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Phytoremediation of soil spiked with crude oil using *Acalypha wilkesiana* plant

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ABSTRACT

In this study, the phytoremediation potential of Acalypha wilkesiana for soil spiked with crude oil was investigated. The plant cuttings were grown on uncontaminated soil and then transplanted to spiked soil mixed with 50 mL of 1500 mg/kg crude oil under greenhouse conditions. The weight and growth rate of A. wilkesiana were not affected by the concentration of crude oil in the soil. The results obtained within 2 - 10 months clearly shows the tolerance of A. wilkesiana grown on crude oil spiked soil with percentage growth of 98.7%. There was an increase in the plant dry biomass, root length, stem height and stem diameter of the samples compared to control samples. A gradual increase in the absorption, accumulation and translocation of aliphatic hydrocarbons in the root and leaves of A. wilkesiana was noticed. The concentration of alkane absorbed on 10 months harvesting were ranged between 1.0-160.0 mg/kg and 1.0-120.0 mg/kg in the root and leaves, respectively. The gradual and moderate absorption rates of aliphatic hydrocarbons in the plant samples implies that A. wilkesiana could be one of the promising plant candidates to be used for the phytoremediation of soil contaminated with a little amount of crude oil.

Keywords: phytoremediation; Aliphatic hydrocarbons; *Acalypha wilkesiana*; crude oil; spiked soil; Soxhlet extractor.

Introduction

Organic pollutants such as petroleum hydrocarbons have contributed significantly to environmental pollution particularly in the last few decades [1]. Organic compounds such as aliphatic hydrocarbons (AHs) and polycyclic aromatic hydrocarbons (PAHs) are regarded as a widespread environmental contaminant. *n*-Alkanes are commonly used to characterize organic matter from different environments due to their source specificity. Hydrocarbon compounds are released into the environment anthropogenically and causes a serious harmful effect to the human and environment because of their toxic effect, hydrophobic nature and its continuation to exist in the environment for a longer period of time. Moreover, hydrocarbon compounds that go in to food chain can be mutagenic and carcinogenic to humans and animals due to their harmful effect [2,3]. Thus, the removal or reduction of these hydrocarbon compounds from contaminated soil and water is one of the major problems in the field of environmental sciences and engineering [4, 5, 6, 7]. The physical and chemical methods for soil remediation usually have many complications due to their administration costs and the harmful effects to the sites [8]. Therefore, to remediate a soil that are polluted with crude oil is one of the most important subjects in environmental engineering [9]. Researchers found some plants species are resistant to petroleum hydrocarbons that can survive and grow in petroleum-polluted sites [1]. Several plants, such as grasses and annual herbs have been used to remediate soils contaminated with organic compounds [10]. Plant species have been used in the removal of organic compounds from contaminated soils, but the remediation efficiency of plants varies from one species to another [11]. A study was aimed to identify total petroleum hydrocarbons (TPHs) to evaluate the phytoremediation potential of native petroleum-resistant plants of Pazanan-E-Gachsaran (Iran). The results revealed that, the contents of TPHs decreases with the distance from the core [1]. A research was conducted to investigate the presence of TPH from soil artificially contaminated with crude oil in the roots and shoots of rice crops (*Oryza sativa* L.). It was observed that the quantity of TPH uptake by the plants has increased as the quantity of crude oil increased gradually in the soil [12]. A research to investigate the uptake and accumulation of AHs in *B. chinensis*

(Chinese cabbage) was carried out. The quantity of AHs determined in *B. chinensis* was 123.7 ± 10.18 mg/kg. AHs percentage accumulation ranged from C_{21}-C_{34} was 85.5%, followed by C_{16} -C_{21} 6.4%, then C_{12} - C_{16} 6.3% and C_{10}-C_{12} 1.8% of the total aliphatic hydrocarbons (TAHs) in the sample [13]. Several plant members of *Anacardiaceous, Annonaceae* and *Asteraceae* were tested for their ability in the phytoremediation of petroleum hydrocarbons. Quantity of AHs in the soil and plant roots was determined, and the mean values were 1493 and 3400 mg/kg, respectively [14]. In a study to assess the effect of hydrocarbons (crude oil) on vegetative traits of *Paspalum scrobiculatum* seedlings, results indicated that the concentration level of crude oil affected the plant height, fresh weight, and plant leaf. As the crude oil concentration increases to 15%, the plant leaf area decreases significantly to 34.07%, while control plant leaf was 68.47% [8]. In an experiment, where the effects of different concentration levels of crude oil on fresh plant weights of *Jatropha curcas* seedlings were investigated. The study was conducted on 5 different concentration levels of crude oil and the results showed that the weight of fresh shoot decreases compared to the shoot fresh weight in control [15].

A study was conducted to investigate the survival, growth and accumulation potential of *A. wilkesiana* in phytoextraction of heavy metals spiked soil [16]. But a limited study on the plant capability to remediate crude oil spiked soil was observed. The plant is a family of Euphorbiaceaece, is fast growing shrub and has a different color combination of leaves depending on cultivation [17]. There is no enough information on the phytodegradation potential of *A. wilkesiana* in the absorption and accumulation of AHs from soil spiked with crude oil.

The objective of this study was to evaluate the phytoremediation potential of the selected plant grown on soil spiked with crude oil. *A. wilkesiana* was grown on the spiked soil to evaluate the survival, growth, accumulation of AHs in the root and translocation of AHs to aboveground parts within the experimental period.

Materials and Methods

Experimental Set up

Crude oil and Spiked Soil Preparation

Crude oil sample was obtained from Miri Sarawak, Malaysia and contained a mixture of AHs. A solution of 50 mL of 1500 mg/kg was used for spiking and for GC-FID

analysis of the chemical composition of crude oil. Soil sample was spiked according to the procedure outlined by [18] with some modification in soil treatment (soil samples were treated with the same concentration of crude oil but different harvesting period). Approximately 1.0 kg of soil sample (each) was spiked with 50 mL of 1500 mg/kg crude oil, mixed thoroughly to ensure homogeneity [19] and the spiked soil was analyzed before it was placed into polyethylene bag. Polybags were placed in a dark fume hood for 7 days to ensure solvent evaporation and equilibration which took place at greenhouse, the spiked soil sample was analyzed again. Approximately 4.0 cm of healthy and well grown *A. wilkesiana* cuttings were transplanted on contaminated soil with five replicates for each harvesting months (2, 4, 6, 8, and 10 months). On each harvesting period, a control plant of five (5) replicate was established [18].

Polyethylene Bag Experiment

Polyethylene bags were set in greenhouse at east campus of Universiti Malaysia Sarawak (Unimas) (N 01^0 33' 03.6'' E 110^0 45' 56.5''), which was covered from the top with transparent polythene sheet and netted from the sides to prevent rain water leaching and birds. *A. wilkesiana* was grown under greenhouse condition without addition of plant growth promoting chemical or fertilizer. The experiment followed a factorial design with one factor: concentration of heavy metals in plant parts (root, stem and leaf) and landfill soil. The polybags (consists of one plant) were arranged in a row and labelled according to the harvesting period (such as 2, 4, 6, 8 and 10 months). Each row of harvesting period contains 5 replicates of exposed plants. Additionally, another five rows (with 5 replicates each) of plants with the same characteristics as above were used as control for each harvesting period (data are presented). Both exposed and control plants were fed with tap water after every 2 days. A total of 50 plants were used for the study.

Plants part Extraction

The plant was harvested on 2, 4, 6, 8 and 10 months (the experiment was terminated at 10 months because enough data to evaluate the capacity of the plant was collected) [18]. The extraction of AHs from the roots and leaves of *A. wilkesiana* was carried out according to [20] with minor modification in the amount of solvent used and the duration of extraction. Approximately 2.0 gram of plant organ in a cellulose thimble was placed in a Soxhlet extractor and then extracted for 8 hours with 200 mL of dichloromethane which was used as a

solvent. Prior to the extraction, 50 µl of 50 µg/g *n*-eicosene was spiked into the sample to serve as an internal standard for AHs. Dichloromethane was evaporated to nearly dryness using a vacuum rotary evaporator to obtain crude extract.

Crude Extract Fractionation

The crude extract was fractionated on a silica gel column chromatography according to procedure described by [21]. A small glass column chromatography was packed with 5.0 g activated silica gel (230-400 mesh) and used to obtain AHs (F1) and others as (F2) fractions. Crude extract was diluted with 1 mL of *n*-hexane and placed on the top of silica gel layer in a column. F1 fraction was subsequently isolated by eluting with 40 mL *n*-hexane and 40 mL mixture of *n*-hexane and dichloromethane (1:1, v/v), respectively. The F1 fraction was collected in a 100 mL pear-shaped flask and evaporated to almost dryness using a vacuum rotary evaporator. The fraction was then dissolved in 1 mL hexane, sonicated and transferred to 5 mL vial using Pasteur pipette. It was then gently evaporated to dryness using purified nitrogen gas and kept in a dark place at 4 ^{0}C temperature until further analysis using GC-FID.

Removal of Elemental Sulphur

An appreciable quantity of sulphur in sample fractions for gravimetric determination of AHs should be removed to avoid interferences. A column prepared with activated Cu was used to remove the elemental sulphur from the sample fraction. A bed of dry activated Cu powder (~3 cm high and ~40 mesh) was packed into a glass chromatographic column to treat the AHs fraction. The sample fraction was diluted with 25 mL dichloromethane and was allowed to elute slowly down the column [22].

Gas Chromatography Analyses

Gas chromatography-Flame ionization detector (GC-FID) was used to analyse AH fractions. Analysis of AHs fraction was carried out on a Hewlett Packard gas chromatograph model 6890 equipped with a flame ionisation detector (FID). Separation was performed on a DB-5 fused silica capillary column coated with a 5% diphenyl and 95% dimethyl polysiloxane stationary phase, with internal diameter 0.25 mm, column length 30 m and film thickness 0.25 µm. Prior to GC-FID analysis, the sample was dissolved in 500 µL *n*-hexane (GC grade). Helium gas with a velocity of 1 mL/min was used as a carrier gas. The oven temperature was programmed at 50 ^{0}C and held for 5 minutes. It was then increased to

300 ^{0}C at the rate of 5 ^{0}C/min and held for 15 minutes. The injector and detector temperatures were set at 250 ^{0}C and 300 ^{0}C, respectively. Exactly 1 µL of sample was injected into the column in split less injection mode. Identification of AHs was carried out by comparing the retention times of individual *n*-alkanes in a sample with those in a mixture of aliphatic hydrocarbons standard.

Quantification of AH in the sample

The quantity of AHs in the plant roots and leaves were obtained by internal standardization method using peak area in chromatogram with *n*-eicosene served as internal standard. The concept of an internal standard (IS) is quite simple, a known amount of the IS was added to every sample, both calibrators and unknowns, the calibration uses the ratio of response between the analyte and the IS [23]. The response factors (RF) for each n-alkane were obtained from the analysis of a mixture of n-alkanes standard. The RF and concentration of *n*-alkanes were obtained by using the equations 1 to 2.

$RF_x = (A_{is} \times C_x) \div (A_x \times C_{is})$ Equation 1

Where RFx is response factor for analyte x; A_x is peak area for x analyte; C_x is concentration of analyte x; A_{is} is peak area for internal standard; C_{is} is concentration of internal standard. This concentration of analyte x is calculated using equation 2 below.

$C_x = RF_x (A_x \times C_{is}) \div (A_{is})$ Equation 2

Statistical analysis

All the experimental data obtained were expressed as average and standard deviation of 5 replicates except where stated. Data statistical analysis was carried out and significant differences between the means were determined by Least Significant Difference (LSD) test ($p<0.05$).

Results

Plant survival and growth in crude oil spiked soil

A. wilkesiana showed a positive response when planted in soil spiked with crude oil by showing a high tolerance and adaptability to the condition of the soil. There was no sign of plant poisoning such as shrinking and yellowing of plant leaves or stunted growth and no plant death was observed from all the replicates. *A. wilkesiana* growth on all the harvesting periods was like the growth of control plant which shows no obstacle as shown in Table 1. Crude oil quantity in the spiked soil and the length of harvesting period did not affect the growth of *A. wilkesiana.*

Plant Biomass

As presented in Table 1, *A. wilkesiana* root height, stem height and stem diameter developed well and increases on each harvesting period in a consistent manner. Among all the 5 harvesting periods, plant root height, stem height, stem diameter, root and leaf weight were significantly increased. The results of root height, stem height and stem diameter are not in agreement with the results obtained by Taheri et al. [8] and Agbogidi et al [15]. The results show no harmful effect on the plant survival and growth, but rather it might improve the plant growth. It was assigned that crude oil spiked soil may have the power to affect the microorganism that have plant growth promoting bacteria within the soil, which generate the plant growth requirement, provide nitrogen and prevent it from soil harmful effect [24]. It was concluded that, certain concentration of crude oil in the soil may be helpful to some green plants [25]. Furthermore, it was noticed that plants are famous in improving soil remediation through biophysical and biochemical procedures. These processes include the enzymes secretion and degradation, uptake and accumulation of contaminants and encourages rhizosphere microorganisms [25].

Table 1: Root length, stem height, stem diameter, dry biomass weight of *A. wilkesiana* plant organs on different harvesting period

Plant organs / Harvesting period	2 months	control	4 months	control	6 months	control	8 months	control	10 months	control
Root length (cm)	4.22±1.05	4.37±0.10	6.85±1.52	6.23±0.12	11.20±2.02	10.11±1.93	15.81±2.22	13.96±1.17	18.07±1.91	17.63±2.64
Stem height (cm)	8.47±2.11	9.13±1.01	12.27±1.91	11.41±1.66	16.47±2.81	14.89±3.11	20.11±2.57	19.06±2.81	26.09±2.63	25.48±3.27
Stem diametercm	0.82±0.01	1.02±0.01	0.97±0.34	1.03±0.12	1.29±0.53	1.04±0.32	1.43±0.68	1.12±1.22	1.62±0.92	1.57±0.89
Dry Biomass Weight										
Root weight (mg)	0.57±0.01	0.68±0.04	1.99±1.00	1.72±0.41	2.49±0.97	2.28±0.51	3.02±0.95	2.98±0.64	3.57±1.00	3.49±1.65
Leaf weight (mg)	0.61±0.14	0.73±1.21	2.73±1.35	2.55±1.27	3.45±1.06	3.11±0.19	4.01±1.21	3.73±0.38	4.64±1.03	4.41±1.11
Average value ±standard deviation (n=5) and control values of the plant organs										

Table 2: The statistical analysis of data

Alpha value	DFW	MSW	t-critical	LSD
0.05	12	26.93	2.18	7.15
Absolute values of square difference of the mean				
Root length (RL) and Stem height (SH)		Root length (RL) and Stem diameter (SD)	Stem height (SH) and Stem diameter r(SD)	
5.46		9.98	15.44	
The absolute value between RL and SH is less than the LSD (7.15), therefore, a statistically significant difference was not found. The absolute value between RL / SD, and SH/SD are greater than the LSD (7.15), therefore, a statistically significant difference has been found in both.				

Statistical analysis of the plant biomass was carried out using analysis of variance (single factor), followed by Least Significant Difference. The calculated p-value (0.002) is less than the alpha value (0.05). The result is presented in Table 2 below. It was found that the calculated p-value of root weight (RW) and leaf weight (LW) is greater than

the alpha value (0.05), therefore, Least Significant Difference was used to determine the statistically significant difference between them. The findings are presented in Table 3.

Table 3: The statistical analysis of Root Weight (RW) and Leaf Weight (LF) data

Alpha value	DFW	MSW	t-critical	LSD
0.05	8	0.97	2.3	1.44
The p-value is 0.16 and absolute value of square difference of the mean is 0.96. The difference between RW and LW is less than the LSD (1.44), therefore the data shows there is no statistically significant difference between them.				

Plant Root Activity

Plant root activity is a relevant and crucial physiological feature which helps to assess the uptake of ions, such as Na^+ and K^+ by the plant root. But in this study, few experiments were obtainable regarding crude oil on plant root activity. There was no significant difference in the roots on all the harvesting period, and this suggest that the root of *A. wilkesiana* had a good endurance to crude oil contaminated soil irrespective of the time of harvest. This was in accordance with the results of plant dry biomass mentioned earlier. Plant root system are very important in maintaining plants life cycle and the importance of plant root system in this experiment is one of the bases for phytoremediation.

Extractable organic matter (EOM)

The quantity of EOM determined from the Soxhlet extraction of *A. wilkesiana* tissues are presented in Figure 1. The quantity of EOM increases consistently with the increasing harvesting period.

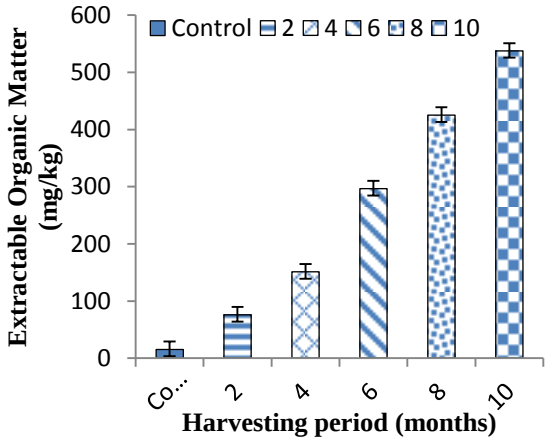

Figure 1: The quantity of Extractable Organic Matter (EOM) determined from *A. wilkesiana* plant within the growth period of 2, 4, 6, 8 and 10 months. The bar on each column represents the standard deviation.

Phytoremediation of Aliphatic Hydrocarbons (AH)

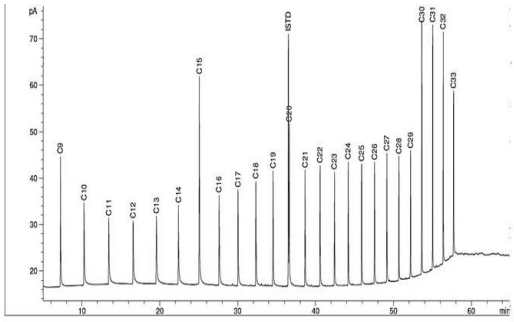

Figure 2 shows a GC-FID chromatogram for *n*-alkanes standard and *n*-eicosene as internal standard. Internal standard is a substance that can be used for calibration by plotting the ratio of the analyte signal to the internal standard signal as a function of the analyte concentration of the standards.

Accumulation of AH in plant root

Plants can absorb organic contaminants from soil or water through their root system and subsequently translocate contaminants to the harvestable parts [26]. Figure 4 shows the accumulation of AHs on the harvesting months. *A. wilkesiana* have absorbed hydrocarbons in the range of C_{11}-C_{33} with concentration ranged between 0.25 - 18.37 mg/kg. A high concentration was observed from C_{18}-C_{23} with concentrations 6.74, 11.02, 14.10, 18.37, 12.03 and 7.28 mg/kg, respectively. The concentration of eicosane was reduced from 6.82 ng/g to 1.86, 1.90 and 2.01 ng/g by *B. napus*, *B. juncea* and *S. Nigrum* plants, respectively, in the phytoremediation conducted for 4 months [27]. It was observed that the GC-FID chromatograms for *A. wilkesiana* contains unresolved complex mixture (Figure 3) which was reduced and resolved at latter stages (such as 4-, 6-, 8- and 10-months harvesting period).

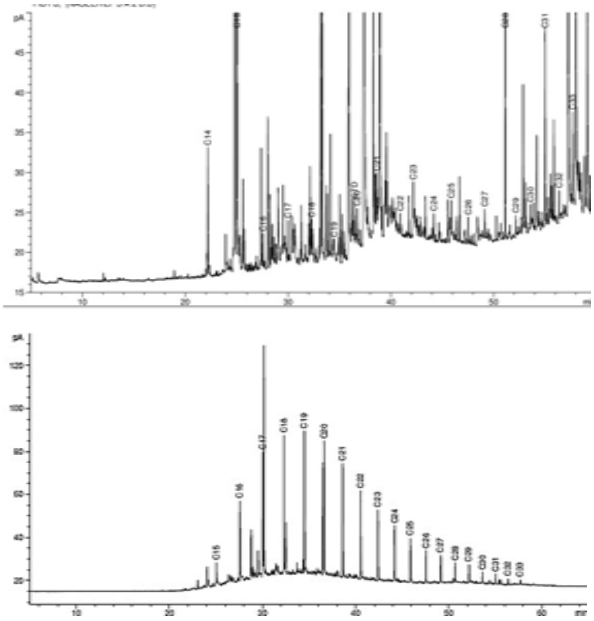

Figure 3. Shows an example of unresolved complex mixtures of aliphatic hydrocarbons chromatograms which were reduced or resolved at latter stages. Figure 3 (A) and (B) contains the unresolved and resolved complex mixtures respectively.

It was also observed an accumulation of low molecular weight hydrocarbons by *Azolla filiculoides Lam* after 15 days grown on crude oil contaminated soil, where alkanes were completely removed from the soil with

concentration of 0.05% crude oil [28]. Alkanes of C_{17}-C_{19} were absorbed by *A. wilkesiana* root with concentrations 11.98, 10.03 and 13.74 mg/kg, respectively. A study revealed that *B. napus*, *B. juncea* and *S. nigrum* were capable to reduce the concentration of octadecane in sewage soil from 9.49 ng/g to 2.53, 2.78 and 2.81 ng/g, respectively [27]. Alkanes C_{11} - C_{33} were absorbed by the root of *A. wilkesiana* with concentration ranged between 0.93- 42.38 mg/kg on the second harvesting period (4 months). It absorbed hydrocarbons C_{16}, C_{17}, C_{18} and C_{19} in the root with concentration 26.06, 42.38, 29.07 and 23.62 mg/kg, respectively. On 6 months harvesting period, *A. wilkesiana* absorbed and accumulated alkanes of C_{11}-C_{33} in the root. It was noticed that the concentration increases consistently from the first to last harvesting period. AHs were absorbed in the root with concentrations ranged between 2.01-65.84 mg/kg. High absorption was noticed on the alkanes of C_{17}, C_{18} and C_{19} with concentrations 56.79, 65.84 and 58.51 mg/kg, respectively. The root system of *A. wilkesiana* also enhanced the accumulation of alkanes C_{11}-C_{33} on 8 months. AHs were absorbed in the root with concentrations ranged between 9.33-110.11 mg/kg. High absorption was noticed on the alkanes of C_{17}, C_{18} and C_{19} with concentrations 110.11, 88.76 and 78.13 mg/kg, respectively. Disappearance of low molecular weight AHs might be due to non-biological factor such as evaporation because alkanes below C_{14} are normally evaporated within few days [29]. Moreover, low molecular weight of AHs (below C_{10}) is not readily soluble in water but are more biodegradable by bacteria and fungi from soil [30]. An appreciable number of AHs were accumulated in the root on 10 months. Light hydrocarbons (C_{12}-C_{23}) are easily to be absorbed by the root of the plant [31]. While, heavy hydrocarbons (C_{23}-C_{40}) are highly hydrophobic and less bioavailable due to high absorptivity onto soil organic matter [32]. AHs in the range of C_{11} to C_{33} were absorbed with concentration ranged between 3.96-173.64 mg/kg. The alkanes of C_{17}, C_{18}, C_{19} and C_{20} were significantly accumulated with concentrations 173.64, 151.23, 138.67 and 129.05 mg/kg, respectively. There was no significant difference in the quantity of AHs in the control sample (not presented) compared *A. wilkesiana* root.

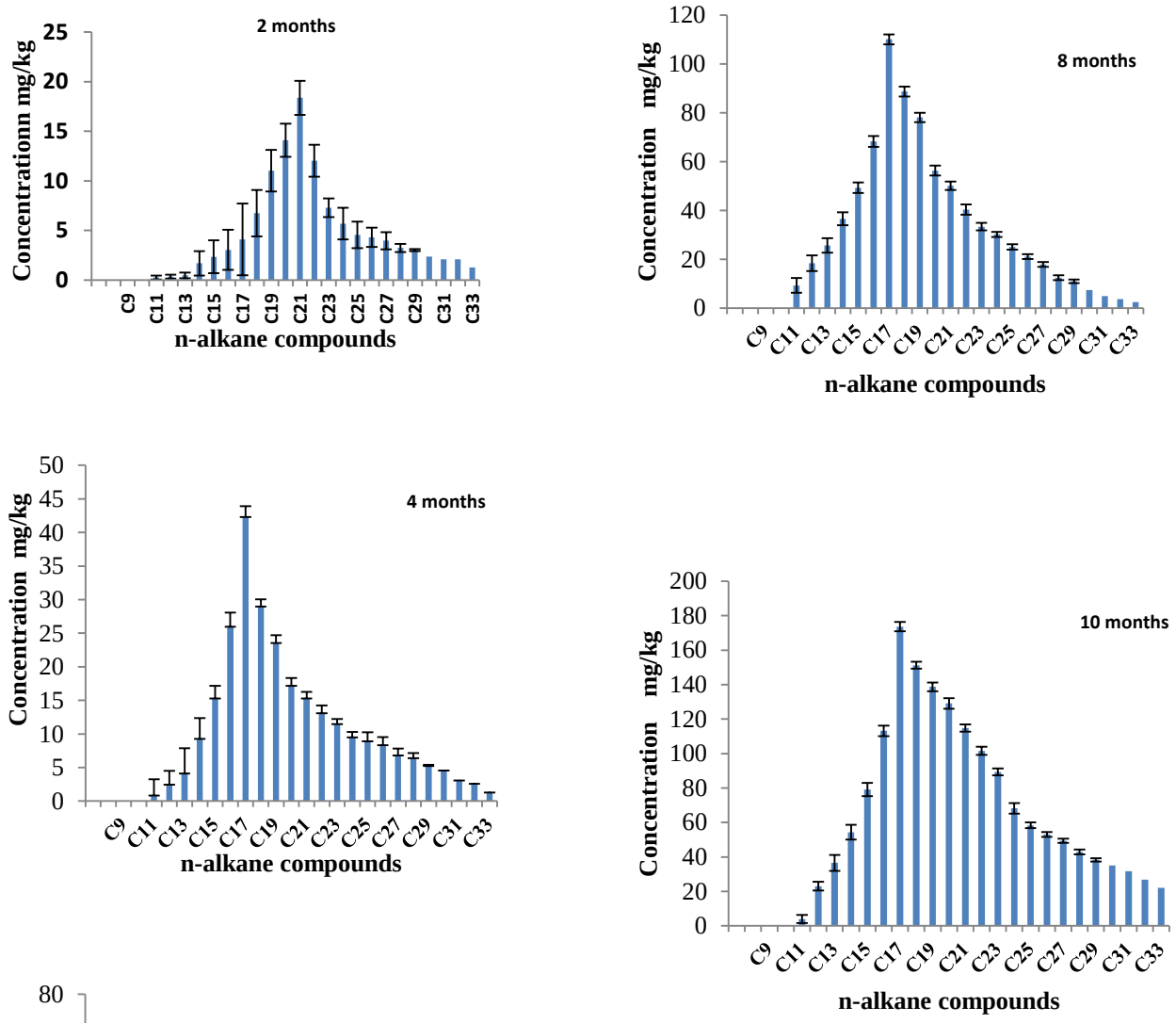

Figure 4. Aliphatic hydrocarbons concentrations (mg/kg) obtained from the root of *A. wilkesiana* plant on harvesting period 2, 4, 6, 8 and 10 months. The bars on each column represents the standard deviation.

Translocation of AH to Plants leaves

A. wilkesiana was able to translocate AHs to its leaves ranged from C_{12}-C_{33} (Figure 5) with concentration ranged between 0.72 to 18.14 mg/kg on the first harvesting period (2

months). High translocation occurred on the alkane of C_{16}-C_{20} with quantity ranged between 10.35-18.14 mg/kg. Hydrocarbons of C_8-C_{11} were not translocated to the leaves by *A. wilkesiana*. Hydrocarbons of C_{12}-C_{33} were translocated to the laves on 4 months and high concentration occurred on the hydrocarbons of C_{17}, C_{18}, C_{19}, and C_{20} with values 38.04, 27.41, 34.26 and 30.08 mg/kg, respectively. Translocation of AHs C_{12}-C_{33} to *A. wilkesiana* leaf was found to increase from 2 months to 4 months. Most of hydrocarbons translocated and accumulated in the root of *A. wilkesiana* on 4 months were 2 or more folds higher than those on 2 months. High concentrations (1×10^{-3} mg/L) of *n*-alkanes in *A. filiculoides* Lam grown on different crude oil spiked soil and the values were C_{17} (116.14), C_{17} (124.72), C_{17} (228.63), C_{19} (35.06), C_{20} (1.23) and C_{21} (7.07) [28]. *A. wilkesiana* also translocated AHs of C_{13}-C_{33} as presented in the figure of 6 months harvesting period. Translocation of C_{17}-C_{20} were significantly translocated to the leaf with quantity ranged between 50.61-65.74 mg/kg. However, hydrocarbons of C_8-C_{12} were not translocated to the leaf of *A. wilkesiana*. *Salix oritrepha* was found to accumulate and translocate some *n*-alkanes to the leaves and high concentration occurred on C_{25} [33]. In this experiment, it was observed that the translocation and accumulation of AHs to the leaves on 8 months was higher than those on 6 months. This may be due to the capability of plant to transfer hydrocarbons to the above ground part. The hydrocarbons of C_{12} to C_{33} were translocated to the leaves of *A. wilkesiana* with concentration ranged between 3.01-86.25 mg/kg on 8 months harvesting period. High translocation was found from hydrocarbons of C_{16} and C_{17} with values 86.25 and 73.91 mg/kg, respectively. A plant of *S. oritrepha* has recorded high peaks of *n*-alkanes with maximum hydrocarbon of C_{27} [34]. Accordingly, *A. wilkesiana* translocated AHs of C_{13}-C_{33} to the leaves with quantity ranged between 14.0-117.02 mg/kg. A significant amount from C_{16}, C_{17}, C_{18} and C_{19} was transferred to *A. wilkesiana* leaf with values 93.42, 117.02, 98.75 and 96.38 mg/kg, respectively. It was revealed that *Ajuga ovalifolia* has the capability to absorb and translocate n-alkane such as the hydrocarbons of C_{27}, C_{29} and C_{33} with maximum concentration [35, 34].

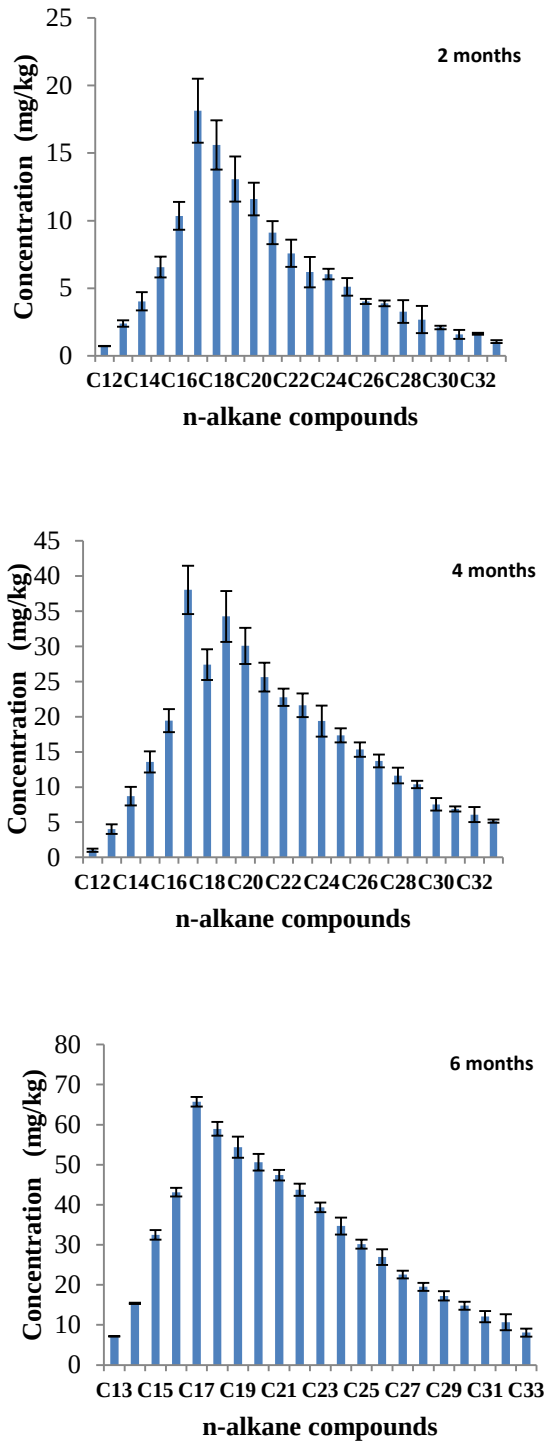

Figure 5. AHs concentrations (mg/kg) translocated to the leaves of *A. wilkesiana* plant on harvesting period 2, 4, 6, 8 and 10 months. The bars on each column represents the standard deviation.

Total aliphatic hydrocarbons (TAH)

It was noticed that the increases in the harvesting period resulted in the increase in the quantity of total *n*-alkanes. The quantity of TAHs in the control samples (not presented) was very low compared to the treated plant on all the harvesting period. This means that *A. wilkesiana* have accumulated more *n*-alkanes in its organs

compared to the control plant and the quantity of TAHs was reduced in a large scale relative to unplanted spiked soil control.

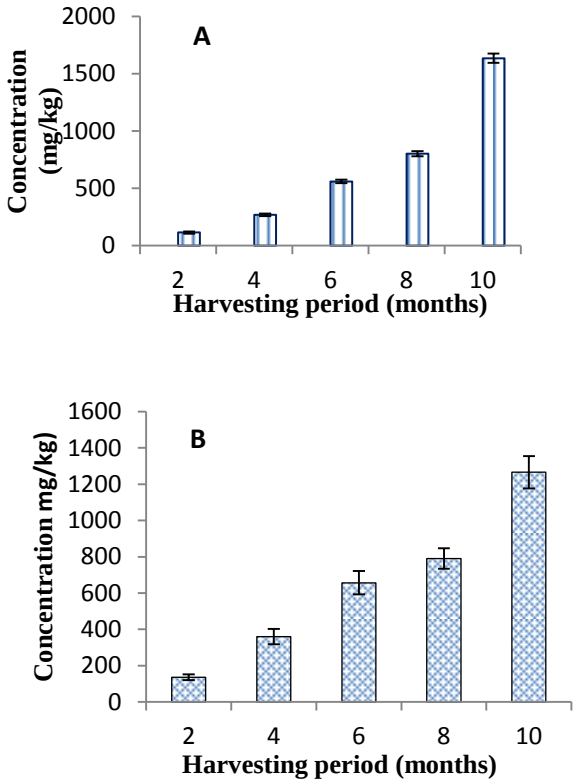

Figure 6. Total aliphatic hydrocarbons (mg/kg) obtained from the root **(A)** and leaf **(B)** of *A. wilkesiana* and bars on each column represents the standard deviation. The results indicate a significant difference from control (not presented) at ($p < 0.05$).

Discussion

Diesel fuel contains a complicated mixture of several hundreds of hydrocarbon and nonhydrocarbon compounds. There are four major groups of petroleum hydrocarbons which include straight-chain alkanes, branched alkanes, cycloalkanes and aromatics [36]. Only little information is available about the degree of hydrocarbons absorption by plants and what particular group of hydrocarbons the plant can absorbed. A hydrocarbon passes through different plant organs such as cell wall and cuticles (which consists of different lipid-like part that shows different attraction to organic pollutants. Some observations shows that, plants have the potential to absorb hydrocarbons from crude oil contaminated soil and water. The plant response towards crude oil contaminants depends on the crude oil concentration and its type [28]. Researches were conducted on the effect of crude oil in the survival and growth of plants. It was reported that *Salicornia fragilis* survived in a medium spiked with 2% and 20% diesel which give a survival percentage of 43% and 10%, respectively [37]. In the study conducted on the survival ability of *Azolla pinnati* found out that the plant can only survive in a medium contaminated with 0.005% diesel [38]. Concentration of crude oil above 0.2% becomes deadly to the plants, but *A. filiculoides* was found to survive in 0.2% oil concentration [28]. A research shows unfavourable effect of diesel fuel on plant growth [39]. In this experiment, *A. wilkesiana* is a promising plant which survives and grow in a soil spiked with 50

mL of crude oil. Plant leaf have made a possible large surface area where gas exchange take place through stomata [40]. Plant leaf are protected from water removal and other diseases due to the presence of waxy cuticle on the surface of plant leaf. And the leaf possesses a lipophilic characteristic due to the waxy cuticle chemical structure. It was noticed hydrocarbons with a lipophilic property can be attached to the surface of plant leaf [41, 42]. At an elevated quantity of diesel oil, a deposition of some oil was noticed on plant leaf which could be due to hydrophobic interactions and responsible for plant death, and crude oil significantly delays the rate of growth in some plant species [28]. Germination is an essential, relevant and crucial stage of plant propagation. Germination ability and early growth stages of seeds or plant species can alter its efficiency in phytoremediation. It was suggested that there is correlation between inadequate germination and improper growth in soil contaminated with hydrocarbons [43]. It was also suggested that the degree of negative outcome of plant growth on contaminated soil mainly relay on the quantity of contaminants [44]. In this experiment, alkanes of C_9-C_{10} were neither absorbed nor transferred into the root and leaf of *A. wilkesiana*, respectively. The harmful outcome of diesel fuel on plant growth may be due to the lethal effect of low molecular weight hydrocarbons in the oil [43]. This happens because low molecular weight goes into cytoplasm via the cell membranes which interrupt the membrane purity and the cell died [39]. There are some factors such as good root system that are responsible for an efficient degradation of contaminants in phytodegradation process. Plants root have influence in an effective degradation process through soil aeration, contaminants separation and reducing contaminants mobility. And also, the major root contribution in phytodegradation of crude oil contaminated site is by encouraging the activity of microorganisms [45]. As far as this experiment is concern, it can be suggested that the degree of tolerance of *A. wilkesiana* to harvesting period ranged between 2-10 months. The absorbed quantity of AHs with C_{16}-C_{20} on the first harvesting period (2 months) in to *A. wilkesiana* root was higher compared to the control (not shown). With respect to the increase in harvesting period the quantity also increases consistently throughout the experiment. Transfer of AHs from roots to leaves should be considered for animal and

humans that use the leaves for comestible purposes. Accumulation of AHs in the root of a plant is of great concern where the root and tubers are consumed by human and animal. Some seeds and grains used for phytodegradation is of great concern also since some parts are comestible. It is expected that low molecular weight and volatile compounds will appear in smaller quantity in *A. wilkesiana* tissues and analysis should be conducted immediately to minimize volatilization in gas exchange processes, but if delayed the compound could not be detected. The concentration of AHs in the plant may reduces due to degradation before being measured. The presence of hydrocarbons of C_{10}-C_{22} in the plant "is an indication of the breaking of long chains of the crude oil affected by plant or oil degrading-bacteria. It is presumed that bacteria at first break weaker bonds in long chains, which is the reason for the increased compounds containing fewer carbon atoms. A reason for the presence of compounds containing fewer carbon atoms is the more availability of more easily degradable materials and unsuitable conditions for the degradation of compounds that are more difficult to degrade, which may become more as time passes" [9]. The absorption of AHs in the plant tissues passes through physical, chemical and biological changes which include biodegradation, volatilization, dissolution and photo-oxidation [46]. It was noticed that within a short period of time the bioavailability, leachability and solubility of AHs reduces once it's in the environment [47]. Some soil microorganism uses hydrocarbons to acquire carbon and unload the pollutant from the soil and make it obtainable for plants to absorb. Large branched AHs are degraded more slowly compared to aromatic and aliphatic (straight chain) hydrocarbons [46]. The absorption of AHs by some plants could be easier due to the presence of microorganism such as rhizospheric in the soil. And there is a good correlation between the microorganism and plant species [48,49].

Conclusions

With regard to the data obtained, it may be suggested that the quantity of crude oil used to spike the soil does not have a significant effect on the survival and growth of *A. wilkesiana*. And also, short or long experimental time of the plant to grow in the spiked soil does not cause any severe symptoms or eventual death of the *A. wilkesiana*. The results obtained about the plant dry biomass, root length, stem height and stem diameter clearly indicate the

tolerance level of *A. wilkesiana* to crude oil (Table 1). When considering the absorption and translocation of some amount of aliphatic hydrocarbons in the plant organs, it can be concluded that *A. wilkesiana* could be quite effective for the reduction of crude oil contaminants. In conclusion, it is known that the use of plants for the removal of crude oil pollutant is more efficient than the unplanted applications. The gradual and moderate rate of aliphatic hydrocarbons reduction observed in this study suggest that *A. wilkesiana* could be used for the phytoremediation of crude oil spiked soil. It is recommended that further study should be conducted to compare the benefits of using *A. wilkesiana* for phytoremediation and how it will affect its use for other purposes.

Conflict of interest

We declare that there is no conflict of interest.

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