

Singapore Journal of

# Scientific Research

ISSN: 2010-006x

**science**  
alert

<http://scialert.net/sjsr>



## Research Article

# Transport and Degradation of Hydrocarbons in a Simulated Crude Oil Contaminated Vadose Zone Due to Nutrient Percolation

<sup>1</sup>Peekate P. Lekiah, <sup>2</sup>Konne J. Lelesi and <sup>3</sup>Abam T.K. Simeon

<sup>1</sup>Department of Microbiology, Faculty of Science, Rivers State University, PMB 5080, Port Harcourt, Nigeria

<sup>2</sup>Department of Chemistry, Faculty of Science, Rivers State University, PMB 5080, Port Harcourt, Nigeria

<sup>3</sup>Institute of Geosciences and Space Technology, Faculty of Science, Rivers State University, PMB 5080, Port Harcourt, Nigeria

## Abstract

**Background and Objective:** Hydrocarbon contaminated vadose zone (HCVZ) is a source of groundwater pollution. Nutrient percolation was investigated for use in remediation of HCVZ. The objectives of the study include determination of the extent of hydrocarbon degradation and vertical transport of hydrocarbons. **Materials and Methods:** Three glass columns loaded with soils from a vadose zone were artificially contaminated with crude oil and labelled start-up concentrations (STC), control (CTL) and treatment (TRT). Soils in STC were used in determination of start-up concentrations, while CTL and TRT were flooded with water and nutrient solution, respectively, at 7 days interval for 4 weeks. Weekly percolated liquids from the setups and soils in the columns at the end of the experiment were analyzed for nutrient, hydrocarbon (TPH) concentrations and populations of selected microbial groups. Results obtained were used in determining transport and degradation of hydrocarbons and in modelling hydrodynamic behaviour in the vadose zone using Hydrus-1D. **Results:** TPH in percolated liquids from TRT and CTL decreased from 4,540-167 and 2,730-183 mg L<sup>-1</sup>, respectively. Hydrocarbon attenuation in TRT and CTL ranged from 50.34-99.06 and 40.22-99.06%, respectively. Calculated hydrocarbon transport into underlying groundwater was 0.202-0.084% for water and 0.335-0.169% for nutrient solution. Simulated hydrodynamic behaviour showed that the vadose zone will lose water more slowly to groundwater than it receives from flooding. **Conclusion:** The study reveals that nutrient percolation through HCVZ will enhance biodegradation of the hydrocarbons and that hydrocarbons entering the underlying groundwater will be minimal.

**Key words:** Petroleum hydrocarbons, vadose zone, nutrient percolation, mass transport of hydrocarbons and hydrodynamic behaviour

**Citation:** Peekate P. Lekiah, Konne, J. Lelesi and Abam T.K. Simeon, 2020. Transport and degradation of hydrocarbons in a simulated crude oil contaminated vadose zone due to nutrient percolation. Singapore J. Sci. Res., 10: 425-437.

**Corresponding Author:** Peekate P. Lekiah, Department of Microbiology, Faculty of Science, Rivers State University, PMB 5080, Port Harcourt, Nigeria  
Tel: +2348063353116

**Copyright:** © 2020 Peekate P. Lekiah *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

The vadose zone, also called the unsaturated zone, is the portion of the earth that extends from the terrestrial surface to the groundwater table<sup>1</sup>. The nature of the vadose zone affects the movement of water, resulting from rainfall or man-made watering, from the land surface to the saturated zone. The saturated zones, commonly called aquifers are the sand/gravel/rock layers beneath the vadose zone that are completely saturated with water<sup>2</sup>. The nature of the vadose zone thus strongly affects the rate of groundwater recharge. Flow rates and biochemical reactions in the vadose zone will thus control the rate of contaminant entry into the underlying groundwater.

Contamination of the vadose zone with hydrocarbons can occur as a result of accidental oil spills from tankers and pipelines carrying crude oil or its product, spills and indiscriminate disposal of by-products generated from artisanal refining of crude oil and leaky underground storage tanks of petroleum products. The contamination can extend to the deep part of the vadose zone and even to underlying groundwater. In an evaluation carried out by United Nations Environment Programme on the extent of petroleum hydrocarbon contamination in selected Ogoni communities located in the Niger Delta area of Nigeria, it was observed that contamination have penetrated deeper than 5 m and has reached the groundwater in many locations<sup>3</sup>. Petroleum hydrocarbon contaminated vadose zone can thus become a long-term source of groundwater contamination. Petroleum hydrocarbons in a contaminated vadose zone may undergo biodegradation and volatilization, persist in the vadose zone or may be transported to the groundwater<sup>4</sup>. The persistence of petroleum hydrocarbons in the vadose zone or its transport to the groundwater will depend on such factors as (1) The rate of biodegradation of the petroleum hydrocarbons in the vadose zone, (2) The nature of the soil horizons making up the vadose zone and (3) Rate of water percolation, resulting from rainfall and man-made watering, through the vadose zone.

Biodegradation of hydrocarbons in petroleum hydrocarbon contaminated vadose zone achieved by instigating biochemical processes that enhances the natural attenuation of the hydrocarbon contaminants. The activities carried out in enhancing natural attenuation of contaminants in an environment through biological means embodied in the term "bioremediation". A typical bioremediation activity consists of (1) Addition of hydrocarbon degrading microorganisms, (2) Application of nutrients, (3) Adjustment of pH, (4) Adjustment of water content, (5) Aeration or (6)

Application of surfactants/biosurfactants<sup>5-10</sup>. Nutrient percolation, through semi-continuous flooding has been suggested and investigated as a means for the *in-situ* bioremediation of petroleum hydrocarbon contaminated vadose zone<sup>11-13</sup>.

Data generated from soil column experiments can be helpful in predicting the fate of hydrocarbons in vadose zone contaminated with crude oil or petroleum products. The aim of this study was the remediation of crude oil contaminated vadose zone through the use of nutrient percolation.

## MATERIALS AND METHODS

### **Vadose zone soil sample collection and on-site geophysical analysis:**

Vadose zone soil samples were collected from a point on a site in Eneka Community of Obio-Akpor LGA, Rivers State, Nigeria. The research project was conducted from September, 2018-September, 2019. The location of the site as determined using the Global Positioning System (GPS) is 4°54'32.7" N, 7°02'41.9" E. Collection of vadose zone soil samples was made possible by drilling a borehole on the chosen site using borehole drilling equipments. Samples were collected based on the soil layers making up the vadose zone, until groundwater was reached. The depth of each soil layer was noted. The soil samples of each soil layer were transferred into large sampling containers and transported to the Microbiology Laboratory of the Rivers State University, Nigeria, for analysis and experimentation.

### **Simulation of petroleum hydrocarbon contaminated vadose zone:**

Cylindrical glass column loaded with vadose zone soil samples was used to simulate the vadose zone environment. The soils in the column were artificially contaminated with crude oil.

### **Column design:**

The cylindrical glass columns used for the study were 1.27 m in height and 0.07 m in diameter. The base of each column had an opening and was shaped like a funnel. Non-absorbent packing material was filled into the base. Each column was supported at the middle and at the base by metal ring support attached to a customized designed stand. The columns were three in number.

### **Loading of columns:**

Soil samples from each soil layer were filled and compacted into the three glass columns based on how they occurred in the sampled vadose zone. The thickness of each soil layer in the column was set relative to the ratio in

Table 1: Composition of the nutrient solution

Components	Quantity
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.4 g
KH <sub>2</sub> PO <sub>4</sub>	0.8 g
Na <sub>2</sub> HPO <sub>4</sub>	1.3 g
NaNO <sub>3</sub>	0.4 g
Tap water	1000 mL

which they occurred in the vadose zone so as to obtain a total vadose zone experimental thickness of 50 cm. The soil columns were labelled STC (setup for determination of start-up concentrations), CTL (control setup) and TRT (setup for nutrient percolation). Each column was artificially contaminated with 150 mL crude oil and Erlenmeyer flasks of 500 mL capacity were placed beneath the base of the columns. The columns were left undisturbed overnight. On the following morning, the volumes of crude oil that have percolated into the different flasks were measured so as to determine the volumes of crude oil that were absorbed in the different columns. The soils in column STC were evacuated and the concentration of petroleum hydrocarbons (TPH), nitrate, phosphate and sulphate in each soil layer was determined. The soil layers were also analyzed for populations of hydrocarbon utilizing bacteria (HUB), anaerobic bacteria (ANB), hydrocarbon-utilizing anaerobic bacteria (HUA), total fungi (TF) and hydrocarbon utilizing fungi (HUF). The population of TF and HUF was determined for only the top soil layer.

**Experimental operation:** A nutrient solution was prepared from selected salts and tap water (Table 1). The pH and concentration of nitrate, phosphate and sulphate of the tap water and the prepared nutrient solution was determined. The population of total heterotrophic bacteria (THB) in the tap water was also determined. Column CTL was flooded with the tap water and column TRT was flooded with the nutrient solution. The time taken for the percolated liquids to start coming out and the volume of liquids consumed were noted. Flooding of the columns was carried out at 7 days interval for 4 weeks, i.e., at day 0, 7, 14, 21 and 28.

**Analysis of vadose zone percolated liquid and soils:** The percolated liquids from the column setups were analyzed for the concentrations of nitrate, phosphate, sulphate and TPH. At the end of the experimental period (day 28), after collection of percolated liquids, the columns were evacuated and soils from the soil layers of each column were analyzed for concentrations of nitrate, phosphate, sulphate and TPH. The soil samples were also analyzed for populations of HUB, ANB

and HUA, while populations of TF and HUF were determined for only the top soil layer.

**Determination of nutrient levels:** The nutrient levels in soil and vadose zone percolated liquid samples were determined in form of nitrate, phosphate and sulphate concentration. The nitrate concentration was determined using the Brucine method<sup>14</sup>, the sulphate concentration was determined using a turbidimetric method (APHA method 4500-SO<sub>4</sub><sup>-2</sup>)<sup>15</sup>, while the phosphate concentration was determined using ascorbic acid method (APHA method 4500-P)<sup>15</sup>.

**Determination of hydrocarbon contamination level:** The concentration of petroleum hydrocarbons (TPH) in soil and percolated liquid samples were analyzed using a spectrophotometric method. In the method, hexane was used in extraction of petroleum hydrocarbons from the samples. The extracts were then subjected to light absorbance measurement at 420 nm using a 721 visible spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). The absorbance values were used to calculate the TPH concentrations with the aid of a previously obtained standard graph of absorbance versus crude oil concentration.

**Determination of population of total fungi and hydrocarbon utilizing fungi:** The population of total fungi (TF) was determined using Sabouraud dextrose agar (SDA) and the spread plate technique. Inoculated plates were incubated at ambient temperature for 3-5 days. The population of hydrocarbon utilizing fungi (HUF) was determined using the modified mineral salts agar medium of Odokuma and Dickson<sup>6</sup> supplemented with 50 µg mL<sup>-1</sup> streptomycin and 50 µg mL<sup>-1</sup> tetracycline. Inoculation was done using the spread plate technique and petroleum hydrocarbons were supplied into the inoculated plate using the vapour phase transfer technique<sup>16</sup>. The plates were incubated at ambient conditions for 5-7 days.

**Determination of the populations of selected bacterial group:** The population of total heterotrophic bacteria (THB) was determined using nutrient agar and the spread plate technique. Inoculated plates were incubated at 37°C for 24 h. For determination of the population of anaerobic bacteria (ANB), nutrient agar and the pour plate technique was used and then inoculated plates were incubated in anaerobic gas jars at ambient temperatures (29-31°C) for 3 days. The population of hydrocarbon utilizing aerobic bacteria (HUB) was determined using the modified mineral salts agar medium

of Odokuma and Dickson<sup>6</sup> supplemented with 1 mg mL<sup>-1</sup> Nystatin. Inoculation was done using the spread plate technique and petroleum hydrocarbons were supplied into the inoculated plate using the vapour phase transfer technique. The plates were incubated at ambient conditions for 7 days. The population of hydrocarbon utilizing anaerobic bacteria (HUA) were also determined using the method used for the HUB population, however the pour plate method was used in place of the spread plate technique and incubation was done in anaerobic gas jars.

**Determination of contaminant transport and degradation:**

Selected data generated from the experimental flooding activity, including the time taken for percolated liquids to start coming out and volume of liquids consumed, were used to determine the transport and degradation of petroleum hydrocarbons in the column setups. The results obtained were used to extrapolate a likely case scenario in the vadose zone studied. The hydrodynamic behaviour in the simulated vadose zone as a result of the flooding activity was modelled using the Hydrus-1D software package (PC-Progress s.r.o, Korunni, Prague, Czech Republic).

**RESULTS AND DISCUSSION**

**Characteristics of the soil layers making up the sampled vadose zone:**

During soil collection from the sampled vadose zone, the zone was observed to be made up of 6 soil layers to a depth of 1200 cm where saturation occurred, saturation occurred about 60 cm deep into the 6th soil layer. In a research carried out by Tamunobereton-ari *et al.*<sup>17</sup> it was observed that vadose zone in some other parts of Rivers State, Nigeria (where the vadose zone for this study was sampled) are made up of 4-5 soil layers to a depth of 1,500 cm. Other extensively studied vadose zones in Rivers State, Nigeria, have been observed to contain 2, 5 and 7 soil layers to a depth of 100-1,200 cm where saturation occurs<sup>3</sup>. It is thus evident that the vadose zone of Rivers State, Nigeria is not a lateral continuous layer and that the number of soil profiles before the saturation zone is reached can range from 2-7. The

observation of 6 soil layers in the vadose zone sampled can thus be acceptable to fall within the range of the number of soil layers that can be found in the vadose zone of an area within Rivers State, Nigeria.

The soil layers from the sampled vadose zone were coded A-F. The approximate thickness of each of the soil layers are as follows: A-40, B-260, C-540, D-210, E-90 and F-60 cm. The characteristic of soil from each of the soil layers are presented in Table 2.

**Thickness of soil layers in the experimental vadose zone:**

Based on the total depth of the soil layers making up the vadose zone (1,200 cm), the glass columns were filled from bottom to top so as to obtain the chosen vadose zone experimental thickness of 50 cm as follows: F-2.5 cm thickness, E-3.8 cm thickness, D-8.8 cm thickness, C-22.5 cm thickness, B-10.8 cm thickness, A-1.7 cm thickness, leading to a total of 50.1 cm in thickness.

**Contamination and nutrient levels:**

Following artificial contamination with 150 mL crude oil for each column, the volumes of crude oil that percolated into their respective flasks was as follow: STC-79, CTL-84 and TRT-81 mL. This indicated that the amounts of crude oil adsorbed in the various columns were, STC-71, CTL-66 and TRT-69 mL.

The petroleum hydrocarbon (TPH) contamination level in each of the soil layers of the setup for determination of start-up concentrations (STC) is presented in Table 3. From the Table, it can be seen that TPH concentration reduced from 56,533 mg kg<sup>-1</sup> at soil layer A (the top soil layer) to 5,407 mg kg<sup>-1</sup> at soil layer E and increased to 9,067 mg kg<sup>-1</sup> at soil layer F (the bottom soil layer). In a related study carried out by Kristensen *et al.*<sup>18</sup>, a decrease in TPH concentration has been observed as one goes down from the sandy-likely loam-gravel layer to the sandy-likely loam layer, followed by an increase from the fine sand layer to the coarse sand layer and then a decrease to the silt loam layer. The reduction in TPH concentration, in this study, from soil layer A (the top soil layer) to soil layer E and then an increase in soil

Table 2: Characteristic of soil from each layer making up the sampled vadose zone

SLC	AT (cm)	Colour	General characteristic	Stickiness	General soil type
A	40	Dark brown	Smooth to the touch	Moderate	Loam
B	260	Brown	Smooth to the touch	Moderate	Clay loam
C	540	Yellowish brown	Smooth to the touch	Sticky	Clay
D	210	Dirty white/greyish	Silty/gritty to the touch, loose soil	Nil	Silty sand
E	90	Light brown/greyish	Silty/gritty to the touch with presence of pebbles	Nil	Sandy Silt
F	60	Light brown/shades of light grey	Silty/gritty to the touch, loose soil	Nil	Silty sand

SLC: Soil layer code, AT: Approximate thickness

Table 3: Start up contaminant and nutrient levels in the experimental vadose zone setup

SLC	TPH (mg kg <sup>-1</sup> )	Nitrate (mg kg <sup>-1</sup> )	Phosphate (mg kg <sup>-1</sup> )	Sulphate (mg kg <sup>-1</sup> )
A	56,533	579	73.92	≤0.67
B	31,733	423	7.44	≤0.67
C	14,413	67	54.14	≤0.67
D	7,793	1.092	27.78	≤0.67
E	5,407	≤2.23	19.96	≤0.67
F	9,067	134	≤0.01	≤0.67

SLC: Soil layer code, TPH: Total petroleum hydrocarbon

Table 4: Selected parameters of the tap water and nutrient solution used in flooding the control and treatment setups

Parameters	THB	pH	Nitrate (mg L <sup>-1</sup> )	Phosphate (mg L <sup>-1</sup> )	Sulphate (mg L <sup>-1</sup> )
Tap water	1.10 × 10 <sup>4</sup>	5.5	≤0.03	6.69	≤0.04
Nutrient solution	-	7.0	202.87	18.42	184.26

THB: Total heterotrophic bacteria

layer F (the bottom soil layer) can be agreed to follow similar pattern with what has been observed by Kristensen *et al.*<sup>18</sup>. The slight variations can be attributed to the differences in the nature of the vadose zones studied.

Results of nutrient determination show that there was presence of nitrate and phosphate in the soils but little or no sulphate (Table 3). Also there was a general decrease in the concentration of nitrate and phosphate from the top to the bottom soil profile. A similar scenario has been observed by Jobbagy and Jackson<sup>19</sup>, phosphorus concentrations in soils were noticed to be far greater than sulphate and phosphorus was also more concentrated in top soils than bottom soil profiles.

#### Physicochemical and bacteriological status of the fluids used in the experimental setups:

Selected physicochemical and heterotrophic bacterial population of the tap water and nutrient solution used in flooding the experimental setups are presented in Table 4. From the Table it can be seen that there was presence of bacteria in the tap water, there was little or no nitrate and sulphate in the tap water, whereas the phosphate concentration was 6.69 mg L<sup>-1</sup>. On the other hand, there was appreciable amount of nitrate, phosphate and sulphate in the nutrient solution used in the study. This is due to the addition of their corresponding salts. The level of nitrate and phosphate in groundwater from neighbouring communities to the place where the tap water was collected has been shown by Festus *et al.*<sup>20</sup> to be ≤0.04 and ≤2.99 mg L<sup>-1</sup>, respectively. In another study conducted by Ukpaka and Ukpaka<sup>21</sup>, the level of sulphate in groundwater from another set of neighbouring communities to the place where the tap water was collected was observed to be between 2.8-9.2 mg L<sup>-1</sup>. With regards to nitrate, the concentration obtained in this study can be said to agree with that obtained by Festus *et al.*<sup>20</sup>. The slight increase in phosphate concentration can be attributed to the detection/

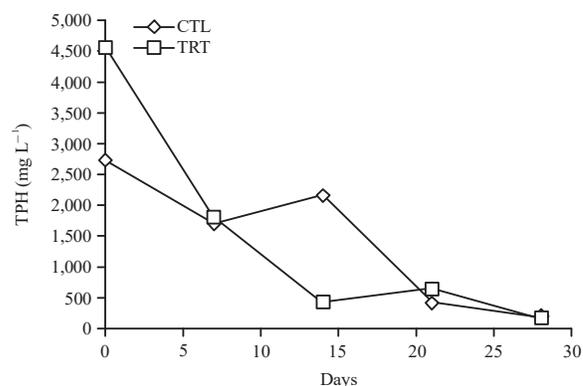


Fig. 1: Total petroleum hydrocarbon (TPH) concentration in the percolated liquids from the experimental setups  
CTL: Control setup, TRT: Setup flooded with nutrient solution

quantification method used. The wide discrepancy in sulphate concentration can be attributed to the nature of the land use around the groundwater source, the tap water used in this study is from groundwater in a University campus, whereas the groundwater studied by Ukpaka and Ukpaka<sup>21</sup> were sourced from crowded busy urban environments.

#### Hydrocarbon and nutrient presence in percolated liquids from the experimental setups:

The concentrations of petroleum hydrocarbons, phosphate, nitrate and sulphate in the percolated liquids from the experimental setups for the duration of the study is presented in Fig. 1-3. In Fig. 1 it can be seen that on day 0 the amount of TPH in the percolated liquid from column TRT (4,540 mg L<sup>-1</sup>) is almost 2 times that in the percolated liquid from column CTL (2,730 mg L<sup>-1</sup>). This may be as a result of a physical property of the nutrient solution which favours some level of desorption of hydrocarbons from soil particles. On day 14 the amount of TPH in the percolated liquid from column TRT (430 mg L<sup>-1</sup>) is about 5 times lower than that in the percolated liquid from column CTL (2,157 mg L<sup>-1</sup>). This can be attributed to

enhanced biodegradation as a result of provision of adequate amount of nutrients for the optimal stimulation of inherent hydrocarbon utilizing microorganisms. The provision of nutrients is evident in the relative high concentration of phosphate, nitrate and sulphate in percolated liquids from column TRT (Fig. 2, 3). Provision of nutrients such as

nitrogen and phosphorus has been shown to enhance biodegradation of petroleum hydrocarbons<sup>22,23</sup>. The observation on day 14 is thus in agreement with the concept of occurrence of enhanced biodegradation. On day 28, the TPH concentration in the treatment and control setup reduced to 167 and 183 mg L<sup>-1</sup>, respectively.

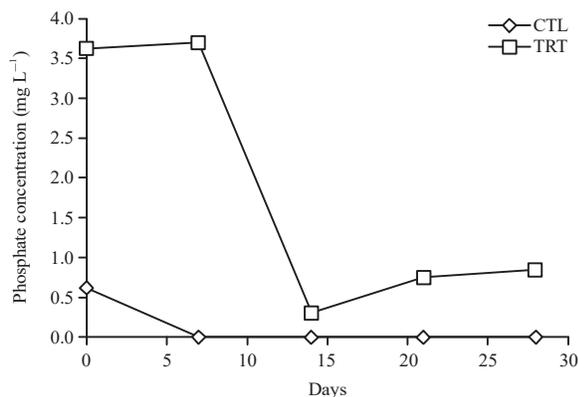


Fig. 2: Phosphate concentration in the percolated liquids from the experimental setups

CTL: Control setup, TRT: Setup flooded with nutrient solution

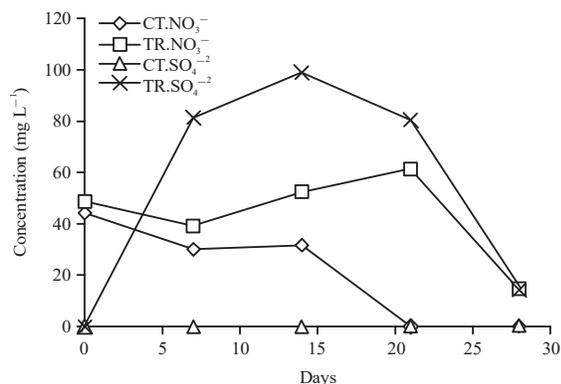


Fig. 3: Nitrate and Sulphate concentration of the percolated liquids from the experimental setups

CT.NO<sub>3</sub><sup>-</sup>: Nitrate concentration in liquid from column CTL, TR.NO<sub>3</sub><sup>-</sup>: Nitrate concentration in liquid from column TRT, CT.SO<sub>4</sub><sup>-2</sup>: Sulphate concentration in liquid from column CTL, TR.SO<sub>4</sub><sup>-2</sup>: Sulphate concentration in liquid from column TRT

**Hydrocarbon attenuation and fluctuation in quantity of nutrients and microorganisms:**

The petroleum hydrocarbon concentration in each soil layer of the experimental setups at the beginning and end of the experimental period and the extent of hydrocarbon attenuation are presented in Table 5. From the Table, it can be seen that hydrocarbon attenuation was achieved in both column CTL and TRT, with a relatively high attenuation in column TRT in soil layers A (92.69), B (93.97) and F (50.34%) as compared to 83.82, 90.52 and 40.22%, respectively in column CTL. Relatively high hydrocarbon attenuation in column CTL occurred in soil layer C (96.72) and E (89.27%) as compared to 95.51 and 60.79%, respectively in column TRT.

The cumulative TPH concentrations, i.e., from day 0-28, in percolated liquids from column CTL and TRT is 7,190 and 7,584 mg L<sup>-1</sup>, respectively. The differences in the cumulative TPH concentration at the beginning and end of the experiment in column CTL and TRT are 106,319 and 111,633 mg kg<sup>-1</sup>, respectively. The TPH concentration in the percolated liquids from both setups is thus far less than the cumulative amount of hydrocarbons lost at the end of the experiment. Therefore much of the reduction in the TPH concentration in both setups can be attributed to degradation by biochemical and probably physical means, rather than to vertical migration resulting from flooding with water or nutrient solution. TPH reduction in the different soil layers in the experimental setups, in terms of biochemical means, can be attributed to enhanced biodegradation as a result of provision of adequate amount of nutrients or moisture for the optimal stimulation of inherent hydrocarbon utilizing microorganisms. The degree of utilization of the provided nutrients and the availability/stimulation of inherent

Table 5: Extent of hydrocarbon (TPH) attenuation at the end of the experimental period

Soil layers	Day 0	Day 28		EHA	
	STC (mg kg <sup>-1</sup> )	CTL (mg kg <sup>-1</sup> )	TRT (mg kg <sup>-1</sup> )	CTL (%)	TRT (%)
A	56,533	9,147	4,133	83.82	92.69
B	31,733	3,007	1,913	90.52	93.97
C	14,413	473	647	96.72	95.51
D	7,793	73	73	99.06	99.06
E	5,407	580	2,120	89.27	60.79
F	9,067	5,420	4,500	40.22	50.34

STC: Start-up concentration, CTL: Control setup, TRT: Nutrient percolation setup, EHA: Extent of hydrocarbon attenuation (initial THC-final THC/initial THC × 100%)

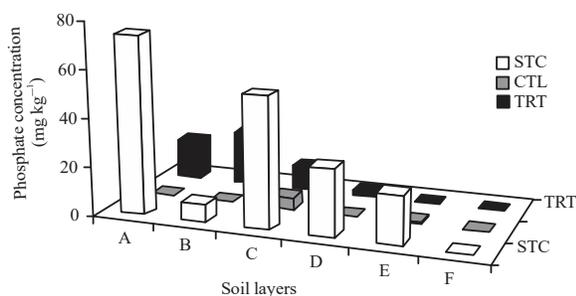


Fig. 4: Phosphate concentration in each soil layer in the column setups at the beginning and end of the experimental period  
STC: Start-up concentration, CTL: Control setup, TRT: Nutrient percolation setup

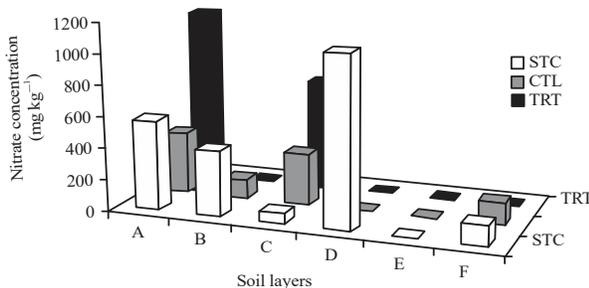


Fig. 5: Nitrate concentration in each soil layer in the column setups at the beginning and end of the experimental period

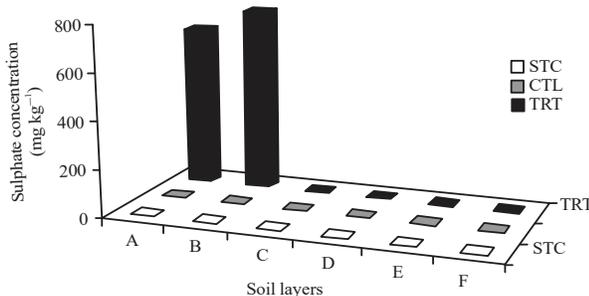


Fig. 6: Sulphate concentration in each soil layer in the column setups at the beginning and end of the experimental period

hydrocarbon utilizing microorganisms can be examined in terms of the amount of nutrients in each soil layer before and after the experimental period (Fig. 4-6) and also in terms of the relative population increase of the investigated microbial groups (Fig. 7-10).

In Fig. 4, it can be seen that in column CTL there was total depletion of phosphate in all the soil layers, except in soil layer C, while in column TRT there was total depletion of phosphate

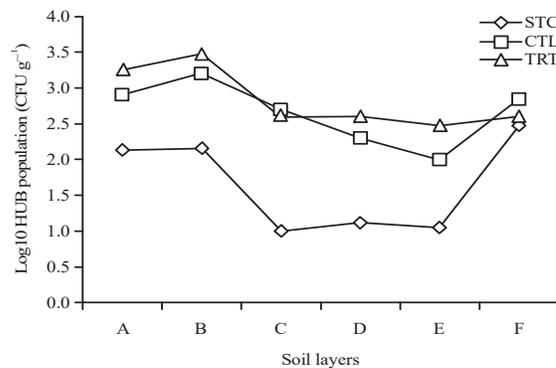


Fig. 7: Hydrocarbon utilizing bacterial (HUB) populations in the different soil layers in the column setups at the beginning and end of the experiment  
STC: Start-up concentration, CTL: Control setup, TRT: Nutrient percolation setup

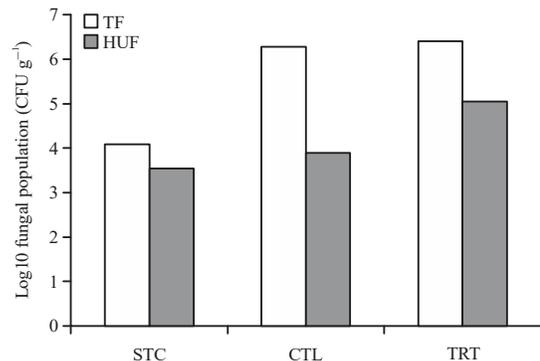


Fig. 8: Population of total fungi (TF) and hydrocarbon utilizing fungi (HUF) in soil layer A in the column setups at the beginning and end of the experimental period

in soil layer E and moderate depletion in soil layer A, C and D. These indicate that phosphate was used up by inherent hydrocarbon utilizing microorganisms for hydrocarbon degradation. Soil layer C in column CTL had the second highest hydrocarbon attenuation percentage, next to soil layer D and still had some amount of phosphate for use by inherent hydrocarbon degraders for hydrocarbon degradation. Soil layer C been clay soil should have a higher biodegradation percentage than soil layer D which is sandy silt, Biodegradation rate has been shown to be higher in clayey soil than in sandy soils<sup>18</sup>. It may thus be reasoned that the higher hydrocarbon attenuation percentage in soil layer D, in both column CTL and TRT, could be as a result of the additional effect of flushing due to flooding with water or nutrient solution. The idea of flushing is based on the fact that hydrocarbon adsorption in fine sands such as clay is higher than hydrocarbon adsorption in coarse sands such as silty

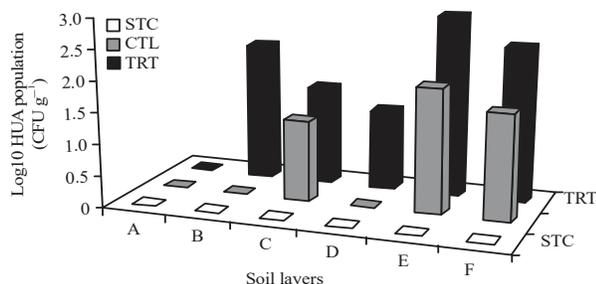


Fig. 9: Hydrocarbon utilizing anaerobic bacteria (HUA) populations in the different soil layers in the column setups at the beginning and end of the experiment  
STC: Start-up concentration, CTL: Control setup, TRT: Nutrient percolation setup

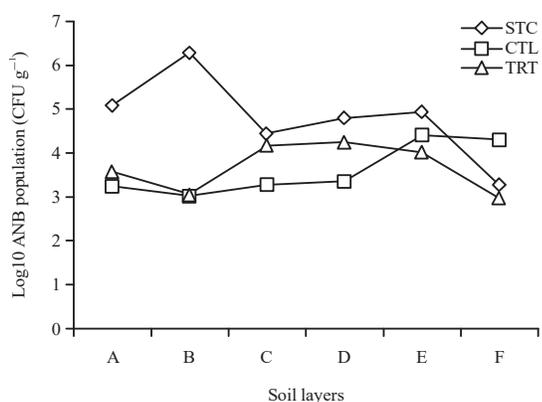


Fig. 10: Anaerobic bacteria (ANB) populations in the different soil layers in the column setups at the beginning and end of the experiment  
STC: Start-up concentration, CTL: Control setup, TRT: Nutrient percolation setup

sands<sup>24</sup>. The role of biodegradation in hydrocarbon attenuation in soil layer D and other soil layers too, cannot be ruled out since there was evidence of increase in the population of hydrocarbon utilizing bacteria (HUB) as depicted in Fig. 7. The increase in HUB population as portrayed in Fig. 7 can be collaborated with the extent of hydrocarbon attenuation in the experimental setups. In Fig. 7, it can be seen that increase in HUB population was more for column TRT than column CTL at soil layers A, B, D and E, while column CTL had more HUB population than column TRT at soil layers C and F. The relative increase in HUB population collaborates with the extent of hydrocarbon attenuation in column CTL and TRT at soil layers A, B and C. Non-collaboration at soil layers E and F of both setups indicates that certain physical means also partook in the hydrocarbon attenuation. The extent of hydrocarbon attenuation in column CTL and TRT at soil layer

A can also be collaborated with the relative increase in the fungal population as depicted in Fig. 8. In the Figure, it can be seen that the increase in the population of hydrocarbon utilizing fungi (HUF) was more in column TRT than in column CTL.

The degree of utilization of nitrate is presented in Fig. 5. From the Figure, it can be seen that in column CTL there was reduction in nitrate in soil layers A and B and complete nitrate reduction in soil layer D, while in column TRT there was complete nitrate reduction in soil layer B, D and F. The reduction in nitrate indicate that there was nitrate utilization by available hydrocarbon utilizing bacteria in degradation of the hydrocarbons and also that nitrate reducing bacteria (NRB) may have partook in the degradation. Nitrate reducing bacteria are facultative anaerobes<sup>25</sup> and are known to partake in the anaerobic degradation of hydrocarbons<sup>26,27</sup>. Anaerobic biodegradation of the hydrocarbons in the experimental setups is indicated by the emergence of hydrocarbon-utilizing anaerobic bacteria (HUA) as depicted in Fig. 9. In the Figure it can be seen that there was emergence and relative increase in HUA population in column TRT in soil layers B-F, while emergence of HUA population in column CTL occurred in soil layers C, E and F. The emergence of HUA population did not occur by spontaneous generation, there was evidence of the presence of anaerobic bacteria as portrayed in Fig. 10. Thus the HUA population that emerged are some of the anaerobic bacteria which developed the ability to utilize the hydrocarbon contaminants.

Nitrate accumulation occurring in soil layer C of both setups and in soil layer A of column TRT (Fig. 5) can be attributed to absence of NRB in soil layer A of column TRT and low population of NRB in soil layer C of both setups. This is evident in the zero population of HUA in soil layer A of column TRT, relatively low HUA population in soil layer C and relatively low population of ANB in soil layers A and C as portrayed in Fig. 9 and 10. Also, it should be noted that a good amount of nitrate was present in the solution (Table 4) intermittently supplied to column TRT. This continual supply can lead to nitrate accumulation.

Sulphate accumulation occurred in soil layers A and B of column TRT as shown in Fig. 6. This could be as a result of little or no bio-utilization of the sulphate in the nutrient solution supplied to column TRT. It can thus be reasoned that the supply of sulphate to column TRT did not play any role in the biodegradation of the petroleum hydrocarbons. However, sulphate infiltration has been shown to enhance biodegradation of petroleum hydrocarbons<sup>28</sup>. The difference in the observations may be attributed to the presence or

Table 6: Transport of petroleum hydrocarbons in the control column

Parameters	Day 0		Day 28	
	CTL	TRT	CTL	TRT
SLH (mg kg <sup>-1</sup> )	56,533	56,533	9,147	4,133
PLH (mg L <sup>-1</sup> )	2,730	4,540	183	167
PSL (%)	4.83	8.03	2.00	4.04
TPE (s)	10	12	9	8
PSZ (%)	0.202	0.335	0.084	0.169
TEZ (s)	239.5	287.4	215.6	191.6

SLH: Hydrocarbon concentration in the top soil layer, PLH: Hydrocarbon concentration in percolated liquid, PSL: Percentage of hydrocarbon concentration of the surface layer in percolated liquid, TPE: Time taken for percolated liquid to emerge from the experimental columns, PSZ: Percentage of hydrocarbon concentration of the top soil layer that will be found in the underlying saturated zone after percolation through the actual thickness of the vadose zone, TEZ: Time taken for percolated liquid to travel into the saturated zone in a real life scenario

absence of sulphate reducing bacteria (SRB). SRB are known to degrade hydrocarbons in anaerobic environments<sup>29</sup> and reduce sulphate under anaerobic condition<sup>30</sup>. In this study, SRB may have been absent in soil layers A and B, therefore the accumulation of sulphates in these layers. However, with regards to soil layer C-F, since there was presence of anaerobes (Fig. 10) and emergence of anaerobic hydrocarbon utilizing bacteria (Fig. 9), some level of hydrocarbon degradation in soil layers C-F may be attributed to the activity of sulphate reducing bacteria.

**Hydrocarbon transport:** Calculation of the transport of petroleum hydrocarbons in the experimental setups is presented in Table 6. In the Table, it can be seen that the percentage of hydrocarbon concentration in the top soil layer in percolated liquids (PSL) is very small for both setups. PSL is calculated using the equation:

$$\text{PSL (\%)} = \frac{\text{Hydrocarbons concentration in percolated liquid}}{\text{Hydrocarbon concentration in the top soil layer}} \times 100$$

In a related research where the experiment was conducted in the field, groundwater pollution resulting from hydrocarbons in the vadose zone was only detected in the upper 30 cm of the underlying groundwater and the concentrations decreased sharply with depth to non-detect at 30 cm depth<sup>31</sup>. The relatively small amount of hydrocarbons crossing over from the vadose zone to the saturation zone can be attributed to retention in the soil layers of the vadose zone and subsequent biodegradation. Adsorption and biodegradation of petroleum hydrocarbons have been shown to be dominant processes occurring during the remediation of contaminated vadose zone<sup>24</sup>.

In calculating the percentage of hydrocarbon concentration in the top soil layer that will be found in the underlying saturated zone or groundwater (PSZ) in a real life scenario, it is assumed that the percentage of hydrocarbon

concentration in the top soil layer that will be found in the saturated zone is inversely proportional to the thickness of the vadose zone (1200 cm). PSZ is thus calculated using the formula:

$$\text{PSZ} = \frac{\text{PSL (\%)} \times 50.1 \text{ cm}}{1200 \text{ cm}}$$

where, 50.1 cm is the distance of percolation or vadose zone thickness in the experimental columns. The calculation showed that about 0.08 and 0.17% of hydrocarbons in the surface layer will be found in the underlying saturated zone after percolation through the vadose zone as a result of flooding with water and nutrient solution, respectively. The percentage may however be lesser because (1) The actual compaction of the soil layers in the vadose zone was not achieved in the experimental setup, (2) All the layers of soil in the experimental setup was contaminated which may not be so in real life scenario and (3) Retention of hydrocarbons and subsequent biodegradation will be taking place in some of the soil layers.

In calculating the time it will take for hydrocarbons to reach the underlying saturated zone in a real life scenario (TEZ), it is assumed that the time taken for percolated liquid to emerge (i.e., time taken for hydrocarbons to enter into the underlying saturated zone) is directly proportional to the thickness of the vadose zone. TEZ is thus calculated using the formula:

$$\text{TEZ} = \frac{1200 \text{ cm} \times \text{TPE}}{50.1 \text{ cm}}$$

where, TPE is the time taken for percolated liquid to emerge from the experimental columns. The calculation showed that it will take about 3.6 and 3.2 min for hydrocarbons in the surface layer to travel to the underlying saturated zone as a result of flooding with water and nutrient

solution, respectively. However in real life scenario, the time may be far lesser due to the compaction and nature of the soil layers and lateral flow of hydrocarbons.

**Hydrodynamic behaviour in the simulated vadose zone:**

Results of the modelling of the hydrodynamic behaviour in the simulated vadose zone as determined using the HYDRUS-1D software package is presented in Fig. 11 and 12 and in Table 7. In Fig. 11, it can be seen that the water content would be high in the various soil layers, except for soil layer D (Silty sand layer). The relatively high water accumulation in soil layers A, B, C, E and F indicates that biodegradation of organic contaminants will be favoured in these layers. Hydrocarbon reduction in soil layer D can thus be attributed more to flushing action that will occur as a result of flooding than to biodegradation.

In Fig. 12, it can be seen that the ease at which water would flow (hydraulic conductivity) through the vadose zone would be higher at a depth of 0-0.5 m and moderate at a depth of 0.5-8 m. This indicates that the hydraulic conductivity

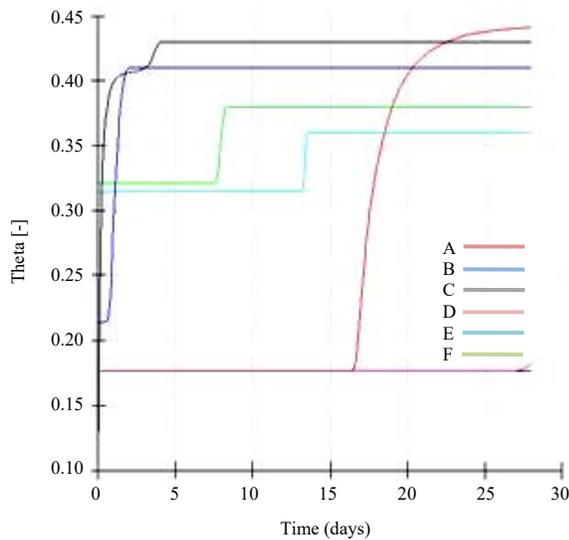


Fig. 11: Change in water content in the different soil layers in the column setups

reduces with depth and thus water supplied through flooding will also flow in lateral directions in addition to percolating to underlying soil layers.

In Table 7, it can be seen that the mean pressure head in the vadose zone will increase with time. This indicates that the vadose zone will become saturated with time as flooding is carried out intermittently. Also it can be seen in the Table that the bottom drainage (Bottom flux) is far lesser than the top drainage (Top flux). This indicates that the vadose zone will lose water more slowly to the underlying groundwater than it receives from the flooding activity. Thus transport of hydrocarbons carried along with water moving from the vadose zone into the underlying groundwater will be very minimal.

**Predicting the duration for drastic reduction in hydrocarbon concentration:**

Attenuation of petroleum hydrocarbons in terms of biodegradation has been shown to follow a first order reaction<sup>32,33</sup>. The equation of first order reaction is given as<sup>34</sup>:

$$\text{Log } [A]_t = \frac{-K}{2.303} + \text{Log } [A]_0$$

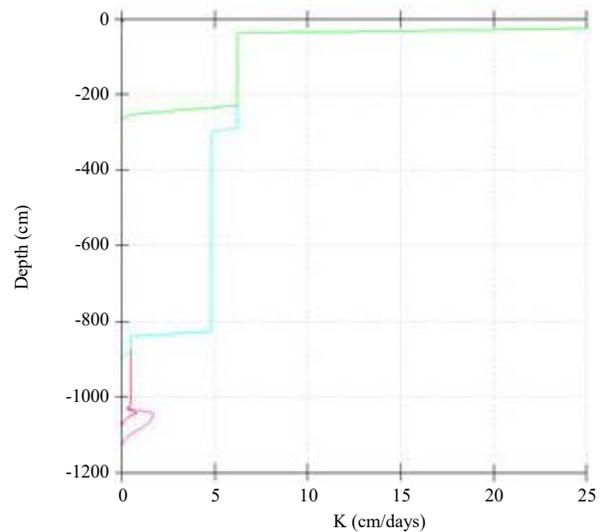


Fig. 12: Hydraulic conductivity in relationship to depth

Table 7: Fluctuations in mass balance variables resulting from water inflow into the vadose zone

Time (day)	.h mean (cm)	Top flux (cm/day)	Bottom flux (cm/day)	WatBalT (cm <sup>3</sup> )	WatBalR (%)
0	-1200.0	-0.00002	-0.0002	-	-
0.08	-1193.5	-1.251	-0.0002	-0.00004	0.006
7	-930.1	-7.423	-0.00011	-0.00004	0.000
14	-207.2	-4.1	-0.00009	-0.00008	0.000
21	145.5	-1.9	-0.00007	-0.00009	0.000
28	195.2	-1.9	-0.00006	-0.00008	0.000

.h mean: Mean pressure head in the entire flow domain, Top flux: Top drainage boundary condition, bottom flux: Bottom drainage boundary condition, WatBalT: Absolute error in the water mass balance of the entire flow domain, WatBalR: Relative error in the water mass balance of the entire flow domain

Table 8: Degradation constants and calculated time for reduction of petroleum hydrocarbons to 1 mg kg<sup>-1</sup> soil

Soil layers	Day 0 (mg kg <sup>-1</sup> )		Day 28 (mg kg <sup>-1</sup> )		K/days		TCD (days)	
	STC	CTL	TRT	CTL	TRT	CTL	TRT	
A	56,533	9,147	4,133	0.07	0.09	156.4	121.6	
B	31,733	3,007	1,913	0.08	0.10	129.6	103.7	
C	14,413	473	647	0.12	0.11	79.8	87.1	
D	7,793	73	73	0.17	0.17	52.7	52.7	
E	5,407	580	2,120	0.08	0.03	107.5	286.6	
F	9,067	5,420	4,500	0.02	0.03	455.7	303.8	

K: Degradation constant as calculated using first order reaction, TCD: Calculated time for hydrocarbon concentrations to reduce to 1 (mg kg<sup>-1</sup>), STC: Start-up concentration, CTL: Control setup

where  $[A]_t$  is the hydrocarbon concentration at time  $t$ ,  $[A]_0$  is the initial hydrocarbon concentration,  $K$  is the degradation constant, and  $t$  is the time taken for  $[A]_0$  to reduce to  $[A]_t$ . The time it will take for hydrocarbon concentration in the different soil layers in the setup columns to reduce to 1 mg kg<sup>-1</sup>, i.e. when  $\text{Log } [A]_t = 0$ , as determined using the first order equation is presented in Table 8. From the Table it can be seen that the time it will take for hydrocarbon concentration in the different soil layers in column CTL to reduce to 1 mg kg<sup>-1</sup> ranges from 53-456 days, while in column TRT it ranges from 53-304 days. This data indicates that drastic reduction in the quantity of petroleum hydrocarbons in contaminated vadose zone as a result of biodegradation will be achieved much quicker with the use of flooding with nutrient solution than with flooding with water.

Investigating the use of nutrient percolation in initiating degradation of hydrocarbons in already contaminated vadose zone will be of immense value in the design of an effective remediation procedure for contaminated vadose zone environments. Effective remediation of contaminated vadose zone environments will serve as a means of curbing hydrocarbon contamination of groundwater.

### CONCLUSION

Study shown that nutrient percolation through a crude oil contaminated vadose zone will enhance biodegradation of the hydrocarbons. Minimal amount of the hydrocarbons will percolate into the underlying groundwater and will be biodegraded as a result of nutrients entering alongside it. There may be not much difference in the extent of hydrocarbon attenuation between the use of water and nutrient solution but due to the much lesser time that will be taken to achieve drastic reduction in hydrocarbon using nutrient solution, the use of nutrient solution is favoured over the use of water.

### SIGNIFICANCE STATEMENT

This study discovers the possible enhancement in biodegradation of petroleum hydrocarbons in simulated contaminated soil layers using nutrient percolation that can be beneficial for remediation procedures in the decontamination of crude oil contaminated vadose zone. The study will help the researcher to uncover critical areas of groundwater contamination resulting from artificial inundation that many researchers were not able to explore. Thus, a new theory on transport of residual hydrocarbons into groundwater during biodegradation resulting from influx of water, nutrient solutions or solutions such as surfactant-nutrient solution into the vadose zone may be arrived at.

### ACKNOWLEDGMENT

The authors are grateful to the Tertiary Education Trust Fund (TETFUND, 2018 grant), Nigeria, which funded the research.

### REFERENCES

1. Holden, P.A. and N. Fierer, 2005. Microbial processes in the vadose zone. *Vadose Zone J.*, 4: 1-21.
2. Maier, R.M. and I.L. Pepper, 2009. *Earth Environments*. In: *Environmental Microbiology*, Maier, R.M., I.L. Pepper and C.P. Gerba (Eds.). 2nd Edn., Chapter 4, Academic Press, Massachusetts, USA., ISBN-13: 9780123705198, pp: 57-82.
3. UNEP., 2011. *Environmental assessment of ogoniland*. United Nations Environmental Programme (UNEP), Nairobi, Kenya, pp: 9-10. [http://postconflict.unep.ch/publications/OEA/UNEP\\_OEA.pdf](http://postconflict.unep.ch/publications/OEA/UNEP_OEA.pdf)
4. Youdeowei, P.O., 2012. *Fate of Subsurface Migration of Crude Oil Spill: A Review*. In: *Crude Oil Exploration in the World*, Younes, M. (Ed.). Chapter 7, InTech Publisher, Rijeka, Croatia, ISBN: 978-953-51-0379-0, pp: 125-134.

5. Abu, G.O. and P.A. Ogiji, 1996. Initial test of a bioremediation scheme for the clean up of an oil-polluted waterbody in a rural community in Nigeria. *Bioresour. Technol.*, 58: 7-12.
6. Odokuma, L.O. and A.A. Dickson, 2003. Bioremediation of a crude oil polluted tropical mangrove environment. *J. Applied Sci. Environ. Manage.*, 7: 23-29.
7. Li, J.L. and B.H. Chen, 2009. Surfactant-mediated biodegradation of polycyclic aromatic hydrocarbons. *Materials*, 2: 76-94.
8. Pradeep, N.V., Anupama, G. Anitha, Renukamma, S. Avvaru and S.S. Afreeen, 2012. Bioremediation of oil contaminated soil using biosurfactant produced by *Pseudomonas aeruginosa*. *J. Res. Biol.*, 2: 281-286.
9. Prince, R.C. and R.R. Lessard, 2004. Crude oil releases to the environment: Natural fate and remediation options. *Encycl. Energy*, 1: 727-736.
10. Thapa, B., K.C.A. Kumar and A. Ghimire, 2012. A review on bioremediation of petroleum hydrocarbon contaminants in soil. *Kathmandu Univ. J. Sci. Eng. Technol.*, 8: 164-170.
11. Nelson, C.H., R.J. Hicks and S.D. Andrews, 1994. An Integrated System Approach for *In-situ* Bioremediation of Petroleum Hydrocarbon Contaminated Soil and Groundwater. In: *Bioremediation Field Experience*, Flathman, P.E., D.E. Jerger and U.H. Exner (Eds.). Chapter 20, Lewis Publishers, Boca Raton, FL, USA, ISBN-13: 9780873717403, pp: 429-433.
12. Widrig, D.L. and J.F. Manning Jr., 1995. Biodegradation of No. 2 diesel fuel in the vadose zone: A soil column study. *Environ. Toxicol. Chem.*, 14: 1813-1822.
13. Zhang, Z.F., L. Zhong, M.D. White and J.E. Szecsody, 2012. Experimental investigation of the effective foam viscosity in unsaturated porous media. *Vadose Zone J.*, Vol. 11, No. 4. 10.2136/vzj2011.0190.
14. EPA., 1971. Method 352.1: Nitrogen, nitrate (colorimetric, brucine) by spectrophotometer. United States Environmental Protection Agency, Washington, DC., USA. [https://www.epa.gov/sites/production/files/2015-08/documents/method\\_352-1\\_1971.pdf](https://www.epa.gov/sites/production/files/2015-08/documents/method_352-1_1971.pdf)
15. APHA., 1992. Standard Methods for the Examination of Water and Wastewater. 18th Edn., American Public Health Association, Washington, DC., USA., ISBN-13: 978-0875532073, pp: 4-115-116, 4-134.
16. Ebuehi, O.A.T., I.B. Abibo, P.D. Shekwolo, K.I. Sigismund, A. Adoki and I.C. Okoro, 2005. Remediation of crude oil contaminated soil by enhanced natural attenuation technique. *J. Applied Sci. Environ. Manage.*, 9: 103-106.
17. Tamunobereton-ari, I., M.A. Alabraba, A.P. Ngeri and A.R.C. Amakiri, 2018. Litho-hydraulic effects on groundwater safety in parts of Rivers State, Niger Delta, Nigeria. *J. Scient. Eng. Res.*, 5: 263-274.
18. Kristensen, A. H., K. Henriksen, L. Mortensen, K.M. Scow and P. Moldrup, 2010. Soil physical constraints on intrinsic biodegradation of petroleum vapors in a layered subsurface. *Vadose Zone J.*, 9: 137-147.
19. Jobbagy, E.G. and R.B. Jackson, 2011. The distribution of soil nutrients with depth: Global patterns and the imprint of plants. *Biogeochemistry*, 53: 51-77.
20. Festus, C., O.S. Etori and I. Abbey-Kalio, 2016. Water quality assessment of boreholes sited near a dumpsite in rumuolumeni port harcourt, rivers state Nigeria. *Applied Sci. Rep.*, 13: 82-87.
21. Ukpaka, C.P. and C. Ukpaka, 2016. Characteristics of groundwater in Port-Harcourt local government area. *Chem. Int.*, 2: 136-144.
22. Amenaghawon, A.N., O. Osunbor and K.O. Obahiagbon, 2014. Impact of nutrients, aeration and agitation on the bioremediation of crude oil polluted water using mixed microbial culture. *Int. J. Scient. Res. Environ. Sci.*, 2: 43-48.
23. Liebeg, E.W. and T.J. Cutright, 1999. The investigation of enhanced bioremediation through the addition of macro and micro nutrients in a PAH contaminated soil. *Int. Biodeterior. Biodegrad.*, 44: 55-64.
24. Yang, M., Y.S. Yang, X. Du, Y. Cao and Y. Lei, 2013. Fate and transport of petroleum hydrocarbons in vadose zone: Compound-specific natural attenuation. *Water Air Soil Pollut.*, Vol. 224, No. 3. 10.1007/s11270-013-1439-y.
25. Govil, T., N.K. Rathinam, D.R. Salem and R.K. Sani, 2019. Taxonomical Diversity of Extremophiles in the Deep Biosphere. In: *Microbial Diversity in the Genomic Era*, Das, S. and H.R. Dash (Eds.). Chapter 35, Academic Press, New York, USA., ISBN: 978-0-12-814849-5, pp: 631-656.
26. Alain, K., J. Harder, F. Widdel and K. Zengler, 2012. Anaerobic utilization of toluene by marine alpha- and gammaproteobacteria reducing nitrate. *Microbiology*, 158: 2946-2957.
27. Zedelius, J., R. Rabus, O. Grundmann, I. Werner and D. Brodkorb *et al.*, 2011. Alkane degradation under anoxic conditions by a nitrate-reducing bacterium with possible involvement of the electron acceptor in substrate activation. *Environ. Microbiol. Rep.*, 3: 125-135.
28. Wei, Y., N.R. Thomson, R. Aravena, M. Marchesi and J.F. Barker *et al.*, 2018. Infiltration of sulfate to enhance sulphate-reducing biodegradation of petroleum hydrocarbons. *Groundwater Monit. Remediat.*, 38: 73-87.
29. Jaekel, U., J. Zedelius, H. Wilkes and F. Musat, 2015. Anaerobic degradation of cyclohexane by sulfate-reducing bacteria from hydrocarbon-contaminated marine sediments. *Front. Microbiol.*, Vol. 6. 10.3389/fmicb.2015.00116.

30. Sigalevich, P., M.V. Baev, A. Teske and Y. Cohen, 2000. Sulfate reduction and possible aerobic metabolism of the sulfate-reducing bacterium *Desulfovibrio oxyclinae* in a chemostat coculture with *Marinobacter* sp. strain MB under exposure to increasing oxygen concentrations. *Applied Environ. Microbiol.*, 66: 5013-5018.
31. Christophersen, M., M.M. Broholm, H. Mosbæk, H.K. Karapanagioti, V.N. Burganos and P. Kjeldsen, 2005. Transport of hydrocarbons from an emplaced fuel source experiment in the vadose zone at Airbase Værløse, Denmark. *J. Contam. Hydrol.*, 81: 1-33.
32. Hohener, P., C. Duwig, G. Pasteris, K. Kaufmann, N. Dakhel and H. Harms, 2003. Biodegradation of petroleum hydrocarbon vapors: Laboratory studies on rates and kinetics in unsaturated alluvial sand. *J. Contam. Hydrol.*, 66: 93-115.
33. Yudono, B., M. Said, Sabaruddin, A. Napoleon and Z. Fanani, 2013. Kinetics approach of biodegradation of petroleum contaminated soil by using indigenous isolated bacteria. *J. Trop. Soils*, 16: 33-38.
34. Manilla, P.N., R.E. Ogali and B.A. Uzoukwu, 2001. *Undergraduate Chemistry: Fundamental Principles*. Timi Hyacinth Enterprises, Lagos, Nigeria, pp: 276-278.