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Assessment of *Vinca rosea* (Apocynaceae) Potentiality for Remediation of Crude Petroleum Oil Pollution of Soil

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Abstract: Petroleum oil pollution is a worldwide problem that results from the continuous exploration, production, and consumption of oil and its products. Petroleum hydrocarbons are produced as a result of natural or anthropogenic practices, and their common source is anthropogenic activities, which impose adverse effects on the ecosystem's nonliving and living components including humans. Phytoremediation of petroleum hydrocarbon-polluted soils is an evolving, low-cost, and effective alternative technology to most traditional remediation methods. The objective of this study is to evaluate the phytoremediation potentiality of *Vinca rosea* for crude oil-contaminated soil by understanding its properties and involvement in the enhanced degradation of crude oil. The remediation potentiality was determined by evaluating the total petroleum hydrocarbon degradation percentage (TPH%) and changes in the molecular type composition of saturated and aromatic hydrocarbon fractions. TPH% was estimated gravimetrically, and changes in the molecular type composition of saturated and aromatic fractions were measured using gas chromatography and high-performance liquid chromatography, respectively. Sulfur concentration was measured using X-ray fluorescence. Cadmium and lead quantification was measured using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES). The results revealed that *V. rosea* enhanced total petroleum hydrocarbon (TPH) degradation and altered the molecular composition of the crude oil. The saturated hydrocarbons increased and the aromatic hydrocarbons decreased. The saturated hydrocarbon fraction in the crude oil showed a wider spectrum of n-paraffin peaks than the oil extracted from unplanted and *V. rosea*-planted soils. Polyaromatic hydrocarbon degradation was enhanced in the presence of *V. rosea*, which was reflected in the increase of monoaromatic and diaromatic constituents. This was parallel to the increased sulfur levels in planted soil. The determination of sulfur and heavy metal content in plant organs indicated that *V. rosea* can extract and accumulate high amounts from polluted soils. The ability of *V. rosea* to degrade TPH and alter the composition of crude petroleum oil by decreasing the toxicity of polyaromatic hydrocarbons in soil, as well as its capability to absorb and accumulate sulfur and heavy metals, supports the use of plant species for the phytoremediation of crude oil-polluted sites.

Keywords: saturated hydrocarbons; aromatic hydrocarbons; n-paraffin compounds; lead; cadmium; sulfur

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1. Introduction

Crude oil pollution has increased worldwide because of the continuous processes of exploration, production, and consumption of oil and its products [1]. Meanwhile, petroleum hydrocarbons (PHC) are produced as a result of natural or anthropogenic practices and activities [2]. PHC impose an adverse effect on the ecosystem and can cause major risks to microorganisms and higher organisms, including humans, as they have highly toxic, carcinogenic, mutagenic, and teratogenic effects [3]. They are complex chemical compounds that are numerous and have different molecular weights, including non-hydrocarbon fractions. The primary hydrocarbons in crude oil are simple aromatics and polycyclic aromatic hydrocarbons, in addition to naphthenes and paraffins as saturated hydrocarbons [4]. The non-hydrocarbon fraction of the oil includes sulfur, nitrogen, and oxygen, as well as trace amounts of heavy metals such as copper, nickel, iron, vanadium, cadmium, and lead. Among the non-hydrocarbon metals, sulfur is the most abundant and is considered the most important for determining the oil's properties and its refinement capacity. Trace metals and nitrogen, on the other hand, may have a significant impact on crude oil characteristics [5].

The crude oil is composed of 16 polycyclic aromatic hydrocarbons (PAHs) classified as code red pollutants, and their removal is considered critical for human health safety and environmental remediation [6]. Crude oil degradation in soils causes the accumulation of heavy metals including cadmium, lead, zinc, chromium, copper, and nickel. Heavy metals pollution in the soil causes adverse effects on the growth and functional traits of plants and affects the activities of the soil microbiota [7]. Plant species may have a crucial role in the remediation of heavy metals in soil, minimizing the pollution threat [8].

Conventional techniques for the remediation of petroleum-contaminated soil include physical and chemical methods, such as chemical oxidation, thermal desorption, soil flushing and washing, and incineration. Despite the fast remediation rate of conventional methods, they cause secondary environmental pollution and are energy-demanding, expensive, and disruptive to soil characteristics and landscape [9]. Therefore, it is essential to have an eco-friendly and cost-effective green technology to remediate petroleum-contaminated soils [10,11]. Phytoremediation is a promising technology that is eco-friendly, relatively cheap, and sustainable for the remediation of petroleum contamination in soil [12]. It involves the use of plants to metabolize, remove, adsorb, or assimilate organic contaminants, including hydrocarbons and hazardous inorganics such as heavy metals from soil [13,14].

Phytoremediation has been used for treating petroleum hydrocarbon pollution using numerous plant species such as *Azolla pinnata* (Azollaceae) [15], tall fescue [16], water hyacinth [17], poplar [18], duckweeds [19], and *Jatropha* [20], Mexican primrose-willow [21], sweet flag [22], grey sedge [23], burning bush [24], and Italian ryegrass [25]. At present, screening for potential hyperaccumulators in plants has become an important tool in phytoremediation research. Nevertheless, many hyperaccumulators, which have high accumulation ability and tolerance to contaminants, are less applicable for practical use because of their restricted growth rate and productivity [26].

Ornamental plants have advantages over other plants as phytoremediators because of their high biomass production, fast growth, and ability to accumulate contaminants separately from food chains, in addition to beautifying the environment while remediating polluted soil [27]. *Vinca rosea* L. (Apocynaceae) (synonymous with *Catharanthus roseus*) is an ornamental plant that is naturally found in tropical countries and has been introduced to subtropical countries and Mediterranean countries. It is known for its applications in medicine due to the use of its extracts for the treatment of numerous diseases [28]. The successful potential for the remediation of heavy metals and diesel exhaust contamination in soil by *V. rosea* was previously reported [29,30]. This study aims to evaluate the potentiality of *V. rosea* for phytoremediation of crude oil-contaminated soil. We hypothesize that the presence of *Vinca rosea* plants enhances the degradation of the heavy long chain n-paraffinic compounds (saturated hydrocarbons) of crude petroleum oil as well as the removal of simple short chain n-paraffinic compounds. The plant uptake and accumulation of crude

oil-associated inorganic compounds in plant tissues increase with increased degradation levels. The saturated and aromatic hydrocarbons and heavy metal accumulation in plant tissues were assessed under different levels of crude oil soil pollution.

2. Materials and Methods

2.1. Experimental Design

Vinca rosea plants were grown in a mixture of sandy–clayey soil under natural conditions in an open greenhouse at the Faculty of Science, Cairo University. The chemical and physical properties of the used soil are found in [31]. Different concentrations of petroleum oil were mixed into the soil in order to obtain the following levels of oil contamination: 1%, 3%, 5%, and 7% crude oil per soil, as this is the expected level of crude petroleum oil pollution of soils where plants survive and perform their growth functions [4,31]. Three different experimental groups were established. The first group had the tested groups of *V. rosea* growing in pots containing sterilized (oven dried at 105 °C) soil polluted with crude oil levels of 1%, 3%, 5%, and 7% oil. The second group contained pots of sterilized polluted soil that had the same concentrations of crude oil as the tested groups but without *V. rosea* plants (unplanted control). The last group included *V. rosea* plants that were planted in unpolluted soil. Five replicates were established for each experimental group. Plants were irrigated regularly using Nile River water to maintain moisture levels when needed. The experiment was conducted over five months, from February to June. Plant shoots and roots were collected at the end of the experiment, washed with water, and dried in an oven at 70 °C. Soil samples were also obtained from each experimental group and stored at −20 °C until use. The phytoremediation potentiality of *V. rosea* was estimated for the determination of petroleum hydrocarbons degradation and the measurement of the residual fractions of total saturated hydrocarbons and aromatic hydrocarbons after a 5-month phytoremediation period. Sulfur concentration was also measured in soil and plant tissues as an indication of remediation progress. The accumulation of the heavy metals, cadmium, and lead in plant shoots and roots was determined. The change in petroleum hydrocarbon composition was investigated by estimating the percentage of normal paraffins and iso-paraffins in total saturated hydrocarbons (SH) and the percentage of polycyclic aromatic hydrocarbons (PAH) in total aromatic hydrocarbons (AH).

2.2. Extraction of Total Petroleum Hydrocarbons (TPH)

Soil samples were allowed to dry in the dark at room temperature and gently homogenized before sieving through 100 mesh. Using 50 mL of dichloromethane, total petroleum hydrocarbon (TPH) was extracted from 10 g of the sieved soil by ultrasonication for 30 min [32]. The supernatant was obtained after 10 min of centrifugation at 3000 rpm. In a clean, dry, and previously weighed conical flask, the supernatant was combined after repeating the extraction cycle three times. The flask was allowed to evaporate dichloromethane in a fume hood until it reached a constant weight. The quantity of extracted TPH was calculated as the difference between the weight of the dried flask holding the extracted hydrocarbons and the weight of the empty pre-weighed flask. The loss of petroleum hydrocarbons from this extraction process is 4.8%.

2.3. TPH Fractionation

The TPH extracts were analyzed and further fractionated using liquid–solid column chromatography followed by gravimetric analysis according to [33]. A glass column with a diameter of 1.3 cm and a height of 130 cm was filled with 30 gm of activated silica gel (60–200 mesh size). The column was then moistened with 100 mL of n-hexane to dissipate the heat of adsorption. The sample was dissolved in a few milliliters of n-hexane and transferred to the column. The column was then eluted with 300 mL of n-hexane, followed by 200 mL of benzene and finally 150 mL of a 1:1 mixture of absolute methanol and benzene. Fractions of 25 mL were taken from the column, the solvent was distilled off, and the refractive index of each fraction was determined. The elutes were classified as

saturated, monoaromatics, diaromatics, and polyaromatics based on refractive index data at 20 °C. The mono-cyclic, bi-cyclic, and polycyclic aromatics have refractive indices from 1.48 to 1.53, 1.53 to 1.59, and higher than 1.59, respectively [15].

2.4. Gas Chromatographic Analysis

The SH were analyzed using PerkinElmer (Clarus 500) gas chromatography (GC) equipped with a hydrogen flame ionization detector (FID) and a capillary column of fused silica, with a 60 m length and 0.32 mm diameter, filled with poly (dimethylsiloxane) HP-1 (nonpolar resin) with a 0.5 µm film thickness. The injector was heated to 350 °C. Nitrogen (oxygen-free) was used as a carrier gas with a 2 mL/min flow rate. The temperature range of the column was 100–300 °C at a constant rate of 3 °C/min. The detector was warmed up to 350 °C, and 0.1 µL of the melted sample was injected into the injector. A mixture of pure normal paraffins (n-paraffins) was used as a standard.

2.5. Sulfur Quantification

An estimation of sulfur concentration in plant shoots and roots and soil was carried out using a X-ray fluorescence sulfur meter (ASTM D-4294-98).

2.6. Cadmium and Lead Quantification

Plant shoots and roots were dried in an oven at 60 °C for 72 h until they reached a constant weight. The tissue was ground and nitric acid digested, followed by metal quantification using Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES).

2.7. Statistical Analysis

The results are presented as mean ± standard errors (n = 3 replications), and the statistical analysis was performed using SPSS 18.0 software for windows. One-way analysis of variance (ANOVA) was used to compare the different treatments using Duncan's multiple range test at $p < 0.05$. For comparison among the different treatments in planted and unplanted soils, two-way analysis of variance (ANOVA) and Duncan's multiple range test ($p < 0.05$) were used [15,31].

3. Results

3.1. Total Petroleum Hydrocarbons

The saturated hydrocarbons (SH) and aromatic hydrocarbons (AH) percentages in crude oil extracted from *V. rosea*-planted soils, compared to the corresponding unplanted soils after a 5-month experimental period, are shown in Figure 1.

In *V. rosea*-planted soils, the SH percentage increased significantly from 41.59% ± 0.56% in crude oil and ranged from 60.71% ± 0.64% to 45.42% ± 0.43% under 1% and 7% crude oil treatment levels, respectively. In the unplanted soils, the SH percentage ranged from 50.75 ± 0.39 to 43.37 ± 0.26 under 1% and 7% crude oil treatment levels, respectively. The increase in SH percentage decreased with the increase in crude oil treatment levels in planted and unplanted soils.

An opposite trend was observed for the AH percentages. The AH percentage decreased significantly from 58.41% ± 0.56% in crude oil and ranged from 39.29% ± 0.64% to 54.58% ± 0.43% under 1%, and 7% crude oil soil treatment levels, respectively. In the unplanted soils, the SH percentage ranged from 49.25 ± 0.39 to 56.63 ± 0.26 under 1% and 7% crude oil soil treatment levels, respectively.

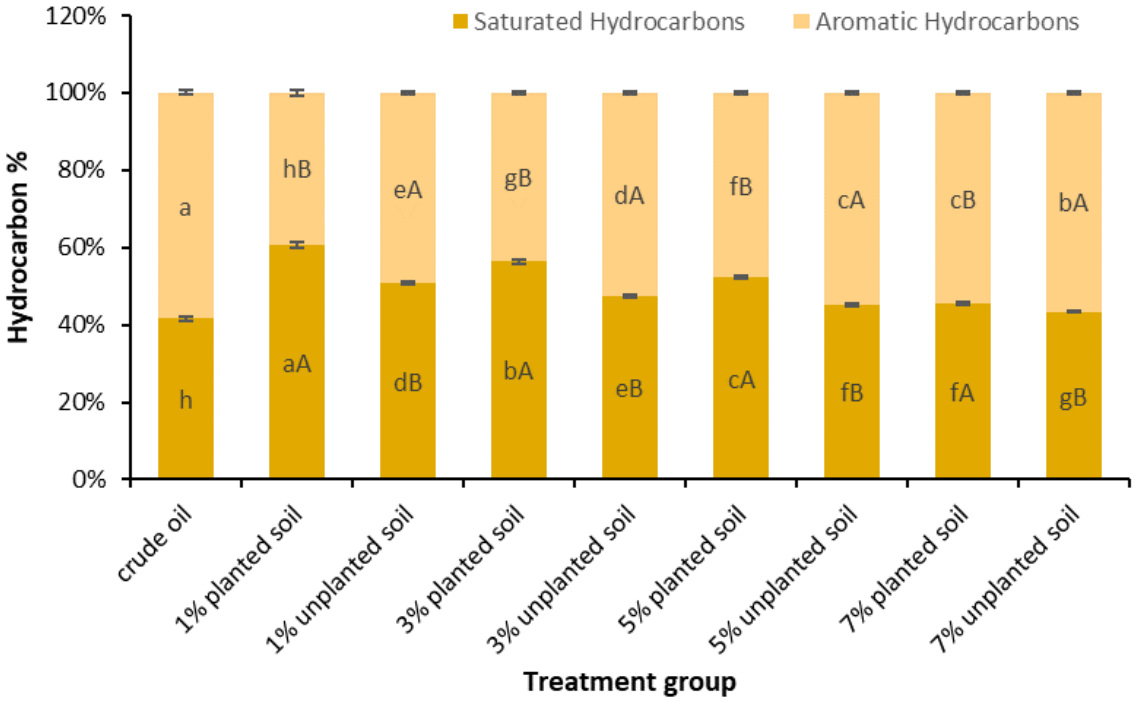

Figure 1. Changes in total saturated and total aromatic hydrocarbons extracted from *Vinca rosea*-planted soils treated with 1%, 3%, 5%, and 7% crude oil after a 5-month experimental period. Results are compared with the initial amounts of crude oil and the residual amounts of crude oil in the unplanted soils as controls. Different lowercase letters indicate significant differences in hydrocarbon percentages between different treatment levels in treated and untreated soils and including crude oil. Different uppercase letters indicate significant differences between the planted and unplanted soil at each crude oil treatment level at $p < 0.05$. Values are expressed as mean ± SE (n = 3).

3.2. Saturated Hydrocarbon

The changes in the relative abundance of SH and its n-paraffin and iso-paraffin compounds are shown in Figures 2 and 3 and Table 1. The experimental groups of 1% and 7% crude oil soil treatment demonstrate the lowest and highest levels of crude oil treatment, respectively.

The initial amounts of SH in crude oil and the residual amounts of hydrocarbons in unplanted soils were considered controls. The chromatograms (Figure 2) show that the SH fraction in the crude oil (control) demonstrated a wider spectrum of n-paraffin peaks than the oil extracted from unplanted and planted soils. Crude oil had 36 n-paraffinic compounds where the number of normal carbon atoms (n-carbon) ranged from 9 (C9) to 44 (C44). After a 5-month experimental period, the compounds in the range of C9 to C15 completely disappeared from planted and unplanted soils at 1% and 7% oil treatment levels (Figure 3, Table 1).

Under the 1% crude oil treatment level, the compounds with C16 and C17 were completely removed but were present in the corresponding unplanted control. Also, a wide range of n-paraffins was significantly reduced in the range of C18 to C34 when compared to the unplanted control. While under the 7% crude oil treatment level, C16 was removed from *V. rosea*-planted soil but appeared in unplanted control, and many compounds in the range of C17 to C35 in *V. rosea*-planted soil have reduced values in comparison to unplanted control.

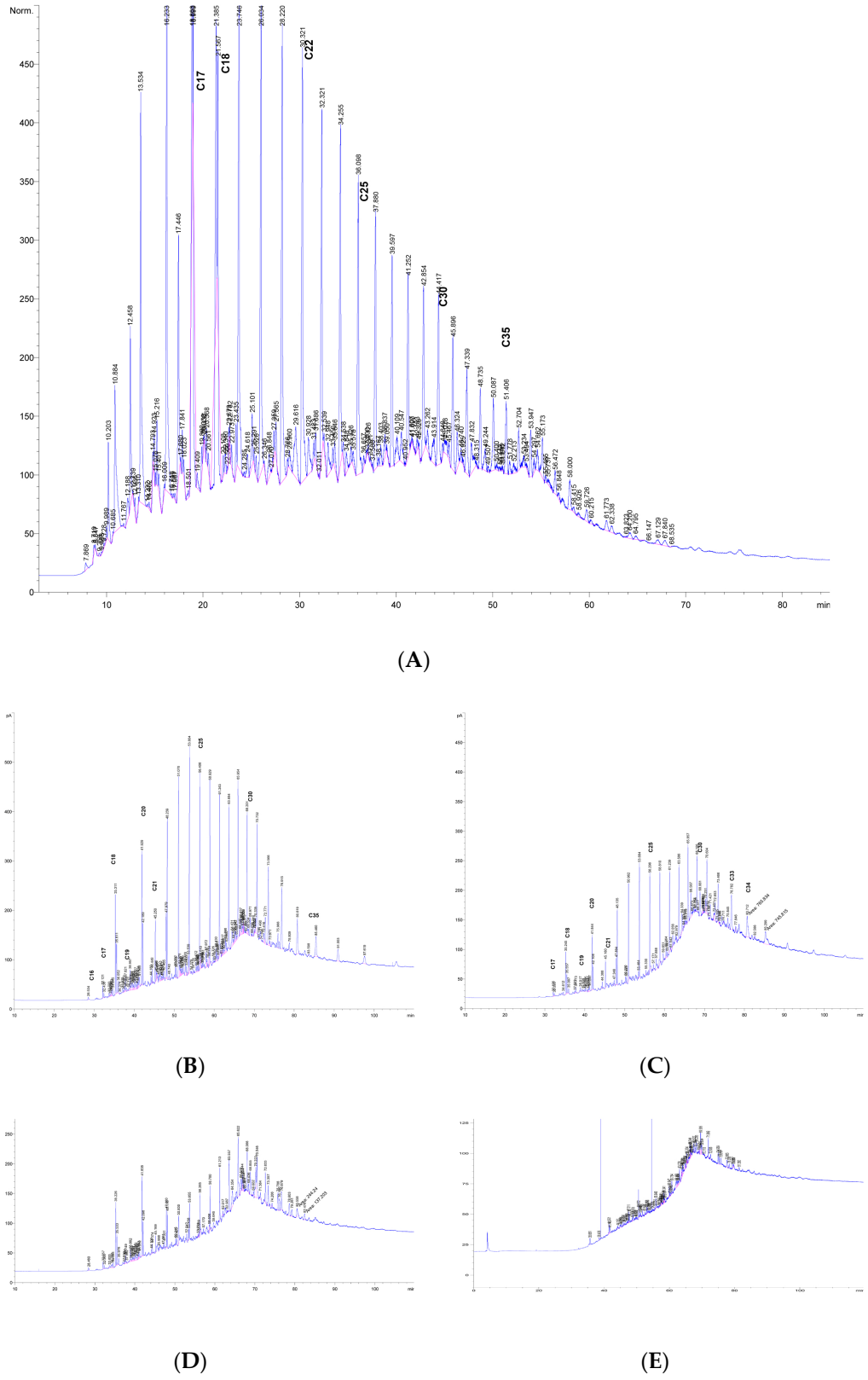

Figure 2. Chromatograms of saturated hydrocarbons extracted from crude oil (**A**), unplanted soil treated with 7% crude oil (**B**), *Vinca rosea*-planted soil treated with 7% crude oil (**C**), unplanted soil treated with 1% crude oil (**D**), and *Vinca rosea*-planted soil treated with 1% crude oil (**E**) after a 5-month experimental period.

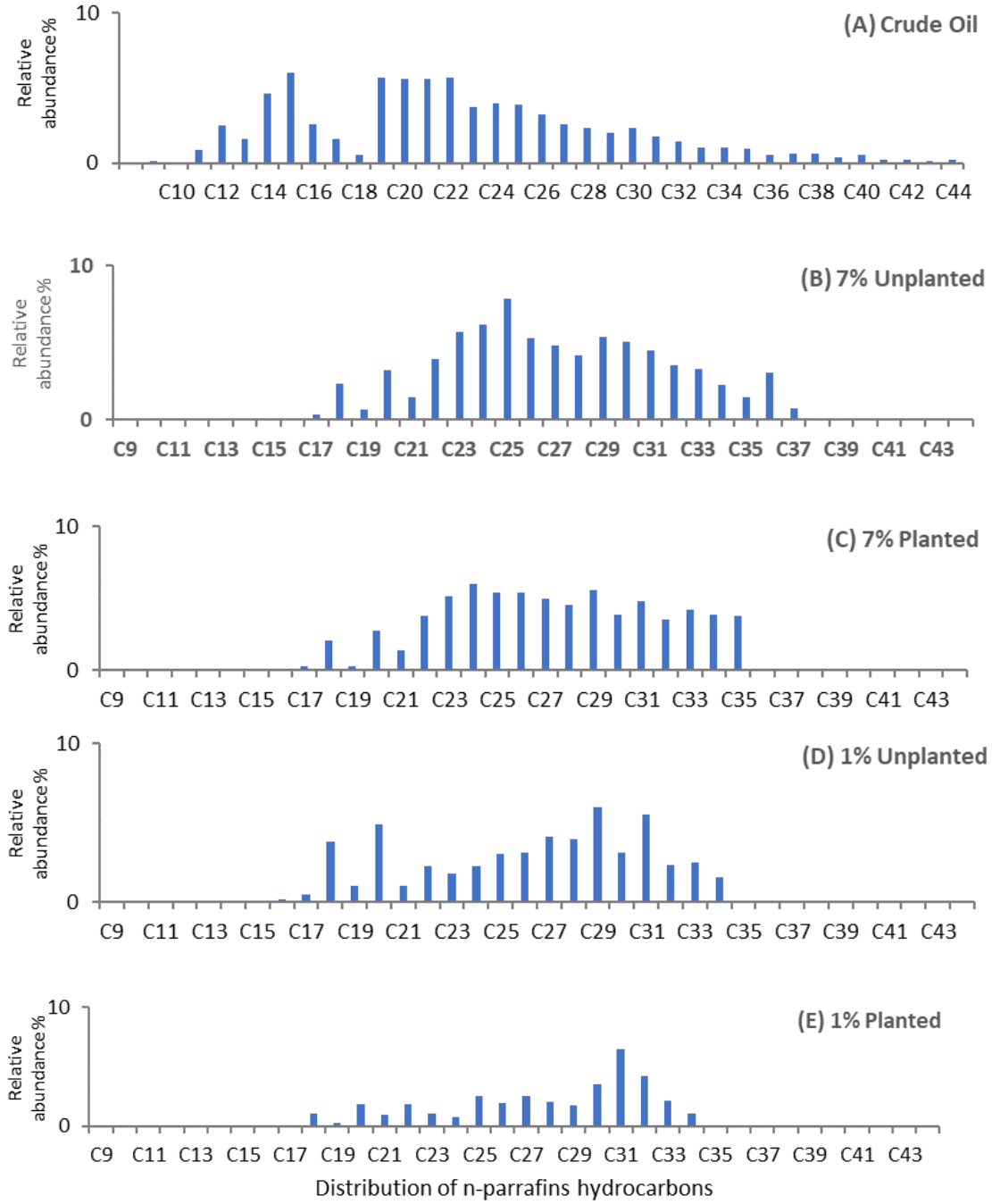

Figure 3. Relative abundance of n-paraffin compounds in the saturated hydrocarbon fractions after a 5-month experimental period extracted from (**A**) crude oil, (**B**) unplanted soil treated with 7% crude oil, (**C**) *Vinca rosea*-planted soil treated with 7% crude oil, (**D**) unplanted soil treated with 1% crude oil, and (**E**) *Vinca rosea*-planted soil treated with 1% crude oil.

The compounds in the range of C38 to C44 completely disappeared from planted and unplanted soils at 1% and 7% treatment levels. In 1% crude oil-treated soils, C35, C36, and C37 compounds were completely removed from planted and unplanted soils. The compounds of C36 and C37 were missed in the spectrum of *V. rosea*-planted soil at a 7% treatment level, while they were present in unplanted soil at the same level of crude oil treatment.

Table 1. Relative quantitative analysis of saturated hydrocarbons extracted from soils treated with 1% and 7% crude oil after a 5-month growth of *Vinca rosea*. The crude oil initial amounts and the residual amounts of crude oil in treated unplanted soils were compared to the results as controls.

Carbon Number	Crude Oil	1%		7%	
		Unplanted	Planted	Unplanted	Planted
C9	0.096	0.00	0.00	0.00	0.00
C10	0.048	0.00	0.00	0.00	0.00
C11	0.856	0.00	0.00	0.00	0.00
C12	2.48	0.00	0.00	0.00	0.00
C13	1.619	0.00	0.00	0.00	0.00
C14	4.622	0.00	0.00	0.00	0.00
C15	6.039	0.00	0.00	0.00	0.00
C16	2.6	0.21	0.00	0.07	0.00
C17	1.578	0.50	0.00	0.30	0.29
C18	0.568	3.85	1.10	2.30	2.12
C19	5.652	1.04	0.32	0.64	0.26
C20	5.562	4.94	1.89	3.19	2.74
C21	5.599	1.06	0.95	1.47	1.39
C22	5.654	2.30	1.89	3.96	3.84
C23	3.753	1.78	1.04	5.71	5.14
C24	3.987	2.29	0.77	6.19	6.06
C25	3.906	3.04	2.55	7.84	5.45
C26	3.21	3.14	1.93	5.27	5.46
C27	2.614	4.12	2.57	4.84	4.98
C28	2.364	3.97	2.10	4.19	4.54
C29	2.041	6.00	1.79	5.38	5.62
C30	2.345	3.10	3.52	5.06	3.85
C31	1.764	5.56	6.48	4.48	4.85
C32	1.4	2.36	4.21	3.56	3.58
C33	1.006	2.49	2.12	3.30	4.26
C34	0.992	1.55	1.10	2.27	3.86
C35	0.981	0.00	0.00	1.48	3.76
C36	0.508	0.00	0.00	3.04	0.00
C37	0.602	0.00	0.00	0.73	0.00
C38	0.608	0.00	0.00	0.00	0.00
C39	0.346	0.00	0.00	0.00	0.00
C40	0.502	0.00	0.00	0.00	0.00
C41	0.233	0.00	0.00	0.00	0.00
C42	0.223	0.00	0.00	0.00	0.00
C43	0.15	0.00	0.00	0.00	0.00
C44	0.172	0.00	0.00	0.00	0.00

Table 1. *Cont.*

Carbon Number	Crude Oil	1%		7%	
		Unplanted	Planted	Unplanted	Planted
n-paraffins %	76.68	53.28	36.35	75.29	72.07
Iso-paraffins %	23.32	46.72	63.65	24.71	27.93
n-paraffins/iso-paraffins	3.29	1.14	0.57	3.05	2.58

n-paraffins–normal paraffins

Generally, there was a significant reduction in n-paraffin compounds in *V. rosea*-planted soils in comparison to crude oil and unplanted controls. Both levels of treatments, 1% and 7%, had a relatively low ratio of n-paraffins to iso-paraffins (0.57 and 2.58, respectively) when compared to their unplanted controls (1.14 and 3.05, respectively), while crude oil showed the relative highest ratio of 3.29.

3.3. Aromatic Hydrocarbons

The individual aromatic constituents of *V. rosea*-planted soils are shown in Table 2. The PAH decreased from 45.22 ± 3.51 in crude oil and ranged from 10.01 ± 0.31 to 21.87 ± 0.35 in *V. rosea*-planted soils treated with 1% and 7% crude oil, respectively, while in unplanted soils the values ranged from 27.44 ± 0.37 to 33.24 ± 0.24, respectively.

Table 2. Aromatic constituents of *Vinca rosea*-planted soils treated with different levels of crude oil (1%, 3%, 5%, and 7%). The results are compared to initial amounts in crude oil and residual amounts in unplanted soils. Values are expressed as mean ± SE (n = 3). Different lower case letters in the same column indicate significant differences between the treatments 1% to 7% at $p < 0.05$ in planted and unplanted soil. Different capital letters in the column indicate significant differences between planted and unplanted soil at $p < 0.05$. Values in planted and unplanted soils are significantly different from the crude oil at $p < 0.05$ levels.

		Aromatic Constituent		
		Monoaromatic	Diaromatics	Polyaromatics
		(Wt. %)	(Wt. %)	(Wt. %)
Crude oil		5.06 ± 0.51	8.13 ± 1.51	45.22 ± 3.51
Planted Soil	1%	19.26 ± 0.22 ^{aA}	10.02 ± 0.91 ^{aA}	10.01 ± 0.31 ^{aA}
	3%	15.74 ± 0.91 ^{bA}	15.08 ± 0.31 ^{bB}	13.81 ± 0.28 ^{bB}
	5%	15.11 ± 0.44 ^{bA}	17.23 ± 0.74 ^{cC}	15.28 ± 0.51 ^{cC}
	7%	13.23 ± 0.21 ^{cA}	19.48 ± 0.51 ^{dD}	21.87 ± 0.35 ^{dE}
Unplanted Soil	1%	11.60 ± 0.64 ^{aB}	10.21 ± 0.85 ^{aA}	27.44 ± 0.37 ^{aB}
	3%	10.21 ± 0.33 ^{aB}	12.15 ± 0.27 ^{bC}	30.29 ± 0.49 ^{bC}
	5%	07.90 ± 0.48 ^{bB}	15.50 ± 0.65 ^{cD}	31.55 ± 0.68 ^{bD}
	7%	06.71 ± 0.43 ^{bB}	16.68 ± 0.87 ^{cE}	33.24 ± 0.24 ^{cD}

The monoaromatic and diaromatic contents of crude oil were 5.06 ± 0.51 and 8.13 ± 1.51, respectively. These values increased to 19.26 ± 0.22 for monoaromatics and 10.02 ± 0.91 for diaromatics in *V. rosea*-planted soil treated with 1% crude oil, while in planted soil treated with 7%, the values were 13.23 ± 0.21 and 19.48 ± 0.51 for monoaromatics and diaromatics, respectively.

The overall indicated trend of the change in aromatic constituents showed that the PAH degradation was enhanced by the presence of *V. rosea* plant in soil, which was also reflected in the increase of monoaromatic and diaromatic constituents in oil-treated planted soils in comparison to controls, i.e., crude oil and unplanted soils.

3.4. Sulfur Content

Sulfur was measured in the unplanted and *V. rosea*-planted soils after a 5-month crude oil treatment period. There was a significant reduction in sulfur in planted soils when compared to their corresponding unplanted soils (Figure 4). In *V. rosea*-planted soils, the sulfur values ranged from 322 ± 1.59 to 433 ± 1.66 under 1% and 7% crude oil treatment levels, respectively. In unplanted soils, the sulfur amount was significantly higher than their corresponding values in planted soils, where the values ranged from 1149 ± 0.50 to 1623 ± 0.46 under 1% and 7% crude oil treatment levels, respectively.

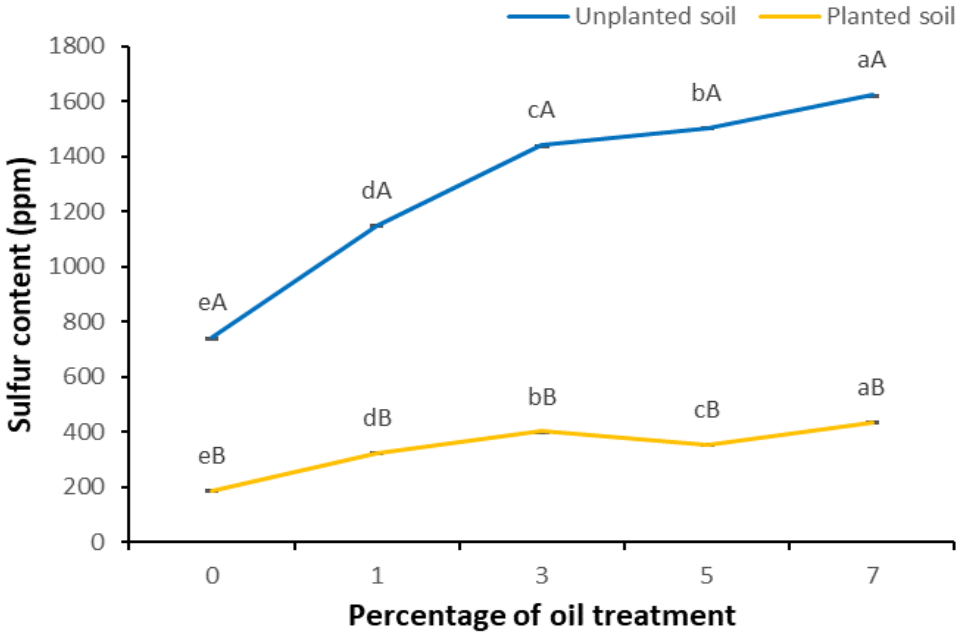

Figure 4. Sulfur content in soil planted with *Vinca rosea* and treated with 0%, 1%, 3%, 5%, and 7% of crude oil after a 5-month experimental period compared to unplanted soil with the same crude oil treatment levels. Different lowercase letters indicate significant differences between different treatments, and different uppercase letters indicate significant differences between the planted and unplanted treatments at each crude oil concentration at $p < 0.05$. Values are expressed as mean ± SE (n = 3).

Sulfur accumulation in *V. rosea* tissues is demonstrated in Figure 5. The accumulated sulfur in plant roots raised in untreated soil was 685 ± 2.94 ppm, while it increased and ranged from 720 ± 0.49 ppm to 975 ± 0.29 ppm in plant roots raised under 1% and 7% crude oil treatments, respectively. The sulfur concentration in the shoots of plants raised under 1% and 7% treatment levels ranged from 300 ± 0.47 ppm to 460 ± 0.49 ppm, respectively, compared to 159 ± 0.24 ppm in the shoots of plants raised in untreated soil. This indicated that the roots of *V. rosea* accumulated more sulfur than the shoots and that sulfur content in plant tissues increased with the increased crude oil treatment level.

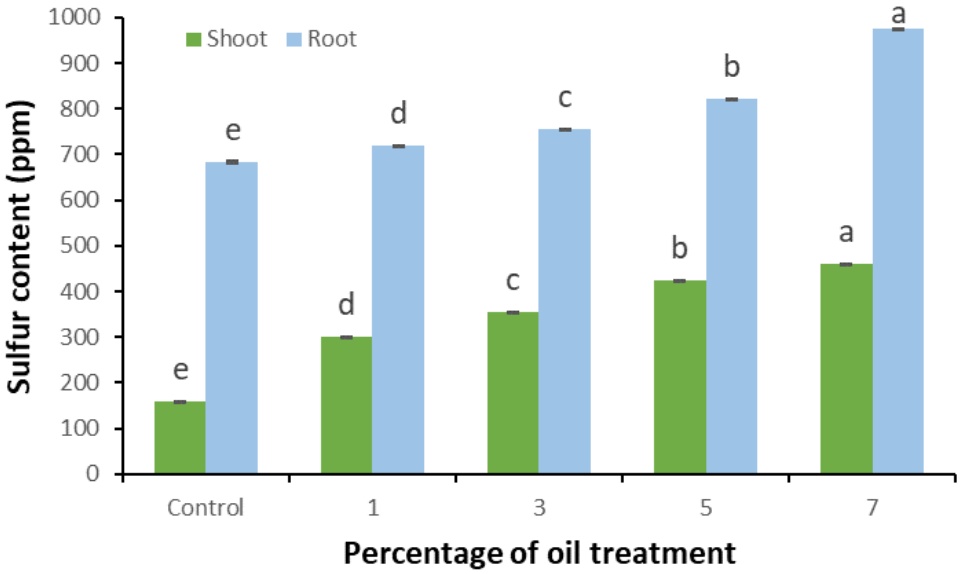

Figure 5. Sulfur content in shoots and roots of *Vinca rosea* plants raised under 0% (control), 1%, 3%, 5%, and 7% treatment levels after a 5-month experimental period. Different letters indicate significant differences between different treatments at $p < 0.05$. Values are expressed as mean ± SE (n = 3).

3.5. Lead Content

The lead content in the shoots and roots of *V. rosea* plants raised under 1%, 3%, 5%, and 7% crude oil treatment levels is shown in Figure 6. In plant shoots, the lead content significantly increased from 22 ± 1.73 µg/g dry weight in plants raised in untreated soil (control) to 69.5 ± 1.44, 82.5 ± 1.44, 77.33 ± 1.45, and 79.33 ± 2.03 µg/g dry weight in plants raised under 1%, 3%, 5%, and 7% crude oil treatment levels, respectively. As for the plant roots, lead content increased from 13 ± 1.55 µg/g dry weight in control plants to 80.67 ± 0.88, 81 ± 1.16, 80.67 ± 3.18, and 80 ± 0.577 in µg/g dry weight in plants raised under 1%, 3%, 5%, and 7% crude oil treatment levels, respectively.

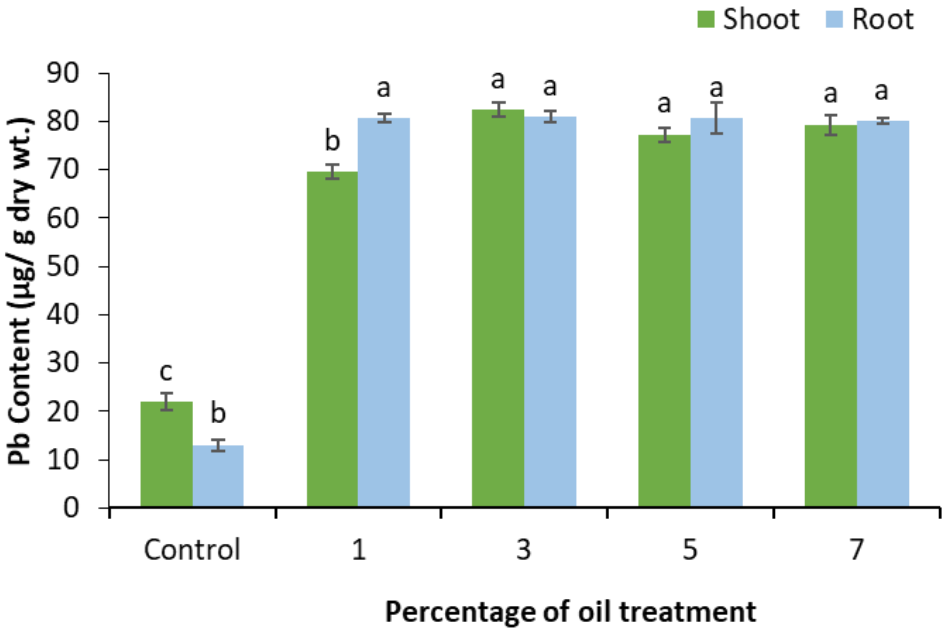

Figure 6. Lead content in shoots and roots of *Vinca rosea* plants raised under 0% (control), 1%, 3%, 5%, and 7% treatment levels after a 5-month experimental period. Different letters indicate significant differences between different treatment levels at $p < 0.05$. Values are expressed as mean ± SE (n = 3).

3.6. Cadmium Content

The cadmium content in the shoots and roots of *V. rosea* raised under different crude oil treatment levels is shown in Figure 7. The cadmium content significantly increased in plant shoots from 10 ± 1.15 µg/g dry weight in plants raised in untreated soil to 40 ± 1.15, 40.67 ± 3.76, 42 ± 1.73, and 45 ± 1.73 µg/g dry weight in plants raised under 1%, 3%, 5%, and 7% crude oil treatment levels, respectively. As for the root, the values ranged from 9.67 ± 0.33 µg/g dry weight in the plants raised in untreated soil to 30.67 ± 1.45, 29.67 ± 0.88, 37 ± 0.58, and 35 ± 0.73 µg/g dry weight in plants raised under 1%, 3%, 5%, and 7% crude oil treatment levels, respectively.

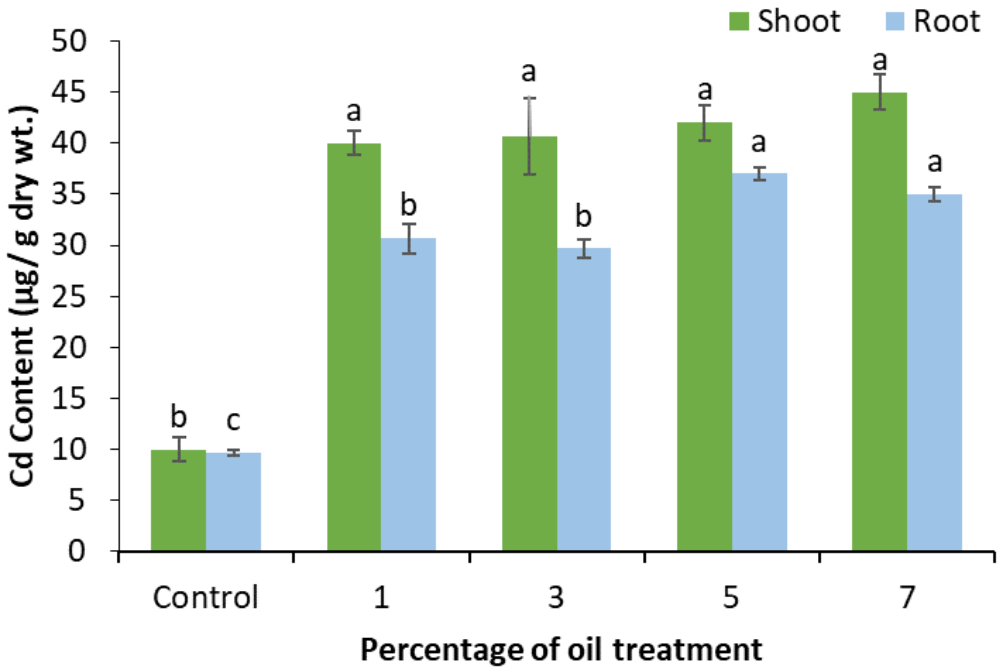

Figure 7. Cadmium content in shoots and roots of *Vinca rosea* plants raised under 0% (control), 1%, 3%, 5%, and 7% crude oil treatment levels after a 5-month experimental period. Different letters indicate significant differences between different treatments at $p < 0.05$. Values are expressed as mean ± SE (n = 3).

4. Discussion

Petroleum hydrocarbon pollution of soils negatively affects its physical and chemical characteristics, the microbial population, plant growth, and functionality, and human and animal health [34]. A broad range of different organic compounds with different molecular weights are found in crude oil. Generally, crude petroleum oil has a complex mixture of hydrocarbons, nitrogen, oxygen, and sulfur compounds, as well as metallic constituents, which may affect the bioavailability of each compound [35].

As shown in our previous study on *V. rosea* [31], the presence of *V. rosea* plants may enhance the total petroleum hydrocarbons degradation in the soil, particularly at low crude oil treatment levels (up to 5%). A lower TPH degradation percentage was recorded in the unplanted control soils treated with 1%, 3%, 5%, and 7% crude oil. The analysis of the extracted petroleum hydrocarbon fractions showed that the SH percentage in the soil planted with *V. rosea* was higher than the percentage of total SH in crude oil and the unplanted control. The reduction in total AH as a consequent effect of plant cultivation was similar to results reported in studies on other plant species, including the ornamental species *Bassia scoparia* [24], *Eichhornia crassipes* [17], *Zea mays* [36], *Vetiveria zizanioides*, *Bidens pilosa*, *Eleusine indica* [37], and fourteen ornamental plant species raised in crude oil-contaminated soil [38]. This reduction may be attributed to the effect of the rhizosphere supported by the fibrous root system of *V. rosea*. The presence of *V. rosea* plants may enhance the rhizosphere microorganisms by supplying exudates, enzymes, and oxygen

through the roots [24]. The decrease in AH with increasing SH may be due to the selective nature of the plant rhizosphere toward different microbial species. *V. rosea* exhibited a greater selection of total hydrocarbon degradation bacteria species, especially those with PAH-degrading capacity.

The rhizosphere is a highly selective environment. This has been shown in many studies such as legume rhizospheres appearing to be good at selecting PAH-degrading microbial communities [39]. The selective property of the rhizosphere towards different organisms has been attributed to the exudation of root-derived carbon sources and other substances such as phenolics, isoflavonoids, and enzymes [40]. Some root exudates have been connected to enhanced degradation of particular hydrocarbons by stimulation of enzymatic pathways or by acting as analogous contaminants with parallel structures [39].

The analysis of the SH fraction showed a potential capacity of the *V. rosea* plant to phytoremediate n-paraffin compounds. The shorter n-paraffins, ranging from C9 to C16, which were present in crude oil, were removed from planted and unplanted soils. This indicates that their removal was a result of environmental weathering factors without the involvement of the plant effect. The amount of n-paraffinic compounds with a chain length between C18 and C34 was decreased by the effect of *V. rosea* presence when compared to the polluted unplanted control. The complete removal of n-paraffinic compounds, especially the heavy compounds, such as C36 and C37 chain length, in the planted soil may be attributed to the enhanced degradation by *V. rosea* and its associated rhizosphere microflora, and this may be due to the phytostimulation process caused by the plant. In the phytostimulation process, plant exudates are released from the roots and enhance the microbial degradation of pollutants [41].

The significant remediation effect of *V. rosea* seems to be due to the biodegradable nature of the n-paraffins, particularly of the short chain lengths like C16 and C17 [42]. The effective degradation of heavy n-paraffins, such as compounds with a C36 to C40 length chain, by the oxidative enzymes of microflora was demonstrated in other studies [43,44], and, on the other hand, the successful potential of *V. rosea* to phytoremediate petroleum hydrocarbons in diesel exhaust was reported by [30].

With respect to the plant effect on the composition of the saturated fraction, a significant reduction in n-paraffins with an increase in iso-paraffins was demonstrated in *V. rosea*-planted soils. The increase in iso-paraffins may be attributed to their nature, as they are more difficult to biodegrade than n-paraffins [45]. The degraded n-paraffin's fate through phytoremediation may be its isomerization, i.e., its conversion to iso-paraffins, which was proved previously [15,24,46]. This study supports this explanation since the fractions of n-paraffins in crude oil and unplanted control were decreased by the effect of *V. rosea* cultivation, while the opposite was found for iso-paraffin fractions, which increased in *V. rosea*-planted soils in comparison to crude oil and unplanted control.

The lower ratios of n-paraffins to iso-paraffins in planted soils indicate that the cultivation of *V. rosea* plants in crude oil-polluted soils enhances the degradation of saturated hydrocarbons. The abundance of PAH was reduced while monocyclic and dicyclic aromatic hydrocarbons were increased in *V. rosea*-planted soils in comparison to crude oil and unplanted controls. The ratio of n-paraffins to iso-paraffins indicates the relative degradation under each treatment level [47]. The larger PAH are converted to lower compounds by the mechanism of oxidative ring cleavage, where one compound's ring is oxidized, producing dihydrodiols that are cleaved to lower PAH derivatives [48]. The increase in the abundance of monoaromatic and diaromatic hydrocarbons may be attributed to the large PAH degradation into smaller aromatic hydrocarbons as reported in previous studies [15,24].

Sulfur analysis showed that the content in *V. rosea*-planted soil was less than in unplanted soil. In addition, the concentration of sulfur in planted and unplanted soil increased with the increase in crude oil concentration. The reduction of sulfur in soil matches the degradation and reduction of high molecular weight PAH. The determination of sulfur content in plant shoots and roots indicated that *V. rosea* can extract and accumulate high amounts of sulfur from polluted soils. The greater sulfur content in the roots than

in the shoots may be attributed to the direct contact of the roots with the soil. When the degradation of petroleum hydrocarbons occurs, sulfur becomes free, and the uptake of sulfur by the plant increases, leading to a decrease in its content in soil and an increase in plant tissues [49]. Other plant species were recorded for their ability to hyperaccumulate sulfur while phytoremediating petroleum hydrocarbons, such as *Eichhornia crassipes* and *Bassia scoparia* [17,24].

The analysis of plant tissues showed that crude oil pollution in soils caused higher concentrations of heavy metals, cadmium and lead, to accumulate in *V. rosea* shoots and roots. Although several metals are necessary for the proper functioning of green plants' metabolic pathways, high concentrations of heavy metals disrupt cellular metabolism and cause toxicity as a result of the inactivation of biological processes [50]. The metal ions Cd^{2+}, Pb^{2+}, Pb^{4+}, Zn^{2+}, Cu^{2+}, and Co^{2+} are among the most toxic to higher plants, especially when present in high concentrations due to environmental contaminants, such as crude oil, which causes the production of free radicals, oxidative stress-causing reactive oxygen species (ROS) [51], disruption of plant metabolism, decreased chlorophyll a/b ratio, and DNA damage [50,52]. Thus, phytoremediation of heavy metal-polluted soils can reduce their toxicity and minimize their potential threat [53].

5. Conclusions

V. rosea is a promising plant species for phytoremediation of soil contaminated with crude oil. This conclusion was proved by the species' capability to decrease the percentage of AH in soil as well as its ability to absorb and accumulate sulfur that is associated with AH. Due to the phytoremediation role of *V. rosea*, the toxicity of high molecular weight PAH decreases in crude oil-contaminated soils. In addition, the plant has the potential to tolerate crude oil-contaminated soil. Further in-depth research is recommended for different other concentrations of pollutants to have a full evaluation of their abilities. Genetic and molecular studies are required for *V. rosea* to determine the stress tolerance genes that are responsible for their ability to withstand and remediate polluted soils.

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