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Assessment of the Phytotoxic Effects and Ecological Risks to *Phaseolus vulgaris* Planted on Crude Oil Spiked Soils

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Abstract

Crude oil can contaminate environmental matrices during extraction, production, refining, loading and offloading. This study evaluated the phytotoxic effects and ecological risks to beans (*Phaseolus vulgaris*) planted on native soils spiked with concentrations of crude oil with a secondary aim to phytoremediate the soil. The results showed a decrease in plant heights (60.80 ± 2.11 to 25.30 ± 1.10 cm), leaf areas (40.00 ± 1.70 to 23.60 ± 1.40 cm^2), leaf number (14.00 ± 0.00 to 8.00 ± 0.00), and stem girth (1.57 ± 0.06 to 1.33 ± 0.06 cm) with increasing crude oil concentrations. The total petroleum hydrocarbons (TPH) indicated that 30.2%, 21.4% and 7.6% of crude oil were removed from 1000 mg/kg (0.1%), 10000 mg/kg (1%) and 100000 mg/kg (10%) crude oil contaminated soil in addition to that taken up by the plants (10.8%, 8.6% and 0), respectively. Considerable differences between the treatment groups and the controls were measured at levels of $P = 0.05$. The plant *Phaseolus vulgaris* had bio-remediative potentials–ability to absorb the pollutants, however, its efficacy to hyper-accumulate will take a considerable period, probably several months to years to phyto-remediate a small percentage of toxicants (crude oil) in the soil.

Keywords: Beans (*Phaseolus vulgaris*), Crude oil, Native soils, Phytoremediation, Total Petroleum Hydrocarbon (TPH).

Introduction

The management of hydrocarbons released into environmental media is a global concern especially in Africa and developing nations. The release of hydrocarbons (crude oil) into environmental matrices could introduce toxic fractions that would affect different organisms inhabiting such media. The decomposition of the discharged oil would depend on some factors, which include concentration, duration, environmental changes and climate; however, the heavier constituents of the oil are usually very vicious and difficultly slow to eliminate from the contaminated medium. Cleaning a contaminated environment using traditional technology may cause damage or harmful effects to organisms in the environment in addition to being tasking, cost intensive and time consuming; hence, attention have been centred on bioremediation techniques (Fingas 2012, Kuo et al. 2014).

Bioremediation is a waste management technique that metabolizes or neutralizes harmful chemicals/substances in the environmental medium using microorganisms. The hazardous substances are broken down into less noxious substances, thereby restoring the medium to its initial state. The microorganisms

metabolize the substance to produce methane, carbon dioxide, water and biomass. Thus, the byproducts can be used as an indication that the bioremediation process was successful (Moreira et al. 2011). Some of the most common types of bioremediation methods include phytoremediation, microbial bioremediation, and mycoremediation, which could be applied as in-situ or ex-situ techniques (Ziemiński K and Frąc 2012).

Phytoremediation is an eco-friendly technique that has been adopted since the 1990s to delineate the removal of toxicants from contaminated medium using plants (McCutcheon and Schnoor 2003, Gerhardt et al. 2009). The technique is an environmentally friendly and cost-effective method that has been considered in green technology and optimized for sustainability. Some plants have an intrinsic tendency to degrade certain pollutants through bioaccumulation, translocation and pollutant storage/elimination (Pirzadah et al. 2015). Hence, for over 30 years now, the capability of plants to degrade pollutants from the environment has been acknowledged and applied in land-farming of wastes (Hidayati et al. 2018).

The study was aimed to evaluate the phytotoxic effects and ecological risks associated with *Phaseolus vulgaris* planted on native soils spiked with varying concentrations of crude oils, with a secondary view to phytoremediate the soil. The choice of the plant *Phaseolus vulgaris* was based on the fact that it has accumulative capability that can take up various types of toxicants, tolerant in different experimental environment/conditions (Nwoko et al. 2007). Though it is highly consumed by humans who obtain a considerable amount of protein from them. This study also shows the potential risks of heavy metal contaminated *Phaseolus vulgaris*. At the end of the experiment *Phaseolus vulgaris* was incinerated.

Materials and Methods

The study area

The study location was Ugbomro, a community in Effurun, in Delta State, Nigeria. The community houses the Federal University of Petroleum Resources, Effurun (FUPRE). The institution has ecological/botanical sites and farms. The geo-references for the study station lie within latitude 5°34′4.908" N and longitude 5°50′26.31"E. The area has two seasons - wet and dry seasons. The wet season starts in April and ends in October, while the dry season begins in November and ends in March. The mean annual temperature varies between 21 °C and 37 °C. The soils in the area are mainly sandy and loamy. Anthropogenic events in the region include crude oil exploration, farming, fishing, combustion of fossil fuels, wood and solid wastes.

Soil sampling

Native (indigenous) soils for the study were randomly collected from farm sites in the Federal University of Petroleum Resources Effurun, Delta State, Nigeria on 20th June 2017. In each case, soils were sampled from the top (surface) and bottom (sub-surface) (0 – 30 cm). Objects such as stone, woods, sticks, dead weed and leaves were prudently removed from the soils after collection.

Preparation of *Phaseolus vulgaris* seedlings

Healthy seedlings of the test species were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan. Prior to the experiment, the seedlings were moistened approximately 24 hours before planting. Three seedlings were planted per test tank.

Physico-chemical characteristics of the soils

The physico-chemical parameters analyzed included: soil pH, total organic carbon (TOC), soil texture, particle size, moisture content, total petroleum

hydrocarbons (TPH) and cation exchange capacity (CEC). The analytical methods used for the determination of the physico-chemical parameters are summarized in Table 1.

Table 1: Soil quality parameters and methods applied for the study

Parameters	Analytical Methods
pH	APHA 4500 H+ pH value (APHA 2017)
Total organic content (%)	Walkey and Black (1934)
Exchangeable bases (Ca, Mg, Na and K)	Extraction using 1 N NH_4OAc and Atomic Absorption Spectrophotometry (AAS) (Shimazu AA7000 model)
Total petroleum hydrocarbons (TPH)	Gas Chromatography-Mass Spectrometry (GC-MSD Agilent - 7890 AGC - 5870 VL MSD model)
Soil texture	Hydrometer method (IITA 1984)
Soil particle size	Hydrometer method (IITA 1984)
Moisture content	Gravimetry

Experimental bioassay procedure for *Phaseolus vulgaris* exposed to crude oil

The experimental procedure was carried out adopting the Organization for Economic Co-operation and Development (OECD) protocol #208 for a period of 7 weeks (OECD 2006). Uncontaminated soil samples were randomly collected into each test vessel spiked with different concentrations of crude oil. Crude oil samples of 1000 mg (0.1%), 10000 mg (1%) and 100000 mg (10%) were accurately weighed and spiked into ten (10) kg of uncontaminated native soils. The triplicate treatment soils were then homogenized (using a mechanical mixer) and allowed to stand for 2–3 weeks for attenuation before the previously moist seedlings were planted in each test tank. The soils in the controls were prepared by homogenizing the substrate with 800 mL of water taking into consideration the water holding capacity of the soil before the seedlings were planted. Observations for germination, leaves, stem and root (germination percent, leaf area, leaf number, stem girth and plant height) were taken daily/weekly depending on the parameter. The results obtained were used to assess the phytotoxic effects, ecological risks as well as the bioremediative potentials of the plants exposed to crude oil.

Determination of Total Petroleum Hydrocarbons (TPH)

Total petroleum hydrocarbons (TPH) concentrations in soils and plants were determined using the method of Adesodun and Mbagwu (2008). Samples (5 g each) were placed in a glass extraction bottle and dehydrated using sodium sulphate. Twenty (20) mL of n-hexane were added and the mixture was vigorously shaken on a shaking bath for 3 h. The resultant extract was filtered into a clean bottle using a glass funnel fitted with glass wool and sodium sulphate at the orifice. The eluent was reduced to one (1) mL concentrated with a stream of nitrogen gas and kept in a cool environment. The extract was re-dissolved in 5 mL n-hexane (or more volume depending on the concentration of the sample). Fresh crude oil was diluted with n-hexane to obtain different known concentrations used for the calibration curves. The TPH concentrations in the standards, samples and references were analyzed with a gas chromatography-mass spectrophotometer (GC-MSD Agilent - 7890 AGC - 5870 VL MSD model).

Degradation of crude oil (percentage)

The percentage degradation of crude oil from contaminated soil for each accession was determined using the following formula (Zahed et al. 2011):

$$\% D = \frac{TPHi - TPHr}{TPHi} \times 100$$

Where: % D = Percentage degradation; TPH_i is the initial TPH concentration, and TPH_r is the residual TPH concentration.

Leaf area

The leaf area (LA) of the plant was measured by multiplying leaf length by leaf width and applying a correction co-efficient (r) of 0.72 (Hoyt and Bradfield 1962, O'Neal et al. 2002).

$$\text{Leaf area (LA)} = L \times W \times r$$

Where: L = leaf length (cm); W = leaf width (cm); r = correlation coefficient (0.72).

Statistical analysis

The means, standard deviations and standard error of the means were used to represent the results for the various assessment endpoints. Significant variation between the crude oil treatments and controls were tested at significance level of $P = 0.05$. The pictorial representation of the assessment endpoints was shown using different graph patterns.

Results and Discussion

The results of the physico-chemical characteristics of the soil, phytotoxic effects and ecological risks on exposure to varying concentrations of crude oil spiked in natural soils for a period of seven (7) weeks are presented in Tables 2–11 and Figures 2–5. Indicators used to monitor and assess the accumulative potential and ecological risk of the plant species include: germination, leaf area, leaf number, stem girth and plant height. Observation for effects on the plant was done on a daily to weekly basis for some of the parameters.

Physico-chemical characteristics of soil

The results of physico-chemical characteristics of soil used in the study are presented in Table 2.

Table 2: Physico-chemical characteristics of soil quality used for the study

Parameter	Results
pH	5.77 ± 0.28
Total organic content (TOC), %	0.49 ± 0.01
Cation Exchange Capacity, CEC (meq/100g)	1.35 ± 0.20
Moisture content, %	1.29 ± 0.20
Water holding capacity, %	7.83± 0.12
Total Petroleum Hydrocarbons (TPH)	<0.001
Sand (%)	89.24
Clay (%)	9.20
Silt (%)	4.56
Soil texture	Sandy

Germination studies

The control and the lowest concentration of crude oil (0.1%) showed 100 ± 0% germination, 1% crude oil concentration exposure showed 88.9 ± 1.2% germination, while no germination was observed in the highest concentration of 10%. This could possibly be due to the effects of the toxicant which prevented the growth at the highest exposure concentration.

Leaf area

The results revealed that the leaf areas of *Phaseolus vulgaris* in the soils at 49 days were 56.1 ± 1.9, 40.0 ± 2.2, 23.6 ± 1.4 and 0.0 ± 0.0 for the control, 0.10%, 1% and 10%, respectively (Table 3). These results showed reduction when compared with the controls. The leaf area reduction could possibly be due to the effects of the toxicant which was not in the control exposure.

Stem girth

The stem girth per treatment for the seedlings was assessed using a thread and a meter rule. At test termination at day 49, the mean results for stem girth values were 1.67 ± 0.06, 1.57 ± 0.06, 1.33 ± 0.06, 0.0 ± 0.0 cm for control, 0.1%, 1%, and 10%, respectively (Table 4).

Senescence

Senescence was estimated by visual counting of the number of dead leaves per seedling per tank. At the end of the experiment (49 days), the results varied for the different concentrations - control (1.0 ± 0.0), 0.1% (3.0. ± 0.5), 1% (4.0 ± 0.5) and 10% (0.0 ± 0.0) likely due to the impact of the toxicant on the plant (Table 5). This research does not extensively involve cell analysis so we treated senescence and programmed cell death as the same. Since they have the same morphological expressions, more so we could not have determined programmed cell death when we never took into note the necessary molecular factors necessary to pinpoint apoptosis. We only considered physical manifestations of leaf death.

Table 3: Bean growth in leaf area (cm^2) under varying crude oil spiked soils

Time (days)	Control	0.10%	1%	10
0	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
7	22.1 ± 1.20	11.9 ± 0.90	11.4 ± 1.10	0.0 ± 0.00
14	25.5 ± 1.30	18.5 ± 1.50	14.9 ± 0.80	0.0 ± 0.00
21	30.6 ± 2.10	26.1 ± 0.90	16.8 ± 0.90	0.0 ± 0.00
28	32.2 ± 1.90	30.3 ± 2.50	19.2 ± 1.10	0.0 ± 0.00
35	34.1 ± 1.90	33.0 ± 2.30	23.6 ± 1.70	0.0 ± 0.00
42	37 ± 1.70	35.6 ± 2.70	23.9 ± 1.20	0.0 ± 0.00
49	56.1 ± 1.90	40.0 ± 2.20	24.2 ± 1.40	0.0 ± 0.00

Data were processed and expressed as mean ± standard deviation of three replicates.

Table 4: Changes in the stem girth (cm) under varying crude oil spiked soils

Days	Control	0.10%	1%	10
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.0 ± 0.00
7	1.17 ± 0.06	1.10 ± 0.10	0.97 ± 0.06	0.0 ± 0.00
14	1.20 ± 0.00	1.13 ± 0.06	1.07 ± 0.06	0.0 ± 0.00
21	1.26 ± 0.06	1.17 ± 0.06	1.07 ± 0.06	0.0 ± 0.00
28	1.43 ± 0.06	1.40 ± 0.00	1.17 ± 0.15	0.0 ± 0.00
35	1.57 ± 0.06	1.46 ± 0.06	1.23 ± 0.12	0.0 ± 0.00
42	1.60 ± 0.10	1.50 ± 0.10	1.30 ± 0.10	0.0 ± 0.00
49	1.67 ± 0.06	1.57 ± 0.06	1.33 ± 0.06	0.0 ± 0.00

Data were processed and expressed as mean ± standard deviation of three replicates.

Table 5: Effects of varying crude oil concentration on leaf senescence of *Phaseolus vulgaris*

Days	Control	0.10%	1%	10%
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
14	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
21	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 0.0	0.0 ± 0.0
28	0.0 ± 0.0	1.0 ± 0.0	3.0 ± 0.5	0.0 ± 0.0
35	0.0 ± 0.0	2.0 ± 0.5	3.0 ± 0.5	0.0 ± 0.0
42	0.0 ± 0.0	3.0 ± 0.5	4.0 ± 0.5	0.0 ± 0.0
49	1.0 ± 0.0	3.0 ± 0.5	4.0 ± 0.5	0.0 ± 0.0

Data were processed and expressed as mean ± standard deviation of three replicates. There was no growth hence no record of senescence (0.0 ± 0.0).

Plant height

Measurement for the plant height was done from the soil level to the shoot apex. At the end of the experiment at 49 days, the mean (± SD) plant heights were 88.2 ± 2.02, 60.8 ± 2.11, 25.3 ± 1.1, 0.0 ± 0.0 cm for control, 0.1%, 1%, 10%, respectively (Figure 1, Table 6) indicating that the plant height was impacted by crude oil exposure as the highest concentration of crude oil resulted in no plant growth. There was significant difference in the treated soils relative to the controls.

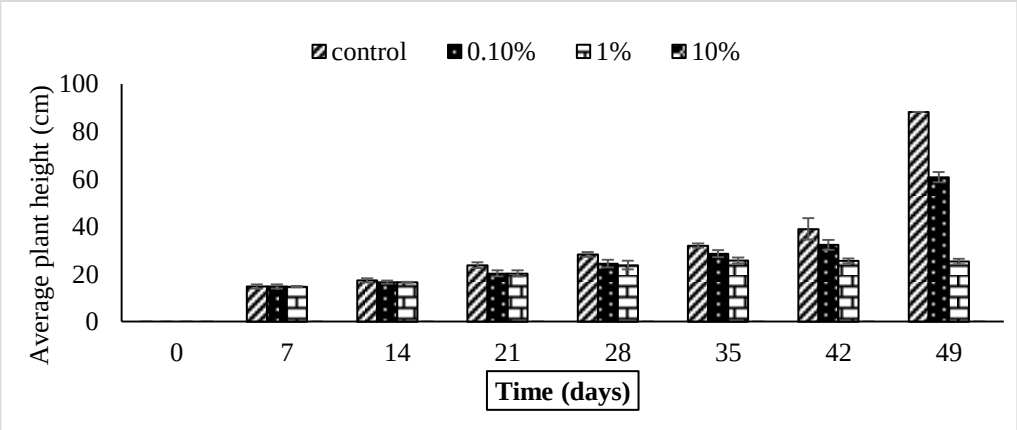

Figure 1 Mean weekly results of the influence of crude oil on plant height.

Leaf number

The numbers of leaves were determined by visual counting per treatment tank. The results at day 7 for each treatment were 2 ± 0.0, 2 ± 0.0, 2 ± 0.0, 0.0 ± 0.0 for the control, 0.1%, 1% and 10%, respectively. The data revealed the emergence of the first leaves at the same time, however, as the days progressed there were variations amongst the treatment groups. At the end of the experiment (49 days), the data varied for the varying concentrations–control (17.00 ± 0.0), 0.1% (14.00 ± 0.0), 1% (8.00 ± 0.0) and 10% (0.0 ± 0.0) (the leaf number was uniform hence the standard deviation is zero) possibly as a result of the likely influence of the crude oil on the plant (Figure 2 and Table 6).

Table 6: Mean growth indicators of the effects of crude oil to *Phaseolus vulgaris* at 7 weeks of planting

Conc. (%)	Leaf number	Senescence	Plant height (cm)	Leaf area (cm^2)	Stem girth(cm)
Control	17.00 ± 0.00	1.0 ± 0.0	88.20 ± 2.02	56.10 ± 1.90	1.67 ± 0.06
0.1%	14.00 ± 0.00	3.0 ± 0.5	60.80 ± 2.11	40.00 ± 1.70	1.57 ± 0.06
1%	8.00 ± 0.00	4.0 ± 0.5	25.30 ± 1.10	23.60 ± 1.40	1.33 ± 0.06
10%	No growth	No growth	No growth	No growth	No growth

Data were processed and expressed as mean ± standard deviation of three replicates.

Necrosis and chlorosis

At the end of the experiment, it was found that in the 1% concentration of crude oil vessel, the three (3) seedlings that emerged were affected by necrosis and chlorosis. This effect could be attributed to the high

concentration of crude oil in the soil, which resulted in the yellowing of leaves (chlorosis). Chlorosis is a condition indicating lack of nutrients (especially phosphorus) in the soil. Necrosis can be considered as a condition when plants cells deteriorate or die and can be identified by dark or wilted leaves and stems, which makes the plant susceptible to diseases (Golstein and Kroemer 2007). In this assessment, the crude oil contamination provided an unfavourable conditions leading to the death of leaves (necrosis). Necrosis and chlorosis were not observed on *Phaseolus vulgaris* in the control and the lowest concentration of 0.1%, however, no growth was measured in the highest concentration of 10% (Figure 3).

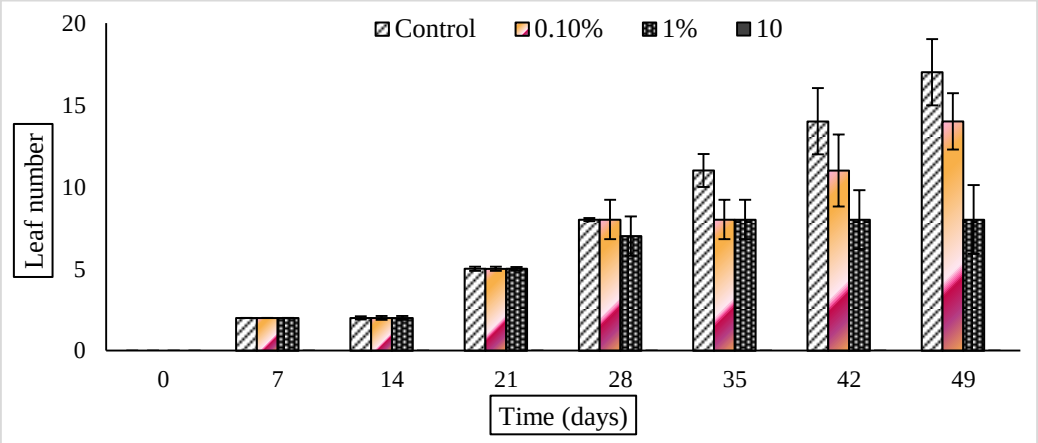

Figure 2: Leaf number of *Phaseolus vulgaris* under varying crude oil spiked soils.

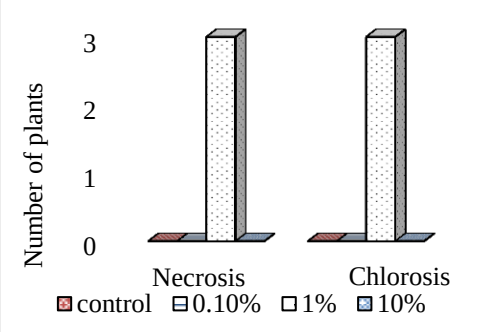

Figure 3: Mean number of plants in each accession affected by necrosis and chlorosis.

Total Petroleum Hydrocarbons

Total Petroleum Hydrocarbons (TPH) was used as an index for assessing the concentrations of crude oil removed from the soil and similar uptake by the plants for the duration of the exposure. The TPH concentrations in contaminated medium at test termination were 698 ± 21 mg/kg, 7860 ± 89 mg/kg, 92400 ± 189 mg/kg for 1000 mg/kg (0.1%), 10000 mg/kg (1%) and 100000 mg/kg (10%), respectively. Similarly, 108 ± 8.9 mg/kg, 860 ± 22 mg/kg and 0 ± 0.0 mg/kg were obtained from the plants in the 0.1%, 1% and 10% concentrations (Table 7). The following concentrations were taken up by the plant representing the percentage removal of 10.8, 8.6, and 0% of crude oil from the polluted soil in the 0.1%, 1% and 10% concentration tanks at the end of the exposure period.

The results of TPH in soil and plants were used in calculating the % degradation of crude oil in the soil and percentage of crude oil accumulated (uptake) in the plant. The results obtained showed that 30.2% and 21.4% of crude oil were removed from 0.1% and 1% crude oil contaminated soil in addition to that taken up by the plants (10.8% and 8.6%), respectively. Concentrations of

59%, 70% and 92.4% of crude oil were left in the contaminated soil of 0.1%, 1% and 10%, respectively. In the 10% spiked soil, the percentage removal was 7.6% indicating that such percentage removal could be attributed to likely microbial activities, evaporation and other environmental factors/conditions (natural attenuation) since no growth of *Phaseolus vulgaris* was observed in that crude oil accession (Table 8) (Kuo et al. 2014).

Table 7: Concentration of crude oil in soil and *Phaseolus vulgaris* at 7 weeks of planting

Concentrations of Total Petroleum Hydrocarbons						
Soil (mg/kg)				*Phaseolus vulgaris* plants (mg/kg)		
Days	0.10%	1.0%	10%	0.10%	1.0%	10%
0	1000 ± 0.0	10000 ± 0.0	100000 ± 0.0	0 ± 0.0	0 ± 0.0	No growth
7	984 ± 32	9702 ± 101	99552 ± 196	9 ± 0.1	14 ± 0.3	No growth
14	954 ± 29	9534 ± 99	98750 ± 209	18 ± 2.0	98 ± 7.1	No growth
21	916 ± 31	9320 ± 76	97860 ± 201	28 ± 3.2	198 ± 9.4	No growth
28	872 ± 28	9120 ± 88	97040 ± 207	37 ± 3.8	245 ± 13	No growth
35	812 ± 26	8913 ± 81	95400 ± 201	52 ± 4.1	456 ± 19	No growth
42	762 ± 25	8323 ± 87	94028 ± 192	74 ± 6.5	602 ± 21	No growth
49	698 ± 21	7860 ± 89	92400 ± 189	108 ± 8.9	860 ± 22	No growth

Data were processed and expressed as mean ± SD of three replicate

Table 8: Mean percentage crude oil degradation in soil and percentage removal by the plant in each accession

Conc. (%)	Initial TPH in soil (mg/kg)	Residual TPH in soil (mg/kg)	TPH uptake in plant (mg/kg)	% of crude oil degraded in soil	% TPH uptake in plant	%TPH of crude oil left in the soil (non-degraded)
0.1%	1000	698 ± 21	108 ± 8.9	30.2	10.8	59
1%	10000	7860 ± 89	860 ± 22	21.4	8.6	70
10%	100000	92400 ± 189	No growth	7.6	0	92.4

Data were processed and expressed as mean ± SD of three replicate

Ecotoxicological Risk Assessments (ERA)

Ecotoxicological Risk Assessment (ERA) on *Phaseolus vulgaris* was evaluated using the information contained in the Ecotoxicological Risk Assessment Matrix (ERAM) (Table 9). On the ERAM, risk levels can be classified as low, medium, or high. If crude oil is spilled in the environment, animals (A), plants (P), environment (E) and community (C) may be affected and classification can be done based on exposure concentration, exposure duration and potency of the toxicant. The risk levels are characterized in a numbered format. Hazard is given a rating that is multiplied by the likelihood that these hazards would occur using the relationship:

Risk level = Hazard severity x likelihood of exposure (Table 10).

Hazard severity are rated as 1 (slight effect), 2 (minor effect), 3 (localized effect or damage). 4 major effect (deaths) and 5 extensive effect (death of population).

Similarly, the likelihood of occurrence or exposure are rated as 1 (seldom–A–yearly), 2 (frequent–B–quarterly), 3 (very likely–C–monthly), 4 (near certain–D–weekly) and 5 (certain–E–daily) (SETAC 1997, USEPA 2015). The ratings for the different concentrations of spiked crude oils and the

likely effects from exposure are represented in Tables 10 and 11.

Fossil fuels (crude oil, heavy oils, coal and natural gas) and refined petroleum products released into environmental matrices (soil, water, and air) could adversely affect the growth and performance of plants. In this study, germination was in accordance with the 3 to 5 days irrespective of the concentrations of the toxicants reported by Anoliefo and Vwioko (1995) and Gbadebo and Adenuga (2012). However, there was no germination in the soil with the highest concentration of 10% crude oil.

The influence of the crude oil on plant heights was comparable with studies described by Odjegba and Sadiq (2002), Kayode et al. (2009) and Njoku et al. (2008a). Some researchers including Nwoko et al. (2007) and Njoku et al. (2008b) have evaluated the effects of crude oil in soil on crop and concluded that contamination with significant levels of crude oil inhibited germination and the plant developments.

The routes by which nutrients are released to the plants are very important; therefore any interference of the pathways will send a negative signal on plant growth. The relatively low germination of the plants in the higher crude oil concentration in this study may have been due to the unfavourable environment created by the presence of crude oil in the soils including asphyxia and hypoxia resulting from competition of the microbial populations/activity (Merkl et al. 2004). The retarded growth observed in the concentration of 1% crude oil, correlates with the findings of (Udo et al. 1995), who reported growth retardation with 0.75% crude oil and no growth at a concentration of 4% and above. It has been reported that extremely high concentrations of contaminants may not allow plants to grow or survive; thus, phytoremediation is likely to be more effective or efficient at lower contaminants concentrations (USEPA 2000, Moreira et al. 2011). The decrease in the leaf areas of the plants in the contaminated soils as observed could imply that the plants did not absorb all the incoming sunlight thereby lacking the ability to converting the energy needed for photosynthesis into biomass (Peter and Ayolagha 2012). *Phaseolus vulgaris* roots can absorb contaminants in the soils and when contaminants are taken up by plant roots, it could weaken the plant, leading to poor yields and metabolic disorders. The uptake and bioaccumulation of toxicants by plants could be serious threats to animals and humans leading to deleterious effects (Almeda et al. 2013).

Table 9: Ecotoxicological Risk Assessment Matrix (ERAM)

Severity			Consequence P	A	E	C	Increasing Probability A	B	C	D	E
							Never experience the chemical in the area	Had been exposed / used in the area	Had been exposed/ used in the area and other locations	Had been exposed / used several times in the area	Had been exposed / used several times in the area and other locations
0	Practically non-toxic	>1000	No injury	No effect	No effect	No impact	Area 1				
1	Practically non-toxic	>1000	Slight injury	Slight effect	Slight effect	Slight impact					
2	Slightly toxic	100-1000	Minor injury	Minor effect	Minor effect	Limited impact					
3	Very toxic	10-100	Major injury	Localized effect	Localized effect	Considerable impact					
4	Extremely toxic	1.0-10	Single fatality	Major effect (deaths)	Major effect	National impact		Area 2		Area 3	
5	Super toxic	<1.0	Multiple fatality	Extensive effect (kills)	Massive effect	International impact					

Abbreviations: LC50 median lethal concentration in ppm. Data from GESAMP (1997), OECD (2003).

Table 10: Ecotoxicological risk assessment for the study

Concentration of crude oil in soil	Frequency of exposure (daily for 49 days) (a)	Hazard severity (b)	Risk level (a X b)	Hazard rating
Control	E	0	E0	E0 or 0 (P,E,C)
0.1%	E	1	E1	E1 or 5 (P,E,C)
1%	E	2	E2	E2 or 10 (P,E,C)
10%	E	5	E5	E5 or 25 (P,E,C)

Table 11: Consequences of the effects of crude oil using Ecotoxicological risk assessment matrix (ERAM)

Concentration of crude oil in soil	Consequences				Toxic consequence
	Plant (P)	Animal (A)	Environment (E)	Community (C)	
Control	No injury	No effect	No effect	No impact	Practically non-toxic
0.1%	Slight injury	Slight effect	Slight effect	Slight impact	Practically non-toxic
1%	Minor injury	Minor effect	Minor effect	Minor impact	Slightly toxic
10%	Multiple fatality	Extensive (kills)	Massive effect	International impact	Super toxic

The low amounts of crude oil degraded in the 10% crude oil polluted soil indicated that there is the possibility of natural degradation (natural attenuation) which occurs rather slowly, and therefore, indicated that other unseen factors in the soil–microorganisms, natural attenuation, environmental factors amongst others may be responsible for the removal of crude oil in the absence of the plants. In addition, the percentage crude oil degradation in the spiked soil of 0.1% and 1% were higher in analogues to that in the 10% crude oil exposure that showed no growth of *Phaseolus vulgaris*. The crude oil degradations were more on the 0.1% and 1% compared to 10% in which no plant was responsible for degrading the crude. In line with this, Abioye et al. (2012) reported higher amounts of crude oil loss in 5% spent motor oil spiked in soils when compared to that of 15%. It is expected that the lower concentration will degrade more since there will be more activities (microbial, natural attenuation) to enhance the degradation process Similarly, in the research carried out by Hidayati et al. (2018), three mangrove species (*Rhizophora sp., Avicennia sp, Bruguiera sp*) used in their study were able to reduce significant levels of TPH in the media after 30 days treatment. Baek et al. (2004), studied the phytotoxic effects of crude oil and oil components on the growth of red beans (*Phaseolus nipponesis* OWH1) and corn (*Zea mays*) and found that the crude oil-contaminated soil of 10000 mg/kg (1%) was phytotoxic to corn and red beans.

Conclusion

We conclude that *Phaseolus vulgaris* was a suitable plant species for absorbing crude oil from the spiked soil; however the plant was ineffective in remediating the soil contaminated with concentrations greater than 10% of crude oil. From the data obtained from this evaluation, *Phaseolus vulgaris* therefore, should not be regarded as a very good crop for the phytoremediation of crude oil above 10% considering the damage and ecological risk many organisms and human would be exposed to and the reduced economic viability of the plant species. In addition, humans mainly rely on the crop as a rich source of protein, and as such, care should be taken so as not to harvest the crop in contaminated sites for consumption because of the likely impact to humans.

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