showing sufficient nitrogen availability to support plant growth and rhizospheric microbial activities essential for effective degradation of petroleum hydrocarbons [27]. Also, the carbon (44.56±0.06 %) and organic matter content (90.45±0.35 %) were high, enabling root exudation, microbial colonization, enzymatic degradation, and pollutant sorption. The cation exchange capacity (CEC) was 37.53±0.03 cmol/Kg. this is indicative of a strong holding ability for essential mineral nutrients that support plant vigor and enzymatic activities of microbes, such as Ca^{2+}, Mg^{2+}, and K^+ [28]. Likely, this high nutrient holding capacity provided a stable environment that supported consistent biochemical activities in the hydrocarbon-polluted soil over the 150-day study period. Electrical conductivity (677.5±0.71 µS/cm) indicates moderate salinity within acceptable limits that didn't induce osmotic stress. The phosphate concentration was high (92.95 ± 0.21 mg/L), suggesting ample availability of phosphorus, essential for root development and energy metabolism in stress environments [29].

Table 1: Physico-chemical analysis of soil sample

Parameter	Mean +sd
Nitrate (mg/l)	53.61±0.15d
Organic matter	90.45±0.35e
organic carbon	44.56±0.06c
pH	9.64±0.00a
Plectrical conductivity µs/an	677.5±0.71g
Phosphate	92.95±0.21f
CEC (ca^+, na^+, k^+,mg^+)	37.53±0.03b

Values represent the Mean ± SD. Means with different letters as superscripts along a column are significantly different at p-value < 0.05. CEC = Cation Exchange Capacity, K^+ = Potassium ion, Na^+ = Sodium ion, Mg^+ = Magnesium ion, Ca^+ = calcium ion

Table 2: Physico-chemical analysis of pollutants (OC and SC)

Parameters	SC	OC
pH	9.91±0.01a	9.51±0.01b
Viscosity mm^2 20 °C	9.33±0.01a	8.16±2.11a
Acid value mg/g	9.37±0.08a	5.67±0.08b
Saponification value (mg/g)	107.7±0.42a	115.00±0.00b
Specific gravity	0.90±0.01a	0.94±0.01b
Electrical conductivity	670.5±2.12a	806.5±2.12b
Flash point (°C)	87.00±0.00a	88.00±0.00b
Total dissolved solid (mg/l)	336.50±0.71a	378.00±0.00b

Values represent the Mean ± SD. Means with different letters as superscripts along a column are significantly different at p-value < 0.05. SC = Spent Engine oil, OC = Crude oil

The physicochemical analysis results of spent engine oil (SC) and crude oil (OC) are presented in Table 2. Results show significant differences that impact their environmental behavior for phytoremediation. While SC has a slightly higher pH (9.91) than OC (9.51), both have strong alkalinity. Soil alkalinity alters root-microbe interactions, which may increase the toxicity of co-contaminants. Also, the availability of essential nutrients such as phosphorus, iron, and manganese is negatively affected by elevated pH levels in the presence of hydrocarbons, impairing plant absorption and rhizospheric microbiota activities [30]. Spent ending oil (SC) had higher viscosity (9.33 mm²/s), compared to crude oil (8.16 mm²/s) representing fluid resistance to flow. This indicates that SC is likely to persist in the upper soil layer by its reduced infiltration and aeration capacity. Consequently, this creates oxygen-deficient conditions that can influence soil microbiota and slow natural biodegradation process [31]. The higher acid value of SC (9.37 mg/g) than OC (5.67 mg/g) further highlights its greater extent of potential oxidation and degradation process owing to initial accumulation of potentially phytotoxic acidic bye-products from engine operation, and thermal stress [32]. Interestingly, SC had lower saponification value (107.7 mg/g) than OC, (115.00 mg/g) showing that OC is more enriched with hydrophobic long chain fatty acid esters susceptible to rhizosphere microbe hydrolysis [33]. This property may be indicative of relative biodegradability of OC than SC, under optimal conditions. However, the specific gravity of OC (0.94) was high than that of SC (0.90), suggesting a higher density that is relative to deeper soil penetration and reduced bioavailability. This property, enhances OC ability to pollute groundwater [34]. Furthermore, OC had higher electricity conductivity (806.5 µS/cm) and total dissolved solid 378.00 mg/L than SC (670.5 and 336.50 mg/L) respectively. This shows that OC can impair development of leaves and root elongation by induce osmotic stress, and reduced water absorption, from its ability to increase soil dissolved ions and salinity [35]. The close flash point of both pollutants (87–88 °C), suggest close flammability risk despite their different environmental persistence and toxicity mode. Owing to high viscosity and acid content, SC more likely will contribute to prolonged soil surface toxicity while OC will contribute to salinity stress and subsurface contamination.

The morphological characteristics of *S. occidentalis* in SEO and crude oil contaminated soil is presented in Table 3. The growth of *S. occidentalis* was optimal in the control soil sample. This is evident in the highest mean value of plant length (PL: 37.43 ± 10.45 cm), stem circumference (SC: 3.44 ± 1.79 cm), internode length (IL: 5.52 ± 2.64 cm), number of leaves (NL: 3.60 ± 5.22), and leaf area (AL: 36.40 ± 22.44 cm²). These values that differ significantly, in the varying concentrations of crude oil and spent engine oil, affirm the capacity of *S. occidentalis* to attain optimal morphological performance under favorable soil conditions, indicating that hydrocarbon contamination, either from spent engine oil, crude oil, or both negatively impact its morphological performance. The plant height showed notable decline with increase in pollutant concentration, reaching 28.15 cm in POC_1 treatment (50 mL crude oil) and 26.43 cm in POC_2 (100 mL crude oil). Similar decline was observed in SEO treatments (PSC_1 and PSC_2) and more pronounced reductions with plant length reaching as low as 16.47 cm, in combined pollution. These findings corroborate previous studies that reported hydrocarbon interference with soil aeration, nutrient availability, and water uptake, inhibiting plant development (36, 37). Similarly, internode length and leaf area decreased from, IL 5.52 cm in the control soil to 2.11 cm in the highest combined pollutant (PSC_2OC_2) treatment and AL decreasing from 36.40 to 16.87 cm^2. Particularly, this leaf area reduction suggests inefficient photosynthesis and overall biomass production, indicative of phytotoxic stress [35]. Leaf count and stem circumference also showed substantial decrease especially under dual pollution, suggesting a possible hydrocarbon toxicity suppression of meristematic activities and cell division essential for the expansion of structural tissues [38]. Though at reduced rate, correlating phytotoxic effects of petroleum hydrocarbon pollution, *Senna occidentalis* sustaining growth despite the evident stress in all concentration levels, demonstrates a degree of tolerance. This resilience, also reported in other phytoremediating leguminous plants, may be as a result of its deep root system, adaptability to poor soils, and nitrogen-fixing potential [33, 29].

Table 3: Morphological characteristics of *S. occidentalis* in SEO and crude oil contaminated soil

	PL	IL	AL	NL	CS
P Only	37.43±10.45a	5.52±2.64a	36.40±22.44a	3.60±5.22a	3.44±1.79a
POC_1	28.15±14.73a	4.23±1.93a	25.6±13.02a	0.7±0.42a	3.78±2.98a
POC_2	26.43±14.22a	3.04±1.23a	22.87±12.70a	0.63±0.41a	2.17±1.33a
PSC_1	31.32±15.43a	3.61±1.91a	23.73±12.46a	0.59±0.40a	3.69±3.13a
PSC_2	23.03±12.01a	3.88±2.14a	21.27±11.41a	1.01±0.57a	2.04±1.33a
PSC_1OC_1	18.3±8.80a	2.28±1.00a	20.53±11.20a	0.55±0.35a	2.18±1.46a
PSC_2OC_2	16.47±9.01a	2.11±1.17a	16.87±11.70a	0.44±0.31a	1.88±1.29a

Values represent the Mean ± SD. Means with different letters as superscripts along a column are significantly different at p-value < 0.05. PL = Plant Length, IL = Internode Length, AL = Average Length, NL = Number of Leaf, CS = Stem Circumference, P only = *S. occidentalis*(P) in unpolluted soil, POC_1 = Plant + 50 ml Crude Oil, POC_2 = Plant + 100 ml Crude Oil, PSC_1 = Plant + 50 ml Spent Engine Oil, PSC_2 = Plant + 100ml Spent Engine Oil, PSC_1OC_1 = Plant + 50 ml Spent Engine Oil + 50 ml Crude Oil, PSC_2OC_2 = Plant + 100 ml Spent Engine Oil + 100 ml Crude Oil.

Table 4: Statistics results for soil enzymes (µg/g/h)

Parameter	Polyphenol oxidase	Succinate dehydrogenase	Alkaline phosphatase	Urease	IAA
50P	15.07±0.32a	16.81±0.66e	33.84±1.24c	1.32±0.04a	4.07±0.16b
$50POC_1$	19.44±0.04c	12.74±0.30c	28.93±0.03a	1.18±0.16a	2.70±0.08b
50 PSC_1OC_1	25.11±0.11f	9.02±0.57a	40.93±1.00f	1.06±0.01a	4.50±0.03b
$50PSC_1$	18.34±0.25b	12.45±0.06c	33.24±0.63bc	1.27±0.16a	3.68±0.05b
100P	20.09±0.40c	17.2±0.12e	32.08±0.45b	1.38±0.01a	6.10±0.24b
$100POC_2$	21.85±0.13d	13.96±0.15d	36.03±0.12d	1.09±0.03a	4.14±0.10b
$100PSC_2OC_2$	26.89±0.08cg	10.34±0.88b	38.24±0.25e	1.47±0.15a	7.52±0.18b
$100PSC_2$	21.77±0.39d	16.06±0.75e	41.48±0.01fg	2.46±0.05a	5.44±0.06b
150P	20.12±0.23c	13.18±0.49cd	29.45±0.17a	2.08±0.18a	7.09±0.05b
$150POC_3$	23.29±0.32e	9.16±0.32a	40.72±0.47f	1.30±0.18a	4.50±0.24b
$150PSC_3OC_3$	20.04±0.77c	12.23±0.54c	42.19±0.31g	1.28±0.30a	6.12±0.04b
$150PSC_3$	22.32±0.52d	13.25±0.39cd	28.38±0.20a	2.45±0.34a	4.70±0.18b

Values represent the Mean ± SD. Means with different letters as superscripts along a column are significantly different at p-value < 0.05 50p = plant only at day 50, 100p= only plant at day 100, 150p= only plant at day 150; POC_1 = Plant + 50 ml Crude Oil, POC_2 = Plant + 100ml Crude Oil, POC_3 = Plant + 150 ml Crude Oil, PSC_1 = Plant + 50 ml Spent Engine Oil, PSC_2 = Plant + 100 ml Spent Engine Oil, PSC_3 = Plant + 150 ml Spent Engine Oil, PSC_1OC_1 = Plant + 50 ml Spent Engine Oil + 50 ml Crude Oil, PSC2OC2 = Plant + 100 ml Spent Engine Oil + 100 ml Crude Oil. PSC_3OC_3 = Plant + 150 ml Spent Engine Oil + 150 ml Crude Oil.

In contaminated environments, soil enzymatic activity indicates its biochemical status and microbial functionality. Table 4 shows results of the five key soil enzymes; polyphenol oxidase (PPO), succinate dehydrogenase (SDH), alkaline phosphatase (ALP), urease, and indole-3-acetic acid (IAA), under the different levels of SEO and crude oil in the presence and absence of *S. occidentalis*. Polyphenol oxidase (PPO) activity increased consistently with pollution levels, with highest record in combined treatment contamination at 26.89 µg/g/h in 100 PSC_2OC_2. Increase in oxidative stress and the activation of plant and microbial defense systems to degrade complex hydrocarbons is commonly associate with elevation in PPO activity [39]. The high record of PPO level in $100PSC_2OC_2$ suggests increase in detoxification activity likely owing to *S. occidentalis* root exudates and rhizospheric microbe interactions. The control soil samples (100P and 50P), and combined pollutant sample ($50PSC_1OC_1$), showed highest value of Succinate dehydrogenase (SDH), (100P 17.2 & 50P:

16.81 μg/g/h), and (9.02 μg/g/h) respectively. This is indicative of metabolic stress and suppressed microbial respiration, likely caused by hydrocarbon toxicity impairing membrane integrity and electron transport chain activity [40].
Moderate hydrocarbon contamination (150PSC$_3$OC$_3$: 42.19 μg/g/h and 100PSC2: 41.48 μg/g/h), increased Alkaline phosphatase (ALP) activity. This enzyme which plays a role in the mineralization of phosphorus, may be stimulated by the adaptive response of rhizosphere microbes to hydrocarbon induced phosphorus immobilization [41]. This increased ALP activity in the polluted treatment may also be owed to the hydrocarbon induced proliferation of oil-degrading bacteria that express phosphatases under stress conditions. Urease activity showed stability across all treatments, ranging from 1.06 to 2.46 μg/g/h to the highest observed value μg/g/h in 100PSC$_2$, suggesting that urease-producing microbes and nitrogen cycle processes actively continued even under hydrocarbon stress. This consistency further supports the role of *S. occidentalis* in the sustenance of microbial function in contaminated soil and may be due to microbial adaptation and the buffering effect of organic matter [29]. The plant growth hormone Indole-3-acetic acid (IAA) known to enhance root elongation and stress tolerance, showed moderate increase in pollution treatments, recording highest concentration in 100PS-$_2$OC$_2$ (7.52 μg/g/h) and 150P (7.09 μg/g/h). The elevation of IAA levels under stress, in moderate contaminant treatments may reflect microbial adaptation and symbiosis with *S. occidentalis* [42]. While hydrocarbon contamination can repress metabolic functions, Table 4 result of enzymatic activities reveals that *S. occidentalis* supports active and functional rhizosphere that can promote biodegradation of hydrocarbons. This supports previous study findings that plants and rhizospheric microbes can potentially enhance and maintain biochemical resilience under soil pollution stress [43, 44].

Confirmatory phytoremediation using GC-MS

The GC-MS result of crude oil (OC) in Table 5 contains 24 hydrocarbon compounds, mostly linear alkanes (straight-chain saturated hydrocarbons) ranging from decane ($C_{10}H_{22}$) to hentriacontane ($C_{31}H_{64}$). The retention time from the crude oil contaminant ranges from 3.98 in decane to 32.20 in Hentriacontane while the relative abundance ranged from 1 in octacosane to 94 in Tridecane. The spent engine oil component (SC) of the PAHs are all products of incomplete combustion typical of used oil, especially from internal combustion engines. Detected compounds may be categorized into 3: (i) the light PAHs including Naphthalene, Acenaphthylene, Acenaphthene, Fluorene, Phenanthrene, Anthracene. These are more volatile compounds often linked to fuel contamination**,** thermal degradation, or short-chain aromatics from fuel combustion. (ii) Medium PAHs including Fluoranthene, Pyrene, Benzo[c]phenanthrene, Benz[a]anthracene, Chrysene, and (iii) Heavy PAHs comprising Benzo[b,j,k]fluoranthene, Benzo[e]pyrene, Benzo[a]pyrene, Indeno[1,2,3-cd]pyrene, etc. Their presence in spent oil reflects long-term accumulation, oil breakdown, and combustion byproducts. These are the heavier and more persistent PAHs, often carcinogenic.

Table 5: GC-MS of contaminated soil after 150 days

Crude Oil (OC)				Spent Engine Oil (SC)		
S/N	**Compounds**	**R.T**	**Q Value**	**Compounds**	**R.T**	**Q Value**
1	Decane	3.980	83	Naphthalene	4.366	1
2	Undecane	5.634	88	Acenaphthylene	7.256	1
3	Dodecane	7.545	86	Acenaphthene	7.589	1
4	Tridecane	9.462	94	Fluorene	8.592	1
5	Tetradecane	11.321	86	Phenanthrene	10.389	63
6	Pentadecane	13.095	60	Anthracene	10.504	66
7	Hexadecane	14.777	89	Fluoranthene	12.710	1
8	Heptadecane	16.425	39	Pyrene	13.119	1
9	Pentadecane,	16.477	41	Benzo[c]phenanthrene	15.146	1
10	Octadecane	17.987	30	Benz[a]anthracene	15.491	1
11	Hexadecane	18.033	58	Chrysene	15.568	15
12	Nonadecane	19.407	23	Benzo[b+j+k]fluoranthene	17.537	1
13	Eicosane	20.820	89	Benzo[e]pyrene	17.915	1
14	Heneicosane	22.067	1	Benzo[a]pyrene	18.017	1
15	Docosane	23.406	75	Indeno[1,2,3-cd]pyrene	19.737	1
16	Tricosane	24.654	24	Dibenz[a,h]anthracene	19.826	53
17	Tetracosane	25.775	78	Benzo[ghi]perylene	20.063	1
18	Pentacosane	26.942	82	Dibenzo(a,l)pyrene	21.681	1
19	Hexacosane	28.007	38	Dibenzo(a,i)pyrene	22.416	1
20	Heptacosane	29.037	50	Dibenzo(a,h)pyrene	22.531	1
21	Octacosane	29.357	1	-	-	-
22	Nonacosane	30.050	1	-	-	-
23	Triacontane	31.057	1	-	-	-
24	Hentriacontane	32.201	47	-	-	-

R.T: Retention Time; OC: Crude Oil; SC: Spent Engine Oil; Q value: abundance

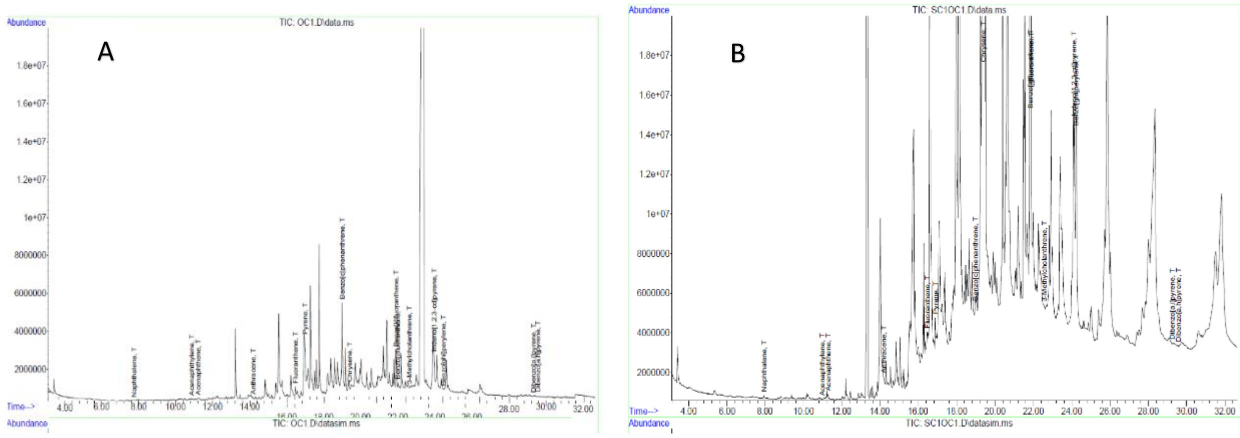

Figure 1a: GC-MS chromatogram of (A) crude oil only (OC) and (B) spent engine oil (SC) only (Control)

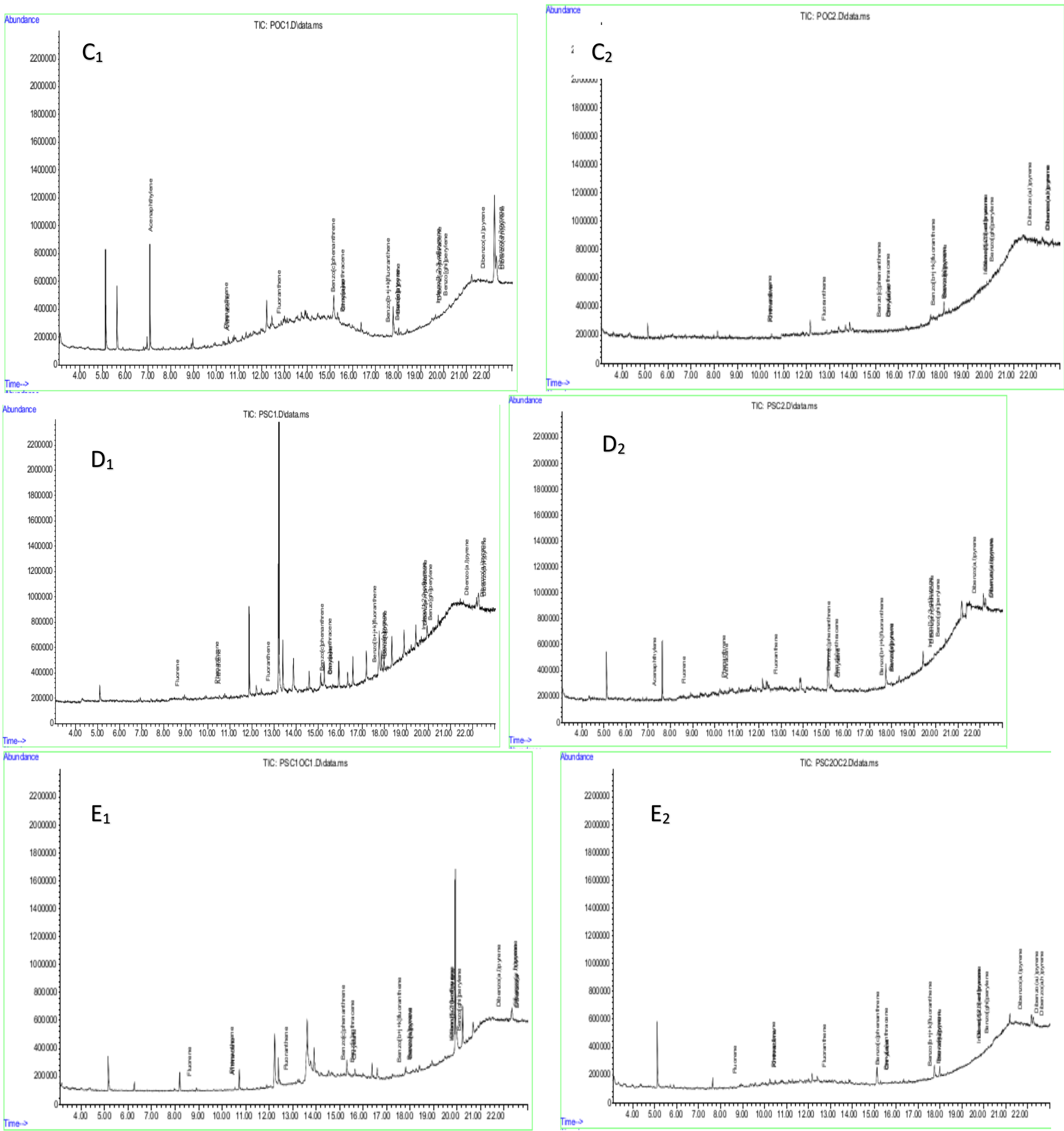

Figure 1b: GC-MS chromatogram of 50 ml crude oil (C_1) and 50 ml SEO (C_2); 100 ml crude oil (D_1) and 100 ml SEO (D_2); 150 ml crude oil (E_1) and 150 ml SEO (E_2)

Organic pollutants are known to resist degradation due to their complex structures leading to environmental persistence and health issues such as cancer, reproductive disorders, immune system alterations, hypertension, and diabetes [9]. The detoxification of organic pollutants by plants involves several key steps, including chemical modification, functionalization, conjugation, and compartmentalization, with enzymes playing a crucial role in either completely mineralizing pollutants or partially degrading them into stable intermediates [6]. In plants, conjugation and compartmentalization are critical steps in xenobiotics detoxification, once inside the cells, they are metabolized by reducing their toxicity before compartmentalization or excretion. The mechanism of phytoremediation by *S. occidentalis* is a combination of Phytovolatilization, Phytodegradation, Rhizofiltration and Rhizodegradation. Degradation of contaminants was seen to decrease with increasing concentration for most of the components of the pollutants as seen in the Fig. 1a & 1b.

Conclusion

Although phytoremediation is an eco-friendly biological process, it is also a slow and gradual process towards generating fewer secondary wastes, minimal associated environmental disturbance, and the ability to leave soils in place and in a usable condition following treatment. The mechanism of phytoremediation by *S. occidentalis* is a combination of Phytovolatilization, Phytodegradation, Rhizofiltration and Rhizodegradation. Degradation of contaminants was seen to decrease with increasing concentration of contaminants.

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