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MONITORING HYDROCARBON LEVELS DURING BIOREMEDIATION BY ENHANCED BIO-STIMULANTS USING GC-FID

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ABSTRACT

The efficacy of bio-stimulants on the remediation of hydrocarbon polluted soil was assessed using Gas Chromatography with Flame Ionization Detector (GC-FID). The enhanced bio-stimulants were discarded melon pulps, discarded breadfruit pulps and poultry droppings. These were applied to the polluted soil in the ratio of 1:5; the blend was observed for sixty days. Inferring from the chromatographs, the carbon compounds present in the polluted and remediated soils ranged from $C_{12} – C_{40}$ with varying concentrations; C_{12} to C_{19} were dominant, C_9 to C_{11} were residual with negligible concentrations. The total petroleum hydrocarbon (TPH) of polluted soil was 42,229.73 mg/kg, and the remediated soils were: biodegraded melon pulp - 23,786.3 mg/kg, biodegraded breadfruit pulp - 15,322.82 mg/kg, and chicken droppings, - 7,314.29 mg/kg. The results indicated that TPH of the polluted soil was reduced by 43.67% in sample remediated with biodegraded melon pulp, 63.71% in sample remediated with biodegraded breadfruit pulp, and 82.67% in sample remediated with chicken droppings. Therefore, a decreasing order of the effectiveness of the bio-stimulants is thus: chicken droppings > biodegraded breadfruit pulp > biodegraded melon pulp. The higher remediation potential of poultry droppings is attributable to high nitrogenous content. The study showed that the aforementioned bio-stimulants are effective in remediation of petroleum polluted soil. GC-FID detected the hydrocarbon present and their concentrations in the polluted and remediated soils. GC-FID is preferred to other analytical techniques due to its precision in identification and quantification of hydrocarbon fractions.

INTRODUCTION

The eco-destruction posed by petroleum spill is enormous and it is attributed to its hydrocarbon composition. Petroleum crude distillates have higher utility values in domestic, industrial and agricultural operations. The spill of the crude petroleum or the distillates on environment has adverse effects on air, water and soil (Asthana and Asthana, 2012). Light and medium fractions of crude petroleum have carbon atom range of one to sixteen ($C_1 – C_{16}$); these fractions are highly volatile due to their low boiling point. When the fractions spill, sometimes microorganisms can degrade it completely (mineralization) into carbon dioxide, water and inorganic compounds (Nilanjana and Preethy, 2010). In some cases, they react with other constituents of the air and form substances that are not eco-friendly. The heavier fractions such as greases and wax tar have complex structures that resist natural attenuation or decomposition; thus, they constitute environmental pollutants when spilled (Asthana and Asthana, 2012). Total petroleum hydrocarbon (TPH) is a term used to describe any mixture of hydrocarbons in

crude oil. Among the contemporary accepted methods for determination of TPH in soil are gravimetry, infrared spectroscopy and gas chromatography (Okparanma, 2013). Gas chromatography (GC) is a common analytical technique used to separate and analyze volatile and semi-volatile compounds in a mixture. The method has the ability to provide some necessary information on the product type (ATSDR, 1999). Identification of analytes is done by comparing the retention time of an individual compound to that of a reference standard. GC techniques have the capacity to identify and quantify hydrocarbon in petroleum polluted samples (Wang, et al, 2003). GC displayed reliable result in post remediation assessment of the residual hydrocarbon in contaminated soil (Solomon et al, 2018). GC-FID is efficient in determination of TPH between 100mg/kg of soil and 10,000mg/kg of soil and as well as hydrocarbon with a boiling point range of 175 ^{0} C to 525 ^{0}C (alkanes, aromatic compounds) (ISO 16703, 2004).GC-FID is comparatively advantageous in assessing TPH due to its ability to identify analyte by comparing retention time.

An important factor in bioremediation operation is the bio-stimulant used. Plant bio-stimulants contain substance(s) and/or microorganisms when used, boost the growth and productivity of a plant (Van Oosten, et al., 2017). According to Dubey (2010), bio-stimulant or bio-fertilizer is a substance that normally contains natural microorganisms and nutrients; the main function of the microorganism is to improve soil nutrient cycle through natural nitrogen and phosphate fixation processes. Microorganisms support ecosystem balance as well as pollution control (Chatterjee, 2010).

Bioremediation involves the application of microorganisms in conversion of contaminants to nontoxic substance (Wami et al., 2008; Ramawat, *et al*., 2009). Microorganisms degrade contaminants either through oxidative or reductive processes. In oxidative process, microorganisms degrade the organic contaminant completely into non-toxic by-product such as carbon dioxide, water or organic acid (Rockne and Reddy, 2003; USEPA, 1991). Bioremediation requires parameters such as water content, pH, oxygen, temperature and plant nutrients (Ola and Ojuri, 2008). Effective bioremediation is determined by the medium, microbes, climatic factors, contaminants and its concentration (Oyoh and Osoka, 2007). The bio-stimulants used in this study were chicken droppings, biodegraded melon pulp, and biodegraded breadfruit pulp. Though chicken droppings have been previously reported by researchers in remediation of hydrocarbon polluted soil (Naowasarn and Leungprasert, 2016), it is used in this work to further ascertain its potency in remediation. Offonry and Achi (1998) monitored the traditional process for the retting of melon pulp and microbiological characteristics in the recovery of melon seeds (*Colocynthiscitrullus* L.). The study indicated that naturally fermented melon pulp had a remarkable microorganism growth (108 - 1010cfu/g). Uzoh, et al. (2018) studied the retting of African breadfruit pulp and microorganisms associated with it. The pulp was sliced and allowed to undergo natural biodegradation which showcased microorganisms' growth. Amusa et al. (2002) studied bio-deterioration of breadfruit pulp in storage and its effects on the nutrient composition. The result revealed that bio-deterioration of breadfruit pulp was associated with microorganisms such as *Aspergillus niger*, *Rhizopus stolonifer*,

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Botryodiplodiatheobromae, and *Penicillium*sp. The study also revealed that mineral contents (phosphorous, potassium, magnesium, calcium, iron) increased during the period of storage. Scientific studies have identified microorganisms that can degrade petroleum hydrocarbon (Hazen et al., 2016).

Previous studies revealed that microorganisms present in biodegraded melon pulps and breadfruit pulps are dominantly hydrocarbon degrading (Amusa et al., 2002; Hazen et al., 2016). Using these earlier studies as baseline, this work reports the remediation of hydrocarbon impacted soil using biodegraded melon pulp, biodegraded breadfruit and chicken droppings. GC-FID was deployed because of its previous reported comparative advantage over other analytical techniques (Ezeani et al., 2022).

METHOD

The selected bio-stimulants for this work were

i. Chicken dropping

ii. Discarded melon pulp (Cucurbitaceae)

iii. Discarded breadfruit pulp (Treculiaafricana)

1kg of melon pulp and 1kg of breadfruit pulp were sliced into small sizes and dried under sun for fourteen days. The dried pulps were pulverized into fine form. These were subsequently wetted by sprinkling with 1 liter of water, covered with cellulous sheet for twenty-one days before application to the polluted soil. The enhanced bio-stimulants were tagged as follow

i. BF1: Biodegraded melon pulp

ii BF2: Biodegraded breadfruit pulp

iii. BF3: Chicken droppings

Remediation Experiment

i. Sample A: Control soil
ii. Sample B: Polluted soil
iii. RE1: Sample B mixed with BF1 at the ration of 5:1 (1.0 kg: 0.2kg)
iv. RE2: Sample B mixed with BF2 at the ratio of 5:1 (1.0 kg: 0.2 kg)
v. RE3: Sample B mixed with BF3 at the ration of 5:1 (1.0 kg : 0.2 kg)

Note RE stands for remediated.

Microbiological analysis.

The pour plate method with oil agar medium was used to analyze the chicken droppings, biodegraded melon pulp, and biodegraded breadfruit pulp. Pure culture of the isolates was Gram stained. The bacteria that took the stain and appeared dark-violet or blue-black were called Gram-positive bacteria.

Table 1. Microorganisms present in the biodegraded bio-stimulants

	BF1	BF2	BF3
1	*Citrobacter* sp.	*Citrobacter* sp.	*Citrobacter* sp.
2	*Klebsiella*sp.	*Klebsiella* sp.	*Klebsiella* sp.
3	*Corynebacterium* sp.	*Corynebacterium* sp.	*Corynebacterium* sp.
4	*Bacillus* sp.	*Bacillus* sp.	*Bacillus* sp.
5	*Escherichia* sp.	*Escherichia* sp.	*Escherichia* sp.
6	*Micrococcus* sp.	*Lactobacillus fermentum*	*Micrococcus* sp.
7	*Lactobacillus* sp.	*Streptococcus faecalis*	*Lactobacillus fermentum*
8	*Saccharomyces cerevisiae*	*Enterobacter*	*Streptococcus* sp.

9	*Aspergillus* sp.	*Enterobacter* sp.
	Rhizopus sp.	*Saccharomyces cerevisiae*
10		*Aspergillus*sp.
11		*Rhizopus*sp.

Gas Chromatography - Flame Ionization Detector (GC- FID) analysis

The GC-FID was operated under the conditions tabulated below

GC	HP 6890 Powered with HP ChemStation Software
Injection Temperature	Splitless
Split Ratio	20:1
Carrier Gas	Helium
Inlet Temperature	250^0C
Column Type	HP 5
Column Dimension	30m x 0.25mm x 0.25µm
Oven Program	Initial temperature at 40^0C for 2 mins Ramp at 10 , 16mins.
Detector	FID
Detector Temperature	320^0C
Hydrogen Pressure	40.0psi
Compressed Air	40.0psi

GC-FID was preferred due to its ability to detect the types of hydrocarbon present.

United States Environmental Protection Agency (USEPA) method 3550C (USEPA, 2007) was adopted for the extraction of petroleum hydrocarbons (PHCs) from the soil samples. 5.00 ±0.001g of homogenized sample was quantitatively transferred into 100ml beaker and thoroughly mixed with about 5.00 ±0.001g of anhydrous sodium sulphate to absorb moisture.

10 ml Dichloromethane was added to the sample inside the beaker. The sample was thereafter sonicated for 20 mins at a constant speed in an ambient temperature. The supernatant was filtered after complete extraction into an extraction vial.

The extract obtained was further concentrated to 1ml prior to GC-FID analysis using USEPA 8015C (USEPA, 2007). After the analysis, Chemstation software was used to integrate the results in relation to Acuu Standard Calibration standards. Samples and the reagents were kept in a cooling system so as to maintain the integrity. The laboratory instruments were properly calibrated prior to use so as to achieve precision measurement.

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RESULTS

The results of the experiment are presented in Table 3.1 and Figures 3.1 to 3.5.

Table 3.1 GC results of TPH in polluted soil and remediated samples at 5:1 blend.

HC	**Polluted soil mg/kg**	**RE1 @60 days (mg/kg)**	**RE2 @60 days (mg/kg)**	**RE3 @60 days (mg/kg)**
C8	*0.00*	0.00	0.00	0.00
C9	*0.985*	0.552	0.27	0.13
C10	*15.06*	3.22	9.00	2.56
C11	*68.49*	20.93	12.05	39.47
C12	*1,764.48*	424.89	1050.16	554.42
C13	*2,603.53*	1,482.83	587.74	364.73
C14	*2,984.53*	1,982.25	552.11	314.16
C15	*3,524.91*	2,147.71	600.97	253.04
C16	*3,142.66*	738.58	618.29	552.51
C17	*4,570.85*	3,374.13	651.32	874.03
Pristane	*2543.73*	1699.52	965.63	284.84
C18	*3,917.51*	2,535.80	802.27	583.67
Phytane	*1,747.74*	1,166.28	807.93	405.77
C19	*3,718.01*	2,421.26	432.24	599.99
C20	*3,131.61*	801.72	3498.93	466.54
C21	*2,579*	1,603.94	1657.38	413.03
C22	*1,997.85*	263.44	935.21	451.74
C23	*1,534.42*	1,112.50	813.06	375.34
C24	*990.07*	772.70	587.53	335.34
C25	*648.25*	535.86	414.52	242.49
C26	*359.09*	319.02	223.63	154.73
C27	*203.48*	190.75	101.47	29.54
C28	*69.98*	71.21	0	13.95
C29	*24.48*	23.66	0	0.21
C30	*49.47*	47.54	0	0.99
C31	*10.30*	10.85	0	0
C32	*22.64*	19.19	0	0
C33	*16.02*	8.50	0	0
C34	*5.77*	2.06	0	0
C35	*12.96*	4.07	1.08	0.76
C36	*7.23*	0.00	0	0
C37	*2.21*	0.00	0	0
C38	*1.26*	0.00	0	0
C39	*8.63*	1.11	0.034	0.31
C40	*19.68*	0.00	0	0
T				
Total	**42,229.73**	**23,786.3**	**15,322.82**	**7,314.29**

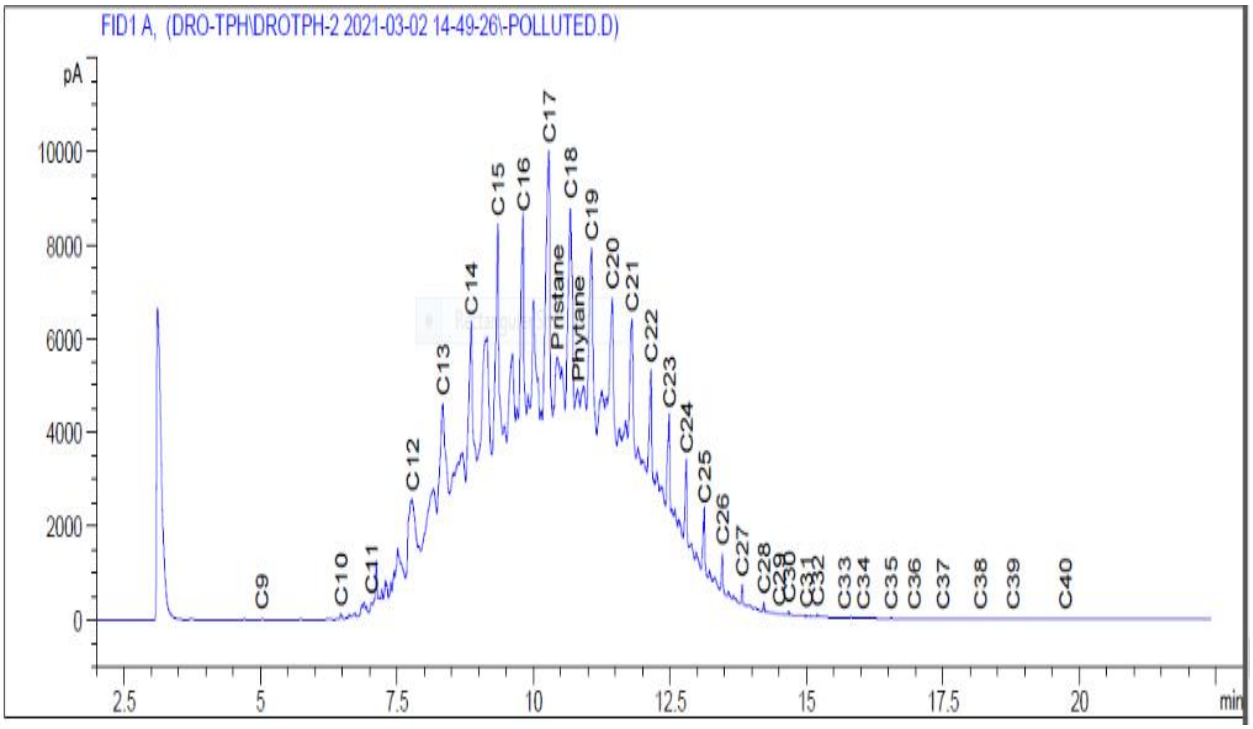

Fig 1: GC Chromatograph for the Polluted soil (TPH: 42,229.73 mg/kg)

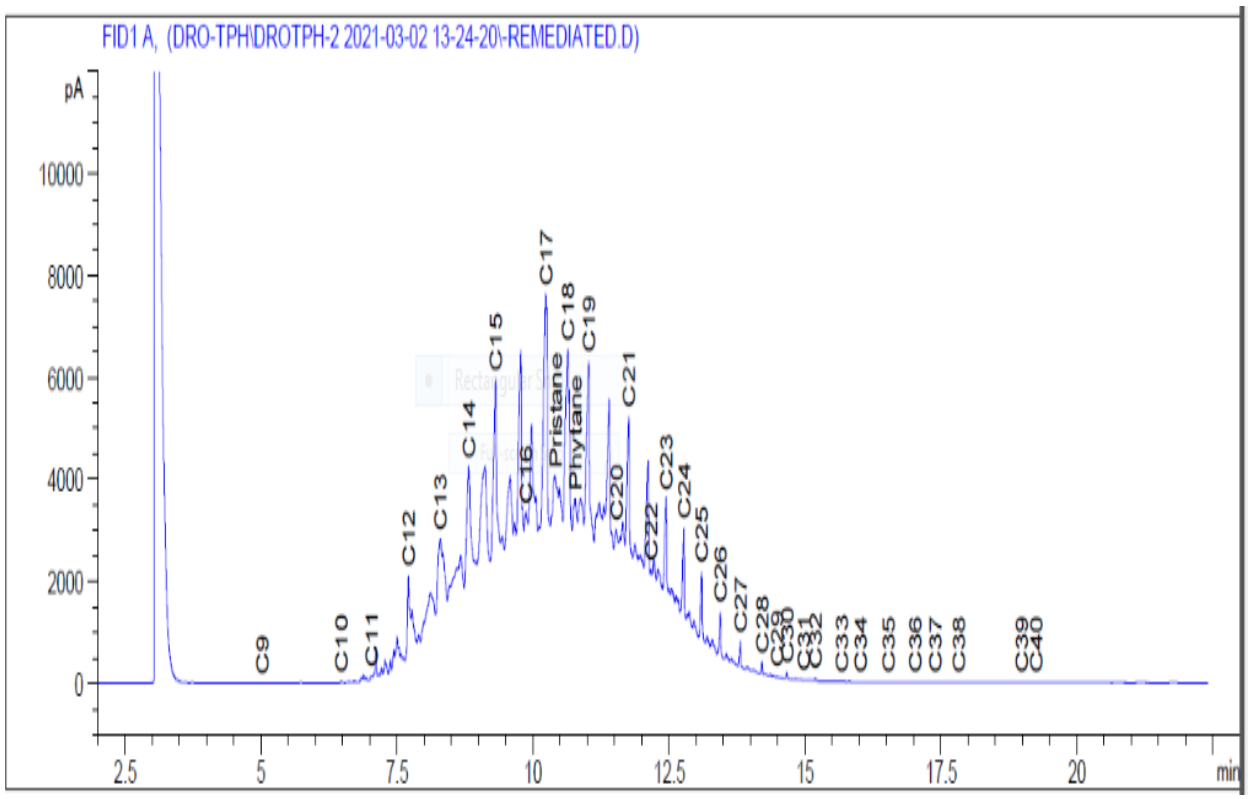

Fig 2: GC chromatograph for bio-remediated soil (RE1) at 60 days duration.

(TPH: 23,786.3 mg/kg)

Scientia Africana, Vol. 21 (No. 2), August, 2022. Pp 139-148 *https://dx.doi.org/10.4314/sa.v21i2.14*

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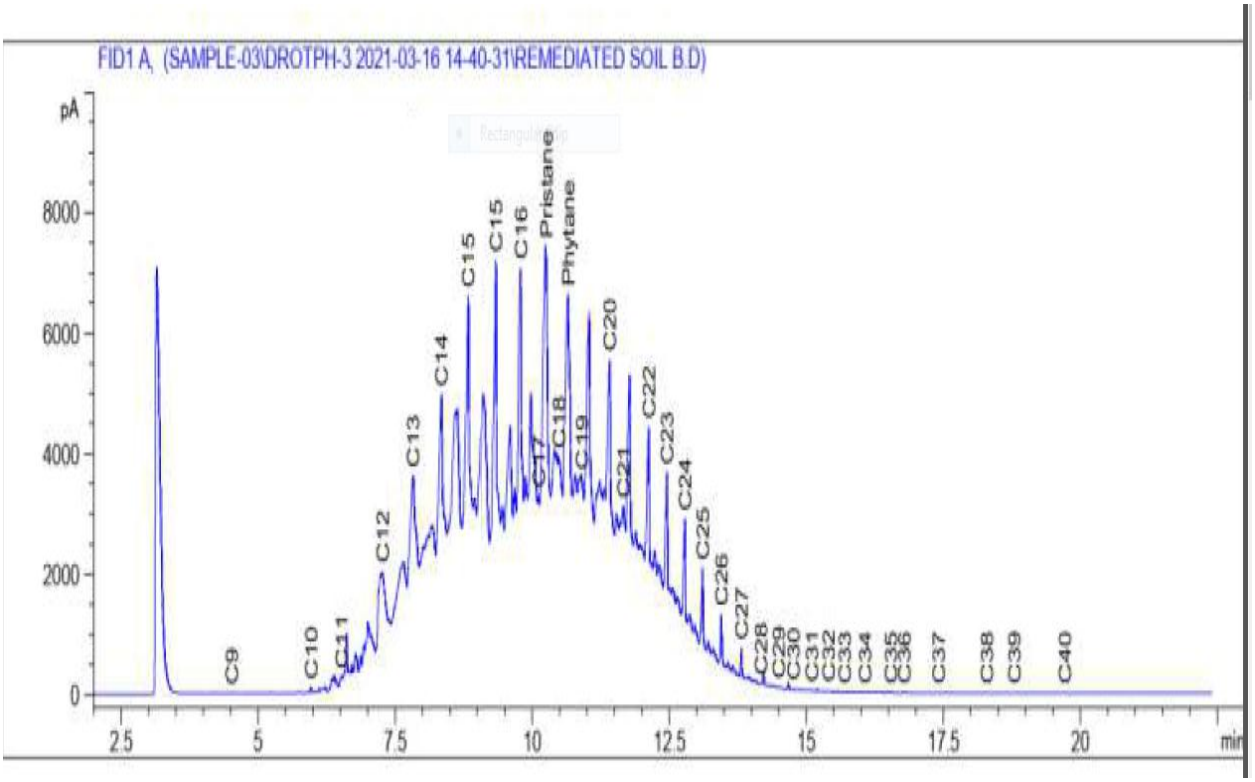

Fig 3: GC chromatograph for bio-remediated soil (RE2) at 60 days duration (TPH: 15,322.82 mg/kg)

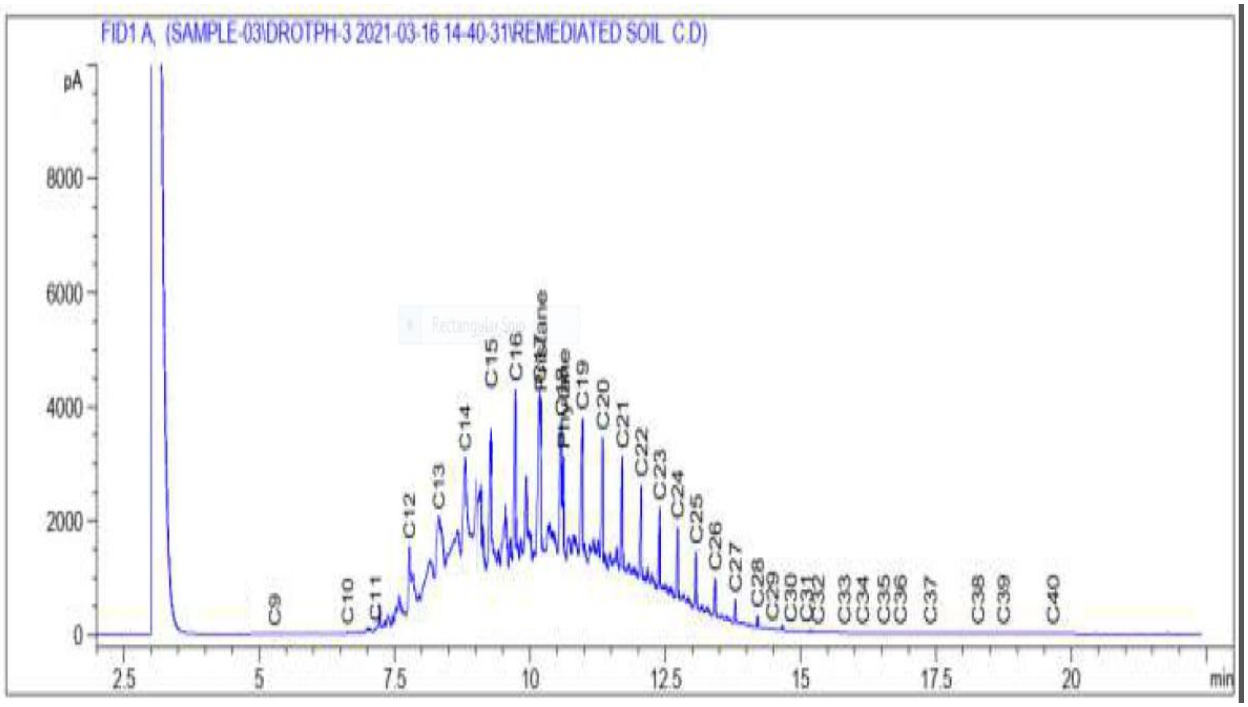

Fig 4: GC chromatograph for bio-remediated soil (RE3) at 60 days duration (TPH:7,314.29 mg/kg).

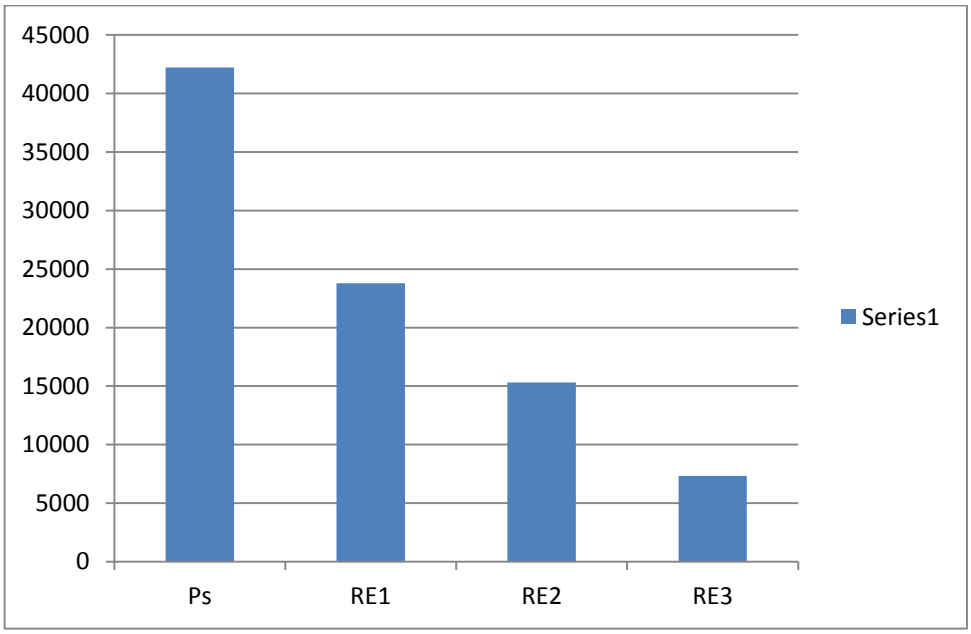

Fig5: TPH of polluted and remediated soil at 60 days duration. (Ps: polluted soil, RE: Remediated)

DISCUSSIONS

GC-FID detected the hydrocarbons and their concentrations in the polluted and remediated soils. Inferring to peaks of the chromatographs, the carbon compounds present in the polluted and remediated soils ranged from C_{12} – C_{40} with varying concentrations; C_{12} to C_{19} were dominant, C_9 to C_{11} were residual with negligible concentrations. The TPH of polluted soil was 42,229.73 mg/kg, and the TPH of the remediated soil at 60 days duration period were thus: RE1 (Biodegraded melon pulp) was 23,786.3 mg/kg, RE2 (Biodegraded breadfruit pulp) was 15,322.82 mg/kg, RE3 (chicken dropping) was 7,314.29 mg/kg. The results indicated that TPH of the polluted sample was reduced by 43.67% in sample remediated with biodegraded melon pulp, 63.71% in sample remediated with biodegraded breadfruit pulp, and 82.67% in sample remediated with chicken dropping. The degradation of hydrocarbon was highest with chicken manure followed by biodegraded breadfruit and the biodegraded melon pulp. The drop in TPH concentration when chicken droppings were used is in conformity with the result of Naowasarn and Leungprasert (2016). Biodeterioration of breadfruit and melon pulps were associated with microorganisms' growth and the mineral contents (phosphorous, potassium, magnesium, calcium, iron) increased during the period of storage (Amusa et al 2002; Offonry et al, 1998). This study affirmed that the microorganisms present in biodegraded melon and breadfruit are dominantly hydrocarbon degrading microbes. The presence of hydrocarbon degrading microbes is responsible for the drop in TPH of the remediated soil.

Department of Petroleum Resources (DPR-EGASPIN, 2018) target value for remediation is 50 mg/kg. With reference to this experiment, if the remediation conditions and rate of degradation remain constant, then the time required by the biodegraded melon pulp, biodegraded breadfruit pulp and chicken droppings to achieve the DPR value (500mg/kg) can be estimated by linear extrapolation. It will take biodegraded melon

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pulp, biodegraded breadfruit pulp and chicken droppings 475 months, 306 months and 146 months respectively. Following the trend of the degradation, if simple proportion is applied, the nutrient estimation can be thus: If 0.2 kg of biodegraded melon is blended with 1kg of the polluted soil and TPH dropped from 42,299.73 to 23, 786 in 60days, then 9.5kg of biodegraded melon pulp may be blended with 1kg of the polluted soil to achieve the acceptable range of 500mg/kg of TPH. Following the same procedure; 6.12 kg of biodegraded breadfruit pulp may be required to achieve the acceptable range while about 3kg of chicken droppings may be needed to achieve the DPR-EGASPIN standard.

CONCLUSION

It can be concluded from the experiment that chicken droppings, biodegraded melon pulp and biodegraded breadfruit pulp contain appreciable hydrocarbon degrading microorganisms and are rich in mineral resources that stimulate growth of microorganisms. Due to remarkable reduction of TPH in the remediated soil, the selected bio-stimulants have proved to be effective in remediation of petroleum hydrocarbon polluted soil. GC-FID displayed effectively the fractions of the petroleum hydrocarbon and their concentrations. This study has affirmed the effectiveness and reliability of GC-FID in determination of TPH in soil.

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