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Comparative Study of Bacterial Response to Nutrient Amendments and Hydrocarbon Removal through Leaching During Remediation

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Abstract:
Introduction: Hydrocarbon pollution is of global concern because of it deleterious effect on the environment and on biodiversity. This study investigated the percentage of hydrocarbon loss through leaching during remediation and how organic-based nutrients impact leaching and the rate of hydrocarbon degradation.
Methods: The investigation involved both microbiological and chemical methods. Total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) was monitored using Gas chromatography – Flame ionization detection (GC - FID). Other physicochemical parameters analysed included, total organic carbon, nitrate, phosphate and the soil pH. Bacterial monitoring was based on a culture dependent assessment of both heterotrophic and hydrocarbon utilizing bacteria. The nutrients used as biostimulant were: soy bean waste, poultry droppings, NPK and a combined treatment of all nutrient sources.
Results: Initial concentrations of TPH and PAHs in the oil-polluted leachate were 2648.3 mg/l and 6320 mg/l respectively while the baseline TPH concentration in the polluted soil was 48, 501 mg/kg. The baseline pH of 7.6 was optimal for microbial multiplication, however nutrient concentrations of nitrate (1.39 mg/kg) and phosphate (2.16 mg/kg) was low.HUB counts in the contaminated leachate was 8.5 x 10^6 (cfu/ml) while that of the pristine soil was 6.1 x 10^6 (cfu/ml). Approximately 6% of TPH and 6.7% of PAHs was lost through leaching from across all the setups during remediation. Nutrient amendment had no direct or significant ($p \leq 0.05$) effect on the rate of hydrocarbon loss via leaching. Treatment C had the fastest rate of hydrocarbon loss (0.0207 d^{-1}) while the least rate of TPH removal (0.0144 d^{-1}) was observed in the unamended control. Analysis of variance revealed there were significant differences ($p = 0.001$) in HUB counts between the nutrient amended treatments and the unamended control treatment. Also, HUB counts significantly correlated ($p = 0.05$ and $R = -0.83$) with TPH removal in all the nutrient amended treatments
Conclusion: The findings from this study will be useful in designing greener remediation strategies and for predicting loss of hydrocarbons via leaching during remediation.

Keywords: *Biostimulation, leaching, hydrocarbons*

1. Introduction

Crude oil spill is a leading and global environmental problem (Ibiene et al. 2011). Though the leading source of energy globally, hydrocarbon exploration and production can lead to severe environmental problems through spills from accidents, sabotage, equipment failure and human error (Nwaguma et al. 2016). Hydrocarbon exploration and production in the Niger Delta dating back to 1948 has led to devastating destruction of farmlands, aquaculture, creeks and wetlands (UNEP 2011; Anejionu et al. 2015). While the leading source of hydrocarbon spills in the Niger Delta can be historically traced to the activities of Multi-national oil companies (UNEP 2011), artisanal refining with little or no regard for the environment has also emerged as a leading and disturbing cause of hydrocarbon spills in the region (Pa et al. 2013; Chikere et al. 2019).

Bioremediation is a cost effective method for dealing with hydrocarbon spills and reducing both its toxicity and risk (Ichor et al. 2014). In the Niger Delta, the environmental conditions (frequent rainfall, suitable temperature) are deemed favourable for accelerated hydrocarbon degradation compared to other regions of the world(Brown et al. 2017). However availability of nutrients can limit microbial proliferation and thus, the rate of hydrocarbon degradation (Azubuike et al. 2016). Nutrients can be supplied during remediation in either organic or inorganic forms (Chikere and Azubuike 2012). The use of organic based nutrient amendment is encouraged as it has the tendency of improving the oil-polluted soil biological and physicochemical properties (Odokuma and Dickson 2004; Zhang et al. 2009). On the other hand, inorganic fertilizers can lead to adverse effect on the soil pH, organic carbon and enzymatic activities in the soil(Li et al. 2017; Ma et al. 2018). In a bid to use greener nutrient sources to enhance hydrocarbon degradation, Salam and Ishaq (2019) used corn steep liquor to achieve over 90% degradation of saturates and 60 – 66% polycyclic aromatic hydrocarbons removal in less than 42 days. Also, a comparative study of the useof both organic (cowdung, poultry droppings) and inorganic nutrients (NPK and Urea) to stimulate microbial proliferation and accelerate hydrocarbon degradation did not reveal significant differences (Chikere et al. 2012). This implies that organic materials can be used as alternative for bio-stimulation during hydrocarbon remediation. This is important since chemical fertilizers can easily contaminate both surface and groundwater through runoffs and leaching (Selberg et al. 2013).

One important means of improving the efficiency and capacity of organic based nutrient is its partial substitution with inorganic fertilizers. The partial substitution of chemical fertilizers with organic manure has been reported to improve the efficiency as well as the physicochemical and biological properties of agricultural soils(Li et al. 2017; Wang et al. 2017; Luan et al. 2019). In a field-scale study on the effect of a partial substitution of chemical fertilizers with pig manure, Zhao et al. (2016) reported that the bacterial composition was such that could promote nitrification and improved metabolism of organic matter. Also, (Ma et al. 2018), compared the effect of partial substitution with the use of only NPK fertilizer on soil microbial community. Their investigation showed that, the use of only NPK fertilizer promoted the abundance of mostly pathogenic fungi that could impact negatively on agricultural products. In yet another study, Zhang et al. (2009) reported that the partial substitution of chemical fertilizers with organic nutrients led to significant improvement of the soil pH and an overall improvement of both corn and wheat yield.

In this study, organic wastes comprising soy bean and poultry droppings were either individually used or partially substituted with chemical NPK fertilizer for the enhancement of microbial growth and biodegradation. The effect of the nutrient addition on hydrocarbon leaching was also investigated in order to predict the potential percentage loss of TPH and PAHs during remediation under field-scale conditions.

2. Methods

2.1. Sample Collection

Crude oil-polluted soil samples were collected from a long-term hydrocarbon polluted site (latitude 7°15 53.552E and 4°37 0.18N, 7°46 10.195E and 4°36 46.164N) in Gokana Local Government Area of Rivers State, Nigeria. Sampling was done using hand auger and involved the collection of both surface (0 – 15 cm) and subsurface (15 – 30 cm) soils. Samples collected from different points of the site were homogenized and transported at 4 °C to the University of Port Harcourt environmental microbiology laboratory for analysis and bioremediation experimentation.

2.2. Experimental Setup for Hydrocarbon Remediation

Bioremediation study involved the use of 5 experimental setups (10 litre capacity) with each containing 4 kg of the oil-polluted soil. The setups were amended with nutrients of varying organic and inorganic sources as described in Table 1 and left exposed to natural environmental conditions. Monitoring of TPH loss was done for 365 days to determine the effect of the different treatments on the rate of hydrocarbon degradation and to determine the fraction lost via leaching. Both changes in bacterial counts and the physicochemical profile of the nutrient amended treatments were compared to an unamended oil-contaminated soil and a pristine soil.

Treatment	Composition
Sample A	4kg soil + 25g Soy bean waste (SBW)
Sample B	4kg soil + 25g NPK fertilizer (NPK)
Sample C	4kg soil + 25g Poultry dropping (PD)
Sample D	4kg soil + 8.4g (SBW, PD and NPK)
Sample CS	4kg Unamended crude oil contaminated soil (COCS)

Table 1: Composition of Bioremediation Experimental Setups

2.3. Determination of physicochemical parameters

Extractable total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAHs), pH, nitrate, phosphate and the moisture content were the physicochemical parameters monitored in this study. pH was monitored using a pH meter, the moisture content was determined using the drying method as described by (Brown et al. 2017). Nitrate was monitored throughout the period of remediation using the brucin method (APHA 4500 – $N0^{3-)}$ while phosphate was determined using the ascorbic method (APHA 4500 – P). Gas chromatography-flame ionization detector (GC-FID) was used to monitor both TPH and PAH. It involved an initial extraction using 20 ml of n-pentane from 10 g of each soil sample. The analysis for both PAHs and saturates using the organic extract was done using Hewlett Packard 5890 series. Helium served as the carrier gas at a linear velocity of 38 cm sec^{-}. Version 10 of the Agilent chemstationchromatograpy was used for data handling.

2.4. Determination of Bacterial Response to Nutrient Amendment

2.4.1. Total Culturableheterotrophic Bacteria Enumeration

To determine the dynamics of heterotrophic bacteria during remediation, 1 ml of leachate sample was homogenised in 0.85% sterile water using a vortexing machine. The suspension was serially diluted (10-fold) and inoculated in duplicates unto nutrient agar (Merk, Germany). The inoculated plates were incubated for 24 hours at 30°C, after which colony counts were obtained from the agar plates (Ichor et al. 2014).

2.4.2. Hydrocarbon Utilizing Bacteria Enumeration And Monitoring

Hydrocarbon utilizing bacteria count in the leachate samples were determined using the method of (Chikere and Azubuike 2012). One ml of leachate was first homogenised with 0.85% sterile water using a vortex machine. Decimal dilutions (10-fold) from the vortexes suspension was inoculated unto Bushnell Haas broth amended with crude oil using the vapour phase transfer method. Precisely 0.1 ml was inoculated and incubated at 30°C for 7 days. Colony counts were taken using the total plate count method to determine the dynamics of HUBs during the 365 days of remediation.

2.3. Statistical Analysis

The data generated from the quarterly monitoring of TPH, PAH and bacterial counts were subjected to One-way analysis of variance using SPSS (v21.0).

3. Results

3.1. Baseline Characterization of the Samples

Prior to commencement of bioremediation, baseline analysis of the oil polluted soil showed TPH and PAHs concentrations in the oil-polluted leachate were 2648.3 mg/l and 6320 mg/l respectively. The baseline pH was 7.6, while analysis of nutrients revealed nitrate was 1.39 mg/kg, phosphate was 2.16 mg/kg and total organic carbon (TOC) was found to be 0.95 %. Comparison of the nutrient content with a pristine soil revealed that nitrate, TOC and phosphate were all higher in the pristine soil. Nitrate content in the pristine soil was found to be 11.5 mg/kg while phosphate and TOC were 12.4 mg/kg and 0.70 mg/kg respectively. On the other hand, ETPH and PAH content in the pristine soil was significantly lower ($p \leq 0.05$) compared to the oil polluted soils. The total culturable heterotrophic bacterial (TCHB) counts and hydrocarbon utilizing bacterial (HUB) counts were found to be higher in the oil contaminated leachate samples. HUB counts in the contaminated leachate was 8.5 x 10^6 (cfu/ml) while that of the pristine soil was 6.1 x 10^6 (cfu/ml). TCHB counts was 5.9 x 10^7 (cfu/ml) in the pristine soil and 3.6 x 10^7 (cfu/ml) in the oil-contaminated soil.

3.2. Observed Changes in TPH as Remediation Progressed

Monitoring of TPH changes across the different treatments revealed a significant loss of hydrocarbons after 365 days. Initialsoil TPH concentration of 48,501 mg/kg reduced to a range of 174 – 28 mg/l in the leachate samples obtained between December 2017 and July 2018 (Fig. 1).During the period of remediation (December 2017 – July 2018), approximately 6% of TPH was lost through leaching across all the setups. No significant differences ($p \leq 0.05$) were observed across the nutrient amended treatments based on ETPH loss profile via leaching. Similarly, significant differences were also not found on comparison of the TPH loss profile of the oil-polluted control and the nutrient amended treatments.

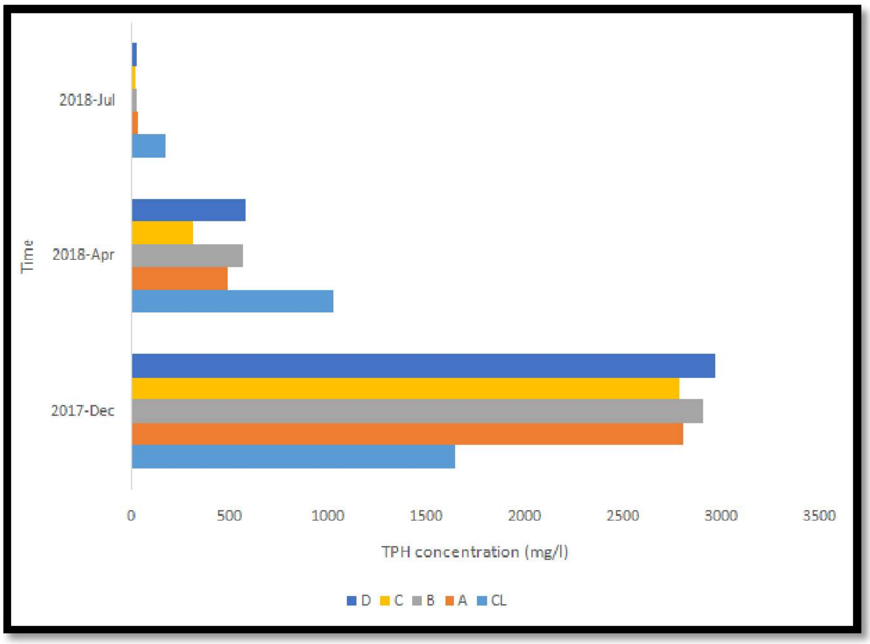

Figure 1: Pattern of TPH Loss via Leaching in All the Investigated Treatments

It was observed that, treatment C had the fastest rate of hydrocarbon loss (0.0207 d^{-1}) with 99% of the observed variation explained by a linear model. Analysis of the degradation half-life revealed it will require 33 days to remove half the concentration of TPH in treatment C via leaching, whereas 48 days will be required in treatment CL under the same environmental conditions. The least rate of TPH removal (0.0144 d^{-1}) was observed in the unamended controlsample and

93% of the variation was also explained by a linear model. Presented in Table 2 is the rate of hydrocarbon degradation, the leaching half-life and explained variation for all the treatments.

Treatment	K (TPH)	Half-Life	Explained Variation
CL	0.0144	48 days	93%
A	0.019	36 days	98%
B	0.0197	35 days	97%
C	0.0207	33 days	99%
D	0.0195	36 days	97%

Table 2: Rate of TPH Loss via Leachate during Remediation and the TPH Loss Half-Life
**K = Rate of Degradation Determined by First Order Kinetics*

3.3. Observed Changes in PAH As Remediation Progressed

Investigation of the dynamics of PAH loss through leachate from the inception of remediation to the end of the study revealed significant differences. Initial PAH detected in the soil samples was 16320 mg/l and this reduced to a range of 7 – 36 mg/l in the leachate samples at the end of the study. Analysis of variance based on the PAH reduction profile across all treatments was not significant at $p \leq 0.05$. Treatments A and C had the shortest degradation half-life of 32 days while the unamended control treatment had the longest degradation half-life of 45 days.Presented in Table 3 is the PAH removal rate kinetics, half-life of PAH loss via leaching and the linear model explained variation.

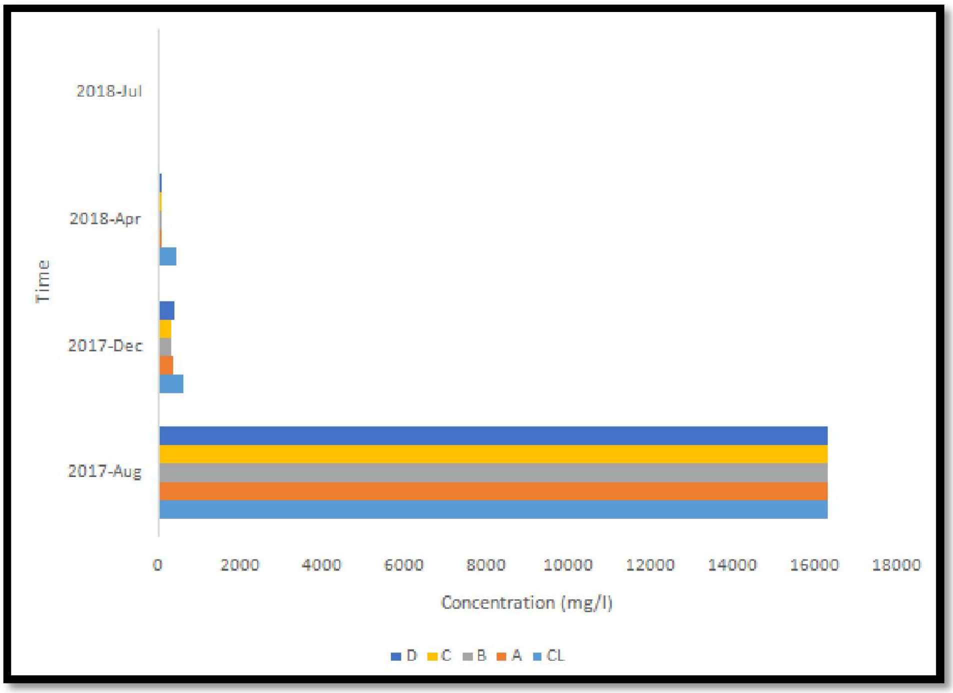

Figure 2: Dynamics of TPH Loss through Leaching in the Different Samples during Remediation

Treatment	K (PAH)	Half-life	Explained variation
CL	0.0153	45 days	92%
A	0.0215	32 days	97%
B	0.0204	34 days	97%
C	0.0216	32 days	96%
D	0.0202	34 days	98%

Table 3: Rate of PAH Loss Via Leachate during Remediation and the PAH Loss Half-Life

3.4. Bacterial Dynamics during Remediation

Enumeration of THB during the period of remediation showed the nutrient amendment significantly led to bacterial increase compared to the unamended control soil at $p \leq 0.05$ (Fig. 3). Treatment D led to the highest increase in bacterial counts with an observed period of total bacteria stability between December 2017 and February 2018. Treatment C also led to significant increase in total bacterial count ($p = 0.03$) compared to the unamended control which had the least bacterial counts throughout the period of remediation. Across all nutrient amended treatments, bacterial counts unanimously peaked between December 2017 and February 2018.

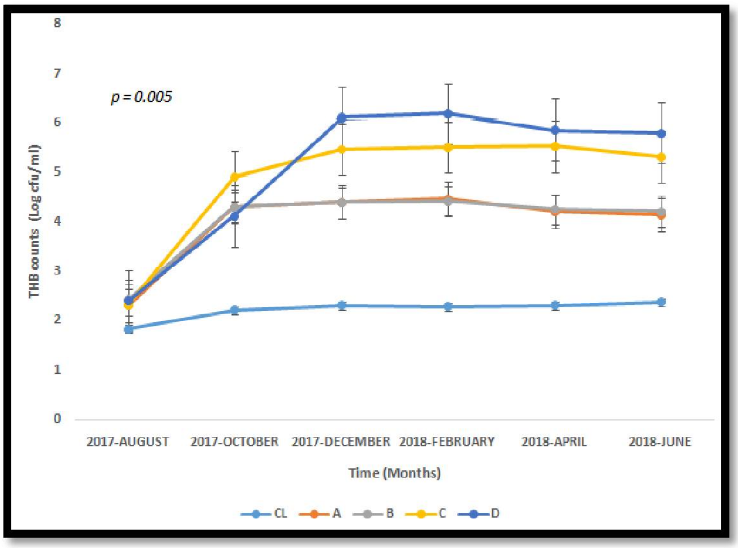

Figure 3: Bacterial Enumeration during Remediation and Across All the Treatments

Similarly, the monitoring of HUB counts during remediation showed that the highest counts were recorded within December 2017 and February 2018. Treatments C and D led to the highest increase in HUB counts throughout the period of remediation. Analysis of variance revealed there were significant differences ($p = 0.001$) in HUB counts between the nutrient amended treatments and the unamended control.HUB counts significantly correlated ($p \leq 0.05$ and $R = -0.83$) with TPH removal in all the nutrient amended treatments.

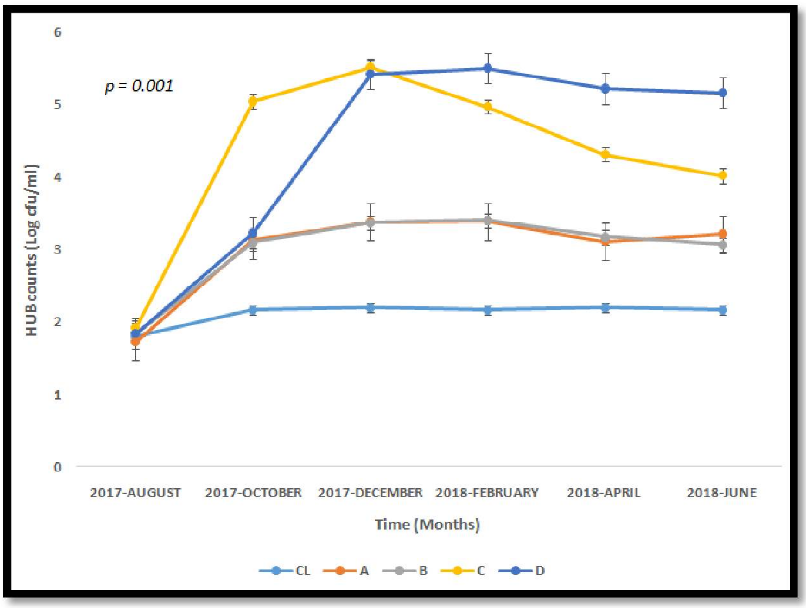

Figure 4: Hydrocarbon Utilizing Bacterial Counts Determined From Leachate Samples during Remediation

4. Discussion

This study investigated the effect of various combinations of organic waste on bacterial richness and the rate of hydrocarbon degradation. The amount of ETPH loss through leaching was investigated by subjecting all experimental setups used for bioremediation under normal environmental conditions. This data will be useful for predicting the amount of hydrocarbons lost through leaching and their potential to contaminate ground water.

Baseline analysis of the oil-polluted soil prior to nutrient addition and leachate monitoring revealed that, concentrations of TPH and PAHs were above the Department of Petroleum Resources (DPR) minimum acceptable standard of 5000 mg/kg as stated in the Environmental guidelines for the petroleum industry in Nigeria (EGASPIN)(Department of Petroleum Resources 2002). The baseline pH of 7.6 was optimal for microbial proliferation (Wu et al. 2017), however nutrient analysis revealed low concentrations of nitrate (1.39 mg/kg) and phosphate (2.16 mg/kg). It has been reported that nutrients for bacterial growth such as nitrogen, phosphorus and potassium are rate limiting nutrients that can influence the overall rate of hydrocarbon degradation in oil-polluted soils (Azubuike et al. 2016). Also, the observed low nutrient concentration is consistent with previous studies of oil-polluted soils (Ezekoye et al. 2015;

Brown et al. 2017). Several studies have reported significant increases in bacterial abundance and the overall hydrocarbon degradation half-life following an amendment of the oil polluted soil with both organic and inorganic nutrients (Chikere et al. 2012; Cerqueira et al. 2014; Kästner and Miltner 2016; Brown et al. 2017).HUB counts revealed that the abundance of potential hydrocarbon degrading bacteria was higher in the oil-polluted soil 8.5 x 10^6 (cfu/ml) than in the pristine soil. This finding is also consistent with other studies of bacterial abundance in oil polluted environments in the Niger Delta including those determined by culture-dependent methods (Ichor et al. 2014; Ekwuabu et al. 2016) and High-throughput metagenomics techniques (Ezekoye et al. 2018; Chikere et al. 2019).

Monitoring of TPH concentration in leachate samples from December 2017 – July 2018 revealed that an approx. 6% of TPH was lost via leaching and that the observed leaching reduced progressively over time. This same observation was made in a 480 days field-scale study on the efficiency of bioremediation in reducing hydrocarbon pollution, toxicity and risk (Chaîneau et al. 2003). They observed very low concentrations of hydrocarbons in leachate samples on the 480th day of the study. On the contrary, significant differences were not observed in the rate of TPH removal across the treatments. This implies that, addition of organic nutrients including its associated increase in organic matter content had no direct effect on hydrocarbon leaching during remediation. Similar to TPH loss pattern via leaching was also the reduction pattern of PAH in all the treatments. Approximately 6.7% of PAH was lost from the oil-polluted soil through leaching and the loss pattern in the nutrient amended treatments compared to the unamended control was insignificant and therefore, had no direct effect on leaching. Jamrah et al. (2007) in a study of the leaching pattern in a crude oil-contaminated soil found that the dilution of the contaminated soil with either asphalt or pristine soil reduced both the leaching of hydrocarbons and heavy metals; thereby preventing the potential contamination of both surface and groundwater.

Unlike the concentration of TPH and PAHs in the leachate, bacterial count in leachate samples significantly increased in the nutrient amended treatments during the period of remediation. HUB and THB counts in the nutrient amended treatments were found to have increased significantly ($p \leq 0.05$) in the leachate following an amendment of the soil with nutrients. Aeration and nutrient addition have been reported to support the proliferation of bacterial cells in oil polluted soils(Brown et al. 2017; Ezekoye et al. 2018). Optimal rate of hydrocarbon degradation and microbial growth was reportedly achieved through amendment of the oil-polluted soil with 25% of poultry droppings (Ezenne et al. 2014). Also, (Salam and Ishaq 2019) applied corn steep liquor as a nutrient amendment to improve bacterial abundance and the degradation of both saturates and aromatic hydrocarbons in a long-term oil-polluted soil. In another study, (Chikere et al. 2012) applied both organic (poultry droppings and cowdung) and inorganic (NPK and Urea) fertilizers to improve microbial growth and the rate of hydrocarbon degradation during remediation conducted in bioreactors. In this study, improved microbial proliferation correlated with increased detection of both HUBs and TCHB in the leachate samples.

5. Conclusions

This study investigated the role of leaching in the reduction TPH and PAHs during hydrocarbon remediation in order to determine what fraction of the pollutant could become a potential source of both surface and groundwater contamination. The findings revealed only an approximate 6% and 6.7% of TPH and PAHs respectively was lost through the process of leaching. Also the addition of nutrients only impacted bacterial abundance in the leachate but not the potential loss of hydrocarbons through leaching as no significant differences was observed in the rate of hydrocarbon leaching between the nutrient amended and unamended treatments. This study did not involve any optimization study to determine if different concentrations of the organic wastes used in this study could have affected hydrocarbon leaching differently. This is therefore recommended for further investigation.

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