Use of Wastewater from Legume Cooking in Bioremediation of Crude-Oil Polluted Soil

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ABSTRACT

Wastewaters generated from the cooking of foods contain nutrients. Such wastewaters may have the potential to be used as nutrients in bioremediation of hydrocarbon polluted environments. In this study, use of wastewaters generated from the cooking of two legumes as nutrient source during bioremediation of hydrocarbon polluted soil was investigated. Crude-oil polluted soils were treated with wastewater from cooking of cowpea (setup B), wastewater from cooking of groundnut-pods (setup G), a mixture of the wastewaters (setup B+G), and tap-water (control setup: setup C). Selected microbial group and physicochemical parameters were determined at seven days interval. The results obtained showed that the extent of hydrocarbon degradation in setup B, G, B+G, and C were 45.5, 23.2, 1.3, and 37.9 % respectively. There was decrease in total organic carbon (TOC), phosphorus, and nitrogen in the setups. However, in setup G there was increase in TOC. Setup C and B had the highest decrease in phosphorus, and setup B had the highest decrease in nitrogen. There was decrease and subsequent increase in percent hydrocarbon-utilizing bacteria in all the setups; the subsequent increase was highest in setup B. There was generally a decrease in percent hydrocarbon-utilizing fungi, except in setup C. The hydrocarbon utilizing microorganisms isolated from the treated soils include Bacillus, Micrococcus, Acinetobacter, Staphylococcus, Pseudomonas, Penicillium, Aspergillus, Candida and Fusarium. Their presence indicates that hydrocarbon degradation was due to biodegradation. It is concluded that wastewater from cooking of cowpeas can be applied in bioremediation of crude-oil polluted soil.

Keywords: *Cowpeas (Vigna unguiculata); Groundnut-pods; Nutrients; Hydrocarbon utilizing microorganisms*

1. INTRODUCTION

Wastewaters generated during the processing of a food will invariable contain some quantity of the nutrients present in the food. Nutrients in the form of nitrates, phosphates, fertilizers, or manure are supplied into hydrocarbon polluted soils during bioremediation studies (Adekunle *et al.*, 2015; Ezeonu *et al.*, 2012; Nwogu *et al.*, 2015). This increases the nutrient level in the polluted soil, thereby enhancing the hydrocarbon degrading ability of hydrocarbon degrading microorganisms present in such soil. Increasing nutrient levels in hydrocarbon polluted soils has been achieved through application of wastes of animal and plant origin (Adams *et al.*, 2017; Agamuthu *et al.*, 2013; El Mahdi & Aziz, 2019), and this has resulted in considerable reduction in the pollutant concentration through biodegradation. Wastes of animal and plant

origin that have been investigated for use as nutrient source during bioremediation of petroleum hydrocarbon polluted soil include chicken manure, cow dung, goat manure, swine wastewater, brewery spent grain, food-waste compost, and wheat bran (Abioye *et al.*, 2010; Adams *et al.*, 2017; Agamuthu *et al.*, 2013; Hara *et al.*, 2013; Nwogu *et al.*, 2015; Zhang *et al.*, 2020). The promising results achieved with the use of such wastes in enhancing the reduction of hydrocarbon concentration in petroleum polluted soils reveals the potential of using wastes in large scale bioremediation of crude oil polluted environments. However, many wastes of animal origin and some wastes of plant origin releases disagreeable odour. Animal wastes also have the potential of introducing pathogens into the environment.

Legumes are flowering plants whose fruits grow into pods containing seeds (Chaturvedi *et al.*, 2011); well-known edible legumes include cowpeas, soybeans, and groundnuts. In some communities in southern Nigeria, cowpeas (*Vigna unguiculata*) are parboiled before the actual cooking. Also, some vendors cook groundnuts (*Arachis hypogaea*) while still in their pods and sell them to the public. Wastewaters generated from the cowpeas parboiling and groundnut-pod cooking processes are disposed off into the environment. Cowpeas and groundnuts are rich in carbohydrate, protein, and in minerals including calcium and phosphorus (Rivas-Vega *et al.*, 2006; Shokunbi *et al.*, 2012). Nitrogen, the fundamental element in protein, and phosphorus are essential nutrients which are utilized by microorganisms during biodegradation of pollutants (Semboung-Lang *et al.*, 2016; Xu *et al.*, 2015). It is possible that wastewater generated from cooking of cowpeas and groundnut-pods will contain adequate quantity of nitrogen and phosphorus. Wastewaters from cooking of cowpeas and groundnut-pods were thus investigated in this study for use in the bioremediation of crude oil polluted soil.

2. MATERIALS AND METHODS

2.1 Collection of the wastewaters

Wastewater from cooking of cowpeas and wastewater from cooking of groundnut-pods were collected from selected households and selected traders, respectively, in Port Harcourt, Rivers State, Nigeria. The wastewaters were collected with the aid of disinfected 1L plastic bottles. The bottled wastewaters were transported to the Microbiology laboratory, Rivers State University, Nigeria, and stored in a refrigerator at 4 °C until required for analysis and experimentation.

2.2 Analysis of the wastewaters

The wastewaters were analyzed for concentrations of carbohydrate, fats/lipids, protein, nitrogen, and phosphorus. The population of total heterotrophic bacteria (THB) and total fungi (TF) in the wastewaters were also determined.

Determination of carbohydrate, fats, protein, nitrogen and phosphorus: The concentration of carbohydrate was determined using the phenol-sulphuric acid method (Nielsen, 2010); fats/lipids were determined through soxhlet extraction and gravimetry (Perera and Brown, 1996); protein and nitrogen was determined using the Kjeldahl method (Bremner & Mulvaney, 1982; Maehre *et al.*, 2018); and phosphorus was determined using the ascorbic acid method (APHA, 1992).

Determination of populations of bacteria and fungi: The populations of total heterotrophic bacteria (THB) and total fungi (TF) were determined by subjecting 1 ml of the samples through ten-fold serial dilution using sterile normal saline. Aliquots of 0.1 ml of the different dilutions were then spread inoculated in duplicates on plates of nutrient agar (NA) as the

culture medium for THB and Sabouraud dextrose agar (SDA) as the culture medium for TF. Inoculated NA and SDA medium were incubated at ambient temperatures (28 – 31 °C) for 24 hours and 4 days respectively. After incubation, ensuing colonies on the NA and SDA medium were counted and used to calculate the populations of THB and TF respectively.

2.3 Bioremediation experiment

Experimental units were set up using 4 glass troughs (length: 30 cm, width: 20 cm, height: 20 cm). The troughs were filled with 1 Kg garden soil, which was then artificially polluted with 100 ml crude-oil. The troughs were labeled C, B, G, and B+G, and then allowed undisturbed at ambient temperatures (28 – 31 °C) for a week. After this period, 40 g composite soil sample was collected from the troughs for determination of pH, total hydrocarbon concentration, total organic carbon, nitrogen, and phosphorus. The populations of THB, TF, hydrocarbon utilizing bacteria (HUB), and hydrocarbon utilizing fungi (HUF) in the soil sample were also determined. After these determinations the crude-oil polluted soils in the troughs were treated as follows: trough B (setup B) with 150 ml wastewater from cooking of cowpea (WCC), trough G (setup G) with 150 ml wastewater from cooking of groundnut-pods (WGP), trough B+G (setup B+G) with 75 ml WCC and 75 ml WGP, and trough C (control setup: setup C) with 150 ml tap-water. Tilling of the soils in the setups was carried out with the aid of a disinfected hand trowel, once a week. Also, 100 ml tap water, WCC, WGP, and 1:1 mixture of WCC and WGP were added to the polluted soils in their respective troughs once in a week. The setups were maintained at ambient temperatures (28 – 31 °C) for 3 weeks.

2.4 Monitoring of the bioremediation experiment

Total hydrocarbon concentration, total organic carbon, and phosphorus concentration in the soils in the bioremediation setups were determined at 7 days interval. Also, the populations of total heterotrophic bacteria (THB), total fungi (TF), hydrocarbon utilizing bacteria (HUB), and hydrocarbon utilizing fungi (HUF) in the soils were determined at 7 days interval. Nitrogen concentration in the soils was determined at the beginning and end of the experiment. Nitrogen, Phosphorus, and population of THB were also determined for the tap water used in the control setup.

Determination of total hydrocarbon concentration: Total hydrocarbon concentration (THC) was determined as follows: about 5 g of polluted soil was placed in 250 ml capacity beaker, followed by addition of 10 ml N-hexane. The resulting mixture was agitated for about 30 seconds, and then filtered through Whatman No. 1 filter paper held in glass funnel into clean 150 ml capacity conical flask. The filtrate was subjected to absorbance measurement using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China) set at 420 nm. Absorbance reading was used to determine THC through extrapolation from previously obtained plot of concentrations of crude-oil in N-hexane against absorbance at 420 nm.

Determination of total organic carbon: Total organic carbon (TOC) was determined using a modification of the colorimetric variant of the Walkley and Black method (FAO, 2020). Firstly, 5, 10, 15, 20, and 25 µg/ml sucrose solutions representing solutions containing 0.21, 0.42, 0.63, 0.84, and 1.05 % carbon were prepared by transferring 5, 10, 15, 20, and 25 ml of 1 g/L sucrose solution into 250 ml conical flasks, followed by the addition of 10 ml potassium dichromate (1N) and 20 ml concentrated sulphuric acid (97 %). The solutions were agitated gently, allowed to cool on asbestos sheet, and then the volumes were made up to 100

ml with distilled water. The absorbance of the resulting solutions now containing 0.21, 0.42, 0.63, 0.84, and 1.05 % carbon was measured at 660 nm using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). The absorbance readings were plotted against the carbon concentrations (% C) to obtain a straight-line graph. The slope (m) of the straight line graph was calculated, and the intercept (c) of the straight line on the y-axis determined. The slope (m) and intercept (c) were then used to generate the equation of the graph following the format for the equation of a straight line graph (y = mx + c). Following the determination of the equation of the graph, the TOC in the soil samples were determined as follows: about 1 g soil was placed in a conical flask and 10 ml potassium dichromate (1N) and 20 ml concentrated sulphuric acid (97 %) were added. The mixture was agitated gently and allowed to cool on asbestos sheet. Afterwards, the volume was made up to 100 ml with distilled water and the resulting mixture allowed for about 10 minutes. After this duration, the mixture was filtered through a Whatman No. 1 filter paper, and the absorbance of the filtrate was measured at 660 nm using a 721 VIS Spectrophotometer (Huanghua Faithful Instrument Co. Ltd, China). Absorbance reading in conjunction with the equation of the previously obtained graph was then used to calculate TOC.

Determination of nitrogen and phosphorus: The concentration of nitrogen was determined using the Kjeldahl method (Bremner & Mulvaney, 1982); and phosphorus was determined using the ascorbic acid method (APHA, 1992).

Determination of populations THB, TF, HUB, and HUF: The populations of THB, TF, HUB, and HUF were determined as follows: 1g soil samples were placed in 10 ml sterile normal-saline solutions to obtain soil/normal-saline mixtures which were serially diluted to 10^{-5} dilutions. Aliquot of 0.1 ml of the dilutions were spread inoculated on plates of appropriate culture medium using sterile bent glass rod. Nutrient agar (NA) was used as the culture medium for THB, Potato dextrose agar (PDA) as the culture medium for TF, mineral salts agar (MSA) containing ketoconazole (100 mg/ml) as the culture medium for HUB, and MSA containing antibiotics (streptomycin and erythromycin, 50 µg/ml each) as the culture medium for HUF. The composition (g/L) of the MSA used is as follows: $MgSO_4.7H_2O$ - 0.42, KH_2PO_4 - 0.83, NaCl - 10.0, KCl - 0.29, Na_2HPO_4 - 1.25, $NaNO_3$ - 0.42, Agar – 15.0 (Source: Odokuma & Dickson, 2003). Inoculated MSA plates were supplied with petroleum hydrocarbons using the vapour phase transfer technique (Ebuehi *et al*., 2005), and incubated at ambient temperatures (28 – 31 °C) for 7 days. Inoculated PDA plates were also incubated at the same temperature but for 4 days, while inoculated NA plates were incubated at 37 °C for 24 hours. After incubation, counts of ensuing colonies on plates of NA, PDA, MSA containing ketoconazole, and MSA containing antibiotics were used to calculate the THB, TF, HUB, and HUF population respectively.

2.5 Extent of hydrocarbon degradation

At the end of the duration (3 weeks) of the bioremediation experiment, the extents of hydrocarbon degradation (EHD) in the setups were determined using the initial and final THC concentrations. This was achieved using the equation below:

$$\text{EHD (\%)} = \frac{(\text{Initial THC} - \text{Final THC}) \times 100}{\text{Initial THC}}$$

2.6 Proportions of hydrocarbon utilizing microorganisms

The proportions of hydrocarbon utilizing microorganisms (% HUB and % HUF) were calculated using the equations below:

$$\% \text{ HUB} = \frac{\text{HUB population}}{\text{THB population}} \times 100; \qquad \% \text{ HUF} = \frac{\text{HUF population}}{\text{TF population}} \times 100$$

2.7 Isolation and identification of hydrocarbon utilizing bacteria and fungi

Bacterial and fungal colonies on enumerated MSA plates were isolated and sub-cultured onto sterile NA and SDA plates respectively, and coded. Isolated bacteria were subjected to Gram staining & microscopic examination, and the following biochemical/physicochemical tests: catalase, oxidase, motility, citrate utilization, indole production, Methyl red (MR), Vogues-Proskauer (VP), starch hydrolysis, and fermentation tests using glucose, lactose, maltose, sucrose, mannitol, xylose, and glycerol. Reaction pattern to these tests in addition to microscopic morphology were used in identifying the isolates by comparing with information on "identification of bacteria" in Madigan *et al*., (2000), Prescott *et al*., (1999), and Skerman (1967). Isolated fungi were subjected to colonial characterization and microscopic examination. Fungal isolates were identified by comparing their colonial characteristics and microscopic morphology with information on "identification of fungi" in Zafar *et al*., (2017).

3. RESULTS

3.1 Organic composition of the wastewaters and tap-water used in the setups

The concentrations of total organic carbon (TOC) and nutrients in the wastewaters and tap-water used in the setups are presented in Table 1. From the Table, it can be seen that wastewater form cooking of cowpea (WCC) had the highest total organic carbon, protein and nitrogen content; wastewater from cooking of groundnut-pods (WGP) had the highest carbohydrate content; and the tap-water used in setup C had the highest amount phosphorus.

Table 1: Organic composition of the wastewaters and tap-water

Organic composition	WCC	WGP	Tap-water
TOC (%)	0.67	0.41	ND
Carbohydrate (% glucose)	1.43	3.52	ND
Fats/lipids (%)	0.04	0.04	ND
Protein (%)	0.76	0.40	ND
Nitrogen (%)	0.12	0.06	0.001
Phosphorus (%)	0.0003	0.0002	0.0021

WCC: wastewater form cooking of cowpea, WGP: wastewater from cooking of groundnut-pods, TOC: total organic carbon, ND: not determined.

3.2 Microbial populations in the wastewaters and tap-water

The total heterotrophic bacterial populations in wastewater form cooking of cowpea (WCC), wastewater from cooking of groundnut-pods (WGP), and tap-water used in the control setup were 6.03×10^4, 2.05×10^3, and 4.40×10^2 CFU/ml respectively. The total fungal populations in WCC and WGP were 2.53×10^3 and 8.50×10^2 CFU/ml respectively.

3.3 Hydrocarbon concentration and extent of hydrocarbon degradation in the setups

The concentrations of hydrocarbon in the bioremediation experimental setups at weekly intervals are presented in Figure 1. In the Figure, it can be seen that there was decline in total hydrocarbon concentration (THC) in the control setup (setup C), setup B, and setup G. There was however, little or no decline in THC in setup B+G.

The extent of hydrocarbon degradation in the setups is presented in Table 2. In the Table, it can be seen that the setup in which WCC was added (setup B) had the highest hydrocarbon degradation (45.5 %), while the setup in which WCC+WGP was added (setup B+G) had the least hydrocarbon degradation (1.3 %).

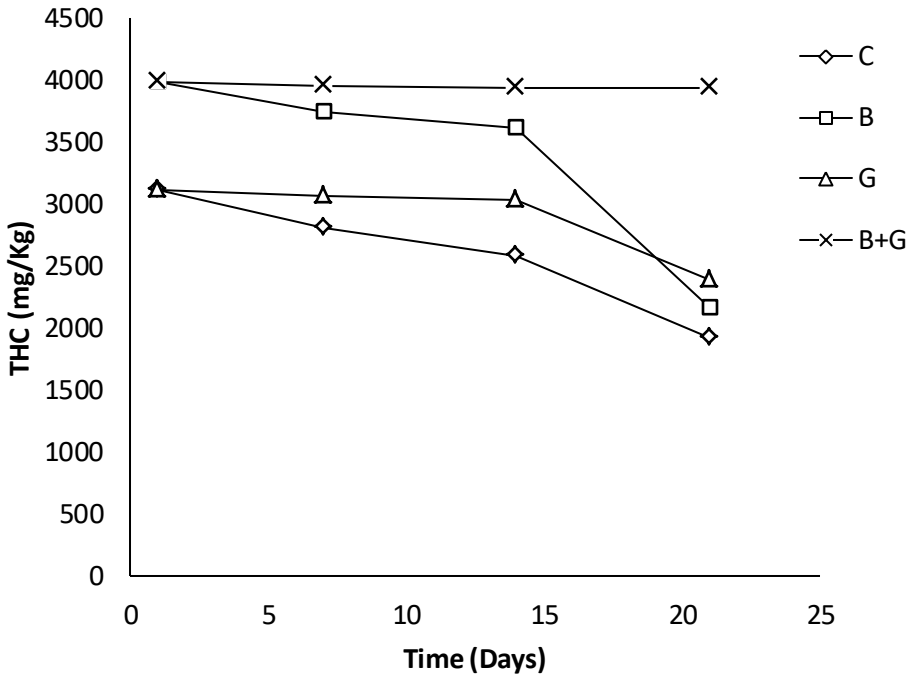

Fig. 1: Total hydrocarbon concentration (THC) in the setups at weekly intervals.

C: setup in which tap water was added (control setup), B: setup in which wastewater from cooking of cowpea (WCC) was added, G: setup in which wastewater from cooking of groundnut-pods (WGP) was added, B+G: setup in which WCC and WGP were added.

Table 2: Extent of hydrocarbon degradation in the setups

Setup	Initial THC (mg/Kg)	Final THC (mg/Kg)	EHD (%)

C	3110	1930	37.9
B	3980	2170	45.5
G	3110	2390	23.2
B+G	3980	3930	1.3

THC: Total hydrocarbon concentration, EHD: Extent of hydrocarbon degraded.

3.4 TOC, nitrogen, and phosphorus concentration in the experimental setups

The TOC and phosphorus concentrations in the setups on day 1, 7, and 21 are presented in Figure 2 and 3. In Figure 2 it can be seen that there was increase in TOC in setup G, and decrease in TOC in the other setups. In Figure 3 it can be seen that from day 1 to 21 there was decrease in phosphorus in all the setups with the decrease been greatest in setup C and B.

The nitrogen concentration in the setups at the beginning and end of the experiment is presented in Figure 4. From the Figure it can be seen that there was reduction in nitrogen in all the setups; the decrease was greatest in setup B, and least in setup B+G.

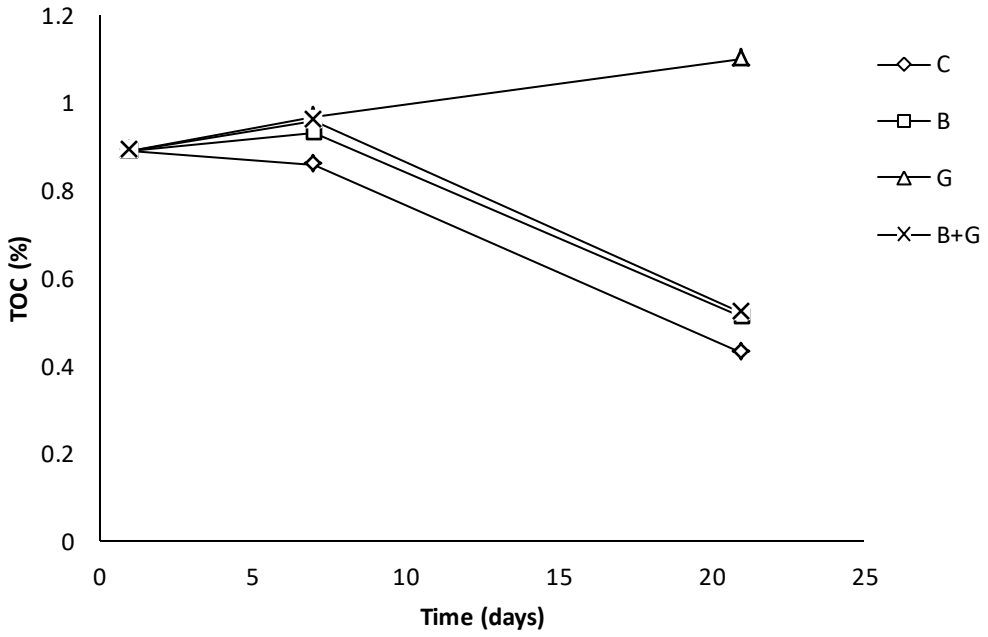

Fig. 2: Total organic carbon (TOC) in the setups on day 1, 7 and 21.

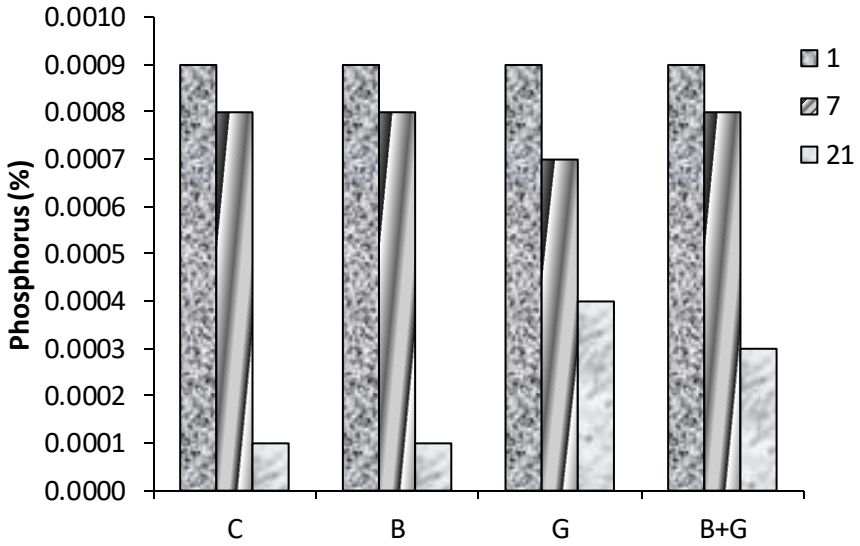

Fig. 3: Phosphorus concentration in the setups on day 1, 7 and 21.

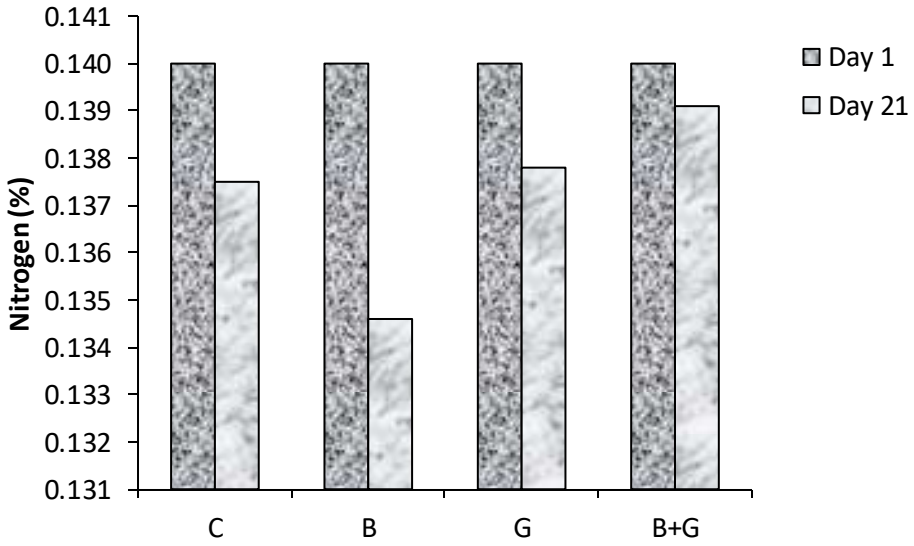

Fig. 4: Nitrogen concentration in the setups at the beginning and end of the experiment.

3.5 Population of THB and HUB in the bioremediation setups

Throughout the duration of the experiment the populations of total heterotrophic bacteria (THB) in setups were relatively high, ranging from 3.1×10^6 to 6.5×10^6 CFU/g (Table 3). The populations of hydrocarbon utilizing bacteria (HUB) were lower than the THB populations; ranging from 2.05×10^4 to 6.30×10^4 CFU/g (Table 4). The percentage HUB in the setups (Figure 5) shows that there was decrease in % HUB which was eventually followed by increase. The decrease was least in setup B, and the ensuing increase was also highest in setup B. On the other hand, the decrease was greater in the control (setup C), and the ensuing increase was also least in the control.

Table 3: Total heterotrophic bacterial populations in the bioremediation setups

Setups	THB (CFU/g)			
	day 1	**day 7**	**day 14**	**day 21**
C	4.84×10^6	5.45×10^6	4.25×10^6	3.85×10^6
B	5.36×10^6	4.03×10^6	5.05×10^6	3.10×10^6
G	5.48×10^6	6.50×10^6	4.80×10^6	3.60×10^6
B+G	5.08×10^6	5.05×10^6	5.75×10^6	3.70×10^6

THB: Total heterotrophic bacteria.

Table 4: Hydrocarbon utilizing bacterial populations in the bioremediation setups

Setups	HUB (CFU/g)			
	day 1	day 7	day 14	day 21
C	4.90×10^4	2.05×10^3	2.10×10^3	2.60×10^4
B	5.55×10^4	3.25×10^4	4.80×10^4	4.60×10^4
G	6.30×10^4	4.75×10^3	2.25×10^3	2.69×10^4
B+G	5.60×10^4	2.05×10^4	5.50×10^4	3.05×10^4

HUB: Hydrocarbon utilizing bacteria

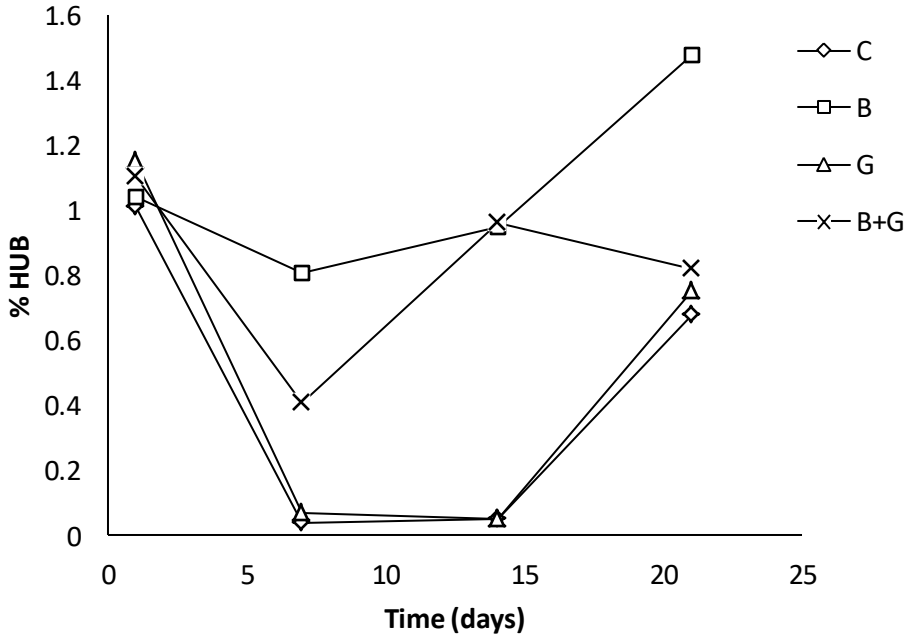

Fig. 5: Percentage hydrocarbon utilizing bacteria (HUB) in the bioremediation setups.

3.6 Population of TF and HUF in the bioremediation setups

The populations of total fungi (TF) in the setups were in the range of 1.23×10^5 to 2.95×10^5 CFU/g (Table 5). The populations of hydrocarbon utilizing fungi (HUF) were lower than the TF populations; ranging from 1.10×10^4 to 3.23×10^4 CFU/g (Table 6). The percentage HUF in the setups (Figure 6) shows that there was decrease in % HUF with time, except in setup B+G and the control (setup C) where an increase occurred from day 7 – 14 and day 14 – 21 respectively.

Table 5: Total fungal (TF) populations in the bioremediation setups

Setups	**TF (CFU/g)**			
	day 1	**day 7**	**day 14**	**day 21**
C	1.65×10^5	1.28×10^5	2.08×10^5	1.24×10^5
B	2.10×10^5	2.53×10^5	1.30×10^5	2.95×10^5
G	1.23×10^5	2.33×10^5	1.98×10^5	2.20×10^5
B+G	2.00×10^5	2.83×10^5	1.63×10^5	2.31×10^5

Table 6: Hydrocarbon utilizing fungal **(**HUF) populations in the bioremediation setups

Setups	**HUF (CFU/g)**			
	day 1	day 7	day 14	day 21
C	2.28×10^4	1.78×10^4	2.60×10^4	2.20×10^4
B	2.48×10^4	3.03×10^4	1.10×10^4	2.13×10^4
G	2.15×10^4	3.23×10^4	2.48×10^4	2.18×10^4
B+G	2.78×10^4	2.40×10^4	2.58×10^4	2.80×10^4

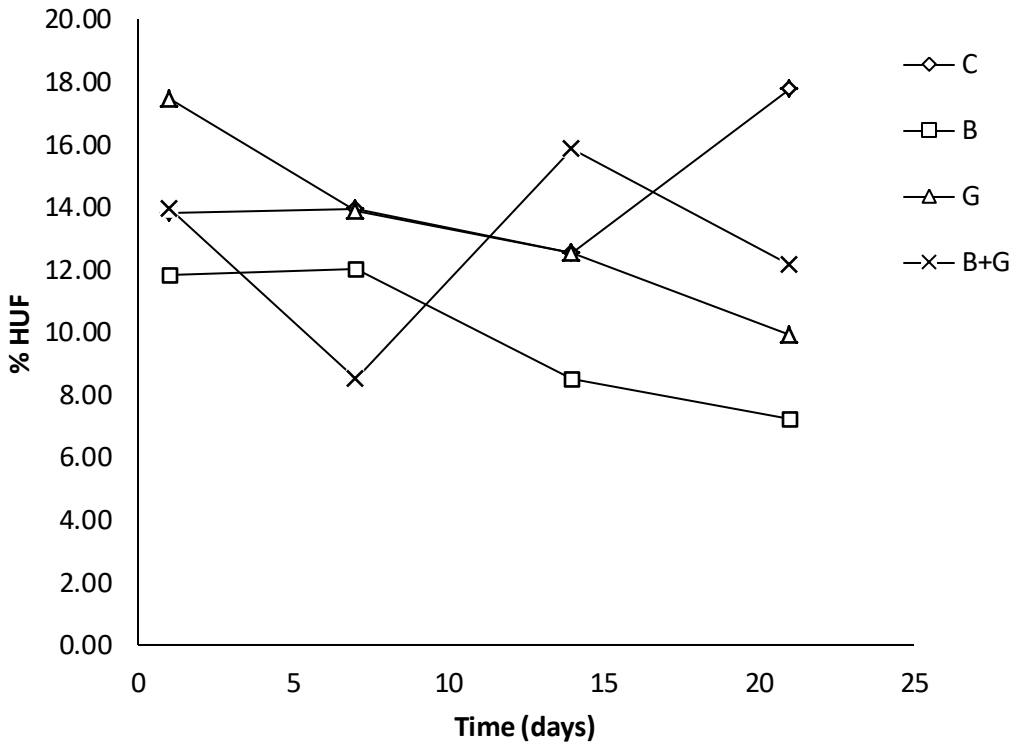

Fig. 6: Percentage hydrocarbon utilizing fungi (HUF) in the bioremediation setups.

3.7 Identity of the hydrocarbon utilizing microorganisms

Hydrocarbon utilizing fungi (HUF) isolated from MSA plates supplemented with antibiotics (streptomycin and erythromycin) were coded as A1-A5. Their colonial characteristics and microscopic morphology are presented in Table 7. The identity of the isolates is suspected as follows: A1 – *Penicillium* sp., A2 & A5 – *Aspergillus* sp., A3 – *Candida* sp., and A4 – *Fusarium* sp.

Hydrocarbon utilizing bacteria isolated from MSA plates supplemented with ketoconazole were coded as H1-H9. Their reaction pattern to the biochemical/physicochemical tests used is presented in Table 8. The identity of the isolates is suspected as follows: H1 – *Bacillus* sp., H2 – *Micrococcus* sp., H3 & H7 – *Acinetobacter* sp., H4, H6 & H8 – *Staphylococcus* sp., and H5 & H9 – *Pseudomonas* sp.

Table 7: Colonial characteristics and microscopic morphology of isolated HUF

Isolate	Colonia characteristic	Microscopic morphology	Suspected organism
A1	Bluish green radially furrowed cottony growth surrounded by white periphery, with reverse white colour	Septate hyphae, with branched conidiophores	*Penicillium* sp.
A2	Black cottony lawny growth, with reverse white colour	Septate hyphae, with non-septate round head	*Aspergillus* sp.

		conidiophores	
A3	Smooth circular glistening colonies	Oval shaped cells	*Candida* sp.
A4	White cottony growth with wrinkled surface and reverse yellow colour	Septate hyphae, with septate un-branched conidiophores and banana shaped conidia	*Fusarium* sp.
A5	Grey-green velvety growth, with reverse white colour	Septate hyphae, with non-septate round head conidiophores	*Aspergillus* sp.

Table 8: Reaction pattern of isolated HUB to selected biochemical/physicochemical tests

	Isolates								
Tests	**H1**	**H2**	**H3**	**H4**	**H5**	**H6**	**H7**	**H8**	**H9**
GS	+	+	-	+	-	+	-	-	-
MM	Rods	Cocci	Rods	Cocci	Rods	Cocci	Rods	Rods	Rods
CT	+	+	+	+	+	+	+	+	+
OX	+	+	-	-	+	-	-	-	+
MT	+	-	-	-	+	-	-	-	+
CU	+	-	-	+	+	+	-	+	+
IN	-	-	-	-	-	-	-	-	-
MR	+	-	-	-	-	-	-	-	-
VP	+	+	-	+	-	+	-	+	-
SH	+	-	-	-	-	-	-	-	-
GF	A	A	0	A	A	A	A	AG	A
LF	A	0	A	AG	0	AG	A	AG	0
MLF	A	0	0	A	0	A	0	A	0
SF	A	0	0	A	0	A	0	A	0
MnF	A	A	0	A	A	A	0	A	A
XF	A	0	0	0	0	0	0	A	A
GyF	A	0	0	A	0	A	0	A	A

SO	*Bacillus*	*Micrococcus*	*Acinetobacter*	*Staphylococcus*	*Pseudomonas*	*Staphylococcus*	*Acinetobacter*	*Staphylococcus*	*Pseudomonas*

GS: Gram stain, MM: microscopic morphology, CT: catalase, OX: oxidase, MT: motility, CU: citrate utilization, IN: indole production, MR: Methyl Red, VP: Vogues Proskauer, SH: starch hydrolysis, GF: glucose fermentation, LF: lactose fermentation, MLF: maltose fermentation, SF: sucrose fermentation, MnF: mannitol fermentation, XF: xylose fermentation, GyF: glycerol fermentation, A: acid produced, AG: acid and gas produced, 0: no acid nor gas produced, SO: suspected organism.

4. DISCUSSION

Addition of nutrients is one of the means by which the hydrocarbon degrading ability of hydrocarbon utilizing microorganisms (HUMs) is enhanced. Nutrients can be found in wastewaters generated during the processing of food crops such as legumes. In this study, wastewaters from cooking of the legumes used were shown to contain nutrients especially nitrogen and phosphorus (Table 1). Nitrogen and phosphorus promotes efficient oxidation of carbon substrates while also accelerating bacterial growth (Sui *et al.*, 2021). Wastewater from cooking of cowpeas (WCC) had the highest nitrogen and phosphorus concentration. This could be the reason why setup B (the setup in which crude-oil polluted soil was treated with WCC) had the highest percent HUB throughout the study period as observed in Figure 5. Also, setup B having the highest percent HUB must have been the reason why it had the highest extent of hydrocarbon degradation (EHD) as observed in Table 3. Also, WCC had the highest microbial load of the wastewaters and tap-water used, thus translating into the highest level of bioaugmentation implemented, in addition to biostimulation. In similar researches (Abioye *et al.*, 2010; Agamuthu *et al.*, 2013; Williams & Amaechi, 2017), it is observed that setups in which the organic waste with the highest concentration of nitrogen and phosphorus (or only nitrogen) was added had the highest count of HUB and also the highest level of hydrocarbon degradation.

The control setup (setup C) had a higher EHD than setup G and B+G (Table 3). This could be attributed to the phosphorus content in the tap-water used in the control setup; it had the highest phosphorus concentration and also some population of bacteria (Table 1 and 2). On the other hand, wastewater from cooking of groundnut-pods (WGP) had the highest carbohydrate (% glucose) content. This translated into the crude-oil polluted soil treated with WGP (setup G) having a gradually increasing TOC as observed in Figure 2; unlike the other setups where TOC gradually reduced, with setup C having the least TOC. Therefore, the HUMs in setup G had a choice between using the carbohydrate made available to them or the petroleum-hydrocarbons as their source of carbon and energy. Just as any bacteria will utilize glucose (a monosaccharide) first before utilizing any other monosaccharide or disaccharides when mixtures of sugars are present in its medium (Todar, 2020), the HUMs in setup G may have followed the same pattern of utilizing the non-toxic bio-molecules (carbohydrates) before utilizing the toxic chemical molecules (petroleum-hydrocarbons). In setup C, petroleum-hydrocarbons were the only source of carbon. Thus the HUMs in setup C had only one choice; to degrade the hydrocarbons. The tap-water used in setup C had the highest

phosphorus concentration, and since phosphorus is among the elements that promotes efficient bio-oxidation of carbon substrates (Sui *et al.*, 2021), the hydrocarbon oxidation and subsequent degrading ability of HUMs in setup C must have been greatly enhanced. This could be the possible reason why higher EHD was observed in setup C as compared to the EHD in setup G and B+G.

At the end of the experimental period, there was reduction in phosphorus and nitrogen concentrations in all the setups, with setup C and B having the lowest phosphorus concentration as observed in Figure 3, and setup B having the lowest nitrogen concentration as observed in Figure 4. This indicates that HUMs present in the setups were utilizing these nutrients for them to be able to degrade the hydrocarbons. It also indicates that due to the higher extent of nitrogen and phosphorus utilization in setup B followed by setup C, therefore the highest EHD observed in setup B followed by setup C. In contrast, setup B+G had the lowest nitrogen utilization, and second to lowest phosphorus utilization, thus it lowest EHD. In other related studies (Ezeonu *et al.*, 2012; Ogugbue *et al.*, 2017; Solomon *et al.*, 2018), higher percentage decrease in nitrogen and phosphorus in crude-oil polluted soils treated with organic-wastes is observed to be associated with higher reduction in total petroleum hydrocarbons.

The pattern of change in percent HUF in the setups did not follow the pattern observed in EHD and nutrient utilization. Rather there was decrease in percent HUF, except in setup C. This could indicate that the HUF population played little role in petroleum-hydrocarbon degradation compared to the HUB population. In a study carried out by Nkwelang *et al* (2008) on diversity, abundance and succession of HUMs in soil polluted with oily sludge, it was shown that the identified HUB were present in the polluted soil throughout the six months experimental period, whereas of the identified HUF, few remained till the end of the experimental period; majority were found only during the first two months. In another such study carried out by Galitskaya *et al* (2021), it was shown that abundance of oil-degrading bacteria became dominant in soils polluted with petroleum, irrespective of the pollution level and soil type, whereas the successions of fungal communities differed between soils.

The hydrocarbon utilizing microorganisms isolated in this study including *Bacillus, Micrococcus, Acinetobacter, Staphylococcus, Pseudomonas, Penicillium*, *Aspergillus, Candida* and *Fusarium* have been shown in other works (Akwukwaegbu *et al.*, 2019; Ali *et al.*, 2011; Chikere *et al.*, 2009; Ewoh & Peekate, 2021) to be microorganisms involved in degradation of petroleum-hydrocarbons in polluted soil and water.

5. CONCLUSION

The study reveals that wastewater from cooking of cowpeas have appreciable amount of nitrogen and phosphorus, and can be applied in the bioremediation of crude-oil polluted soil. On the other hand, wastewater from cooking of groundnut-pods have high amount of carbohydrate and will add its carbohydrate content to the soil thereby limiting hydrocarbon degradation. Also, it is discovered that tap-water containing high phosphorus concentration has potential for use in bioremediation of crude-oil polluted soil. The use of food processing wastewaters having appreciable amount of nitrogen and phosphorus in bioremediation of crude-oil polluted soil can reduce the expenses associated with the use of compounded nutrient sources, and circumvent the potential problems associated with the use of wastes of animal origin which include large-scale introduction of pathogens into the environment.

Conflict of interest

The authors declare that there is no conflict of interests.

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