Phyto-Tolerance Degradation of Hydrocarbons and Accumulation of Heavy Metals by of *Cajanus cajan* (Pigeon Pea) in Petroleum-Oily-Sludge-Contaminated Soil

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Abstract: A pot experiment was conducted to measure the phyto-tolerance and accumulation of heavy metals in petroleum oil sludge POS by *Cajanus cajan* (pigeon pea) on soils treated with five different concentrations (1%, 2%, 3%, 4%, and 5% w/w) of the POS. The response of the plant to oily sludge varied significantly from the untreated control and among the various treatments. The growth of *C. cajan* was slightly (but not significantly) influenced by the oily sludge in soil; growth of *C. cajan* at relatively lower concentrations of POS (1 to 3%) was greater than in the treatments with relatively higher concentrations POS (4 to 5%). A significant interaction was observed in the relative growth rates (RGRs) of *C. cajan*, which significantly increased in the treatments with relatively low POS (1 to 3%) and decrease significantly at higher POS concentrations. The heavy metal content of the plant roots as the POS concentrations were increase show that the concentration of all heavy metals in the roots increased accordingly. Cu showed the highest accumulation with an increase from 1.9 to 6.8 mg/kg followed by Pb, Zn, Ni, Mn, and Cr, which was the least-accumulated. Heavy metal analysis in *C. cajan* tissues indicated a considerable accumulation of the metals Pb, Zn, Ni, Mn, Cu, and Cr in the root and stem of the plant, with negligible metal concentrations detected in the plant leaves, suggesting a low translocation factor but indicating that *C. cajan* is resistant to heavy metals. As the search for more eco-friendly and sustainable remediating green plant continues, *C. cajan* shows great potential for reclaiming POS-contaminated soil due to the above properties including resistance to toxic heavy metals from oily sludge. These findings will provide solutions to polluted soils and their subsequent re-vegetation.

Keywords: phytoremediation; petroleum oily sludge; *Cajanus cajan*; heavy metals; legumes

1. Introduction

The petroleum industry has, over the years, led to the production of a considerable amount of oily sludge which contaminates the environment and is toxic to human and environmental health [1]. Several components of petroleum oily sludge POS are toxic, carcinogenic, and mutagenic [2]. The components in oil include n-alkanes, branched alkanes, cycloalkanes, polycyclic aromatic hydrocarbons (PAH), nitrogen, sulfur, and oxygen-containing heterocyclics, asphaltenates, and resins [3]. Likewise, the heavy metal component of POS is a serious threat for both terrestrial and aquatic ecosystems [4]. Many respiratory, renal, and central nervous system disorders are due to the effect of n-alkanes,
branched alkanes, and cycloalkanes. Among the methods available for cleaning up oily-sludge-contaminated environments biologically is bioremediation thought is efficient but had limitations, which phytoremediation provides solutions to. These solutions include the accumulation and uptake of non-degradable heavy metals by the plant [4]. Phytoremediation, a subgroup of bioremediation, utilizes special plants to remediate contaminated soils, sediments, and surface and ground waters [5]. Phytoremediation has been increasingly considered because of its low-cost and environment-friendly features such as thermochemical decomposition (pyrolysis) [5]. Some plant uptake contaminants into the root tissues only, while other plants translocate them from the root to the upper parts of the plant; the plant is then harvested for further appropriate disposal [6]. The contaminants include metals such as Ag, Cd, Co, Cr, Cu, Hg, Mn, Mo, Ni, Pb, and Zn, metalloids (As, Se) [7], radionuclides (Sr, Cs, U), and nonmetals (B) [8]. Plants that uptake a large number of heavy metals (hyperaccumulators) have the capacity to accumulate higher amounts than normal plants [9]. The collection of these hyperaccumulators in phytoextraction is used in the new technology known as phytomining. Other authors refer to it as bio-mining [5]. When such a plant is used to extract a metal of economic importance, it is known as phytomining which is a included under phytoremediation and is fast-growing technology [10]. The legume plant is used to immobilize metalloid or heavy metal contamination in a particular environment. In this process, the plant will not produce the necessary uptake of the contaminant but by precipitation or sorption of the metal will make it inactive through this biological process [11]. It can do this with both contaminated soil and water and it can occur through sorption, precipitation, complexation, or metal valence reduction [12]. This process works by reducing the amount of water percolation in the soil. Therefore, phytoremediation may expose a new opportunity for a more efficient and sustainable solution for the remediation of POS-contaminated soils [13]. Various plants have been identified for their potential to facilitate the phytoremediation of sites contaminated with petroleum hydrocarbons and the majority of studies singled out grasses and legumes as having higher potential [14,15]. Legumes are known to have an advantage over non-leguminous plants in phytoremediation because of their ability to fix nitrogen [16], and thus, they do not have to compete with microorganisms and other plants for limited supplies of available soil nitrogen at oil-contaminated sites. Common desirable characteristics of these plants are the ability to fix nitrogen (a major limiting factor for effective degradation of pollutants) and drought tolerance [16].

*Cajan cajan* (pigeon pea) (kacang dhal) in Bahasa Malaysia is a common legume crop in tropical countries, and it serves as a very important source of protein in human diets. It has a long root system which withstands different soil conditions and properties [17,18]. It resists pH over a wide range and temperatures from 10 to 35 °C. Perennial pigeon pea is used as a multi-purpose species for agroforestry systems in India where its numerous uses include food, fodder, manure, and firewood [19]. In addition, pigeon pea improves soil fertility by nutrient cycling and biological nitrogen fixation. In Africa, tall perennial pigeon pea is often used as live fences, barriers, and in soil conservation and is also favored for use as fuelwood and basket weaving in African villages [20]. Previously, we reported on the ability of this plant to grow and tolerate POS and showed the changes in the metagenomic property of the rhizome in adapting to a high concentration of POS [21]. The objectives of this study were to determine the tolerance of *C. cajan* to POS at different concentrations, using several *C. cajan* biomass markers as indicators of toxicity, and to determine the accumulation of heavy metals in the plant.

## 2. Materials and Methods

### 2.1. Experimental Setup

The POS used in this study was obtained from the Shell refinery center in Port Dickson, Negeri Sembilan, Malaysia in 2014. The oil sludge was collected in a clean jerry can and transported to the biotechnology garden of the University Putra Malaysia. Agricultural soil was collected from University Putra Malaysia agricultural farm while seeds of *Cajan s
cajan was also bought from a local market. The experimental design consisted of five treatments in replicates. The five treatments were: (CR1) 1% oil sludge + C. cajan; (CR2) 2% oil sludge + C. cajan; (CR3) 3% oil sludge + C. cajan; (CR4) 4% oil sludge + C. cajan; (CR5) 5% oil sludge + C. cajan; (CN1%, 2%, 3%, 4%, and 5%) oil sludge without C. cajan; and (UR) uncontaminated with oil sludge + C. cajan [21].

2.2. Treatment of Soil with Petroleum Oil Sludge

Soil was air-dried for a period of one week, homogenized, and filtered by passing through a 4 mm mesh sieve. Then, 3 kg of soil was measured into clean dry plastic pots and moistened to 20% with distilled water to ensure proper mixing with the sludge. Oil sludge of 5%, 4%, 3%, 2%, and 1% concentration was properly agitated and thoroughly mixed to ensure uniform soil contamination, together with an uncontaminated control. The pots were allowed to remain undisturbed for 7 days. The soil samples were taken for physicochemical analysis before treatment. After the 7 days, seeds of C. cajan was planted accordingly [21].

The viability of the seeds of C. cajan and was tested by the floatation method. The seeds were tested by soaking in distilled water for 5 min. The floating seeds (nonviable) were removed and the water was drained immediately from the seeds that sank (viable seeds). Since both seeds are dormant due to their coat, germination of the seeds was enhanced by softening or weakening the seed coat (scarification) using warm water overnight [14]. The seeds were sterilized in 70% ethanol for 2 min then rinsed twice with sterile water before planting [14]. The seed was sown to a depth of 2.5 to 5 cm; five seeds were planted in a hole. After sowing, the soil was irrigated every day and emergence was observed subsequently.

2.3. Sampling and Measurements

The shoot number and height were measured weekly, from the 1st week after plant establishment to the 4th week. The plants were harvested after 30 days of growth for determination of dry/wet weight. The uprooted plants were washed with de-ionized water to remove any adhering sediments and cut into the shoot (parts above soil level) and roots [22,23]. All samples were dried to constant weight at 70 °C for 5 days in a forced-air cabinet and weighed for the dry weight (DW). The relative growth rate (RGR) was determined using a formula [24].

2.4. Heavy Metal Analysis

Rhizosphere soil, root, shoot, and leaf samples from different treatment were collected and oven-dried at 70 °C for 2 days [24]. One gram of the samples collected was mixed with 15 mL of HNO₃ (15.7 M and 70% purity), H₂SO₄ (18.4 M and 98% purity), and HClO₄ (11.4 M and 70% purity) in the ratio (5:1:1) at 100 °C and we waited until a clear solution appeared. Whatman No. 42 filter paper was used to filter the digested samples, and the filtrates were diluted by 50 mL of deionized water and then stored for further analysis. Concentrations of six heavy metals in both samples of the dried soil and plant tissues were determined by atomic absorption spectrophotometer (AAS). The bioconcentration factor (BCF) and translocation factor (TF) of heavy metals by C. cajan was calculated using the following formula [24]:

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BCF = \frac{\text{mean heavy metal concentration in the plant/heavy metals in the soil}}{}
\]

\[
TF = \frac{\text{concentration above ground}}{\text{concentration in root}}
\]

2.5. Extent of Degradation of Hydrocarbon

The extent of degradation was determined by gas chromatography; 1 mL of the extracted oil was diluted with 1 mL n-hexane and transferred directly to a gas chromatography (GC) vial and analyzed by GC-FID using capillary column gas chromatography (Agilent Technologies, Model 7890A, GC System, Santa Clara, CA, USA), with a HP-5 5% phenyl methyl siloxane column (30 m × 0.32 mm × 0.25 m) and nitrogen as the carrier gas.
The column temperature was held at 50 °C for 1 min, and then increased by 15 °C per min to 320 °C for 10 min [4].

2.6. Statistical Analysis

The data generated from the experiments were analyzed using INSTAT 3 statistical package. One-way analysis of variance (ANOVA) was used. Tukey’s post-hoc analysis was applied to test for significance between means.

3. Results

3.1. Physicochemical Analysis of Soil and Emergence of C. cajan

The physicochemical analysis of the soil sample revealed that the pH value was 6.8. The pH remained slightly acidic to neutral, the soil temperature was 36 °C. Other physicochemical properties analyzed were percentage carbon, nitrogen, and phosphorus as shown in Table 1. Emergence started about four and six days after sowing. The number of plants that emerged was unaffected by the treatment with POS except for the highest concentration tested (CR5) where a significant reduction (p < 0.05) of 28.6% was observed (Figure 1).

| Soil | pH 6.8 ± 0.2 | Moisture (%) 12.1 ± 1.1 | Temperature (°C) 36 | Total carbon (%) 1.75 ± 0.05 | Total Nitrogen (%) 0.16 ± 0.01 | Total phosphorus (%) 0.07 ± 0.02 |
|------|-------------|-------------------------|----------------------|-----------------------------|-------------------------------|-------------------------------|

Figure 1. Percentage emergence of different treatments at various POS concentrations. Different letters are significantly different based on Tukey’s post-hoc analysis (p < 0.05). Bars represent means ± standard deviation (n = 3). UR is an uncontaminated rhizosphere and CR 1 to CR5 represent contaminated rhizosphere with POS from 1 to 5% w/w).

3.2. Growth and Response of C. cajan to POS

3.2.1. Effect of POS on Number of Shoots Per Pot

ANOVA analysis reveals that C. cajan exposed to the highest POS concentration of 5% (w/w) (CR5) showed a significant reduction of the number of shoots per pot (p < 0.05) as early as 7 days after emergence followed by POS concentration of 4% (w/w) (CR4), which showed a significant reduction of the number of shoots per pot (p < 0.05) at day 14 onwards. The reductions in the number of shoots for CR4 and CR5 were 40.6 and 53.1%, respectively. Other POS concentration did not significantly affect the number of shoots per pot at the end of the experimental period (Figure 2a). The same trend was observed
for plant height (Figure 2b). The reduction in plant height for CR4 and CR5 was 13.8 and 44.2%, respectively.

Figure 2. *C. cajan* number of shoots (a) and height (b) grown in POS-spiked soil. Bars represent means ± standard deviation (*n* = 3). UR is an uncontaminated rhizosphere and CR 1 to CR5 represent contaminated rhizosphere with POS from 1 to 5% *w*/*w*).

3.2.2. Effect of POS to Growth Rate

The relative growth rate of *C. cajan* at various POS concentrations showed a significant reduction at higher concentrations of POS (CR4 and CR5) at day 30 of growth while there was no significant difference in growth rate compared to control (UR) for other POS concentrations. However, there was a significantly higher growth rate observed at POS concentrations of 2 and 3% (*w*/*w*) (CR2 and CR3, respectively), for both periods of growth at day 60 and 90. The increments observed for CR2 and CR3 were 35.6 and 29.1% and 34.2 and 31.2% for day 60 and 90, respectively. At the same time, there was a significantly lower growth rate observed at POS concentrations of 4 and 5% (*w*/*w*) (CR4 and CR5, respectively), for both periods of growth at day 60 and 90 (Figure 3).
3.2.3. Effect of POS on Dry and Fresh Weights of Root and Shoot

At the relatively low POS concentrations of 1, 2, and 3% (w/w) represented by CR1, CR2, and CR3, respectively, a significant increase ($p < 0.05$) in the dry and fresh weights of the shoot/pot and root/pot was observed especially at days 60 and 90, with a more pronounced increase observed for dry weight compared to fresh weight. CR1, CR2, and CR3 treatments increased dry weight of the root at 44.0, 34.9, and 48.8% and of the shoot of 44.4, 71.0, and 69.4% at day 60, respectively, and an increase of dry weight of the root of 67.3%, 70.9, and 72.7% and of the shoot of 40.0, 69.3 and 68.0% at day 90, respectively. At the relatively high POS concentrations of 4 and 5% (w/w) represented by CR4 and CR5, respectively, a significant but small decrease ($p < 0.05$) in the dry weight root/pot of 18.1% was observed at day 90 while the dry weight of root/pot showed no significant changes at day 90 (Figure 4a–c). The stimulatory effect of POS at 2 and 3% (CR2 and CR3) to C. cajan measured by fresh weights of root and shoot/pot was significant at days 60 and 90 but was not more than 20%. On the other hand, the inhibitory effect of POS at 4 and 5% (CR4 and CR5) to C. cajan to the fresh weights of root and shoot/pot was significant at days 30, 60, and 90. CR4 treatment resulted in a reduction in the fresh weight of root at days 30, 60, and 90 of 35.6, 40.4, and 25%, respectively, and a reduction in the fresh weight of shoot at days 30, 60, and 90 of 30.3, 14.1, and 28%, respectively. CR5 treatment resulted in a larger reduction in the fresh weight of root at days 30, 60, and 90 of 62.5, 62.8, and 53.4%, respectively, and a reduction in the fresh weight of shoot at days 30, 60, and 90 of 37.1, 37.2, and 55.8%, respectively (Figure 4d–f).

3.3. Heavy Metal Analysis

3.3.1. Accumulation of Heavy Metals in C. cajan

The results reveal the ability of C. cajan to accumulate metals in the root. These are later translocated into the stem of the plant in an insignificant transfer. In the root of C. cajan grown in POS spiked soil after 90 days, at the concentration of POS of 1% (CR1), the results show accumulation of Pb to be 0.04 mg/kg, Zn was 2.13 mg/kg, Ni was 13.0 mg/kg, Mn was 1.93 mg/kg, and Cr was 0.03 mg/kg. Treatment CR2 showed Pb accumulation in the root to be 0.06 mg/kg, Zn was 2.70 mg/kg, Ni was 1.80 mg/kg, Mn was 0.53 mg/kg, Cu was 2.80 mg/kg, and Cr was 0.09 mg/kg. CR3 showed Pb to be 0.08 mg/kg, Zn was 3.40 mg/kg, Ni was 2.0 mg/kg, Mn was 0.86 mg/kg, Cu was 4.03 mg/kg, and Cr was
0.13 mg/kg. At the relatively higher concentration of CR4, the results show accumulation of Pb to be 0.12 mg/kg, Zn was 3.8 mg/kg, Ni was 1.93 mg/kg, Mn was 0.4 mg/kg, Cu was 5.66 mg/kg, and Cr was 0.08 mg/kg. CR5 shows Pb accumulation in the root to be 0.18 mg/kg, Zn was 4.16 mg/kg, Ni was 2.06 mg/kg, Mn was 0.53 mg/kg, Cu was 6.8 mg/kg, and Cr was 0.09 mg/kg. In summary, the results of the heavy metal analysis in *C. cajan* tissues indicated a considerable accumulation of the metals (Pb$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, Cu$^{2+}$, and Cr$^{6+}$) in the root and stem of the plant, with negligible metal concentrations detected in the plant leaves. Cu$^{2+}$, Zn$^{2+}$, Ni$^{2+}$, Mn$^{2+}$, and negligible Pb$^{2+}$ and Cr$^{6+}$ (Figure 5).

**Figure 4.** Effects of POS after 30, 60, and 90 days of plant growth to the dry (a–c) and fresh weight (d–f) biomass of *C. cajan*, respectively. Different letters to *C. cajan* root and shoot organs are significantly different based on Tukey post hoc analysis ($p < 0.05$). Bars represent means ± standard deviation ($n = 3$). UR is an uncontaminated rhizosphere and CR 1 to CR5 represent contaminated rhizosphere with POS from 1 to 5% *w/w*. 
Figure 5. Heavy metal concentrations in roots of *C. cajan*. Bars represent means ± standard deviation (*n* = 3). UR is an uncontaminated rhizosphere and CR 1 to CR5 represent contaminated rhizosphere with POS from 1 to 5% *w/w*).

Heavy metal concentration in the stem of *C. cajan* at POS of 1% or CR1 shows Pb to be 0.003 mg/kg, Zn was 0.6 mg/kg, Ni was 0.36 mg/kg, Mn was 0.16 mg/kg, Cu was 1.0 mg/kg, and Cr was 0.001 mg/kg. CR2 shows Pb to be 0.003 mg/kg, Zn was 0.96 mg/kg, Ni was 0.36 mg/kg, Mn was 0.33 mg/kg, Cu was 1.9 mg/kg, and Cr was 0.004 mg/kg. CR3 shows Pb to be 0.07 mg/kg, Zn was 1.56 mg/kg, Ni was 0.5 mg/kg, Mn was 0.20 mg/kg, Cu was 1.86 mg/kg, and Cr was 0.002 mg/kg. At the relatively higher concentration of POS at CR4, the results show Pb to be 0.07 mg/kg, Zn was 1.66 mg/kg, Ni was 0.60 mg/kg, Mn was 0.27 mg/kg, Cu was 2.0 mg/kg, and Cr was 0.002 mg/kg. CR5 also shows Pb to be 0.11 mg/kg, Zn was 2.63 mg/kg, Ni was 0.93 mg/kg, Mn was 0.43 mg/kg, Cu was 2.36 mg/kg, and Cr was 0.07 mg/kg. In summary, heavy metals in the stem, followed, in descending order of concentration, by Zn$^{2+}$, Cu$^{2+}$, Ni$^{2+}$, and Mn$^{2+}$, with negligible Pb$^{2+}$ and Cr$^{6+}$ concentrations (Figure 6).

Figure 6. Heavy metal concentrations in the stem of *C. cajan*. UR: Bars represent means ± standard deviation (*n* = 3). UR is an uncontaminated rhizosphere and CR 1 to CR5 represent contaminated rhizosphere with POS from 1 to 5% *w/w*).
3.3.2. Bioconcentration Factor (BCF) and Translocation Factor (TF) of Heavy Metals in *C. cajan*

TF and BCF were determined to quantify the uptake of metal into the above-ground part of the plant. The translocation factor (TF) shows the ability of the plant to relocate the metals from the roots of the plant to stem or leaves. The TF value of Zn was 0.329 in the CR1 oily sludge concentration and was 1.993 in the CR5 oily sludge concentration; Cu also showed a similar increase in TF value at higher concentrations of oily sludge. The BCF shows the ability of the plant to accumulate metals from soil to the plant tissues. The result shows BCF in all treatments and metals to be less than 1 with Zn showing a value of 0.329 for CR1 and 0.642 for CR5. Likewise, Pb showed a value of 0.052 in CR1 and for CR5, it was 0.220. (Table 2).

3.4. Degradation Analysis

The result of oily sludge degradation in the soil shows the effectiveness of *C. cajan* in the plant–microbe bioremediation process (Figures 7–11). It was observed that the reduction in area and height of the chromatographic peaks was more pronounced in the contaminated rhizosphere of (CR1) (Figure 7a) than in the contaminated non-rhizosphere (Figure 7b). The contaminated rhizosphere treatment (CR2) (Figure 8b) also showed a reduced chromatographic peak area and height compared to the contaminated non-rhizosphere (Figure 8a). A similar pattern of reduction was obtained for CR3 (Figure 9). In CR4, there was a partial reduction of chromatographic peak compared to the contaminated non-rhizosphere control (Figure 10a). There was little reduction in peak area and height compared to the contaminated non-rhizosphere control in the CR5 treatment (Figure 11a). Similarly, these results corresponded to the one obtained in the gravimetric analysis where the rate of degradation followed an almost similar pattern (Data not shown).

![Figure 7](image1.png)

*Figure 7.* Comparative chromatograph of (a) contaminated non-rhizosphere (CN) and (b) contaminated rhizosphere (1% oily sludge with *C. cajan*) after 90 days.

![Figure 8](image2.png)

*Figure 8.* Comparative chromatograph of (a) contaminated non-rhizosphere (CN) and (b) contaminated rhizosphere (2% oily sludge with *C. cajan*) after 90 days.
Table 2. Bioconcentration factor (BCF) and translocation factor (TF) of heavy metals in C. cajan-remediated soil.

| Treatments | Pb (BCF) ± SE | Pb (TF) ± SE | Zn (BCF) ± SE | Zn (TF) ± SE | Ni (BCF) ± SE | Ni (TF) ± SE | Mn (BCF) ± SE | Mn (TF) ± SE | Cu (BCF) ± SE | Cu (TF) ± SE | Cr (BCF) ± SE | Cr (TF) ± SE |
|------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|---------------|--------------|
| CR1        | 0.052 ± 0.01  | 0.0026 ± 0.01| 0.329 ± 0.02  | 0.0026 ± 0.01| 0.317 ± 0.01  | 0.119 ± 0.06  | 0.25 ± 0.053 | 0.35 ± 0.014 | 0.12 ± 0.038 | 0.31 ± 0.005 | 0.96 ± 0.004 | 0.31 ± 0.001 | 0.001 ± 0.01 |
| CR2        | 0.072 ± 0.02  | 0.0033 ± 0.01| 0.427 ± 0.02  | 0.0150 ± 0.013| 0.967 ± 0.03  | 0.37 ± 0.030  | 0.077 ± 0.026 | 0.23 ± 0.09  | 0.449 ± 0.001| 1.40 ± 0.001| 0.81 ± 0.001 | 0.004 ± 0.01 |
| CR3        | 0.104 ± 0.02  | 0.0733 ± 0.01| 0.524 ± 0.04  | 0.01636 ± 0.036| 1.567 ± 0.01  | 0.50 ± 0.063  | 0.125 ± 0.019 | 0.21 ± 0.016| 0.646 ± 0.001| 1.87 ± 0.002| 0.54 ± 0.001 | 0.002 ± 0.01 |
| CR4        | 0.144 ± 0.05  | 0.0702 ± 0.01| 0.586 ± 0.04  | 1.667 ± 0.012| 1.667 ± 0.01  | 0.60 ± 0.01   | 0.058 ± 0.011 | 0.28 ± 0.01  | 0.908 ± 0.002| 1.90 ± 0.002| 0.69 ± 0.006 | 0.002 ± 0.01 |
| CR5        | 0.220 ± 0.08  | 0.0866 ± 0.01| 0.643 ± 0.016 | 1.993 ± 0.013| 1.9722 ± 0.062| 0.93 ± 0.025  | 0.077 ± 0.014 | 0.43 ± 0.007 | 1.10 ± 0.01  | 1.97 ± 0.01  | 0.81 ± 0.003 | 0.070 ± 0.01 |
4. Discussion

4.1. Emergence of C. cajan and Response to POS

The inhibition to the percentage of emergence at 5% POS indicates significant toxicity of POS to C. cajan. Similar results have been reported in Cajanus cajan, Vigna subterranea, and Phaseolus vulgaris exposed to high concentrations of spent engine oil [19]. However, it is also very important to note that as the plant grows the tolerance of C. cajan to the contaminant may increases as reported by [25]. A high concentration of hydrocarbon is reported to be deleterious to plant growth and causes damages to the plant at the early
Aside from the chemical toxicity of oily sludge, another important mechanism of plant inhibition is related to the physical property of high oil content soil that leads to the minimal water-holding capacity of the sludge, resulting in a reduction in water absorption by the seeds of *C. cajan* similar to a previous finding [27] that reported a decrease in the germination per cent of *V. unguiculata* in oily-sludge-contaminated soil. Similarly, the common grass used in the phytoremediation of *Scirpus grossus* in soil contaminated with 0.25% (v/w) of diesel showed signs of phytotoxicity, with the death of some *S. grossus* plants recorded after 42 days [28]. In another study, plants in soil contaminated with 2.5% of used lubricating oil showed high symptoms of phytotoxicity with deaths of at least one *Jatropha curcas* plant in the subjected treatments [24,29]. In the present study, the comparatively lower concentration treatments tended to increase growth rate at the relatively low concentrations of POS (1 to 3% w/w). This suggests that the *C. cajan* rhizome system increases nutrients to the plants by degrading and assimilating the hydrocarbons in the oily sludge, a feat we observed in our previous publication [21]. However, at higher concentrations, the toxicity of the components of the oily sludge inhibited this activity, and, together with oily sludge’s inherent toxicity to plants resulted in a decreased growth at the relatively high concentrations but no plant death was recorded. After 28 days, the shoot number and height for POS, from 1 to 3%, were not significantly different from the control but higher POS concentrations caused significant phytotoxicity with a dramatic decline in plant height and a number of shoots similar to several previous reports involving plant exposure to hydrocarbons at elevated concentrations [26–32].

The RGR of lower concentrations (1, 2, and 3%) at 60 to 90 d showed higher growth rates compared to control (Figure 3), which were significant ($p < 0.05$) while it was not significant ($p > 0.05$) between the 30th day of growth and the uncontaminated control, which reiterates the results obtained from Figures 4–6. This result agrees with the findings of [22] and [28] that as the concentration of oil increases, the RGR reduces, but slightly varies from the findings of [33] who reported that RGR is higher for plants growing in oil-contaminated soil than in uncontaminated soil.

As opposed to the 30-days observation, it appears that the wet and dry weights of the shoot and root of the plant were increased compared to control up to 3% of POS while higher concentrations were inhibitory to plant growth after 60 and 90 days of growth. This increase in plant growth parameters in the presence of oily sludge may indicate assimilation of the hydrocarbons into growth either directly or indirectly in the form of microorganism growth that in turn promotes the growth of the plant. This phenomenon was also reported in several studies where the subsequent utilization of the hydrocarbon degradation products for plant metabolism was observed; as microorganisms actively metabolize hydrocarbon, they are able to release growth-promoting hormones to the plant [27,34].

The beneficial species in the rhizosphere are aided by plant exudates, while the non-beneficial organisms benefit from the metabolic activity of root populations. Furthermore, plants obtain a number of mineral nutrients as well as vitamins, auxins, cytokinins, and gibberellins, and other complex molecules due to the use of microbial populations, which improve the solubilization and recycling of minerals [26–32]. This increase in biomass and plant acclimation to the contaminant can be connected to the activity of plant’s rhizosphere community that degrade organic substances [25,35] while the inhibitory effects of oily sludge at higher concentrations are probably due to oxidative stresses as discussed previously. However, further fundamental work is needed to confirm this finding and to uncover the mechanistic detail of this activation. However, this is beyond the scope of this study.

There is a direct connection between RGR and contaminant found in plant rhizosphere as when the concentration of the contaminant is at the level tolerable by the plant, the RGR of the plant is higher than the uncontaminated control [22]. The relative growth rate (RGR) indicates the tolerance of plants during a treatment period. The RGRs shows a significant difference in both time and the different treatments. This result indicates that the growth of *C. cajan* was not affected by lower concentrations in a given time; even the high concentration was tolerated with no plant death recorded though growth was slow at
relatively high concentrations. As many plants have different tolerance levels to a specific contaminant it is reported that *C. cajan* is able to grow under varied conditions and has the ability to withstand adverse toxicity. This plant is worth evaluating to remove metals from waste leachate [36]. The investigators also noted that forage yield, plant height, and maturity of plants in the contaminated soil improved in the later stages of the study, that is, after 90 days, as this was when the concentration of POS may have been reduced. This strongly supports our findings as the plant used in this study was a pea plant.

### 4.2. Accumulation of Heavy Metals by *C. cajan*

The results of metal accumulation are similar to those in another study [37] where it is reported that the accumulation of metals in the root of the plant is followed by subsequent translocation to the stem, but no metals were found in the leaves of the plant (*H. cannabis*). This is probably because of the type of plant used in this study; legumes, are said to be efficient immobilizers of metals in the soil leading to phytostabilization [37]. The pattern of accumulation of the heavy metals observed in this study is similar to that observed in one study that reported three legume plant symbioses that accumulated heavy metals essentially in the roots, and poorly in shoots with a pattern showing Zn > Pb in terms of accumulation [11]. Though all the heavy metals were found in the root and stem of *C. cajan*, the levels were lower when compared to the maximum permissible concentration of metals allowed in the plant by regulatory organizations [38]. The higher accumulation of Zn may be due to the fact that Zn is an essential element in plant metabolism unlike Pb and Cr. However, a high concentration of Zn can become toxic to plants and, similar to Cu, is a potential hazard to human. The result however shows a higher concentration of Zn followed by Cu, although the concentrations were not high enough to be at toxic levels that can lead to damages to the root and shoot of the plant. These results show that heavy metals were mainly accumulated in root tissues which are underground, revealing, in general, poor metal translocation from roots to shoots indicating that *C cajan* is not a good accumulator. The accumulation of heavy metals by plants differs not only according to plant species but soil property and types of metal. It is reported that heavy metal accumulation in alfalfa follows the order: (shoot) Zn > Pb > Cu and (root) Zn > Cu > Pb, with a variation in the accumulation pattern of Pb [39]. However, our result showed a similar pattern of accumulation of heavy metals for both the root and stem.

### 4.3. Bioconcentration Factor (BCF) and Translocation Factor (TF) of Heavy Metals in *C. cajan*

Our results showed TF values in all treatment to be less than 1 for Ni, Mn, Cr, and Pb, while TF values greater than 1 were encountered for Zn and Cu, which slightly contradicted the findings of [39] and [24] who reported TF values of less than 1 in *H. cannabinus* and *M. sativa* for these metals. This may be due to the difference in the accumulation mechanism utilized by plants. The BCF value was similar to a previous study [24] that reported a BCF value of less than 1 for the 2.5% concentration of spent lubricating oil using *H. cannabinus*. But the results are in contrast to another study [40] that reported a higher value of BCF in water mimosa and water hyacinth tested with 0.5 mg/L of Pb with a BCF value of 177.1. This is due to the difference in the plant as these plants are known hyperaccumulators of metals [41]. The result shows that heavy metals were slightly taken up by the root and translocated to the stem of the plant in a preferential pattern. *C. cajan* bioaccumulated a significant quantity of metals in its roots. Hyperaccumulator plants accumulate metals more efficiently at a higher quantity than tolerant plants [42], with the subsequent translocation of metal to the aerial parts of the plant [36]. For this reason, *C. cajan* is classified as a tolerant plant similar to sunflower and alfalfa [43,44]. Both the accumulation and the transfer of metals in root and other tissues are important. The translocation factor (TF) for Zn and Cu shows a (TF) value greater than 1.0 but for Pb, Ni, Mn, and Cr, the values are comparatively insignificant. This value is an indication of the restricted internal transport of the metal to the aerial part, but there is still an option to consider *C. cajan* for phytoextraction. The phytoextraction ability of a plant relies on the total amount of metal that can be taken up,
which depends on the metal concentration in the plant tissue and plant biomass [45]. The fact that heavy metals were mainly accumulated in roots and the scarce plant biomass results showed negligible total heavy metal uptake by C. cajan, hence a low phytoextraction ability. However, the fact that heavy metals were accumulated to a certain extent in the plant roots but with a low TF could lead to a phytostabilization of heavy metals, which is another important aspect of phytoremediation.

4.4. Degradation Analysis Using Gas Chromatography Analysis

Total petroleum hydrocarbons (TPH) is a term used to describe hydrocarbon compounds derived from petroleum sources like POS [3,46]. Lighter-end TPH compounds (i.e., fewer than 16 carbon atoms) tend to be more mobile due to greater solubility, greater volatility, and lower organic partitioning coefficients [3]. Lightweight aromatic compounds, such as benzene, are also more toxic, making them of greater concern if released into the environment [32]. Heavier TPH compounds typically have opposing properties, tending to adsorb into the organic fraction of soil. Heavier aromatic compounds, referred to as polycyclic aromatic compounds (PAHs), can also have higher toxicity and are typically more persistent in the environment [47]. The larger compounds are less water-soluble and less volatile (i.e., less prone to evaporate) [31]. TPHs, without being activated, are toxic to higher plants only when applied at high concentrations [3].

The trend of TPH removal was relatively high but differed significantly for the different treatments. The analyzed result indicates the ability of C. cajan to enrich and stimulate degradation of TPH and tolerate the varying concentrations of POS-treated soils. Statistical analysis was performed between treatments. Due to the interaction between the plant and its rhizobacteria, endophytic bacteria are inclusive especially with C. cajan which are in symbiotic interaction, hence aiding the degradation of hydrocarbons. As stated by [48], the mechanisms of TPH phytoremediation in the soil include volatilization, leaching, photodegradation (contaminated at the surface), plant uptake, biodegradation, and other abiotic losses [49]. However, the main mechanism of TPH phytoremediation in contaminated soils is assumed to be rhizodegradation [50–52]. The rhizodegradation is the stimulation of bacteria in the rhizosphere zone to degrade and enhance removal of TPHs and subsequent accumulation of heavy metals [53–56].

5. Conclusions

After 90 days of a pot experiment (mesocosm setting), it was demonstrated that C. cajan has the ability to survive, tolerate, and provide good conditions for its corresponding rhizosphere to degrade petroleum hydrocarbon at all investigated POS concentrations (1%, 2%, 3%, 4%, and 5%). C. cajan was able to uptake a significant quantity of heavy metals in the root with no significant translocation to the aerial parts of the plant as shown in the TF values. Although most of the reported legume–rhizobia symbioses were used for phytostabilization, which was safer for consumption, translocation from roots to stem still occurred for metals like Zn and Cu. In addition, root exudates enhanced the growth of microbes that participated in oily-sludge degradation. Hence, the C cajan, plant due to its rich rhizosphere and phyto-tolerance is an excellent candidate for the restoration of oily-sludge-contaminated sites. This provides an eco-friendly restoration alternative method for removing oil contaminants from soil. We recommend a field study of the above finding where other environmental factors can be considered.

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